

# Utilization of Cathodic Hydrogen as Electron Donor for Chloroform Cometabolism by a Mixed, Methanogenic Culture

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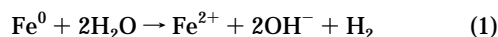
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Currently there is considerable interest in using elemental iron ( $\text{Fe}^0$ ) for treatment of highly chlorinated organic compounds. Early studies found little microbiological contribution to degradation in laboratory and field tests. Most work since then has focused on abiotic processes. In studies conducted with a mixed, methanogenic culture, however, pseudo-first-order rate coefficients for chloroform degradation were at least 3.6 times greater in serum bottle incubations containing 40 mesh iron filings and live cells as compared to incubations containing  $\text{Fe}^0$  and killed cells,  $\text{Fe}^0$  and cell-free mineral medium, or  $\text{Fe}^0$ -free incubations with live cells. CF cometabolism and methanogenesis was apparently supported using cathodic hydrogen produced by anaerobic corrosion of the added  $\text{Fe}^0$ . The use of selective microbial inhibitors showed that  $\text{H}_2$ -consuming methanogens and not homoacetogens were responsible for CF degradation. The sustainability of the process was established in a 60-day column study using steel wool as support for microbial growth. The observation that cathodically produced  $\text{H}_2$  can support reductive dechlorination by anaerobic bacteria may have significant practical implications.

## Introduction

Chlorinated aliphatic hydrocarbons (CAHs) are frequent groundwater contaminants (1). They are susceptible to biotransformations catalyzed by pure and mixed methanogenic (2-9) and nonmethanogenic, anaerobic cultures (5, 10-13). Electron donors that have been investigated for methanogenic biotransformation of CAHs include methanol (7, 8), acetate (2, 3, 9), and hydrogen (14). Hydrogen is a more thermodynamically favorable electron donor than either methanol or acetate (15), and its high diffusivity through biofilms ensures that the target CAHs would be flux-limiting to optimize removal rates. Nevertheless, practical problems exist with regard to the use of hydrogen due to its low solubility in water, about 1.6 mg/L at 20 °C, based on a Henry's constant of  $6.83 \times 10^4 \text{ atm/mol}$  (16).

One way that hydrogen can be introduced into aqueous solution involves the use of iron metal. When  $\text{Fe}^0$  is immersed in anoxic water, hydrogen is produced (17):



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Pure cultures of methanogenic (18-21), homoacetogenic (19), and sulfate-reducing bacteria (19) have demonstrated the ability to use cathodic hydrogen as an energy source for growth in short-term batch experiments. Also, anaerobic bacteria are commonly associated with the corrosion of metals (22, 23). However, the ability of methanogens to sustain growth on cathodic hydrogen in the presence of CAHs has not been established. This is particularly important because many CAHs, especially chloroform (CF), extremely inhibitory to methanogens (9, 24-29).

The biotransformation of CAHs using cathodic hydrogen as electron donor is confounded by the direct, abiotic reduction of CAHs by  $\text{Fe}^0$  (30-33). In the presence of 100-mesh iron powder, carbon tetrachloride (CT) underwent sequential dehalogenation to CF and then to dichloromethane (DCM), which was not further degraded (31).

Thus it is well-known that anaerobic bacteria can catalyze the reductive dechlorination of CAHs, that CAHs can be transformed abiotically using  $\text{Fe}^0$ , and that  $\text{Fe}^0$  can support the growth of anaerobic bacteria. Furthermore, there are limitations to the biotic process because of potential toxicity of parent CAHs and their metabolites and to the abiotic process because of the potential buildup of "dead-end" products. We hypothesize that the presence of an active methanogenic consortium in the presence of  $\text{Fe}^0$  may enhance the rate and extent of degradation of selected CAHs. "Proof-of-concept" experiments have been reported elsewhere (34). The objectives of the research described in this paper were (1) to determine if anaerobic bacteria could utilize cathodic hydrogen as the electron donor for biotransformation of CF, (2) to identify the microbial group responsible for CF degradation using microbial inhibitors, and (3) to evaluate the sustainability of the process in a continuous-flow column reactor. CF was used in the present study because of the lack of reactivity of DCM with  $\text{Fe}^0$ , which simplified the kinetic analysis, and because previous research demonstrated that CF could be transformed by an unacclimated, mixed, methanogenic culture available in our laboratory (35).

