Apparent minimum free energy requirements for methanogenic archaea and sulfate-reducing bacteria in an anoxic marine sediment

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Abstract

Among the most fundamental constraints governing the distribution of microorganisms in the environment is the availability of chemical energy at biologically useful levels. To assess the minimum free energy yield that can support microbial metabolism *in situ*, we examined the thermodynamics of H_2-consuming processes in anoxic sediments from Cape Lookout Bight, NC, USA. Depth distributions of H_2 partial pressure, along with a suite of relevant concentration data, were determined in sediment cores collected in November (at 14.5°C) and August (at 27°C) and used to calculate free energy yields for methanogenesis and sulfate reduction. At both times of year, and for both processes, free energy yields gradually decreased (became less negative) with depth before reaching an apparent asymptote. Sulfate reducing bacteria exhibited an asymptote of -19.1 ± 1.7 kJ·(mol SO_4^{2-})$^{-1}$, while methanogenic archaea were apparently supported by energy yields as small as -10.6 ± 0.7 kJ·(mol CH_4)$^{-1}$.

Key Words: Anoxic sediments, Hydrogen, Critical Free Energy

1. Introduction

Currently understood mechanisms of energy conservation require that, in order to be biologically useful, energy must be available at levels not less than one-third to one-fifth of the energy needed to phosphorylate ADP to ATP [1]. The existence of this "biological energy quantum" means that a significant amount of the chemical energy available on Earth cannot be exploited by life. Likewise, the absolute magnitude of the biological energy quantum must be a critical parameter in determining the distribution of microbial life in the environment. This is particularly relevant for oligotrophic settings, such as deep sea sediments and crustal rocks, which are now thought to potentially harbor a vast "deep biosphere" [2-5].

Extensive research based on organisms in culture provides a detailed understanding of energy conservation in chemotrophic anaerobes [6-10]. These studies, combined with theoretical considerations, suggest a quantum of about -20 $kJ \cdot mol^{-1}$ as the minimum energy that can be exploited by living cells [1]. A clear next step in understanding microbial energy conservation is to examine the apparent energy requirements of microorganisms in natural ecosystems, where they are often obligated to function at the energetic fringe.

This study sought to investigate *in situ* bioenergetic requirements by monitoring the free energy yields obtained by H_2-consuming microorganisms in anoxic marine sediments from Cape Lookout Bight (CLB), NC, USA. In such sediments, H_2 is produced via bacterial fermentation of complex organic matter [11, 12], and is

subsequently consumed by sulfate-reducing bacteria (SRB) or, when sulfate is absent, methane-producing archaea (MPA) [13-16]:

$$SO_4^{2-} + 4H_2 \xrightarrow{SRB} S^{2-} + 4H_2O \quad (1)$$

$$CO_2 + 4H_2 \xrightarrow{MPA} CH_4 + 2H_2O \quad (2)$$

The coupling between production and consumption of H_2 in CLB is very close, resulting in very low partial pressures and very short residence times for H_2 [17], as is typical for sedimentary environments [18].

Previous studies have shown that in such closely coupled systems, H_2 concentrations are controlled by the H_2-consuming organisms in a pattern that reflects the availability of free energy. Specifically, H_2-consuming organisms catalyzing more exergonic processes can generally maintain H_2 partial pressures at lower levels. This effect has been demonstrated by varying the terminal electron acceptor (e.g., SO_4^{2-} –vs- CO_2) [17, 19, 20] or the temperature [21-24], both of which affect the free energy yield of H_2 consumption. Subsequently, we demonstrated that the relationship between H_2 partial pressures and intracellular bioenergetics in CLB sediments is *quantitative* temperature and sulfate concentration [17]. Based on this finding, we utilized measurements of H_2 partial pressures in CLB sediment cores to examine the bioenergetics of methanogenesis and sulfate reduction in a natural ecosystem.

2. Methods

2.1. Study Site and Sampling Methodology

Sediments for laboratory studies and depth profiling were obtained from Cape Lookout Bight, a 10-m deep back barrier island lagoon located in coastal North Carolina,

USA. Due to high rates of sedimentation [25] and organic carbon loading [26], the sediments are free of bioturbation and completely anoxic below 2 mm [27]. Sulfate reduction is the dominant terminal electron-accepting process in the upper portion of the sediment column; below the depth of sulfate depletion, which varies seasonally between 8 and 25 cm, methanogenesis is the dominant process [28].

