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Single-Well, "Push-Pull" Test for In Situ Determination of Microbial Activities

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Abstract

A single-well, "push-pull" test method is proposed for the in situ determination of microbial metabolic activities in ground-water aquifers. The method consists of the pulse-type injection ("push") of a test solution into the saturated zone of an aquifer through the screen of an existing monitoring well followed by the extraction ("pull") of the test solution/ground-water mixture from the same well. The test solution contains a tracer and one or more reactive solutes selected to investigate specific microbial activities. During the injection phase, the test solution flows radially away from the monitoring well into the aquifer. Within the aquifer, biologically reactive components of the test solution are converted to various products by the indigenous microbial community. During the extraction phase, flow is reversed and solute concentrations are measured to obtain breakthrough curves, which are used to compute the quantities of reactant(s) consumed and/or product(s) formed during the test and reaction rates. Tests were performed to determine rates of aerobic respiration, denitrification, sulfate reduction, and methanogenesis in a petroleum contaminated aquifer in western Oregon. High rates of oxygen, nitrate, nitrite, and hydrogen utilization and nitrite, and carbon dioxide production support the hypothesis that petroleum contamination has resulted in an increase in microbial activity in the anaerobic portion of the site. The results suggest that the push-pull test method should be useful for obtaining quantitative information on a wide range of in situ microbial processes.

Introduction

Quantitative information on microbial activities is needed to improve our understanding of biological processes in ground-water aquifers, to quantify rates of natural microbial attenuation of ground-water contaminants, and to design and evaluate the effectiveness of in situ bioremediation technologies. A wide variety of methods have been used to quantify microbial activities in the subsurface including: rate calculations based on monitoring well data (McAllister and Chiang, 1994), batch, column, and microcosm reactor studies (Wilson et al., 1983), direct observation and culture techniques (Harvey et al., 1984), biochemical marker techniques (Balkwill et al., 1988), molecular methods (Bowman et al., 1993), analysis of geochemistry data (Lovley and Goodwin, 1988), and ecological modeling (Kelly et al., 1988). The relative advantages and disadvantages of many of these methods are discussed in Chapelle (1993). For example, some disadvantages of microcosm studies include the need for core

material, the typically small and potentially unrepresentative volume of individual core samples, the potential disturbance and contamination of core material during collection, the difficulty in reproducing field conditions in an artificial laboratory environment, and the potential for selective growth (e.g., some methods may select the fastest-growing species which may not be the most important ones for a specific transformation of interest). Additional disadvantages of other methods can include the inability to provide quantitative information on reaction rates, the inability to compute mass balances for reactants and products, and the requirement for highly specialized and expensive laboratory equipment.

The test method presented here is an extension of a group of related methods that have been developed to determine physical characteristics of aquifers in situ using measurements performed in monitoring wells. In situ test methods are generally considered more representative of actual subsurface conditions than laboratory methods based on core samples because they can be designed to investigate a larger (and presumably more representative) volume of aquifer material than can typically be obtained by coring. Test methods that can be performed in monitoring wells are desirable because they allow use of the large number of existing monitoring wells available at most sites. Single-well test methods are particularly desirable because they provide the opportunity to determine site-scale variability in aquifer characteristics (e.g., by comparing test results obtained from many wells at a single site).

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Single-well tests for determining the hydraulic conductivity of an aquifer (slug tests and pumping tests) have been standard practice for many years (Dawson and Istok, 1991). The borehole-dilution method, developed to measure ground-water velocity, is another type of single-well test (Leap and Kaplan, 1988). A novel type of single-well test method, which we will refer to as a "push-pull" test, is potentially useful for the quantitative determination of a wide range of aquifer physical, chemical, and biological characteristics. A push-pull test consists of the controlled injection of a prepared test solution into a single monitoring well followed by the recovery of the test solution/ground-water mixture from the same well. The test solution consists of water containing a tracer and one or more reactive solutes; the type, combination, and concentration of these solutes is selected to investigate specific aquifer characteristics. In general, a push-pull test consists of two phases. During the *injection phase*, the test solution is injected ("pushed") into the aquifer where it flows radially outward and penetrates an approximately cylindrical volume of aquifer material centered about the well. During the *extraction phase*, the test solution/ground-water mixture is pumped ("pulled") from the same well and concentrations of the tracer, reactants, and possible reaction products are measured in the extracted water as a function of time to determine breakthrough curves for each solute. Aquifer properties are determined by an analysis of these breakthrough curves. In some cases a *drift* or *reaction phase* may also be included between the injection and extraction phases, during which there is no pumping and the test solution plume is allowed to "drift" downgradient in the direction of the regional ground-water flow field prior to the start of the extraction phase.

The push-pull test method was perhaps first used by Sternau et al. (1967) to study the degree of mixing of injected water with ground water in an application related to artificial ground-water recharge. However, the method has been most widely used to determine residual oil saturation in petroleum reservoirs (Tomich et al., 1973). In a typical application the test solution contains a known concentration of ethyl acetate. Within the reservoir, a portion of the injected ethyl acetate hydrolyzes to ethanol and the concentrations of both solutes are measured during the extraction phase. If residual oil is present in the portion of the reservoir investigated by the test, transport of ethyl acetate (which readily partitions into the stationary oil phase) is retarded relative to ethanol. The residual oil saturation can be computed from the differences in arrival times for the two solutes (Tomich et al., 1973). Push-pull tests have also been proposed to measure physical characteristics of ground-water aquifers. For example, Gelhar and Collins (1971) derived an equation that can be used to determine the longitudinal dispersivity of an aquifer using the extraction well breakthrough curve for an injected tracer. Beauheim (1987) used single-well tracer tests to study mass transfer processes in a dolomite aquifer. Hall et al. (1991) derived equations that can be used to determine the effective porosity and regional ground-water velocity from the results of a push-pull tracer with a drift phase, which Hall et al. referred to as a "drift-pumpback" test.

Trudell et al. (1986) perhaps first applied a simple form of push-pull test to study microbial processes in the subsurface. In their study a modified drive sampler was used to inject and extract fluids from a specific depth interval in a specially prepared borehole to assay for denitrification in an alluvial aquifer. Evidence for denitrification was obtained by comparing nitrate,

nitrite, and tracer concentrations in water samples collected during the extraction phase. A controlled-release experiment with some features of a push-pull test was recently performed to study hydrocarbon degradation under denitrifying and sulfate-reducing conditions by Reinhard et al. (1995). In their approach, relatively large volumes (750 to 900 l) of test solutions were injected into the saturated zone of an aquifer using an existing well. Evidence for hydrocarbon degradation was obtained by periodically collecting water samples from the same well and analyzing them for tracer, hydrocarbon, and electron acceptor concentrations. However, because only a small portion of the injected volume was recovered, mass balances and reaction rates for reactants and products could not be accurately computed.

The objective of this paper is to present a modified form of push-pull test that can be used for the in situ determination of microbial metabolic activities in existing monitoring wells. In this approach, the test solution contains a nonreactive tracer and one or more reactive solutes to assay for the activity of specific microbial enzyme systems. Typically the reactive solutes serve as electron acceptors or electron donors for a particular microbially mediated oxidation-reduction reaction (see below). Within the aquifer, some of these solutes may be converted to products (e.g., injected nitrate may be reduced to nitrite by denitrifying bacteria; sulfate may be reduced to hydrogen sulfide by sulfate-reducing bacteria); the extent of the conversion of reactant to product provides a quantitative measure of the microbial activity controlling the reaction. Breakthrough curves for tracer, reactants, and products are measured during the extraction phase and are used to compute a mass balance for all solutes, empirical reaction stoichiometries, and reaction rates.

Description of Push-Pull Test Method

A push-pull test consists of the controlled injection of a prepared test solution into a single monitoring well, followed by the extraction of the test solution/ground-water mixture from the same well. During the injection phase the test solution flows radially outward from the well casing, through the well screen and sand pack (if present) and into the saturated zone of the aquifer (Figure 1a). The total volume of aquifer investigated by the test (V_t) is determined by the total injected volume (V_i), the volume of water stored in the well casing (V_w) and sand pack (V_s), and the effective porosity of the aquifer (ϕ) and sand pack (ϕ_s):

$$V_t = \frac{V_i - V_w}{\phi} - \frac{V_s}{\phi_s} \quad (1)$$

The test solution may be injected and extracted across the entire saturated thickness or within a specific depth interval (e.g., by using a pair of inflatable packers to isolate a portion of the well screen). The injected test solution penetrates an approximately cylindrical volume of aquifer centered at the well but the exact shape of the penetrated zone depends on several factors including the presence of aquifer heterogeneities (e.g. layers or lenses with different hydraulic conductivities), the initial saturated thickness, well screen length, injection rate and duration, and well construction and development methods.

