ESTCP
Cost and Performance Report

(ER-9921)

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

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TABLE OF CONTENTS

Page

TABLE OF CONTENTS (continued)

Page

FIGURES

TABLES

ACRONYMS AND ABBREVIATIONS

ACFEE	Air Force Center for Environmental Excellence
Br	bromine
BTEX	benzene, toluene, ethylbenzene, and xylenes
CAH	chlorinated aliphatic hydrocarbon
CAS	cometabolic air sparging
CF	chloroform
cis-DCE	cis-1,2-dichloroethene
1,1-DCE	1,1-dichloroethene
DO	dissolved oxygen
DoD	Department of Defense
EGDY	East Gate Disposal Yard
EPA	Environmental Protection Agency
ESTCP	Environmental Security Technology Certification Program
FID	flame ionization detector
GC	gas chromatography
HP	Hewlett-Packard
IC	ion chromatography
LEL	lower explosive limit
McAFB	McClellan Air Force Base
MCL	maximum contaminant level
Na_2CO_3	sodium carbonate
$NaHCO_3$	sodium bicarbonate
$NaNO_3$	sodium nitrate
NETTS	National Environmental Technologies Test Site
NO_3^-	nitrate
O_2	oxygen
OSU	Oregon State University
P&T	purge and trap
PCE	tetrachloroethene / perchloroethene
PID	photo ionization detector
SERDP	Strategic Environmental Research Development Program
SF_6	sulfur hexafluoride

1,1,1-TCA	1,1,1-trichloroethane
TCE	trichloroethene
trans-DCE	trans-1,2-dichloroethene
VC	vinyl chloride
VOA	volatile organic analysis

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We would also like to thank Fort Lewis allowing us access to the wells on base, and special thanks to Kira Lynch of the U.S. Army Corps of Engineers for her logistical support.

1.0 EXECUTIVE SUMMARY

Aerobic cometabolism is a promising technology for 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 3-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 at Fort Lewis Logistics Center, Washington, using toluene as the cometabolic substrate. McAFB was selected as the demonstration site since it has significant CAH groundwater contamination, and it was 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.

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 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.

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 taken periodically. 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.

In the McAFB demonstration, propane was added as the cometabolic substrate, and ethylene and propylene were used as surrogate 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-1,2-dichloroethene (cis-DCE), and trichloroethene (TCE). Transport push-pull tests showed 80 to 90% of the injected tracer; substrates and surrogated could be recovered upon extraction, and little or no transformation or retardation occurred during transport.

Biostimulation tests showed initial rates of propane utilization to be 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 that 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 that 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 was 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 that monooxygenase enzymes were involved.

The Fort Lewis tests demonstrated that indigenous toluene utilizers 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-1,2-dichloroethene (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, 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.

1.1 BACKGROUND INFORMATION

Aerobic cometabolism is a promising technology for in situ remediation of chlorinated CAH at DoD sites. Low-cost methods are needed for generating the data required to design field-scale systems. This task is complicated by the complexity of the cometabolic process and the various cometabolic substrates from which to choose. The contaminants and their concentration are also important considerations, along with the transformation abilities of the indigenous microorganisms that are stimulated on a specific substrate. This report describes a newly developed single-well, push-pull test field technology for evaluating the feasibility of using in situ aerobic cometabolic processes to treat groundwater contaminated with chlorinated solvent mixtures. The test consists of the controlled injection of a prepared test solution into an aquifer followed by the recovery of the test solution/groundwater mixture from the same location. The test solution consists of water containing nonreactive tracers such as bromide, the cometabolic substrate of interest, dissolved oxygen, and reactive solutes that are designed to permit the estimation of the in situ transformation rates of the CAHs of interest.

The ESTCP supported 3-year laboratory and field studies to investigate single-well push-pull tests to evaluate the potential for aerobic cometabolism of CAHs at McAFB, California, and Lewis Logistics Center, Washington. McAFB was selected as the demonstration site for a variety of reasons: (1) McAFB has significant CAH groundwater contamination; (2) previous studies by Oregon State University (OSU) demonstrated that indigenous bacteria at McAFB could utilize propane as a growth substrate and support cometabolic CAH degradation; and (3) McAFB is a member of the Strategic Environmental Research Development Program (SERDP) National Environmental Technologies Test Site (NETTS) program and expressed interest in supporting this unique technology. At Fort Lewis, the ability of the push-pull test to detect and quantify in situ rates of aerobic cometabolism of chlorinated ethenes was demonstrated in a TCE and cis-DCE contaminated aquifer. At this site, toluene was used as a cometabolic growth substrate, and different surrogates and inhibitors were evaluated in push-pull field activity tests.

The layout of this report is as follows: Section 1 provides an introduction to the technology, including background information, objectives of the demonstration, and regulatory drivers. Section 2 describes the technology, process description, strengths and weaknesses of the technology, and major factors influencing cost and performance. Section 3 describes demonstration design, the test site and facilities, 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 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. Section 7 lists references used. Appendix A provides points of contact for the study.

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 TCE using gaseous cometabolic substrates such as propane and soluble substrates such as toluene. Specific objectives were:

- 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 surrogate compounds for evaluating the cometabolic potential
- To optimize combinations of injected nutrients and substrates to maximize rates of contaminant transformation
- To evaluate various cometabolic substrates and different methods of substrate addition.

Table 1 in Section 3.1 presents the performance objectives and criteria, and the expected and actual performance.

1.3 REGULATORY DRIVERS

The target CAH compounds for the single-well test technology include the chlorinated ethenes—TCE, cis-DCE, 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 (MCL) governed under the Safe Drinking Water Act (42 U.S.C. s/s 300f et seq. 1994). The U.S. Environmental Protection Agency (EPA) has set an 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 STAKEHOLDERS/END USER ISSUES

The demonstration provides information on how to conduct and analyze push-pull tests for evaluating potential aerobic cometabolism as a potential remediation process. Various methods are evaluated for conducting the tests, including activity tests and natural gradient "drift" tests and gas sparge tests. This provides the end user with options for selecting test methods most appropriate for the site of interest and that fit best 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 end users are provided surrogate compounds for the different cometabolic substrates and agents to block the enzyme activity. An end user issue to be addressed is obtaining regulatory approval to add CAHs, such as TCE and cis-DCE to the test solutions. This is required since it proved difficult to assess their transformation based on background concentrations in the site groundwater. A protocol document was written to aid the end user in the future application of technology.

2.0 TECHNOLOGY DESCRIPTION

2.1 DESCRIPTION

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 (Figure 1). 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. 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. The natural-drift test differs from the push-pull test in that the test solution is not extracted after a given time to react, but is allowed to drift under natural gradient conditions in the aquifer, and samples are taken periodically.

