

Flowpath Independent Monitoring of Reductive Dechlorination Potential in a Fractured Rock Aquifer

by Paul M. Bradley, Pierre J. Lacombe, Thomas E. Imbrigiotta, Francis H. Chapelle, and Daniel J. Goode

Abstract

The flowpath dependent approaches that are typically employed to assess biodegradation of chloroethene contaminants in unconsolidated aquifers are problematic in fractured rock settings, due to difficulties defining discrete groundwater flowpaths in such systems. In this study, the variation in the potential for chloroethene biodegradation with depth was evaluated in a fractured rock aquifer using two flowpath independent lines of field evidence: (1) the presence of the three biochemical prerequisites [electron donor(s), chloroethene electron acceptor(s), and chlororespiring microorganism(s)] for efficient chloroethene chlororespiration and (2) the in situ accumulation of chloroethene reductive dechlorination daughter products. The validity of this approach was assessed by comparing field results with the results of [1, 2-¹⁴C] *cis*-DCE microcosm experiments. Microcosms were prepared with depth-specific core material, which was crushed and emplaced in discrete packer intervals for 1 year to allow colonization by the indigenous microbial community. Packer intervals characterized by significant electron donor concentrations, elevated numbers of chlororespiring microorganisms, and high reductive dechlorination product to parent contaminant ratios correlated well with the production of ¹⁴C-labeled reductive dechlorination products in the microcosm experiments. These results indicate that, in the absence of information on discrete groundwater flowpaths, a modified approach emphasizing flowpath independent lines of evidence can provide insight into the temporal and spatial variability of contaminant biodegradation in fractured rock systems.

Introduction

Assessing natural attenuation processes in shallow, chloroethene-contaminated aquifers has been an environmental imperative as perchloroethene and trichloroethene (TCE) were designated priority groundwater pollutants in the 1970s (Bradley 2003; Häggblom and Bossert 2003; Löffler et al. 2003). Efforts to assess the natural attenuation of chloroethene contaminants in unconsolidated aquifers employ the U.S. EPA multiple lines of evidence protocol with an emphasis on changes in biogeochemical parameters along identified groundwater flowpaths (U.S. EPA 1997; Wiedemeier et al. 1998). Direct application of such flowpath dependent approaches in fractured rock aquifers is problematic, however, due to difficulties defining discrete groundwater flowpaths in such systems. Thus, comparatively little is known about the spatial and temporal variability of natural attenuation processes such as biodegradation in fractured rock systems. Improved methods for character-

izing groundwater flowpaths and/or emphasis on flowpath independent lines of evidence is needed to assess the variability of natural attenuation processes in fractured rock systems.

In this study, the basic U.S. EPA framework was modified to assess the potential for chloroethene reductive dechlorination in the shallow, fractured rock aquifer at the Naval Air Warfare Center (NAWC) Trenton, New Jersey (Lacombe 2000, 2002; Williams et al. 2007) using flowpath independent lines of evidence. The installation of a multilevel, borehole straddle-packer system (Shapiro 2001) in 2005 provided an opportunity to assess the variation in chloroethene biodegradation potential that can occur with depth in fractured rock. Because of the uncertainties in the magnitude and direction of groundwater flows in this system, the potential for chloroethene biodegradation was evaluated in discrete packer intervals using two flowpath independent lines of field evidence: (1) the presence of the biochemical prerequisites (electron donor, chloroethene compounds, and chlororespiring microorganisms) for efficient chloroethene reductive dechlorination and (2) the in situ accumulation of chloroethene reductive dechlorination daughter products. Conclusions of the flowpath independent field study were

validated by comparison with ^{14}C -*cis*-DCE degradation experiments conducted using crushed aquifer material that had been colonized in situ for a year.

Methods

Study Site

The NAWC Trenton site is a decommissioned, military jet engine testing facility in west-central New Jersey, which operated from the mid-1950s to the late 1990s. The site is underlain by fractured, Triassic-age mudstone shales and sandstones that dip 15° to 70° to the northwest from a fault zone with an approximate strike of $\text{N}65^\circ\text{E}$ (Lacombe 2000, 2002). Overlying the fractured bedrock is a soil and rubble zone that varies between 1 and 5 m in thickness. A stratigraphic cross section of the shallow aquifer near the test borehole (68BR) is provided in Figure 1 (Lacombe 2000). The depth to the water table at 68BR is typically between 2 and 4 m below land surface. The general direction of groundwater flow at this location is southwest toward well 15BR, which is continuously pumped at a rate of approximately 35 L/min (9 gal/min).

Groundwater contamination in the vicinity of 68BR is the result of historical losses from a TCE storage and handling facility. Primary groundwater contaminants are TCE and its degradation products, *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC). Dissolved concentrations in excess of 10 mg/L have been documented for all three contaminants in groundwater in this area of the site.

Borehole Packer System

Borehole 68BR was drilled with continuous core collected in May 2005. Borehole 68BR had a nominal diameter of 15 cm and a total depth of 52 m below land surface. Hydraulic testing was conducted using the Bedrock-Aquifer Transportable Testing Tool packer testing system (Shapiro 2001) and using single- and cross-hole borehole flowmeter testing (Williams et al. 2007). Packer testing results indicated that transmissivity in tested sections ranged from 1.6

$\times 10^{-4} \text{ m}^2/\text{s}$ to below the detection limit of less than $1 \times 10^{-10} \text{ m}^2/\text{s}$ (Shapiro, A.M., C.R. Tiedeman, and R. Rosman 2005, written communication). The highest transmissivity was for the 4- to 16-m depth interval. Six other depth intervals were identified with transmissivities ranging from 1×10^{-6} to $3.7 \times 10^{-6} \text{ m}^2/\text{s}$. Borehole flow testing identified three transmissive zones present at approximate depths of 12, 29, and 44 m below land surface (Williams et al. 2007).

