Environ. Sci. Technol. **1998**, *32,* 1817−1824

Enrichment of High-Rate PCE Dechlorination and Comparative Study of Lactate, Methanol, and Hydrogen as Electron Donors To Sustain Activity

CYNTHIA S. CARR AND JOSEPH B. HUGHES*

Department of Environmental Science and Engineering, George R. Brown School of Engineering, Rice University, 6100 Main − MS 317, Houston, Texas, 77005-1892

The influence of three electron donors on the ability to sustain rapid tetrachloroethene (PCE) dechlorination in mixed cultures was investigated. Experiments were performed in recycle columns containing either a high-rate PCE dechlorinating culture developed from granular sludge or a (1:1) mixture of this culture with an enrichment culture derived from soil cores taken from a chlorinated ethene contaminated site. Columns were fed PCE (aqueous concentration of 5 mg/L) and either lactate, methanol, or H_2 as their electron donor. Comparisons between electron donors were formulated using the observed rates of PCE disappearance and the extent of dechlorination achieved in each column. Over a period exceeding 1 year, the observed PCE half-life in each column was found to decrease from approximately 13 h to slightly less than 2 h, regardless of electron donor fed. Although initial mass balance studies demonstrated that the extent of dechlorination varied between inoculum and electron donor, over time, the recycle columns behaved similarly, and PCE was recovered as approximately 80% vinyl chloride and 20% ethene in all systems. Rates and extents of PCE dechlorination were improved over time in systems containing high H_2 partial pressures (0.8 atm) in the presence of an active methanogenic community.

Introduction

The reductive dechlorination of tetrachloroethene (PCE) by anaerobic bacteria has been a topic of research for nearly two decades. PCE dechlorination is known to occur through both cometabolic (*1*−*3*) and respiratory processes (*4*−*11*). The most common end products of dechlorination are trichloroethene (TCE), dichloroethene isomers (DCEs), vinyl chloride (VC), and ethene (*12*−*17*), although ethane (*18*) and carbon dioxide (*19*, *20*) have also been observed.

Studies demonstrating the ability to enrich rapid rates of complete PCE dechlorination via respiratory processes have heightened interest in the application of anaerobic bioremediation at chlorinated ethene contaminated sites (*21*). Due to the propensity for PCE to exist as a nonaqueous-phase liquid (NAPL), the success of an in situ reductive dechlorination system will be contingent upon the ability to enrich PCE dechlorination and sustain activity for the duration of clean up. Recent studies have indicated that the selection of electron donor(s) may impact the ability to sustain dechlorination activity in situ (*17*, *22*).

The central concern regarding electron donor selection is the possible competition between dechlorinators and other H_2-utilizing microorganisms for H_2 produced during the fermentation of organics (*14*, *23*). In particular, half-velocity constants calculated for dechlorinators and H_2-utilizing methanogens have indicated that H_2-utilizing methanogens may have the potential to out-compete dechlorinators for H_2 when present at high partial pressures (*17*, *22*). Thus, the use of fermentable substrates that maintain low levels of H_2 may offer a competitive advantage for H_2-utilizing dechlorinators.

For this reason, Fennell et al. (*24*) investigated the ability of four fermentable substrates to sustain PCE dechlorination long-term (i.e., approximately 4 months). The choice of organic substrates was based upon their rates of fermentation and the H_2 partial pressures that could be developed and maintained. Despite the difference in the resulting H_2 partial pressures (ranging from approximately 1×10^{-5} to 3×10^{-3} atm), no long-term effect on dechlorination was observed. This result may indicate that either low H_2 partial pressures were not required to maintain a competitive dechlorinating community or, since several isolated PCE respiring bacteria do not utilize H_2 as an electron donor (*8*−*10*), H_2 was not the source of PCE-reducing equivalents in all systems tested.

Studies presented herein focus on the enrichment of a high-rate PCE dechlorinating culture and the long-term sustainability of its dechlorination activity when fed fermentable substrates (lactate and methanol) or H_2 (directly) at high partial pressures (ca., 0.8 atm). Enrichment cultures were prepared with a granular sludge with no known exposure to chlorinated solvents and from aquifer solids obtained at a historically contaminated site. Further studies were conducted to assess the long-term sustainability of dechlorination—measured as the rate of PCE disappearance and the distribution of dechlorination products—when cultures were fed different substrates. These studies suggest that similar dechlorination activity can be stimulated and sustained with the three electron donors tested and that dechlorinating bacteria were not sensitive to high H_2 partial pressures developed through direct H_2 addition.

Materials and Methods

Chemicals. The following chemicals were obtained in liquid form: tetrachloroethene (ACS reagent, 99.5+%; Sigma-Aldrich); trichloroethene (HPLC grade, 99.9+%; Sigma-Aldrich); and *cis*-1,2-dichloroethene (*cis*-DCE) (99% purity; Supelco). Vinyl chloride (7.99%) in nitrogen was purchased from Air Liquide. Fluorobenzene, 2000 mg/mL in methanol, was purchased from Supelco. Sodium lactate (60 wt % aqueous solution), pentane (HPLC grade), and methanol (certified ACS, spectranalyzed) were obtained from Acros Organics, Fisher Scientific. Methane (99.0%), propane (99.5%), and ethene (99.5%) were procured from Scott Specialty Gases. Custom-made gases, N_2/CO_2 (90/10%, v/v) and H_2/CO_2 (80/20%, v/v), were prepared by TriGas.

Nutrient Medium. Reagent-grade chemicals were used in nutrient medium preparation. The nutrient medium consisted of the following: 400 mg/L NH_4Cl, 400 mg/L KCl, 400 mg/L $MgCl_2 \cdot 6H_2O$, 80 mg/L $(NH_4)_2HPO_4$, 25 mg/L $CaCl_2 \cdot 2H_2O$, 10 mg/L $(NaPO_3)_{13}$, 2.5 mg/L KI, 2.5 mg/L $CoCl_2 \cdot 6H_2O$, 0.5 mg/L $MnCl_2 \cdot 4H_2O$, 0.5 mg/L NH_4VO_3, 0.5 mg/L $ZnCl_2$, 0.5 mg/L $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg/L H_3BO_3, 0.5 mg/L $NiCl_2 \cdot$

* Author to whom correspondence should be addressed. Fax: (713) 285-5203, e-mail: hughes@rice.edu.

S0013-936X(97)00985-1 CCC: $15.00
Published on Web 05/01/1998

$6H_2O$, 200 mg/L yeast extract, and $NaHCO_3$ as buffer when needed. Except where noted, the nutrient medium was made anaerobic by the addition of 300 mg/L $Na_2S{\cdot}9H_2O$ and 40 mg/L $FeCl_2{\cdot}4H_2O$, and 1 mg/L resazurin was added as a redox indicator.

