Microbial Ecology

Changes in Organic Matter Biodegradability Influencing Sulfate Reduction in an Aquifer Contaminated by Landfill Leachate

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Abstract

In situ experiments were conducted to measure sulfate reduction rates and identify rate-limiting factors in a shallow, alluvial aquifer contaminated with municipal landfill leachate. Single-well, push–pull tests conducted in a well adjacent to the landfill with >8 mM dissolved organic carbon (DOC) exhibited a sulfate reduction rate of 3.2 μmol SO_4^{-2} (L sediment)$^{-1}$ day^{-1}, a value in close agreement with laboratory-derived estimates. Identical tests conducted in wells located 90 m downgradient where DOC levels remained high (>3 mM) showed no detectable sulfate consumption, and laboratory assays confirmed this observation. However, the rates of sulfate reduction in sediment samples obtained from this site were three times larger when they were amended with filter-sterilized groundwater from the upgradient location. The effect of various amendments on sulfate reduction rates was further examined in laboratory incubations using sediment collected from the downgradient site amended with ^{35}S sulfate. Unamended sediments showed only weak conversion of the tracer to ^{35}S sulfide (5 to 7 cpm/cm^2), whereas the addition of *Desulfovibrio* cells increased ^{35}S sulfide production to 44 cpm/cm^2. However, the application of heat-killed *Desulfovibrio* had a similar stimulatory effect, as did a lactate amendment. Collectively, these findings indicate that the lack of measurable sulfate reduction at the downgradient site was not due to the absence of the necessary metabolic potential, the presence of lower sulfate concentration, or the quantity of electron donor, but by its biodegradability. The findings also indicate that field bioaugmentation attempts should be interpreted with caution.

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Introduction

The microbial decomposition of organic matter coupled with the reduction of sulfate is an important mechanism governing carbon and energy cycling in many anaerobic environments. In anoxic marine sediments up to half of the total organic carbon is mineralized by sulfate-reducing microorganisms [11, 15]. Freshwater environments also harbor active populations of sulfate reducers that dominate carbon and energy metabolism even when sulfate concentrations are low [20, 32]. Sulfate-reducing bacteria utilize an impressive array of organic molecules and hydrogen to support their metabolic activity [1, 10, 18, 28, 33]. Often, electron acceptor availability is considered as a dominant factor controlling the activity of this group of organisms [12, 16, 19, 29]. However, the distribution and supply of suitable electron donors can also be a critical variable affecting sulfate reduction. Indeed, electron donor supply exerts a powerful influence on microbial activities in a variety of environments [14, 17, 23, 26]. In turn, microbial activities play a prominent role influencing physical and geochemical processes in aquifers [6]. Thus, organic matter degradation in contaminant plumes both depends on and contributes to the evolution of groundwater quality along aquifer flow paths. Landfill leachate plumes typically demonstrate decreasing dissolved organic carbon (DOC) concentrations with increasing distance from the source [4, 5, 8]. Nevertheless, the distal portions of these plumes often retain DOC levels that are diminished by only one-half to one-third of the concentration measured at the landfill source. For example, at the Grindsted landfill, organic carbon concentrations were >6 mM near the source and remained elevated at 60 m distance [27]. Thus, it is not unusual for elevated DOC levels to persist along aquifer flow paths despite geochemical evidence of extensive microbial degradation.

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We examined the influence of organic matter degradability on sulfate reduction along an aquifer flow path. At an upgradient location near the landfill mound, *in situ* rates of sulfate reduction in the aquifer could be measured and even stimulated with formate in the presence of high (>8 mM) DOC levels in the groundwater. In contrast, sulfate reduction was not measurable in comparable assays conducted at a downgradient location, although DOC levels were still relatively high (~3 mM). Our results indicate that sulfate reduction rates in the more distal location are restricted by the biodegradability rather than the concentration of DOC in the groundwater.

