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In situ assessment of microbial sulfate reduction in a petroleum-contaminated aquifer using push–pull tests and stable sulfur isotope analyses

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Abstract

Anaerobic microbial activities such as sulfate reduction are important for the degradation of petroleum hydrocarbons (PHC) in contaminated aquifers. The objective of this study was to evaluate the feasibility of single-well push–pull tests in combination with stable sulfur isotope analyses for the in situ quantification of microbial sulfate reduction. A series of push–pull tests was performed in an existing monitoring well of a PHC-contaminated aquifer in Studen (Switzerland). Sulfate transport behavior was evaluated in a first test. In three subsequent tests, we injected anoxic test solutions (up to 1000 l), which contained 0.5 mM bromide (Br^-) as conservative tracer and 1 mM sulfate (SO_4^{2-}) as reactant. After an initial incubation period of 42.5 to 67.9 h, up to 1100 l of test solution/groundwater mixture was extracted in each test from the same location. During the extraction phases, we measured concentrations of relevant species including Br^-, SO_4^{2-} and sulfide (S(-II)), as well as stable sulfur isotope ratios ($\delta^{34}S$) of extracted, unconsumed SO_4^{2-} and extracted S(-II). Results indicated sulfate reduction activity in the vicinity of the test well. Computed first-order rate coefficients for sulfate reduction ranged from 0.043 ± 0.013 to 0.130 ± 0.015 day^{-1}. Isotope enrichment factors (ε) computed from sulfur isotope fractionation of extracted, unconsumed SO_4^{2-} ranged from $20.2 \pm 5.5‰$ to $22.8 \pm 3.4‰$. Together with observed fractionation in extracted S(-II), isotope enrichment factors provided strong evidence for microbially mediated sulfate reduction. Thus, push–pull tests combined with stable sulfur isotope

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analyses proved useful for the in situ quantification of microbial sulfate reduction in a PHC-contaminated aquifer.

Keywords: Bioremediation; Microbial activity; Sulfate reduction; Single-well test; Stable isotopes; Petroleum hydrocarbons

1. Introduction

Microbial sulfate reduction is an important metabolic activity in many petroleum hydrocarbon (PHC)-contaminated aquifers (Lovley, 1997; Wiedemeier et al., 1999). During dissimilatory sulfate reduction, bacteria reduce sulfate (SO_4^{2-}) to sulfide (S(-II), defined here as the sum of H_2S, HS^- and S^{2-}). Concurrently, PHC and other indigenous organic compounds are oxidized and often mineralized to carbon dioxide (CO_2) and water. Thus, microbial sulfate reduction contributes to the removal of PHC constituents from contaminated aquifers (Thierrin et al., 1993; Vroblesky et al., 1996; Reinhard et al., 1997; Anderson and Lovley, 2000). Quantitative information on microbial sulfate reduction is needed to assess its contribution to overall PHC removal at a site.

Over the last decade, it has become increasingly apparent that in situ test methods are required to accurately assess subsurface microbial activities (Gillham et al., 1990; Madsen, 1991, 1998). Recently, single-well injection–withdrawal tests, which we call "push–pull" tests, have been used for the in situ quantification of microbial activities in PHC-contaminated aquifers (Istok et al., 1997; Reinhard et al., 1997; Schroth et al., 1998). In a push–pull test, a prepared test solution that contains a non-reactive, conservative tracer and one or more reactive solutes (reactants) is injected ("pushed") into the aquifer through an existing well. During the following initial incubation period (i.e., a rest phase without pumping), indigenous microorganisms consume reactants and generate metabolic products. Thereafter, the test solution/groundwater mixture is extracted ("pulled") from the same location. Rates of microbial activities are then determined from an analysis of solute breakthrough curves obtained by measuring concentrations of tracer, reactants and/or metabolic products at the injection/extraction well during the extraction phase of the test (Haggerty et al., 1998; Snodgrass and Kitanidis, 1998). So far, push–pull tests have been employed to quantify several microbial processes in PHC-contaminated aquifers including aerobic respiration, denitrification, sulfate reduction and methanogenesis (Istok et al., 1997), and degradation of PHC constituents under nitrate- and sulfate-reducing conditions (Reinhard et al., 1997). In addition, push–pull tests were used to assess spatial variability in aerobic respiration and denitrification (Schroth et al., 1998). However, despite their efforts Istok et al. (1997) were unsuccessful in determining rates of microbial sulfate reduction, as essentially none of the injected SO_4^{2-} was consumed during their tests, possibly due to the relatively short incubation periods ($\sim$ 7 h) employed.

Quantification of microbial sulfate reduction based on reactant (SO_4^{2-}) consumption or product (S(-II)) formation may be obscured by concurrent abiotic transformations, e.g., by dissolution/precipitation of gypsum ($CaSO_4$, Stumm and Morgan, 1981), or by

precipitation of S(-II) as iron sulfides (Anderson and Lovley, 2000). As a tool to discern microbial activity from abiotic transformations, stable isotope analyses have found increasing application in recent years. For example, sulfur in natural environments consists largely of two stable isotopes: ^{32}S (95.02% natural abundance) and ^{34}S (4.21% natural abundance) (Hoefs, 1997). Microbial sulfate reduction usually results in significant isotope fractionation, i.e., an enrichment of ^{34}S in unconsumed SO_4^{2-} coupled to an enrichment of ^{32}S in produced S(-II) (Krouse, 1980; Clark and Fritz, 1997; Hoefs, 1997). Sulfur isotope fractionation in groundwater was previously observed in forest hydrology studies (Robertson and Schiff, 1994; Alewell and Giesemann, 1996) as well as in contaminated aquifers, e.g., at a waste disposal site (Bottrell et al., 1995). Thus, sulfur isotope fractionation appears to be a valuable indicator for microbial sulfate reduction in various environments. Unfortunately, little is known about sulfur isotope fractionation in PHC-contaminated aquifers.

