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Kinetics of sulfate reduction in a coastal aquifer contaminated with petroleum hydrocarbons

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Abstract An integrated field and laboratory study was conducted to quantify the effect of environmental determinants on the activity of sulfate reducers in a freshwater aquifer contaminated with petroleum hydrocarbons (PHC). Within the contaminated zone, PHC-supported in situ sulfate reduction rates varied from 11.58 ± 3.12 to 636 ± 53 nmol cm^{-3} d^{-1} and a linear increase (R^2=0.98) in reduction rate was observed with increasing in situ sulfate concentrations suggesting sulfate limitation. Half-saturation concentration (K_s) for sulfate reduction coupled to PHC mineralization was determined for the first time. At two different sites within the aquifer, maximum sulfate reduction rate under non-limiting conditions (R_{max}) was 5,000 nmol cm^{-3} d^{-1}, whereas the retrieved K_s values were 3.5 and 7.5 mM, respectively. The K_s values are the highest ever reported from a natural environment. Furthermore, the K_s values were significantly higher than in situ sulfate concentrations confirming sulfate limited growth. On addition of lactate and formate, sulfate reduction rate increased indicating that reactivity and bioavailability of organic substrate may also have played a role in rate inhibition in certain parts of the aquifer. Experiments with sulfide amendments show statistically minor decrease in sulfate reduction rates on addition of sulfide and analogous increase in sulfide toxicity with increasing sulfide concentrations (0.5–10 mM) was not apparent.

Keywords Redox reactions · Half-saturation constant · Monod kinetics · Hydrocarbon · Biodegradation · Groundwater contamination · Coastal aquifer · Sulfide toxicity

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Introduction

Sulfate reduction has been studied extensively in natural and impacted environments since early twentieth century, but it is only in the last decade that limited studies have shown that sulfate reducers can metabolize petroleum hydrocarbons (PHC) to innocuous compounds (Beller et al. 1992; Edwards et al. 1992; Schmitt et al. 1996; Hunkeler et al. 1998; Elshahed and McInerney 2001; Somsamak et al. 2001; Coates et al. 2002; Rothermich et al. 2002). PHC are known to degrade more efficiently under aerobic environments, however, anoxic conditions normally develop in aquifers contaminated with PHC forcing their degradation through anaerobic pathways. Although sulfate reduction is

thermodynamically less favorable, stoichiometrically it is one of the most efficient anaerobic pathways for degrading PHC (see Roychoudhury and Merrett 2006). As a result, anaerobic pathways in general, and sulfate reduction in particular, are increasingly being investigated as a means of developing aquifer clean-up strategies to manage organic waste.

Most of the studies on PHC mineralization made so far have focused on (i) identifying the microbial community structure capable of degrading PHC (Grishchenkov et al. 2000; Kao et al. 2001; Kleikemper et al. 2002b; Nakagawa et al. 2002), (ii) evidence for mineralization of specific PHC compounds by sulfate reducers (Beller et al. 1992; Edwards et al. 1992; Somsamak et al. 2001; Coates et al. 2002), (iii) rate of oxidation of PHC with various terminal electron acceptors (Hunkeler et al. 1998; Baker et al. 2000; Johnson et al. 2003; Villatoro-Monzón et al. 2003; Maliyekkal et al. 2004) or (iv) the rate of sulfate reduction with PHC as electron donor (Aharon and Fu 2000; Shin et al. 2000). Furthermore, many of these studies are based on enrichment culture work under optimum laboratory conditions for microbial growth, which may not be relevant to field situations.

Before sulfate reduction can be seriously considered as an option for in situ bioremediation of PHC in groundwater aquifers, a mechanistic understanding of sulfate reduction process in the presence of PHC under aquifer conditions is required. To identify the controls, an integrated field and laboratory investigation of sulfate reduction was conducted at a shallow, unconfined, coastal aquifer contaminated with PHC. In particular, kinetic parameters for sulfate respiration coupled to PHC degradation were quantified in order to elucidate the influence of electron donor, substrate availability and the presence of inhibitors on the metabolic activity of sulfate reducers. The experiments were conducted either in situ, or in the laboratory using homogenized sediment slurries under conditions as close as possible to those present in the aquifer, using intrinsic microbial population and aquifer matrix contaminated with PHC. Therefore any quantitative estimates for the kinetic process derived here depict a communal response of inherent sulfate reducing consortia to a mixture of hydrocarbon substrate.

Materials and methods

Site description

The study site has been described in detail previously (Roychoudhury and Merrett 2006). Briefly, the aquifer is unconfined and shallow with approximate depth of the bedrock close to 10 m in the area. In September 2000, 101,560 l of 97-octane petrol leaked from a ruptured underground storage tank into the surrounding groundwater system. For monitoring of the plume, 45 three-meter long PVC cased and capped wells were put in place (Fig. 1). The bottom 0.2 m of the PVC casing was slotted to allow water flow in the wells. Three monitoring wells (48, 49, and 50) were placed up-gradient as per the direction of regional hydraulic flow to ascertain background conditions (Fig. 1). Because of the lack of a significant gradient, the contaminant plume was practically stagnant over the 4 years it had been under observation (Ross Campbell *personal communication*) and was confined within an area of approximately 15,000 m^2 close to the spillage location. Minor westward movement of the plume along the regional hydraulic gradient was evident, though (Fig. 2c).

Groundwater sampling and analyses

Groundwater samples were collected in September 2004 following the procedure described previously (Roychoudhury and Merrett 2006). Immediately after collecting each sample, pH, Eh, dissolved oxygen, electrical conductivity and temperature was measured using a WTW® multiparameter meter and associated electrodes. Each electrode was calibrated every day prior to the start of sampling and at random intervals throughout the day.

For the analyses of Fe^{2+}, alkalinity and $\sum H_2S$, samples were fixed in the field to avoid equilibration with the atmosphere. Using Eppendorf® pipettes, 250 µl, 2 ml and 5 ml of filtered sample was disbursed into three separate vials pre-charged

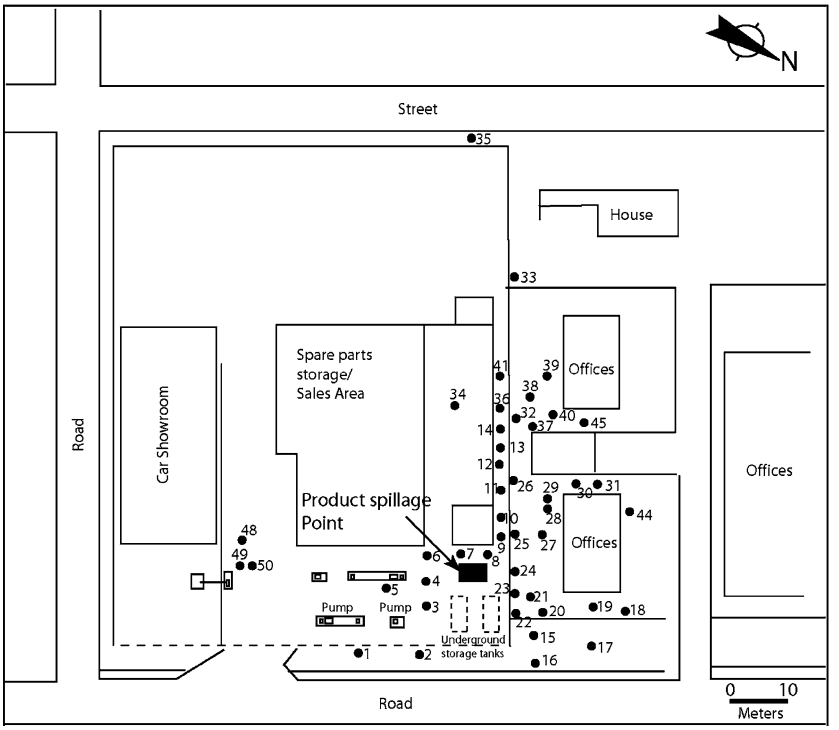

Fig. 1 Site map and location of boreholes used for collection of water samples (modified from Roychoudhury and Merrett (2006))