PCE Saturated Aqueous Solution, PCE_{aq}. Two milliliters of PCE was added to 100 mL of deionized water in a 120 mL serum bottle. After sealing with a Teflon-lined butyl rubber septum and aluminum crimp cap, the PCE/water solution was vigorously shaken, then allowed to sit quiescently for a period of 24 h. The resulting aqueous-phase PCE concentration was consistently measured to be 200 mg/L (the aqueous solubility of PCE at ca. 25 °C). The aqueous phase was used for PCE addition in selected experiments as described in the following sections.

Analytical Methods. A variety of gas chromatography techniques were established to quantify and identify chlorinated ethenes, nonchlorinated end products, and methane. Quantification of chlorinated ethenes and ethene required correction for partitioning between aqueous and gas phases. Dimensionless Henry's Gas Law constants (H_c) reported for PCE, TCE, the DCE isomers, and VC at 24.8 °C were used (*25*). A dimensionless Henry's Gas Law constant for ethene was measured in the lab as outlined in ref *25* and was found to be 7.75 ± 0.46 (95% confidence interval). Throughout this text, all reported aqueous and gas-phase concentrations have been corrected for partitioning.

Two methods were used for PCE and TCE quantification. The first method allowed for low level analysis of PCE, TCE, and DCE isomers. The second method was used for samples with high concentrations of PCE and TCE, but was not effective for the analysis of DCE isomers.

In the first method (method I), aqueous samples were analyzed with a gas chromatograph (GC) (Hewlett-Packard) equipped with a mass selective detector (MS). Samples were introduced to the GC following concentration by purge and trap (O. I. Analytical) equipped with a VOCARB 3000 trap (Supelco). Separation was achieved with a 60 m × 0.25 mm i.d., 1.5 mm film VOCOL capillary column (Supelco). The detector and injection port temperatures were 275 and 220 °C, respectively. The oven temperature program was as follows: 40 °C, hold 4 min, 4 °C/min to 190 °C, no hold. Helium, the carrier gas, was split 13.3:1. Nominal detection limits for PCE, TCE, and *cis*-DCE were 46, 22, and 46 μg/L, respectively. The program for the purge and trap was as follows: purge 6 min, dry purge 7 min, desorb 4 min at 250 °C, and bake 20 min at 270 °C. Ultrahigh purity helium was used as the purge gas. All samples were obtained in a 5 mL purge and trap syringe (Hamilton). Fluorobenzene was used as the internal standard. The purge and trap/GC-MS system was calibrated daily.

In the second method (method II), aqueous samples (1 mL) were extracted in 5 mL of pentane and analyzed by direct injection (1 μL) with a GC (Hewlett-Packard) equipped with an electron capture detector (ECD) and a VOCOL capillary column (60 m × 0.75 mm i.d., 1.5 μm film; Supelco). The oven temperature was isothermal (100 °C), the detector temperature was 300 °C, and the injection port temperature was 200 °C. Helium (8 mL/min) was used as the carrier gas, and nitrogen (56 mL/min) was used as the auxiliary and anode purge gas. Standards were prepared by adding known volumes of methanol containing PCE and TCE to vials containing 5 mL of pentane and 1 mL of deionized water. The extraction efficiencies of PCE and TCE were determined to be 92.2 ± 10% and 95 ± 7%, respectively. Nominal detection limits for PCE and TCE were 12 and 59 μg/L, respectively.

Headspace analysis was used to quantify VC, ethene, and propane. Headspace samples (100 μL) were injected into a GC (Hewlett-Packard) equipped with a flame ionization detector and a packed column (6 ft × $^1/_8$ in o.d.) containing 60/80 Carbopack B/1% SP-1000 (Supelco). The oven program was as follows: 40 °C, hold 2 min, 20 °C/min to 150 °C, no hold, 10 °C/min to 200 °C, hold 10 min. The detector and injection port temperatures were 275 and 200 °C, respectively. The gas flow rates were 12 mL/min helium, 40 mL/min H_2, and 460 mL/min zero air. Standards were prepared in serum bottles (60 mL) closed with aluminum crimp caps and Teflon-lined butyl rubber stoppers. The liquid-to-gas ratio in the serum bottles was consistent with the liquid-to-gas ratio in the systems being evaluated. Known volumes of VC, ethene, and propane were added to the serum bottles using gastight, locking syringes (Dynatech). Gas standards were kept inverted to minimize loss through the septum.

Analyses of methane and H_2 were conducted by direct injection of headspace samples (100 μL) into a GC (EG&G Chandler Engineering) equipped with a thermal conductivity detector (TCD) and a molecular sieve 5A column (6 ft × $^1/_8$ in o.d., 8100 mesh, EG&G Chandler Engineering). The oven was operated isothermally at 102 °C. Nitrogen was the carrier gas, and the flow rate was 30 mL/min. Standards were prepared by volumetric dilutions of certified gas standards.

The pH of cultures was routinely checked with a pH meter and probe (Accumet). The pH meter was calibrated daily using a certified buffer solution of pH 7 (Fisher Scientific).

Culture Enrichment. Two PCE-dechlorinating methanogenic mixed cultures were developed and used in further studies. One was enriched on methanol as its primary electron donor and is referred to as the methanol/PCE enrichment culture. The other culture was derived from aquifer solids, referred to as the aquifer-solids-derived suspended growth enrichment culture, and fed both lactate and H_2 as its primary electron donors. The inoculum for the methanol/PCE enrichment culture (a gift from Shell Development, Westhollow Research Center, Houston, TX) was an anaerobic granular sludge obtained from an upflow sludge blanket reactor used for the treatment of wastewater "generated" during the synthesis of polyester with no previous exposure to chlorinated ethenes (water is a product of the reaction between monomeric subunits of polyester and is condensed with ethylene glycol as a wastewater, thus, chlorinated solvents were never introduced into the system). The aquifer solids used in the aquifer-solids-derived suspended growth enrichment culture were obtained from a chlorinated ethene contaminated site in Texas. These samples were obtained through Groundwater Services, Inc., Houston, TX. The following section describes how both cultures were developed and maintained.

For the methanol/PCE enrichment culture, anaerobic granular sludge was packed into an airtight, glass column (8.1 cm in diameter, 35 cm long, empty bed volume of 1800 mL) fitted with three sampling ports at 5, 17, and 24 cm from the base. Nutrient medium (without $Na_2S{\cdot}9H_2O$, $FeCl_2{\cdot}4H_2O$, and resazurin) was continuously delivered to the base (2 mL/min) via a peristaltic pump (Cole Parmer). The resulting empty bed hydraulic retention time was 15 h. A syringe pump (Harvard Apparatus) continuously dispensed a methanol/PCE solution to the bulk medium flow near the base of the column. Influent PCE concentrations, reported after the mixing of the methanol/PCE solution with the nutrient medium, were incrementally increased from an initial concentration of 0.5 mg/L (2.8 μM) to 86 mg/L (483 μM) as dechlorination activity increased. During days 357−455, PCE was replaced by an equimolar amount of TCE (483 μM) to confirm TCE dechlorination. After this time, the influent chlorinated ethene was changed back to PCE. The concentration of methanol fed to the column (after mixing with medium) was 6.5 mM until the influent PCE concentration was increased to 86 mg/L and was then raised to 52 mM. To avoid adsorption losses, inert tubing (i.e., stainless steel and

glass) was used from the point of PCE addition to the column effluent port.

