

Cellular energy conservation and the rate of microbial sulfate reduction

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ABSTRACT

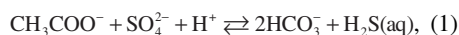
Microbial sulfate reduction is subject to a thermodynamic limit arising from the microorganisms' need to save energy for maintenance and growth, and this limit prevents the process from proceeding until the supply of electron donor or sulfate has been consumed, as would be expected from commonly applied kinetic theory. In pure culture experiments, acetotrophic sulfate reduction stops when the energy liberated by the reaction falls to ~33–43 kJ·(mol SO₄²⁻)⁻¹, and an overlapping range of 40–56 kJ·(mol SO₄²⁻)⁻¹ is observed where sulfate reduction has ceased in experiments with microbial consortia, as well as in nature, in lacustrine, marine, and aquifer sediments. These observations correspond to an energetic requirement of 33–47 kJ·(mol SO₄²⁻)⁻¹ calculated on the basis of the cellular physiology of sulfate reducers. In sediments underlying Lake Washington, USA, variation of pore-water chemistry with depth can be explained by a reactive transport model accounting for cellular energy conservation, whereas a model in which thermodynamics are neglected predicts an unrealistic pattern. Energy availability constitutes a primary, if commonly overlooked, control on the distribution and rate of microbial sulfate reduction in nature and helps resolve apparent contradictions observed in the laboratory and natural environment.

INTRODUCTION

Microbial sulfate reduction is a dominant process in natural environments, accounting for perhaps half the degradation of organic matter in anoxic settings (Jørgensen, 1977). Sulfate-reducing microorganisms play roles in a spectrum of biogeochemical processes, ranging from nutrient cycling to environmental contamination and remediation (Jensen et al., 1995; Barkay and Schaefer, 2001; Kirk et al., 2004). An understanding of the controls on the distribution and rate of microbial sulfate reduction, then, is central to the study of a variety of scientific and practical problems.

Sulfate-reducing microorganisms in nature face a special challenge: little chemical energy is available in many environments because the oxidation of various electron donors by sulfate reduction is favored only moderately by thermodynamics. However, a certain amount of the energy liberated by the reaction needs to be conserved in a cell's cytoplasm as ATP (adenosine triphosphate), to be used by the cell for maintenance and growth (Jin and Bethke, 2007). The thermodynamic drive f for a microbe's catabolism is the difference $\Delta G_A - \Delta G_C$, i.e., the difference between the energy available ΔG_A in the environment to drive the reaction and the energy conserved by the cell ΔG_C (Jin and Bethke, 2002, 2003). These quantities for microbial sulfate reduction are commonly of similar magnitude, and so, accounting for the cell's need to derive energy, the thermodynamic drive is in many cases quite small.

Most current approaches to describing the rate of sulfate reduction employ kinetic laws such as the dual-Monod equation (Widdel, 1988). Lacking an accounting of energetics, this equation predicts that sulfate reduction will proceed unimpeded until either the electron donor or sulfate has been depleted. Previous studies have shown that sulfate reducers, at least those oxidizing H₂, require a minimum amount of chemical energy to proceed (Hoehler et al., 1998; Jakobsen and Cold, 2007), suggesting that the reaction might in fact cease before the electron donor or sulfate has been consumed. In this paper, we show that the requirement of cellular energy conservation exerts a critical control on the distribution and rate of acetotrophic sulfate reduction,



which is a microbially mediated reaction of primary importance in anaerobic environments. Specifically, we consider the way in which the amount of energy available in laboratory experiments and various natural environments affects where the reaction proceeds, and how quickly it proceeds.

MATERIALS AND METHODS

Batch Reactor Experiments

We conducted batch reactor experiments on *Desulfobacter hydrogenophilus* (DSM3380) at 30 °C in 160 mL serum bottles. Bottles containing 100 mL of sterile saltwater growth media (Widdel and Bak, 1999) were degassed with a mixture of 80% N₂ and 20% CO₂, capped with rubber stoppers, and sealed with aluminum caps. We inoculated ~10⁸ cells into each of the bottles

and incubated them in the dark without shaking. We carried out each experiment in triplicate.