with 5 ml of ferrozine buffer (0.2 g ferrozine + 12 g Hepes in 1 l water, pH = 7), 2 ml of mixed reagent (50 mg l^{-1} Bromophenol blue + 0.1 M formic acid) and five drops of zinc acetate (20% wt/vol), respectively. At the end of each day, samples were analyzed using a UV–Vis spectrophotometer (Aquamate, Thermospectronic) by colorimetric methods for Fe^{2+} (Stookey 1970), alkalinity (Sarazin et al. 1999) and $\sum H_2S$ (Cline 1969), along with SO_4^{2-} by turbidimetric method (Tabatabai 1974). In addition, total dissolved organic carbon was measured in samples collected from background boreholes (48–50) using a CHN-S elemental analyzer (Thermo-Finnigan, Flash EATM 1112).

Separate groundwater samples were collected for BTEX (Benzene, Toluene, Ethylbenzene and Xylene) and MTBE (Methyl *t*-butyl ether) analyses using a Teflon® bailer and preserved in 500 ml amber glass bottles with no head space. The samples were sent to an ISO/IEC accredited analytical laboratory for the analyses where BTEX and MTBE were analyzed using gas chromatography following a purge and trap method. A flame- or photo-ionization detector was connected at the end of the GC column and the detection limit was 1 μg l^{-1}.

Sediment sampling and characterization

For experimental work, sediment samples were collected from depths below the sediment-water interface, usually within 2.5–3 m from the ground surface using a hand augur. Water/PHC saturated sediments were retrieved from five sites close to boreholes 19, 24, 27, 28, and 48 for the determination of in situ sulfate reduction rates. The sampling sites were chosen based on the variation in hydrogeochemistry of the site characterized *a priori* (Roychoudhury and Merrett 2006). Separate sediment samples for amendment experiments were collected on several occasions near borehole 27 and 28 between September and November 2004. After collection, sediments were immediately transferred in polyethylene bags and were stored in a BBL® anaerobic jar where anaerobic conditions were maintained by placing wet Anaerocult® A tablets (Merck chemicals). The jars were kept on ice in the field and during transportation to the laboratory. At the laboratory

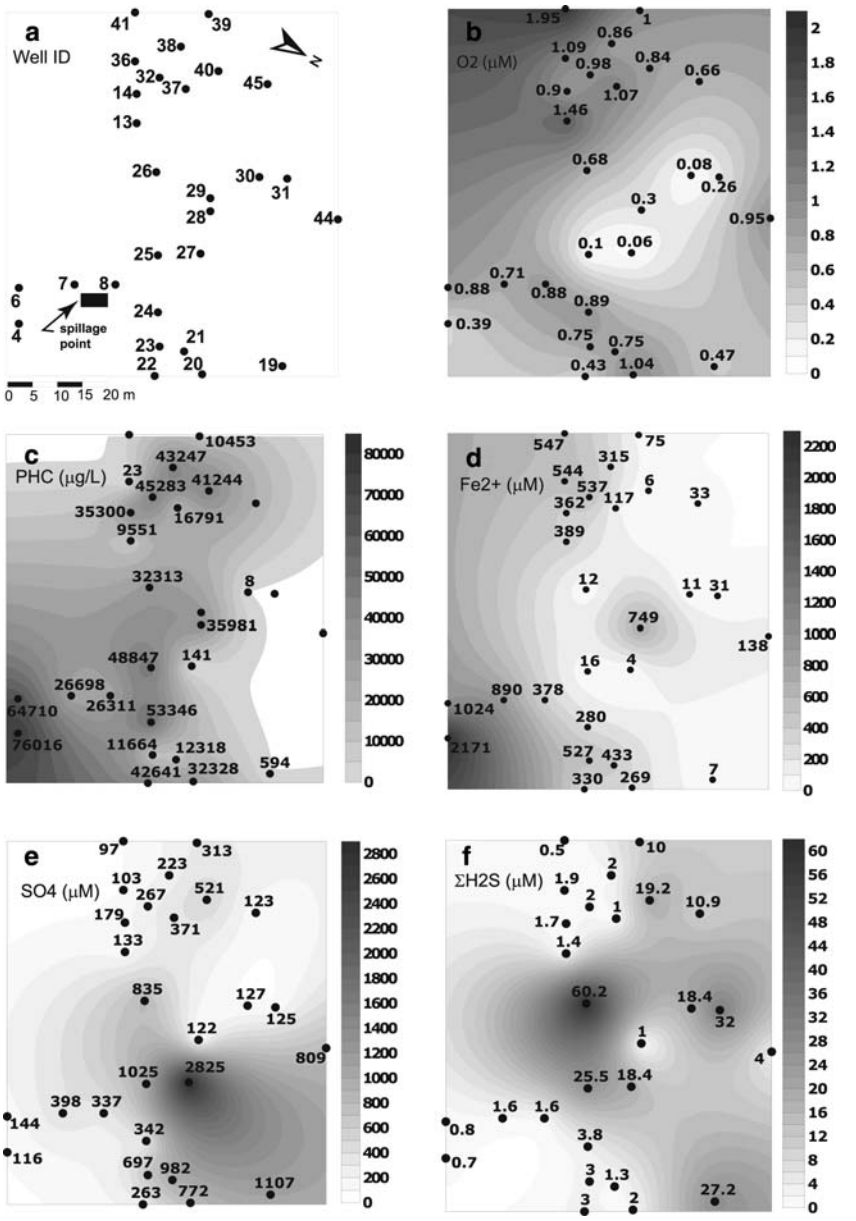

Fig. 2 Figure shows the spatial distribution of measured chemical species at the study site within the Cape Flats Aquifer. The panel (**a**) shows the location and the identity number of the sampled boreholes within the contaminant plume. The black rectangle denotes the point of discharge. Please note that the background boreholes (48, 49 and 50) are not plotted here; however, the contour plots for the measured properties in panels (**b**)–(**f**) were generated by taking into consideration the measured concentration in the background boreholes. The contour maps were plotted based on the interpolation of spot measurements at each sampled well using Point Kriging with a linear drift. Numerical values in panels (**b**)–(**f**) denote the actual measured concentrations of chemical species present in respective boreholes

the samples were transferred inside an anaerobic chamber with an automatic airlock and nitrogen atmosphere (Coy labs). Carbon dioxide and hydrogen were avoided in the anaerobic chamber as both can interfere with PHC degradation mediated by sulfate-reducing consortia (Sørensen et al. 1981; Grabić-Galić and Vogel 1987; Meckenstock 1999; Coates et al. 2002; Matias et al. 2005). The sediments were then processed immediately as per the requirements of individual amendment experiments described below.

Unsaturated and uncontaminated sediments (depth < 0.5 m) were also collected using a hand augur from five different sites for the characterization of background properties. The sediment samples were analyzed for total and organic carbon using a CHN-S elemental analyzer, and for iron and manganese content using dithionite extraction as described in Roychoudhury et al. (2003a). Sediment carbon content was not measured on PHC saturated sediments. Sediment grain size was determined by mechanical sieving.

In water-saturated sediment, porosity was determined by measuring the weight loss of a saturated sediment sample packed in a 1 ml syringe after oven-drying at 60°C. Sediment density was calculated by measuring the weight of sediment in the same syringe used for porosity determination.

