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Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture

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Use of an appropriate hydrogen level is necessary to favor dehalogenation of chlorinated solvents, such as tetrachloroethene (PCE) and trichloroethene (TCE), over other hydrogen using processes. This study examined the competition between dehalogenators and other microorganisms occurring in a benzoate-acclimated dehalogenating methanogenic mixed culture. Results show that the dehalogenators competed best against methanogens and homoacetogens when the hydrogen level was maintained between 2 and 11 nM. The 2 nM hydrogen concentration represents the lower threshold value found here for *cis*-1,2-dichloroethene (*cis*-DCE) dehalogenation. The usefulness of this hydrogen range was further confirmed with both batch-fed and continuously-fed reactors. In batch studies, three times more ethene was produced from dehalogenation of *cis*-DCE using propionate than benzoate as electron donor, while benzoate produced three times more methane than propionate. A three times greater hydrogen utilization efficiency for dehalogenation was obtained with a CSTR than with batch reactors when benzoate was used as substrate because a constant hydrogen concentration in the appropriate range could be maintained with the CSTR. These results suggest different approaches that might be used to favor dehalogenators in competition with other microorganisms.

Introduction

Chloroethenes, such as tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC), can be sequentially dehalogenated by microorganisms under anaerobic conditions via reductive dehalogenation (*1*, *2*). Because this process results in complete dehalogenation to harmless ethene (ETH), anaerobic dehalogenation is recognized as a useful method for remediation of sites contaminated by chlorinated ethenes, either naturally through intrinsic remediation (natural attenuation) or through engineered approaches. Nevertheless, this strategy is often hindered because of incomplete dehalogenation of PCE to ETH. Rates of the reduction from PCE to *cis*-DCE are generally high, and the requirement for electron donor is relatively small, while the subsequent reduction steps from *cis*-DCE to VC and ETH are much slower, and often require much more electron donor to drive the reactions (*3, 4*). All pure cultures isolated to date can only reductively dehalogenate PCE to *cis*-DCE (*5*−*8*), except strain 195 (*9*), which can carry out the complete reduction to ETH. Because of the general need to obtain complete dehalogenation, better understanding is yet needed of the factors affecting the rate and extent of the last critical steps of dehalogenation, that is, from *cis*-DCE to ETH.

Among the factors affecting the practical application of dehalogenation is competition for electron donors between dehalogenators and other microorganisms in anaerobic mixed environments (*10*, *13*). Various studies have shown that many different electron donors can sustain reductive dehalogenation. However, growing evidence indicates that hydrogen is a key electron donor used in dehalogenation of *cis*-DCE and VC to ETH, and organic electron donors appear to serve mainly as primary precursors to supply the needed hydrogen via fermentation (*11*, *12*). This suggests that the dehalogenating microorganisms occupy a niche in an anaerobic system similar to that occupied by hydrogen-utilizing methanogens, homoacetogens and sulfidogens (*13*), all of which compete for hydrogen in mixed cultures. Because of such competition, much more electron donor is generally needed to achieve complete dehalogenation than would be suggested by the stoichiometric requirement for dehalogenation alone. This is a potential hindrance to economical application of anaerobic reductive dehalogenation.

Lovley, Chapelle, and co-investigators (*14*−*18*) systematically studied the competition existing among different hydrogen-consuming microorganisms in natural aquatic sediment systems. Sulfate-reducing bacteria were found to outcompete methanogenic bacteria mainly by maintaining the concentration of hydrogen below the minimum threshold necessary for methane production (*17*, *18*). Similar competition existed between Fe(III)-reducing, Mn(IV)-reducing, nitrate-reducing, sulfate-reducing, and methanogenic bacteria (*14*−*16*, *19*, *20*). Hydrogen concentrations associated with the various terminal electron-accepting reactions in sediments were as follows: methanogenesis, 7−10 nM; sulfate reduction, 1−1.5 nM; Fe(III) reduction, 0.2 nM; Mn(IV) and nitrate reduction, less than 0.05 nM. The results suggested that each terminal electron-accepting reaction has a unique threshold hydrogen concentration associated with it, which is primarily dependent upon the physiological characteristics of the hydrogen-consuming microorganisms, and the energy yield from hydrogen oxidation as affected by the electron acceptor used. Organisms using acceptors associated with greater energy production (more electrochemically positive) have lower hydrogen concentration thresholds than organisms using electron acceptors that yield less energy from hydrogen oxidation.