The main objective of this study was to evaluate the feasibility of push–pull tests in combination with stable sulfur isotope analyses for the in situ quantification of microbial sulfate reduction in a PHC-contaminated aquifer. Sulfate reduction was quantified based on sulfate consumption observed during push–pull tests. In addition, stable sulfur isotope analyses of extracted, unconsumed SO_4^{2-} and of extracted S(-II) were used to determine isotope enrichment factors, which served as indicators for microbial sulfate reduction.

2. Materials and methods

2.1. Field site description

The study was conducted in a heating oil-contaminated aquifer in Studen, Switzerland (Fig. 1a), which was characterized in detail by Bolliger et al. (1999). In 1993, a spill from a leaking underground heating oil pipe was discovered at the site. Engineered remediation was limited to the removal of free-phase heating oil (~ 34 m^3) by partial excavation of contaminated soil and by pumping until 1996. At that time, engineered remediation was terminated and monitored natural attenuation was selected as the follow-up remediation strategy.

The 20- to 25-m-thick unconfined aquifer consists of unconsolidated glaciofluvial outwash deposits with interbedded layers of poorly sorted silt, sand and gravel. The ground water table is generally between 2 and 4 m below ground surface. Hydraulic conductivity ranges from 1.0×10^{-4} to 9.3×10^{-3} m s^{-1}, porosity is estimated at 0.19, and the average pore water velocity is ~ 0.4 m day^{-1} (Bolliger et al., 1999).

Push–pull tests described in this paper were conducted in monitoring well PS3, which is located within the contaminant source zone (free-phase PHC present) (Fig. 1a). Well PS3 is constructed of 11.5 cm I.D. polyvinyl chloride casing and partially penetrates the aquifer to a depth of ~ 1 m below the ground water table. Compared to the uncontaminated, upgradient well P20 (Fig. 1a), groundwater in PS3 exhibited reduced conditions and contained up to 1 mg l^{-1} dissolved PHC (Bolliger et al., 1999). Monitoring of geochemical parameters along a center flow line revealed that dissolved oxygen (not shown) and nitrate were almost completely depleted and SO_4^{2-} was

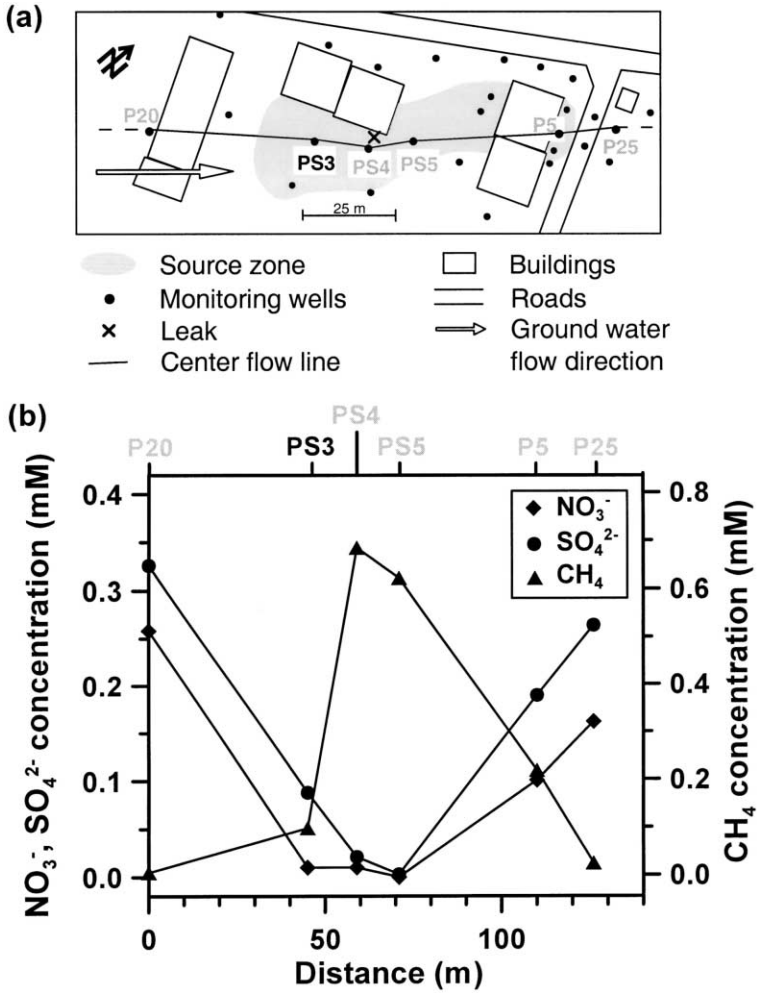

Fig. 1. (a) Site map of the heating oil-contaminated aquifer in Studen, Switzerland, and (b) concentrations of selected geochemical parameters along the center flow line as determined by Bolliger et al. (1999). Push–pull tests described in this paper were conducted in monitoring well PS3.

partially depleted by the time groundwater reached PS3 (Fig. 1b, adopted from Bolliger et al., 1999). Conversely, methane (CH_4) concentrations increased considerably downgradient from PS3. Long-term monitoring for this site provided additional evidence that PS3 is located within a transition zone where both sulfate-reducing and methanogenic conditions are found (Bolliger et al., 2000).

