ground water

Push-Pull Tests for Assessing In Situ Aerobic Cometabolism

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Abstract

Three types of single-well push-pull tests were developed for use in assessing the feasibility of in situ aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs). These included transport tests, biostimulation tests, and activity tests. Transport tests are conducted to evaluate the mobility of solutes used in subsequent tests. These included bromide or chloride (conservative tracers), propane (growth substrate), ethylene, propylene (CAH surrogates), dissolved oxygen (electron acceptor), and nitrate (a minor nutrient). Tests were conducted at an experimental wellfield of Oregon State University. At this site, extraction phase breakthrough curves for all solutes were similar, indicating apparent conservative transport of the dissolved gases and nitrate prior to biostimulation. Biostimulation tests were conducted to stimulate propane-utilizing activity of indigenous microorganisms and consisted of sequential injections of site ground water containing dissolved propane and oxygen. Biostimulation was detected by the increase in rates of propane and oxygen utilization after each injection. Activity tests were conducted to quantify rates of substrate utilization and to confirm that CAH-transforming activity had likely been stimulated. In particular, the transformation of injected CAH surrogates ethylene and propylene to the cometabolic byproducts ethylene oxide and propylene oxide provided evidence that activity of the monooxygenase enzyme system, responsible for aerobic cometabolic transformations of CAHs, had likely also been stimulated. Estimated zero-order transformation rates decreased in the order propane > ethylene > propylene. The series of push-pull 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.

Introduction

Site-specific data are needed to perform feasibility assessments and remedial design for in situ bioremediation of chlorinated aliphatic hydrocarbon (CAH) contamination in ground water. Site-specific data are needed because treatment effectiveness is determined by the metabolic capabilities of native microorganisms, the combinations and concentrations of contaminants, and a variety of other factors, such as trace nutrient availability. For example, the use of aerobic cometabolism to oxidize CAH compounds like trichlorethene (TCE) to harmless byproducts requires the selection of a site-specific cometabolic growth substrate (e.g., propane, methane, or toluene).

Laboratory microcosm tests performed with sediment samples collected by coring are often used for this purpose (Hopkins et al. 1993; McCarty et al. 1998). In previous field applications, microcosm tests were followed by pilot-scale studies using well-to-well recirculation tests (Hopkins et al. 1993). Although the approach has been successfully applied at a few sites (McCarty et al. 1998), it has several disadvantages that limit its routine use for feasibility assessment and remedial design. For example, core samples are often difficult to obtain, and may be too small to provide representative information on subsurface conditions. Well-to-well recirculation tests provide more representative information, but are expensive and logistically complicated.

This study adapts the single-well push-pull test for use in conducting rapid, low-cost feasibility assessments for in situ enhanced aerobic cometabolism of CAHs. Push-pull tests have been used to obtain quantitative site-specific

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information on a variety of aquifer physical, chemical, and microbiological characteristics (Istok et al. 1997; Reinhard et al. 1997; Schroth et al. 1998; Kleikemper et al. 2002; McGuire et al. 2002) including the anaerobic transformation of CAHs (Hageman et al. 2001) and radionuclides (Senko et al. 2002). A push-pull test consists of the controlled injection of a prepared test solution into an aquifer followed by the extraction of the test solution/ground water mixture from the same location. The injected test solution consists of ground water containing a nonreactive tracer and one or more biologically reactive solutes selected to investigate specific processes of interest. The test solution is injected (pushed) into the aquifer where it flows radially outward from the well and penetrates a volume of aquifer material adjacent to the well. During the extraction phase, flow is reversed; the test solution/ground water mixture is extracted (pulled) from the same location, and concentrations of tracer, reactants, and reaction products are measured as a function of time. Reaction rate coefficients are computed from the mass of reactant consumed and/or product formed (Istok et al. 1997; Haggerty et al. 1998). A rest phase (with no pumping) may be included between the injection and extraction phases to allow time for a particular reaction to proceed. Alternatively, the injected test solution may be allowed to simply drift downgradient with the regional ground water flow; in this case, breakthrough curves are constructed by periodically sampling the injection well over time (Hageman et al. 2001; Senko et al. 2002)

In this study, we developed a series of push-pull tests for assessing the feasibility of in situ aerobic cometabolism of CAHs by indigenous propane-oxidizing bacteria. Propane-oxidizers have the ability to cometabolically transform a wide range of CAHs, including chlorinated methanes (e.g., chloroform), chlorinated ethanes (e.g., 1,1,1-trichloroethane), and chlorinated ethylenes (e.g., *cis*–1,2-dichloroethylene or c-DCE, and trichloroethylene or TCE) (Wackett et al. 1989; Kim 1996; Tovanabootr and Semprini 1998; Timmins et al. 2001). Phenol- and toluene-oxidizers also effectively transform chlorinated ethylenes, but they are much less effective in transforming chlorinated methanes and ethanes. Although methane-oxidizers also have an ability to transform a wide range of CAHs, the CAH-transforming rates significantly decrease in the presence of trace metals (e.g., copper), which are common in the subsurface (Lontoh and Semrau 1998). In addition to CAHs, propane-oxidizers also have an ability to transform methyl *tert*-butyl ether (Steffan et al. 1997). Therefore, push-pull test methods developed to assess the aerobic cometabolism of propane-oxidizers have potential application to a wide range of commonly occurring ground water contaminants.

