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Single-well, gas-sparging tests for evaluating the in situ aerobic cometabolism of *cis*-1,2-dichloroethene and trichloroethene

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Abstract

This study developed single-well, gas-sparging tests for assessing the feasibility of in situ aerobic cometabolism of trichloroethene (TCE) and *cis*-1,2-dichloroethene (*cis*-DCE) using propane and methane as growth substrates. Tests were performed in groundwater contaminated with TCE (100–400 $\mu g\,l^{-1}$) and *cis*-DCE (20–60 $\mu g\,l^{-1}$). A series of gas-sparging tests was performed by first sparging (''bubbling'') gas mixtures in a well fitted with a ''straddle'' packer and then periodically sampling groundwater from the same well to develop concentration profiles and to estimate transformation rate coefficients. Evidence that gas-sparging of propane (or methane) and oxygen had stimulated organisms expressing a propane (or methane) monooxygenase enzyme system and the capability to transform TCE and *cis*-DCE included: (1) the transformation of sparged ethylene and propylene to their corresponding cometabolic by-products, ethylene oxide and propylene oxide, (2) the transformation of both *cis*-DCE and TCE in the propane-sparged well, (3) the transformation of *cis*-DCE in the methane-sparged well, and (4) the inhibition of ethylene and propylene transformations in the presence of acetylene, a known monooxygenase inactivator. At a well sparged with propane, first-order rate coefficients for propane utilization and ethylene and propylene transformation were similar, ranging from 0.007 to 0.010 h^{-1}. At the well sparged with methane, the propylene first-order transformation rate coefficient was 0.028 h^{-1}, a factor of 1.8 and 1.6 greater than methane and ethylene, respectively. The results demonstrated that gas-sparging tests are a rapid, low-cost means of assessing the potential for the in situ aerobic cometabolism of *cis*-DCE and TCE.

Keywords: Single-well, gas-sparging tests; CAH aerobic cometabolism; Ethylene-oxide; Propylene-oxide; Acetylene inhibition

1. Introduction

In situ aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs) is a promising technology for the remediation of groundwater plumes with contaminant concentrations above the drinking water standards (Semprini, 1997). Several gaseous substrates (including methane, propane, and butane) have been studied and used in practice for stimulating microbial growth capable of cometabolism of CAHs (Semprini, 1997; Alvarez-Cohen and Speitel, 2001; Arp et al., 2001). These substrates and the required oxygen can be added to the subsurface through the use of conventional air sparging technology (Travis and Rosenberg, 1997; Tovanabootr et al., 2001; Connon et al., 2005), but effective application of this technology requires in situ methods for determining the effectiveness of specific substrates for biostimulating indigenous cometabolic activity.

In previous studies, we developed single-well push–pull tests as a tool for detecting and quantifying aerobic cometabolic transformations of CAHs (Kim et al., 2006). In these tests, site groundwater was extracted from the aquifer, amended with growth substrates, reinjected into the aquifer, and then monitored by periodic sampling from the

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same well. Although that approach was successful, it was logistically complicated and time-intensive. For example, to stimulate propane utilization, six successive injections of 500 l of site groundwater containing known concentrations of propane, oxygen, and nitrate were required to stimulate indigenous cometabolic CAH transformation.

In this study, we investigated an alternative test format that involved adding gaseous growth substrates (propane or methane) and gaseous reactive tracers (ethylene or propylene) directly to the aquifer by sparging gas mixtures in the saturated zone using a monitoring well. This approach eliminates the need to repeatedly extract, mix, and inject large volumes of aqueous solutions that were used to stimulate microbial activity in previous studies.

Nontoxic reactive tracers that can be added to subsurface to study the cometabolic process are also of interest. Cometabolism of ethylene and propylene to ethylene oxide and propylene oxide, respectively, by microorganisms expressing monooxygenase enzyme has been reported in laboratory studies (Hou et al., 1983; Stephen and Dalton, 1987) and in field tests (Kim et al., 2004, 2006). Propane monooxygenase has also been shown to initiate transformation of CAHs (Vanderberg and Perry, 1994; Vanderberg et al., 1995). The ability to cometabolize ethylene and propylene to their corresponding oxides has also been observed with CAH-transforming methanotrophic cultures in laboratory studies (Hou et al., 1979; van Hylckama Vlieg et al., 1996). For example, van Hylckama Vlieg et al. (1996) found that both *cis*-1,2-dichloroethene (*cis*-DCE) and trichloroethene (TCE) were transformed to their corresponding epoxide by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase (sMMO). Both ethylene and propylene were also rapidly cometabolized to their respective epoxides (Hou et al., 1979), and Woods and Murrell (1989) and De Bont and Beck (1980) reported that most propane-oxidizing microorganisms cannot grow on ethylene or propylene. In addition, microorganisms utilizing alkenes (e.g., ethylene and propylene) as sole carbon and energy sources express an enzyme, epoxidase, to further metabolize the corresponding epoxides (Ensign, 1996; Allen and Ensign, 1998). Thus, monitoring the transformation of injected ethylene to ethylene oxide or propylene to propylene oxide provides direct evidence of the presence and activity of propane- or methane-utilizing microorganisms with cometabolic transformation abilities, and these same microorganisms should also have the ability to aerobically transform CAHs. Ethylene and propylene have the additional advantage that they are nontoxic and not normally present in ground water at high concentrations and thus are well suited for use in field tests. In addition ethylene and propylene are gaseous substrates and are therefore suitable for introduction into the subsurface using gas-sparging techniques.

The ability to selectively inhibit cometabolic processes is important for confirming that observed transformations are microbially mediated. Acetylene is an irreversible inactivator of methane monooxygenase from *Methylococcus capsulatus* (Bath) (Prior and Dalton, 1985), ammonia monooxygenase from *Nitrosomonas europaea* (Keener and Arp, 1993), butane monooxygenase from butane-grown *Pseudomonas butanovora*, an environmental isolate, CF8 (Hamamura et al., 1999), and propane monooxygenase from propane-grown *Mycobacterium vaccae* JOB5 (Vanderberg and Perry, 1994). Acetylene inhibition has also been observed in studies with mixed cultures grown on methane and propane (Alvarez-Cohen and McCarty, 1991; Tovanabootr and Semprini, 1998). Radiolabelled [^{14}C] acetylene was also used to identify the active site protein of monooxygenase and to evaluate mechanisms of monooxygenase inactivation (Hamamura et al., 1999). In this study, acetylene was used to evaluate the involvement of monooxygenases in propane and methane degradation and transformation of reactive tracers and CAHs. Since acetylene is also a gas under typical field conditions, it is also compatible with our proposed gas-sparging test method and procedures were developed to use acetylene as an inhibitor of monooxygenase activity during in situ testing.

