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Geochimica et Cosmochimica Acta 74 (2010) 3938–3947
Geochimica et Cosmochimica Acta
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Gibbs energies of reaction and microbial mutualism in anaerobic deep subseafloor sediments of ODP Site 1226

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Received 1 April 2009; accepted in revised form 29 March 2010; available online 7 April 2010

Abstract

In situ Gibbs energies of reaction (ΔG) for acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction, and acetoclastic methanogenesis, and sulfate-reducing methanotrophy are consistently negative and relatively constant throughout most of the sediment column at the eastern equatorial Pacific Ocean Drilling Program (ODP) Site 1226. The energy yields ($-\Delta G$) closely match the values (for acetate-oxidizing sulfate reduction and acetoclastic methanogenesis) in published culturing experiments with actively growing cells and, for sulfate-reducing methanotrophy, in other environments.

Although microbes mediating these reactions compete for substrates, mutualistic interactions between them appear to sustain their co-existence in deep subseafloor sediments for millions of years (the interval over which the sediments have been deposited). These competing and mutualistic interactions collectively constitute a highly coupled reaction network where relative rates of reaction are regulated by the in situ Gibbs energies of reaction.

1. INTRODUCTION

Microbial cells are abundant (Parkes et al., 2000) and active (Schippers et al., 2005) in deeply buried subseafloor sediments. These microbial communities catalyze a variety of oxidation–reduction (redox) reactions throughout sediment columns that span hundreds of meters and millions of years of deposition (D'Hondt et al., 2004). Although only about 1% of marine organic production reaches deep-sea sediments (Suess, 1980), it appears to fuel most of the microbially catalyzed reactions in anoxic subseafloor sediments (D'Hondt et al., 2004; Lipp et al., 2008).

Redox reactions that often appear sequentially with depth in shallow marine sediments, consistent with the sequence of their standard Gibbs energy yields ($-\Delta G^0$) (e.g., Martens and Berner, 1977; Froelich et al., 1979) co-exist in anoxic deep subseafloor sediments, despite the extremely low availability of organic matter and competition for limited electron donating substrates. Sedimentary pore-fluid chemical profiles reflect widespread co-occurrence of methanogenesis and sulfate reduction in deep sediments throughout the ocean (Mitterer et al., 2001; Bralower et al., 2002; D'Hondt et al., 2002, 2004). Sulfate reduction, iron reduction and methanogenesis co-exist throughout most of the hundreds-of-meter-thick sediment column at eastern equatorial Pacific sites (D'Hondt et al., 2004; Wang et al., 2008). Based on a numerical technique that calculates net reaction rates from pore water chemical and physical property data, considering diffusion and sediment burial, net rates of sulfate reduction at Site 1226 range from about 2.5×10^{-5} mol m^{-3} yr^{-1} in the uppermost portion of the sediment column [0–40 m below seafloor (mbsf)] to approximately 6.6×10^{-7} mol m^{-3} yr^{-1} in the lowermost 145 m of the column (275–420 mbsf) (Wang et al., 2008). It has been inferred that the net rate of iron reduction in the subseafloor sediments of Site 1226 is at least half the net rate of sulfate reduction throughout the sediment column because 96% of the dissolved sulfide produced by sulfate reduction

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doi:10.1016/j.gca.2010.03.034

is precipitated in the sediments and concentrations of dissolved Fe(II) remain low in the sediment column (Wang et al., 2008).

The co-occurrence of terminal respiration reactions has been observed in other subsurface environments. In aquifer systems, similar occurrence of concomitant microbial respiration reactions has been discussed (Postma and Jakobsen, 1996; Jakobsen and Postma, 1999; Kirk et al., 2004; Park et al., 2006; Bethke et al., 2008). This co-occurrence has been explained by a partial equilibrium approach, where the fermentative step is rate limiting, while the terminal respiration processes occur at close to equilibrium conditions (Postma and Jakobsen, 1996; Jakobsen and Postma, 1999), and using population models in which rates are parameterized by non-linear irreversible thermodynamics (Bethke et al., 2008).

To assess the energetic constraints that may sustain the co-existence of competing metabolic pathways in deeply buried marine sediments, we have calculated the in situ Gibbs energy of reaction (ΔG) for acetate-oxidizing sulfate reduction and iron reduction, acetoclastic methanogenesis, and sulfate-reducing methanotrophy throughout the sediment column of ODP Site 1226 at depths where total dissolved sulfide ($\sum H_2S = S^{2-} + HS^- + H_2S_{(aq)}$) is detectable (7–275 mbsf).