Since obtaining regulator's approval to inject CAHs to probe for CAH-transforming activity may be difficult and since the presence of CAH transformation products in the aquifer may confound the interpretation of test data, the transformation of injected CAH surrogate compounds was evaluated. In a previous study, Hageman et al. (2001) proposed the use of trichlorofluoroethene as a fluorinated surrogate for use in assessing in situ anaerobic transformations of TCE. In this study, ethylene and propylene were evaluated as surrogates to probe in situ aerobic transformations of CAHs. Cometabolism of ethylene and propylene to their corresponding epoxides, ethylene oxide and propylene oxide respectively, by the propane monooxygenase enzyme has been reported in laboratory studies (Hou et al. 1983; Stephen and Dalton 1986). Propane monooxygenase has also been shown to initiate transformation of CAHs (Vanderberg et al. 1995; Vanderberg and Perry 1994). The ability to cometabolize ethylene and propylene to their corresponding oxides has also been observed with CAH-transforming methanotrophic cultures (Hou et al. 1979; van Hylckama Vlieg et al. 1996). For example, van Hylckama Vlieg et al. (1996) found that both *cis*-DCE and TCE were transformed to their corresponding epoxide by *M. trichosporium* OB3b expressing soluble methane monooxygenase (sMMO). Both ethylene and propylene are also rapidly cometabolized to their respective epoxides (Hou et al. 1979). Woods and Murrell (1989) and deBont and Beck (1980) have also reported that most propane-oxidizing microorganisms cannot grow on ethylene or propylene. In addition, microorganisms utilizing alkenes (e.g., ethylene and propylene) as the sole carbon and energy source express an enzyme, epoxidase, to further metabolize the corresponding epoxides (Ensign 1996; Allen and Ensign 1998). Thus, the detection of the epoxides should indicate the ethylene and propylene have been cometabolized. By monitoring the transformation of injected ethylene-to-ethylene oxide or the transformation of injected propylene-to-propylene oxide, evidence of the presence of propane-utilizing microorganisms with cometabolic transformation abilities will be obtained. These same microorganisms should also likely have the ability to aerobically transform CAHs. Ethylene and propylene have the additional advantages that they are nontoxic and not normally present in ground water at high concentrations, and thus are well suited for use in field tests.

The objective of this study was to develop a series of rapid, low-cost push-pull tests for use in evaluating the site-specific potential for aerobic cometabolism of CAHs. The tests may be conducted in a single well or in a series of wells to assess the site-scale spatial variability in these processes. The test series consists of (1) a short-duration (~8 hours) transport test to evaluate the mobility of the substrates and CAH surrogates in the absence of biological activity, (2) long-term (weeks) biostimulation tests to evaluate the ability of propane additions to stimulate propane-utilizers; and (3) intermediate-term (~24 hours) activity tests to quantify rates of substrate utilization and CAH surrogate transformation.

Materials and Methods

Site Description

Push-pull tests were performed in an experimental wellfield at Oregon State University. The aquifer at this site is not contaminated with CAHs, the ground water is aerobic (~2 mg/L dissolved oxygen), and had not been exposed to propane prior to the start of these tests. The aquifer consists primarily of alluvial deposits, and is unconfined with a water table depth ranging from 130 to 280 cm below

ground surface. The aquifer porosity and bulk density are 0.3 and 1.85 g/cm^3, respectively. The regional hydraulic gradient is ~0.002 m/m, and the average ground water (Darcy flux) velocity is ~0.35 m/day. Tests were conducted in a single monitoring well constructed of 5.1 cm polyvinyl chloride casing with a 1.5 m long screen.

Push-Pull Tests

A single transport test, three biostimulation tests, and three activity tests were conducted for this study (Table 1). Field equipment consisted of compressed or liquefied gases, gas flow meters, a carboy to contain the prepared test solution, and a peristaltic pump to inject the test solution into the well (Figure 1). Test solutions were prepared from site ground water, and contained known concentrations of bromide (KBr, Spectrum Chemical Mfg. Corp., Gardena, California) or chloride (NaCl, Mallinckrodt Chemical Inc., Paris, Kentucky) to serve as nonreactive tracers, one or more dissolved gases (propane [99.5%], ethylene [> 99.9%], propylene [> 99.0%] and/or oxygen [Airgas Inc., Randor, Pennsylvania]) to probe for microbial activity, and nitrate ($NaNO_3$ [Mallinckrodt Chemical Inc., Paris, Kentucky]) as a trace nutrient. Specified dissolved gas concentrations were achieved by controlling the flow rates of each gas to ceramic sparging stones placed in the bottom of the carboy. Gas flow rates were controlled using rotameters fitted to a gas proportioner multitube frame that contained direct reading flow tubes (Cole-Parmer Instrument Co., Vernon Hills, Illinois). Preliminary experiments indicated that dissolved gas concentrations in the test solution stabilized after ~2.5 hours of sparging. After dissolved gas concentrations had stabilized, test solutions were injected into the well through 1.9 cm braided nylon tubing (Kuryama Co., Santa Fe Springs, California) using a Masterflex peristaltic pump (Barnanat Co., Barrington, Illinois).