Dehalogenating microorganisms also compete with the above organisms for hydrogen. Recent studies (*10*, *21*) on hydrogen affinity constants for dehalogenators clearly indicated that dehalogenating microorganisms are capable of effective utilization of hydrogen at very low concentrations. On the basis of free energy considerations alone (*22*, *23*), the dehalogenators should be similar to nitrate reducers in their affinity for hydrogen, that is, one might expect a hydrogen threshold on the order of 0.05−0.1 nM (*20*). Smatlak et al. (*10*) mentioned that the hydrogen threshold for the PCE dechlorinators in their mixed culture was less than 2 nM. However, in other studies, dehalogenators could compete effectively for hydrogen with methanogens but not with organisms using iron oxide, manganese oxide, and nitrate (*24*). Thus, considerations other than energetics alone appear involved. Site studies by Chapelle (*25*) showed that reductive dehalogenation was mostly favored in methanogenic and sulfate-reducing zones. Clearly, determination of the hy-

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drogen threshold for dehalogenation is central to help clarify the nature of the competition for electron donors between dehalogenators and other anaerobic microorganisms. Here, we report studies directed toward finding a hydrogen concentration range and threshold level that favors dehalogenators in competition with other possible hydrogen-utilizing microorganisms within a methanogenic mixed culture.

Materials and Methods

Chemicals. Liquid PCE, TCE, and *cis*-1,2-DCE (Aldrich Chemical Co., Milwaukee, WI) were used for preparing stock feed solutions and analytical standards. VC, ETH, and methane gases (99+%, Scott Specialty Gases, Alltech Associates, Inc., Deerfield, IL) were used as analytical standards. Benzoate (sodium salt, 99%, Aldrich Chemical Co.), hydrogen (99.99%, Scott Specialty Gases), and acetate (analytical reagent, J. T. Baker Chemical Co., Phillipsburg, NJ) were used as electron donors and to develop analytical standards. Yeast extract (Difco Laboratories, Detroit, MI) was used as a nutrient source.

Culture and Growth Medium. Digested sludge was collected from a municipal wastewater treatment plant and used directly for an initial comparative evaluation of the hydrogen threshold for methanogenesis. For all subsequent evaluations, a dehalogenating source culture was developed in a closed continuous stirred-tank reactor (CSTR) (total volume 4.3 L, liquid volume 3.6 L) initially seeded with aquifer material from a PCE-contaminated groundwater site in Victoria, TX, where dehalogenation was occurring. The main microorganisms that resulted in this culture were dehalogenators, which completely transformed PCE to ETH, and hydrogen-utilizing methanogens. Little acetoclastic activity was observed. The reactor was maintained at 28 (± 2) °C and remained anaerobic as indicated by absence of color from 1 mg/L resazurin added in the feed. A continuous anaerobic feed consisting of 1.7 mM sodium benzoate, 20 mg/L yeast extract, 0.98 mM PCE (near saturation), and trace nutrients in basal medium was syringe pumped at 100 mL/day, resulting in a 36-day detention time. Every 2 days, 200 mL of liquid was removed to bring the reactor back to 3.6 L and for use as the seed culture in batch studies. The basal medium contained the following constituents per liter of Milli-Q water: 0.5 g of K_2HPO_4, 1.5 g of Na_2CO_3, 20 mL of minerals solution (40 g of NaCl, 50 g of NH_4Cl, 5 g of KCl, 5 g of KH_2PO_4, 5 g of $MgCl_2 \cdot 6H_2O$, and 2 g of $CaCl_2 \cdot 2H_2O$ L^{-1}), 5 mL of trace metal solution (1 g of $FeCl_2 \cdot 4H_2O$, 1 g of $MnCl_2 \cdot 4H_2O$, 0.2 g of $CoCl_2 \cdot 6H_2O$, 0.12 g of H_3BO_3, 0.02 g of $ZnCl_2$, 0.02 g of $CuCl_2 \cdot 2H_2O$, 0.02 g of $NiCl_2 \cdot 6H_2O$, 0.02 g of $Na_2MoO_4 \cdot 2H_2O$, 0.02 g of Na_2SeO_4, 0.02 g of $Na_2WO_4 \cdot 2H_2O$, 0.04 g of $Al_2(SO_4)_3 \cdot 18H_2O$, and 10 mL of 1 N HCl L^{-1}), 1 mL of filter-sterilized vitamin stock solution (0.02 g of biotin, 0.02 g of folic acid, 0.1 g of pyridoxine, 0.05 g of riboflavin, 0.05 g of thiamine, 0.05 g of nicotinic acid, 0.05 g of pantothenic acid, 0.05 g of *p*-aminobenzoic acid (PABA), 0.05 g of cyanocobalamine, and 0.05 g of thioctic acid L^{-1}), and 5 mg of Na_2S as a sulfur source and reductant.