2.2. Experimental and Analytical Methodology

Free energy calculations are based on concentration data originally reported in [17], which includes a detailed description of methodology for the laboratory and field investigations discussed here. A brief synopsis of the experimental design follows:

Temperature Experiments: Actively methane-producing sediments from Cape Lookout Bight were slurried with a sterile anoxic solution of either NaCl (salinity = 34‰) or NaCl + Na_2SO_4 to a final porosity of approx. 0.9 (a 25% dilution of the whole sediment). The final sulfate concentration in the first treatment was <10μM and methanogenesis remained active; in the second, the sulfate concentration was 35 mM and sulfate reduction supplanted methanogenesis as the dominant terminal metabolic process. The methanogenic slurry was divided among 20 serum vials and the sulfate-reducing slurry among 16 vials, and these replicates were divided evenly among four constant temperature baths (9.0, 15.5, 23.4, and 29.8°C) for a 2-week incubation in the dark. At the end of the period, previously described methods were employed for analysis of H_2, CH_4, ΣCO_2, ΣH_2S, and SO_4^{2-} in each sample vial [17].

Sulfate Concentration Experiment: Actively methanogenic sediments from Cape Lookout Bight were slurried with a sterile anoxic solution containing NaCl +

Na_2SO_4 to a final porosity of approx. 0.9 (a 25% dilution of the whole sediment). A final sulfate concentration of 6 mM was measured and sulfate reduction became the dominant mode of terminal metabolism. The slurry was dispensed into 15 serum vials, which were subsequently amended with concentrated anoxic solutions of NaCl and/or Na_2SO_4 to yield final sulfate concentrations of 6, 11, 25, 52, and 104 mM (n=3 replicates each). The vials were incubated in the dark at 22°C for one week, after which each sample vial was analyzed for H_2, ΣH_2S, and SO_4^{2-} using previously described methods [17]. Two of the 104 mM samples were compromised during the incubation period, so that the H_2 partial pressure reported at this concentration reflects a single sample.

Depth Profiles: Cores of sediment were taken from Cape Lookout Bight at each of two sampling times (August, 40-cm long core, sediment temp. = 27°C; and November, 70-cm long core, sediment temp. = 14.5°C). Sediments were subsampled at desired depth intervals, transferred anaerobically to serum vials without slurrying, and incubated in the dark at *in situ* temperature for 5 days. During the incubation, the sediment was disturbed as little as possible in order to avoid disrupting *in situ* microbial assemblages. At the end of the incubation, each sample was analyzed for H_2, CH_4, and SO_4^{2-} using previously described methods [17].

Concentrations of ΣCO_2 and ΣH_2S were not measured but were instead taken as the average of previously measured depth profiles at the same site, time of year, and temperature. For ΣH_2S, we averaged 4 July/August profiles taken over 3 years and 2 November profiles taken over 2 years (from [29]); for ΣCO_2, we used 3 July/August profiles taken over 3 years and 2 November profiles taken over 2 years (from [30] and [31]). Year-to-year variation ($\pm 1\sigma$) in ΣCO_2 was always less than $\pm$ 15% at all depths in

the sediment column. For ΣH_2S, variation was less than ± 20% at all depths, except for the upper 3 cm of the sediment column in November, where it was ± 74%.

2.3. Thermodynamic Calculations

Free energy values for H_2-based sulfate reduction (ΔG_{SR}) or methanogenesis (ΔG_{MP}) under in situ conditions were calculated from

$$\Delta G_{SR} = \Delta G^{\circ}_{(T)\text{-}SR} + RT \cdot \ln\left(\frac{\{S^{2-}\}}{\{SO_4^{2-}\}(P_{H_2})^4} \right) \quad (3)$$

or

$$\Delta G_{MP} = \Delta G^{\circ}_{(T)\text{-}MP} + RT \cdot \ln\left(\frac{P_{CH_4}}{P_{CO_2}(P_{H_2})^4} \right) \quad (4)$$

where {} denotes activity; P denotes partial pressure; R is the universal gas constant; T is absolute temperature; and $\Delta G^{\circ}_{(T)\text{-}SR}$ and $\Delta G^{\circ}_{(T)\text{-}MP}$ are the standard free energies of reaction for sulfate reduction and methane production, corrected to ambient temperature using the Gibbs function, $\Delta G = \Delta H - T\Delta S$ [32]. Standard free energies of formation of products and reactants (used to calculate ΔG°_{SR} and ΔG°_{MP}) were taken from [32] and [33]. Partial pressures differ from fugacities by less than 0.1% under the P-T conditions of our experimental systems [32], and fugacity coefficients are thus neglected in the present treatment.