The composition of the test solution depends on the purpose of the test but will typically consist of water containing known concentrations of a tracer and one or more reactive solutes selected to assay the activity of specific microbial enzyme

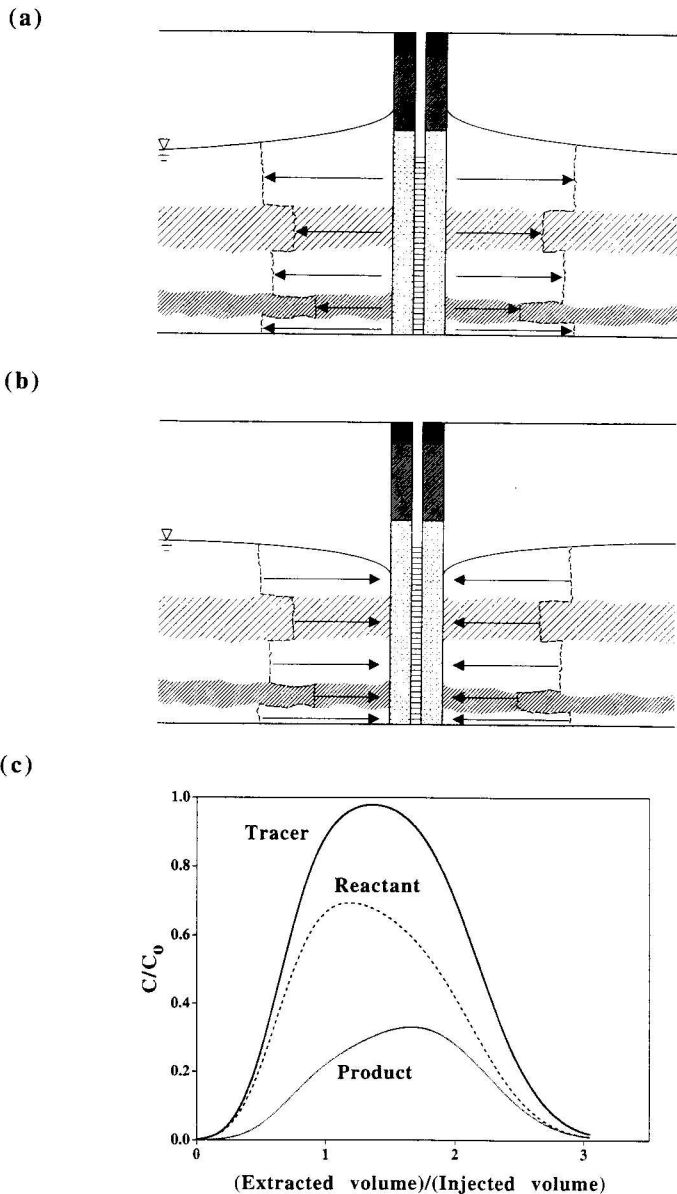


Fig. 1. Single-well, "push-pull" test for in situ determination of microbial activities (a) injection phase, (b) extraction phase, and (c) idealized breakthrough curves for injected tracer and reactant, and product formed in situ.

systems (see below). The volume of the injected test solution and the total quantity of all injected solutes is known. During the extraction phase, flow is reversed and the test solution/groundwater mixture flows radially inward toward the well (Figure 1b). Water samples are collected periodically during the extraction phase and the concentrations of tracer, reactive solutes, and any products formed by microbial metabolism are measured and used to develop breakthrough curves for each solute. The tracer breakthrough curve is used to quantify solute losses due to physical processes of advection, dispersion, and diffusion; breakthrough curves for the reactive solutes and products are used to quantify microbial activities. Idealized breakthrough curves for a simulated push-pull test in a hypothetical aquifer illustrate these concepts (Figure 1c). In Figure 1c, the injection and extraction of a simple test solution consisting of a tracer and a single reactive solute (labeled "reactant") which is transformed within the aquifer by a first-order reaction mechanism to a single product was simulated using the finite-difference scheme in

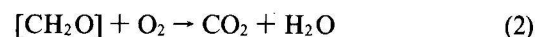
Hoopes and Harleman (1967). In these simulations, the aquifer was assumed homogeneous and isotropic and sorption of tracer, reactant, and product and transport by regional groundwater flow were ignored. Breakthrough curves for the extraction phase show the relative concentration C/C_0 for each solute, where C is the measured concentration and C_0 is the injected concentration, vs. the (cumulative) extracted volume when the sample was collected divided by the total injected volume of test solution. In this simulation, the test solution injection was followed by the injection of a small volume of solute-free water so that initial solute concentrations during the extraction phase are zero. The total quantity of each solute recovered can be obtained by integrating the area under each breakthrough curve. Reaction rates (e.g., zero- or first-order rate constants) are computed by comparing the breakthrough curves for reactants and/or products with the tracer breakthrough curve.

The proposed test method has many potential advantages over existing methods for determining in situ microbial activities. The test can be performed in existing wells, eliminating the need for core samples (and the sometimes extremely elaborate field procedures used to obtain cores aseptically or in an oxygen-free atmosphere) used in laboratory microcosm studies. The test can investigate a large volume of aquifer material (tens to hundreds of liters) and can therefore potentially provide more representative information than laboratory studies performed on typically much smaller core samples. A series of sequential push-pull tests can be performed in a single well to quantify the activity of different enzyme systems or to determine the response of the microbial community to various treatment processes (e.g. the addition of substrates to stimulate growth), changing environmental conditions, or changing composition or concentrations of contaminants. Alternatively, a series of identical push-pull tests can be performed in several wells at a site to quantify site-scale variability in these processes.

Push-pull tests can potentially be designed to investigate a wide variety of metabolic activities; the specific activity investigated depends on the test solution composition and conditions within the aquifer. In this paper we will limit our discussion of the push-pull test method to applications related to processes of bacterial respiration, the biological oxidation of an electron donor coupled to the reduction of an electron acceptor. In a later section we will describe the use of a series of push-pull tests to quantify rates of both aerobic and anaerobic respiration at a petroleum contaminated site. Although these microbial activities are well-known (see e.g., Brock and Madigan, 1991; and Gottschalk, 1986), a brief review is presented here to provide the rationale for test design.

Aerobic Respiration

The utilization of molecular oxygen as a terminal electron acceptor is the most energetically favorable form of microbial respiration. During aerobic respiration, electrons derived from the catabolism of an electron donor are used to reduce oxygen (the electron acceptor) to water. As a representative example, the aerobic oxidation of a generic carbohydrate [CH_2O] to carbon dioxide (CO_2) can be summarized by the reaction:

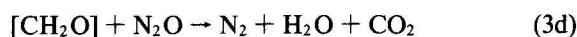
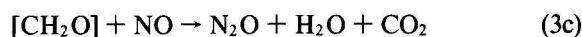
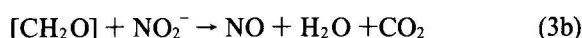
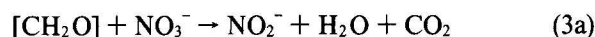


(note that this and following reactions are used for illustration only and are not balanced). Aerobic respiration can be investigated in a push-pull test by injecting a test solution containing a

suitable electron donor and dissolved oxygen and measuring concentrations of the electron donor, oxygen, and carbon dioxide in the test solution/ground-water mixture during the extraction phase. The extent of the reaction can be determined from either the decrease in quantity (injected mass-recovered mass) of electron donor or oxygen or the increase in quantity of carbon dioxide (extracted mass-injected mass). It should be noted that many heavily contaminated ground waters contain an excess of organic compounds to serve as electron donors for this and following respiration processes, and it may therefore not be necessary to supply an electron donor in the test solution.

Denitrification

The oxides of nitrogen can serve as electron acceptors when molecular oxygen is absent or present at very low concentrations (i.e., under anaerobic or anoxic conditions). This process is called denitrification and is the most energetically favorable of the microbial anaerobic respiration processes. Denitrification involves the stepwise reduction of nitrate (NO_3^-) to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and molecular nitrogen (N_2). Although denitrification requires the production of specialized enzymes and electron transport components, the underlying catabolic pathways allow for the complete oxidation of organic electron donors to CO_2 . The degradation of a carbohydrate under denitrifying conditions is therefore similar to the scheme presented for aerobic respiration except for the multiplicity of electron acceptors and can be represented by the series of reactions:



Denitrification can be investigated in a push-pull test by injecting an oxygen-free test solution containing a suitable electron donor and nitrogen oxide(s) to serve as electron acceptor(s), and measuring concentrations of the electron donor, nitrogen oxide(s), and dissolved carbon dioxide in the test solution/ground-water mixture during the extraction phase. The extent of the reactions can be determined from the decrease in quantity of electron donor or nitrogen oxide consumed [e.g. NO_3^- in equation (3a)], an increase in quantity of carbon dioxide, or an increase in quantity of nitrogen oxide [e.g., NO_2^- in equation (3a)] or dinitrogen produced.

Sulfate Reduction

Sulfate reduction couples the oxidation of electron donors such as hydrogen, lactate, or acetate to the reduction of sulfate (SO_4^{2-}) to hydrogen sulfide (H_2S). The major respiratory activities of sulfate-reducing bacteria when hydrogen serves as the electron donor can be summarized by the reaction:

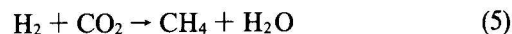


Sulfate reduction can be investigated in a push-pull test by injecting an oxygen-free test solution containing a suitable electron donor and sulfate and measuring concentrations of the electron donor, sulfate, hydrogen sulfide, and carbon dioxide in the test solution/ground-water mixture during the extraction phase. The extent of the reaction can be determined by computing the decrease in the quantity of electron donor, the increase in

the quantity of carbon dioxide (if formed by the reaction), the decrease in the quantity of sulfate, or the increase in the quantity of hydrogen sulfide.

