Exploring the Correlation between Halorespirer Biomarker Concentrations and TCE Dechlorination Rates

Marcio L. B. Da Silva¹ and P. J. J. Alvarez²

Abstract: Whether the concentration of selected genetic biomarkers could be correlated to the rate of trichloroethene (TCE) reductive dechlorination was investigated. Samples from a pilot-scale aquifer that had been bioaugmented with a halorespiring mixed culture to promote bioremediation of a chlorinated solvent source zone were used for this purpose. Dechlorination rates were determined in batch microcosms, and real-time quantitative polymerase chain reaction (qPCR) analyses were used to estimate the concentration of phylogenetic 16S rRNA for total Bacteria and for Dehalococcoides spp., and the catalytic gene tceA and its expressed mRNA. The first-order dechlorination rate coefficient (k') obtained from the mixed culture used to bioaugment the pilot scale aquifer ranged from 0.033 to 0.662 h⁻¹. Samples collected from the source zone showed the highest k' value (0.03 h⁻¹ ± 0.01; n=6) as well as the highest concentration of the biomarkers tested: 2.2 ± 0.5 × 10⁷ genes/µL for total Bacteria, 1.97 ± 0.1 × 10⁷ genes/µL for Dehalococcoides spp.; 9.6 ± 1.2 × 10⁷ genes/µL for tceA; and 1.44 ± 0.2 × 10⁷ genes/µL for the tceA mRNA. Apparently, the inoculated halorespiers thrived near the dense nonaqueous phase liquid, which is important to enhance source zone remediation. Surprisingly, the strongest biomarker correlation (r² = 0.86) with TCE dechlorination rate coefficients (k') was obtained with the universal biomarker for total Bacteria. No significant correlations (p > 0.05) were obtained between k' and the concentration of specific biomarkers for Dehalococcoides 16S rRNA (r² = 0.02), the tceA gene (r² = 0.40), or its transcriptome (mRNA) (r² = 0.11). Therefore, although qPCR is an important tool to verify the presence and distribution of halorespiers, its use to estimate dechlorination rates based on the tested biomarker concentrations was unreliable.

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CE Database subject headings: Chlorination; Biodegradation; Kinetics; TCE; Correlation.

Introduction

The remediation of aquifers contaminated with chlorinated solvents such as tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC) is a significant challenge for industry and state and federal government (Stroo et al. 2003). Bioremediation and monitored natural attenuation (MNA) are among the most cost-effective approaches to manage soil and groundwater contamination by such hazardous pollutants. However, these environmental biotechnologies are not universally applicable and may be marginally effective when the necessary microbial catalytic capacity is not present or expressed. Thus, regulatory and public approval of bioremediation and MNA requires extensive site characterization and documentation of contaminant degradation, which can be a labor-intensive process that is subject to considerable uncertainty.

Molecular microbial ecology techniques such as quantitative polymerase chain reaction (qPCR) might offer simple and relatively inexpensive evidence that desirable microbiological processes are operating, and characterize their spatial distribution to enhance MNA performance assessment. For example, naphthalene catalytic gene frequency (naph) was found to be a good indicator of polyaromatic hydrocarbon (PAH) biodegradation (Fleming et al. 1993). Similarly, Fe (III) reduction activity was correlated to the expression of 16S rRNA from the iron-reducing bacterium Geobacter sulfurreducens (Chin et al. 2004). Direct correlations between levels of mercuric reductase (merA)-specific transcripts and Hg (II) volatilization rates have also been observed (Jeffrey et al. 1996; Nazaret et al. 1994). Molecular techniques have also been used to assess reductive dechlorination (Löfler et al. 2000; Hendrickson et al. 2002; Holmes et al. 2006; He et al. 2003a,b; Duhannel et al. 2004; He et al. 2005; Lu et al. 2006; Ritalahti et al. 2006).