The objective of this study was to develop a series of gas-sparging tests for use in evaluating the site-specific potential of indigenous microbial communities to transform CAHs by aerobic cometabolic pathways. The test series consisted of (1) biostimulation tests to evaluate the ability to stimulate propane- and methane-utilizing bacteria; (2) activity tests to evaluate the abilities of the stimulated microorganisms to utilize growth substrate and transform the reactive tracers, ethylene and propylene, to their respective oxides; and (3) acetylene inhibition tests to evaluate the involvement of monooxygenase in observed growth substrate utilization and reactive tracer transformation. As part of this study we also compared substrate utilization and reactive tracer transformation results from the gas-sparging tests with those from modified liquid injection push–pull tests performed by injecting amended site groundwater. Evidence for *cis*-DCE and TCE cometabolism was investigated by comparing their concentration responses to those of the conservative and reactive tracers.

2. Materials and methods

2.1. Site description

Tests were performed in existing monitoring wells at former McClellan Air Force Base, CA. The aquifer consists primarily of alluvial sediments including medium graded materials of silty or clayey sand or gravel, and is unconfined with a water table depth ranging from 28 to 30 m below ground surface. The average pore-water velocity is 0.12 m d^{-1}. Tests were conducted in two monitoring wells (MW1 and MW2) constructed of 5.1 cm polyvinyl chloride casing screened between 30 and 33 m below ground surface. A sand filter pack (14 × 40 mesh, 0.5 mm) was installed surrounding the screened interval. Propane

sparging tests were conducted at MW1, which had not been previously exposed to propane, and methane sparge tests were conducted at MW2, which had not been previously exposed to methane. The chemical composition of groundwater in all wells prior to testing was similar. Groundwater at this site is aerobic (dissolved oxygen 6 mg l^{-1}) and contaminated mainly with *cis*-DCE (20–60 μg l^{-1}) and TCE (100–400 μg l^{-1}) and contained about 1 mg l^{-1} of nitrate (as N).

2.2. Transport tests

Transport characteristics of bromide, sulfur hexafluoride (SF_6), oxygen, propane, methane, and nitrate in the study aquifer were evaluated in push–pull tests performed prior to the sparging tests. In these tests, site groundwater was extracted and amended with these solutes, reinjected into the well, and then periodically sampled during continuous extraction pumping (e.g., Kim et al., 2004, 2006). Bromide and SF_6 were used for evaluating the retardation of dissolved solutes due to sorption and mass transfer from a liquid phase to a gas phase trapped in the aquifer, respectively. SF_6 transport is expected to be highly retarded in the presence of trapped gas bubbles due to relatively high volatility (dimensionless Henry's law constant at 20 °C = 169.7, Wilson and Mackay, 1993).

Transport tests were performed by collecting groundwater into a large plastic tank (250 l), a plastic carboy (50 l), and a collapsible metalized-film gas-sampling bag (30 l) (Chromatography Research Supplies, Addison, IL) using a submersible pump (GRUNDFOS Pumps Co., Fresno, CA). The 250 l test solution was bubbled with oxygen to achieve a dissolved oxygen concentration of approximately 30 mg l^{-1}; potassium bromide was also added to achieve a bromide concentration of 100 mg l^{-1}. The 50 l test solution was first bubbled with nitrogen to remove oxygen, TCE and *cis*-DCE and then bubbled with a mixture of propane, ethylene, propylene and nitrogen to achieve the targeted concentrations of all solutes. Another 30 l of test solution was prepared by direct addition of SF_6 gas (0.13 l) to the bag followed by manual mixing to achieve a SF_6 concentration of 1 mg l^{-1}.

During injection the three test solutions were blended by adjusting pumping rates to achieve the desired solute concentrations. The 250 l test solution was injected at approximately 2 l min^{-1} with a peristaltic pump, and the 50 and 30 l test solutions were separately injected at 0.2 l min^{-1} with two piston pumps. Measured injected average concentrations (±95% confidence interval) entering the aquifer were 2.0 ± 0.04 mg l^{-1} for propane, 3.8 ± 0.15 mg l^{-1} for ethylene, 2.8 ± 0.26 mg l^{-1} for propylene, 7.7 ± 0.27 mg l^{-1} for methane, 23 ± 1.4 mg l^{-1} for oxygen, 80 ± 1.2 mg l^{-1} for bromide, and 0.10 ± 0.01 mg l^{-1} for SF_6. After a 2 h rest phase, the test solution/groundwater mixture was extracted by continuous pumping at 3.8 l min^{-1} for 3 h; samples were collected and analyzed to develop extraction phase breakthrough curves for all injected solutes.

2.3. Sparging procedure

For safety considerations, propane and methane concentrations in gas mixtures used for sparging were maintained below the lower explosive limits (LELs) for propane (2.1% by volume in air) and methane (5% by volume in air) by blending with argon. An on-line LEL detector was used to monitor well headspace during gas-sparging and was equipped with an alarm and an automatic gas shut-off valve, which stopped flow if the gas composition reached 90% of the LEL (Fig. 1). During the field tests, the gas composition never reached 90% of the LEL. The LEL detector was calibrated daily with a propane or methane calibration standard gas (Scott Specialty gases, Longmont, CO). Lines from the propane (or methane), oxygen and argon tanks were fitted with check values to prevent backflow of the gases into the tanks.

Gas mixtures were introduced into the well using a "straddle" packer system; the upper and lower packers were pressurized with air to isolate a portion of the well screen for testing (Fig. 1). Rotameters were used to regulate the flow of gases to achieve the desired composition. The rotameters were installed on a gas proportioner multitube frame fitted with calibrated direct reading flow tubes (Cole-Parmer Instrument Co., Vernon Hills, IL). The outflow line (Masterflux Tygon® lab tube) from the gas proportioner terminated at a porous ceramic sparging stone (similar to those used in aquariums) placed near the lower packer, which allowed the gas mixtures to bubble within the water column between the two packers.

2.4. Biostimulation tests

A series of biostimulation tests were performed to determine if sparging the well with gaseous substrates could stimulate the activity of indigenous propane- and methane-utilizing bacteria; ethylene and propylene were not introduced into the aquifer during biostimulation tests (Table 1). The gas mixture was sparged in the well for 6 h. After sparging, groundwater samples were collected periodically over several weeks as substrates were transported away from the vicinity of the well by regional groundwater flow under natural gradient conditions. Samples were analyzed for all injected solutes.

