Final Report

Push-Pull Tests for Evaluating the Aerobic Cometabolism of Chlorinated Aliphatic Hydrocarbons

by

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ABBREVIATIONS AND ACRONYMS

ACFEE	Air Force Center for Environmental Excellence
CAH	chlorinated aliphatic hydrocarbon
$CaCl_2$	calicium chloride
cis-DCE	*cis*-1,2-dichloroethene
$CoCl_2$	cobalt chloride
$CuSO_4$	copper sulfate
DO	dissolved oxygen
DoD	Department of Defense
ESTCP	Environmental Security Technology Certification Program
$FeCl_3$	ferric Chloride
FID	flame ionization detector
GC	gas chromatograph
HP	Hewlett Packard
H_3BO_3	acid boric
KH_2PO_4	potassium dihydrogen phosphate
McAFB	McClellan Air Force Base
MCLs	maximum contaminant levels
$MgSO_4$	magnesium sulfate
MW	monitoring wells
NA	not analyzed
Na_2EDTA	ethylenediaminetetraacetic acid disodium salt
Na_2MoO_4	sodium molibdate
ND	not detected
NETTS	National Environmental Technologies Test Site program
NH_4NO_3	ammonium nitrate
NO_3	nitrate
O_2	oxygen
OSU	Oregon State University
P&T	purge and trap
PCE	tetrachloroethene
	perchloroethene
PID	photoionization detector
QA/QC	quality assurance/quality control
TCE	trichloroethene
trans-DCE	*trans*-1,2-dichloroethene
U.S. EPA	United States Environmental Protection Agency
VC	vinyl chloride
VOA	volatile organic analysis
USAF	United States Air Force
$ZnSO_4$	zinc sulfate

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EXECUTIVE SUMMARY

The single-well test methods were developed and demonstrated to determine: 1) the transport characteristics of nutrients, substrates, and CAHs and their transformation products; 2) the capability of indigenous microorganisms capability to utilize selected substrates and transform targeted contaminants and surrogate compounds; 3) the rates of substrate utilization and contaminant transformation; and 4) the combinations of injected nutrients and substrates that maximize rates of contaminant transformation.

In the McAFB demonstration, propane was added as the cometabolic substrate, and ethylene and propylene were used as surrogates compounds. The transformation of these compounds to their oxides is diagnostic of the presence of microorganisms with the targeted cometabolic activity. Test solutions were prepared from site groundwater, which was amended with a bromide tracer and combinations of propane, oxygen, nitrate, ethylene, propylene, cis-DCE, and TCE. Transport push-pull tests showed 80 to 90 % of the injected tracer; substrates and surrogated could be recovered upon extraction and little or transformation or retardation occurred during transport. Biostimulation tests showed the initial rates of propane utilization were very low, and rates increased substantially following five sequential additions of dissolved propane and oxygen over a period of 75 days. Push-pull activity tests and natural drift activity tests provided similar results and showed injected propane and oxygen were consumed, and that injected ethylene and propylene were transformed to ethylene and propylene oxide. Transformation of cis-DCE and TCE proved more difficult to assess, since they were present in the injected groundwater at concentrations lower than were present in the aquifer. However, normalization with respect to the background concentrations indicated that cis-DCE was transformed. In a final test the utilization of propane and the transformation of cis-DCE and ethylene were inhibited by acetylene, a known inhibitor of the propane monooxygenase enzyme.

The effectiveness of gas sparging to stimulate indigenous propane utilizers or methane utilizers was evaluated in the second McAFB demonstration, also using single well test methods. Transport tests showed sulfur hexafluoride (SF_6) was transported similarly to coinjected bromide tracer, indicating conservative transport of dissolved gases in the absence of microbial transformations. A series of biostimulation tests were performed by sparging propane (or methane)-oxygen-argon-SF_6 gas mixture at specific depth intervals using a “straddle” packer. Biostimulation was demonstrated with repeated gas sparging tests, where the time to deplete methane and propane concentrations decreased compared to SF_6. Propane (or methane) utilization, oxygen consumption, and ethylene and propylene cometabolism were demonstrated in gas sparging activity tests, with ethylene oxide and propylene oxide observed as cometabolic by-products. When acetylene was included in the gas mixture, propane and methane utilization and ethylene and propylene transformation were effectively blocked, indicating monooxygenase enzymes were involved.

The Ft. Lewis tests demonstrated that indigenous tolueneutilizers could be stimulated. The sequence and methodology for the tests was similar to that of the first demonstration at McAFB. Biostimulation test solutions contained dissolved toluene substrate, hydrogen peroxide, bromide, and nitrate. During the biostimulation tests, decreases in toluene concentration and the production of o-cresol as an intermediate oxidation product indicated the simulation of toluene-utilizing microorganisms containing an ortho-monooxygenase enzyme. Transformation tests

demonstrated that indigenous microorganisms have the capability to transform the surrogate compound (e.g. isobutene) and both cis-DCE and trans-DCE. Isobutene was transformed to isobutene oxide, indicating transformation by a toluene ortho-monooxygenase, and both cis-DCE and trans-DCE were added to the injected fluid and were transformed at similar rates. Similar rates of toluene-utilization, and cis-DCE, and isobutene transformation were achieved using the push-pull activity tests and the natural-gradient tests. In a final test, the utilization of toluene, and the transformation of isobutene, cis-DCE, and trans-DCE were all inhibited in the presence of 1-butyne, a known inhibitor of the toluene ortho-monooxygenase enzyme.

The demonstrations showed that single-well tests can be a cost effective method for evaluating the potential for in situ cometabolism. The method is less costly than well-to-well tests, and can be applied to standard monitoring wells. A guidance document was written on test protocols that will help with the transition of this technology into practice.

1. Introduction

1.1 Background

Aerobic cometabolism is a promising technology for the in situ remediation of chlorinated aliphatic hydrocarbons (CAH) at Department of Defense (DoD) sites. Low-cost methods are needed for generating the data required to design field-scale systems. This report describes a newly developed single-well technology for evaluating the feasibility of using in situ aerobic cometabolic processes to treat groundwater contaminated with chlorinated solvent mixtures.

The Environmental Security Technology Certification Program (ESTCP) supported a three-year field study to investigate single-well tests to evaluate the potential for aerobic cometabolism of CAHs. Tests were performed at McClellan Air Force Base (McAFB), California, using propane as the cometabolic substrate, and Fort Lewis Logistics Center, Washington, using toluene as the cometabolic substrate. McAFB was selected as the demonstration site since significant CAH contamination of groundwater exists, and it was also the site of the ESTCP demonstration of cometabolic air sparging (CAS) with propane as a growth substrate. In the Fort Lewis demonstration, toluene was evaluated as a cometabolic growth substrate and different surrogates and inhibitors were evaluated.

A single well push-pull test consists of the controlled injection ("push") of a prepared test solution into an aquifer using an existing monitoring well followed by the extraction ("pull") of the test solution/groundwater mixture from the same location after allowing time for reactions to occur. A second type of test is a natural-drift test which differs from the push-pull test in that the test solution is not extracted over a short period, but is allowed to drift under natural gradient conditions in the aquifer and samples are periodically taken. A typical field setup used to conduct single-well tests required only simple components, such as pumps to extract groundwater from the test wells, plastic tanks and carboys to hold prepared test solutions, and standard groundwater sampling equipment. The injected test solution consists of water containing one or more conservative (i.e., nonreactive) tracers and one or more reactive solutes; the type, combination, and concentration of reactive solutes are selected to investigate specific aquifer characteristics.

The layout of this report is as follows: Section 1 provides an introduction to the technology including background information, objectives, regulatory drivers, and previous testing of the technology. Section 2 describes the technology, process description, strengths and weaknesses of the technology, and major factors influence cost and performance. Section 3 describes demonstration design, test site description and facilities, the demonstration approach, sampling and monitoring methods, and field and analytical methods. The performance assessment is described in Section 4, which provides an interpretation of the results of the demonstration. The cost assessment in is included in Section 5 and implementation issues, such as cost and performance observations, lessons learned, and approaches to regulatory compliance and acceptance are discussed in Section 6. References are included in Section 7 and points of contact are listed in Section 8.

1.2 Objectives of the Demonstration

The purpose of this demonstration was to evaluate the potential of the push-pull test for determining in situ aerobic cometabolism of CAHs, such as trichloroethene (TCE), using gaseous cometabolic substrates such as propane and soluble substrates, such as toluene. The push-pull method is described and was evaluated to obtain the following site-specific information:

1) To determine the transport characteristics of nutrients, substrates, and CAHs and their transformation products,

2) To determine whether indigenous microorganisms have the capability to utilize selected substrates and transform targeted contaminants,

3) To determine rates of substrate utilization and contaminant transformation, and

4) To optimize combinations of injected nutrients and substrates to maximize rates of contaminant transformation.

1.3 Regulatory Drivers

The target CAH compounds for the single-well test technology include the chlorinated ethenes [trichloroethene (TCE), cis-1,2-dichloroethene (*cis*-DCE) and trans-1,2-dichloroethene (*trans*-DCE), 1,1-dichlorothene (1,1-DCE), and vinyl chloride (VC)]; the chlorinated ethanes [(1,1,1-trichloroethane (1,1,1-TCA) and the lower chlorinated ethane isomers]; and the chlorinated methanes [chloroform (CF) and the lower chlorinated methanes]. The regulatory drivers for these environmental contaminants are maximum contaminant levels (MCLs) governed under the Safe Drinking Water Act (42 U.S.C s/s 300f et seq. 1994). The U.S. EPA has set a maximum contaminant level (MCL) of 0.005 mg/L for TCE, 0.07 mg/L for cis-DCE, 0.1 mg/L for trans-DCE, and 0.002 mg/L for VC (Source: http://www.epa.gov/safewater/mcl.html#3).

1.4 Stakeholder/End-User Issues

The demonstration provides information on how to both conduct and analyze push-pull tests for evaluating the potential aerobic cometabolism as a potential remediation process. Different methods are evaluated for conducting the tests, including activity tests and natural gradient "drift" tests, as well as gas sparge tests. This provides the end user with options for selecting test methods that might be most appropriate of the site of interest and that best fits with the logistical support for conducting the tests. For example, if on site support of daily sampling is available, and the groundwater velocity is slow enough, then natural gradient "drift" tests might be the test of choice, since they are easier to perform than the activity tests. Tests were also developed for the three most common cometabolic substrates: methane, propane, and toluene. Thus the end users have been provided with surrogate compounds for use with the different cometabolic substrates as well as agents to block the enzyme activity. A protocol document has been written to aid the end-user in the future application of technology.

2. Technology Description

2.1 Technology Development and Application

The recently developed push-pull method was successfully used to evaluate the potential of in situ aerobic cometabolic processes to treat groundwater contaminated with chlorinated solvent mixtures. The technology is called the single-well push-pull test. A push-pull test consists of the controlled injection ("push") of a prepared test solution into an aquifer followed by the extraction ("pull") of the test solution/groundwater mixture from the same location. Tests may be performed in existing monitoring wells or multilevel samplers. The injected test solution consists of water containing one or more conservative (i.e., nonreactive) tracers and one or more reactive solutes; the type, combination, and concentration of reactive solutes are selected to investigate specific aquifer characteristics. During the *injection phase*, the test solution is injected into the aquifer where it flows approximately radially outward and penetrates a roughly cylindrical volume of aquifer material centered about the well (Figure 2.1A). During the *extraction phase*, flow is reversed and the test solution/groundwater mixture is pumped from the same location and concentrations of tracer, reactive solutes, and possible reaction products are measured as a function of time (Figure 2.1B).

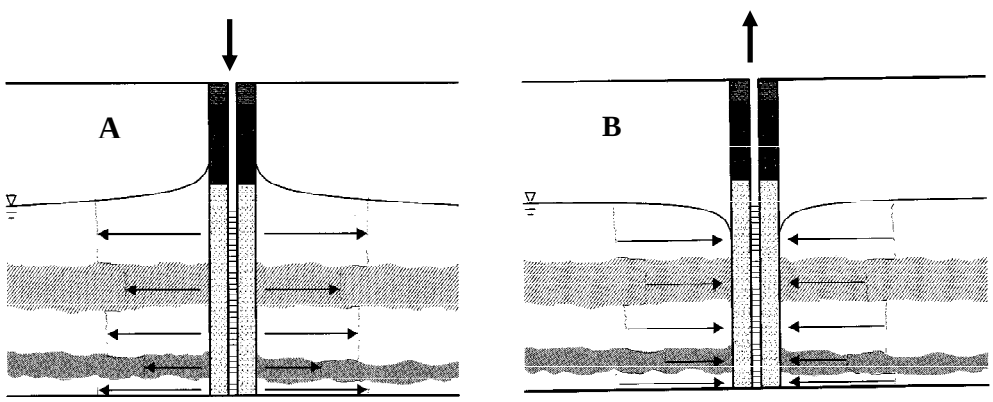

Figure 2.1. Injection and extraction phases of a "push-pull" test

Tracer concentrations are used to adjust concentrations of reactive solutes and reaction products for dilution. Mass balances are computed by integrating dilution-adjusted concentrations during the extraction phase. Reaction rates are computed from the mass of reactive solute consumed and/or product formed.

The push-pull method is simple, inexpensive and may be more representative of the degradative activity of the resident microbial population compared to microcosm tests that use small size samples of aquifer material and are performed under laboratory conditions that mimic in situ conditions. The method requires only simple components, such as pumps to extract groundwater from the test wells, plastic tanks and carboys to hold prepared test solutions, and standard groundwater sampling equipment.

2.2 Previous Testing of the Technology

Push-pull tests have been previously used to obtain quantitative information on a variety of aquifer physical, chemical, and microbiological characteristics (Istok et al., 1997; Schroth et al., 1998; Istok et al., 1999; Schroth et al. 2001; Hageman et al., 2001). Currently, the push-pull method is under investigation as a tool for measuring in situ rates of microbially mediated uranium reduction (Istok et al., 2004) and of anaerobic BTEX degradation (Reusser et al., 2002).

2.3 Factors Affecting Cost and Performance

When comparing push-pull tests with alternative technologies, cost and performance of push-pull tests are less costly than well-to-well recirculation tests. Push-pull tests are more costly to perform than microcosm tests, however microcosm tests require site core materials which are expensive to obtain. Push-pull tests are conducted under in situ conditions and can be used at existing monitoring wells. The single-well, push-pull test provides rapid in situ means of generating data needed for designing in situ cometabolic treatment systems. Test can be performed over a period of about two months. The method may eliminate the need for obtaining core samples and conducting microcosm studies. Compared to microcosm tests, this in situ test will be more representative of the actual environmental conditions that will be encountered in the field. The method should permit for more rapid deployment of this treatment process at DoD sites.

Push-pull tests can be performed at lower costs than well-to-well recirculation tests or at more discrete locations at the site. Limitation of the tests includes the need for successive biostimulation injections, especially when the groundwater velocities may be high. Also personnel must be well trained to conduct these tests.

2.4 Advantages and Limitations of the Technology

The push-pull method offers a number of advantages over microcosm studies. It can be used on site at existing monitoring wells and consequently explores a much larger volume of sediment and groundwater.It is simple, inexpensive and may be more representative of the degradative activity of the resident microbial population. A technical risk is that this technology will not work at a site where microbes are not present to aerobically metabolize CAHs. If this is the case, bioaugmentation of microorganisms that have this ability to grow on CAHs may be a necessary step. Recently in situ bioaugmentation for cometabolic degradation of environmental contaminants has been tested by OSU researchers at the Moffett field site, CA. This method could be applied with push-pull tests where desired bacteria populations are not available at test site.

A major problem limiting the widespread use of aerobic cometabolism for treating chlorinated aliphatic hydrocarbons (CAHs) contamination in groundwater is the need for site-specific data for use in feasibility assessment and remedial design. Currently, the approach used to obtain this information consists of preliminary laboratory microcosm tests performed on core samples followed by pilot-scale well-to-well recirculation tests (Semprini et al., 1992). Although this approach has been successfully applied in a limited number of field demonstrations, it has

several disadvantages that limit its routine use. For example, sediment samples are difficult to obtain and samples obtained by coring may be too small to provide representative information on subsurface conditions. Well-to-well recirculation tests interrogate a larger volume of the subsurface and thus have the potential to provide more representative information but are expensive and logistically complicated.

3. Demonstration Design

3.1 Performance Objectives

The primary performance objective for this study was to demonstrate push-pull tests to assess the potential for aerobic cometabolism of CAHs, such as trichloroethene (TCE), using gaseous and liquid cometabolic substrates such as propane and toluene, respectively. A series of push-pull tests are described (Table 3.1) that can be used to obtain the following site-specific information:

Table 3.1: Performance Objectives

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Actual Performance
Quantitative	Determine Transport Characteristic of nutrients, substrates, CAHs, and transformation products	Similar transport and recovery as bromide the conservative tracer	Transport and recovery was similar to bromide the conservative tracer
Quantitative	Biostimulation can be achieved through successive additions of substrate, dissolved oxygen and nutrients under natural gradient conditions	Biostimulation will be achieved as indicated by increasing rates of substrate and dissolved oxygen utilization	Biostimulation was achieved as indicated by increasing rates of substrate and dissolved oxygen utilization
Quantitative	Activity tests can be used to determine rates of substrate utilization and surrogate transformation	Rates of substrate utilization and surrogate transformation can be estimated from activity tests	Rates of substrate utilization and surrogate transformation where estimated from activity tests
Quantitative	Products formed from surrogate transformation can be tracked and quantified	Products could be detected and quantified	Products were detected and quantified
Quantitative	Transformation of CAHs in the site's GW could be determined	Concentrations decreases would be observed in push-pull tests	Decreased in concentrations of background CAHs were not observed and rates could not be determined
Quantitative	Rates of transformation of CAHs can be determined when added above the injected concentrations	CAH concentrations decreases can be used to estimate rates of transformation	CAH concentration decreases were observed and rates of transformation were estimated
Quantitative	Natural drift tests yield similar rate estimates as activity tests	Rates can be determined from natural drift tests	Similar rates were determined in drift tests as activity tests
Quantitative	Biological transformation can be selectively blocked with mechanistic based inhibitors	Blocking agents would inhibit substrate utilization, oxygen consumption and the transformation of CAHs	Blocking agents inhibited substrate utilization, oxygen consumption, and CAH transformation

3.2 Selecting Test Site

Our first and second site demonstrations evaluated aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs) using propane as a cometabolic substrate were performed at McAFB. The McAFB site had relatively high TCE concentrations and a wide distribution of CAH compounds. This study was conducted at Operating Unit A (OU A). The site chosen was the site of the ESTCP demonstration of cometabolic sparging. Upon first inspection, OU A appeared to have relatively permeable zones and to be geologically suitable for air sparging. The site also was remotely located at the southern end of the base in an area of low vehicular traffic and minimal above ground obstructions. Our third site demonstration evaluated aerobic cometabolism of CAHs using toluene as a cometabolic substrate. The demonstration was performed at Fort Lewis Logistics Center,WA. Groundwater samples from these wells had TCE and cis-DCE concentrations ranging from 50-500 ug/L and dissolved oxygen concentrations were around 5-6 mg/L.

3.3 Test Site Description

3.3.1 McClellan Site Description

Field tests were performed at the site of the former McClellan Air Force Base near Sacramento, CA. Dissolved propane was added as the cometabolic substrate, and ethylene and propylene were used as surrogates compounds. The site was that used for the ESTCP demonstration or cometabolic sparging (Tovanabootr et. al., 2001). The aquifer consists primarily of alluvial deposits, and is unconfined with a water table depth ranging from 30 to 32 m below ground surface. In the first demonstration, push-pull tests were performed in two monitoring wells (MW2 and MW3) at McAFB, CA. The aquifer at this site is mainly contaminated with cis-DCE (20 – 40 µg/L) and TCE (200 – 400 µg/L), and is aerobic (~ 6.2 mg/L dissolved oxygen). The concentration of cis-DCE, TCE, DO, NO_3^-, Cl^-, and SO_4^{-2} at MW1, MW2, MW3, and MW4 wells are given in Table 3.2. The aquifer consists primarily of alluvial deposits, and is unconfined with a water table depth ranging from 30 m to 32 m below ground surface. The monitoring wells were constructed of 5.1 cm polyvinyl chloride casing with a 2.9 m long screen.

In the second demonstration both propane and methane were evaluated as cometabolic substrates. In these tests the substrate, oxygen, and surrogate compounds were directly sparged into the aquifer. The tests with propane were conducted in well MW1 and with methane in well MW2. Figure 3.1 is showing the demonstration locations at McAFB.

Table 3.2. Background Groundwater Sample Composition from Monitoring Wells from McClellan AFB.

Well Location	cis-DCE (µg/L)	TCE (µg/L)	Oxygen (mg/L)	NO_3^- (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)
MW1	37 2.5±	408 ±24	7.9 ±0.7	1.0 ±0.03	18 ±0.3	2.3 ±0.35
MW2	26 ±2.7	92 ±25	6.1 ±0.2	3.6 ±0.6	15 ±0.4	2.1 ±0.26
MW3	22 ±4.2	270 ±33	5.4 ±0.6	1.1 ±0.1	15 ±0.4	1.7 ±0.1
MW4	1420	1860	-	-	-	-

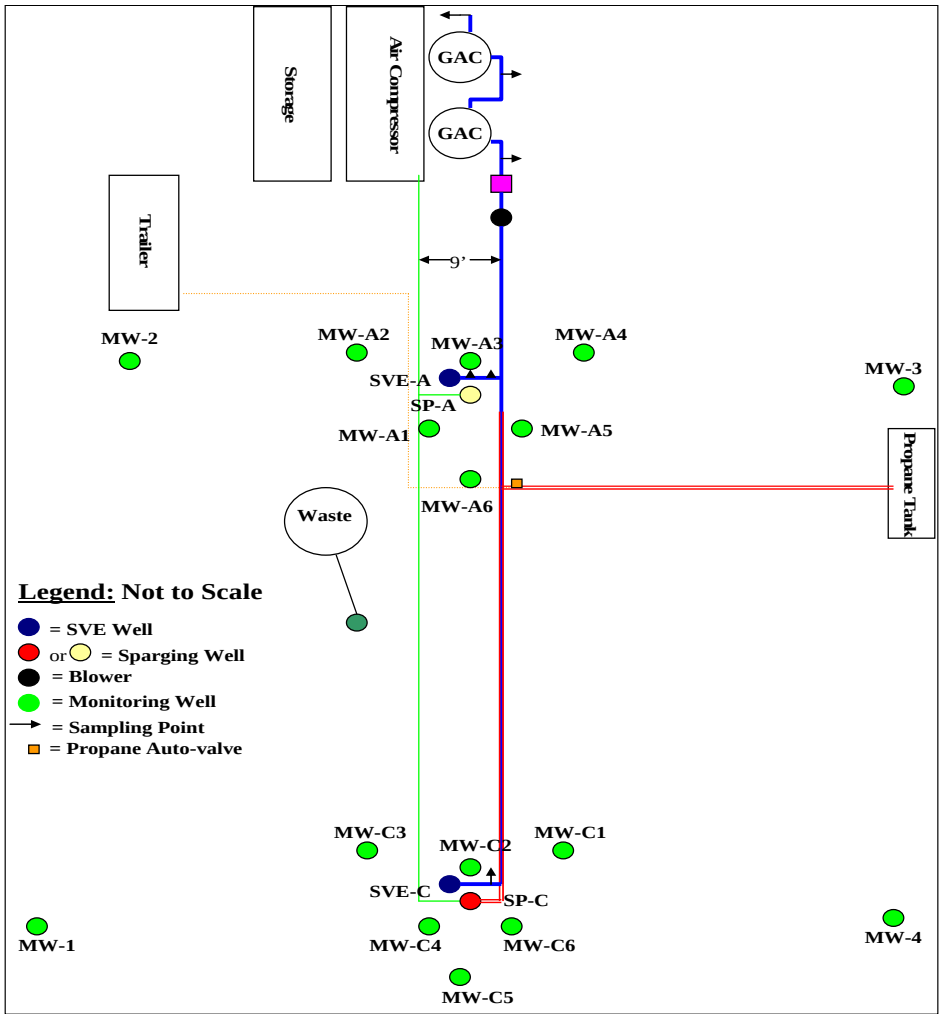

Figure 3.1. CAS Site Layout at McAFB

3.3.2 Fort Lewis Site Description

Tests were conducted in a shallow alluvial aquifer in the area of Fort Lewis known as the East Gate Disposal yard (EGDY), formerly known as Landfill 2. The EGDY site consists of approximately 29 acres of which 13.5 acres is fenced area (U.S. Army, 2002). The EGDY was used as a disposal site for TCE between 1940 and 1970 (U.S. Army, 2002). The depth of groundwater at the site is approximately 10 feet and groundwater velocities across EGDY range from 0.25 to 0.75 feet per day (ESTCP, 2001). LC191 and LC192 were multi-port monitoring wells selected for the push-pull tests. Each multi-port well installed at the EGDY was made of continuous, multi-channel, extruded polyethylene tubing called CMT tubing manufactured by Solinst Canada Ltd. CMT well tubing is 1.7 inches in outer diameter and is customized with up to seven individually screened intervals, called ports, from which groundwater samples are collected (U.S. Army, 2002). The six outside chambers of a CMT well were 7/16-inch in diameter, while the inside chamber was 3/8-inch in diameter. The multi-port monitoring wells were of interest since they allow for the use of smaller injection volumes, which simplified test logistics. The concentration of cis-DCE, TCE, DO, NO_3^-, Cl^-, and SO_4^{-2} at the multipoint locations are given in Table 3.3. The aquifer was aerobic in the region of these tests. cis-DCE and TCE concentrations were generally below 500 ug/L, which is ideal for aerobic cometabolism. Figure 3.2 is showing the demonstration locations at Fort Lewis.

The series of transport, biostimulation, and activity tests were performed in Ports 1 and 2 (P1 and P2) (25 ft and 35 ft depths, respectively) of each well (LC191 or LC192). Test solutions were prepared with groundwater extracted from each port and amended with a suite of solutes and injected into the same location. Bromide was used as a non-reactive tracer in all tests. Reactive solutes included the dissolved growth substrate (toluene), hydrogen peroxide, as a source of dissolved oxygen, non-toxic surrogates (isobutene), and nutrient (NO_3^-). All of these compounds and cis-DCE and TCE present in the groundwater were measured during the injection and extraction phases.

Table 3.3. Background Groundwater Sample Composition from Injection Wells from the Fort Lewis Field Data.

Well Location	cis-DCE (µg/L)	TCE (µg/L)	Oxygen (mg/L)	NO_3^- (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)
LC191-P1	281 ± 22	118 ± 9	5.6 ± 0.6	3.1 ± 0.1	6.3 ± 0.3	9.9 ± 0.3
LC191-P2	161 ± 13	112 ± 9	4.9 ± 0.5	1.85 ± 0.09	4.3 ± 0.2	10.3 ± 0.3
LC192-P1	60 ± 5	460 ± 37	6.6 ± 0.7	3.00 ± 0.1	3.9 ± 0.2	9.7 ± 0.3
LC192-P2	47 ± 3	514 ± 41	6.4 ± 0.5	2.51 ± 0.12	3.2 ± 0.16	11 ± 0.3

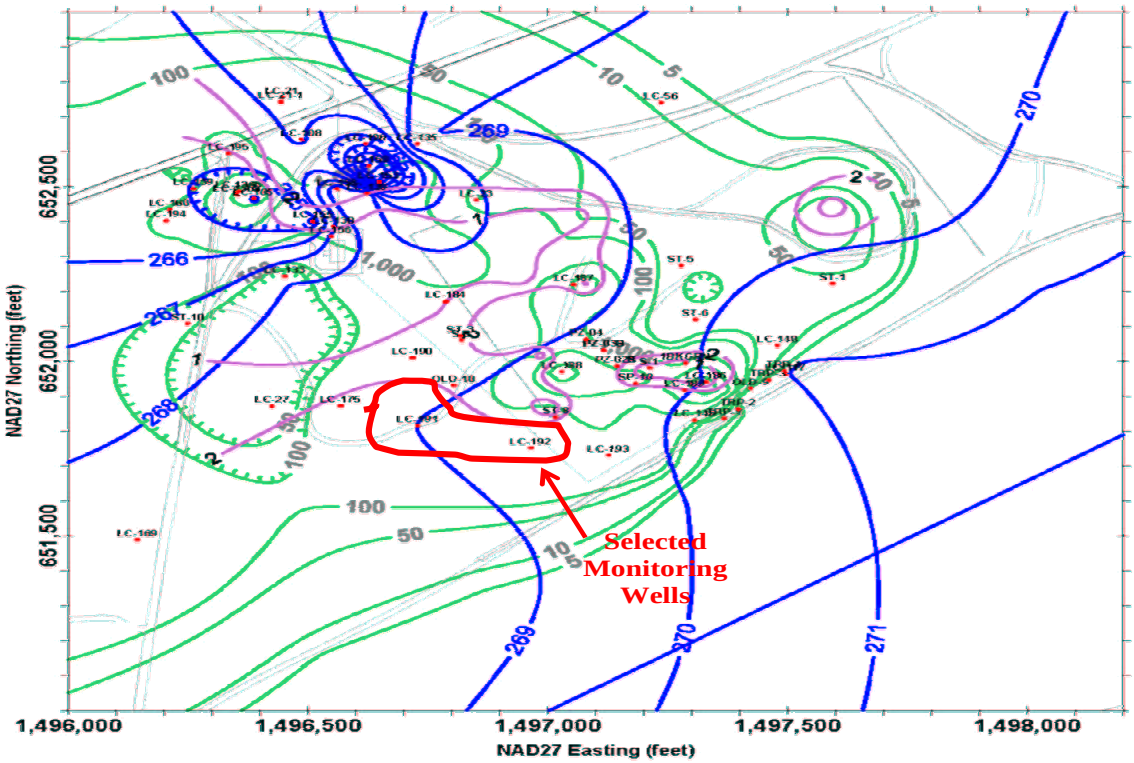

Figure 3.2. Fort Lewis site view showing selected LC191 and LC192 wells for the pushpull tests.

3.4 Pre-Demonstration Testing and Analysis

Previously, a series of single-well drift and push-pull tests were conducted at McAFB, CA, in two monitoring wells, where aquifer is contaminated mainly with cis-DCE and TCE. Successive push-pull activity tests were performed after biostimulation was achieved using the same procedures as the transport tests. To probe transformation activity on cis-DCE and TCE, which transformed to produce cis-DCE epoxide and TCE expoxide, ethylene and propylene were used as the surrogates of two CAHs. Propane utilization, DO consumption, and ethylene and propylene cometabolism were well-demonstrated in successive push-pull tests. The stimulated propane utilizers cometabolized ethylene and propylene to produce ethylene oxide and propylene oxide, cometabolic by-products. Zero-order rates were estimated and ranked from the highest to the lowest as follows: propane > ethylene > propylene.

Previous microcosm laboratory tests showed cis-DCE was cometabolized more rapidly than TCE. Thus, if CAH cometabolism is occurring, the concentration ratios of cis-DCE/TCE should reflect this rate difference. A greater decrease in the ratio was observed during the propane activity tests than during the ethylene and propylene activity tests, as expected since propane was rapidly removed and served as an energy source to enhance cometabolism. The result indicates that cis-DCE cometabolism is likely occurring.

