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Single-well reactive tracer test and stable isotope analysis for determination of microbial activity in a fast hydrocarbon-contaminated aquifer

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"Capsule": *Reaction rates calculated by push-pull tests are not uniformly distributed in time and space.*

Abstract

Single-well reactive tracer tests, such as the push-pull test are useful tools for characterising in-situ bioattenuation processes in contaminated aquifers. However, the analytical models that are used to interpret push-pull data may be over-simplified, and potentially overlook important processes responsible for the frequent discrepancy between predicted and observed results obtained from push-pull tests. In this study, the limitations underlying the push-pull test methodology were investigated and were supported with results from a push-pull test conducted in a sulphate-reducing aquifer contaminated by crude oil. Poor (<7%) mass recovery was achieved when the push-pull test was performed in a fast-flowing aquifer, preventing a quantifiable reaction rate to be determined. Breakthrough curve data were unexplainable using simplified analytical models, but exhibited trends analogous with tests conducted by others, when >20% mass recoveries were achieved. Push-pull test data collected from sulphate-reducing aquifers indicate that the assumption of a well-mixed batch reactor system is incorrect and that reaction rates obtained from push-pull tests in such systems may be affected by the extraction regime implemented. Evidence of microbial respiration of the reactive tracer was provided by stable sulphur isotope analysis, from which an isotope fractionation factor of $+9.9 \pm 8.1$‰ was estimated. The stable isotope data support the argument that reaction rates calculated using push-pull tests are not uniformly distributed in space and time and are likely to be influenced by heterogeneities in the flow field.

Keywords: Push-pull test; Monitored natural attenuation; Sulphate reduction; Aquifer; Stable isotope

1. Introduction

Single-well reactive tracer tests serve as useful tools for demonstrating bioattenuation processes perceived to be active in contaminated groundwater systems. Such tests are especially pertinent in contaminated aquifers where monitored natural attenuation is proposed as a viable remediation option and supporting evidence of attenuative capacity is required to strengthen a conceptual model.

One such test is the single-well push-pull test, presented by Istok et al. (1997) for the determination of microbial respiration rates. The push-pull test has been applied to evaluate zero- and first-order microbial respiration rates in aerobic and anaerobic aquifer systems impacted with various hydrocarbon contaminants (e.g. Istok et al., 1997; Schroth et al., 1998; Schroth et al., 2001). The major assumptions of the push-pull test data interpretation methodology are (Haggerty et al., 1998) (i) that the injected tracers are instantaneously and completely mixed in the aquifer formation, (ii) that the reactive and non-reactive tracers exhibit identical transport properties, such as retardation, and (iii) that the system under study operates as a well-mixed batch reactor with spatially and temporally uniformly distributed reaction rates.

Zero-, or first-order reaction rates for the reactive solute are calculated from breakthrough curve data,

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which are interpreted using simplified analytical models (Haggerty et al., 1998; Snodgrass et al., 1998). A drawback of the simplified models is that the calculated reaction rates tend to be constrained by the above simplifying assumptions and particularly by the latter one, which neglects assessment for possible non-linear microbial kinetics. During reactive well tests, the hydraulic and chemical equilibria of the system are disturbed, thus it is likely that the biological activities will also be dynamic. Zero- or first-order kinetics may accurately approximate Monod kinetics over certain biogeochemical ranges (Bekins et al., 1998); however, in order to characterise these ranges, the half-saturation constant and maximum utilisation parameters of the Monod expression must be determined. For this reason, Monod kinetics are seldom considered in practice (Wiedemeier et al., 1999).

A practical constraint of the push-pull test is that due to lack of hydraulic control during the incubation period of the test, the injected tracers are allowed to drift with the background advective groundwater flux. In aquifers possessing a high groundwater velocity, poor recovery rates are likely to be experienced. According to published results, mixed success has so far been achieved from push-pull tests conducted in sulphate-reducing aquifers (Istok et al., 1997; Schroth et al., 2001). Istok et al. (1997) did not observe any reactive loss of their sulphate tracer; however the longest time any tracer remained in the aquifer during the test was 8 h, probably too short for any reaction to occur. Further, Schroth et al. (2001) measured a reactive loss of sulphate from tests of between 73.0 and 119.9 h duration. Significant scatter was observed in Schroth's data (see Fig. 4 therein), to an extent that may question the applicability of a first-order reaction model used to describe the results. Of particular interest in Schroth's data, was that the pattern of the reactive tracer breakthrough curve data, from which reaction rates were calculated, appeared to have been determined somewhat by the duration of abstraction.

The results of any bioattenuation assessment made using data collected in-situ are subject to the inherent uncertainties associated with hydraulic, geochemical and biological parameters, which are uniquely determined by the heterogeneous nature of the system. However, when implementing natural attenuation as a remediation strategy it is most important to demonstrate that phenomena monitored in the field are attributed to biotic processes rather than geochemical or physical processes, which generally do not result in actual mass destruction of the contaminants of concern (Madsen, 1991). On this front, stable isotope analysis provides a powerful tool for identifying biologically mediated reactions due to preferential assimilation of lighter isotope fractions in biologically mediated reactions (Thode et al., 1951; Chambers et al., 1979).