Batch Experiments. Batch studies were conducted using 160 mL serum bottles, with 60 mL of headspace filled with a gas mixture (80% N_2, 20% CO_2) and 100 mL liquid volume. Basal medium (containing 20 mg/L yeast extract) was anaerobically (using gas purging) and aseptically delivered to each bottle. The source cultures were inoculated with syringes. In the first experiment to determine the threshold hydrogen concentration for methanogenesis, 10 mL of digested sludge was added to each bottle. Substrates (acetate, benzoate, butyrate, formate, methanol, and phenol) were added in an amount equivalent to 30 μmol of benzoate (900 microelectron equivalents). No chlorinated ethenes were added. In the second experiment to determine the threshold hydrogen concentration for dehalogenation, 10 mL of the PCE-dehalogenating culture from the CSTR, 8 μmol of *cis*-DCE, and either 30 μmol of benzoate or 410 μmol of hydrogen were added to each bottle. Control bottles without benzoate and hydrogen were included. In a third comparative study with benzoate and propionate, 50 mL of the dehalogenating source culture was used. Benzoate and propionate were added at 30 and 65 μmol, respectively, as single doses at the start of the experiment; 5 μmol of *cis*-DCE was added initially and then supplemented repeatedly whenever it became depleted.

To maintain anaerobic conditions, rubber stoppers and aluminum crimp caps were used to seal the bottles. Adsorption of chlorinated compounds on the rubber stoppers was minimal after the first few days so that good mass balances could be maintained throughout the studies. All the bottles were continuously mixed at a rate of 100 rpm on a shaker table (Lab-Line Instruments, Inc., Melrose Park, IL). Hydrogen concentration was tracked over time to ensure that it had stabilized at some steady-state concentration. Duplicates were used in each experiment, and each experiment was repeated at least twice to ensure reproducibility.

Analytical Methods. Measurement of PCE, TCE, *cis*-DCE, VC, ETH, CH_4, and H_2 were performed by gas chromatography (GC), using 250 μL headspace samples. Compounds were identified by comparison of their retention times with that of external standards. Total amount of gaseous compounds and the concentration of H_2 in liquid were calculated by using Henry's law constants published by Gossett (*26*) and verified in our laboratory. PCE, TCE, *cis*-DCE, and VC were determined with a Fractovap 2900 series GC (Carlo Erba Strumentazione, Milan, Italy) equipped with a model PI-52-02A photoionization detector (PID) (10.2 eV lamp; HNU Systems, Inc., Newton, MA), operated isothermally at 40 °C. ETH and CH_4 were measured with a model 5730A GC (Hewlett-Packard, Palo Alto, CA) equipped with a flame ionization detector (FID) operated isothermally at 90 °C. H_2 was analyzed with a reduction gas detector (RGD) (Trace Analytical, Inc., Menlo Park, CA), having a detection limit just below 0.1 Pa (corresponding to 0.8 nM liquid concentration), which was measured by comparing the response of blanks with standards. All hydrogen concentrations reported thereafter refer to the liquid-phase concentration. Benzoate and acetate analyses were performed with a series 4000i ion chromatography (IC) (Dionex, Sunnyvale, CA) equipped with a conductivity detector, a Dionex IonPac AS4A column (4 × 250 mm), and a AG4A guard column (4 × 50 mm), operated with sodium tetraborate (5 mM, pH = 9.3) as eluant.

Results

Threshold Hydrogen Concentration in Batch Bottles in the Absence and Presence of *cis*-DCE. The hydrogen threshold concentration for the process of methanogenesis alone was investigated first with batch studies using digested sludge as seed. Various substrates including acetate, benzoate, butyrate, formate, methanol, and phenol were added initially. Except for phenol, the substrates were consumed within 2 weeks. About 2 weeks were required to adapt to phenol, and its fermentation was complete within another 2 weeks. Shown in Figure 1 is the hydrogen concentration change over time following substrate addition. The eventual hydrogen threshold concentrations reached were all in the range of 10−13 nM (11.5 ± 1.6 nM). This is just above the reported value of 7−10 nM for methanogenesis (*20*).