Methods

Field Site. The study site is a closed municipal landfill occupying about 12 hectares on the Canadian River floodplain south of the town of Norman in central Oklahoma [3]. The alluvium consists of fluvial sediments 10–12 m thick with a water table that is typically 1.0 to 2.5 m below land surface. Solid municipal waste was deposited at the site beginning in 1922 and continued unrestricted until 1985 when the landfill was closed and covered with local clay and silt. The landfill contains no liner or leachate collection devices. As a result, leachate emanating from the refuse comprises a complex waste stream that contaminates the local aquifer to at least 1.5 km from the base of the mound [7]. The aquifer is uniformly anoxic (O_2<5 μM), with elevated alkalinity and ferrous iron concentrations; iron reduction, sulfate reduction, and methanogenesis are important microbially catalyzed processes occurring at the site [3, 8, 12]. Sulfate reduction was examined at two locations (identified as sites 35 and 47 in Fig. 1). The upgradient site near the landfill (site 35) is characterized by high DOC levels and low sulfate concentrations, whereas the downgradient site (47) contains lower levels of DOC and higher sulfate concentrations (Table 1). Each location has a series of wells located along a transect that is perpendicular to the direction of groundwater flow. This arrangement allowed us to conduct simultaneous push–pull tests in separate wells that intercepted zones of comparable groundwater geochemistry. The wells at each location had a single 30-cm-long screen centered at 3.5 m (site 35) and 1.8 m (site 47) below land surface.

Push–Pull Tests. Sulfate reduction rates were estimated by using the push–pull test procedure [13]. This procedure involves injection of a test solution containing a reactant and conservative tracer into the aquifer. The solution is extracted and samples are taken for determination of reactant and tracer loss. Ratios of extracted/injected concentrations (C/C_0) are used to interpret reactant loss relative to that of the tracer, thereby correcting for dilution losses due solely to groundwater flow, and to estimate *in situ* microbial activity. In this study, test solutions were prepared using 50 L of groundwater amended with sodium sulfate (0.3 mM at site 35; 70 μCi carrier-free $Na_2{}^{35}SO_4$ at site 47) and sodium bromide (1.2 mM at both sites) as reactant and tracer, respectively. The solution was extracted from the test well into a plastic carboy and sparged with N_2/CO_2 (4:1) for 15 min prior to the start of each test. The presence of 20% CO_2 in the gas phase served to buffer the system with naturally occurring carbonates in the sediment, resulting in a circumneutral pH throughout the tests. To start the tests, the test solution was injected into the aquifer by using a peristaltic pump. Once the injection phase was complete, the solution was extracted from the same well over a period of 23 days. In addition, some test solutions included added formate (20 mM) as a potential electron donor. During extraction, liquid samples (5 mL) were taken at discrete time intervals and analyzed by high-performance liquid chromatography (HPLC, Dionex, Sunnyvale, CA, USA) for sulfate, bromide, and formate.

We anticipated difficulty in detecting sulfate reduction against the high background levels of sulfate at the downgradient location (Table 1). Therefore, we included 70 μCi $Na_2{}^{35}SO_4$ in the test solutions to provide a more sensitive means of assessing *in situ* microbial activity. The

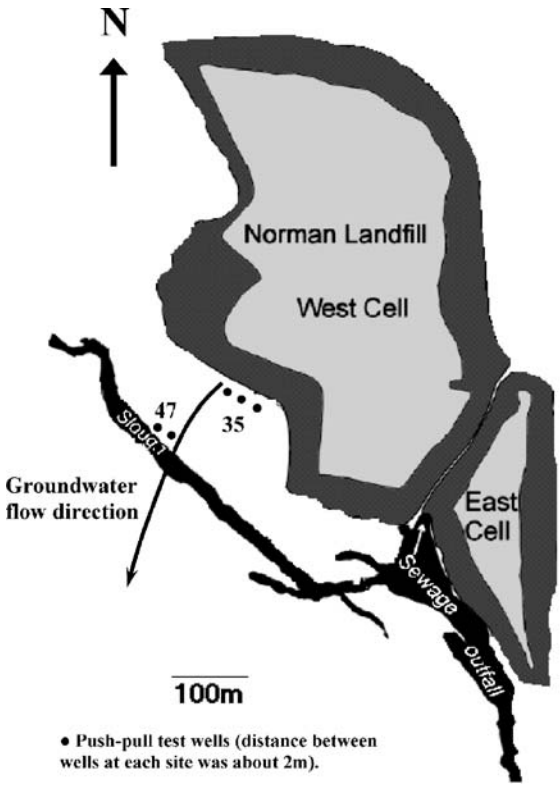

Figure 1. Map of the study area. *Numbers* refer to well sites along the groundwater flow path. Site characteristics can be found at http://csdokokl.cr.usgs.gov/norlan/.