Previous research has yielded mixed results regarding the potential to correlate dechlorination activity to dehalorespiers biomarker concentrations. Encouraging early results include reports of TCE dechlorination activity increasing with the presence of 16S rRNA from Dehalococcoides spp. (Löfler et al. 2000; Hendrickson et al. 2002) and its catalytic dehalogenerate genes (Ritalahti et al. 2006; Holmes, et al. 2006). On the other hand, Lu et al. (2006) did not find a significant correlation between the concentration of Dehalococcoides spp. (phylogenetic) 16S rRNA and dechlorination activity. Dehalococcoides was targeted in that study because it is the only genus known to reductively dechlorinate PCE and TCE beyond DCE to VC and ethane (He et al. 2003a,b; Cupples et al. 2003; Maynard-Gatell et al. 2001). However, the genus-level phylogenetic probe used in that study cannot discern the potential to dechlorinate different chloroorganic elec-
tron acceptors used by different *Dehalococcoides* species, let alone the expression of such activity (Ritalahti et al. 2006). Thus, *Dehalococcoides* strain-specific reductive dehalogenase (RDase) genes were later identified and used to assess dechlorination activity, according to their assigned function. These include tetrachloroethene reductive dehalogenase (pceA) for PCE to TCE (Rahn et al. 2006; Fang et al. 2007), trichloroethene reductive dehalogenase (tecA) for TCE to dichloroethene, vinyl chloride reductase dehalogenase (vcrA and vcaA) for VC to ethene (Rahn et al. 2006; He et al. 2005; Hölscher et al. 2003; Krajmalnik-Brown et al. 2004; Magnusson et al. 2000; Müller et al. 2004; Sung et al. 2006), hydroxynitrile (H₃-ases) for hydrogen substrate utilization, and several others respiration-associated oxidoreductase genes (Rahn et al. 2006). These catabolic biomarkers complemented existing 16S rRNA gene-based approaches to more comprehensively assess the physiological activity of *Dehalococcoides* spp. in enriched cultures. Nonetheless, the usefulness of such biomarkers to directly estimate in situ dechlorination rates was not evaluated.

The estimation of in situ dechlorination rates is important to decrease the risk of selecting MNA as a remedial action because the degradation rate is an important determinant of plume length and longevity, and thus, the likelihood and duration of exposure. Rahn and Richardson (2008) recently explored the correlation between the concentration of *Dehalococcoides* 16S rRNA, formate dehydrogenase (Fdh), the hydrogenases (H₂-ases) Hup, and reductive dehalogenases (TceA, PceA, and DET1545) genes with dechlorination rates in a mixed culture containing *Dehalococcoides* ethenogenes strain 195. A significant correlation was found with the catabolic biomarkers tested at lower PCE feeding rates (1.5–4.8 μmol/L/h), but not at higher PCE feeding rates (4.8–9.2 μmol/L/h). Thus, the applicability and limitations of biomarker-rate correlations for forensic analysis of bioremediation and MNA require further study.

This paper compares the usefulness of PCR-based 16S rRNA phylogenetic biomarkers (for total *Bacteria* and *Dehalococcoides* spp.), the catabolic tceA gene, and its transcribed mRNA to quantitatively query for the presence of dehalorespiring organisms in a near field scale aquifer system, and to test the potential for these halorespirer biomarkers to infer on TCE dechlorination rates.

**Material and Methods**

**Mathematical Analysis of Dechlorination Rates**

Fate-and-transport models that are used to predict the extent to which contamination will spread and estimate the time required to achieve cleanup goals often describe dechlorination kinetics as a first-order decay process with respect to the contaminant concentration (*C*):

\[
\frac{dC}{dt} = -k'C
\]

(1)

where *k'*=first-order rate coefficient (in this case, for reductive dechlorination). This first-order kinetic assumption was then used as it is usually an appropriate simplification to describe dechlorination in aquifers for two reasons: (1) mass transfer limitations that could be rate-limiting are often first-order (Fickian) processes (Simoni et al. 2001); and (2) the target pollutants are often present at lower concentrations than the corresponding Monod's half saturation coefficient (*Kₛ*), which results in first-order kinetics.