Transport Test

A single transport test was conducted to compare the relative mobility of bromide, nitrate, and dissolved propane, oxygen, propylene, and ethylene in the aquifer prior to the biostimulation and activity tests which will be described (Table 1). 40 L of test solution (prepared as previously described) were injected at 1 L/min. Eight samples of the injected test solution were collected during the injection phase and analyzed to determine test solution composition. The extraction phase began immediately after the end of the injection phase to minimize the time available for microbial transformations of injected nitrate or dissolved gases. During the extraction phase, the test solution/ground water mixture was extracted from the well using a PVC bailer (Forestry Suppliers Inc., Jackson, Mississippi). 1 L was bailed from the well every five minutes to yield a time-averaged extraction rate of ~0.2 L/min. Samples collected during the extraction phase were analyzed and used to prepare breakthrough curves for each injected solute. The entire test was completed in ~10 hours.

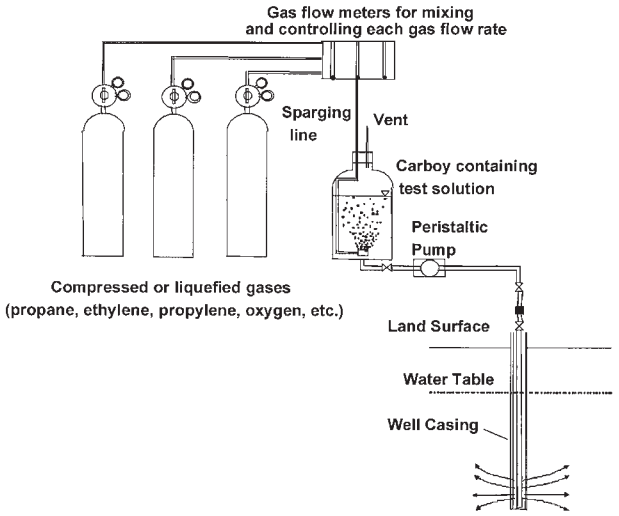

Figure 1. Field setup for single-well push-pull tests.

Biostimulation Tests

Three biostimulation tests were conducted to stimulate the activity of indigenous propane-oxidizing bacteria. Test solutions were prepared and injected as previously described and contained known concentrations of Br^- or Cl^- tracer, dissolved propane and oxygen, and nitrate (Table 1). Since commercial grade propane can contain ethylene and propylene, high purity propane (99.5%) was used to insure the stimulation of propane-utilizing microorganisms, and not ethylene-utilizing or propylene-utilizing microorganisms.

Table 1
Sequence of Field Push-Pull Tests

Test Type	Volume Injected (L)	Volume Extracted (L)	Concentration of Solute Injected ± 95% Confidence Interval (mg/L)						
			Propane	Ethylene	Propylene	[1]Oxygen	[2]NO_3^- (as N)	Cl^-	Br^-
Transport	40	80	5.2 ± 0.2	0.30 ± 0.1	50 ± 0.5	29 ± 0.4	10 ± 0.1	0	108 ± 1
1st Biostimulation	100	140	8.3 ± 0.2	[3]NI	NI	32 ± 0.7	11 ± 0.2	0	110 ± 2
2nd Biostimulation	100	70	7.2 ± 0.2	NI	NI	36 ± 0.7	10 ± 0.1	0	115 ± 1
3rd Biostimulation	100	31	8.7 ± 0.2	NI	NI	34 ± 0.8	11 ± 0.1	0	119 ± 2
Activity (propane)	40	80	2.4 ± 0.1	NI	NI	16 ± 0.2	13 ± 0.1	2.7 ± 0.1	120 ± 1
Activity (ethylene)	40	77	NI	3.5 ± 0.4	NI	16 ± 0.4	13 ± 0.2	109 ± 0.2	22 ± 1.3
Activity (propylene)	40	74	NI	NI	4.7 ± 0.2	21 ± 0.7	14 ± 0.3	17.4 ± 0.2	122 ± 3

[1]Background oxygen concentration of 2mg/L
[2]Background NO_3^- (as N) concentration of 0.4 mg-N/L
[3]NI indicates not included

Unlike the transport test, however, the test solution/ground water mixture was not immediately extracted. Instead, discrete samples were collected from the well periodically for up to 25 days following injection to allow time for microbial utilization of injected propane and oxygen to proceed. Samples were collected using a PVC bailer and analyzed to develop concentration profiles for all injected solutes.

Activity Tests

After the biostimulation tests were completed, a series of three activity tests was conducted to quantify rates of propane and oxygen utilization, and rates of ethylene and propylene transformation (Table 1). Test solutions were prepared and injected as previously described. Injected solutions were allowed to reside in the aquifer ~12 hours before the start of the extraction phase to allow time for propane utilization and surrogate transformation to occur. Samples were collected using a PVC bailer and analyzed to develop breakthrough curves for all injected solutes and transformation products formed in situ.