2.5. Activity tests

After microbial activity had been stimulated by the biostimulation tests, a series of activity tests were conducted to measure rate coefficients of propane and methane utilization and rate coefficients of ethylene and propylene transformation in the same wells. Gas mixtures were prepared by controlling flow rates of the various gases as described above and listed in Table 1; after sparging, groundwater samples were collected periodically over a period of approximately 12 d and samples were analyzed

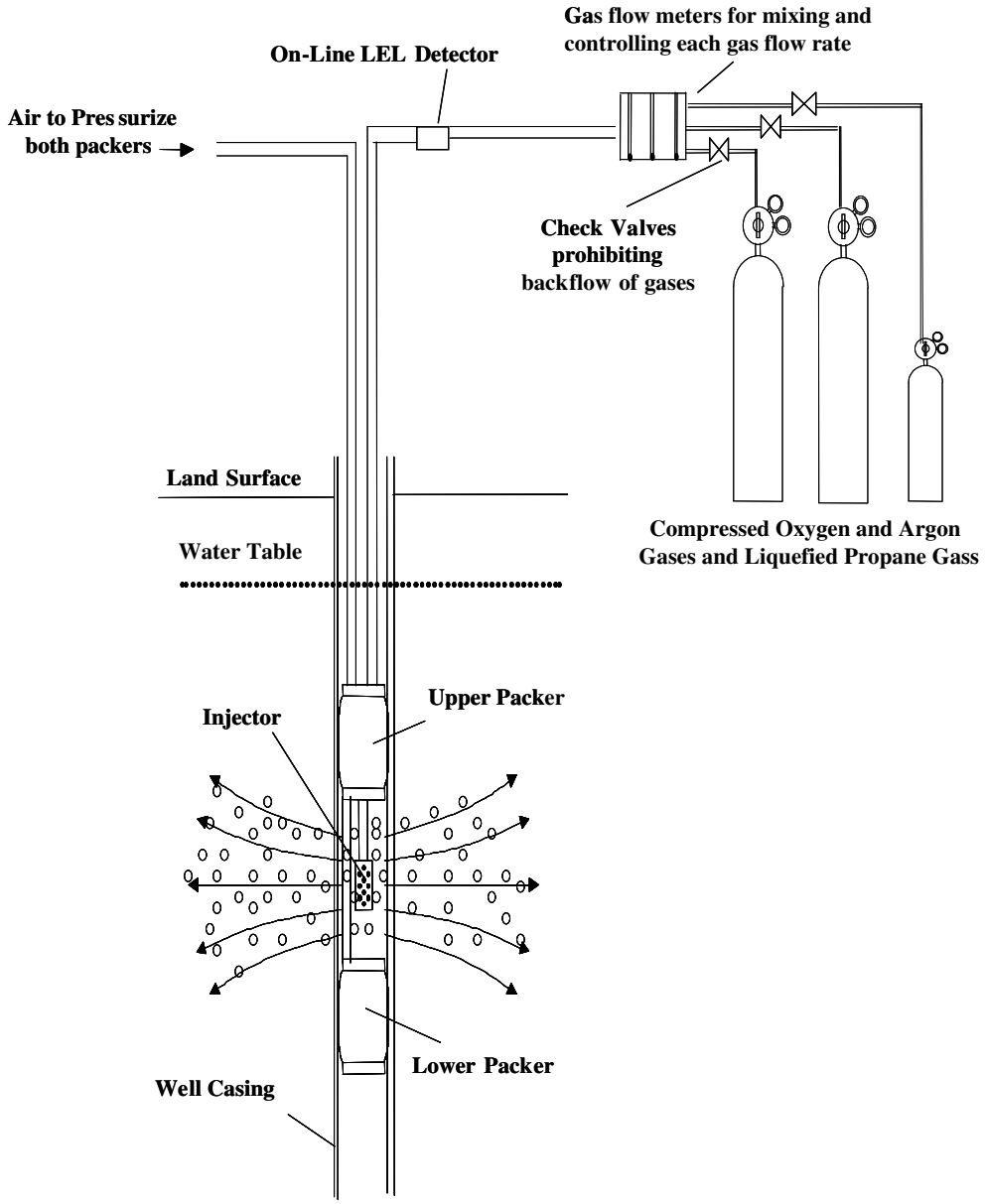

Fig. 1. Experimental set-up for direct gas-sparging into the saturated aquifer.

for injected tracer and gaseous substrates and for ethylene oxide and propylene oxide formed in situ.

2.6. Modified push–pull activity tests

To evaluate if trapped gas is present in the aquifer tested, push–pull transport tests were performed prior to modified push–pull activity tests. Procedures were the same as for the push–pull transport tests described above.

Modified liquid injection push–pull activity tests were performed after the sparging activity tests (Table 1). Four hundred liters of site groundwater were extracted from the well and amended with bromide, SF_6, oxygen, propane (or methane), ethylene, and propylene, reinjected into the well and then periodically sampled over the period of approximately 12 d, as the injected test solution drifted away from the well under natural gradient conditions. Modified push–pull activity test performed in this study involves a "push-phase" followed by a "drift-phase".

2.7. Acetylene inhibition tests

Procedures and gas composition were the same as for the gas-sparging biostimulation and activity tests described above except that acetylene was added to the gas mixture sparged in the well (Table 1).

2.8. Sample analysis

In all tests, groundwater samples were collected using a submersible pump (GRUNDFOS Pumps Co., Fresno, CA). Groundwater samples were collected using a gas tight

Table 1
Test sequence, compositions of aqueous tests solutions (in mg l^{-1}) used in liquid injection transport and activity tests, and gas flow rate (in l min^{-1}) used in gas-sparging, biostimulation activity and acetylene inhibition tests