3.5 Testing and Evaluation Plan

Typically, a series of parallel tests is conducted in adjacent wells to examine the effects of physical or chemical heterogeneity on microbial activity or to evaluate various treatment alternatives. A series of push-pull tests can be used to obtain the following site-specific information:

- To determine the transport characteristics of nutrients, substrates, and CAHs and their transformation products,
- To determine whether indigenous microorganisms have the capability to utilize selected substrates and transform targeted contaminants,
- To determine rates of substrate utilization and contaminant transformation, and
- To optimize combinations of injected nutrients and substrates to maximize rates of contaminant transformation.

3.5.1 Series of Tests to be Performed

A series of tests (Figure 3.3) are conducted in a single monitoring well. Transport characteristics (e.g., retardation factors) of substrates, contaminants, and, in some cases their transformation products are needed to compute substrate utilization and contaminant transformation rates and are also needed as input to site-scale groundwater flow and contaminant transport modeling and these are obtained using *Transport Tests*. Transport Tests are conducted in a way that minimizes the potential for substrate utilization or contaminant transformation.

Biostimulation Tests are designed to stimulate microbial activity. Rate of substrate utilization and contaminant transformation are determined using *Activity Tests,* which are conducted under conditions that promote the expression of indigenous microbial activity. transport tests are conducted first,then a series of biostimulation tests is conducted to stimulate microbial activity.

Activity tests are conducted to demonstrate aerobic cometabolic activity of the indigenous microorganisms by monitoring the rate of consumption of injected nutrients (e.g., nitrate) and gaseous substrates (e.g., propane and oxygen), the production of defined products from injected surrogate compounds (e.g. the production of ethylene oxide from injected ethylene and the production of propylene oxide from injected propylene), and the production of defined CAH oxidation products (e.g. the production of cis-DCE epoxide).

The final test is an *inhibition test*, where a mechanism based inhibitor of the enzyme of interest is added to inhibit the transformations observed in the previous activity test and to confirm that observed reactions are microbially mediated.

Direct gas sparging (e.g., propane and methane) into an aquifer, as an alternate method for introducing gaseous substrates was tested in our second demonstration at McAFB. This method involves direct gas injection where potentially only one addition is made with a prolonged release of the dissolved gaseous substrate and oxygen. Direct injection of a gaseous substrate/air mixture may result in formation of gaseous bubbles in the aquifer and sand pack around well casing. It is likely that even if most of the bubble resides in the sand pack around well, they will slowly dissolve, and propane/methane and oxygen will be transported into the aquifer under natural gradient conditions.

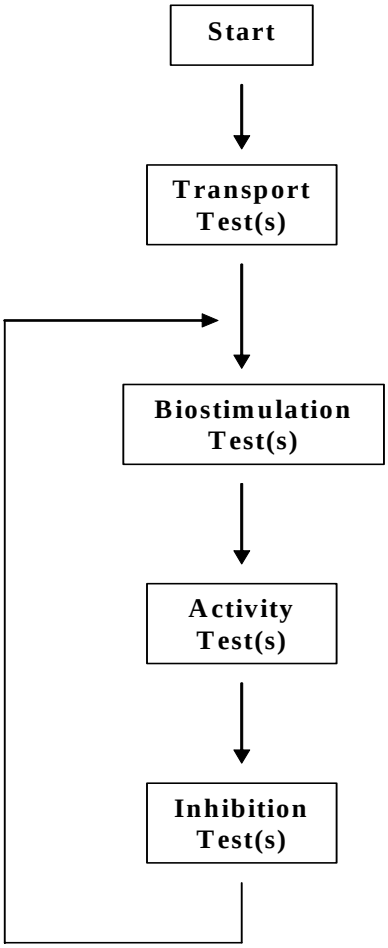

Figure 3.3. Push-pull test sequence

3.5.2 Period of Operation

The three demonstrations were conducted of three seasons of testing. Typically a period of six to eight months was required to complete the tests described in Figure 3.3. Table 3.4 presents the period of operation for conducting the tests.

Table 3.4. Period of Operation

McClellan Propane Tests	Test	Duration
	Transport	1/05/01 - 2/05/01
	Biostimulation	2/05/01 - 4/15/01
	Activity Tests (Push-pull)	4/15/01 - 7/15/01
	Natural Drift Activity	7/15/01 - 8/15/01
	Blocking Tests	8/15/01 - 9/15/01
McClellan Sparging Tests	Transport	12/01/01 -1/05/01
	Biostimulation	1/05/02 - 2/15/02
	Activity Tests – Sparge Tests	2/15/02 - 4/15/02
	Blocking Tests	4/15/02 - 5/15/02
Fort Lewis Toluene Tests	Transport	1/05/03 - 2/05/03
	Biostimulation	2/05/03 - 4/15/03
	Activity Tests (Push-pull)	4/15/03 - 7/15/03
	Natural Drift Activity	7/15/03 - 8/15/03
	Blocking Tests	8/15/03 - 9/15/03

3.6 Experimental Design

A typical field setup used to conduct push-pull tests is shown in Figure 3.4. The method requires only simple components, such as pumps to extract groundwater from the test wells, plastic tanks and carboys to hold prepared test solutions, and standard groundwater sampling equipment. The injected test solution consists of water containing one or more conservative (i.e., nonreactive) tracers and one or more reactive solutes; the type, combination, and concentration of reactive solutes are selected to investigate specific aquifer characteristics. The following paragraphs describe how a series of push-pull tests are conducted.

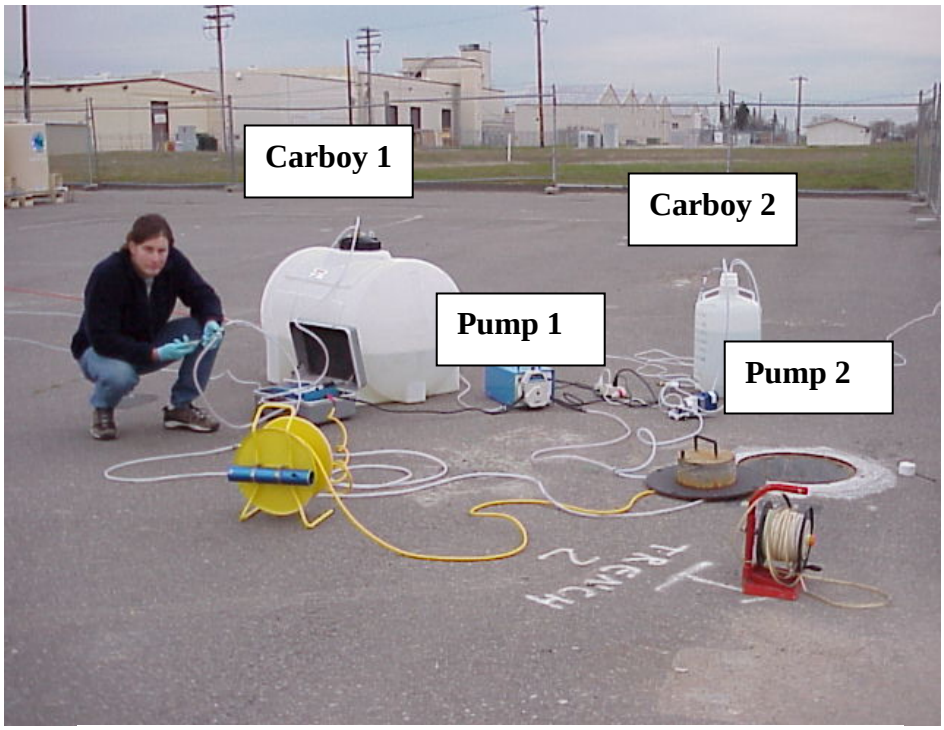

Figure 3.4. Typical field setup for push-pull tests

3.6.1 Transport Tests

Test solutions for transport tests contain a tracer and additional solutes (either substrates, CAH surrogates, or CAHs) for which transport information is desired. Note that it is also possible to simultaneously obtain transport information for additional solutes present in site groundwater components if these are *not* present in the injected test solution but are analyzed for during the extraction phase. Transport tests are conducted under conditions selected to minimize the opportunity for microbial transformation of injected solutes. This is usually accomplished by selecting injection and extraction pumping rates that minimize the total time that the test solution is in contact with the aquifer. For example, the composition of the injected test solution may be adjusted, for example by removing a necessary nutrient (e.g., NO_3^-) or substrate (e.g., O_2). The volume of injected test solution is selected to interrogate a sufficient volume of aquifer so that representative results are obtained. Samples of the test solution are collected during the injection phase so that the initial concentrations of all solutes are known. Additional samples are collected during the extraction phase to develop breakthrough curves for all injected solutes and, if desired, solutes present in the site groundwater that were not included in the injected test solution. In a transport test, extraction pumping continues until approximately twice the injection volume has been recovered, which is usually sufficient to recover a substantial portion of the injected test solution.

3.6.2 Biostimulation Tests

Biostimulation tests are designed to expose the indigenous microbial community to nutrients and substrates for extended periods of time (days to weeks) to stimulate growth and activity. The injected test solutions contain only tracer, nutrients, and gaseous substrates or soluble substrates (no surrogates or CAHs). This approach utilizes aqueous solutions to deliver dissolved substrates and nutrients to the aquifer.

The extraction phase of a biostimulation test consists of discrete sampling events instead of the continuous extraction phase pumping and sampling used for transport and activity tests. The frequency of the sampling events is selected to provide sufficient data to monitor changing concentrations of substrate during the test. Biostimulation tests are often repeated until the resulting increase in activity is large enough to be detected by an activity test (Figure 3.3). The biostimulation test data are interpreted using the method of Haggerty et al. (1998), which involves plotting dilution-adjusted solute concentrations as a function of residence time. Dilution adjustments are performed using measured concentrations of the bromide tracer of the injected solute in the aquifer (for solutes with retardation factors equal to one) or with retardation factors estimated from transport tests (for solutes with retardation factors greater than one). The sample residence time is defined as the elapsed time from the midpoint of the injection phase to the time the sample was collected.

3.6.3 Activity Tests

Unlike transport tests, activity tests are conducted under conditions that allow microbial activity to be detected. Thus, injected test solutions contain all nutrients and substrates required for a particular reaction to proceed. Samples are collected during the injection phase of an activity test so that initial concentrations of all solutes are known. A drift phase with no pumping is typically included between the injection and extraction phases. The duration of the drift phase is selected to be long enough to permit detectable consumption of injected substrates (e.g., O_2, propane or toluene), surrogates (e.g., ethylene, isobutene), or CAHs (e.g., cis-DCE) and detectable production of surrogate or CAH transformation products (e.g., ethylene oxide or isobutene oxide). The duration of the drift phase must also be selected to be sufficiently short that a substantial portion of the injected test solution can be recovered during extraction phase pumping. Regional groundwater flow will eventually transport injected test solutions away from the well and reduce measured solute concentrations below detection limits. The effect of regional flow can be mitigated by injecting a larger volume of test solution, reducing the duration of the drift phase, or increasing the extraction phase pumping rate.

Activity test data are interpreted using the method of Haggerty et al. (1998), which involves plotting dilution-adjusted solute concentrations as a function of sample residence times. Dilution adjustments are performed using measured concentrations of the bromide tracer (for solutes with retardation factors equal to one) or with retardation factors estimated from transport tests (for solutes with retardation factors greater than one). The sample residence time is defined as the elapsed time from the midpoint of the injection phase to the time the sample was collected.

Example is in Section 4.1.3. Activity tests are typically conducted before--and after--biostimulation tests so that increases in microbial activity resulting from biostimulation may be detected and quantified. Typically rates of nutrient and substrate utilization and surrogate and/or CAH transformation increase following biostimulation and thus it may be desirable to decrease the duration of the rest phase as microbial activity increases.

3.6.4 Inhibition Tests

The final test to be performed is the inhibition test. The inhibition test is the same as an activity test, except a mechanistic based inhibitor of the monooxygenase enzyme of interest is added along with the substrates of interest. For propane utilizers, acetylene is used as the inhibitor of the oxygenase enzyme, while 1-butyne was used as an inhibitor of the toluene ortho-monooxygenase enzyme. Test procedures are exactly the same as used in the activity test so direct comparison between the test can be made. If effective inhibition is achieved the results from inhibition test should be similar to those observed in the transport test.

3.7 Test Solution Preparation

3.7.1 Conservative Tracer and Nutrients

Although many conservative (i.e., nonreactive) tracers have been used in groundwater studies, bromide at a concentration of 100 mg/L was used as a conservative tracer for push-pull tests. This concentration was selected as a compromise between analytical detection limits (~ 1 mg/L for Br^- by ion chromatography) and the desire to avoid injecting test solutions with densities substantially larger than that of site groundwater. Bromide is added as potassium bromide (KBr). If background Cl^- concentrations are below a few mg/L, Cl^- (added as NaCl) is an acceptable alternative. Alternative tracers may be used if their conservative transport behavior is demonstrated (e.g., by performing a transport test with coinjected bromide) and if their chemical and microbial stability can be assured for the duration of activity and biostimulation tests. Nitrate in the form of sodium nitrate ($NaNO_3$) may be added as a nutrient in some tests. Both KBr and $NaNO_3$ are highly water-soluble; thorough mixing of added KBr and $NaNO_3$ is accomplished during the gas sparging used to introduce gaseous substrates and surrogate compounds to the test solution.

3.7.2 Gaseous Substrates and Surrogate Compounds

Gaseous substrates (propane and oxygen) and surrogate CAHs (propylene and ethylene) were introduced into the test solution by bubbling (sparging) the test solution contained in plastic carboys with a defined mixture of compressed gases (Figure 3.5). Sparging also serves to thoroughly mix the test solution with respect to added KBr and $NaNO_3$. Site groundwater was used to prepare three solutions: 1) 500-L with known concentrations of bromide (KBr, Spectrum Chemical Mfg. Corp. Gardena, CA) to serve as a nonreactive tracer, nitrate ($NaNO_3$, Mallinckrodt Chemical, Inc. Paris, KY) as a trace nutrient, and dissolved oxygen as an electron acceptor; 2) 50-L with known concentrations of high purity (> 99.0 %) dissolved propane, ethylene, and/or propylene (>99.0%) (Airgas Inc., Randor, PA) to probe for microbial activity; and 3) 5 L with known concentrations of high purity (99.6 %) dissolved acetylene (Airgas Inc.,

Randor, PA) in a collapsible metalized-film bag for use in inhibition tests. Specified dissolved gas concentrations in the carboys were achieved by controlling gas flow rates to ceramic sparging stones placed in the bottoms of the carboys. 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, IL). Specified dissolved gas concentrations in the metalized -film bag were achieved by injecting known volumes of gas into the bag through a septum. After dissolved gas concentrations had stabilized, the contents of the carboys and metalized-film bag were combined to obtain the desired solute concentrations using calibrated peristaltic and piston pumps and injected into the well. Specified aqueous concentrations of substrate and surrogate CAHs were achieved by controlling the flow rate of each gas to the sparging lines. The flow rates were selected to achieve a desired partial pressure of each gas in the carboy headspace; from the partial pressures, the aqueous concentration of each gas may be determined using the solution temperature and Henry's law constant for the gas. Gas flow rates were controlled and gases were mixed using gas flowmeters (Cole-Parmer Instrument Co., Vernon Hills, IL), which were calibrated for each specific gas used. For all tests it was necessary to avoid creating an explosive gas mixture in the carboy headspace. To avoid this problem, a portion of the test solution was contained in one carboy and sparged with the flammable gases (propane, ethylene, and/or propylene) and a portion was contained in a second carboy and sparged with oxygen (Figure 3.5). The two portions of test solution were combined by pumping from each carboy into a single injection line and mixed with a mixing coil prior to injection. The resulting dissolved gas composition of the injected test solution was therefore controlled by the partial pressure of each gas in the two carboys and the two pumping rates. Samples of the injected test solution were collected using a syringe and sampling valve during the injection phase and analyzed for aqueous gas concentrations so that the composition of the injected test solution was well known. The composition of the test solution was monitored during and after injection by collecting samples from the well using a submersible pump.

3.7.3 Gas Sparging

Sparging gas mixtures including propane or methane and oxygen may cause a safety issue with injecting a gas above the lower explosive limit (LEL). For safety considerations, propane (or methane) concentration in injected gas mixture was maintained below LEL (2.1% for propane and 5% for methane). The flammable gas level was monitored using an LEL detector on the site during gas sparging. An on-line automatic gas shut-off valve connected to LEL detector with alarms was also installed. The electric valve will shut off gas flow at 90% of LEL. The LEL detector was calibrated with calibration gases of 90% LEL (1.8% for propane and 4.5% of methane). Lines from the propane, oxygen and argon tanks were fitted with check values that prohibit backflow of the gases into the tanks.

Rotameters were used to regulate the flow of argon, propane, and oxygen to achieve the desired injection concentrations of the sparge gases. The three gases were mixed into one line at the surface so that a controlled concentration mixture below the LEL is achieved and monitored throughout the sparging event. The system used is shown in Figure 3.6. The rotameter was installed on a gas proportioner multitube frame fitted with calibrated direct reading flow tubes

(Cole-Parmer Instrument Co., Vernon Hills, IL). The gas-flow line (Masterflux Tygon® lab tube) from a rotameter was directly connected to the gas purging line installed between the packers. The gas purging line consisted of gas-flow lines connected with a purging stone near the lower packer.

The propane (or methane)/oxygen/argon gas mixture was sparged at specific depth intervals using the “straddle” packer system shown in Figure 3.4. The upper and lower packers were pressurized with air to isolate a specific depth interval, permitting the injected gases to be transported into the aquifer. The lower packer was placed near bottom of the well to stimulate propane (or methane) utilizers over whole screen interval, since gas bubbles move upward through the formation.

3.7.4 Liquid Substrates and Surrogate Compounds

The test solution was prepared with groundwater extracted from the well or well port where push-pull tests solution was to be injected. Bromide was used as a non-reactive tracer. Reactive solutes include the dissolved growth substrate (toluene), hydrogen peroxide (DO), non-toxic dissolved surrogate isobutene, and nitrate as a nutrient. Groundwater needed for making the inject solution was pumped from the wells using a Masterflex peristaltic pump (Barnant Co., Barrington, IL). The test solution was prepared by adding bromide, nitrate and hydrogen peroxide in a plastic carboy and thoroughly mixed. Toluene was added to a collapsible Teflon bag and to achieve a desired concentration. Isobutene solution was prepared in a plastic carboy with the same method as described in Section 3.7.2. The different injection solutions were mixed together at different flow rates to achieve the desired injection concentration. Figure 3.7 shows a schematic of equipment used to introduce liquid substrates and surrogates in single push-pull field tests.

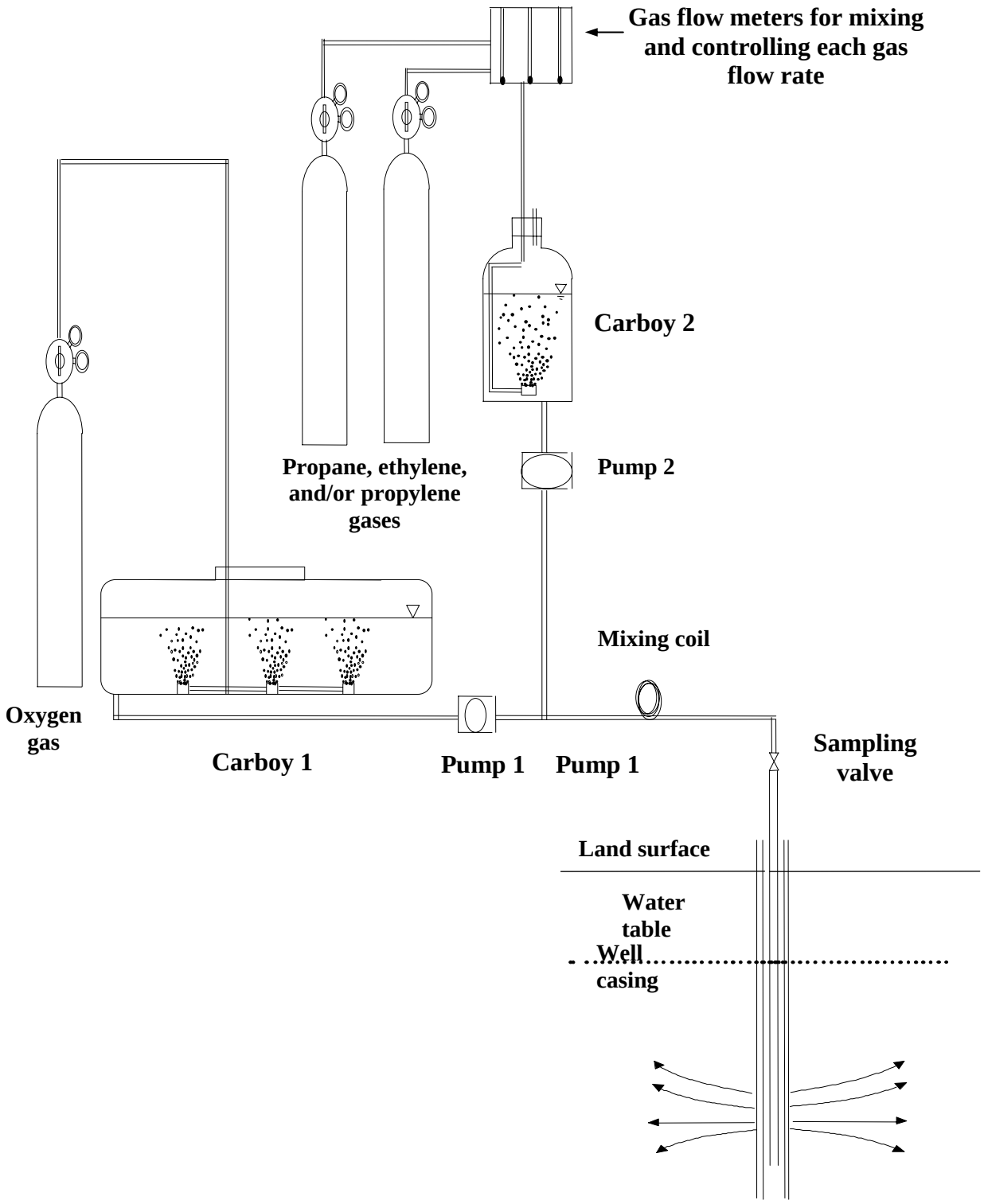

Figure 3.5. Equipment used to introduce gaseous substrates and surrogates into injected test solutions.

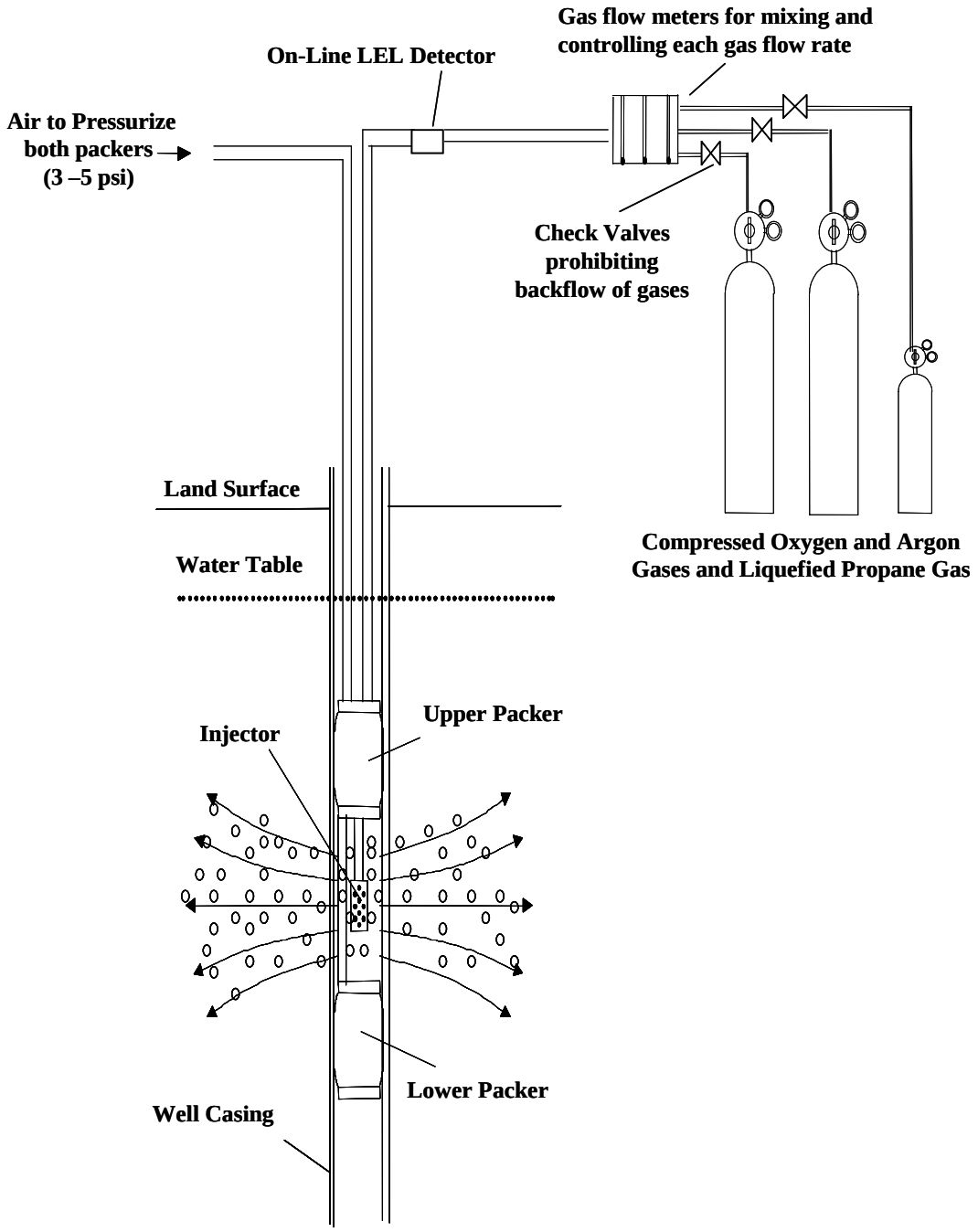

Figure 3.6. Set-up for direct gas sparging into saturated aquifer at injection rates of 110 L/min for 6 hours.

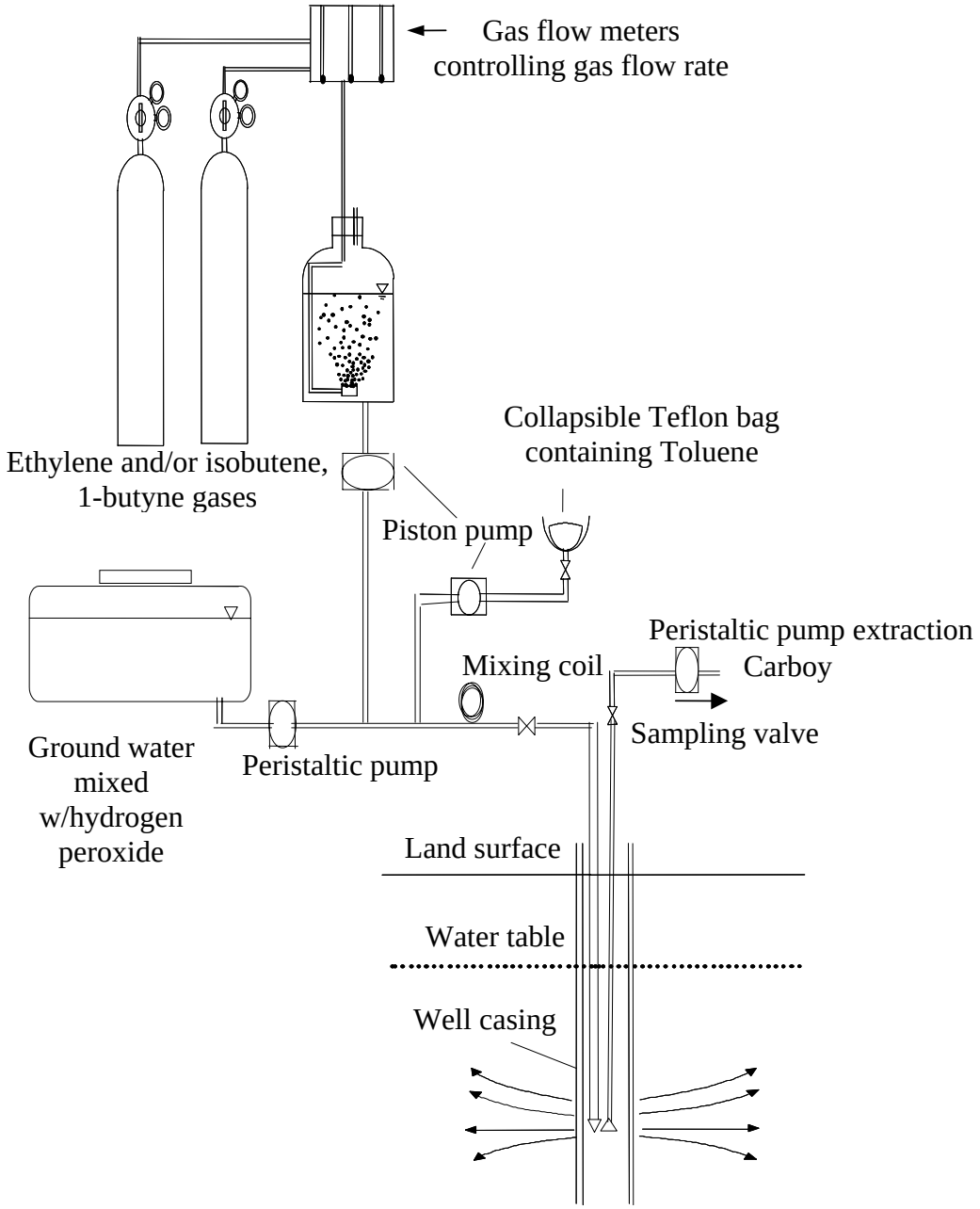

Figure 3.7. Schematic of equipment used to introduce liquid substrates and surrogates in single push-pull field tests.

3.8 Sampling, Monitoring, and Analytical Procedures

3.8.1 Sample Collection

Liquid samples were required for analysis of injected tracer, nutrient, substrate, surrogates, CAHs, and their transformation products. A sampling valve equipped with a syringe adapter was used to collect samples during the injection and extraction phases of all tests. To collect a sample, a gas-tight syringe was fitted to the sampling valve, purged several times, and then aspirated to obtain a liquid sample. The time of sample collection was also recorded. The contents of the syringe were dispensed into sample vials as follows: A 1 mL sample was collected in a plain glass vial for tracer (Br^-) and nutrient (NO_3^-) analyses by ion chromatography (IC). A 2 mL sample was collected in a syringe for dissolved oxygen analysis in the field by oxygen electrode. A 40 mL sample without headspace was collected in brown bottles equipped with a septa and a screw cap for substrate, CAHs, and transformation product analyses by gas chromatography (GC). Samples were not preserved with acid, since abiotic transformations of the potential cometabolic by-products ethylene oxide, propylene oxide, and isobutene oxide are acid catalyzed. IC and GC samples were stored at 4 ºC until analyzed.