On the basis of hydraulic-testing results, in June 2005 a straddle-packer system consisting of five inflatable packers was installed in 68BR to create six discrete monitoring intervals (Figure 1). Interval A extended from the base of the steel surface casing, approximately 4 m below land surface, to 16 m below land surface. Depths corresponding to intervals B to F were approximately 17 to 19, 20 to 27, 28 to 30, 31 to 45, and 46 to 52 m below land surface, respectively. Interval A had much higher transmissivity than the other five intervals, and the water level generally decreased from interval A to F. The three zones identified by borehole flow testing correspond to intervals A (12 m below land surface; shallow zone), B (29 m; intermediate zone), and the bottom of interval E along with interval F (44 m; deep zone) (Williams et al. 2007).

The straddle-packer system was removed from 68BR in June 2006, in order to recover the in situ sampling devices described below. The packer system was immediately reinstalled with polyethylene tubing extending from the surface to the A, C, D, and F packed intervals. Pumped sampling to assess the long-term electron donor pool in the A, C, D, and F packed intervals was conducted in August 2006.

In Situ Sampling Devices

The variation in the potential for biodegradation of chloroethene contaminants with depth in 68BR was investigated by assessing the presence within each packed interval of the biochemical ingredients deemed necessary for efficient chloroethene reductive dechlorination. In situ chloroethene reductive dechlorination can be viewed as a biochemical reaction triangle, with efficient biodegradation requiring three critical components: electron acceptor, electron donor, and a biochemical catalyst. The presence and relative concentration of each critical component were assessed using targeted passive sampling devices that were deployed with the packer string and emplaced for a period of approximately 1 year.

Electron Acceptor Concentration

The chloroethene molecule serves as the electron acceptor for microbial reductive dechlorination of chloroethene contaminants in groundwater. The concentration of chloroethene contaminants in each packed interval was assessed using polyethylene-based passive diffusion bag samplers as described elsewhere (Vroblesky 2001). Passive diffusion bag samplers were attached to the stem of the packer string within each packed interval and deployed at depth for 1 year. On recovery of the packer string, the diffusion samplers were detached from the stem of the packer assembly and their contents were transferred to glass VOC vials for subsequent analysis by the USGS laboratory in New Jersey

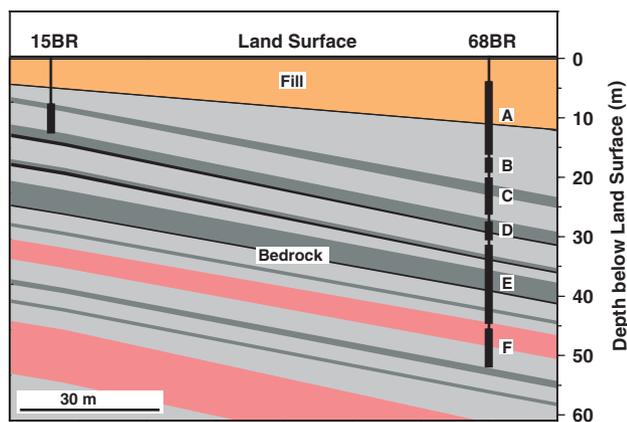


Figure 1. Cross section of the fractured rock aquifer at NAWC Trenton showing the relative location of the borehole packer system in 68BR and the pumping well, 15BR. Letters A to F identify discrete packer intervals.

using gas chromatography with ion selective mass spectrometry (GC/MS).

Electron Donor Concentration

Hydrogen is generally considered to be the ultimate electron donor for respiratory microbial chloroethene reductive dechlorination (Bradley 2003; Häggblom and Bossert 2003; Löffler et al. 2003). The dissolved hydrogen concentration within each packed interval of 68BR was assessed using simple hydrogen diffusion syringe samplers as described elsewhere (Vroblesky et al. 2007). Duplicate high-density polyethylene syringe samplers were attached to the stem of the packer string within each packed interval and deployed at depth for 1 year. Upon string recovery, the syringes were detached from the packer assembly and their contents were analyzed by direct injection reduction gas detection gas chromatography (Chapelle et al. 1997; Vroblesky et al. 2007) within 30 min of recovery.

Over the long term, chloroethene biodegradation depends on the supply of fermentable carbon substrate(s) and a microbial community capable of fermenting that substrate to H₂ for subsequent use in microbial reductive dechlorination. U.S. EPA guidelines for assessing natural attenuation as a viable long-term mechanism for in situ remediation of chloroethene contamination recommend quantifying the total and dissolved organic carbon (TOC and DOC, respectively) present in the aquifer as a rough indication of the ongoing presence and potential persistence of electron donor capable of supporting in situ chloroethene reductive dechlorination (Wiedemeier et al. 1998). However, TOC and DOC are typically measured by oxidation to carbon dioxide. Thus, these properties provide no information about the chemical nature of the constituent organic compounds and, often, are poor indicators of the bioavailable organic carbon in groundwater. Dissolved concentrations of total hydrolysable neutral sugars (THNS) and total hydrolysable amino acids (THAA) have been suggested as promising indicators of the bioavailable fraction of organic carbon in aquatic environments (Amon et al. 2001; Benner 2002, 2003; Gremm and Kaplan 1998; Volk et al. 1997; Weiss and Simon 1999), including groundwater systems (Chapelle et al. 2009). Thus, to gain some insight into the variation in the long-term electron donor supply with depth in 68BR, DOC, THNS, and THAA concentrations were determined as described previously (Chapelle et al. 2009) in water pumped from intervals A, C, D, and F in August 2006.