Test		Test period[g] (d)	Propane[a]	Methane[b]	Ethylene	Propylene	Oxygen	SF_6	Argon	Acetylene
Traditional transport test (aqueous test solution injection)[f]		2 (2)	2.0 ± 0.04	7.7 ± 0.27	3.8 ± 0.15	2.8 ± 0.26	23 ± 1.4	0.10 ± 0.01	NI	NI
Biostimulation tests (gas-sparging)	1st	22 (22)	2.9	2.6	NI[c]	NI	20	NI	110	NI
	2nd	15 (20)	2.9	2.6	NI	NI	20	NI	110	NI
	3rd	24 (15)	2.9	2.6	NI	NI	20	0.30[d]	110	NI
	4th[e]	0 (22)	0.0	5.2	NI	NI	100	0.30	30	NI
Activity tests (gas-sparging)		12 (12)	1.4	3.1	0.52	0.21	7.8	0.96	110	NI
Modified activity test (aqueous test solution injection)[f]		12 (12)	1.3 ± 0.09	0.57 ± 0.07	0.43 ± 0.05	0.58 ± 0.06	23 ± 2.2	0.48 ± 0.04	NI	NI
Acetylene inhibition test (gas-sparging)		6 (6)	1.4	3.1	0.52	0.21	7.8	0.96	110	0.3

[a] Propane was sparged into the MW1 aquifer.
[b] Methane was sparged into the MW2 aquifer.
[c] NI indicates not included.
[d] SF_6 was sparged into the MW1 aquifer, not into the MW2 aquifer.
[e] Gas mixtures were sparged only into the MW2 aquifer.
[f] Average aqueous concentrations ±95% confidence interval in the injected test solution were presented.
[g] Numbers in parenthesis are test period for methane-sparging tests.

syringe connected to a valve in the pump discharge tubing. Samples for dissolved gas analyses were collected in 40-ml VOA vials having a teflon/neoprene septum and a polypropylene-hole cap (Supelco, Bellefonte, PA); samples for ion chromatography were collected in 1 ml glass vials. Samples were stored at 4 °C and analyzed within 1 week.

Dissolved oxygen concentrations were measured in the field with a Clark (Yellow Springs, Ohio) style O_2 electrode mounted in a glass water-jacketed cell to maintain a constant temperature. To convert oxygen saturation values to concentration units (mg l^{-1}), the temperature and oxygen saturation of a reference sample (distilled water sparged with oxygen) were measured before each sample measurement.

Bromide and nitrate concentrations were determined using a Dionex (Sunnyvale, CA) model DX-120 ion chromatograph equipped with an auto-sampler, an electrical conductivity detector and a Dionex AS14 column. Calibration curves were developed using external standards.

Propane, methane, ethylene, propylene, TCE, *cis*-DCE, and their transformation products were determined by a modified EPA 8000 purge and trap method. A 1 or 5 ml aqueous sample was taken from a VOA vial using a gas tight luer lock syringe (Supelco Co., Bellefonte, PA). The sample was then added into a purge tube installed in HP 7695 Purge & Trap. A Tenax/silica gel/charcoal trap was used as the purge trap (Supelco, Bellefonte, PA). A sample purge time of 15 min was used to increase the removal of the less effectively trapped compounds, such as ethylene, and to detect low concentrations of the less volatile transformation products, such as ethylene epoxide and propylene epoxide. Chromatographic separation was achieved with a 30-m megabore GSQ-PLOT column from J&W Scientific (Folsom, CA) installed on a HP 6890 series gas chromatograph (GC) connected to a photo ionization detector (PID) followed by a flame ionization detector (FID) operated at 250 °C. The GC temperature program was as follows: initial oven temperature, 40 °C for 3 min; 4 °C min^{-1} increase to 70 °C; 5 °C min^{-1} increase to 220 °C. The GC was operated in the splitless inlet mode with a carrier gas (He) flow of 15 ml min^{-1}, a H_2 flow to detectors of 35 ml min^{-1}, an air flow to the detectors of 165 ml min^{-1} and a FID detector makeup gas (He) flow of 15 ml min^{-1}. The retention time of each compound under this GC method was as follows: ethylene (3.3 min); propylene (9.8 min); propane (10.2 min); ethylene oxide (14.9 min); propylene oxide (21.9 min); *cis*-DCE (28.8 min); and TCE (33.7 min). Ethylene, propane, ethylene oxide, and propylene oxide were quantified by FID; propylene, *cis*-DCE and TCE were quantified by PID. Calibration curves for the compounds were developed using external standards.

Ethylene oxide and propylene oxide were identified by retention time comparisons with authentic ethylene oxide (>99.5%, Aldrich, Milwaukee, WI) and propylene oxide (>99.5%, Fluka, Milwaukee, WI) standards. Under the same GC operating conditions as described above, the retention times for ethylene oxide and propylene oxide with standards were 14.9 and 21.9 min, respectively. To supplement this identification, authentic standards were assayed with a HP 624 capillary column under the same GC operating conditions. The retention times for ethylene oxide and propylene oxide were 6.31 and 7.98 min, respectively. To further confirm compound identification, the method of standard addition was used where specific amounts of authentic standards were added to the test samples, and resulting concentration increase measured.

SF_6 analysis was performed using the method of Wilson and Mackay (1993). After creating a headspace in a 40-ml VOA vial by extracting 10 ml of aqueous sample from the

vial, the vial was inverted and placed on a rotary shaker at 20 °C to achieve an equilibrium concentration in the headspace. SF_6 analysis of the headspace samples was performed on a HP 6890 series GC equipped with an electron capture detector connected to a HP 624 capillary column. Calibration curves for the compounds were developed using external standards.

2.9. Data analysis

Measured concentrations of injected solutes were plotted as relative concentrations, C/C_o, where C is the measured aqueous concentration of any solute and C_o is the average aqueous concentration of the same solute in the injected test solution. Dilution-adjusted concentrations, C^*, were computed using

$$C^* = 1 - \left[\frac{(C - C_{BG})}{(C_0 - C_{BG})}\right] \quad (1)$$

where C_o is average aqueous concentration of a solute in the injected test solution and C_{BG} is the aqueous concentration of the same solute in the background (pre-test) groundwater. The adjustment for the background concentration is especially important for TCE and *cis*-DCE, since they were present in the site groundwater at higher concentrations than in the injected test solutions, or were present in test solutions at lower concentrations than in site groundwater because test solutions were sparged with various gases, which partially removed some TCE and *cis*-DCE initially present in site groundwater.

First-order reaction rate coefficients (h^{-1}) for the sparging activity tests and the modified push–pull activity tests were computed by nonlinear regression fitting the ratio of the natural log of the dilution-adjusted concentrations of the reactant consumed or product formed versus the time since the end of injection as described by Haggerty et al. (1998).

$$\ln\left(\frac{C^*_{solute}}{C^*_{tracer}}\right) = \ln\left[\frac{(1 - e^{-kt_{inj}})}{kt_{inj}}\right] - kt \quad (2)$$

where k is the rate coefficient, t_{inj} is the finite duration of test solution injection and t is the time elapsed since the end of test solution injection. Using Eq. (2), we can obtain an estimate for the rate coefficient k by measuring the relative concentration of a solute C^*_{solute} (i.e., the measured concentration divided by average solute concentration injected) and the relative concentration of a tracer (C^*_{tracer}) during the tests and then fitting Eq. (2) to a plot of $\ln(C^*_{solute}/C^*_{tracer})$ versus t using nonlinear regression.