Table 1. Values of selected chemical and physical parameters from a background location, the well adjacent to the landfill (site 35), and the well located 90 m downgradient (site 47)

	Well designation		
Constituent[a]	*Background site*	*Upgradient site 35*	*Downgradient site 47*
DOC (mM)	0.24	>8	3.3
Specific conductance ($\mu S\ cm^{-1}$)	1570	4990	5940
Methane (μM)	0.03	396	45
Sulfate (mM)	1.2	0.038	7.1
Chloride (mM)	5.1	9.7	13.6
Hydrogen (nM)	ND	1.6	ND
pH	7.0	7.0	6.9
Oxygen (μM)	ND	<10	<10

ND: not determined.
[a]DOC and specific conductance for sites 35 and 47 are from [6] and Isabelle Cozarrelli (pers. comm.), respectively. Parameters for the background site are from [29].

radiotracer allowed us to monitor the reduction of both injected ^{35}S sulfate and ^{32}S sulfate. Furthermore, any ^{35}S sulfate reduced during the test would precipitate in the aquifer as stable iron sulfides that could be subsequently quantified in the laboratory via autoradiographic analysis (InstantImager, Packard Instrument Co., Downers Grove, IL, USA) of intact cores obtained from the area impacted by the push–pull tests. Initially, the distribution of both ^{35}S sulfate and ^{35}S sulfide was determined in unwashed samples of intact cores taken from the zone impacted by the push–pull test. The unreacted ^{35}S sulfate was then removed from the cores by an anoxic water wash to allow the determination of precipitated ^{35}S sulfide in the sediment.

Sediment Core Collection. Sediment cores were collected with a Geoprobe sampling device (Geotech, Inc., Salina, KS, USA). Cores were flushed with N_2 immediately after collection and transported to the laboratory where the samples were processed in an anaerobic glove bag.

Sulfate-Reduction Activity in Sediment. Filter-sterilized groundwater from the respective sites was used to supply soluble organic carbon to serve as electron donor for sulfate reduction in sediment samples. Serum bottle (160 mL) incubations containing 50 g of sediment as the inoculum and 75 mL filter-sterilized groundwater were amended with sulfate (where necessary) to an initial concentration of 7 mM. Sediment from near and distal sites were used in all possible combinations with filter-sterilized groundwater from the various locations. The bottles were sealed with butyl rubber stoppers and incubated for 7 days under a N_2/CO_2 (4:1, 1 atm) headspace at 20°C in the dark. Sulfate depletion in slurries was determined by HPLC.

Electron Donor Amendments to Cores. Sulfate reduction as a function of electron donor amendment was examined in sediment cores. Cores were transported within 2 h of extraction and placed inside a N_2-flushed glove bag where they were sectioned to produce segments ($20 \times 5 \times 0.5$ cm) to be used in radioisotope experiments. Sulfate reduction was assessed by monitoring the conversion of ^{35}S sulfate to ^{35}S sulfide on the surface of the core segments. The incubation was started by uniformly applying an anoxic sterile solution of 100 μCi of $Na_2{}^{35}SO_4$ in 15 mL water to the face of each core segment. The segments were incubated in gastight containers in the dark at 20°C under a headspace of N_2/CO_2 (4:1) for 14 to 90 days. Once the incubation was complete, the core segments were washed with anoxic water to remove unreacted ^{35}S sulfate while leaving the precipitated ^{35}S sulfide unaltered. After the segments were washed, the distribution of ^{35}S sulfide was visualized by autoradiography. To investigate the effect of various amendments on sulfate reduction, core segments were prepared as above and subdivided into three sections of similar area. The first third was amended with lactate by adding 5 mL of a sterile lactate solution (10 mM) containing 33 μCi ^{35}S sulfate. Amendments that included washed preparations of *Desulfovibrio* G11 were prepared by centrifuging log-phase cells at $15{,}000 \times g$ for 20 min. The cell pellet was resuspended in sterile anoxic water and centrifuged again. The cycle was repeated three times with fresh water to thoroughly wash media components from the cells. The final cell pellet was resuspended in 10 mL anoxic water and divided into two aliquots. The first aliquot was used as a live-cell preparation; the second was boiled for 20 min to heat-inactivate the cells. Radiolabeled sulfate was added to each of these aliquots to a final activity of 33 μCi. Then, 5 mL of each cell suspension (4.5 mg protein mL^{-1}) was applied to the appropriate third of the core section. All amendments and cell manipulations were done in a N_2-flushed anaerobic glove bag.