The objectives of this study were (i) to apply a single well push-pull test in a fast-flowing, hydrocarbon-contaminated aquifer to determine rates of sulphate reduction, (ii) to use stable sulphur isotope analysis to demonstrate that sulphate reduction in the aquifer was attributed to microbial processes, and (iii) to highlight limitations of the push-pull methodology when used for determination of microbial rates, in particular, but not exclusive to cases where the test is applied in aquifers where a fast groundwater velocity is present.

2. Materials and methods

2.1. Site description

The study was conducted in part of the Po river plain aquifer at Trecate in the Piemonte region of Italy. In 1994 the site was the scene of an inland crude oil spill following an oil well blowout from an ENI-Agip-operated exploration well, designated Trecate 24. Details of the incident, which resulted in approximately 15,000 m^3 of middleweight crude oil being released overland contaminating both soil and groundwater, together with descriptions of the subsequent site remediation have been reported elsewhere (e.g. Reisinger et al., 1996, Brandt et al., 2002). Since 1998, natural attenuation has been monitored in groundwater at the site as a follow-up remediation strategy. The main zone of hydrocarbon contamination at the site covers approximately 96,000 m^2 and is characterised by an anoxic, electrochemically reductive groundwater plume. In the plume, levels of the electron acceptors oxygen, nitrate and sulphate are depleted and levels of reduced products–ferrous iron and sulphide, are elevated with respect to conditions hydraulically up-gradient of the site (Table 1). These data indicate that sulphate-reduction is the terminal electron acceptor process dominant at the site.

The Po river plain aquifer at Trecate comprises an extensive, unconfined sand and gravel unit in excess of 60 m thickness beneath the site. The hydraulic properties of the aquifer in the Trecate region were determined from a 71-h continuous rate pumping test performed in 1994 using six wells located 250 m from the study site. A pumping test was not conducted closer to the study site in order to minimise disturbance of the contaminated site conditions. The test data yielded average hydraulic conductivity, transmissivity and specific yield values of 56.5 ± 5.1 md^{-1}, 2700 ± 240 m^2d^{-1} and 0.29, respectively (ENI-Agip, unpublished data). Groundwater levels at the site fluctuate by 6 m seasonally, with higher levels experienced during the summer period due to surficial recharge from agricultural irrigation practices. Groundwater at the site flows in an easterly direction towards the Ticino River. Monitored natural attenuation

data for three of the thirty-nine existing monitoring wells at the study site are presented in Table 1.

The push-pull test was performed from a 50 mm diameter, PVC, partially penetrating well (BC) located at the centre of the main, sulphate-reduced contaminant plume (Fig. 1). The well screened from 9.37 to 13.87 m below ground level (bgl), and the water table rested 7.53 m bgl. Over this depth range, the aquifer comprised a gravelly, fine to medium grained sand. A local hydraulic gradient of between 0.001 and 0.003 was measured at the time of the study, with an estimated background groundwater velocity of between 0.21 and 0.64 md^{-1}.

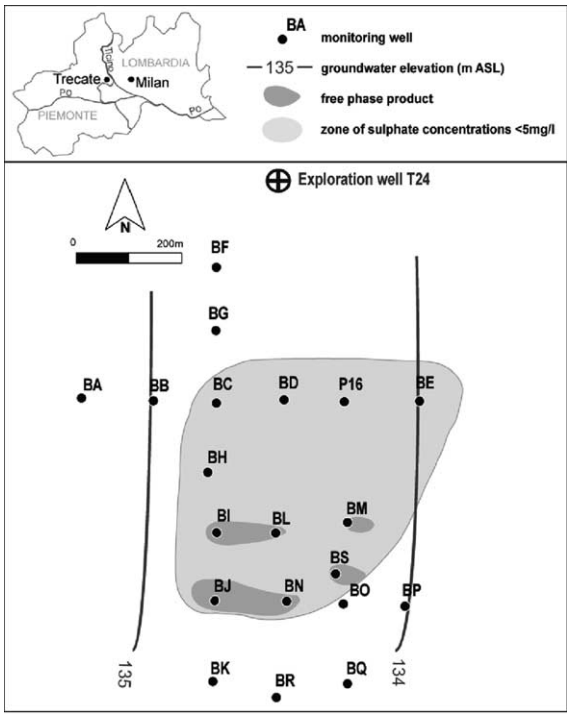

Fig. 1. Map of the crude oil contaminated site near Trecate, Novara, Italy. The site location is in the inset. Note the location of the oil well T24, source of the contamination. The push-pull test was conducted in well BC.

2.2. Push-pull test methodology

A single well push-pull test of the type presented by Istok et al. (1997) was performed in well BC in July 2001. An anoxic test solution was prepared in a polyethylene tank at the wellhead by spiking native groundwater extracted from the well with a non-reactive tracer (chloride, prepared with NaCl) and a reactive, electron acceptor tracer (sulphate, prepared with K_2SO_4) to yield concentrations of 69.0 mgl^{-1} Cl^- and 90.6 mgl^{-1} SO_4^{2-}. The test solution was continuously sparged with nitrogen gas to maintain anoxic conditions. A centrifugal pump (model MP1, Grundfos, Fresno, CA, USA) was used to inject 900 l of the test solution into the aquifer via the well over a period of 1 h. A further 25 l of unspiked groundwater were injected as a chaser to flush the solute from the well casing into the aquifer formation. Assuming the injected test solution exhibited radial symmetry, it was calculated that the test solute travelled 0.11 m beyond the well casing during injection.