2.2. Push–pull tests and sample collection procedures

Four push–pull tests, denoted PPT1 through PPT4, were performed over a 9-month period from August 1999 until April 2000. First, PPT1 was performed to evaluate

sulfate transport behavior during a push–pull test. Then PPT2, PPT3 and PPT4 were performed to repeatedly quantify rates of microbial sulfate reduction. For each push–pull test, groundwater was first withdrawn from PS3 using a submersible pump (Grundfos MP-1, Grundfos Pumpen, Fällanden, Switzerland) and collected in 500-l plastic carboys. Test solutions were then prepared by adding bromide (Br^-, prepared from KBr, Fluka, Buchs, Switzerland) as non-reactive, conservative tracer and SO_4^{2-} (prepared from K_2SO_4, Fluka) as reactant to achieve final concentrations of 0.5 mM Br^- and 1.0 or 2.0 mM SO_4^{2-} (Table 1). In PPT2, PPT3 and PPT4, the carboys were continuously sparged with nitrogen gas to minimize O_2 dissolution from air into test solutions during preparation and subsequent injection.

For each push–pull test, injection of a specified volume of test solution into PS3 began at time $t = 0$ h and was completed within 0.92 to 2.83 h using gravity drainage (Table 1). In PPT1, continuous extraction began 1.1 h after the injection was terminated. Longer initial incubation periods were used in PPT2, PPT3 and PPT4 before continuous extraction was initiated in PPT2 and stepwise extraction over four consecutive days (batches of 200 to 300 l each per day) was employed in PPT3 and PPT4. In this fashion, we extracted between 380 and 1100 l of test solution mixed with native groundwater in each test at nearly constant flow rates. Total test duration varied from 4.1 h in PPT1 up to 119.9 h in PPT3 (Table 1).

Water samples for chemical/isotope analyses were obtained during the collection of groundwater in carboys (background concentrations), during the injection of test solutions (injection concentrations), and at regular intervals during the extraction phase of the push–pull tests. Samples for Br^- and SO_4^{2-} were filtered in the field using 0.45-μm polyvinylidenefluoride filters (Millipore, Bedford, USA) and stored in 12-ml plastic vials. Samples for alkalinity and pH were collected without headspace in 117-ml serum bottles using butyl rubber stoppers. All samples were stored at 4°C prior to analysis. Samples collected for dissolved O_2, S(-II) and ferrous iron (Fe(II)) determination were analyzed immediately in the field (see below), as was groundwater temperature using an appropriate electrode (Cyberscan pH100, Eutech Cybernetics, Singapore) fitted to a flow cell.

Samples for sulfur isotope measurements of SO_4^{2-} (PPT2–PPT4) were collected in 1-l glass bottles acidified with 2 ml of 32% HCl (Fluka). Sulfate was subsequently precipitated as $BaSO_4$ by replacing 10 ml of sample with 10 ml of a 1.2-M $BaCl_2$

Table 1
Summary of experimental conditions during four push–pull tests performed to evaluate sulfate transport behavior (PPT1) and microbial sulfate reduction (PPT2, PPT3, and PPT4) in a PHC-contaminated aquifer

Test	SO_4^{2-} injection concentration (mM)	Injection volume (l)	Injection duration (h)	Initial incubation period (h)	Total extracted volume (l)	Total test duration (h)
PPT1	2.0	190	0.92	1.1	380	4.1
PPT2	1.0	500	1.83	67.9	1000	73.0
PPT3	1.0	1000	2.83	44.1	1100	119.9
PPT4	1.0	1000	2.17	42.5	1100	117.3

solution. Samples for sulfur isotope measurements of S(-II) (PPT4 only) were collected in 25-l plastic carboys with 5 l of headspace. Immediately after collection, samples were acidified using 180 ml of 1 M HCl and vigorously sparged with nitrogen gas for at least 30 min. During sparging, S(-II) was precipitated as Ag_2S in a gas trap. The trap contained 40 ml of 12.75 mM $AgNO_3$ buffered at pH = 4 using 100 mM acetic acid/20 mM sodium acetate (Moncaster and Bottrell, 1991).

2.3. Analytical methods

Bromide and SO_4^{2-} concentrations were determined using a DX-100 ion chromatograph system equipped with a conductivity detector (Dionex, Sunnyvale, CA, USA). Alkalinity was measured by potentiometric titration using Gran plots for graphical determination of the end point (Stumm and Morgan, 1981) and pH was measured in the laboratory with a MP 225 pH meter equipped with an InLab407 electrode (both Mettler-Toledo, Schwerzenbach, Switzerland). Dissolved inorganic carbon (DIC, sum of H_2CO_3, HCO_3^- and CO_3^{2-}) concentrations were calculated from alkalinity and pH (Stumm and Morgan, 1981). Dissolved O_2, S(-II) and Fe(II) were measured colorimetrically using a DR/890 colorimeter (Hach, Loveland, CO, USA) following standard protocols.