3. Results

3.1. Transport tests

Mass balance calculations showed that 92% of the injected bromide and 81% of the injected SF_6 were recovered during the extraction phase for transport tests in MW1 and MW2; recoveries for injected propane, methane, ethylene, and propylene were also very high (103%, 93%, 90%, and 99%, respectively), and were comparable to bromide and slightly higher than SF_6. These results confirmed that bromide and SF_6 could be used as conservative tracers to perform dilution adjustments in subsequent activity tests. These results also suggest that only minor amounts of trapped gas were present in the aquifer as partitioning of SF_6 into trapped gas would result in greater spreading of the SF_6 elution curve relative to bromide, see e.g., Schroth et al. (2001), and that substrate utilization during the transport tests (conducted prior to biostimulation) was negligible.

3.2. Biostimulation tests

Results of repeated growth substrate additions are presented in Fig. 2. (Note that SF_6 was added as a conservative gaseous tracer only in the last gas-sparging test.) Substrate utilization is indicated by the decreasing concentrations for propane or methane compared to the SF_6 tracer. By the third and fourth additions of propane and

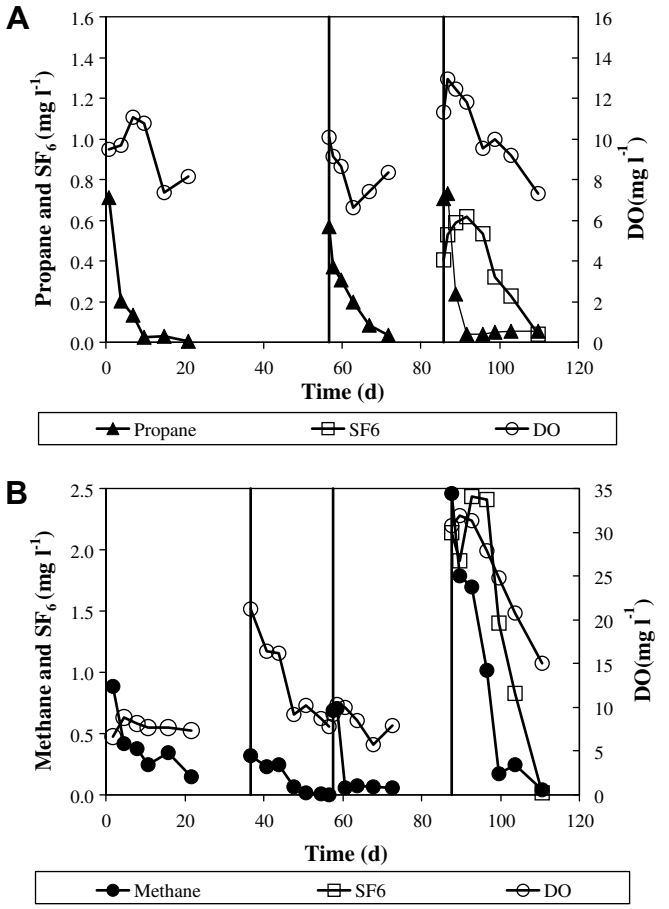

Fig. 2. Concentration histories of methane or propane, DO, and SF_6 at the monitoring wells of MW1 (A) and MW2 (B) during the gas-sparging biostimulation tests.

methane, respectively, injected propane or methane were completely consumed and oxygen was reduced to background concentrations within 10–15 d after injection. The more rapid decrease in propane and methane concentration compared to SF_6 indicates utilization of these substrates.

First-order substrate utilization rate coefficients were estimated using the method reported by Haggerty et al. (1998). Estimated first-order rate coefficients were 0.022 ± 0.002 and $0.006 \pm 0.001\ h^{-1}$ for propane and methane, respectively (Table 2).

3.3. Activity tests

Gas-sparging activity tests conducted after the series of biostimulation tests showed the complete utilization of injected growth substrates and the partial transformation of injected reactive tracers (ethylene and propylene) to diagnostic cometabolic products (Fig. 3). For example, complete utilization of injected propane was observed within 12 d after sparging during an activity test conducted in MW1; by comparison the corresponding dilution loss of the SF_6 tracer was only 40% (Fig. 3A). Ethylene oxide and propylene oxide were detected after 4 d (Fig. 3B), which is consistent with the initiation of ethylene utilization (Fig. 3A). Propylene was transformed more rapidly than ethylene (Fig. 3A), and propylene oxide reached a maximum concentration of 0.01 mg l^{-1} after 6 d, while ethylene oxide reached at maximum concentration of about 0.08 mg l^{-1} after 8 d (Fig. 3B).

Similar results were observed during the methane activity test conducted in MW2 (Fig. 3C and D). Essentially complete utilization of methane, and transformation of ethylene and propylene were observed after 7 d (Fig. 3C), with more rapid decreases than the SF_6 tracer. The stimulated methane utilizers transformed ethylene and propylene to ethylene oxide and propylene oxide, respectively, which were detected after 4 d (Fig. 3D). Consistent with the results of the propane activity tests, the maximum concentration of propylene oxide (0.01 mg l^{-1}) was lower than the