Following a 38.5-h incubation period, the diluted test solution was continuously pumped from the well at a rate of between 720 and 960 lh^{-1}, until the equivalent of 4.25-injection volumes had been recovered.

2.3. Analytical methods

A total of 40 samples (P1-P40) of the extracted water were collected at the wellhead at 6-min intervals for chloride and sulphate ion analyses, and analysis of stable sulphur isotopes in unconsumed sulphate. Samples for sulphate and chloride analysis were 0.2 μm filter-sterilised, stored in sterile 10 ml polyethylene vials

Table 1
Geochemical and thermodynamic data from long term monitoring of selected wells along contaminant plume transect at Trecate (ENI-Agip)

	Well								
	BA			BC			BE		
	1996	1999	2000	1996	1999	2000	1996	1999	2000
TPH (μg l^{-1})	139	97	nd	1905	2019	3012	1682	555	1867
Temperature (°C)	16	17.1	16.5	15.2*	17	16.8	15.5	21	20.6
PH	6.9	7.3	7.2	7.1*	7.4	6.8	6.9	7	7
Conductivity (μS cm^{-1})	406	369	425	347*	373	475	462	583	514
Eh (mV)	153	256	291	81	−41	−25	90	266	305
Dissolved oxygen (mg l^{-1})	8.3	7	8.2	0.7*	0.4	0.1	2.4	0.3	2.2
Nitrate (mg l^{-1})	6.8	5.9	5.3	0.8	2.3	2.4	1.2	1.2	1.5
Ferrous iron (mg l^{-1})	0.07	0.01	0.02	1.05	>3.3	>3.3	0.11	0.01	0.03
Sulphate (mg l^{-1})	44	36	43	4	12	13	2	9	18
Sulphide (mg l^{-1})	nd	nd	nd	0.02	0.01	nd	0.01	0.02	Nd

Well BA is hydraulically up-gradient of the main study zone and well BE down-gradient. Well locations are shown in Fig. 1. All data are for the month of July, except where marked *, where data are from August. '–' denotes not analysed, 'nd' indicates concentration below method detection limit. TPH = Total Petroleum Hydrocarbons, determined using method EPA-8015B.

and analysed by ion chromatography (model 4000i, Dionex, Sunnyvale, CA, USA) using an AS4A column with carbonate:bicarbonate eluent (51.4:48.6). Detection limits of 0.45 mgl^{-1} sulphate and 0.26 mgl^{-1} chloride were achieved.

Samples for stable isotope analysis were collected in 1 l glass, amber bottles. Analysis was conducted by precipitating sulphate with barium as $BaSO_4$, which was subsequently dried in a vacuum and converted to SO_2 through combustion with CuO at 1100 °C. The SO_2 was purified on a high vacuum extraction line and subsequently analysed for isotopic composition using a mass spectrometer (model Delta Plus, Thermo Finnigan MAT, Bremen, Germany) calibrated against a Carlo Erba sulphur standard, which was referenced against the Vienna-Canyon Diablo Triolite (V-CDT) standard (precision ±0.2‰).

Isotope data are reported in the conventional δ-notation relative to the V-CDT standard, defined as:

$$\delta^{34}S(0/00) = \left(\frac{^{34}S/^{32}S_{sample} - ^{34}S/^{32}S_{V\text{-}CDT}}{^{34}S/^{32}S_{V\text{-}CDT}}\right) \times 1000 \quad (1)$$

All samples were stored under refrigerated conditions (<4 °C) and analyses were completed within 2 weeks from the time of sampling.

Potential variations in dissolved oxygen, Eh, pH, temperature and conductivity of the groundwater were monitored throughout the extraction phase of the test using Global Water Quality Sensors (Global Water, Gold River, CA, USA) set in a flow-through cell. Conductivity data were used as a real-time qualitative indicator of the progress of recovery of the saline test solution. Biogeochemical parameters including alkalinity, total sulphide and ferrous iron were also measured in the field during the test at less frequent intervals. Alkalinity was determined by the GRAN titration method and together with pH was used to determine the dissolved inorganic carbon (DIC) concentration (sum of CO_2, H_2CO_3, HCO_3^-, CO_3^{2-}). Dissociation constant values for the CO_2-system were corrected for temperature and ionic strength effects (approximated using the conductivity measurements) (Mackreth et al., 1989). Total sulphide (H_2S, HS^-, S^{2-}) and ferrous iron were measured by the methylene blue and phenanthroline methods, respectively (HACH, 1997) using a portable spectrophotometer (Model DR/2000, HACH, Loveland, USA). An increase in levels of DIC, alkalinity and sulphide would be expected if biodegradation by dissimilatory sulphate reduction was active.

2.4. Push-pull data analysis

The chloride breakthrough curve data collected during the extraction phase of the test were corrected for background chloride levels and used as a reference point that discounted for hydraulic phenomena of the test, such as dilution. It was assumed in the test that chloride and sulphate possessed similar transport properties. The chloride and sulphate concentrations that were employed were significantly below levels at which density-driven effects were likely to have occurred (Ward et al., 1998), and below levels at which any inhibitory effects to freshwater sulphate-reducing bacteria were expected (Beller et al., 1992; Friedrich Widdel, Max Planck Institute, Germany, personal communication).

