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Complete biological dehalogenation of chlorinated ethylenes in sulfate containing groundwater

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Abstract

The ability of dehalogenating bacteria to compete with sulfate reducing bacteria for electron donor was studied in microcosms that simulated groundwater contaminated with both chlorinated ethylenes and fuel hydrocarbon compounds. Results demonstrate that reductive dehalogenation of perchloroethylene to ethylene can proceed in the presence of $>$ 100 mg l^{-1} sulfate. The hydrogen concentration, which was 2.5 nM in the presence of approximately 150 mg l^{-1} sulfate and in the absence of chlorinated compounds, decreased to 0.7 nM during the dechlorination of trichloroethylene and increased to 1.6 nM during the dechlorination of *cis*-dichloroethylene and vinyl chloride. With only sediment associated donor ("historical" donor) present, dechlorination of trichloroethylene proceeded slowly to ethylene (on a time scale of several years). Addition of toluene, a model hydrocarbon compound, stimulated dechlorination indirectly. Toluene degradation was rapid and linked to sulfate utilization, and presumably formed fermentable substrates that served as hydrogen donors. Dehalogenation was inhibited in soil free microcosms containing 5 mM sulfide, but inhibition was not observed when either aquifer sediment or 5 mM ferrous chloride was added.

Introduction

Numerous sites are contaminated with chlorinated ethylenes, fuel hydrocarbons (including benzene, toluene, ethylbenzene, and xylenes (BTEX)) and non-chlorinated solvents (Committee on Ground Water Cleanup Alternatives 1994). Typically, biodegradation of BTEX depletes both oxygen and nitrate, leading to sulfate-reducing or fermentative conditions. Once fermentative conditions are established, dechlorination of perchloroethylene (PCE) and trichloroethylene (TCE) can proceed rapidly to ethylene (Bouwer & McCarty 1983; De Bruin et al. 1992; Holliger et al. 1998; Maymo-Gatell et al. 1997). In the presence of sulfate, however, dechlorination has been reported to cease at *cis*-dichloroethylene (*cis*-DCE) or vinyl chloride (VC) (Bagley & Gossett 1990; Boopathy & Peters 2001; Cabirol et al. 1998; Harkness et al. 1999; Pavlostathis & Zhuang 1993). To devise cost-effective remediation strategies, it is important to know how the dechlorination process is affected by sulfate ions and sulfate reducing bacteria (SRB), and how the addition of degradable organic co-contaminants, such as petroleum hydrocarbons, sustain the dehalogenation process. The recommended approach is to enhance attenuation by first completely removing the background electron acceptors, i.e., oxygen, nitrate, and sulfate (ESTCP 2001), an approach that may be expensive and difficult to implement. This study shows that removal of sulfate may not be necessary to completely dechlorinate *cis*-DCE and VC and that natural attenuation may be a viable alternative.

Various PCE and TCE dechlorinating organisms have been identified that can consume electron donors other than hydrogen, such as acetate and pyruvate (He et al. 2002; Holliger et al. 1998). So far, however, all known organisms that reductively dehalogenate *cis*-DCE and VC appear to be hydrogenotrophic, i.e., require hydrogen as the electron donor. Growth of hydrogenotrophic organisms, such as dehalogenating

bacteria (DHB), sulfate reducing bacteria, and methanogenic bacteria (MB) (Figure 1), in natural environments is generally limited by the availability of hydrogen. Observed hydrogen concentrations therefore represent a steady-state hydrogen concentration that is determined by the organism with the lowest hydrogen threshold level (Fennell et al. 1997; Löffler et al. 1999; Yang & McCarty 1998). Hydrogen threshold levels are determined by the physiology of the organism and are independent of the rate of hydrogen production or consumption (Lovley & Goodwin 1988). Thermodynamic considerations suggest that hydrogen threshold levels for DHB should be lower then those for SRB (Löffler et al. 1999). However, experimental results (Table 1) indicate that hydrogen threshold levels associated with *cis*-DCE and VC dehalogenation can be lower or higher than those for SRB (Löffler et al. 1999; Lu et al. 2001; Yang & McCarty 1998). This suggests that, in certain environments, DHB are able to utilize hydrogen at lower concentrations than SRB. Metabolic dehalogenation of chlorinated ethylenes might thus limit or prevent growth of SRB at steady-state hydrogen concentrations. Such conditions are expected when electron donors are limiting and hydrogen production is slow. Mazur & Jones (2001) reported lower hydrogen concentrations and less sulfate reduction during continuous PCE dehalogenation as compared to sulfate reduction in the absence of PCE, but did not address dehalogenation of TCE, *cis*-DCE, and VC in the presence of sulfate.