Analytical Methods

Aqueous samples were collected using a plastic syringe. A 1 mL sample was collected for ion chromatographic (IC) analysis. A 2 mL sample without headspace was collected in 2 mL amber vials having a Teflon®/neoprene septum and a polypropylene hole cap (Supelco, Bellefonte, Pennsylvania) for dissolved gaseous substrates analysis. Samples were stored at 4ºC and analyzed within one week. A separate 2 mL sample was collected for field measurement of dissolved oxygen.

Bromide, chloride, and nitrate concentrations were determined using a Dionex (Sunnyvale, California) model DX–120 ion chromatograph equipped with an auto-sampler, an electrical conductivity detector, and a Dionex AS14 column that utilized an eluent consisting of a mixture of Na_2CO_3 and $NaHCO_3$. A 0.6 mL sample was transferred to Dionex Polyvials™ with filter caps for use in the auto-sampler. The auto-sampler was programmed to deliver an injection volume of 50 μL. Calibration curves were developed daily using external standards.

The U.S. Environmental Protection Agency 502.2 purge-and-trap method (Slater and Ho 1986) was adapted for use in determining the dissolved concentrations of gaseous substrates. A 250 or 500 μL sample was added into a HP 7695 purge-and-trap system, and the volatile compounds were sorbed onto a tenax/silica gel/charcoal trap (Supelco, Bellefonte, Pennsylvania). A sample purging time of 15 minutes was used. Chromatographic separations were achieved with a 30 m megabore GSQ-PLOT column from J&W Scientific (Folsom, California) installed on a HP5890 series gas chromatograph (GC) connected to a photo ionization detector (PID) followed by a flame ionization detector (FID). The GC was operated at an initial oven temperature of 40°C for three minutes, 4°C/min up to 70°C; and 5°C/min up to 220°C. The GC was operated in the splitless inlet mode with a carrier gas (helium) flow of 15 mL/min, a H_2 flow to detectors of 35 mL/min, an air flow to the detectors of 165 mL/min, and a FID detector makeup gas (helium) flow of 15 mL/min. Calibration curves for the compounds were developed daily using external standards.

Ethylene oxide and propylene oxide were identified by retention time comparisons with authentic ethylene oxide (> 99.5%) (Aldrich, Milwaukee, Wisconsin) and propylene oxide (> 99.5%) (Fluka, Milwaukee, Wisconsin) standards. Under the same GC operating conditions as previously described, the retention times for ethylene oxide and propylene oxide with standards were 14.4 and 21.7 minutes, respectively. To supplement this identification, authentic standards were assayed with a HP624 capillary column under the same GC operating conditions. The retention times for ethylene oxide and propylene oxide were 6.31 and 7.98 minutes, respectively. To further confirm the identification of test samples, the method of standard addition was used where specific amounts of authentic standards were added to the test samples, and resulting concentration increase measured.

Dissolved oxygen concentrations were measured in the field with a Clark (Yellow Springs, Ohio) style O_2 electrode mounted in a glass water-jacketed reaction vessel (1.8 mL) to maintain a constant temperature. To convert oxygen saturation values to concentration units (mg/L), the oxygen saturation of a reference sample was measured before each sample measurement. The reference sample consisted of distilled water sparged with oxygen gas. The dissolved oxygen concentration of a sample was determined using the measured oxygen saturation for the sample, the measured oxygen saturation for the reference sample, the measurement temperature, and a value for oxygen solubility in distilled water at the measurement temperature.

Data Analysis

Mass balance calculations were performed by integrating measured solute concentrations and injection and extraction volumes. Dilution-adjusted solute concentrations were computed by dividing measured concentrations of nitrate and the gaseous substrates and products by the relative concentration for the bromide or chloride tracer, C/C_o, where C is the measured tracer concentration and C_o is the average concentration of the tracer in the injected test solution. Dilution-adjusted concentrations generally decreased linearly as a function of time during these tests, and were well fit by a simple zero-order reaction model. Overall zero-order reaction rates (r) for injected solutes were calculated using the method of Istok et al. (1997):

$$r = \frac{M_{inj} - \{M_{ext}/R_{tracer}\}}{(V_{inj})(t^*)} \quad (1)$$

where M_{inj} is total mass of solute injected, M_{ext} is total mass of solute extracted, V_{inj} is volume of injected test solution (L), R_{tracer} is the mass recovery fraction of the conservative tracer (extracted tracer mass divided by injected mass), and t^* is mean residence time defined as the elapsed time from the midpoint of the injection phase to the centroid of the conservative tracer breakthrough curve during the extraction phase. Additional details about the reaction rate calculations are in Istok et al. (1997) and Haggerty et al. (1998).

Results

Transport Test

At this site extraction phase breakthrough curves for dissolved oxygen, the gaseous substrates, and nitrate were all very similar to the breakthrough curve for the bromide tracer, indicating conservative transport of all solutes during the transport test (Figure 2). In Figure 2, results are displayed as relative concentrations, C/C_o, where C is the measured solute concentration in a sample and C_o is the average concentration of the same solute in the injected test solution. This result is important because it means that measured tracer concentrations can be used to adjust dissolved oxygen, gaseous substrate, and nitrate concentrations measured during biostimulation and activity tests for dilution. Mass balance calculations indicated that between 50% and 65% of injected solute mass was recovered during the extraction phase of the transport test (Table 2). Loss of injected tracer mass is due to several processes including advective transport with regional ground water flow, dispersion, heterogeneities in aquifer properties, buoyancy-induced vertical flow (sinking) of the injected test solution, injection and extraction rates, well construction details, and other factors. Because it is not possible to quantify these processes in a mechanistic way, it is assumed that these processes are adequately accounted for by the tracer breakthrough curves in all tests, and that substrate utilization observed during the biostimulation and activity tests can be detected and quantified by adjusting substrate, surrogate, and product concentrations for dilution using measured relative concentrations of the Br^- tracer.