The sulphate breakthrough data were similarly corrected for background sulphate levels using the dilution factor calculated from the chloride analyses. The differences in mass balances between the normalised sulphate and chloride data were assumed to be attributed to microbial respiration of the reactive electron acceptor (sulphate) tracer. Thus, the rate of microbial respiration was reflected in the rate of change in the mass deficit. Sulphate depletion rates were assessed for zero-order degradation kinetics according to the model of Snodgrass and Kitanidis (1998):

$$\hat{C}_r(t^*) = C_r^0\left(\frac{C_r(t^*)}{C_r^0} - \frac{C_t(t^*)}{C_t^0} + 1\right) \quad (2)$$

Where C_i (t^*) denotes concentration of tracer i, at time t^* after the end of injection and C_i^0 denotes the initial (injected) tracer concentration. Subscripts r and t denote reactive (sulphate) and conservative (chloride) tracers, respectively. For a zero-order reaction rate, a plot of Eq. (2) against time should yield a straight-line, the slope of which equals the zero-order decay constant.

Assessment for first-order degradation kinetics was made according to the model of Haggerty et al. (1998):

$$\ln\left[\frac{(C_r(t^*)/C_r^0)}{(C_t(t^*)/C_t^o)}\right] = \ln\left[\left(\frac{(1-e^{-kt_{inj}})}{kt_{inj}}\right)\right] - kt^* \quad (3)$$

Where t_{inj} is the length of time of injection and k is the first-order reaction rate for the reactive tracer.

2.5. Analysis of isotope enrichment

$\delta^{34}S(SO_4^{2-})$ values measured for groundwater during the extraction phase represented the sum of isotopic compositions of sulphate from the injected solute (+9.0‰) mixed with sulphate from native groundwater (−0.1‰), together with any isotope fractionation effects. The contribution of mixing effects with native groundwater on measured $\delta^{34}S(SO_4^{2-})$ values were adjusted for, using chloride data as a measure of dilution to yield corrected $\delta^{34}S(SO_4^{2-})$ values, denoted by $^c\delta^{34}S$.

A per mil enrichment factor, ϵ(‰) was calculated for sulphur isotopes from $^c\delta^{34}S(SO_4^{2-})$ data, using the Rayleigh distillation equation, given by Marriotti et al. (1981) as:

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

Where $\delta^{34}S(SO_4^{2-})_0$ is the initial isotope composition of sulphate in the injected test solution and f is the unreacted fraction of sulphate (from $(C_r/C_r^0)/(C_t/C_t^0)$). A value of ϵ(‰) was obtained from the slope of a straight-line, fit by least-squares linear regression to the corrected data set. The expression in (4) holds for unidirectional reaction systems, where reaction products do not isotopically re-equilibrate with the reactants.

3. Results

3.1. Field geochemistry

Thermodynamic and geochemical properties of background groundwater and injected solution are presented in Table 2. Groundwater at the test well was significantly depleted in sulphate (3.0 mgl^{-1}) relative to levels measured hydraulically up-gradient of the contaminant plume (well BA 38.0 mgl^{-1}, see Table 1). Elevated levels of ferrous iron (3.09 to >3.3 mgl^{-1}) and sulphide (0.03 mgl^{-1}) were also recorded in well BC, however dissolved sulphide concentrations were low in comparison to the mass of sulphate reduced in the aquifer. This is not an uncommon feature in anoxic groundwater environments since sulphide may react with species such as ferrous iron to form metal-sulphide complexes that can precipitate out of solution (Matsunaga et al., 1993; Hunkeler et al., 1998). Additional qualitative evidence of sulphidogenic conditions was obtained from olfactory evidence of sulphide gas emitted from groundwater drawn from the well. These phenomena have consistently been observed throughout the monitored natural attenuation study of the site (see Table 1), confirming that anaerobic, sulphate-reducing conditions were prevalent in the study zone of the aquifer (Chappelle, 2001). A background concentration of 10.8 mgl^{-1} chloride was measured for the aquifer.

3.2. Reactive tracer test

During the extraction phase of the push-pull test, sulphate and chloride concentrations in groundwater were reduced to background levels within 20 samples (equivalent to the extraction of approximately 1.8-injected test solution volumes) (Fig. 2). The shapes of the breakthrough curves were closely matched, suggesting that our assumption that both solutes possessed similar retardation characteristics was valid. Chloride data exhibited a much larger variation than sulphate data, probably due to chloride having a greater spatial variability in the aquifer, although this was not quantified directly. Breakthrough data indicated that only 6.6% of the total mass of injected chloride tracer and 5.5% of the total mass of the injected sulphate tracer were recovered during the push-pull test. The discrepancy between recovered masses agrees with the hypothesis that sulphate supplemented to the aquifer in the test solution was consumed as a result of sulphate-reducing microbial respiration, whereas chloride was not involved in any biogeochemical reactions. The recovered volume of test solution exceeded the combined volume of the well and well filter pack, indicating that at least half of the recovered solute had entered the aquifer formation, even if the effectiveness of the chaser was neglected.