In situ sulfate reduction rate determination

At the site, PHC-saturated sediments were homogenized with a spatula and immediately used to completely fill 5 ml vials in triplicate. About 5 µl of carrier-free $^{35}SO_4^{2-}$ (1 μCi/µl) was injected into each sample longitudinally using a glass microsyringe while the needle was slowly removed from the sediment. The vials were then capped tightly to avoid head spaces, and then incubated for 2 h by lowering them back into the boreholes below the water-table to maintain them at in situ temperature. Following incubation, sediment samples were fixed with 10 ml of 20% (wt/vol) zinc acetate solution and immediately frozen on ice to terminate any microbial activity. For each site, triplicate control samples were prepared following exactly the same procedure except that instead of incubating them for 2 h, they were fixed and frozen immediately after the injection of the radioisotope. Later the samples were analyzed in the laboratory for measurement of ^{35}S incorporated in the reduced fraction using a one-step distillation process modified after Fossing and Jørgensen (1989). Sulfate reduction rates were calculated based on the amount of ^{35}S incorporated in the total reduced sulfide fraction.

Effects of organic substrate addition

Slurry incubations were conducted to deduce the effect of alternate electron donors on the activity of sulfate reducers in the presence of PHC. Sediment samples were amended with salts of low-molecular-weight organic acids that are presumably more bioavailable and are also some of the intermediate compounds produced during PHC degradation (Cozzarelli et al. 1994; Kleikemper et al. 2002a). Sediments collected from borehole 27 were transported to the laboratory and thoroughly mixed inside the anaerobic chamber using a spatula. Nine aliquots were prepared by taking approximately 2 ml of sediment slurry into 4 ml polypropylene vials. Triplicate vials were injected with an appropriate amount of sodium formate (HCOONa), sodium acetate (CH_2COONa), or sodium lactate ($CH_3CHOHCOONa$) to adjust their final concentration to 1 mM. Tightly capped vials were left inside an incubator at in situ temperature for acclimatization for an hour. Afterwards the vials were recovered and each sample was injected with 5 μCi of carrier free $^{35}SO_4^{2-}$. The vials were again incubated at in situ temperature for 2 h. The same procedure discussed previously was followed for fixing and analyzing the samples to determine the rate of sulfate reduction.

Effect of sulfide addition

Excess sulfide can be toxic to sulfate reducers themselves (Okabe et al. 1992; Reis et al. 1992; Vavilin et al. 1994; O'Flaherty et al. 1998). Therefore, in engineered systems where sulfate is supplied in excess for clean-up of PHC, there is a potential for generation of excess sulfide that may be detrimental to the catabolic process. In order to ascertain critical sulfide levels that impede activity of sulfate reducers, slurry incubations were conducted with amendments of increasing sulfide concentrations. Sediments collected from borehole 27 were thoroughly homogenized inside the anaerobic chamber and 4 ml of sediment was dispensed in each of the 15 vials. Triplicate samples with final sulfide concentration of 0.5, 1, 3, 5 and 10 mM were prepared by treating with appropriate amount of sodium sulfide stock solution. The vials were left at in situ temperatures for 1 h for acclimatization and then injected with 5 μCi of carrier free $^{35}SO_4^{2-}$. Following the injection of radiotracer, the samples were again incubated for 2 h at in situ temperatures after which they were fixed and analyzed to determine the rate of sulfate reduction as described above.

Determination of Monod kinetics parameters

Kinetic parameters describing substrate-dependent microbial activity were derived following the Monod kinetic model (Monod 1949),

$$R = \frac{R_{max}[SO_4^{2+}]}{K_s[SO_4^{2-}]} \quad (1)$$

where R_{max} is the maximum rate when sulfate concentration, $[SO_4^{2-}]$, is not limiting and K_s is the half-saturation constant.

Slurry incubations were conducted in order to observe the effect of sulfate concentration on microbial activity. Samples were collected from boreholes 27 and 28 that had very different in situ sulfate and PHC concentrations. The samples were homogenized and left at the in situ temperature for one day for depletion of porewater sulfate. Previous incubation experiments had shown that porewater sulfate is completely exhausted within that time (Roychoudhury and Merrett 2006). After 24 h, homogenized sediments were split into 30 vials, each containing approximately 4 ml of sediment. Triplicate vials containing the sediment sub-samples were then treated with 2 ml of degassed sulfate solutions of increasing concentrations of 0.1, 0.25, 0.5, 1, 3, 5, 10, 15, 20 and 28 mM, respectively. The vials were capped and shaken. The whole procedure was performed inside the anaerobic chamber in the laboratory. The vials were then left for 2 h for the microbial population to adjust to the new sulfate concentrations. Following acclimatization, individual samples were injected with 5 μCi of the radioisotope. Further incubation at in situ temperature of the samples followed for 2 h. Subsequently, the samples were fixed sequentially with zinc acetate solution and frozen. Later they were distilled in the laboratory to determine the sulfate reduction rates. The reciprocal of the rate was plotted versus the reciprocal of sulfate concentration on a Lineweaver-Burk plot (Roels 1983). The half-saturation constant, K_s, and maximum sulfate reduction rate, R_{max}, were derived from the slope and the intercept of the best-fit line obtained from linear regression.

Statistical analysis

Impact of amendments (organic acid and sulfide addition experiments) on sulfate reduction rate was statistically analyzed using the software STATISTICA (version 7). For organic acid addition experiments, one-way ANOVA was carried out to determine if the amendments resulted in a statistically significant change in the reduction rate. To verify if the measured sulfate reduction rates are significantly different after addition of individual organic acids compared to in situ reduction rates, student *t*-test was performed for each pair, i.e., in situ rate and the rate measured on addition of each organic acid. A regression analysis was used to evaluate the effect of sulfide concentration on sulfate reduction. Since this regression was not significant (see Results), we pooled all sulfide amended rates and compared them to the in situ rate using the student *t*-test.

Results

Aquifer characteristics and hydrogeochemistry

Limited hydrogeochemical parameters important for the interpretation of the experiments were measured for the contaminated site during September 2004 (Table 1, Fig. 2). Detailed groundwater geochemistry of the site from previous surveys has been described in detail elsewhere and shows that iron and sulfate reduction are the major anaerobic pathways coupled to PHC mineralization (Roychoudhury and Merrett 2006). Results show marked variation within the plume and between the plume and the background (Table 1, Fig. 2). In the background boreholes (48–50), groundwater was fairly oxygenated (E_h 132–209 mV; $[O_2]$ 3–5 mg l^{-1}) and sulfate concentrations varied from 1.07 to 1.36 mM. Dissolved organic carbon was found to be below detection limit (<0.1 mg l^{-1}) as were the PHC concentrations (<1 µg l^{-1}). In the background sediments, particulate organic carbon was negligible and varied between 0.083 and 0.093 wt%. The solid phase iron and manganese concentrations were 31.9 and 0.04 nmol cm^{-3}, respectively. Aquifer sediments were composed dominantly of sand (97.4%) with a porosity of 35% and a density of 2.1 g cm^{-3}.