A variety of fermentable organic materials can be precursors for hydrogen formation, including commonly encountered aromatic pollutants such as benzene, toluene, and xylenes (Figure 1). In this study, toluene and unidentified organic material (including other contaminants and bacterial cells) associated with the sediment or groundwater, termed here "historical" donor, were evaluated as potential electron donors. Toluene was selected because it is frequently encountered at contaminated sites and readily degraded under sulfidogenic conditions (Edwards et al. 1992), it can be degraded under fermenting conditions (Grbic-Galic & Vogel 1987), and it has been shown to support reductive dehalogenation of PCE under methanogenic conditions (Harkness et al. 1999; Sewell & Gibson 1991). Minimum hydrogen concentrations were detected for SRB and compared to TCE, *cis*-DCE, and VC dehalogenating consortia. Sulfate reduction and dehalogenation were monitored when an excess of hydrogen was present, and suspected sulfide toxicity for DHB was investigated in the presence and absence of aquifer sediment and ferrous iron.

This study was undertaken to (i) evaluate the ability of DHB to compete against SRB under simulated site conditions, (ii) investigate the effect of toluene on dehalogenation of chlorinated ethylenes in the presence of sulfate, (iii) determine hydrogen threshold levels during sulfate reduction and dechlorination of TCE, *cis*-DCE, and VC, and (iv) investigate dehalogenation of chlorinated ethylenes under simulated site conditions when sulfate has been depleted.

Materials and methods

Chemicals

All compounds used were 99+% pure unless noted otherwise, and used as received. Toluene, PCE, TCE, and *cis*-DCE (97%) (Aldrich Chemical Co., Milwaukee, WI) were used as amendments and to prepare analytical standards. Nitrogen and hydrogen (Praxair Inc., Danbury, CT) were used as headspace gases. VC, ethylene, methane, and hydrogen gases (Scott Specialty Gases, Alltech Associates, Inc., Deerfield, IL) were used as analytical standards. Final concentrations of toluene, chlorinated solvents, and VC were determined with gas chromatography (GC) and flame ionization detection (FID) analysis. Sodium salt solutions of sulfate and acetate (J.T. Baker Chemical Co., Phillipsburgh, NJ) were used as amendments and/or to prepare analytical standards.

Aquifer sediment and enrichment culture

Aquifer sediment and groundwater were collected from a depth of 15 feet in a borehole at Hunter's Point Shipyard, San Francisco, CA. This site was contaminated with multiple pollutants, including PCE, TCE, and fuel hydrocarbons. The sulfate concentration in the site groundwater was 1.98 mM (190 mg l^{-1}). The presence of *cis*-DCE, VC, ethylene, and ethane at the site, and the absence of PCE and TCE, suggested that dechlorination of PCE and TCE to *cis*-DCE, VC, and ethylene was ongoing at the site. Toluene and other BTEX compounds were absent in the groundwater, and had presumably been removed through anaerobic biodegradation prior to sampling.

A culture dehalogenating TCE to ethylene was enriched from Moffett Field, Mountain View, CA. This site was contaminated with multiple pollutants,