2. DATA AND METHODS

2.1. Study site

ODP Site 1226 is located in the eastern equatorial Pacific Ocean (3°5.7′S, 90°49.08′W) near the present-day boundary between the South Equatorial Current and the Peru Current at a water depth of 3297 m. The sediment column is 420 m thick and ranges in age from 0 to 16.5 Ma (Shipboard Scientific Party, 2003). Seawater flows laterally through the underlying basalt, driving sulfate toward seawater concentrations at the bottom of the column (D'Hondt et al., 2003; Bekins et al., 2007). Site 1226 is within 100 m of ODP Site 846 (3°5.7′S, 90°49.1′W) (Shipboard Scientific Party, 1992). Based on detailed stratigraphic studies of Site 846, the upper 275 m of sediments were deposited over the last 7 million years (Shipboard Scientific Party, 1992). The principal sedimentary components of these sites are biogenic calcite and biogenic opal (Shipboard Scientific Party, 2003). High-resolution pore water chemical and physical property data throughout the entire sediment column (Shipboard Scientific Party, 2003) make Site 1226 an ideal location for the study of metabolic activities in anoxic deep subseafloor sediments. In situ downhole temperature (t, °C) is given by,

$$t = 1.74 + 0.0572 \times z \quad (1)$$

where z is the sediment depth in mbsf (Shipboard Scientific Party, 2003). In situ pressure (P) is assumed to be hydrostatic and approximated by

$$P = (h + z)/10 \quad (2)$$

where P is in 10^5 Pa, and h is the water depth, 3297 m (Shipboard Scientific Party, 2003).

2.2. In situ pH

Energy yields of subsurface microbially-mediated redox reactions often depend on in situ pH. However, in situ pH differs from pH measured on the ship due to pressure and temperature changes during core recovery and sampling and carbonate precipitation during sample recovery and handling (Gieskes, 1974).

To calculate in situ pH, we developed a method that relies on the fact that at equilibrium, if [DIC], [alkalinity], and [Ca^{2+}] are known, in situ pH and [CO_2aq], [HCO_3^-], and [CO_3^{2-}] are mathematically over-determined if there is carbonate saturation. This over-determined system allows the calculation of the amount of carbonate precipitated during core recovery and sampling and hence the determination of in situ pH. A detailed description of the method is given in Electronic Annex EA-1. The method of calculation for the activities of other pH-dependent species is also given in EA-1.

2.3. Mineral solubility calculations

To identify potential controls of dissolved Fe^{2+} at this site, the degree of saturation (Ω) of Fe(II), FeS (mackinawite), was calculated. Degree of saturation is defined as

$$\Omega = \log_{10} \frac{IP}{K_{sp}} \quad (3)$$

where IP is the ion product, $\frac{\prod_i \{product\}^{\lambda_i}}{\prod_i \{reactant\}^{\lambda_i}}$, in which λ_i is the stoichiometric coefficient of the species in the reaction, {} represents the activity of chemical species, and K_{sp} is the equilibrium solubility constant for in situ conditions. The activity of chemical species, i, is expressed as, $\{i\} = \gamma_i[i]$, where γ_i is the activity coefficient, and $[i]$ represents the concentration of chemical species i. The activity coefficients were calculated for an ionic strength of 0.7 M using the IUPAC Specific Interaction Theory (SIT) program (IUPAC, 2003). Because no calculation was available for the activity coefficient of Fe^{2+} in this program, the activity coefficient of Fe^{2+} was taken as that of Mn^{2+} due to their similarity in terms of solution chemical properties. For uncharged species, activities were taken as 1. The ionic strength of 0.7 M was calculated from pore water chemical compositions at Site 1226, in which Cl^- is the dominant anion with the concentration of about 0.56 M (Shipboard Scientific Party, 2003).

FeS dissolution is governed by,

$$FeS(s) + H^+ = Fe^{2+} + HS^- \quad (4)$$

The thermodynamic calculations and data for in situ temperature and pressure corrections are compiled in Electronic Annex EA-2. Free Fe^{2+} activities were calculated considering complexes with CO_3^{2-}, OH^-, Cl^-, SO_4^{2-}, and HCO_3^-. The detailed calculations of free Fe^{2+} activities are described in Electronic Annex EA-3.

2.4. Gibbs energy of reaction calculations

For the ΔG calculations at Site 1226, we focused on acetate as the representative electron donor because (i) it is

present throughout the sediment column (Fig. 1a), (ii) it is one of the most common microbial fermentation products (Madigan et al., 2000), and (iii) it is one of the two main substrates in anoxic microbial respiration reactions (Winfrey and Ward, 1983; Lovley, 1987). In situ ΔG values were calculated for four reactions:Acetate-oxidizing sulfate reduction

$$SO_4^{2-} + C_2H_3O_2^- = HS^- + 2HCO_3^- \quad (5)$$

Acetate-oxidizing iron reduction with hematite (Fe_2O_3) as the reactant iron phase

$$4Fe_2O_3 + C_2H_3O_2^- + 15H^+ = 8Fe^{2+} + 2HCO_3^- + 8H_2O \quad (6)$$

Acetoclastic methanogenesis

$$C_2H_3O_2^- + H_2O = CH_4 + HCO_3^- \quad (7)$$

Sulfate-reducing methanotrophy

$$SO_4^{2-} + CH_4 = HS^- + H_2O + HCO_3^- \quad (8)$$

We chose to write the reactions with HCO_3^- rather than CO_2aq or CO_3^{2-} since it is the major inorganic carbon species at in situ pH values. This choice does not affect the ΔG as equilibrium between the carbonate species is assumed. The choice of HS^- rather than H_2S or S^{2-} similarly does not affect the calculated ΔG.