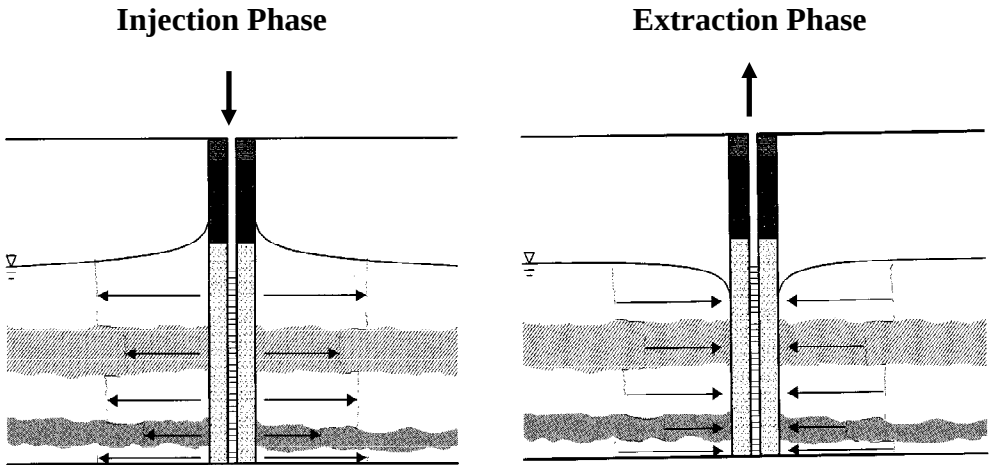

Figure 1. Injection and Extraction Phases of a Push-Pull Test.

2.1.1 Technology Background, Development, Function, and Intended Use

A major problem limiting the widespread use of aerobic cometabolism for treating CAHs contamination in groundwater is the lack of site-specific data for feasibility assessment and remedial design. The current approach for obtaining 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. A comparison of advantages and disadvantages of push-pull tests compared to interwell tests is provided in Table 4.5.1 of the Final Report.

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. The push-pull method is simple, inexpensive and, because tests are conducted in situ, provides a more representative description of microbial activity of indigenous organisms. 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.

2.1.2 Theory of Operation

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. As shown in Figure 2 and described in Section 3.2 of this report, a series of push-pull tests conducted in a single monitoring well can be used to obtain the following site-specific information.

Transport tests are used to determine transport characteristics (e.g., retardation factors) of substrates, contaminants, and, in some cases their transformation products. These are used to compute substrate utilization and contaminant transformation rates and to provide input to site-scale groundwater flow and contaminant transport modeling. 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 through successive injections of site groundwater containing growth substrate and dissolved oxygen.

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), substrates, dissolved oxygen; the production of defined products from injected surrogate compounds (e.g., ethylene oxide from injected ethylene and propylene oxide from injected propylene); and the production of defined CAH oxidation products (e.g., cis-DCE epoxide).

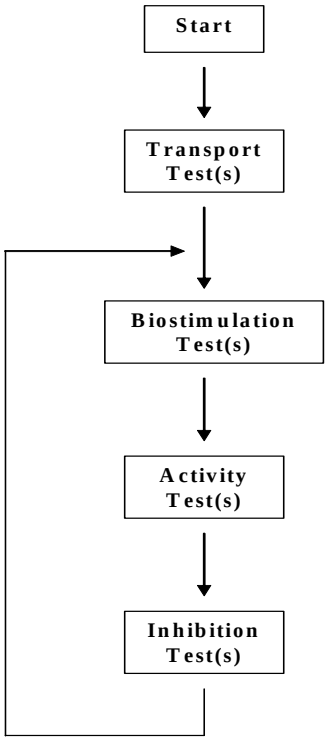

Figure 2. Push-Pull Test Sequence.

Inhibition tests are conducted to confirm that observed reactions are microbially mediated. In this type of test, a mechanism-based inhibitor (e.g., acetylene) of the enzyme of interest is added to inhibit the transformations observed in the previous activity test. Activity loss in the presence of inhibitor confirms that transformations observed during biostimulation and activity tests are microbially mediated.

Direct gas sparging (e.g., propane and methane) into an aquifer (as opposed to aqueous injections) is an alternate method for introducing gaseous substrates that 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 gaseous substrate and oxygen into the groundwater.

2.2 PROCESS DESCRIPTION

A typical field setup used to conduct push-pull tests is shown in Figure 3. The method requires 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 sections describe tests how a series of push-pull tests are conducted.

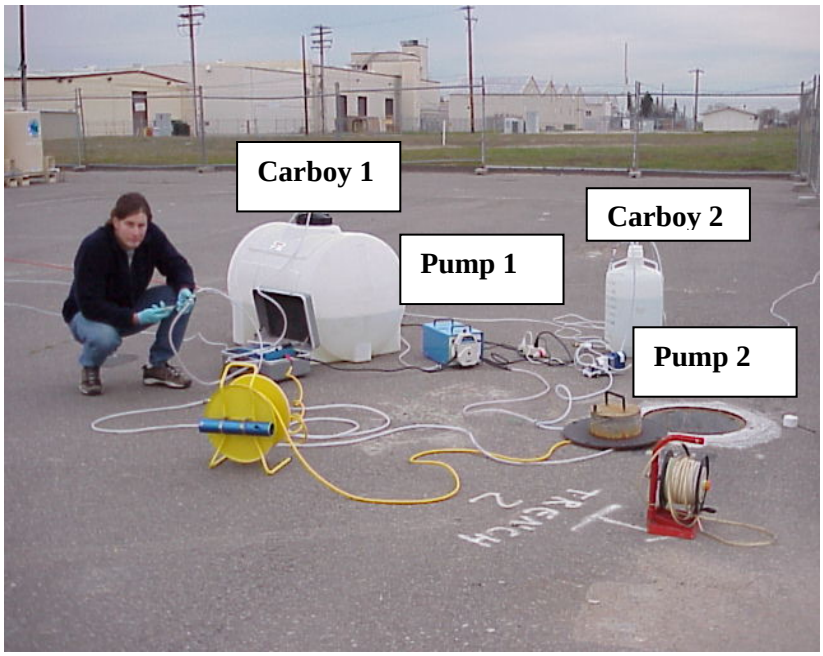

Figure 3. Typical Field Setup for Push-Pull Tests.

2.2.1 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. Transport tests are conducted under conditions selected to minimize the opportunity for microbial transformation of injected solutes, which is usually accomplished by selecting injection and extraction pumping rates that minimize the total time the test solution is in contact with the aquifer. For example, the composition of the injected test solution may be adjusted by removing a necessary nutrient (e.g., nitrate [NO_3^-]) or substrate (e.g., oxygen [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 to ensure that the initial concentrations of all solutes are known. 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.