Table 1 Selected physico-chemical parameters measured in groundwater samples collected from different boreholes in Cape Flats Aquifer, South Africa

Borehole	pH	E_h (mV)	Temp (°C)	EC (μS cm^{-1})	Alkalinity (mM)	Benzene* (μg l^{-1})	Toluene* (μg l^{-1})	Ethylbenzene* (μg l^{-1})	Xylene* (μg l^{-1})	MTBE (μg l^{-1})
4	6.7	–135	19.4	1,289	12.7	12,354	29,091	3,326	18,832	12,413
6	6.8	–128	22	1,270	9.5	16,223	29,303	2,415	15,623	1,146
7	6.7	–108	18.6	1210	11.1	2,612	11,464	2,611	8,276	1,735
8	6.7	–98	18.3	1,160	10.9	6,214	14,590	1,205	4,068	234
13	6.8	–117	20.7	1,177	6.9	2,815	3679	154	1,660	1,243
14	6.8	–85	17.5	1,140	9.7	4,490	20,449	2,250	7,960	151
19	6.7	–172	19.5	1,272	4.4	22	10	2	1	559
20	6.7	–105	19.5	1,285	7.1	3,444	20,752	847	7,156	129
21	7.0	–133	19.8	1,432	10.3	1,851	4,206	565	5,527	169
22	6.7	–191	21	1,096	7.1	6,167	27,322	1,334	6,870	948
23	6.6	–119	20.6	991	8	2,441	6,563	354	1,992	314
24	6.7	–302	21.2	1,039	8.1	13,010	27,807	1,011	11,300	218
25	6.9	–299	21.9	1,498	9	6,160	29,162	2,130	11,198	197
26	6.8	–247	21.3	1,426	9.4	7,715	17,522	1,293	4,254	1,529
27	7.2	–195	20	2,160	9.3	7	15	2	7	110
28	7.1	–292	20.3	1,622	12.1	8,242	16,360	1,523	8,347	1,509
30	6.8	–277	19.3	1031	7.2	4	3	0	1	0
31	6.8	–269	19.1	1,597	9.5	n.d	n.d	n.d	n.d	n.d
32	6.8	–138	19.9	1,155	9.5	3,329	29,322	1,181	11,165	286
34	6.6	–113	20.8	1,328	8	n.d	n.d	n.d	n.d	n.d
35	7.1	2	19.9	288	1.2	n.d	n.d	n.d	n.d	n.d
36	6.7	–85	17.9	817	7.3	0	3	0	1	19
37	6.6	–57	19.2	1,232	7.4	6,081	5,610	49	4,211	840
38	6.8	–130	19.5	1,033	8.3	7,346	25,218	2,310	8,027	346
39	6.9	–153	19.1	1,417	10.9	4,556	2,540	24	2,810	523
40	6.9	–243	18.4	1397	9.4	3,038	25,374	2,639	10,055	138
41	6.6	–62	17.6	854	6.6	n.d	n.d	n.d	n.d	n.d
44	6.8	–207	18.5	1,240	5.8	n.d	n.d	n.d	n.d	n.d
45	6.7	–246	19	1,471	9.6	n.d	n.d	n.d	n.d	n.d
48	7.1	150	19.9	1,182	9.2	<1	<1	<1	<1	<1
49	7.0	209	18.1	911	7.8	<1	<1	<1	<1	<1
50	7.1	132	19	925	7.6	<1	<1	<1	<1	<1

*BTEX data reproduced from Roychoudhury and Merrett (2006); n.d: not determined

In the contaminated zone, oxygen concentrations throughout the plume were very low (0.6–1.95 μM) confirming the development of dysoxic to anoxic conditions at the spill site (Fig. 2b). BTEX and MTBE, the most hazardous components of fuels, were measured as a proxy for PHC. In the majority of the wells, MTBE was depleted to below 500 μg l^{-1} with high concentrations clustered in two areas close to the spill point (Table 1). In contrast, BTEX concentrations (the sum of benzene, toluene, ethylbenzene and xylene concentration) were high and more evenly dispersed (Table 1; Fig. 2c). Their combined concentrations when plotted as a contour plot show that the PHC plume is dispersed along the regional hydraulic gradient in the westerly direction with high concentrations measured in majority of the boreholes (Fig. 2c). High dissolved iron concentrations were measured close to the spill point and in general in the southern section of the site (Fig. 2d). In contrast, high sulfate concentrations were measured predominantly in the northern corner of the site with highest concentration (2.85 mM) measured in borehole 27 (Fig. 2e). Sulfide concentrations were significantly lower than expected compared to the amount of sulfate depleted at various locations on the site, and ranged between 0.5 and 60 μM (Fig. 2f). Although we did not measure methane, methanogenesis, especially at low sulfate concentrations, might be important at the site.

Sulfate reduction rates

In situ sulfate reduction rates varied considerably at the site ranging from practically no reduction (1.75 ± 1.04 nmol cm^{-3} d^{-1}; ± 1σ, n = 3) near the background borehole 48 to highest rates (636 ± 53 nmol cm^{-3} d^{-1}; ± 1σ, n = 3) measured at borehole 27 (Fig. 3a). At borehole 19, rates were found to be similar when measured on two separate occasions in triplicate samples (Fig. 3a). Within the contaminated zone sulfate reduction rates were observed to increase linearly (R^2=0.98) with increasing in situ sulfate concentration (Fig. 3b).

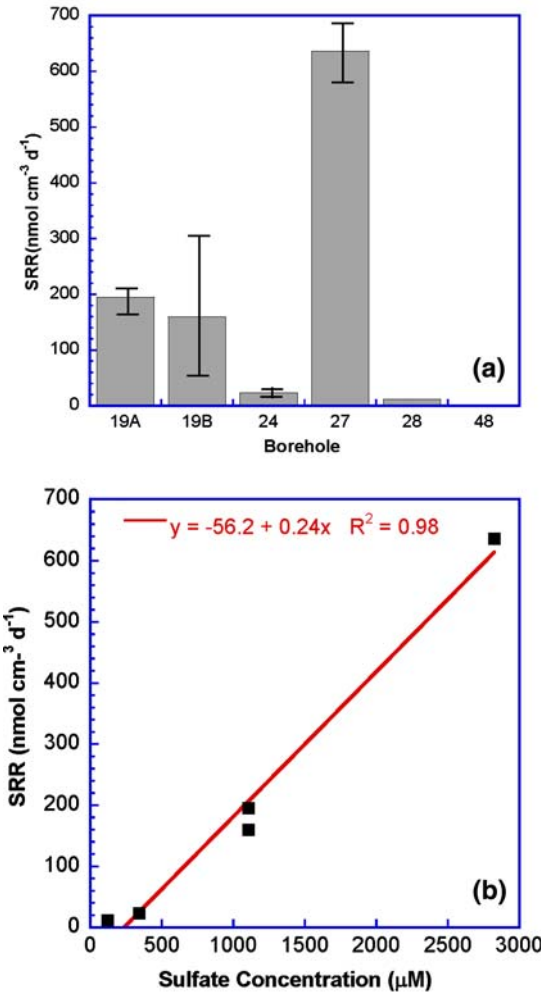

Fig. 3 In situ sulfate reduction rates measured at different sites are plotted as a function of (**a**) borehole location and (**b**) sulfate concentration measured within the PHC contaminant plume. Note that each bar in panel (**a**) represents an average sulfate reduction rate (n = 3) and the error bars denote the maximum and minimum rate measured in triplicate samples. The rates are given after subtracting the rates measured in control samples

Addition of alternate electron donor

One-way ANOVA suggests that addition of organic acid affected in situ sulfate reduction rates (P = 0.025). In general, rates increased on addition of alternate electron donors to the sediment samples from borehole 27. When lactate was added as an alternate substrate, sulfate reduction rate increased significantly (1462 ± 49 nmol cm^{-3} d^{-1}; ± 1σ, n = 3; t-test, P = 0.00003). On addition of formate the reduction rates were somewhat suppressed but still significantly higher (P = 0.0002) than the in situ rate (Fig. 4). Addition of acetate, however, had no impact (P = 0.9) on the sulfate reduction rates (Fig. 4).