Chlororespiratory Microbial Community

Although co-metabolic reductive dechlorination of chloroethene contaminants is considered common in anoxic chloroethene-contaminated groundwater systems, this process is generally inefficient and not considered a reliable mechanism for in situ contaminant remediation (for review of this subject, see Bradley 2003; Häggblom and Bossert 2003; Löffler et al. 2003). In contrast, respiratory microbial reductive dechlorination (i.e., chlororespiration) of chloroethene compounds is considered an efficient and environmentally important mechanism for in situ contaminant remediation (for review of this subject, see Bradley

2003; Häggblom and Bossert 2003; Löffler et al. 2003). In the environment, complete chlororespiration of TCE to the nonchlorinated, nontoxic product, ethene, appears to involve a consortium of microorganisms. *Dehalococcoides*-type microorganisms capable of reductive dechlorination of TCE to DCE and VC are relatively common in anoxic, chloroethene-contaminated, shallow aquifer systems. Efficient dechlorination of VC to ethene appears to involve a specific subset of *Dehalococcoides*-type microorganisms capable of VC chlororespiration. Thus, to assess the relative variation in the chlororespiratory “catalytic” capacity in 68BR with depth, quantitative polymerase chain reaction (Q-PCR) analyses targeted at both TCE and VC chlororespiratory populations were employed.

A number of earlier reports (e.g., Alfreider et al. 1997; Harvey et al. 1984; Hazen et al. 1991) suggested that the biodegradation capacity of shallow, unconsolidated aquifer systems primarily reflects the activity of the indigenous, attached microbial community. Recent investigations, however, suggest that both attached and planktonic communities contribute significantly to contaminant degradation in groundwater systems (Lehman et al. 2001a, 2001b). Thus, although the difference in microbial community structure and activity between planktonic and attached populations in fractured rock systems is less understood, these investigators have advocated assessment of both populations (Lehman et al. 2001a, 2001b).

In this study, polyvinyl chloride beakers (2.5-cm inner diameter; 50-cm length) were filled with rock core, which was collected from the respective depth interval during coring of 68BR and crushed by hammer to yield material of 0.5 to 1.5 cm maximum dimension. Filled beakers were attached to the stem of the packer string within each packed interval and deployed at depth for 1 year to allow the water in the beaker to equilibrate to the formation water and to allow colonization by the indigenous microorganisms. On recovery of the packer string, the beakers were detached from the stem of the packer assembly, and their contents (water and crushed core material) were transferred to sterile glass containers and stored on ice for subsequent DNA extraction and use in microcosm activity experiments.

Enumeration of Chlororespiring Microbial Community

The variation in the total number of prokaryotes (bacteria and archaea) and in the numbers of chlororespiring bacteria associated with core material, which was incubated at depth over a year period, was assessed using the Q-PCR. DNA for Q-PCR analyses was extracted from triplicate 1-mL samples of core material and associated water using the UltraClean Soil DNA Kit (MO BIO Laboratories Inc., Solana Beach, California) as described (Lendvay et al. 2003). Each 1-mL sample contained approximately 1 g dry weight of core material and 0.5 mL of associated formation water.

Q-PCR targeted at the 16S rRNA genes of prokaryotic groundwater microorganisms was carried out using the degenerate universal prokaryotic primer pair Uni340F (5'-CCT ACG GGR BGC ASC AG-3') and Uni806R (5'-GGA CTA CNN GGG TAT CTA AT-3') to yield an rDNA amplicon of approximately 450 bp (Takai and Horikoshi 2000). Q-PCR targeted at the 16S rRNA gene

sequence of *Dehalococcoides* spp. was carried out using the primer pair DHE750F (5'-AAG GCG GTT TTC TAG GTT GTC AC-3') and DHE1155R (5'-CGT TTC GCG GGG CAG TCT-3') to yield an amplicon of approximately 400 bp (Löffler et al. 2000). However, because not all *Dehalococcoides* spp. are capable of chlororespiration of VC to the nontoxic product, ethene, the relative importance of indigenous VC chlororespiring bacteria was also assessed in order to evaluate the potential for complete biological detoxification of chloroethene contaminants at the NAWC Trenton site. Q-PCR targeted at the VC reductive dehalogenase gene of BAV1-type *Dehalococcoides* was carried out using the primer pair *bvcAF* (5'-TGC CTC AAG TAC AGG TGG T-3') and *bvcAR* (5'-ATT GTG GAG GAC CTA CCT-3') to yield an approximately 840 bp amplicon (Krajmalnik-Brown et al. 2004).

Universal prokaryotic and *Dehalococcoides*-specific DNA targets were amplified by two-step SYBR Green Q-PCR (Bradley et al. 2005a) on a Prism 7000 Sequence Detection System (Applied Biosystems Inc., Foster City, California) according to the following protocol: 10 min activation at 94°C, followed by 50 cycles of 15 s at 94°C, 60 s at 60°C, and a final extension step of 10 min at 72°C. Q-PCR was calibrated by serial dilution of genomic DNA standards as described (Lendvay et al. 2003), and had an effective detection limit (10² copies/g dry core material) similar to that reported previously for aquifer material (Lendvay et al. 2003) and soil (Sleep et al. 2006). PCR products were confirmed by 2% agarose-gel-electrophoresis and UV visualization with ethidium bromide as well as by melting curve analysis between 72°C and 94°C. The results of the Universal prokaryotic primer analyses were compared with acridine orange direct counts (Hobbie et al. 1977) and found to agree within one order of magnitude.

