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Dynamics of reductive TCE dechlorination in two distinct H_2 supply scenarios and at various temperatures

A.C. Heimann*, A.K. Friis, C. Scheutz & R. Jakobsen
*Institute of Environment & Resources, Bygningstorvet, Technical University of Denmark, Building 115, DK-2800, Lyngby, Denmark (*author for correspondence: e-mail: axh@er.dtu.dk)*

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Abstract

Anaerobic microbial dechlorination of trichloroethene (TCE) by a mixed, *Dehalococcoides* containing culture was investigated at different temperatures (4–60 °C) using propionate and lactate as a slow- and fast-releasing hydrogen (H_2) source, respectively. Distinct temperature-dependent dynamics of substrate fermentation and H_2 levels could explain observed patterns of dechlorination. While varying the temperature caused changes in rate, the overall pattern of dechlorination was characteristic of the supplied electron donor. Feeding cultures with a rapidly fermentable substrate such as lactate generally resulted in high H_2 concentrations and fast and complete dechlorination accompanied by rapid methanogenesis. In contrast, low H_2 release rates resulting from fermentation of propionate were associated with 2 to 3−fold longer time frames necessary for complete dechlorination at intermediate temperatures (15–30 °C). A lag-phase prior to dechlorination of *cis*-dichloroethene (*c*DCE), together with a characteristic build-up of H_2 and methane, was consistently observed at slow H_2 supply. At temperatures of 10 °C and lower, the system remained in this lag phase and no dechlorination past *c*DCE was observed within the experimental time frame. However, when lactate was the substrate, complete dechlorination of TCE occurred within 74 days at 10 °C, accompanied by methane production. The choice of fermentable substrate decisively influenced the rate and degree of dechlorination at an electron donor/TCE ratio as high as 666:1. Temperature-dependent H_2 levels resulting from fermentation of different substrates could be satisfactorily explained through thermodynamic calculations of the Gibbs free energy yield assuming a constant metabolic energy threshold of −20 kJ/(mol reaction).

Introduction

Due to their adverse health effects chlorinated ethenes pose a serious problem at many contaminated sites (Wiedemeier et al. 1999). Chlorinated ethenes such as trichloroethene (TCE) can be microbially reduced yielding non-toxic compounds like ethene (DiStefano et al. 1991; Freedman & Gossett 1989). As we currently understand it, complete microbial dechlorination is restricted to the bacterial genus *Dehalococcoides* which require molecular hydrogen (H_2) as their ultimate electron donor (Löffler et al. 2003). In mixed microbial communities this can lead to competition for H_2 produced by fermentation of organic substrates reflected by low aqueous H_2 levels of a few nmol per liter (Löffler et al. 1999; Yang & McCarty 1998).

A wide variety of organic compounds can be used as substrates in dechlorinating consortia either by producing H_2 via fermentation or as direct electron donors. Various studies have elucidated the role and applicability of these different electron donors to microbial dechlorination encompassing short-chain acids (formate, acetate, propionate, lactate, butyrate, isobutyrate), alcohols (methanol, ethanol), and carbohydrates (glucose) (Aulenta

et al. 2005; Carr & Hughes 1998; Fennell et al. 1997; Freedman & Gossett 1989; Lu et al. 2002; Schöllhorn et al. 1997). However, the choice of an adequate electron donor in engineered dechlorinating systems remains delicate, weighing rapid and complete cleanup against adverse side effects of adding surplus donor, such as bioclogging or explosion hazards through excess methane production (Fennell & Gossett 1999; Lee et al. 2004). Moreover, the aforementioned studies focusing on the role of different electron donors were all conducted at constant temperature levels, ranging from 14 to 35 °C. Thus, the understanding of the response of the dechlorination pattern to different H_2 supply rates at varying temperatures is quite limited. He et al. (2003) studied the important final step in the chloroethene dechlorination succession, reduction of vinyl chloride (VC) to ethene, at temperatures between 4 and 35 °C using different substrates. For higher chlorinated ethenes, some knowledge exists on perchloroethene (PCE) dechlorination at different temperatures, however, here only acetate was used as electron donor (Zhuang & Pavlostathis 1995). Thus a consistent study of the combined effects of both donor conditions and temperature on sequential dechlorination is presently lacking.

The aim of this study was (i) to explore the effects of two distinct electron donors on the rate and especially the pattern of dechlorination at a set of different temperatures, (ii) to closely follow the dynamics of the added organic substrates, H_2, and methane under these different settings, and from this, (iii) to determine factors that appear favorable for complete and rapid TCE biodegradation. To this end, we conducted batch experiments using the mixed anaerobic culture KB-1™ containing *Dehalococcoides* bacteria capable of complete TCE dechlorination (Duhamel et al. 2002). Dechlorination patterns were studied combining low and high rate H_2 release, respectively, with incubations at different temperatures, ranging from values commonly found in aquifers (4–20 °C) to values favorable for bioengineered approaches (30–40 °C). Two electron donors were chosen to represent two extreme conditions with respect to H_2 release: (1) Lactate exemplifying a very transient system with high buildup of H_2, and (2) propionate yielding much lower H_2 production rates and representing a system closer to steady state (with respect to H_2 production and consumption). The different H_2 levels attainable through substrate utilization are based on the Gibbs free energy yield (ΔG_r) of the respective fermentation reaction (Table 1, reactions 1 and 2; please note that for the sake of uniformity all species entering the calculation of ΔG_r^0 and ΔH_r^0 are expressed as aqueous species, including H_2). A ΔG_r value of around -20 kJ (mol reaction)$^{-1}$ is commonly viewed as the minimum energy yield necessary to maintain microbial metabolism (Canfield et al. 2004; Conrad 1999). Figure 1 visualizes the effect of this threshold energy gain on the theoretical maximum H_2 level at different temperatures under representative conditions of our study. H_2 concentrations exceeding values of lines (1) and (2) render the respective reaction unfavorable whereas the opposite is true for H_2 concentrations that plot below the lines. The experimental data shown in Figure 1 as well as the relevance of reaction (3) will be discussed in the Results and Discussion section. The temperature optimum of this culture for dechlorination in the context of bioremediation following thermal treatment is discussed in a companion study of this experiment (Friis et al. submitted).