## Materials and Methods

**Experimental Design. Batch Reactors.** Batch experiments were conducted in the dark at 20 °C using 25 mL liquid volume in sealed, 38-mL serum bottles. Duplicates were used for all treatments. Bottles were incubated in an inverted position on a circular action shaker table (Lab-Line) at 200 rpm. Initial batch experiments examined the effect that amending methanogenic incubations with  $\text{Fe}^0$  had on the kinetics of CF transformation. Iron filings (2 g, 40 mesh, specific surface area 0.237  $\text{m}^2/\text{g}$ , Malinkrodt) were weighed into selected bottles. Bottles were then filled with DI water, sealed with Teflon-coated rubber septa (West Co., Phoenixville, PA), and capped with aluminum crimp caps. Bottles were flushed with  $\text{N}_2/\text{CO}_2$  gas (80:20, v/v) through the septa to displace the water. A 100-mL glass, gas-tight syringe (Scientific Glass Engineering, Australia) was used to inject bottles with 25 mL of either freshly prepared mineral medium, cell suspension from a stock culture reactor, or autoclaved cell suspension (120 °C for 20 min). The headspace gas exited through a 25-gauge needle. The mineral medium used in these experiments was the same as that supplied to the stock culture reactor. Transformation experiments were initiated by injecting a volume of CF stock solution through the septum with a 10- $\mu\text{L}$  syringe. CF-free controls containing iron and live cell suspension were used to investigate the inhibition of methanogenesis by CF. Bottles were sampled periodically for CF, DCM, hydrogen, and methane. The liquid-gas mass

transfer coefficient,  $K_L a$ , was determined using the method of Tatara et al. (36).

An experiment was conducted to elucidate the possible roles of methanogens and homoacetogens in cathodic hydrogen consumption and CF degradation. Two microbial inhibitors were used for this purpose: bromoethanesulfonic acid (BESA) and vancomycin. BESA is a specific methanogenic inhibitor (37). Vancomycin is an antibiotic that inhibits eubacterial cell wall formation (38). Incubations containing 2 g of iron filings were amended with neither, one, or both of the inhibitors at concentrations of 50 mM BESA (39, 40) or 100 mg/L vancomycin (14, 40–42).  $\text{Fe}^0$ -free incubations with a  $\text{N}_2/\text{CO}_2$  headspace received 2 mL of  $\text{H}_2/\text{CO}_2$  gas and neither, one, or both of the inhibitors at the same concentrations. The bottles were sampled daily for hydrogen and methane. When hydrogen reached low levels in some of the  $\text{H}_2$ -amended incubations, the headspace of all of the  $\text{H}_2$ -amended incubations was purged with  $\text{N}_2/\text{CO}_2$  gas, and an additional 2 mL of  $\text{H}_2/\text{CO}_2$  gas was injected into the serum bottles. This cycle was repeated once more, and this time a volume of CF-saturated stock was also injected into the bottles.