2.2.2 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). 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 under natural gradient conditions 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 amount of substrate utilization and dissolved oxygen consumption is observable. 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 and the measured concentration of the substrate and oxygen.

2.2.3 Activity 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. Surrogate compounds that result in the production of easily detected intermediate products can be added. For example, ethylene, propylene, and isobutene were added as surrogate compounds, and their cometabolic transformation products (ethylene oxide, propylene oxide, and isobutene oxide) were easily detected. 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 or isobutene) or CAHs (e.g., cis-DCE) and detectable production of surrogate 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.

2.2.4 Inhibition Tests

An inhibition test is the same as an activity test except a mechanistic based inhibitor of the targeted monooxygenase enzyme system is added with the substrates of interest. Acetylene was used as the inhibitor of the propane 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 comparisons between the tests can be made. If effective inhibition is achieved, the results from the inhibition test should be similar to those observed in the transport test.

2.3 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 benzene, toluene, ethylbenzene, and xylenes (BTEX) degradation (Reusser et al., 2002).

2.4 STRENGTHS, ADVANTAGES, AND WEAKNESSES OF THE TECHNOLOGY

The push-pull method offers a number of advantages over microcosm studies. It can be used on site with existing monitoring wells and consequently explores a much larger volume of aquifer solids and groundwater. Thus it may be more representative of the degradative abilities of the resident microbial population. Tests can be performed over a period of about 2 months. It would, however, prove to be more costly to conduct push-pull tests to evaluate a broad matrix of conditions compared to microcosm tests.

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

2.5 FACTORS INFLUENCING COST AND PERFORMANCE

The ambient groundwater flow velocity is a factor influencing the performance of the push-pull test method. When the groundwater flow is too rapid, it can be difficult to biostimulate microbial activity by adding substrates. The residence time of injected substrate near the well may be too short for effective biostimulation to succeed. Successive additions are needed to provide enough substrate at high enough concentrations to achieve biostimulation. This increases the cost of performing the tests. Natural-gradient activity tests can be conducted to increase the residence of the injected solutes near the well to better assess transformation rates. It is also difficult to assess the transformation of the CAH when they are present in the aquifer as background contaminants. The addition of surrogate compounds is therefore useful in assessing the transformation potential. The design of tests where one chemical analysis can be used to determine the concentration of all the injected solutes will also help in the reduction of the analytical costs.

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3.0 DEMONSTRATION DESIGN

3.1 PERFORMANCE OBJECTIVES

The primary performance objective for this study was to demonstrate push-pull tests for assessing the potential for aerobic cometabolism of CAHs such as TCE using gaseous and liquid cometabolic substrates such as propane and toluene, respectively. Table 1 shows the performance objectives and criteria, and the expected and actual performance.

Table 1. Performance Objectives.

Type of Performance Objective	Primary Performance Criteria	Expected Performance	Actual Performance
Quantitative	Determine transport characteristics of nutrients, substrates, CAHs, and transformation products.	Similar transport and recovery will be achieved with bromide as the conservative tracer	Transport and recovery was similar to bromide as 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 were 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 groundwater could be determined.	Concentration decreases would be observed in push-pull tests.	Decreases 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 concentration 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 inactivity tests.
Quantitative	Biological transformation can be selectively blocked with mechanistic-based inhibitors.	Blocking agents would inhibit substrate utilization, oxygen consumption, and CAH transformation.	Blocking agents inhibited substrate utilization, oxygen consumption, and CAH transformation.

3.2 SITE/FACILITY DESCRIPTION

3.2.1 Background and Test Site Selection

The first and second demonstrations were performed at McAFB at Operating Unit A. This was the site of the ESTCP demonstration on cometabolic sparging (ESTCP, 2001, http://www.estcp.org/documents/techdocs/). Propane was evaluated as a cometabolic substrate in the first demonstration, though the injection of propane dissolved in groundwater. In the second demonstration, both propane and methane were evaluated, with substrates added by direct gas addition via sparging. Our third site demonstration evaluated aerobic cometabolism of CAHs using toluene as a cometabolic substrate. The demonstration was performed at Fort Lewis Logistics Center, Washington.

3.2.2 McClellan Site Description

Field tests were performed at the site of the former McAFB near Sacramento, California. This site was also used for the ESTCP demonstration of cometabolic sparging (Tovanabootr et al., 2001; ESTCP, 2001). The aquifer consists primarily of alluvial deposits, and is unconfined with a water table depth 30–32m below ground surface. The aquifer at this site is mainly contaminated with cis-DCE (20–40 µg/L) and TCE (200–400 µg/L) and is aerobic (~ 6.2mg/L dissolved oxygen). The aquifer consists primarily of alluvial deposits, and is unconfined with a water table depth 30–32m below ground surface. The monitoring wells MW1 and MW2 used in the tests were constructed of 5.1cm polyvinyl chloride casing with a 2.9-m-long screen.

3.2.3 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 depth of groundwater at the site is approximately 10 ft, and groundwater velocities across EGDY range from 0.25 to 0.75 ft per day (U.S. Army, 2002). The aquifer is aerobic in the region of these push-pull tests in which multiport monitoring wells were used. Cis-DCE and TCE concentrations were generally below 500 ug/L, which is ideal for aerobic cometabolism.

3.3 TEST SOLUTION PREPARATION

3.3.1 Conservative Tracer and Nutrients

Bromide at a concentration of 100 mg/L was used as a conservative (i.e., nonreactive) tracer for push-pull tests. This concentration was selected as a compromise between analytical detection limits (~ 1mg/L for bromine (Br) by ion chromatography [IC]) and the desire to avoid injecting test solutions with densities substantially larger than that of site groundwater. Nitrate in the form of sodium nitrate ($NaNO_3$) may be added as a nutrient in some tests.

3.3.2 Gaseous Substrates and Surrogate Compounds

Gaseous substrates (propane and oxygen) and surrogate compounds (propylene, ethylene and isobutene) were introduced into the test solution by bubbling (sparging) the groundwater contained in plastic carboys with a defined mixture of compressed gases. 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. Specified dissolved gas concentrations of dissolved acetylene were achieved by injecting known volumes of acetylene gas into a metalized film bag through a septum. The different injection solutions were mixed together at different flow rates to achieve the desired injection concentration. Details of the preparation of the groundwater solutions are provided in Section 3.7.2 of the Final Report.

3.3.3 Gas Sparging

Direct sparging of gas substrates was evaluated in the second demonstration at McAFB. For safety considerations, the propane (or methane) concentration in the injected gas mixture was maintained below lower explosive limit (LEL), 2.1% for propane and 5% for methane. During gas sparging the flammable gas level was monitored using an LEL detector on the site. 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 was achieved and monitored throughout the sparging event. The propane (or methane)/oxygen/argon gas mixture was sparged at specific depth intervals using a "straddle" packer system (see Section 3.7.3 of the Final Report).

