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THE THERMODYNAMIC LADDER IN GEOMICROBIOLOGY

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ABSTRACT. A tenet of geomicrobiology is that anaerobic life in the subsurface arranges itself into zones, according to a thermodynamic ladder. Iron reducers, given access to ferric minerals, use their energetic advantage to preclude sulfate reduction. Sulfate reducers exclude methanogens in the same way, by this tenet, wherever the environment provides sulfate. Examining usable energy—the energy in excess of a cell's internal stores—in subsurface environments, we find that in groundwater of near neutral pH the three functional groups see roughly equivalent amounts. Iron reducers hold a clear energetic advantage under acidic conditions, but may be unable to grow in alkaline environments. The calculations fail to identify a fixed thermodynamic hierarchy among the groups. In long-term bioreactor experiments, usable energy did not govern microbial activity. Iron reducers and sulfate reducers, instead of competing for energy, entered into a tightly balanced mutualistic relationship. Results of the study show thermodynamics does not invariably favor iron reducers relative to sulfate reducers, which in turn do not necessarily have an energetic advantage over methanogens. The distribution of microbial life in the subsurface is controlled by ecologic and physiologic factors, and cannot be understood in terms of thermodynamics alone.

Key words: Geomicrobiology, thermodynamics, iron reduction, sulfate reduction, methanogenesis, microbial redox processes, mutualism

INTRODUCTION

The concept of a thermodynamic ladder has over the past three decades almost indelibly colored the environmental scientist's view of the distribution of microbial activity in groundwater flows. Respiring microbes live by trapping some of the energy liberated when they catalyze the transfer of electrons from a reduced species such as acetate or dihydrogen to an oxidized species like dioxygen, nitrate, ferric iron, or sulfate. They use the energy they trap to carry out life functions such as cell maintenance, and to create biomass.

The initially reduced species consumed by a respirer is the electron donor, and the oxidized species is the acceptor. As they transfer electrons, microbes oxidize the donor species and reduce the acceptor, directly regulating the redox state and hence the quality and chemical properties of the groundwater in which they live. Geomicrobiologists and geochemists, for this reason, would like to understand more fully the factors controlling the distribution of microbial activity in natural environments, pristine and contaminated (for example, Banfield and Nealson, 1997; Chapelle, 2001; Kovacik and others, 2006; Heimann and others, 2010).

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The idea that microbial activity in aquifers is distributed according to an energetic hierarchy of electron accepting processes—a thermodynamic ladder—was formalized by Champ and others (1979). Noting that oxidation potential, as measured by platinum electrode, decreases along groundwater flow paths, they suggested that groundwater passes through a series of redox zones in which electron acceptance yields progressively less energy. Groundwater recharges an area of aerobic oxidation and denitrification, where chemical energy is abundant. Once $O_2(aq)$ and NO_3^- are depleted, water passes through a less energetically favored interval of iron and manganese reduction, they suggested, before entering a zone of sulfate reduction, a process that liberates comparatively little energy. About this time, Froelich and others (1979) proposed a thermodynamic ladder operates in marine sediments, as did Patrick and Henderson (1981), for soils.

Chapelle and Lovley (1992) used the thermodynamic ladder to explain the chemical zoning of groundwater in the Middendorf aquifer in South Carolina, USA, invoking the concept of competitive exclusion. By this idea, a functional group of microbes on a high rung of the ladder can maintain an advantage over the groups on lower rungs that compete for the same electron donor (Lovley and Phillips, 1987; Hoehler and others, 1998; Heimann and others, 2010). Iron reducers, for example, might drive down the concentration of acetate to a level sufficient to provide themselves energy, but too low for acetotrophic sulfate reducers to live.

Upstream in the Middendorf aquifer, a zone of non-marine sediments containing ferric iron holds groundwater rich in Fe^{2+}, interpreted to result from iron reducers working to the exclusion of sulfate reducers (Lovley and others, 1990). Downstream, where the aquifer is composed of marine sediments lacking ferric iron, and hence where iron reduction is not possible, a zone of iron-poor water is apparently dominated by sulfate reducers. Water flows next into a zone depleted in sulfate where methane accumulates. Lacking competition from iron and sulfate reducers, methanogens thrive here (Chapelle and Lovley, 1992). Following the groundbreaking work on the Middendorf, the idea of competitive exclusion has been applied broadly to explain geochemical zoning in pristine and contaminated aquifers (fig. 1; Lovley and others, 1994; Lovley and Chapelle, 1995; Watson and others, 2003; Cozzarelli and Weiss, 2007; McMahon and Chapelle, 2008; Canfield and Thamdrup, 2009; Chapelle and others, 2009; Heimann and others, 2010).

In this paper we consider the distribution of energy in confined aquifers in light of the requirements of energy capture by anaerobic organisms. Specifically, we calculate

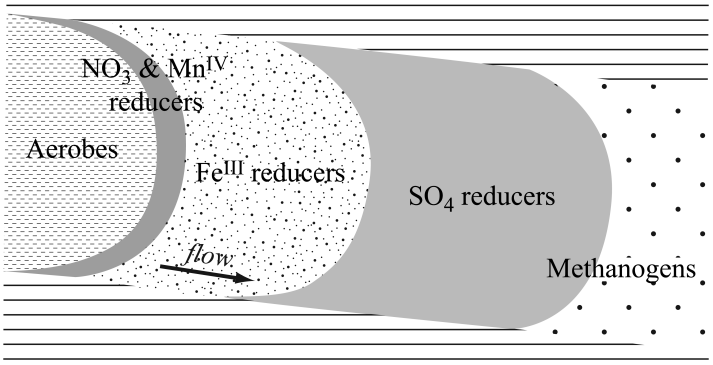

Fig. 1. Microbiological zoning as envisioned to occur in a pristine aquifer, showing arrangement of zones according to a thermodynamic ladder of electron accepting processes, after Lovley and others (1994).

not only the energy available to various functional groups in anoxic groundwater, but the usable energy, the portion of the available energy microbes can take advantage of to drive their metabolisms. We calculate how for the various groups the usable energy changes with water composition, and how it affects microbial reactions. Finally, we consider in detail how the distribution of usable energy affects microbial activity in long-term bioreactor experiments, and how it might control the microbial ecology of the subsurface.

We formalize our discussion in terms of confined aquifers transmitting pristine groundwater. Much of the consideration herein nonetheless might be generalized to apply to other zoned biogeochemical environments, such as contaminated aquifers (for example, Bekins and others, 1999; McGuire and others, 2002), river and lake sediments (Ingvorsen and Brock, 1982; Conrad and others, 1987; Christensen and others, 1989; Kuivila and others, 1989), marine sediments (Canfield and others, 1993; Murray and others, 1995), microbial mats (Canfield and Des Marais, 1993; Minz and others, 1999; Hoehler and others, 2001; Fike and others, 2008), and soils and wetlands (Patrick and Jugsujinda, 1992; Roden and Wetzel, 2003; Baez-Cazull and others, 2008; Kocar and Fendorf, 2009).

Our focus is the lowermost rungs on the ladder. Aerobes live only in oxic waters, commonly found near the surface, in unconfined aquifers. Nitrate is produced in soils by nitrifying bacteria, but because it is in biologic demand there, little escapes to the realm of deep groundwater. Where nitrate is found in groundwater, it is commonly due to pollution from agriculture, or by sewage (for example, Bohlke, 2002, 2003). Oxyanions of arsenic and selenium such as $HAsO_4^{2-}$ and SeO_4^{2-} are effective environmental oxidants (Stolz and Oremland, 1999; Rittmann and McCarty, 2001), but again we are concerned with uncontaminated groundwater. Manganese oxides, also high on the ladder, are quickly depleted from aquifers under anoxic conditions. Manganese is less abundant than iron in sediments and, where in contact with groundwater, its oxides reduce spontaneously to Mn^{2+} by abiotic reaction with Fe^{2+} as well as H_2S (Burdige and Nealson, 1986; Lovley and Phillips, 1988; Postma and Appelo, 2000). We are, then, most interested here in the least energetically favored of the functional groups: the iron reducers, sulfate reducers, and methanogens.

MICROBIAL CATALYSIS

A functional group of microorganisms is a collection of microbes that trap energy released by a specific reaction, such as the oxidation of acetate by sulfate. The various organisms that make up a group might each catalyze the full reaction, or different organisms may catalyze specific steps in the overall reaction, together forming a symbiotic community. A respiring microbe's net reaction is an electron donating half-cell reaction coupled to an electron accepting half-reaction. Acetoclastic methanogenesis is likewise the stoichiometric sum of two half-cell reactions, as described below, but in this case no interspecies electron transfer is involved. Table 1 shows various donating and accepting half-reactions important in the subsurface.[1]

The electron donors available to respiring and acetoclastic organisms in aquifers are the simple reduced species, primarily the acetate (CH_3COO^-) and dihydrogen [$H_2(aq)$] produced in the subsurface as organic matter ferments (fig. 2; see for example, Postma and Jakobsen, 1996). Oxidation of these species by acetotrophy and hydrogentrophy can be coupled to a number of electron accepting processes, as listed in table 1. Combining the half-reactions for acetotrophy and hydrogentrophy with

[1] Reactions in this paper are balanced on an eight electron basis, corresponding to the consumption of one acetate or four dihydrogens, and all thermodynamic quantities cited correspond to this convention.

