



Quantifying the effects of fumarate on in situ reductive dechlorination rates

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Abstract

In situ methods are needed to evaluate the effectiveness of chemical amendments at enhancing reductive dechlorination rates in groundwater that is contaminated with the priority pollutant, trichloroethene (TCE). In this communication, a method that utilizes single-well, “push–pull” tests to quantify the effects of chemical amendments on in situ reductive dechlorination rates is presented and demonstrated. Five push–pull tests were conducted in each of five monitoring wells located in a TCE-contaminated aquifer at the site of a former chemical manufacturing facility. Rates for the reductive dechlorination of the fluorinated TCE-surrogate, trichlorofluoroethene (TCFE), were measured before (test 1) and after (test 5) three successive additions (tests 2–4) of fumarate. Fumarate was selected to stimulate the growth and activity of indigenous microorganisms with the metabolic capability to reduce TCFE and TCE. In three wells, first-order rate constants for the reductive dechlorination of TCFE increased by 8.2–92 times following fumarate additions. In two wells, reductive dechlorination of TCFE was observed after fumarate additions but not before. The transformation behavior of fumarate was also monitored following each fumarate addition. Correlations between the reductive dechlorination of TCFE and the reduction of fumarate to

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succinate were observed, indicating that these reactions were supported by similar biogeochemical conditions at this site.

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1. Introduction

Significant research efforts have been devoted to the development of in situ bioremediation as an approach for remediating groundwater contaminated with the priority pollutant, trichloroethene (TCE). In anaerobic environments, this approach depends on the metabolic capability of indigenous subsurface microorganisms to catalyze the reductive dechlorination of TCE to the dichloroethene (DCE) isomers, chloroethene (CE) and ethene (Fig. 1a) (Vogel et al., 1987; McCarty, 1997; Bradley, 2000). Engineered approaches are needed where natural attenuation does not result in the complete conversion of TCE to ethene or where rates are too slow to meet risk management goals.

A common approach for enhancing in situ reductive dechlorination is to stimulate the growth of indigenous dechlorinating microorganisms with the addition of chemical amendments. A wide variety of chemicals and chemical mixtures have been evaluated for their suitability as amendments for enhancing reductive dechlorination. Lee et al. (1997) reviewed results from laboratory tests that were designed to assess the effectiveness of potential amendments such as complex organic mixtures (molasses, wastewater, cheese whey permeate, corn steep liquor and manure tea), metabolic intermediates (benzoate, lactate, propionate, acetate and butyrate), alcohols (methanol and ethanol), molecular hydrogen, sulfate, nitrate, vitamins and micronutrients. While many of these amendments were effective, disadvantages were associated with each and none were universally effective at stimulating reductive dechlorination in groundwater at all sites. To the best of our knowledge, fumarate (*trans*-1,2-ethenedicarboxylate) has not previously been tested as a chemical amendment for enhancing reductive dechlorination rates. There is evidence that a number of dechlorinating microorganisms use fumarate as an alternative electron acceptor (Neumann et al., 1994; Neumann et al., 1995; Scholz-Muramatsu et al., 1995; Gerritse et al., 1996; Krumholz et al., 1996; Krumholz, 1997; Miller et al., 1997; Gerritse et al., 1999) and that certain dechlorinating organisms grow faster on fumarate than on chlorinated ethenes (Gerritse et al., 1999). Some dechlorinating microorganisms are also known to use fumarate (Kengen et al., 1999) or its reduction product, succinate (1,2-ethanedicarboxylate) (Gerritse et al., 1996), as electron donors during reductive dechlorination. Hence, an objective of this work was to evaluate the effectiveness of fumarate at enhancing in situ reductive dechlorination rates.

The effectiveness of a chemical amendment is generally evaluated by comparing reductive dechlorination rates measured with and without the chemical amendment in laboratory experiments with pure or mixed cultures of microorganisms. Experiments conducted in actual aquifers are not as common because field experiments, especially

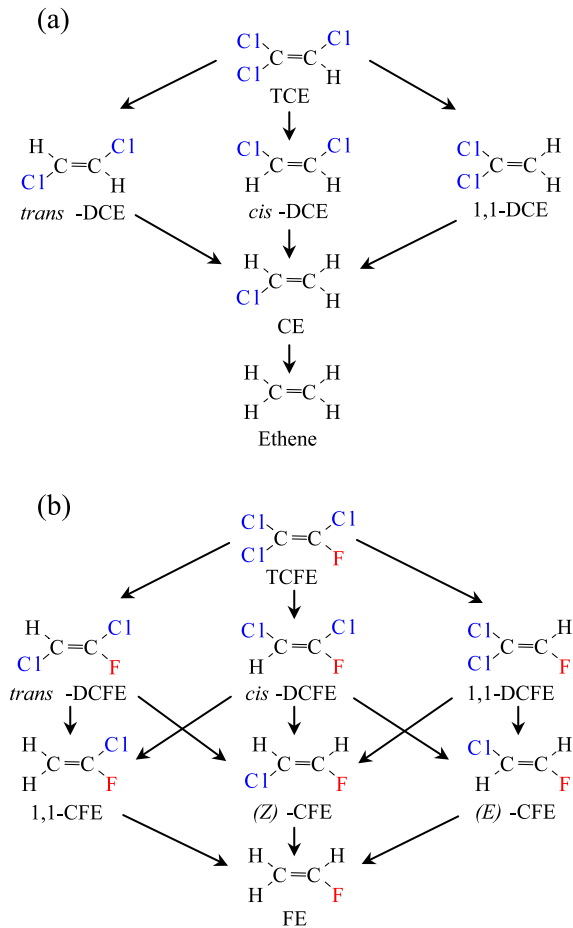


Fig. 1. Analogous reductive dechlorination pathways for (a) TCE and (b) its fluorinated surrogate, TCFE.

