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Assessment of *In-Situ* Reductive Dechlorination Using Compound-Specific Stable Isotopes, Functional-Gene Pcr, and Geochemical Data

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Abstract

Isotopic analysis and molecular-based bioassay methods were used in conjunction with geochemical data to assess intrinsic reductive dechlorination processes for a chlorinated-solvent contaminated site in Tucson, Arizona. Groundwater samples were obtained from monitoring wells within a contaminant plume comprising tetrachloroethene and its metabolites trichloroethene, cis-1,2-dichloroethene, vinyl chloride, and ethene, as well as compounds associated with free-phase diesel present at the site. Compound specific isotope (CSI) analysis was performed to characterize biotransformation processes influencing the transport and fate of the chlorinated contaminants. PCR analysis was used to assess the presence of indigenous reductive dechlorinators. The target regions employed were the 16s rRNA gene sequences of *Dehalococcoides* sp. and *Desulfuromonas* sp., and DNA sequences of genes *pceA*, *tceA*, *bvcA*, and *vcrA*, which encode reductive dehalogenases. The results of the analyses indicate that relevant microbial populations are present and that reductive dechlorination is presently occurring at the site. The results further show that potential degrader populations as well as biotransformation activity is non-uniformly distributed within the site. The results of laboratory microcosm studies conducted using groundwater collected from the field site confirmed the reductive dechlorination of tetrachloroethene to dichloroethene. This study illustrates the use of an integrated, multiple-method approach for assessing natural attenuation at a complex chlorinated-solvent contaminated site.

Introduction

Chlorinated aliphatic hydrocarbons, such as tetrachloroethene, trichloroethene, and carbon tetrachloride, are among the most common groundwater contaminants in the USA due to their prior widespread use for numerous industrial and commercial applications. As such, major effort is being focused on characterization and remediation of sites contaminated by these

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Supporting Information: Additional information regarding the materials and methods used for this study are available in the Supporting Information published online. In addition, several Figures and Tables containing supplemental information are available therein.

compounds (e.g., 1,2). Recently, great interest has developed in using monitored natural and enhanced attenuation (MNA) for the management of chlorinated-aliphatic groundwater contaminant plumes. Robust site-characterization methods are required to accurately evaluate the feasibility of applying MNA to a site. Microbial transformation processes (e.g., reductive dechlorination for chlorinated aliphatics) are typically the key, predominant factor contributing to attenuation of organic contaminants. Thus, characterizing the occurrence, magnitude, and rate of microbial transformation processes is a critical element of evaluating MNA feasibility. Standard methods of site characterization, such as geochemical profiling and microcosm studies, typically have a high degree of associated uncertainty. Therefore, recent research has focused on alternative characterization methods, such as analysis of shifts in the isotopic composition of specific compounds and molecular-based assays of microbial communities.

Compound specific isotope (CSI) analysis allows differentiation between microbial and non-microbial mediated contaminant attenuation, as well as characterization of the extent of biotransformation (e.g., 3,4). This method is based on the isotopic fractionation that occurs during biotransformation of organic compounds due to differential reaction rates. For example, given the dynamics of isotopic fractionation, the products of microbial transformation are initially more depleted in ^{13}C and more enriched in ^{12}C than the parent compounds. Conversely, the parent compounds become enriched in ^{13}C and depleted in ^{12}C as they biodegrade. Compound specific isotope analysis has been used to assess the occurrence and extent of biotransformation for both aromatic hydrocarbons (e.g., 5,6) and chlorinated aliphatics (e.g., 7-10) for laboratory and field systems.