3.3.4 Liquid Substrates and Surrogate Compounds

For the third demonstration at Fort Lewis, the test solution was prepared with groundwater extracted from the well port where push-pull test solution was to be injected. Bromide was again used as a nonreactive tracer. Reactive solutes include the dissolved growth substrate (toluene), hydrogen peroxide, nontoxic 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 Company, Barrington, Illinois). The test solution was prepared by thoroughly mixing bromide, nitrate, and hydrogen peroxide in a plastic carboy. Toluene was added to a collapsible Teflon bag, and to achieve a desired concentration. Isobutene solution was prepared in a plastic carboy by the same method described in Section 3.6.3. The different injection solutions were mixed together at different flow rates to achieve the desired injection concentration. Details can be found in Section 3.7.4 of the Final Report.

3.4 SAMPLING, MONITORING, AND ANALYTICAL PROCEDURES

3.4.1 Sample Collection

Groundwater samples were required for analysis of injected tracer, nutrient, substrate, surrogates, CAHs, and their transformation products. A sampling valve equipped with 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. A 1-mL sample was collected in a plain glass vial for tracer (Br) and nutrient (NO_3) analyses by IC. A 2mL sample was collected in a syringe for dissolved oxygen (DO) analysis in the field by oxygen electrode. A 40mL sample without headspace was collected in brown volatile organic analysis (VOA) bottles equipped with a Teflon/neoprene

septa and a polypropylene-hole cap (Supelco, Bellefonte, Pennsylvania) 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 4ºC and analyzed within 1 week.

3.4.2 Determination of Inorganic Anions by Ion Chromatography

Concentrations of inorganic anions (Br and NO_3^-) were determined with a Dionex DX-500 (Sunnyvale, California) IC equipped with electrical conductivity detector and a Dionex AS14 column. The eluent consisted of 3.5mM sodium carbonate (Na_2CO_3) and 1.0 mM sodium bicarbonate ($NaHCO_3$) and the eluent flow rate was 1.5 mL/min.

3.4.3 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. Hydrogen peroxide concentrations were monitored using thiocyanate colorimetric method developed by CHEMetrics, Inc. This method covers hydrogen peroxide concentrations of 0-1,000 mg/L. Details are provided in Section 3.8.3 of the Final Report.

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

The purge-and-trap method was used in determining the dissolved concentrations of propane, toluene, ortho-cresol, ethylene, isobutene and their transformation products, and CAHs. Five-mL aqueous samples from the VOA vials were introduced into an Hewlett-Packard (HP) 7695 purge-and-trap system, and the volatile compounds were sorbed onto a Vocarb-3000 trap. Chromatographic separations were achieved with two 30-m megabore GSQ-PLOT and HP-624 columns from Agilent (New Castle, Delaware) installed on an HP 6890 series GC connected to a photo ionization detector (PID) followed by a flame ionization detector (FID). 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 40mL 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 SF_6 standards were made for calibration of GC. Details of the GC methods can be found in Section 3.8.4 of the Final Report.

4.0 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 CAHs at McAFB and Fort Lewis. 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. Tables 2 and 3 present performance criteria, expected performance, and performance confirmation methods for the demonstration.

Table 2. Performance Criteria.

Performance Criteria	Description	Primary Or Secondary
Transport characteristic of nutrients, substrates, CAHs, and transformation products	Demonstrate that the substrates, surrogates, and nutrients are transported like bromide, the conservative tracer	Primary
Biostimulation can be achieved through successive additions of substrate, DO, 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 groundwater 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 with different substrates and surrogate compounds	Primary
Scale-up constraints	Used standard monitoring well	Secondary

Table 3. 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 and mass recovery	Determine concentration breakthrough curves and mass balances	Breakthrough curves were similar to bromide tracer; mass recovery was similar to bromide tracer
Biostimulation can be achieved through successive additions of substrate, DO, 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, and rate estimates	Concentrations decreased compared to the prior transport tests. Rate 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, and rate estimates	Products were produced, and mass balances permitted production rates to be measured
Transformation of CAHs in the site's groundwater could be determined	Decrease concentrations in breakthrough curves bromide conservative tracer	Determine concentration breakthrough curves, mass balances, and 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, and rate estimates	Concentrations decreased compared to the prior transport tests and the bromide tracer. Rate 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, and rate estimates	Concentrations decreased compared to the bromide tracer. Rate 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, and rate estimates	Concentrations did not decrease compared to the bromide tracer and prior activity test
Factors affecting the technology performance	Similar metrics as above	Similar metrics as above	Tests work at high groundwater 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 least one highly 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

4.1 EXAMPLE RESULTS FROM FIELD PUSH-PULL TESTS CONDUCTED AT McCLELLAN AFB, CALIFORNIA

Transport tests were conducted in each well. Based on mass balances on the injected solutes, the recovery rate of bromide was 99%, while the recovery rates of other injected solutes were slightly higher or similar (Table 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 results of a transport test conducted at McAFB are shown in Figure 4.

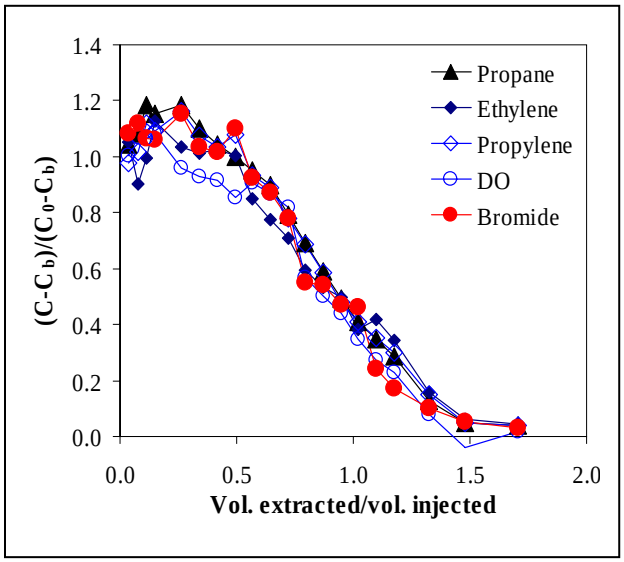

Figure 4. Extraction Phase Breakthrough Curves in a Push-Pull Transport Test Conducted at the McAFB, California (MW2) Field.

The transport of all the solutes injected was similar, indicating that little transformation or sorption to the aquifer solids was occurring. Details of the transport tests are provided in Section 4.1.1 of the Final Report.