**Column Reactors.** An experiment employing column reactors was conducted over a 60-d period to determine if the CF degradation activity observed with batch reactors was sustainable. Three glass chromatography columns (2 cm i.d.  $\times$  20 cm) were used to study the transformation of CF under continuous-flow conditions. Steel wool (0.0075  $\text{m}^2/\text{g}$ , Medium 1, Rhodes/American, Chicago, IL) was used as an iron source and physical support for the attachment of bacteria. The chemical composition of the steel wool, as reported by the manufacturer, was (in %) Fe (52), Si (30), C (16), Mn (1.25), P (0.7), and S (0.05). Two columns were packed end-to-end with 5.5-g sections of an unrolled steel wool pad. The third column was used as a sterile (autoclaved) control to evaluate volatilization losses and was filled with 5-mm-diameter glass beads. One steel wool-filled column was seeded with two 100-mL aliquots of cell suspension from the stock culture reactor using a 100-mL glass, gas-tight syringe. The microorganisms were allowed to colonize the steel wool for 2 days with no flow to the column. The columns were then operated for 2 weeks with CF-free influent, using the same medium that was supplied to the stock culture reactor, but without the addition of acetic acid. The medium was buffered by adding 1 mL of 1 N HCl to 200 mL of fresh medium and then adjusting the pH to 6.7 with  $\text{NaHCO}_3$ . After 2 weeks, CF was added to the feed solution from the CF stock solution using a 10- $\mu\text{L}$  syringe. Influent was pumped into the columns through Teflon tubing from 25-mL glass, gas-tight syringes (Hamilton) with a syringe pump (Harvard Apparatus). The columns were fed upflow at a volumetric flow rate of 7.9 mL/d. The porosity of the steel wool- and glass bead-filled columns was 0.90 and 0.30, respectively, resulting in superficial velocities of 2.8 and 8.4 cm/d and hydraulic retention times of 7.2 and 2.4 d, respectively. Effluent samples were taken with disposable syringes attached to Teflon tubing, which extended to the top of the steel wool or glass beads.

**Chemicals and Stock Solutions.** CF (high-performance liquid chromatography (HPLC) grade) and DCM (certified American Chemical Society (ACS) grade) were purchased from Fisher Scientific (Pittsburgh, PA). Stock aqueous solutions of CF and DCM were prepared by adding about 5 mL of each chemical to 25 mL of autoclaved, distilled deionized water in 43-mL glass serum bottles sealed with Teflon-lined rubber septa and aluminum crimp caps. Other chemicals used included acetic acid (glacial, Malinkrodt), methane gas (100%, Scott Specialty Gases), 2-bromoethanesulfonic acid (98%, Aldrich Chemical Company, Milwaukee, WI), and vancomycin (Sigma Chemical Company, St. Louis, MO).

**Stock Culture Reactor.** The source of organisms was a magnetically stirred, 9.5-L glass reactor containing an acetate-enriched methanogenic cell suspension volume of 8 L. The

reactor was maintained at 20 °C with a 40-d hydraulic retention time, such that 200 mL of cell suspension was removed daily and replaced with fresh medium. The medium recipe has been listed previously (9). The medium was buffered with  $\text{NaHCO}_3$  as needed to maintain a reactor pH of  $6.9 \pm 0.1$ . The volatile suspended solids concentration of the reactor averaged  $245 \pm 20$  mg/L ( $n = 5$ ) at the time the experiments were conducted.

**Analytical Methods.** CF, DCM,  $\text{H}_2$ , and  $\text{CH}_4$  were determined by gas chromatography (GC) using headspace analysis. Headspace samples were withdrawn using a locking, gas-tight syringe (Precision Sampling Corp., Baton Rouge, LA) equipped with a 22-gauge side-port needle and then injected into a GC. The headspace of 38-mL batch reactors was directly sampled by this method. For the column reactors, a 1-mL aqueous sample was taken from an effluent sample port with a 3-mL disposable plastic syringe and, using a 25-gauge needle, injected into a 5-mL glass vial sealed with a Teflon-coated, rubber septum and screw cap. The headspace of this bottle was then injected into a GC.

CF was analyzed using an HP 5890 Series II GC equipped with an electron capture detector and a DB-5 capillary column (J&W Scientific, Folsom, CA). DCM and  $\text{CH}_4$  were analyzed on a HP 5890 Series II GC equipped with a flame ionization detector and a DB-WAX capillary column (J&W Scientific).  $\text{H}_2$  was analyzed on a HP 5890 Series II GC equipped with a thermal conductivity detector using a Haysep Q packed column (Alltech Associates). For the 38-mL batch reactors, sample sizes for CF, DCM,  $\text{H}_2$ , and  $\text{CH}_4$  were 100, 500, 100, and 100  $\mu\text{L}$ , respectively, with corresponding detection limits of 1.7, 15.3, 188, and 9.3 nmol/bottle, respectively. For the column reactors, a 500- $\mu\text{L}$  sample size was used for CF, DCM, and  $\text{CH}_4$ , and detection limits were 0.07, 0.78, and 2  $\mu\text{M}$ , respectively.

