Effect of competitive terminal electron acceptor processes on dechlorination of *cis*-1,2-dichloroethene and 1,2-dichloroethane in constructed wetland soils

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Abstract

Thermodynamic calculations were coupled with time-series measurements of chemical species (parent and daughter chlorinated solvents, H_2, sulfite, sulfate and methane) to predict the anaerobic transformation of *cis*-1,2-dichloroethene (*cis*-1,2-DCE) and 1,2-dichloroethane (1,2-DCA) in constructed wetland soil microcosms inoculated with a dehalorespiring culture. For *cis*-1,2-DCE, dechlorination occurred simultaneously with sulfite and sulfate reduction but competitive exclusion of methanogenesis was observed due to the rapid H_2 drawdown by the dehalorespiring bacteria. Rates of *cis*-1,2-DCE dechlorination decreased proportionally to the free energy yield of the competing electron acceptor and proportionally to the rate of H_2 drawdown, suggesting that H_2 competition between dehalorespirers and other populations was occurring, affecting the dechlorination rate. For 1,2-DCA, dechlorination occurred simultaneously with methanogenesis and sulfate reduction but occurred only after sulfite was completely depleted. Rates of 1,2-DCA dechlorination were unaffected by the presence of competing electron-accepting processes. The absence of a low H_2 threshold suggests that 1,2-DCA dechlorination is a cometabolic transformation, occurring at a higher H_2 threshold, despite the high free energy yields available for dehalorespiration of 1,2-DCA. We demonstrate the utility of kinetic and thermodynamic calculations to understand the complex, H_2-utilizing reactions occurring in the wetland bed and their effect on rates of dechlorination of priority pollutants.

Introduction

Reductive dechlorination of chlorinated solvents such as trichloroethene (TCE), *cis*-1,2-dichloroethene (*cis*-1,2-DCE) and 1,2-dichloroethane (1,2-DCA) are commonly linked to the utilization of molecular H_2 as an electron donor (Ballapragada *et al*., 1996; Smatlak *et al*., 1996; Fennel & Gossett, 1998; Maymó-Gattel *et al*., 1999, 2001; Kassenga *et al*., 2004) by dehalorespiring microbial populations. A number of microbial groups compete for the available H_2, and therefore production and utilization of H_2 is one of the most important factors that control the kinetics and thermodynamics of reductive dechlorination and other terminal electron-accepting processes in anaerobic systems (Hoehler *et al*., 1998; Jacobsen *et al*., 1998; Conrad, 1999). Complicating the influence of H_2 on reductive dechlorination is the ability of other H_2-utilizing anaerobic populations (i.e. methanogens, acetogens and sulfate reducers) to dechlorinate solvents cometabolically (Fathepure & Boyd, 1988; Terzenbach & Blaut, 1994; Cole *et al*., 1995).

Microbial populations, specifically the H_2 consumers and the H_2 producers, can be limited in their metabolic activities by the H_2 concentrations in their natural habitats (Lovley & Klug, 1983; Robinson & Tiedje, 1984; Cord-Ruwisch *et al*., 1988; Lovley & Goodwin, 1988; Lovley & Phillips, 1987; Fennel & Gossett, 1998; Yang & McCarty, 1998). This limitation is due to the thermodynamics of their metabolic reactions, which are highly sensitive to H_2 concentrations. Terminal electron-accepting reactions can be affected when H_2 concentrations become too low. This may result in inhibition of H_2-linked metabolic reactions (Zehnder & Brock, 1979; Hoehler *et al*., 1994, 1998). The dominant microbially mediated terminal electron-accepting process can limit H_2 within a very narrow range, and, on this basis, the H_2 concentration has been used as an indicator of the specific electron-accepting process (Lovley & Goodwin,

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1988; Jacobsen & Postma, 1999). A characteristic of dehalorespiring populations is their ability to operate effectively at very low H_2 concentrations (< 0.3 nM) (Fennel & Gossett, 1998; Kassenga *et al.*, 2004).

H_2 levels can be a useful tool for indicating the redox conditions if a given process is exceedingly dominant (assuming steady-state conditions and complete competitive exclusion), otherwise interpretation of H_2 concentrations may sometimes be ambiguous, as Jacobsen & Postma (1999) observed. In the presence of higher potential oxidants, O_2 and NO_3^-, Washington *et al.* (2004) observed that H_2 was not diagnostic but was predictive once these oxidants were reduced. Owing to limitations of using H_2 alone, Jacobsen *et al.* (1998) proposed an alternative approach whereby measured reactant and product concentrations may be used to determine actual potential *in situ* energy yield, which, in turn, will indicate the potential for a given H_2-oxidizing terminal electron-accepting process. This approach can be used to interpret H_2 from systems where steady-state and complete exclusion is not achieved (Jacobsen & Postma, 1999). In the present study this approach is extended to chlorinated solvent degradation in wetland peat soils. H_2 concentrations are coupled with time-course measurements of parent and daughter compounds and free energy calculations to evaluate the dominant electron-accepting processes in these complex anaerobic systems.

Treatment wetlands have been proposed and tested for biological treatment of chlorinated solvents (Pardue *et al.*, 1999; Kassenga *et al.*, 2003, 2004) as natural wetlands appear effectively to support attenuation of these compounds (Lorah & Olsen, 1999; Lorah & Voytek, 2004). For wetlands to be a reliable treatment alternative, dehalorespiring organisms need to compete successfully with other H_2-utilizing organisms for available H_2. Owing to their abundance of organic carbon, wetlands are an environment where anaerobic populations capable of cometabolic degradation of chlorinated solvents may play an important role. Simultaneous measurements of H_2, chlorinated solvents, methane and the associated microbial populations have helped to explain the complex relationships between dehalorespirers and methanogens in wetland soil microcosms (Kassenga *et al.*, 2004). Thermodynamic calculations may be useful in predicting whether reactions are feasible based on real *in situ* concentrations of H_2, reactants and metabolites involved in the dechlorination reactions. In particular, the effect of alternative electron acceptors such as sulfate and sulfite are of interest because these may be present in many coastal environments where this treatment approach is of interest. The main objective of the study was to investigate the use of thermodynamic calculations coupled with direct measurements of chemical species (parent and daughter compounds, H_2, sulfite, sulfate and methane) to predict the feasibility and patterns of transformation of *cis*-1,2-DCE and 1,2-DCA in the wetland bed. A number of studies have focused on the biodegradation of 1,2-DCA and *cis*-1,2-DCE (Holliger *et al.*, 1990; Ballapragada *et al.*, 1996; Maymó-Gattel *et al.*, 1999; Arp *et al.*, 2001) by dehalorespirers and cometabolic degrading populations such as methanogens and acetogens.