During the biostimulation tests, five sequential additions of groundwater containing propane and oxygen were made to each well to stimulate the activity of indigenous propane oxidizing bacteria. In the first biostimulation test, the trends in concentration changes of the three compounds were very similar, showing gradual decreases over 25 days. In each subsequent test, the rates of propane, oxygen (DO), and nitrate utilization (not shown) increased (Figure 5). 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.

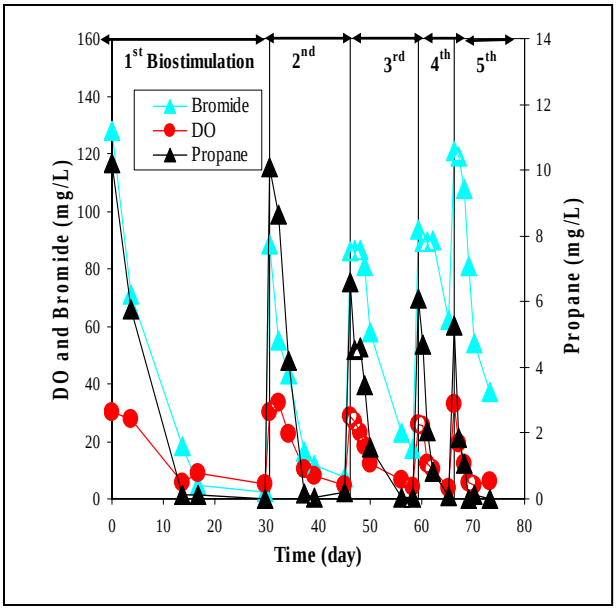

Figure 5. Measured Propane, Oxygen, and Bromide Concentrations During Five Field Biostimulation Tests Conducted at the McAFB, California (MW2) Field.

The ethylene and propylene activity tests were performed to demonstrate cometabolism by propane utilizers, with ethylene and propylene acting as surrogate compounds for the CAHs. After injecting the solution containing ethylene or propylene, oxygen, nitrate and chloride, the solution was permitted to react in the aquifer for

12.4 hours and then extracted over a period of 7.3 hours. During the extraction phase, by-products having the same retention time on the GC as ethylene oxide and propylene oxide were detected. The build-up of the product was associated with ethylene and propylene transformation via cometabolism. In one set of activity tests ethylene or propylene were added in the absence of propane, and in another, they were added with propane. The results of an activity test using ethylene, when propane was also added, are shown in Figure 6. The production of ethylene oxide corresponding to the transformation of ethylene is clearly shown.

The final test was performed in the presence of acetylene to further evaluate monooxygenase activity. The push-pull activity tests were repeated using the same procedures as the prior activity tests. Results of an acetylene test where both propane and ethylene were present are shown in Figure 7. In the presence of acetylene, substrate utilization was essentially completely inhibited, and very little ethylene oxide was produced. The ratio ethylene oxide formed to ethylene transformed was ~ 0.12 % (Table 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). The strong inhibition by acetylene indicates that a propane monooxygenase enzyme is likely responsible for propane degradation and the cometabolism of ethylene.

Zero-order rates were estimated for the activity tests using methods described by Istok et al. (1997). Details of the rate estimates are provide in Section 4.1 of the Final Report. Estimated zero-order rates of propane utilization were similar between wells MW2 and MW3 (Table 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). The computed zero-order rate of propylene transformation at MW2 was approximately a factor of 1.5 lower than the ethylene transformation rate, while both rates are comparable at MW3 (Table 4). Rates of propane utilization and ethylene transformation were greatly reduced in the presence of acetylene.

A series of gas-sparging biostimulation tests were also performed by sparging propane (or methane)/oxygen/argon/SF_6 gas mixtures at specific depth intervals using a “straddle” packer. Gas-sparging activity tests were similar to the 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. Details of the results of the sparging tests are provided in Section 4.2 of the Final Report. In general, similar results were obtained as observed in the tests where dissolved solutes were added to the injected fluid. With successive additions, more rapid utilization of propane or methane was observed. For the sparging demonstration, SF_6 served as an effective conservative tracer. In activity tests, ethylene and propylene transformation was achieved by microorganisms stimulated on either propane or methane and ethylene oxide and propylene oxide, as cometabolic by-products. Detailed descriptions of results for each test type are described in Section 4.2 of the Final Report.

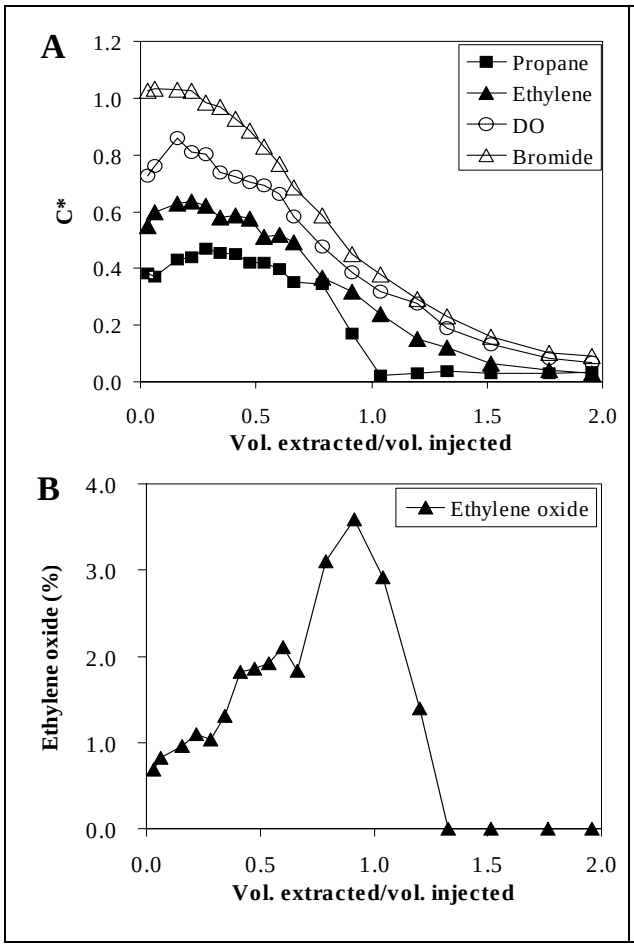

Figure 6. Extraction Phase Breakthrough Curves from Well MW3 During the Fourth Propane Activity Test. ([A] injected solutes [B] ethylene oxide concentrations expressed as a percentage of average ethylene concentration in injected test solution)