A distinct peak in all data at time when extracted volume/injected volume equals 1.8, corresponded to an event during the extraction phase when power to the pump was temporarily lost and water levels in the well recovered to initial conditions. This action resulted in oxidation of the system (3 mgl^{-1} according to dissolved oxygen data, data not shown) probably as a result of aerobic mixing in the pump discharge line and with the

Table 2
Geochemical and thermodynamic data for background groundwater measured at well BC and for injected test solution

Measurement	Background	Injected
Sulphate (mg l^{-1})	3.0 (±1.2)	90.7 (±1.5)
Chloride (mg l^{-1})	10.8 (±1.4)	69.1 (±1.6)
Sulphides (mg l^{-1})	0.03	ND
Ferrous iron (mg l^{-1})	3.09–>3.3	2.69
Alkalinity (meq l^{-1})	3.85	4.22
pH	6.83	7.28
Eh (mV)	−164.88	ND
Temperature (°C)	16.94	ND
Dissolved oxygen (%)	1.68 (<0.2 mg l^{-1})	7.52 (<0.6 mg l^{-1})[a]
Conductivity (μS cm^{-1})	306	650[b]
Dissolved inorganic carbon (mmol l^{-1})	5.03	4.64[a]
$\delta^{34}S(SO_4^{2-})$ (‰)	−0.1 (±0.7)	9.0 (±0.0)

ND indicates parameter not determined.
[a] Indicates measurement calculated using background groundwater temperature value.
[b] Indicates parameter measured in the laboratory as opposed to in the field.

(aerobic) capillary fringe. The observation that chloride levels also increase at this period suggests that part of the injected solute that may have been trapped within the well casing, filter pack or aquifer was flushed out during this event by the transient vertical flow. This feature reflects the unconfined nature of the aquifer, in which pores near to the water-table would have become dewatered during pumping and vertical flow gradients would have occurred. Vertical flow gradients would also have been pronounced by the partial-penetrative nature of the well. The combined effect of vertical flow fields and physical aquifer heterogeneities (as have subsequently been observed from an unpublished ground penetrating radar survey of the site) could have contributed to the incomplete vertical mixing of the test solution in the aquifer.

Results of sulphide and alkalinity analyses exhibited little variation over the duration of the test. Part of the reason for the invariance in the sulphide and alkalinity results may have been attributed to analytical errors associated with measuring these particularly unstable parameters, such as degassing of hydrogen sulphide and carbon dioxide from the samples during sampling and analysis. Equally, the failure to measure a distinct increase in sulphide in the retrieved test solution could be explained by sulphide precipitation reactions highlighted in Section 3.1. The high levels of ferrous iron (consistently >3.3 mgl^{-1} in samples collected during the extraction phase) and thermodynamic conditions at the site are favourable for the Trecate aquifer being oversaturated with respect to FeS (Davison, 1991). Despite the analytical uncertainty associated with these data, an increase in DIC of between 0.2 and 0.92 mmol was calculated throughout the extraction phase using the alkalinity results (data not shown) and was a positive indicator that microbial respiration occurred during the test.

The data from the push-pull test were unamenable to either of the simplified zero- or first-order degradation models [Eqs. (2) and (3)]. The results of a first-order model assessment of the data are shown in Fig. 3. In this model, the non-linear regression fit of Eq. (3) was performed only using tracer data for which concentrations were above background (samples P1–P20). Attention should be drawn to the fact that in the case of the first-order model, the intercept of the line of regression is forced by Eq. (3) to pass through the vertical axis at:

$$\ln\left[\frac{(C_{\mathrm{r}}(t^*)/C_{\mathrm{r}}^0)}{(C_{\mathrm{t}}(t^*)/C_{\mathrm{t}}^o)}\right] = \ln\left[\left(\frac{(1-\mathrm{e}^{-kt_{\mathrm{inj}}})}{kt_{\mathrm{inj}}}\right)\right] \quad (5)$$

The tracer data from the push-pull test tend to indicate that the loss of sulphate observed by the test was not constant throughout the duration of the test and that sulphate removal processes may have been amplified during the extraction phase of the test. As a consequence, the results of any model fit to the data would be subjective according to the selection of data points to which the model was fit and to the period of the extraction phase to which these data points were related. This feature is analogous with that seen in the first-order rate coefficient assessment of PPT3 and PPT4

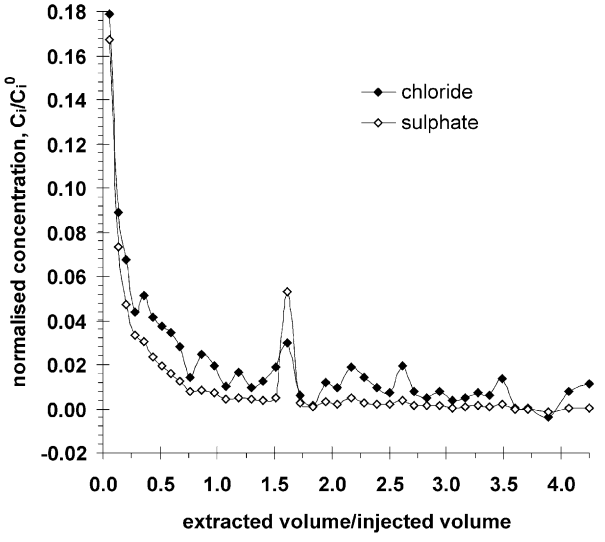

Fig. 2. Chloride and sulphate breakthrough curve from the extraction phase of the push-pull test. Normalised tracer concentrations have been corrected for background concentrations. The large peak for which the normalised sulphate concentration surpasses the normalised chloride concentration corresponds to the period when pumping was momentarily interrupted.