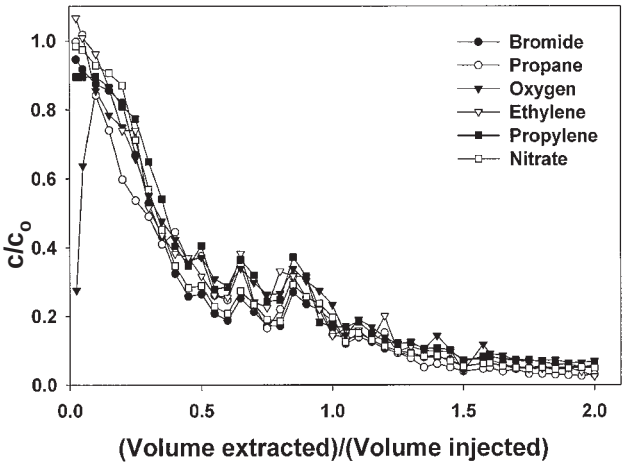

Figure 2. Extraction phase breakthrough curves for short-term transport test showing conservative transport of dissolved gases and nitrate.

Biostimulation Tests

During the first biostimulation test, dilution-adjusted concentrations of propane (electron donor) and oxygen (electron acceptor) decreased simultaneously over a period of 25 days as injected substrates were utilized by indigenous microorganisms (Figure 3). In the first test, initial dilution-adjusted propane concentrations exceeded 1.0, which is physically unreasonable. The only plausible explanation is that propane concentrations in the injected test solution (C_o) were measured inaccurately (underestimated) so that C/C_o values for propane were overestimated. This

Table 2
Summary of Mass Balance and Rate Calculations for Field Push-Pull Tests

Test Type	Quantities	Propane	Ethylene	Propylene	Oxygen	NO_3^--N	Br^-	Cl^-
Transport	Injected mass (mmol)	4.7	0.43	46	37	6.4	54	
	Extracted mass (mmol)	2.4	0.24	28	23	3.4	27	
	% recovery	50	57	61	64	53	50	
	Zero-order rate (μmol/L/hr)	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	—	
Activity (propane)	Injected mass (mmol)	2.2			20	8.2	60	
	Extracted mass (mmol)	0.01			8.9	3.8	32	
	% recovery	0.24			45	46	53	
	Zero-order rate (μmol/L/hr)	[2]»3.6			»5.0	»1.7	—	
Activity (ethylene)	Injected mass (mmol)		5.0		19	8.6		123
	Extracted mass (mmol)		1.6		17	4.0		58
	% recovery		32		87	46		47
	Zero-order rate (μmol/L/hr)		2.6		[1]NA	0.39		—
Activity (propylene)	Injected mass (mmol)			4.3	27	9.0	61	
	Extracted mass (mmol)			2.0	20	4.5	34	
	% recovery			45	76	51	56	
	Zero-order rate (μmol/L/hr)			1.4	NA	1.4	—	