Culture-independent molecular techniques are reliable tools for characterizing complex microbial communities, tracking specific species in environmental systems, and monitoring community level dynamics both spatially and temporally (e.g., 11-13). The presence of indigenous microbial communities with the potential to perform reductive dechlorination has been evaluated at the field scale by assaying samples from contaminated sites for known dechlorinators, using polymerase chain reaction (PCR) to amplify specific 16s rRNA gene sequences (e.g., 14-18). This approach identifies the presence of specific populations, but does not determine whether or not the detected microbial populations have the specific gene sequences coding for reductive dechlorination of a particular compound (e.g., 12). For example, members of *Dehalococcoides* sp., which includes VC dechlorinators and non-VC dechlorinators, have a high degree of similarity (>98% identity) in their 16S rRNA sequence (19). Thus, screening for the presence of the key genes involved in reductive dechlorination may yield more specific information regarding biotransformation capability (12). The gene sequences involved in reductive dechlorination of tetrachloroethene (PCE), trichloroethene (TCE), *cis*-1,2-dichloroethene (DCE), and vinyl chloride (VC) have been recently published (Table S1), allowing the use of PCR primers to detect specific functional genes involved in reductive dechlorination.

The objective of this study was to evaluate the combined use of CSI and molecular-based bioassay methods for characterizing reductive dechlorination activity for a chlorinated-solvent contaminated site. Compound specific isotope analysis was used to differentiate between microbial and non-microbial attenuation processes, and to characterize the extent of biodegradation of a compound (DCE) that is itself a biotransformation product of other contaminants (PCE/TCE). This study affords the opportunity to evaluate the efficacy of CSI analysis for assessing reductive dechlorination of lower chlorinated hydrocarbons produced by the biotransformation of PCE and TCE for conditions wherein the original compounds remain present and are continuing to undergo biotransformation. In conjunction with the CSI analysis, PCR was used to assay for the presence of bacteria capable of reductive dechlorination of the chlorinated contaminants. Conventional 16s rRNA gene analysis was supplemented with

functional-gene PCR analysis targeting reductive dehalogenases to enhance characterization of the microbial community.

Materials and Methods

Site Description

The Park-Euclid WAQRF site (Figure 1) is located in Tucson, Arizona, and has been used for industrial dry cleaning purposes since 1947. Use of PCE, the only solvent reportedly employed, began at least in 1964 and ended in 1985. Chlorinated-solvent contamination appears to have reached a perched aquifer via disposal, unintentional spills, and pipe leaks. The perched aquifer is located 24 to 30 m below ground surface (bgs) and its thickness varies from a few centimeters to approximately 2.4 m, depending on aquitard topography. Although affected by seasonal fluctuations, the local groundwater flow is generally to the N-NE, with a mean hydraulic gradient of approximately 0.01. Hydraulic conductivity ranges from 2.6×10^{-7} to 4.9×10^{-5} m/sec and the average total organic carbon content of the sediments is 0.3% (20,21).

An aqueous-phase contaminant plume of approximately 450 m² is present within the aquifer, with maximum dissolved concentrations that have exceeded 40 mg/L for PCE and 100 mg/L for TCE and DCE within the source zone. Vinyl chloride, 1,1-DCE, and *trans*-1,2-DCE are also present at lower concentrations. A layer of free-phase liquid comprised of a mixture of diesel components (e.g. BTEX, PAH's, ketones) and chlorinated aliphatics (PCE, TCE, DCE, VC) is floating on top of the water table in a portion of the subsurface. Seventeen monitoring wells have been emplaced at the site since 1990 for characterization purposes. A soil vapor extraction (SVE) system consisting of four wells operated for several years in the vicinity of the dry-cleaning facility.

Geochemical Data and Groundwater Sample Analysis

Information for several geochemical parameters including groundwater temperature, conductivity, pH, oxidation-reduction (redox) potential, and Fe²⁺ is collected as part of the site monitoring program and are available from the Arizona Department of Environmental Quality (ADEQ) data base. Dissolved oxygen values for three of the monitoring wells were measured with a probe during the last sampling campaign of 2005. Data for dissolved volatile organic components have been collected since 1994.

The groundwater monitoring program does not include analysis for dissolved hydrogen, methane, ethane, or ethene. We therefore collected samples for their analysis. Groundwater samples were collected in duplicate in glass vials (headspace free), and preserved with acid (except for hydrogen samples). Samples were packed in ice and shipped via overnight delivery to Columbia Analytical Services, Air Quality Laboratory, CA. Dissolved gas analyses were conducted using method RSK-175 (22). Another set of duplicate groundwater samples were collected, filtered (0.4- μ m pore membrane) to remove microorganisms, and shipped on ice to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University for compound specific isotope analysis. Groundwater samples from selected monitoring wells were also collected for PCR analysis. Three sterile 40-ml glass vials were filled with groundwater (headspace free) from each well and placed in coolers after collection. The water samples were stored at 4°C for later use. Samples for most of the data analysis were collected using disposable bailers in November 2005. Samples for bioassay and dissolved gases tests were also collected in May 2005 and/or March 2006. A second round of samples for isotopic analysis was collected in December 2007.