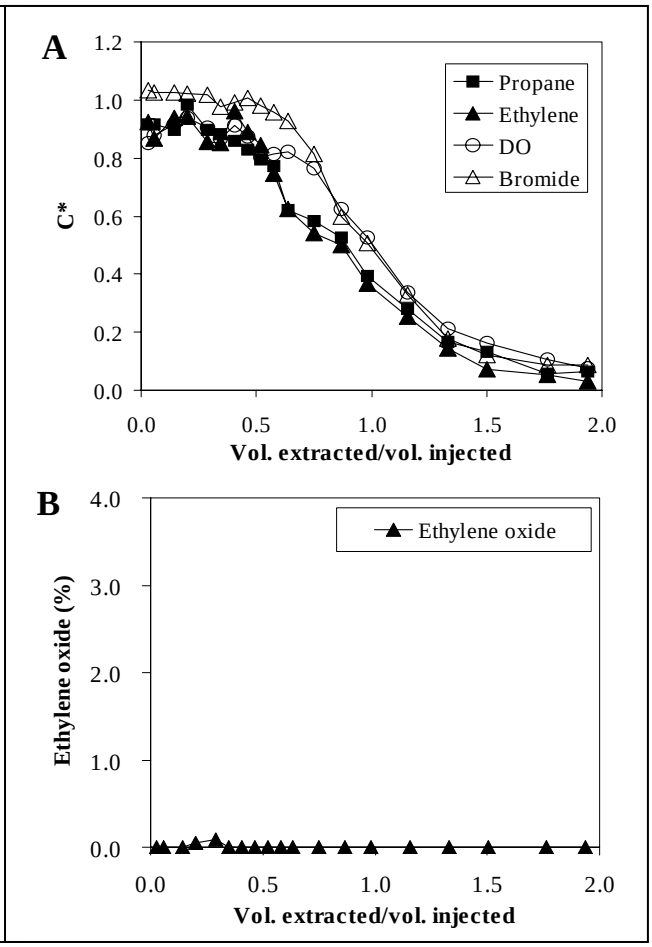

Figure 7. Extraction Phase Breakthrough Curves from Well MW3 During the Acetylene Blocking Test. ([A] injected solutes [B] ethylene oxide concentrations expressed as a percentage of average ethylene concentration in injected test solution)

Table 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	-	-	-	-	107[2]	92
	rate (µmol/L/hr)	1.1	0.8	-	-	-	-	-	-
Ethylene activity test	% recovery	-	-	59 (3.1%)[1]	75 (3.8%)[1]	-	-	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	-	-	-	-	75 (2.3%)[1]	69 (0.45%)[1]	92	88
	rate (µmol/L/hr)	-	-	-	-	0.34	0.46	-	-
Fourth propane activity test	% recovery	-	40	-	60 (5.2%)[1]	-	-	-	107
	rate (µmol/L/hr)	-	0.82	-	1.2	-	-	-	-
Acetylene blocking test	% recovery	-	90	-	86 (0.12%)[1]	-	-	-	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 Rtracer in an equation is assumed as 1.00.

4.2 EXAMPLE RESULTS FROM FIELD PUSH-PULL TESTS CONDUCTED AT FORT LEWIS, WASHINGTON

Our third demonstration conducted at Fort Lewis evaluated aerobic cometabolism of CAHs using toluene as a cometabolic substrate. Transport characteristics of injected solutes, including bromide, toluene, isobutene, oxygen, and NO_3^- push-pull tests were evaluated in transport tests, as previously discussed. Table 5 shows a summary of recoveries and rates achieved in the transport tests for toluene, isobutene, oxygen, nitrate, and bromide for one of the test wells. In the transport tests, the recoveries were lower, approximately 30%, compared to 88 to 100% achieved in the McAFB tests. The results show faster groundwater flow at Fort Lewis than at McAFB.

Biostimulation test results showed decreases of injected toluene concentration, and the production of o-cresol as an intermediate oxidation product indicated the stimulation of toluene utilizing microorganisms containing an ortho-monooxygenase enzyme. A small fraction of utilized toluene was observed as o-cresol. Toluene oxidation to o-cresol by the toluene ortho-monooxygenase pathway was also observed at the Moffett field site by Hopkins et al. (1995) and Fries et al. (1997). Push-pull activity tests, like those performed at McAFB, were also performed at Fort Lewis. For these tests, the solutes were allowed to reside in the aquifer for about 20 hrs prior to being extracted. In the isobutene activity test, reduced mass recoveries of toluene, isobutene, DO, and nitrate were observed (Table 5). Essentially complete toluene utilization was observed in the isobutene activity test. Isobutene was transformed and isobutene oxide was produced as an intermediate oxidation product. The production of isobutene oxide indicated the stimulation of toluene utilizing microorganisms containing an ortho-monooxygenase enzyme, consistent with the observation of the production of o-cresol. In isobutene activity tests, additional cis-DCE (500 µg/L) was added to increase cis-DCE concentrations and to monitor its potential transformation. TCE was not added, and its transformation was not detected in the toluene or surrogate compound activity tests in the presence of high background TCE concentrations.

When cis-DCE was added above its background level, its cometabolic transformation was observed. The corresponding zero-order-rates transformation are provided in Table 5. The results indicated that cis-DCE was transformed by toluene utilizers, but at a slower rate than the isobutene, the surrogate substrate. Cis-DCE, however, was present at a lower concentration than isobutene and would affect the zero-order rate estimate (Table 5) and likely the actual rate of transformation.

Natural drift activity tests were also performed in the Fort Lewis demonstration. Natural drift tests were similar to activity tests except that no extraction pumping was performed, and samples were collected periodically. This permitted a longer residence time for transformation reactions to occur. In the natural drift activity test, trans-DCE (500 µg/L), which was not present as a groundwater contaminant, was also added with cis-DCE 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 8A).

Table 5. Summary of Quantities of Injected and Extracted Solute Mass and Percent Recovery in Transport, Biostimulation, and Activity Tests Conducted at Fort Lewis.

Test	Quantities	Toluene	o-Cresol	Isobutene	Isobutene oxide	Cis-DCE	Trans-DCE	DO	NO_3^- N	Br
Transport LC191-P1	Mass recovery (%)	30.1	NA[1]	36.5	NA	NA	NA	29.3	31.1	32.9
	Rate (µmol/L/hr)	0.35	NA	≈ 0	NA	NA	NA	--	--	--
Biostimulation LC191-P1	Mass recovery (%)	26.6	NA	NA	NA	NA	NA	26.3	21.6	33.1
	Rate (µmol/L/hr)	0.83	0.02	NA	NA	NA	NA	--	--	--
Isobutene activity LC191-P1	Mass recovery (%)	2.58	NA	21.0	NA	NA	NA	18.7	18.7	25.0
	Rate (µmol/L/hr)	0.81	NA	0.73	0.22	0.08	NA	--	--	--
Drift activity LC191-P1	Rate (µmol/L/hr)	1.27	NA	1.12	0.18	0.12	0.11	--	--	--
Inhibition LC191-P1	Rate (µmol/L/hr)	≈ 0	NA	≈ 0	NA	≈ 0	≈ 0	--	--	--

[1] NA: Not applicable

The normalized isobutene and trans-DCE concentrations gradually decreased to zero 48 and 30 hours after the injection, respectively. When isobutene was utilized, isobutene oxide was observed as an intermediate oxidation product. Cis-DCE concentrations were gradually reduced and reached background levels (Figure 8A). The dilution-normalized concentrations of toluene, isobutene, cis-DCE, and trans-DCE, and DO were lower than unity, as shown in Figure 8B, indicating that these compounds were utilized or cometabolically transformed.