Materials and methods

Anaerobic microcosms

Degradation kinetics of *cis*-1,2-DCE and 1,2-DCA were investigated under sulfite- and sulfate-reducing conditions and methanogenic conditions in microcosms constructed with a wetland soil mixture and deionized water. Microcosms were constructed under a nitrogen atmosphere in a glove-bag from a mixture of Latimer peat (Latimer's Peat Moss Farm, West Liberty, OH), a compost product, Bion Soil (Dream Maker Dairy, Cowlesville, NY) and sand mixed at a ratio of 1.3 : 1.1 : 1 (Bion Soil : Latimer : sand) by weight (Kassenga *et al.*, 2003). This mixture has been proposed as a construction material for treatment wetlands for chlorinated solvents (Kassenga *et al.*, 2003). The organic matter content of the soil mixture was ~30%. The soil components were mixed with deionized water to achieve a slurry of *c.* 250 g L^{-1}. The slurry (145 mL) was dispensed into 160 mL serum bottles that were subsequently sealed with Teflon-lined, butyl rubber septa and aluminum crimp caps. The microcosms were inoculated with 5 mL of slurry taken from microcosms, which previously demonstrated complete degradation of the test compounds. In a previous study (Kassenga *et al.*, 2004), these slurries were confirmed to contain *Dehalococcoides* sp., a dehalorespiring bacteria capable of degrading *cis*-1,2-DCE completely to ethane via vinyl chloride and ethene and 1,2-DCA to vinyl chloride and ethene (Maymó-Gattel *et al.*, 1999; Hendrickson *et al.*, 2002).

The following treatments were used during the experiments: (1) *cis*-1,2-DCE (methanogenic conditions), (2) 1,2-DCA (methanogenic conditions), (3) 1,2-DCA and sulfite, (4) 1,2-DCA and sulfate, (5) *cis*-1,2-DCE and sulfite, and (6) *cis*-1,2-DCE and sulfate. Aqueous stock solutions of chemicals of interest were added to bottles using a gas-tight syringe to arrive at the desired concentrations. Initial concentrations of *cis*-1,2-DCE and 1,2-DCA in all experiments were *c.* 70 μM. To establish sediments in which sulfate and sulfite reduction were the predominant terminal electron-accepting processes, sodium sulfate and sodium sulfite were added into the bottles at a final concentration of *c.* 2.5 mM from aqueous stock solutions. Sterile controls were included in the study to monitor for nonbiological losses of the test chemicals. Reaction mixtures were prepared

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as described above, and were adjusted to contain 1% formalin. After spiking with appropriate chemicals the microcosms were incubated in an inverted position under static conditions at 25 °C in the dark. Resazurin (0.0002%) was added as the redox indicator. To ensure reproducibility, triplicate microcosms were used in each experiment. Microcosms were not amended with either hydrogen donor or nutrients.

Temporal monitoring of concentrations of parent compounds and daughter products was performed until the concentrations of the contaminants dropped below the detection limits of the analytical methods. Samples for measurement of chlorinated ethenes and ethanes, ethane, ethene, methane, carbon dioxide, hydrogen, pH and oxidation–reduction potential (ORP) were withdrawn from the bottles at appropriate intervals of time as determined by the rates of degradation of test chemicals and analysed immediately without storage. Aqueous sulfate, sulfite and sulfide concentrations were also monitored.

Degradation kinetics

Modeling of *cis*-1,2-DCE and 1,2-DCA monitoring data in anaerobic microcosms was performed assuming that degradation follows pseudo first-order kinetics:

$$C_t = C_o e^{-kt} \quad (1)$$

where C_t [ML^{-3}] is the concentration at any time t, C_o [ML^{-3}] is the initial concentration and k [T^{-1}] is the pseudo first-order reaction rate constant. When significant losses (greater than 5%) were observed in the sterile microcosms, this was subtracted from the concentration in the nonsterile microcosms to adjust for abiotic losses before the first-order rate constant was calculated.

Thermodynamic calculations for the terminal electron-accepting processes

The amount of free energy, ΔG_r, obtained from a hypothetical biologically mediated reaction aA+bB → cC+dD for the given environmental conditions is calculated from Dolfing & Harrison (1992), Thauer *et al.* (1977):

$$\Delta G_r = \Delta G_r^{o'} + RT \ln \frac{[C]^c [D]^d}{[A]^b [B]^b}. \quad (2)$$

The value $\Delta G_r^{o'}$ is obtained from the value ΔG^o by making the appropriate corrections for pH = 7 and temperature. Table 1 gives the equations and thermodynamic values used for calculating *in situ* Gibb's free energies for terminal electron-accepting processes of interest in the present study. Thermodynamic values were calculated for 25 °C and pH 7, which are the approximate temperature and pH at which the experiments were conducted.

Analytical procedures

Volatile organic compounds (VOCs) were analysed using US EPA Method 8260B. The gas chromatograph–mass spectrometer (Agilent 6890-5972A) was equipped with a 30 m × 0.25 mm × 0.25 μm film thickness of Agilent-5MS (5% phenyl methyl siloxane capillary column) (Agilent, Palo Alto, CA). Daily blanks, calibration checks and surrogates were run to ensure that the analytical method was stable. Methane, ethene, ethane and carbon dioxide were measured using gas chromatography/Flame ionization detector (FID). Headspace gas was withdrawn using a gas-tight syringe and injected onto the gas chromatograph with flame ionization detector (Agilent 5890 Series II) equipped with a 2.4 m × 0.32 mm inner diameter column packed with Carbopack b/1% SP-1000 (Supelco, Bellefonte, PA). The column temperature was held at 40 °C isothermally for 6.5 min, and the injector and detector temperatures were 375 and 325 °C, respectively. The carrier gas was ultrahigh-purity nitrogen (BOC Gases, Baton Rouge, LA) at a flow rate of 12 mL min^{-1}.

Organic acids (lactate, acetate, propionate, butyrate, formate, succinate and benzoate) were measured using a high-pressure liquid chromatograph (Agilent 1090 Series II Liquid Chromatograph) with 0.1% H_3PO_4 as a mobile phase and a diode array detector. A Supelcogel C-610 H column was utilized at a flow rate of 0.5 mL min^{-1} and temperature of 30 °C. A series of external standards of each organic acid were utilized for quantitation. Hydrogen was analysed using reduction gas analyser (Trace Analytical, Menlo Park, CA) equipped with a reduction gas detector. Headspace samples were injected into a 1 mL gas sampling loop and were separated with a molecular sieve analytical column (Trace Analytical) at an oven temperature of 40 °C. The detection limit for H_2 under these conditions was 0.01 μL L^{-1}. Aqueous H_2 concentrations were calculated using the following equation adopted from Löffler *et al.* (1999): $[H_{2,aq.}] = LP/RT$, where $[H_{2,aq.}]$ is the aqueous concentration of H_2 (in mol L^{-1}), L is the Ostwald coefficient for H_2 solubility (0.01913 at 25 °C, unitless), R is the universal gas constant (0.0821 L atm K^{-1} mol^{-1}), P is the pressure (in atmospheres) and T is the temperature (K). Preparation of standards was performed using a certified 10 μL L^{-1} standard in N_2 (BOC Gases). The standards were diluted in serum bottles containing H_2-free N_2 at ambient temperature and pressure. Hydrogen was sampled at a sufficient interval of time (72–240 h) to ensure complete equilibrium during the continuous production and consumption of aqueous hydrogen in the sediment material