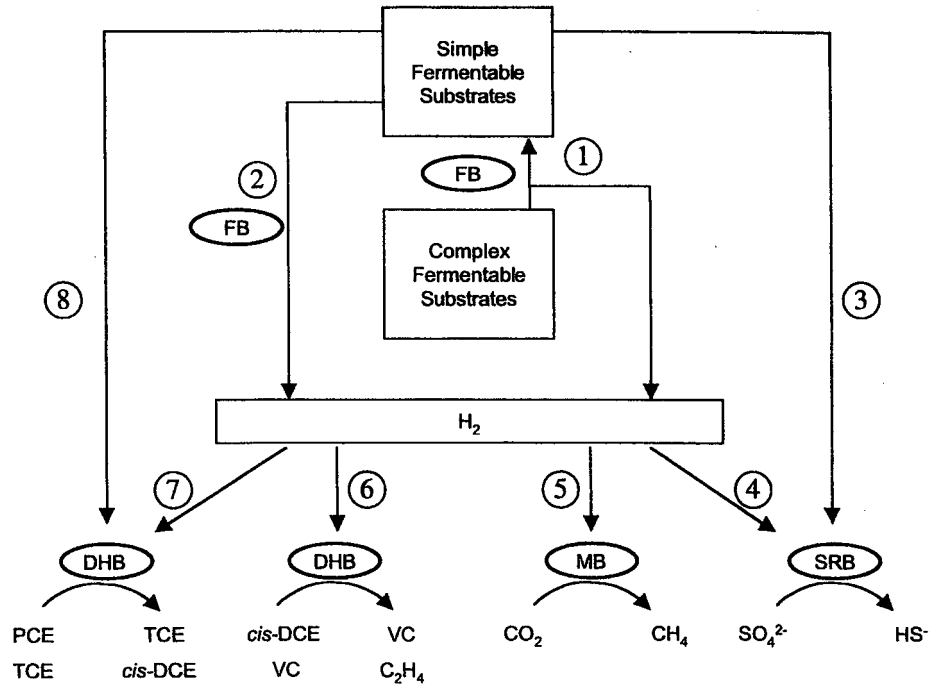

Figure 1. Simplified scheme showing the main pathways for hydrogen production and utilization in natural environments in the presence of chlorinated ethylenes: degradation of complex fermentable substrates (e.g. bacterial cell compounds) (1) or simple fermentable substrates (e.g. organic acids, alcohols, and aromatics including BTEX compounds) (2) by fermentative bacteria (FB), direct consumption of simple fermentable substrates by sulfate-reducing bacteria (SRB) (3), competitive hydrogen consumption by SRB (4), methanogenic bacteria (MB) (5), and dehalogenating bacteria (DHB) (6, 7), and direct consumption of simple fermentable substrates by certain DHB (8).

including PCE, TCE, and fuel hydrocarbons, and contained approximately 2.5 mM sulfate (250 mg l^{-1}). The culture was grown in a methanogenic medium as described previously (Holliger et al. 1993) and used for microcosm studies to investigate sulfide toxicity. Aquifer sediment used for these experiments was obtained from the same site.

Microcosm studies

Microcosms were constructed in duplicate or triplicate in an anaerobic glove box by adding 20 g aquifer sediment and 110 ml groundwater from the site in 160 ml serum vials. Oxygen was removed from groundwater by degassing under vacuum followed by purging with nitrogen gas. Vials were sealed with butyl rubber stoppers and aluminum crimp seals, and the headspace (about 40 ml) was replaced with nitrogen. The microcosms were incubated at room temperature during the course of the experiment. Sorption of PCE to the sediment and rubber stoppers was assumed to be completed during the time period between the construction of the microcosms (Day 0) and the first analyses (Day 46). The measured concentrations at Day 46 in the microcosms are given in Table 2. The microcosms were amended with either sulfate and PCE (microcosm SP), sulfate and toluene (microcosm ST), or sulfate, toluene and PCE (microcosm STP). An autoclaved control (microcosm TPC) was amended with toluene and PCE. Toluene and PCE were added to the microcosms in neat form with a 10 μl Hamilton syringe at concentrations of approximately 200 μM and 150 μM (aqueous, assuming no partitioning to gas and solid phases), respectively. Sulfate was added at 500 μM using a stock solution of 275 mM. The autoclaved control study (microcosm TPC) was conducted to determine abiotic reactions and system losses. Because duplicate microcosms showed consistent results, only one data set is discussed for each microcosm pair. Methane concentrations did not exceed 25 μM with the exception of microcosm STP, which was amended with hydrogen after approximately 48 months. Ethane was not formed in any of the microcosms.

Analytical methods

Headspace samples (250 μl) were withdrawn from microcosms using a 500 μl valved precision gas-tight

Table 1. Hydrogen threshold concentrations associated with various terminal electron acceptors. Data in μM

	Lovley & Goodwin 1988	Yang & McCarty 1998	Löffler et al. 1999	Lu et al. 2001	Mazur & Jones 2001	This study
PCE				0.6–0.9	0.5	
TCE				0.6–0.9	0.5	0.7
cis-DCE		2	<0.3	0.1–2.5		1.6
VC		2	<0.3	2–24		1.6
Methanogenesis	7–10	>11		2.5–24		
Sulfate reduction	1–1.5			1.5–4.5	0.8	2.5

Table 2. Initial concentrations of sulfate, toluene, chlorinated ethylenes, ethylene, and methane in microcosms after amendments of toluene, PCE, and sulfate on Day 46. Data in μM

Experiment	TPC[1]	SP[2]	ST[2]	STP[2]
Sulfate	2000	2480	2440	2420
Toluene	161	0	162	161
PCE	83.4	113.5	0	59.0
TCE	0.0	0.0	0.0	0.0
cis-DCE	1.2	2.2	2.0	1.3
VC	1.1	0.9	1.0	0.6
Ethylene	0.4	0.3	0.4	0.2
Methane	11.6	12.1	13.5	8.6

Concentrations are calculated for the aqueous phase assuming no partitioning into the gaseous and solid phases. Sulfate has been detected directly through liquid phase analysis (IC), all other concentrations have been calculated from the gas phase concentration (GC/FID).
[1]Control study, autoclaved prior to chemical amendments.
[2]Amended with 0.50 mM sodium sulfate at Day 0.