The culture from the PCE-dehalogenating CSTR was then used to determine the threshold hydrogen concentration in the absence and presence of *cis*-DCE. Benzoate and hydrogen were used as electron donors in this study. For each donor, two bottles had no *cis*-DCE added to represent the process of methanogenesis alone, and another two had

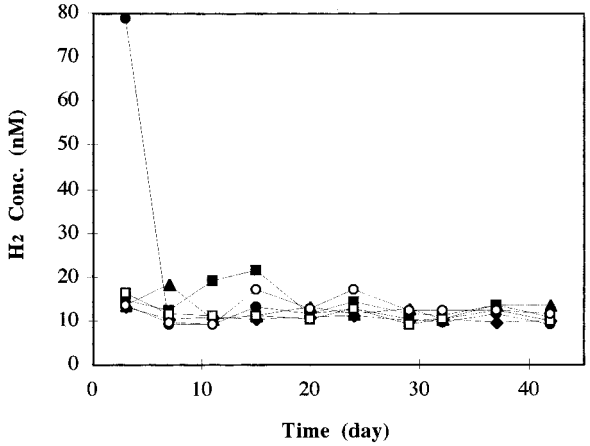

FIGURE 1. Hydrogen concentration changes over time following addition of various substrates to a methanogenic culture seed from digested sludge: acetate (♦), benzoate (■), butyrate (▲), formate (●), methanol (□), and phenol (○).

cis-DCE added for methanogenesis in combination with dehalogenation.

The formation and consumption of various constituents with benzoate as electron donor are depicted in Figure 2. Without *cis*-DCE added, the hydrogen concentration reached about 120 nM during active benzoate fermentation. This initial high hydrogen concentration was partially a result of fermentation of yeast extract that was added as a necessary nutrient for dehalogenation. Three weeks later when benzoate was depleted, hydrogen decreased to 11 nM and remained at that level, even after several months of incubation. Biphasic methanogenesis (*27*) occurred. Methanogenesis during the first three weeks coincided with the higher hydrogen level, this is believed to be the result of hydrogen-utilizing methanogenesis. Methanogenesis after the hydrogen level dropped to 11 nM is believed to be the result of acetate-utilizing methanogenesis as acetate then decreased as well. These results are consistent with the control study.

In comparison, hydrogen levels in the bottles to which *cis*-DCE was added were different. Hydrogen concentration reached about 180 nM during benzoate fermentation but decreased to and remained at about 2 nM 4 weeks later, lower than in the absence of *cis*-DCE. Methane production ceased when the hydrogen level decreased. Little acetate was used. This is consistent with the absence of acetoclastic activity observed in the source culture that was fed PCE (shown later). Since acetoclastic activity eventually resulted in the absence of *cis*-DCE (Figure 2), it appears that chloroethenes might be responsible for the inhibition of acetate-utilizing methanogenesis in the *cis*-DCE fed batch cultures. There was much less apparent inhibition of hydrogen-associated methanogenesis at the levels of *cis*-DCE used.

Dehalogenation of *cis*-DCE and VC was detectable at the 2 nM hydrogen level. Following the disappearance of the chlorinated ethenes, the hydrogen concentration increased