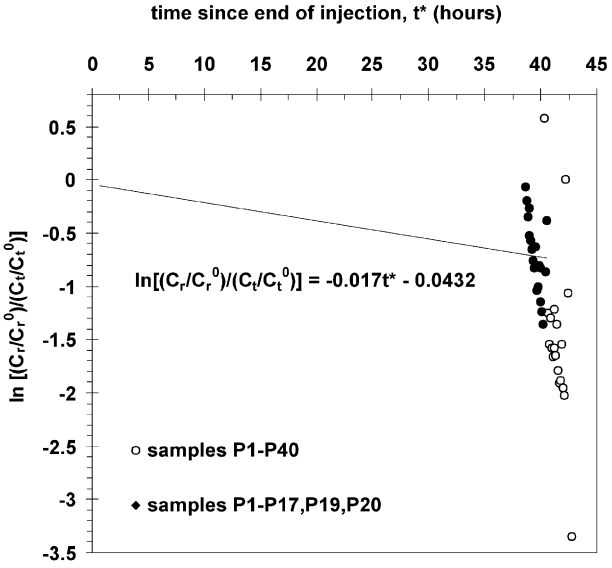

Fig. 3. Example of fit of first-order degradation model (solid line) [Eq. (3)] to field data. Selected data points (samples P1–P19 and P20) that correspond to when tracer concentrations were above background levels and when conditions were anaerobic, were used to fit the model.

data of push-pull tests conducted by Schroth et al. (2001) (see Fig. 4 therein).

3.3. Sulphur isotope fractionation

The results of the sulphur isotope analyses in unconsumed sulphate over the period of abstraction are presented in Fig. 4. For all except the first four samples, $^{c}\delta^{34}S(SO_4^{2-})$ values indicated that sulphate in the recovered solute became enriched in ^{34}S. $^{c}\delta^{34}S(SO_4^{2-})$ values as high as 31.2‰ were measured in the test. The $\delta^{34}S(SO_4^{2-})$ breakthrough curve displayed no specific temporal trend, but this may partly be attributed to heterogeneities in the flow field. Such effects may have caused tracer to be recovered at times non-reflective of the time spent in the aquifer. This phenomenon is likely to be more enhanced in aquifers where a strong regional flow field is present, as at Trecate. The first four extracted samples suggested that the sulphate became enlightened in ^{34}S, contrary to expectations.

An enrichment factor of $+9.9\pm8.1$‰ was computed for the breakthrough curve data from the Rayleigh distillation equation [Eq. (4)] (Fig. 5). In this calculation the first four samples (for which an enlightenment in S^{34} was observed), the sample relating to when pumping was temporarily interrupted (P18) and any samples collected after tracer concentrations dropped below background (after sample P20) were omitted.

4. Discussion

4.1. Push-pull test

As anticipated, owing to the high groundwater velocity at Trecate, very little (6.6%) of the injected solute in the push-pull test was retrieved. This result demonstrated the difficulty of selecting a suitable test duration, for which the incubation period of the injected solute was of sufficient length for bacterial reduction of the reactive tracer by a measurable amount and yet short enough to have avoided complete loss of tracer due to the background advective flux. Incubations generally need to be longer for anaerobic systems than aerobic systems due to slower microbial reaction rates in anoxic systems. For the range of published first-order sulphate removal rate constants-0.02 to -0.08 day^{-1}, measured from in-situ and laboratory microcosm systems where hydrocarbons constituted the electron donor (Chappelle et al., 1996; Bölliger et al., 2001), a sulphate residence time of between 35 and 131 h would be required to measure a 10% reduction in sulphate, attributed to reactive loss. Clearly a residence time of 131 h in a fast aquifer, such as at Trecate poses significant recovery problems and data interpretation problems as has been demonstrated by this study.

Since only 6.6% of the injected test solution was recovered, it has been unjustifiable to quantitatively speculate reaction kinetics observed from the push-pull

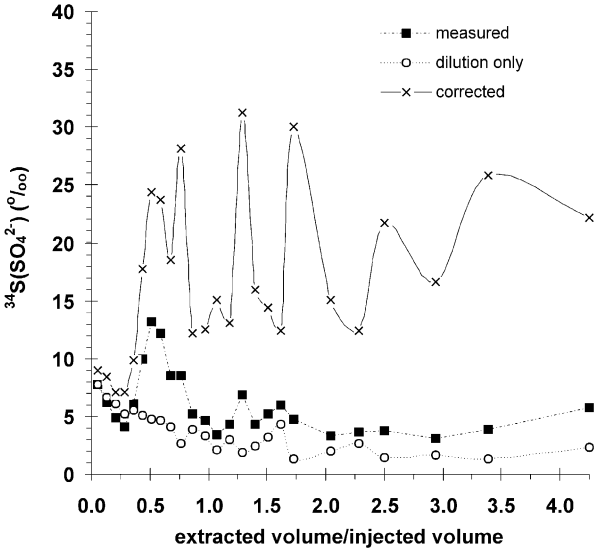

Fig. 4. Breakthrough curve of $\delta^{34}S(SO_4^{2-})$ values. 'Dilution only' values are $\delta^{34}S(SO_4^{2-})$ values expected if test solution ($+9.0$‰) was diluted with native background groundwater (-0.1‰) according to ratios given by normalised concentration data measured for the non-reactive chloride tracer in the push-pull test. 'Corrected' values reflect $\delta^{34}S(SO_4^{2-})$ values of recovered reactive tracer, after effects of dilution with native groundwater have been accounted for in measured values.