Methanogenesis

The production of methane (CH_4) as a consequence of microbial metabolism is called methanogenesis. Methanogenic organisms are extremely oxygen sensitive, and thus methanogenesis only occurs under highly anaerobic environments. Although methane production from hydrogen and carbon dioxide does not involve a conventional electron transport chain as described for aerobic respiration, denitrification, and sulfate reduction, the process can be conveniently viewed as a form of respiration where hydrogen acts as an electron donor and carbon dioxide acts as an electron acceptor, as shown in the reaction:



In this simplified form, methanogenesis can be investigated in a push-pull test by injecting an oxygen-free test solution containing dissolved hydrogen and a source of dissolved carbon dioxide (e.g. carbonate ion, CO_3^{2-}) and measuring the concentration of H_2 , CO_2 , and CH_4 in the test solution/ground-water mixture during the extraction phase. The extent of the reaction can be determined from the decrease in the quantity of hydrogen or carbon dioxide, or the increase in the quantity of methane.

Example Application

Site Description

A series of push-pull tests was conducted at the site of a former gasoline bulk terminal located in Corvallis, OR (Figure 2a). A portion of the site was contaminated with petroleum hydrocarbons by a leaking underground fuel storage tank and surface spills between 1986 and 1993. The terminal was closed and all buildings, surface installations, and pavement were removed between May and November 1994. Eight wells (MW-1, etc.) were installed for use in site characterization, monitoring, and remediation in December 1994 (Figure 2a). The wells were constructed of 5.1 cm (2 in.) polyvinyl chloride (PVC) inside a 10.2 cm hole created with a hollow-stem auger. The wells were screened across approximately 5 m of the sand and gravel deposit that forms the principal aquifer in this area; the annular space was backfilled with washed, uniform medium sand across the screened interval (Figure 2b). Short duration (~2 hours) pumping tests were performed in wells MW-1, RW-1, and MW-8 immediately following well construction.

Regional ground-water flow is from the southwest to the northeast and discharges to the Willamette River approximately 100 m northeast of the site (Figure 2a). The hydraulic conductivity of the unconfined sand and gravel aquifer, determined by pumping tests, is fairly high, ranging from 0.6 to 1 m/d. Estimated pore-water velocities range from 0.02 to 0.03 m/d. Water-table depths range from 3 to 4 m across the site.

The results of soil and water sampling indicated that gasoline had penetrated the unsaturated zone and reached the unconfined alluvial aquifer at depths up to 8 m with the highest concentrations occurring in the central and northeastern portions of the site; the southeastern portion of the site, located upgradient, was apparently not contaminated. Remediation at the site was performed beginning in November 1994 and consisted of removal of the fuel storage tank and contaminated surface soils to a depth of approximately 2 to 3 m, followed by a

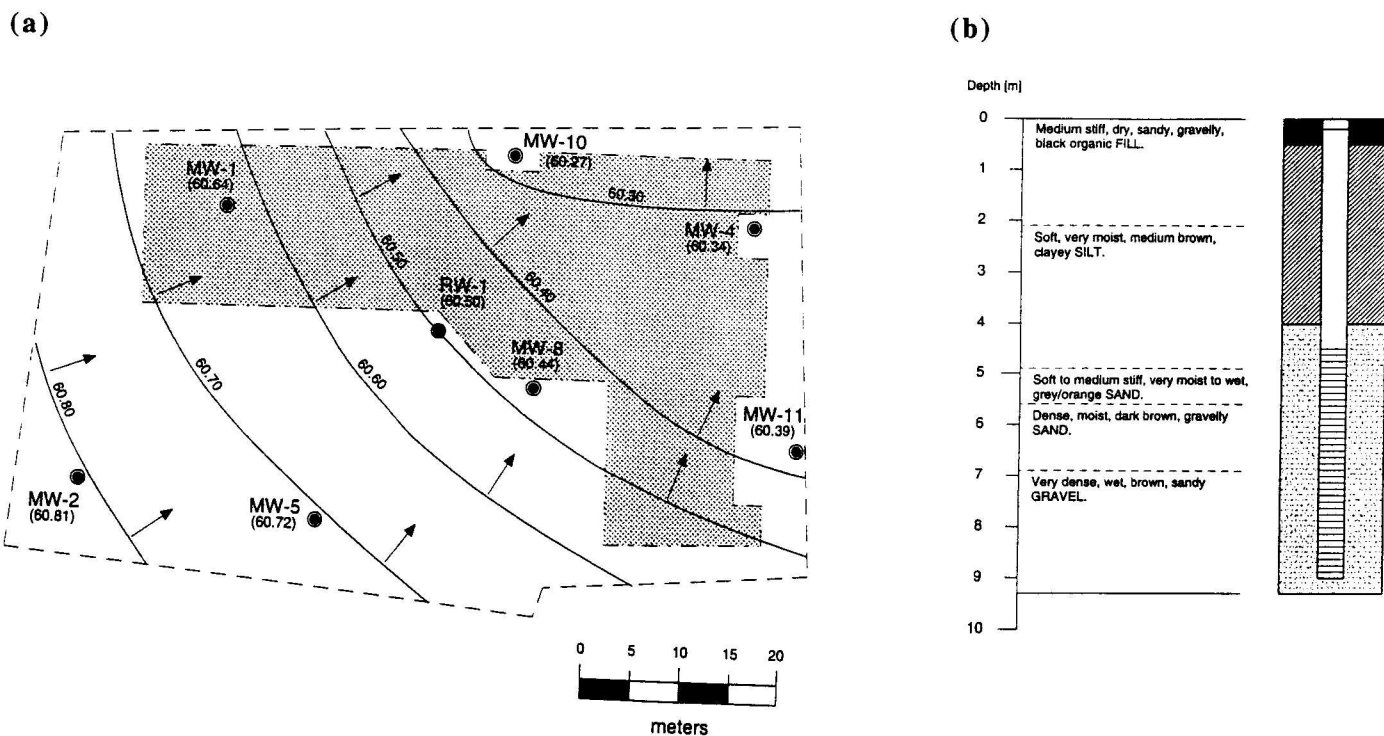


Fig. 2. (a) Map of study area showing location of monitoring and recovery wells, water-table elevations (m), boundaries of soil excavation (shaded area), and direction of ground-water flow. Monitoring well MW-2 is located upgradient from the leaking tank and surface spills; monitoring well MW-4 is within the BTEX plume. (b) Construction details and geologic log for monitoring well MW-2.

brief period of pumping from a single recovery well (RW-1) located in the center of the site (Figure 2a). The tank was located between 1 and 5 m north of RW-1. A small volume of free product was recovered from RW-1; dissolved phase recovery continued from November 1994 to January 1995. Initial BTEX (benzene, toluene, ethyl benzene, and xylenes) concentrations in ground water ranged from 10 to 100 mg/l. Ground-water sampling and testing was performed bimonthly from December 1994 to March 1995 and the results were consistent with the assumption that automotive gasoline was the principal source of contamination at the site. Since the end of pumping, BTEX concentrations have ranged from nondetect to around 50 $\mu\text{g/l}$.

Push-Pull Test Design

Push-pull tests were performed in two monitoring wells. Monitoring well MW-2 is located in the southwestern portion of the site and is upgradient of the leaking tank and fuel spills in a portion of the aquifer that has previously had no detectable concentrations of BTEX or other petroleum hydrocarbons. Monitoring well MW-4 is located in the northeastern portion of the site in a portion of the aquifer that has historically had relatively high BTEX concentrations, although current concentrations are less than or equal to 2 $\mu\text{g/l}$. Measured concentrations of selected water quality parameters for the two wells are summarized in Table 1. The ground water at MW-2 is aerobic with a dissolved oxygen concentration of approximately 8 mg/l, the ground water at MW-4 is anaerobic with a dissolved oxygen concentration of approximately 10 $\mu\text{g/l}$. The observed decrease in oxygen, nitrate, nitrite, and sulfate concentrations and increase in iron (II), carbon dioxide, and methane concentrations as ground water flows through the contaminated zone (from MW-2 to MW-4) suggests that the types or rates of microbial activity in the two zones may be different. Our initial

hypotheses were that: (1) aerobic respiration should dominate microbial activity in the aquifer at MW-2 and rates should be limited by the availability of a suitable electron donor (reduced organic carbon), and (2) anaerobic processes (denitrification, sulfate reduction, and/or methanogenesis) should dominate microbial activity in the aquifer at MW-4 and rates should be limited by the availability of suitable electron acceptor.

An identical series of push-pull tests was performed in MW-2 and MW-4; at least three replications were performed for each type of test in each well. For each test, between 90 and 130 l of test solution were injected. This volume was selected to be equivalent to approximately three times the combined volume of ground water stored in the well casing and sandpack at each well, and represents the volume of water required to penetrate a radial distance into the aquifer of approximately 25 cm beyond the

Table 1. Summary of Water Quality Data for Monitoring Wells Used in Push-Pull Tests

	MW-2	MW-4
Temperature ($^{\circ}\text{C}$)	19	21
pH	7.0	7.1
Benzene ($\mu\text{g/l}$)	¹ ND	2.0
Toluene ($\mu\text{g/l}$)	ND	0.5
Ethyl benzene ($\mu\text{g/l}$)	ND	1.0
Xylenes ($\mu\text{g/l}$)	ND	0.8
Oxygen (mg/l)	8.0	<0.01
Nitrate (mg-N/l)	0.10	<0.01
Nitrite (mg-N/l)	0.02	<0.01
Sulfate (mg/l)	16	<1
Methane ($\mu\text{g/l}$)	ND	67
Carbon dioxide (mg/l)	ND	4

¹ND = Not detected (<0.5 $\mu\text{g/l}$).

outer edge of the sand pack. The test solution was followed by the injection of between 30 and 45 l of clean water (water with no added solutes) intended to displace test solution remaining in the well casing and sand pack into the aquifer. These volumes were computed using the well construction records and the measured saturated thickness in the well prior to the start of the test. The saturated thickness ranged from 3 to 4 m at MW-2 and from 4 to 5 m at MW-4.