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Table 1. Equations and thermodynamic values used for calculating *in situ* Gibbs free energy yields for terminal electron-accepting processes

Terminal electron-accepting process	Equation used for calculating ΔG_r	$\Delta G_r^{o'}$* (kJ/mol)	$\Delta G_r^{o'}$ (kJ/mol H_2)
Hydrogenotrophic methanogenesis: $HCO_3^- + 4H_2(aq) + H^+ \leftrightarrow CH_4(aq) + 3H_2O$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([CH_4]/[H_2]^4[HCO_3^-][H^+])$	–194.5	–48.6
Acetoclastic methanogenesis: $CH_3COO^- + H_2O \leftrightarrow CH_4(aq) + HCO_3^-$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([CH_4][HCO_3^-]/[CH_3COO^-])$	–14.4	
Sulfate reduction: $SO_4^{2-} + 4H_2(aq) + H^+ \leftrightarrow HS^- + 4H_2O$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([HS^-]/[H_2]^4[SO_4^{2-}][H^+])$	–222.5	–55.6
Sulfite reduction: $SO_3^{2-} + 3H_2(aq) + H^+ \leftrightarrow HS^- + 3H_2O$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([HS^-]/[H_2]^3[SO_3^{2-}][H^+])$	–225.7	–75.2
cis-1,2-DCE dechlorination: $C_2H_2Cl_2 + H_2(aq) + H^+ \rightarrow C_2H_3Cl + Cl^- + 2H^+$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_3Cl][Cl][H^+]/[H_2][C_2H_2Cl_2])$	–156.9	–156.9
Vinyl chloride dechlorination: $C_2H_3Cl + H_2(aq) + H^+ \rightarrow C_2H_4 + Cl^- + 2H^+$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_4][Cl][H^+]/[H_2][C_2H_3Cl])$	–167.0	–167.0
Ethene reduction: $C_2H_4 + H_2(aq) \rightarrow C_2H_6$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_6]/[H_2][C_2H_4])$	–116.3	–116.3
Sum (sequential dechlorination of *cis*-1,2-DCE)		–440.0	–440.0
1,2-DCA dechlorination (dihaloelimination): $C_2H_4Cl_2 + H_2 + H^+ \rightarrow C_2H_4(aq) + 2Cl^- + 3H^+$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_4][Cl]^2[H^+]^2/[H_2][C_2H_4Cl_2])$	–205.6	–205.6
Consecutive hydrogenolysis: $C_2H_4Cl_2 + 2H_2 + H^+ \rightarrow C_2H_5Cl + Cl^- + 4H^+$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_5Cl][Cl][H^+]^3/[H_2]^2[C_2H_4Cl_2])$	–264.8	–132.4
$C_2H_5Cl + H_2 + H^+ \rightarrow C_2H_6 + Cl^- + 2H^+$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_6][Cl][H^+]/[H_2][C_2H_5Cl])$	–169.4	–169.4
Sum (consecutive hydrogenolysis of 1,2-DCA) $C_2H_4Cl_2 + 3H_2(aq) + 2H^+ \rightarrow C_2H_6(aq) + 2Cl^- + 6H^+$	$\Delta G_r = \Delta G_r^{o'} + RT\ln([C_2H_6][Cl]^2[H^+]^4/[H_2]^3[C_2H_4Cl_2])$	–434.2	–144.7

cis-1,2-DCE, *cis*-1,2-dichloroethene; 1,2-DCA, 1,2-dichloroethane.
Thermodynamic data on solutes are from Dolfing & Janssen (1994) and Stumm & Morgan (1996).
*$\Delta G_r^{o'}$ values were calculated according to Dolfing & Janssen (1994): organic substrate + H_2 → organic product + H^+ + Cl^-. Aqueous ΔG_r° values were used for organic substrates at 25 °C, pH 7. $\Delta G_r^\circ(H^+) = -39.9$ kJ mol^{-1}; $\Delta G_r^\circ(Cl^-) = -131.3$ kJ mol^{-1}.

(Hoehler *et al.*, 1998). In addition, before analysis, the bottles were shaken to equilibrate dissolved H_2 with the gas phase. All hydrogen data are reported as the aqueous phase concentration.

Sulfur and chloride were analysed using a colorimetric method with a portable spectrophotometer (DR 2010; Hach Co., Loveland, CO). Whenever possible samples were immediately analysed after being collected, otherwise sulfide samples were filtered and spiked with four drops of 2 N zinc acetate per 100 mL and stored at 4 °C for preservation prior to analysis. The HCO_3^- concentration was calculated from CO_2 partial pressure in the microcosm headspace using the Henry's constant for CO_2, the p*K*a for HCO_3^- (Westermann, 1994) and the *in situ* pH (Conrad *et al.*, 1986). HS^- concentrations were calculated from the measured values of hydrogen sulfide using the ionization fraction parameters approach ($pK_{a1} = 7.02$ and $pK_{a2} = 13.9$ at 25 °C) (Morel & Hering, 1993). Redox potential and pH were determined using an Ultrameter 6P (Myron L Company, Carlsbad, CA). The instrument was standardized in commercially prepared buffer solutions for pH (4.0 and 7.0) (Hach Co.) and saturated quinhydrone buffer solutions for redox potential.

Results

Dechlorination of *cis*-1,2-DCE and 1,2-DCA under methanogenic conditions

Degradation kinetics of 1,2-DCA and *cis*-1,2-DCE and associated H_2 concentrations were determined under methanogenic conditions (Figs 1 and 2). To establish that methanogenic conditions were present, microcosm porewater was analysed for redox-sensitive substances including ferric iron, ferrous iron, nitrate, nitrite, sulfate, sulfite and methane. Except for methane, which was detected at aqueous concentrations in excess of 1000 μM in all microcosms, all other redox-sensitive compounds were present in trace amounts in these highly organic wetland soils. With the exception of one instance of acetate at 1.8 μM, we did not observe the presence of detectable concentrations of organic anions (detection limit ~1 μM).