Compound Specific Isotope Fractionation

Stable carbon isotope analyses were performed via gas chromatograph combustion isotope-ratio mass spectrometry (GC-C-IRMS) to determine compound specific $^{13}\text{C}/^{12}\text{C}$ concentrations in headspace. These analyses were conducted by the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University. The data were analyzed using the commonly employed Rayleigh equation (e.g., 23,24) to evaluate enrichment and to determine fractionation factors (the equations are presented in Supporting Information). Isotope fractionation factors for chlorinated ethenes determined in laboratory experiments have been reported for a range of microorganisms and consortia (8,9,25-30). The degree of isotope fractionation depends, among other factors, on bacterial species and geochemical conditions. The published values range from 0.994 to 0.998.

For field applications, it is advantageous to calculate the extent of biodegradation B (%) using isotopic data. An advantage of this approach is that the calculation of B is independent of the contaminant concentration measured in the field (e.g., 3). The methods used for these calculations are presented in Supporting Information.

PCR Analysis and Sequencing

DNA-PCR analyses were conducted to assay for bacteria capable of reductive dechlorination. *Dehalococcoides* ethenogenes strain 195 was the first reported isolate capable of dechlorinating PCE all the way to VC and to ethene (31). *Dehalococcoides ethenogenes* strain 195 and strain FL2 dechlorinate PCE to VC and ethene in a slow, cometabolic process that leads to VC accumulation (32,33). *Dehalococcoides* sp. strains BAV1 and VS dechlorinate all DCE isomers and VC to ethene; however, these strains do not appear to metabolize PCE or TCE (34). *Dehalococcoides* sp. strain GT has been shown to completely dechlorinate TCE to ethene (35). Conventional 16S rRNA gene-based analysis was used to target the most important group of known dechlorinators, *Dehalococcoides* sp., as well as *Desulfuromonas* sp. In addition, a comprehensive set of dehalogenase-gene-targeted assays were conducted, focusing on function-related markers, in an attempt to identify specific strains present (noted in Supporting Information).

The target sequences employed in this study include the 16s rRNA gene specific for *Dehalococcoides* sp. and *Desulfuromonas* sp., and those for the functional genes available through publication at the time of the analysis which are genes *pceA* found in five of the most recognized chlororespiring microorganisms, and *tceA*, *bvcA*, and *vcrA* found in *Dehalococcoides* sp. DNA was extracted directly from the groundwater samples collected from the site. The samples were prepared as discussed in the Supporting Information. Sequences of the primers used in these reactions are listed in Table S1, Supporting Information. PCR products were purified (QIAquick PCR Purification Kit, Qiagen, Valencia, CA) prior to sequencing at the University of Arizona's facility. Automated sequencing was performed with an Applied Biosystems 3730xl DNA Analyzer, using the specific primers previously described. Nucleotide sequences were compared with EMBL and GenBank databases at NCBI using BLAST (36, 37). The approach employed provides information useful for qualitative assessment of reductive dechlorination activity at a site. Quantitative characterization of specific populations and their activity was beyond the scope of the present study.

Microcosm Experiments

Laboratory microcosm experiments were conducted to characterize reductive dechlorination of tetrachloroethene. Given the unavailability of aquifer sediment, groundwater samples collected from the field site were used as the source of indigenous microorganisms. While this approach has been used in prior studies (e.g., 38,39,40), it is recognized that the results obtained may not fully reflect system composition or behavior (e.g., 41). The experiments were

conducted using standard procedures. Subsamples of groundwater collected from three locations (SVE-101, SVE-103, and PEP-9) were used as the source of microorganisms for the experiments. Detailed description of materials used and methods employed for the microcosm experiments are presented in Supporting Information. For selected experiments, aqueous subsamples collected from the microcosms at the completion of the experiments were subjected to PCR analysis using methods described above.