The test solution compositions are summarized in Table 2. Each test solution consisted of tap water containing 100 mg/l Br⁻ (prepared with KBr) to serve as a tracer. The test solution also contained specified concentrations of other solutes to serve as electron acceptors or donors. Specified NO₃⁻, NO₂⁻, and SO₄²⁻ concentrations were prepared with NaNO₃, NaNO₂, and Na₂SO₄. Specified CO₃²⁻ concentrations were prepared with K₂CO₃. Prior to injection, the test solution was saturated with a selected gas by passing the test solution through a small volume, flow-through gas absorption column located on a cap installed on the top of the well casing (Figure 3a). The test solution entered the column through a spray nozzle that distributed the flow over the top of the ceramic ring packing material. The solution then trickled downward through the packing and exited the column through tubing attached to the lower end that extended to an injector resting on the bottom of the well. Compressed gas (either O₂, N₂, or H₂ depending on the test, see Table 2) entered the gas absorption column near its base. A portion of the compressed gas flowed upward through the column packing and exited the top of the column through a pressure relief valve and a portion of the compressed gas flowed downward through the tubing to the bottom of the well and bubbled up within the water column inside the well casing, eventually exiting the well through a pressure relief valve attached to the cap. Dissolved gas measurements (data not shown) performed on water samples collected from a sampling port at the base of the gas adsorption column

and from various depths within the well casing indicated that the column was effective in saturating the test solution with the selected gas and that the bubbling action of the gas within the well casing was effective in vertically mixing the test solution across the entire screened interval. In all tests, the clean water injected after the test solution consisted of nitrogen saturated tap water containing no added solutes. Test solution and clean-water injection rates ranged from 0.8 to 1.2 l/min and were adjusted in the field to keep the water-table buildup below 20 cm during the injection phase. The injection phase of a test was typically completed in about two hours.

Breakthrough Curve Determination

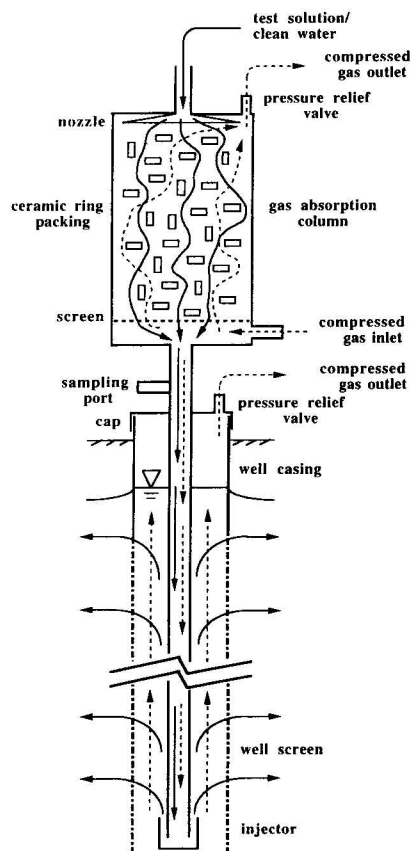
The extraction phase of the push-pull tests began immediately after the end of the test solution and clean-water injections. The gas adsorption column, tubing, and injector were removed from the well casing and a submersible pump (Model 1A106003, Grundfos, Dierikon, Switzerland) was inserted with the pump intake located approximately 25 cm above the base of the well (Figure 3b). The pump was connected to a hose reel and a variable speed flow controller (REEL E-Z model 200, Instrumentation Northwest, Inc., Richmond, WA) which allowed adjustment of the pumping rate during a test. Extraction rates for the test solution/ground-water mixture ranged from 0.8 to 1.2 l/min and were adjusted in the field to keep water-table drawdown below 20 cm during the extraction phase. The extraction phase of a test was typically completed in about five hours. The discharge from the pump was first passed through a glass jacketed cell (used in dissolved oxygen and hydrogen measurements, described below) and then collected in 5 l plastic containers. Water samples were collected from the discharge stream for analysis every 5 or 10 l. The elapsed time and cumulative volume extracted were recorded for each sample. Pumping continued

Table 2. Summary of Test Solution Compositions for Push-Pull Tests at Field Site

Microbial activity	Monitoring well	[Br ⁻]	[O ₂]	[N ₂]	[H ₂]	[NO ₃ ⁻]	[NO ₂ ⁻]	[SO ₄ ²⁻]	[CO ₃ ²⁻]
Aerobic respiration	MW-2	100 mg/l (1.25 mM)	¹ sat						
	MW-4	100	sat						
Denitrification (NO ₃ ⁻ injection)	MW-2	100		sat		5 mg/l (0.36 mM)			
	MW-4	100		sat		5			
Denitrification (NO ₂ ⁻ injection)	MW-2	100		sat			2 mg/l (0.14 mM)		
	MW-4	100		sat			2		
Sulfate reduction	MW-2	100			sat			80 mg/l (0.83 mM)	
	MW-4	100			sat			80	
Methanogenesis	MW-2	100			sat				120 mg/l (2.00 mM)
	MW-4	100			sat				120

¹ Test solution was saturated with the indicated gas by flow through the gas adsorption column prior to injection. Approximate range of dissolved gas concentrations for O₂ = 40 to 45 mg/l (1.25 to 1.41 mM), for N₂ = 18 to 20 mg/l (0.64 to 0.71 mM), and for H₂ = 1.6 to 1.7 mg/l (0.80 to 0.85 mM).

(a)



(b)

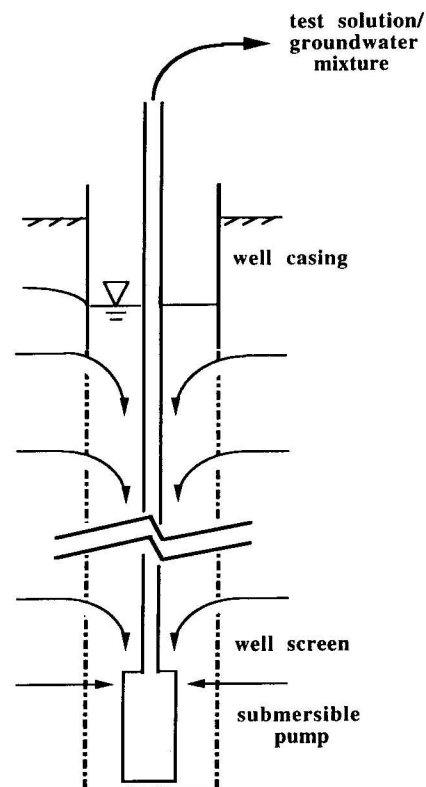


Fig. 3. Field equipment used to perform push-pull tests: (a) injection phase, (b) extraction phase.

until the extracted volume equaled at least three times the total (combined test solution and clean water) injected volume.

Bromide concentrations were measured using a combination glass body bromide electrode (Model 27502-05, Cole Parmer Instrument Co., Niles, IL) and ion-specific meter (Accumet Model 25, Denver Instrument Co., Arvada, CO) which displayed probe potential in millivolts. Millivolt readings were converted to Br^- concentrations using a standard curve developed from potential measurements performed on Br^- standards cooled to the ground-water temperature ($\sim 15^\circ\text{C}$).

Dissolved oxygen concentrations were measured using two methods. For dissolved oxygen concentrations greater than 1 mg/l, a Clark type polarographic probe and meter (Models 5331 and 5300, Yellow Springs Instrument Co., Yellow Springs, OH) were used. Measurements were performed in a glass jacketed cell that surrounded the probe and sample chamber with ground water discharged from the submersible pump to keep the probe and sample at the same temperature as site ground water. Dissolved oxygen concentrations below 1 mg/l were determined using a colorimetric assay based on Rhodazine D reduction (CHEMetrics, Inc., Calverton, VA), which has a detection limit of $5\ \mu\text{g/l}$.

Dissolved hydrogen concentrations were determined using an identical glass jacketed cell and the same Clark type polarographic probe used in the dissolved oxygen measurements except that the probe was preconditioned using an oscillator circuit described by Sweet et al. (1980) and the polarity of the probe was reversed as described by Hanus et al. (1980). The detection limit for this system was approximately $15\ \mu\text{g/l}$.

Nitrate and nitrite concentrations were determined by a colorimetric assay based on cadmium reduction and organic dye formation (CHEMetrics, Inc.). Sulfate concentrations were determined using a colorimetric/turbidimetric assay based on the formation of barium sulfate crystals on reaction with barium chloride and a portable colorimeter (HACH Co., Loveland, CO). Hydrogen sulfide concentrations were determined using a colorimetric assay based on methylene blue formation (CHEMetrics, Inc.). Dissolved carbon dioxide concentrations were determined by a titration/colorimetric assay based on phenolphthalein end-point detection (CHEMetric, Inc.). The detection limit for all these assays was approximately 0.1 mg/l.