Effect of sulfide addition

On addition of sulfide, the measured mean sulfate reduction rates varied between 306 and 515 nmol cm^{-3} d^{-1}. Regression analysis showed that sulfate reduction rate did not decrease significantly with increasing sulfide concentration (P = 0.59; R^2=0.29; Fig. 5). Although sulfide amended sediments generally appeared to have

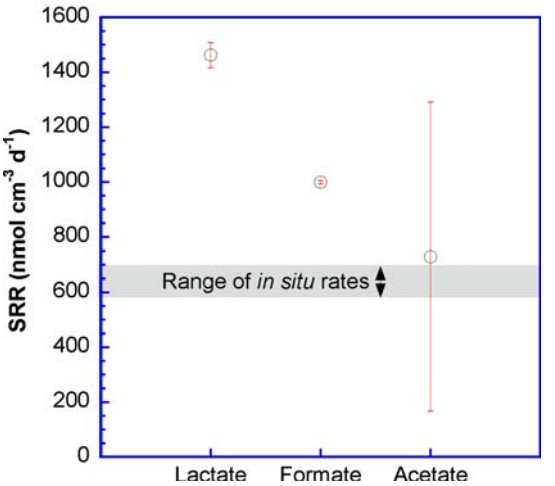

Fig. 4 Average sulfate reduction rate (n = 3; ± 1σ) measured during organic substrate addition experiments at borehole 27. The grey rectangle denotes the range of sulfate reduction rates measured under in situ environmental conditions

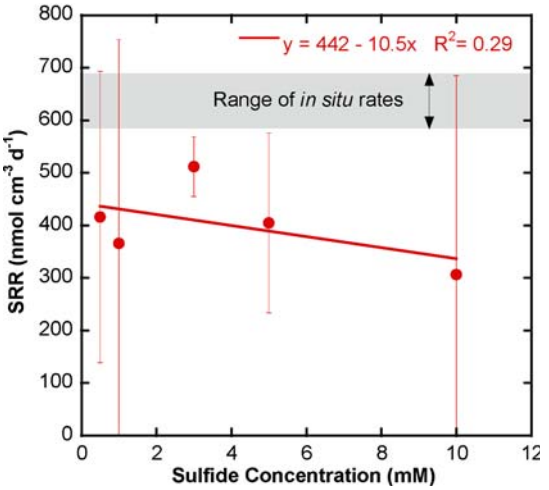

Fig. 5 Average sulfate reduction rate ($n = 3$; $\pm 1\sigma$) measured during sulfide addition experiments at borehole 27. The grey rectangle denotes the measured range of in situ sulfate reduction rate with no sulfide addition

lower sulfate reduction rates compared to those measured in situ (Fig. 5), this pattern was not significant (*t*-test, $P = 0.08$).

Monod kinetic parameters

Effect of sulfate concentration on sulfate reduction rate was deduced following Monod kinetics (see Eq. (1)). The R_{max} and K_s values were calculated by plotting the reciprocal of the sulfate reduction rate measurements against the reciprocal of sulfate concentration on a Lineweaver-Burk plot (Fig. 6). For borehole 27, a R_{max} value of 5,000 nmol cm^{-3} d^{-1} and a K_s value of 7.5 mM was calculated. At borehole 28, values for R_{max} and K_s were 5,000 nmol cm^{-3} d^{-1} and 3.5 mM, respectively (Fig. 6).

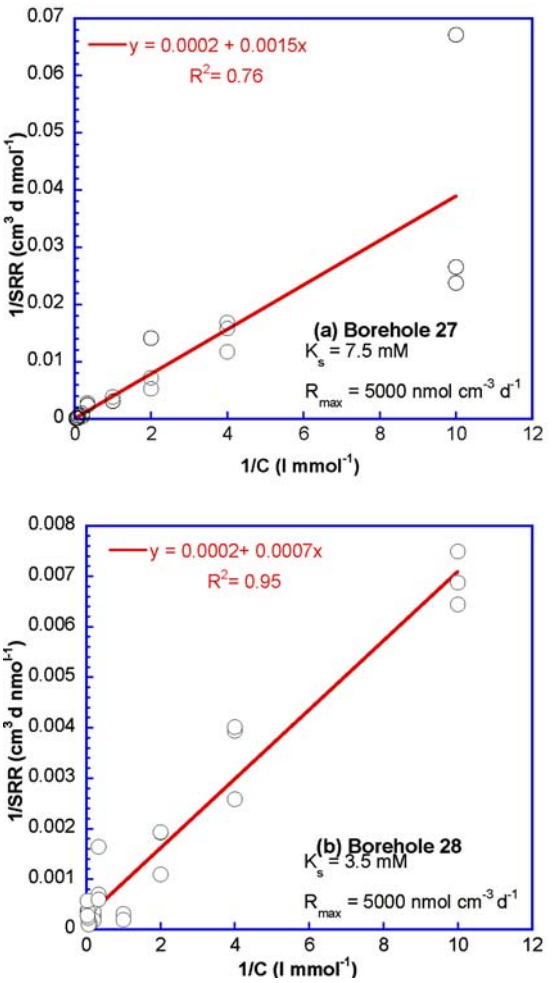

Fig. 6 Triplicate sulfate reduction rates measured in samples from (**a**) borehole 27 and (**b**) borehole 28, amended with increasing sulfate concentration, are plotted on a Lineweaver-Burk plot. A least square fit of the experimental data is used to retrieve the K_s and R_{max} values in the Monod kinetic expression (Eq. (1))

Discussion

Site characteristics

Subsequent to leakage of gasoline from an underground storage tank, suboxic to anoxic conditions had developed in the Cape Flats Aquifer site triggering in situ anaerobic activity. Given that the background concentration of particulate or dissolved organic carbon is negligible within the aquifer, it is believed that PHC constitute the principal electron donor for sustaining anaerobic metabolism. Measurement of BTEX and MTBE concentration in the groundwater shows that the PHC contaminant plume did not disperse homogeneously (Fig. 2c). As a result, significant geochemical differences within the aquifer were observed. As expected, oxygen concentrations were low (few micromoles) throughout the contaminated zone (Fig. 2b). Even though oxygen was present in the groundwater, the concentrations seems to be limiting as evident by the widespread occurrence of anaerobic reactions

(see Roychoudhury and Merrett 2006). Reduced iron and sulfate concentrations were higher in distinctly separate zones (Fig. 2d and e). Whereas dissolved iron dominates in the southern region overlapping the PHC plume, most of the sulfate was consumed in that zone (Fig. 2e). It is only in the northern corner of the site that comparatively high sulfate groundwater, similar to background concentrations, exists (Fig. 2e). Accumulation of iron in the southern part of the aquifer is helped by the lack of free sulfide and by dissolution of iron coatings on sand grains that are replenished annually when oxygenated rain water percolates in the unconfined aquifer. In addition, dissolved iron is also transported with the groundwater flowing through the iron rich Table Mountain Group sandstones found in the vicinity (Roychoudhury and Merrett 2006).

Correspondingly, higher sulfide concentrations were also observed in the northern end reflecting the presence of active sulfate reducing population in the northern section of the site (Fig. 2f). Sulfide concentrations; however, do not reflect the stoichiometry consistent with sulfate reduction probably because of their oxidation to sulfate or precipitation with dissolved iron as suggested by low dissolved iron concentrations in the northern corner of the site (Fig. 2d). Furthermore, although PHC degradation is dominantly coupled to sulfate reduction at the site (Roychoudhury and Merrett 2006), it was not possible to establish the amount of PHC degraded over time from the distribution of sulfate as the sulfate concentrations in the aquifer are constantly modified seasonally by rain and evapotranspiration (Roychoudhury and Merrett 2006), for which no quantitative data is present.