TABLE 1

Electron accepting and donating processes arranged from most to least favored, as defined by free energy change[a] in a nominal anoxic geochemical environment[b]

Electron donating half-reactions		ΔG_{don} (kJ mol^{-1})
Acetotrophy	$CH_3COO^- + 4\,H_2O \rightarrow 2\,HCO_3^- + 9\,H^+ + 8\,e^-$	–216
Hydrogentrophy	$4\,H_2(aq) \rightarrow 8\,H^+ + 8\,e^-$	–185
Electron accepting half-reactions		ΔG_{acc} (kJ mol^{-1})
Denitrification	$8\,e^- + \frac{8}{5}\,NO_3^- + \frac{48}{5}\,H^+ \rightarrow \frac{4}{5}\,N_2(aq) + \frac{24}{5}\,H_2O$	–550
Mn^{IV} reduction[c]	$8\,e^- + 4\,MnO_2 + 16\,H^+ \rightarrow 4\,Mn^{2+} + 8\,H_2O$	–417 to –383
Birnessite red'n	$8\,e^- + \frac{2}{3}\,Mn_8O_{14}\cdot 5H_2O + \frac{56}{3}\,H^+ \rightarrow \frac{16}{3}\,Mn^{2+} + \frac{38}{3}\,H_2O$	–385
Mn^{III} reduction[d]	$8\,e^- + 8\,MnOOH + 24\,H^+ \rightarrow 8\,Mn^{2+} + 16\,H_2O$	–347 to –333
Ammonification	$8\,e^- + NO_3^- + 10\,H^+ \rightarrow NH_4^+ + 3\,H_2O$	–297
$Fe(OH)_3$ red'n[e]	$8\,e^- + 8\,Fe(OH)_3 + 24\,H^+ \rightarrow 8\,Fe^{2+} + 24\,H_2O$	–4 to 96
Sulfate reduction	$8\,e^- + SO_4^{2-} + 9\,H^+ \rightarrow HS^- + 4\,H_2O$	150
Goethite red'n	$8\,e^- + 8\,FeOOH + 24\,H^+ \rightarrow 8\,Fe^{2+} + 16\,H_2O$	155
Methanogenesis	$8\,e^- + HCO_3^- + 9\,H^+ \rightarrow CH_4(aq) + 3\,H_2O$	184
Magnetite red'n	$8\,e^- + 4\,Fe_3O_4 + 32\,H^+ \rightarrow 12\,Fe^{2+} + 16\,H_2O$	231

[a] Free energies in this paper were derived from the LLNL thermodynamic dataset (Delany and Lundeen, 1989), with entries for soil ferrihydrite from Lindsay (1979) and geothite from Bigham and others (1996).
[b] 25 °C; pH 7; 1 mmol kg^{-1} Ca^{2+}, $CO_2(aq)$ + HCO_3^-, SO_4^{2-}, NO_3^-, Fe^{2+}, and Mn^{2+}; 1 μmol kg^{-1} CH_3COO^-, $CH_4(aq)$, HS^-, and NH_4^+; 1 nmol kg^{-1} $H_2(aq)$; $N_2(aq)$ at atmospheric saturation.
[c] Energies cited depend on whether Mn^{IV} is supplied by γ-MnO_2 or pyrolusite (β-MnO_2).
[d] For Mn^{III} from manganite (γ-MnOOH) or bixbyite (Mn_2O_3).
[e] For Fe^{III} supplied by freshly precipitated $Fe(OH)_3$ or soil ferrihydrite.

electron accepting half-reactions from this list gives the net reactions for the various functional groups, as shown in table 2.

Acetotrophic sulfate reducers, for example, are respiring microbes that catalyze electron transfer from acetate to sulfate ions, producing carbonate and sulfide species (for example, Muyzer and Stams, 2008). Combining the half-reaction from table 1 for acetotrophy with that for sulfate reduction gives

$$CH_3COO^- + SO_4^{2-} \rightarrow 2\,HCO_3^- + HS^- \qquad (1)$$

which is the group's net reaction. Iron reducing bacteria can use a variety of minerals, including ferric oxides and oxyhydoxides and Fe^{III}-bearing clays, as an oxidant. The net reaction

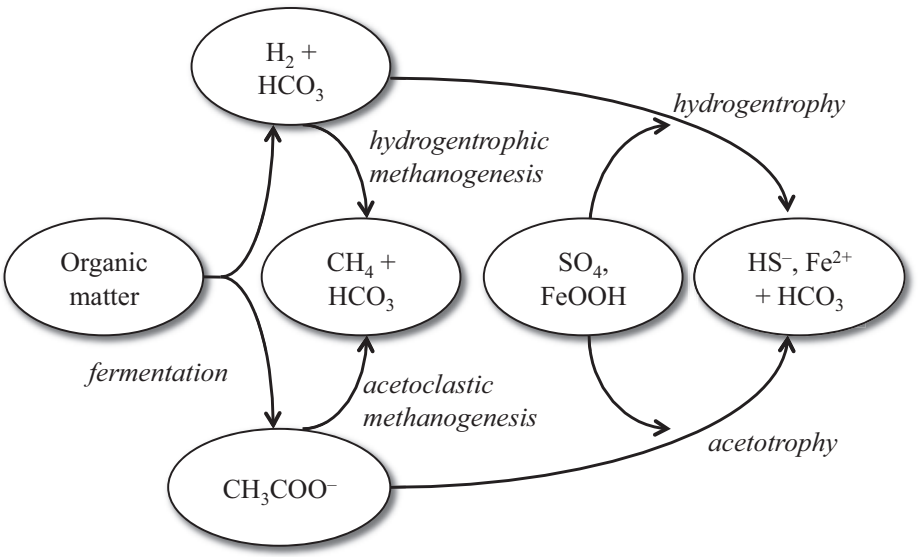

Fig. 2. Schematic diagram of the degradation and oxidation of natural organic matter in the subsurface, showing the principal microbial processes considered in this study. Fermenting microorganisms degrade natural organic matter to bicarbonate (HCO_3^-), dihydrogen (H_2), and simple organic compounds, such as acetate (CH_3COO^-). Hydrogentrophic and acetoclastic methanogens produce methane (CH_4) from these compounds, whereas sulfate reducers and iron reducers oxidize them by respiration. Diagram does not show fermentation products of secondary importance, such as formate and lactate, and omits various known microbial processes, including autotropic acetogenesis, anaerobic acetate oxidation, and methanotrophy, by which mass can cycle among the reservoirs shown.

$$CH_3COO^- + 8\ FeOOH(s) + 15\ H^+ \rightarrow 8\ Fe^{2+} + 2\ HCO_3^- + 12\ H_2O \quad (2)$$

is the half-reaction for acetotrophy linked to electron acceptance by goethite (FeOOH).

Acetoclastic methanogens derive energy from acetoclastis, the dismutation of acetate

$$CH_3COO^- + H_2O \rightarrow CH_4(aq) + HCO_3^- \quad (3)$$

as a result of cleaving the molecule's carbon-carbon bond. One of the carbon atoms goes to form methane and the other to carbonate, so acetate serves in the reaction as electron donor as well as acceptor. Acetoclastis (Reaction 3) is the stoichiometric sum of the half-reaction for acetotrophy combined with that for methanogenesis.

The electron donating half-reactions produce hydrogen ions and the accepting half-reactions consume them (table 1). Acetotrophy produces 9 H^+, or 8 below pH 6.3, where the reaction produces more $CO_2(aq)$ than HCO_3^- and hydrogentrophy produces 8 H^+. Sulfate reduction consumes about the same number of H^+ as acetotrophy and hydrogentrophy produce: 9 where pH > 7, or 10 under acidic conditions, where more sulfide is present as $H_2S(aq)$ than HS^-. Similarly, methanogenesis consumes 9 H^+, or 8 below pH 6.3. As a result, the net reactions for the sulfate reducers and methanogens involve few hydrogen ions and hence depend weakly, if at all, on pH. Electron acceptance by the Mn^{III}, Mn^{IV}, and Fe^{III} oxides and oxyhydroxides, in contrast, consumes from 16 to 24 H^+, and magnetite reduction (Kostka and Nealson, 1995) consumes 32 H^+. The net reactions for manganese and iron reduction, then, are strongly acid consuming and highly pH-dependent.

TABLE 2

Net reactions for microbial metabolisms occupying low rungs on the thermodynamic ladder, the reactions' available and usable energies and thermodynamic potential factor in a nominal geochemical environment[a]

Acetotrophy		ΔG_A (kJ mol^{-1})	ΔG_U (kJ mol^{-1})	F_T
Goethite red'n	$CH_3COO^- + 8\,FeOOH + 15\,H^+ \rightarrow 8\,Fe^{2+} + 2\,HCO_3^- + 12\,H_2O$	61	5	.22
SO_4 reduction	$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2\,HCO_3^-$	66	21	.76
Methanogenesis	$CH_3COO^- + H_2O \rightarrow CH_4(aq) + HCO_3^-$	32	21	.99
Magnetite red'n	$CH_3COO^- + 4\,Fe_3O_4 + 23\,H^+ \rightarrow 12\,Fe^{2+} + 2\,HCO_3^- + 12\,H_2O$	–15	–70	0
Hydrogentrophy				
Goethite red'n	$4\,H_2(aq) + 8\,FeOOH + 16\,H^+ \rightarrow 8\,Fe^{2+} + 16\,H_2O$	30	–15	0
SO_4 reduction	$4\,H_2(aq) + SO_4^{2-} + H^+ \rightarrow HS^- + 4\,H_2O$	35	–10	0
Methanogenesis	$4\,H_2(aq) + HCO_3^- + H^+ \rightarrow CH_4(aq) + 3\,H_2O$	1	–10	0
Magnetite red'n	$4\,H_2(aq) + 4\,Fe_3O_4 + 24\,H^+ \rightarrow 12\,Fe^{2+} + 16\,H_2O$	–46	–91	0

[a] Environmental conditions are defined in footnote to table 1. Usable energies and thermodynamic factors calculated from ATP numbers m and average stoichiometric numbers χ in table 3, taking ΔG_P to be 45 kJ mol^{-1}.

AVAILABLE ENERGY

The energy ΔG_A available to a functional group in its environment is the free energy liberated by the group's net reaction. This quantity is the negative of ΔG_r, the reaction's free energy change. Since ΔG_r is the sum $\Delta G_{don} + \Delta G_{acc}$ of the energies for the donating and accepting half-reactions, the available energy is given

$$\begin{aligned} \Delta G_A &= -\Delta G_r \\ &= -\Delta G_{don} - \Delta G_{acc}. \end{aligned} \tag{4}$$

Table 1 shows ΔG_{don} and ΔG_{acc} calculated for various half-reactions in a nominal geochemical environment, arranged in order of decreasing energy yield. By equation (4), the more negative the free energy ΔG_{acc} of an electron accepting half-reaction, the more energy is available to microorganisms. Arranging the electron accepting processes in reverse order of ΔG_{acc} produces the environment's thermodynamic ladder, as shown in table 1.

The energies in table 1 represent conditions in a nominal environment composed of a pH 7 groundwater in contact at 25 °C with iron and manganese oxyhydroxide minerals. The water contains 1 mmol kg^{-1} dissolved carbonate, sulfate, nitrate, Fe^{II}, and Mn^{II}; 1 μmol kg^{-1} acetate, methane, sulfide, and ammonium; and 1 nmol kg^{-1} dihydrogen. The calculations account for mineral solubility, species' activity coefficients, and the distribution of dissolved mass. Of most direct importance, it distributes carbonate among the species CO_2(aq), HCO_3^-, CO_3^{2-}, and their ion pairs; sulfide to H_2S(aq) and HS^-; and metals to their free ions and hydroxy complexes.