For stable sulfur isotope measurements, $BaSO_4$ and Ag_2S were separately recovered on 0.45-μm HVLP membrane filters (Millipore). After drying at 80°C, ~0.7 mg $BaSO_4$ or Ag_2S were weighted in tin cups together with ~1.4 mg vanadium pentoxide (added as catalyst). Sulfur isotope ratios ($^{34}S/^{32}S$) were subsequently measured on an Optima mass spectrometer (Fisons, Middlewich, Cheshire, UK) coupled in continuous-flow to an elemental analyzer (Carlo Erba Instruments, Milan, Italy). The system was calibrated using international standards IAEA NZ1, IAEA NZ2, and NBS127 (IAEA, 1995). Analytical reproducibility of the measurements was ±0.3‰. Isotope data are reported in the conventional δ-notation relative to the Vienna-Canyon Diabolo Troilite (V-CDT) standard using:

$$\delta^{34}S\,(‰) = \frac{R_{\text{sample}} - R_{\text{V-CDT}}}{R_{\text{V-CDT}}} \times 1000 \qquad (1)$$

where R_{sample} and $R_{\text{V-CDT}}$ are $^{34}S/^{32}S$ sulfur isotope ratios in sample and V-CDT standard, respectively.

2.4. Determination of first-order rate coefficients

First-order rate coefficients for sulfate reduction were determined from sulfate consumption using the method of Haggerty et al. (1998). This method is based on an analysis of tracer and reactant transport in the alternating diverging/converging radial flow field surrounding a monitoring well during a push–pull test. The method assumes that the injected reactant is transformed within the aquifer according to the first-order type reaction $dC_r/dt = -kC_r$, where C_r is the reactive solute concentration and k is the rate coefficient. The method also assumes that the injected test solution is well mixed within the aquifer and that the advection–dispersion–sorption transport properties of

tracer and reactant are similar. With these assumptions, the rate coefficient may be determined from (Haggerty et al., 1998):

$$\ln\left(\frac{C_r^*(t^*)}{C_{tr}^*(t^*)}\right) = \ln\left[\frac{(1-e^{-kt_{inj}})}{kt_{inj}}\right] - kt^* \quad (2)$$

where C^* is relative concentration (i.e., measured concentration divided by the concentration in the injected test solution), subscripts r and tr denote reactant and tracer, respectively, t^* is time elapsed since the end of the test solution injection, and t_{inj} is duration of the test solution injection. Hence, a plot of $\ln(C_r^*/C_{tr}^*)$ versus t^* generates a straight line with a slope $-k$ and an intercept $\ln[(1-e^{-kt_{inj}})/kt_{inj})]$. A nonlinear least-squares routine was used to fit Eq. (2) (both slope and intercept) to experimental breakthrough data to obtain estimates of first-order rate coefficients for sulfate reduction. The 95% confidence interval for k ($k \pm 2\sigma_k$) was computed from the variance of the estimated k, σ_k^2 using (Schroth et al., 1998):

$$\sigma_k^2 = \sigma^2\left\{\sum_{i=1}^{n}\left[\frac{1-e^{kt_{inj}}+kt_{inj}}{k(e^{kt_{inj}}-1)} - t^*\right]^2\right\}^{-1} \quad (3)$$

with $i = 1$ to n, where n is the total number of observations, and σ^2 is the variance of errors in $\ln(C_r^*/C_{tr}^*)$.

2.5. Determination of isotope enrichment factors

Sulfur isotope fractionation was quantified by computing isotope enrichment factors, ε (in ‰). In a closed system, enrichment factors can be determined by fitting Rayleigh distillation equations to experimental data (Mariotti et al., 1981). Specifically, enrichment factors of extracted, unconsumed SO_4^{2-} and extracted S(-II) may be determined from measured $\delta^{34}S$ values using (Böttcher et al., 1999):

$$\delta^{34}S(SO_4^{2-}) = \delta^{34}S(SO_4^{2-})_0 + \varepsilon \ln f \quad (4)$$

$$\delta^{34}S(S(-II)) = \delta^{34}S(SO_4^{2-})_0 - \varepsilon(f \ln f)/(1-f) \quad (5)$$

where f is the fraction of extracted, unconsumed SO_4^{2-}, and $\delta^{34}S(SO_4^{2-})_0$ is the initial isotope composition of sulfate in the injected test solution.

3. Results and discussion

3.1. Push–pull tests

Breakthrough curves for Br^- and SO_4^{2-} showed a gradual decline in C^* as extracted test solution was increasingly diluted with native groundwater during PPT1 (Fig. 2). In addition, relative concentrations of SO_4^{2-} and Br^- were nearly identical throughout the extraction phase. This indicates that SO_4^{2-} transport behavior in general was similar to that of Br^- and that SO_4^{2-} sorption to aquifer solids in particular was negligible during

PPT1 (Schroth et al., 2000). Thus, results from PPT1 confirmed the assumption of similar transport behavior for tracer and reactant, which is required for the accurate computation of rate coefficients in subsequent tests using the method of Haggerty et al. (1998). Moreover, by the end of PPT1 78% of the injected Br^- mass and 77% of the injected SO_4^{2-} mass was recovered (computed by integrating solute breakthrough curves shown in Fig. 2). Recovery of similar relative solute masses clearly indicates that the total test duration (4.1 h, Table 1) was sufficiently short to prevent notable SO_4^{2-} consumption during PPT1.

Extraction phase breakthrough curves for Br^- and SO_4^{2-} during PPT2, PPT3 and PPT4 (Fig. 3) showed gradual declines in C^* similar to that observed during PPT1 (Fig. 2). However, relative SO_4^{2-} concentrations were smaller than relative Br^- concentrations during most of PPT2–PPT4 extraction phases, which indicated that sulfate was consumed during those tests, presumably due to microbial activity. Relative concentrations of SO_4^{2-} slightly larger than those of Br^- were only observed in samples collected near the end of PPT2–PPT4 extraction phases (Fig. 3). This is due to SO_4^{2-} contained in native groundwater (~ 0.03 mM at the time the tests were conducted), which was extracted together with the injected test solutions. To account for SO_4^{2-} contained in native groundwater, we computed corrected sulfate concentrations ($^cSO_4^{2-}$) using Br^- breakthrough curves as a measure of dilution of test solution with native groundwater and assuming constant SO_4^{2-} background concentrations for each test (Schroth et al., 1998). As a consequence, relative $^cSO_4^{2-}$ concentrations were smaller than relative Br^- concentrations throughout PPT2–PPT4 extraction phases (Fig. 3). Note that in PPT1 no correction of SO_4^{2-} data was necessary due to the higher SO_4^{2-} concentrations employed in this test.