Increases in methane concentration, depletion of 1,2-DCA and accumulation of ethene were simultaneously observed in microcosms, indicating that dechlorination of 1,2-DCA and methanogenesis were co-occurring (Fig. 1). 1,2-DCA concentrations decreased from ~75 to < 1 μM

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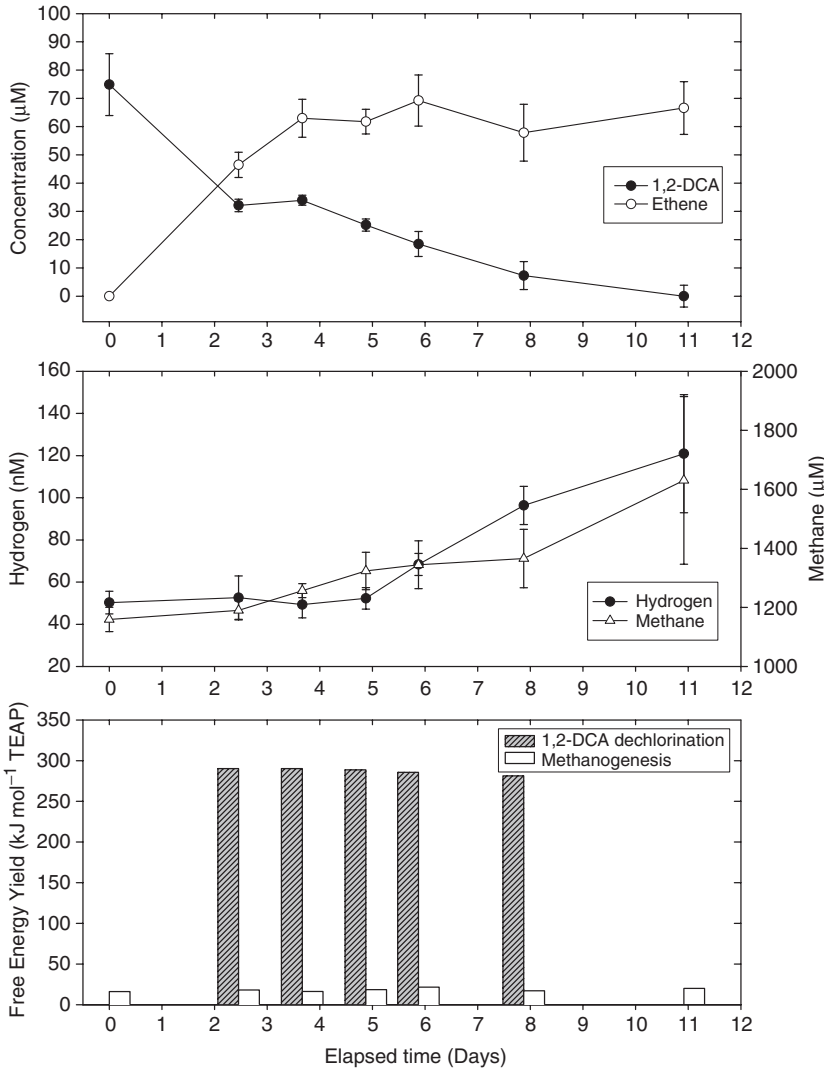

Fig. 1. Dechlorination of 1,2-dichloroethane and energy yields of the terminal electron-accepting processes under methanogenic conditions.

while methane increased from ~1200 to ~1600 μM. Rate constants for 1,2-DCA dechlorination averaged 0.25 ± 0.02 day^{-1} (Table 2). Thermodynamic calculations supported the observed simultaneous dechlorination of 1,2-DCA and methanogenesis (1,2-DCA dechlorination, 273–270 kJ mol^{-1} free energy yield; hydrogenotrophic methanogenesis, 18.3–20.8 kJ mol^{-1} free energy yield). H_2 concentrations were greater than 50 nM during the experimental run (50.2 ± 2.9 nM), which ensured that positive free energy yields were simultaneously possible for both processes.

By contrast, simultaneous dechlorination of *cis*-1,2-DCE and methanogenesis did not occur (Fig. 2). The onset of dechlorination of *cis*-1,2-DCE coincided with a drastic decrease in H_2 concentration from about 55 nM to about 6 nM within 12 h (first-order decay constant = 8.6 ± 4.6 day^{-1}), and concentrations stayed nearly constant at 5.62 ± 0.83 nM (± SEM) during the period of

Table 2. Degradation rate constants and H_2 concentrations during dechlorination of *cis*-1,2-DCE and 1,2-DCA

Competing electron-accepting process	First-order rate constant, day^{-1}* (hydrogen concentrations, nM)†	
	cis-1,2-DCE	1,2-DCA
Sulfite	0.05 ± 0.01	0‡
	(0.41 ± 0.12)	(106.2 ± 43)
Sulfate	0.28 ± 0.01	0.19 ± 0.06
	(4.41 ± 0.92)	(27.8 ± 21)
Methanogenic	0.62 ± 0.19	0.25 ± 0.02
	(5.62 ± 0.83)	(50.2 ± 2.9)

cis-1,2-DCE, *cis*-1,2-dichloroethene; 1,2-DCA, 1,2-dichloroethane.
* ± 1 standard error of estimate.
†Estimated as the average H_2 concentration during dechlorination ± 1 standard deviation.
‡Dechlorination of 1,2-DCA occurred with a rate constant of 0.29 ± 0.02 day^{-1} only after sulfite was completely depleted (day 35).

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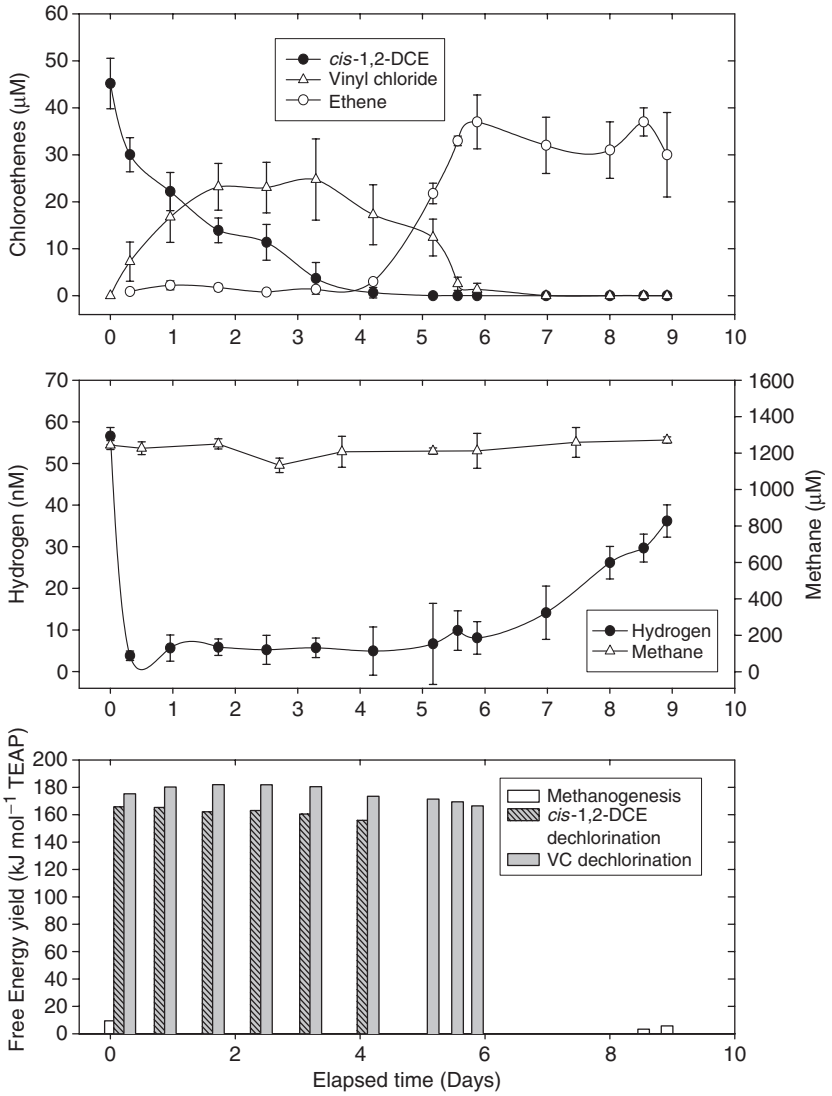