Column effluent exited the column through glass tubing and passed through a series of inverted Erlenmeyer flasks in route to a waste container. Liquid effluent samples were taken directly from the top of the column, before entering the first flask, and were monitored for the presence of PCE and TCE using method II. Influent PCE and TCE concentrations were also quantified by this method until the concentrations became too high to measure accurately with an ECD (or by purge and trap/GC-MS system). Beyond that point, the reported influent concentrations were calculated values. Gas produced by the system was trapped in the first flask, which was fitted with two sidearms capped with 20 mm Teflon-lined butyl rubber septa (Supelco) and aluminum crimp caps (Wheaton). Periodically, the gas was sampled for headspace analysis (i.e., DCE isomers, VC, and ethene) using a gastight, locking syringe.

The aquifer-solids-derived suspended growth enrichment culture was maintained in a thick walled, glass bottle (1.1 L) modified with a sidearm and septum. In an anaerobic glovebox (100% N_2), 800 g of aquifer solids was added to the bottle and diluted with 280 mL of nutrient medium. The bottle was then sealed with a heavy rubber stopper, removed from the glovebox, and purged with H_2/CO_2. On a daily basis, H_2/CO_2 was added as needed using a 60 mL syringe. Every 2 days, 20 mL of culture was removed, and 20 mL of fresh nutrient medium was added (hydraulic retention time was 58 days) containing 4.6 mM lactate and sodium bicarbonate as needed. PCE was added every 2 days as PCE_{aq}. The initial PCE addition was 0.5 mg/L, but was gradually raised to 4.3 mg/L as the culture demonstrated the ability to dechlorinate higher concentrations. Glass tubing, which extended through the stopper, was used for medium removal and addition and for liquid sampling. Every fourth day, the culture was purged with H_2/CO_2 to remove dechlorination products. This culture was operated for 126 days, after which it was used for other experiments. The culture was maintained at room temperature (ca. 25 °C) and was continuously stirred with a magnetic stirrer (Fisher Scientific). Aquifer solids were allowed to settle prior to use as an inoculum source in other systems.

Comparison of Electron Donors: Recycle Column Experiments. The effects of various electron donors on the extent and rate of PCE dechlorination were investigated in recycle column experiments. Six glass columns were assembled, each having an inner diameter of 1.5 cm and a length of 16.7 cm. The empty bed volume was 27 mL, but was reduced to 12 mL by the addition of 3 mm glass beads (Fisher Scientific). The columns were operated in an upflow mode, with contents of the system being continuously recirculated through the column. Column effluent was circulated to a medium reservoir then pumped back to the base of the column via a peristaltic pump. Nutrient medium, vigorously mixed via a magnetic stirrer, was pumped through the column at a flow rate of 2 mL/min. The medium reservoir consisted of an Erlenmeyer flask (250 mL) modified with a sidearm and septum. The medium reservoir was sealed at the top with a rubber stopper and silicon sealant. A syringe port connected to the recycle line via a two-way valve (Swagelock) allowed access to the contents of the medium reservoir and was used for liquid sampling. All of the fittings (Swagelock) and tubing, except the piece of Viton (Cole Parmer) tubing used in the peristaltic pump, were composed of stainless steel. The columns were maintained at 25 °C.

Two control studies were performed in the recycle columns before inoculation to ensure that losses of chlorinated ethenes and nonchlorinated end products would not occur due to design or construction materials. The most highly sorptive analyte, PCE, and the analyte with the highest Henry's constant, ethene, were added to a recycle column along with deionized water (60 mL). Sorptive losses of PCE were quantified over a 4 day period. The initial PCE concentration was 1.61 mg/L [number of observations (n) = 1], and the average concentration (n = 3) over the next 96 h was 1.51 mg/L (standard deviation = 0.24). The percent recovery of ethene in the recycle column after 24 h was determined to be 102% (n = 3), indicating that the recycle column system was not subject to volatile losses.

Three columns were inoculated with 12 mL of methanol/PCE enrichment culture, and the other three were inoculated with 6 mL of methanol/PCE enrichment culture and 6 mL of aquifer-solids-derived suspended growth enrichment culture. At the time of inoculation, the methanol/PCE enrichment culture and the aquifer-solids-derived suspended growth enrichment culture had been enriched on PCE for a period of 645 and 111 days, respectively. One column from each inoculum subset was fed methanol, lactate, or H_2 as electron donor. The methanol-, lactate-, and H_2-fed columns containing the methanol/PCE enrichment culture only were named using the suffix "I" (i.e., MeOH-I, Lact-I, and H_2-I, respectively). The three columns containing the methanol/PCE enrichment culture and the aquifer-solids-derived suspended growth enrichment culture were named likewise using the suffix "II" (i.e., MeOH-II, Lact-II, and H_2-II).

Nutrient medium (60 mL) was added to the medium reservoir of all six columns. The headspace in the medium reservoir of the lactate- and methanol-fed columns was purged with N_2/CO_2, while H_2/CO_2 was used to purge the headspace in H_2-fed columns. H_2 (as H_2/CO_2) was added daily as needed in the H_2-fed columns to relieve the negative pressure in the medium reservoir. The average uptake of H_2 in H_2-I and H_2-II was determined during the initial startup of the columns and was found to be 8.5 mequiv/day (130 mL of H_2/CO_2). The average H_2 uptake was checked again 1 month later and was found to be consistent. The methanol- and lactate-fed columns were fed 17 mequiv of methanol and lactate, respectively, every 2 days to ensure that electron donor additions were equivalent in all systems [equivalent calculations were based on the conversion of electron donor to CO_2, (*26*)]. Yeast extract was not considered a source of reducing equivalents since control studies demonstrated that, in systems where only yeast extract was added (i.e., no lactate, methanol, or H_2), dechlorination activity was indistinguishable from systems where no electron donor was added (i.e., no yeast extract, lactate, methanol, or H_2). H_2 consumption was observed to be consistent over the next 472 days, except for occasions when the pH dropped below 6.8. On these days, the amount of H_2 added was less than 8.5 mequiv/day. The average daily consumption from days 0 to 472 was 4.7 and 5.3 mequiv for H_2-I and H_2-II, respectively. Every day, the positive pressure in the methanol- and lactate-fed columns was measured and released.