Materials and methods

Chemicals

The following chemicals were obtained in liquid form: trichloroethylene (GC grade 99.5 + %,

Table 1. Relevant H_2 (1, 2) and acetate (3) producing reactions, and their Gibbs free energies (ΔG_r^0) and enthalpies (ΔH_r^0)

Reaction[a]	ΔG_r^0 (kJ/mol rxn)[b]	ΔH_r^0 (kJ/mol rxn)[b]
(1) $Propionate^-_{(aq)} + 3H_2O \rightarrow HCO_3^-{}_{(aq)} + Acetate^-_{(aq)} + H^+_{(aq)} + 3H_{2(aq)}$	169.2	178.7
(2) $Lactate^-_{(aq)} + 2H_2O \rightarrow Acetate^-_{(aq)} + HCO_3^-{}_{(aq)} + H^+_{(aq)} + 2H_{2(aq)}$	71.2	71.9
(3) $2\ HCO_3^-{}_{(aq)} + 4H_{2(aq)} + H^+_{(aq)} \rightarrow Acetate^-_{(aq)} + 4H_2O$	−214.7	−228.6

[a] All species, including H_2, are expressed as activities in aqueous solution.
[b] Calculated from Gibbs free energies of formation (ΔG_f^0) and enthalpies of formation (ΔH_f^0) of the compounds given in the literature (Kohn & Boston 2000; Lide 2003; Stumm & Morgan 1996; Thauer et al. 1977).

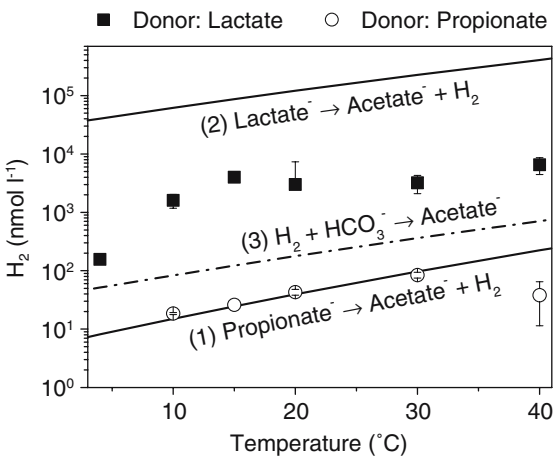

Figure 1. Dissolved hydrogen (H_2) concentrations at different temperatures for the three reactions introduced in Table 1 (lines) corresponding to a Gibbs free energy yield of −20 kJ/(mol reaction), and maximum measured H_2 concentrations in this study, using propionate (circles) and lactate (squares), respectively, as substrate. Lines were constructed using Gibbs free energy yields (Table 1) at the given temperature (calculated by van't-Hoff's equation), and activities of reactants representative of our experimental conditions; $-\log[HCO_3^-] = 2.0$, pH = 6.41–6.54, $-\log[Acetate^-] = 5.3$–2.7, $-\log[Propionate^-] = 2.3$, $-\log[Lactate^-] = 2.7$ (Acetate concentrations were below the detection limit in most of the propionate fed batches; calculations were thus performed assuming a small, rapidly cycled acetate pool of 5 μmol l^{-1}; varying this concentration by a factor of 5 only changes the calculated H_2 levels by a factor of 0.6 and 1.7, for a decrease and increase in acetate, respectively). Data points falling below lines (1) and (2), as well as data exceeding line (3) render the respective reaction thermodynamically favorable.

Merck), 1,1-dichloroethene (GC grade 99.5 + %, Fluka), trans-dichloroethene (GC grade 97 + %, Fluka), *cis*-dichloroethene (97%, Acros). Vinyl chloride was purchased from Gerling, Holz & Co. (99.97%), ethane was obtained as pure gas from Mikrolab, Aarhus. DL-lactic acid sodium salt solution (Fluka; purum, 50% in water) and propionic acid, sodium salt (Sigma Chemical Company, USA; 99% purity) were used as electron donors.

Setup of experimental batches

The anaerobic microbial consortium KB-1™ Dechlorinator (KB-1™) was kindly provided by SiREM (Guelph, Ontario) and was used as inoculum for batch experiments. This enrichment culture was originally derived from TCE contaminated soil and groundwater and contains bacteria phylogenetically related to *Dehalococcoides ethenogenes* (Duhamel et al. 2002).

Sulfate-free mineral salts medium was prepared according to Heimann et al. (2005) to exclude potential competition by sulfate-reducing bacteria. The absence of sulfate in all bottles was confirmed by ion chromatography. Batches were set up in 120 ml serum bottles which were filled with 104 ml mineral medium and sealed with 1-cm-thick butyl rubber stoppers and aluminum crimp caps; headspace gas was N_2/CO_2 (80/20%). Triplicates were prepared for both lactate and propionate experiments at 10, 20, 30, and 40 °C, single batches were stored at 4, 15, 50, 60 °C for both setups. The 10 °C batches were unintentionally kept at room temperature between day 1 and 3.

Each batch was initially spiked with 0.8 μmol TCE establishing an aqueous concentration of 7.2 μmol l^{-1}. The initial electron donor concentrations were 4.1 mmol l^{-1} of lactate and 4.8 mmol l^{-1} of propionate. Accordingly, on an equivalent basis the electron donor was over two orders of magnitude in excess of TCE, the ratios being 378:1 (lactate:TCE) and 666:1 (propionate:TCE), assuming fermentation to acetate and complete dechlorination of TCE. The experiment was initiated by inoculation with 300 μl of KB-1™ suspension (10^{11} *Dehalococcoides* cells ml^{-1}). Bottles were incubated upside down in the dark without agitation. pH values were between 6.3 and 6.7.