In situ ΔG values depend on pressure, temperature, ionic strength and chemical concentrations. These were explicitly taken into account in our calculations. Over the in situ temperature range, 2–17 °C, and pressure range, $3.02-3.28 \times 10^7$ Pa, changes in thermodynamic properties are relatively small, allowing ΔV and $\Delta\kappa$ in Eq. (9) and ΔH (all defined below) in Eq. (11) each to be taken as constant. Thus we used simplified estimation approaches to calculate

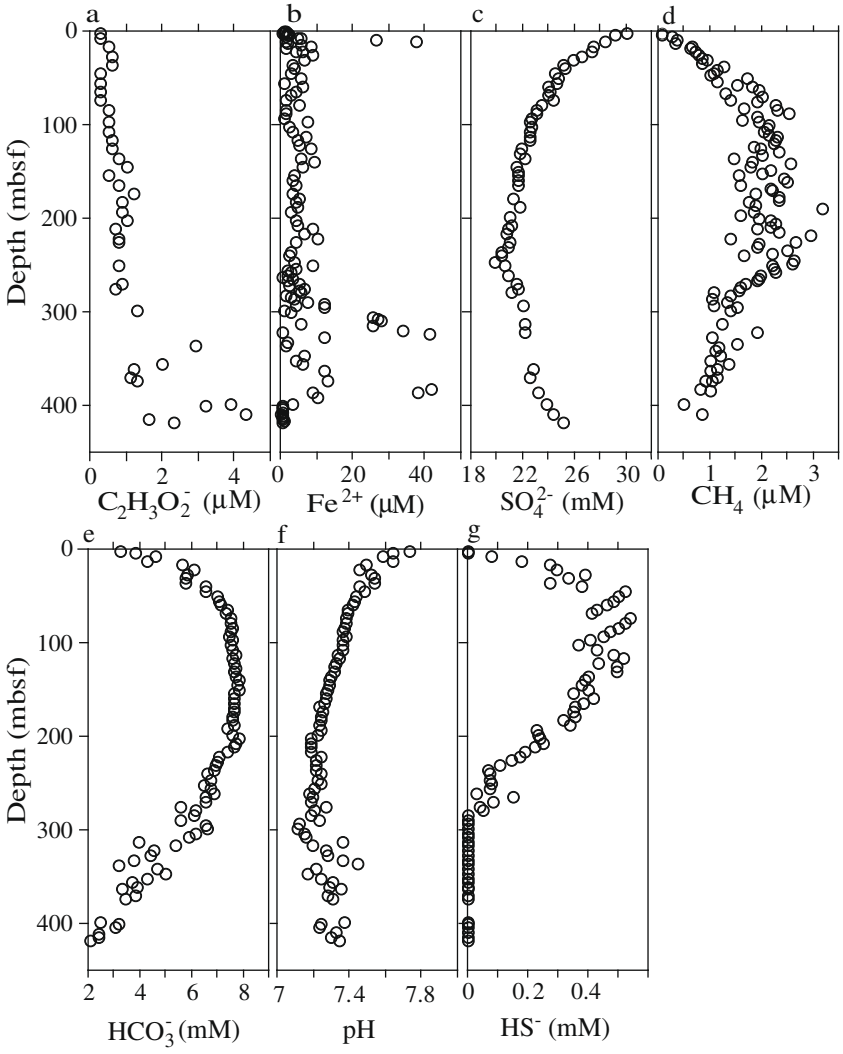

Fig. 1. Profiles of pore water chemical concentrations and in situ pH at Site 1226: (a) $C_2H_3O_2^-$, (b) Fe^{2+}, (c) SO_4^{2-}, (d) CH_4, (e) in situ HCO_3^-, (f) in situ pH, and (g) in situ HS^-. Concentrations of $C_2H_3O_2^-$, Fe^{2+}, SO_4^{2-} and CH_4 are from Shipboard Scientific Party (2003). In situ HCO_3^-, pH, and HS^- were calculated as described in the text.

temperature and pressure dependences of ΔG. The effect of pressure on ΔG was approximated as,

$$\Delta G^0(T_r,P) = \Delta G^0(T_r,P_r) + \Delta V^0 \times (P - P_r) - \frac{\Delta \kappa^0}{2} \times (P - P_r)^2 \quad (9)$$

where $\Delta G^0(T_r, P)$ is the Gibbs energy of reaction at the reference temperature (T_r, 298.15 K) and in situ pressure (P), $\Delta G^0(T_r, P_r)$ is the standard Gibbs energy of reaction at the reference temperature and reference pressure, P_r, 1×10^5 Pa, P is the in situ pressure (in 10^5 Pa), ΔV^0 is the partial molar volume change of reaction at the reference temperature and pressure, given by Eq. (EA-2-1), and $\Delta \kappa^0$ is the partial molar compressibility change of reaction at the reference temperature and pressure, given by Eq. (EA-2-2) (e.g., Millero, 1983). $\Delta G^0(T_r, P_r)$ was calculated using the definition,