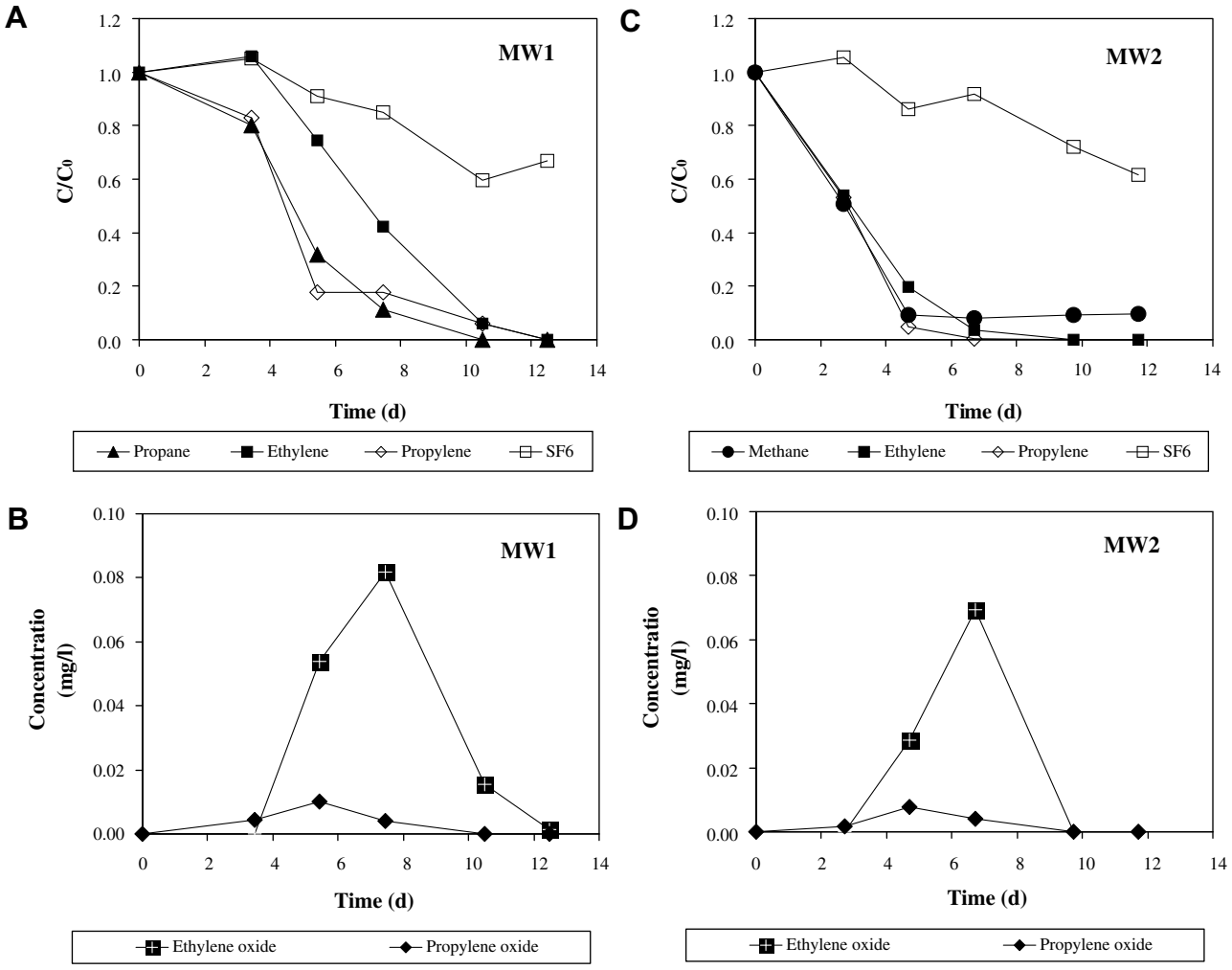

Fig. 3. Normalized concentration histories of propane or methane, ethylene, propylene, and SF_6 and concentrations of transformation by-products of the reactive tracers at the monitoring wells of MW1 (A and B) and MW2 (C and D) during gas-sparging activity tests.

Table 2
First-order degradation rate coefficients (in h^{-1}) with standard error values for each standard least-square regression for propane, methane, ethylene, and propylene in liquid injection push–pull tests and in gas-sparging tests

Test	Well tested	Propane	Methane	Ethylene	Propylene
Biostimulation tests (gas-sparging)	MW1	0.022 ± 0.002	–	–	–
	MW2	–	0.006 ± 0.001	–	–
Activity tests (gas-sparging)	MW1	0.010 ± 0.001	–	0.007 ± 0.001	0.010 ± 0.001
	MW2	–	0.016 ± 0.002	0.017 ± 0.003	0.028 ± 0.004
Modified activity test (aqueous test solution injection)	MW1	0.009 ± 0.001	–	0.012 ± 0.002	0.021 ± 0.003
	MW2	–	0.010 ± 0.002	0.010 ± 0.001	0.013 ± 0.002

maximum concentration of ethylene oxide ($0.07\ mg\ l^{-1}$). Ethylene and propylene oxide concentrations gradually reduced to a nondetectable level 10 d after sparging (Fig. 3D).

First-order substrate utilization rate coefficients for propane, methane, ethylene, and propylene were estimated from gas-sparging activity tests (Table 2). In MW1 the estimated rate coefficients (±standard error) for propane utilization and ethylene and propylene transformation were similar, ranging from 0.007 ± 0.001 to $0.010 \pm 0.001\ h^{-1}$. In MW2, the estimated propylene transformation rate coefficient was 0.028 ± 0.004, compared to 0.016 ± 0.002 and $0.017 \pm 0.003\ h^{-1}$ for methane and ethylene, respectively. Good fits were obtained to the first-order model of Haggerty et al. (1998) with standard errors ranging from 10% to 18%.

3.4. Modified push–pull activity tests

The results of push–pull transport tests performed prior to liquid injection push–pull activity tests showed similar recoveries for bromide and SF_6 at both MW1 and MW2 (data not shown). These results suggest that bromide and SF_6 could be used as conservative tracers to perform dilution adjustments in subsequent modified liquid injection push–pull activity tests.

For comparison with our previous studies (Kim et al., 2006) modified liquid injection push–pull activity tests were also performed. Injected growth substrates, reactive tracers, TCE, and *cis*-DCE were transformed during push–pull activity tests conducted after the gas-sparging activity tests (Fig. 4). In Fig. 4, extraction phase breakthrough curves for propane (Fig. 4A), methane (Fig. 4B), ethylene, propylene, *cis*-DCE, TCE, SF_6, and bromide are plotted as $1 - C^*$, that is, $1 - [(C - C_{BG})/(C_o - C_{BG})]$ because, unlike the growth substrates and reactive tracers, *cis*-DCE and TCE concentrations were present in the injected test solution at a lower concentration than in site groundwater. Values of $1 - C^*$ for the nonreactive tracers (bromide and SF_6), growth substrate and reactive tracers added to groundwater should be close to zero during the very early phase of extraction, as concentrations are near the injected concentrations, and should increase to 1 as the test solution is gradually transported away from the well. Utilization of growth substrate and reactive tracers are indicated by the more rapid increase of $1 - C^*$ to 1 compared to the nonreactive tracers. Values of $1 - C^*$ for *cis*-DCE or TCE, which have higher concentrations in the site groundwater than in the injected test solution can be less than 0 during early phase of the test, and if transformation is occurring, the values will remain below those of the non reactive tracers as the test proceeds.