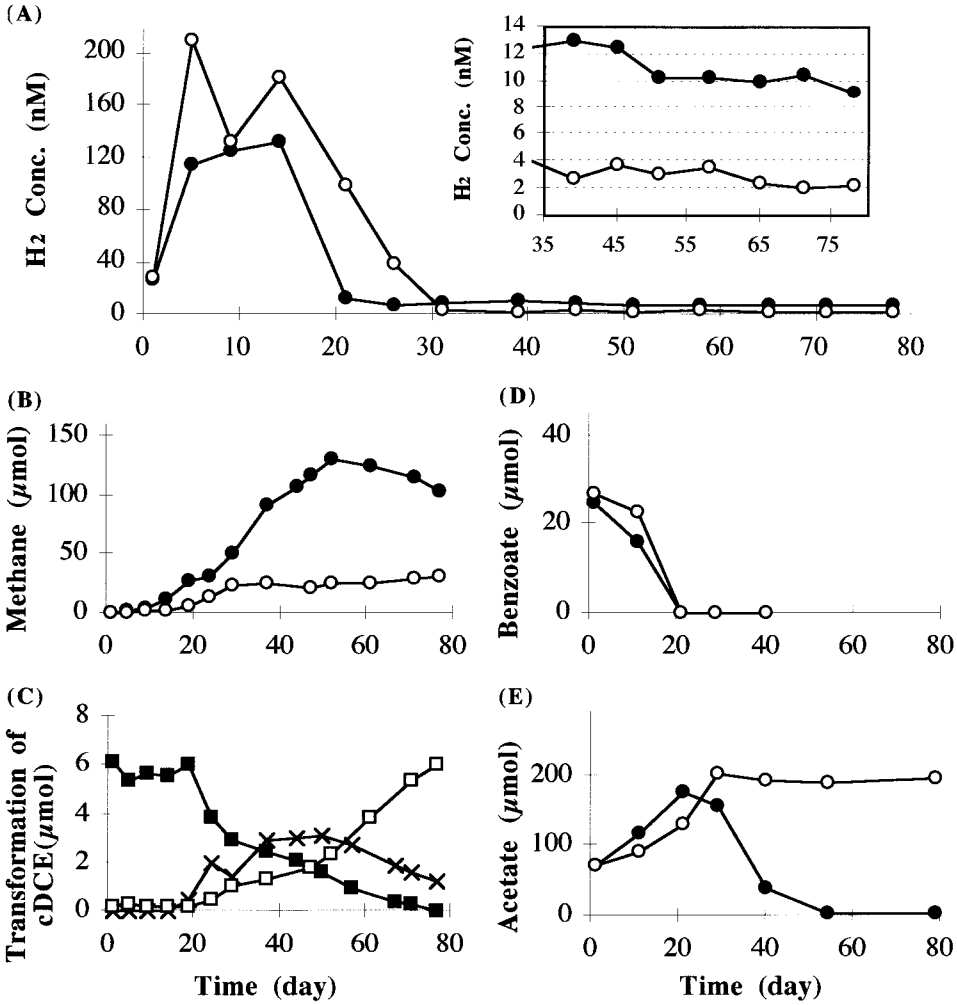

FIGURE 2. Results of a batch study with benzoate as electron donor performed in the absence (●) and presence (○) of *cis*-DCE, respectively. Shown only on panel C are *cis*-DCE (■) and its transformation products VC (×) and ETH (□).

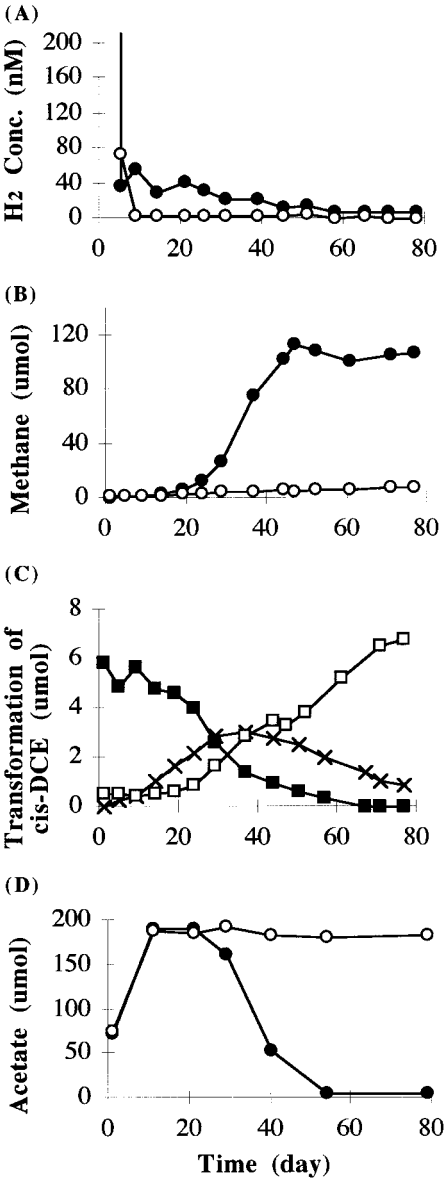

FIGURE 3. Results of a batch study with hydrogen as electron donor performed in the absence (●) and presence (○) of *cis*-DCE, respectively. Shown only on panel C are *cis*-DCE (■) and its transformation products VC (×) and ETH (□).

to a similar level of about 11 nM as in the absence of *cis*-DCE, but a subsequent respiking with *cis*-DCE then caused the hydrogen level to return to about 2 nM (data not shown), confirming that this lower level of hydrogen is related to the process of dehalogenation.

With benzoate as electron donor, a mass balance (considering the reducing equivalents of 15 μmol as H_2 for dehalogenation contributed by the 2 mg of yeast extract added, as determined in separate studies) indicates that about 91% and 9% of the assumed hydrogen intermediate product was used for the processes of methanogenesis and dehalogenation, respectively. However, dehalogenation became the main hydrogen consumer after the hydrogen concentration was reduced below 11 nM.