Analytical methods

For the determination of chlorinated ethenes, and ethene, acidified aqueous samples (1 ml) were analyzed with a gas chromatograph (Agilent 6890N) equipped with a mass selective detector (MS, Agilent 5973). Samples were introduced to the GC following pre-heating to 80 °C and headspace gas sampling. Separation was performed on a 25.0 m × 320 μm × 1.00 μm (nominal) capillary column (J&W GSQ) with helium (class 2) as carrier gas. Chloroform was used as internal standard (0.5 ml of a 10 ppmv solution). Aqueous concentrations were converted to concentrations per batch using Henry's law constants at different temperatures for chlorinated ethenes (Staudinger & Roberts 2001) and ethene (Wilhelm et al. 1977). Detection limits in μmol l^{-1} were: TCE 0.077, *c*DCE 0.043, *t*-DCE 0.065, 1,1-DCE 0.109, VC 0.035, Ethene 1.533.

Samples for formate, acetate, propionate, and lactate were filtered through 0.45 μm nylon filters, acidified with 50 μl 17% H_3PO_4 per ml of sample and kept frozen until analysis by suppressed ion chromatography on a Dionex ICE-AS1 9×250 mm ion exclusion column (eluent: 4 mM heptafluorobutyric acid; chemical suppression: 10 mM tetrabutyl ammonium). Detection limits for all compounds were between 0.3 and 0.8 mg/l. Sulfate was analyzed by suppressed ion chromatography on a Dionex Ion Pac AS14 4×250 mm column with 3.5 mM Na_2CO_3/1 mM $NaHCO_3$ as eluent and a detection limit of 4.5 μmol/l.

Headspace samples for methane (CH_4) and on several occasions for the analysis of ethene and VC, were injected into a Shimadzu 14A gas chromatograph equipped with a packed column (3% SP/500 Carbopack B) and a flame ionization detector (FID). Detection limits in (μmol/l_{gas}) were: methane 0.05, ethene 0.02, and VC 0.04. Headspace hydrogen (H_2) was analyzed by a reduction gas detector (Trace Analytical RGD2) with a detection limit of 0.1 ppmv. As samples for both parameters (H_2 and CH_4) were allowed to attain atmospheric pressure prior to injection, measured concentrations of H_2 and CH_4 were corrected for overpressure in the batches as determined with a portable manometer (Manofix X30D) to account for this loss (e.g. a sample degassing from 1.1 to 1.0 atm experiences a loss of around 10%). Headspace concentrations were converted to aqueous-phase concentrations using tabulated Henry's law constants at different temperatures (Wilhelm et al. 1977). Error bars on all graphs represent ± one standard deviation from the average value.

Data analysis and presentation

Interpretation of dechlorination rates of polychlorinated compounds such as TCE can be complicated by the sequential nature of the process in which reaction products such as *c*DCE subsequently act as reactants for further dechlorination. A useful technique to overcome this difficulty and to enable the direct comparison of several experiments in a single graph is to calculate the degree of dechlorination on the basis of the inferred amount of chloride produced from dechlorination. The amount of chloride ($n_{chloride}$) produced via degradation of TCE and its products is calculated according to Equation (1):

$$n_{chloride} = f_{cDCE} + 2f_{VC} + 3f_{ETH} \quad (1)$$

f_{cDCE}, f_{VC}, and f_{ETH} representing molar fractions of *c*DCE, VC, and ethene, respectively. For each time step we determined the fractional contribution of each ethene species to the overall mass balance (sum of all ethene species) resulting in $n_{chloride}$ values between 0 (= no dechlorination) and 3 (= complete dechlorination of TCE).

Results and discussion

For both electron donors, lactate and propionate, no dechlorination took place at temperatures higher than 40 °C, TCE dechlorination to *c*DCE only occurred within the first three days at 40 °C. Complete dechlorination of TCE to ethene was observed in lactate fed batches between 4 and 30 °C, whereas incubations with propionate only showed complete dechlorination between 15 and 30 °C. Dechlorination halted at *c*DCE in propionate batches at 4 and 10 °C. When complete dechlorination occurred it generally took 2–3 times longer with propionate as compared to lactate as substrate. An overview of dechlorination end products and time frames involved in the different setups is given in Table 2.

While dechlorination rates showed a strong dependence on temperature, the patterns of dechlorination revealed the influence of the employed donor, i.e. the rate of H_2 supply. As an illustrative example of this pattern, Figures 2 and 3 show sequential dechlorination at 20 and 30°C, respectively, for both substrates.

In both setups dechlorination of TCE to *c*DCE proceeded without any lag phase within the first 10 days (Figures 2A, B and 3A, B). Thereafter, incubations with lactate displayed immediate and rapid dechlorination of *c*DCE and vinyl chloride (VC). In contrast, the setup featuring propionate as electron donor showed a lag period of 15–20 days prior to further dechlorination of *c*DCE (Figures 2B and 3B). As expected, concentrations of H_2 and methane were characteristic of the fermentation kinetics, i.e. rapid lactate utilization resulting in a large buildup of a few thousand nmol l^{-1} of H_2 accompanied by active methano-

Table 2. Dechlorination products and time frames for incubation at different temperatures with either lactate or propionate as substrate

Electron donor	Incubation temperature (°C)	Main ethene species at end of incubation	Time for complete dechlorination (days)[a]
Lactate	4	*c*DCE	neg.
Propionate	4	*c*DCE	neg.
Lactate	10	Ethene	74
Propionate	10	*c*DCE	neg.
Lactate	15	Ethene	34
Propionate	15	Ethene	74
Lactate	20	Ethene	26
Propionate	20	Ethene	58
Lactate	30	Ethene	20
Propionate	30	Ethene	74[b]
Lactate	40	TCE	neg.
Propionate	40	TCE	neg.
Lactate	50	TCE	neg.
Propionate	50	TCE	neg.
Lactate	60	TCE	neg.
Propionate	60	TCE	neg.