syringe equipped with a side-port needle, and analyzed for toluene, PCE, TCE, *cis*-DCE, *trans*-DCE, 1,1-DCE, VC, ethylene, ethane, and methane using GC/FID (HP 5890). Samples were injected splitless at 225 °C and separated using a 30-m megabore GSQ-PLOT column (J&W). With PCE present, the temperature program was 60 °C for 1 min, increase to 150 °C at 30 °C min^{-1}, and isothermal at 150°C for 30 min. Without PCE present, GC conditions were 60 °C for 1 min, increase to 220 °C at 70 °C min^{-1}, and isothermal at 220 °C for 3 min. Volatile compounds were identified and quantified by comparing retention times and peak areas with those of external standards. Headspace concentrations and Henry's constants (Gossett 1987; Stumm & Morgan 1996) were used to calculate the total amount in a microcosm, assuming that solid-phase partitioning was insignificant. Volatile compound concentrations are expressed as if the volatile fraction is present in the liquid phase, i.e., as total mass present per volume of water. Sulfate, benzoate, and acetate were analyzed using a Dionex series 4000i ion chromatograph (IC) equipped with a conductivity detector, a Dionex IonPac AS4A column (4 × 250 mm), and an AG4A guard column (4 × 50 mm). Sodium bicarbonate was the eluent and an external standard was used for quantification. Samples (200 μl) were centrifuged and diluted (50 times) prior to analysis. Hydrogen was analyzed by direct headspace injection on a Trace Analytical gas chromatograph model RGA3 (Menlo Park) equipped with a reduction gas detector (RGD) and quantified using an external standard. The detection limit for hydrogen in the gas phase was 10 ppb, which corresponds to an aqueous hydrogen concentration of approximately 0.01 nM.

Results and discussion

Controls consisted of autoclaved microcosms (TCP) that were amended with toluene and PCE. Over the course of the experiment, concentrations of (chlorinated) ethylenes, toluene, sulfate, and methane remained essentially constant, indicating that abiotic PCE transformation and system losses were insignificant (Figure 2).

Dehalogenation in the presence of sulfate with only historical electron donor present

In microcosm SP, which contained approximately 2.5 mM sulfate and only historical electron donor, PCE was slowly transformed to VC and traces of ethylene (Figure 3). Sulfate reduction was not observed. VC

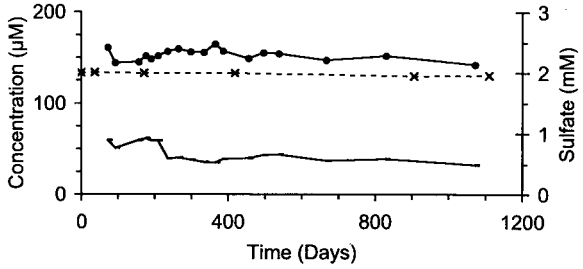

Figure 2. Microcosm TPC: Control study. Microcosm was autoclaved prior to amendment of toluene and PCE. Toluene, PCE, and sulfate do not change significantly, indicating that abiotic processes can be ignored. Toluene (●), PCE (−), and sulfate (×).

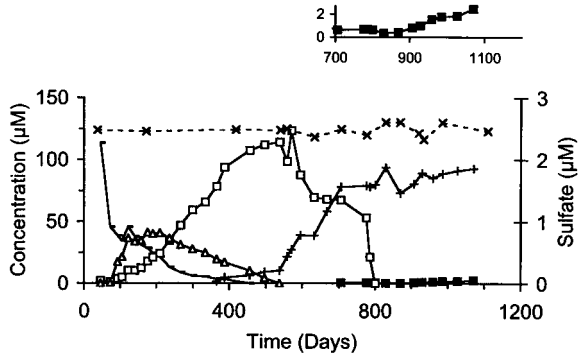

Figure 3. Microcosm SP: Dehalogenation of PCE to VC and traces of ethylene without a donor added and without noticeable sulfate reduction. PCE (−), TCE (△), *cis*-DCE (□),VC (+), ethylene (■), and sulfate (×).

formation was slow but noticeable when TCE was present and increased markedly after TCE was depleted. Ethylene formation commenced at Day 801 only after *cis*-DCE was completely removed at a slow rate (approximately 0.01 μM day^{-1}) (insert Figure 3). The increase in ethylene was clearly measurable (final concentration of 2.5 μM), although the amount of ethylene formed was too small to result from a measurable decrease in the VC concentration.