When hydrogen was added as the electron donor, a similar steady-state hydrogen concentration was observed for *cis*-DCE dehalogenation and methanogenesis (Figure 3). But unlike with benzoate, a sharp drop in the hydrogen level initially was primarily the result of homoacetogenesis (88%), which was indicated by a significant rise in acetate concentration, rather than methanogenesis (8%) and dehalogenation (4%). However, dehalogenation was again the main hydrogen consumer when the hydrogen level was reduced below 11 nM.

TABLE 1. Mass Balance for CSTR over a 2-Day Period

parameter	mass change[d] (μmol)	electron equiv factor	electron equiv consumed (μequiv)	electron equiv formed (μequiv)
benzoate	−323	30	−9690	
yeast extract[a]	−35	20	−700	
acetate	900	8		7200
biomass[b]	18	20		360
methane	161	8		1288
ethene[c]	114	8		912
total			−10390	9760

[a] Calculated by assuming yeast extract as $C_5H_7O_2N$. [b] The empirical formula of $C_5H_7O_2N$ for bacterial cells was used in the calculation of biomass. Biomass concentration in the CSTR was 10 mg (dry weight) L^{-1}. [c] The electron equivalents for dehalogenation were calculated from ethene production rather than from PCE addition as some PCE may have been lost through rubber tubing during feeding. [d] Negative and positive values represent consumption and production, respectively.

Similar threshold hydrogen concentrations were obtained with other substrates such as acetate and yeast extract alone. The statistical average values for all substrates are 10.9 ± 3.3 nM ($n = 40$) and 2.2 ± 0.9 nM ($n = 32$) in the absence and presence of *cis*-DCE, respectively.

CSTR Evaluation. The PCE dehalogenating CSTR was operated continuously for over 2 years prior to this study. In this reactor, 980 μM PCE was dehalogenated almost completely to ETH, and the liquid concentrations of TCE, *cis*-DCE, and VC intermediates inferred from headspace analysis were typically below 1 μM. The hydrogen concentration in the CSTR, also inferred from headspace analysis, maintained itself at 2.6 ± 0.7 nM, which is just slightly above the threshold concentration for dehalogenation found in the batch studies.

Table 1 contains a mass balance summary for the CSTR over a typical 2-day cycle. Assuming benzoate undergoes normal fermentation as follows (*28*−*30*):

$$C_6H_5COO^- + 6H_2O \rightarrow 3CH_3COO^- + CO_2 + 2H^+ + 3H_2$$

3 mol each of acetate and hydrogen would be formed from each mole of benzoate. On that basis, the 2.6 mol of acetate production from 1.0 mol of benzoate consumption in the reactor indicates that only a small amount of the acetate formed was used in methanogenesis in the reactor. Hydrogen utilization in contrast was essentially complete, and most of the methane production and PCE reduction appear associated with this. Ignoring the smaller contributions of acetate and yeast extract to methane or ethene production, it appears that the hydrogen produced had been used about 58% for methane production and 42% for dehalogenation.

Subsequently, the benzoate concentration in the feed to the CSTR was reduced 25% to 1.28 mM while the PCE concentration was maintained at 0.98 mM. No significant reduction in PCE dehalogenation occurred, except for a slight increase in VC concentration from 1 μM to between 5 and 10 μM. Here, the fraction of hydrogen intermediate used for dehalogenation was increased to well over one-half of the available hydrogen.

Two puzzles are associated with the CSTR results. One is the apparent production of methane from hydrogen, even though the liquid hydrogen concentration (based upon headspace analysis) remained below the 11 nM threshold required for its conversion to methane. The second is the apparent lack of production of methane from acetate, even though the concentrations of chlorinated ethenes (<1 μM) were well below likely inhibitory levels. One hypothesis for

these occurrences is mass-transfer limitations, which could result in localized high concentrations of hydrogen and chlorinated ethenes before the concentrated feed solution entering the reactor became completely mixed with the CSTR liquid contents. Also, ethene at the level found in the CSTR headspace (about 2.5%) has been reported to be inhibitory to methanogenesis (*31*), although the apparent selective inhibition of acetoclastic methanogenesis only, as suggested here, has not been reported.

Comparison between Benzoate and Propionate as Electron Donor for *cis*-DCE Dehalogenation. The study of hydrogen threshold concentration showed that the dehalogenators can use hydrogen when at a lower concentration than the competitive methanogens and homoacetogens. Therefore, a slowly degrading substrate that produces hydrogen slowly is expected to favor dehalogenation over methanogenesis and homoacetogenesis (*12*). Propionate was selected for this purpose for two reasons. First, propionate was not a major intermediate in the CSTR, and so the propionate-using population was small, thus propionate utilization was expected to be very slow. Also, from an energetic viewpoint, propionate conversion is endergonic at high hydrogen concentration (*12*). The upper hydrogen concentration at which propionate-degrading bacteria can still obtain energy is 70 nM under the experimental conditions. In contrast, benzoate can result in a hydrogen concentration about 10 times higher.