those involving well-to-well tests, are perceived as complicated, expensive and/or time-consuming in comparison to laboratory experiments. In addition, in situ transformation rates are difficult to obtain because solute concentrations in groundwater are affected by both transformation and transport (advection, dispersion and sorption) processes. Nevertheless, the need for field methods that can be used to evaluate the effectiveness of chemical amendments is becoming increasingly apparent as concerns about discrepancies between laboratory and field results mount (Chapelle and Lovely, 1990; U.S. EPA, 1998; Suarez and Rifai, 1999; Washington and Cameron, 2001). Field pilot tests designed to determine in situ reductive dechlorination rates in the presence of chemical amendments (e.g. acetate, nitrate and sulfate) were reviewed by Lee et al. (1998). In all but 1 of the 13 reported pilot tests, in situ reductive dechlorination rates were determined using well-to-well tests that involved recirculating amended groundwater between injection and extraction wells.

An alternative to using well-to-well tests to determine in situ transformation rates is to use single-well, push–pull tests (Istok et al., 1997; Haggerty et al., 1998; Schroth et al., 1998; Hageman et al., 2001; Schroth et al., 2001; Kleikemper et al., 2002; Pombo et al., 2002). Push–pull tests are conducted by injecting (“pushing”) an aqueous test solution containing a nonsorbing, nonreactive tracer and one or more reactants into the saturated zone of an aquifer via a monitoring well. Samples of the test solution/groundwater mixture are then extracted (“pulled”) from the same well over time and analyzed for tracer, reactant and product concentrations. The in situ transformation rate of an injected reactant is then determined by removing the effects of transport processes from measured reactant concentrations using a data processing technique. Push–pull tests are cost-effective relative to well-to-well tests because push–pull tests require only one groundwater well per test. Additional advantages are that push–pull tests take less time to conduct than well-to-well tests since injected solutes do not have to be transported between wells and that push–pull tests can be conducted simultaneously in different wells to assess spatial variability in transformation rates. Hageman et al. (2001) described the development of push–pull tests for determining in situ reductive dechlorination rates and demonstrated the technology by conducting push–pull tests in a TCE-contaminated aquifer. The injected test solution contained trichlorofluoroethene (TCFE), which was used as a surrogate for TCE based on evidence that it undergoes reductive dechlorination by a pathway analogous to that of TCE while retaining the fluorine label (Fig. 1b) (Vancheeswaran et al., 1999). TCE itself was not injected into TCE-contaminated groundwater because mixing of the injected test solution with native groundwater would have rendered it impossible to distinguish injected and background TCE.

The overall objective of the project described herein was to use the approach presented by Hageman et al. (2001) to quantify the effects of fumarate additions on in situ reductive dechlorination rates of TCFE. To this end, TCFE reductive dechlorination rates were measured before and after three consecutive additions of fumarate in five wells located in a TCE-contaminated aquifer. Additionally, the transformation behavior of fumarate was monitored after each fumarate addition so that correlations between reductive dechlorination and fumarate transformation behavior could be assessed.

2. Experimental

2.1. Chemicals

TCFE (97% pure, containing 0.1% *cis*-DCFE and 0.3% *trans*-DCFE), *cis/trans*-1,2-dichloroethene (DCFE) (98%) and *E/Z*-1-chloro-2-fluoroethene (97%) were obtained from SynQuest Laboratories (Alachua, FL). Fluoroethene (FE) (98%) was obtained from Lancaster Synthesis (Pelham, NH). Sodium fumarate (98%) and sodium succinate (99%) were obtained from Aldrich (Milwaukee, WI). Potassium bromide (99.7%) and sodium formate (99.6%) were obtained from Fisher Scientific (Fair Lawn, NJ). For use as an internal standard, 1-chloropropane was obtained from Matheson (Cincinnati, OH).

Table 1
Biogeochemical indicator concentrations in tested wells

	Concentration (μM) ^a				
	A-zone wells		C-zone wells		
	10A	9A	15C	16C	21C
Total organic carbon	15,000	1200	ND ^b	ND	ND
Dissolved oxygen	130	7.8	5.6	13.1	18
Total dissolved iron	95	218.4	ND	5.6	ND
Nitrate	ND	ND	ND	ND	ND
Sulfate	960	25,000	490	8300	170
Methane	18	46.25	0.26	0.36	ND

^a Samples were collected in May and June 1999. Total organic carbon by EPA 9060, dissolved oxygen by membrane electrode probe, total dissolved iron by EPA 6010B, nitrate and sulfate by EPA 9056, and methane by RSK-175.

^b Not detected.