[1]NA indicates not applicable
[2]Greater than

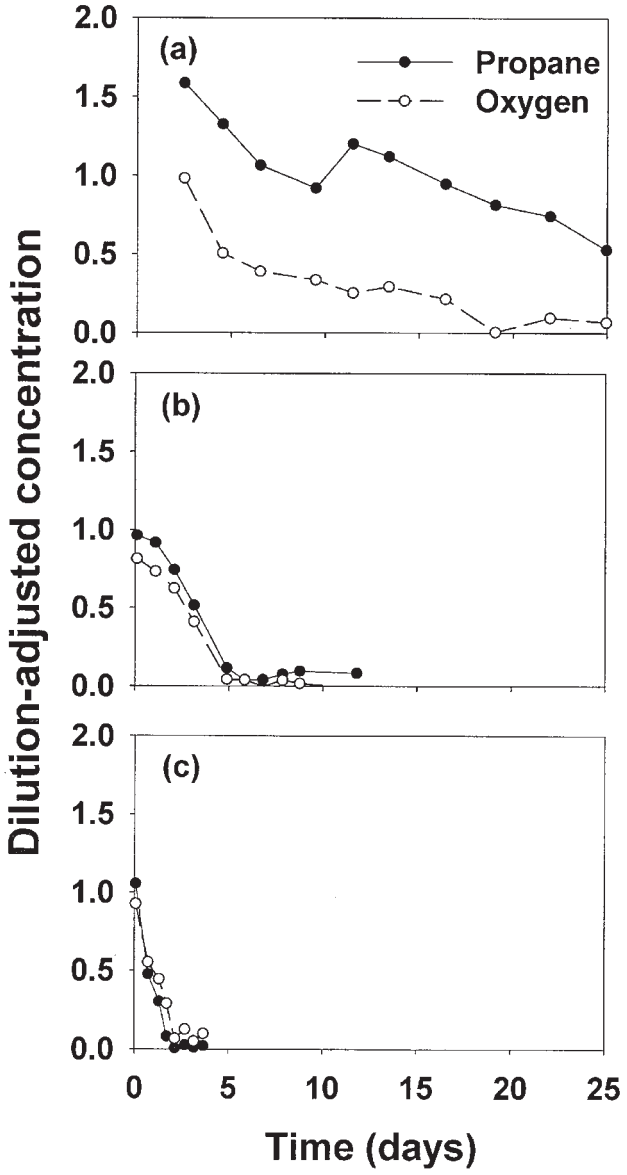

Figure 3. Dilution-adjusted propane and oxygen concentrations during the (a) first, (b) second, and (c) third biostimulation tests.

could have occurred, for example, if an incorrect (smaller) sample volume was delivered to the purge-and-trap so that propane concentrations computed from GC peak areas were smaller than would have been the case if the correct sample volume had been used. In the second and third biostimulation tests, rates of propane and oxygen utilization significantly increased (Figure 3), indicating that the activity of propane-utilizing microorganisms was stimulated by successive injections.

Zero-order rates of oxygen and propane utilization during biostimulation tests were computed from dilution-adjusted concentrations as described in the Data Analysis section. Computed rates increased in each successive biostimulation test (0.4, 1.0, and 4.7 μmol/L/hr, respectively, for propane; and 2.8, 6.0, 16 μmol/L/hr, respectively, for oxygen), indicating progressive biostimulation of propane-utilizers. Computed values of the ratio (oxygen utilization rate)/(propane utilization rate) decreased in each successive test (7.1, 5.9, and 3.5, respectively) and were higher than the theoretical value of 1.8 computed using the energetic model of Rittmann and McCarty (2001), which assumes biomass growth. Alternatively, a theoretical value of 3.5 would be required to completely oxidize injected propane to CO_2 and H_2O. Computed values of the ratio > 3.5 suggest that a portion of the injected oxygen is being utilized to oxidize other components in the ground water or aquifer solids—for example, reduced iron or organic matter.

Activity Tests

The results of the propane activity test conducted following the three biostimulation tests indicated essentially complete utilization of injected propane with simultaneous utilization of co-injected oxygen and nitrate (Figure 4). Propane was utilized during the 12-hour period the injected fluid resided in the aquifer prior to extraction. Normalized concentrations of dissolved oxygen increased after ~1 injection volume was extracted as the injected test solution was diluted with oxygenated ground water from outside the zone of influence of the injected test solution. The computed value of the ratio (oxygen utilization rate)/(propane utilization rate) was 1.4, which is similar to the theoretical value 1.8 (mole ratio) obtained from the energetic model, when biomass growth is occurring.

The results of the ethylene activity test indicated that injected ethylene was transformed in situ to ethylene oxide (Figure 5). In Figure 5, ethylene oxide concentrations are presented as normalized concentrations (expressed as a percentage), which are defined as the dilution-adjusted ethylene oxide concentration divided by the average ethylene concentration in the injected test solution. The ethylene activity test was conducted in the absence of added propane, and oxygen utilization during this test was minimal (Table 2). Oxygen mass recovery was greater than bromide recovery due to dissolved oxygen being present in the ambient ground water. Minimal oxygen utilization in the ethylene activity test indicates that ethylene was cometabolically transformed by enzymes produced previously during microbial oxidation of propane. The computed rate of ethylene transformation was ~60% of the computed rate

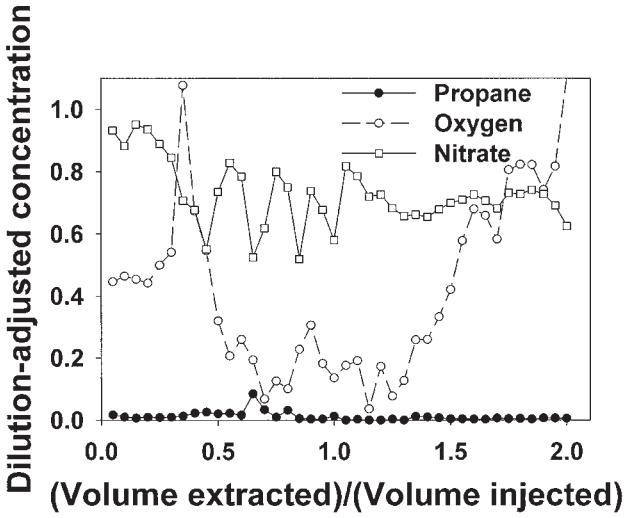

Figure 4. Dilution-adjusted concentrations of injected propane, oxygen, and nitrate during propane activity test.