In situ sulfate reduction rates

Measured sulfate reduction rates varied considerably throughout the site reflecting the heterogeneous environment developed within the contaminant plume. Despite relatively high sulfate concentrations, no sulfate reduction (1.75 ± 1.04 nmol cm^{-3} d^{-1}; $\pm 1\sigma$, $n = 3$) was observed around the background borehole 48 because of the lack of available organic substrate. In contrast, highest rates were observed around borehole 27 where sulfate concentrations were the highest but PHC concentrations were among the lowest measured. At borehole 28, where sulfate concentrations were low but high PHC concentrations were measured, the reduction rates were close to the minimum measured rates. Intermediate values for the measured reduction rates were observed for boreholes 19 and 24 (Fig. 3).

A range of sulfate reduction rates were measured in triplicate samples in each case. Despite homogenization, such fluctuations are common in slurry experiments under in situ or laboratory conditions (Meier et al. 2000; Fishbain et al. 2003; Roychoudhury 2004). The variations are primarily caused by millimeter scale heterogeneity of the sediments, imperfect homogenization of slurry and variations in the activity and the size of the microbial population in different aliquots. Regardless of the caveats, homogenization was preferred for incubation experiments because of the sandy nature of our samples and the difficulties with obtaining undisturbed cores from the aquifer. Mixing ensures (1) the availability of porewaters despite easy separation of water from sands during sampling and storage and, (2) similar matrix in replicate samples.

A number of factors may have played a role in controlling the rate of sulfate reduction at the site. At first glance, sulfate concentration may be the limiting factor for sulfate reduction within the contaminated plume as the measured rates increased linearly (R^2=0.98) with increasing sulfate concentration in different boreholes (Fig. 3b). Interestingly, an inverse relationship is observed with dissolved iron and PHC concentrations. It is possible that iron reducers may be competing for the organic substrate and are able to out-compete sulfate reducers at high PHC concentrations. This scenario; however, is unlikely because in a previous study concomitant iron and sulfate reduction was observed in bag incubations with sediments from borehole 26 and 27 with high and low PHC concentrations, respectively (Roychoudhury and Merrett 2006). The toxic nature of the contaminants may also have impacted the activity of the microbes as it has been shown that the activity of certain microbes is inhibited beyond a critical concentration of BTEX (Shim

and Yang 1999; Maliyekkal et al. 2004). We do not know of any prior study that shows toxicity effect of high BTEX concentrations specifically on sulfate reducers, however. Furthermore, it has also been shown that different species of sulfate reducing bacteria preferably use specific organic substrate (Kleikemper et al. 2002a). Therefore, the observed variation in reduction rate may also be reflective of the variability in organic compounds, their concentration and subsequently different sulfate reducing microbial consortia active under those specific conditions.

Organic substrate addition

Sulfate reduction rates increased on addition of lactate and formate (Fig. 4). Lactate seems to be the preferred organic substrate as the rates almost doubled on its addition (see Fig. 6a). Although somewhat suppressed, rates also increased on addition of formate. For acetate, null hypothesis could not be ruled out using the student *t*-test ($P = 0.9$), i.e., addition of acetate did not impact the activity of sulfate reducer. A lack of preference for acetate by freshwater sulfate reducers has been documented before (Oude Elferink et al. 1998). Furthermore, in presence of acetate, methane oxidizing bacteria are known to outcompete sulfate reducers (Yoda et al. 1987).

We are unaware of any prior published work that quantifies the response of sulfate reducers, acclimatized to PHC, on addition of alternate electron donors. However, on addition of alternate electron donor, increase in metabolic activity is expected if the system is limited with respect to organic substrate. The argument for organic substrate limitation at borehole 27 is strengthened by the fact that higher K_s value was measured at borehole 27 compared to borehole 28 despite the presence of higher sulfate concentrations. Note that the PHC concentrations were much lower in borehole 27 when compared to borehole 28. Another possibility for the increase in the measured reduction rate may be related to sulfate reducing community structure. Lactate has been identified to be an important carbon source for sulfate reduction in freshwater environments (Cappenberg and Prins 1974; Hordijk and Cappenberg 1983). Therefore it is possible that the increase in sulfate reduction rate observed here is simply reflective of sulfate reducing population which is similar to the microbes from other freshwater habitats that prefer lactate as the carbon source. In one study, Kleikemper and coworkers (Kleikemper et al. 2002a) show that the sulfate reducing community structure changes when sediments contaminated with PHC were stimulated by adding these low molecular weight organic salts. Certain sulfate reducing species that were originally inactive in the system became active after addition of specific salts such as, lactate and acetate (Kleikemper et al. 2002a).

Sulfide addition

Bioremedial processes dependent on sulfate reduction are bound to produce sulfide, a known toxin. A 50% reduction in growth rate (IC_{50}) of sulfate reducers has been documented previously when sulfide was supplied in the range of 3–17 mM at a pH of 6.8–7.2 (Okabe et al. 1992; Reis et al. 1992; Vavilin et al. 1994; O'Flaherty et al. 1998). It is believed that sulfide toxicity in sulfate reducers can result either through a direct process due to intrinsic toxicity of sulfide, or from an indirect pathway where iron, needed for cell constituents such as ferrodoxine and cytochrome C, is precipitated with sulfide externally or sulfide reacts with cytochrome iron inside the cell causing electron transport system to cease activity (Okabe et al. 1992; Reis et al. 1992).

We did not observe a progressive decrease in reduction rates with increasing sulfide concentrations (e.g., Reis et al. 1992; Fig. 5). Furthermore, although the mean sulfate reduction rates observed in sulfide amended sediments were lower than the mean in situ rate (Fig. 5), the response to the addition of sulfide was not significant within the 95% confidence interval (*t*-test, $P = 0.08$). One of the reasons for the observed lack of response to sulfide toxicity may be the precipitation of significant amount of sulfide with iron during the incubation. The aquifer material contains 32 nmol cm^{-3} of particulate iron, which would mean that a maximum of 128 nmol of Fe was available for reaction with sulfide or a maximum of 256 nmol of sulfide would have been consumed during the incubation experiments.

Furthermore, in a previous study conducted at the same site, a maximum of 4 μM $cm^{-3}_{wet\ sediment}$ h^{-1} of dissolved iron accumulated over an incubation period of 36 h (Roychoudhury and Merrett 2006). Given the low concentration of dissolved and particulate iron in the system, this precipitation scenario as sole explanation for low sulfide toxicity seems unlikely. A more likely possibility is the role of sulfide speciation on imparting toxicity. It has been shown previously that non-ionized sulfide (H_2S) is more toxic to sulfate reducers and the sensitivity of sulfate reducing bacteria to sulfide toxicity increases substantially at lower pH (<7.0) (O'Flaherty et al. 1998). However, at in situ and experimental pH of 7.2, HS^- is the dominant species which is not easily transported across the cell membrane, leading to the reduced toxicity effect.

Monod kinetic parameters

As per our knowledge, the present work is the first attempt to quantify Monod Kinetics parameters for sulfate reduction coupled to PHC mineralization. The measured K_s values are significantly higher than in situ sulfate concentrations and clearly suggest that the activity of sulfate reducers at the site is substrate limited. The fact that sulfate reduction rates increased linearly with increasing sulfate concentration in various boreholes further supports the substrate limited growth model (see Fig. 3b). Such information is critical to the development of clean-up technologies for in situ treatment of PHC contamination. For example, at the studied site, based on the K_s value, if sulfate is supplied at concentrations ≥15 mM, sulfate reduction rate and hence PHC degradation can be significantly enhanced.

As far as the authors are aware, the K_s values obtained here (3.5–7.5 mM) are the highest of any known reported value from a natural ecosystem. The measured K_s values at the aquifer are 2–36 times higher than the K_s values (204 μM–1.63 mM) reported from marine environments (Boudreau and Westrich 1984; Roychoudhury et al. 1998; Roychoudhury et al. 2003b), seven orders of magnitude larger than those reported (10–70 μM) from low-sulfate freshwater environments (Ingvorsen et al. 1981; Smith and Klug 1981; Lovely and Klug 1986; Urban et al. 1994) and one to seven times higher than the K_s values (1.24–3.17 mM) reported from hydrothermal springs (Roychoudhury 2004).