Acetate concentrations were determined by HPLC analysis using a PRP-X300 column (Hamilton), Gilson Model 306 pump, and Model 805 manometric module (pulse dampener). Peak areas were integrated using Gilson 712 Controller Software version 1.2. The detection limit was approximately 0.08 mM.

Biomass was measured as volatile suspended solids using Method 2540 E in *Standard Methods* (43). The pH was measured with a pH meter (Beckman Model F 72) and combination electrode (Fisher Scientific, Pittsburgh, PA).

## Results

**Batch Reactors.** CF was transformed most rapidly in the incubations containing live cells and  $\text{Fe}^0$  (Figure 1a). DCM was detected only in the treatments containing cells and  $\text{Fe}^0$  (Figure 1b). The  $\text{H}_2$  concentration in the incubations containing live cells and  $\text{Fe}^0$  differed sharply depending on the presence of CF.  $\text{H}_2$  behavior in the incubation containing live cells,  $\text{Fe}^0$ , and CF was similar to  $\text{H}_2$  behavior in the treatment containing mineral medium and iron until the CF concentration in the live cell- $\text{Fe}^0$ -CF treatment reached low levels, whereas  $\text{H}_2$  in the CF-free incubation containing live cells and  $\text{Fe}^0$  remained at low levels throughout (Figure 1c). Likewise, CF affected methane production in the live cell- $\text{Fe}^0$  treatments; low levels of methane were measured in the live cell- $\text{Fe}^0$  incubation containing CF until the CF concentration reached low levels, while methane was steadily produced at a faster rate in the CF-free live cell- $\text{Fe}^0$  incubation (Figure 1d).

CF was rapidly transformed and DCM was produced when  $\text{Fe}^0$ -free incubations containing live cells were amended with 2 mL of  $\text{H}_2/\text{CO}_2$  (80/20; v/v) gas (Figure 2). The slow disappearance of CF in the  $\text{H}_2$ -free control containing live cells indicated that an external electron donor was required for rapid CF transformation. Furthermore, adding  $\text{H}_2$  to incubations containing autoclaved cell suspension had little

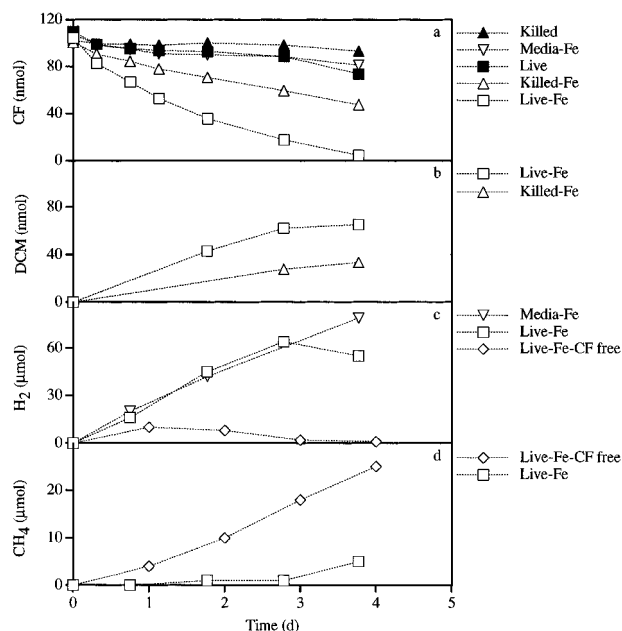


FIGURE 1. CF degradation (a), DCM formation (b), hydrogen evolution and utilization (c), and methane production (d) in batch reactors containing 2 g of iron filings and killed or live cells, or mineral medium. Filled symbols indicate treatments that were not amended with iron. All incubations received CF except those designated CF free.

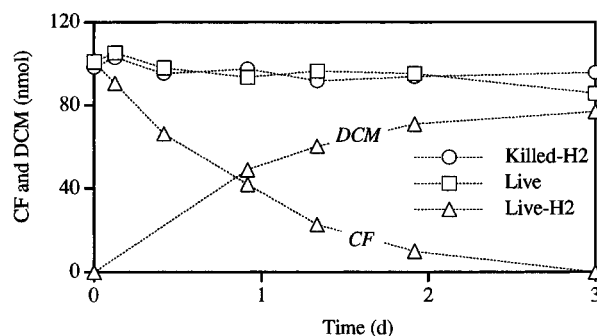


FIGURE 2. CF degradation and DCM production in batch reactors amended with hydrogen.

effect on CF transformation, indicating that CF transformation using  $H_2$  as an electron donor was enzymatic.