During the extraction phases of PPT2, PPT3 and PPT4, we recovered between 25% and 31% of the injected Br^- mass and between 20% and 23% of the injected SO_4^{2-}

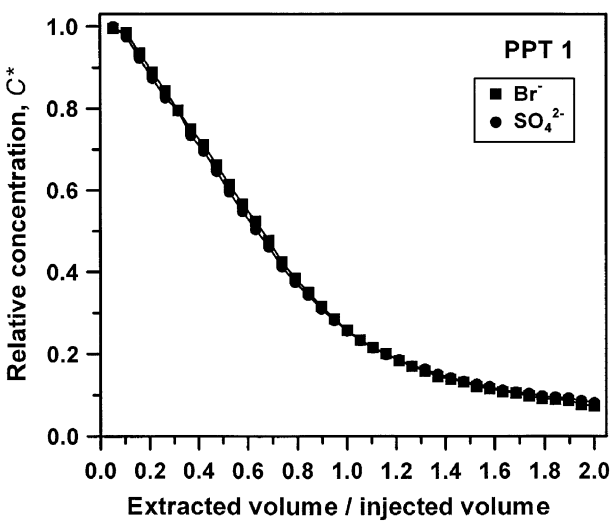

Fig. 2. Breakthrough curves for Br^- and SO_4^{2-} obtained during the extraction phase of PPT1, which was performed to assess SO_4^{2-} transport behavior during a push–pull test.

mass. Differences between recovered relative Br^- and SO_4^{2-} masses ($21.3 \pm 7.2\%$ on average for PPT2–PPT4) illustrate that total test durations (Table 1) were sufficiently long to allow notable sulfate consumption during these tests. Conversely, mass recovery of injected Br^- tracer in PPT2–PPT4 (25–31%) was poor compared to that in PPT1 (78%). This is likely due to longer test durations in PPT2–PPT4 (Table 1) in combination with a fairly high average pore water velocity (~ 0.4 m day^{-1}) at the site. Thus, during PPT2–PPT4, a significant portion of test solution migrated beyond the radius of influence of PS3. However, it is important to note that no complete tracer mass recovery is required during push–pull tests for an accurate quantification of rate coefficients (Haggerty et al., 1998; see next section).

Sulfide increased from background concentrations (9.3–16.8 μM) to maximum concentrations ranging from 14.0 to 50.9 μM during PPT2–PPT4 extraction phases (not shown). For each test, produced S(-II) mass was computed by integrating measured S(-II) concentrations after they were corrected for background and injected test solution S(-II) concentrations. Produced S(-II) mass recovered during PPT2–PPT4 ranged from 2.3 to 14.9 mmol, which is much less than expected based on measured SO_4^{2-} consumption (41.4–50.0 mmol). Loss of produced S(-II) during our tests was presumably due to FeS precipitation, as Fe(II) concentrations up to 59.1 μM were measured during the extraction phases (solubility product for FeS(s) is $K_{s0} = 10^{-18.1}$ at 25°C; Stumm and Morgan, 1981). Increased Fe(II) concentrations are commonly encountered within reduced zones of contaminated aquifers (e.g., Lovley, 1997; Lovley and Anderson, 2000), often rendering S(-II) data useless for the quantification of microbial sulfate reduction.

Other geochemical parameters did not vary significantly during PPT2–PPT4 extraction phases (not shown). In particular, calculated DIC concentrations ranged from 11.1 to 13.2 mM during the extraction phases without an obvious trend and were even slightly smaller than DIC background concentrations (13.3 to13.8 mM) in PS3. This is at least in part due to somewhat smaller DIC injection concentrations (9.7 to 13.3 mM, due to sparging of test solutions prior to and during injection) compared to DIC background concentrations. Moreover, based on measured SO_4^{2-} consumption and given the following reaction stoichiometry (Bolliger et al., 1999):

$$1.37CH_{1.85} + SO_4^{2-} + 2H^+ \rightarrow 1.37CO_2 + H_2S + 1.26H_2O \quad (6)$$

we would expect minimal DIC production during our tests (up to 68.5 mmol DIC in 1000 l of extracted test solution) compared to DIC contained in test solutions and native groundwater. Thus, DIC data obtained during our tests did not provide additional information for the quantification/verification of microbial sulfate reduction. This result contrasts results for DIC analyses performed across entire contaminated sites, which often provide useful information on overall metabolic activities (e.g., Chapelle et al., 1996; Bolliger et al., 1999; Hunkeler et al., 1999).