The energies were computed from the LLNL thermodynamic dataset (Delany and Lundeen, 1989), with data added from Lindsay (1979) for soil ferrihydrite and Bigham and others (1996) for goethite, using version 8.0.10 of The Geochemist's Workbench® software (Bethke, 2008).[2] We consider later in this paper how the energies vary with fluid composition. It is not necessary to be overly concerned at this point with the precise chemical composition we assign to our nominal environment, since free energy varies with the logarithm of species activity. Changing acetate concentration by a full order of magnitude, for example, affects the free energy for acetotrophy by just $\pm 2.303\ RT_K$. The gas constant R is 8.314 J mol^{-1} K^{-1}, and taking absolute temperature T_K as 298 K, this quantity amounts to only ± 5.7 kJ mol^{-1}.

Under the geochemical conditions considered in table 1, ΔG_{don} for acetotrophy is -216 kJ mol^{-1}, and ΔG_{acc} for sulfate reduction is 150 kJ mol^{-1}. In our nominal environment, then, 66 kJ is available by reaction (1) to sulfate reducers, per mole of acetate consumed (table 2). As a second example, the energy available to acetoclastic methanogens by reaction (3) can be calculated from the free energies for acetotrophy and methanogenesis. For the acetoclasts, $\Delta G_A = 216 - 184$, or 32 kJ per mole of acetate consumed. Table 2 shows the energies available in our nominal environment for net reactions central to discussion in this paper.

The energy available to manganese and iron reducers depends on the mineralogic form the metal takes in the aquifer (for example, Curtis, 2003; Larsen and others, 2006), as shown in table 1. Metal oxides and oxyhydroxides in contact with groundwater are primarily the products of sediment weathering and of the subsurface oxidation of dissolved Mn^{2+} and Fe^{2+} (for example, van der Zee and others, 2003). The minerals commonly form as hydrous, disordered precipitates, but over time they ripen and re-precipitate into progressively more stable phases (for example, Banfield and others, 2000; Zachara and others, 2002; Cornell and Schwertmann, 2003; Hansel and others, 2003; Yee and others, 2006), rendering them less energetically suitable as electron

[2] The GWB input files used in this study are available in the Supplemental Appendix.

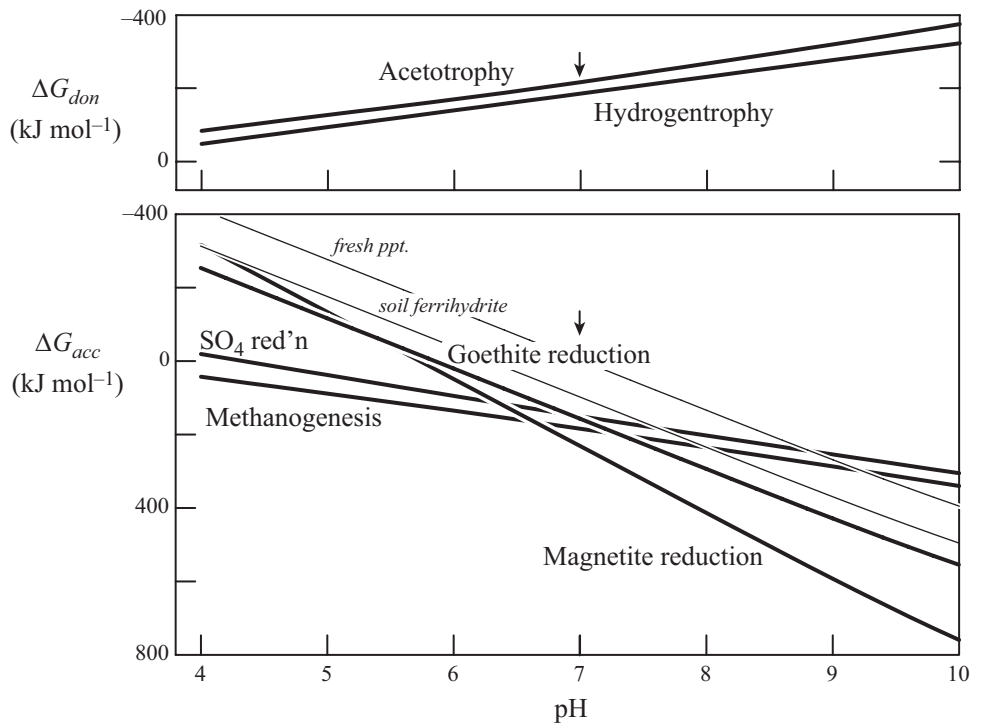

Fig. 3. Free energy change of various electron donating (ΔG_{don}, top) and accepting (ΔG_{acc}, bottom) half reactions important to microbial metabolisms in pristine aquifers, as a function of pH. The vertical axes of the plots are reversed, so the most energetically favorable reactions fall toward the top. Calculation assumes the nominal geochemical environment, except pH (arrows show nominal value), listed in footnote to table 1.

acceptors. In the case of ferric iron, where the metal is present in fresh form as soil ferrihydrite [$Fe(OH)_3$], iron reduction is favored in our nominal environment over sulfate reduction (table 1). But goethite (α-FeOOH) is probably a better proxy than soil ferrihydrite for the ferric iron in most aquifers (Cutting and others, 2009), and in our discussions in this paper we will refer to this mineral. In this case, iron reducers in our nominal environment lose their advantage over sulfate reducers, or even over the methanogens. Magnetite (Fe_3O_4) yields in this environment the least energy of the acceptors considered.

Figure 3 shows how the various donating and accepting processes contribute to available energy, calculated as a function of pH. The donating processes contribute hydrogen ions, as noted in the previous section, and the processes of methanogenesis and sulfate reduction consume them in approximately equal numbers. The contributions $-\Delta G_{don}$ of acetotrophy and hydrogentrophy increase with pH, then, about as rapidly as $-\Delta G_{acc}$ for those acceptors decreases. There is little net effect of pH on available energy for the methanogens and sulfate reducers. The energies liberated by metal reducing half-reactions, in contrast, decrease along a much steeper slope, reflecting the fact that they are highly acid consuming (table 1). Under conditions more acidic than about pH 6.5 to 7, goethite reduction is favored over sulfate reduction and methanogenesis, and below pH 5.3, magnetite yields more energy than either of those minerals. The converse is true in alkaline groundwater: sulfate reduction and methanogenesis yield more energy there than iron reduction.

USABLE ENERGY

As a microbe catalyzes its net reaction, it traps a portion of the energy liberated and stores it within its cytoplasm. It captures the energy by phosphorylating adenosine diphosphate, or ADP,

$$ADP^{2-} + H_2PO_4^- \rightarrow ATP^{3-} + H_2O \qquad (5)$$

to form the metastable molecule adenosine triphosphate, ATP. The ATP serves as the cell's primary store of chemical energy. If a cell creates m ATPs per turnover of the net reaction and ΔG_P is the free energy change of ATP synthesis (reaction 5), the amount of energy captured is $m \times \Delta G_P$. The microbe uses this energy to perform its life functions: synthesizing biomolecules, maintaining its biomass, reproducing, and so on.

The remaining portion of the energy liberated, the part not captured by the cell, provides the thermodynamic drive for the cell's metabolism (Jin and Bethke, 2002). This quantity, which we refer to as usable energy ΔG_U, drives forward the microbe's net reaction (reaction 1, 2, or 3, for example) coupled to ATP synthesis (reaction 5). In the absence of usable energy, the coupled reaction cannot proceed and no ATP can be produced. The cell in this case would not be able to derive energy from its environment, and hence could live only in a dormant state.

Put another way, a microbe can run its metabolism only where the energy available outside the cell exceeds its internal store (Jin and Bethke, 2009). The usable energy ΔG_U, then, is the difference between the energy available in an environment and that maintained by a cell; it is given from the available energy ΔG_A by

$$\Delta G_U = \Delta G_A - m\Delta G_P \qquad (6)$$

(Jin and Bethke, 2002, 2003, 2005, 2007). Under environmental conditions, ΔG_P within the cytoplasm is about 45 kJ (mol ATP)$^{-1}$ (Thauer and others, 1977; Schink, 1997; Jin and Bethke, 2009).

The ATP number m for a microorganism can be determined from knowledge of its physiology, when the microbe's respiratory chain has been studied in detail (Jin and Bethke, 2007). Alternatively, the number can be estimated by identifying laboratory experiments or natural environments where respiration has ceased. In this case, m is the energy remaining, in ratio to ΔG_P (Jin and Bethke, 2003, 2009). ATP numbers vary somewhat among organisms within a functional group, and a microbe may be able to adjust its ATP yield to some extent, depending on its environment (Jin and Bethke, 2003). Table 3 shows values of m we take as representative of various functional groups under environmental conditions. Iron reducers and sulfate reducers, by these data, capture per mol of electron donor consumed more energy than methanogens. But by capturing less energy than the other groups, as we can see from equation (6), the methanogens better drive forward their metabolism where available energy is limited. Since $m = \frac{5}{4}$ for acetotrophic iron reducers, but only $\frac{1}{4}$ for methanogens, the latter can live where available energy is $(\frac{5}{4} - \frac{1}{4}) \times 45 = 45$ kJ mol^{-1} less than needed to sustain the former.

Table 2 lists the usable energies for various functional groups under the nominal environmental conditions, and figures 4 and 5 show how the energies vary with pH and groundwater composition. Acetoclastic methanogenesis and acetotrophic sulfate reduction see nearly equivalent usable energies over a broad range in pH (fig. 4). In neutral and alkaline water, ΔG_U does not vary with pH, because H^+ does not appear in the net reactions for these groups (table 2). Below pH 6.3, acetoclastis is slightly favored as pH decreases, because the net reaction written in terms of CO_2(aq) consumes one H^+. Likewise, for acetotrophic sulfate reduction, usable energy increases as water trends acidic, since carbonate is present as CO_2(aq) and sulfide as H_2S(aq). The reaction

$$CH_3COO^- + SO_4^{2-} + 3\ H^+ \rightarrow 2\ CO_2(aq) + H_2S(aq) + 2\ H_2O \qquad (7)$$

TABLE 3

Representative ATP numbers and average stoichiometric numbers assumed in calculations, for various functional groups of anaerobic microbes[a,b]

	m	χ
Ac-trophic Fe^{III} reducers	$\frac{5}{4}$	8
H_2-trophic Fe^{III} reducers	1	8
SO_4 reducers	1	6
Methanogens	$\frac{1}{4}$	2

[a] Expressed per mol turnover of reactions written in terms of consumption of one acetate or four H_2.
[b] Compiled as described in Jin and Bethke (2009) from references therein as well as Thauer and Badziong (1981).

consumes 3 H^+ (or 2 past pH 4.7, where acetate is mostly acetic acid, CH_3COOH), yielding increasing energy with decreasing pH.