PCE was added to the recycle column systems as PCE_{aq} every 4 days. The target PCE concentration in the columns was 5 mg/L (approximately 6.7 μmol or 0.054 mequiv), resulting in an electron donor to PCE ratio (ED:PCE) of 630:1 mequiv. The initial PCE concentration was not able to be measured in the column due to the rapid dechlorination by the cultures. Therefore, at every PCE addition, the initial PCE concentration was estimated using a set of four serum bottles, each containing deionized water at the same gas-to-liquid ratio as the recycle column system. In two serum bottles, a corresponding volume of PCE_{aq} was added to achieve the equivalent PCE concentration targeted in the column. The remaining serum bottles were maintained as blanks, each amended with the same volume of deionized water as PCE_{aq}. All four bottles were stored inverted for a period of 24 h. Aqueous samples (1 mL) from each bottle were analyzed by method I. After correcting for background

PCE found in the blanks, the PCE concentrations in the other two serum bottles were averaged to reflect the initial PCE concentrations in the columns.

On a daily basis, medium was removed (1 mL) and analyzed for PCE, TCE, and DCE isomers using method I. Headspace samples were routinely analyzed for VC and ethene, and pH was checked periodically (if needed, the pH was increased to 6.8−7.2 by adding $NaHCO_3$). Every fourth day, the headspace was purged with either N_2/CO_2 (methanol- and lactate-fed systems) or H_2/CO_2 (H_2-fed systems), nutrient medium was completely removed via the syringe port, and fresh nutrient medium was added. Propane (290 μL, 1 atm) was used as an internal standard to evaluate volatile losses from the system. The cultures were enriched on their respective ED and PCE for nearly 100 days before detailed experiments commenced.

Evaluation of H_2 as the Final Electron Donor for PCE Dechlorination. After 474 days of operation, an experiment was performed to evaluate whether H_2 could serve as the primary electron donor for dechlorination in the methanol- and lactate-fed columns. All four columns were operated normally until gas production ceased, nutrient medium was replaced, and the medium reservoirs were flushed with H_2/CO_2. The columns were then spiked with PCE_{aq} to a concentration of 5 mg/L, and operated in the same manner identical to H_2-I and H_2-II.

PCE Dechlorination at High H_2 Partial Pressures. An experiment was performed to compare the flux of H_2 between methanogens and dechlorinators in homogeneous batch systems at high H_2 partial pressures. For this experiment, the H_2-I column was sacrificed (on day 472) and used as inoculum for batch cultures.

In an anaerobic chamber (100% N_2), the column contents were placed in an Erlenmeyer flask (flask had syringe port and sampling port) and diluted with anaerobic medium (60 mL). The flask was sealed from the atmosphere and purged with H_2/CO_2. The culture was stored on a stir plate and homogenized. For a period of 1 week, H_2 was fed daily and PCE was added on a 4 day interval (analogous to recycle columns). After 8 days, aliquots (5 mL) of the homogenized culture were transferred to duplicate serum bottles (120 mL) containing anaerobic medium (55 mL) sealed with Teflon-lined butyl rubber septa and aluminum crimp caps. Headspaces were flushed with H_2/CO_2 to remove any residual PCE or reduced end products. Propane (50 μL, 1 atm) was added as an internal standard to assess any volatile losses, and cultures were spiked with PCE_{aq} to a concentration of 5 mg/L.

Fifteen minutes prior to headspace and aqueous sampling, H_2/CO_2 was added as needed to maintain an atmospheric pressure of 1 atm within the bottles. Cultures were sampled approximately every 3 h, and the liquid and gas samples were analyzed for methane, H_2, PCE, and TCE (method I), and other dechlorination end products. After 14 h, the cultures were sacrificed to compare biomass levels. Cultures were filtered with 47 mm glass fiber filters (Gelman Scientific), and the filtered residue was dried to a constant weight at 120 °C.

Results

Methanol/PCE Enrichment Culture. Figure 1 shows the influent and effluent PCE and TCE concentrations from the methanol/PCE enrichment culture column during the first 100 days of operation. Although this culture had not previously been exposed to PCE, dechlorination commenced within 24 h as indicated by the production of TCE. TCE concentrations increased for the first 20 days, as effluent PCE concentrations dropped, then TCE concentrations decreased rapidly to approximately 0.5 μM. Effluent concentrations fluctuated for the next 40 days, but on day 62,

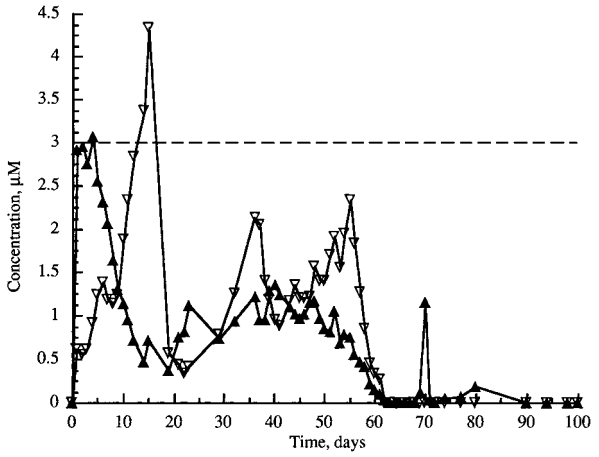

FIGURE 1. Results from the first 100 days of operation of the methanol/PCE enrichment culture. Values shown represent the effluent concentrations of PCE (▲) and TCE (▽) with a target influent PCE concentration of 2.97 μM (0.5 mg/L) shown by the dashed line.

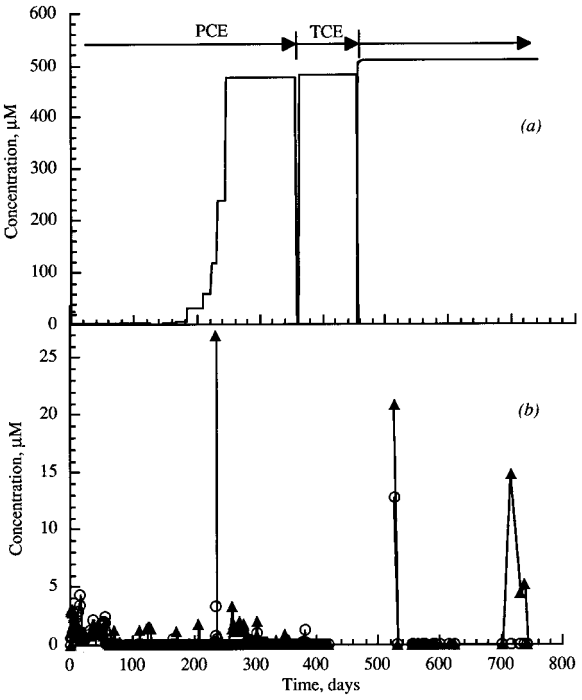

FIGURE 2. Influent (a) and effluent (b) concentrations of PCE (▲) and TCE (○) from the methanol/PCE enrichment culture during period of step increases in PCE influent concentration.

both PCE and TCE concentrations dropped below detectable limits. For the remaining 38 days, effluent concentrations of PCE and TCE were typically below detection limits. Elevated PCE effluent concentration on day 70 was caused by a failure in the peristaltic pump system that caused PCE to flow into the column undiluted.