Results

In Situ Field Sulfate-Reduction Rates. Measured sulfate consumption rates in push–pull tests conducted at site 35 adjacent to the landfill (Fig. 1) were ~3 μmol $SO_4{}^{-2}$ (L sediment)$^{-1}$ day^{-1} (Fig. 2). Previous work indicated that sulfate reduction was an active microbial respiratory process occurring in the area [8, 12, 29]. The steady-state dissolved hydrogen concentration in wells at this site was consistently 1.6–2.0 nM, supporting the contention that sulfate reduction in this location was a dominant terminal electron-accepting process [8]. The estimated rate determined in the push–pull test was similar to that observed in unamended laboratory incubations of intact core material obtained from the

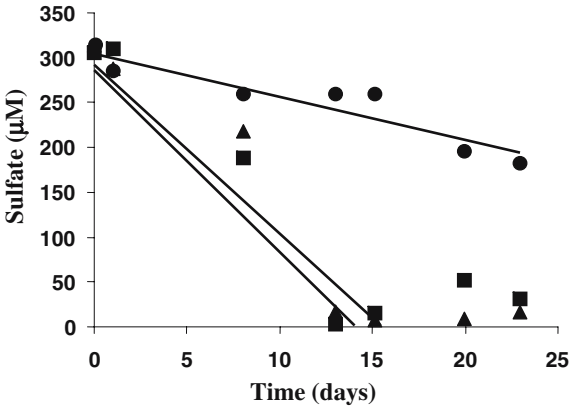

Figure 2. Microbial sulfate consumption at site 35 in push–pull tests amended with sulfate only (●) and in the presence of added sulfate and formate (replicate tests, ▲ and ■). Sulfate data have been corrected for dilution via sodium bromide values.

same depth (Fig. 3). When two subsequent push–pull tests were conducted with 20 mM added formate, sulfate consumption rates increased by a factor of about 4 to ~14 µmol SO_4^{-2} (L sediment)$^{-1}$ day^{-1} (estimated over the first 15 days of the tests, before sulfate was depleted). The increased rate due to the presence of formate was comparable to that measured in laboratory incubations of material taken from near the water table (Fig. 3).

In contrast, measured rates of sulfate reduction in push–pull tests conducted at site 47, located downgradient of the source, were not detectable. Breakthrough curves for ^{35}S sulfate and bromide were identical, indicating that observed decreases in sulfate concentration were due simply to dilution as the injected test solution gradually drifted from the well (Fig. 4A). Recoveries of ^{35}S sulfate and bromide were also nearly

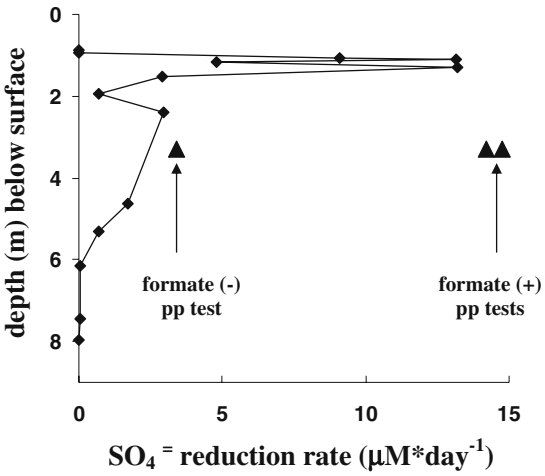

Figure 3. Comparison of sulfate-reduction rates in intact cores (♦) determined from a previous study [6] to those estimated from push–pull tests (▲) at site 35 in this study. The *y*-axis values for push–pull test rates correspond to the depth where tests were done.