The hydrogentrophic reactions for methanogenesis and sulfate reduction consume one H^+ (table 2), or two H^+ for the sulfate reducers below pH 5.7. As such, they yield somewhat less usable energy as pH rises (fig. 4). Under the nominal conditions chosen, neither group sees positive usable energy. Sulfate reduction, however, becomes energetically favored at low pH, and both groups are favored where $H_2(aq)$ concentration exceeds about 3 nmol kg^{-1} (fig. 5). As well, the sulfate reducers gain usable energy as sulfate concentration rises, and the methanogens benefit from decreasing methane levels. The environmental range of the hydrogentrophs, therefore, is somewhat more limited than that of the acetotrophs, as would be expected from the free energy changes of the electron donating reactions (fig. 3).

Iron reduction, in contrast to the other groups, is promoted strongly by acid (table 2). Acetotrophic and hydrogentrophic reduction of goethite can proceed in our nominal groundwater where pH is less than about 6.8 to 7. Below about pH 6.5, goethite reducers attain a thermodynamic advantage of tens to hundreds of kJ mol^{-1} over sulfate reducers and methanogens (see also Postma and Jakobsen, 1996). Under alkaline conditions, conversely, there is no usable energy in the environment to drive goethite reduction and the process cannot proceed. Near neutral pH, there is a striking convergence of the usable energies for iron reducers, sulfate reducers, and methanogens.

BIOREACTOR EXPERIMENTS

We analyzed the results of long-term bioreactor experiments to better understand how sulfate reducers, iron reducers, and methanogens interact in environments limited in energy. For each functional group we calculated how usable energy and reaction rate varied over the course of experiments conducted by Kirk (ms, 2008) and Kirk and others (2010).[3] They inoculated 600 ml of synthetic groundwater in each of three semi-continuous flow reactors with 3 g of fine grained sediment from an alluvial aquifer, along with its natural microbial consortium; a fourth reactor inoculated with autoclaved sediment was maintained as a sterile control. Each reactor had an internal volume of one liter and was held under anaerobic conditions. The water was oxygen-

[3] See Supplemental Appendix.

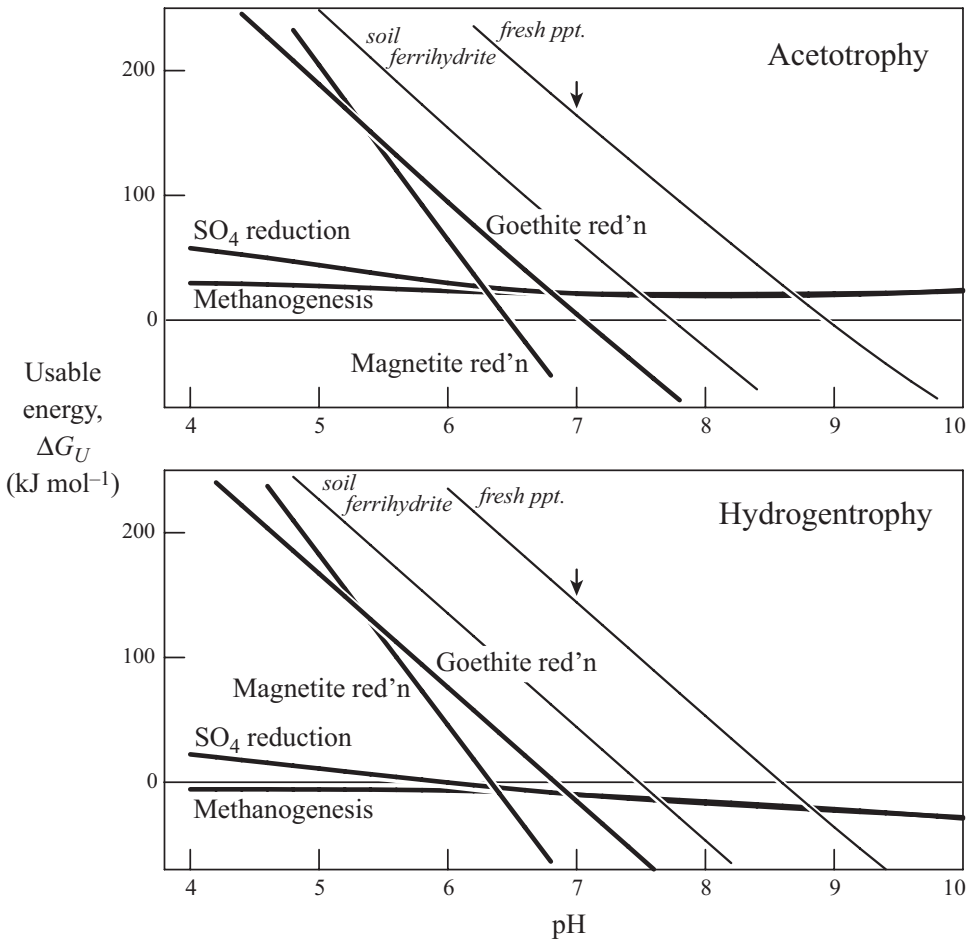

Fig. 4. Usable energy available to acetotrophic (top) and hydrogentrophic (bottom) iron reducers, acting on various ferric minerals, and sulfate reducers, and to acetoclastic and hydrogentrophic methanogens, as functions of pH. Calculation assumes the nominal geochemical environment, except pH (arrows show nominal value). A functional group's metabolism can proceed only where ΔG_U is positive.

free, initially sterile, and buffered to pH 7.3. As detailed in table 1 of Kirk and others (2010), the water contained: background electrolytes; 0.8 mmol kg^{-1} acetate, to serve as the electron donor; arsenate, the subject of the original study; and small amounts of NH_4Cl, minerals, and vitamins, to facilitate microbial growth.

Every seven days Kirk and others removed one-third of the fluid from each reactor, reserved it for immediate chemical analysis, and replaced it with fresh synthetic groundwater. They sampled gas in the headspace of each experiment every two to eight weeks. From the partial pressure of methane and Henry's law, they calculated the concentration of dissolved CH_4. The reactors were agitated after each sampling event, to promote thorough mixing.

Experiments

In one experiment, the water contained 1.1 mmol kg^{-1} sulfate as the electron acceptor for sulfate reducers. Water used in a second experiment was free of sulfate,

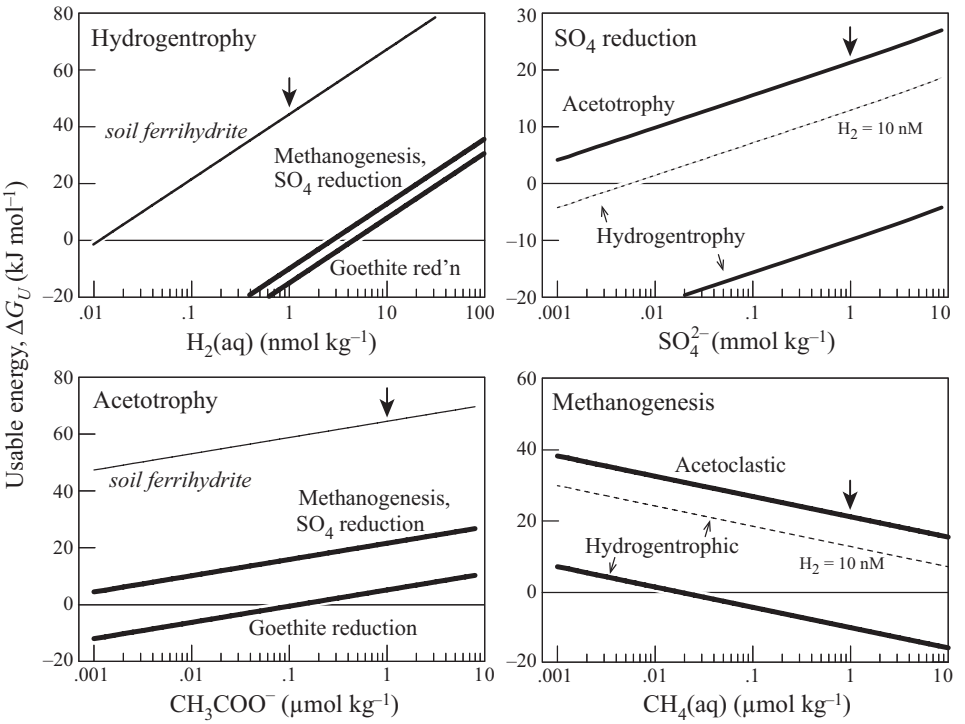

Fig. 5. Variation with groundwater composition in the energy usable by various functional groups of microorganisms, depending total concentrations of dissolved dihydrogen, sulfate, acetate, and methane. Arrows show positions of the nominal environment. Broken lines show usable energies for hydrogentrophic sulfate reducers and methanogens, when the H_2(aq) concentration is taken as 10 nmol kg^{-1}, rather than 1 nmol kg^{-1}. To live, microbes require positive amounts of usable energy in their environment.

but Kirk and others added 6 mmol of sterile, freshly precipitated FeOOH to the reactor at the onset of the run, to allow iron reduction. The FeOOH was identified by x-ray diffraction as goethite. A third experiment contained 1.1 mmol kg^{-1} sulfate in the feed water as well as FeOOH in the sediment, providing for both sulfate and iron reduction. We refer to the three configurations, respectively, as the SO_4-only, FeOOH-only, and FeOOH+SO_4 experiments.

Acetate could be oxidized by sulfate reducing bacteria in the SO_4-only and FeOOH+SO_4 experiments, and by iron reducing bacteria in the FeOOH-only and FeOOH+SO_4 experiments. Methanogenesis was possible in each of the experiments, since acetoclasts require only acetate and water to derive energy. Kirk and others (2010) maintained the reactors for about 300 days, but after 216 days they altered the feed composition in the FeOOH+SO_4 experiment for reasons unrelated to this study, and results past that point are not reported here.