3.2. Quantification of microbial sulfate reduction

First-order rate coefficients for sulfate reduction were computed for PPT2, PPT3 and PPT4 based on Br^- and $^cSO_4^{2-}$ breakthrough data (Fig. 3) using the method of

Haggerty et al. (1998) (Fig. 4). Note that best fit lines in Fig. 4 are intentionally extended to the *y*-axis intercept (not forced through the origin) as both slope and *y*-axis intercept were fitted (Eq. (2)). Simultaneous fitting of slope and intercept effectively forces k to be the same throughout an entire push–pull test, i.e., during injection as well as during subsequent incubation and extraction. Also note that PPT2 results were unamenable for display due to the different extraction mode used in that test. Computed values of k ranged from 0.043 to 0.130 day^{-1} with 95% confidence intervals ranging from 0.010 to 0.015 day^{-1} (Table 2). Thus, values of k varied by less than a factor of four between the tests. On the other hand, variations in k were significant to the 95%

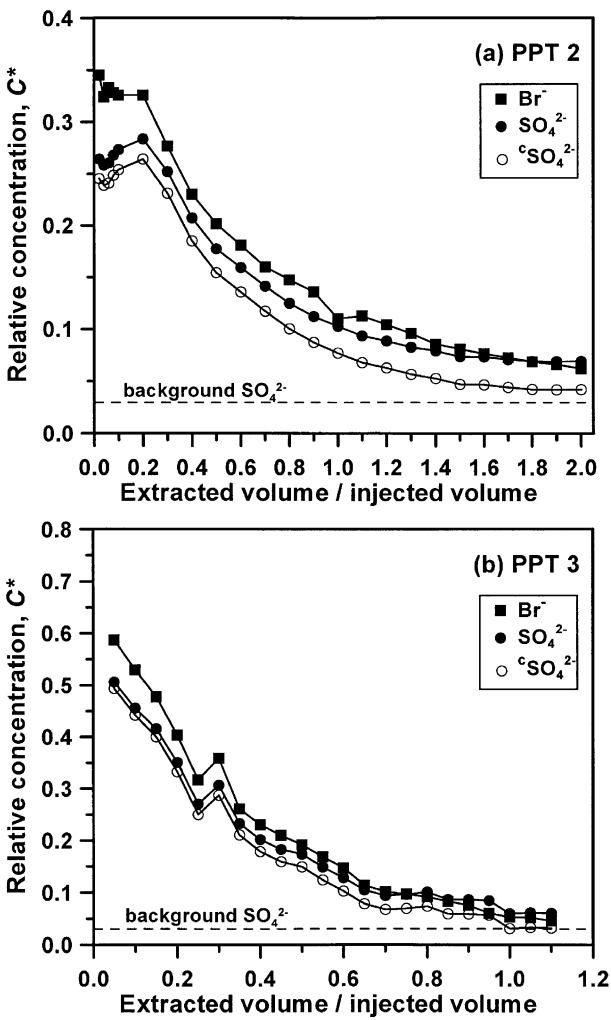

Fig. 3. Breakthrough curves for Br^- and SO_4^{2-} obtained during extraction phases of (a) PPT2, (b) PPT3, and (c) PPT4. Open symbols show corrected SO_4^{2-} concentrations ($^cSO_4^{2-}$), which were computed using Br^- data as a measure of dilution between test solutions and native groundwater.

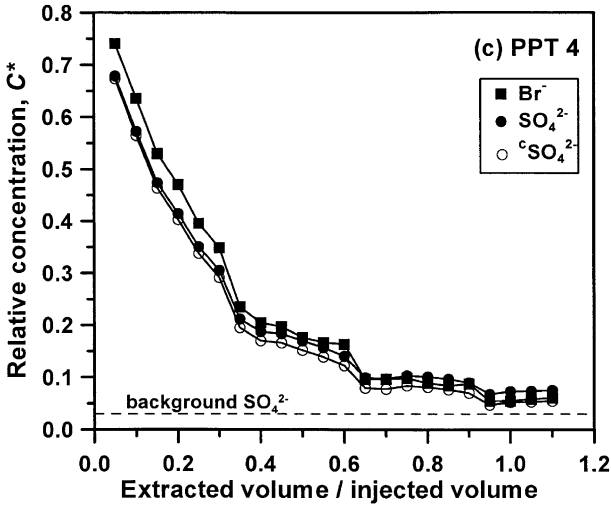

Fig. 3 (*continued*).

confidence level and may at least in part be attributed to the difference in groundwater temperature at the time each test was conducted (Table 2). Specifically, values of k increased with increasing temperature in our tests, which is the generally expected response of microorganisms to changes in temperature within a specific interval suitable for growth (Madigan et al., 2000). Conversely, some scatter in the experimental data are visible in Fig. 4, which may indicate that the method's underlying assumption of

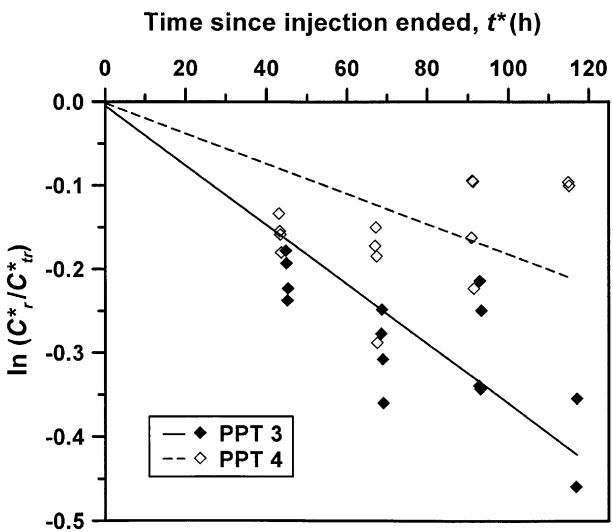

Fig. 4. Determination of first-order rate coefficients of microbial sulfate reduction for PPT3 and PPT4. Lines show the best fit of Eq. (2) to experimental data obtained using the method of Haggerty et al. (1998).