2.2. Site description

Push–pull tests were conducted at the site of a former chemical manufacturing facility near San Francisco, CA where reductive dechlorination has been monitored in recent years (Buscheck et al., 1997; Buscheck, 1998; Hageman et al., 2001). Tests were conducted in two distinct aquifer zones. The A-zone is an unconfined shallow layer composed mainly of placed fill over Bay Mud. The water table lies within 3 meters of the ground surface. Groundwater velocities range from 1.5 to 6 m/year. The C-zone underlies the Bay Mud, is characterized by alluvial fan deposits, and is located approximately 6–23 m below the ground surface. Groundwater velocities range from 6 to 31 m/year. Biogeochemical indicator concentrations for each well are given in Table 1.

2.3. Push–pull tests

A series of five push–pull tests were conducted in each of two A-zone wells (10A and 9A) and in each of three C-zone wells (15C, 16C and 21C) between December 1999 and February 2001 (Table 2). All test solutions contained ~1.3 mM of bromide, which served as a conservative tracer. All test solutions injected during test 1, which was designed to

Table 2
Test solution compositions

Well	Test 1 (12/99)	Test 2 (9/00)	Test 3 (10/00)	Test 4 (12/00)	Test 5 (2/01)
10A	16 μM TCFE	0.81 mM fumarate	0.12 mM fumarate	0.75 mM fumarate	9.4 μM TCFE
9A	6.9 μM TCFE, 0.75 mM fumarate	0.87 mM fumarate	0.13 mM fumarate	0.74 mM fumarate	4.3 μM TCFE, 0.70 mM fumarate
15C	20 μM TCFE, 8.3 mM formate	0.79 mM fumarate	0.13 mM fumarate	0.74 mM fumarate	15 μM TCFE, 6.2 mM formate
16C	14 μM TCFE, 8.0 mM formate	0.81 mM fumarate	0.14 mM fumarate	0.73 mM fumarate	11 μM TCFE, 6.3 mM formate
21C	34 μM TCFE	0.81 mM fumarate	0.12 mM fumarate	0.70 mM fumarate	29 μM TCFE

measure initial rates for TCFE reductive dechlorination, contained TCFE (Table 2). Additionally, fumarate was included in the test solution injected into well 9A. While the focus of this project was not to measure the effects of formate on reductive dechlorination rates, formate was included in test solutions injected into wells 15C and 16C because previous measurements indicated that electron donors in the C-zone may limit reductive dechlorination rates. Rates obtained during test 1 in wells 10A, 15C and 21C were previously reported (Hageman et al., 2001); however, they differ from the rates reported herein because a different data processing techniques was used. The objectives of tests 2–4 were to amend the groundwater with fumarate and then to monitor the fumarate transformation behavior following each addition. Thus, test solutions injected during tests 2–4 contained fumarate (Table 2). The objective of test 5 was to measure reductive dechlorination rates after the fumarate additions so that rate changes due to fumarate could be quantified. Therefore, test solutions injected during test 5 contained TCFE, fumarate and formate at concentrations similar to those used in test 1 (Table 2).

The experimental design was identical to that described in Hageman et al. (2001), where a diagram of the experimental set-up and further information can be found. Test solutions were prepared on-site by adding bromide and fumarate or formate (Table 2) to argon-sparged tap water. In tests 1 and 5, a concentrated aqueous solution of TCFE was metered into the injection line. The TCFE solution was stored and pumped from a collapsible metallized-film gas-sampling bag to prevent volatilization loss during injection. In A-zone wells, which were 2.5 cm in diameter, 25–50 l of test solution were injected into the bottom of the well at a rate of ~0.2 l/min. In C-zone wells, which were 10 cm in diameter and capable of accepting a greater injection flow rate, ~250 l of test solution were injected at a rate of ~2 l/min between a pair of inflatable packers used to isolate a meter-long section of the well screen. Following the injection, samples of the test solution/groundwater mixture were collected approximately once per week for up to 85 days during tests 1 and 5 and on a varying schedule, with a maximum of four samples per day, for up to 20 days during tests 2–4. Samples were collected by extracting water with a peristaltic pump from the bottom of A-zone wells or from between the inflatable packers in C-zone wells. Volatile organic analysis vials (40 ml) were filled without headspace after purging A-zone and C-zone wells of 0.3 and 12 l of water, respectively. Samples were collected in duplicate or triplicate, shipped on ice and stored at 4 °C until analysis. All samples except those for bromide were preserved in 0.75% (v/v) concentrated HCl. Duplicate analyses were performed on ~10% of samples.

2.4. Analytical methods

Chromatographic separation and detection of TCFE, DCFE, CFE and FE were achieved using a gas chromatography/mass spectrometry method that was previously described (Hageman et al., 2001). The analyte introduction method was either headspace analysis (test 1 samples) or purge-and-trap (test 5 samples). The purge-and-trap system was composed of a Tekmar-Dohrmann (Cincinnati, OH) 3100 sample concentrator and an AQUA Tek 70 liquid analyzer. Quantification was performed in selected ion monitoring mode with 1-chloropropane as an internal standard. Chromatographic separation and detection of organic acids in test series 1–4 samples were performed on a Waters Alliance

(Milford, MA) high performance liquid chromatograph equipped with a photodiode array detector. Separations were achieved on a Phenomenex (Torrance, CA) Luna C18 column. Test series 5 samples were analyzed on a Dionex (Sunnyvale, CA) DX-320 ion chromatograph equipped with a conductivity detector and AS11 column. Bromide concentrations were measured with either a Dionex DX-120 or Dionex DX-320 ion chromatograph equipped with a conductivity detector and Dionex AS14 or AS11 column.