Usable Energy and Reaction Rate

We computed from the chemical analyses of the water and gas samples[4] the usable energies available to iron reducers, sulfate reducers, and methanogens over the course of the experiments, at the beginning and end of each reaction interval. The energies were determined as elsewhere in this paper, using The Geochemist's Workbench®

[4] See Supplemental Appendix.

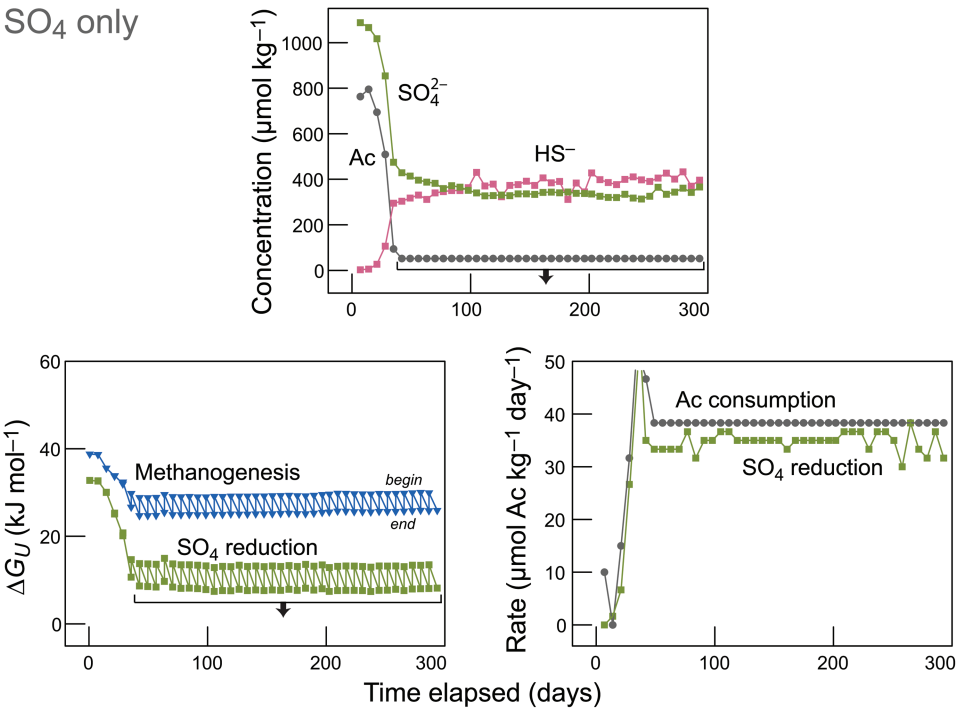

Fig. 6. Results of a long-term experimental study of microbial growth in a semi-continuous flow reactor containing sulfate but not goethite (the SO_4-only experiment), plotted as a function of time. Figure shows: concentrations in solution of key species at the end of each reaction interval, the usable energy for acetoclastic methanogenesis and acetotrophic sulfate reduction at the beginning and end of each interval, and the rates of acetate comsumption and sulfate reduction. Brackets delimit where acetate concentration falls below the detection limit of about 50 μmol kg^{-1}. Here, the usable energies are upper bounds calculated using the limiting concentration. As described in text, the bounding values are significant in a relative but not absolute sense.

software, the parameters in table 3, and the stability of goethite determined by Bigham and others (1996).

Where acetate concentration fell below detection, we report the energy corresponding to the nominal detection limit of 50 μmol kg^{-1} (3 mg kg^{-1}), and mark it as a maximal bounding value. Significantly, each chemical reaction considered is written in terms of the consumption of one acetate. For this reason, the relative values of energy calculated this way are meaningful, whether an absolute energy is stated, or just a limiting value. Where the usable energy for one group is reported as $<$20 kJ mol^{-1}, for example, and that for another is $<$10 kJ mol^{-1}, the first group would be expected to find 10 kJ mol^{-1} more usable energy in its environment than the second.

We also calculated the overall rate of acetate consumption, the rates at which the sulfate reducers and iron reducers consumed acetate, and approximate reaction rates for the acetoclastic methanogens. We determined the rates (mol Ac consumed kg^{-1} day^{-1}) from the amounts of reactant and product species in the unreacted water and their concentrations in the reactors at the beginning and end of each reaction interval, as described in the Appendix.

Results

Figures 6, 7 and 8 show how concentrations of key species, usable energy, and reaction rates vary over the courses of the three experiments. In the SO_4-only

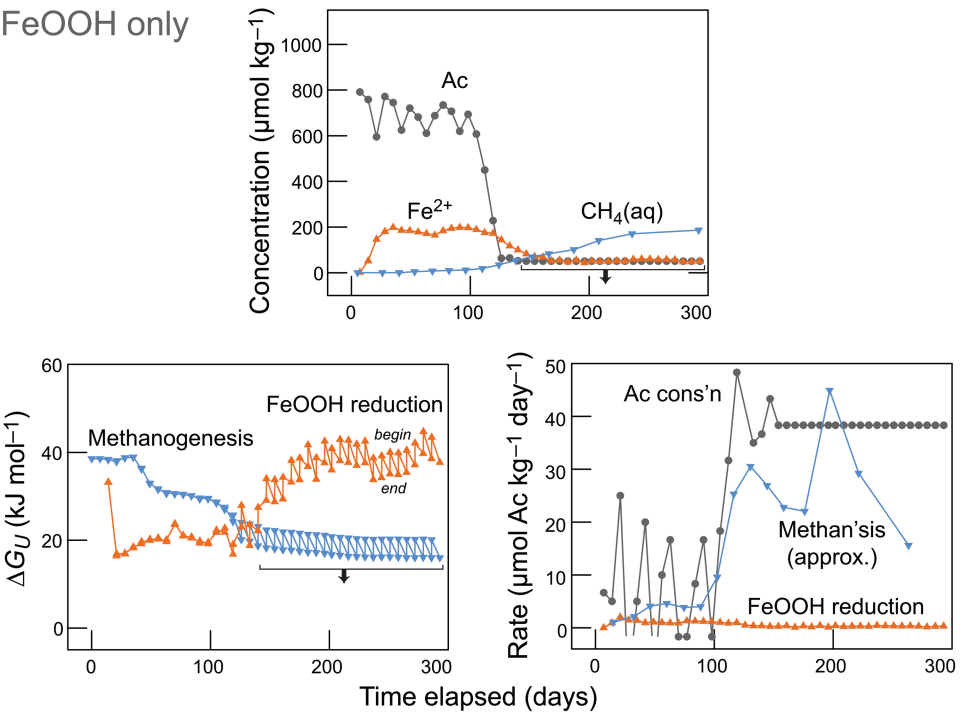

Fig. 7. Results of a long-term experimental study of microbial growth in a semi-continuous flow reactor containing goethite but not sulfate (the FeOOH-only experiment). Diagram is plotted as described in caption for figure 6.

experiment (fig. 6), sulfate and acetate concentrations decreased and dissolved sulfide increased for about a month, as the microbial community established itself in the reactor. After this interval, the community held acetate concentration below the detection limit, about 50 μmol kg^{-1}. About 4 μmol kg^{-1} of methane accumulated in the fluid. Once acetate fell below detection, the usable energy for the sulfate reducers and methanogens did not exceed about 14 kJ mol^{-1} and 30 kJ mol^{-1}, respectively. Throughout the experiment, the rate of sulfate reduction balanced that of acetate consumption. In light of this result and the small amount of methane in the reactor, acetoclastis in the experiment was unimportant. Consistent with this result, Oremland and Polcin (1982) found that sulfate reducers living on acetate in natural sediments can outcompete methanogens.

The FeOOH-only experiment (fig. 7) took longer than the first, about four months, to reach stable conditions. Acetate varied in concentration over the first 100 days, perhaps reflecting cyclic behavior of the microbial community, or an unknown issue with analysis for the compound. As in the previous case, acetate concentration eventually decreased to below detection. Ferrous iron accumulated in solution for about a month, then decreased in concentration as iron reduction slowed and unreacted replaced reacted water.

As Fe^{2+} was depleted from solution, methane began to accumulate, marking the onset of methanogenesis. In contrast to the other two experiments, almost 200 μmol kg^{-1} of dissolved methane built up in the fluid, and almost twenty times this much partitioned into the headspace. Methane production was rapid enough in the latter half of the experiment that at one point gas had to be vented from the reactor.

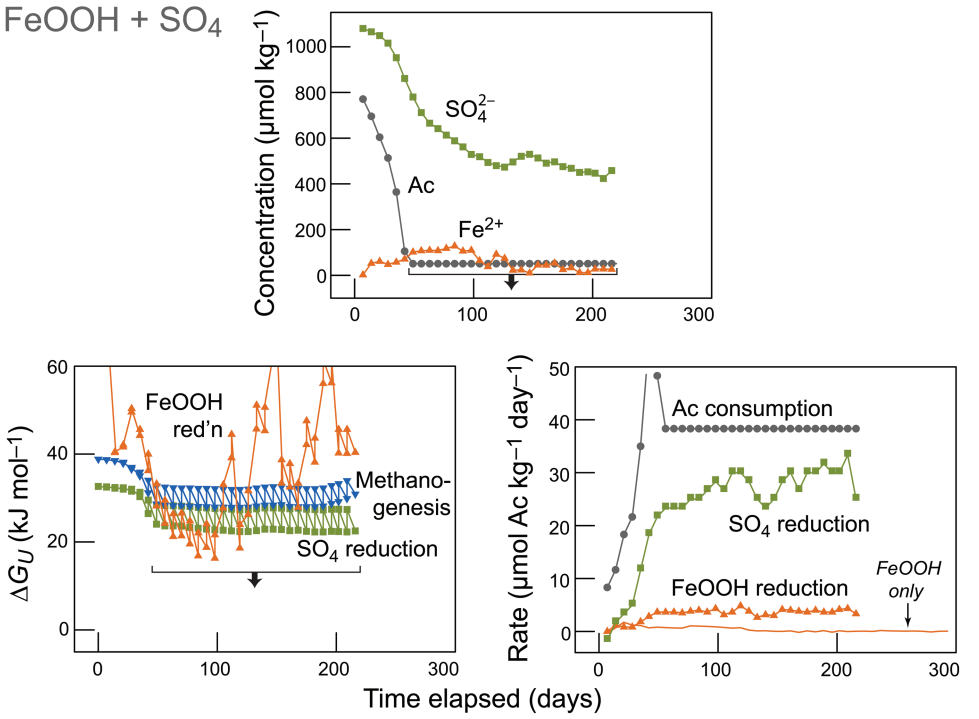

Fig. 8. Results of a long-term experimental study of microbial growth in a semi-continuous flow reactor containing goethite and sulfate (the FeOOH+SO_4 experiment). Diagram is plotted as described in caption for figure 6.