Dehalogenation of chlorinated ethylenes at the site was incomplete at the time of sampling (*cis*-DCE was present at approximately 1.7 μM (165 μg l^{-1})). It is interesting to speculate why *cis*-DCE appeared to persist at the site even though historical electron donor was present. Assuming the rates observed in microcosm SP, it would have taken an estimated three more years to complete the dechlorination of 1.7 μM *cis*-DCE. Perhaps, historical donor was unavailable in situ, and breaking up soil particles in microcosm SP made this donor available to fermenting bacteria.

Complete dehalogenation in toluene amended microcosms

The effect of toluene on sulfate reduction and dehalogenation was studied in microcosms ST (Figure 4A/B) and STP (Figure 5A/B). Both microcosms were amended with approximately 160 μM toluene on Day 0 and respiked at Day 558. In both cases, toluene transformation was accompanied by sulfate removal. In a separate microcosm study, toluene was replenished until the sulfate was depleted (data not shown). The toluene (25 μM) remaining after sulfate depletion appeared persistent, suggesting that toluene was utilized directly by SRB, as observed in previous experiments (Beller et al. 1996). Toluene transformation in microcosm STP commenced after a lag-time of 209 days (Figure 5A), whereas no apparent lag-time was observed in microcosm ST (Figure 4A). We suspect that the long lag phase in microcosm STP was due to PCE toxicity because the onset of toluene transformation in microcosm STP coincided with the rapid drop in PCE (Figure 5B). The lag-time was only 37 days after respiking, when VC was the only chlorinated ethylene present.

Historically present *cis*-DCE and VC were completely dechlorinated to ethylene in microcosm ST (Figure 4B), and, as in microcosm SP, dehalogenation occurred in the presence of moderately high sulfate (1.36 mM or 131 mg l^{-1}). Dechlorination of *cis*-DCE to VC commenced shortly after the onset of toluene degradation and was complete within 176 days. VC dechlorination was slow until Day 387 when ethylene was produced at a rate of approximately 0.02 μM day^{-1} until VC was nearly depleted. To confirm the complete conversion of VC to ethylene, the microcosm was flushed with nitrogen gas and amended with 45.2 μM VC (Day 830). To better approximate site conditions, sulfate was added to obtain 2 mM. Without a detectable lag, VC was completely transformed to ethylene in 241 days (Figure 4C), producing ethylene at a rate as high as 1 μM day^{-1}.

Dehalogenation in microcosm STP (Figure 5B) proceeded all the way to ethylene and occurred significantly faster than in microcosm SP (containing only historical donor). PCE, TCE, and *cis*-DCE were completely transformed to VC in 387 days, compared to 801 days in microcosm SP, demonstrating that toluene addition stimulated dehalogenation. Dechlorination of PCE and TCE proceeded at similar rates as in SP until the onset of toluene transformation. Thereafter, dehalogenation rates increased significantly. Once *cis*-

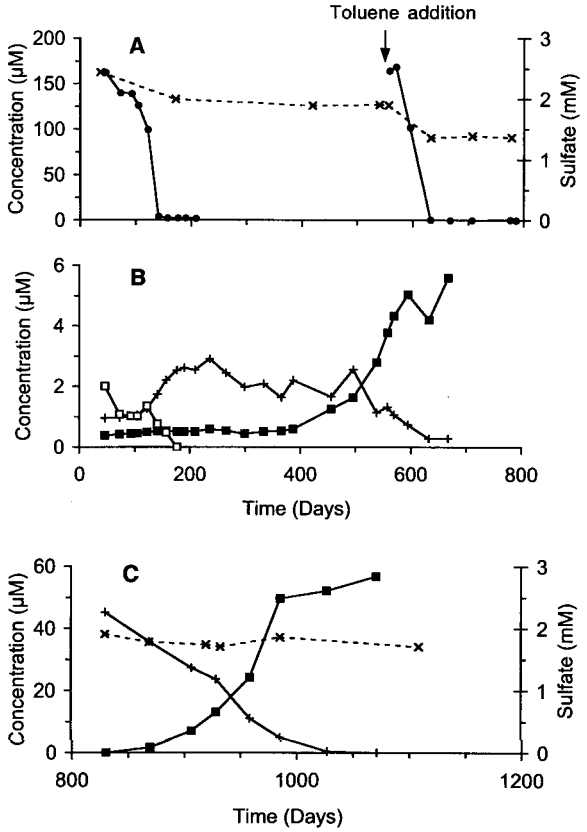

Figure 4. Microcosm ST: (A) Concurrent removal of toluene and sulfate in the absence of PCE. (B) Conversion of historically present *cis*-DCE and VC to ethylene in the presence of sulfate (>1.3 mM). (C) Conversion of VC to ethylene without noticeable sulfate reduction. Toluene (●), *cis*-DCE (□), VC (+), ethylene (■), and sulfate (×).