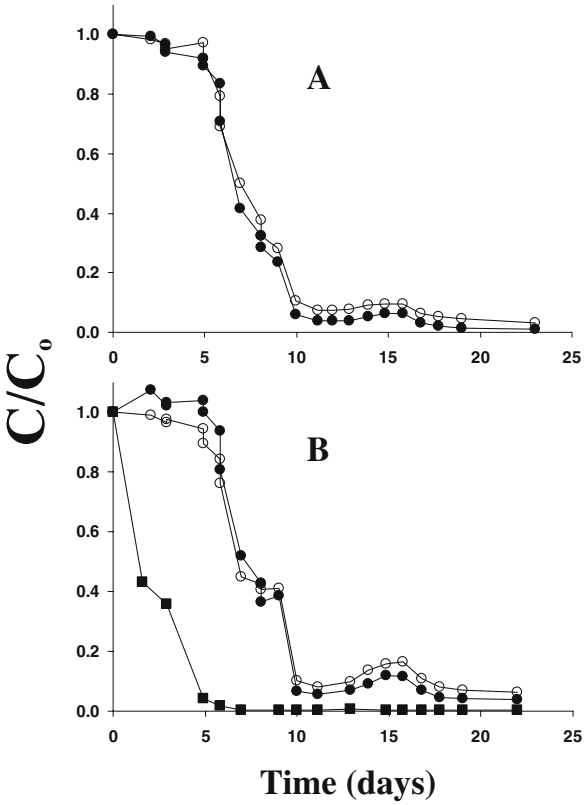

Figure 4. Breakthrough curves of sulfate (●), bromide (○), and formate (■) in an unamended (A) and formate-amended (B) push–pull test at the downgradient location (site 47).

identical at 68.4 and 63.4%, respectively. Furthermore, the addition of 20 mM formate did not stimulate sulfate reduction at this site even though this potential electron donor was consumed within 5 days after injection (Fig. 4B). In the formate-amended test, recoveries of ^{35}S sulfate (79.2%) and bromide (80.7%) were almost identical. Very little sulfate reduction was detected in slurries from the downgradient site. Although the residual radioactive signal was uniformly distributed in samples of cored material (data not shown) indicating that injected ^{35}S sulfate from the push–pull test contacted the portion of the aquifer sampled, there was no evidence of the accumulation of ^{35}S sulfide. These findings confirm that little or no sulfate reduction occurred at the site distal from the landfill despite ample concentrations of sulfate and DOC (Table 1).

Sulfate-Reduction Activity in Cores. Possible explanations for the lack of sulfate reduction at the downgradient site include the lack of organisms with the necessary metabolic potential, the presence of an inhibitory substance, and the lack of suitable electron donors. To explore these possibilities in more detail, laboratory incubations were conducted by using sediment cores collected from this site. Results showed

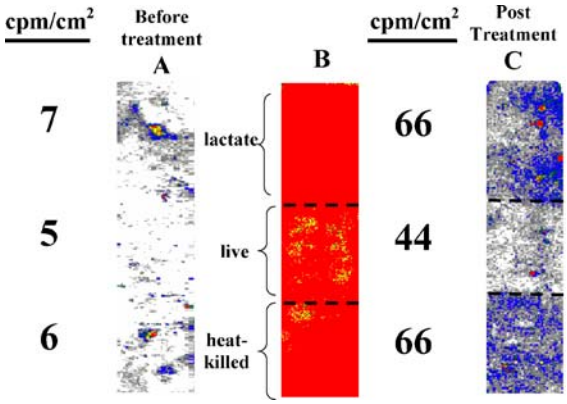

Figure 5. Sulfate-reduction activity distributions in a core segment incubated with ^{35}S sulfate and amendments of lactate and *Desulfovibrio* preparations. (A) Represents sparse distribution of ^{35}S sulfide in an unamended core segment incubated for 90 days in the presence of radiolabelled sulfate. (B) The same segment analyzed immediately after lactate, ^{35}S sulfate, and *Desulfovibrio* amendments showing uniform distribution of the radiotracer. (C) The same segment after 17 days of incubation representing precipitated ^{35}S sulfide remaining after unreacted ^{35}S sulfate was removed by washing.