Zero-order rates of toluene, isobutene, and cis-DCE transformation rates were all a factor of 1.5 times greater in the natural drift activity test than in the isobutene activity test (Table 5). The results indicate that faster toluene utilization rates are associated with faster rates of cometabolism. Cis-DCE and trans-DCE were transformed at very similar rates. These results indicate that indigenous micro-organisms were able to cometabolize cis-DCE and trans-DCE after stimulation on toluene. The results also demonstrated that natural-drift tests yielded results similar to push-pull activity tests. Results of these tests are described in detail in Section 4.3 of the Final Report.

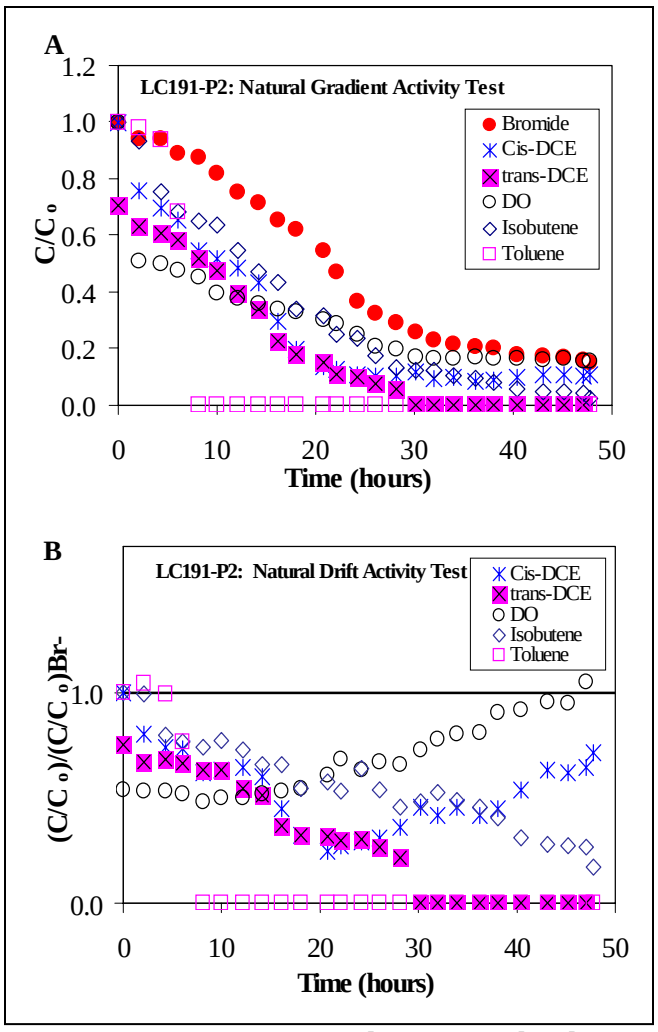

Figure 8. Extraction Phase Normalized Concentrations in LC191-P2 with nutrients (A) and dilution-adjusted concentrations of injected solutes (B) in natural drift activity tests.

An inhibition test was also performed under natural gradient conditions. 1-butyne has been shown to be an effective inhibitor of the toluene monooxygenase enzyme (Yeager et al., 2002; Hicks, 2002). 1-butyne completely blocked the utilization toluene and transformation of isobutene, cis-DCE, and trans-DCE. Extraction breakthrough curves for toluene, isobutene, 1-butyne, cis-DCE, trans-DCE, and DO achieved during the inhibition test were very similar to the breakthrough curve of the bromide tracer, indicating conservative transport and negligible transformation of any of the injected solutes. Neither isobutene oxide nor o-cresol was detected during the 1-butyne inhibition tests, and cis-DCE and trans-DCE were not transformed, indicating an ortho-monooxygenase enzyme was likely involved in their transformation. The rates of transformation were greatly reduced in the presence of 1-butyne, as shown in Table 5.

4.3 DATA ASSESSMENT

The data described here in Section 4 and in more detail in Section 4 of the Final Report provide a realistic assessment of the demonstration objectives at McAFB and Fort Lewis, respectively.

Figures and tables of results are shown for tests performed in the saturated zone at McAFB using propane as a cometabolic substrate, while toluene tests were performed at Fort Lewis, Washington.

The effectiveness of dissolved substrate addition to stimulate the indigenous propane utilizers and toluene utilizers was evaluated in standard monitoring wells. Bromide served as an effective conservative tracer to study transport characteristics of dissolved solutes and to normalize for dilution effects resulting from groundwater transport. Propane and toluene utilization as growth substrates was 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 hrs and then was 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, and nontoxic surrogates were added to probe for CAH transformation activity in the propane studies at McAFB, while isobutene was added in the toluene studies at Fort Lewis. 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 is 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, 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 single-well tests were also performed at McAFB in the second demonstration. The sparging method was fairly simple to apply and yielded results similar to those obtained by dissolving the solutes in the injected groundwater. The sparge tests indicated the stimulation of methane- and propane-oxidizing microorganisms and 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.

When cis-DCE and TCE were present as background contaminants, it was difficult to clearly observe their transformation in the push-pull or natural drift tests. This results from the groundwater with background contamination mixing with the injected solution during the test. When combined with their slower rates of transformation, and the lack of production of an easily detected product, this makes the detection of their transformation difficult. There was some evidence of cis-DCE transformation in tests prior to its addition to the test solution. Upon adding cis-DCE to the test solution to achieve concentrations above background, transformation was observed in both push-pull activity tests and natural gradient drift tests. Trans-DCE when added

to the injection solution was also transformed in a natural gradient drift test. Thus, obtaining regulatory permission to add the contaminants of interest to the injected fluid may be required to demonstrate directly their transformation and to quantify transformation rates. The demonstration, however, clearly demonstrated that surrogate compound transformation and product formation could be used to predict CAH transformation potential.

The tests at Fort Lewis demonstrated that even under conditions of rapid groundwater flow, biostimulation could be achieved and cometabolism potential could be assessed. Transformation was easier to observe under natural gradient drift test conditions compared to push-pull conditions because of the longer residence time and the ability to monitor the process over a period of several days. In application it might also be easier to apply natural drift tests since, on injection of the test solution, periodic samples are collected over time, simplifying the test procedures.