Figure 3 shows the impact of the inhibitors on cathodic hydrogen and methane levels in the incubations containing cells and iron. The behavior of the incubations was controlled by the methanogenic inhibitor BESA: bottles containing BESA alone or BESA and vancomycin responded similarly, while bottles containing vancomycin alone or no inhibitor behaved similarly. Hydrogen levels were similar in all bottles for the first 2 days. After this, the  $H_2$  concentration in the BESA-amended incubation sets continued to increase, while the concentration of  $H_2$  in the vancomycin or inhibitor-free incubations decreased, eventually becoming nondetectable (Figure 3a). Similarly, methanogenesis was inhibited in both incubation sets containing BESA as compared to the inhibitor-free incubations or the incubations containing vancomycin only (Figure 3b).

Figure 4 shows the impact of the inhibitors on hydrogen consumption, methane production, and CF transformation in incubations supplied with  $H_2$ . In general, the behavior of the incubations was controlled by BESA, and vancomycin alone had no effect. During the first 2 days, neither of the inhibitors appeared to affect  $H_2$  consumption. After this, however, the rate of  $H_2$  consumption in the incubations containing vancomycin or no inhibitor remained steady, while

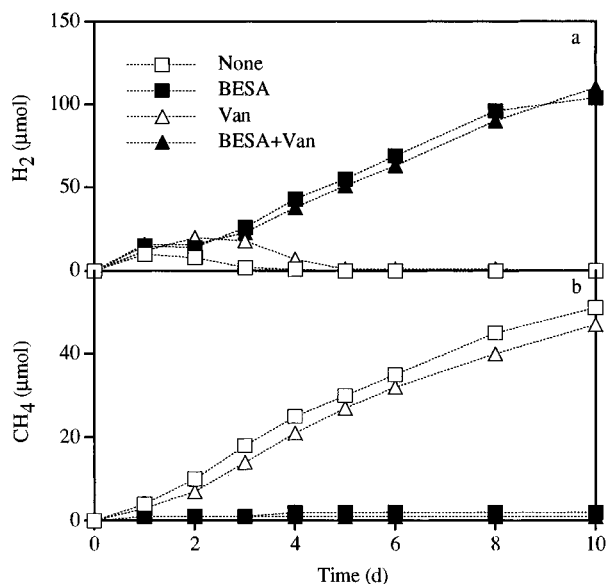


FIGURE 3. Hydrogen evolution and utilization (a) and methane production (b) in batch reactors containing 2 g of iron filings. None, inhibitor-free control; BESA, 50 mM BESA; Van, 100 mg/L vancomycin; BESA+Van, 50 mM BESA and 100 mg/L vancomycin.