Radiochemicals

The variation in general microbial activity in 68BR packed intervals was investigated using carboxyl-labeled [1-¹⁴C] acetate (49 µCi/µM; Sigma-Aldrich Biochemicals, St. Louis, Missouri). The variation in the potential for chloroethene biodegradation was investigated using uniformly labeled [1,2-¹⁴C] *cis*-DCE (4 µCi/µM; Moravek Biochemicals, Brea, California). The radiochemical purities of the ¹⁴C-substrates were evaluated by direct injection radiometric detection liquid chromatography (HPLC/RD) or gas chromatography (GC/RD) and found to be greater than 98% pure.

Microcosm Assessment of Chloroethene Biodegradation

Microcosm studies were conducted as described previously (Bradley and Chapelle 1999a, 1999b; Bradley et al. 2005b). In general, microcosms were composed of 10 mL serum vials containing 5 ± 0.5 g of depth-specific core material; 2 mL deoxygenated, depth-specific formation water; and an atmosphere of ultrahigh purity nitrogen. Microcosms were assembled under an atmosphere of nitrogen and subsequently flushed three separate times with 1000 times the headspace volume of pure nitrogen, which had been passed through a heated column of reduced copper filings to remove trace oxygen as described previously (Lovley and Phillips 1986). Replicate experimental (three microcosms), auto-

claved control microcosms (two microcosms), and a single matrix-free, container control microcosm were prepared for each depth. All controls were autoclaved three times for 1 h at 15 PSI and 121°C. All microcosms were pre-incubated in the dark for 5 days prior to the addition of ¹⁴C-substrates. Microcosms were amended with ¹⁴C-substrates to yield initial dissolved substrate concentrations of 300 µg/L and 680 µg/L for [1-¹⁴C] acetate and [1,2-¹⁴C] *cis*-DCE, respectively. Microcosms were incubated in the dark, at room temperature (circa 23°C) for 230 days. Anoxic conditions (microcosm dissolved oxygen concentrations less than 20 µg/L analytical detection limit) were confirmed throughout the study by headspace, thermal conductivity detection (TCD) gas chromatography.

Analytical Methods

Analytical methods were as described previously (Bradley and Chapelle 1999a, 1999b; Bradley et al. 2005b). Headspace concentrations of ¹⁴CH₄, ¹⁴CO₂, ¹⁴C-ethene, ¹⁴C-ethane, and ¹⁴C-VC were monitored by analyzing 0.5 mL of headspace using packed column gas chromatography with radiometric detection and TCD. The headspace sample volumes were replaced with pure nitrogen. Dissolved phase concentrations of ¹⁴C-analytes were estimated based on experimentally determined Henry's partition coefficients as described previously (Bradley et al. 2002). The radiometric detector was calibrated by liquid scintillation counting using H¹⁴CO₃⁻. Anoxic conditions (microcosm dissolved oxygen concentrations less than 20 µg/L) were confirmed throughout the study by headspace, TCD gas chromatography.

Results and Discussion

In this study, the change in the potential for chloroethene biodegradation with depth was evaluated using two flow-path independent lines of field evidence: (1) the presence of the three biochemical prerequisites [(electron donor(s), chloroethene electron acceptor(s), and chlororespiring microorganism(s))] for efficient chloroethene chlororespiration and (2) the in situ accumulation of chloroethene reductive dechlorination daughter products. The validity of the field conclusions was assessed with microcosm ¹⁴C-DCE biodegradation experiments.

Variability of Chlororespiration Prerequisites with Depth

Potential chlororespiration electron acceptors were detected in all six packed intervals in 68BR (Figure 2). Dissolved concentrations of the parent contaminant (TCE) increased substantially with a depth from approximately 0.5 µM (approximately 60 µg/L) in intervals A to C up to greater than 155 µM (greater than 20 mg/L) in intervals D to F. The greater than two order of magnitude increase in TCE concentrations over the 4 m between sampler locations in intervals C and D illustrates the substantial spatial variability that can characterize fractured bedrock aquifer systems (Figure 2). The observed increase in chloroethene contamination with depth is consistent with a density-driven component to contaminant transport, but may also reflect preferential contaminant transport within the "deep" zone