Chemical Analysis

We measured sulfide and bicarbonate concentrations immediately after the sampling using the methylene blue and Gran titration methods, respectively, and we analyzed acetate using a high-pressure liquid chromatograph (HPLC) and a Hamilton™ HC-75 H⁺ form column. We determined pH using a pH probe and measured sulfate with an ion chromatograph and a Dionex™ IonPac AS11-HC-4 mm column. We also measured sulfate concentration as total sulfur using an inductively coupled plasma–emission spectrophotometer, adding zinc acetate before analysis to remove sulfide. We monitored biomass by measuring total protein concentrations using a Coomassie Plus™ protein assay kit (Pierce, Rockford, Illinois, United States).

Energy Available

We calculated the energy available, ΔG_A , in kJ·(mol SO₄²⁻)⁻¹, the negative value of the Gibbs free energy change ΔG of reaction 1, according to

$$\Delta G = \Delta G_T^0 + RT \ln \prod_i (\gamma_i \times m_i)^{\nu_i}, \quad (2)$$

where ΔG_T^0 is the standard value of ΔG at temperature T (K), R is the gas constant (J·mol⁻¹·K⁻¹), and γ_i , m_i , and ν_i are activity coefficients (molal⁻¹), molal concentrations, and reaction coefficients of chemical species involved in reaction 1, respectively. Values of ΔG_T^0 were evaluated using The Geochemist's Workbench® software package (Bethke, 2008) and the LLNL (Lawrence Livermore National Laboratory) Thermodynamic Database (Delany and Lundeen, 1990). The software used an extended form of the Debye–Hückel equation (Helgeson, 1969) to evaluate activity coefficients, and calculated the concentrations of chemical species from the fluid's analyzed composition.

RESULTS

Laboratory Experiments

Figure 1 shows the variation of fluid composition and biomass concentration with time in a typical batch reactor experiment. As the experiment progressed, the *D. hydrogenophilus* culture consumed acetate and sulfate, produced sulfide and bicarbonate, and synthesized biomass. About 6 d into the experiment, acetate and sulfate concentrations decreased to constant values of 0.10 ± 0.03 and 1.23

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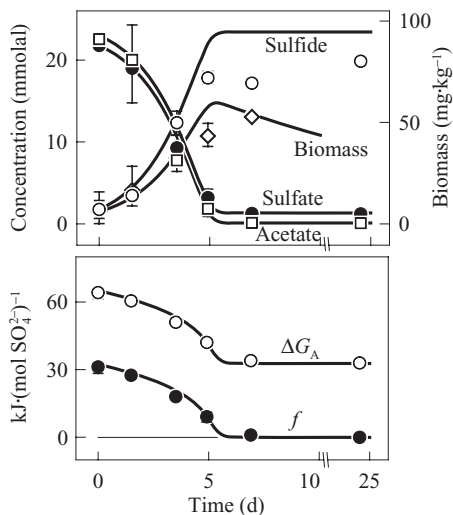


Figure 1. Results of batch reactor experiment tracing sulfate reduction by *Desulfobacter hydrogenophilus*. Concentrations are averages of replicate analyses, and error bars show range observed. Data points for energy available (ΔG_A) were computed from concentrations of chemical species, as described in text; thermodynamic drive f was calculated assuming value of $33 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$ for ΔG_C (energy conserved by the cells). Lines show simulation resulting from integrating Equations 3 and 4.

$\pm 0.13 \text{ mmolal}$, respectively. The experiment continued past this point for several weeks, but no significant changes in the concentrations were observed, indicating sulfate reduction ceased after 6 d. As the fluid composition changed, the chemical energy available to the microbes (ΔG_A) decreased sharply (Fig. 1). At the onset of the experiment, $64.2 \pm 2.5 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$ of energy was available, but by 6 d, the energy available had decreased to $33.0 \pm 1.1 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$, after which it remained roughly constant.

To test how these results might be affected by chemical composition at the onset of the experiment, we ran a series of 14 batch reactors similar to the one already presented (Fig. 1), using an initial acetate concentration of 15 mmolal and sulfate concentrations ranging from 2 to 15 mmolal. The final pH and concentrations of acetate, sulfate, sulfide, and bicarbonate varied among the experiments, as shown in Figure 2, but the energy available after the reaction had ceased stayed within a relatively narrow range of 33.1 ± 0.6 to $42.8 \pm 0.3 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$. Sulfide concentrations in the experiments did not reach a level high enough to fully inhibit the organism's metabolism (Okabe et al., 1995).