Dissolved methane concentrations were determined using Gas Chromatography (GC) with Flame Ionization Detection (FID). A syringe was used to collect a 10 ml water sample from the pump discharge stream and inject it into a sealed 40 ml glass serum vial, which was followed by headspace analysis. Nitrous oxide concentrations were determined using an identical sampling and analysis procedure used for methane except that the GC was equipped with an Electron Capture Detector.

Breakthrough curves were plotted using measured solute concentrations. Concentrations for solutes that were in the injected test solution (i.e., Br^- , O_2 , NO_3^- , NO_2^- , SO_4^{2-} , or H_2) are reported as relative concentrations, C/C_0 by dividing the measured concentration of the solute in the sample, C by the prepared concentration of the same solute in the injected test solution, C_0 . Concentrations of solutes that were not injected but were produced within the aquifer during the test (i.e., CO_2 , NO_2^- , H_2S , or CH_4) are reported as measured solute concentration in

the sample in mg/l. No adjustments were made to measured concentrations to account for initial solute concentrations in the aquifer.

For each test, total quantities of all injected solutes in mmol were computed from measured solute concentrations and the volumes of injected test solution. The total quantity of all recovered solutes in mmol were obtained by numerically integrating breakthrough curves. Recovery percentages for injected solutes were computed as 100% x (extracted mass)/(injected mass). Zero-order reaction rates (mmol/l/hr or mM/hr) for reactants and products were computed by dividing the quantity of reactant consumed (injected mass-extracted mass) or product formed (extracted mass) by the test solution injection volume and by the mean residence time for the test solution in the aquifer. For this calculation, the mean residence time was defined as the elapsed time from the midpoint of the injection phase to the centroid of the bromide breakthrough curve (the time when one-half of the quantity of injected bromide had been recovered). The minimum reaction rate that can be determined by these tests is controlled

by the overall precision of the various concentration measurements (which typically ranged from 0.5 to 1%), the volume of injected test solution, and the frequency of concentration measurements during the extraction phase. We estimate that the minimum zero-order reaction rate we can confidently detect in these tests is approximately 0.001 mM/hr. It should be noted that an alternative method for interpreting push-pull test breakthrough curves to obtain first-order reaction rates has been developed and will be presented in a future paper.

An important consideration in the design of push-pull tests is the selection of an appropriate residence time for the test solution in the aquifer. If rates of a particular microbial process are anticipated to be "slow," a longer residence time should be selected to insure that a detectable quantity of reactant is consumed (or product formed) during the test. Alternatively, if rates are anticipated to be "fast," a shorter residence time should be selected to insure that only a portion (but not all) of the injected reactant is consumed during the test. It is also important to note that if the ground-water velocity is fast relative to an anticipated

Table 3. Summary of Quantities of Injected and Extracted Solutes (mmol), % Recovery, and Zero-Order Reaction Rates (mM/hr) for Push-Pull Tests

<i>Microbial activity</i>	<i>Monitoring well</i>		Br^-	O_2	CO_2	NO_3^-	NO_2^-	N_2O	H_2	SO_4^{2-}	H_2S	CH_4
Aerobic respiration	MW-2	injected	76	84	0							
		extracted	73	83	0							
		% recovery rate	96	99	—							
	MW-4	injected	143	159	0							
		extracted	142	16	107							
		% recovery rate	99	10	—							
			—	0.50	0.37							
Denitrification (NO_3^- injection)	MW-2	injected	68			18	0	0				
		extracted	65			15	0	0				
		% recovery rate	96			84	—	—				
	MW-4	injected	122			29	0	0				
		extracted	121			6	0.3	—				
		% recovery rate	99			20	—	—				
			—		0.08	0.001	—					
Denitrification (NO_2^- injection)	MW-2	injected	80				2.0	0				
		extracted	70				1.4	0				
		% recovery rate	88				78	—	—			
	MW-4	injected	100				3.0	0				
		extracted	98				0.2	0				
		% recovery rate	98				7	—	—			
			—			0.01	—					
Sulfate reduction	MW-2	injected	80						50	54	0	
		extracted	71						26	58	0	
		% recovery rate	89						52	107	—	
	MW-4	injected	147						92	89	0	
		extracted	141						32	93	0	
		% recovery rate	96						33	105	—	
			—					0.22	—	—		
Methanogenesis	MW-2	injected	80						48			0
		extracted	70						24			0
		% recovery rate	88						50			—
	MW-4	injected	127						94			0
		extracted	116						38			0.5
		% recovery rate	91						40			—
			—					0.20			0.002	

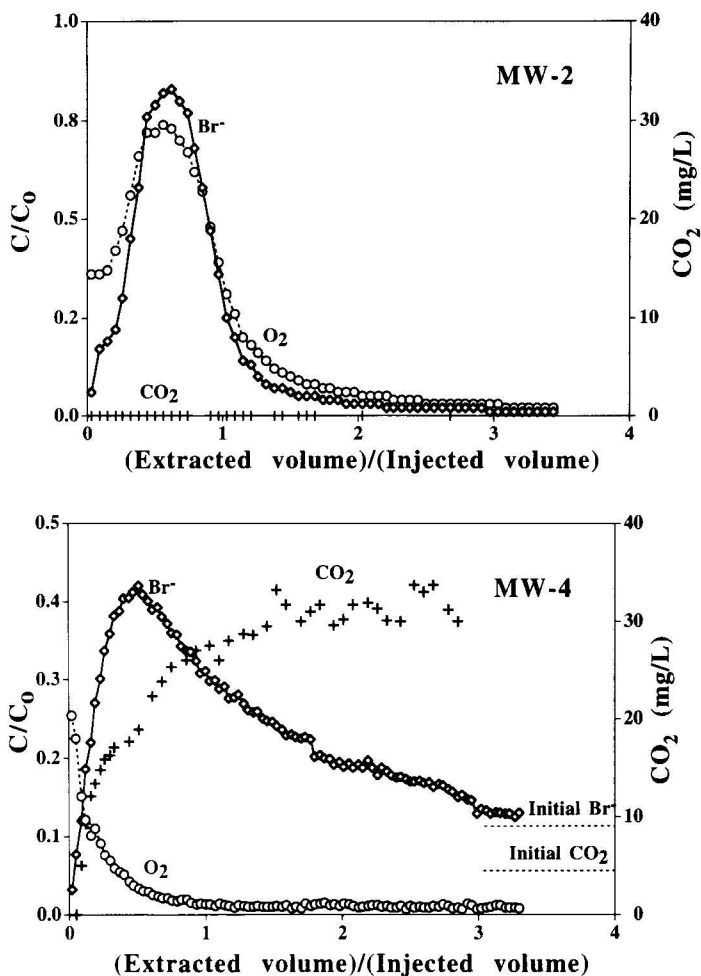


Fig. 4. Push-pull test results for aerobic respiration experiment in (a) monitoring well MW-2, and (b) monitoring well MW-4.

reaction rate the test solution may be swept downgradient before a measurable quantity of reactant is consumed (or product formed).

Results of Field Experiments

Aerobic Respiration

Aerobic respiration was assayed by injecting an oxygen saturated test solution containing a Br^- tracer (Table 2) and measuring the concentrations of Br^- , O_2 , and CO_2 during the extraction phase. In tests performed in MW-2, breakthrough curves for Br^- and O_2 were nearly identical, and CO_2 concentrations remained below 0.1 mg/l indicating that no detectable carbon dioxide production occurred during the test (Figure 4a). Recovery percentages for Br^- and O_2 were high and similar (Table 3) indicating that if aerobic respiration is occurring in the aquifer at MW-2, the rate of O_2 utilization is very slow compared to the test duration. The O_2 breakthrough curves had higher initial concentrations, slightly lower peak concentrations, and a longer tail than the Br^- curve (Figure 4a). This is attributed to the retardation of O_2 transport caused by the partitioning of dissolved O_2 into naturally occurring trapped gas in the sand pack and aquifer created by seasonal water-table fluctuations (Fry et al., 1995).

In aerobic respiration tests performed in MW-4, however, breakthrough curves for Br^- and O_2 were substantially different from those observed in MW-2; O_2 concentrations decreased

rapidly and remained low while measured CO_2 concentrations increased above the background (Figure 4b). Only 10% of injected O_2 was recovered during the extraction phase and the computed zero-order rate for O_2 consumption was 0.5 mM/hr (Table 3). The quantity of O_2 consumed during this test was $159 - 16 = 143$ mmol (Table 3). Based on the stoichiometry of aerobic respiration, we would expect an equal quantity of CO_2 to be produced for each mole of O_2 consumed, if complete conversion has occurred. However, only 107 mmol of CO_2 (or 75% of the expected amount based on O_2 consumption) was recovered during the test (Table 3). However, referring to the breakthrough curves for this test (Figure 4b), we see that the recovered quantity of CO_2 would probably have been larger if the test had not been discontinued before CO_2 concentrations returned to initial levels (Figure 4b).