The influent PCE concentration was maintained at 2.97 μM (0.5 mg/L aqueous concentration) to day 165, after which it was incrementally increased. Changes in PCE influent concentration and resulting effluent PCE and TCE concentrations for the culture are shown in Figure 2, panels a and b, respectively. Influent PCE concentration was increased by a factor of more than 160 within a period of 81 days with no effect on the effluent PCE and TCE concentrations. Occasional increases in PCE effluent concentrations above 5 μM corresponded to days in which problems with the peristaltic pump system occurred.

TABLE 1. Calculated[a] PCE Half-Lives ($t_{1/2}$ in Hours) for Recycle Columns

	day 98			day 106			day 120			day 129			day 474[c]		
column	$t_{1/2}$	r^2	*n*	$t_{1/2}$	r^2	*n*	$t_{1/2}$	r^2	*n*	$t_{1/2}$	r^2	*n*	$t_{1/2}$	r^2	*n*
MeOH-I	12.3	0.89	4	12.5	0.96	3	7.9	0.99	3	4.4	0.99	3	1.8	0.84	3
MeOH-II	14.8	0.96	5	18.7	0.84	4	14.0	0.90	3	9.3	0.98	3	2.1	0.73	3
Lact-I	17.3	0.95	5	14.4	0.84	3	9.4	0.97	3	4.8	*b*	*b*	1.7	0.67	3
Lact-II	13.8	0.91	4	10.9	0.94	3	8.7	0.96	3	6.6	0.95	3	1.9	0.66	3
H_2-I	5.6	0.98	3	8.2	0.98	3	7.5	0.96	3	3.5	*b*	*b*	*c*	*c*	*c*
H_2-II	16.1	0.98	5	12.5	0.97	4	8.6	0.98	3	7.8	0.9	3	*c*	*c*	*c*
avg $t_{1/2}$	13.3			12.9			9.3			6.0			1.9		
STDEV	±4.2			±3.5			± 2.4			± 2.2			±0.2		

[a] Observed rates of PCE disappearance were modeled using first-order kinetics. Resulting kinetic coefficients were used to calculate the half-life of PCE in the systems. The Pearson's r^2 value obtained from the linear regression and the number of data points (*n*) used in the regression are listed for each $t_{1/2}$ value. [b] Rates of PCE disappearance in these cultures were so rapid that by the third sampling, PCE concentrations were below the detection limit. In these instances, data analysis was performed using only two data points. [c] On day 474, experiments were performed in lactate- and methanol-fed columns with H_2 added as electron donor.

During days 358−456, TCE was substituted for PCE in equimolar amounts (483 μM) to confirm TCE dechlorination by the methanol/PCE enrichment culture. Effluent TCE concentrations during this time were below detectable limits. On day 457, the influent contaminant was returned to PCE (519 μM). The influent concentration remained at this level, as further increases in concentration were limited by the aqueous solubility of PCE.

Periodic headspace sampling of gas trapped in the first Erlenmeyer flask showed that *cis*-DCE and VC were the major reduced end products being formed. Ethene was never observed in appreciable quantities. Because the column was maintained as an open system, completing a mass balance on all products from this system was not attempted.

Rate and Extent of PCE Dechlorination in Recycle Columns. PCE dechlorination was observed in all systems throughout the experimental period (ca. 474 days), although its rate of disappearance and the distributions of dechlorination products changed during the study. Columns were monitored throughout the experimental period to ensure dechlorination activity and to evaluate dechlorination products. Occasionally, studies were conducted to obtain rate information and quantify product distributions. Results of these more extensive monitoring studies are presented in Tables 1 and 2.

Table 1 presents the observed PCE half-lives ($t_{1/2}$) in all columns throughout the experimental period. Half-lives were calculated from observed first-order rate coefficients. Pearson's r^2 values obtained from the linearization of PCE disappearance data are shown along with the number of observations (*n*) used for the analysis. In early experiments, PCE disappearance was slower, allowing for more data to be used in approximating $t_{1/2}$. Over time, the observed PCE disappearance rates increased such that PCE concentrations dropped below detectable limits within several hours after PCE addition. In these experiments, there are three or fewer data points. Half-lives are not shown for day 427 because PCE concentrations decreased to below detectable levels before samples were taken, corresponding to an estimated $t_{1/2}$ value of 2 h or less.

The distribution of PCE and reduced end products recovered in these experiments after 4 days of incubation are presented in Table 2. On day 98, the primary products of dechlorination were *cis*-DCE and VC. In columns inoculated from the methanol/PCE enrichment culture column only (e.g., MeOH-I, H_2-I, and Lact-I), *cis*-DCE was the primary reduced end product in systems fed methanol and H_2, while VC was the primary reduced end product in Lact-I. On day 98, VC was observed in all columns inoculated with both enrichment cultures, and only MeOH-II had measurable levels of *cis*-DCE. By day 120, VC was observed in all columns, without detectable levels of ethene, and then by day 129, ethene was produced in measurable quantities in all six systems. By day 427, VC and ethene were the only products observed in all columns. Ethene accounted for approximately 12−22% (on a molar basis) of the initial PCE added in each column.

TABLE 2. Percent Molar Distribution of Dechlorination End Products in Recycle Columns