To consider the effect of environmental conditions in a different way, we calculated the energy available at the cessation of sulfate reduction by *Desulfobacter postgatei* and *Desulfotomaculum acetoxidans* in experiments

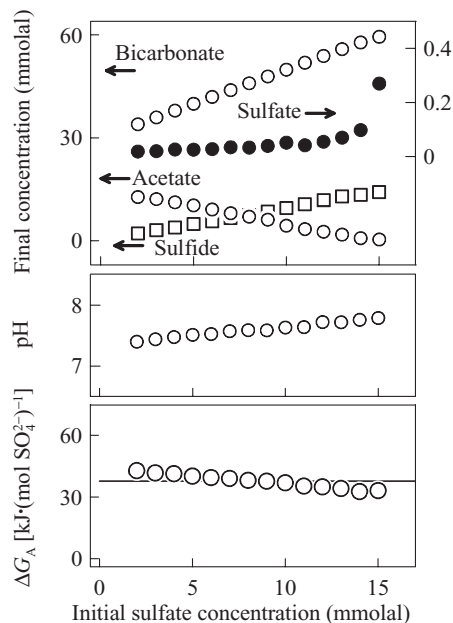


Figure 2. Results of batch reactor experiments after sulfate reduction had ceased for a suite of experiments like that shown in Figure 1, but with differing initial sulfate concentrations. Solid line represents average value.

reported previously (Ingvorsen et al., 1984; Su, 2004). Unlike *D. hydrogenophilus*, which inhabits marine sediments (Widdel, 1987), *D. postgatei* lives in brackish sediments (Widdel and Pfennig, 1981), and *Desulfotomaculum acetoxidans* occurs in both freshwater and marine sediments (Widdel and Pfennig, 1977). The energy available to *D. postgatei* at the cessation of sulfate reduction ranged from 36.4 to $43.3 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$; the energy remaining after sulfate reduction by *Desulfotomaculum acetoxidans* had ceased was $43.1 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$. This behavior of pure cultures is observed as well in tests using natural microbial consortia. Smith

and Klug (1981, their figure 5) used batch reactors to study sulfate reduction in live lake sediments. Sulfate additions to the sediments stimulated sulfate reduction and, given the absence of reactive metals in the experiment, drove up sulfide concentration. After 1 h, sulfide maintained a constant level; the sulfate concentration at this point was $\sim 1 \text{ mmolal}$, acetate was $40 \mu\text{molal}$, bicarbonate was 15 mmolal , and pH was 7 (Lovley and Klug, 1982, 1983; Smith and Klug, 1981). The energy available at the cessation of sulfate reduction held steady at $56.0 \pm 1.0 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$.

Natural Environments

We compiled previous studies in which the radiotracer method (Jørgensen, 1978) was used to identify subsurface environments worldwide where sulfate reduction proceeds at near-zero rates. We then used the reported pore-water compositions to calculate the energy available in those environments. The environments include lake sediments, marine sediments, and freshwater aquifers. As shown in Table 1, even though sulfate reduction has ceased at each location, finite amounts of acetate and sulfate remain unreacted. Trophic state varies broadly among the locations, but the energy available (ΔG_A) for sulfate reduction falls within a relatively narrow range of ~ 40 – $54 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$, with an average value of $47.3 \text{ kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$.

Physiological Requirement

Since the thermodynamic drive f for a microbially catalyzed reaction is the difference between the energy available and that conserved (i.e., $\Delta G_A - \Delta G_C$), we can figure the minimum level of available energy that can support acetate oxidation by sulfate reducers from the mechanism of cellular energy conservation. Rabus et al. (2006) estimated that the catabolism of *Desulfobacter* spp. synthesizes two-thirds of an ATP per sulfate reduced. The synthesis of ATP

TABLE 1. LOCATIONS IN THE NATURAL ENVIRONMENT WHERE SULFATE REDUCTION PROCEEDS AT NEAR-ZERO RATES

Environment (trophic state)	Temperature (°C)	Acetate (μmolal)	Sulfate (μmolal)	ΔG_A $\text{kJ} \cdot (\text{mol SO}_4^{2-})^{-1}$
Lake sediment				
Lake Kaiike, Japan (oligotrophic)*	20.0	3.80	2.15×10^4	53.5
Lake Vechten, Netherlands (eutrophic)†	5.0	3.0	10.4	39.6
Marine sediment				
Fjord sediment, Denmark (eutrophic)§	10.9 ± 3.5	4.5	13.9	47.5
	14.2 ± 0.4	1.8	11.6	41.0
	17.9 ± 1.4	45.5	4.4	42.7
Aquifer				
Asserbo aquifer, Denmark (oligotrophic)#	8.0	1.9	90.0	53.4
Rømø aquifer, Denmark (oligotrophic)**	8.0	0.24 ± 0.05	8.84 ± 5.58	53.2 ± 2.7

*Koizumi et al. (2004, their figure 2).