only a sparse distribution of sulfate-reduction activity in the core segments despite incubations of up to 7 weeks (data not shown). For example, a core taken from the downgradient site and incubated for 90 days in the presence of ^{35}S sulfate showed little overall sulfate reduction (Fig. 5). The small amount of activity that was noted was spatially localized (Fig. 5). The same core was subsequently divided into three subsections and supplemented with lactate (10 mM), an inoculum of *Desulfovibrio* G11 (22.5 mg protein), or a heat-killed treatment of the same organism. After a 17-day incubation, the core segments were assayed again (Fig. 5). The presence of a suitable inoculum was ensured by the addition of live *Desulfovibrio* to the core segment, and this treatment resulted in a ninefold increase in sulfate reduction. However, the heat-killed preparation as well as the lactate amendment stimulated sulfate reduction to a comparable degree (Fig. 5). The stimulation in sulfate reduction by both the live and heat-killed cell treatments suggested that the former served largely as an equivalent nutritional augment. We questioned if the live inoculum was capable of sulfate reduction in the core or if microbial activity was affected by an unknown inhibitor. The ability of the cells to reduce sulfate was confirmed in incubations of twice-autoclaved sediment (5 g) with live inoculum (1 mL of a washed cell suspension, 4.5 mg protein/mL). Sulfate was consumed (>2 mM) in these incubations and the sediment turned black in a few hours (data not shown). The addition of heat-killed *Desulfovibrio* G11 (1 mL of the same cell suspension, boiled) to twice-autoclaved slurries did not result in sulfate depletion or a black precipitate.

Effect of Groundwater Quality on Sulfate Reduction. We examined the ability of filter-sterilized groundwater from either the upgradient or downgradient site to supply electron donors for sulfate reduction in an additional series of laboratory incubations. Combinations of sediment and groundwater from site 35 consumed sulfate at the fastest rates, designated for comparative purposes as 100% (Fig. 6). The activity was diminished to only 18% when groundwater from the distal site was used as the source of electron donor. The diminished rate was similar to the 19% value observed in incubations prepared using sediment and groundwater from the distal location. Groundwater from near the landfill was able to enhance the sulfate reduction rate by the organisms in the downgradient sediments to about 59%.

We considered whether the presence of an unknown inhibitory substance in downgradient groundwater reduced the level of activity in the samples. However, this did not appear to be the case. If sediment from near the landfill was incubated with no addition of groundwater, we would predict a sulfate reduction rate of only about 8% of the maximum observed in Table 1 (based on the residual amount of water in the sediment). However, addition of downgradient groundwater resulted in a rate of about 18%, more than twice the expected level in absence of water from the distal site. Therefore, the