column	ethenes recovered[a]	day 98	day 106	day 120	day 129	day 427	day 474[b]
MeOH-I	% PCE	0.0	2.2	2.4	0.1	0.0	0.0
	% TCE	0.0	0.0	0.8	0.3	0.0	0.0
	% *cis*-DCE	100.0	97.8	21.5	11.0	0.0	0.0
	%VC	0.0	0.0	75.5	82.6	77.6	71.8
	% ethene	0.0	0.0	0.0	6.0	22.4	28.2
MeOH-II	% PCE	19.3	59.2	18.3	4.1	0.0	0.0
	% TCE	0.0	0.0	2.9	0.8	0.0	0.0
	% *cis*-DCE	44.4	6.3	40.2	19.3	0.0	0.0
	%VC	36.3	34.5	38.6	70.3	86.2	88.9
	% ethene	0.0	0.0	0.0	5.5	13.8	11.1
Lact-I	% PCE	26.7	3.3	4.3	0.0	0.0	0.0
	% TCE	3.8	0.0	1.5	0.0	0.0	0.0
	% *cis*-DCE	0.0	0.0	8.7	0.0	0.0	0.0
	%VC	69.5	96.7	85.5	95.2	77.8	74.5
	% ethene	0.0	0.0	0.0	4.9	22.2	25.55
Lact-II	% PCE	4.9	5.7	2.8	0.9	0.0	0.0
	% TCE	0.0	0.0	0.5	0.5	0.0	0.0
	% *cis*-DCE	0.0	0.0	3.2	4.9	0.0	0.0
	%VC	95.1	94.3	93.5	84.5	80.5	74.8
	% ethene	0.0	0.0	0.0	9.2	19.5	25.2
H_2-I	% PCE	0.0	0.0	1.2	0.0	0.0	*b*
	% TCE	0.0	0.0	0.3	0.2	0.0	*b*
	% *cis*-DCE	95.9	100.0	52.0	8.7	0.0	*b*
	%VC	4.1	0.0	46.6	86.7	80.2	*b*
	% ethene	0.0	0.0	0.00	4.4	19.8	*b*
H_2-II	% PCE	5.9	3.1	1.8	1.2	0.0	*b*
	% TCE	0.0	0.0	0.1	0.2	0.0	*b*
	% *cis*-DCE	0.0	0.0	5.6	3.2	0.0	*b*
	%VC	94.1	96.9	92.5	83.1	75.9	*b*
	% ethene	0.0	0.00	0.00	12.5	24.1	*b*

[a] Percent molar distribution of PCE, TCE, *cis*-DCE, VC, or ethene. The average percent molar recovery and standard deviation for the experiments reported above were MeOH-I = 74.1% ± 15.9; MeOH-II = 66.4% ± 22.2; Lact-I = 89.5% ± 18.9; Lact-II = 95.1% ± 6.4; H_2-I = 82.8%, ± 20.0; H_2-II = 105.9% ± 19.9. [b] On day 474, experiments were performed in lactate- and methanol-fed columns with H_2 added as electron donor.

H_2 Addition to Methanol-Enriched and Lactate-Enriched Recycle Columns. On day 474 of operation, H_2 was added to the lactate- and methanol-fed recycle columns to evaluate the potential for H_2 to serve as the electron donor for dechlorination in these cultures. Except for the change in electron donor added, studies were performed identically to those done previously and results are presented in Tables 1 and 2. In all four columns, PCE dechlorination occurred as

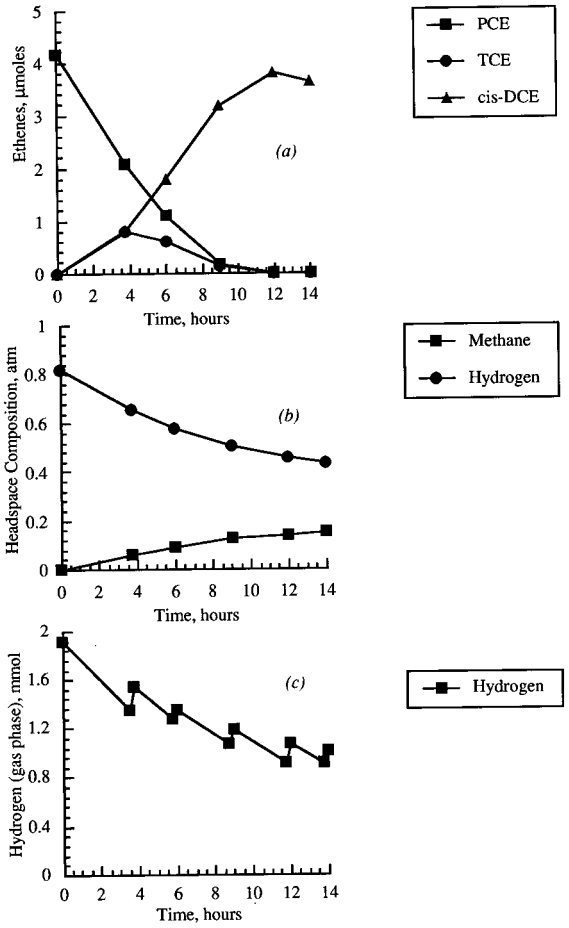

FIGURE 3. Results of batch experiments of PCE dechlorination in batch studies using the H_2-I column inoculum. (a) PCE (■) transformation and accumulation of TCE (●) and *cis*-DCE (▲). (b) Concentration of hydrogen (●) after supplemental gas additions and corresponding methane concentrations (■) during dechlorination study. (c) Range of hydrogen concentrations in the gas-phase (■) throughout the period of study. All points represent the average of duplicate systems.

observed previously. The observed half-lives of PCE in these cultures were similar to previous experiments in which methanol or lactate was used (Table 1)—ranging from 1.7 to 2.1 h—and the distribution of end products remained relatively unchanged as compared to previous results with lactate or methanol (Table 2).

Dechlorination at High H_2 Partial Pressures. Figure 3 presents results of batch experiments where PCE dechlorination, H_2 uptake, and methane production were monitored using a diluted inoculum from the homogenized H_2-I column. All results are shown as the average between two duplicates, which behaved similarly (dry biomass in duplicate systems were 160.4 and 155.6 mg, respectively).

As expected, the observed PCE dechlorination rate (shown in Figure 3a) in these cultures was slower than in recycle columns due to the dilution of biomass. Under these conditions, TCE was observed as an intermediate, and 91% of the PCE was recovered as *cis*-DCE after 12 h. VC and ethene were not observed in this time frame.

H_2 utilization and methane production are shown in Figure 3b. After 12 h, the partial pressure of H_2 dropped from 0.8 to 0.46 atm. Methane production was found to occur simultaneously with PCE dechlorination, and after 12 h, the methane partial pressure in the cultures was 0.15 atm. To maintain atmospheric pressure within the systems, H_2 was added as needed 15 min prior to headspace and liquid sampling. The amount of H_2 added was used to calculate the amount of H_2 consumed between sampling. Changes in the mass of H_2 in the headspace that occurred between sampling is shown in Figure 3c. Using Henry's Law constant for H_2, 7.06×10^4 atm/mol fraction at 25 °C, changes in the aqueous H_2 concentration were calculated. Throughout the duration of the experiment, the calculated aqueous H_2 concentration ranged from 645 to 306 μM.