There were also interesting differences in the shapes of Br^- breakthrough curves in tests performed in MW-2 and MW-4 (Figure 4). At MW-2, Br^- curves were narrower and reached peak relative concentrations that were higher than those observed at MW-4, although the % recovery for Br^- exceeded 88% in all tests at both wells (Table 3). The explanation for these differences is unknown, since drilling logs indicated that the overall character of the aquifer material at the two sites was generally similar. However, only visual/manual logging methods were used to prepare the geologic descriptions (one example is shown in Figure 2b) and it is quite likely that these methods were unable to detect subtle heterogeneities in aquifer properties that could cause the observed differences in Br^- breakthrough curves. Additional possible explanations include undocumented differences in drilling, well construction, or development methods used to install the two wells or undocumented excavation or other soil disturbance that extended into the aquifer near one of the wells. In addition to the differences in breakthrough curve shape, MW-4 had a background Br^- concentration of about 10 mg/l whose source is unknown.

Denitrification

Denitrification was assayed by injecting a nitrogen saturated test solution containing a Br^- tracer and either NO_3^- or NO_2^- (Table 2) and measuring the concentrations of Br^- , NO_3^- , NO_2^- , and N_2O during the extraction phase. Breakthrough curves for Br^- and NO_3^- were very similar during NO_3^- injection tests in MW-2 (Figure 5a) and 84% of injected NO_3^- was recovered (Table 3). In addition, NO_2^- concentrations remained at or below background levels during the extraction phase and no N_2O production was observed. The results also suggest that, in the absence of nitrate reduction, NO_3^- transport is essentially conservative, which is expected for an anion in this coarse-grained alluvium (and was observed for Br^-). Similar results were obtained for NO_2^- injection experiments in MW-2 (Figure 6a) where most injected NO_2^- (88%) was recovered and no N_2O formation was observed (Table 3).

In the anaerobic conditions at MW-4, however, breakthrough curves for Br^- and NO_3^- were substantially different (Figure 5b). Nitrate concentrations remained below Br^- concentrations during the extraction phase indicating substantial NO_3^- loss within the aquifer. Only a portion of the injected NO_3^- (6 mmol or 20%) and the computed zero-order reaction rate for NO_3^- consumption was 0.08 mM/hr. In soils and aquatic sediments, NO_3^- is often completely reduced to dinitrogen, without the significant accumulation of intermediates in the denitrifica-

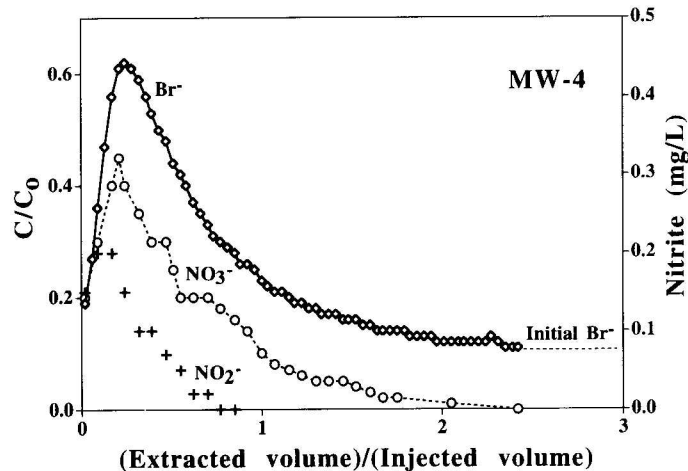
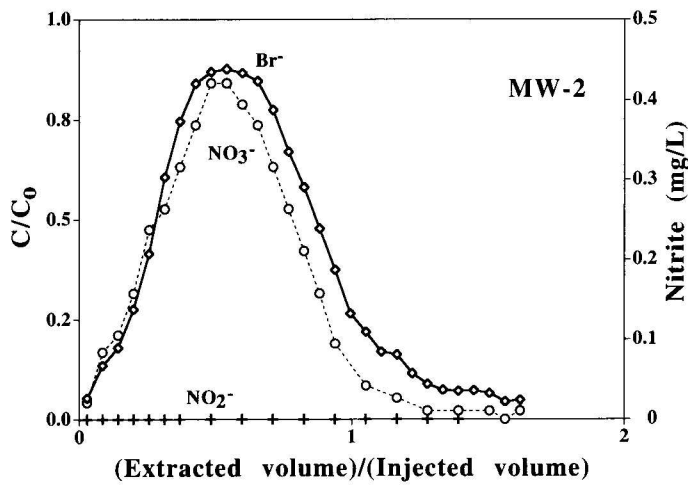


Fig. 5. Push-pull test results for denitrification experiment (NO_3^- injection) in (a) monitoring well MW-2, and (b) monitoring well MW-4.

tion pathway. However, to further characterize the consumption of NO_3^- we investigated the possible accumulation of two denitrification intermediates, NO_2^- and N_2O . We did not observe N_2O accumulation during tests in MW-4. However, we did detect the accumulation of low levels of NO_2^- (0.03 mmol) although this was insufficient to account for the observed NO_3^- loss. While NO_2^- accumulation is unusual, it is certainly consistent with denitrification-dependent NO_3^- consumption and could be accounted for by an excess of NO_3^- -reducing activity relative to the NO_2^- , NO , and N_2O -reducing activities. This interesting accumulation of NO_2^- certainly justifies additional study of the fate of denitrification intermediates in future tests.

Sulfate Reduction

Sulfate reduction was assayed by injecting a hydrogen saturated test solution containing a Br^- tracer and SO_4^{2-} (Table 2) and measuring concentrations of Br^- , SO_4^{2-} , H_2S , and dissolved H_2 during the extraction phase. Breakthrough curves for Br^- and SO_4^{2-} were similar during tests in MW-2 (Figure 7a), except that the breakthrough curve for SO_4^{2-} was somewhat broader, which could be due to ion exchange or some other mechanism that could cause retarded SO_4^{2-} transport in the aquifer in the vicinity of MW-2. However, Br^- and SO_4^{2-} breakthrough curves for tests performed in MW-4 were very similar (Figure 7b) and give no indication of SO_4^{2-} retardation in the aquifer near that well. In any event, essentially all injected SO_4^{2-} was recovered and no

H_2S production was observed during sulfate reduction in tests performed in either well (Table 3). Although no sulfate reduction appears to be occurring in MW-2, only 52% of injected H_2 was recovered and the computed zero-order rate for hydrogen consumption was 0.13 mM/hr (Table 3).

Indirect evidence that sulfate reduction is occurring at this site is provided by the decrease in SO_4^{2-} concentration in ground water from MW-2 to MW-4 (Table 1). However, breakthrough curves for Br^- and SO_4^{2-} were identical at MW-4 and all injected SO_4^{2-} was recovered with no observed H_2S production (Figure 7b, Table 3). However, only 33% of injected H_2 was recovered (Table 3), which is smaller than the quantity of H_2 recovered in SO_4^{2-} injection tests in MW-2 and resulted in a larger utilization rate (0.22 mM/hr).

Methanogenesis

Methanogenesis was assayed by injecting a hydrogen solution containing Br^- tracer and CO_3^{2-} (to serve as a source of CO_2) (Table 2) and measuring the concentrations of Br^- and dissolved H_2 and CH_4 during the extraction phase. The results show that although no CH_4 production was observed, only 50% of the injected H_2 was recovered (Figure 8a, Table 3). In the anaerobic conditions at MW-4, the recovery of injected H_2 was smaller than at MW-2 (Table 3), but CH_4 concentrations were not

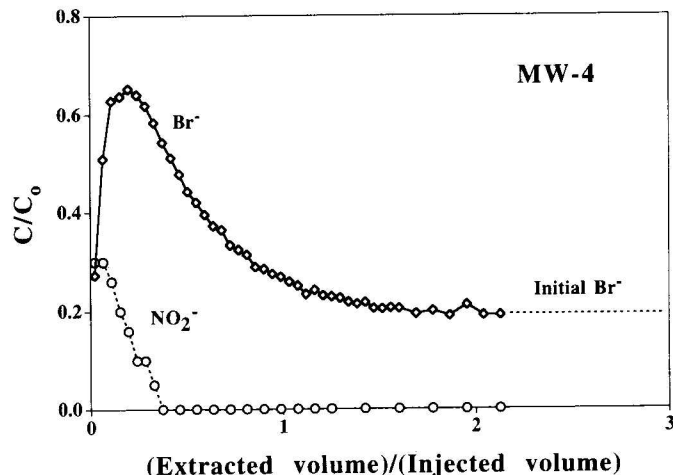
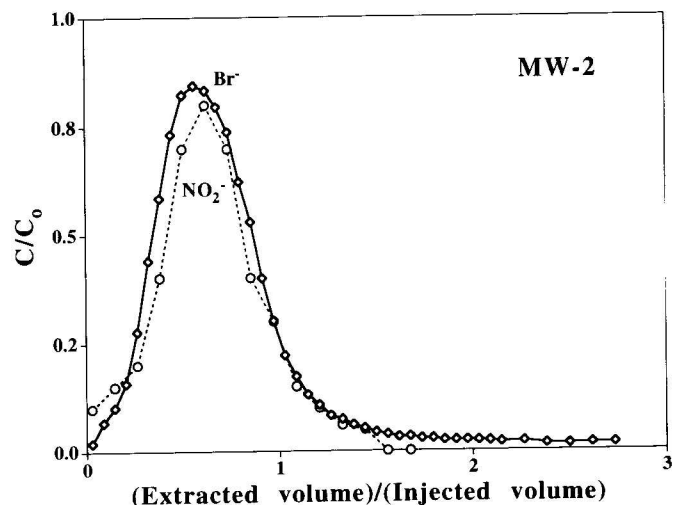


Fig. 6. Push-pull test results for denitrification experiment (NO_2^- injection) in (a) monitoring well MW-2, and (b) monitoring well MW-4.