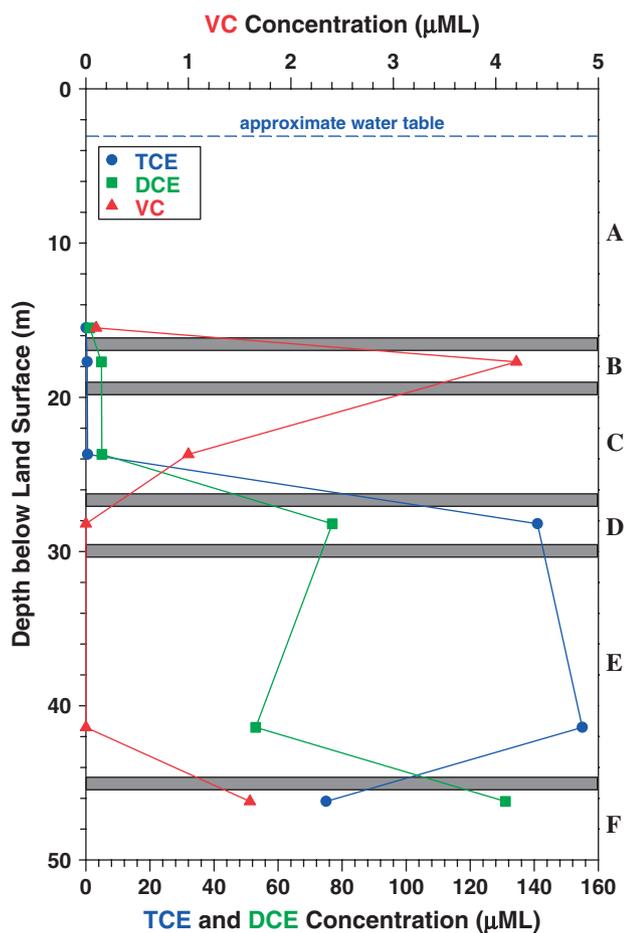


Figure 2. Variation in the concentrations of potential chlororespiration electron acceptors with depth in 68BR. Dissolved concentrations ($\mu\text{M/L}$) of chloroethene compounds are from passive diffusion devices placed in each packed interval of 68BR for 1 year.

located approximately 44 m below land surface in 68BR (Williams et al. 2007). The presence and concentration of chloroethene compounds in each interval in 68BR were consistent with a potential for chloroethene chlororespiration in the fractured aquifer system at NAWC Trenton and indicated that this potential may increase with depth.

The range of dissolved H_2 concentrations observed throughout 68BR also was consistent with the potential for efficient microbial reductive dechlorination of chloroethene contaminants at NAWC Trenton (Figure 3). Hydrogen is the ultimate electron donor for most chlororespiring microorganisms (Bradley 2003; Häggblom and Bossert 2003; Löffler et al. 2003). All currently identified *Dehalococcoides* organisms, which include all known VC chlororespirers, are strict hydrogenotrophic chlororespirers (Cupples 2008; Futagami et al. 2008; Furukawa 2006; Löffler and Edwards 2006). The dissolved H_2 concentrations observed throughout 68BR were in the range previously reported to support chlororespiration of chloroethene compounds in sediment and in liquid cultures (Löffler et al. 1999; Mazur and Jones 2001; Smatlak et al. 1996; Yang and McCarty 1998). The substantially elevated dissolved H_2 concentrations detected in packed interval B may indicate an enhanced supply of

electron donor and a greater potential for chlororespiration in this zone.

The concentration of DOC is commonly employed as an upper estimate of the electron donor supply in groundwater systems (Wiedemeier et al. 1998) but is an unreliable indicator of the bioavailable electron donor fraction (see Benner [2003] for review). More recently, dissolved concentrations of THNS and THAA have been employed as useful indicators of the bioavailable electron donor supply in groundwater systems (Chapelle et al. 2009). Both the 120 to 190 μM (approximately 1.4 to 2.3 mg/L DOC as carbon) range in DOC concentration and the 0.25% to 1.25% THNS and THAA fractions in 68BR were consistent with an adequate electron donor supply for chloroethene chlororespiration in packed intervals A, C, D, and F (Figure 3). Intervals B and E were not pump accessible and were not sampled for DOC, THNS, and THAA. Combined with the observed distribution of chloroethene compounds and H_2 concentrations, these results suggest a potential for contaminant biodegradation throughout 68BR, if a suitable microbial catalyst exists in situ.

To assess the presence and relative importance of chlororespiring microorganisms in 68BR, depth-appropriate core material was placed within each packed interval for 1 year to allow colonization by the indigenous microorganisms. Q-PCR analyses (universal) and acridine orange direct counts of the colonized core material indicated a total microbial population on the order of 10^6 to 10^7 cells/g of dry core material with the greatest density occurring in the intermediate and deep zones (packer intervals B and F; Figure 4). The lack of detectable amplification (detection limit of 10^2 copies/g) of interval A DNA extracts using the *Dehalococcoides*-specific DHE primer pair was consistent with the relatively low concentration of chloroethene compounds in interval A and indicated that efficient chlororespiration of these contaminants in the shallow flow zone at 68BR was unlikely. However, DHE-specific amplification was significant in DNA extracts from packed intervals B to F. Q-PCR analyses of DNA extracts from intervals B to F indicated *Dehalococcoides* populations on the order of 10^4 to 10^5 cells/g (assuming one copy per cell) of core material, with the greatest densities occurring in the intermediate and deep zones (intervals B and F; Figure 4). Based on these Q-PCR results, *Dehalococcoides* spp. comprised 0.2% to 2.0% of the microbial community that colonized core material from 68BR. The densities of *Dehalococcoides* observed in intervals B to F in this study are comparable to those reported previously for biostimulation and bioaugmentation test plots in a shallow, chloroethene-contaminated aquifer (Lendvay et al. 2003) and are consistent with a significant potential for chloroethene chlororespiration in intervals B to F. The approximate order of magnitude greater densities of *Dehalococcoides* spp. in samples from packer intervals B and F indicated that the highest microbial potential for chloroethene chlororespiration existed in these zones at NAWC Trenton at the time of sample collection.