Results and Discussion

Contaminant and Geochemical Parameter Distribution

The distribution of dissolved-phase PCE and its metabolites at the study site is illustrated in Figure 1. The TCE and DCE plumes encompass larger regions relative to the PCE plume. Conversely, the VC plume is smaller than the other three and is confined to the area of the SVE system and well PEP-9. In general, PCE has been replaced by DCE (*cis*-1,2-DCE) as the primary contaminant in the groundwater plume. A concentration-time series is presented in Figure S1, Supporting Information, for well PEP-9. The observed behavior is representative of that obtained for all monitoring locations where decreases in concentrations of PCE and TCE and increases in concentrations of DCE and VC occur (Table S2 in Supporting Information). The PCE concentration for PEP-9 has decreased from 500 to 21 µg/L during the period from 2000 to 2005. The concentration of TCE has decreased from 2700 to 540 µg/L during that period, while the DCE concentration has increased from 270 to 2800 µg/L. The presence of dissolved VC was first detected in March 2001 and its concentration has since varied up to a maximum of 540 µg/L in February 2004. The presence of ethene (from ~1 to 2800 mg/L) has been determined in samples collected from several wells (see Table S3 in Supplemental Information). The presence and observed concentrations of PCE biodegradation byproducts are evidence of the occurrence of reductive dechlorination reactions.

Temporal variability of the spatial distribution of the contaminants is illustrated in Figure S2 of Supporting Information, which shows DCE concentrations for the A-A' cross-section (primary flow direction). The concentration of DCE increased from 2000 to 2004, and decreased in 2005. The total concentration of chloroethenes is observed to increase after the middle of 2004 (Figure S1). This may indicate increased mass flux of contaminants from the organic-liquid phase (diesel/chlorinated-solvent mixture), which acts as a long-term source of chlorinated aliphatics for the dissolved phase. Possible changes in mass fluxes, which may be caused by changes in water levels and mean-gradient directions, complicate site characterization and data analysis. It has been previously estimated that approximately 10,000 kg of contaminant were initially present in the subsurface (20).

The aqueous geochemistry of the local, uncontaminated groundwater is predominated by calcium bicarbonate, and comprises moderate alkalinity (280 mg/L), neutral pH, and an average redox potential (ASTM method A2580B) of 144 mV. The background concentrations of dissolved oxygen (DO), chloride, sulfate, and ferrous iron (Fe²⁺) are approximately 4 mg/L, 20-100 mg/L, 20-150 mg/L, and 1.8 mg/L respectively. Concentrations of selected geochemical parameters within the contaminant plume relative to background are presented in Figure S3, Supporting Information for the A-A' cross-section. Chloride, which is produced during reductive dechlorination of chloroethenes, is significantly higher than the background (Figure S3a). Iron reducing conditions, indicated by increased levels of dissolved ferrous iron (Figure S3b), and increased alkalinity, indicated by total CaCO₃ (Figure S3c), support the presence of reducing conditions within the contaminant plume (e.g., 42).

Concentrations of dissolved gases within the contaminant plume are presented in Table S3 in Supporting Information. The DO values are below background (Figure S3d) and may indicate development of anaerobic microenvironments at the center of the plume. As noted above,

ethene, a product of reductive dechlorination of chlorinated aliphatics, is present. This suggests that reductive dechlorination is an active process within the study domain. Dissolved hydrogen was not detected in any of the samples and ethane concentrations were not significant. The presence of methane may be related to biodegradation of diesel-related compounds via methanogenesis. These concentrations indicate strongly reducing conditions and a water geochemistry favorable for reductive dechlorination (e.g., 42, 43).

Laboratory Microcosm Study

A representative set of results for the microcosm experiments is presented in Figure 2. Tetrachloroethene, the sole original compound, was biodegraded to below detection within approximately 400 hours. Concomitantly, DCE was produced at significant levels, whereas TCE accumulated temporarily to a small extent during the process. Vinyl chloride was not observed. These results demonstrate the reductive dechlorination of PCE to DCE in the presence of microorganisms collected in groundwater from the field site. The findings are in general agreement with the large scale conversion of PCE and TCE to DCE observed at the site.