Specifically, in this case we can ignore *C* in the denominator of Monod's equation, obtaining a linear (first-order) equation:

\[
\frac{dC}{dt} = -\frac{k'XC}{Kₛ+C} \approx -\frac{k'X}{Kₛ}C \quad \text{when } C \ll Kₛ
\]

(2)

Here, *k'*=maximum specific substrate utilization rate (g substrate/g cells/day) and *X*=active microbial concentration (g cells/L), determined as the specific biomarker concentration. Thus, when mass transport is not rate limiting, *k'* can be explained in terms of Monod's parameters. Specifically, a comparison of Eqs. (1) and (2) reveals that

\[
k' = \frac{k}{Kₛ}X
\]

(3)

Thus, *k'* is directly proportional to the concentration of the active biomass, estimated by the concentration of specific biomarkers (*X*), which is the basis for the postulated correlation. This analysis reflects that *k'* is not a constant, but a coefficient that can vary in time and space due to microbial population shifts and changes in environmental conditions (Alvarez and Illman 2005).

**Microcosms**

Dechlorination rates were initially obtained from a dechlorinating culture developed from an anaerobic methanogenic consortium that had shown dechlorination activity for over 9 years in the laboratory (Zheng et al. 2001). This culture is capable of rapid and complete dechlorination of PCE to ethene (240 μmol/L/day) and was previously used for bioaugmentation of a PCE source zone (Adamson et al. 2003; Da Silva et al. 2006). This culture (150 mL) was transferred anaerobically to 250 mL serum bottles (Wheaton, Ringoes, N.J.) inside an anaerobic chamber (Coy, Grass Lake, Mich.) and then capped with Teflon stoppers and aluminum crimps (Sigma-Aldrich, St. Louis). Three ten-fold dilutions were made (10⁻¹, 10⁻², and 10⁻³) using anaerobic sterile synthetic groundwater (Zheng et al. 2001). Prior to addition of TCE (Sigma-Aldrich, 99.9%); the microcosms were purged with an anaerobic gas mixture (5% H₂, 20% CO₂, balanced N₂) for 30 min to purge any trace of chlorinated compounds and provide hydrogen as the electron donor. The initial concentration of TCE (1 mg/L) was selected to be smaller than *Kₛ* [which has commonly been reported to range between 10 and 108 mg/L; Barrio-Lage et al. (1987), Neumann et al. (1996), Sponza (2001)] to validate the first-order kinetics assumption [Eq. (2)], and to ensure easy monitoring as TCE degrades. To discern volatilization losses, a sterile microcosm (negative control) was used. The concentration of TCE was measured over time.

**Determination of *k’***

Values of *k’* were determined in batch microcosms seeded with samples from a pilot aquifer tank (11 m³ volume) used to demonstrate dense nonaqueous phase liquid (DNAPL) source zone bioremediation (Adamson et al. 2003; Da Silva et al. 2006). This tank had been bioaugmented with a mixed culture capable of dechlorinating PCE to ethene (Adamson et al. 2003; Da Silva et al. 2006), and dilutions from this culture were also used to estimate *k’* values. For all microcosms, a plot of ln(*C*) versus time linearized the data, and *k’* was determined as the slope of the best-fitting line (with its associated standard error) using linear regression.
Serial dilutions of the dechlorinating culture were first used to determine the lowest dilution that resulted in measurable dechlorination over a 4 day incubation period (pure culture or ten-fold dilution) and discern the lowest rate that could be reliably measured and be statistically discernible from control losses. Samples that showed dechlorination activity (i.e., significantly lower final TCE concentrations than in abiotic controls, at the 95% significant level) were used to evaluate whether $k'$ was correlated with $X$.