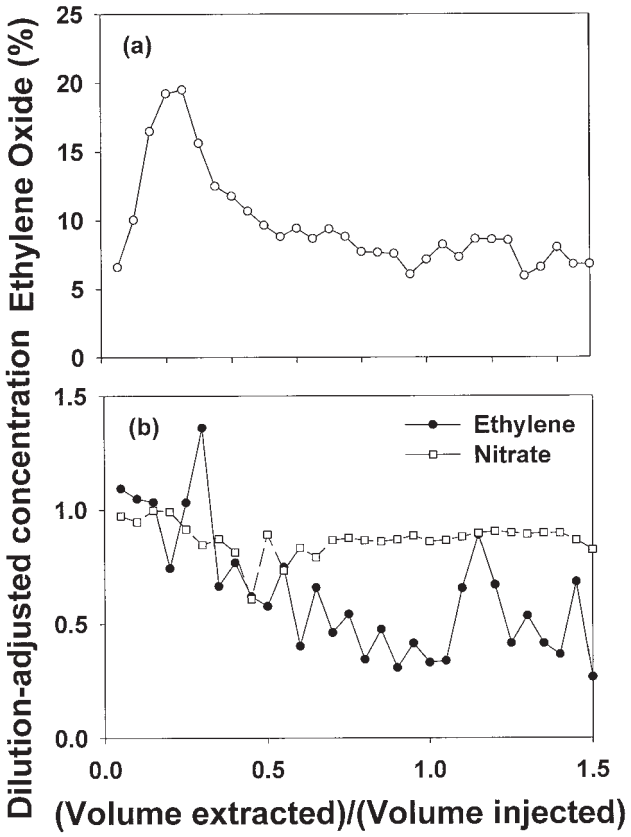

Figure 5. (a) Ethylene oxide concentrations in the extracted ground water as a percent of average ethylene concentration in injected test solution; (b) dilution-adjusted concentrations for ethylene and nitrate during ethylene activity test.

of propane utilization (Table 2). It should be noted that ethylene was injected at a higher concentration than propane. This was confirmed by mass balance calculations; the total mass of ethylene oxide produced was ~33% of the mass of injected ethylene transformed during the test. One possible explanation for the apparent incomplete transformation of injected ethylene-to-ethylene oxide is that a portion of the ethylene oxide formed was further biologically transformed to nondetected products. The abiotic transformation of ethylene oxide in sterilized ground water samples was observed in a laboratory test, but computed half-lives were quite large (~22 days) indicating that abiotic transformation of ethylene oxide is not likely occurring in this aquifer at a rate sufficient to effect ethylene oxide concentrations during these tests. Alternatively, a portion of the ethylene oxide formed could have been sorbed to aquifer sediments. However, the high aqueous solubility of ethylene oxide makes this unlikely, especially since breakthrough curves for the less-soluble gases (oxygen, propane, propylene, and ethylene) were essentially identical to that for bromide during the transport test. Van Hylckama Vlieg et al. (1996) showed that the epoxide formed during *cis*–1,2-dichloroethylene transformation was likely being biologically transformed. Thus, the biological transformation of ethylene oxide was possibly occurring in our test.

The results of the propylene activity test indicated that injected propylene was transformed to propylene oxide (epoxide). The ratio of mass of propylene oxide formed to propylene transformed was ~15%, which is smaller than the ~33% observed for ethylene oxide during the ethylene activity test. One possibility is that propylene oxide is degraded more quickly than ethylene oxide. However, the small concentrations of ethylene and propylene oxides observed in these tests combined with the limited transformation of propylene permits only a qualitative comparison. Nevertheless, the results of the series of activity tests showed that propane-utilizers stimulated by successive propane additions were able to cometabolize the injected CAH surrogates ethylene and propylene, indicating cometabolism was occurring and that the propane-utilizers in this aquifer would likely have the ability to cometabolize CAHs. The computed zero-order rate of propylene transformation was about a factor of two lower than the ethylene transformation rate (Table 2).

Discussion

Previous applications of the push-pull test have focused on anaerobic transformations of petroleum hydrocarbons (Schroth et al. 1998; Reinhard et al. 1997), CAHs (Hagemen et al. 2001), and radionuclides (Senko et al. 2002). This study demonstrates that the method can also be used to examine microbial processes involved in the aerobic cometabolism of CAHs. Aerobic cometabolism is based on an entirely different biochemistry and involves entirely different classes of microorganisms. Reaction rates, degradation products, and the response of the microbial community to substrate additions are expected to be completely different under aerobic conditions. To our knowledge, this study is the first to measure in situ rates of propane and oxygen utilization during aerobic cometabolism in any aquifer system and the first to demonstrate in situ transformations of nontoxic CAH surrogate compounds for an aerobic cometabolic process. This study also demonstrates that push-pull tests can be successfully conducted with highly volatile gaseous substrates and that biostimulation tests can successfully stimulate propane-utilizing activity to the point that it can be detected in tests lasting only a few tens of hours.

In this aquifer, injected test solutions remained in the vicinity of the well for a sufficient length of time to allow for the detection and quantification of microbial activity, even though reported Darcy velocities for the site are fairly high (0.35 m/day). Because the Darcy velocity was computed using K values obtained by slug tests (accuracy ±100%), one possibility is that reported velocity values are overestimates. However, a few calculations with an analytical solution to the advective/dispersion equations readily shows that dispersion/dilution of the test solution during advective transport is sufficient to cause detectable concentrations of a conservative tracer to remain in the well casing for many days, even if the ground water velocity was as large as 1 m/day. Thus, these tests are feasible at most sites. At sites with even higher ground water velocities, or at sites where rates of substrate utilization are much smaller, data can still be obtained if the volume of injected test solution is increased to compensate for the increased rate of drift.