The results of the PCR analyses conducted for aqueous subsamples collected from selected microcosm experiments were generally consistent with the results obtained from analysis of the field samples (to be discussed below). For example, the 16s rRNA gene amplification product (434 pb) specific for *Dehalococcoides* sp. was detected in samples from all three microcosm sets (source water originating from SVE-101, SVE-103, and PEP-9, respectively). Similarly, the gene sequence *vcrA*, which codes for VC-Rdase involved in the transformation of DCE, was found only in samples from the microcosm set with water originating from well SVE-103, as was observed for the field analyses. Despite the observed presence of VC-reductase genes in the microcosms, transformation of DCE to VC was not observed during the experiment. Given the uncertainty associated with use of groundwater as the source, it is unclear whether an insufficient number of microorganisms or experimental conditions may explain the observation that VC was not generated in the microcosm experiments, whereas it is observed in the field.

Carbon Isotope Analysis

Measures of $\delta^{13}\text{C}$ were obtained for DCE for all sampling locations. Values for PCE and TCE were obtained for only a few locations due to analytical constraints. Thus, the discussion will focus on DCE. Isotope values for DCE ranged from -28.5 to -31.9‰ across the site. This range of values is indicative of the impact of biotransformation. The carbon isotope composition for well SVE-101 (-31.90‰) is the least enriched in ^{13}C , indicating it is the least biodegraded of all samples. The aqueous fraction of DCE for this location is greater than 80% (Figure S4) and there are no indications of significant further biotransformation at this location given that production of VC or ethene is not evident. Thus, the data for this well was selected to represent the source values for $\delta^{13}\text{C}$ (R_0) and concentration (C_0) for DCE for the Rayleigh and extent-of-biodegradation analyses.

It has been observed that isotopic fractionation during reductive dechlorination of chloroethenes follows the Rayleigh Model, wherein enrichment of ^{13}C corresponds to decreases in concentration of the compound (e.g., 8-10). Data from the 2005 sampling campaign are plotted as natural logarithms of C_t/C_0 vs. R_t/R_0 (Figure 3), according to equation 1 in Supporting Information. A least-squares regression resulted in a fractionation factor (α) of 0.999, with $R^2 = 0.78$, which indicates a moderate correlation between the enrichment in ^{13}C and the lessening DCE concentration. The value for well SVE-103 appears to be an outlier, most likely due to the presence of free-phase organic liquid in that well. Removing the

SVE-103 data point from the regression analysis had no measurable impact on the α value while significantly increasing R^2 .

Isotopic analyses for complex field sites such as the focus of this study are influenced by uncertainty to a significantly greater extent than typical laboratory studies. Potential sources of uncertainty include the presence of organic liquid, spatial variability, and simultaneous production and consumption of multiple compounds. To partly address this uncertainty, a second data set was collected from the same location more than two years after the first. These data are also plotted in Figure 3. Comparing the two data sets, it is evident that DCE has become further enriched in ^{13}C while the aqueous concentrations have declined. An α value of 0.999 ($R^2 = 0.73$) was obtained for the second data set. It appears that the data point for well SVE-101 is an outlier, again likely due to effects associated with the presence of free-phase organic liquid. As for the first data set, removing the SVE-101 data point from the regression analysis had no measurable impact on the α value while greatly increasing R^2 . The results obtained for the two sampling rounds are very consistent, supporting the robustness of the CSI analysis. The fractionation factor obtained from this study is somewhat larger than values reported in prior studies for DCE, which have ranged from 0.980 to 0.986 (8,9,25-28).

As noted above, CSI data can be used to estimate extent of biodegradation. This was done for DCE using the equation presented in Supporting Information. The alpha values obtained from the Rayleigh analysis of the field data was used for one set of estimates, and a second set of estimates was obtained for comparison using a representative alpha value (0.986) from the literature (26). Values for the extent of biodegradation (B) for samples from 2005 ranged from 92 to 98% and 17 to 24% for the estimates obtained using α values of 0.999 and 0.986, respectively. Extents of biodegradations of approximately 70% were reported for two prior field studies involving chlorinated-ethene compounds (8, 9).