Speciation in the ΣCO_2, ΣH_2S, and ΣH_2SO_4 systems was calculated based on a measured average pH of 7.2 (which remained approximately constant with depth and temperature in the sediments) and using acidity and Henry's law constants from [32] and

[34]. Errors reported for the free energy values are calculated through a standard error propagation routine, utilizing the standard deviation among replicates (for all measured quantities) and an assumed standard deviation of ± 20% for ΣCO_2 and ΣH_2S in the down-core profiles (see section 2.2). For the uppermost sample of the November profile, a standard deviation of ± 75% for ΣH_2S was assumed, per the discussion in section 2.2.

3. Results and Discussion

3.1. Temperature and Sulfate Concentration Experiments

Two previously published experiments demonstrate that H_2 partial pressures in CLB sediments are controlled quantitatively by the bioenergetics of H_2-consuming microorganisms [17]. In the first, H_2 partial pressures were monitored in sulfate-reducing and methanogenic sediments that were subjected to variations in temperature over the range of 10 to 30°C. If the concentrations of all products and reactants remained constant, this temperature increase would change the *in situ* free energy yield for both processes (ΔG_{SR} and ΔG_{MP} in eq. 3 and 4) by approximately +17 $kJ \cdot mol^{-1}$, meaning much less free energy is available at higher temperature. However, in both treatments, the H_2 partial pressure increased with increasing temperature (Figure 1a), similar to previous observations in methanogenic [21-24] and sulfate-reducing [23] systems. Relative to the initial sediment temperature of 22°C, the H_2 partial pressure either increased *or* decreased in a way that was exactly sufficient to offset the temperature-induced change in ΔG, and thereby maintain a constant (± 3%) free energy yield for the H_2-consuming organisms: $\Delta G_{MP} = -15.8 \pm 0.5$ $kJ \cdot mol^{-1}$ in methane-producing sediments and $\Delta G_{SR} = -24.7 \pm 0.6$ $kJ \cdot mol^{-1}$ under sulfate-reducing conditions (Figure 1b).

In a second experiment, H_2 partial pressures in an actively sulfate-reducing sediment were found to decrease in response to increasing sulfate concentrations (Figure 2a). According to equation 3, an increase in sulfate concentrations makes ΔG_{SR} more negative (more available free energy), which could allow for more efficient utilization of H_2. Solving equation 3 for P_{H2}:

$$P_{H_2} = \left(\frac{\{S^{2-}\}}{\{SO_4^{2-}\}} \cdot \exp\left(\frac{\Delta G^{\circ}_{(T)SR} - \Delta G_{SR}}{RT} \right) \right)^{\frac{1}{4}} \quad (5)$$

shows that, in order for sulfate reducers to maintain a constant ΔG_{SR} (as in the temperature experiment), the H_2 partial pressure must change according to the relationship $P_{H2} \propto \{SO_4^{2-}\}^{-0.25}$, given constant T and approximately constant $\{S^{2-}\}$, as maintained in this experiment. The experimental results agreed closely, exhibiting the relationship $P_{H2} \propto \{SO_4^{2-}\}^{-0.26 \pm 0.01}$, with $r^2 = 0.996$ (Figure 2a). Thus, as in the temperature experiment, the variation in H_2 was exactly sufficient to offset the sulfate-induced change in ΔG_{SR} and maintain a constant energy yield ($\Delta G_{SR} = -22.8 \pm 0.8$ kJ·mol^{-1}) for sulfate-reducing bacteria (Figure 2b).

In both experiments, the variation in H_2 returns the system to a specific value of ΔG_{SR} or ΔG_{MP}, regardless of whether the environmental change (i.e., in temperature or sulfate) is positive or negative with respect to the free energy yield. This can be explained if:

1) H_2-consuming organisms draw down the H_2 partial pressure to as low a level as is physiologically possible.

2) The physiologic limitation in this system is a bioenergetic one, so that the H_2 partial pressure is consistently held at a level corresponding to biologically-critical free energy yield.

The second point implies that measurements of H_2 partial pressure in CLB sediment cores can be used to quantitatively assess the energy requirements of methanogens and sulfate reducers *in situ*. In order to do so, however, it is critical to consider how the spatial distribution of H_2-consuming organisms affects the partial pressure of H_2 in the bulk pore fluid – for it is this pool of H_2 that is sampled by the H_2 measurement technique, and which is ultimately used to calculate *in situ* energy yields.