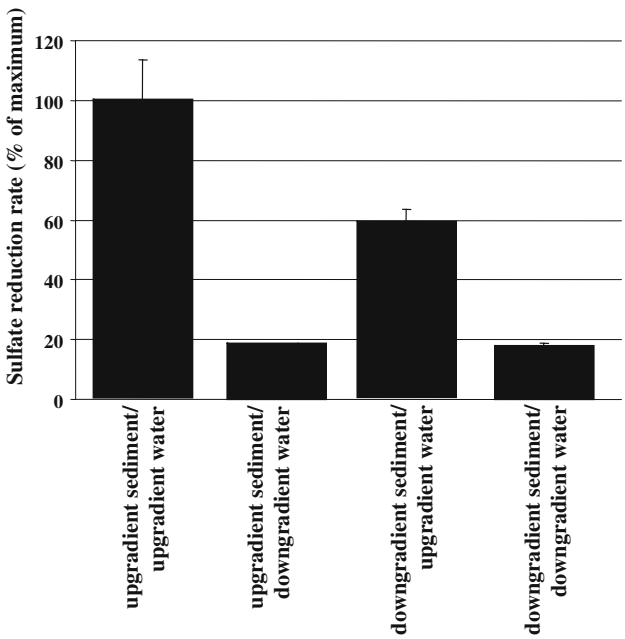

Figure 6. Sulfate-reduction rates in sediment slurries at saturating (7 mM) sulfate concentrations and in the presence of different groundwaters as potential electron donor sources. Slurries containing sediment and groundwater from the upgradient site consumed sulfate at the highest rate [156 nmol SO_4^{-2} (g wet wt.)$^{-1}$ day^{-1}] and were designated as 100%. Rates for the other treatments are relative to 100%. The values are the means of triplicate incubations ± SE.

presence of an inhibitor could not explain the reduced rate observed when groundwater from the distal site was used. We further considered whether the presence of reactive iron oxides could be limiting sulfate reduction. To address this issue, sulfate concentrations were monitored for more than 150 days (data not shown) to allow time for the depletion of iron oxides. No sulfate consumption was detected in the slurries upon extended incubation, suggesting the presence of iron oxides was not a factor limiting sulfate reduction at the distal site.

We examined the ability of hydrogen and formate to serve as potential electron donors for sulfate reduction in laboratory incubations (data not shown). Sulfate reduction was not stimulated by either potential electron donor in incubations using sediment from the upgradient site. In contrast, sulfate reduction in sediment from the distal site was stimulated by a factor of 5 in the presence of added hydrogen and by a factor of two in the presence of added formate. In the presence of added formate, a small amount of methane was produced in slurries from the downgradient site. However, sulfate consumption, methane, and acetate production accounted for <10% of the formate that was consumed, suggesting an alternate fate for formate exists at this site, a result that was consistent with the field observations (Fig. 2).

Discussion

Endogenous electron donors in the sediment/groundwater closest to the landfill supported measurable *in situ* rates of sulfate reduction (Fig. 2). However, these rates could be increased by the addition of a labile electron donor like formate. Presumably, the stimulated effect observed in field tests was due to the proliferation of formate utilizing sulfate-reducing bacteria. This rate is comparable to those obtained in laboratory incubations using sediments from near the water table (1.5 m depth, Fig. 3) where the increased rates are supported by higher sulfate concentrations that result from seasonal oxidation of iron sulfides [29].

Sulfate reduction was not detected in field tests conducted at the downgradient site regardless of formate amendment (Fig. 4). Dissolved oxygen concentrations were uniformly low throughout the tests, indicating that aerobic conditions were not the reason for the lack of sulfate consumption. In addition, the lack of activity was not due to sulfate limitation, as the concentration of this anion remained nonlimiting during the course of the push–pull tests. One explanation for the lack of sulfate reduction in push–pull tests at the downgradient site was competition for formate by microorganisms other than sulfate reducers. Although sulfate consumption was not detected, added formate was rapidly degraded over the first 5 days of the test indicating the presence of an active microbial community capable of metabolizing this compound. Similar to field results, added formate was rapidly consumed in slurries from the downgradient site but resulted in only a slight increase in the sulfate reduction rate (data not shown). After determining an electron balance in the slurries, less than 10% of the consumed formate was accounted for by sulfate reduction, methanogenesis, and acetogenesis, further suggesting an alternative fate for formate exists at this site.