In a multiple-contaminant system, the isotopic signatures of biodegradation products can be affected by both production and consumption simultaneously. This complicates characterizing the degree of isotopic fractionation involved in each step of the dechlorination sequence. Specifically, additional production of the degradation product will contribute to ^{12}C enrichment, thus reducing the apparent rate of enrichment of ^{13}C due to biodegradation of that compound. The impact of simultaneous production and consumption is illustrated by examining the $\delta^{13}\text{C}$ values for PCE, TCE, and DCE obtained for well SVE-103. It is assumed that the value obtained for PCE (-28.7‰) indicates microbially-mediated enrichment, given that PCE was the sole compound used and considering the presence of metabolites (i.e., PCE currently composes less than 1% of the total mass of chloroethenes in the dissolved phase at that location; Figure S4, Supporting Information). PCE $\delta^{13}\text{C}$ values of -27 and -33.9‰ were reported for samples collected from the sources of contamination at two field sites (8, 9). It is also assumed that the value for TCE (-23.6‰) indicates substantial enrichment in ^{13}C given the comparatively low aqueous fraction of TCE present (Figure S4), which suggests that substantial biotransformation of TCE has occurred. The isotopic value for DCE (-28.8‰) suggests less enrichment in ^{13}C in comparison to TCE, which would be consistent with the fact that DCE was produced via TCE transformation and that microbial transformation of DCE is still ongoing. A simpler case is observed for well SVE-101, wherein only two compounds, TCE and DCE, comprise the majority of the total contamination (combined aqueous fraction of 0.99, see Figure S4). In this situation, the isotopic fractionation of TCE is no longer significantly influenced by production associated with PCE biodegradation. The TCE $\delta^{13}\text{C}$ value (-25.4‰) is again assumed to reflect substantial enrichment in ^{13}C given TCE's small remaining aqueous fraction (<20%). Conversely, the value for DCE (-31.9‰) is much less enriched, consistent with its apparent ongoing production from TCE biodegradation and minimal subsequent biotransformation to VC.

DNA-PCR analysis

The 16s rRNA gene amplification product (434 pb) specific for *Dehalococcoides* sp. was detected in groundwater samples from all three wells tested at the Park-Euclid site (Figure S5, Supporting Information). The 16s rRNA gene region specific to *Desulfuromonas* sp. was found in groundwater collected from one well (SVE-101). Sequence analysis of these PCR amplification products confirmed that the products were *Dehalococcoides* sp. and *Desulfuromonas* sp. 16s rRNA gene sequences as listed in Table 1. The former results confirm the presence of members of the *Dehalococcoides* group. However, as noted above, this analysis does not provide information regarding which specific members are present.

Genes encoding for synthesis of PCE-RDase were detected in all of the groundwater samples analyzed (Table 1), which confirms the presence of microorganisms with the genetic capacity to use PCE as an electron acceptor and biotransform it into DCE. This is consistent with the significant extent of conversion of PCE to DCE observed in the microcosm studies and in the field. Sequence analysis of the PCR amplification products confirmed the identity of these products. The gene sequence *pceA* in *Dehalospiillum multivorans* was confirmed in samples from well SVE-103 and detected once in well SVE-101. Meanwhile, the *pceA* sequence in *Dehalobacter restrictus* and *Desulfitobacterium* sp. Strain Y51 was detected in samples from all three wells. Thus, a moderate biodiversity of PCE-RDases appears to exist at the site. In addition, the gene sequence *tceA* in *Dehalococcoides* sp. was detected in samples from wells SVE-103 and PEP-9. The gene sequence *vcrA*, which codes for VC-Rdase involved in the transformation of DCE and VC into ethene, was found only in samples from well SVE-103. This is consistent with the observation of high concentrations of ethene in samples collected from this well, much higher than observed for any of the other wells. The gene sequence *bvcA* was not detected in any of the samples (Table 1).