The pilot tank (Fig. 1) consisted of a metal tank (5.49 m long, 2.13 m wide, 1.83 m high) open to the atmosphere and packed with fine masonry sand (from a quarry in New Caney, Tex.). Multiple internal sampling or injection points were installed using 1.3 or 0.6 cm inner diameter stainless steel tubing, placed during the packing of the system. The tank was fed continuously using a source of potable water supply that consisted of a mix of surface water and groundwater. 1 L PCE (99+% Sigma-Aldrich, St. Louis) was injected 30 cm below surface to establish a DNAPL source zone. Hydrogen releasing compound (HRC, Regenesis, San Clemente, Calif.) was added continuously in the influent of the tank as a prehydrolized (diluted) mixture consisting of 50:50 v/v HRC: deionized water. The tank was bioaugmented after 120 days of operation by adding 15 L of the anaerobic dechlorinating consortium, directly into the source zone, as described elsewhere (Da Silva et al. 2006).

Groundwater (GW) samples were collected from the bioaugmented tank after 240 days of operation to assess the spatial distribution and concentration of the biomarkers and estimate their correlation with dechlorination rates. Four locations along the Experimental Controlled Release System (ECRS) were used to collect the samples (Fig. 1): upgradient from the source, DNAPL source zone, downgradient from the source, and the effluent line. Viton tubes (Cole-Parmer, Vernon Hills, Ill.) were attached to a 100 mL gas tight syringe and used to pull the GW samples from the sampling wells. GW samples were pulled from the water table and transferred directly to 1 L glass bottles previously capped with Teflon septa and aluminum crimp and purged with anaerobic gas mix (5% H₂, 20% CO₂, balanced N₂). Once in the laboratory, aliquots of 150 mL were transferred anaerobically inside the anaerobic chamber Coy to 250 mL serum bottles and capped as described earlier. The remaining 850 mL of the samples collected were used for DNA and mRNA extraction.

We recognize that most subsurface microorganisms live attached to surfaces rather than in groundwater (Lehman et al. 2001). Thus, this sampling effort was not comprehensive. Nevertheless, the quantification of specific biomarkers in groundwater samples yielded valuable insight into the bio Remediation processes that may develop near chlorinated solvent DNAPLs.

**TCE Analysis**

TCE (99+% Sigma-Aldrich) concentrations in aqueous samples were determined using headspace analysis as described previously in Zheng et al. (2001). Briefly, 100 µL of headspace sample was injected directly into a GC HP5890 (Hewlett Packard, Ramsey Minn.) equipped with a flame ionized detector. Standards were prepared by adding TCE in methanol to serum bottles (250 mL) containing deionized water (150 mL).

**DNA and mRNA Extraction**

DNA and mRNA were extracted from all the samples at the beginning of the experiment (time zero) using MoBio kits (Mo Bio Laboratories, Inc., Carlsbad, Calif.) according to the manufacturer's protocols. Approximately 850 mL of GW samples were filtered through a 0.22 µm nylon membrane filter (Osmonics Inc., Minnetonka, Minn.) secured in a 47 mm glass microanalysis filter holder assembly (Fisher Scientific, Mt. Holly, Pa.) equipped with a vacuum pump (Gast Manufacturing Inc., Benton Harbor, Mich.). The membrane filters were used as a matrix for DNA/ RNA extraction using the MoBio PowerSoil kit according to manufacturer's instructions. A bead-beating device (Mini Beadbeater-8, Biospec, Bartlesville, Okla.) was utilized for cell lysis. The concentration and purity of the DNA and mRNA was measured ($A_{260}/A_{280}$) using a spectrophotometer (Amersham Bio-
<table>
<thead>
<tr>
<th>Target biomarker</th>
<th>Sequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria (16S rRNA)</td>
<td>BACT1369F 5'-CGGTGATAATCCGTTCCG</td>
<td>Beller et al. 2002</td>
</tr>
<tr>
<td></td>
<td>PROK1492R 5'-GywTACCTGGTACGACC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TM1389F 5'-CTTCACTGATCCACGCGGC</td>
<td>He et al. 2003a,b</td>
</tr>
<tr>
<td></td>
<td>Dhc1200F 5'-CTATGCTAATCCACGAG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dhc1271R 5'-CAATTCTGACGGCGCC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dhc1240</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5'-FAM-TTCCTAGTTCGAGTGCTGCTAA-TAMRA</td>
<td></td>
</tr>
<tr>
<td>Dehalococcoides spp.</td>
<td>TceA1270F 5'-ATCCAGATTATGACCCCCGTTGGA</td>
<td>Johnson et al. 2005</td>
</tr>
<tr>
<td></td>
<td>TceA1336R 5'-GCGCTATATTTAGGATGCTTCT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TceA1294</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5'-FAM-TGGGCTATGGCGACGCACGGA-TAMRA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forward 5'-ACGCGCAGCGGGGATG</td>
<td>Beller et al. 2002</td>
</tr>
<tr>
<td>tceA (also mRNA)</td>
<td>Reverse 5'-AGAGACACGAAAACGCGGTTT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe TET-5'ACCTTGCGGCTGTATGCTGCCG-TAMRA</td>
<td></td>
</tr>
<tr>
<td>Bacteriophage λ</td>
<td>Forward TACAAACACCCGCAAACATGCGA</td>
<td>Johnson et al. 2005</td>
</tr>
<tr>
<td></td>
<td>Reverse GGAAGTTGCACCGGCGGTCAT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Probe V1C-CGCGCCGTGCGCAGGTCTCC-6-TAMRA</td>
<td></td>
</tr>
</tbody>
</table>