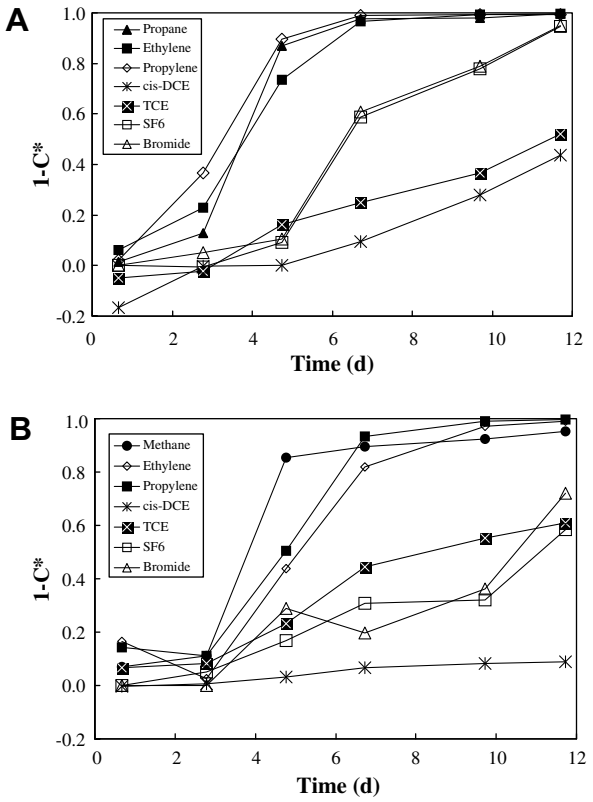

Fig. 4. Dilution-adjusted concentrations of *cis*-DCE, TCE, SF_6, and bromide during modified liquid injection push–pull activity tests in monitoring wells of MW1 (A) and MW2 (B).

In the propane-sparged well (MW1), normalized concentrations ($1 - C^*$) for bromide and SF_6 were very similar during the extraction phase, indicating that very little trapped gas is present in the aquifer. Thus it is unlikely that *cis*-DCE and TCE concentration profiles were affected by retardation due to trapped gas partitioning. The $1 - C^*$

normalized values for propane, ethylene, and propylene were slightly greater than zero during the early phase of extraction, and increased more rapidly with time to 1 than the non reactive tracers (Fig. 4A), indicating significant utilization of propane and transformation of ethylene and propylene during the test. Ethylene oxide and propylene oxide were detected, but at lower concentrations than in the previous gas-sparging activity tests (data not shown). Normalized *cis*-DCE and TCE concentrations were lower than those of bromide and SF_6, (6–12 d) indicating transformation of *cis*-DCE and TCE during the test.

Similar results were obtained for the push–pull test conducted in the methane-sparging well (MW2). The $1 - C^*$ values for methane, ethylene, and propylene increased more rapidly than those for bromide or SF_6 indicating substantial utilization and transformation of these substrates (Fig. 4B); ethylene and propylene oxide were also detected but not quantified during the test (data not shown). The rate coefficients of growth substrate utilization and ethylene and propylene transformation were lower in the methane stimulated well than in the propane stimulated well (Table 2). Normalized TCE concentrations were similar to those for the bromide and SF_6 tracers, while normalized *cis*-DCE concentrations were lower than the tracers suggesting that *cis*-DCE, but not TCE, was being cometabolically transformed. It is interesting to note that the normalized *cis*-DCE concentrations remained near zero throughout the test, indicating continued transformation of *cis*-DCE.

First-order rate coefficients were also estimated using the results of the modified liquid injection push–pull activity tests (Table 2). At MW1, the estimated rate coefficients for propane, ethylene, and propylene ranged from 0.009 ± 0.001 to $0.021 \pm 0.003\ h^{-1}$, while in MW2, rate coefficients for methane, ethylene, and propylene were similar, ranging from 0.010 ± 0.001 and $0.013 \pm 0.002\ h^{-1}$. Here again good fits were obtained with the first-order model Haggerty et al. (1998), with standard errors ranging from 10% to 20%. The rate coefficients obtained from the gas sparge tests were comparable to those obtained using the liquid injection push–pull tests, indicating that comparable information can be obtained with both types of tests.

3.5. Acetylene inhibition tests

Acetylene inhibition tests were performed using the sparge test method with similar conditions as those used to generate the data in Fig. 3, but with acetylene present. In the presence of acetylene, the utilization of methane and propane and the transformation of ethylene and propylene were essentially completely inhibited (Fig. 5A and B). Acetylene acts as a mechanism-based inactivator for most of the oxygenases expressed by methane- and propane-oxidizing bacteria (Prior and Dalton, 1985; Hamamura et al., 1999), thus these results provide compelling evidence that propane and methane monooxygenase enzymes were responsible for the observed transformations of ethylene, propylene, *cis*-DCE, and TCE.

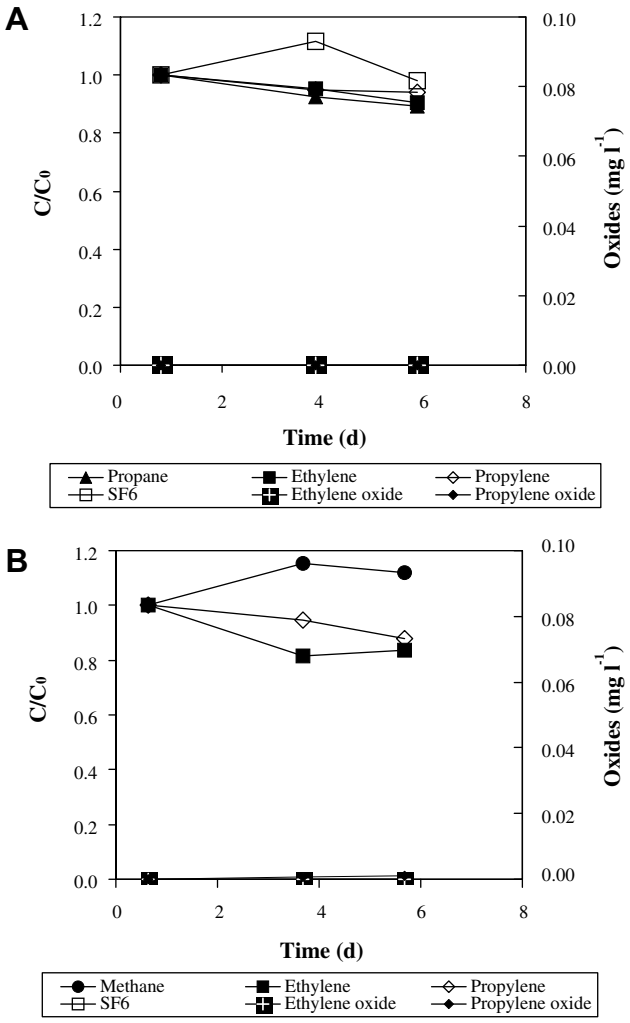

Fig. 5. Normalized concentration histories of propane or methane, ethylene, propylene, SF_6, transformation by-products of the reactive tracers at the monitoring wells of MW1 (A) and MW2 (B) during acetylene inhibition tests.