2.5. Data analysis

In situ rates for the reductive dechlorination of TCFE were determined by removing the effects of transport processes from measured aqueous concentrations of TCFE using a data processing technique called “forced mass balance” (FMB) (Hageman et al., 2003). TCFE transformation products were also treated with the FMB technique so that the distribution of transformation products formed in situ could be readily compared between tests. FMB was selected over other available data processing techniques (Haggerty et al., 1998; Snodgrass and Kitanidis, 1998) because it was designed for use with sorbing solutes. TCFE and its products were expected to undergo sorption during push–pull tests in both A- and C-zones based on results from short-term transport tests conducted at the selected site (Hageman et al., 2001) as well as estimated retardation factor (R) values.

The FMB technique (Hageman et al., 2003) was conducted by first calculating total (aqueous plus sorbed) concentrations for TCFE and each transformation product by multiplying the respective R values for each solute by their molar aqueous concentrations measured in push–pull test extraction samples. The R values estimated for TCFE, DCFE, CFE and FE were 11.5, 4.89, 2.41 and 1.69, respectively, in the A-zone and 2.05, 1.39, 1.14 and 1.07, respectively, in the C-zone. These values were estimated (Schnoor, 1996) using K_{om} values for TCFE, DCFE, CFE and FE of 90.5, 33.5, 12.2 and 5.99 l/kg, respectively; a bulk density of 2.32 kg/l; an aquifer porosity of 0.2; and fraction organic matter values of 0.01 (A-zone) and 0.001 (C-zone). The K_{om} values were estimated using the Estimations Program Interface Suite (Syracuse Research, 2000), while the other values were selected based on measurements made on aquifer sediments collected at the site.

A “transport-process” adjustment factor (Σ/Σ_o , where Σ and Σ_o are the sums of the total concentrations of TCFE and its transformation products in the extraction sample and in the well at the end of the test solution injection, respectively) was then calculated for each extraction sample. Next, total concentrations were divided by corresponding Σ/Σ_o values to remove the effects of transport processes (including sorption) and obtain FMB-adjusted concentrations. Finally, nonlinear least squares analysis was used to derive the equation that best described changes in FMB-adjusted concentrations of TCFE over time. Reviews of reported half-velocity coefficients for reductive dechlorination indicate that it is appropriate to assume first-order kinetics when chlorinated ethene concentrations are several μM or lower (Haston and McCarty, 1999; Suarez and Rifai, 1999). Since all measured aqueous concentration of TCFE were less than 2 μM except for those measured in the first two extraction samples collected in wells 10A and 21C, TCFE transformation was assumed to be governed by first-order kinetics. Based on evidence that sorbed-phase reactants are not bioavailable (Lyman et al., 1992), it was assumed that only TCFE in the

aqueous phase (and not in the sorbed phase) underwent transformation. Thus, equations used to describe changes in FMB-adjusted concentrations of TCFE were of the form

$$[TCFE]_{\text{FMB}} = [TCFE]_{\text{FMB},0} \exp(-kt/R(TCFE)) \quad (1)$$

where $[TCFE]_{\text{FMB},0}$ is the FMB-adjusted concentration of TCFE in the well at the end of the test solution injection, k is the first-order rate constant describing the rate of change in total concentrations of TCFE due to transformation alone, t is time and $R(TCFE)$ is the retardation factor for TCFE. The validity of the FMB technique was evaluated by quantifying errors in rates derived by applying FMB to push–pull test data generated by a numerical model (Hageman et al., 2003). Since the error analysis indicated that the in situ rate for the reductive dechlorination of TCFE was underestimated relative to the actual in situ rate by 10%, all rates reported herein are expected to be underestimated by a similar magnitude.

The effects of transport processes were removed from measured aqueous concentrations of fumarate, succinate and formate using a data processing technique developed for use with nonsorbing solutes that is referred to herein as “tracer-normalization” (Haggerty et al., 1998; Snodgrass and Kitanidis, 1998). Sorption was assumed to have a minimal effect on fumarate, succinate and formate concentrations since these solutes are negatively charged and highly water-soluble. Tracer-normalized concentrations for each solute were obtained by dividing their measured aqueous concentrations by the transport-process adjustment factor ($[Br]/[Br]_0$, where $[Br]$ and $[Br]_0$ are the measured concentrations of the co-injected bromide tracer in an extraction sample and in the injected test solution, respectively).

3. Results and discussion

3.1. Reductive dechlorination rates and product distribution ratios

Reductive dechlorination of TCFE occurred following its first injection into well 10A (test 1) as indicated by decreasing aqueous TCFE concentrations and increasing aqueous *cis*-DCFE concentrations (Fig. 2a). *Trans*-DCFE and (*E*)-CFE were also detected at relatively low concentrations ($<0.05 \mu\text{M}$). FMB-adjusted concentrations were calculated for each solute (see Section 2.5) (Fig. 2b). The exponential equation that best described the change in FMB-adjusted concentrations of TCFE over time,

$$[TCFE]_{\text{FMB}} = 180 \mu\text{M} \exp(-0.012t/11.5), \quad (2)$$

indicated that the in situ rate (k) for the transformation of TCFE to DCFE was 0.012 day^{-1} (Table 3). FMB-adjusted concentrations for TCFE and its transformation products obtained for test 1 in well 10A were also plotted in a stacked area graph (Fig. 3a) to facilitate visualization of TCFE/product distribution ratios.