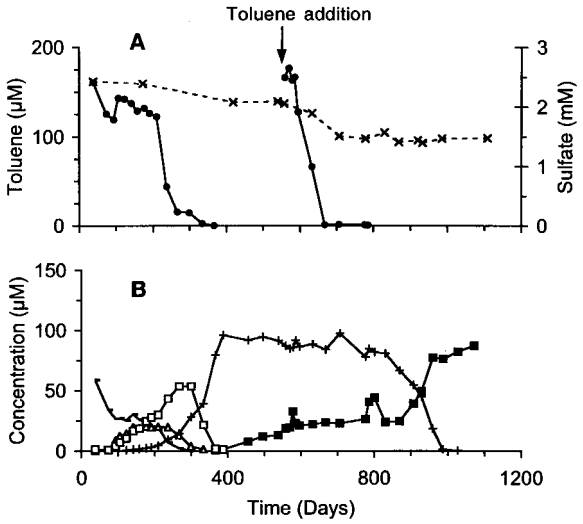

Figure 5. Microcosm STP: PCE dehalogenation to ethylene stimulated by toluene. (A) Concurrent removal of toluene and sulfate. (B) Complete conversion of PCE to ethylene. Toluene (●), PCE (−), TCE (△), *cis*-DCE (□), VC (+), ethylene (■), and sulfate (×).

DCE was completely removed, conversion of VC to ethylene began at a rate of approximately 0.09 μM ethylene day^{-1}. The microcosm was respiked with toluene on Day 558 in an effort to stimulate the dechlorination of VC. Although toluene was removed rapidly, the stimulatory effect was not evident for another 270 days. During this time the production of ethylene slowed down for unknown reasons. Conversion of VC to ethylene resumed around Day 830 at an accelerated rate (approximately 0.6 μM ethylene day^{-1}) and went to completion. Ethylene formed (87 μM) exceeded the amount of PCE (59 μM), which is attributed to desorption and subsequent dechlorination of historically present chlorinated ethylenes.

Enhanced dehalogenation occurred only after toluene depletion, thus toluene can be excluded as a direct electron donor. The underlying mechanisms for the stimulating effect of toluene on dehalogenation reactions are unknown, but may involve fermentation of anabolic products formed during toluene transformation. Yang & McCarty (2000) suggested that biomass could be a slow hydrogen release substrate, and showed that bacterial cells can serve as an electron donor for dehalogenation of *cis*-DCE.

Hydrogen threshold levels

The observation that dehalogenation of PCE to ethylene proceeded without noticeable sulfate removal in microcosm SP suggests that the responsible DHB can compete with SRB for available electron donor, presumably by consuming hydrogen at lower levels than the SRB. This is consistent with thermodynamic considerations (Löffler et al. 1999) and hydrogen threshold levels determined in previous studies (Table 1). To verify this hypothesis, hydrogen concentrations were monitored in microcosm STP after respiking with approximately 30 μM TCE, and compared to microcosms SP and ST. Acetate concentrations were non-detectable (< 0.5 mg l^{-1}) at the beginning and the end of the experiment for all three microcosms. Figure 6A shows sequential transformation of TCE to ethylene in microcosm STP. The hydrogen concentrations in microcosms STP, ST, and SP are depicted in Figure 6B. The hydrogen level of 2.5 ± 0.3 nM (average and standard deviation of three injections) in microcosm ST, which was depleted in chlorinated compounds, represents the minimum hydrogen con-

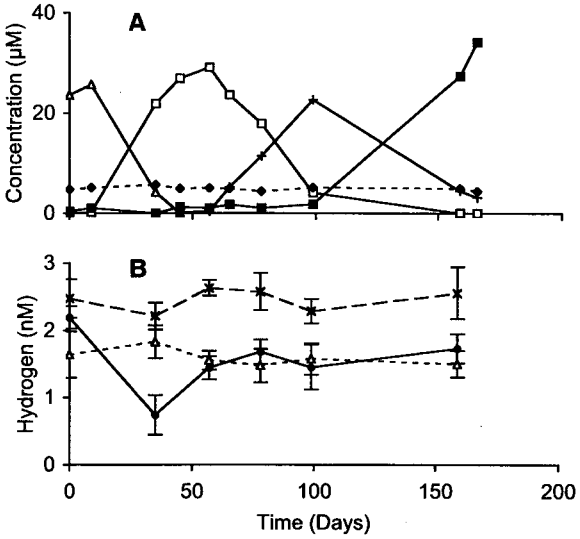

Figure 6. Microcosms SP, ST, and STP: Dehalogenation and hydrogen concentrations. (A) TCE dehalogenation in microcosm STP without noticeable sulfate reduction. TCE (△), *cis*-DCE (□), VC (+), ethylene (■), and methane (⧫). (B) Hydrogen threshold levels for sulfate reduction (2.5 ± 0.3 nM), *cis*-DCE and VC dehalogenation (1.6 ± 0.2 nM), and TCE dehalogenation (0.7 ± 0.2 nM). Error bars indicate the standard deviation of 3 injections. Microcosm ST (×), SP (△), and STP (●).