4. Discussion

In this study, gas-sparging tests were developed for assessing the feasibility of in situ aerobic cometabolism of TCE and *cis*-DCE using propane and methane as growth substrates. A series of tests were performed by first sparging (bubbling) gas mixtures in a well fitted with a "straddle" packer and then periodically sampling groundwater from the same well to develop concentration profiles and to estimate transformation rate coefficients. Evidence that gas-sparging of propane (or methane) and oxygen had stimulated organisms expressing a propane (or methane) monooxygenase enzyme system and the capability to transform TCE and *cis*-DCE included: (1) the transformation of sparged ethylene and propylene to their corresponding cometabolic by-products, ethylene oxide and propylene

oxide, (2) the transformation of both *cis*-DCE and TCE in the propane-sparged well, (3) the transformation of *cis*-DCE at the methane-sparged well, and (4) the inhibition of ethylene and propylene transformations in the presence of acetylene, a known monooxygenase inactivator.

Although the involvement of monooxygenase enzyme in the degradation of growth substrates and reactive tracers was indicated with stimulation on propane (MW1) and methane (MW2), the microbial activity was similar with respect to ethylene and propylene transformation, but differed with respect to TCE and *cis*-DCE transformation. During the push–pull activity tests, *cis*-DCE and TCE were transformed in the propane test (MW1) while only *cis*-DCE was transformed in the methane test (MW2) (Fig. 4). In a previous cometabolic air sparging demonstration conducted at the same site using propane as a substrate (Tovanabootr et al., 2001; Connon et al., 2005), *cis*-DCE was transformed faster than TCE. Similar observations were achieved in microcosm studies performed with aquifer solids and groundwater from the site (Timmins et al., 2001). One possible explanation for the lack of TCE transformation with stimulation on methane is that the methane utilizers that were stimulated expressed particulate methane monooxygenase (pMMO) and not sMMO. In in situ tests with indigenous microorganisms stimulated on methane, *cis*-DCE was transformed, but not TCE (Semprini et al., 1991). Semprini (1997) hypothesized methane utilizers that expressed pMMO were likely stimulated in these tests, because copper is not limiting in the subsurface, which is a requirement for expressing sMMO. Our tests indicate that ethylene and propylene as reactive tracers were not able to predict that TCE would be transformed after stimulation on propane, but not on methane, because similar rate coefficients and extents of product formation were observed in both tests. Therefore developing reactive tracers that might distinguish between the stimulations of sMMO and pMMO is of importance.

cis-DCE continued to be transformed in the methane stimulated well (Fig. 4). It is possible that microbes were stimulated that can potentially grow on *cis*-DCE. Connon et al. (2005) using a microbial sample from the same site as our tests, sequenced a clone that was 98.2% similar to the 16S rRNA of the isolate JS666 which is known to use *cis*-DCE as a sole source of carbon and energy. The sample was obtained from an area stimulated on propane that also showed prolonged transformation of *cis*-DCE. The use of molecular methods for characterizing the composition of stimulated microbial communities would be the important subject of future studies.

Our previous studies utilized modified liquid injection push–pull tests to assess the feasibility of aerobic cometabolism of *cis*-DCE and TCE using propane as a cometabolic substrate. The tests were performed by injecting liquid test solution containing dissolved components of propane and oxygen (Kim et al., 2006). Although the tests were successful, collecting, preparing, and injecting the large volumes of required groundwater was very time-intensive. For example, 500 l of test solution prepared from site groundwater were injected five times every 2 weeks for about 75 d to stimulate propane utilizers. In the current study three or four direct gas-sparging additions into an aquifer were as effective in stimulating the targeting microorganisms. After sparging ended propane (or methane) and oxygen in the trapped bubbles slowly dissolved, and were transported into the aquifer under natural gradient conditions. Thus a slow, prolonged release of growth substrates was probably achieved.

A gas-sparging test has some disadvantages over a modified liquid injection push–pull test. In a modified liquid injection push–pull test, the aqueous test solution penetrates the formation, assaying a certain predictable volume of aquifer material. In a gas-sparging test, it is not entirely clear to what degree gas bubbles penetrate the formation. It is be possible that one may be assaying only a relatively small volume (e.g., the filter pack surrounding a well) rather than the formation. This should be the subject of future work.

First-order substrate transformation rate coefficients obtained from the modified liquid injection push–pull test and gas mixture sparging activity tests were comparable, so the gas-sparging method was found useful for qualitatively and quantitatively estimating the feasibility for in situ aerobic cometabolism of CAHs. The analytical approach of Haggerty et al. (1998) provided by Eq. (2) yielded good fits to the field observations and proved to be a useful method to quantitatively estimate transformation rate coefficients.

The direct gas-sparging method appears to be more effective in stimulating indigenous microorganisms in the single-well tests than the addition of groundwater with dissolved gaseous growth substrates. The gas-sparging activity tests and acetylene inhibition tests were also easy to implement and effectively demonstrated that cometabolic transformation was being achieved. This method would also be effective for testing other gaseous substrates, such as butane, which can be used to stimulate microorganisms that can cometabolically transform a broad range of CAHs, including 1,1-dichloroethene, 1,1-dichloroethane, and 1,1,1-trichloroethane (Kim et al., 2000, 2002; Semprini et al., 2007). Traditional liquid phase push–pull tests have been successfully performed with toluene as a cometabolic substrate, with isobutene as a reactive tracer and 1-bytene as an inhibitor (Azizian et al., 2006). All of these compounds could be added as gaseous substrates as well. The series of gas-sparging tests developed and field tested in this study should prove useful for conducting rapid, low-cost feasibility assessments for in situ aerobic cometabolism of CAHs with a broad range of cometabolic substrates, reactive tracers and inhibitors.

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