$$\Delta G^0(T_r,P_r) = \sum_i \lambda_i G^0_{f(product)} - \sum_i \lambda_i G^0_{f(reactant)} \quad (10)$$

where G^0_f is the Gibbs energy of formation of the chemical species (kJ mol^{-1}). The effect of temperature was approximated using the Van't Hoff equation (e.g., Stumm and Morgan, 1995):

$$\Delta G^0(T,P) = \left[\Delta H^0 \times \left(\frac{1}{T} - \frac{1}{T_r}\right) + \frac{\Delta G^0(T_r,P)}{T_r}\right] \times T \quad (11)$$

where T is the in situ temperature (K), $\Delta G^0(T, P)$ is the Gibbs energy of reaction under the condition (T, P), and ΔH^0 is the partial molar enthalpy change of reaction. Gibbs energies of formation, partial molar volumes, partial molar compressibility, and partial molar enthalpies compiled from the literature are listed in Table EA-2-1.

Lastly, the effect of chemical concentrations on ΔG was considered:

$$\Delta G = \Delta G^0(T,P) + 2.3RT\log_{10}\left(\frac{\prod_i \{product\}^{\lambda_i}}{\prod_i \{reactant\}^{\lambda_i}}\right) \quad (12)$$

where ΔG is the in situ Gibbs energy of reaction, and R is the gas constant (8.314 kJ mol^{-1} K^{-1}). As with the mineral solubility calculations, the activity coefficients were calculated for an ionic strength of 0.7 M using the IUPAC SIT program. The activity coefficient of Fe^{2+} was taken as that of Mn^{2+} and the activities of the uncharged solids were taken as 1. For uncharged dissolved gases their activity coefficients can be calculated using an empirical equation in Millero (2000):

$$\ln \gamma = 0.0938 + 0.3404\ k_s(\mathrm{NaCl}) \quad (13)$$

where k_s(NaCl) is the salting coefficient of the non-electrolyte in NaCl solution, values of which are provided in Millero (2000). For methane, k_s(NaCl) of 0.319 from Millero (2000) was used.

2.5. Data

Acetate increases near the seafloor from 0.3 μM to about 1 μM at around 200 mbsf and is relatively constant to 300 mbsf. It then increases to 4 μM at 400 m depth with increasing temperature (Fig. 1a), similar to what has been observed in the deep sediments of Blake Ridge, Atlantic Ocean (3–17 °C) (Paull et al., 1996) by Wellsbury et al. (1997). Concentrations of dissolved iron are relatively low with a few peaks near the seafloor and deep in the sediment column (Fig. 1b), even though it has been inferred that iron reduction occurs in this sediment column at rates comparable to sulfate reduction (Wang et al., 2008). Sulfate concentration decreases from 29 mM near the seafloor to about 20 mM at around 250 mbsf (Fig. 1c). Then it increases downcore, as sulfate diffuses up from seawater flowing through the underlying basalt (D'Hondt et al., 2004). Methane, on the other hand, increases from 0.07 μM near the seafloor to 3 μM in the middle of the column, and then decreases to 0.8 μM near the bottom of the sediment column (Fig. 1d). Net methane production has been shown to occur in the depth intervals where sulfate reduction occurs in this sediment column (D'Hondt et al., 2004; Hinrichs et al., 2006; Wang et al., 2008). $\sum H_2S$ is below the analytical detection limit (0.17 μM) at depths shallower than 7 mbsf and depths greater than 275 mbsf (Shipboard Scientific Party, 2003). For this reason, Gibbs energies of reaction and degrees of saturation were not calculated at depths shallower than 7 mbsf or depths greater than 275 mbsf. For SO_4^{2-} and HCO_3^-, complexes with Fe^{2+} were ignored because concentrations of Fe^{2+} (Fig. 1b) are at least two orders of magnitude smaller than concentrations of SO_4^{2-} (Fig. 1c) and HCO_3^- (Fig. 1e).

We selected hematite as the oxidized iron phase in the iron reduction reaction for three reasons. First, amorphous Fe(III) oxides are depleted rapidly near the sediment surface (Haese et al., 2000) due to their biological reactivity. Second, hematite is not highly reactive with hydrogen sulfide (Canfield, 1989; Canfield et al., 1992; Poulton et al., 2004); consequently, it is expected to persist in depth intervals where concentrations of $\sum H_2S$ are relatively low. Third, hematite was detected in the lower portion of this sediment column (Shipboard Scientific Party, 2003) and might reasonably be expected to be present in other depth intervals. The detection of hematite at depth in the Site 1226 sediments is consistent with the general observation that substantial amounts of crystalline Fe(III) oxides persist into deep sediments (Canfield, 1989; Haese et al., 2000) and with observations of hematite in deep (100 mbsf) methanogenic layers at other sites (Shipboard Scientific Party, 1998; König et al., 2000). As for potential iron reduction in clays, even though its rates may be comparable to those of iron oxide reduction (Kostka et al., 2002), because the clay-bound iron species remain trapped in the clay matrix within the sediment layer where the reduction occurs (König et al., 1999), it cannot be addressed using the currently available data in this study.