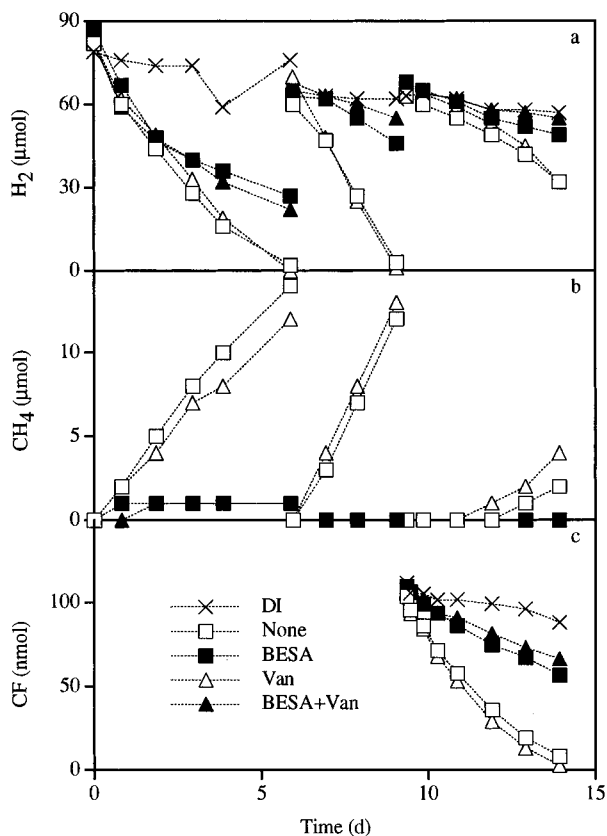


FIGURE 4. Hydrogen evolution and utilization (a), methane production (b), and CF degradation in batch reactors amended with hydrogen. DI, deionized water control; None, inhibitor-free control; BESA, 50 mM BESA; Van, 100 mg/L vancomycin; BESA+Van, 50 mM BESA and 100 mg/L vancomycin.

the rate of  $H_2$  consumption in the incubations amended with BESA alone or BESA and vancomycin decreased (Figure 4a). The relatively constant  $H_2$  concentration in the live cell incubations was confirmed that the decrease in the  $H_2$  levels in the live cell incubations was not due to volatile losses. Acetate was not detected ( $<0.08$  mM) in liquid samples taken from the incubations during the first 3 days. Methane production in the incubations containing BESA alone or BESA and van-

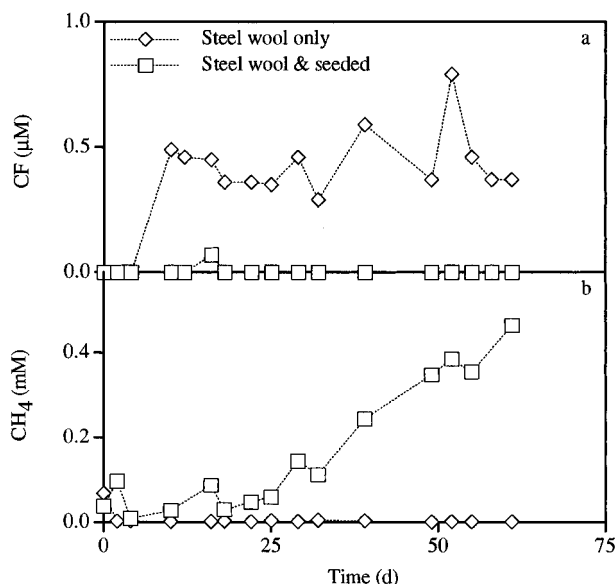


FIGURE 5. CF (a) and methane (b) concentrations in the column reactor effluents. The average influent CF concentration was  $1.61 \pm 0.49 \mu\text{M}$ , and the average effluent CF concentration in the glass bead control column was  $1.29 \pm 0.85 \mu\text{M}$ .

comycin was severely inhibited in comparison to methane production in the vancomycin- or inhibitor-free incubations (Figure 4b). When bottles were resupplied with  $\text{H}_2$ , vancomycin had no effect on either  $\text{H}_2$  consumption or methane production, while BESA severely inhibited both (Figure 4a,b). Bottles were resupplied with  $\text{H}_2$  on the ninth day and spiked with CF. Vancomycin alone had no impact on CF transformation (Figure 4c). Moreover, the rate of CF transformation in incubations amended with BESA alone or BESA and vancomycin was similar and lower than in BESA-free incubations. Hydrogen consumption and methane production were inhibited in the presence of CF.

**Continuous-Flow Column Studies.** The CF concentration in the effluent from the abiotic, steel wool column, averaging  $0.41 \pm 0.17 \mu\text{M}$ , was consistently greater than that from the methanogenic, steel wool column, which averaged  $0.00 \pm 0.02 \mu\text{M}$  CF (Figure 5a). In fact, on only one occasion was CF detectable in the methanogenic column effluent. A small peak on chromatograms was visible at the DCM elution time (2.3 min) in samples from these two columns on several sampling events; however, this peak was not integrated. The effluent from the glass bead control column,  $1.29 \pm 0.85 \mu\text{M}$  CF, was not statistically different (at the 95% level) from the influent CF concentration,  $1.61 \pm 0.49 \mu\text{M}$  CF. This indicated that the loss of CF in the steel wool columns did not result from sorption or volatilization. Methane in the methanogenic column averaged  $0.79 \pm 0.48 \text{ mg of CH}_4/\text{L}$  over the first 25 days after CF addition was initiated and increased 1 order of magnitude during the next 36 days to  $7.43 \text{ mg of CH}_4/\text{L}$  on day 61 (Figure 5b). The pH of the effluent from the methanogenic, steel wool column and the abiotic, steel wool column was 8.3 and 8.7, respectively.