[a]Time at which ethene was the only remaining daughter product. Incubation time was 74 days at 4–30 °C, incubation was stopped at temperatures >30 °C after 34 days of no TCE dechlorination; neg. = negative, i.e. not observed in the experimental time frame.
[b]One of the triplicates still contained ~2 μmol/l vinyl chloride.

genesis (Figures 2C and 3C). In comparison, H_2 concentrations in the propionate setup were two orders of magnitude lower and showed a conspicuous increase during the *c*DCE lag phase (Figures 2D and 3D). This increase was accompanied by the onset of methane production. Equivalent patterns were observed at 15 °C. The onset of methane production was always positively correlated with rapid dechlorination of *c*DCE. As a matter of fact, the small amount of VC produced from day 15–26 in Figure 2B is attributable to a single out of three triplicates displaying methane production at that time.

The difference in H_2 concentration between both setups is also visualized in Figure 1 where the maximum observed H_2 level at temperatures between 4 and 40 °C is shown. In the propionate setup H_2 seems to be controlled by the minimum energy gain of propionate fermentation (reflected by the experimental values coinciding with the calculated line in Figure 1). In contrast, H_2 concentrations observed in the lactate experiment are, while considerably higher than in the other setup, still orders of magnitude below levels that would limit the transformation of lactate to acetate (Figure 1). This reflects the active role of hydrogen-consuming processes such as methanogenesis, acetogenesis and dechlorination.

Lactate fermentation proceeded rapidly with complete depletion occurring after a time frame ranging from 6 days (30 °C) to 74 days (4 °C). At temperatures between 4 and 30 °C lactate was converted to acetate at an average molar ratio of 1:1.3 (Figures 2E and 3E), indicating that acetate was not only produced directly from lactate, but also through hydrogenotrophic acetogenesis which becomes feasible at high H_2 concentrations (Hoehler et al. 1999). This is also visualized in Figure 1 (Reaction 3) showing that all measured H_2 concentrations in the lactate setup greatly exceed the level required for rendering this equation energetically favorable. At 40 °C the ratio increased to approximately 1:1 showing that the latter process did not occur at higher temperatures, or that part of the acetate was used by acetotrophic methanogens. Lactate was only transformed into propionate at 30 °C (up to 0.15 mmol l^{-1}). No lactate fermentation was observed at temperatures >40 °C. Utilization of propionate occurred at very low rates (Figures 2F and 3F) and propionate conversion was incomplete at all temperatures showing highest turnover rates at 30 °C (16% converted after 74 days). Accordingly, only very little acetate was produced through propionate fermentation (maximum 0.30 mmol l^{-1} at 30 °C). It appears