Implications

The results of this study produced strong evidence that natural attenuation is occurring at the Park-Euclid site, and that reductive dechlorination constitutes the primary natural attenuation mechanism. Application of compound-specific isotope analysis appeared to be successful for characterizing the reductive dechlorination process. As discussed above, the study site represents a complex system, and such complexity may have influenced the CSI analysis. For example, while the data reported above indicate a predominance of anaerobic conditions at the site, sporadic episodes of aerobic conditions may develop in localized areas due for example to changes in groundwater levels, thereby allowing additional biotransformation processes to occur. However, such episodes may not have a significant impact on the overall CSI analysis given that isotopic fractionations measured for aerobic biodegradation of TCE, DCE, and VC have been shown to be significantly smaller than those reported for reductive dechlorination (e.g., 45,46). Another complicating factor for the study site is the presence of free-phase organic liquid, which serves as a long-term source of compounds to the aqueous phase via mass-transfer processes. Temporal and spatial variability in mass-transfer processes may further complicate analysis of the isotope data. In addition, simultaneous biotransformation of multiple precursor compounds and associated degradation products is actively ongoing at the site.

The uncertainty inherent to this field-scale application of CSI analysis was addressed by conducting a second sampling effort more than two years after the first, the results of which corroborated the initial results. In addition, applying functional-gene PCR allowed the identification of specific microorganisms present that are able to reductively dechlorinate PCE and its metabolites. Temporal and spatial variations in the distribution of the dehalogenators were observed, and would be expected for a heterogeneous, complex field site such as this. The results of this study illustrate that an integrated, multiple-method approach may enhance characterization of reductive dechlorination potential and activity for complex sites.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Park-Euclid site map showing the locations of the monitoring wells, the extent of the floating free product (diesel and chlorinated solvent mixture), the November 2005 PCE, TCE, DCE, and VC contours based on the maximum contaminant level (MCLs) for each respective compound, and the location of the A-A' cross-section.

Figure 2.

Illustrative results for the laboratory microcosm experiments. The abiotic (A) control consisted of reactors prepared in the same way as the treatments but with no microorganisms; they were subjected to the same autoclaving schedule as the treatment samples (autoclaved two times, the first after 48 hrs of incubation and the second 24 hrs later). The blank control consisted of reactors containing PCE dissolved in deionized water. Groundwater collected in March 2006 from SVE-101 was used for the experiment for which the data are shown. Error bars (95% confidence interval) are shown only when their size is larger than the symbols.

Figure 3.

Results of Rayleigh analysis of compound specific isotope data for DCE. The two data points considered to be outliers are identified by adjacent labels. Regression lines with and without outliers considered are included. Error bars (95% confidence interval) are shown only when their size is larger than the symbols.

Table 1

PCR amplification results for reductive dehalogenase gene and 16s rRNA gene sequences in groundwater samples collected from wells within the contaminant plume at the Park-Euclid site. (na) not analyzed; (+) positive response; (-) negative response.

Well	Date	<i>Dehalococcoides</i> sp.			<i>Desulfuromonas</i> sp. Strain BB1 and <i>D. chloroethenica</i>		<i>Dehalospirillum multivorans</i>		<i>Dehalobacter restrictus</i>		<i>Desulfitobacterium</i> sp. strain Y51	
		16s rRNA	<i>tce A</i>	<i>bvc A</i>	<i>ver A</i>	16s rRNA	<i>pceA</i>	<i>pceA</i>	<i>pceA</i>	<i>pceA</i>	<i>pceA</i>	<i>pceA</i>
SVE-103	05/05	+	-	-	+	-	-	-	-	-	-	-
	11/05	+	+	-	+	-	+	+	+	+	+	+
	03/06	+	+	-	-	-	+	+	+	+	+	+
SVE-101	05/05	na	na	na	na	na	na	na	na	na	na	na
	11/05	-	-	-	-	+	-	-	-	-	+	+
	03/06	+	-	-	-	+	+	+	+	+	+	+
PEP-9	05/05	+	na	-	-	-	-	+	+	+	+	+
	11/05	+	+	-	-	-	-	+	+	+	+	+
	03/06	+	+	-	-	-	-	+	+	+	+	+