3.8.2 Determination of Inorganic Anions by Ion Chromatography

Concentrations of inorganic anions (Br^- and NO_3^-) were determined with a Dionex DX-500 (Sunnyvale, CA) ion chromatograph equipped with electrical conductivity detector and a Dionex AS14 column. The eluent consisted of 3.5 mM Na_2CO_3 and 1.0 mM $NaHCO_3$ and the eluent flow rate was 1.5 mL/min. A 0.6-mL sample was transferred to Dionex Polyvials ™ with filter caps for auto-sampler injection; the auto-sampler was programmed to deliver an injection volume of 50 µL. Run time was approximately 10 minutes. External calibration was performed using five standards with anion concentrations between 5 and 100 mg/L; the approximate quantitation detection limit was 0.5 mg/L.

3.8.3 Determination of Dissolved Oxygen by Oxygen Electrode

Dissolved oxygen was determined in the field using a Clark (Yellow Springs, Ohio)-style oxygen electrode and meter. The electrode was mounted in a glass water-jacketed vessel to maintain a stable electrode temperature; the temperature of the water was recorded with a mercury thermometer. The electrode contacts the sample within a small (1.8 mL) volume chamber mounted inside the vessel. To perform a dissolve oxygen measurement, a water sample collected from the sampling valve was dispensed from the syringe into the chamber (filling it to overflowing), which was then closed with a glass plug. A small stir bar within the chamber and an external magnetic stirrer were used to mix the sample during measurement. After the meter reading stabilizes, the oxygen saturation value from the meter was recorded. The sample was then removed from the chamber using a plastic syringe.

To convert oxygen saturation values to concentration units (mg/L), the oxygen saturation of a reference sample was measured immediately after each sample measurement using the same procedure. The reference sample consists of oxygen saturated distilled water, which was

prepared by sparging a 1 L bottle 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 handbook value for oxygen solubility in distilled water at the measurement temperature. Hydrogen peroxide concentrations were monitored using thiocyanate colorimetric method developed by CHEMetrics, Inc. The thiocyanate method consists of ammonium thiocyanate and ferrous iron in acid solution. Hydrogen peroxide oxidizes ferrous iron to the ferric state, resulting in the formation of a red thiocyanate complex. This method covers hydrogen peroxide concentrations of 0-1000 mg/L.

3.8.4 Determination of Gaseous and Liquid Substrates, Surrogate Compounds, and CAHs by Gas Chromatography

Test samples were collected in 40-mL VOA vials with a Teflon/neoprene septum and a polypropylene-hole cap (Supelco, Bellefonte, PA). Samples were not preserved with acid, since the transformation of potential cometabolic by-products, ethylene oxide, propylene oxides, and isobutene oxide are acid catalyzed. Samples for laboratory analysis were stored at 4 ºC and analyzed within one week.

Gaseous substrates, surrogates, and CAHs and their transformation products: Gaseous substrates, surrogates, and CAHs and their transformation products were determined by a modified EPA 8000 purge and trap GC analysis. A 1 or 5 mL aqueous sample was taken from a VOA vial using a S. G. E. 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 a purge trap (Supelco, Bellefonte, PA). A sample purge time of 15 min was used, rather than the standard 5 min, to increase the removal of the less effectively trapped compounds, such as ethylene, and to detect low concentrations of the less volatile metabolic 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 HP6890 series GC connected to a photo ionization detector (PID) followed by a flame ionization detector (FID) operated at 250 °C. The GC was operated at the following conditions: initial oven temperature, 40 °C for 3 min; 4 °C/min up to 70 °C; 5 °C/min up to 220 °C. The GC was operated in the splitless inlet mode with a carrier gas (He) 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 (He) flow of 15 mL/min. 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). The sensitivity of PID and FID on each compound was different, so that each compound was quantified by a more sensitive detector. Ethylene, propane, ethylene oxide, propylene oxide were quantified by FID, and propylene, cis-DCE and TCE were quantified by PID. Calibration curves for the compounds were developed using external standards.

SF_6 analysis method was adapted report by Wilson and McCay (1993). After creating a headspace in a 40-mL VOA vial by extracting 10 mL of aqueous sample from the vial, the vial

was inversely placed and then shaken on a rotary shaker at 20 °C to achieve an equilibrium concentration in the headspace. SF_6 analysis was performed on a GC equipped with an electron capture detector by injecting gaseous samples. A series of SF6 standards were made for calibration of GC.

Liquid substrates, surrogates, and CAHs and their transformation products: The Purge-and-trap method was used in determining the dissolved concentrations of toluene, ortho-cresol, ethylene, isobutene and their transformation products, and CAHs. Five mL aqueous samples from the VOA vials were introduced into an HP 7695 purge-and-trap system, and the volatile compounds were sorbed onto a Vocarb-3000 trap (Supelco, Bellefonte, PA). Optimizing P&T Cycle Time Experimentation yielded a time of 11 min to provide the optimal sample purge for the determination of toluene and o-cresol. Under equivalent conditions, a 5-min purge time did not adequately separate cis-DCE and isobutene oxide. A 2-min desorption time of 250°C accommodated sharp initial peaks and provided good separation. Chromatographic separations were achieved with two 30-m megabore GSQ-PLOT and HP-624 columns from Agilent (New Castle, DE) installed on a HP6890 series GC connected to a photo ionization detector (PID) followed by a flame ionization detector (FID). The GC was operated splitless inlet mode with He carrier gas flow, a H_2 flow to FID detectors of 35 mL/min, an air flow to the detectors of 165 mL/min and a detector makeup gas (He) flow of 15 mL/min. For the GSQ-PLOT column, the GC was operated at the following conditions: column flow 15 mL/min; initial oven temperature, 50°C; 4 °C/min up to 150 °C hold for 3 min; 10 °C/min up to 220 °C. The retention time of each compound under this GC method was as follows: ethylene (3.1 min); isobutene (9.71 min); isobutene oxide (19.97 min); cis-DCE (20.24 min); TCE (25.9 min); toluene (31.81 min); and o-Cresol (34.06 min). The HP624 megabore column was used to better separate the isobutene oxide and cis-DCE with the close retention times of 19.97 min and 20.24 min on GSQ-PLOT column, respectively. Using the HP624 column also supplements the identification of o-cresol and isobutene oxide with authentic standards which were assayed with chromatographic separation. For the HP624 column, the GC was operated at the following conditions: column flow 5 mL/min; the initial oven temperature, 40 °C for 5 min; 3 °C/min up to 100 °C hold for 2 min. The retention time of each compound under this GC method was as follows: isobutene (7.04 min); 1-butyne (7.62 min); trans-DCE (12.28 min); isobutene oxide (12.69 min); cis-DCE (14.87 min); TCE (19.19 min); toluene (23.28 min); and o-Cresol (25.15 min). Calibration curves for the compounds were developed using external standards.

4. Performance Assessment

The performance assessment provides an evaluation of the demonstration of single-well, push-pull tests for feasibility assessments for the aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs) at McAFB and Fort Lewis fields. The demonstrations consisted of a series of push-pull and natural-gradient drift tests, conducted in a logical sequence, so that they were rationally interpreted. Presented in Tables 4.1 and 4.2 are performance criteria, expected performance, and performance confirmation methods for the demonstration.

Table 4.1. Performance Criteria

Performance Criteria	Description	Primary or Secondary
Transport characteristic of nutrients, substrates, CAHs, and transformation products	Demonstrate the substrates, surrogates and nutrients are transported like bromide the conservative tracer	Primary
Biostimulation can be achieved through successive additions of substrate, dissolved oxygen and nutrients under natural gradient conditions	Demonstrate consumption of substrate and the uptake of oxygen in successive push-pull tests	Primary
Activity tests can be used to determine rates of substrate utilization and surrogate transformation	Rates of substrate utilization and surrogate transformation can be estimated using activity tests	Primary
Products are formed from surrogate transformation	Products can be detected and quantified	Primary
Transformation of CAHs in the site's GW could be determined	Concentrations decreases would be observed in push-pull tests	Primary
Rates of transformation of CAHs can be determined when added above the background concentrations	CAH concentrations decreases can be used to estimate rates of transformation	Primary
Natural drift tests yield similar rate estimates as activity tests	Rates can be determined from natural drift tests	Primary
Biological transformation can be selectively blocked with mechanistic based inhibitors	Blocking agents inhibit substrate utilization, oxygen consumption and the transformation of CAHs	Primary
Factors affecting the technology performance	GW flow velocity Depth to groundwater Dissolved oxygen, pH, contaminant concentrations,	Primary
Reliability	Tests can be performed at different sites and different well types	Secondary
Ease of Use	Number and skills of people required to perform tests	Primary
Versatility	Use at several locations. Use with different substrates and surrogate compounds	Primary
Scale-up Constraints	Used standard monitoring well	Secondary

Table 4.2. Expected Performance and Performance Confirmation Methods

Performance Criteria	Expected Performance Metric	Performance Confirmation Method	Actual
Transport characteristic of nutrients, substrates, CAHs, and transformation products	Breakthrough curves similar to bromide tracer; Mass recovery similar to bromide tracer	Determine concentration breakthrough curves; mass balances	Breakthrough curves were similar to bromide tracer; mass recovery were similar to bromide tracer
Biostimulation can be achieved through successive additions of substrate, dissolved oxygen and nutrients under natural gradient conditions	Increased rates of utilization with successive additions	Measurement of concentrations temporally under natural drift conditions	Rates of utilization increased with successive additions
Activity tests can be used to determine rates of substrate utilization and surrogate transformation	Decreased concentrations in breakthrough curves compared to prior transport tests	Determine concentration breakthrough curves; mass balances. Rate estimates	Concentrations decreased compared to the prior transport tests. Rates estimates were made.
Products are formed from surrogate transformation	Products are produced and are apparent in breakthrough curves	Determine product concentration breakthrough curves; mass balances; rate estimates	Products were produced and mass balances permitted production rates to be measured
Transformation of CAHs in the site's GW could be determined	Decrease concentrations in breakthrough curves bromide conservative tracer	Determine concentration breakthrough curves; mass balances. Rate estimates	Decreases in concentration were not evident, and rates could not be determined
Rates of transformation of CAHs can be determined when added above the background concentrations	Decrease concentrations in breakthrough curves compared to the bromide conservative tracer.	Determine concentration breakthrough curves; Mass balances. Rate estimates	Concentrations decreased compared to the prior transport tests and the bromide tracer. Rates estimates were made.
Natural drift tests yield similar rate estimates as activity tests	Decrease concentrations in breakthrough curves compared to the bromide conservative tracer.	Determine concentration breakthrough curves; mass balances. Rate estimates	Concentrations decreased compared to the the bromide tracer. Rates estimates were made.
Biological transformation can be selectively blocked with mechanistic based inhibitors	Concentrations do not decrease compared to the bromide tracer and prior activity test	Determine concentration breakthrough curves; mass balances. Rate estimates	Concentrations did not decreased compared to the the bromide tracer and prior activity test
Factors affecting the technology performance	Similar metrics as above	Similar metrics as above	Tests work as high GW velocities compared to lower and at greater depth compared to shallower depth.
Ease of Use	Personnel required; tests conducted per day	Number and training of personnel	Required at less one high trained technician with field expertise and analytical skills. Up to four well tests conducted per day
Versatility	Similar metrics as above	Similar metrics as above	Method worked well at two different sites; with two different well types; and with three different cometabolic substrates
Scale-up Constraints	Conducted at full scale	Conducted at full scale	Conducted at full scale

A summary of the study results from both sites was presented in Section 4.1 through 4.3, followed by a data assessment presented in Section 4.4, and a technology comparison in Section 4.5.

4.1 Example Results from Field Push-pull Tests Conducted at the McCellan AFB, CA

To illustrate additional details about the push-pull test methodology, a series of single-well-push-pull tests were performed to assess the feasibility of in situ aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethylene (TCE) and cis-1,2-dichloroethylene (cis-DCE), using propane and toluene as growth substrates. Propane and methane tests were performed in the saturate zone at the McAFB, CA, while toluene tests were performed at Fort Lewis, WA. The sequence of field tests followed the flow chart given in Figure 3.3; additional experimental details for each test are given in Tables 4.3, 4.5, 4.6, and 4.7. A transport test was conducted first followed by a series of biostimulation tests and then a series of activity tests. Detailed descriptions of test methodology and test results for each test type are described in the following sections.

4.1.1 Transport Tests

Push-pull tests were performed in two monitoring wells (MW2 and MW3) at McAFB, CA. The aquifer at this site was mainly contaminated with cis-DCE (20 – 40 µg/L) and TCE (200 – 400 µg/L), and was aerobic (~ 6.2 mg/L dissolved oxygen). The aquifer consists primarily of alluvial deposits, and was unconfined with a water table depth ranging from 30 m to 32 m below ground surface. In the first demonstration the tests were conducted in two monitoring wells (MW2 and MW3) constructed of 5.1 cm polyvinyl chloride casing with a 2.9 m long well screen.

Transport tests were conducted in each well. These tests were followed by a biostimulation period consisting of five sequential additions of propane and dissolved oxygen to each well, followed by a series of activity tests and acetylene blocking tests (Table 4.3). Field equipment consisted of compressed or liquefied gases, gas flow meters, two carboys (500 L and 50 L), a collapsible metalized-film gas-sampling bag (Chromatography Research Supplies, Addison, IL), a peristaltic pump to inject the test solution into the well, and a submersible pump (GRUNDFOS Pumps Co, Fresno, CA) to extract groundwater from the same well (Figures 3.5 and 3.6). Site groundwater was used to prepare three solutions: 1) 500-L with known concentrations of bromide (KBr, Spectrum Chemical Mfg. Corp. Gardena, CA) to serve as a nonreactive tracer, nitrate ($NaNO_3$, Mallinckrodt Chemical, Inc. Paris, KY) as a trace nutrient, and oxygen as an electron acceptor; 2) 50-L with known concentrations of one or more dissolved gases [(propane (99.5%), ethylene (>99.9%), and/or propylene (>99.0%); Airgas inc., Randor, PA] to probe for microbial activity; and 3) 5-L with known concentrations of dissolved acetylene (99.6%, Airgas inc., Randor, PA) in a collapsible metalized-film gas-sampling bag. Specified dissolved gas concentrations in the 500 L and 50 L carboys were achieved by controlling the flow rates of each gas to ceramic sparging stones placed in the bottom of the carboys. 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, IL).

Table 4.3. Test Solution Composition for Push-pull Tests Conducted Demonstration 1 at the McAFB, CA (MW2) Field.

Test Type	Injection Volume (L)	Propane (mg/L)	Propylene (mg/L)	Ethylene (mg/L)	[1]Oxygen (mg/L)	[2]NO_3^-N (mg/L)	Br^- (mg/L)	[3]cis-DCE (µg/L)	[3]TCE (µg/L)
Transport Test	264	2.0 ± 0.1	4.0 ± 0.2	4.1 ± 0.2	22 ± 0.8	[3]NI	34 ± 1.5	3.7 ±1.0	27 ±5.1
Biostimulation Period (5 sequential additions)	498 ± 15	7.6 ± 3.0	[7]NI	NI	30 ± 3.5	7.7 ± 0.6	108 ± 20	2.5 ±0.5	28 ±2.5
[5]First Propane Activity Test	238	2.4 ±0.1	NI	NI	30 ±0.8	1.9 ±0.1	40 ±1.5	4.4 ±1.1	54 ±1.1
Second Propane Activity Test	250	1.3 ±0.1	NI	NI	16 ±0.6	4.4 ±0.2	22 ±0.1	2.1 ±0.2	36 ±3.2
Ethylene Activity Test	255	NI	NI	0.67 ±0.02	17 ±0.45	5.8 ±0.3	68 ±1.5	1.3 ±0.01	32 ±2.0
Third Propane Activity Test	251	1.6 ±0.1	NI	NI	18 ±1.0	6.0 ±0.2	122 ±4.3	1.4 ±0.1	31 ±2.6
Propylene Activity Test	255	NI	1.6 ±0.1	NI	16 ±0.6	4.9 ±0.1	228 ±3.5	1.4 ±0.2	33 ±2.1
Fourth Propane Activity Test	317	1.6 ±0.2	NI	2.0 ±0.18	35 ±0.95	3.8 ±0.1	37 ±1.4	5.2 ±0.8	44 ±4.2
[6]Acetylene Blocking Test	346	1.2 ±0.9	NI	2.2 ±0.15	31 ±2.2	7.3 ±0.4	77 ±3.2	4.8 ±0.3	35 ±1.0

[1]: Background average dissolved oxygen concentration of 6.3 mg/L. [2]: Background average NO_3^- (as N) concentration of 1.1 mg-N/L. [3]: Average concentrations of cis-DCE and TCE concentrations in the injected test solution (C_0). [4]: Average values obtained during First through Fifth Biostimulation tests. [5]: The First propane activity test was performed just prior to the second biostimulation test. [6]: Tests were performed in MW3 only. Injected acetylene concentration was ~ 0.5 mM (10 mg/L). [7]: NI indicates not included.

After dissolved gas concentrations had stabilized, the contents of the carboys and metalized bag were combined to obtain the desired solute concentrations using calibrated peristaltic and piston pumps and injected into the well. The composition of the test solution was monitored during injection by collecting samples from the well using a submersible pump.

Samples of the injected test solution were collected by pumping the groundwater from the wells using a Grundfos pump placed down-hole in the screened interval of the well. Thus, the actual concentration of entering the aquifer was monitored. This down-hole sampling method provided very reproducible concentrations of the dissolved gases injected fluid.

A short-duration transport test was conducted in each well to compare the relative mobility of bromide, nitrate, and dissolved propane, oxygen, propylene, and ethylene in the aquifer prior to subsequent tests (Table 4.3). Two hundred sixty liters of test solution (prepared as described above) were injected at 2 L/min. After a 16 hr rest phase with no pumping, the test solution/ground water mixture was extracted from the well at a rate of 2.5 L/min. Samples collected during the extraction phase were analyzed and used to prepare breakthrough curves for each injected solute.

The transport characteristics of all the substrates were very similar to bromide, showing no retardation (Figure 4.1A). Based on mass balances on the injected solutes, the percent recovery of bromide was 99%, while the recovery of other injected solutes were slightly higher or similar to bromide (Table 4.4). Nitrate and dissolved oxygen had recoveries greater than 100% since they are present in the native groundwater. The results demonstrate that the solutes can be effectively injected and recovered using the push-pull method that was developed, even at the aquifer depth of 30 m at the McAFB site. The dilution adjusted concentrations are all near unity (Figure 4.1B) indicating no reaction or retardation.

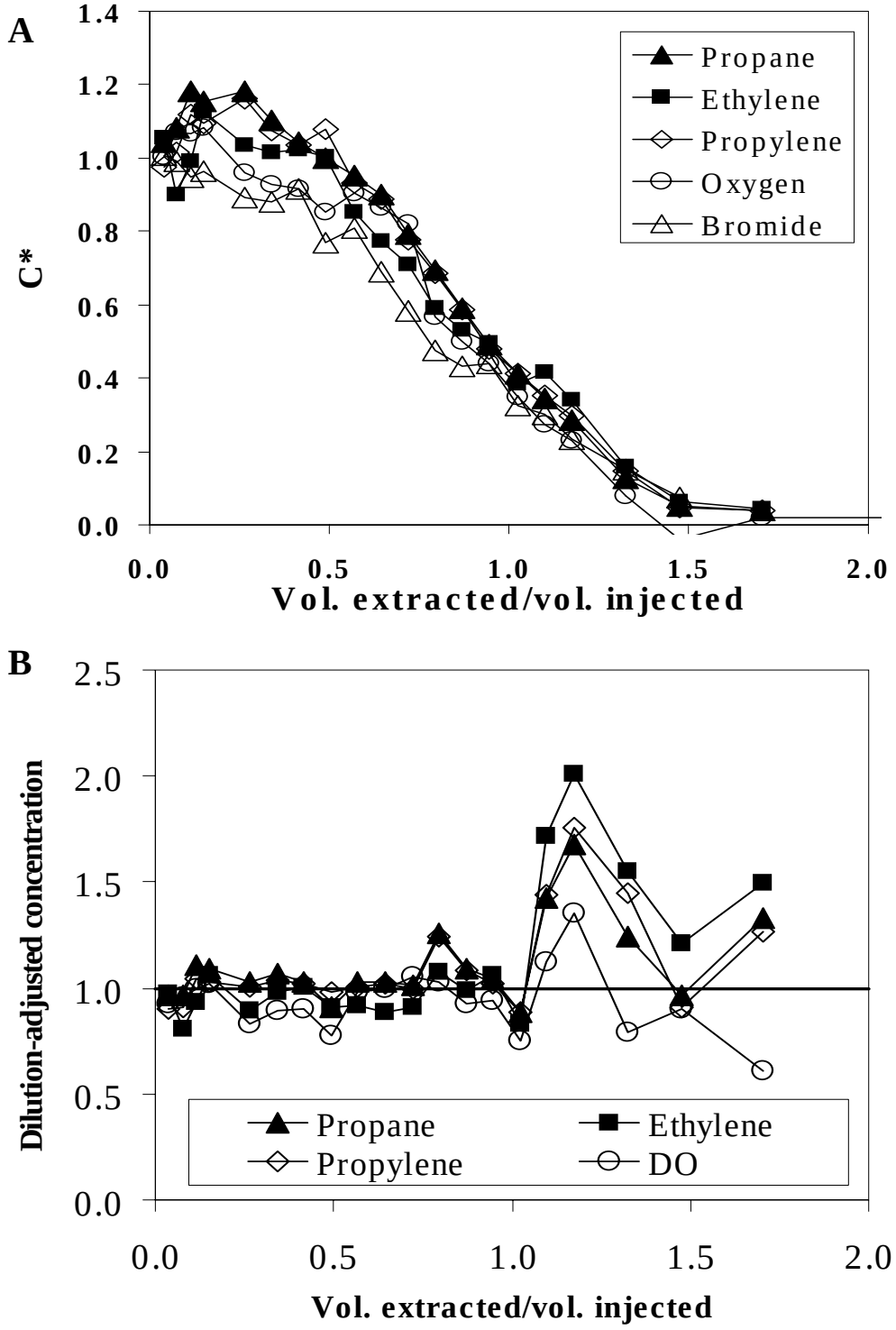

Figure 4.1. Extraction phase breakthrough (A) and dilution adjusted (B) curves in a push-pull transport test conducted at the McClellan AFB, CA (MW2) field.

Table 4.4. Summary of Quantities of Injected and Extracted Solutes Mass, Percent Recovery, and Zero-Order Rate for Push-Pull Tests for MW2 and MW3.

Test Type	Quantities	Propane		Ethylene		Propylene		Br^-	
		MW2	MW3	MW2	MW3	MW2	MW3	MW2	MW3
Transport Test	% recovery	104	105	99	99	103	105	99	98
	rate(µmol/L/hr)	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	≈ 0	-	-
First Propane Activity Test	% recovery	94	94	-	-	-	-	96	88
	rate (µmol/L/hr)	0.09	≈ 0	-	-	-	-	-	-
Second Propane Activity Test	% recovery	31	7	-	-	-	-	[2]107	92
	rate (µmol/L/hr)	1.1	0.8	-	-	-	-	-	-
Ethylene Activity Test	% recovery	-	-	[1]59 (3.1%)	[1]75 (3.8%)	-	-	102	90
	rate (µmol/L/hr)	-	-	0.51	0.35	-	-	-	-
Third Propane Activity Test	% recovery	44	17	-	-	-	-	99	90
	rate (µmol/L/hr)	1.0	1.8	-	-	-	-	-	-
Propylene Activity Test	% recovery	-	-	-	-	[1]75 (2.3%)	[1]69 (0.45%)	92	88
	rate (µmol/L/hr)	-	-	-	-	0.34	0.46	-	-
Fourth Propane Activity Test	% recovery	-	40	-	[1]60 (5.2%)	-	-	-	107
	rate (µmol/L/hr)	-	0.82	-	1.2	-	-	-	-
Acetylene Blocking Test	% recovery	-	90	-	[1]86 (0.12 %)	-	-	-	107
	rate (µmol/L/hr)	-	≈ 0	-	≈ 0	-	-	-	-

[1]: Numbers in parenthesis indicate percentage of the oxide mass extracted to the mass of ethylene transformed. [2]: When bromide recovery is greater than 100%, a value of R_{tracer} in an equation 1 s assumed as 1.00.

4.1.2 Biostimulation Tests

During the biostimulation period, five sequential additions of groundwater containing propane and oxygen were performed in each well to stimulate the activity of indigenous propane oxidizing bacteria. Test solutions were prepared and injected as described above and contained known concentrations of bromide, dissolved propane and oxygen, and nitrate (Table 4.3). Since commercial grade propane can contain ethylene and propylene, high purity propane (99.5%) was used to ensure the stimulation of propane-utilizing microorganisms, and not ethylene-utilizing or propylene-utilizing microorganisms. Periodic sampling of the test solution/groundwater mixture was used to quantify rates of propane and oxygen utilization.

The extraction phase consisted of discrete sampling events distributed over 3-25 days following test solution injection. For each sampling event, groundwater samples were collected and analyzed propane, dissolved oxygen, nitrate, and bromide. In the first biostimulation test, the trends in concentration changes of the three compounds were very similar, showing gradual decreases over 25 days (Figure 4.2). In tests four and five the rates of propane, oxygen (DO), and nitrate utilization increased. The simultaneous decrease in concentrations of the injected electron donor (propane), electron acceptor (oxygen) and nutrient (nitrate) provide evidence that the biostimulation tests were successful in stimulating activity of propane oxidizing bacteria in the subsurface.

The concentration trends in Figure 4.3 can be more clearly seen if the data are adjusted for the dilution of the test solution as a result of groundwater flow. Because the transport test confirmed that propane, oxygen, and nitrate are transported identically to bromide in the absence of microbial utilization, concentrations of these solutes in each sample were adjusted for dilution by dividing measured concentrations for the relative bromide concentration (i.e. the measured bromide concentration divided by the bromide concentration in the injected test solution) for that sample. In biostimulation test five, the normalized concentrations decreased following injection and the rate of utilization increased in subsequent tests (Figures 4.3A and 4.3B). These results suggest the stimulation of propane-utilizing microorganisms was achieved in the repeated push-pull tests. By the fifth test (Figure 4.3B), propane was completely consumed, while oxygen was partially consumed. Incomplete utilization of oxygen and difficulty in clearly observing nitrate utilization resulted from the background oxygen and nitrate concentrations of regional groundwater that mixed with the injected solution.

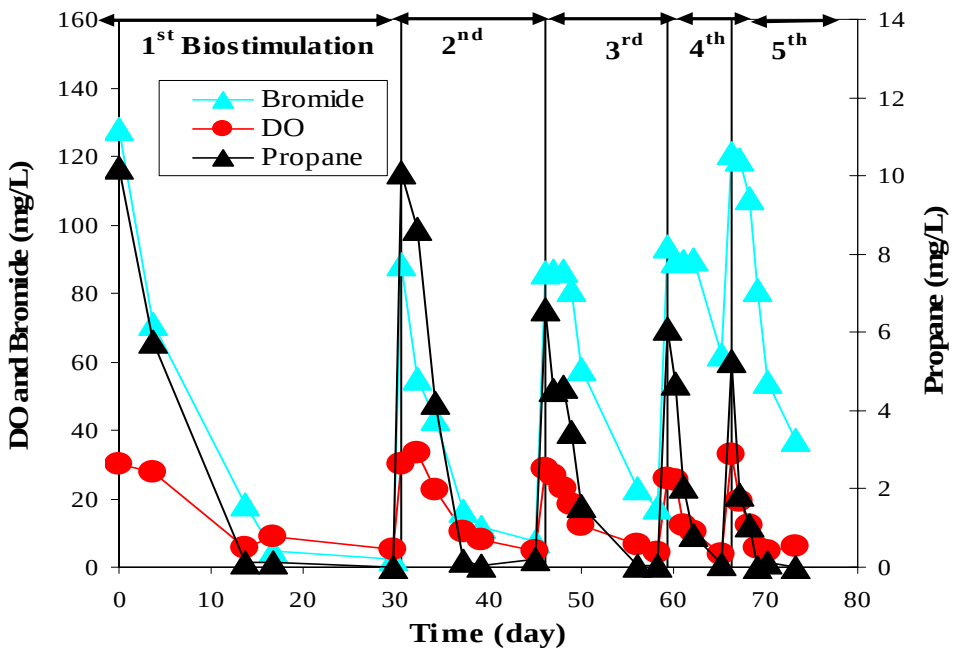

Figure 4.2. Measured propane, oxygen (DO), nitrate, and bromide concentrations during five field biostimulation tests conducted at the McAFB, CA (MW2) field.

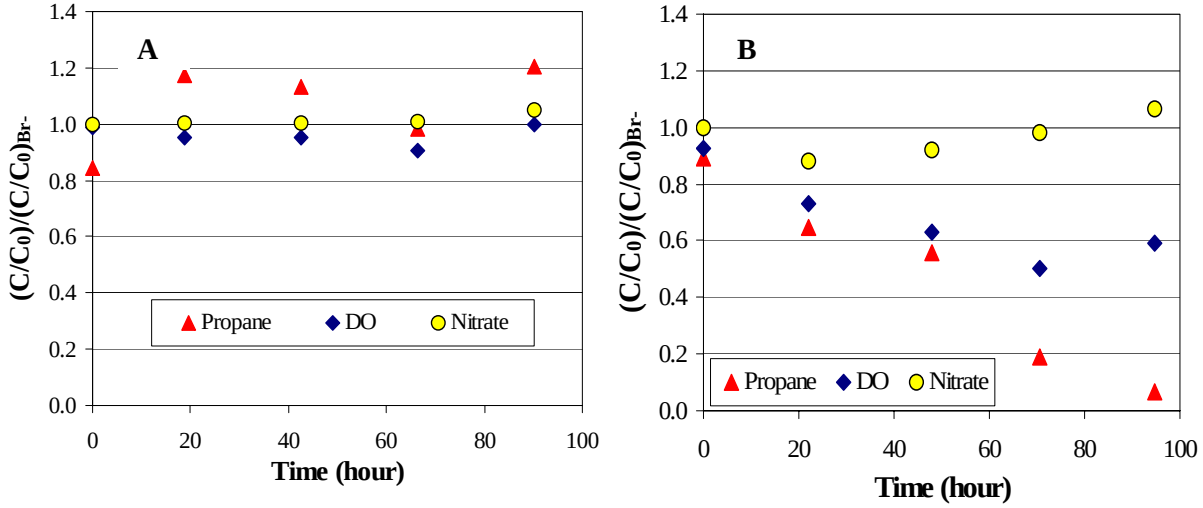

Figure 4.3. Normalized concentrations of propane, oxygen (DO), and nitrate during the third- biostimulation (A) and fifth biostimulation (B) propane biostimulation push-pull tests (MW2) "Drift Test".

4.1.3 Activity Tests

Following the biostimulation period, a series of five activity tests were conducted to quantify rates of propane utilization, ethylene and propylene transformation, and cis-DCE and TCE transformation (Table 4.3). Test solutions were prepared and injected as described above. After rest phase of 12 to 16 hrs with no pumping, the test solution/groundwater mixture was extracted from the well at a rate of 2.5 L/min. Samples collected during the extraction phase were analyzed and used to prepare breakthrough curves for each injected solute and transformation products formed in situ.