As expected, significantly different results were obtained with the two selected substrates (Figure 4). Within 2.5 months, more than three times the amount of ethene was produced in the propionate-fed bottles as in the benzoate-fed ones, while the amount of methane produced from benzoate was three times that from propionate. Figure 4D illustrates the hydrogen concentration changes over time. In benzoate-fed bottles, after an initial burst, the hydrogen level dropped rapidly to and was maintained thereafter at about 2 nM. The initial hydrogen burst corresponded with the rapid fermentation of benzoate and yeast extract that were completed within 3 weeks. Little hydrogen production was available after that for dehalogenation. In contrast, propionate was used slowly over about 3 months. As a result, a much higher hydrogen level (but below 11 nM most of the time) and dehalogenation rate were maintained for a longer period.

Discussion

This study of hydrogen threshold concentrations indicates that the dehalogenators present in the studied mixed culture were able to use hydrogen when at a lower concentration than can be used by the competitive homoacetogens and methanogens. The hydrogen threshold concentration observed in the batch reactors was 2.2 ± 0.9 nM for dehalogenators and 10.9 ± 3.3 nM for methanogens. The small difference in these values, regardless of the electron donors used, suggests that the hydrogen threshold concentration is independent of the electron donor used and the processes of hydrogen production.

Theoretically the threshold concentration of hydrogen for a given reaction in which it is the electron donor is influenced by the energy yield available from its oxidation; an inverse correlation should exist between the free energy of the reaction and the steady-state hydrogen concentration (*32*, *33*). This partially explains the lower hydrogen threshold concentration for dehalogenators as the free energy from the dehalogenation reaction is much more than that from the methanogenic and homoacetogenic processes. The free energy available to each group of microorganisms at its apparent hydrogen threshold is listed in Table 2. Obviously, methanogens and homoacetogens are very efficient in energy conservation because at their threshold concentration the

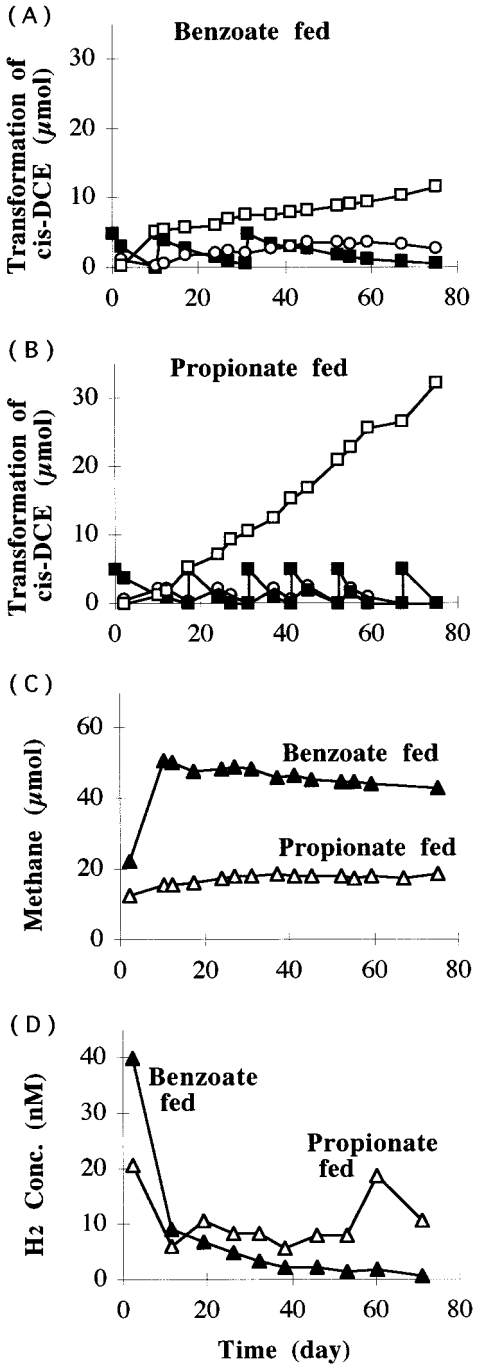

FIGURE 4. Batch study with benzoate and propionate. Symbols in panels A and B represent *cis*-DCE (■) and its transformation products VC (○) and ETH (□).

energy available is very close to the reported lowest net energy required by microorganisms for growth (*34*). Dehalogenators, however, have more energy available to them from hydrogen oxidation at their threshold concentration. This may mean that they are not as efficient at capturing the energy available from hydrogen oxidation as the other organisms.