Discussion

The rapid onset of PCE dechlorination (within 24 h after PCE addition) observed in the methanol/PCE enrichment culture was not anticipated since the culture had no prior history of chlorinated ethene exposure. It is likely that this activity was due to cometabolic processes. Over time, the culture exhibited an increase in the extent of dechlorination observed. For a period of approximately 2 years, the primary dechlorination end product in the methanol/PCE enrichment culture was *cis*-DCE, with very little or no VC or ethene being detected. After this time, VC was observed followed by the production of small amounts of ethene. Production of end products not associated with the process of reductive dechlorination but reported in other cases, e.g., carbon dioxide (*19*, *20*) and ethane (*18*), was not investigated, although the formation of these compounds cannot be ruled out. Dechlorination was sustained in the methanol/PCE enrichment culture throughout the experimental period, and dechlorination rates were rapid enough to accept large increases in the influent PCE concentration and maintain PCE/TCE concentrations below detection limits in the effluent. Throughout this time, daily gas production levels fluctuated from near zero (it never ceased indefinitely) to greater than 500 mL. Interestingly, no changes in dechlorination were observed during periods without gas production.

Dechlorination was observed to commence immediately upon PCE addition in the aquifer-solids-derived suspended growth enrichment culture. Like the methanol/PCE enrichment culture, it had a significant population of methanogens as indicated by the production of methane. Observed dechlorination end products included TCE, *cis*-DCE, and VC.

At the time of the recycle column inoculation, the methanol/PCE enrichment culture was not producing detectable levels of VC or ethene. Thus, the aquifer-solids-derived suspended growth enrichment culture was added as an additional inoculum to the second set of recycle columns since it was capable of this higher degree of dechlorination. Although VC production did commence earlier in systems inoculated with both cultures, over the duration of the experiments all of the columns developed the ability to dechlorinate beyond *cis*-DCE with similar levels of VC and ethene formation. (Six months after the inoculation of the recycle columns, the methanol/PCE enrichment culture also began producing large amounts of vinyl chloride, indicating that the culture was still undergoing the process of acclimation.) Rates of PCE disappearance were initially higher in the recycle columns containing only the methanol/PCE enrichment culture, but by day 474, the half-life of PCE in all six columns was approximately 2 h. Thus, inoculum was not found to be particularly influential in either the extent or the rate of PCE dechlorination.

Methanol (*12*–*15*) and fatty acids (*12*, *17*–*19*, *23*, *27*–*30*) have been shown to sustain dechlorination in various types of experimental systems. Results from several investigations have implied that H_2, produced by the metabolism of fermentative and acetogenic substrates, served as the final electron donor for dechlorination (*14*, *23*). This study represents a long term, comparative analysis of electron donors for sustaining PCE dechlorination in which H_2 was fed directly. Results from recycle column experiments

demonstrated that electron donor did not influence the extent or the rate of dechlorination. The H_2-fed column, H_2-I, had smaller half-lives for PCE initially, but over time, the half-lives in all six columns converged to approximately 2 h. The extent of dechlorination achieved was not dependent upon electron donor added either, since PCE was recovered as approximately 80% VC and 20% ethene with all three electron donors on day 427, with the MeOH-II column lagging slightly behind in ethene production. Thus, with time, rates of PCE disappearance increased collectively, and the extent of dechlorination progressed to favor less chlorinated ethenes with all three electron donors.

The average percent recovery of PCE and dechlorination products for all experiments in the recycle columns is presented at the bottom of Table 2. The percent of mass recovered for individual four day experiments varied between columns and throughout the study. In some of the early experiments with methanol-fed columns (days 98 and 106), only 30−50% recovery was obtained. Analytical problems were not believed to be attributed to the low mass recovery since all of the mass was recovered in Lact-II and H_2-II on the same days. Although the reason for this occurrence is not known, it is possible that these particular cultures may have been dechlorinating PCE to a product not analyzed (e.g., CO_2, ethane). The percent of mass recovered was also affected by the loss of volatiles during gas release in methanol- and lactate-fed cultures. Lastly, mass balances were observed to get better over time, i.e., recovery would increase significantly from day 2 to day 4, possibly due to uptake and release by biomass in the system.

The inability of these cultures to completely dechlorinate all of the PCE fed to ethene may have been indirectly caused by the design of the recycle columns. Possibly, the large headspace volume in the medium reservoir may have led to contact limitations between VC and the culture in the column. The Henry's Law constant for VC is relatively high, thus, the majority of the VC mass remained in the headspace and only that which was dissolved in the aqueous phase came into contact with the culture. This phenomenon may have led to the slow development of a culture capable of VC dechlorination.

Data observed in the recycle columns supports the results obtained by Fennell et al. (*24*), who investigated several electron donors as potential H_2 donors for PCE dechlorination. The authors concluded that over long-term experiments, dechlorination could be equally sustained and maintained regardless of electron donor fed. In their research, however, the electron donors (ethanol, butyric acid, lactic acid, and propionic acid) were added in equivalent amounts according to the number of H_2 equivalents that would be released upon fermentation, whereas in the research presented here, electron equivalent calculations were based upon the complete oxidation of each electron donor.

The role of H_2 as the final electron donor for PCE dechlorination was investigated by adding H_2 to the methanol- and lactate-fed recycle columns. The extent and rate of PCE dechlorination were relatively unchanged, indicating that the dechlorinating organisms in these cultures were capable of utilizing H_2 for energy purposes. The average uptake of H_2 in these four columns (1.9 mequiv/day MeOH-I; 2.2 mequiv/day MeOH-II; 2.3 mequiv/day Lact-I; and 2.5 mequiv/day Lact-II) during this experiment was considerably less than that of the recycle columns H_2-I and H_2-II, although it continued to increase daily. The low but increasing H_2 uptake observed may have been attributed to an increase in the growth of H_2-utilizing, nondechlorinating microorganisms stimulated by the increase in hydrogen availability. As a result, these data indirectly support the role of H_2 as the final electron donor for dechlorinators in systems fed organic substrates, although it is possible that when fed methanol or lactate, these cultures utilized an electron donor other than H_2.

To date, no attempts have been made to isolate a PCE-respiring bacterium from cultures used in these studies, but the rapid rates of dechlorination and the products observed are similar to mixed cultures from which PCE-respiring organisms have been isolated (*15*). In mixed culture systems exhibiting high dechlorination rates, others have reported the potential for methanogens to out-compete dechlorinating bacteria for H_2 under conditions of high H_2 partial pressures (*17*, *22*). In recycle column systems fed H_2, this was not observed (e.g., rate and extent of dechlorination increased with time). A possible reason that this competition was not observed in H_2-fed column systems—as compared to suspended growth systems—is the spatial heterogeneity of H_2 and/or PCE in column systems that may have resulted in the spatial stratification of individual communities and organisms. For example, growth of dechlorinating organisms could have been favored at the top of the column, where aqueous H_2 concentrations would have been lower than at the base, or the concentrations of PCE fed could have been inhibitory to methanogens at the base of the column yielding the inverse. Thus, the purpose for homogenizing one of the H_2-fed column systems, and comparing the flux of H_2 equivalents to dechlorination and methanogenesis at high H_2 partial pressure with results from column studies, was to evaluate this hypothesis.