Fig. 2. Dechlorination of *cis*-1,2-dichloroethene and energy yields of the terminal electron-accepting processes under methanogenic conditions.

dechlorination (Fig. 2). Rate constants for *cis*-1,2-DCE dechlorination were $0.62 \pm 0.19\,\text{day}^{-1}$ (Table 2). Energy yields associated with *cis*-1,2-DCE and vinyl chloride reduction were calculated from the microcosm data and found to range between 166.5 and 155.6 kJ mol^{-1} and between 181.9 and 166.4 kJ mol^{-1}, respectively. Upon depletion of *cis*-1,2-DCE and vinyl chloride, H_2 concentrations gradually increased to levels comparable with the initial concentrations. During the time of continuous dechlorination of *cis*-1,2-DCE and vinyl chloride, methane concentration was noted to be nearly constant, with a negative free energy yield $< -10\,\text{kJ mol}^{-1}$ for hydrogenotrophic methanogenesis. Concentrations of *cis*-1,2-DCE used in this study were well below concentrations of chloroethenes demonstrated to be directly inhibitory to methanogenesis (Yu & Smith, 2000).

Dechlorination of 1,2-DCA under sulfite- and sulfate-reducing conditions

Time courses of dechlorination of 1,2-DCA and reduction of sulfite and the corresponding energy yields of the electron-accepting processes are shown in Fig. 3. After addition of sulfite, H_2 concentrations dropped from ~160 nM to less than 2 nM. During this period of depressed H_2 concentrations, 1,2-DCA dechlorination and methanogenesis did not occur. Once the sulfite was depleted, H_2 levels rapidly increased and increased headspace methane concentrations were observed. During the same period, complete dechlorination of 1,2-DCA to ethene was observed (Fig. 3) with a rate constant of $0.29 \pm 0.02\,\text{day}^{-1}$ (Table 2), which was not statistically different from rate constants observed under methanogenic conditions.

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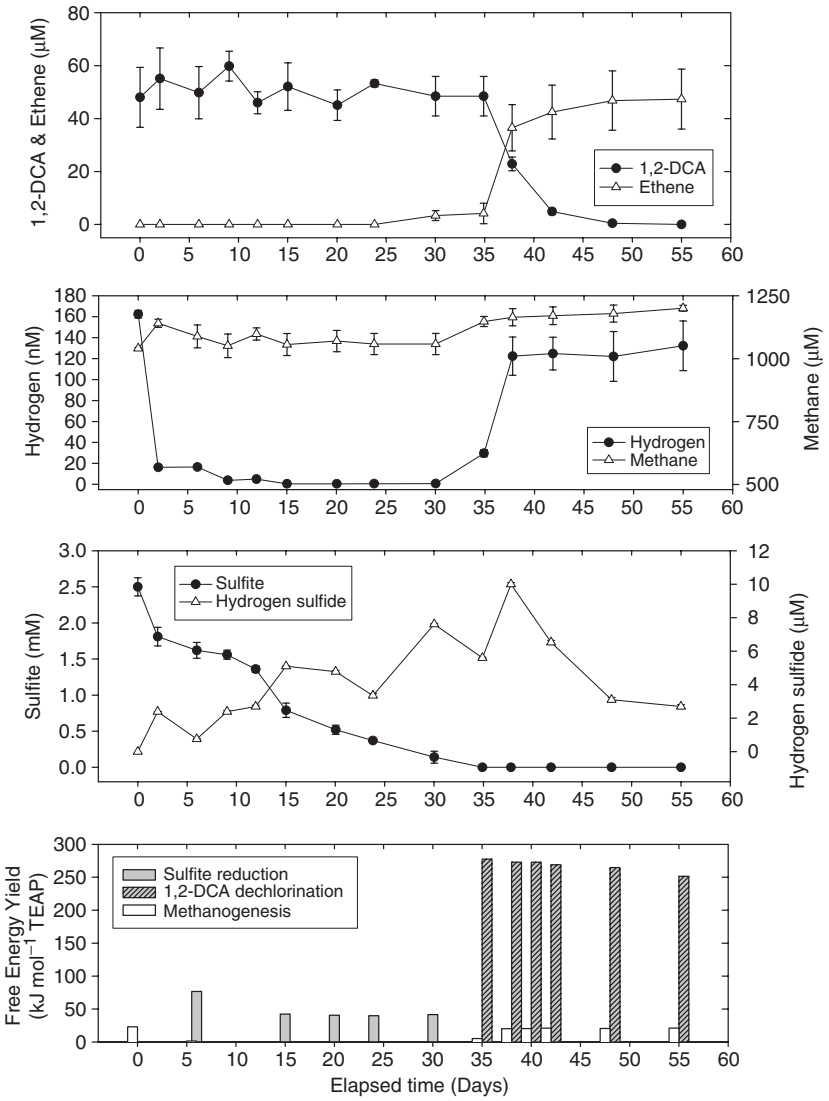

Fig. 3. Dechlorination of 1,2-dichloroethane and energy yields of the terminal electron-accepting processes under sulfite-reducing conditions.

Sulfite reduction was energetically favorable (14–38 kJ mol^{-1} free energy yield) initially and hydrogenotrophic methanogenesis was initially not favorable (−1 to −38 kJ mol^{-1}) due to the drawdown of H_2. Methanogenesis became favorable (>15 kJ mol^{-1}) after depletion of sulfite and the increase in H_2 concentrations that followed. Calculated free energy yields for 1,2-DCA dechlorination were also significant (>200 kJ mol^{-1}) following the increase in H_2 concentrations. Prior to day 35, ethene was below detection (<1 μg L^{-1}); therefore, Gibb's free energies could not be directly calculated from the microcosm data. Free energy calculations performed after assuming some typical ethene concentrations indicated that free energy yields would be consistently >200 kJ mol^{-1} throughout the experiment.

Figure 4 shows time courses of 1,2-DCA and sulfate reduction, H_2 and methane concentrations, and free energy yields. 1,2-DCA degradation began immediately and complete dechlorination to ethene was observed by day 12. 1,2-DCA degraded with a rate constant of 0.19 ± 0.06 day^{-1} (Table 2), a value that was not statistically different from rate constants observed under methanogenic conditions. Decreases in sulfate concentrations began after a lag period of 5 days, even though decreases in H_2 were observed almost immediately. 1,2-DCA-degrading populations appeared to be able to compete with sulfate reducers for H_2 in contrast to the sulfite reducers. After sulfate was depleted, H_2 concentration increased to levels that were thermodynamically favorable for methanogenesis (Fig. 4).