Real-time Quantitative PCR and Reverse-Transcriptase PCR

Real-time quantitative PCR (qRT-PCR) analyses were performed to estimate the concentrations of total bacteria (16S rRNA), *Dehalococcoides* spp., TCE dehalogenase (*tceA*), and mRNA (*tceA*) genes. Each PCR reaction mix contained 1× Taqman PCR Master Mix (Applied Biosystems, Foster City, Calif.); 0.5 μM forward and reverse primers, 0.25 μM probe, 2 μL sample DNA or cDNA, and sterile DNAase-free water to make up a final volume of 25 μL. Primers and probe used in this work (Table 1) were obtained from Integrated DNA Technologies (Coralville, Iowa). PCR reactions were performed using a Sequence Detector (Model ABI 7500, Applied Biosystems) with the following reaction conditions: 30°C for 2 min, followed by 95°C for 10 min and 40 cycles at 95°C for 15 s, and 60°C for 1 min.

The extracted mRNA was immediately reverse-transcribed (RT-PCR) into cDNA using Taqman Reverse-transcription reagents kit (Applied Biosystems) according to the manufacturer's protocol. To avoid DNA contamination in the RNA analysis, RNAase-free DNase was added (DNase kit, Promega, Madison, Wis.) in all mRNA extractions prior to RT-PCR. Each 50 μL reaction volume contained 8 μL of mRNA and 0.5 μM of the *tceA* or the internal standard luciferase reverse primer. The reaction mixture was incubated for 30 min at 55°C followed by 5 min at 95°C. Reactions were performed at 55°C to increase the stringency of primer-template binding and therefore improve RT-PCR specificity (Freeman et al. 1996; Fuchs et al. 1999). After RT-PCR, the concentration of cDNA was measured in the real-time PCR ABI Prism 7000 Sequence Detection System (Applied Biosystems). qRT-PCR reactions were prepared as described earlier using 2 μL cDNA as template. Luciferase (1 mg/mL; Promega) was used as the internal standard to account for the variability during the RT-PCR step as described elsewhere (Johnson et al. 2005). The recovery for the internal mRNA ranged from 3.1 to 5.3%.

The biomarkers concentrations in each sample were estimated using Gene/μL = [(1 μg DNA/μL)/(5.3 × 10^6 bp/genome)] × (9.1257 × 10^14 bp/μg DNA) × (genes/genome) This equation assumes the size of the *Escherichia coli* genome [5.3 × 10^14 base pairs (bp)] used as the standard in the calibration curves and there are approximately 9.1257 × 10^14 bp/μg of DNA (http://www.genomesonline.org), and that there was 1 gene per genome (http://rnn.db.cme.msu.edu). Calibration curves were prepared using (log DNA concentration versus a set cycle threshold value) ten-fold serial dilutions of pure culture genomic DNA of *E. coli* or plasmid DNA carrying either a cloned *Dehalococcoides* 16S rRNA gene or *tceA* of *Dehalococcoides* sp. strain FL2 (Ritalahti et al. 2006). Calibration curves (10^{-1} to 10^{10}) yielded r values of 0.998 or greater. The limits of detection for the biomarkers tested were 2 copies/μL for *Bacteria* 16S rRNA, 15 copies/μL for *Dehalococcoides* spp. 16S rRNA, 2 copies/μL for *tceA*, and 2 copies/μL for its mRNA.