Propane Activity Test. After injecting groundwater containing propane, oxygen, nitrate and bromide (Table 4.3), the solution was permitted to react in the aquifer for 12.1 hours and then extracted over a period of 6.5 hours. Propane utilization was not detected during the first propane activity test (conducted just prior to the second biostimulation test) as normalized concentrations of injected propane, oxygen, and bromide were all similar (Figure12A). However, substantial propane and oxygen utilization were observed during the second propane activity test (conducted after the fifth biostimulation test) (Figure 4.4B). Similar results were observed in tests at MW3 (data not shown). Estimated zero-order rates of propane utilization were also similar between wells MW2 and MW3 (Table 4.4).

Ethylene Activity Test. The ethylene activity test was performed to demonstrate cometabolism by propane utilizers, with ethylene acting as a surrogate compound for the CAHs. After injecting the solution containing ethylene, oxygen, nitrate and chloride (Table 4.3), the solution was permitted to react in the aquifer for 12.4 hours and then extracted over a period of 7.3 hours. Chloride was used as a conservative tracer rather than bromide to identify the test solution from the previously injected solution. As shown in Figures 4.5A and 4.5D, ethylene was transformed at a much slower rate than propane was utilized in the previous propane test. Very little uptake of nitrate was observed. During the extraction phase, a by-product having the same retention time on the GC as ethylene oxide was detected (Figure 4.5B). The build-up of the product was associated with ethylene transformation via cometabolism. In Figure 4.5C, extraction phase breakthrough curves for cis-DCE, TCE, and bromide are plotted as 1-C*, that is, $1-[(C - C_{BG})/(C_o - C_{BG})]$. The transformation of cis-DCE and TCE proved more difficult to assess, since they were present in the injected groundwater at concentrations lower than were present in the aquifer. The results indicate decreases below the bromide curve at later time, which indicates some transformation may be occurring. Their transformation at earlier time might have been inhibited by the presence ethylene. Extensive transformation, however, of cis-DCE and TCE was not observed in the 24-hr activity test. It is possible that the presence of ethylene inhibited cis-DCE and TCE transformation.

Propylene Activity Test. A propylene activity test was then performed to demonstrate cometabolism by propane utilizers, with propylene acting as a surrogate compound for CAHs. The injected test solution containing propylene, oxygen, nitrate and bromide, was permitted to react in the aquifer for 13.2 hours and then extracted over a period of 6.7 hours. As shown in Figures 4.6A and 4.6D, propylene was transformed at a slower rate than that of propane or ethylene. Very little uptake of nitrate or oxygen was observed. During the extraction phase, a byproduct having the same retention time on the GC as propylene oxide was detected (Figure 4.6B). The build-up of the product was associated with propylene cometabolism. In Figure 4.6C, extraction phase breakthrough curves for cis-DCE, TCE, and bromide are plotted as 1-C*.

Neither cis-DCE or TCE transformation was indicated from the activity data at early time, with the trends following those of bromide. Some decreases in the normalized cis-DCE and TCE, below the bromide curve, were observed at later time that might be associated with transformation. The presence of propylene at early time may have inhibited their transformation. The activity test results clearly showed that propane utilizers stimulated with repeated push-pull tests were able to cometabolize ethylene and propylene resulting in the formation of the byproducts ethylene oxide and propylene oxide.

Several factors likely contributed to TCE and cis-DCE only being marginally transformed. The residence time for the activity tests were only 24-hrs and the reactions rates were too slow to see significant changes. Also the presence of ethylene and propylene likely inhibited the rates of cis-DCE and TCE transformation, since it was not until later time, when they were reduced to low concentrations, that there was some evidence for TCE and cis-DCE transformation. cis-DCE appeared to be more rapidly transformed than TCE. This result is consistent with the results of the cometabolic air sparging demonstration that was conducted at the same site (Tovanabootr et al. 2001) and the results of microcosm studies performed with aquifer solids and groundwater from the site (Timmins et al. 2001). One possible improvement in the protocol is to conduct some tests where cis-DCE or TCE is added to the test solutions above background concentrations. Results of such tests are presented in the Ft. Lewis demonstration.

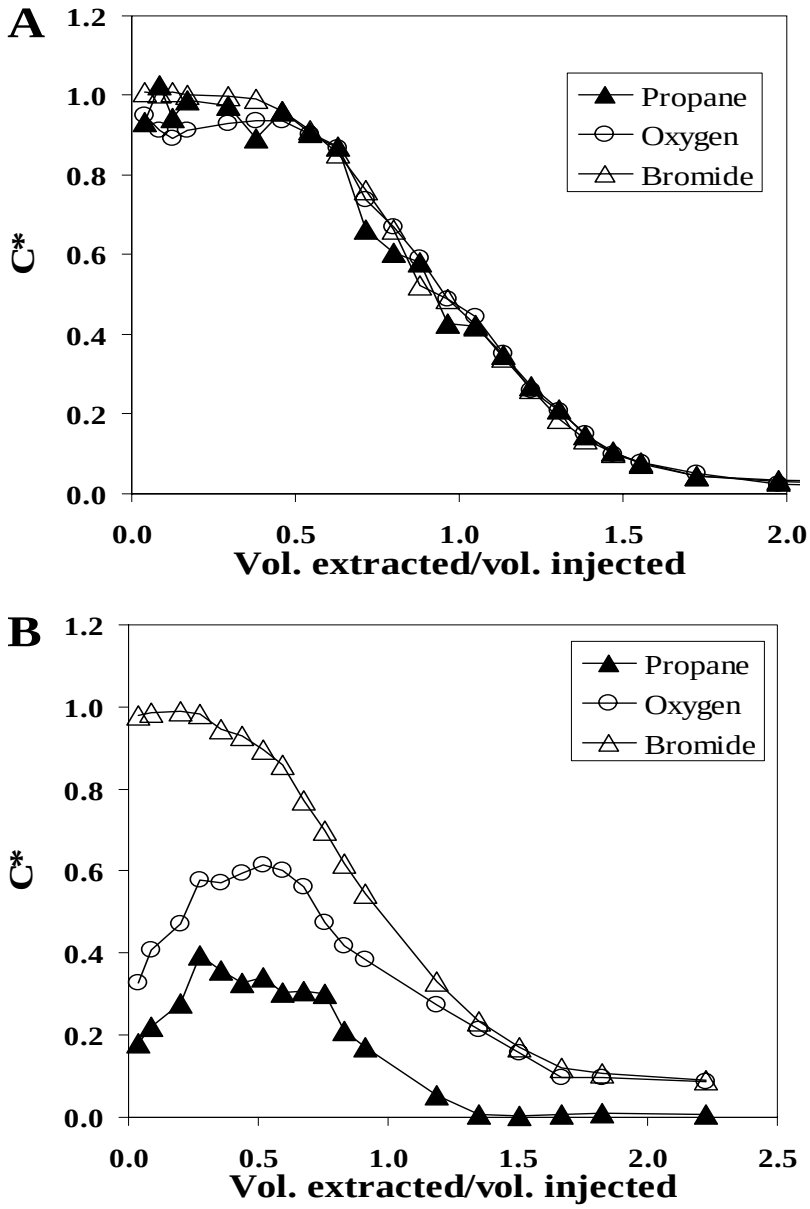

Figure 4.4. Extraction phase normalized concentrations in well MW2 during (A) first propane activity test, and (B) second propane activity test.

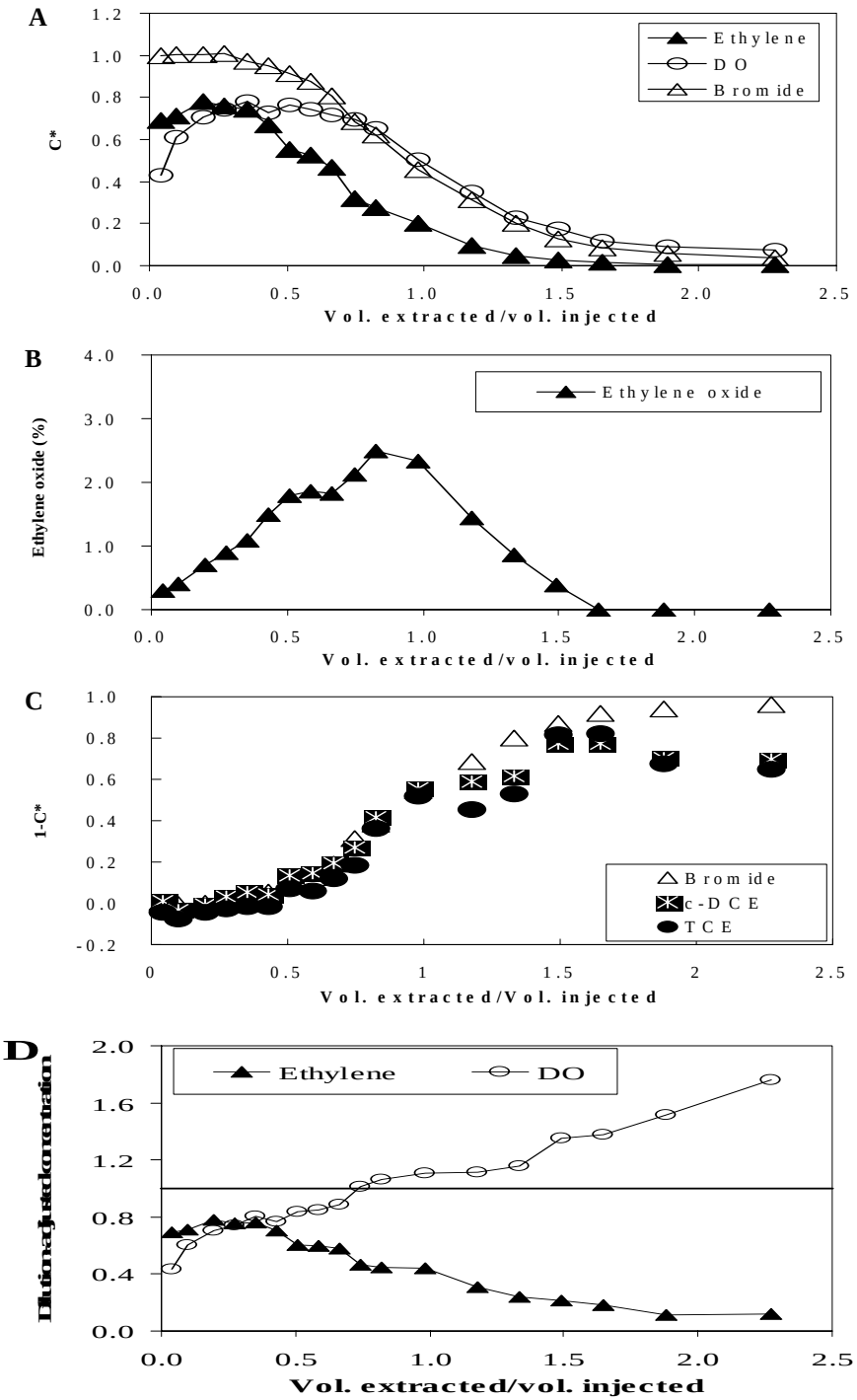

Figure 4.5. (A) Normalized concentrations for ethylene, oxygen, and bromide in well MW2 during the ethylene activity test, (B) ethylene oxide concentrations in the extracted groundwater as a percentage of average ethylene concentration in injected test solution (C) cis-DCE and TCE concentrations in the extract groundwater, and (D) dilution adjusted concentrations of ethylene and DO.

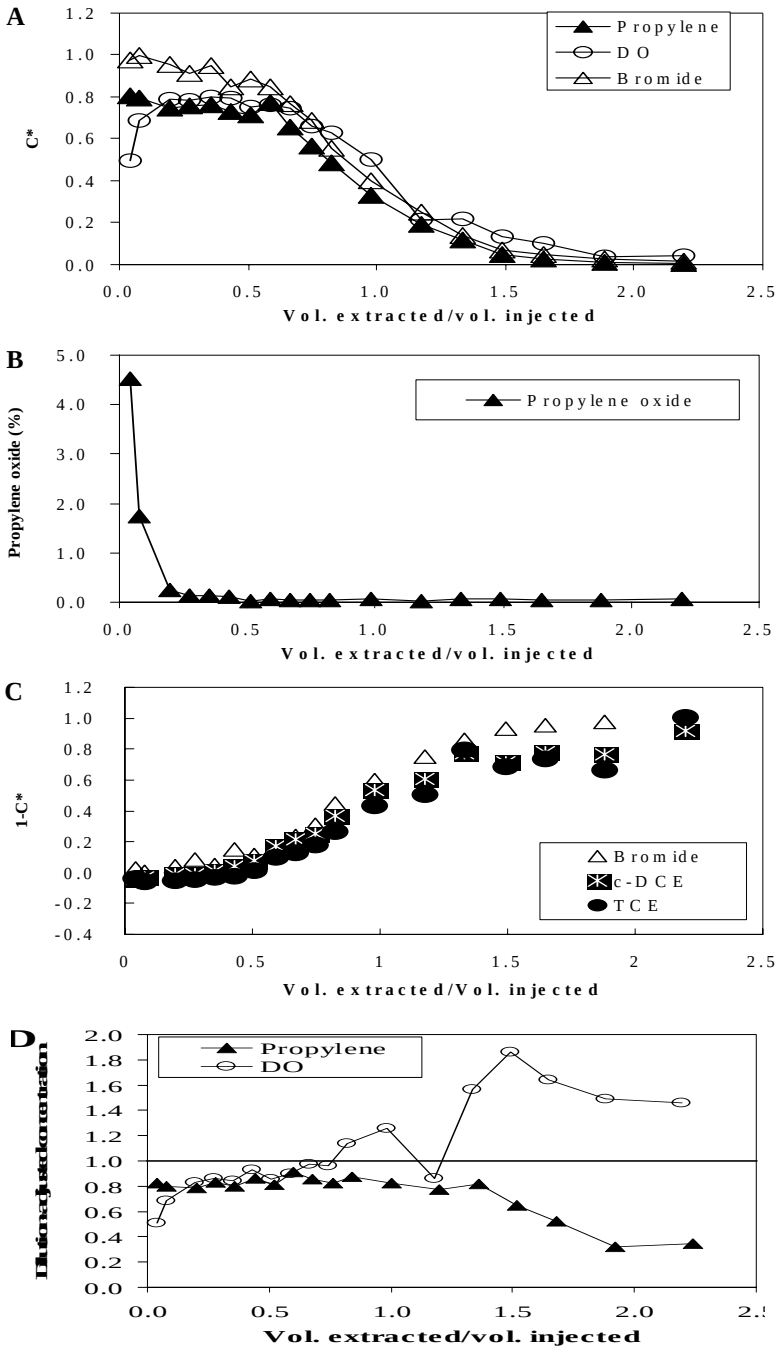

Figure 4.6. (A) Normalized concentrations for propylene, DO, and bromide in well MW3 during the propylene activity test and (B) propylene oxide concentrations in the extracted groundwater as a percentage of average propylene concentration in injected test solution (C) cis-DCE and TCE concentrations in the extract groundwater, and (D) dilution adjusted concentrations of propylene and DO.

Zero-order rate estimation. In order to calculate the rates of substrate utilization and surrogate compound transformation, a method for estimating zero-order reaction rates developed by Istok et al. (1997) was adapted. For the field test, the total quantities of all injected solutes (TM_i) in µmol, were calculated using equation 1

$$TM_i = C_s * V_{inj} \quad (1)$$

where C_s is measured solute injection concentration (µM) and V_{inj} is volume of the injected solution (L). The total quantity of all recovered solutes (TM_e) in µmol, was obtained by integrating breakthrough curves using equation 2

$$TM_e = \sum (V_{ext}^* * C_{ext}^*) \quad (2)$$

where V_{ext}^* is a volume of test solution/groundwater mixture extracted between the measurements and C_{ext}^* is an average concentration between the measurements. Recovery percentages (R) for injected solutes were computed using equation 3

$$R = \frac{TM_e}{TM_i} * 100 \quad (3)$$

The zero-order reaction rate (r_0) in µmol/L/hr for reactants was calculated using equation 4.

$$r_0 = \frac{TM_i - \{TM_e / 0.01 R_{tracer}\}}{(V_{inj})(t^*)} \quad (4)$$

where R_{tracer} is recovery percentage for tracer and t* is mean residence time (hour). The 0.01 factor in equation 4 converts from percentage into fractional numbers. The mean residence time (t*) was defined as the elapsed time from the midpoint of the injection phase to the centroid of the bromide breakthrough curve. Table 4.4 summarizes the masses of injected and extracted solutes, the percent recovery of the injected solutes upon extraction, and zero-order rate estimates using the method previously described. In the transport test, recovery percentages for dissolved gaseous substrates, oxygen, and nitrate were slightly higher or similar to those achieved with bromide. The result demonstrates that the solutes can be effectively recovered using the push-pull method that was developed. The results also indicate that partitioning due to entrapped gas, or sorption was minimal in the aquifer. Thus, in the transport test the zero-order reaction rate was approximately zero. These results indicate that there was no biological and abiotic loss of substrates in the aquifer.

In the propane activity test, the recovery percentage of bromide was slightly higher than that of oxygen and nitrate, and much higher than propane. The propane degradation rate calculated in Table 4.4 is a conservative estimate, because all the propane that was injected was degraded within 12.1 hours (Figure 4.2). Thus, the actual rate is likely larger than that reported. During the extraction phase, oxygen present in the native groundwater was introduced into the extraction groundwater. Despite this introduction a high zero-order reaction rate of oxygen was estimated (data not shown). The zero-order rate is higher than propane, which is consistent with the great stoichiometric amounts required for the oxidation of propane.

In the ethylene activity test, the recovery percentage of chloride (conservative tracer) was slightly higher than that for ethylene, and was similar to that for nitrate. The zero-order reaction rate for ethylene transformation was slower than propane degradation rate. The oxygen recovery percentage was much higher than that for chloride, indicating that the oxygen consumption was minimal and oxygen from the ambient groundwater was introduced into the extraction solution.

In the propylene activity test, slightly lower recovery percentage for propylene and nitrate was observed than bromide. A higher oxygen recovery percentage was again observed. The zero-order reaction rate for propylene transformation was smaller than rates of propane degradation and ethylene transformation.

Estimated zero-order rates of propane utilization were similar between wells MW2 and MW3 (Table 4.4). In both wells the estimated zero-order rate of ethylene transformation was ~ 45% of the estimated zero-order rate of propane utilization obtained from the second propane activity test at both wells (Table 4.4). The computed zero-order rate of propylene transformation at MW2 was about a factor of 1.5 lower than the ethylene transformation rate, while both rates are comparable at MW3 (Table 4.4).

4.1.4 Acetylene Blocking Tests.

The fourth propane activity test was performed with both propane and ethylene present in the injected groundwater. Simultaneous utilization of propane, ethylene, and oxygen were observed (Figure 4.7A), and ethylene oxide was again produced with a ratio ethylene oxide formed to ethylene transformed of ~ 5.2 % (Figure 4.7B). The zero-order rate of ethylene oxidation was about a factor of three greater than achieved in the earlier test in MW3, while the propane utilization rate was similar to that achieved in the second propane activity test. It may be that the presence and utilization of propane resulted in an increase in the rate of ethylene oxidation. Ethylene concentrations were also a factor of three higher, which likely affected the zero-order rate estimate. The presence of ethylene may have also inhibited the rates of propane utilization, since the zero-order rate of propane utilization is slower than achieved in the third propane activity test. Since the activity tests were performed sequentially, it is difficult to make strong conclusions related to inhibition and the causes of the changes in rates. Transformation of cis-DCE and TCE proved more difficult to assess, since they were present in the injected groundwater at concentrations lower than were present in the aquifer. However, normalization with respect to the background concentrations indicated that cis-DCE was likely transformed (Figure 4.7B), however TCE was not.

In a final test the utilization of propane and the transformation of cis- DCE and ethylene were inhibited by acetylene, a known inhibitor of the propane monooxygenase enzyme. An acetylene blocking test was then performed using the same conditions was the fourth propane activity test, but with acetylene added to the injection solution. Acetylene was injected at a concentration of ~ 0.5 mM (10 mg/L). In the presence of acetylene, substrate utilization was essentially completely inhibited (Figure 4.8A), and very little ethylene oxide was produced. The ratio ethylene oxide formed to ethylene transformed was ~ 0.12 % (Table 4.4). Zero-order rates of propane-utilzation and ethylene oxidation decreased by a factor of 4.7 and 2.4, respectively, in the acetylene blocking test compared to the fourth propane activity test (Table 4.4). The strong inhibition by acetylene indicates that a propane monooxygenase enzyme is likely responsible for propane degradation and the cometabolism of ethylene.

Concentrations of cis-DCE and TCE in the injected and extracted fluids were also measured during the activity tests. The relationship between ethylene and propylene as surrogates for CAH transformation can also be evaluated. In Figure 4.9, extraction phase breakthrough curves for propane, ethylene, cis-DCE, TCE, and bromide are plotted as 1-C*, that is, $1-[(C - C_{BG})/(C_o - C_{BG})]$. This method of plotting was used because, unlike the other substrates, cis-DCE and TCE

concentrations were lower in the injected test solution than in the background groundwater as a result of the sparging of groundwater with oxygen and the other gas prior to injection. For a non-reactive compound, such as bromide, this method of normalization should result in zero values during the early phase of an extraction and should increase to unity as during the latter phase of extraction. A reactive component with an injection concentration much greater than background (i.e. propane or ethylene) should yield values greater than zero, but it was less than unity, during the early phase of extraction, and then increasees to unit as extraction proceeds. For reactive compounds with high background concentrations in the aquifer (cis-DCE or TCE) compared to the injection concentration, negative values could result during the early phase of extraction, with values potentially remaining below unity as extraction proceeds.

During the fourth propane activity test, the propane and ethylene values were greater than zero during the early phase of extraction and increased to unity as extraction continued, indicating significant degradation of propane and ethylene occurred during the rest phase. cis-DCE values were lower than those of bromide, indicating that cis-DCE was cometabolically transformed during the test. TCE values were essentially identical to those of bromide, suggesting that no detectable TCE transformation occurred (Figure 4.7). During the acetylene blocking test, values for all solutes showed similar trends as bromide (Figure 4.8A). Here cis-DCE values approached unity towards the end of the test indicating cis-DCE transformation was also inhibited by acetylene.

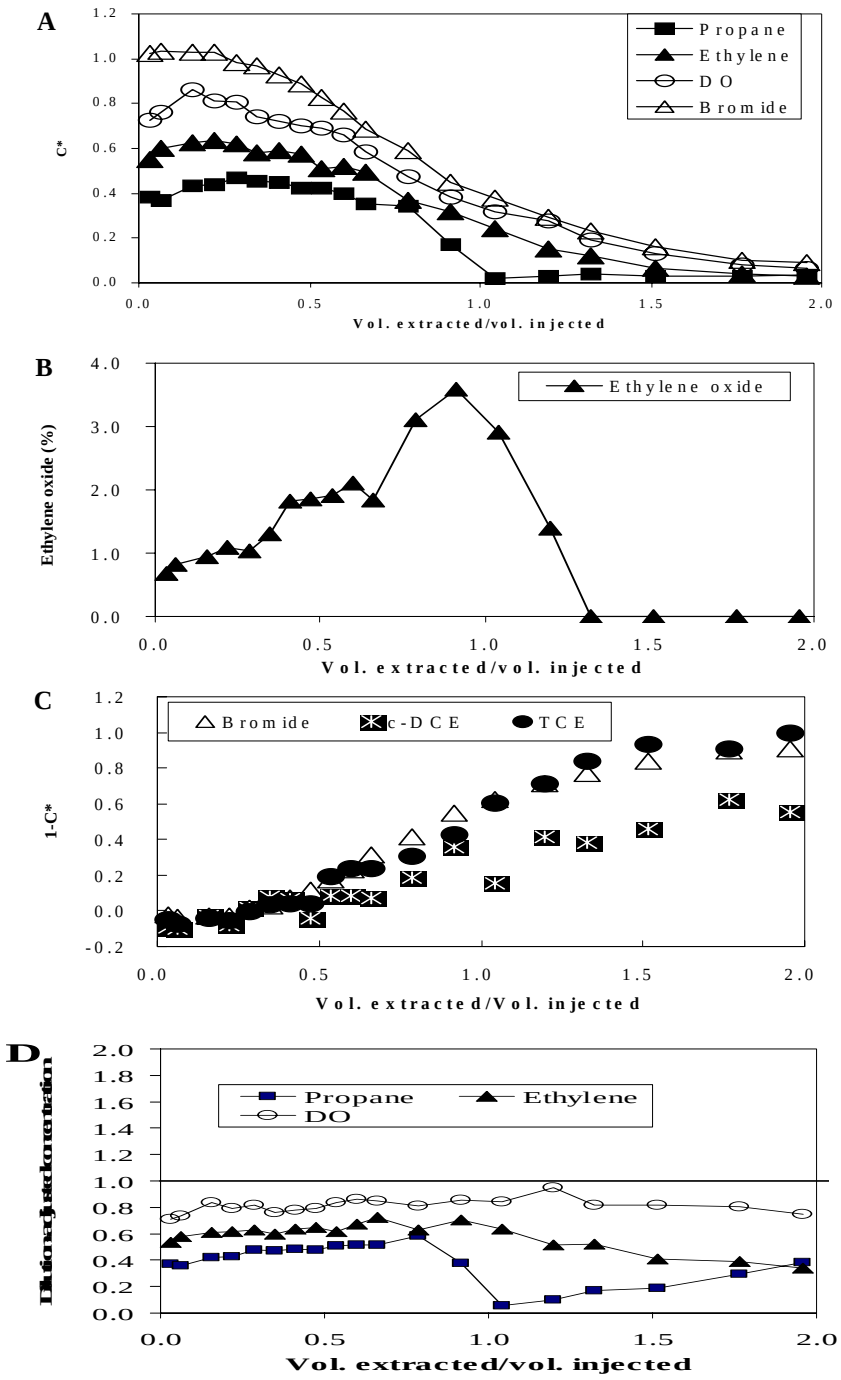

Figure 4.7. Extraction phase breakthrough curves from well MW3 during the fourth propane activity test (A) injected solutes (B) ethylene oxide concentrations expressed as a percentage of average ethylene concentration in injected test solution, (C) cis-DCE and TCE concentrations in the extract groundwater, and (D) dilution adjusted concentrations of propane, ethylene, and DO.

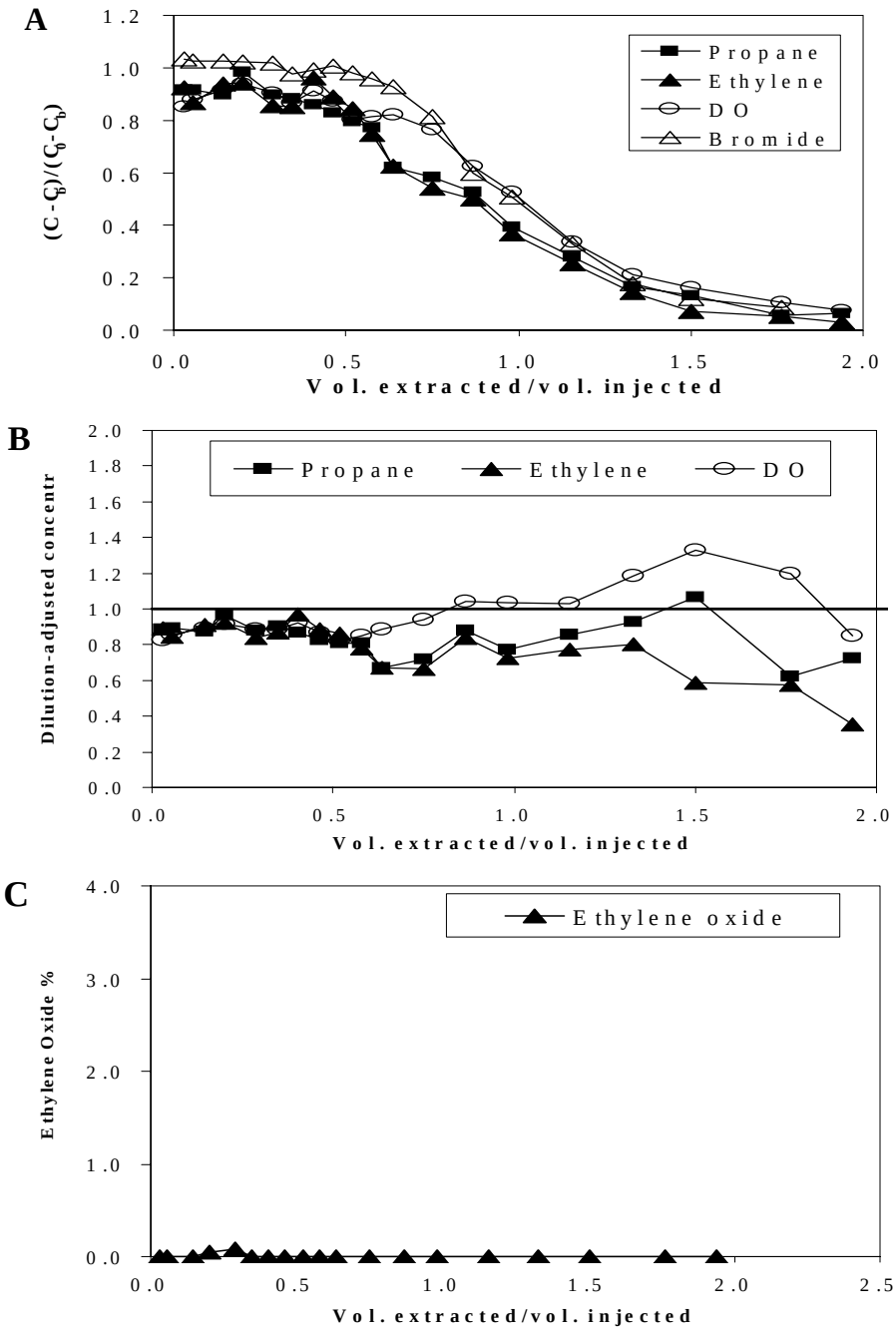

Figure 4.8. Extraction phase breakthrough curves from well MW3 during the acetylene blocking test (A) injected solutes (B) dilution adjusted concentrations propane, ethylene, and DO (C) ethylene oxide concentrations expressed as a percentage of average ethylene concentration in injected test solution.