Sulfate reduction was energetically favorable even as H_2 concentrations were depressed. Again, dechlorination of 1,2-DCA to ethene had very substantial free energy yields (>200 kJ mol^{-1}) irrespective of the drawdown of H_2. Hydrogenotrophic methanogenesis had a positive energy

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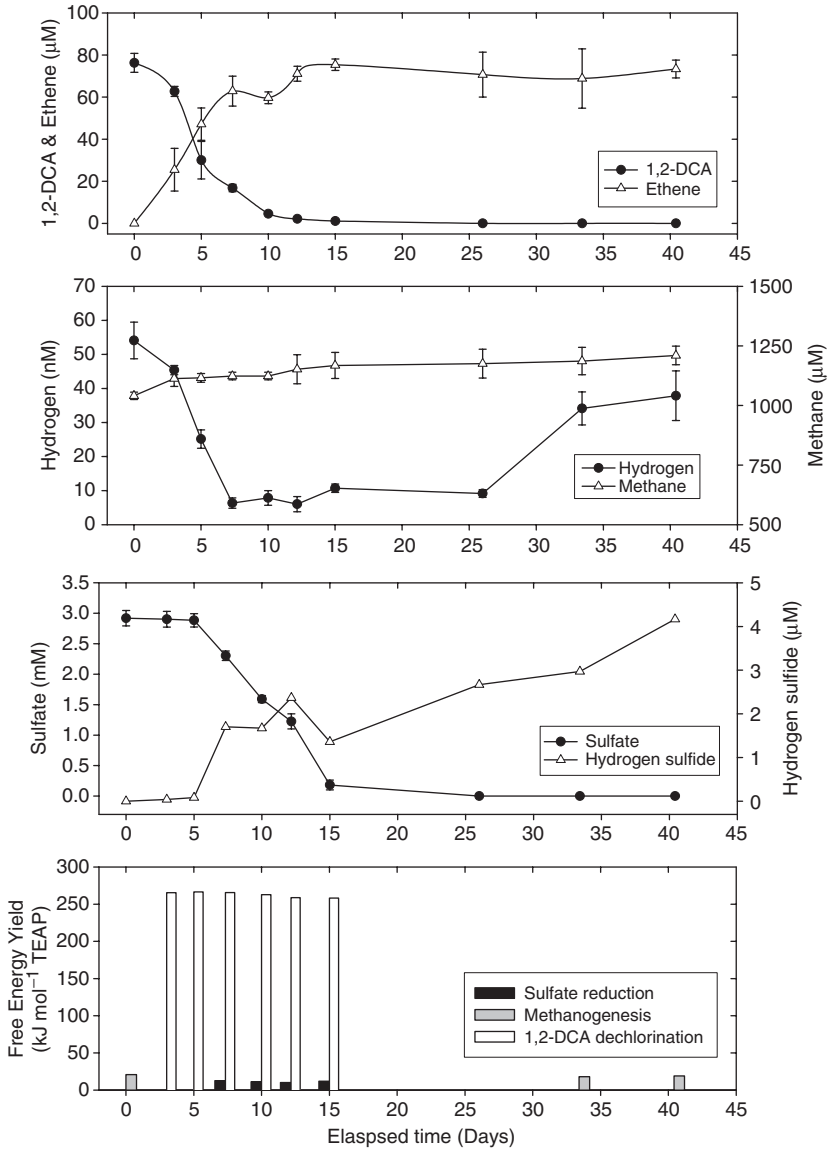

Fig. 4. Dechlorination of 1,2-dichloroethane and energy yields of terminal electron-accepting processes under sulfate-reducing conditions.

yield initially but as sulfate and 1,2-DCA were added the yield became negative as H_2 was depressed. Only when H_2 concentrations increased above 30 nM did a positive yield become re-established in the microcosms.

Dechlorination of *cis*-1,2-DCE under sulfite- and sulfate-reducing conditions

Transformation of *cis*-1,2-DCE in the presence of sulfite and the associated concentrations of H_2 and methane are shown in Fig. 5. *cis*-1,2-DCE dechlorinated without lag to vinyl chloride and further to ethene. By day 50, complete dechlorination to ethene was observed. Sulfite reduction occurred simultaneously with *cis*-1,2-DCE dechlorination with accumulation of sulfide. Dechlorination rate constants for *cis*-1,2-DCE in the presence of sulfite (0.05 ± 0.01 day^{-1}) were an order of magnitude lower than under methanogenic conditions (0.62 ± 0.19 day^{-1}; Table 2). H_2 concentrations were also substantially lower in the presence of sulfite (0.41 ± 0.12 nM) than under methanogenic conditions (5.6 ± 0.8 nM). Based on Gibbs free energy yield, vinyl chloride dechlorination was thermodynamically the most favorable reaction, followed closely by *cis*-1,2-DCE dechlorination, sulfite reduction and hydrogenotrophic methanogenesis (Fig. 5).

Methane production was inhibited by *cis*-1,2-DCE dechlorination and sulfite reduction (Fig. 5). Inhibition of methane production was paralleled by a decrease in the

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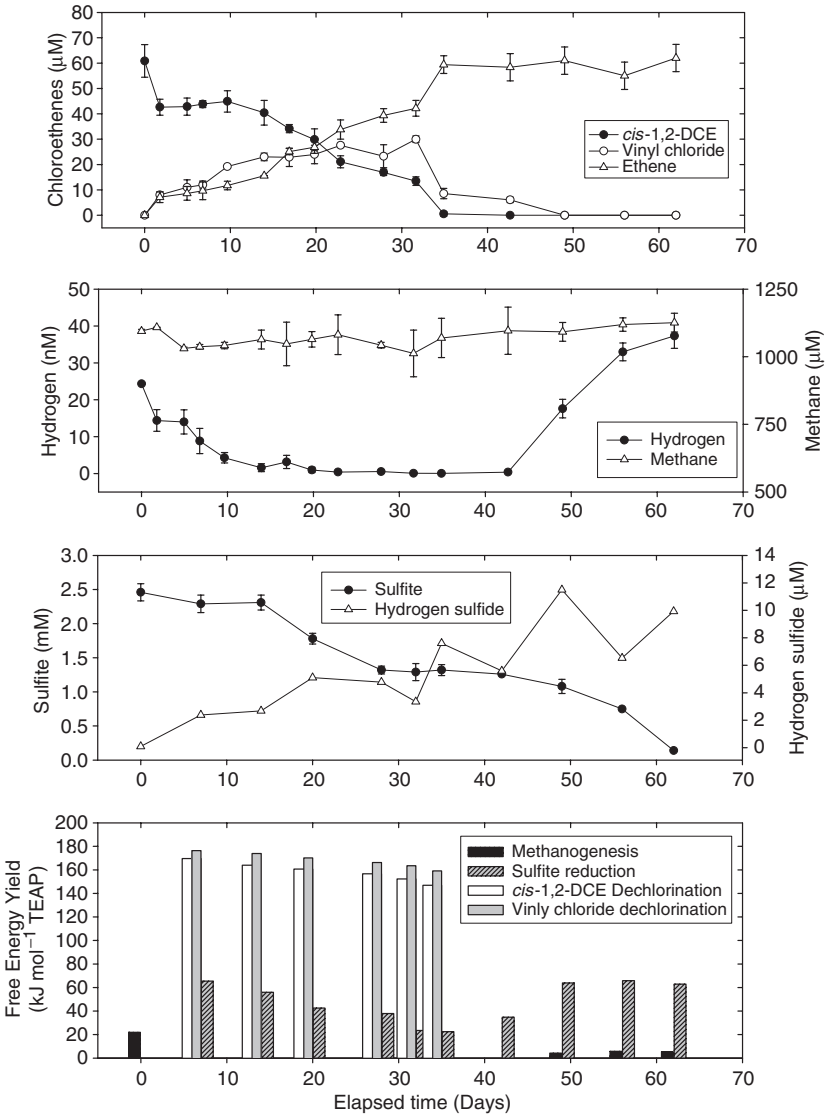