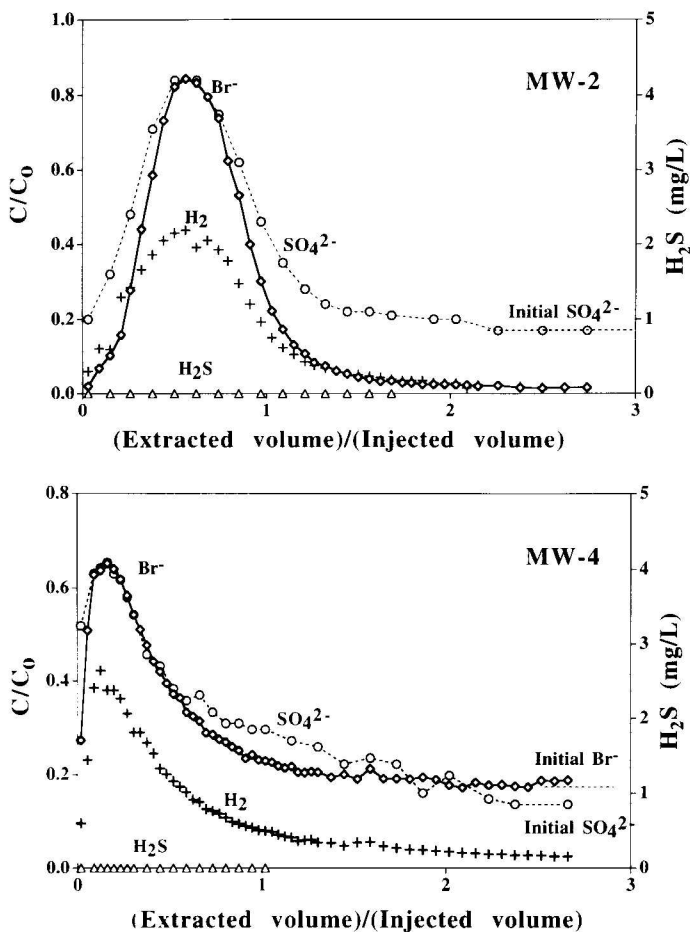


Fig. 7. Push-pull test results for sulfate reduction experiment in (a) monitoring well MW-2, and (b) monitoring well MW-4.

observed to increase above background (Figure 8a) and the quantity of recovered CH_4 was small (Table 3). It is interesting to note that computed rates of H_2 consumption were similar during sulfate reduction and methanogenesis tests in MW-4 (Table 3). Indirect evidence that methanogenesis is occurring at this site is seen in the observed increase in CH_4 concentration in ground water from MW-2 to MW-4 (Table 1).

Discussion

The results of the series of experiments described in this study have clearly demonstrated that the push-pull test method can detect short-term changes in the concentrations of compounds which are often associated with normal microbial metabolic activities. Furthermore, the differential distribution of these activities in MW-2 and MW-4 is consistent with our hypothesis that microbial activities in these two sites are electron donor and electron acceptor limited, respectively.

The most conclusive results obtained in this preliminary study are the observations that O_2 consumption and CO_2 production both occurred during tests in MW-4 in the contaminated portion of the site, while neither process occurred during tests in MW-2 in the uncontaminated portion of the site. It is possible that chemical processes such as the oxidation of ferrous iron in aquifer minerals could be responsible for observed O_2 reduction at MW-4. However, the concurrent production of CO_2 strongly suggests that the reduction in O_2 concentration can be accounted for in whole, or in part, by microbial aerobic respiration supported by organic electron donors. An obvious further refine-

ment of this experiment would be to include an inhibitor of aerobic respiration or a sterilizing agent to estimate the relative proportions of biological and abiological processes. The concentration of dissolved iron could also be measured to estimate the relative contribution of iron oxidation to the observed oxygen loss.

The push-pull tests aimed at examining denitrifying activity also demonstrated a differential distribution of activities with high rates of nitrate consumption observed during tests in MW-4 with little or none observed during tests in MW-2. The production of nitrite from nitrate and the independent demonstration of nitrite consumption in MW-4 are both compatible with biological denitrification. The fact that we did not observe nitrous oxide production in these tests does not exclude denitrification as the processes responsible for these transformations. For example, we did not observe nitrous oxide during our attempts to establish a background concentration for this gas at MW-4, and it is reasonable to suppose that both nitrate and nitrite are fully reduced to nitrogen at this site. However, while nitrate is relatively unreactive in soils, there are several abiological transformations of nitrite which are described collectively as chemodenitrification. These processes could contribute to the observed loss of nitrite and therefore lead to an overestimation of the rate of biological denitrification. Once again, future experiments need to determine the relative contributions of biological and abiological reactions. Undoubtedly, the detection of carbon dioxide production during a denitrification test would provide additional strong support for a biologically mediated process.

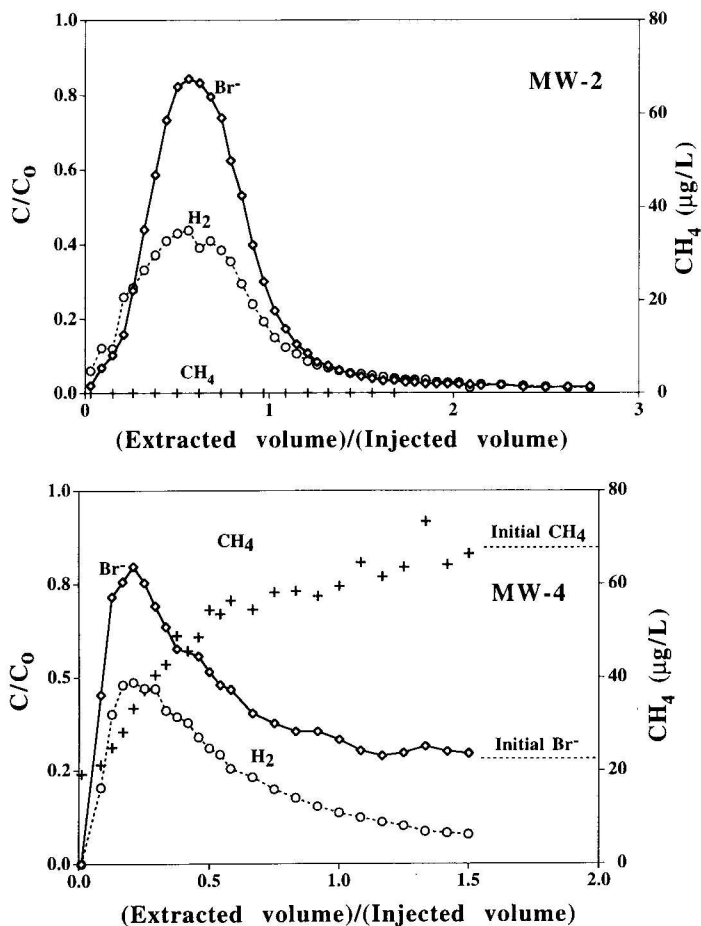


Fig. 8. Push-pull test results for methanogenesis experiment in (a) monitoring well MW-2, and (b) monitoring well MW-4.

Concurrent nitrate consumption and CO₂ evolution was used as a measure of subsurface denitrification in an earlier form of a push-pull technique described by Trudell et al. (1986). Likewise, the use of acetylene to block the biological reduction of nitrous oxide to nitrogen would be an appropriate approach for examining the stoichiometry of nitrate and nitrite consumption. The accumulation of nitrous oxide in the presence of acetylene would provide strong evidence that the relevant biological processes are responsible for the disappearance of these compounds.

Both the aerobic respiration and denitrification tests were designed to measure rates of consumption of added electron acceptors (either O₂ or NO₃⁻). Referring to equations (2) and (3), it is interesting to speculate on the organic compound(s) that served as electron donors for these processes. It is well-known that BTEX in the ground water at MW-4 could serve as an electron donor for either process, but the reported concentrations (Table 1) are too small to provide a sufficient quantity of BTEX to balance observed rates of O₂ and NO₃⁻ consumption. However, several reasonable candidates for the electron donors which supported the observed microbial activity are dissolved and sorbed metabolic byproducts of BTEX degradation which are known to accumulate, often to high concentrations, under denitrifying conditions. For example, Evans et al. (1992) have shown that nearly 20% of toluene-derived carbon accumulates extracellularly during the degradation of toluene under denitrifying conditions in laboratory cultures. While these compounds do not support the growth of denitrifiers, they can likely be utilized as electron donors by aerobes.

Compared to the aerobic respiration and denitrification test, the results we obtained for the sulfate reduction and methanogenesis tests were more equivocal. The strongest evidence we have obtained for the presence of these processes is the apparent consumption of hydrogen during tests in MW-4. However, the significance of this observation is compromised by the fact that we also observed an apparent consumption of hydrogen during tests in MW-2, although a larger percentage was recovered in MW-2 than in MW-4. A variety of explanations for the limited recovery of hydrogen at both sites exists. For example, hydrogen is an excellent electron donor under both anaerobic and aerobic conditions and the poor recovery of hydrogen at MW-2 may reflect a biological process. It should be noted that the addition of hydrogen to MW-2 is the only instance in this study when an exogenous electron donor, rather than an electron acceptor, was added to the uncontaminated site.