†Hordijk et al. (1984), Hordijk et al. (1994), Hordijk and Cappenberg (1983), and Steenbergen and Verdouw (1982).

§Holmer and Kristensen (1996, their figures 2, 5, 6, 7, and 10).

#Jakobsen and Cold (2007, their figures 2, 3, and 4).

**Hansen et al. (2001, their figure 6.3).

from ADP (adenosine diphosphate) and phosphate in a cell's cytoplasm requires between 50 and 70 kJ·(mol ATP)⁻¹ (Schink, 1997; Thauer et al., 1977). We thus expect sulfate reducers to require between 33 and 47 kJ·(mol SO₄²⁻)⁻¹ of energy to proceed.

REACTIVE TRANSPORT MODEL

To better understand the way in which the requirement of energy conservation might affect natural systems, we constructed a reactive transport model of microbial activity in the sediments beneath Lake Washington, Washington, USA, using pore-water compositions reported by Kuivila et al. (1989, their figure 4). Fermenting microbes in the lake-bottom sediment degrade organic matter, producing acetate and other reduced compounds that serve as electron donors for respiring microbes and methanogens. Aerobic respirers are active in the top few centimeters, but pore waters below this level lack O₂ and so host only anaerobes. The concentration profile of H₂, as well as in situ rate measurements indicate that at depths greater than 3 cm, the dominant microbial processes besides fermentation are sulfate reduction and methanogenesis (Kuivila et al., 1989; Lovley and Goodwin, 1988); the activities of other microbial groups, such as iron and manganese reducers, are of secondary importance.

Given the relatively homogeneous distribution of organic matter within the sediment (Hedges et al., 1982), we assumed that acetate was produced at a uniform rate and consumed by aerobic respiration, sulfate reduction, and acetoclastic methanogenesis. The rates r (mol·s⁻¹) of the latter three processes were computed in the simulation from the thermodynamically consistent form of the dual-Monod equation (Jin and Bethke, 2005, 2007)

$$r = k[X] \frac{m_D}{K_D + m_D} \frac{m_A}{K_A + m_A} \left[1 - \exp\left(-\frac{f}{\chi RT}\right) \right], \quad (3)$$

where k is a rate constant (mol·mg⁻¹·s⁻¹), $[X]$ represents biomass concentration (mg·kg⁻¹), m_D and m_A are the molal concentrations of electron donor and sulfate, K_D and K_A are half-saturation constants (molal), and χ is the reaction's average stoichiometric number. This rate law differs from the dual-Monod equation in that it rigorously accounts for the effects of the thermodynamic drive f on reaction rate. Biomass concentration in the simulation varies according to

$$\frac{d[X]}{dt} = Y \cdot r - D \cdot [X], \quad (4)$$

where Y is growth yield (mg·mol⁻¹), and D is the decay constant (s⁻¹).

In light of the absence of significant bioturbation and the slow sedimentation rate of ~0.3 cm·a⁻¹ (Edmondson and Allison, 1970), which translates to a boundary Péclet number of ~0.03, the model accounts for transport through the sediment column by diffusion alone. We used the reactive transport simulator X1t from version 7.0.4 of The Geochemist's Workbench software package (Bethke, 2008) to solve for the steady-state distribution of pore-water chemistry and microbial activity in the sediment column. Details of how we constructed the simulation, the values we assumed for kinetic parameters, and our input files are available in the GSA Data Repository.¹

In the calculation results (Fig. 3), sulfate reducers oxidize acetate using sulfate that diffuses downward from the lake, producing sulfide that diffuses upward. As a result, sulfate concentration decreases from ~105 μmolal at the sediment-water interface to 16 μmolal ~20 cm below the interface; below this depth, it remains roughly constant. The energy available (ΔG_A) from reaction 1 is greatest, over 100 kJ·(mol SO₄²⁻)⁻¹, at the sediment-water interface and decreases sharply downward (Fig. 3) in response to the changing concentrations of the chemical species. Below 8 cm, the energy available approaches the amount of energy conserved, and hence the thermodynamic drive approaches zero. Even though acetate and sulfate remain in finite concentrations, sulfate reduction rates here approach zero because of the small thermodynamic drive. If we had not accounted for the requirement of cellular energy conservation, we would have arrived at different results. When the thermodynamic term is omitted from Equation 3, the calculation predicts that, contrary to observation, sulfate would disappear at depths below 12 cm (broken line in Fig. 3).