Fig. 5. Dechlorination of *cis*-1,2-dichloroethene and energy yields of terminal electron-accepting processes under sulfite reducing conditions.

steady-state concentration of H_2 to a level that produced a negative free energy yield. Unlike in the 1,2-DCA/sulfite-emended microcosms, methanogenesis did not resume in bottles spiked with *cis*-1,2-DCE and sulfite, even after all chlorinated ethenes had been degraded, because the experiment was prematurely terminated before all sulfite and ethene (electron acceptors with higher energy yields than CO_2) were completely depleted. Note from Figure 5 that after *cis*-1,2-DCE and vinyl chloride were completely depleted, hydrogen concentrations started to increase, which caused sulfite reduction to become an even more energetically favorable reaction.

Dechlorination of *cis*-1,2-DCE, reduction of sulfate and the associated H_2 and methane concentrations and energy yields are shown in Fig. 6. *cis*-1,2-DCE dechlorinated via vinyl chloride to ethene and finally to ethane without lag. Complete dechlorination was observed after 40 days with a rate constant of $0.28 \pm 0.01\ \text{day}^{-1}$; this value is lower than the rate constant observed under methanogenic conditions, but significantly higher than the rate constant observed under sulfite-reducing conditions (Table 2).

Discussion

Simultaneous analysis of thermodynamic and kinetic parameters revealed several trends that help to explain the degradation of chlorinated solvents in these wetland soils. Dechlorination reactions all possessed large free energy yields (highly negative ΔG values) for these reactant and product concentrations. This indicates that these dechlorination reactions operate far from equilibrium, emphasizing

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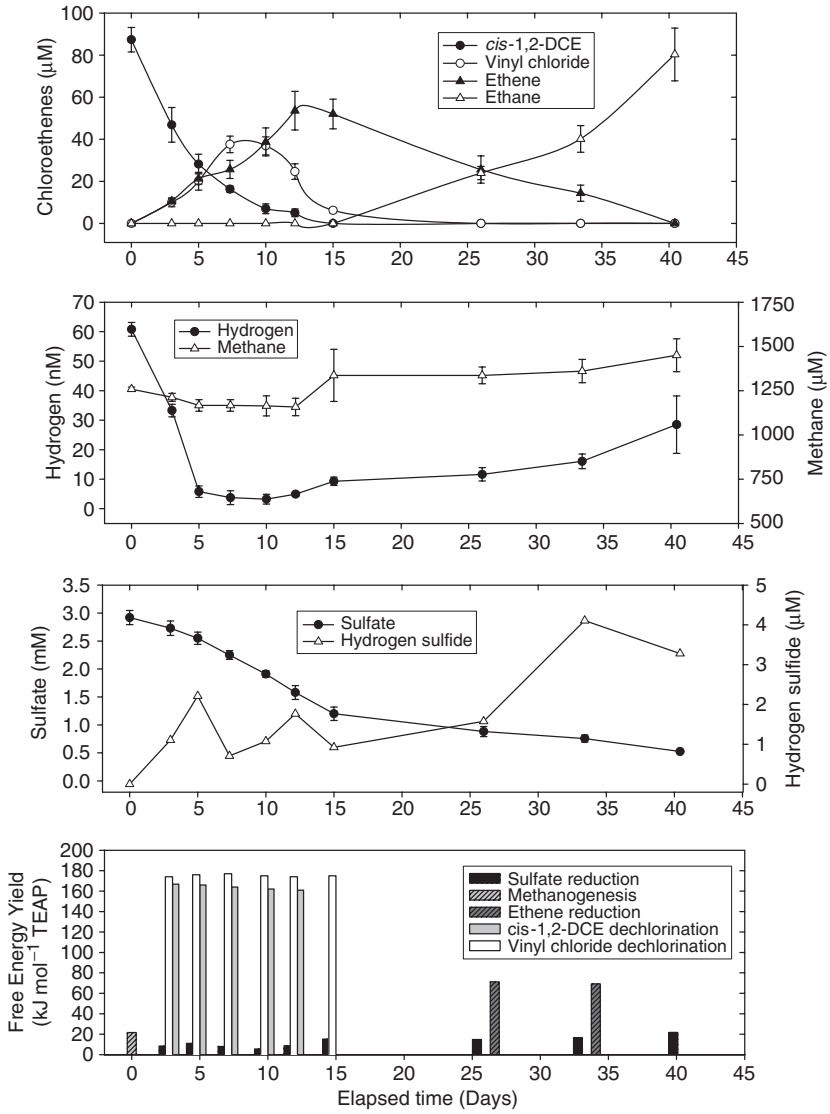

Fig. 6. Dechlorination of *cis*-1,2-dichloroethene and energy yields of terminal electron-accepting processes under sulfate-reducing conditions.

the importance of the kinetics of degradation. The energy yield of dechlorination reactions is relatively insensitive to H_2 concentrations, as observed in the equations presented in Table 1. For dechlorination, ΔG varies with H_2 to the first power, in contrast to that for methanogenesis, which varies to the power of 4. This indicates that although dehalorespirers may competitively exclude other hydrogen-utilizing reactions (i.e. hydrogenotrophic methanogenesis) by drawing down the H_2 concentration, they are unlikely to be competitively excluded themselves. This also ensures that if dehalorespirers are not present with the enzymatic capabilities to degrade these compounds, competitive exclusion may influence populations of organisms responsible for the cometabolic dechlorination of *cis*-1,2-DCE or 1,2-DCA. These populations include methanogens and acetogens, both of which are highly influenced by H_2 concentration. Thus, competition for H_2 would lead to complex patterns of degradation within the wetland bed.