Most important from a contaminant remediation point of view, a substantial percentage of the *Dehalococcoides* organisms that were detected in 68BR intervals B to F appeared to be positive for VC reductive dehalogenase (*bvcA*;

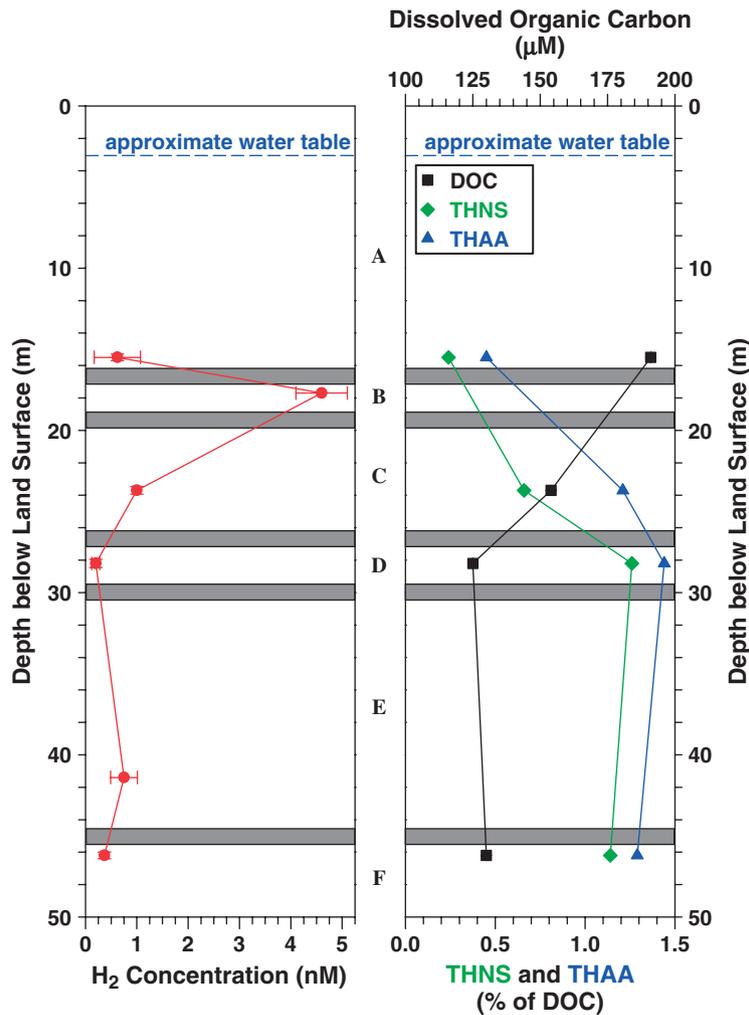


Figure 3. Variation in the concentrations of potential electron donors with depth in 68BR. Dissolved concentrations of H_2 (mean \pm SD in nM) are from duplicate passive diffusion syringe samplers employed in each packed interval for 1 year. Dissolved concentrations of organic carbon (DOC in μM carbon), THNS (as a percentage of DOC), and THAA (as a percentage of DOC) are from single samples pumped from intervals A, C, D, and F in 2006.

Figure 4). Microorganisms containing the *bvcA* gene represented $10 \pm 0.1\%$ of the *Dehalococcoides* spp. detected in interval B samples, assuming one copy of the *bvcA* gene target per cell. In samples from packer intervals C to F, the estimated number of *bvcA* VC reductive dehalogenase gene copies was equivalent to 50% or more of the detected *Dehalococcoides* population, assuming one copy per cell. These results indicate that the potential for complete chlororespiration of chloroethene contaminants to nontoxic ethene was substantial in 68BR intervals B to F, with the apparent maximum microbial potential for VC chlororespiration occurring in the deep zone (packer interval F).

The preceding assessment of the biochemical prerequisites [electron donor(s), chloroethene electron acceptor(s), and chlororespiring microorganism(s)] for efficient chloroethene chlororespiration in 68BR supported the following conclusions. The potential for chloroethene chlororespiration at 68BR appeared to vary significantly with depth over the range of 0 to 50 m below land surface. In toto, the evidence indicated little potential existed for efficient chloroethene contaminant biodegradation in the shallow

zone (packer interval A) at 68BR. In contrast, the cumulative geochemical and microbiological evidence indicated a significant potential for chloroethene chlororespiration existed below packer interval A with the highest potentials occurring in the intermediate and deep zones. Q-PCR results indicated that reductive dechlorination to ethene was likely below interval A, particularly in the intermediate and deep zones.

Variability of Reductive Dechlorination Products with Depth

The presence of chlorinated daughter products provides compelling evidence for reductive degradation of chloroethene contaminants and has long been emphasized as a line of evidence for natural attenuation of chloroethene contamination in unconsolidated shallow aquifer systems (Bradley 2003). Similarly, the detection of DCE and VC throughout 68BR indicates that reductive dechlorination of TCE occurred in the vicinity of or upgradient from all 68BR packed intervals because DCE and VC were not original contaminants at the NAWC Trenton site (Figure 2). How-