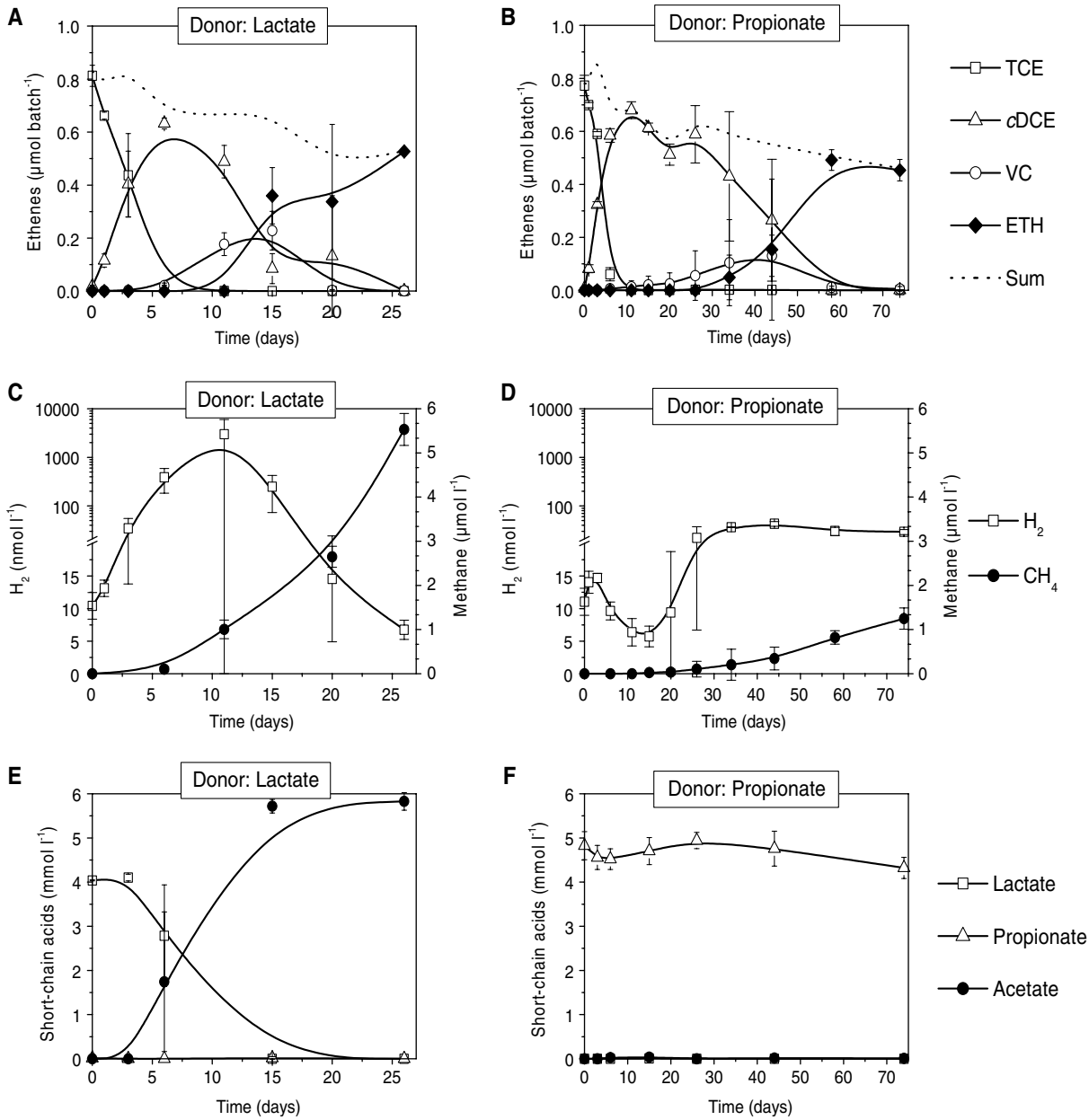

Figure 2. Concentrations of ethenes (A, B), H_2 and methane (C, D), and short-chain acids (E, F) at an incubation temperature of 20 °C. The left column refers to experiments using lactate the right to experiments using propionate as electron donor. Curves are smoothed using a cubic B-spline connection. (Note different time scales of the two setups, and the combined linear-log scales for H_2. In the lactate setup the standard deviation for H_2 at Day 11 exceeded the average value producing a range encompassing negative values, therefore the standard deviation was set equal to the average at this data point.)

that initially propionate is utilized to reduce TCE (either directly or via H_2 production), after which H_2 levels display a characteristic increase, followed by the onset of further dechlorination (Figures 2D and 3D). In the period of maximum H_2 levels, a time lag prior to *c*DCE dechlorination is observed, implying that organisms capable of *c*DCE dechlorination need time to grow and/or benefit in some way from the onset of methane production. This again implies that H_2 produced through propionate fermentation will not be used, before either H_2-consuming *c*DCE-

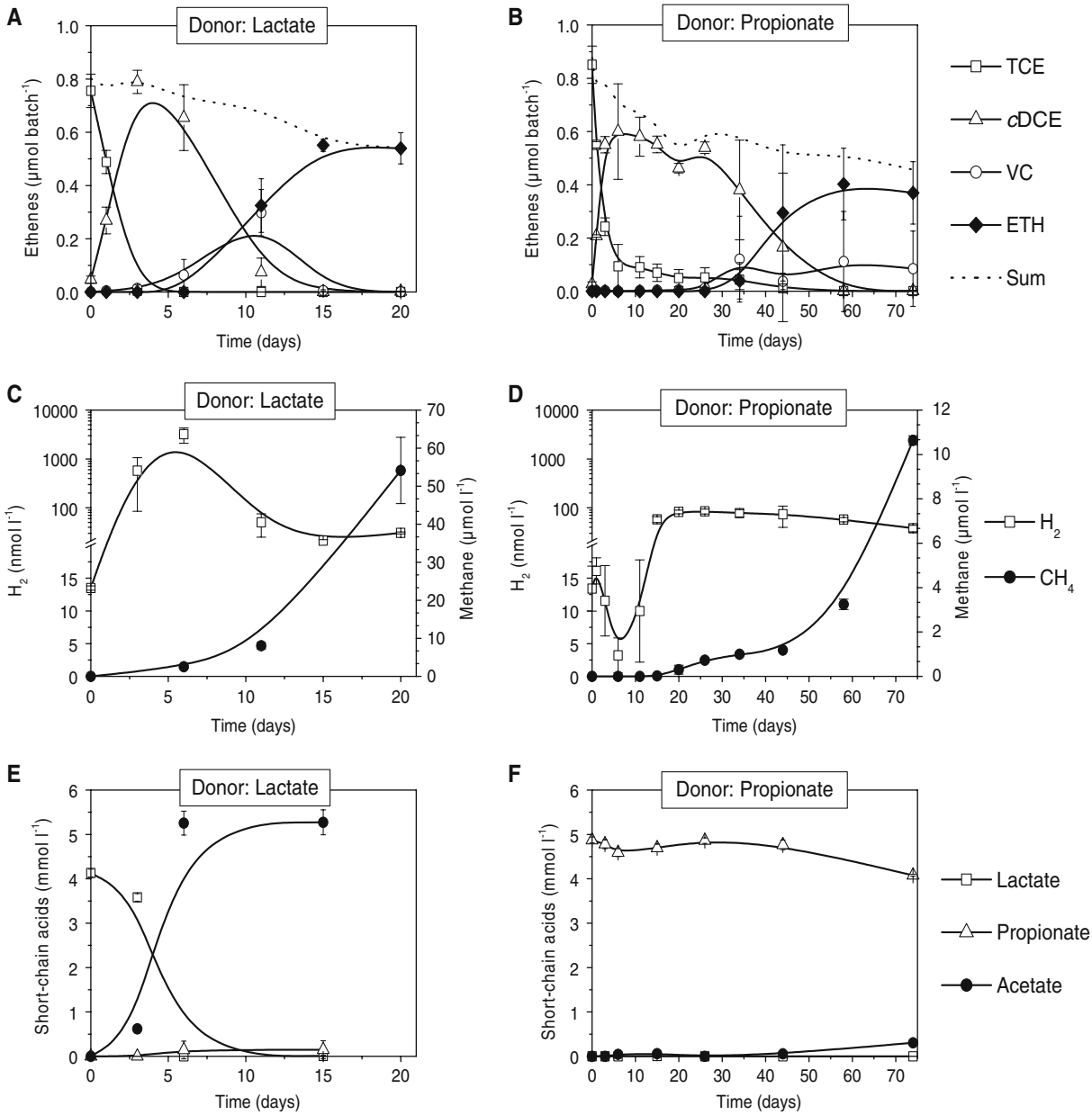

Figure 3. Concentrations of ethenes (A, B), H_2 and methane (C, D), and short-chain acids (E, F) at an incubation temperature of 30 °C. Figures in the left and right columns display results obtained with lactate and propionate as electron donor, respectively (note different time scales of the two setups, the different *y*-axis scales for methane, and the combined linear-log scales for H_2). Curves are smoothed using a cubic B-spline connection.

dechlorinators or methanogens appear. This course of events is nicely supported by the thermodynamic equilibrium calculations presented in Figure 1.