The method was found to yield consistent results in repeated tests and with different methods of testing. Push-pull activity tests at Ft. Lewis yielded similar rates of transformation at the four locations tested. Also, rates determined in natural drift activity tests were 1.5 times the rates achieved in the push-pull activity tests, despite the different conditions of the tests. The faster rates for substrate utilization were correlated to faster rates of surrogate and cis-DCE transformation.

Simple procedures were used to estimate rates of substrate utilization and contaminant and surrogate transformation using mass balances approaches. For comparison purposes, zero-order kinetics was applied in making rate estimates. The rates, however, are likely concentration-dependent. The use of more complicated rate expressions such as monod kinetics would require numerical analysis using transport codes, which is beyond the scope of this work.

4.4 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 they are expensive and logistically complicated. Push-pull tests and single well drift tests permit in situ testing but with a much simplified method. The method can also be applied to a number of locations at site. For example, a two-person crew at Fort Lewis could conduct activity tests at four well locations over a period of 3 days.

Different methods of biostimulation and activity testing were evaluated. Direct sparging of gaseous substrates greatly simplified test procedures, but results were less quantitative than when solutes were dissolved in groundwater and injected. Tests using sparged gas addition, however, might be used to obtain qualitative information, including biostimulation potential, surrogate transformation, and production of products from surrogate transformation.

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5.0 COST ASSESSMENT

Implementation costs for the push-pull tests at McAFB and Fort Lewis are shown in Table 6. 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 had this been done by commercial vendors, as shown in Table 7. Higher costs with commercial vendors are associated with the higher analytical costs of purge and trap (P&T) GC system analysis and IC system analysis, as shown in Table 7. OSU average estimated cost for each site is about $160,000, while the same operation costs for commercial vendors would be about $260,000, or about 62% higher than OSU costs.

Savings would be realized in equipment costs by using the same equipment at several sites with only the cost for maintenance. 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 would likely suffice. The breakthrough curves are very reproducible, and the similar shapes would be constructed with 10 or 20 samples. Costs for conducting activity tests and drift tests are high since they 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, were significant, assuming one or two persons need to travel out of state, (e.g., at McAFB site) or if no storage is established at the site, as at the Fort Lewis site, and all the equipment has to be hauled back and forth. Costs could be reduced in practice if local on-site personnel are used and if travel and shipping costs can be reduced.

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6.0 IMPLEMENTATION ISSUES

6.1 COST OBSERVATIONS

Factors that affected project costs were the sites selected and the level of analysis needed. 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. The multiport monitoring wells at Fort Lewis were a cost factor since they allowed for the use of smaller injection volumes, which simplified test logistics and costs. The major cost factor with a commercial vendor is for analytical analysis. A reduction in the number of samples, tests, and type of analysis performed would lower these costs. It might also be possible to eliminate transport tests and proceed directly to push-pull activity tests or single-well drift tests. The number of tests could also be reduced significantly by adding the growth substrate, surrogate compounds, and possible CAHs together in a single activity or drift test. Costs presented in Tables 6 and 7 are based on tests as conducted in the demonstration project, which would be greater than those likely to be applied in practice.

If is somewhat difficult to compare costs with current practice since push-pull tests would be compared with either microcosm tests or interwell tests, which differ greatly. A comparison of the advantages and disadvantages of push-pull tests compared to interwell tests, for example, is provided in Table 4.5.1 of the Final Report. The cost of push-pull tests would likely be lower than interwell tests, but interwell tests provide information that would be more useful for scaling up to full-size remediation systems. Push-pull tests, would cost more than microcosm studies but would provide information under conditions that more closely mimic in situ conditions and are conducted using standard monitoring wells. Our experience with microcosm studies is that a good study would cost approximately $50,000, which is lower that our estimated cost for push-pull tests, presented in Table 6. The cost of push-pull tests would be in the range of interwell tests, but the cost would likely be less if a commercial vendor specialized in conducting these tests. Cost reductions could be realized by lowering the cost of mobilization, reusing equipment, conducting CAH analysis, and reporting as the method was optimized.

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 microorganisms 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. Direct evidence for cis-DCE and TCE transformation could not be obtained when they were present as background contaminants. This

was a limitation of the method. When cis-DCE was added above background concentrations, its transformation rate could be determined. Thus, obtaining regulatory approval to add the CAH of interest to the injected groundwater above background concentration is recommended. Acetylene effectively blocked both propane utilization and ethylene transformation, further indicating the stimulation of propane monooxygenase activity. Toluene utilization, isobutene, cis-DCE, and trans-DCE transformation were effectively inhibited by 1-butyne, and indicated ortho-monooxygenase activity.

Table 6. McAFB and Fort Lewis Demonstration Costs.

Cost Category	Subcategory	Site 1[(a)] Costs ($)	Site 2[(a)] Costs ($)	Site 3[(b)] Costs ($)
Fixed Costs				
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				
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	1,000	1,000	1,000
	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
	– CAH 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
Subtotal	$**100,900**		**100,900**	**83,900**
Total Costs				
Total Technology Cost: $**477,700**				
Unit Cost: **$159,233/Site**				

[(a)] McAFB, California, demonstration site costs
[(b)] Fort Lewis, Washington, demonstration site costs

Table 7. Estimated Demonstration Costs by Commercial Vendor.

Cost Category	Subcategory	Costs ($)
Fixed Costs		
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		
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
Subtotal	$**188,400**	
Total Costs		
Total Technology Cost**:** **$260,900**		
Unit Cost: $**260,900/Site**		

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. No direct evidence for TCE transformation was observed.

At Fort Lewis, the effectiveness of toluene additions in stimulating aerobic cometabolic activity of indigenous microorganisms was demonstrated by 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.

6.3 SCALE-UP

Push-pull tests were performed on the same 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 realized by reducing the number of samples taken for CAHs and tracer analyses. Tracers may be used that could be determined by the same GC method for CAH analysis, thus eliminating the need for bromide tracer IC 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 the greater depth, the potential for volatilization of dissolved gas component was greater. The shallow aquifer at Fort Lewis and the multiport 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.

Detecting CAH transformation proved difficult when the concentrations injected were below background concentrations. The addition of cis-DCE to the injected groundwater to concentrations well above background permitted its transformation to be detected and quantified.

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. When additional cis-DCE was added to the injected solution, its transport could be easily tracked and the transformation rate could be estimated.

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 is 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 CAH bioremediation through aerobic cometabolism. The activity test is performed by injecting site groundwater amended with propane or toluene as a cometabolic substrate. The ease of obtaining regulatory approval to inject nontoxic 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 nonreacted tracers are removed during the extraction phase and subsequent sampling.

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APPENDIX A

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