Usable energy for acetoclasts in the reactor fell with time to a level less than about 20 kJ mol^{-1}. The iron reducers, in contrast, saw little decline in usable energy, because the acetate and Fe^{2+} concentrations decreased roughly in tandem. By the time acetate fell below detection, iron reducers could have seen as much as 45 kJ mol^{-1} of usable energy. The rate of iron reduction increased initially, as the microbial community developed, but then declined gradually over the remainder of the experiment. Iron reducers accounted for little of the acetate consumed in the latter half of the run; most of the acetate was taken up by acetoclasts.

The FeOOH+SO_4 experiment (fig. 8) stabilized within about two months, after which the acetate concentration, as in the other experiments, dropped to below detection. Sulfate reducers accounted for the majority of the respiration in the experiment, oxidizing acetate about 6 to 9 times as rapidly as the iron reducers. Jakobsen and Postma (1999), for comparison, observed iron reduction occurring together with sulfate reduction in sediments from a sandy unconfined aquifer in Denmark. Iron reduction can be driven indirectly by sulfate reducers, if sulfide reacts spontaneously with goethite

$$4\ HS^- + 8\ FeOOH(s) + 20\ H^+ \rightarrow 4\ S(s) + 8\ Fe^{2+} + 16\ H_2O \qquad (8)$$

to produce elemental sulfur, S(s). This does not appear to be the case here, because zero-valent sulfur was not observed in the precipitate, and the amount of sulfate consumed balanced the sulfide mineral that formed. About 1 µmol kg^{-1} of CH_4(aq) built up in the reactor fluid, indicating a nearly complete lack of methanogenesis.

Mackinawite (FeS) precipitated over the course of the experiment from the Fe^{2+} liberated by the iron reducers and the HS^- generated by sulfate reduction (Kirk and others, 2010). Most of the Fe^{2+} and all of the detectable HS^- produced were taken up by the precipitation; only a small amount of Fe^{2+} remained in solution. As in the other experiments, usable energy for acetoclasts and sulfate reducers declined as acetate was depleted, falling to levels of no more than about 34 kJ mol^{-1} and 28 kJ mol^{-1}, respectively. Precipitation of the iron sulfide held Fe^{2+} concentration low, so the limiting values of usable energy for the iron reducers was at the end of the experiment most often higher than for the other two groups.

DISCUSSION

Thermodynamics exerts direct control on microbial catalysis in the subsurface. Functional groups of microbes live by catalyzing reactions that release usable energy, which is the chemical energy contained in the aquifer in excess of the microbes' internal stores (Jin and Bethke, 2005, 2007). In the absence of usable energy, where ΔG_U is zero, electrons pass forward and backward through a cell's transport chain at equal rates, precluding net reaction and preventing the cell from deriving energy from its environment. As ΔG_U trends positive, the ratio of forward to backward electron flow increases, providing a net positive reaction and thereby allowing the microbe to trap energy. For large enough ΔG_U, backward electron flow is negligible relative to forward flow and reaction proceeds unfettered by thermodynamics.

Thermodynamic Potential Factor

The thermodynamic potential factor F_T provides the quantitative link between the usable energy in an aquifer and the rate of microbial reaction there (Jin and Bethke, 2002, 2003, 2005, 2007). The factor varies with usable energy according to

$$F_T = 1 - \exp\left(-\frac{\Delta G_U}{\chi R T_K}\right) \tag{9}$$

where χ is the average stoichiometric number, the number of times the rate determining step, commonly trans-membrane proton translocation, occurs in the overall reaction. The stoichiometric number can be taken as the number of protons transferred by the most sluggish translocation step (Jin and Bethke, 2007).

A microorganism catalyzes its net reaction at a rate that varies in direct proportion to the reaction's F_T. Where $\Delta G_U = 0$, F_T by this equation is zero as well and microbial reaction ceases. For sufficiently large ΔG_U, in contrast, the exponential term vanishes and F_T approaches one. Microbial reaction in this case proceeds constrained by kinetic but not energetic limits. Figure 9 shows how F_T varies with ΔG_U, calculated using values of χ (table 3) representative of several functional groups of anaerobes.

There are, then, two factors that control the extent to which the energy ΔG_A available in an aquifer can support the activity of a functional group of microbes. The ATP number m accounts for the requirement that ΔG_A exceed the group's cellular energy stores. And the average stoichiometric number χ determines how much of this excess, the usable energy ΔG_U, is needed to create an effective thermodynamic drive for the group's net reaction. Both m and χ vary among the functional groups, as shown in table 3, so the groups' energetic requirements differ.

Taking m for the acetotrophic iron reducers to be $\frac{5}{4}$ and ΔG_P as 45 kJ (mol ATP)$^{-1}$, the group requires 56 kJ mol^{-1} of energy in the environment to balance its internal stores. The sulfate reducers ($m = 1$) need just 45 kJ mol^{-1} and the acetoclasts ($m = \frac{1}{4}$) require a ΔG_A of only 11 kJ mol^{-1}. Rearranging equation (9),

$$\Delta G_U = -\chi R T_K \ln(1 - F_T) \tag{10}$$

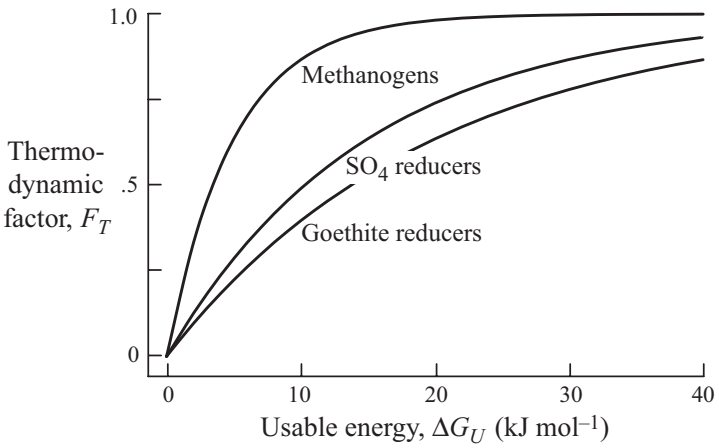

Fig. 9. Variation with usable energy ΔG_U of the thermodynamic potential factor F_T for iron reducers (χ = 8), sulfate reducers (χ = 6), and methanogens (χ = 2), calculated according to equation (9). Each functional group in a given environment encounters a distinct usable energy, depending on its overall metabolic reaction.

we can figure from the data in table 3 how much usable energy a group needs to run its metabolism unhindered by thermodynamics. Taking χ for the iron reducers to be 8, F_T exceeds 80 percent, for example, where $\Delta G_U > 32$ kJ mol^{-1}. A representative stoichiometric number for the sulfate reducers is 6, so this group needs just 24 kJ mol^{-1} to achieve such a value for F_T, whereas the acetoclasts, for which χ is taken as 2, need only 8 kJ mol^{-1}. The acetoclastic methanogens, then, derive more usable energy from a given supply of available energy than the sulfate reducers, and drive their metabolisms effectively with a smaller amount of usable energy. Sulfate reducers, in turn, need less available energy to live and thrive than iron reducers.

Thermodynamic Controls

In light of these results, we find unexpected thermodynamic controls. We commonly assume an energetic sequence in which iron reducers are favored relative to sulfate reducers, which themselves have an advantage over methanogens. For acetotrophs in our nominal environment (table 2), however, we observe the inverse: the acetoclastic methanogens (F_T = .99) are less limited thermodynamically than the sulfate reducers (F_T = .76), which hold a significant edge relative to the goethite reducers (F_T = .22). As pH decreases from pH 7, goethite reducers and then magnetite reducers gain the thermodynamic advantage; the value predicted for F_T for the two groups quickly approaches one (fig. 10). Under alkaline conditions, in contrast, there is no energetic drive for the microbial reduction of either goethite or magnetite, and F_T is zero.

For the hydrogentrophs, the thermodynamic factors F_T in the nominal environment (table 2) are zero for the goethite and sulfate reducers, and the methanogens, indicating each of these groups lacks a thermodynamic drive for its net reaction. As pH decreases from 7, as shown in figure 10, an energetic drive develops first for the goethite and magnetite reducers, then for the sulfate reducers. Increasing the H_2(aq) concentration in the nominal environment (at pH 7) enables first methanogenesis and sulfate reduction, then goethite reduction (fig. 10). Lovley and Goodwin (1988) observed methanogenesis and sulfate reduction occurring in the field at roughly comparable H_2(aq) concentrations.