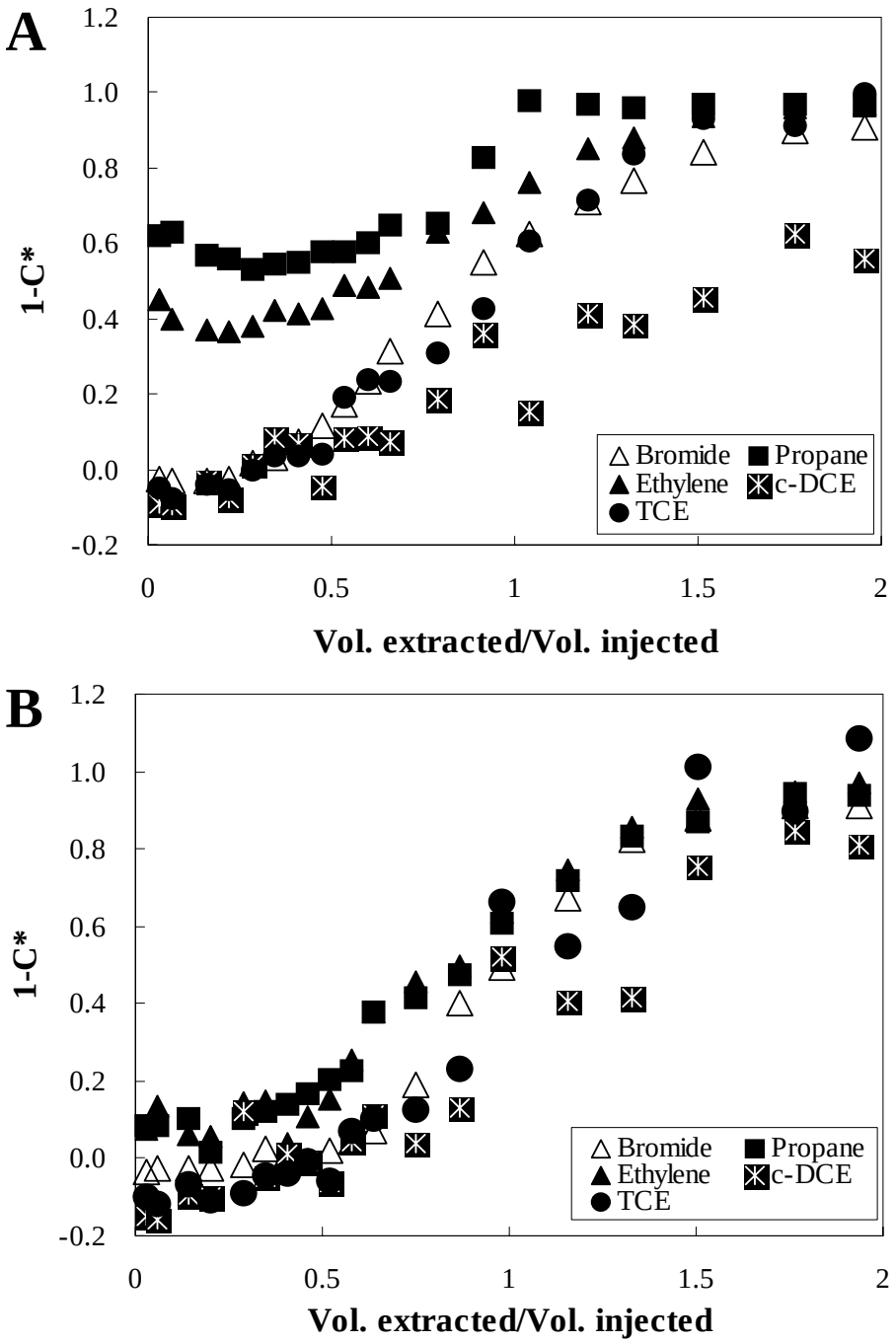

Figure 4.9. Extraction phase breakthrough curves during from well MW3 (A) the fourth propane activity test, and (B) acetylene blocking test.

4.2 Example Results from Gas Sparging Tests Conducted at the McCellan AFB, CA

Single-wellgassparging tests were developed for assessing the feasibility of in situ aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs), such as TCE and cis-DCE, using propane and methane as growth substrates. The tests were performed at the McAFB test site.

To evaluate transport characteristics of dissolved solutes [sulfur hexafluoride (SF_6) or bromide (non-reactive tracers), propane or methane (growth substrate), ethylene, propylene (nontoxic surrogates to probe for CAH transformation activity), and DO], push-pull transport tests were performed. A series of gas-sparging biostimulation tests were performed by sparging propane (or methane)/oxygen/argon/SF_6 gas mixtures at specific depth intervals using a "straddle" packer. Temporal groundwater samples were obtained from the injection well under natural gradient "drift" conditions. Gas-sparging activity tests were performed using the same procedures as the gas-sparging biostimulation tests, except that ethylene and propylene were included in the sparging gas mixtures. Gas-sparging acetylene blocking tests were performed by sparging gas mixtures including acetylene to demonstrate the involvement of monooxygenase enzymes. A summary of injected gaseous substrate concentrations for biostimulation, activity, and inhibition tests is listed in Table 4.5. The transport test was conducted by injecting substrates in solution rather than sparging gases. Detailed descriptions of methodology and results for each test type are described in the following sections.

4.2.1 GasSparging Transport Test

To evaluate transport characteristics of injected dissolved solutes, such as a non-reactive tracer (Br^- and SF_6), dissolved growth substrates (propane or methane), dissolved oxygen (DO), non-toxic CAH surrogates (ethylene and propylene) and nutrients (NO_3^-), push-pull tests were performed by measuring all these compounds and CAHs during the injection and extraction phase. Both bromide and SF_6 were used as a tracer to assess the absence or presence of trapped gas bubbles in the test zone before gas-sparging biostimulation. In contrast to bromide, SF_6 would be highly retarded by the presence of trapped gas bubbles due to relatively high volatility (dimensionless Henry's law constant @ 20 C = 151).

Site groundwater was used as an injection solution. Approximately 340 L of groundwater was pumped from the each well using a submersible pump. A 50-L test solution in a 50 L carboy was purged with nitrogen gas for half an hour to remove oxygen, TCE and cis-DCE from the solution and headspace. The solution was then purged with propane, ethylene, propylene, and nitrogen for one hour to achieve a specific aqueous concentration of each gas. The gas mixtures in the headspace were recirculated using a Masterflex peristaltic pump for three hours. A 250-L test solution was prepared separately in a 500-L carboy, and purged with oxygen to achieve a concentration of approximately 30 mg/L of DO. A 30-L of test solution was also prepared in a collapsible metalized-film gas-sampling bag. SF_6 gas (0.13 L) was added to the bag to achieve the aqueous concentrations of about 1 mg/L. Potassium bromide was added into a 500-L carboy to achieve a concentration of 100 mg/L of bromide. The test solution in the big carboy was injected at ~ 2 L/min with a Masterflex peristaltic pump, and each test solution in the small carboy and a collapsible bag was injected at 0.2 L/min with a piston pump.

Table 4.5. Gas composition for gas sparging tests conducted at the McAFB, MW1 and MW2 Field.

Test		Quantities	[1]Methane	[2]Propane	Ethylene	Propylene	Oxygen	SF_6	Argon	Acetylene
Transport		Flow (L/min) Volume (L) Conc. (mg/L	[3]NI	NI	NI	NI	NI	NI	NI	NI
Gas Sparging Biostimulation	1st	Flow (L/min) Volume (L) Conc. (mg/L)	2.6 50 ~2.0	2.9 50 ~2.0	0	0	20 250 25	0	10	0
	2nd	Flow (L/min) Volume (L) Conc. (mg/L)	2.6 50 ~2.0	2.9 50 ~2.0	0	0	20 250 25	0	10	0
	3rd	Flow (L/min) Volume (L) Conc. (mg/L)	2.6 50 ~2.0	2.9 50 ~2.0	0	0	20 250 25	0	10	0
	4th	Flow (L/min) Volume (L) Conc. (mg/L)	2.6 50 ~2.0	-	0	0	20 250 25	0.30 30 ~0.1	10	0
Gas-Sparging Activity		Flow (L/min) Volume (L) Conc. (mg/L)	3.1 50 ~2.4	1.4 50 ~1.0	0.52 50 ~2.0	0.21 50 ~2.0	7.8 250 ~10	0.96 30 ~0.3	2.6	0
Gas-Sparging Acetylene-Blocking		Flow (L/min) Volume (L) Conc. (mg/L)	3.1 50 ~2.4	1.4 50 ~1.0	0.52 50 ~2.0	0.21 50 ~2.0	7.8 250 ~10	0.96 30 ~0.3	2.6	0.3

[1]: Methane was sparged into the MW2 aquifer. [2]: Propane was sparged into the MW1 aquifer. [3]: NI indicates not included (The transport test was conducted by injecting a test solution rather than sparging gases).

The injection solution of the combined flows was injected into the aquifer over a 2-hour period. Based on dilution ratio, injected aqueous concentrations of each solute into the aquifer were ~ 2.0 mg/L for propane, ethylene and propylene, ~ 25 mg/L for DO, ~ 80 mg/L for bromide and ~ 0.1 mg/L for SF_6. Samples for the injected test solution were taken from the well using a submersible pump placed at the same level as injection line end. After 4 L (3 times of injection line volume) of groundwater is extracted, samples were taken. The procedure was shown to yield very reproducible samples of the injected fluid. After an 18-hour rest phase in the aquifer the fluid was extracted. Approximately 700 L of test solution/groundwater mixtures was extracted at a flow rate of 3.8 L/min for a total extraction phase duration of three hours. Samples of the injected test solution and the extracted solution were collected during injection and extraction phase. The concentration histories of the injected and extracted solutes permitted the assessment of the recovery of the solutes in the extracted fluid, dispersion in the aquifer, and potential retardation during transport. This 1st transport test is important prior to gas mixture sparging biostimulation, since the later 2nd transport test and activity test was conducted under similar conditions for comparison.

Mass balance showed about 90% of the injected bromide and about 80% of the injected SF_6 were recovered, and the recoveries of other solutes were comparable with bromide and slightly higher than SF_6. The transport tests showed that bromide and SF_6 could be used as conservative tracers for biological activity tests at MW1 and MW2 (Figure 4.10). The tests showed little loss of the dissolved gaseous substrates prior to biostimulation, and that negligible trapped gas was present in the aquifer.

4.2.2 Biostimulation Test by Gas Mixture Sparging

After the transport test, the sequential biostimulation tests were performed 1) to evaluate if the propane and methane utilizers could be stimulated in the aquifer; and 2) if so, to develop a method of data analysis that confirms the stimulation of propane and methaneutilizers. The propane (or methane)/oxygen/argon gas mixture for sparging was formulated to stimulate indigenous propane (or methane) utilizers, while maintained the gas below the LEL. For safety considerations check valves prohibiting backflow of gas were installed on gas lines between each gas tank and the rotameter, and on-line LEL detector and a valve for shutting off gas flow upon exceeding 90% of LEL were used throughout the sparging tests (Figure 3.6). The LEL detector was calibrated daily with propane (or methane) calibration standard gas (Scott Specialty gases, Longmont, CO). The gas mixture was injected at a rate of approximately 35 L/min for 6 hours to create a slow dissolving source of propane and oxygen. After sparging, temporal groundwater samples was taken and analyzed for substrates, DO and CAHs.

The results suggest the stimulation of methane and propane-utilizing microorganisms was achieved in the repeated push-pull tests. By the third test, propane and methane was completely consumed at MW1 and MW2, respectively (Figure 4.11), while oxygen was partially consumed. Incomplete utilization of oxygen resulted from the background oxygen concentration of regional groundwater that mixed with the injected solution. With repeated gas sparging tests depletion

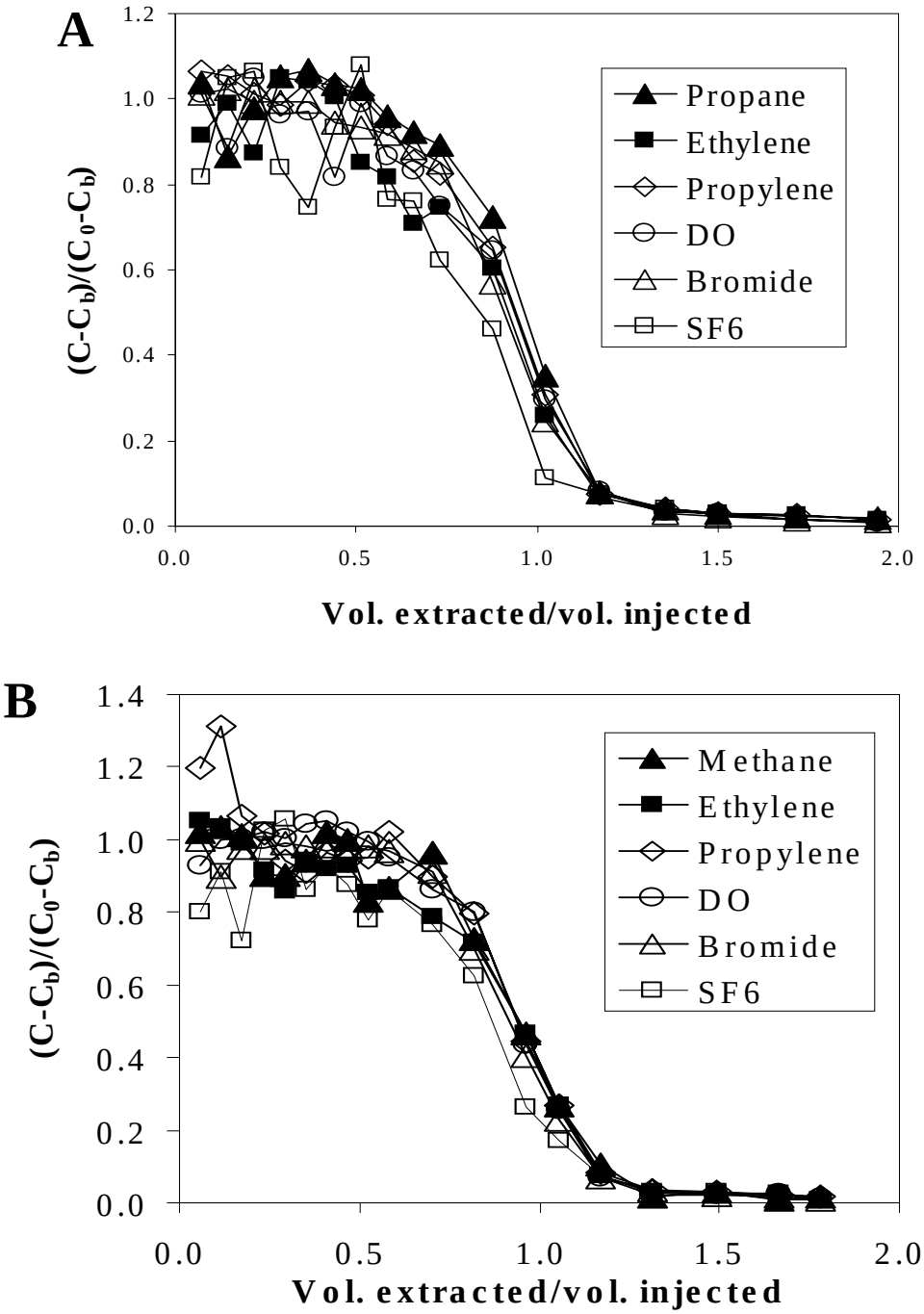

Figure 4.10. Pull phase normalized concentrations at MW2 (A) and MW1 (B) during the activity control test (Rest phase = 18 hours) showing conservative transport of dissolved gases.

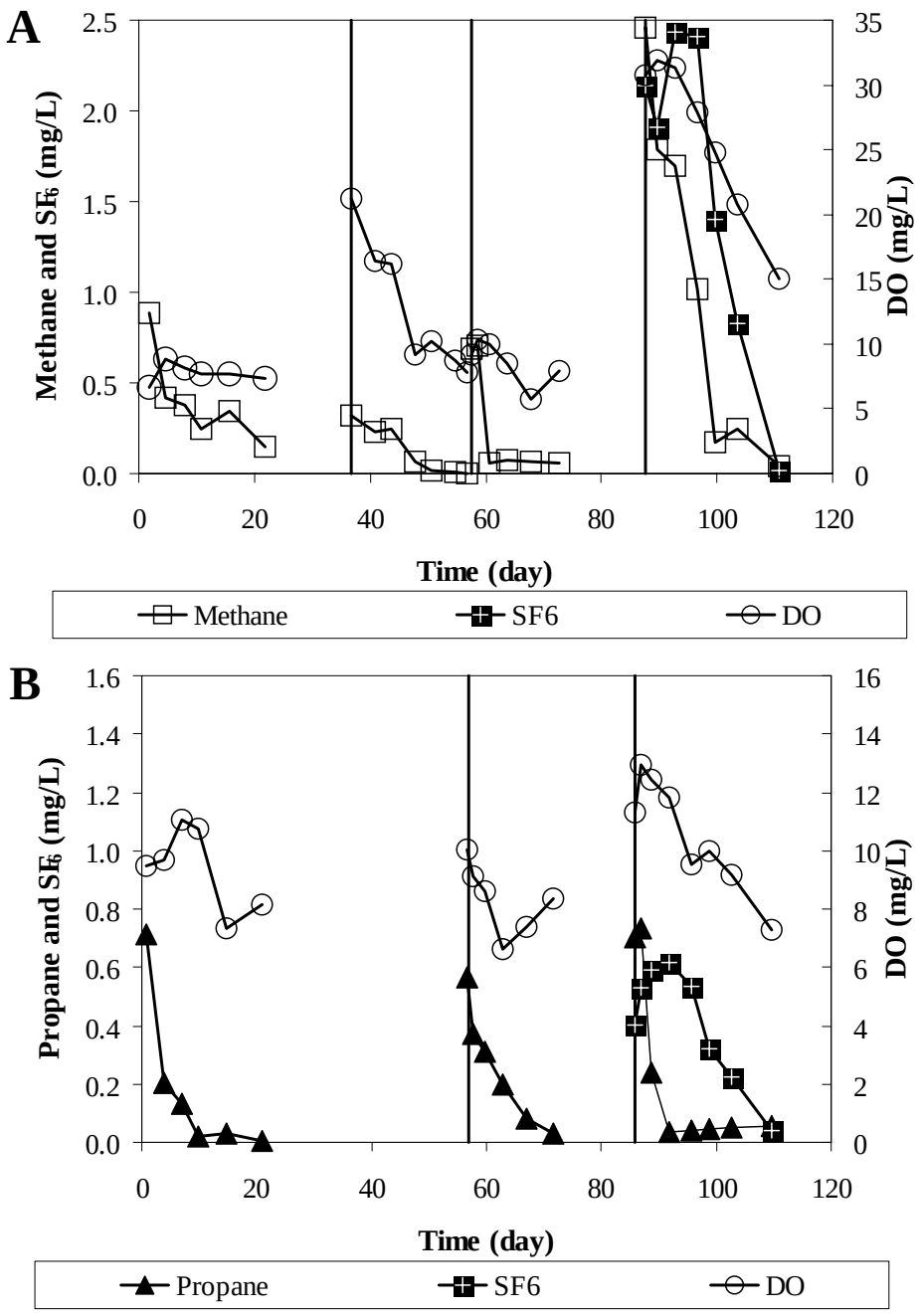

Figure 4.11. Temporal concentrations of methane or propane, DO and bromide at the monitoring wells of MW2 (A) and MW1 (B) during the transport and biostimulation drift tests.

times of methane and propane decreased from 30 to 12 days and from 10 to 5 days, respectively, while SF_6 was depleted after 20 days at both wells (Figure 4.11). The decreased time for growth substrate depletion and the longer time to deplete SF_6 as a conservative tracer indicate the progress of biostimulation.

4.2.3 Gas-Sparging Activity Tests

The propane (or methane)/ethylene/propylene/oxygen/SF_6/argon gas mixture sparging was performed to evaluate relative utilization rates of propane and transport of ethylene and propylene under natural gradient conditions. We maintained total flammable gas below the LEL of propylene that has the lowest LEL of 2% among the flammable gases. The gas mixture was injected at the following flow rates: propane (1.4 L/min), methane (3.1 L/min), ethylene (0.52 L/min), and propylene (0.21 L/min), oxygen (7.8 L/min), SF_6 (0.96 L/min), and argon (2.6 L/min) (Table 4.5). After sparging, temporal groundwater samples were taken and analyzed for all of gaseous compounds, DO, CAHs, and potential metabolic products, such as ethylene oxide and propylene oxide.

Complete utilization of methane, ethylene, and propylene was observed at MW2 5 days after injection, while SF_6 concentration reduced about 20% (Figure 4.12A). By-products having the same retention time on the GC as ethylene oxide and propylene oxide were detected (Figure 4.12B). The stimulated methane utilizers cometabolized ethylene and propylene to produce ethylene oxide and propylene oxide, as cometabolic by-products. Ethylene oxide and propylene oxide were observed after three days of residence in the aquifer and then increased and reached maximum concentrations of about 0.01 mg/L and 0.07 mg/L, respectively, after seven days (Figure 4.12B). Ethylene and propylene oxide concentrations gradually reduced to non-detect after a residence time of 10 days (Figure 4.12B). Similar results were obtained in tests at MW1, where propane was injected, however rates of propane utilization and ethylene and propylene transformation were slower (Figure 4.13A). The stimulated propane utilizers cometabolized ethylene and propylene to produce ethylene oxide and propylene oxide, as cometabolic by-products. Ethylene oxide and propylene oxide were observed after two days of residence in the aquifer, and then increased and reached maximum of about 0.03 mg/L and 0.04 mg/L, respectively, after six days (Figure 4.13B).

The activity test results showed that methane and propaneutilizers stimulated with repeated push-pull gas sparging tests were able to cometabolize ethylene and propylene, resulting in the formation of the by-products ethylene oxide and propylene oxide.

4.2.4 Gas-Sparging Inhibition Tests

Inhibition tests were performed as another demonstration that the observed uptake of propane, ethylene, propylene, and CAH transformation are biologically catalyzed reactions and not the result of abiotic processes (*e.g.* sorption or volatilization). Acetylene which acts as a mechanism-based inactivator for most of the oxygenases expressed by methane- and propane-oxidizing bacteria (Hamamura et al., 1999; Prior and Dalton, 1985) was used as an inactivator of both propane and ethylene uptake. Gas-sparging activity tests with propane (or methane),

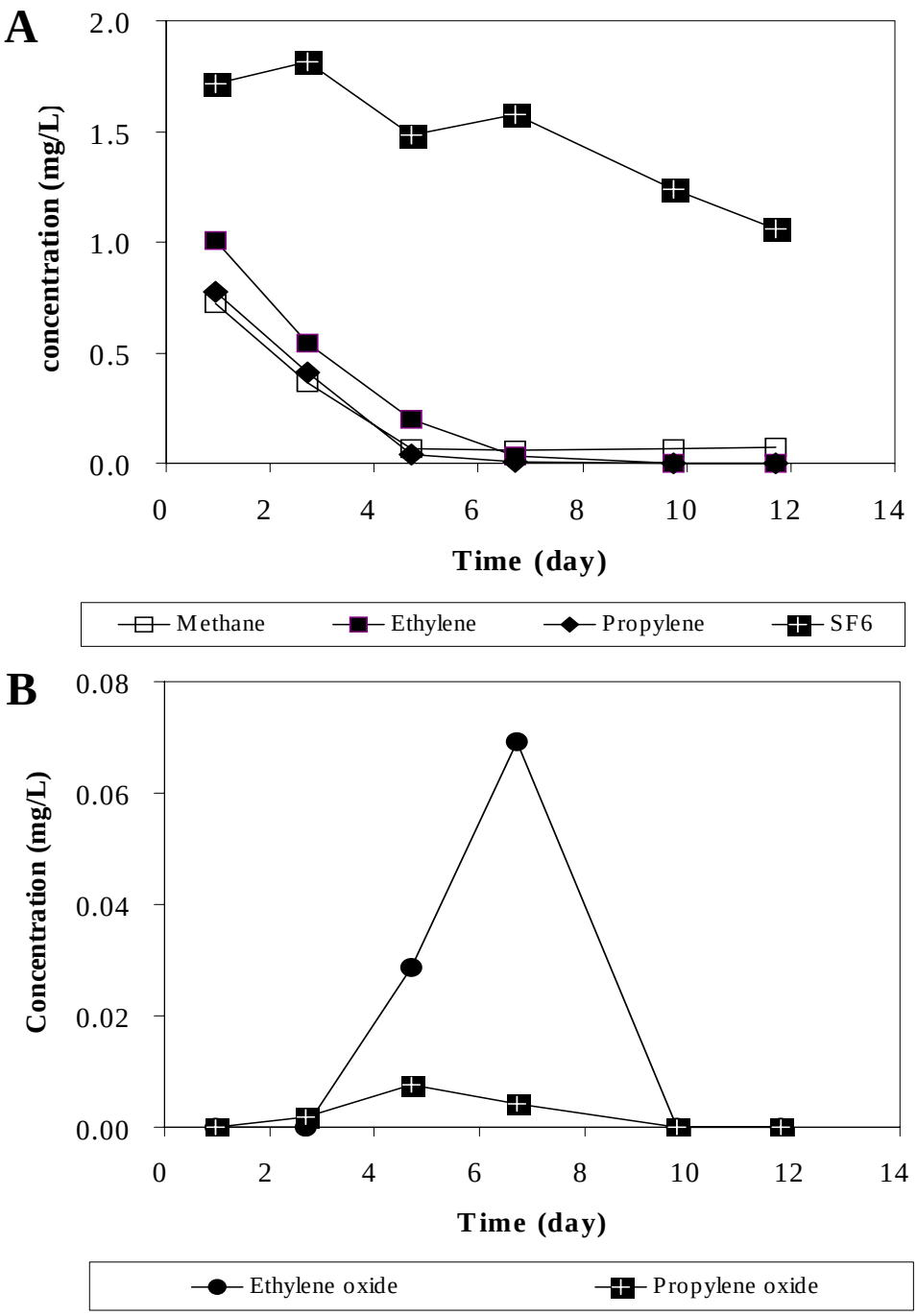

Figure 4.12. Temporal concentrations of methane, ethylene, propylene, and SF6 (A) and transformation by-products (B) at the monitoring wells of MW2 during the gas-sparging activity tests.

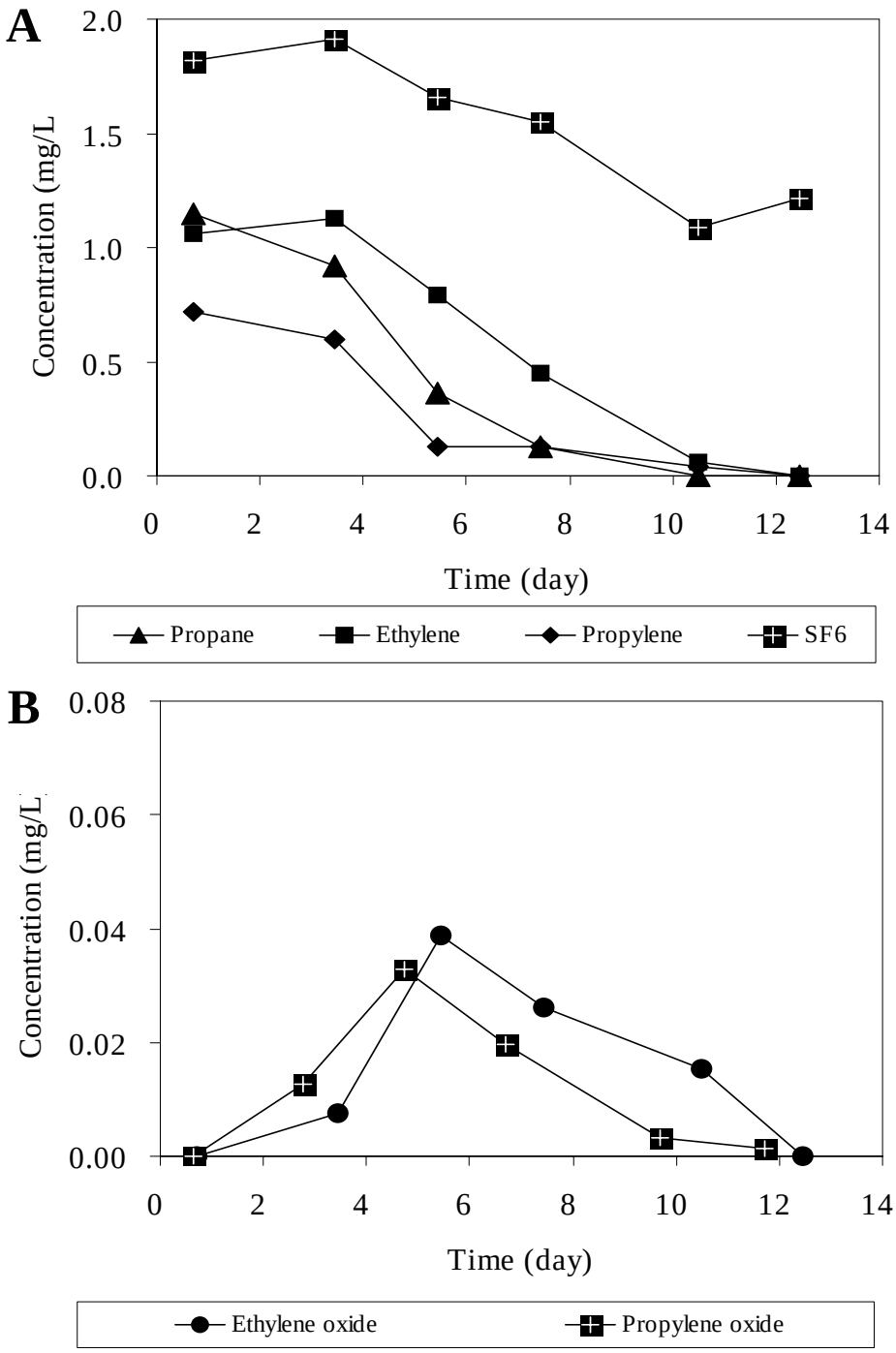

Figure 4.13. Temporal concentrations of propane, ethylene, propylene, and SF6 (A) and transformation by-products of the CAH surrogates (B) at the monitoring wells of MW1 during the gas-aparging activity tests.

propylene and ethylene sparging *without acetylene injection*, and propane (or methane), propylene and ethylene sparging *with acetylene injection* were repeated in successive tests to determine if inhibition of biological activity occurred.

Propane utilization and ethylene and propylene oxidation were essentially completely inhibited in the presence of acetylene, and no production of the corresponding oxides was also observed in both MW1 and MW2 wells as shown in Figures 4.14A and 4.14B. The results when compared with those obtained in the activity tests (Figures 4.12 and 4.13) further demonstrate propane and methane monooxygenase enzymes were responsible for the transformation of ethylene and propylene.