3. RESULTS

3.1. Chemical species profiles

Profiles of calculated values of in situ pH, HCO_3^- and HS^- are shown in Fig. 1. In situ pH varies between 7.1 and 7.8 (Fig. 1f). This is between the in situ pK_{1a} and

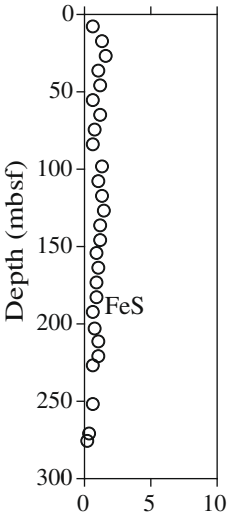

Fig. 2. Degrees of saturation of iron sulfide, mackinawite, at Site 1226.

pK_{2a} of carbonic acid thus the concentration of HCO_3^- (Fig. 1e) is approximately equal to that of DIC throughout the column, increasing with depth until approximately 200 mbsf, below which it decreases to 2 mM at the bottom of the sediment column. The decrease toward the bottom of the sediment column implies that the seawater flowing through the underlying basalt is slightly depleted in DIC compared to regional bottom water, potentially due to the precipitation of carbonate in the crust which results from the retrograde solubility of $CaCO_3$ (Bekins et al., 2007). The pH is close to the in situ pK_{1a} of hydrogen sulfide, thus $[HS^-]$ (Fig. 1g) is approximately equal to half of $\sum[H_2S]$.

3.2. Degree of saturation of Fe^{2+}-sulfide

Formation of metal sulfides is the principal sink of dissolved sulfides and dissolved metals in sulfidic sediments (Berner, 1970). At Site 1226, the sediment is supersaturated with respect to mackinawite and presumably pyrite is present throughout the sediment column (Shipboard Scientific Party, 2003). For mackinawite, Ω is greater than 0 at depths where dissolved $\sum H_2S$ is above the detection limit (Fig. 2). Mackinawite is slightly supersaturated with Ω varying between 0.2 and 1.5 and thus appears to be the phase that controls Fe activities. These degrees of the observed saturation state of iron monosulfide appear to explain the lack of a significant dissolved sulfide flux from the open ocean sediment column of ODP Site 1226. There is only about 1% of the reduced sulfur diffusing out of the column (D'Hondt et al., 2004), despite a fairly high rate of net sulfate reduction in the sediment [ranging from about 9.1×10^{-7} to 2.5×10^{-5} mol m^{-3} yr^{-1} (Wang et al., 2008)].

3.3. Gibbs energies of reaction for anaerobic respiration reactions

The in situ Gibbs energy of reaction for acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction, acetoclastic methanogenesis, and sulfate-reducing methanotrophy are consistently negative (Fig. 3), indicating that all of the reactions are energetically favourable throughout the sediment column where $\sum H_2S$ is detectable. The uncertainty in ΔG due to uncertainty in the concentrations of dissolved chemical species is calculated by

$$\sqrt{\sum_i \left(\frac{\partial(\Delta G)}{\partial([product]_i)}\delta_i\right)^2 + \sum_i \left(\frac{\partial(\Delta G)}{\partial([reactant]_i)}\delta_i\right)^2},$$

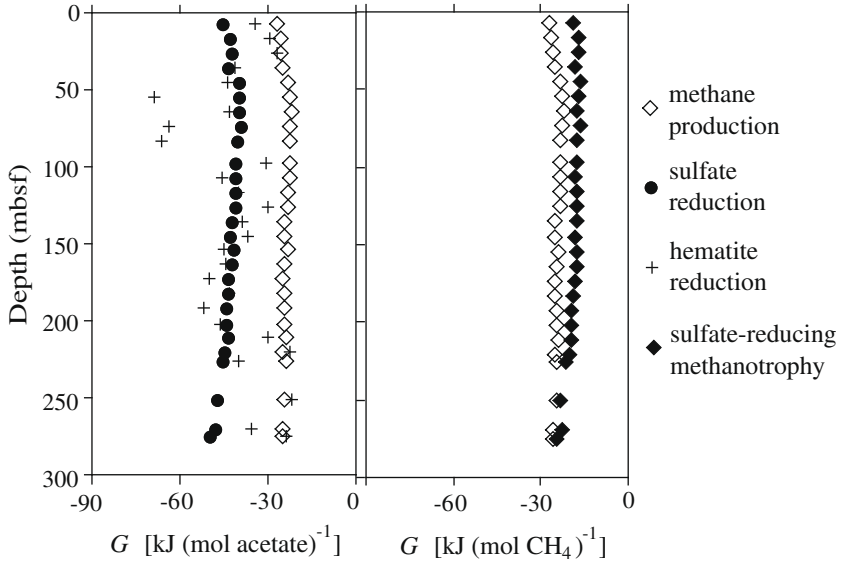

Fig. 3. Profiles of in situ Gibbs energies (ΔG) of reaction for respiration reactions at Site 1226 at depths where $\sum H_2S$ is detectable. (a) ΔG for acetoclastic methanogenesis, acetate-oxidizing sulfate reduction, and acetate-oxidizing hematite reduction. (b) ΔG for acetoclastic methanogenesis and sulfate-reducing methanotrophy.