Bacteriophage λ (500 bp, Sigma-Aldrich, St. Louis) was used as an internal standard. The bacteriophage λ was added in the samples prior to DNA extraction for the determination of DNA efficiency recovery (Beller et al. 2002). Bacteriophage λ recoveries lower than 100% were normalized to the fraction recovered. The DNA recovery ranged from 0.2 to 1.1%. Such low DNA recovery from natural systems is commonly reported (Mygind et al. 2003; Zhou et al. 1996) and are probably due to the binding of sample impurities (e.g., humic acids) that interfere with the activity of *Taq* polymerase during PCR analysis (Porteous et al. 1997).

Results and Discussion

Dechlorination Rates

The dechlorination rate coefficients obtained from the culture dilutions ranged from 0.033 ± 0.0014 h^{-1} (ten-fold dilution; n = 10) to 0.662 h^{-1} (undiluted culture; which removed all the TCE within 14 h) (Table 2). Due to the relatively short incubation period for determining rates (4 days), TCE removal in the 100-fold dilution was not statistically discernible from the controls (abiotic losses). Thus k = 0.03 h^{-1} (half-life of about 1 day) was the lower limit for reliable activity measurements by this rapid incubation approach. TCE metabolites (DCE and VC) were both occasion-
Table 2. Dechlorination Rate Coefficients Measured in the Microcosms

<table>
<thead>
<tr>
<th>Culture dilutions</th>
<th>( k' (\text{h}^{-1}) )</th>
</tr>
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<tbody>
<tr>
<td>Undiluted</td>
<td>0.662</td>
</tr>
<tr>
<td>( 10^{-1} )</td>
<td>0.033</td>
</tr>
<tr>
<td>( 10^{-2} )</td>
<td>ND</td>
</tr>
<tr>
<td>( 10^{-3} )</td>
<td>ND</td>
</tr>
<tr>
<td>Pilot aquifer tank</td>
<td>0.014</td>
</tr>
<tr>
<td>Upgradient</td>
<td>0.030</td>
</tr>
<tr>
<td>DNAPL source zone</td>
<td>ND</td>
</tr>
<tr>
<td>Downgradient</td>
<td>0.014</td>
</tr>
<tr>
<td>Effluent</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Note: ND = significant dechlorination activity (assessed per TCE removal relative to abiotic controls) was not observed during the 4-day degradation assay.

Fig. 2. Correlation between dechlorination rate \( (k') \) and the concentration of biomarkers \( (X) \) in copies/mL: 16S rRNA (A); Dehalococcoides spp. (B); tceA (C), and mRNA (tceA) (D). Circles (dilutions of the culture used for bioaugmentation; crosses ECRS upgradient; triangles ECRS source zone; diamonds ECRS downgradient; and squares ECRS effluent.

Biomarker Correlations

The correlations between the concentrations of these biomarkers \( (X) \) and the corresponding \( k' \) values are presented in Fig. 2.

The universal bacterial biomarker for total Bacteria showed the strongest correlation \( (r^2=0.85) \) with TCE dechlorination rate coefficients (Fig. 2), yielding the only correlation that was significant at the 95% confidence level. This was unexpected because this biomarker targets many species incapable of dechlorinating TCE. Even though the genus Dehalococcoides is the only one known to dechlorinate TCE to ethane, its phylogenetic biomarker yielded a weaker correlation \( (r^2=0.02) \). This may reflect that the presence of Dehalococcoides spp. does not guarantee the expression of their dehalogenase genes, and corroborates the lack of significant correlation between dechlorination activity and the concentration of Dehalococcoides 16S RNA genes reported by Lu et al. (2006).