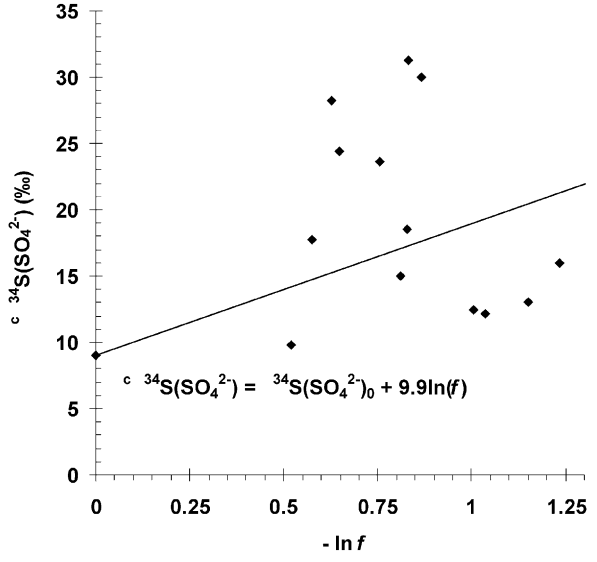

Fig. 5. Rayleigh distillation model (solid line) fit using least squares linear regression to corrected $\delta^{34}S(SO_4^{2-})$ values. The isotopic enrichment factor ϵ is given by the slope of the line. f is the fraction of unconsumed reactive sulphate tracer remaining. Data from early samples (P1–P4) for which S^{34} enlightenment was measured have been neglected in the model.

test. However, the breakthrough data exhibited several similarities to the data collected by Schroth et al. (2001) from their series of push-pull tests conducted in a sulphate reducing aquifer- Schroth et al. (2001) recovered 25–31% of the solute mass added from their injected test solutions. It was apparent in Schroth's data and from the results presented in this push-pull test study that relative decreases measured for sulphate over the incubation period of the tests were not explained by simplified zero- or first-order reaction rates and that the assumption of a well mixed batch reactor was invalid. Firstly, when tracer data were semi-log-transformed and plot against the time of the push-pull test as for interpretation using the first order model [Eq. (3)], a lag phase to the onset of sulphate reaction is evident (Fig. 3). This time lag feature is ignored entirely by the simplified analytical models [Eqs. (2) and (3)] due to the constraint of forcing the y-intercept at time zero to equal either zero in the case of the zero-order model, or equal the y-axis value given by Eq. (5) in the case of the first-order model. Secondly, during the extraction phase, the semi-log-transformed data indicated reaction rates that may somehow be linked to the solute recovery phase itself. Schroth et al. (2001) applied a step-wise abstraction protocol in two tests (PPT3 and PPT4). A direct comparison of the trend exhibited by data transformed according to Eq. (3) between this test (Fig. 3) and their tests (Fig. 4 in Schroth et al., 2001) highlights this phenomenon.

The failure of zero- and first-order models to fit the data may be due to the true in-situ reaction rates being non-linear and non-constant. Any effects of non-linear microbial reaction kinetics are likely to be enhanced during the push-pull test, due to the sudden supply of electron acceptor to an electron-acceptor-limited system, combined with hydraulic mixing of substrates and nutrients required for microbial metabolism.

Haggerty et al. (1998) demonstrated hypothetically in a sensitivity analysis that push-pull test breakthrough curve data were not strongly affected ($<10\%$ error in k estimates) by physical aquifer characteristics such as porosity and dispersivity or heterogeneities in the aquifer matrix. It is our belief that the effects of heterogeneities in the flow field will inevitably determine the reaction rates observed from reactive well tests and are to some extent responsible for the failure for the simplified analytical push-pull models to match observed data. Heterogeneities in the flow field are controlled by heterogeneities in the physical aquifer matrix, but also by velocity variations over travel distance from the injection/abstraction well. Reaction rates will be dependent upon the flow field, since this will govern supply and mixing of electron acceptors, nutrients and substrates to the microbial community of the aquifer.

It has been shown (Miralles-Wilhelm et al., 1996; Scholl, 2000) that where microbial decay rates were non-uniformly distributed and were directly correlated with hydraulic conductivity, considerable errors in biodegradation rate predictions resulted. Aeolien (2000) provided direct evidence using microcosm studies that microbial activity and grain-size may be correlated. The silt and clay fraction of a hydrocarbon-contaminated aquifer was demonstrated to yield lower biodegradation activity than sand fractions from the same aquifer. These factors are not considered in the push-pull test methodology and are as of yet uncharacterised for the test, but may partly be responsible for the results obtained herewith.