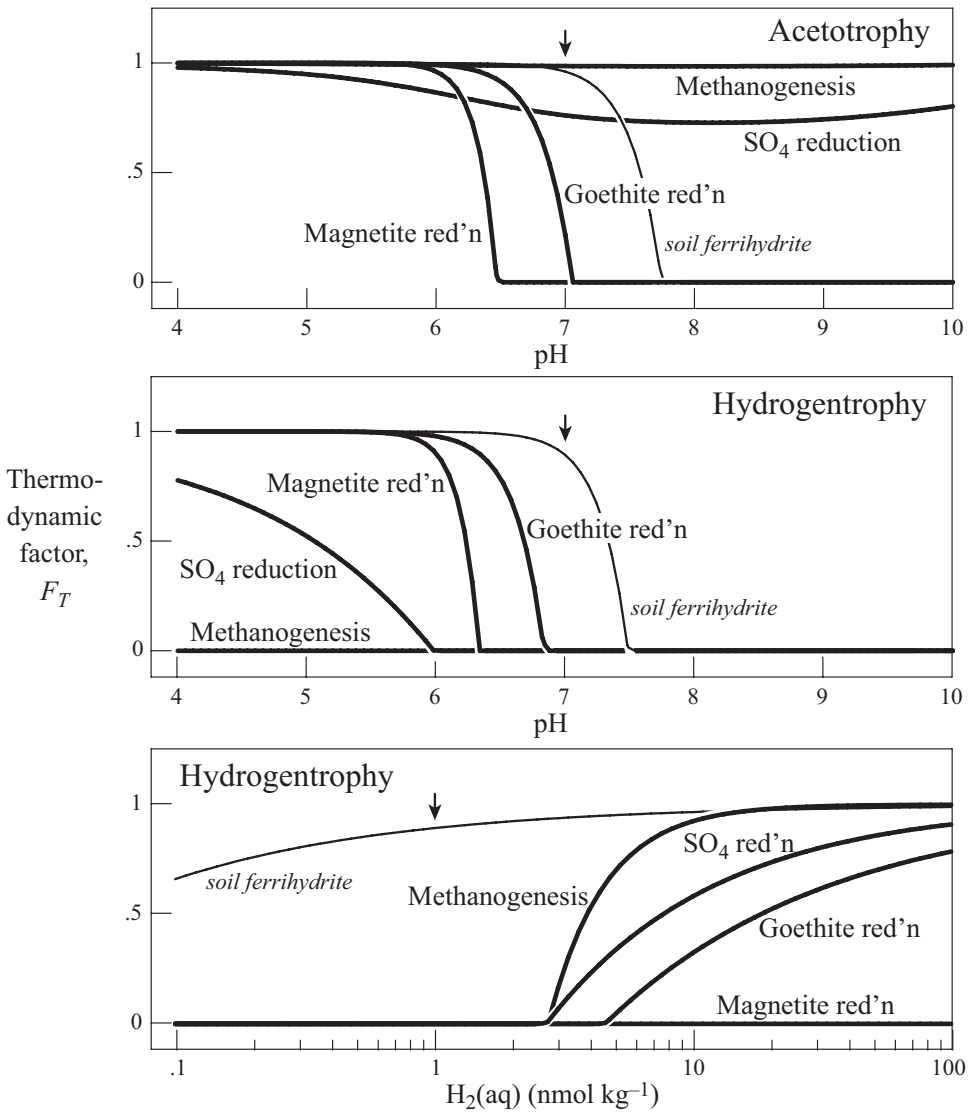

Fig. 10. Variation of thermodynamic potential factor for acetotrophs with pH (top), and hydrogentrophs with pH (middle) and with dihydrogen concentration (bottom). Arrows show positions of the nominal environment.

Provenance of Iron Reduction

The usable energy supplied in the environment to iron reducers depends on the chemical stability of the ferric mineral, as shown in figure 4. Acetotrophs, according to the calculations, can respire goethite in our nominal environment at pH $\leq$ 7. The iron reducers might use soil ferrihydrite, which is less stable than goethite, however, where pH $\leq$ 7.8, and freshly precipitated ferric hydroxide if pH $\leq$ 9. Studies of the solubility products for goethite compiled by Cornell and Schwertmann (2003) report solubility products that range over more than two log units. The range likely reflects not only the difficulty of determining K_{sp} experimentally, but variations in crystallinity and compo-

sition among natural and synthetic goethite samples used in the studies. The value used here to compute usable energy, from Bigham and others (1996), is near the high-solubility (that is, low-stability) end of the range, suggesting that iron reducers in nature might be more restricted in provenance than the calculations in this paper imply.

The result that goethite reducers lack an effective thermodynamic drive under alkaline conditions helps explain instances in which ferric iron is preserved in contact with anoxic groundwater. The Mahomet aquifer system, a well-studied regional water supply in central Illinois (Kempton and others, 1991; Panno and others, 1994), for example, hosts widespread microbial activity fed by the degradation of organic matter trapped in its young glacial sediments (Kirk and others, 2004; Kelly and others, 2005; Flynn and others, 2008). Even deep reaches of the aquifer contain prominent zones marked by bright orange-red ferric iron (Sanford and others, 2009). The distinctive color, characteristic of hematite (Fe_2O_3), indicates the ferric surfaces have not been subjected to chemical reduction, perhaps owing to the occurrence in the Mahomet of non-acidic groundwater, the pH of which falls mostly in the range 7 to 8.

Acidophilic Iron Reduction

As shown in figure 4, the usable energy provided to the goethite reducers at pH $\leq$ 6, according to calculations made using m and χ from table 3, is much more than needed to provide an effective thermodynamic drive. Acetotrophic and hydrogentrophic magnetite reducers similarly find an overabundance of usable energy at pH $\leq$ 5.5. Under fully acidic conditions the excess energy is considerable, amounting to hundreds of kJ mol^{-1}. Any such excess energy would be dissipated to the environment as the microbial reaction is catalyzed, and hence lost.

Given that the microbiologic realm abhors wasting chemical energy, we might surmise that iron reducers adapted to living in fully acidic waters (for example, Kappler and Straub, 2005; Lu and others, 2010) trap more energy per electron transferred than those living at circum-neutral pH. If so, the values of m representing acidophilic iron reduction will prove larger than that shown in table 3.

Implications for Laboratory Study

In light of discussion in this paper, we see that misleading interpretations might be drawn from laboratory studies of iron reduction, when considering microbial activity in natural aquifers. A great many experimental studies have been conducted using an aqueous iron source such as ferric citrate ($FeC_6H_5O_7$) as the electron acceptor (for example, Komlos and Jaffe, 2004; Lovley and others, 2004; Brown and others, 2005). The net reaction in these cases

$$CH_3COO^- + 8FeC_6H_5O_7 + 8Ca^{2+} + 4H_2O \rightarrow 2HCO_3^- + 8Fe^{2+} + 8CaC_6H_5O_7^- + 9H^+ \quad (11)$$

is acid-producing and hence favored energetically under alkaline conditions. The microbial reduction of ferric hydroxide and oxyhydroxide minerals (table 2), in contrast, is energetically distinct from the homogeneous reaction because it consumes acid (for example, Ehrenreich and Widdel, 1994). Chelating ions such as citrate are not abundant in pristine aquifers; ferric iron in aquifers is commonly found in mineralogic form, so iron reduction there is favored under acidic rather than alkaline conditions.

Other studies of bacterial iron reduction (for example, Zavarzina and others, 2006) have used as the electron acceptor freshly precipitated $Fe(OH)_3$, which is less stable than the ferrihydrite and goethite commonly observed in the natural environment. Iron reduction in such experiments, for this reason, can proceed under

conditions considerably more alkaline than might be expected in the field, as shown in figure 4.

Importance of Microenvironments

The results here serve to emphasize the likely significance within aquifers of microbial microenvironments. Hydrogentrophic methanogens and sulfate reducers by our calculations require H_2(aq) concentrations of several nmol kg^{-1}, somewhat higher than observed in the bulk water of many aquifers. Confined aquifers commonly contain mildly alkaline water, as a second example, but the calculations here predict that goethite reducers need pH-neutral or acidic conditions to live.

Fermentation of natural organic matter (represented here as $C_n{\cdot}mH_2O$) generates acetate and acid

$$2\ C_n{\cdot}mH_2O \rightarrow n\ CH_3COO^- + n\ H^+ + 2(m-n)\ H_2O \tag{12}$$

as well as dihydrogen

$$C_n{\cdot}mH_2O \rightarrow 2n\ H_2(aq) + n\ CO_2(aq) + (m-2n)\ H_2O. \tag{13}$$

A biofilm surrounding a fragment of fermenting organic matter could trap those compounds, creating a microenvironment. Even where hydrogentrophs are excluded from the bulk of the aquifer, they might thrive in the microenvironment, living on the fermentation products. Goethite reducers might thrive there also, taking advantage of the acid generated. The latter might respire by transferring electrons to ferric surfaces outside the biofilm using shuttles (Straub and Schink, 2003; Roden and others, 2010) or nanowires (Reguera and others, 2005; Gorby and others, 2006). Perhaps part of the reason microbes have developed a broad array of mechanisms for extracellular respiration (Gralnick and Newman, 2007) is to exploit the variety of chemical microenvironments found in nature.

Bioreactor Experiments

In the SO_4-only experiment (fig. 6), acetoclastic methanogens after a month saw about 17 kJ mol^{-1} more usable energy than the sulfate reducers. Sulfate reduction dominated microbial activity, nonetheless; almost no methanogenesis was observed. Reactive transport models of the growth of microbial populations in aquifers calculated by Bethke and others (2008) show a parallel result. A zone developed in the models where sulfate reducers dominated methanogens, even though the latter were thermodynamically favored. Methanogens failed to grow in the model because they died more quickly than they reproduced. Raskin and others (1996) observed a similar phenomenon in biofilms: when they supplied sulfate to their experiment, methanogenesis ceased. Much of the methanogenic population had apparently died, because when they switched back to a sulfate-free medium, it took almost two months for methanogenesis to resume.

Such a situation, known in ecology as an extinction vortex, arises in mathematical models wherever acetate concentration falls below a critical point:

$$m_{Ac} < \frac{D}{Y}\cdot\frac{K_D}{k_+ F_r} \tag{14}$$

(Bethke and others, 2008). Here, D is the decay coefficient (s^{-1}), Y is the growth yield (mg mol^{-1}), K_D is the half-saturation constant (mol kg^{-1}), and k_+ is the rate constant (mol mg^{-1} s^{-1}); as before, F_T is the thermodynamic potential factor. Taking values for D, Y, K_D, and k_+ from table 1 in that paper, and using the F_T calculated above, equation (14) predicts acetoclasts require acetate in concentrations of at least about 1 μmol kg^{-1} to maintain a viable population. After the first month, concentration in the

bioreactor at the end of each reaction interval fell below the detection limit of 50 μmol kg^{-1}, consistent with the idea that acetoclasts in the SO_4-only experiment were unable to reproduce rapidly enough to achieve net population growth.

In the FeOOH-only experiment, in sharp contrast, methanogens dominated microbial activity. Iron reduction increased early in the experiment, as the population of iron reducers grew. But the rate of iron reduction began to slow after about a month, and most of the acetate supplied to the reactor went unused. Only several months later did a population of acetoclastic methanogens develop of sufficient size to consume the available acetate. The acetoclasts dominated microbial activity for the remainder of the experiment, even though the iron reducers were strongly favored thermodynamically, seeing an advantage in usable energy of about 20 kJ mol^{-1}.