Table 2
Computed first-order rate coefficients (k) for sulfate reduction, isotope enrichment factors (ε) for remaining, unconsumed SO_4^{2-}, and measured groundwater temperature during three consecutive push–pull tests performed to assess microbial sulfate reduction in monitoring well PS3

Test	First-order rate coefficient, $k \pm 2\sigma_k^a$ (day^{-1})	Isotope enrichment factor, $\varepsilon \pm 2\sigma_\varepsilon^a$ (‰)	Groundwater temperature (°C)
PPT2	0.130 ± 0.015	–[b]	16.2
PPT3	0.085 ± 0.010	22.8 ± 3.4	12.5
PPT4	0.043 ± 0.013	20.2 ± 5.5	9.4

[a] 95% confidence interval.
[b] No ε computed, but isotope fractionation in SO_4^{2-} was qualitatively observed.

first-order kinetics was not entirely valid during the tests. However, attempts to employ an alternate method (Snodgrass and Kitanidis, 1998) to determine zero-order rates of sulfate reduction from our data yielded unsatisfactory results.

Values of k determined in our study agreed well with those determined by Chapelle et al. (1996), who obtained $0.02 \leq k \leq 0.08$ day^{-1} for sulfate reduction by fitting a form of the advection–dispersion equation to sulfate concentrations measured across an entire PHC-contaminated site. Conversely, Lu et al. (1999) determined $k = 0.004$ day^{-1} for sulfate reduction in another PHC-contaminated aquifer by fitting a flow and transport model to geochemical data. In this context, it is important to note that the k values we obtained represent local measurements made in the vicinity of a monitoring well known to be within a sulfate-reducing zone rather than an average value across an entire contaminated site. On the other hand, we computed $0.003 \leq k \leq 0.004$ day^{-1} for unamended and enhanced sulfate reduction in another gasoline-contaminated aquifer from data presented by Reinhard et al. (1997), who employed a test similar in design to ours for quantifying degradation rates of specific petroleum constituents. Obviously, many factors such as, e.g., SO_4^{2-} concentration, specific types and concentrations of substrates, temperature and pH may contribute to differences in observed rates of microbial sulfate reduction. Thus, an unequivocal comparison between sulfate reduction rates obtained from different studies is difficult. In addition, while not measured here, we would expect substantial variation in k across our site due to spatial variability in microbial activities ("hot spots"). Existence of "hot spots" in contaminated aquifers has been extensively documented in the literature (e.g., Harvey et al., 1984; Chiang et al., 1989; Adrian et al., 1994; Schroth et al., 1998).

3.3. Verification of microbial sulfate reduction

Increases in $\delta^{34}S(SO_4^{2-})$ compared to $\delta^{34}S(SO_4^{2-})_0$ were observed in samples collected during PPT2–PPT4 extraction phases (Fig. 5, PPT2 data not shown). In Fig. 5, $\delta^{34}S(SO_4^{2-})$ is plotted against f, which was computed from experimental breakthrough data using $f = (C_r^*/C_{tr}^*)$. Note that $f > 0.65$ in all samples due to the limited amount of SO_4^{2-} consumed during the tests (Fig. 5). To account for the isotope composition of background SO_4^{2-}, we computed corrected isotope ratios ($^c\delta^{34}S(SO_4^{2-})$) using Br^-

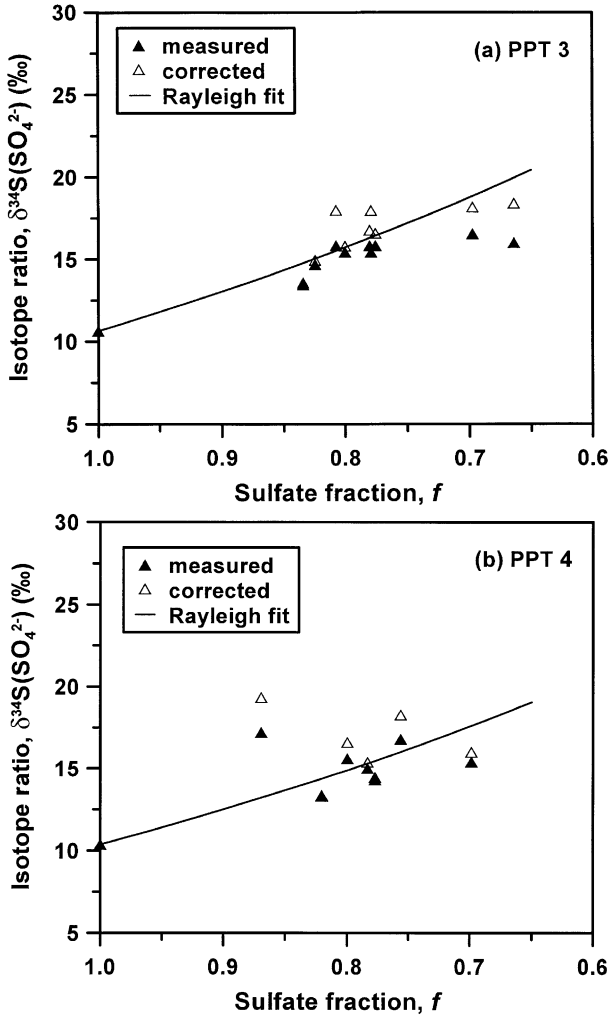

Fig. 5. Stable sulfur isotope ratios of remaining, unconsumed SO_4^{2-} obtained during (a) PPT3 and (b) PPT4 extraction phases. Corrected isotope ratios ($^c\delta^{34}S(SO_4^{2-})$) were computed using Br^- data as a measure of dilution between test solutions and native groundwater. The solid lines represent Rayleigh distillation curves (Eq. (4)), which were fitted to $^c\delta^{34}S(SO_4^{2-})$ data using linear regression analyses.