Microbial growth directly depends on nutrient uptake. In the case of sulfate reducers, sulfate and organic substrates are transported across cell membranes and the corresponding sulfate reduction rate, a proxy for specific growth rate, is often modeled as a function of limiting sulfate concentration (Brezonik 1994). This type of growth is called transport limited and the Monod kinetic model used here to depict corresponding microbial activity assumes single substrate limitation.(Monod 1949). Sulfate limitation alone; however, can not explain the high K_s values measured in our system as considerably lower K_s values have been obtained previously from other freshwater low-sulfate environments. A number of factors may have played a role in the high K_s values measured at the PHC contaminated site.

The diversity observed in K_s values measured in natural systems increasingly suggest that the assumption of single substrate limitation to depict sulfate reduction may not be sufficient and the role of organic substrate must be looked at more closely. At present the impact of organic matter on K_s values for sulfate reduction can only be speculated as little is known about the uptake mechanism of various organic substrates by the sulfate reducers. The role of organic substrate on rate limitation can be very complex and may depend on its bioavailability, i.e., the ease with which it can be assimilated within a microbial cell, and reactivity, i.e., the ease with which the organic molecule can be broken down inside the cell (Brezonik 1994). At the study site, barring acetate, sulfate reduction rates increased on addition of low molecular weight organic salts suggesting that the recalcitrant nature of PHC, i.e., their low reactivity inside the microbial cell, and availability must have contributed to the high measured K_s values. Furthermore, because of the complex molecular structure of PHC, it is possible that their uptake by sulfate reducing bacteria is inefficient and given that sulfate reduction is limited by the transport of substrate across the cell membrane, the inefficient uptake of PHC

may also have contributed to the measured high K_s values.

Most K_s values documented in the literature are in the μM range (Ingvorsen and Jørgensen 1984; Ingvorsen et al. 1984; Fukui and Takii 1994; Roychoudhury et al. 1998; Sonne-Hansen et al. 1999; Roychoudhury et al. 2003b) and have been obtained either in the laboratory with pure cultures under ideal growth conditions, or from natural environments where labile carbon was present in excess. A few high K_s values that have been obtained are from natural environments only (Boudreau and Westrich 1984; Roychoudhury 2004) and the data is not sufficient to make concrete conclusions as no one has looked systematically at the reason for variations in the K_s values. At this point we can only speculate, as we have done here, that the reactivity of organic matter is contributing to the higher K_s values. If true, then the K_s values obtained here using Monod Kinetic model to analyze presumably bisubstrate rate data do not represent true K_s values but apparent K_s values that are a function of the reactions's intrinsic kinetic parameters and availability and concentration of organic substrate that is assumed not to vary.

Conclusions

A quantitative understanding of the activity of sulfate reducers acclimatized to PHC has emerged by combining a comprehensive hydrogeochemical dataset generated from field characterization with systematic laboratory experiments. In a PHC-contaminated freshwater aquifer, in situ activity of sulfate reducers varied considerably. The sulfate-reducing microbial consortia seem to be responding to the variability in hydrogeochemical parameters, mainly supply of electron donors and acceptors. Addition of sulfide in the range of 0.5–10 mM slightly suppressed the activity of sulfate reducers. The response, however, was homogenous irrespective of sulfide concentration and no increase in toxicity was observed with increasing sulfide concentration. The empirical behavior of sulfate reducers on manipulating environmental variables further suggests that in situ bioremediation of PHC mediated by sulfate reducers is a distinct possibility at the studied aquifer.

Laboratory experiments with PHC-contaminated aquifer matrix and inherent microbial population suggest that in situ sulfate reduction rates are inhibited because of sulfate and organic substrate limitation and reactivity of organic compounds. A major implication being that the often used Monod Kinetic formulation, that depicts microbial growth under single substrate limitation, will provide an apparent K_s value when used to model bisubstrate dependent rates and may not be useful for retrieving intrinsic kinetic constants for sulfate reduction where organic carbon is an additional variable. However, further research is required to conclude the role of organic matter in controlling sulfate reduction and hence the value of half-saturation constant.

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References

Aharon P, Fu B (2000) Microbial sulfate reduction rates and sulfur and oxygen isotope fractionations at oil and gas seeps in deepwater Gulf of Mexico. Geochimica et Cosmochimica Acta 64(2):233–246

Baker RJ, Baehr AL, Lahvis MA (2000) Estimation of hydrocarbon biodegradation rates in gasoline-contaminated sediment from measured respiration rates. J Contam Hydrol 41(1–2):175–192

Beller HR, Grabić-Galić D, Reinhard M (1992) Microbial degradation of Toluene under sulfate-reducing conditions and the influence of iron on the process. Appl Environ Microbiol 58(3):786–793

Boudreau BP, Westrich JT (1984) The dependence of bacterial sulfate reduction on sulfate concentration in marine sediments. Geochimica et Cosmochimica Acta 48:2503–2516

Brezonik PL (1994) chemical kinetics and process dynamics in aquatic systems. Lewis, Boca Raton, 754pp

Cappenberg TE, Prins RA (1974) Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of fresh-water lake. III. Experiments with ^{14}C-labelled substrates. J Microbiol 40:457–469

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Coates JD, Chakraborty R, McInerney MJ (2002) Anaerobic benzene biodegradation—a new era. Res Microbiol 153(10):621–628

Cozzarelli IM, Baedecker MJ, Eganhouse RP, Goerlitz DF (1994) The geochemical evolution of low-molecular-weight organic acids derived from the degradation of petroleum contaminants in groundwater. Geochimica et Cosmochimica Acta 58(2):863–877

Edwards EA, Wills LE, Reinhard M, Grabić-Galić D (1992) Anaerobic degradation of Toluene and Xylene by aquifer microorganisms under sulfate-reducing conditions. Appl Environ Microbiol 55(3):794–800

Elshahed MS, McInerney MJ (2001) Is interspecies hydrogen transfer needed for toluene degradation under sulfate-reducing conditions? FEMS Microbiol Ecol 35(2):163

Fishbain S, Dillon JG, Gough HL, Stahl DA (2003) Linkage of high rates of sulfate reduction in Yellowstone hot springs to unique sequence types in the dissimilatory sulfate respiration pathway. Appl Environ Microbiol 69(6):3663–3667

Fossing H, Jørgensen BB (1989) Measurement of bacterial sulfate reduction in sediments: Evaluation of a single-step chromium reduction method. Biogeochemistry 8(3):205–222

Fukui M, Takii S (1994) Kinetics of sulfate reduction by free living and particle-associated SRB. FEMS Micirobiol Ecol 13(4):241–247

Grabić-Galić D, Vogel TM (1987) Transformation of toluene and benzene by mixed methanogenic cultures. Appl Environ Microbiol 53:254–260

Grishchenkov VG, Townsend RT, McDonald TJ, Autenrieth RL, Bonner JS, Boronin AM (2000) Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions. Proc Biochem 35:889–896

Hordijk KA, Cappenberg TE (1983) Quantitative high-pressure liquid chromatography-fluorescence determination of some important lower fatty-acids in lake sediments. Appl Environ Microbiol 46:361–369

Hunkeler D, Jorger D, Haberli K, Hohener P, Zeyer J (1998) Petroleum hydrocarbon mineralization in anaerobic laboratory aquifer columns. J Contam Hydrol 32(1–2):41–61

Ingvorsen K, Jørgensen BB (1984) Kinetics of sulfate uptake by freshwater and marine species of Desulfovibria. Arch Microbiol 139(1):61–66