It is unlikely that it is merely the concentration of DOC that limits sulfate reduction at the downgradient site. The level of DOC near the landfill mound is relatively high (>8 mM), similar to measurements in other contaminated aquifers including those polluted with landfill leachate [2, 5, 22, 24], and at least an order of magnitude higher than that typically found in aquifers upgradient from landfills [5, 27, 29]. In slurries from the Grindsted landfill, microbial iron reduction could not be stimulated by additions of amorphous iron hydroxides alone, despite the presence of comparably high DOC levels in the leachate [21]. However, iron reduction was stimulated in the presence of acetate. These results are similar to those obtained in this study, suggesting that the relatively recalcitrant nature rather than the quantity of electron donor is the primary factor limiting the rate of sulfate respiration. Dissolved organic matter emanating from the Norman landfill has been fractionated and examined previously [24]. The hydrophobic fraction was found to contain primarily highly branched, cyclic aliphatic compounds that likely represent sizing agents released during the biodegradation of cellulose from paper. Less refractory organic matter such as polysaccharides, cellulose, and proteins were either not detected or present at very low levels. Presumably, these compounds are present in the refuse deposited in the landfill but are degraded rapidly, leaving the more recalcitrant molecules to migrate downgradient to the sampled area. Thus, the high degree of aliphaticity of the leachate organic matter along with the lack of more labile carbon structures is consistent with the inability of the DOC fraction to support maximum rates of sulfate reduction. Moreover, DOC at the downgradient location (3.3 mM) is still 13 times higher than background concentrations [29]. If dilution were a significant factor in attenuating DOC levels along the flow path, we would expect the concentration of other dissolved constituents to approach those found in background water. However, chloride and specific conductance determinations at the downgradient site remain three times higher than background levels [29] and are not diminished relative to the upgradient site (Table 1), indicating that dilution alone cannot account for the decreased DOC concentrations along the 90-m flow path. These results suggest that microbial activity is responsible for decreasing

organic matter concentrations along the flow path. Indeed, several microbial processes have been detected in the aquifer that can contribute to the degradation of DOC along the groundwater flow path [8]. Nevertheless, substantial amounts of DOC persist even at a distance of 90 m from the landfill. We hypothesize that microbial degradation along the flow path results in a diminished biodegradability of the DOC such that sulfate reduction is limited by electron donor at the distal site.

In an effort to elucidate factors limiting sulfate consumption downgradient from the landfill, radiotracer experiments were done in cores from the distal site (Fig. 5). The ability of lactate, as well as a heat-killed preparation of *Desulfovibrio*, to stimulate sulfate reduction indicated the lack of a suitable inoculum was not the reason for the lack of activity in the core. Active populations of sulfate-reducing bacteria are clearly present in the sediment, but their activity could only be realized if they were supplied with a more labile form of electron donor. Moreover, this electron donor limitation could be supplied by lactate or a heat-killed cell preparation.

To further explore the lack of activity at the distal site, the ability of filter-sterilized groundwaters to support microbial sulfate consumption was examined in slurries that were replete with this anion. These experiments demonstrated the presence of active sulfate-reducing microorganisms in the sediment from both sites sampled when groundwater from near the landfill supported relatively rapid rates of sulfate consumption whether the inoculum source was sediment from either site (Fig. 6). Thus, despite the obvious presence of a capable sulfate-reducing community in these sediments, their activity was diminished to similar levels as the result of the inferior biodegradability of electron donor supplied by groundwater from the distal site.

These results are especially interesting in the context of using bioaugmentation to stimulate *in situ* bioremediation in contaminated aquifers. Several studies have examined microbial inoculation as a strategy to remediate contaminated aquifers [9, 25, 31]. However, in addition to acting as a catalytic entity, the microbial inoculant can also serve as a labile source of electron donor. Indeed, microbial inoculation experiments demonstrate various degrees of success, in part because of the lack of survival of the injected microorganisms [30]. The inactivation and subsequent lysis of inoculated cells would provide a rich source of electron donor in the form of cell debris. This electron donor then becomes available to the native microbial community driving the catalysis of various processes that may include the very transformation the original inoculation was intended to produce. This is illustrated by the control treatment in Fig. 5 where the heat-killed preparation of *Desulfovibrio* is included. A similar control containing an inactivated cell preparation is also necessary in field studies where microbial inoculation is employed as a remediative strategy. However, this type of treatment is seldom carried out and, thus, the true nature of the contribution made by the microbial inoculant is not clear.

In summary, the lack of sulfate reduction at the distal site was not due to a lack of a suitable inoculum, sulfate limitation, the presence of an unknown inhibitory substance, or the DOC quantity. Rather, the results are more consistent with a limitation in DOC biodegradability.

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