breakthrough data as a measure of dilution between test solutions and native groundwater (Fig. 5). By fitting the Rayleigh equation (Eq. (4)) to $^c\delta^{34}S(SO_4^{2-})$ data, we then obtained ε values of 22.8 ± 3.4‰ for PPT3 and 20.2 ± 5.5‰ for PPT4 (Table 2). Note that we were unable to compute $^c\delta^{34}S(SO_4^{2-})$ data for PPT2 due to an unreliable measurement of the background SO_4^{2-} isotope composition for the time this test was conducted. Consequently, no ε value was computed for this test.

Enrichment factors computed for our tests agreed well with ε values obtained by others for microbial sulfate reduction in different environments, e.g., ε values of 16‰

to 42‰ (Habicht and Canfield, 1997), 19‰ (Böttcher et al., 1999), and 21.4‰ to 28‰ (Asmussen and Strauch, 1998). Moreover, ε values we obtained were significantly larger than those commonly observed during physical/chemical transformations of SO_4^{2-} (Krouse, 1980), and they were within a range that is indicative for microbial sulfate reduction ($\sim$ 20‰ to 40‰, Clark and Fritz, 1997). Hence, stable sulfur isotope fractionation of extracted, unconsumed SO_4^{2-} provided strong evidence for microbial sulfate reduction during our tests. On the other hand, the magnitude of sulfur isotope fractionation depends on many environmental parameters (e.g., Clark and Fritz, 1997; Böttcher et al., 1999). Thus, a fully quantitative interpretation of ε values obtained in natural systems such as PHC-contaminated aquifers, including the quantification of reaction rate coefficients based on isotope enrichment factors (Aggarwal et al., 1997), is not feasible to date. Consequently, we cannot unequivocally exclude the possibility that a portion of injected SO_4^{2-} may have been abiotically consumed during our tests. However, inorganic sulfate reduction is usually not significant in ground waters (Clark and Fritz, 1997), and, at least for PPT1, SO_4^{2-} precipitation did not appear to play a major role during the test (Fig. 2).

Concurrent to an increase in $\delta^{34}S(SO_4^{2-})$, a decrease in $\delta^{34}S(S(-II))$ was measured in samples collected during the extraction phase of PPT4 (not shown). This would be expected for a closed system, in which enrichment of ^{34}S in unconsumed SO_4^{2-} must lead to a depletion of ^{34}S in produced S(-II). However, due to the small quantity of produced S(-II) recovered during the test and the relatively high S(-II) background concentration in native groundwater (see above), computation of ε was not straightforward in this case. Depending on the (unknown) composition of extracted S(-II) (produced or background S(-II)), we computed hypothetical values of ε by fitting Eq. (5) to experimental data. Computed values of ε ranged from 14.8 ± 2.7‰ (for the case that samples were composed of produced S(-II) only) to 30.1 ± 6.5‰ (for the case that samples were composed of 100% background S(-II) and variable amounts of produced S(-II)). For the case that samples were composed of 50% produced/50% background S(-II), we calculated $\varepsilon = 22.9 \pm 5.4$‰. The latter value of ε in particular agreed well with values of ε calculated from ${}^{c}\delta^{34}S(SO_4^{2-})$ data for PPT3 and PPT4 (Table 2), but an unbiased comparison between the different ε values was obviously impossible. Nevertheless, the general observation of isotope fractionation in extracted S(-II) during PPT4 provided additional qualitative evidence for microbial sulfate reduction.

4. Summary and conclusions

Microbial sulfate reduction was quantified in situ in a PHC-contaminated aquifer based on sulfate consumption observed during single-well push–pull tests. In three consecutive tests, calculated first-order rate coefficients for sulfate reduction varied by less than a factor of four. These variations were attributed in part to observed differences in groundwater temperature between the tests. However, variations in other parameters such as e.g., substrate concentration and pH may also have contributed to differences in measured rates of sulfate reduction between consecutive tests. Although some scatter in the field data was observed, we were able to determine rate coefficients for sulfate

reduction with reasonable accuracy, as indicated by relatively small 95% confidence intervals. Additional studies should be conducted at common field sites to compare rate coefficients for sulfate reduction obtained by our method with those obtained by other in situ methods such as, e.g., in situ microcosms.

We demonstrated that quantification and/or verification of microbial sulfate reduction based on metabolic product formation (S(-II), DIC) was not feasible during our push–pull tests. On the other hand, we provided strong evidence for microbial sulfate reduction during our tests using stable sulfur isotope analyses. From measured isotope fractionation of extracted, unconsumed SO_4^{2-}, we computed enrichment factors that suggested microbial activity as the major mechanism for SO_4^{2-} consumption. Further evidence for microbial sulfate reduction during one push–pull test was obtained from stable isotope measurements of extracted S(-II), even though a straightforward analysis of $\delta^{34}S(S(-II))$ data was hampered by the small quantity of produced S(-II) compared to the high S(-II) background concentration. Further experiments will be required to interpret computed isotope enrichment factors resulting from microbial sulfate reduction in a more quantitative fashion. Nonetheless, push–pull tests combined with stable sulfur isotope analyses proved useful for the in situ quantification of microbial sulfate reduction in a PHC-contaminated aquifer.

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