After three successive additions of fumarate (tests 2–4), which were made 1–2 months apart, TCFE was injected into well 10A for a second time (test 5). Reductive dechlorination of TCFE to *cis*-DCFE, *trans*-DCFE, CFE (*E*>1,1>*Z*) and FE occurred (Fig. 3b) with TCFE being transformed at a maximum rate of 1.1 day^{-1} between days 30

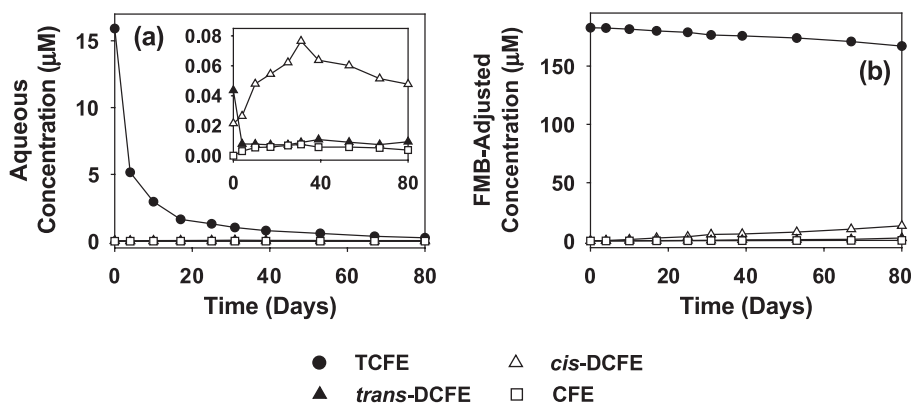


Fig. 2. Test 1 in well 10A. (a) Measured aqueous concentrations indicating concentration changes due to transformation and transport processes (with expanded view in inset) and (b) forced mass balance (FMB)-adjusted concentrations (see data analysis section) indicating concentration changes due to transformation only.

and 84 (Table 3). Thus, the maximum transformation rate was ~ 92 times greater after fumarate additions than before. Moreover, the extent of dechlorination increased, as indicated by detection of the less chlorinated products. The formation of FE had important implications for bioremediation at this site because it is the fluorinated analog of ethene (Hageman et al., 2001) (Fig. 1) and ethene is the desired end product for reductive dechlorination due to its low toxicity.

Following the first injection of TCFE into well 9A (test 1), TCFE underwent reductive dechlorination primarily to *cis*-DCFE although *trans*-DCFE was also formed (Fig. 3c). The rate of TCFE transformation was 0.021 day^{-1} (Table 3). Note that fumarate was co-injected with TCFE during the initial test conducted in well 9A but not during the initial test conducted in well 10A. After three additional injections of fumarate (tests 2–4) to well 9A, TCFE and fumarate were co-injected into well 9A for a second time (test 5). TCFE underwent reductive dechlorination primarily to *cis*-DCFE although *trans*-DCFE and CFE ($E>1,1>Z$) were also formed (Fig. 3d). TCFE was transformed at a maximum rate of 1.6 day^{-1} between days 7 and 50 (Table 3). Thus, the maximum transformation rate was ~ 76 times greater during test 5 than during test 1. While TCFE transformation rates obtained

Table 3
TCFE transformation rates

Well	Maximum first-order rate constants for transformation of TCFE to DCFE (day^{-1}) ^a	
	Test 1 (12/99)	Test 5 (2/01)
10A	0.012 (0–80 days)	1.1 (30–84 days)
9A	0.021 (0–82 days)	1.6 (7–50 days)
15C	0.017 (55–82 days)	0.14 (0–84 days)
16C	– ^b	0.051 (49–84 days)
21C	–	0.15 (16–84 days)

^a Transformation rates were calculated from FMB-adjusted concentrations over the period of days shown in parenthesis. Reported rates are expected to be underestimated relative to actual rates by $\sim 10\%$ based on results from a computer modeling study (Hageman et al., 2003) (see Section 2.5).

^b Transformation of TCFE to DCFE was not observed.