where δ_i is the uncertainty associated with analysis and sampling of the chemical species (Taylor, 1997). The relative uncertainty of each dissolved chemical species is taken as 1%, which is a conservative estimate based on the analysis of replicate samples and the deviations between measured and smoothed profiles. The calculated uncertainties for acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction, acetoclastic methanogenesis, and sulfate-reducing methanotrophy are greater than 1, indicating the sensitivity of in situ Gibbs energies of reaction to concentrations of dissolved reactants and products. The in situ Gibbs energies of reaction for sulfate reduction, methanogenesis, and sulfate-reducing methanotrophy are nearly constant throughout the sediment column. The ΔG for acetate-oxidizing iron reduction is also relatively constant, but more variable than the values for the other reactions. The average ΔG for acetate-oxidizing hematite reduction is -41.6 ± 12.4 (1σ, $n = 27$) kJ (mol-acetate)$^{-1}$ where σ is the standard deviation and n is the number of samples. The ΔG for acetate-oxidizing sulfate reduction is -42.9 ± 2.7 (1σ, $n = 27$) kJ (mol-acetate)$^{-1}$. Acetoclastic methanogenesis has an average ΔG of -23.7 ± 1.2 kJ (mol-acetate)$^{-1}$ (1σ, $n = 27$), slightly more than half as much energy per mole of acetate as sulfate reduction or iron reduction (Fig. 3a). The ΔG for sulfate-reducing methanotrophy averages -19.2 ± 2.0 (1σ, $n = 27$) kJ (mol-methane)$^{-1}$ (Fig. 3b).

4. DISCUSSION

4.1. Biologically-controlled Gibbs energies of reaction for the co-occurring reactions

The in situ Gibbs energies of acetate-oxidizing sulfate reduction and iron reduction, acetoclastic methanogenesis, and sulfate-reducing methanotrophy in the deep subseafloor sediments at Site 1226 are very close to the values observed in actively growing populations that carry out the same reactions at similar temperatures. For acetate-oxidizing sulfate reduction, the yield ($-\Delta G$) is 42.9 ± 2.7 kJ (mol-acetate)$^{-1}$, nearly indistinguishable from a chemostat value, 41.2 kJ (mol-acetate)$^{-1}$ (Seitz et al., 1990) and within the range of an energetic requirement of 33–47 kJ (mol-acetate)$^{-1}$ calculated on the basis of the cellular physiology of sulfate reducers (Jin and Bethke, 2009). In pure culture experiments the energy limit for sulfate reducers falls to 33–43 kJ (mol-acetate)$^{-1}$ (Jin and Bethke, 2009). The yield of acetoclastic methanogenesis, 23.7 ± 1.2 kJ (mol-acetate)$^{-1}$, closely matches values observed in syntrophic batch cultures, 22 kJ (mol-acetate)$^{-1}$ (Lee and Zinder, 1988). Similar yields were also observed in culture experiments with rice paddy soils where the yields ranged from 23 to 35 kJ (mol-acetate)$^{-1}$ (Chin and Conrad, 1995) and reached a constant value of about 25 kJ mol^{-1} after 30 days of incubation (Roy et al., 1997). However, the yield exceeds the value in shallow marine sediments of Cape Lookout Bight (CLB), North Carolina; CLB acetoclastic methanogenesis exhibits a stable ΔG of -11 kJ (mol-acetate)$^{-1}$ at subseafloor depths of a few tens of cm (Hoehler et al., 2001). Possible reasons for this difference include differences in community composition or physiological state at the two sites and/or analytical biases between the two studies. The yield of sulfate-reducing methanotrophy, 19.2 ± 2.0 kJ (mol-methane)$^{-1}$, is consistent with the value in shallow sediments of the Baltic Sea/North Sea region, 22 kJ (mol-methane)$^{-1}$ (Harder, 1997) and the modelled value at the low-temperature end of a mixing zone between cold seawater and a high-temperature vent, approximately 25 kJ (mol methane)$^{-1}$ (McCollom and Shock, 1997).

The in situ Gibbs energy yields of these subseafloor reactions are nearly equal to or greater than the standard estimate of the minimum energy that can be biologically utilized, 20 kJ per mole of proton transported across the membrane (Schink, 1997). This indicates that these reactions are biologically exploitable. The similarities of the yields of these reactions in these sediments to the yields in laboratory cultures and other environments strongly imply that these metabolic pathways are active throughout the sediment column. The relative constancy of the energy yield profiles is consistent with these reactions being driven to the yields close to the usable energy limit by metabolic utilization of a limiting substrate.