Ingvorsen K, Zehnder AJB, Jørgensen BB (1984) Kinetics of sulfate and acetate uptake by Desulfobacter postgatei. Appl Environ Microbiol 47(2):403–408

Ingvorsen K, Zeikus JG, Brock T D (1981) Dynamics of bacterial sulfate reduction in a eutrophic lake. Appl Environ Microbiol 42:1029–1036

Johnson SJ, Woolhouse KJ, Prommer H, Barry DA, Christofi N (2003) Contribution of anaerobic microbial activity to natural attenuation of benzene in groundwater. Eng Geol 70(3–4):343–349

Kao CM, Chen SC, Liu JK, Wang YS (2001) Application of microbial enumeration technique to evaluate the occurrence of natural bioremediation. Water Res 35(8):1951–1960

Kleikemper J, Pelz O, Schroth MH, Zeyer J (2002a) Sulfate-reducing bacterial community response to carbon source amendments in contaminated aquifer microcosms. FEMS Microbiol Ecol 42(1):109–118

Kleikemper J, Schroth MH, Sigler WV, Schmucki M, Bernasconi SM, Zeyer J (2002b) Activity and Diversity of Sulfate-Reducing Bacteria in a Petroleum Hydrocarbon-Contaminated Aquifer. Appl Environ Microbiol 68:1516

Lovely DR, Klug MJ (1986) Model for the distribution of methane production and sulfate reduction in freshwater sediments. Geochimica et Cosmochimica Acta 50:11–18

Maliyekkal SM, Rene ER, Philip L, Swaminathan T (2004) Performance of BTX degraders under substrate versatility conditions. J Hazard Mater 109(1–3):201–211

Matias PM, Pereira IAC, Soares CM, Carrondo MA (2005) Sulphate respiration from hydrogen in Desulfovibrio bacteria: a structural biology overview. Progr Biophys Mol Biol 89(3):292–329

Meckenstock RU (1999) Fermentative toluene degradation in anaerobic defined syntrophic cocultures. FEMS Microbiol Lett 177:67–73

Meier J, Voigt A, Babenzien HD (2000) A comparison of S-35-SO42- radiotracer techniques to determine sulphate reduction rates in laminated sediments. J Microbiol Methods 41(1):9–18

Monod J (1949) The growth of bacterial cultures. Ann Rev Microbiol 3:371–394

Nakagawa T, Sato S, Yamamoto Y, Fukui M (2002) Successive changes in community structure of an ethylbenzene-degrading sulfate-reducing consortium. Water Res 36(11):2813–2823

O'Flaherty V, Mahony T, O'Kennedy R, Colleran E (1998) Effect of pH on growth kinetics and sulfide toxicity thresholds of a range of methanogenic, syntrophic and sulphate-reducing bacteria. Proc Biochem 33(5):555–569

Okabe S, Nielsen PH, Characklis WG (1992) Factors affecting microbial sulfate reduction by *Desulfovibrio desulfuricans* in continuous culture: Limiting nutrients and sulfide concentration. Biotechnol Bioeng 40:725–734

Oude Elferink SJWH, Luppens SBI, Marcelis CLM, Stams AJM (1998) Kinetics of acetate oxidation by two sulfate reducers isolated from anaerobic granular sludge. Appl Environ Microbiol 64(6):2301–2303

Reis MAM, Almeida JS, Lemos PC, Carrondo MJT (1992) Effect of hydrogen sulfide on growth of sulfate reducing bacteria. Biotechnol Bioeng 40:593–600

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier Biomedical Press, Amsterdam

Rothermich MM, Hayes LA, Lovley DR (2002) Anaerobic, sulfate-dependent degradation of polycyclic aromatic hydrocarbons in petroleum-contaminated harbor sediment. Environ Sci Technol 36:4811–4817

Roychoudhury AN (2004) Sulfate respiration in extreme environments: a kinetic study. Geomicrobiol J 21(1):33–43

Roychoudhury AN, Kostka JE, Van Cappellen P (2003a) Pyritization: a palaeoenvironmental and redox proxy reevaluated. Estuar Coast Shelf Sci 57(5–6):1183–1193

Roychoudhury AN, Merrett GL (2006) Redox pathways in a petroleum contaminated shallow sandy aquifer: iron and sulfate reduction. Sci Total Environ doi: 10.1016/j.scitotenv.2005.10.024

Roychoudhury AN, Van Cappellen P, Kostka JE, Viollier E (2003b) Kinetics of microbially mediated reactions: dissimilatory sulfate reduction in saltmarsh sediments (Sapelo Island, Georgia, USA). Estuar Coast Shelf Sci 56(5–6):1001–1010

Roychoudhury AN, Viollier E, Van Cappellen P (1998) A plug flow-through reactor for studying biogeochemical reactions in undisturbed aquatic sediments. Appl Geochem 13(2):269–280

Sarazin G, Michard G, Prevot F (1999) A rapid and accurate spectroscopic method for alkalinity measurements in sea water samples. Water Res 33(1):290–294

Schmitt R, Langguth H-R, Puttmann W, Rohns HP, Eckert P, Schubert J (1996) Biodegradation of aromatic hydrocarbons under anoxic conditions in a shallow sand and gravel aquifer of the Lower Rhine Valley, Germany. Organ Geochem 25(1–2):41–50

Shim H, Yang S-T (1999) Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by a coculture of *Pseudomonas putida* and *Pseudomonas fluorescens* immobilized in a fibrous-bed bioreactor. J Biotechnol 67(2–3):99–112

Shin WS, Pardue JH, Jackson WA (2000) Oxygen demand and sulfate reduction in petroleum hydrocarbon contaminated salt marsh soils. Water Res 34(4):1345–1353

Smith RL, Klug MJ (1981) Reduction of sulfur compounds in the sediments of a eutrophic lake basin. Appl Environ Microbiol 41:1230–1237

Somsamak P, Cowan RM, Haggblom MM (2001) Anaerobic biotransformation of fuel oxygenates under sulfate-reducing conditions. FEMS Microbiol Ecol 37(3):259–264

Sonne-Hansen J, Westermann P, Ahring BK (1999) Kinetics of sulfate and hydrogen uptake by the thermophilic sulfate-reducing bacteria Thermodesulfobacterium sp Strain JSP and Thermodesulovibria sp Strain R1Ha3. Appl Environ Microbiol 65(3):1304–1307

Sørensen J, Christensen D, Jørgensen BB (1981) Volatile fatty acids and hydrogen as substrates for sulfate-reducing bacteria in anaerobic marine sediments. Appl Environ Microbiol 42:5–11

Stookey LL (1970) Ferrozine-A New spectrophotometric reagent for iron. Analyt Chem 42(7):779–781

Tabatabai MA (1974) A rapid method for determination of sulfate in water samples. Environ Lett 7(3):237–242

Urban NR, Brezonik PL, Barker LA, Sherman LA (1994) Sulfate reduction and diffusion in sediments of Little Rock Lake, Wisconsin. Limnol Oceanogr 39(4):797–815

Vavilin VA, Vasiliev VB, Rytov SV, Ponomarev AV (1994) Self-oscillating coexistence of methanogens and sulfate-reducers under hydrogen sulfide inhibition and the pH-regulating effect. Bioresource Technol 49(2):105–119

Villatoro-Monzón WR, Mesta-Howard AM, Razo-Flores E (2003) Anaerobic biodegradation of BTEX using Mn(IV) and Fe(III) as alternative electron acceptors. Water Sci Technol 48(6):125–131

Yoda M, Kitagawa M, Miyaji Y (1987) Long term competition between sulfate-reducing and methane-producing bacteria for acetate in an anaerobic biofilm. Water Res 21:1547–1556