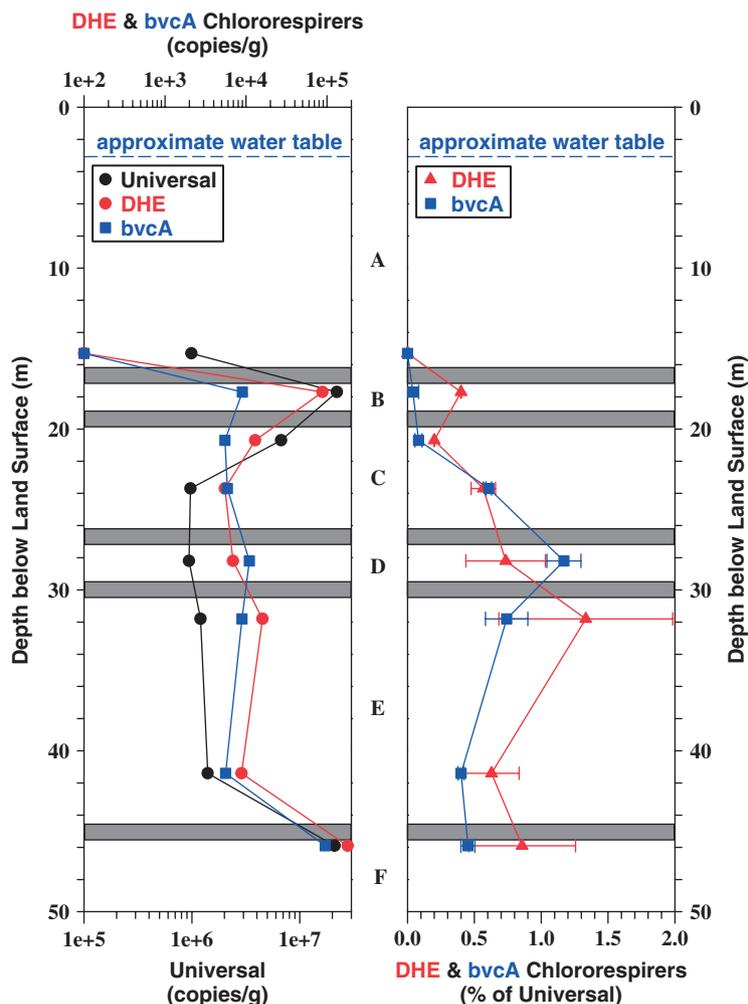


Figure 4. Variation in general and chlororespiration-specific microbial populations with depth in 68BR. Density estimates are means from triplicate samples of depth-specific core material colonized in situ for 1 year. The coefficient of variation for density estimates was 50% or less. Error bars for the relative number of DHE and bvcA chlororespirers are \pm one standard deviation.

ever, an examination of the product-parent ratio, the ratio of reductive dechlorination daughter products (DCE plus VC) to the parent compound (TCE), in each packed interval supports the earlier conclusion that the efficiency of chloroethene reductive dechlorination at 68BR varies significantly with depth.

Chloroethene contaminants were present only at low concentrations (cumulative concentration of TCE, DCE, and VC was less than $1.3 \mu\text{M}$ or 131 ppb) in interval A samples. However, the fact that the product-parent molar ratio was greater than 20:1 indicated that the chloroethene contamination detected in interval A had undergone significant reductive dechlorination in the vicinity of or upgradient from 68BR interval A. This observation is in apparent contrast to the lack of detection of DHE and bvcA Q-PCR targets in interval A samples, which indicated a poor potential for efficient reductive dechlorination of chloroethene contaminants in the shallow flow zone at 68BR. The discrepancy between these observations raises the possibilities that (1) the contamination detected in interval A was transported from an upgradient zone of active dechlorination; (2)

reductive dechlorination occurred in the vicinity of interval A as the result of microorganisms that were not detected by the Q-PCR methods employed in this study; or (3) the chloroethenes detected in interval A were attributable to cross contamination from the underlying packed interval, which was characterized by substantially higher contaminant concentrations but with the same product-parent ratio.

The product-parent molar ratios of 20:1 and 11:1 observed in intervals B and C, respectively (Figure 2) indicated that the chloroethene contamination in these zones had undergone significant reductive dechlorination. These observations are consistent with the chlororespiration prerequisites line of evidence, which indicated a substantial geochemical and microbiological potential for chlororespiration in this intermediate zone. Likewise, the substantial daughter product accumulation and the 2:1 product-parent molar ratio observed in packed interval F are consistent with the chlororespiration prerequisites evaluation for the F interval and support the conclusion that chlororespiration is efficient in the deep zone at 68BR. In light of the similar density of *Dehalococcoides* spp. in the intermedi-

ate (packed interval B) and deep (packed interval F) zones and the 20-fold greater accumulation of daughter products in the deep (packed interval F) zone, the lower product-parent molar ratio of interval F is not consistent with a lower dechlorination efficiency in the deep zone than in the intermediate zone but is consistent with a shift toward saturation kinetics and a concomitant decrease in the relative rate of dechlorination. In contrast, because the product-parent ratios observed in intervals D and E were substantially lower (0.4:1) than in interval F even though all had similar total contaminant concentrations does suggest a lower dechlorination efficiency and is consistent with the Q-PCR results for packed intervals D and E indicating a substantially lower microbiological potential for chlororespiration (i.e., order of magnitude lower population of chlororespirers) in the low flow zone.

Microcosm Evaluation of Field Results

Both lines of field evidence (the accumulation of daughter products and the prerequisites for chlororespiration) provided a consistent indication of effective chlororespiration activity in the intermediate (packed interval B) and deep (packed interval F) zones with low to poor potential for

chlororespiration in the intervening zone (packed intervals C and D) and in the shallow zone (interval A). To test the validity of this flowpath independent field approach, anoxic microcosms were prepared using colonized core material from 68BR (Figure 5).

Activity observed in the [1-¹⁴C] acetate treatments indicated a substantial potential for microbial activity in all packed intervals in 68BR with 10% to 100% mineralization to ¹⁴CO₂ within 24 h (Figure 5). The minimum [1-¹⁴C] acetate mineralization observed in interval B microcosms and the elevated dissolved H₂ concentrations observed in the B packed interval are consistent with the earlier hypothesis that the supply of electron donor is elevated in the B interval. However, intervals B and E were not instrumented for pumped sampling and, consequently, the availability of electron donors other than H₂ was not assessed.