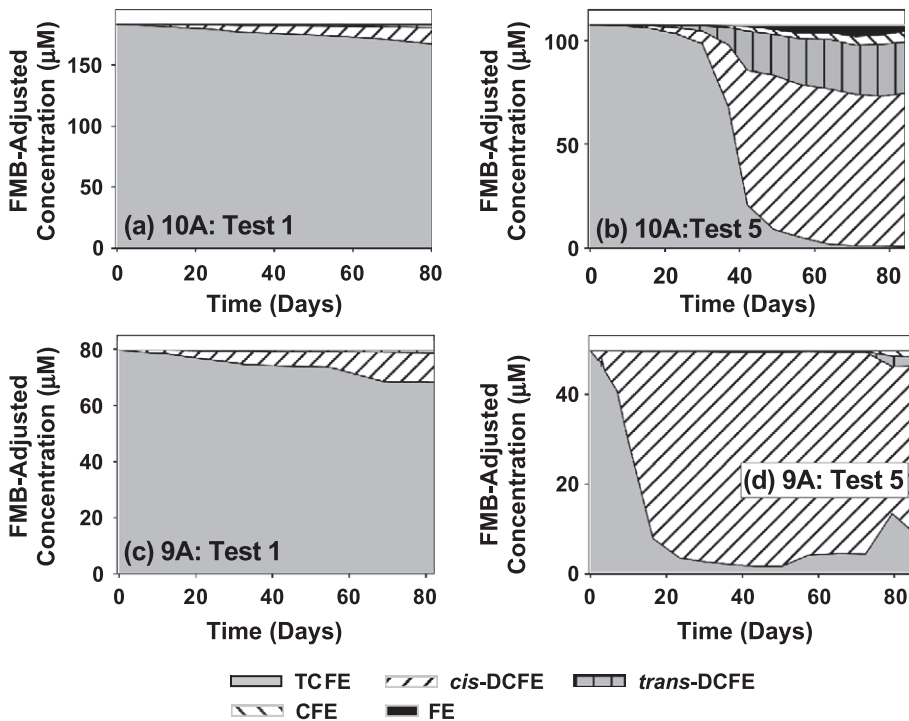


Fig. 3. Stacked area plots of FMB concentrations depicting reductive dechlorination of TCFE during tests conducted before (test 1) and after (test 5) fumarate additions in A-zone wells.

during test 5 in wells 10A and 9A were similar, the extent of dechlorination observed in these wells was remarkably different. In well 9A, *cis*-DCFE was formed almost exclusively (Fig. 3d), while in well 10A, the dechlorination of TCFE to FE was observed (Fig. 3b). This indicates that the microbial populations available for stimulation by fumarate were different in the two wells even though the wells were located within 25 m of each other and in the same horizontal subsurface zone.

Significant reductive dechlorination was not observed until 55 days after the first injection of TCFE into well 15C (test 1) (Fig. 4a). At that point, TCFE underwent reductive dechlorination primarily to *cis*-DCFE although *trans*-DCFE was also formed. TCFE was transformed at a rate of 0.017 day^{-1} between days 55 and 82 (Table 3). To increase the likelihood that reductive dechlorination would occur, formate was co-injected as an electron donor with TCFE during this test. Co-injected formate was completely degraded by day 27 (data not shown), indicating that an active community of formate-utilizing organisms was present. However, acetate, which would have been formed during acetogenesis of injected formate, was not detected. After three successive injections of fumarate (tests 2–4), TCFE and formate were co-injected into well 15C for a second time (test 5). Reductive dechlorination of TCFE to primarily *cis*-DCFE and CFE ($E \approx 1,1>Z$) occurred (Fig. 4b) although *trans*-DCFE was also formed. TCFE was transformed at a maximum rate of 0.14 day^{-1} between days 0 and 84 (Table 3). Thus, the maximum

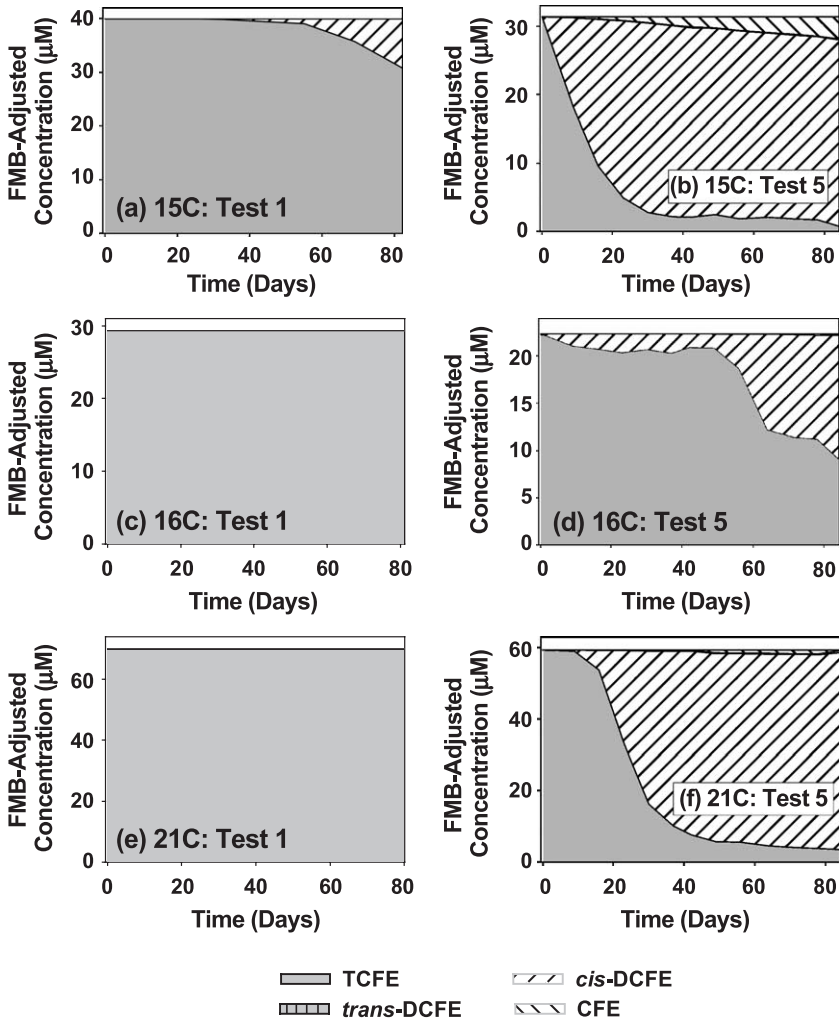


Fig. 4. Stacked area plots of FMB concentrations depicting reductive dechlorination of TCFE or lack thereof during test conducted before (test 1) and after (test 5) fumarate additions in C-zone wells.

transformation rate was ~8.2 times greater after fumarate additions than before. However, it may be of greater significance that the transformation reaction began immediately during test 5 instead of being delayed until late in the test as it was during test 1. Co-injected formate was not detected in any samples, indicating that the formate-utilization rate also increased between tests. This observation is consistent with the expectation that electron-donor utilization and reductive dechlorination rates should increase together. Again, acetate was not detected. Note that because formate was added as an amendment with TCFE in tests 1 and 5, the project was not designed to evaluate the effect of formate on reductive dechlorination rates.