The collapse of iron reduction in the reactor coincides with the buildup of Fe^{2+} in solution. The ion sorbs strongly to goethite surfaces

$$\equiv FeOH + Fe^{2+} \rightarrow \equiv FeOFe^{+} + H^{+} \qquad (15)$$

where $\equiv FeOH$ and $\equiv FeOFe^{+}$ are sorbing sites in uncomplexed and complexed states. The reaction is known to interfere with bacterial iron reduction (Urrutia and others, 1999; Roden and Urrutia, 2002; Hansel and others, 2004). When ferrous ions sorb to a ferric surface, valence electrons are taken up and conducted toward high potential sites (Williams and Scherer, 2004; Larese-Casanova and Scherer, 2007). The electrons lower the effective redox potential of the surface, degrading its ability to act as an electron acceptor (Handler and others, 2009; Rosso and others, 2010). In the FeOOH-only experiment, the iron reducers' own waste, it seems, undermined their ability to derive energy, despite its abundant supply.

The FeOOH+SO_4 experiment is extraordinary in that the iron reducers, confronted with sulfate reducers competing for a limited supply of acetate, respired more rapidly than they did in the absence of sulfate reducers. Over the final three months of the experiment, the iron reducers were fourteen times more active on average than they were during this interval (126 to 216 days) in the FeOOH-only experiment.

Evidence of Mutualism

Bethke and others (2008) suggested iron reducers and sulfate reducers might live in aquifers in a mutualistic relationship, taking advantage of the precipitation of iron sulfide

$$Fe^{2+} + HS^{-} \rightarrow FeS(s) + H^{+} \qquad (16)$$

(for example, Neal and others, 2001). The reaction would promote iron reduction by preventing Fe^{2+} from accumulating and fouling the ferric surface, according to reaction (15). Sulfate reducers, seeing less HS^{-} in solution, would in turn benefit from a reduced level of product inhibition. If such a mutualistic relationship existed in the experiment, it would be revealed by a one:one ratio of Fe^{2+} production to HS^{-} generation, reflecting the stoichiometry of sulfide precipitation (reaction 16).

Iron reducers produce eight Fe^{2+} per acetate respired (reaction 2), whereas sulfate reducers make only one HS^{-} (reaction 1), so stoichiometric balance occurs when $8 \times r_{FeR}/r_{SR} = 1$. Figure 11 shows how this ratio varied over the course of the FeOOH+SO_4 experiment. The ratio is greater than one initially, reflecting the rapid onset of iron reduction. Within a month, however, the ratio approaches a value of about one and remains near unity for the duration of the run. This result provides compelling evidence that iron reducers and sulfate reducers in the reactor lived in a close mutualistic relationship, maintaining their respiration rates in a delicate balance, so neither group outpaced the other.

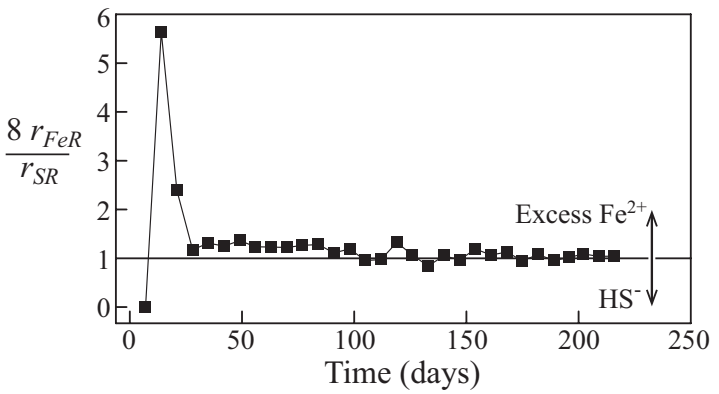

Fig. 11. The ratio of eight times the rate of iron reduction to the rate of sulfate reduction in the $FeOOH+SO_4$ experiment, as a function of time. When the ratio is one, iron reducers generate Fe^{2+} at the rate sulfate reducers produce HS^-. Convergence to a value of one suggests the two functional groups balance their respiration rates to produce FeS(s), providing compelling evidence of mutualism in the experiment.

CONCLUDING REMARKS

The analysis in this paper fails to identify a fixed thermodynamic hierarchy among the methanogens, sulfate reducers, and iron reducers. For one group to dominate another by thermodynamic exclusion, the first group needs to hold down usable energy for the second. But under typical natural conditions groundwater provides usable energy to methanogens in amounts almost indistinguishable from those afforded sulfate reducers. Perhaps not surprisingly, the methanogens are well adapted to environments that provide little energy. The group compensates for its apparent thermodynamic disadvantage by trapping little energy per mole of electron donor consumed, maximizing usable energy.

Iron reducers too see about the same usable energy as methanogens and sulfate reducers, where pH is near neutral. They assume a notable advantage under acidic conditions, but find little or no usable energy where groundwater is alkaline. The idea that the three groups fall into a fixed sequence on a thermodynamic ladder, however well entrenched in our thinking, stands up poorly to scrutiny. Energetics does not invariably favor iron reducers over sulfate reducers, which in turn are not necessarily preferred relative to the methanogens.

To be sure, a group that captures more energy than another derives ecologic advantages. Iron reducers and sulfate reducers, as shown in table 3, conserve in their ATP pools four or five times more energy than methanogens, per mole of electron donor consumed. Acidophilic iron reducers, as noted above, may capture energy even more effectively. The more energy a microbe traps, the larger a growth yield it can achieve (Roden and Jin, 2011). A large growth yield can give a group an ecologic upper hand, because the number of cells an environment can carry at steady state varies directly with yield (Lovley and Klug, 1986). A high yield also serves to protect a group against entering an extinction vortex, as can be seen from the form of equation (14).

Long-term bioreactor experiments shed further light on the thermodynamic question. In one of the experiments we analyzed, methanogens saw considerably more usable energy than sulfate reducers. The sulfate reducers came to dominate the experiment, nonetheless, apparently because the methanogenic cells could not reproduce as quickly as they died. Providing sulfate to another experiment increased the rate of iron reduction by more than an order of magnitude, relative to the rate achieved in the absence of sulfate reduction.

The two groups in this experiment, contrary to the idea of competitive exclusion, were not found to be competing for the limited supply of electron donor. Instead, the iron reducers and sulfate reducers worked in a delicate balance to produce Fe^{2+} and HS^- in the one:one stoichiometric ratio needed to precipitate FeS(s). The bioreactor experiments yield compelling evidence that ecologic factors such as population viability and mutualism, rather than thermodynamics alone or simple competition, govern the distribution of microbial activity in the laboratory, and perhaps in nature.

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Appendix

The overall rate r_{Ac} of acetate consumption in the bioreactor experiments is given by the concentrations m_{Ac}^{start} and m_{Ac}^{end} (mol kg^{-1}) of acetate observed before and after an interval, and its concentration m_{Ac}^{in} in the input fluid (0.8 mmol kg^{-1}), according to

$$r_{Ac} = \frac{\frac{2}{3}\, m_{Ac}^{start} + \frac{1}{3}\, m_{Ac}^{in} - m_{Ac}^{end}}{\Delta t} \tag{17}$$

where Δt is interval length (~7 days). The rate r_{SR} of sulfate reduction is given by the parallel relation

$$r_{SR} = \frac{\frac{2}{3}\, m_{\mathrm{SO_4}}^{start} + \frac{1}{3}\, m_{\mathrm{SO_4}}^{in} - m_{\mathrm{SO_4}}^{end}}{\Delta t} \tag{18}$$

in which $m_{\mathrm{SO_4}}^{in}$ is 1.1 mmol kg^{-1}.

The rate r_{FeR} of iron reduction is

$$r_{FeR} = \frac{1}{8}\left(\frac{m_{\mathrm{Fe^{2+}}}^{end} - \frac{2}{3}\, m_{\mathrm{Fe^{2+}}}^{start}}{\Delta t} + r_{SR}\right) \tag{19}$$

because 8 Fe^{2+} are produced for each Ac respired. The input water contained no Fe^{2+}, so an m^{in} term does not appear in the equation. The r_{SR} term is applied in considering the FeOOH+SO_4 experiment only, because one FeS(s) precipitated from each HS^- generated by sulfate reduction, as was verified by extracting acid-volatile sulfide from each bioreactor after the experiments concluded. Ferrous minerals such as siderite or vivianite are unlikely to have formed in the experiment, because the fluid contained little carbonate or phosphate, and indeed after the experiment only sulfides were observed under the electron microscope.

Ferrous ions undoubtedly sorbed to the goethite at the onset of iron reduction, so the rates reported for early in the experiment may understate the actual rate of iron reduction. Considering the goethite surface area resulting from the synthesis procedure (Schwertmann and Cornell, 2000), the site density (Villalobos and others, 2003), and the competition for sorbing sites (Appelo and others, 2002; Dixit and Hering, 2006), no more than 100 μmol kg^{-1} could have been taken up. The amount is less than was produced in the first two weeks of the FeOOH-only and FeOOH+SO_4 experiments, so the potential effect of sorption on the calculations is small.

We calculated the rate r_{Meth} of methanogenesis accounting for CH_4 in gaseous as well as dissolved form. One CH_4 is produced from each Ac consumed, and no methane is present in the input water. In light of the gas law, then, the rate is given

$$r_{Meth} = \frac{1}{(\Delta t)_g}\left[m_{\mathrm{CH_4}}^{end} - m_{\mathrm{CH_4}}^{start} + \frac{1}{3}\sum_s m_{\mathrm{CH_4}}^{s} + \frac{V_g}{n_w \cdot RT_K}\left(P_{\mathrm{CH_4}}^{end} - P_{\mathrm{CH_4}}^{start}\right)\right]. \tag{20}$$

In this equation, $(\Delta t)_g$ is the time interval (days) between gas samplings, which occurred less frequently and at different points in time than the fluid samplings; $m_{\mathrm{CH_4}}^{s}$ is methane concentration at each fluid sampling event s within the gas sampling interval; V_g and n_w are the gas volume (400 cm^3 = .0004 m^3) and fluid mass

(0.6 kg) in the reactor, respectively; and $P_{CH_4}^{start}$ and $P_{CH_4}^{end}$ are the partial pressures (Pa) of methane observed in the headspace before and after the reaction interval. As before, R is the gas constant and T_K is absolute temperature.

The methanogenesis rate could be determined only approximately. Methane concentration is known only when gas was sampled, so the concentrations $m_{CH_4}^s$ at the fluid sampling events had to be estimated by interpolation. 208 days into the study, furthermore, enough methane had built up in the FeOOH-only experiment that gas needed to be vented from the headspace. An unknown amount of methane was lost at that time, affecting the rate calculation for the interval in question.

Supplemental Appendix

http://earth.geology.yale.edu/~ajs/SupplementaryData/2011/01BethkeAppendix.pdf

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