The use of dilution-adjusted concentrations based on concentrations of a conservative tracer like bromide is a

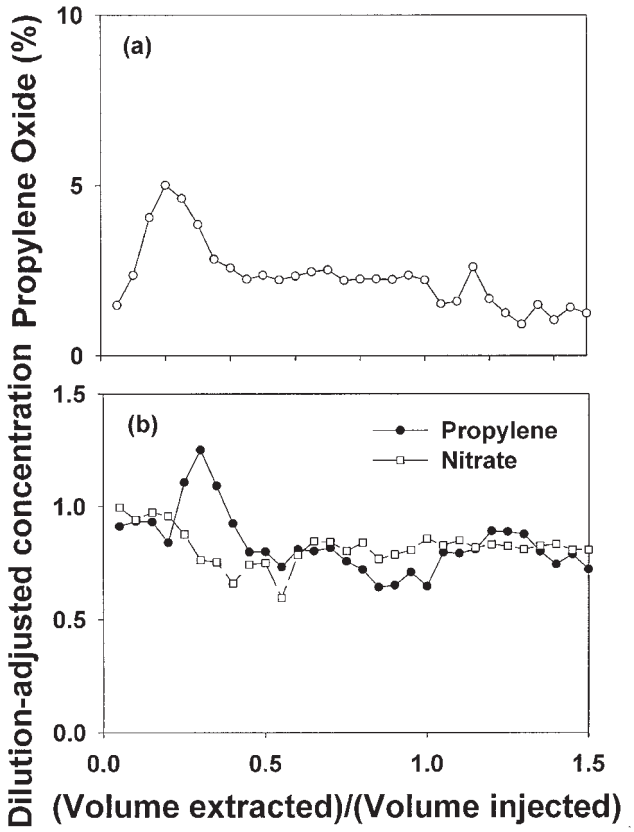

Figure 6. (a) Propylene oxide concentrations in the extracted ground water as a percent of average propylene concentration in injected test solution; (b) dilution-adjusted concentrations for propylene and nitrate during propylene activity test.

simple and easy way to distinguish between concentration decreases due to dilution by ground water flow and biological utilization of injected substrates (Figure 4). Stimulation of indigenous propane utilizers was clearly demonstrated in the increased rates of propane and oxygen utilization observed during successive biostimulation tests. The ratio of growth substrate (propane) mass utilized to oxygen mass consumed could also be estimated from mass balance calculations. Dilution-adjusted concentrations can also be used to compute initial rates for propane and oxygen utilization. However, this approach assumes that tracer and gaseous substrates are transported similarly, which was confirmed for this site by the results of the transport test.

Activity tests were performed in the sequence (1) propane, (2) ethylene, and (3) propylene, and computed transformation rates decreased in the order propane > ethylene > propylene. Ethylene and propylene appeared to be good CAH surrogates to probe for cometabolic activity, since the in situ production of ethylene oxide and propylene oxide could be readily detected and quantified during activity tests. However, care needs to be taken in comparing relative transformation rates of ethylene and propylene, since the sequence of the tests can affect the cometabolic activity of the microorganisms. For example, microorganisms might have lost some of their cometabolic transformation capacity using energy reserves to transform ethylene, resulting in a lower propylene transforming activity. To overcome this issue, we recommend that additional biostimulation tests be performed between ethylene and propylene activity tests, allowing transformation rates of ethylene and propylene to be normalized to measured propane utilization rates. This would permit a more accurate comparison of the rates of ethylene and propylene transformation.

Although zero-order kinetics described the data reasonably well, it is recognized that a mechanistic description of substrate utilization and aerobic cometabolism observed in these tests would likely require a more complicated kinetic expression (i.e., first- or second-order or some mixed expression). Zero-order kinetics may be appropriate in these tests as initial substrate concentrations were likely higher than the saturation constant for this system, obscuring any rate dependence on concentration. However, it is recognized that the data collected in these tests are not sufficient to develop a complete kinetic model for either substrate utilization or aerobic cometabolism of the surrogate CAH compounds. In this paper, computed rates are used simply to facilitate comparisons between tests and to allow order-of-magnitude comparisons to be made between rates obtained from these tests and those reported in the literature for very different experimental systems.

Although this study demonstrated a method for assessing feasibility for in situ aerobic cometabolism activity, the tests were performed in an aquifer that was not contaminated with CAHs. An extensive series of tests at a CAH-contaminated site is in progress to verify the results reported here. In addition, it would be useful to examine the relationship between measured rates of ethylene and propylene transformation and measured rates of CAH transformation. Blocking tests using monooxygenase inactivators, such as acetylene, could also be performed to demonstrate monooxygenase activity. In addition, it would be interesting to evaluate a suite of potential growth substrates such as propane, methane, and toluene at a single site.

The push-pull test method presented here has several potential advantages over traditional methods such as microcosm tests and well-to well tests. Tests can be performed in situ using existing monitoring wells. Injected test solution also interrogates a larger and likely more representative volume of aquifer material than can be obtained by coring. Moreover, tests can be completed more rapidly and at lower cost compared to larger scale well-to-well recirculation tests.

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