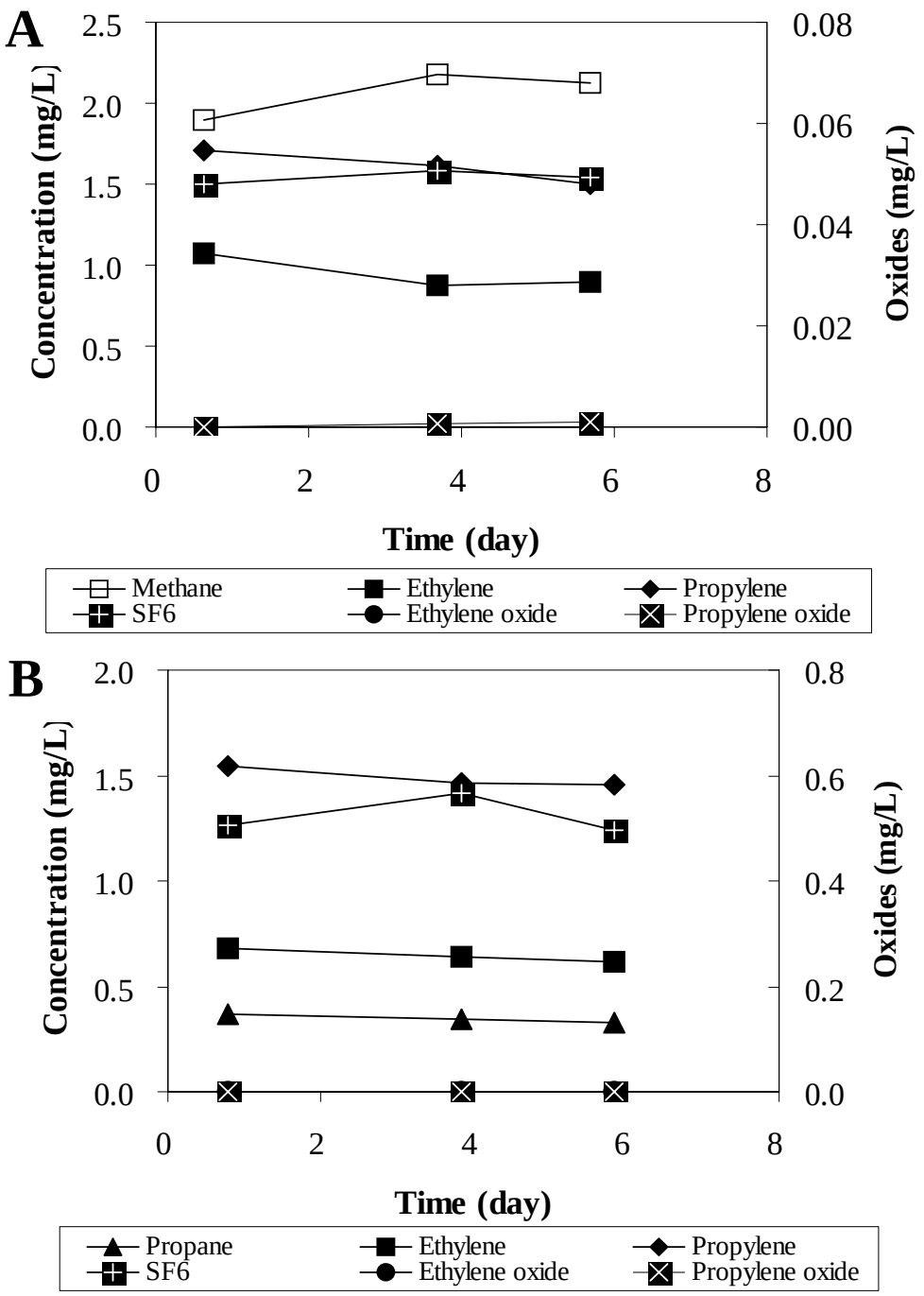

Figure 4.14. Temporal concentrations of propane or methane, ethylene, propylene, SF6, transformation by-products of the CAH surrogates at the monitoring wells of MW2 (A) and MW1 (B) during the gas-sparging acetylene-blocking tests.

4.2.5 Summary Results from Field Push-pull Tests Conducted at the McCellan AFB, CA

In situ aerobic cometabolic transformations of ethylene, propylene, and cis-DCE were examined in groundwater contaminated with cis-DCE and trichloroethylene (TCE). In situ measurements were performed by conducting field push-pull tests, which consisted of injecting site groundwater amended with a bromide tracer and combinations of propane, oxygen, nitrate, ethylene, propylene, cis-DCE, and TCE into existing monitoring wells and sampling the same wells over time. Mass balance and transformation rate calculations were performed after adjusting for dilution losses using measured tracer concentrations. Initial rates of propane utilization were very low; rates increased substantially following sequential additions of dissolved propane and oxygen. Evidence that propane and oxygen additions had stimulated organisms expressing a propane monoxygenase enzyme system and the capability to transform CAHs included: (1) the transformation of injected ethylene and propylene to the cometabolic byproducts ethylene oxide and propylene oxide, (2) the transformation of cis-DCE, and (3) the inhibition of these transformations in the presence of coinjected acetylene, a known monoxygenase mechanism-based inactivator.

Transformation of cis-DCE and TCE proved more difficult to assess, since they were present in the injected groundwater at concentrations lower than were present in the aquifer. Several factors likely contributed to TCE and cis-DCE only being marginally transformed. The residence time for the activity tests were only 24 hrs and the reactions rates were too slow to see significant changes. Also the presence of ethylene and propylene likely inhibited the rates of cis-DCE and TCE transformation, since it was not until later time, when they were reduced to low concentrations, that there was some evidence for TCE and cis-DCE transformation. cis-DCE appeared to be more rapidly transformed than TCE. This result is consistent with the results of the cometabolic air sparging demonstration that was conducted at the same site (Tovanabootr et al. 2001) and the results of microcosm studies performed with aquifer solids and groundwater from the site (Timmins et al. 2001). One possible improvement in the protocol is to conduct some tests where cis-DCE or TCE is added to the test solutions above background concentrations. Results of such tests are presented in the Ft. Lewis demonstration.

This study also developed single-well-gas-sparging tests for assessing the feasibility of in situ aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs), such as TCE and cis-DCE, using propane and methane as growth substrates. A series of gas-sparging biostimulation tests were performed by sparging propane (or methane)/oxygen/argon/SF_6 gas mixtures at specific depth intervals using a “straddle” packer. With repeated gas sparging tests depletion times of methane and propane decreased from 30 to 12 days and from 10 to 5 days, respectively, while SF_6 cocnetration was reduced after 20 days in both wells. The decreased time for growth substrate depletion and the longer time to decrease SF_6 concentration as a conservative tracer indicate the progress of biostimulation. Propane (or methane) utilization, DO consumption, and ethylene and propylene cometabolism were well demonstrated. The stimulated propane- and methane-utilizers cometabolized ethylene and propylene to produce ethylene oxide and propylene oxide, as cometabolic by-products. Gas-sparging acetylene blocking tests demonstrated the involvement of monooxygenase enzymes methane and propane utilization and ethylene and propylene transformation was essentially completely inhibited in the presence of acetylene, and no production of the corresponding oxides was also observed. The gas-sparging

tests support the stimulation of methane- and propane- oxidizing microorganisms and cometabolic transformation of ethylene and propylene by the enzyme responsible for methane and propane degradation.

These results suggest that a series of push-pull tests performed with nontoxic surrogate probes can be useful for detecting and monitoring in situ aerobic cometabolism of CAHs. 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.

4.3 Example Results from Field Push-Pull Tests Conducted at Fort Lewis, WA

Our third site demonstration evaluated aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs) using toluene as a cometabolic substrate. The demonstration was performed at Fort Lewis Logistics Center, WA. The effectiveness of dissolved substrate addition to stimulate indigenous toluene utilizers was evaluated in multi-level monitoring wells. Reactive solutes included the dissolved growth substrate (toluene), hydrogen peroxide, as a source of dissolved oxygen, non-toxic surrogates (isobutene), and nutrient (NO_3^-). In biostimulation, activity, and inhibition tests, nutrients (modified G4 Minimal Media) (Yeager, 2001) were added to P1 and P2 at LC192 and LC191, respectively. cis-DCE and trans-DCE were also added in selected tests. cis-DCE was added to achieve injection concentration above background levels in the groundwater. Trans-DCE was not present in the groundwater and was added as a contaminant to evaluate its transformation.

A series of push-pull tests was performed to evaluate transport characteristics, biostimulation, and transformation activity of the injected solutes. A single transport test was conducted in each well port prior to biostimulation. For these tests the injected solution was allowed to reside in the aquifer for about 20 hrs, and was then extracted at a rate of 1 L/min for 3.3 hrs. Biostimulation activity tests were performed like transport tests by injecting toluene, hydrogen peroxide, and nitrate in order to increase the biomass of toluene-utilizing microorganisms. Biostimulation was monitored by measuring dissolved concentration of toluene, nitrate, and oxygen in the selected well port. After biostimulation was achieved, push-pull activity tests were performed evaluating the reactivity of the stimulated microorganisms on isobutene as a surrogate compound. Isobutene was selected as a surrogate compound since laboratory studies by Hicks (2002) indicated that isobutene epoxide would be formed when an ortho monooxgygenase enzyme is expressed. Inhibition tests were performed in another phase of the test where 1-butyne (as an inhibitor) was added and utilization and the inhibition of the transformation of toluene, isobutene, and CAH was monitored. Studies of Hicks (2002) also showed 1-butyne was an inhibitor of toluene-ortho monooxgygenase enzymes

Two types of tests were performed upon the injection of the test solution. Activity tests were performed as previously described. In the Fort Lewis tests, the rest phase was about 20 hr (no pumping). Approximately 200-L of groundwater was then extracted at a flow rate of 1 L/min. A second type of activity test, called a natural drift test, was performed. These tests are similar to activity tests except groundwater was not extracted after a rest period. Samples instead were collected periodically from the injection location as the test solution drifted downgradient. The natural drift tests were performed at Fort Lewis since groundwater velocities were faster than McAFB. The test permitted for longer reaction times in the aquifer. Rate estimates between activity test and the natural drift test can be also compared. Samples collected from both types of tests were analyzed for injected tracer and potentially reacting solutes, as well as reaction products formed in situ. Section 3.8.2 and 3.8.4 above summarizes the analytical methods used to measure concentrations of tracer, nutrients, substrates, chlorinated solvents and their transformation products in the test samples. A summary of injected solute concentrations for transport, biostimulation, activity, and inhibition tests is listed in Tables.4.6 and 4.7.

Table 4.6. Test Solution Composition for Push-pull Tests Conducted at Fort Lewis.

Test Type	Injection Volume (L)	[1]Toluene (mg/L)	[1]Isobutene (mg/L)	[2]cis-DCE (µg/L)	[2]TCE (µg/L)	[1]DO (mg/L)	[3]NO_3^--N (mg/L)	Br^- (mg/L)
Transport Test (push-pull)	125	10.4 ±0.4	5.7 ±0.4	40-242	92-379	40 ±4	8.6 ±0.2	95.9 ±4
First Biostimulation (natural drift)	105	10.2 ±0.2	NI	23-170	160-429	40 ±4	8.5 ±0.2	103 ±4
Second Biostimulation (natural drift)	105	10.2 ±0.2	NI	21-145	160-429	40 ±4	8.5 ±0.2	103 ±4
Third Biostimulation (natural drift)	200	21.7 ±0.4	NI	22-99	123-380	40 ±4	9.9 ±0.2	110 ±4
Fourth Biostimulation (natural drift)	200	19.9 ±0.4	NI	488-506	114-399	40 ±4	9.7 ±0.2	110 ±4
Toluene Activity Test (push-pull)	105	10.2 ±0.2	NI	15-88	106-367	40 ±4	10.2 ±0.2	108 ±4
Fifth Biostimulation (natural drift)	200	20.7 ±0.4	NI	21-76	112-378	40 ±4	9.6 ±0.2	112 ±4

[1]: Average concentrations in the injected test solution (C_0) in Ports 1 and 2 in LC191 and LC192. [2]: Range of cis-DCE and TCE concentrations in the injected test solution (C_0). [3]: Nitrate as a nutrient was added to Port 1 in LC191 and in Port 2 in LC192, while nutrients (modified G4 minimal media) were added to Port 2 in LC191 and Port 1 in LC192.

Table 4.7. Test Solution Composition for Push-pull Tests Conducted at Fort Lewis.

Test Type	Injection Volume (L)	[1]Toluene (mg/L)	[1]Isobutene (mg/L)	1-Butyne (mg/L)	[2]cis-DCE (µg/L)	trans-DCE (µg/L)	[2]TCE (µg/L)	[1]DO (mg/L)	[3]NO3-N (mg/L)	Br- (mg/L)
First Isobutene Activity Test (push-pull)	125	2.2 ±0.1	3.1 ±0.1	NI	448-483	NI	150-300	40 ±4	9.1 ±0.2	103 ±4
Second Isobutene Activity Test (natural drift)	125	2.1 ±0.1	3.08 ±0.1	NI	435-517	NI	130-150	40 ±4	8.9 ±0.2	105 ±4
Sixth Biostimulation (1 additions)	200	20.2 ±0.4	NI	NI	10-76	NI	132-403	40 ±4	9.8 ±0.2	102 ±4
Third Isobutene Activity Test (natural drift)	125	3.3 ±0.1	2.96 ±0.1	NI	514-540	492-510	180-290	40 ±4	8.7 ±0.2	109 ±4
Seventh Biostimulation (1 additions)	200	21.4 ±0.4	NI	NI	23-89	NI	144-466	40 ±4	9.2 ±0.2	102 ±4
Inhibition Test (natural drift)	125	3.0 ±0.1	3.1 ±0.1	20 ±0.4	502-540	484-497	180-280	40 ±4	8.6 ±0.2	103 ±4

[1]: Average concentrations in the injected test solution (C_0) in Ports 1 and 2 in LC191 and LC192. [2]: Range of cis-DCE and TCE concentrations in the injected test solution (C_0). [3]: Nitrate as a nutrient was added to Port 1 in LC191 and in Port 2 in LC192, while nutrients (modified G4 minimal media) were added to Port 2 in LC191 and Port 1 in LC192. [4]: NI indicates not injected.

Detailed descriptions of methodology and results for each test type are described in the following sections.

4.3.1 Transport Test

Transport characteristics of injected solutes, including bromide, toluene, isobutene, DO, and NO_3^- push-pull tests were evaluated in transport tests as previously discussed. Experimental methods were essentially identical to the McAFB tests except smaller injection volumes were used. The injection system is shown in Figure 3.7. Groundwater (125-L) needed to make the injection solution was extracted from the LC191 and LC192 wells ports at a flow rate of ~ 2 L/min using a Masterflex peristaltic pump (Barnant Co., Barrington, IL). The test solution (100-L) was prepared by adding bromide (125 mg/L), nitrate (50 mg/L) and hydrogen peroxide (105 mg/L). Groundwater (20-L) in the 50-L carboys was purged at controlled flow with isobutene gas for one hour to achieve aqueous concentrations of approximately 35 mg/L. The isobutene gas in the headspace is recirculated using a Masterflex peristaltic pump for one hour to help equilibrate the system. Groundwater (5-L) was added to a collapsible Teflon bag and toluene was added to achieve a concentration of 250 mg/L.

The injection solutions were pumped at different flowrates and mixed together to achieve the desired injection concentration. The rates were as follows: 100-L solution, ~1 L/min; 20-L solution, 0.2 L/min; and 5 L toluene solution, 50 mL/min. A series of metering pumps were used. The aqueous injected concentrations are presented in Table 4.6. The solution was injected into the aquifer over a 1.67 hour period. After a residence period of 20 hours, approximately 200 L was extracted (over a period of 3.3 hours) at a flow rate of 1 L/min. Samples of the injected test solution were taken from the well using a peristaltic pump placed at the same level in the well as injection line end. After extracting 1 L (3 times of injection line volume) of groundwater the samples were taken.

Extraction phase breakthrough curves, as a function of relative concentration (C/C_o), for bromide, toluene, isobutene, and nitrate are plotted in Figure 4.15A for P1 in well LC192. Extraction breakthrough curves for toluene, isobutene, and nitrate tests were very similar to the bromide tracer, indicating conservative transport of all injected solutes prior to biostimulation. The dilution adjusted concentrations of $[(C/C_o)/(C/C_o)Br^-]$ of toluene, isobutene, and nitrate are also shown in Figure 4.15B. The dilution adjusted concentrations are all near unity indicating no reaction or retardation. These results at the other three test locations were very similar to those observed at LC192 in P1.

A summary of measured concentrations and computed masses achieved in the transport tests for toluene, isobutene, DO, nitrate, and bromide for all four test locations are shown in Table 4.8. Mass balances indicated 30-50% of injected mass of the different solutes were recovered at LC191 in P1 or P2 well ports, while 56-65% were recovered at LC192 in P1 or P2 (Table 4.8). The similar percent recoveries indicated similar transport characteristics of the conservative tracer and reactive solutes at both LC191 and LC192 well ports. The lower recovery at the LC191 well ports indicates higher groundwater velocities compared to the

LC192 well ports. The shallower LC191 P1 had a lower recovery than the deeper P2 well port, indicating a faster groundwater in the shallower zone. The results show faster groundwater transport at Fort Lewis than at McAFB, where recoveries were 88-100%. A summary of recoveries and rates achieved in the transport tests for toluene, isobutene, DO, nitrate, and bromide for all four locations is shown in Table 4.8. The aquifer at the test site is alluvial, and thus spatial variability to hydraulic conductivity is expected. Groundwater extraction was also occurring in the aquifer, which could have resulted in spatial variability in groundwater velocities. The bromide tests indicated that differences in groundwater velocity existed at the different test locations, and groundwater was flowing faster in the shallower zone (~ 25 ft) compared to the deeper zone (~ 35 ft).

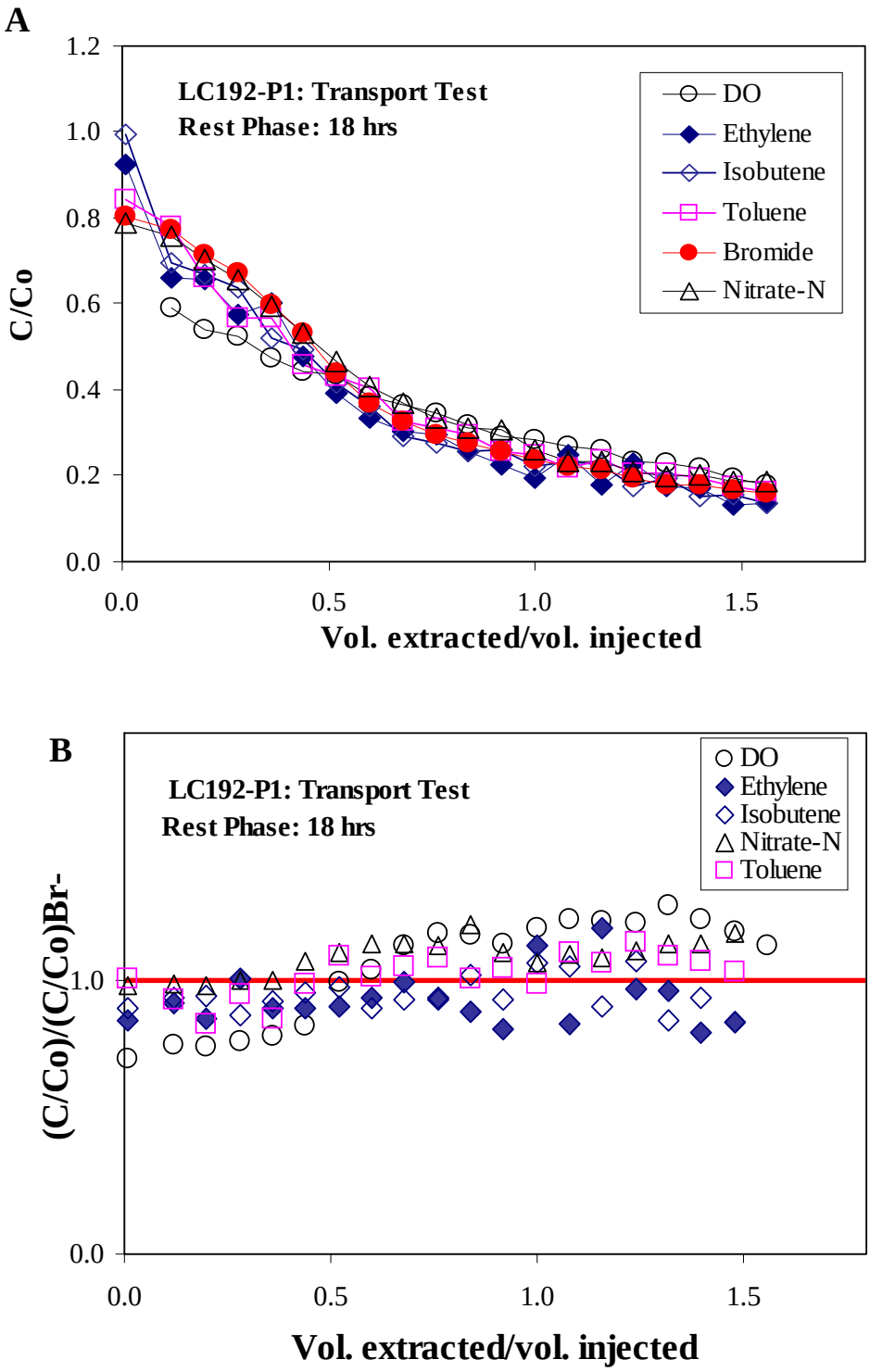

Figure 4.15. Extraction phase breakthrough curves for push-pull transport tests at Port 1 in LC192 (A). Dilution-adjusted concentrations are presented in (B).

Table 4.8. Summary of Quantities of Injected and Extracted Solute Mass and Percent Recovery in Transport Tests.

Test Location	Quantities	Toluene	Isobutene	DO	NO_3^--N	Br^-
Transport LC191-P1	Mass recovery (%)	30.1	36.5	29.3	31.1	32.9
	Rate (µmol/L/hr)	0.35	≈ 0	--	--	--
Transport LC191-P2	Mass recovery (%)	44.8	47.7	41.4	41.4	46.1
	Rate (µmol/L/hr)	0.18	≈ 0	--	--	--
Transport LC192-P1	Mass recovery (%)	58.3	56.17	58.1	56.3	59.5
	Rate (µmol/L/hr)	0.10	≈ 0	--	--	--
Transport LC192-P2	Mass recovery (%)	61.9	57.1	60.2	55.4	66.1
	Rate (µmol/L/hr)	0.33	≈ 0	--	--	--

4.3.2 Biostimulation by Injecting Dissolved Substrates

Biostimulation tests were performed by injecting a test solution containing dissolved toluene substrate, hydrogen peroxide, bromide, and nutrients in order to increase biomass of toluene-utilizing microorganisms. Injected solute compositions for biostimulation tests are summarized in Table 4.6. Nutrients, modified G4 Minimal Media, Yeager, (2001), were added to P1 and P2 at LC192 and LC191, respectively, while only nitrate was added to P1 and P2 at LC191 and LC192 wells. The modified minimal media contained (per 105 liters) 3.15 g NH_4NO_3, 2.0 g $MgSO_4$.7H2O, 0.11 g $CaCl_2.2H_2O$, 0.11 g Na_2EDTA, 0.05 g $FeCl_3$, 0.05 M KH_2PO_4/K_2HPO_4, 0.15 g H_3BO_3, 0.11 g $MgSO_4$.7H2O, 0.03 g $ZnSO_4$.7H2O, 0.01 g $CoCl_2.4H_2O$, 0.008 g $CuSO_4.5H_2O$, and 0.005 g $Na_2MoO_4.2H_2O$. This resulted, for example, in an injection concentration of NH_4NO_3 as N of 10.5 mg/L or 78.7 mmoL as N.

For biostimulation tests, groundwater (100-L) containing 105 mg/L bromide, 42 mg/L nitrate, and 89 mg/L of hydrogen peroxide and a 5-L of 210 mg/L toluene in a collapsible Teflon bag was injected to increase biomass. The test solution was injected into the aquifer and then was transported under natural-gradient conditions. Over a period of one month, five sequence additions of groundwater amended with toluene (10 and 20 mg/L, Table 4.6) were made to increase biomass of toluene-utilizing microorganisms. Samples were taken from the injected test solutions immediately after each addition and again after one week of residence in the aquifer. All samples were analyzed for toluene, o-cresol, DO, nitrate, cis-DCE and TCE. Results from these experiments showed that complete utilization of toluene and significant reduction in cis-DCE concentrations. DO, nitrate, and TCE concentration were near groundwater background concentrations. Interestingly, a trace amount of o-cresol was observed in the samples collected after one week of residence in the aquifer.

Biostimulation activity tests were then performed using the same procedures as the earlier transport tests, where the injected solution was allowed to reside in the aquifer for 20 hours and then extracted over a period of 3.3 hrs. Biostimulation test results showed decreases of injected toluene concentration and the production of o-cresol as an intermediate oxidation product, indicating the stimulation of toluene-utilizing microorganisms contain an ortho-monooxygenase enzyme. o-cresol was identified by retention time comparisons with an authentic o-cresol standard. Under the GC operating conditions as described in section 3.7.2, the retention time for o-cresol was 25.12 min. Toluene and o-cresol formation concentration in P1 and P2 at LC191-P1 and LC192-P2, respectively, are plotted in Figures 4.16A and 4.16B. A small fraction of utilized toluene was observed as o-cresol. The o-cresol represent range from 0.1 to 0.3% of the total toluene mass injected (Table 4.9). The small mass of o-cresol produced could be due to the rate of formation and microbial utilization of the o-cresol. Toluene oxidation to o-cresol by the toluene ortho-monooxygenase pathway was also observed by Hopkins et. al., (1995) and Fries et. al., (1997) at the Moffett field site.

Extraction breakthrough curves of normalized concentrations and dilution-adjusted curves for LC192-P1 are presented in Figures 4.17A and 4.17B, respectively. Figure 4.17A shows a decrease in concentrations of injected solutes, toluene, nitrate, and DO compared to the bromide

tracer after approximately 25 hrs of residence in the aquifer. The decrease in toluene concentrations are most evident, especially when compared with the transport tests conducted prior to biostimulation (Figure 4.15A). Figure 4.17B shows a decrease to less than unity in toluene, nitrate, and DO concentrations. Toluene utilization is most pronounced, especially when compared to the transport test results shown in Figure 4.15B. The toluene injected concentration ranged from 10 to 11 mg/L for these tests. Thus several mg/L were removed during the tests. Dilution-adjusted DO and nitrate concentrations slightly increased at the end of the test since DO and nitrate are present in the regional groundwater that mixed with the injected solution. Figure 4.18A shows background adjusted concentration [$(C-C_b)/(Co-C_b)$] versus (vol. extracted/vol. injected), for nitrate, DO. Figure 4.18B shows C/Co for bromide, cis-DCE, and TCE concentrations. cis-DCE concentrations in injected and extracted solution were reduced almost to zero due to biostimulation activity by addition of toluene and TCE concentrations remained unchanged and no degradation was observed during biostimulation tests.

Transformation of cis-DCE and TCE proved more difficult to assess, since they were present in the injected groundwater at concentrations lower than were present in the aquifer. However, normalization with respect to the background concentrations indicated that cis-DCE was transformed. The results indicate that the toluene-utilizers stimulated would have the ability to cometabolize cis-DCE, however, TCE transformation was not clearly demonstrated. In previous field studies using toluene as a cometabolic substrate, cis-DCE was transformed more rapidly than TCE (Semprini et al. 1994; Hopkins and McCarty, 1995). Results from the push-pull tests are consistent with these past well-to-well field tests. The reaction time of about 24 hrs may not have been long enough for TCE transformation to be observed. It is also possible that the presence of isobutene may have inhibited TCE transformation. These observations indicate that assessing TCE cometabolic transformation potential, when background TCE is already present, may prove difficult using the push-pull method described here.

A summary of measured concentrations and computed masse recoveries for toluene, DO, nitrate, and bromide for Biostimulation Activity Test are summarized in Table 4.9. Mass balance calculations indicated similar bromide mass recoveries between transport and biostimulation tests were achieved in all test ports (Tables 4.8 and 4.9). Nitrate mass recoveries in the biostimulation tests were 21.6 and 27.3 in P1 and P2 in LC191 and 32.9 and 43% in P1 and P2 at LC192, respectively (Table 4.9), which were less than the nitrate recoveries in the transport tests (Table 4.3). Similarly, toluene mass recoveries in the toluene activity tests range from 57 to 83% of the bromide recoveries compared to 90 to 98% in the toluene transport tests (Table 4.8). The toluene activity tests provided evidence of the stimulation of toluene-utilizing microorganisms. o-cresol formation was observed indicating that organisms expressing an ortho mono-oxgygenease enzyme were formed, and decrease toluene breakthrough curves, demonstrate toluene utilization occurred.

The rates of toluene removals were higher in LC192-P1 and P2 than the LC191-P1 and P2. The higher rates therefore were not correlated with nutrient addition, since nutrients were added to LC191-P2 and LC192-P1. The LC192 ports had higher bromide recoveries both in the transport and toluene activity tests. Thus groundwater flow was slower in the region of these wells. One

possibility for the higher rates is that a grater toluene-utilization biomass was stimulated with longer residence time for toluene during the biostimulation phase of these tests. It is also interesting to note that rates of o-cresol formation were lower at those locations, possibly indicating a more robust microbial community had developed.

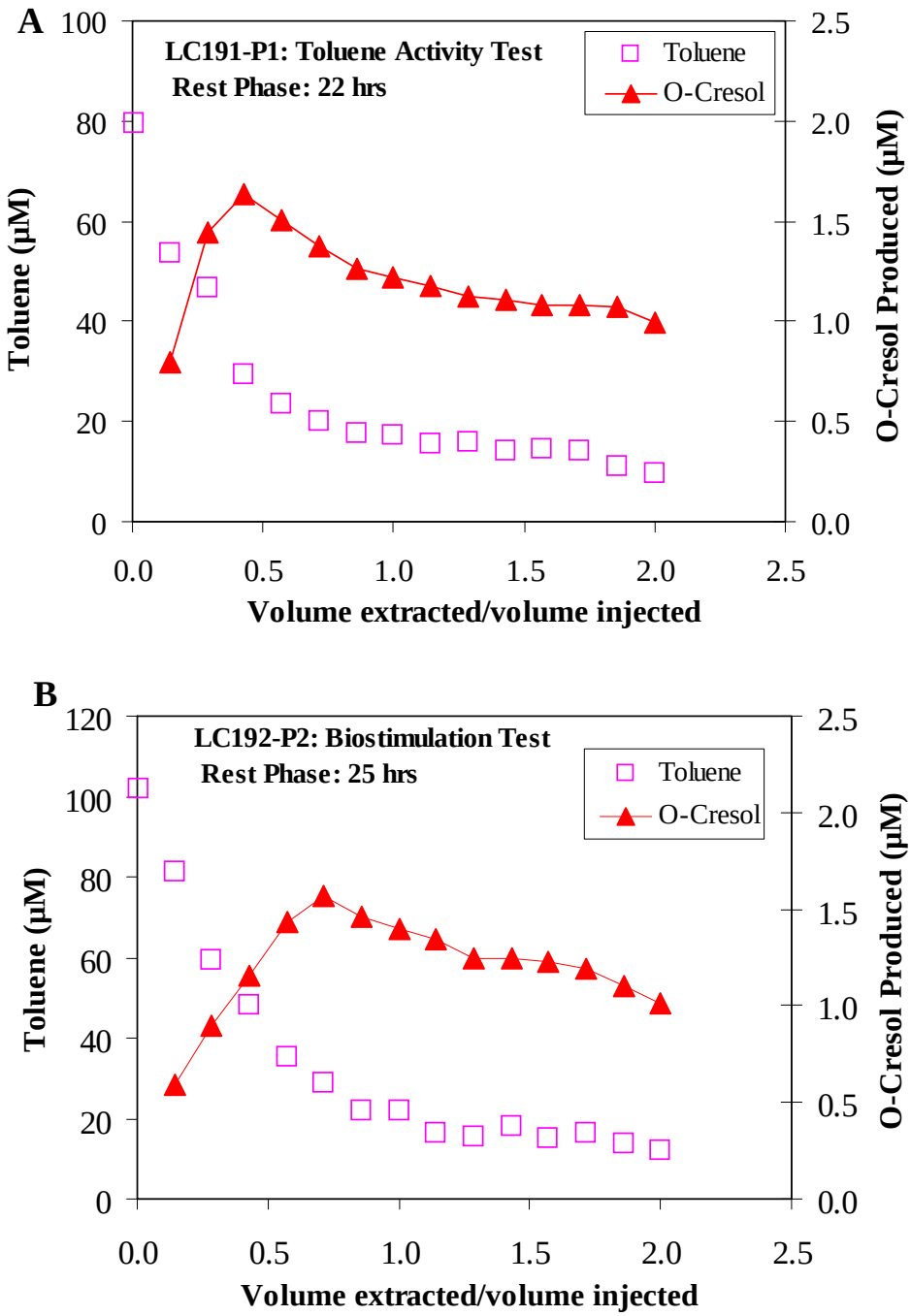

Figure 4.16. Toluene and o-cresol concentrations in the extracted groundwater during the toluene biostimulation test in wells LC191-P1 and LC192-P2 (without nutrient).