The results of the [1, 2-¹⁴C] *cis*-DCE experiment confirmed significant variation in microbial reductive dechlorination activity with depth. No recovery of degradation products was observed in microcosms prepared with material from intervals A, C, or D. However, substantial reductive dechlorination of ¹⁴C-DCE was observed in microcosm treatments prepared with material from intervals B and F as

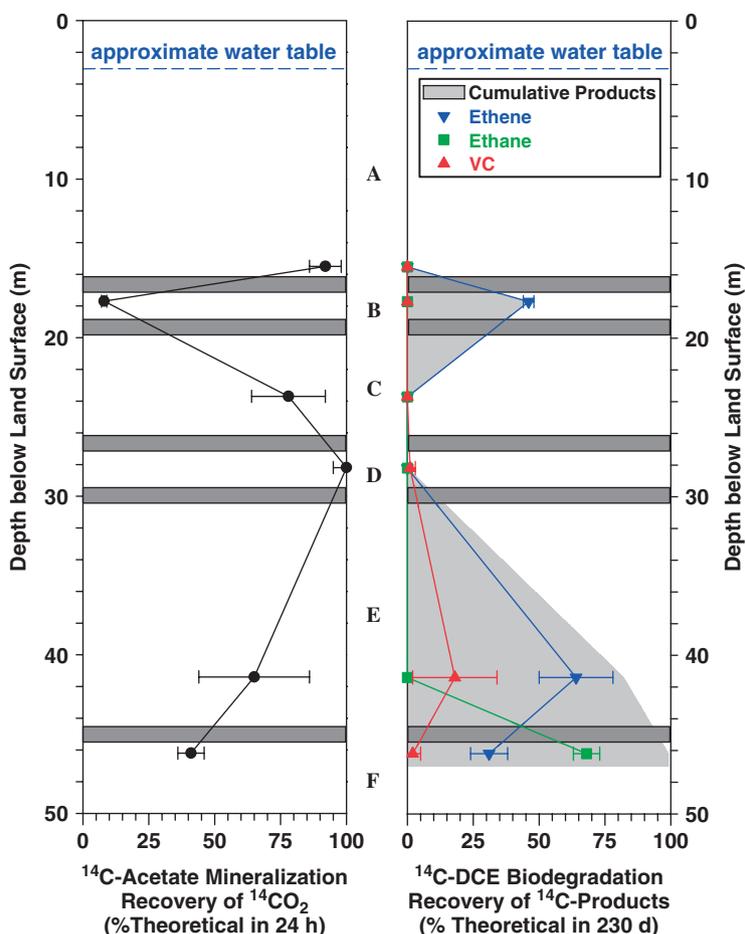


Figure 5. Variation in microbial activity with depth in 68BR. Data are means \pm SD for triplicate anaerobic microcosms amended with ¹⁴C-acetate or ¹⁴C-DCE. For ¹⁴C-DCE results, the shaded area represents the total accumulation of degradation products (VC, ethene, plus ethane) detected in each depth treatment. No significant accumulation of degradation products was observed in autoclaved control treatments for either substrate.

well as the lower part of interval E. Thus, the microcosm biodegradation experiments confirmed the field conclusions (based on daughter product accumulation and chlororespiration prerequisites lines of evidence) that a significant potential for in situ reductive dechlorination existed in 68BR in the B and F intervals.

Implications for Assessing Chloroethene Biodegradation in Fractured Rock

Significant improvements in subsurface flow characterization techniques are necessary before fractured bedrock systems can be assessed using the flowpath dependent approaches that are typically employed to assess biodegradation of chloroethene contaminants in unconsolidated aquifers. In the interim, a modified approach emphasizing flowpath independent lines of evidence can be employed to assess the temporal and spatial variability of contaminant biodegradation in fractured rock systems. In this study, the change in the potential for chloroethene biodegradation with depth was evaluated using two flowpath independent lines of field evidence: (1) the presence of the three biochemical prerequisites [electron donor(s), chloroethene electron acceptor(s), and chlororespiring microorganism(s)] for efficient chloroethene chlororespiration and (2) the in situ accumulation of chloroethene reductive dechlorination daughter products. A comparison of the field results with the results of microcosm [1, 2-¹⁴C] *cis*-DCE biodegradation experiments indicates that the flowpath independent lines of evidence approach is a useful framework for assessing chloroethene biodegradation in a fractured rock setting. Improved understanding of groundwater flowpaths in fractured rock systems is needed, however, to allow extrapolation of such field evidence to the intervening aquifer volume between adjacent monitoring wells/boreholes.

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Biographical Sketches

Paul M. Bradley, corresponding author, is a research ecologist at U.S. Geological Survey, South Carolina Water Science Center, Columbia, SC 29210; (803) 750-6125; fax (803) 750-6181; pbradley@usgs.gov.

Pierre J. Lacombe is a hydrologist at U.S. Geological Survey, New Jersey Water Science Center, West Trenton, NJ 08628; (609) 771-3942; fax (609) 771-3915; placombe@usgs.gov.

Thomas E. Imbrigiotta is a hydrologist at U.S. Geological Survey, New Jersey Water Science Center, West Trenton, NJ 08628; (609) 771-3914; fax (609) 771-3915; timbrig@usgs.gov.

Francis H. Chapelle is a research hydrologist at U.S. Geological Survey, South Carolina Water Science Center, Columbia, SC 29210; (803) 750-6116; fax (803) 750-6181; chapelle@usgs.gov.

Daniel J. Goode is a hydrologist at U.S. Geological Survey, Pennsylvania Water Science Center, Exton, PA 19341; (610) 321-2434; fax (610) 321-2509; djgoode@usgs.gov.