There was no significant correlation \( (p>0.05) \) between \( k' \) and the concentration of the catabolic gene tceA \( (r^2=0.40) \) or its expression as measured by its mRNA \( (tceA) \) \( (r^2=0.11) \) (Fig. 2). Rahman and Richardson (2008) demonstrated that correlations between PCE respiration rates with the biomarkers Fdh, H2ase, TceA, or PceA were only achieved when an highly enriched culture was fed PCE at 1.5 to 4.8 \( \mu \text{mol/L/h} \) but not when fed at a higher rate of 4.8–9.2 \( \mu \text{mol/L/h} \). Predictive quantitative correlations between catabolic gene concentrations and biotransformation rates for other compounds have been reported for some bacteria (Chin et al. 2004; Fleming et al. 1993), but not for others (Tseng et al. 1994; Lee et al. 2006). In our case, the absence of a direct relationship between tceA concentration and rates of dechlorination activity could be due to the fact that other RDase genes not tested in this work were responsible for the activity, such as Dehalococcoides spp. vcrA or non-Dehalococcoides spp. reductases (Seshadri et al. 2005; Christiansen et al. 1998; Schumacher et al. 1997). For example, a study reported that most of the RDase genes detected in field samples (>50%) were not associated with Dehalococcoides spp. (Ritalahti et al. 2006). It remains to be determined whether more reliable correlations between biomarker concentrations and dechlorination rates may be achievable through characterizing TCE RDase gene sequences from a wider range of bacteria and considering the diversity of this gene for the development of new degenerate primers-probe (biomarker) sets.

This study evaluated the usefulness of dechlorination-associated biomarker genes (i.e., phylogenetic and catabolic) available in the literature as a potential tool to estimate TCE dechlorination rates in environmental samples. Overall, none of the halorespiro biomarkers tested yielded a significant correlation with TCE dechlorination rates.

We recognize that in addition to the possibility that genes that were not targeted were responsible for some dechlorination, there may be numerous site-specific factors that could confound the
correlation between biodegradation rates and the concentration of specific degraders (reflected by the concentration of specific biomarkers). These include factors seldom present in lab experiments with enriched cultures, such as bioavailability and mass transfer limitations, nutrients and electron acceptor influx, redox conditions, suboptimal pH or temperature, and the presence of inhibitory compounds. For example, Johnson et al. (2005) demonstrated that the level of tecA expression is independent of the concentration of chlorinated ethenes, electron donor (hydrogen), and electron acceptors (fumarate, sulfate, nitrate, etc.) but highly dependent on incubation temperature. Thus, future work is needed to investigate how hydrogeologic and geochemical factors affect rate–biomarker correlations. Whether the temporal and spatial scales under consideration could influence the usefulness of such correlations may also be worthwhile to explore because phylogenetic and catalytic biomarkers may experience long-term enrichment as a result of bacterial growth on the target pollutant (i.e., bioremediation), making it plausible for some DNA-based biomarkers to be correlated to dechlorination activity over the large temporal and spatial scales that are relevant to MNA.

Conclusions

There is a growing need for improved performance assessment and biodegradation rate estimation for monitored natural attenuation and bioremediation of aquifers contaminated with recalcitrant pollutants. The use of molecular approaches to establish correlations with specific biomarker concentrations (X) could represent a potential breakthrough to estimate λ and characterize its spatial distribution in a cost-effective and reliable manner. In this work, however, no statistically significant correlations were found between first-order dechlorination rate coefficients (k) and the concentration of Dehalococcoides spp. or its dehalogenase tecA catabolic gene and its transcriptome (mRNA). Overall, the results suggest that qPCR-based analyses of genetic biomarkers might be useful to establish that specific dechlorinating organisms are present, and that their concentrations are higher in the treatment zone compared to background samples, which is an important line of evidence to demonstrate that bioremediation is working. However, the concentration of these biomarkers may not satisfactorily correlate with instantaneous dechlorination rates, due to the high uncertainty that would be associated with such rate predictions.

References