In the presence of alternative electron acceptors, sulfate and sulfite, *cis*-1,2-DCE and 1,2-DCA exhibited very different behavior. For *cis*-1,2-DCE, degradation proceeded completely to ethene via vinyl chloride in the presence of sulfate and sulfite without lag. However, the rate constant decreased from 0.62 day^{-1} for methanogenic conditions, to 0.28 day^{-1} for sulfate-reducing conditions and to 0.05 day^{-1} for sulfite-reducing conditions. This decrease in the rate constant was inversely related to the energy yield of the competing alternative electron acceptor. Sulfate reduction maintained energy yields of 5–10 kJ mol^{-1} whereas sulfite had energy yields of 40–60 kJ mol^{-1} during the period of dechlorination. Methanogenesis had a negative energy yield after the rapid drawdown of H_2, so no competition for H_2 occurred

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from this process. The dechlorination rate constant was also closely correlated with the rate constant for the drawdown of H_2. The larger the rate constant of H_2 decrease, the more rapid the dechlorination of *cis*-1,2-DCE (log k_{H2} vs. k, $R^2 = 0.94$). This rapid drawdown of H_2 and low H_2 threshold is a common feature of dehalorespirers (Löffler *et al.*, 1999). Sulfite is known strongly to inhibit dechlorination by *Dehalococcoides* strain FL2 (He *et al.*, 2005) and does not support growth of *Dehalococcoides* strain 195 as an alternative electron acceptor (Maymó-Gattel *et al.*, 1997). By contrast, sulfate was not inhibitory to *Dehalococcoides* strain FL2 (He *et al.*, 2005) and also does not support growth of strain 195 (Maymó-Gattel *et al.*, 1997). Therefore, inhibition of *cis*-1,2-DCE dechlorination in the presence of sulfite may be due to specific inhibition of the dechlorination process rather than the indirect effect of H_2 competition.

For 1,2-DCA, dechlorination occurred in the presence of sulfate reduction and methanogenesis but did not occur until all of the sulfite was depleted. 1,2-DCA dechlorination was superseded by sulfite reduction, an electron acceptor with a lower energy yield. Several possibilities exist for this contradiction. The absence of a microbial population linking 1,2-DCA respiration with energy (dehalorespiration) could explain these results. In the absence of an organism with this enzymatic capability, 1,2-DCA could only degrade cometabolically. A number of organisms including methanogenic bacteria, notably *Methanosarcia barkeri* and *Methanococcus mazei,* have been observed to degrade 1,2-DCA cometabolically, producing the same daughter product, ethene, observed here (Holliger *et al.*, 1990). In the current study, only when the H_2 level increased to levels favorable to methanogenesis did 1,2-DCA degrade. Cometabolic degradation of 1,2-DCA is consistent with data from previous studies using similar microbial populations (Kassenga *et al.*, 2004).

A second possibility is the presence of a specific 1,2-DCA dehalorespirer that does not use H_2 as an electron donor. *Desulfitobacterium dichloroeliminans* strain DCA1 is a dehalorespiring organism that can degrade 1,2-DCA to ethene (De Wildeman *et al.*, 2003) using H_2 and other electron donors such as formate. Stoichiometric considerations suggest that formate should be present well in excess of the 1 μM detection limit if it was the donor utilized in 1,2-DCA dechlorination. However, identification of *Desulfitobacterium dichloroeliminans* or dehalorespiring populations other than *Dehalococcoides* was not performed. *Desulfitobacterium dichloroeliminans* can use sulfite as an electron acceptor but not sulfate (De Wildeman *et al.*, 2003). Sulfite significantly inhibited dechlorination of tetrachloroethene in *Desulfitobacterium frappieri* TCE1 (Gerritse *et al.*, 1999), suggesting that sulfite may directly inhibit dechlorination in this group of dehalorespirers rather than indirectly through changes in H_2 concentration. Therefore, the absence of H_2 drawdown cannot be used conclusively to link the dechlorination of 1,2-DCA with energy production as other non-H_2-utilizing dehalorespirers may be involved.

Interestingly, once degradation began, rates of 1,2-DCA dechlorination were not statistically different under methanogenic, sulfite-reducing or sulfate-reducing conditions. This suggests that H_2 competition was not affecting the rate of dechlorination of 1,2-DCA. Under sulfate-reducing, sulfite-reducing and methanogenic conditions, degradation of 1,2-DCA appeared to proceed directly to ethene. Two mechanisms of 1,2-DCA microbially mediated reductive dechlorination have been reported: hydrogenolysis, which involves the sequential replacement of a chlorine atom by hydrogen, and dihaloelimination, in which two adjacent chlorine atoms are removed and replaced by a carbon double bond to form ethene. Only ethene was identified as an end-product in the current study, strongly suggesting that dihaloelimination was the operative pathway. Dihaloelimination requires only 0.5 mol of reducing equivalents per mol of chlorine removed whereas 1 mol of reducing equivalents per mol of chlorine removed is required for dechlorination via hydrogenolysis (Dolfing, 1999; Lorah & Olsen, 1999; Dyer *et al.*, 2000). Based on thermodynamic considerations, dihaloelimination yields about 60% more Gibb's free energy than hydrogenolysis, suggesting that the former dechlorination mechanism is energetically more favorable than the latter mechanism (Dolfing, 1999).

Throughout this paper, H_2 has been implicitly assumed to be the only source of reducing equivalents for methanogens and dehalorespirers. Although dechlorination of *cis*-1,2-DCE to ethene is, to date, exclusively linked to H_2, methanogens utilize acetate, and dechlorination of 1,2-DCA can occur via *Desulfitobacterium* sp. using other electron donors such as formate as described above. Although typically two-thirds of methane production would occur via acetate, conditions for acetoclastic methanogenesis were not optimal during these incubations. Temperatures for the incubations were high (25 °C), which favors an increasing percentage of hydrogenotrophic methanogenesis (Fey & Conrad, 2000). Concentrations of acetate at the beginning of the incubations were low (below detection at <1 μM) and measured concentrations of H_2 were below published thresholds (336–3640 nM) for acetogenesis (Cord-Ruwisch *et al.*, 1988; Breznak, 1994). These data suggest that acetate was in short supply during the incubations. Despite these low concentrations, Gibb's free energy yield of acetoclastic methanogenesis was >15 kJ mol^{-1}, if acetate was assumed to be at the detection limit of 1 μM. Therefore, the absence of acetate detection cannot eliminate the possibility that acetoclastic methanogenesis was occurring. In other studies, the regulating function of hydrogenotrophic methanogenesis over acetoclastic methanogenesis has been demonstrated (Fey & Conrad, 2000; Yao & Conrad, 1999). On this

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basis, even though other donors may have been available at low concentrations, H_2 appeared to regulate methanogenic and dehalorespiring processes in these wetland soils.

In conclusion, simultaneous consideration of thermodynamics and kinetics was required to understand the factors controlling dechlorination of 1,2-DCA and *cis*-1,2-DCE in this wetland soil. Thermodynamics represents the ultimate control for any chemical transformation, whether biologically mediated or not. This control was often not expressed in these soils because it was superseded by kinetic considerations was or obscured by the effects of bacteria operating far from equilibrium conditions (Hoehler *et al.*, 1998), such as occurred during the degradation of these chlorinated solvents. In highly organic wetland soils, consideration of both cometabolic and dehalorespiration processes may be important owing to the large anaerobic populations supported by the diagenesis of organic matter in these systems.

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