The calculated chloride release rates and patterns, calculated according to Equation (1), for both electron donors at temperatures from 4–30 °C are shown in Figure 4 along with the concentrations of methane and H_2. The lag phase in the propionate systems is reflected by stable values of around 1 (= *c*DCE stage) from day 10–25. With lactate as electron donor not only were the dechlorination rates higher but there was also no lag phase at temperatures of 15–30 °C. Only at

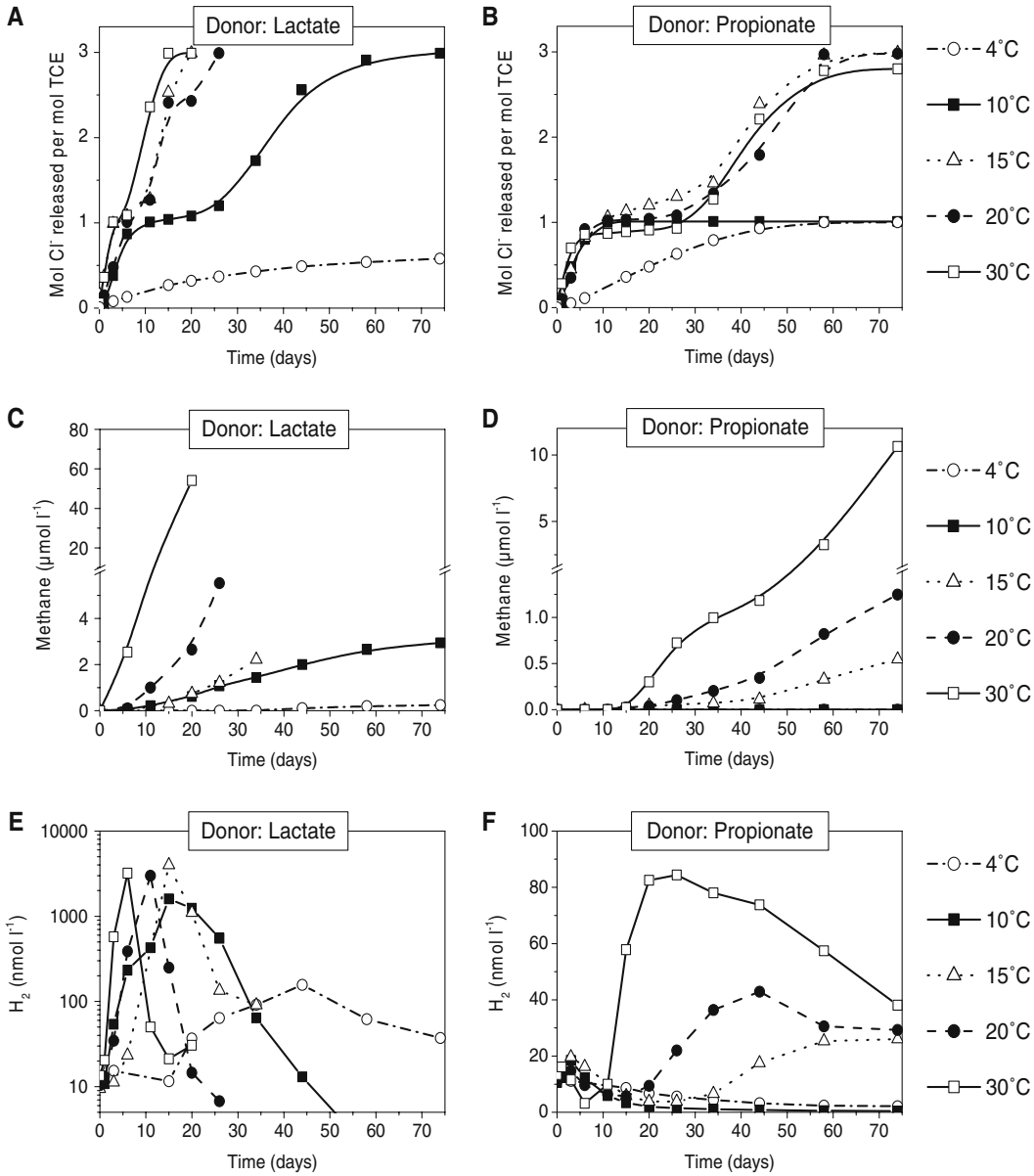

Figure 4. Moles of chloride produced per mol of TCE (A, B), concentrations of methane (C, D), and H_2 (E, F) at various incubation temperatures. Figures in the left and right columns display results obtained with lactate and propionate as electron donor, respectively (note different *y*-axis scales on figures C–F, and the combined linear-log scales for CH_4). Curves in A–D are smoothed using a cubic B-spline connection.

10 °C did the lactate setup follow a course similar to the propionate setup. At 4 °C both setups showed slow and incomplete dechlorination. The temperature-dependence of the overall dechlorination process is a combination of two different temperature effects, one on the fermentation reaction and the other one on the terminal dechlorination reaction (i.e. reduction of a given chlorinated ethene species with H_2). The lactate-fed cultures at 10–30 °C all exhibited high H_2 concentrations of well above 1000 nmol l^{-1}, implying H_2 is not limiting the dechlorination process. This suggests that in these cultures, the observed temperature-dependence of the overall dechlorination reflects the temperature-dependence of the terminal dechlorination reaction (as

discussed in Friis et al. submitted). In contrast, the propionate-fed culture experienced low hydrogen levels compared to previously determined half-velocity constants for H_2-utilizing dehalogenators (7–100 nmol l^{-1}; Ballapragada et al. 1997; Cupples et al. 2004; Smatlak et al. 1996). Thus, the observed temperature dependence in the propionate-fed cultures is likely an inseparable combination of temperature effects on both propionate fermentation and the terminal dechlorination reaction.