4.2. Stable isotope analysis

$^{c}\delta^{34}S(SO_4^{2-})$ values in Fig. 4 exhibited a large temporal variability, which may be a reflection of the true breakthrough of the recovered test solution. Such effects are likely to be more pronounced for the condition described here, where recovered tracer concentrations were low and where solute transport during the incubation phase due to a fast groundwater velocity would have disrupted any radial symmetry of the injected test solution. The effect of disrupting the radial symmetry of the system would be to cause an offset in the temporal axis of the breakthrough curve, whereby the earliest samples in the breakthrough data would no longer reflect tracer that has spent the shortest residence time in the aquifer, instead, the mass of tracer in each sample would be composed of a composite mix of tracer of varying age, that would have been determined by the system hydraulics. If reaction rates were temporally and spatially non-uniformly distributed as we have suggested above, then the additional effect of asymmetric spatial distribution of the drifting test solution about the well would be to impose further non-uniformity to the breakthrough curve. Furthermore, in the case of stable isotope fractionation reactions interpretation is complicated by the fact that the specific conditions that determine the effects of microbially mediated isotope fractionation remain unclear (see Detmers et al., 2001 for a review). It has recently been proven that there is no common rule that relates isotope fractionation to specific rate of sulphate reduction (Detmers et al., 2001; Bölliger et al., 2001, Canfield, 2001) hence there is no consistent correlation between fractionation and physical and chemical properties. Detmers et al. (2001) suggested that fractionation depends on the species-specific cell physiology. Interestingly, of the 32 sulphate reducing strains studied by Detmers, mesophilic bacteria from freshwater aquifers were not represented. Canfield (2001) identified five different responses to sulphate fractionation observed when substrate type; substrate concentration; sulphate concentration and temperature were altered in a controlled manner. In one of the earlier isotope studies involving freshwater sulphate reducing

bacteria, Harrison et al. (1958) observed low fractionation (<4‰) at sulphate concentrations below 57 mgl^{-1}. In light of these responses, the variable conditions under which any fractionation measured during our experiment occurred cannot be inferred.

There also exists the possibility that the variable fractionation values measured during the experiment were due to the failure of this system to follow a Rayleigh distillation, which is only valid for closed systems involving unidirectional reactions. The high levels of ferrous iron present in groundwater at the site inevitably indicate that a source of ferric iron must be present in the aquifer, probably in the form of Fe(III) oxyhydroxide minerals. It is viable that oxidation of sulphide by reaction with Fe(III) or with oxygen during sample handling and analysis would remove evidence of sulphate reduction, returning isotopically light sulphur to the sulphate pool. The push-pull test system may be open in terms of the sulphur cycle as external sources of sulphide may include diffusion into the test zone of the aquifer from deeper groundwater or from residual sulphide trapped in pore waters. The inverse fractionation observed in the isotope data of samples collected at the early part of the extraction phase may partly have been attributed to sulphate reaction of the injected solute trapped within the well casing and not solution that mixed with the aquifer matrix.

Despite the large uncertainty, the enrichment factor of +9.9‰ calculated in this study was within range of average values +9.7 to +23.5‰ found by others for microbially mediated sulphate reduction in hydrocarbon-contaminated aquifers (Bölliger et al., 2000; Schroth et al., 2001; Spence et al., 2001). Thus, although it has not been possible to quantify a sulphate reduction rate, there exists strong qualitative evidence that biodegradation processes within the main contaminant plume at Trecate are active under sulphate-reduced conditions.

5. Conclusions

In this study a single-well push-pull test of the type described by Istok et al. (1997) was applied to determine microbial activity in a highly transmissive, sulphate-reducing aquifer contaminated by crude oil. The findings revealed several weaknesses of the push-pull test, some of which have not been highlighted before. These were: (i) that the push-pull test methodology is unsuited to sites where a high background groundwater velocity is prevalent, especially where a long residence time may be required such as under highly reduced, sulphidogenic conditions; (ii) that the well-mixed batch reactor assumption underlying the push-pull test methodology is not correct in all cases; (iii) that zero- and first-order reaction rates successively fail to describe reaction rates measured from push-pull tests performed in sulphate-reduced aquifers. Attempts to fit such rates using simplified models are highly constrained, resulting in subjective calculations; (iv) that reaction rates calculated from push-pull tests appear to be sensitive to the extraction regime implemented. This was best seen in results of other studies such as Schroth et al. (2001). A consequence is that predicted reaction rates may not reflect in-situ degradation processes, and (v) the assumption of uniformly distributed reaction rates in space and time is probably an invalid assumption, the effects of which are likely to be accentuated by heterogeneities in the flow-field. These factors are not accounted for in the existing push-pull methodology but may manifest themselves in the breakthrough data.

From the use of stable sulphur isotope analysis conducted in parallel with the push-pull test, it has been concluded that: (i) reduced sulphate concentrations measured in the plume of crude oil contamination, within the aquifer at Trecate were attributed to microbial activity and thus, biodegradation of the crude oil, and (ii) $\delta^{34}S$ analysis of unreacted sulphate from the push-pull test yielded an isotope enrichment, ϵ, of $+9.9 \pm 8.1$‰.

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