The greater downhole variation in the ΔG of iron reduction (relative standard deviation of 30%) may be explained by one or more of the following reasons. (1) It may be caused by vertical variability in the iron-containing phases available for reaction. For example, one or more iron minerals other than hematite may be present in these sediments with variable relative abundances, differing degrees of crystallinity, and different particle sizes. In this case the values used for hematite in the free energy calculation may not reflect the other iron phases present. A more constant ΔG for iron reduction would be expected if iron phases available for reaction were known at each depth. (2) It may result from the downcore variability of dissolved iron concentrations, which are raised to the power of 8 in the free energy calculation (as can be seen in Rxn. (6) and Eq. (12). (3) The downcore variability in pH may play a part since it is raised to the power of 15 in the calculation.

Acetate is a major fermentation product and substrate in anaerobic terminal respiration reactions in marine sediments (Winfrey and Ward, 1983; Lovley, 1987). Our data do not allow us to determine how much acetate is consumed via a particular pathway. But considering that sulfate reduction is the predominant respiration reaction at Site 1226 (Wang et al., 2008), the approximate magnitude of the turnover time for acetate can be estimated. The turnover time is given by the abundance divided by the consumption rate. The magnitude of the estimated consumption rate is equivalent to the sulfate reduction rate, which on average in the topmost 40 m interval is 2.5×10^{-5} mol m^{-3} yr^{-1}, and the average acetate concentration is 6×10^{-4} mol m^{-3}. This gives a turnover time of 24 years. At depths where the rate may be higher, e.g., near the seafloor, a much shorter turnover time is expected. The scale of the turnover time also shows that diffusion does not play a significant role in determining the acetate profile on length scales greater than a few meters since the diffusion length scale is given by $(Dt)^{1/2}$, i.e., 0.7 m, where D is the diffusion coefficient, 0.02 m^2 yr^{-1}, and t is the turnover time. That is, acetate concentration is

predominantly controlled by local production and consumption (on the scale of a meter) rather than transport.

In summary, at Site 1226, the ΔG for acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction, acetoclastic methanogenesis, and sulfate-reducing methanotrophy are stable and poised near biological usable limits for actively growing cells throughout a sediment column that spans more than 200 meters and was deposited over millions of years.

4.2. Maintenance of a complex ecosystem by feedbacks between microbes mediating the reactions

The discovery that the in situ Gibbs energy yield by each of these reactions is poised near a biologically usable limit builds on discoveries that hydrogen concentrations in microbial experiments with shallow near-shore sediment vary with changes in electron acceptor concentration, temperature, and pH to maintain a constant Gibbs energy yield for the predominant hydrogen-oxidizing electron-accepting process (Hoehler et al., 1998). More recent experiments demonstrate that fermentative bacteria similarly adjust their hydrogen production rates to maintain constant Gibbs energy yields despite experimental changes in reactant concentrations, product concentrations, and temperature (Adams et al., 2006), while Jin and Bethke (2003) have showed how rates of reaction may be directly dependent on the net Gibbs energy of respiration reactions combined with ATP synthesis.

In the subseafloor ecosystem of Site 1226, concentrations of the reactants and products of multiple reactions are simultaneously regulated through negative and positive feedbacks that maintain nearly constant Gibbs energy yields presumably throughout the 7-million-year history of sedimentation represented by the upper 275 m of the sediment column. These feedbacks are driven by the dependence of the energy yields on the concentrations of common substrates and reaction products.

For example, yields of acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction and acetoclastic methanogenesis depend on acetate concentrations while yields of acetate-oxidizing sulfate reduction and sulfate-reducing methanotrophy depend on sulfate concentrations. It is expected that if only negative feedbacks, via competition for a common substrate, are involved, then one pathway would dominate. The pathway that would dominate would be the one that has biologically utilizable energy yields and drives the energy yield of the competing pathways below utilizable values by depleting the common substrate.

However, despite this competition, no single metabolic pathway has gained the competitive advantage necessary to drive the other reactions from this subseafloor sedimentary ecosystem. Instead, acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction, and the combination of acetoclastic methanogenesis and sulfate-reducing methanotrophy all yield nearly identical free energies per mole of acetate {respectively 42.9 ± 2.7 kJ, 41.6 ± 12.4 kJ, and 42.9 ± 2.4 kJ [(23.7 ± 1.2) kJ + (19.2 ± 2.0) kJ}.

We hypothesize that the co-occurrence of these reactions in this ecosystem is sustained by the addition of positive feedbacks between microbes mediating these pathways. Some of these feedbacks are syntrophic. For example, acetoclastic methanogenesis provides the electron donor for sulfate-reducing methanotrophy. A positive feedback exists between microbes mediating these processes because consumption of methane helps to maintain energetic favorability of methanogenesis by removing the product of methanogenesis from the system. Similar syntrophic relationships are well known from culturing experiments and other environments (Schink, 2002; Dale et al., 2008), but have not been previously recognized in hundreds-of-meter deep subseafloor ecosystems.