Although the hydrogen-producing processes have no apparent effect on the hydrogen threshold, the available hydrogen level is a determining factor for the activity of the different competing microorganisms. With benzoate as the primary electron donor in the batch experiments, the resulting initial hydrogen level was much higher than the threshold concentrations for either methanogens or dehalogenators but lower than that required thermodynamically for homoacetogenesis. Thus methanogens and dehaloge-

TABLE 2. Threshold H_2 Concentration and Energetics of H_2 Utilization Reactions

biological processes	equation	threshold H_2 concn in liquid phase (nM)	energy available at threshold H_2 concn[b,c] (kJ/mol H_2)
dehalogenation	*cis*-DCE + H_2 → VC + H^+ + Cl^-	2	−115.5
	VC + H_2 → ETH + H^+ + Cl^-		−129.3
methanogenesis	$0.25CO_2 + H_2 \rightarrow 0.25CH_4 + 0.5H_2O$	11	−5.8
homoacetogenesis	$0.5CO_2 + H_2 \rightarrow 0.25CH_3COO^- + 0.25H^+ + 0.5H_2O$	400[a]	−6.8

[a] Data for *Acetobacterium woodii* (*31*). [b] Under experimental condition when P_{CO_2} = 0.2 atm, P_{CH_4} = 0.05 atm, [acetate] = 2 mM, [Cl^-] = 0.02 M, [cDCE] = 1 μM (in headspace), [VC] = 20 μM (in headspace). [H_2] = H_2 threshold concentration. [c] All calculations are based upon published tables (*22*, *23*).

nators were the main consumers of hydrogen, with methanogenesis dominating. In contrast, with hydrogen as the primary electron donor, homoacetogens became the dominant group in hydrogen utilization with their advantageous kinetic properties.

Comparative studies with benzoate and propionate further confirm the importance of the hydrogen level. When benzoate was used as substrate, its rapid fermentation and thereby rapid hydrogen production and a higher than methanogenic hydrogen threshold concentration resulted over the first few weeks. Most of this hydrogen therefore was consumed by the methanogens. Subsequently, a lower hydrogen level and dehalogenation rate resulted. In contrast, the small propionate-utilizing population and thermodynamic regulation of propionate fermentation caused an initially slower release of hydrogen, but one that was maintained much longer. The net result was a higher long-term hydrogen production rate that produced a persistent hydrogen level just below the threshold for methanogens. This not only limited methanogenesis but also resulted in a higher rate of dehalogenation. This result is consistent with the short-term studies by Fennel et al. (*12*).

The manner in which the electron donor is delivered to the microorganisms is also a major factor affecting the outcome of competition for hydrogen as suggested by comparison results between the CSTR and the batch reactors. In the batch study with benzoate, its rapid fermentation caused hydrogen to accumulate above the threshold concentration for methanogens. This gave the methanogens the opportunity to obtain a far greater share of available reducing equivalents than the dehalogenators, which obtained only 9%. However, with continuous benzoate feed to a CSTR, hydrogen remained at a low steady-state concentration near the dehalogenation threshold of about 2 nM. Here, dehalogenation of PCE to ETH was complete, a process that consumed over one-half of the potentially available hydrogen. Just considering the conversion of *cis*-DCE to ETH, about one-quarter of the available hydrogen was consumed for this conversion as compared with 9% in the batch study.

These results suggest approaches that may be used to impart a competitive advantage to dehalogenating microorganisms in bioremediation. When methanogens are the main competitors for hydrogen, efficient use of hydrogen for dehalogenation can be obtained by strategies that maintain the hydrogen concentration between 2 and 11 nM. This might be realized in either of three ways: first, by adjusting the delivery rate of hydrogen precursors, such as benzoate, to the microorganisms in order to achieve an effect similar to that obtained in the CSTR; second, through the use of an appropriate hydrogen precursor that is slowly degradable so that it can release hydrogen in a slow manner to maintain the ideal hydrogen concentration as found here with propionate; and third, by use of a hydrogen precursor as the primary electron donor that requires a very low hydrogen partial pressure thermodynamically for the fermentation to occur, perhaps also somewhat similar to the case here with propionate.

Acknowledgments

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