In contrast to what was observed in all previously described wells, reductive dechlorination was not observed after the first injection of TCFE into well 16C (test 1) (Fig. 4c). Formate, which was again co-injected as an electron donor with TCFE, underwent degradation slowly such that it was still detected in samples collected at the end of the test (data not shown). This observation is consistent with the expectation that a slow formate-utilization rate would accompany a slow or non-detectable TCFE reductive dechlorination rate. Acetate formation via the potential acetogenesis pathway was not detected. After three successive additions of fumarate (tests 2–4), TCFE and formate were co-injected into well 16C for a second time (test 5). Reductive dechlorination of TCFE primarily to *cis*-DCFE occurred although *trans*-DCFE and CFE ($E \approx 1,1 > Z$) were also formed (Fig. 4d). TCFE was transformed at a maximum rate of 0.051 day^{-1} between days 49 and 84 (Table 3). Co-injected formate was not detected, indicating that formate-utilization and TCFE reductive dechlorination rates increased together, as was observed in well 15C. Although relatively high concentrations of TCFE persisted until the end of the test, it is of particular significance that TCFE reductive dechlorination was stimulated (test 5) (Fig. 4d) in a well where it had not initially been observed (test 1) (Fig. 4c).

Well 21C is located upgradient from the contaminant plume in an uncontaminated location. Reductive dechlorination of TCFE was not observed following the first injection of TCFE (test 1) (Fig. 4e), which is consistent with the expectation that dechlorinating microorganisms would not be active in the absence of chlorinated compounds. After three successive additions of fumarate (tests 2–4), TCFE was injected for a second time (test 5). Reductive dechlorination of TCFE primarily to *cis*-DCFE occurred although *trans*-DCFE and CFE ($E \gg 1,1 \approx Z$) were also formed (Fig. 4f). TCFE was transformed at a maximum rate of 0.15 day^{-1} between days 16 and 84 (Table 3). The occurrence of TCFE reductive dechlorination in well 21C during test 5 is significant because it indicates that reductive dechlorination was stimulated in an uncontaminated well.

3.2. Correlations between fumarate and TCFE transformation behavior

Three types of fumarate transformation behavior were observed during tests 2–4. The first type of behavior is classified as that in which fumarate was reduced to succinate upon the first fumarate injection and the rate at which fumarate was reduced to succinate increased upon each additional fumarate injection. This type of behavior was observed in wells 10A and 9A. In well 10A, for example, fumarate concentrations decreased more slowly in test 2 than in test 4 (Fig. 5a). Likewise, succinate formation was observed later in test 2 than in test 4. In both tests 2 and 4, succinate concentrations decreased to undetectable levels, indicating that microbial populations utilized succinate. Based on results from laboratory experiments (Gerritse et al., 1996), it is likely that succinate was utilized as an electron donor. In wells 10A and 9A, the transformation behavior exhibited by TCFE was similar to that exhibited by fumarate in that reductive dechlorination rates increased between tests 1 and 5. The similarities between TCFE and fumarate transformation behaviors indicate that reductive dechlorination and fumarate reduction may be supported by similar biogeochemical conditions in the A-zone. Note that, by plotting tracer-normalized concentrations in Fig. 5a, changes in concentration due only to transformation are depicted (see Section 2.5); however, in contrast to what was observed in

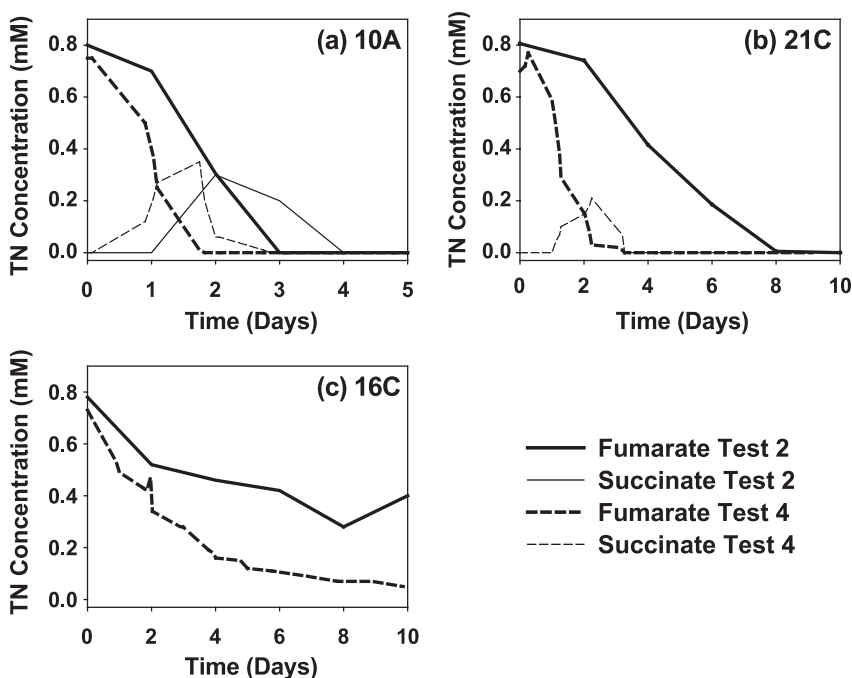


Fig. 5. Tracer-normalized (TN) concentrations (see Section 2.5) of fumarate and its reduction product, succinate.