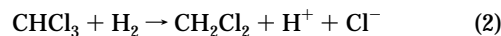
## Discussion

When the data shown in Figure 1a was plotted as  $\ln(M/M_0)$  versus  $t$  [where  $M$  is the total mass of CF (nmol of CF) in at bottle at time  $t$ , and  $M_0$  is the initial mass of CF (nmol of CF)], linear plots were produced, indicating that CF transformation rates followed first-order kinetics. Slopes of these plots, i.e., pseudo-first-order rate coefficients,  $k$  ( $\text{d}^{-1}$ ), were then compared. The CF transformation rate coefficient for the treatment containing  $\text{Fe}^0$  and live cells,  $0.72 \text{ d}^{-1}$  ( $r^2 = 0.96$ ), was greater than the value of  $k$  for the treatment containing live cells only,  $0.10 \text{ d}^{-1}$  ( $r^2 = 0.81$ ); killed cells and  $\text{Fe}^0$ ,  $0.20$

$\text{d}^{-1}$  ( $r^2 = 0.99$ ); and mineral medium and  $\text{Fe}^0$ ,  $0.08 \text{ d}^{-1}$  ( $r^2 = 0.63$ ). The large value of the gas-liquid mass transfer coefficient,  $K_L a$ ,  $95 \text{ d}^{-1}$ , ensured that CF transformation kinetics were not limited by the rate of mass transfer between the liquid and gas phase.

Interestingly, combining  $\text{Fe}^0$  and live cells was synergistic with respect to CF degradation; the rate coefficient for the combined  $\text{Fe}^0$  and live cell treatment was 2.4 times the sum of the rate coefficients for the separate treatments containing  $\text{Fe}^0$  or live cells. Hence, abiotic and microbial CF degradation processes were not independent when  $\text{Fe}^0$  and cells were combined in a single reactor. We propose that this synergism resulted from the production of hydrogen as a result of the oxidation of  $\text{Fe}^0$  (17), which was then used as a primary substrate for CF cometabolism. The detection of hydrogen in the incubations to which  $\text{Fe}^0$  was added (Figure 1c) and the stimulation of CF degradation when hydrogen was added directly support this hypothesis (Figure 2).

Reductive dechlorination is an electron-consuming process, the stimulation of which by the addition of an exogenous electron donor is common (8, 41, 44). Although  $\text{H}_2$  stimulated CF degradation, CF inhibited hydrogen consumption and methane production (Figure 1c,d), which is a common effect of CF on methanogenic systems (9, 24–29). It should be noted that, while the consumption of  $\text{H}_2$  in incubations containing CF is not noticeable (Figure 1c), only 1 nmol of  $\text{H}_2$  is required for the reduction of 1 nmol of  $\text{CHCl}_3$  to  $\text{CH}_2\text{Cl}_2$ , according to



Consequently, during the first 3 days, for example, a negligible amount (about 0.1%) of the  $\text{H}_2$  produced (at least  $60 \mu\text{mol}$  of  $\text{H}_2$ ) would have been required for the degradation of about 80 nmol of CF.