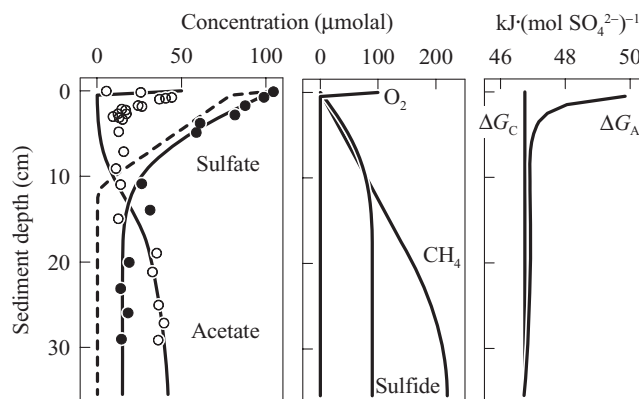
DISCUSSION

Results of this study help resolve an apparent contradiction observed in the laboratory and natural environment: why microbial sulfate reduction fails to proceed actively where supplies of electron donor and sulfate remain unreacted. Sulfate reducers capture the energy they need for growth and cell maintenance by coupling the sulfate reduction reaction to the synthesis of ATP in their cytoplasm. This requirement of cellular energy conservation diminishes the reaction's thermodynamic drive, influencing the rate of sulfate reduction. Where the energy available matches the amount conserved in the cell, the thermodynamic drive disappears, and, as a consequence, microorganisms stop catalyzing the reaction.

This theoretical framework, laboratory experiments by ourselves and others, and field observations all portray microbial sulfate reduction as a process that ceases when available energy falls within a certain range. From their physiology, we expect sulfate reducers to require between 33 and 47 kJ·(mol SO₄²⁻)⁻¹ of energy. In experiments, sulfate reduction stopped once energy available fell in the range 33–56 kJ·(mol SO₄²⁻)⁻¹. In addition, where sulfate reduction by diverse sulfate reducers is observed in natural environments to have ceased (Table 1), the energy available ranges from 40 to 54 kJ·(mol SO₄²⁻)⁻¹. The notable overlap in these ranges offers strong support to the idea that the availability of chemical energy, rather than simply the supply of electron donor and sulfate, limits the distribution and rate of sulfate reduction in the laboratory and in nature.

Calculations of sulfate reduction rates by conventional means can give misleading results because rate laws in common use, such as the dual-Monod equation, take no accounting of cellular energetics. Where little energy in excess of that required by the microbes is available in the

Figure 3. Variation in fluid composition, and energy available (ΔG_A) from reaction 1, in pore waters from lake-bottom sediments in central basin of Lake Washington (Washington, United States), plotted versus depth below sediment-water interface. Data points are chemical analyses for acetate and sulfate, as reported by Kuivila et al. (1989). Solid lines show results of reactive transport model accounting for diffusion and microbial activity; dashed line represents sulfate concentration predicted by model calculated without accounting for requirement of cellular energy conserved (ΔG_c took a value of 46.75 kJ·(mol SO₄²⁻)⁻¹).



¹GSA Data Repository item 2009259, detailed description of the reactive transport model, is available online at www.geosociety.org/pubs/ft2009.htm, or on request from editing@geosociety.org or Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301, USA.

environment, conventional analysis significantly overestimates the rate of sulfate reduction. More dramatically, rate laws in common use predict that a reaction will proceed in the presence of a negative thermodynamic drive, in which case, the cell would need to expend the internal energy stores on which it depends to remain alive, in order to catalyze sulfate reduction.

The ultimate control on the rate of sulfate reduction in a given environment, then, is not necessarily the kinetic factors considered in traditional analysis: the rate constant and the concentrations of electron donor and sulfate, relative to the respective half-saturation constants. Instead, the rate of sulfate reduction can be constrained by the rate at which chemical energy enters the local environment. Energy might be introduced by creation of electron donor species, through the fermentation of natural organic matter, for example, or by the influx of sulfate by diffusion or groundwater flow. The requirement of cellular energy conservation is a central, if commonly overlooked, factor controlling the rate of microbial sulfate reduction in the natural environment.

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