Fig. 2 for aqueous and FMB concentrations, aqueous and tracer-normalized concentrations were not significantly different. For example, on day 1 during test 4 in well 10A, measured aqueous concentrations of fumarate and succinate were 0.35 and 0.20 mM, respectively, while corresponding tracer-normalized concentrations were 0.37 mM and 0.21 mM.

The second type of fumarate transformation behavior is classified as that in which the reduction of fumarate to succinate was not observed after its first addition (test 2) but was observed after later additions (e.g. test 4). This type of behavior was observed in wells 15C and 21C. In well 21C, for example, fumarate concentrations decreased without the concomitant detection of succinate in test 2 (Fig. 5b); however, decreases in fumarate concentrations were accompanied by succinate detection in test 4. While it is possible that succinate was not detected because it was rapidly utilized, the detection of succinate in test 4 indicates that it is likely that microbial populations capable of reducing fumarate to succinate were stimulated by successive fumarate additions. The TCFE transformation behavior observed in wells 15C and 21C was consistent with that observed for fumarate since TCFE reductive dechlorination was either observed only after a significant lag time or not observed at all in these wells during the first TCFE injection (test 1). Yet, TCFE reductive dechlorination was observed in these wells during the second TCFE injection (test 5). This correlative behavior indicates that reductive dechlorination and fumarate reduction may be supported by similar biogeochemical conditions in the C-zone.

The third type of fumarate transformation behavior is classified as that in which the reduction of fumarate to succinate was not observed during any of the fumarate additions

(tests 2–4). This type of behavior was observed in well 16C only (Fig. 5c). The hypothesis that reductive dechlorination and fumarate reduction were supported by similar biogeochemical conditions is further supported by observations in well 16C since the reductive dechlorination rate obtained during test 5 in well 16C was lower than that obtained in any other well during test 5 (Table 3).

Although TCFE and fumarate displayed parallel transformation behaviors in all five wells at this site, further investigation is needed to determine what processes were responsible for increases in TCFE reductive dechlorination rates. An important consideration, however, is that in all wells (except well 16C) both injected fumarate and succinate (formed in situ) were degraded to undetectable concentrations within 10 days after each fumarate injection (tests 2–4) (Fig. 5). Since neither fumarate nor succinate was present in the groundwater during the final TCFE injection (test 5), it was not the presence of either of these compounds that caused reductive dechlorination rates to increase. Rather, the consecutive additions of fumarate appeared to have altered the biogeochemical conditions in a way that favored reductive dechlorination. Since previous reports indicate that a number of dechlorinating microorganisms utilize fumarate as an alternative electron acceptor (Neumann et al., 1994, 1995; Scholz-Muramatsu et al., 1995; Gerritse et al., 1996, 1999; Krumholz et al., 1996; Krumholz, 1997; Miller et al., 1997) and that at least one of them grows faster on fumarate than on chlorinated ethenes (Gerritse et al., 1999), it is possible that fumarate additions stimulated the growth of microbial populations that utilize both fumarate and chlorinated ethenes. Therefore, a change in microbial community structure could have been responsible for the enhancement of reduction rates for both TCFE and fumarate. However, it is also possible that changes in microbial populations were induced by the presence of succinate (formed in situ), which can be used as an electron donor (Gerritse et al., 1996), or by fumarate acting as an electron donor instead of as an electron acceptor (Kengen et al., 1999). Note that background total organic carbon, which includes compounds that can potentially serve as natural electron donors, was present at a higher concentration than injected fumarate (1.3 mM) in well 10A, at a similar concentration in well 9A, and was not detected in C-zone wells (Table 1). Other potential scenarios are that fumarate acted as an electron donor to reduce the concentrations of indigenous electron acceptors or that TCFE itself stimulating the growth of dechlorinating microorganisms. On-going research in our group with soil microcosms is designed to further characterize the relation between fumarate and TCE/TCFE reductive dechlorination rates.

4. Conclusions

This communication describes the application of a methodology for quantifying changes in in situ reductive dechlorination rates due to the addition of chemical amendments. This methodology has the potential to improve bioremediation technologies because it can be used to measure the effectiveness of bioremediation technologies in the field. To the best of our knowledge, this communication also describes the first use of fumarate in a field method designed to enhance reductive dechlorination rates. TCFE reductive dechlorination rates increased from 8.2 to 92 times in wells where reductive

dechlorination was initially observed. Reductive dechlorination activity was stimulated in wells where it was not initially observed. Similarities in the transformation behaviors of TCFE and fumarate indicated that reductive dechlorination and fumarate reduction were supported by similar biogeochemical conditions.

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