Other feedbacks in the deep subseafloor sedimentary ecosystem of Site 1226 are mutualistic, but not necessarily syntrophic. For example, iron reducers and sulfate reducers influence the energetics and kinetics of the reactions mediated by each other, both of which control the respiration rates in energy-limited sediments (Jin and Bethke, 2005, 2007). Products of these reactions (ferrous iron and sulfide) co-precipitate as iron sulfide. This co-precipitation of dissolved products keeps both reactions energetically favorable, forming a positive feedback. Under the control of this feedback, if iron reducers respire iron at a higher rate, increased dissolved iron causes more dissolved sulfide to precipitate. This decrease in the concentration of dissolved sulfide causes sulfate reduction to be energetically more favourable. The sulfate reducer can then catabolize sulfate at a higher rate until its minimum usable energy is again reached (and vice versa).

Iron reduction in clays is expected to have a negative feedback on the other reactions because it competes for acetate. However, because the clay iron species [(Fe(II) or Fe(III)] remains trapped in the clay matrix where the reduction occurs (König et al., 1999) and does not precipitate dissolved sulfide, iron reduction in clays renders no positive feedback on the energetics of sulfate reduction. Thus, iron reduction in clays is not a major player in the maintenance of multiple metabolic pathways in this sedimentary ecosystem.

These mutual energetic feedbacks form a web through which this complex ecosystem that utilizes multiple metabolic pathways may be maintained under an energy-limited condition for millions of years. Under such condition the thermodynamic driving force on the respiration rates is expected to be dominant (Jin and Bethke, 2005, 2007). As a result of these feedbacks, the relative rates of these metabolic reactions adjust until their in situ ΔG reach biologically usable limits. Because sulfate is the principal terminal electron acceptor for organic matter oxidation in terms of the amount of carbon oxidized [1 mol of sulfate is required for the oxidation of 2 mol of oxidation state 0 organic carbon while 1 mol of Fe(III) oxidizes 1/4 mol of oxidation state 0 organic carbon] in this ecosystem and iron reduction occurs at high enough rates to remove most dissolved sulfide from the ecosystem (Wang et al., 2008), the feedbacks between iron reducer and sulfate reducer appear to predominate in the feedback web of this system. Thus, concentrations of acetate and bicarbonate are mainly

controlled by the relative rates of iron reduction and sulfate reduction in this subseafloor system (because the relative rates of those reactions control the concentration of dissolved $\sum H_2S$ and the energy yields of both reactions remain close to the biologically usable energy limits).

Although acetate-utilizing reactions constitute only a few of the redox reactions mediated by microbes in subseafloor sediments, they serve as a model for the microbial energetics of the entire anoxic subseafloor sedimentary ecosystem. Similar feedbacks presumably exist for reactions that utilize other electron donors (e.g., formate and lactate). Because the latter reactions utilize many of the same reactants [e.g., SO_4^{2-} and Fe(III)] and generate many of the same products as the acetate-utilizing reactions (e.g., dissolved sulfide, dissolved Fe(II) and DIC], we predict that the entire deeply buried sedimentary ecosystem is a highly coupled reaction network where metabolic diversity is maintained by a complex web of positive and negative feedbacks. Compared to surface or energy-rich ecosystems, such as shallow marine sediments, the complexity of the energy-poor deep subseafloor ecosystem may be not as high in the sense that the feedback web may be not so densely formed. However, our results reveal that the reaction network may be similarly highly coupled, if not more highly coupled, to maintain the metabolic diversity to their energy limits in such a food-limiting environment.

5. CONCLUSIONS

Throughout most of the sediment column at ODP Site 1226, the Gibbs energies of reaction for acetate-oxidizing sulfate reduction, acetate-oxidizing iron reduction, acetoclastic methanogenesis, and sulfate-reducing methanotrophy are relatively constant and close to the ΔG for the same reactions in laboratory cultures and other environments.

Although microbes that mediate these reactions compete for substrates, syntrophic and mutualistic interactions appear to sustain the co-existence of these reactions for millions of years (the interval over which the sediments have been deposited).

These competing, syntrophic, and mutualistic interactions collectively constitute a highly coupled reaction network where dissolved chemical concentrations are regulated by the energetics of biologically mediated reactions operating near their biologically minimum energy yield. Because the deep sediment of Site 1226 is not physically or chemically exceptional in any obvious way, microbial interactions are likely to constitute similar reaction networks that are likely to occur in deep anoxic sediment throughout the subseafloor ocean.

ACKNOWLEDGMENTS

We greatly appreciate the thoughtful comments of Andrew Dale, David Burdige, Wolfgang Bach, Craig Bethke, and two other anonymous reviewers that have significantly improved the manuscript. We thank the NASA Astrobiology Institute for funding this study. We also thank Edward Roggenstein for measuring some of the pore water Ca^{2+} concentrations.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2010.03.034.

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Associate editor: David J. Burdige