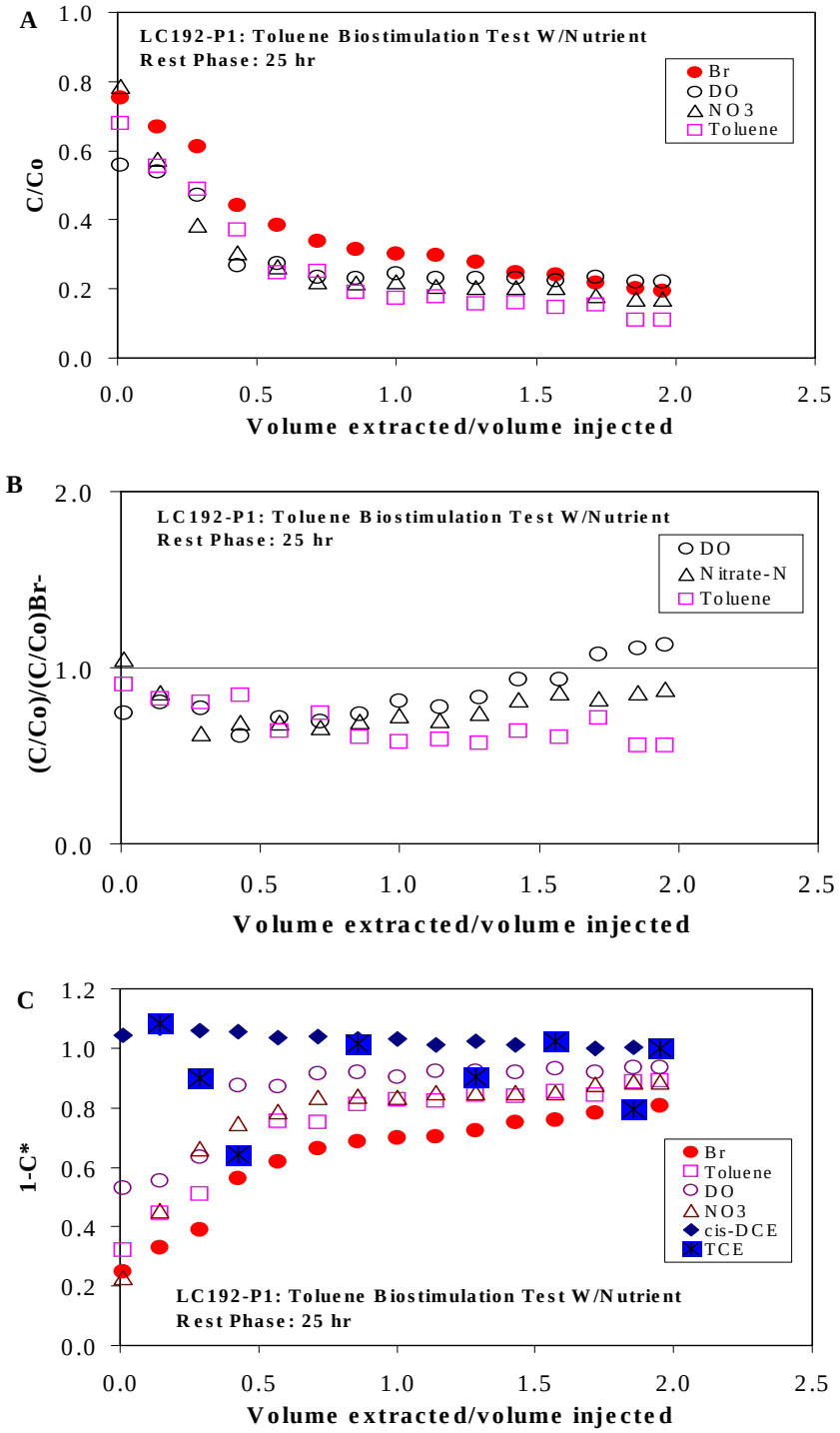

Figure 4.17. Extraction phase breakthrough curves of toluene biostimulation tests in LC192-P1 (A). Dilution-adjusted concentration of toluene, DO, and nitrate are shown in (B).

Table 4.9. Summary of Quantities of Injected and Extracted Solutes Mass, Percent Recovery, and Zero-Order Rate Estimates in Toluene Activity Tests

Test Location	Quantities	Toluene	o-Cresol	DO	NO_3^--N	Br^-
Biostimulation LC191-P1	Mass recovery (%)	26.6	[2]NA	26.3	21.6	33.1
	Rate (µmol/L/hr)	0.83	0.02	--	--	--
Biostimulation[1] LC191-P2	Mass recovery (%)	32.4	NA	35.4	27.3	38.9
	Rate (µmol/L/hr)	0.80	0.04	--	--	--
Biostimulation[1] LC192-P1	Mass recovery (%)	33.2	NA	40.7	32.9	53.0
	Rate (µmol/L/hr)	1.53	0.01	--	--	--
Biostimulation LC192-P2	Mass recovery (%)	37.8	NA	45.9	42.9	67.2
	Rate (µmol/L/hr)	1.79	0.01	--	--	--

[1]Nutrients (modified G4 Minimal Media) were added to ports 1 and 2 at LC192 and LC191, respectively. [2]NA**:** Not applicable

4.3.3 Push-Pull Activity Tests with Isobutene as a Surrogate Compound

Activity tests with isobutene added as a surrogate compound were then performed. Activity tests were performed by injecting a test solution containing dissolved toluene substrate, isobutene as the surrogate compound, and the bromide tracer to estimate utilization and transformation rates (Table 4.7). Isobutene was selected as a surrogate compound since laboratory studies by Hicks (2002) indicated that isobutene epoxide would be formed when an toluene ortho monooxgygenase enzyme is expressed. The injected solution for the activity tests was prepared using the same procedures described in the transport and biostimulation tests.

Two types of tests were conducted: 20-hr activity tests and natural-drift activity tests. In the push-pull activity tests, injected groundwater was permitted to reside in the aquifer for 20 hrs before extraction. 200-L of groundwater was then extracted and samples were taken over time. In the natural-drift test, the identical activity test solution was injected into the aquifer. However, the solution was left in place and samples taken under natural gradient conditions every 2hrs for a period of 48 hrs. Activity tests involve injecting test solutions containing toluene, isobutene, cis-DCE, bromide, H_2O_2, and nitrate and measuring the concentrations of the original compounds, metabolic products, and CAHs during the injection and extraction phases. In isobutene activity tests additional cis-DCE (500ug/L) was added to increase cis-DCE concentrations and to monitor its potential transformation. Only in the third activity test (*natural drift)*, trans-DCE (500 ug/L) was also added along with cis-DCE (Table 4.7).

Push-Pull Activity Test Results: A summary of measured concentrations and computed masses for toluene, isobutene, DO, nitrate, and bromide for push-pull activity tests are summarized in Table 4.10. Results of activity tests conducted after biostimulation showed similar bromide mass recoveries between transport and activity tests (Tables 4.9 and 4.10). Results from push-pull activity tests showed that concentrations of toluene, isobutene, DO, and nitrate were reduced during the extraction phase. The injected toluene concentration was deceased from approximately 10 mg/L in the previous toluene activity test to 2 mg/L for these tests. This was done to observe a greater fraction of toluene removal, and to limit inhibition of isobutene transformation. The decrease of normalized concentrations of isobutene, toluene, cis-DCE, DO, and nitrate in LC192-P1after 22.5 hours of residence in the aquifer are shown in Figure 4.18A. Essentially complete toluene utilization was observed at the lower injection concentration. Normalized cis-DCE concentrations were also greatly reduced and isobutene was also reduced compared to bromide.

The dilution-normalized concentrations are shown in Figure 4.18B. The results showed concentrations less than unity for all solutes indicating biological transformations occurred. A reduction of approximately 50% in isobutene concentration was observed during initial 50-60 L of extraction phase, which was coincided with maximum reduction in DO concentrations (Figure 4.18A). Dilution-normalized concentrations of DO decreased to 0.6 immediately during the initial extraction phase, and gradually increased to the background oxygen concentration (Figure 4.18B). Significant reductions in cis-DCE concentrations were observed during the initial extraction phase, however, the dilution-adjusted cis-DCE concentration increased as extraction

proceeded, which is due to the background cis-DCE in the native groundwater (Figure 4.18B). TCE removal was minimal in the toluene and surrogate compound activity tests, while results indicated transformation of cis-DCE in biostimulation and activity tests as shown in background adjusted plots of 1-C* in Figure 4.18C. Transformation of cis-DCE and TCE proved more difficult to assess, since they were present in the injected groundwater at concentrations lower than were present in the aquifer. However, normalization with respect to the background concentrations indicated that cis-DCE was transformed. These results indicate that the tolueneutilizers stimulated would have the ability to cometabolize cis-DCE.

Toluene mass recoveries in activity tests relative to bromide were 8.5 and 5.5% in P1 and P2 in LC191 and 3.7 and 8.4% in P1 and P2 in LC192 (Table 4.10). Toluene concentrations were reduced to almost non-detect after 20 hours. o-cresol was not detected during the activity tests, likely since the injected toluene concentration was only 2-3 mg/L. Isobutene mass recoveries ranged from 61 to 73% of the bromide recovery in the activity test (Table 4.10) compared to 86.3 to 110% in the transport tests (Table 4.9), indicating transformation occurred. When isobutene was utilized, isobutene oxide was observed as an intermediate oxidation product. Isobutene oxide was identified by retention time comparisons with an authentic isobutene oxide standard. Extracted isobutene concentrations and observed isobutene oxide concentrations (uM) in P1 in LC191 and LC192 are plotted Figures 4.19A and 5.19B, respectively. The ratios of mass of isobutene oxide produced to the isobutene mass injected were 2.8 and 3.1% in P1 and P2 in LC191 and 4.6 and 4.9% in P1 and P2 in LC192, respectively (Table 4.10). Reduction in isobutene concentrations and the production of isobutene oxide as an intermediate oxidation product indicated the stimulation of toluene-utilizing microorganisms containing an ortho-monooxygenase enzyme. Similar results for isobutene oxidation by toluene-utilizing microorganisms were observed in laboratory culture studies of Hicks (2002).

The estimated zero-order rates for the injected solutes upon extraction are summarized in Table 4.10. The estimated zero-order rates for isobutene transformation in the activity tests of P1 and P2 in LC191 were 0.73 and 0.63 µmol/L/h and 0.80 and 0.93 µmol/hr/L in P1 and P2 in LC192, respectively (Table 4.10). Results indicated that about 20% higher transformation activities for isobutene in LC192 compared to LC191. The estimated zero-order rates for toluene transformation in the isobutene activity tests were 0.81 and 0.79 µmol/L/h in P1 and P2 in LC191 and 1.02 and 1.11 µmol/hr/L in P1 and P2 in LC192 (Table 4.10). Similar rates for toluene were observed in P1 and P2 in LC191 in the toluene activity tests, but slightly higher rates were observed in P1 and P2 in LC192 (Table 4.9). These are conservative estimates of toluene utilization rates since essentially all the toluene added was transformed. Thus the higher rates in P1 and P2 in LC192 reflect the greater amount of toluene added. It is also possible that the greater amount of toluene degraded promotes faster rates of isobutene transformation. Higher transformation activities for toluene and isobutene in LC192 compared to LC191 are consistent with results of transport tests which indicate relatively higher groundwater velocities and lower residence time for microbial activity in LC191 compared to the LC192 well.

Table 4.10. Summary of Quantities of Injected and Extracted Solutes Mass, Percent Recovery, and Zero-Order Rate Estimates in Isobutene Activity Tests.

Test Location	Quantities	Toluene	Isobutene	Isobutene oxide	cis-DCE	DO	NO_3^--N	Br^-
LC191-P1	Mass recovery (%)	2.58	21.0	NA	18.7	18.7	25.0	30.5
	Rate (µmol/L/hr)	0.81	0.73	0.22	0.08	--	--	--
LC191-P2	Mass recovery (%)	2.25	30.0	NA	18.4	21.9	28.9	40.9
	Rate (µmol/L/hr)	0.79	0.63	0.19	0.11	--	--	--
LC192-P1	Mass recovery (%)	1.87	34.0	NA	23.8	19.3	40.7	50.6
	Rate (µmol/L/hr)	1.02	0.80	0.22	0.12	--	--	--
LC192-P2	Mass recovery (%)	5.19	37.62	NA	32.1	26.9	42.4	62.0
	Rate (µmol/L/hr)	1.11	0.93	0.19	0.10	--	--	--

NA, not applicable

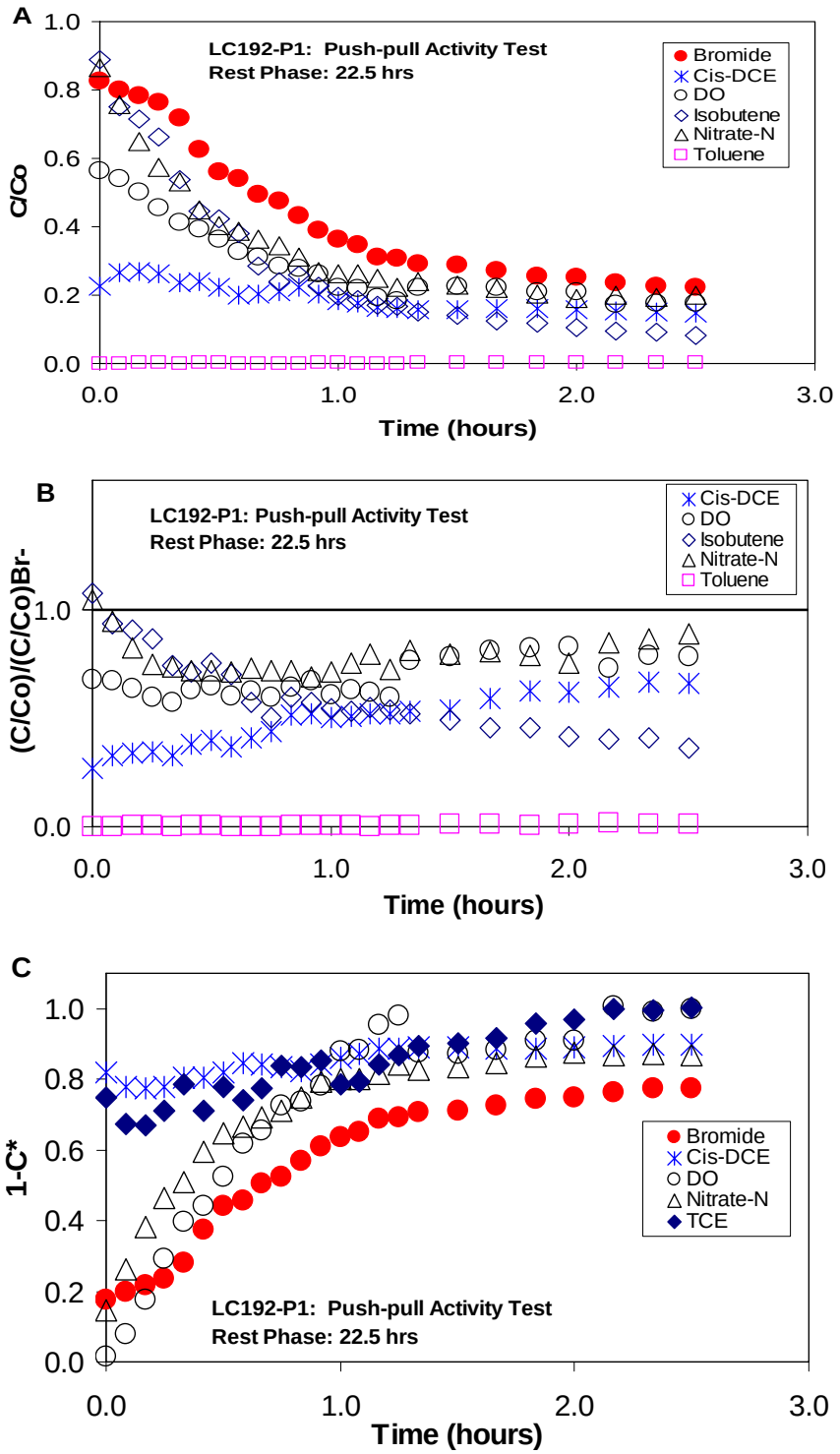

Figure 4.18. Extraction phase breakthrough curves of isobutene activity tests at Port 1 in LC192 (A). (B) dilution-adjusted concentration and (C) toluene, isobutene, cis-DCE, TCE and DO and (C) background adjusted plots 1-C*.

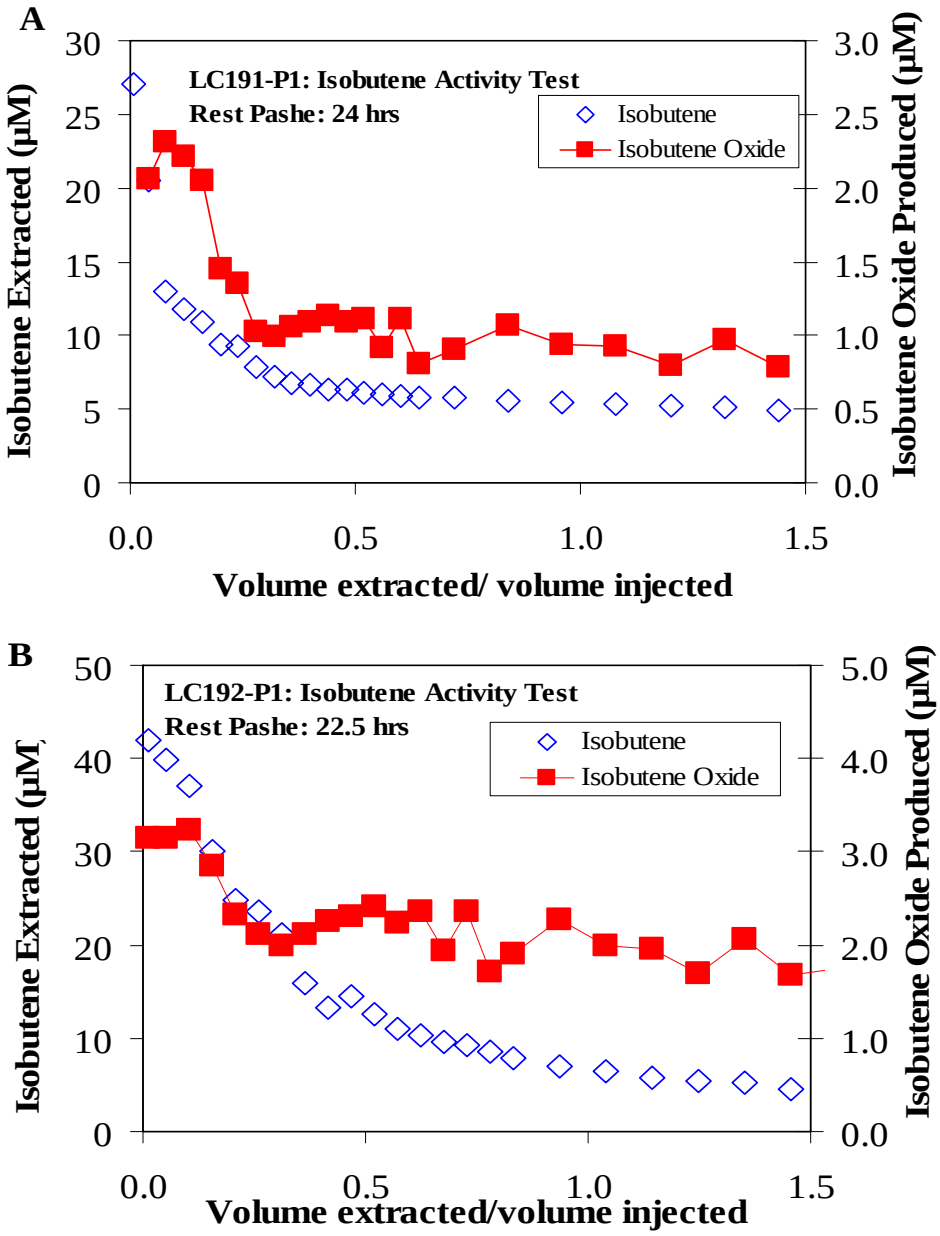

Figure 4.19. Isobutene transformation and isobutene oxide formation in isobutene activity tests in LC191-P1 (without nutrients) and LC192-P1 (with nutrients).

The aquifer at the test site is alluvial, and thus spatial variability to hydraulic conductivity is expected. Groundwater extraction was also occurring in the aquifer, which could have resulted in spatial variability in groundwater velocities. The bromide tests indicated that differences in groundwater velocity existed at the different test locations, and groundwater was flowing faster in the shallower zone (~ 25 ft) compared to the deeper zone (~ 35 ft).

The estimated zero-order rates for cis-DCE transformation in the activity tests in P1 and P2 in LC191 were 0.08 and 0.11 µmol/L/hr, which are about the same rates values of 0.12 and 0.1 µmol/L/hr estimated for P1 and P2 in LC192. These are about 10% to 13% of the computed zero-order rates of toluene and isobutene. The results indicated chlorinated ethenes (e.g. cis-DCE) were transformed by toluene utilizers, but at a slower rate compared to the isobutene surrogate substrate. cis-DCE however was present at a lower concentration than isobutene, which would affect the zero-order rate estimate, and likely the actual rate of transformation.

4.3.4 Natural Drift Activity Tests with Isobutene as a Surrogate Compound

Natural drift tests were performed similar to activity tests except that no extraction pumping was performed and samples periodically collected. Natural drift activity tests involved injecting a test solution containing toluene, isobutene, cis-DCE, trans-DCE, H_2O_2, and nitrate (Table 4.7). In natural drift tests, trans-DCE, which was not present as a background contaminant, was also added to further confirm the cometabolic transformation. Breakthrough curves for toluene, isobutene, cis-DCE, trans-DCE, and DO during natural drift activity tests were all lower than bromide (Figure 4.20A). For example, the normalized concentrations of bromide decreased from 1 to 0.2 during the 48 hrs following injection in P2 in LC191, while toluene concentrations were reduced to essentially zero, eight hours after the injection (Figure 4.20A). o-cresol was not detected during natural drift activity tests, likely because the injected toluene concentration was only 3.3 mg/L. The normalized isobutene concentrations gradually decreased to zero 48 hrs after the injection. When isobutene was utilized, isobutene oxide was observed as an intermediate oxidation product. Isobutene oxide was observed after 10 hours of residence in the aquifer and then increased and reached to maximum of about 0.3 mg/L after 24 hours as shown in Figure 4.21. Isobutene oxide concentrations gradually reduced to non-detectable at the end of 48 hrs of isobutene residence in the aquifer (Figure 4.21). cis-DCE concentrations were gradually reduced and reached background levels (Figure 4.20A). Trans-DCE concentrations also decreased and reached zero after 30 hours of residence, as shown in Figure 4.20A.

The dilution-normalized concentrations of toluene, isobutene, cis-DCE, and trans-DCE and DO were lower than unity, as shown in Figure 4.20B, indicating that these compounds were utilized or cometabolically transformed. An increase of cis-DCE concentrations after 40 hrs of residence in the aquifer resulted from the presence of the background cis-DCE and DO in the aquifer (Figure 4.20A). The results at the other three test locations were essentially the same as those observed at LC192 in P1.

In natural drift activity tests, mass balances of the injected solutes were calculated by integrating the area under the breakthrough curve (C/C_o), as presented in Table 4.11. This was done since

unlike the push-pull activity tests, the extraction phase of the natural drift tests consists of discrete sampling events instead of the continuous extraction phase pumping and sampling used for push-pull activity tests.

Similar bromide areas under the breakthrough curve were observed for all four locations (Table 4.11). These results differ from the push-pull activity tests, which showed lower bromide mass recoveries in P1 and P2 in LC191 compared to P1 and P2 in LC192. This may have been caused by seasonal changes in groundwater velocities, since natural drift tests were conducted in mid September 2003, compared to push-pull activity tests which were conducted in early June 2003. The integrated breakthrough areas for isobutene were similar in both ports in LC191 and LC192, showing these recoveries of about 70% of those observed for bromide (Table 4.11). The integrated areas under isobutene oxide concentration curve (Figure 4.21) for P1 and P2 in LC191 were greater than those of well LC192. One possible explanation is that abiotic transformation of isobutene oxide may have occurred with longer time for LC192 samples prior to analysis. The integrated breakthrough areas for cis-DCE and trans-DCE very similar in both ports in LC191 and LC192 (Table 4.11), indicates that cis-DCE and trans-DCE both were transformed to similar extents.

The estimated zero-order reaction rates for the injected solutes were calculated by multiplying dilution-adjusted concentrations $(C/C_o)/(C/C_o)Br^-$ by the corresponding initial concentration (C_o). Zero-order transformation rates were estimated by the slope of linear regression of decreasing dilution-adjusted concentrations $(C)/(C/C_o)$ Br^- versus time (Figures 4.22A and 4.22B). The estimated zero-order rates of the injected solutes in the natural drift tests are summarized in Table 4.11. Zero-order rates of toluene and isobutene transformation were relatively higher than those observed in the push-pull activity tests (Table 4.10). Isobutene shows a fairly linear decrease in the normalized concentration. cis-DCE and trans-DCE showed very similar rates of decrease in concentration. cis-DCE rates were determined with data collected during the first 20 hrs, before the normalized concentrations began to increase as a result of background groundwater. cis-DCE transformation rates were about 0.1µmol/L/h, which were similar to those of the push-pull activity tests (Table 4.10). These results indicate indigenous microorganisms were able cometabolize cis-DCE and trans-DCE after stimulation on toluene.

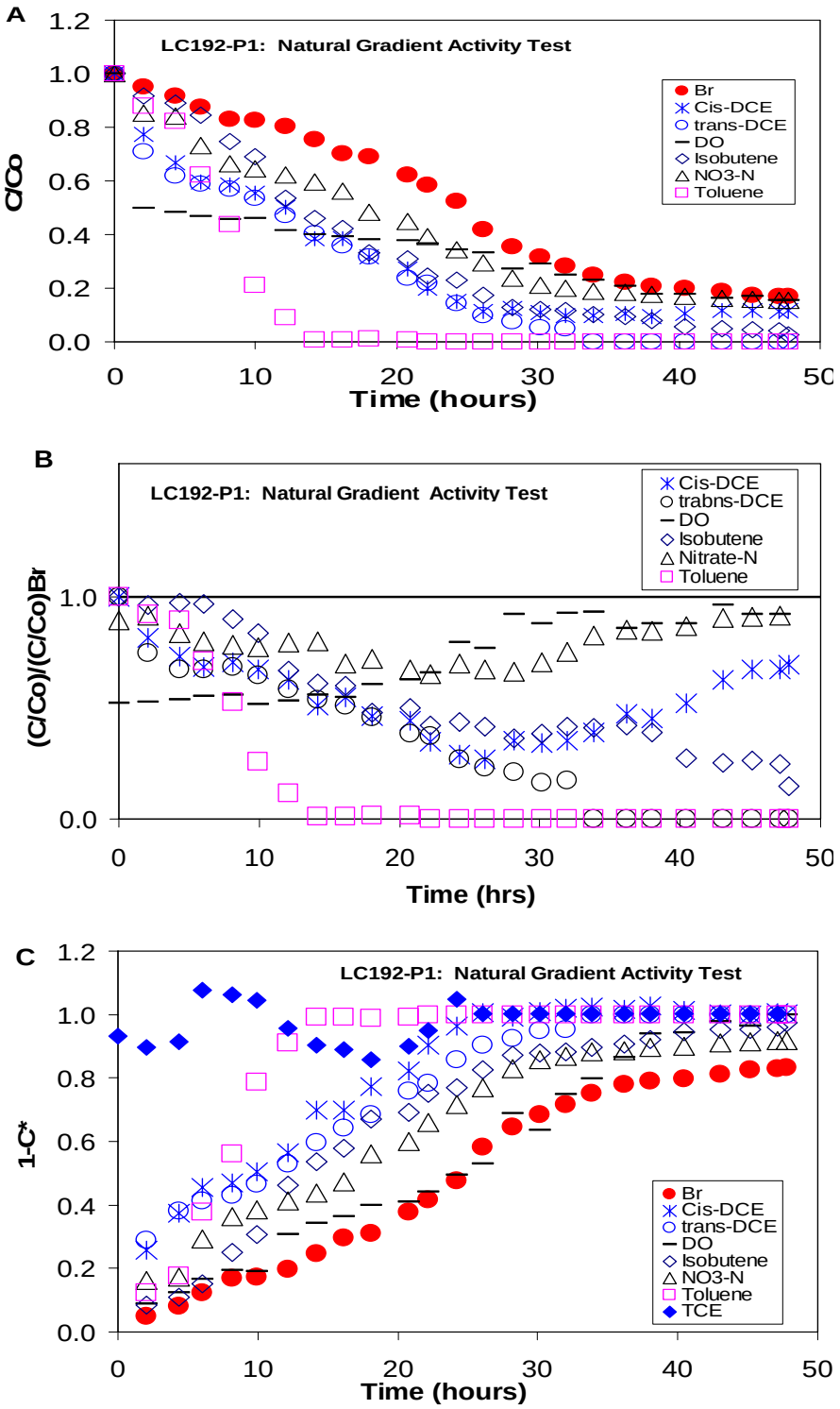

Figure 4.20. Extraction phase normalized concentrations in LC191-P2 (with nutrients) (A) and dilution-adjusted concentrations of injected solutes (B) in natural drift activity tests.

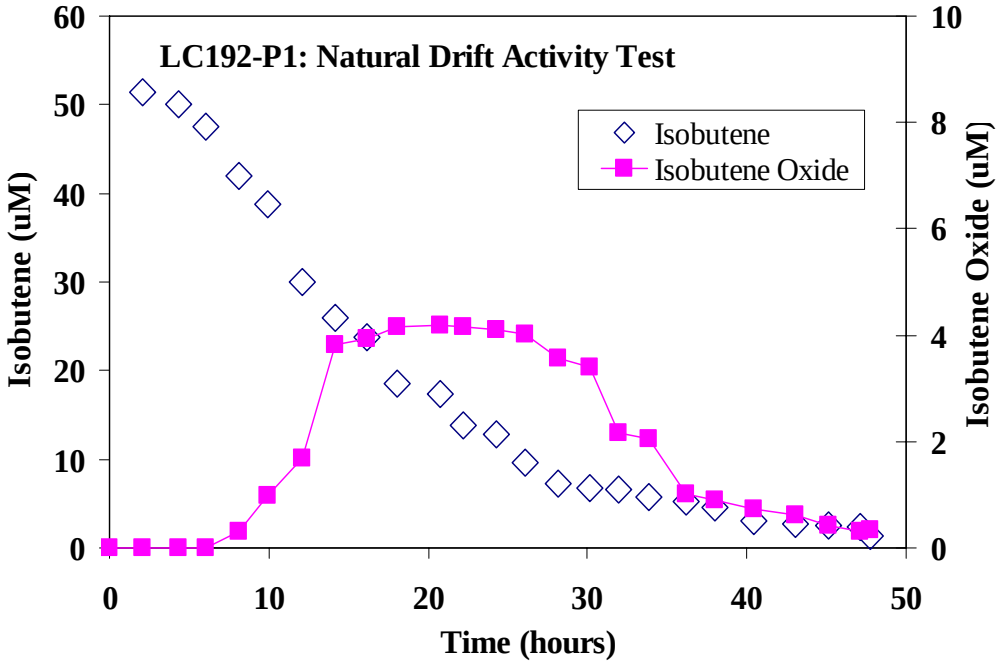

Figure 4.21. Isobutene transformation and isobutene oxide formation in natural gradient activity tests in LC192-P1.