centration for SRB in our microcosms. During the course of this experiment minor quantities of VC were converted to ethylene in microcosm SP in the presence of approximately 2.5 mM sulfate (data not shown). This microcosm had a significantly lower average hydrogen concentration of 1.6 ± 0.2 nM, indicating that the VC dehalogenating bacteria consume hydrogen at a lower level than the SRB. The hydrogen concentration in microcosm STP, which was depleted in chlorinated compounds until the beginning of the experiment, was 2.2 ± 0.2 nM at Day 0. This is close to the hydrogen concentration observed in microcosm ST. The hydrogen level dropped to 0.7 ± 0.3 nM while TCE was converted to *cis*-DCE. After TCE depletion the hydrogen level increased to 1.6 ± 0.2 nM during dehalogenation of *cis*-DCE and VC, consistent with hydrogen concentrations observed in microcosm SP.

These data clearly show that in our system DHB have a lower hydrogen threshold level than the SRB. This means that DHB can outcompete SRB and complete dechlorination can occur without losing reducing equivalents to sulfate reduction. The analytical variation in the sulfate data, however, is too large to draw the conclusion that sulfate reduction did not occur at all. The significantly lower hydrogen concentration associated with TCE dehalogenation compared to *cis*-DCE and VC dehalogenation may explain the consistent sequential dehalogenation patterns observed in our study. After TCE depletion, the hydrogen concentration increased from 0.7 nM to 1.6 nM before significant dehalogenation of *cis*-DCE and VC was observed. The depressed hydrogen concentrations during TCE conversion may explain why rapid VC formation only occurred in the absence of higher chlorinated compounds.

Rapid dehalogenation in microcosms amended with hydrogen

Microcosm STP was amended with hydrogen to investigate the effect of excess of electron donor on sulfate reduction and dehalogenation reactions. After complete transformation of approximately 60 μM PCE (Figure 5B) and 25 μM TCE (Figure 6A) in the presence of sulfate, microcosm STP was flushed with hydrogen gas (Day 0) and respiked with 129 μM PCE. Hydrogen was kept in excess during the course of the experiment.

Sulfate removal (Figure 7A) began shortly after the addition of hydrogen and was depleted in less than four days. Significant methane production started after a lag-time of seven days. PCE transformation started immediately after addition of hydrogen (Figure 7B). Dehalogenation of PCE, TCE, *cis*-DCE, and VC occurred concurrently and resulted in rapid and complete conversion to etylene. This observation was in contrast to the previous experiments with this microcosm, where hydrogen was limiting and dechlorination was sequential (Figure 6A). The short lag-time for sulfate reduction and the immediate onset of dechlorination suggests that active hydrogenotrophic dehalogenating and sulfate-reducing communities were already present. In contrast, the seven-day lag phase for methanogenesis indicates that methanogens had been present but unable to develop in the presence of sulfate, as expected.

Dehalogenation was rapid although 2 mM sulfate was reduced and inhibitory levels of sulfide could have formed. A possible explanation for the apparent absence of sulfide inhibition is that sulfide was precipitated with ferrous iron contained in the sediment, as suggested previously for toluene degradation under sulfate reducing conditions (Beller & Reinhard 1995). To further investigate the effect of sulfide, ferrous iron, and aquifer sediment, dehalogenation of TCE was monitored in soil-free microcosms using a dehalogenating enrichment culture grown in a meth-