The experiments conducted with microbial inhibitors support the hypothesis that methanogenic bacteria were responsible for cathodic hydrogen consumption and CF dechlorination using cathodic hydrogen as electron donor. Vancomycin, shown by Murray and Zinder (45) to inhibit eubacteria at the concentration used in this study, 100 mg/L, had little or no impact on cathodic hydrogen consumption or methanogenesis when added alone to bottles containing iron (Figure 3). In contrast, BESA, a methanogenic inhibitor, inhibited both of these processes to a similar degree regardless if added alone or with vancomycin (Figure 3). Similar results were seen in treatments to which hydrogen was added directly: vancomycin had no impact on hydrogen consumption or methanogenesis when added alone, while both of these processes were inhibited in bottles that contained BESA alone or BESA and vancomycin (Figure 4a,b). Utilization of hydrogen by methanogens for metabolic purposes may account for the decrease in hydrogen concentration in the BESA-inhibited incubations in the early stage of this study. Acetate, a product of homoacetogens, was not detected. Furthermore, vancomycin by itself had no impact on CF degradation as compared to an inhibitor-free control (Figure 4c). In contrast, CF degradation was inhibited to the same degree in bottles containing BESA alone or BESA and vancomycin, compared to CF degradation in the inhibitor-free control (Figure 4c). The slow rate of CF degradation in the BESA-inhibited bottles supports the hypothesis that methanogens were responsible for CF degradation. BESA is a structural analogue of coenzyme M (2-mercaptoethane sulfonic acid) (37), which is unique to methanogens (46). BESA inhibits the conversion of methyl coenzyme M to methane by binding to methyl coenzyme M methyl reductase in place of coenzyme M (47). The prosthetic group of methyl reductase is coenzyme  $\text{F}_{430}$  (48), a nickel porphyrinoid that facilitates the reduction of CF and other chlorinated aliphatics in abiotic experiments when a bulk reducing agent such as

titanium(III) citrate or dithiothreitol is provided (6, 49). Methanogens contain other metallocoenzymes, such as cobalt corronoids, that are not directly affected by BESA and that have also been shown to reduce CF and other chlorinated aliphatics (49–56). Hence, although BESA may completely inhibit methanogenesis and  $F_{430}$ -mediated CF reduction, BESA may not stop the degradation of CF by pure or mixed methanogenic cultures. Experimental results to the contrary are lacking. These results do not argue against the ability of homoacetogens to utilize hydrogen for reductive dechlorination, but rather that, in these cultures, their involvement was not witnessed.

The extent of CF removal in the microbial steel wool column was consistently greater than in the abiotic column during the 60-d experimental period. This illustrates that CF cometabolism by anaerobic bacteria using cathodic hydrogen as the sole electron donor is sustainable, a finding that was uncertain based on the batch experiments where methanogenesis was strongly inhibited in the presence of CF (Figure 1). The increase in the concentration of methane in the effluent from the microbial steel wool column no doubt reflects the growth of hydrogenotrophic methanogens within the column as well as acclimation to CF. Based on the presumed increase in methanogenic biomass during the study, it is reasonable to assume that higher influent CF concentrations than those studied here could be reduced to nondetectable levels in the effluent. The limits of this technology will have to be determined for field-scale systems.

This research illustrates that microbial metallic iron treatment systems may offer advantages over abiotic zero-valent iron schemes. In a field-scale application of this technology, cells grown in a fermenter or sludge from an anaerobic digester could be injected into a permeable, iron-containing, reactive barrier similar to that proposed by Blowes et al. (57). For some compounds, combined microbial abiotic systems may accelerate the rate of transformation and extent of mineralization; dichloromethane, a CF dechlorination product, does not undergo measurable abiotic transformation by iron (31) but can be utilized as a growth substrate by acclimated anaerobes (58–61). Conversely, anaerobic bacteria reduce TCE to ethene or ethane via dichloroethylene and vinyl chloride (41, 62), while abiotic iron systems reduce TCE to ethene and ethane without the production of these intermediates (33). Another potential negative consequence of bacteria is biofouling. The proliferation of bacteria in an improperly designed reactive barrier could reduce the hydraulic conductivity of the barrier, thereby hindering the flow of groundwater through it. These observations indicate that further research is needed to optimize the use of hydrogenotrophic anaerobic bacteria and metallic iron for the reductive treatment of CAHs.

Although hydrogen is one of the most thermodynamically favorable anaerobic substrates, its use as an electron donor for bioremediation purposes has been limited by its low solubility. The observation that cathodic hydrogen, a product of anaerobic iron oxidation, can support reductive dechlorination by anaerobic bacteria may have significant practical applications.

## Acknowledgments

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