Table 4.11. Summary of Quantities of Injected and Extracted Solutes Area Under Breakthrough Curves in Natural Drift Activity Tests.

Test Location	Quantities	Toluene	Isobutene	Isobutene oxide	cis-DCE	trans-DCE	DO	NO_3^--N	Br^-
Drift Activity LC191-P1	Area under Breakthrough Curve	7.1	18.0	13.3[1]	13.2	9.3	16.2	20.0	24.5
	Rate (µmol/L/hr)	1.27	1.12	0.18	0.12	0.11	--	--	--
DriftActivity LC191-P2	Area under Breakthrough Curve	6.3	15.7	8.2[1]	13.3	11.4	14.1	19.9	23.3
	Rate (µmol/L/hr)	5.16	0.75	0.11	0.15	0.10	--	--	--
Drift Activity LC192-P1	Area under Breakthrough Curve	7.3	16.4	6.75[1]	14.4	12.0	16.1	20.7	25.0
	Rate (µmol/L/hr)	2.14	1.37	0.09	0.09	0.08	--	--	--
Drift Activity LC192-P2	Area under Breakthrough Curve	10.6	17.7	4.98[1]	13.5	11.7	14.9	19.4	24.7
	Rate (µmol/L/hr)	1.07	1.26	0.07	0.10	0.09	--	--	--

[1] Area under isobutene oxide concentration curve (Figure 4.20)

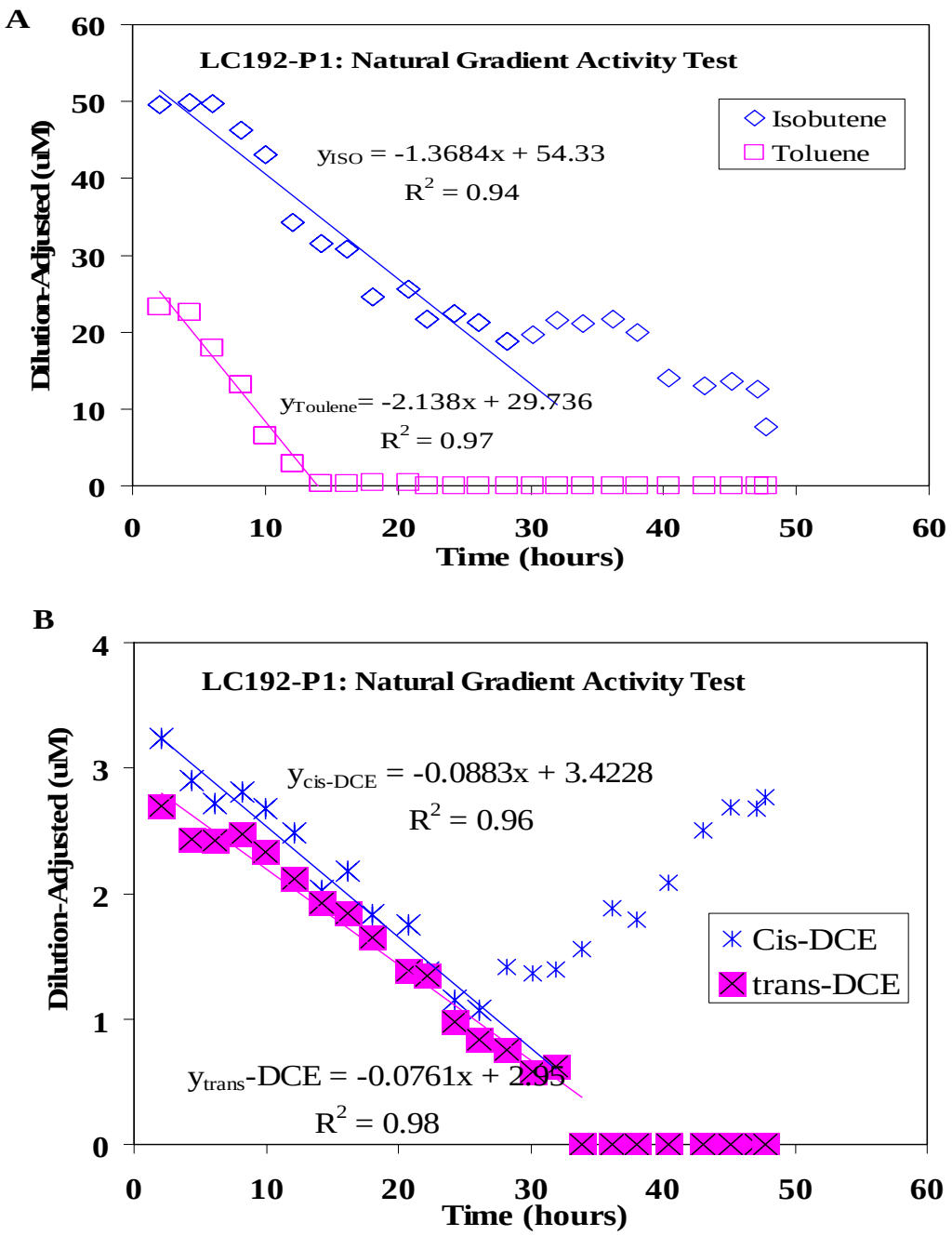

Figure 4.22. Estimated zero-order rates of injected solutes in natural gradient activity of tests in LC192-P, isobutene and toluene (A), cis-DCE and trans-DCE (B).

4.3.5 Inhibition Tests

Inhibition tests were performed as the final phase of the demonstration. The injected solution included 1-butyne, which acts as a mechanism-based inactivator of the ortho-monooxygenases expressed by toluene-oxidizing bacteria (Yeager, 2002). The inhibition tests were performed under natural gradient flow conditions using the same procedures and solutes as the activity tests. The concentration of 1-butyne in the injection solution was 20 mg/L (370 μM). Groundwater (105-L) containing dissolved hydrogen peroxide, toluene, and nitrate was injected into the aquifer to stimulate toluene utilizers prior to the inhibition tests.

Push-Pull Inhibition Test Results: 1-butyne completely blocked the utilization toluene and transformation of isobutene, cis-DCE, and trans-DCE (Figures 4.23A). Extraction breakthrough curves for toluene, isobutene, 1-butyne, cis-DCE, trans-DCE, and DO the during inhibition test were very similar to the breakthrough curve of the bromide tracer, indicating conservative transport and no transformation of any of the injected solutes (Figure 4.23A). This is directly in contrast with the results of natural drift activity tests shown in Figure 4.20A, where transformation was observed. Figure 4.23B also shows no decrease in the dilution-adjusted concentrations, with all the concentrations centered around unity. Similar results were obtained at the other test locations. o-cresol and isobutene oxide were not detected during the inhibition tests, and cis-DCE and trans-DCE transformation was also blocked by 1-butyne, indicating an ortho-monooxygenase enzyme was likely involved in their transformation.

In the natural drift inhibition tests, the integrated areas under the breakthrough curve were determined. The inhibition tests results showed similar areas for each injected solute at all four locations (Table 4.12). Similar areas under the breakthrough curve of bromide between the natural drift activity and inhibition tests were observed in both ports in LC191 and LC192 (Tables 4.11 and 4.12). The results clearly show that at all locations microbial utilization of toluene, DO, and nitrate was essentially completely inhibited by 1-butyne, as well as the transformation of toluene and isobutene, cis-DCE, and trans-DCE. The results when compared with those obtained in the activity tests (Table 4.11) demonstrate that the microbial utilization and transformation observed in the activity tests.

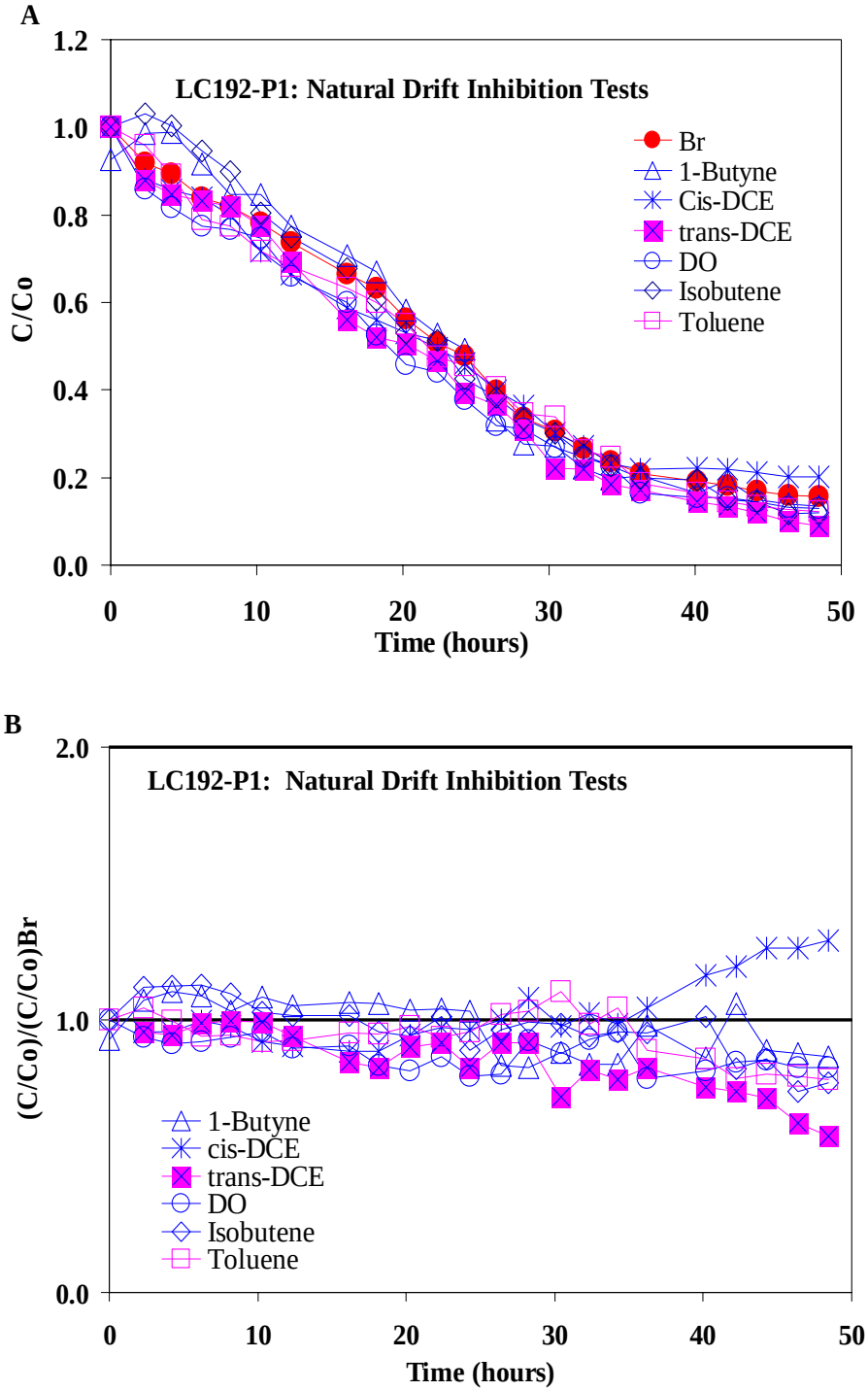

Figure 4.23. Extraction phase normalized concentrations in LC192-P1 (without nutrients) (A) and dilution-adjusted concentrations of injected solutes in natural gradient inhibition tests.

Table 4.12. Summary of Quantities of Injected and Extracted Solutes Area under Breakthrough Curves in Inhibition Tests.

Test Type	Quantities	Toluene	Isobutene	1-Butyne	cis-DCE	trans-DCE	DO	NO_3^--N	Br^-
Inhibition LC191-P1	Area under Breakthrough Curve	26.4	22.3	21.6	22.0	21.9	22.9	22.7	21.4
Inhibition LC191-P2	Area under Breakthrough Curve	20.7	22.7	21.7	21.7	21.7	22.6	23.2	22.2
Inhibition LC192-P1	Area under Breakthrough Curve	22.8	24.2	24.2	21.2	21.4	21.3	24.3	23.8
Inhibition LC192-P2	Area under Breakthrough Curve	21.3	24.5	23.9	23.9	23.3	22.7	24.6	24.4

4.3.6 Summary Results from Field Push-Pull Tests Conducted at Fort Lewis, WA

Single-well push-pull tests were performed to assess the feasibility of in situ aerobic cometabolism of the chlorinated aliphatic hydrocarbons (CAHs) trichloroethene (TCE), cis-1,2-dichloroethene (cis-DCE), and trans-1,2-dichloroethene (trans-DCE) using toluene as a growth substrate. Tests were performed in a CAH-contaminated groundwater aquifer at Fort Lewis, WA. Transport characteristics of dissolved solutes were evaluated by comparing breakthrough curves of injected substrates and CAHs to those of a co-injected bromide tracer, and indicating conservative transport of all solutes in the absence of microbial transformations. Microbial utilization of injected toluene as a growth substrate was indicated by decreases in dilution-adjusted toluene concentrations and by the production of o-cresol as an intermediate oxidation product.

Evidence that injected toluene stimulated organisms with the ortho-monoxygenase enzyme system was provided by the oxidation of injected isobutene to isobutene oxide and by the inhibition of toluene and isobutene oxidation in the presence of a coinjected 1-butyne inhibitor. Evidence was also obtained for the in situ transformation of injected cis-DCE and trans-DCE, but not TCE. The results demonstrated that push-pull tests can be used to evaluate the potential for in situ cometabolic metabolism of chlorinated ethenes.

4.4 Data Assessment

The data described in Sections 4.1, 4.2, and 4.3 provide a realistic assessment of the demonstration objectives at McAFB and Fort Lewis, respectively. Figures and tables of results were shown for propane and methane tests performed in the saturate zone at the AFB, while toluene tests were performed at Fort Lewis, WA.

The effectiveness of dissolved substrate addition to stimulate the indigenous propane utilizers and toluene utilizers was evaluated in standard monitoring wells. Transport characteristics of dissolved solutes were evaluated using bromide as a conservative tracer. Propane and toluene utilization as growth substrates were evaluated by observing repeated uptake under both natural gradient flow conditions, and during push-pull activity tests. For the push-pull activity tests the injected solution was amended with the substrates of interest, and after injection was permitted to reside in the formation for 19 to 24-hours and then extracted. Decreases in propane and toluene concentrations, normalized to bromide as a conservative tracer, indicated utilization of these growth substrates. When toluene was utilized, ortho-cresol was observed as an intermediate oxidation product.

Ethylene, propylene, nontoxic surrogates to probe for CAH transformation activity, was added in the propane tests, while isobutene was added in the toluene tests. The stimulated propane utilizers cometabolized ethylene and propylene to produce ethylene oxide and propylene oxide as cometabolic by-products. The stimulated toluene utilizers produced isobutene oxide, which provides evidence that microorganisms with an ortho-monooxygenase were stimulated. Propane results confirmed that microorganisms with a propane monooxygenase enzyme were stimulated.

In order to further demonstrate the involvement of monooxygenase enzymes, acetylene blocking tests were also performed. Propane utilization and ethylene and propylene oxidation were essentially completely inhibited by the presence of acetylene. Toluene utilization, isobutene, cis-DCE, and trans-DCE transformation were inhibited by 1-butyne. Inhibition by 1-butyne indicates transformation by an ortho-monooxygenase enzyme. The Gas- sparging tests support the stimulation of methane- and propane- oxidizing microorganisms, cometabolic transformation of ethylene and propylene by the enzyme responsible for methane and propane degradation. 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. The results at both sites demonstrated that push-pull tests can be used to evaluate the potential of in situ cometabolic treatment

4.5 Technology Comparison

The push-pull test may be comparable to well-to-well recirculation tests (Semprini et al., 1992). Although the well-to-well recirculation approach has been successfully applied in a limited number of field demonstrations, it has several disadvantages that limit its routine use. Well-to-well recirculation tests interrogate a larger volume of the subsurface and thus have the potential to provide more representative information, but are expensive and logistically complicated. Provide in Table 4.13 is a comparison of well-to-well and push-pull tests, and a relative ranking.

Table 4.13. Comparison Well-to-Well Tests versus Push-Pull Test (+ less of an advantage; +++ more of an advantage)

Well-to-Well Tests	Rank	Push-Pull Tests	Rank
Injection, extraction, monitoring wells required	+	Single monitoring well required	+++
Continuous injection and extraction	+	Pulse injection	+++
Injected fluid continuous made-up with nutrient, substrates, CAHs of interest	+	Injected fluid made up in a batch for addition	+++
Large volume of fluid extracted	+	Small volume of fluid extracted	+++
Larger masses of regulated chemical are added	+	Small masses of regulated chemicals are added	+++
Continuous monitoring	+	Less frequent monitoring	+++
Sample a large volume of aquifer	+++	Samples a small volume of aquifer	+
Easier to biostimulate	++	More difficult to biostimulate	+
Representative of actual treatment	+++	Not representative of actual treatment	+
Groundwater flow less impact	++	Groundwater flow a greater impact	+
Steady-state treatment can be achieved	+++	Steady-state treatment cannot be achieved	+
Higher Cost	+	Lower Cost	++
Usually conducted at a single location	+	Can be conducted at multiple locations	++
More amendable to modeling	++	Less amenable to modeling	+
Single substrate tests	+	Multiple substrate tests	++
Other processes can be observed, such as sorption	++	Difficult to observe other processes, such as sorption	+

5. Cost Assessment

Implementation costs for the push-pull tests at McAFB and Fort Lewis are shown in Table 4.8. Costs include fixed and variable costs. Various major costs included travel costs for distance sites and labor associated with the significant analytical load of the demonstration (estimated at approximately $58,000). Higher costs are expected if this would have been done by commercial vendors as shown in Table 5.1. Higher costs with commercial vendors are associated with the higher analytical costs of P&T gas chromatograph (GC) system and ion chromatograph (IC) system as shown in Table 5.2. OSU average estimated cost for each site is about $160,000, while same operation costs for commercial vendors would be about $260,000 or about 62% higher than OSU costs.

Equipment costs such as Groundfos pumps and Peristaltic pumps, flow meters, and DO meters are high. Savings would be realized in equipment costs by using the same equipment at several sites with only the cost for maintenance. Purchase of equipment requires a large initial investment, but long-term savings are realized over time as the equipment is used at all of the sites. The cost of buying equipment for several sites is significantly less than buying pumps, flow meters, etc. for each individual site.

Analytical costs for transport and activity tests could be reduced by 50% in practice, compared with the demonstrations performed at McAFB or Fort Lewis. For example instead of taking 20 samples in transport and activity tests to construct breakthrough curves, 10 samples will likely suffice. This is because the breakthrough curve is fairly predictable, and the same shape will be constructed with 10 or 20 samples. Costs for conducting activity tests and drift tests are high since require taking samples more often over a period of several days to a week. Costs also could be reduced by using local or on-site personnel.

Travel costs, especially for the distance sites was significant, assuming one or two persons need to travel out of state, e.g. at McAFB site or if you have to haul all equipment back and forth if no storage is established at the site, e.g. at the Fort Lewis site. Costs could be reduced in practice if local on-site personnel are used and if travel and shipping costs can be reduced.

6. Implementation Issues

6.1 Cost Observations

Factors that affected project costs were the selected sites. At McAFB the depth of groundwater in injection wells was about 100 ft, which required special pumps (i.e. Groundfos), while at Fort Lewis the depth of groundwater was about 10 ft and only peristaltic pumps were required to conduct the push-pull tests. The multi-port monitoring wells at Fort Lewis were a cost factor since they allow for the use of smaller injection volumes, which simplified test logistics and costs.

Table 5.1. McAFB and Fort Lewis Demonstration Costs

Cost Category	Sub Category	Site 1[(a)] Costs ($)	Site 2[(a)] Costs ($)	Site 3[(b)] Costs ($)
FIXED COSTS				
1.CAPITAL COSTS	Mobilization/demobilization	$10,000	$10,000	$10,000
	Planning/Preparation	$20,000	$20,000	$20,000
	Site investigation and testing			
	- Field work preparation	$5,000	$5,000	$5,000
	- Other	$2,000	$2,000	$2,000
	Equipment Cost			
	- Groundfos Pumps	$4,000	$0,000	$0,000
	- Peristaltic Pumps	$3,500	$0,000	$0,000
	- DO meter	$3,500	$0,000	$0,000
	Start-up and Testing	$5,000	$2,000	$2,000
	Other			
	- Carboys, Tubings	$4,500	$2,500	$2,000
	- Chemicals, Gas supplies	$5,000	$5,000	$5,000
	- Sampling vials, labels	$5,000	$5,000	$5,000
Sub-Total $67,500			$49,500	$49,000
VARIABLE COSTS				
2. OPERATION AND MAINTENANCE	Labor			
	- Field personnel	$5,000	$5,000	$0,000
	- Travel	$15,000	$15,000	$10,000
	- Lodging	$10,000	$10,000	$8,000
	Materials and Consumables	$1,000	$1,000	$1,000
	Utilities and Fuel	$1000	$1000	$1000
	Equipment Rentals			
	- Trailer	$1,500	$1,500	$1,500
	- Analytical tank rentals	$1,000	$1,000	$1,000
	- Other rentals	$500	$500	$500
	Performance Testing/Analysis			
	- Tracer analysis	$8,000	$8,000	$8,000
	- CAHs analyses	$50,000	$50,000	$50,000
	- Data analyses	$5,000	$5,000	$5,000
	- Report preparation	$10,500	$10,500	$5,000
	- Other	$2,500	$2,500	$2,500
	Other direct costs	$400	$400	$400
Sub-Total	$100,900		$100,900	$83,900
TOTAL COSTS				
TOTAL TECHNOLOGY COST:				**$477,700**
Unit Cost ($):				**$159,233/Site**

[(a)] McAFB, CA demonstration site costs, [(b)] Fort Lewis, WA demonstration site costs

Table 5.2. Estimated Demonstration Costs by Commercial Vendor

Cost Category	Sub Category	Costs ($)
FIXED COSTS		
1. CAPITAL COSTS	Mobilization/demobilization	$10,000
	Planning/Preparation	$20,000
	Site investigation and testing - Field work preparation - Other	 $10,000 $2,000
	Equipment Cost - Groundfos Pumps - Peristaltic Pumps - DO meter	 $4,000 $3,500 $3,500
	Start-up and Testing	$5,000
	Other - Carboys, Tubing - Chemicals, Gas supplies - Sampling vials, labels	 $4,500 $5,000 $5,000
Sub-Total $72,500		
VARIABLE COSTS		
2. OPERATION AND MAINTENANCE	Labor - Field personnel - Travel - Lodging	 $10,000 $15,000 $15,000
	Materials and Consumables	$1,000
	Utilities and Fuel	$1000
	Equipment Rentals - Trailer - Analytical tank rentals - Other rentals	 $1,500 $1,000 $500
	Performance Testing/Analysis - Tracer analysis (IC) - CAHs analyses (GC) - Data analyses - Report preparation - Other	 $10,000 $100,000 $10,000 $20,500 $2,500
	Other direct costs	$400
Sub-Total		**$188,400**
TOTAL COSTS		
TOTAL TECHNOLOGY COST:		**$260,900**
Unit Cost ($):		**$260,900/Site**

6.2 Performance Observations

This study demonstrated that single-well, “push-pull” tests can be used to assess the potential for stimulating in situ aerobic cometabolism using existing monitoring wells. The method requires only simple components, such as pumps, to extract groundwater from the test wells, plastic tanks and carboys to hold prepared test solutions, and standard groundwater sampling equipment. Typically, a series of parallel tests were conducted in adjacent wells to examine the effects of physical or chemical heterogeneity on microbial activity or to evaluate various treatment alternatives.

At McAFB, it was possible to stimulate propane utilizing microorgansims under aerobic conditions in a CAH contaminated aquifer by sequential additions of propane and oxygen dissolved in groundwater. Moreover, in situ rates of propane utilization, ethylene, and propylene transformation could be quantified. After biostimulation, injected ethylene and propylene were transformed to ethylene oxide and propylene oxide, respectively, which provides direct evidence that these substrates are being cometabolized, and provides indirect evidence that these organisms could similarly transform CAHs. Acetylene effectively blocked both propane utilization and ethylene transformation, further indicating the stimulation of propane monooxygenase activity.

Evidence that propane and oxidation additions in these field tests stimulated indigenous propane utilizers with the capability to aerobically cometabolize cis-DCE and TCE using a monooxygenase enzyme system are: (1) the observed simultaneous utilization of propane and oxygen during the Biostimulation period, (2) the transformation of ethylene and propylene to ethylene and propylene oxide, respectively during the activity test, (3) transformation of cis-DCE during the activity test, and (4) complete inhibition of propane utilization, and ethylene and cis-DCE transformation during the acetylene block test. Since TCE was present as a background contaminant, the mixing with the background groundwater resulted in our inability to demonstrate that TCE was transformed. The results indicated that the rates of TCE transformation were likely slow under the conditions of the tests. Additions of TCE above the background concentrations would likely be required to better assess rates of TCE transformation.

At Fort Lewis the effectiveness of toluene additions in stimulating aerobic cometabolic activity of indigenous microorganisms was demonstrated by a an extensive series of single-well tests conducted in existing multilevel monitoring wells. Transport tests demonstrated the feasibility of injecting and recovering complex solute mixtures from a contaminated aquifer and verify that bromide concentrations can be used to compute dilution-adjusted concentrations for the other substrates. The detection of o-cresol during activity and natural drift tests confirmed that injected toluene was being transformed by microorganisms containing an ortho-monooxygenase enzyme. Further evidence that toluene additions stimulated aerobic cometabolic activity were obtained by the in situ transformation of injected isobutene to isobutene oxide, the complete inhibition of substrate utilization in the presence of coinjected 1-butyne, and by the observed transformation of cis-DCE, and trans-DCE. TCE was not added in these tests, and like our observations at McAFB, direct evidence for TCE transformation was not obtained at Fort Lewis. When

background concentrations of TCE are present, the rates of TCE transformation are slow enough so that TCE transformation is difficult to observe using the push-pull method that was developed.

6.3 Scale-Up

Push-pull tests were performed at the scale that they would be implemented within practice. Cost reductions would be realized by sharing equipment among injection wells (i.e., pumps and carboys). Cost reductions for the push-pull field demonstration would be seen by reducing number of samples taken for CAHs and tracer analyses. Tracers might be used that could be determined by the same GC method used for CAH analysis, thus eliminating the need for bromide tracer ion chromatograph analysis.

Push-pull activity tests or natural drift activity tests could be performed with all the solute and surrogates added together. Separate tests for each component are more cumbersome and do not add to the overall interpretation of the results. Thus the number of tests could be reduced significantly.

6.4 Lessons Learned

Working in the shallow aquifer at Fort Lewis was much easier than the deeper aquifer at McAFB. While working at depth the potential for volatilization of dissolved gas component was greater. The shallow aquifer at Fort Lewis and the multi-port monitoring wells simplified test logistics. The use of multiport wells was also desirable because of the smaller dead volume in the casing, resulting in less mixing. Smaller volumes of fluid could be injected as a result of the shorter screened intervals.

In some tests it may be desirable to include a *drift phase* (with no pumping) between injection and extraction phases to increase the residence time of the test solution in the aquifer and allow more time for microbial transformations to proceed. During the drift phase, transport of the injected test solution is dominated by the regional groundwater flow field. Drift phase durations may range from hours to months, depending on the type of test and site conditions. For example, long drift phases are generally desirable if targeted transformations are likely to be slow. However, if the duration of the rest phase is too large, excessive dilution of the injected test solution may occur, lowering concentrations of tracer, reactants, and products below detection limits.

6.5 End-User Issues

The recently developed push-pull technique has been used successfully to measure in situ rates of aerobic cometabolism of chlorinated solvents. More work is needed relating rates of surrogate transformation to the rates of CAH transformation. It also proved difficult to estimate rate of transformation of cis-DCE that was already present in the aquifer, without adding additional cis-DCE. Once additional cis-DCE was added to the injected solution, its transport could be easily tracked and transformation rate could be estimated.

Obtaining regulatory permission to add cis-DCE and TCE may prove to be problematic at DoD sites. We obtained regulatory approval to add cis-DCE at Fort Lewis by meeting with the regulators and discussing the test plan. The regulators gave permission based on the following criteria: the aquifer was already contaminated with cis-DCE in the area where the tests were preformed; very small quantities of cis-DCE were needed to be added in push-pull tests; concentrations added were low (~ 250 μg/L); and the down gradient plume was being captured by a pump-and-treat system. Although obtaining regulatory approval will differ on a case by case basis, the above criteria are likely important in obtaining regulatory approval. In the absence of obtaining regulatory approval, surrogate compound addition is recommended. More studies however are needed to relate rates of surrogate compound transformation to rates of cis-DCE and TCE transformation.

This method could be expanded to demonstrate the ability of the push-pull test to detect and quantify in situ rates of intrinsic aerobic metabolism of cis-DCE and/or VC. Such a capability would be of direct benefit to the assessment of monitored natural attenuation as a treatment alternative for dilute-plumes of aerobic CAH-contaminated groundwater, which are widespread within the DoD complex.

6.6 Approach to Regulatory Compliance and Acceptance

The push-pull activity test method developed in this study is useful for evaluating the feasibility for in situ CAHs bioremediation through aerobic cometabolism. The activity test is performed by injecting site groundwater amended with propane or toluene as a cometabolic substrate, CAHs, and ethylene, propylene, and isobutene as a reactive CAH surrogate added to the injected groundwater. The ease of obtaining regulatory approval to inject non-toxic surrogate compounds (ethylene, propylene, and isobutene during push-pull tests at field sites is an important advantage of this method. Regulatory approval for injecting toluene, cis-DCE, and trans-DCE was facilitated by the recognition that injection volumes and tracer quantities are small and much of the unreacted tracers are removed during the extraction phase, subsequent sampling, and by performing tests within an pump-and-treat capture zone.

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8. Points of Contact

Points of contact are listed in the following table.

Table 8.1. Points of Contact

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Mr. Ficklen Holmes	AFCEE/ERT 3207 North Road Brooks, AFB, TX 78235-5363	(210) 536-4366 (210) 536-4330 holmes.ficklen@brooks.af.mil	Project Manager
Kira Lynch	US Army Corps Seattle District, 4735 East Marginal Way South, Seattle, WA	(206)-764-6918 (206)-764-3706 kira.p.lynch@usace.army.mil	Fort Lewis Point of Contact