While methane was produced right from the beginning in incubations with lactate (at temperatures >4 °C) it took about 15 days for the initiation of methanogenesis in the propionate setup (Figure 4C, D). The onset of methane production coincided with the *c*DCE lag phase and an increase in H_2 concentration in the propionate setup (Figure 4F). In all experiments, only two single batches (at 15 and 20 °C, respectively) displayed slow *c*DCE turnover without concomitant methane production within the first 10–15 days of the experiment with a rate of 5.5–9.4 mmol chloride produced per mol of *c*DCE and day. This is about one order of magnitude lower compared to the average *c*DCE dechlorination rate after the lag phase (90.6 ± 28.8 mmol chloride per mol *c*DCE and day; propionate setup at 15–30 °C) which was accompanied by methanogenesis.

It was a general feature that rapid dechlorination past *c*DCE did not occur when methanogenesis was absent. Dechlorination either ceased completely or resumed only after a lag phase in which H_2 concentrations increased and methanogenesis was initiated. Presumably, a shift in community structure occurred during transition from the first dechlorination step (TCE $\rightarrow$ *c*DCE) to dechlorination of lower chlorinated ethenes such as *c*DCE. Different dechlorinating organisms responsible for the degradation steps leading to *c*DCE and the following dechlorination steps, respectively, have been previously related to accumulation of *c*DCE (Maymó-Gatell et al. 2001). For the KB-1 culture, a recent study indicated shifts in population composition between different subcultures enriched on TCE and *c*DCE, respectively (Duhamel et al. 2002). Similar results were obtained studying microbial communities in *c*DCE and VC fed subcultures from several perchloroethene (PCE) enriched cultures derived from river sediments (Flynn et al. 2000). The characteristic increase in the H_2 concentration in our propionate fed batches might reflect a similar shift. When TCE has been depleted, while *c*DCE consumption has not yet started, the H_2 concentration is presumably being controlled at a higher level by the minimum energy gain for propionate fermentation (Figure 1). It appears that growth of dehalogenators capable of *c*DCE and VC dechlorination is a key factor in achieving complete dechlorination in these H_2-limited environments (i.e. propionate-fed cultures).

The dechlorinating culture KB-1 was enriched from a contaminated site by repeated transfers into TCE- and methanol-amended medium (Duhamel et al. 2002), and is currently maintained on TCE/methanol in a 1:5 electron equivalent ratio (sometimes ethanol and hydrogen are supplemented; SiREM, pers. communication). However, Duhamel et al. (2002) found that methanol, ethanol, hydrogen, lactate, and propionate could all serve as electron donor for sustaining dechlorination in KB-1. All their experiments were performed at 25 °C, which is close to the optimum temperature for dechlorination we found here for both substrates (30 °C, Friis et al. submitted). Our data show that the inoculum we used did not lack propionate-utilizers as dechlorination in the propionate/TCE-cultures was complete at all temperatures between 15 and 30 °C. However, it could be argued that the initial number of propionate utilizers may have been too low to allow for observation of dechlorination past *c*DCE at low temperatures (4–10 °C) within the experimental period of 74 days. Therefore we prolonged the incubation of one out of three replicates of the 10 °C propionate/TCE-culture. It was monitored for an additional 44 days (total of 118 days), and did not exhibit any dechlorination of *c*DCE even after this prolonged incubation, suggesting that time was not the limiting factor here. This culture was then inoculated (0.7% v/v) with the 10 °C lactate/TCE culture, which previously exhibited complete dechlorination of TCE to ethene and which, at the time of inoculation, was devoid of any lactate (all lactate had been converted to acetate within the first 26 days of incubation). Shortly after inoculation, production of vinyl chloride started, followed by production of ethene (data not shown). After a total of 250 days dechlorination to ethene was complete, again emphasizing the merits of the lactate/TCE-culture (containing the organisms necessary for complete

dechlorination) as compared to the propionate/TCE-culture at low temperatures. Similarly, no dechlorination past *c*DCE was observed in the 4 °C propionate/TCE culture even after a prolonged observation period of 329 days, leaving ample time for potential growth.

Apart from the choice of substrate, another important factor controlling the relative rates of dechlorination and methanogenesis is the initial population structure of the involved microorganisms, as was demonstrated in simulations by Lee et al. (2004). This implies that the observed patterns may not be generally valid, but could be a function of the initial composition of a given mixed dehalogenating culture.

The exact role of methanogenesis in our experiments remains debatable. In contrast to the findings presented here, previous studies showed dechlorination of *c*DCE and VC by KB-1 and other enrichment cultures to occur in the absence of methanogenesis (Duhamel et al. 2004; Flynn et al. 2000; He et al. 2002). While in some cases H_2 levels at least three orders of magnitude higher than in our experiments (propionate setup) might explain this disagreement (Duhamel et al. 2004; Flynn et al. 2000), He et al. (2002) observed VC dechlorination proceeding at steady-state H_2 levels of below 0.5 ppmv in what appeared to be a non-methanogenic, syntrophic acetate-oxidizing population. Thus, methanogenic activity is not a prerequisite for dechlorination of lower chlorinated ethenes, but could rather have been triggered by the increasing H_2 levels which may be necessary for the dechlorination of lower chlorinated ethenes (higher H_2 thresholds for dechlorination of *c*DCE and VC were reported by Lu et al. 2001, Luijten et al. 2004, and Hoelen & Reinhard 2004).