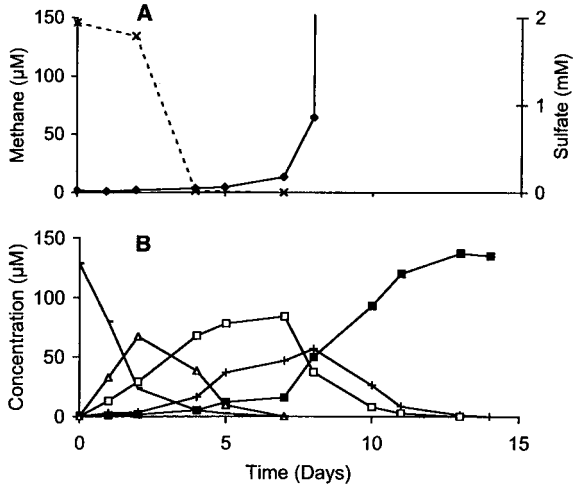

Figure 7. Microcosm STP: Rapid PCE dechlorination to ethylene in the absence of sulfate and with hydrogen in the headspace. (A) Rapid sulfate depletion after the addition of hydrogen. Methanogenesis starts 7 days after hydrogen addition. (B) Rapid dehalogenation of PCE. PCE (−), TCE (△), *cis*-DCE (□), VC (+), ethylene (■), methane (♦), and sulfate (×).

Table 3. Effect of sulfide on dehalogenation rates

	Day 0	Day 21			
	TCE	TCE	*cis*-DCE	VC	ETH
No amendments	23.2	0.0	0.1	2.3	23.7
Sediment	27.0	0.0	0.0	0.0	29.1
5 mM Na_2S	19.7	3.9	9.4	8.3	2.3
Sediment + 5 mM Na_2S	17.6	0.0	0.2	0.7	19.8
5 mM FeCl2 + 5 mM Na_2S	21.2	0.0	0.0	2.6	22.5

An enrichment culture dehalogenating TCE to ethylene grown in a methanogenic medium was used for this experiment. The addition of 5 mM sulfide inhibited TCE dehalogenation, but this effect was not observed when either 5 mM ferrous iron or aquifer sediment was added.

anogenic medium. Results were compared to similar microcosms amended with sulfide, sulfide and aquifer sediment, or sulfide and ferrous chloride (Table 3). Dehalogenation was inhibited in soil free microcosms containing 5 mM sulfide. However, this effect was suppressed when either aquifer sediment or 5 mM ferrous chloride was added. This experiment shows that sulfide toxicity can be avoided when sufficient aquifer sediment is present. This may explain why sulfide inhibition has not been observed at field experiments where large quantities of sulfate were reduced (Lee et al. 1998). However, the capacity of aquifer sediment to neutralize sulfide may be site specific. Continuous sulfide production from new groundwater during long-term field applications may exhaust the sulfide neutralizing capacity of the sediment, resulting in sulfide accumulation and inhibition of dehalogenation reactions.

Conclusions and implications for site remediation

Data developed here show that dehalogenation of chlorinated ethylenes can be complete even in the presence of moderately high sulfate concentrations (100–250 mg l^{-1}). This finding is consistent with hydrogen measurements, which indicate lower threshold levels for DHB than for SRB. The measured hydrogen threshold concentration for TCE dehalogenation (0.7 nM) is significantly lower than for sulfate reduction (2.5 nM) and dehalogenation of *cis*-DCE and VC (1.6 nM). These threshold levels may explain the frequently made observation that *cis*-DCE and VC accumulate at sulfidogenic sites. Hydrogen produced in the presence of TCE is efficiently used by TCE dehalogenators and unavailable to SRB and *cis*-DCE and VC dechlorinating bacteria. By contrast, when *cis*-DCE and VC are present, hydrogen is consumed by SRB thereby lowering the hydrogen availability to the *cis*-DCE and VC dechlorinating bacteria. When hydrogen is present at concentrations exceeding the threshold level of the SRB, sulfate consumption and dehalogenation proceed rapidly and dehalogenation of TCE to ethylene is complete, consistent with the hypothesis that TCE dehalogenation inhibits the SRB by lowering the hydrogen concentration.

The fact that TCE dechlorination may proceed to completion even in the presence of sulfate is significant from a practical view point. It indicates that natural processes may lead to complete dehalogenation at sulfidogenic sites if historical donor is present and that complete removal of sulfate to enhance anaerobic dechlorination may not be necessary.

Sulfide produced from sulfate reduction was shown to inhibit dehalogenation. This effect can be mitigated by aquifer sediment or ferrous iron that decreases sulfide toxicity by binding hydrogen sulfide. However, the sulfide binding capacity of the sediments is likely limited and the effect in field applications is likely temporary. DHB can compete efficiently with SRB for hydrogen when hydrogen is formed slowly by the fermentation of some relatively persistent compounds. Toluene has been found effective to stimulate dechlorination, presumably by serving as a precursor

to fermentable substrates, possibly biomass. We expect other alkyl benzenes to be similarly effective. Natural attenuation might therefore be expected at sites contaminated with both chlorinated ethylenes and fuel hydrocarbons. The underlying mechanisms, such as the hypothesized hydrogen release from bacterial cells, as well as possible field applications merit further study.

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