It should also be noted that other, abiological processes may also be responsible for a portion of the hydrogen loss observed during sulfate reduction and methanogenesis tests. For example, it is possible that some dissolved hydrogen was lost from solution by diffusion of hydrogen through the walls of the submersible pump discharge line (Figure 3b). However, calculation of hydrogen diffusion rates through the 6 mm thick walls indicate that only extremely small amounts of hydrogen can be lost by this process during the short time (~3 minutes) that water is in this tubing. It is also possible that hydrogen transport, which has a much larger diffusion coefficient in water than the other solutes used in these tests, was influenced by matrix diffusion effects.

While the exact causes of hydrogen disappearance remain to be determined, the geochemical data shows decreased concentrations of both sulfate and nitrate, in addition to detectable levels of methane at MW-4 relative to MW-2. These data suggest

increased anaerobic activities such as sulfate reduction and methanogenesis are occurring in the subsurface areas surrounding MW-4. Our inability to accurately detect these activities using our current protocols may reflect a lack of sensitivity or inappropriate assay conditions. For example, longer periods between injection and withdrawal might be required to compensate for slow rates of activity. Likewise, alternative electron donors might produce faster rates of activities. By the same argument, our limits of resolution may also be limited by the actual numbers of active microorganisms at the site. In support of this argument, we have detected very high levels of carbonate-dependent methane production at several more highly contaminated sites in Oregon using exactly the same procedures described in this report.

Summary

This study has described a single-well, "push-pull" test method for use in the in situ determination of microbial metabolic activities. In an example application, a series of push-pull tests were performed to determine rates of aerobic respiration, denitrification, sulfate reduction, and methanogenesis in aerobic and anaerobic portions of a petroleum contaminated aquifer. Measurements of the rates of oxygen, nitrate, nitrite, and hydrogen utilization and nitrite and carbon dioxide production support the hypothesis that petroleum contamination has resulted in an increase in microbial activity in the anaerobic portion of the site. However, nonbiological processes may contribute to a portion of observed oxygen, nitrite, and hydrogen consumption. At this field site we have demonstrated that the degradation rate in the contaminated zone is limited by the availability of electron acceptors. Since electron acceptors are added during the tests, the reported reaction rates overestimate the actual degradative activity or intrinsic rate. However, they can be considered to be a measure of the activity potential. Essentially, the subsurface microbial populations are "primed" to use any oxygen or nitrate that enters the contaminated aquifer. The high measured rates relative to ground-water flow support the instantaneous oxygen utilization assumption used in a model such as BIOPLUME II. The measures of a site's microbial activities provided by push-pull tests will improve our ability to predict the efficacy of remediation technologies, such as air sparging or peroxide addition, which are based on supplying electron acceptors.

Although some elements of the method presented here are contained in experiments performed by earlier investigators (especially Trudell et al., 1986 and Reinhard et al., 1995), the modifications to the single-well test method for quantifying in situ microbial metabolic processes reported here substantially increase the range of potential applications of this approach (and, we feel, justifies the use of the new name "push-pull" test). Unlike the injection/extraction test method of Trudell et al. (1986), which relied on the use of a specially modified drive point sampler, the method presented here can be performed in any existing monitoring well using standard, commercially available field equipment. In contrast to the approach used by Reinhard et al. (1995), which utilized a large-volume injection followed by periodic water sampling without an extraction phase, our method uses much smaller injection volumes and includes an extraction phase so that complete mass balances can be performed and accurate reaction rates can be computed for all solutes. Our ability to recover essentially all injected tracer and nonreactive solutes should help speed the widespread approval

of the method by regulatory agencies including the addition of contaminants to the test solution so that true in situ bioremediation rates can be determined.

Acknowledgments

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References

- Balkwill, D. L., F. R. Leach, J. T. Wilson, J. F. McNabb, and D. C. White. 1988. Equivalence of microbial biomass measures based on membrane lip and cell wall components, adenosine, triphosphate, and direct counts in subsurface aquifer sediments. *Microbial Ecology*. v. 16, pp. 73-84.
- Beauheim, R. L. 1987. Interpretation of single-well hydraulic tests conducted at and near the Waste Isolation Pilot Plant (WIPP) site, 1983-1987. Sandia National Laboratories, Albuquerque, NM. SAND87-0039.
- Bowman, J. P., L. Jimenez, I. Rosario, T. C. Hazen, and G. S. Sayler. 1993. Characterization of the methanotrophic bacterial community present in a trichloroethylene-contaminated subsurface groundwater site. *Appl. Environ. Microbiol.* v. 59, pp. 2380-2387.
- Brock, T. D. and M. T. Madigan. 1991. *Biology of Microorganisms*, 6th ed. Prentice Hall, Englewood Cliffs, NJ.
- Chapelle, F. H. 1993. *Ground-Water Microbiology and Geochemistry*. John Wiley & Sons, Inc., New York.
- Dawson, K. J. and J. D. Istok. 1991. *Aquifer Testing: Design and Analysis of Pumping and Slug Tests*. Lewis Publishers, Inc.
- Evans, P. J., W. Ling, B. Goldschmidt, E. R. Ritter, and L. Y. Young. 1992. Metabolites formed during anaerobic transformation of toluene and *o*-xylene and their proposed relationship to the initial steps of toluene mineralization. *Applied and Environmental Microbiology*. v. 58, no. 2, pp. 496-501.
- Fry, V. A., J. D. Istok, L. Semprini, K. T. O'Reilly, and T. E. Buscheck. 1995. Retardation of dissolved oxygen due to a trapped gas phase in porous media. *Ground Water*. v. 33, no. 3, pp. 391-398.
- Gelhar, L. W. and M. A. Collins. 1971. General analysis of longitudinal dispersion in nonuniform flow. *Water Resources Research*. v. 15, no. 6, pp. 1511-1521.
- Gottschalk, G. 1986. *Bacterial Metabolism*. Springer-Verlag, New York.
- Hall, S. H., S. P. Luttrell, and W. E. Cronin. 1991. A method for estimating effective porosity and ground-water velocity. *Ground Water*. v. 29, no. 2, pp. 171-174.
- Hanus, F. J., K. R. Carter, and H. J. Evans. 1980. Techniques for measurement of hydrogen evolution by nodules. *Methods in Enzymology*. v. 69, pp. 731-739.
- Harvey, R. W., R. L. Smith, and L. George. 1984. Effect of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod, Mass., aquifer. *Applied and Environmental Microbiology*. v. 53, no. 12, pp. 2992-2996.
- Hoopes, J. A. and D.R.F. Harleman. 1967. Dispersion in radial flow from a recharge well. *Journal of Geophysical Research*. v. 72, no. 14, pp. 3595-3607.
- Kelly, F. X., K. J. Dapsis, and D. A. Lauffenburger. 1988. Effect of bacterial chemotaxis on dynamics of microbial competition. *Microbial Ecology*. v. 16, pp. 115-131.
- Leap, D. I. and P. G. Kaplan. 1988. A single-well tracing method for estimating regional advective velocity in a confined aquifer: Theory and preliminary laboratory verification. *Water Res.* v. 23, no. 7, pp. 993-998.
- Lovley, D. R. and S. Goodwin. 1988. Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochemica et Cosmochimica*. v. 52, pp. 2993-3003.
- McAllister, P. M. and C. Y. Chiang. 1994. A practical approach to evaluating natural attenuation of contaminants in groundwater. *Ground Water Monitoring Review and Remediation*. v. XIV, no. 2, pp. 161-173.
- Reinhard, M., G. D. Hopkins, E. Orwin, S. Shang, and C. A. LeBron. 1995. In situ demonstration of anaerobic BTEX biodegradation through controlled-release experiments. In: R. E. Hinchee, J. A. Kittel, and H. J. Reisinger (eds.). *Applied Bioremediation of Petroleum Hydrocarbons*. Battelle Press, Columbus, OH. pp. 263-270.
- Sternau, R., J. Schwarz, A. Mercado, Y. Harpaz, A. Nir, and E. Halevy. 1967. Radioisotope tracers in large-scale recharge studies of groundwater. *Proceedings of the Symposium on Isotopes in Hydrology*, International Atomic Energy Agency in Cooperation with the International Union of Geodesy and Geophysics. Vienna, November 14-18, 1967. pp. 489-505.
- Sweet, W. J., J. P. Houchin, P. R. Rosen, and D. J. Arp. 1980. Polarographic measurement of H₂ in aqueous solutions. *Analytical Biochemistry*. v. 107, pp. 337-340.
- Tomich, J. F., R. L. Dalton, H. A. Deans, and L. K. Shallenberger. 1973. Single-well tracer test method to measure residual oil saturation. *Journal of Petroleum Technology*. Feb., pp. 211-218.
- Trudell, M. R., R. W. Gillham, and J. A. Cherry. 1986. An in situ study of the occurrence and rate of denitrification in a shallow unconfined sand aquifer. *J. Hydrol.* v. 83, pp. 251-268.
- Wilson, J. T., J. F. McNabb, D. L. Balkwill, and W. C. Ghiorse. 1983. Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. *Ground Water*. v. 21, pp. 134-142.