Consequently, there are two possible conclusions: (1) the relationship between methanogenesis and the rapid overcoming of the lag-phase prior to *c*DCE dechlorination is non-causal, but rather associated with rising H_2 values due to a microbial community shift, or (2) methanogens play a key role in facilitating the transition to this *c*DCE dechlorination phase under low, steady state H_2 supply. Preliminary findings from follow-up experiments using the lactate/TCE-culture (at 20 °C) as inoculum indicated that the latter may be true. In these experiments we investigated dechlorination of VC at different chloride/bromide ratios ($Cl^- + Br^- = 105$ mmol l^{-1}), using acetate as substrate (a condition that corresponds to the situation after rapid conversion of lactate to acetate, as observed after 15 days at 20 °C; see Figure 2). Figure 5 shows VC dechlorination rates versus rates of methanogenesis for 12 culture bottles monitored in this experiment. While different chloride/bromide ratios neither influenced methane production rates nor dechlorination (data not shown), VC degradation to ethene was positively correlated with methane production.

Positive influence of methanogenesis on dechlorination has been described for degradation of chlorinated ethenes (Adamson et al. 2003; Bradley & Chapelle 1999; Freedman & Gossett 1989; Skeen et al. 1995), polychlorinated biphenyls (Kim & Rhee 1999), chlorinated phenols (Perkins et al. 1994) and chloroform (Yu & Smith 1997), the latter potentially accounted for by cofactors such as F_{430} or vitamin B_{12}. However, interpretation of these findings has been complicated by the fact that the methanogenic inhibitor 2-Bromoethanesulfonate (BES) which is frequently employed to evaluate the role of methanogens can also be an inhibitor of reductive dechlorination (Löffler et al. 1997). In contrast to the literature mentioned above, several studies emphasize the adverse effects of increased methane production on dechlorination in bioaugmentation approaches, such as competition for electron donor, potentially

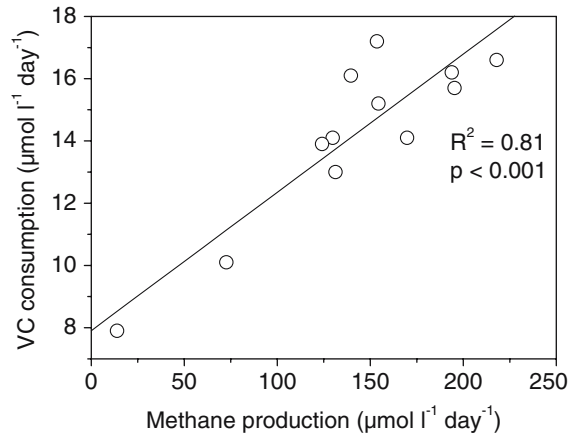

Figure 5. Vinyl chloride (VC) consumption rates versus methane production rates in 12 acetate-fed KB-1 cultures during a 7-day period following VC addition (average initial aqueous concentration of 106 μmol l^{-1}). Rates represent the difference between initial and final amounts present in both the aqueous phase and the headspace, divided by the volume of the aqueous solution, and corrected for loss by sampling.

leading to wastage of substrate, thus promoting bioclogging and residual organics in the aquifer (Yang & McCarty 2000, 2002, 2003). Accordingly, the very modest substrate conversion of the propionate-fed cultures examined here may be beneficial to an efficient and cost-effective bioaugmentation, despite the slower, and sometimes incomplete dechlorination as compared to the lactate-fed cultures. This viewpoint is again challenged by others arguing that rapid and complete dechlorination is the prior goal in bioaugmentation and that dechlorination is not inhibited by methanogenesis (Carr & Hughes 1998; Lutes et al. 2003). As pinpointed by the findings we present here, further investigations should focus on (i) exactly how methanogens influence the transition from dechlorination of TCE to lower chlorinated ethenes, and how this influence relates to the H_2 level, and on (ii) how growth kinetics of *c*DCE/VC dehalogenators depend on H_2 supply, temperature and the abundance of methanogens as controlling factors.

Conclusions and implications for bioremediation

It is well-known that dechlorinators are capable of outcompeting methanogens at low H_2 concentrations both on a kinetic level in terms of half-velocity constants (Ballapragada et al. 1997; Smatlak et al. 1996) as well as on the basis of metabolic H_2 thresholds (Löffler et al. 1999; Lu et al. 2001; Yang & McCarty 1998). Thus, it has been suggested that slowly fermentable substrates should be used to exploit this competitive advantage at low H_2 levels (Fennell et al. 1997; Smatlak et al. 1996). Our results suggest that utilization of slowly fermentable substrates such as propionate may result in incomplete dechlorination at low temperatures or significantly longer time frames necessary for complete dechlorination. However, more rapid and complete dechlorination with a slow H_2-releasing substrate such as propionate may be achievable by increasing the starting population of syntrophic propionate utilizers.

Nonetheless, this finding is interesting considering the high donor/TCE ratio in our experiments (666:1 in the propionate setup; equivalent basis considering only fermentation to acetate). Previously, it has been argued that this ratio could be decisive in explaining contradictory findings on the influence of electron donor on dechlorination outcome (Carr & Hughes 1998, 1999; Fennell & Gossett 1999; Fennell et al. 1997), the line of reasoning being that choice of donor only matters at low donor/chloroethene ratio but becomes negligible at values as high as 630:1. Our data show that this is not necessarily true even at the high ratio employed in this study. However, it should be kept in mind that the addition of large amounts of rapidly fermentable substrate, such as lactate, could, while ensuring rapid and complete dechlorination, be counterproductive to bioaugmentation goals by creating methane levels that are of environmental concern. Therefore, successful bioaugmentation will depend on a thoughtful trade-off between degrading contaminants rapidly and simultaneously avoiding environmental problems caused by excessive methane production and substrate addition.

The experiments presented here revealed original patterns as to the rate and degree of dechlorination at different temperatures and different H_2 levels, supported by thermodynamic equilibrium calculations. These findings may be significant for understanding the outcome of bio-engineered contaminant removal at sites polluted with chlorinated ethenes, thus adding to current understanding of microbial anaerobic dechlorination as a remedial process.

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