During the experiment, dechlorination and methanogenesis occurred simultaneously, and neither process appeared to be inhibited by the other. In the batch systems, it was calculated that less than 1% (0.4%) of the H_2 equivalents consumed (uptake as measured by GC) was utilized for dechlorination, and that 69% of the H_2 equivalents was accounted for by methane production. The remaining 30% was presumably shunted to cell growth and/or acetogenesis. The small percentage of H_2 equivalents being used for dechlorination is consistent with results from the H_2-I column, in which it was determined that 0.36% of H_2 equivalents added were being utilized for dechlorination (based on cumulative moles of H_2 and PCE added). The electron donor to PCE ratio in batch systems was less than that in the recycle column system (114 mequiv H_2:1 mequiv PCE—due to the smaller headspace), but resulting aqueous-phase H_2 concentrations were similar in both cases. The influence of H_2-utilizing acetogens on methane production or dechlorination was not evaluated.

For a period exceeding 1 year, it was demonstrated that PCE dechlorination in mixed cultures could be sustained equally using equivalent amounts of methanol, lactate, or H_2. Furthermore, it was observed that dechlorination was not impacted by competition for electron donor at high H_2 partial pressures by other H_2-utilizing microorganisms, particularly methanogens. These results imply that issues such as cost and method of delivery may dictate the electron donor (i.e., fermentable substrates vs H_2) selected for stimulation of anaerobic in situ bioremediation systems.

Acknowledgments

This research was supported by Rice University, Houston, TX, through the Energy and Environmental Systems Institute (EESi) and an Eleanor and Mills Bennett Fellowship. The authors would like to thank Shell Development, Westhollow Research Center, and Groundwater Services, Inc., for donating the anaerobic granular sludge and aquifer solids, respectively, used in this research.

Literature Cited

(1) Fathepure, B. Z.; Nengu, J. P.; Boyd, S. A. *Appl. Environ. Microbiol.* **1987**, *53* (*11*), 2671−2674.

(2) Fathepure, B. Z.; Boyd, S. A. *Appl. Environ. Microbiol.* **1988**, *54* (12), 2976−2980.
(3) Fathepure, B. Z.; Boyd, S. A. *FEMS Microbiol. Lett.* **1988**, *49*, 149−156.
(4) Holliger, C.; Schraa, G.; Stams, A. J. M.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1993**, *59* (9), 2991−2997.
(5) Holliger, C.; Schumacher, W. *Antonie van Leeuwenhoek* **1994**, *66*, 239−246.
(6) Neumann, A.; Scholz-Muramatsu, H.; Diekert, G. *Arch. Microbiol.* **1994**, *162*, 295−301.
(7) Scholz-Muramatsu, H.; Neumann, A.; Messmer, M.; Moore, E.; Diekert, G. *Arch. Microbiol.* **1995**, *163*, 48−56.
(8) Sharma, P. K.; McCarty, P. L. *Appl. Environ. Microbiol.* **1996**, *62* (3), 761−765.
(9) Krumholz, L. R.; Sharp, R.; Fishbain, S. S. *Appl. Environ. Microbiol.* **1996**, *62* (11), 4108−4113.
(10) Gerritse, J.; Renard, V.; Gomes, T. M. P.; Lawson, P. A.; Collins, M. D.; Gottschal, J. C. *Arch. Microbiol.* **1996**, *165*, 132−140.
(11) Maymó-Gatell, X.; Chien, Y.-t.; Gossett, J. M.; Zinder, S. H. *Science* **1997**, *276*, 1568−1571.
(12) Freedman, D. L.; Gossett, J. M. *Appl. Environ. Microbiol.* **1989**, *55*, 2144−2151.
(13) DiStefano, T. D.; Gossett, J. M.; Zinder, S. F. *Appl. Environ. Microbiol.* **1991**, *57* (8), 2287−2292.
(14) DiStefano, T. D.; Gossett, J. M.; Zinder, S. F. *Appl. Environ. Microbiol.* **1992**, *58* (11), 3622−3629.
(15) Tandoi, V.; DiStefano, T. D.; Bowser, P. A.; Gossett, J. M.; Zinder, S. H. *Environ. Sci. Technol.* **1994**, *28* (5), 973−979.
(16) Maymó-Gatell, X.; Tandoi, V.; Gossett, J. M.; Zinder, S. H. *Appl. Environ. Microbiol.* **1995**, *61* (11), 3928−3933.
(17) Ballapragada, B. S.; Stensel, H. D.; Puhakka, J. A.; Ferguson, J. F. *Environ. Sci. Technol.* **1997**, *31* (6), 1728−1734.
(18) de Bruin, W. P.; Kottermann, M. J. J.; Posthumus, M. A.; Schraa, G.; Zehnder, A. J. B. *Appl. Environ. Microbiol.* **1992**, *58* (6), 1996−2000.
(19) Vogel, T. M.; McCarty, P. L. *Appl. Environ. Microbiol.* **1985**, *49* (5), 1080−1083.
(20) Bradley, P. M.; Chapelle, F. H. *Environ. Sci. Technol.* **1997**, *31* (9), 2692−2696.
(21) McCarty, P. L. *Science* **1997**, *276*, 1521−1522.
(22) Smatlak, C. R.; Gossett, J. M.; Zinder, S. H. *Environ. Sci. Technol.* **1996**, *30* (9), 2850−2858.
(23) Gibson, S. A.; Sewell, G. W. *Appl. Environ. Microbiol.* **1992**, *58* (4), 1392−1393.
(24) Fennell, D. E.; Gossett, J. M.; Zinder, S. H. *Environ. Sci. Technol.* **1997**, *31* (3), 918−926.
(25) Gossett, J. M. *Environ. Sci. Technol.* **1987**, *21* (2), 202−208.
(26) McCarty, P. L. *Stoichiometry of Biological Reactions*; Presented at the International Conference Toward a Unified Concept of Biological Waste Treatment Design; Atlanta, GA, October 6, 1972.
(27) Bouwer, E. J.; McCarty, P. L. *Biotechnol. Bioeng.* **1985**, *27*, 1564−1571.
(28) Bouwer, E. J.; McCarty, P. L. *Appl. Environ. Microbiol.* **1983**, *45* (4), 1286−1294.
(29) Gibson, S. A.; Roberson, D. S.; Russell, H. H.; Sewell, G. W. *Environ. Toxicol. Chem.* **1994**, *13* (3), 453−460.
(30) Scholz-Muramatsu, H.; Szewyzk, R.; Szewyzk, U.; Gaiser, S. *FEMS Microbiol. Lett.* **1990**, *66*, 81−86.

Received for review November 7, 1997. Revised manuscript received March 27, 1998. Accepted April 1, 1998.

ES970985T