If H_2-consuming organisms are distributed randomly in relation to discrete sources of H_2, the supply of H_2 must be drawn from the bulk pore fluid (Figure 3a). In order to maintain mass transport into the cell, a gradient in H_2 between the cell surface and the bulk pore fluid is required [35]. Because the H_2 partial pressure measured in the bulk pool would thus always be higher than that at the cell surface, the actual energy yield obtained by H_2-consuming organisms would always be overestimated by pore fluid measurements. Such a scenario cannot simultaneously account for the quantitative link between intracellular thermodynamics and extracellular (bulk fluid) H_2 partial pressures that is evident in both the temperature and sulfate experiments, unless the magnitude of the H_2 concentration gradient is very small in comparison to the measured partial pressures.

Alternatively, H_2-consuming organisms could be localized around discrete sources of H_2 in order to intercept the outgoing flux (Figure 3b). Conrad [36] has previously shown that such assemblages may account for a high percentage of H_2 cycling in natural

ecosystems. For any given H_2 source, the impetus for continued colonization by H_2-consumers would persist until the H_2 efflux was reduced to a level that was no longer biologically accessible. Assuming a bioenergetic limitation on H_2 consumption, the H_2 partial pressure outside such clusters (in the bulk pore fluid, where measurements are made) would then be fixed at levels corresponding precisely to the minimum energy requirements of the H_2 consumers. In this mechanism, changes in temperature or sulfate concentration would automatically adjust pore water H_2 to a partial pressure exactly equivalent to the critical ΔG_{SR} or ΔG_{MP}, as observed for the experimental treatments.

It is important to note that in such a scenario, the measured H_2 partial pressure would not necessarily reflect the energetic environment experienced by all, or even most, H_2-consuming organisms in the system. Instead, it must reflect the minimum energetic yields that can still drive metabolism of H_2 in the outermost "shell" of H_2-consuming clusters – potentially only a small fraction of the H_2 consuming population, but presumably having the most efficient organisms. With this in mind, we sought to use down-core H_2 measurements as a means of probing the *in situ* energy requirements of methanogens and sulfate reducers in an intact sediment microbial ecosystem.

3.2. Depth Profiles of ΔG_{SR} and ΔG_{MP} in Cape Lookout Bight Sediments

Depth distributions of H_2 partial pressure were measured in CLB sediments and used to determine down-core variations in ΔG_{SR} and ΔG_{MP} for natural populations of sulfate-reducing and methanogenic bacteria. Here, we present data from two months: August, when warmer temperatures (27.0°C) result in higher sulfate reduction rates and sulfate depletion at shallow depths (~10 cm ; Fig. 4a); and November, when relatively

cool temperatures (14.5°C) allow for somewhat deeper sulfate penetration (~15 cm; Fig. 4c). At both times of year, energy yields for sulfate-reducing bacteria increase (become less favorable) with depth before reaching an apparent asymptote. In August, an average value of -20.3 ± 0.6 kJ·mol^{-1} is reached by 4 cm depth and maintained until the depletion of sulfate at approximately 10 cm (Fig. 4b); under cooler conditions, an average of -19 ± 1.8 kJ·mol^{-1} is maintained between 7 cm and the sulfate depletion depth of 16 cm (Fig. 4d). For methanogens, ΔG_{MP} values were generally greater than zero when the sediments contained sulfate, consistent with H_2-based competitive exclusion of methanogenesis by sulfate reducers[15, 16]. Below the sulfate depletion depth (where methanogenesis becomes active [23]) ΔG_{MP} values increase with depth before reaching average values of -10.9 ± 0.3 (August; Fig. 4b) and -10.4 ± 0.8 kJ·mol^{-1} (November; Fig. 4d) below about 25 cm. Interestingly, this 25 cm "inflection point" marks the horizon above which the methanogenic community is seasonally disrupted by infiltration of sulfate (above 25 cm), but below which the community can adapt to permanently methanogenic conditions.

Thus, sulfate reducers in CLB sediments function with energy yields close to the biologically useful minimum of -20 kJ·mol^{-1} that is suggested by culture-based studies [1]. Methanogens in these sediments can apparently support metabolism of H_2 with energy yields as low as -10 kJ·mol^{-1}. This value is significantly lower than has been calculated for several other environments [37], but is comparable, in general, to the findings of Westermann [24]. In support of the low energy yields observed for H_2-based methanogenesis, we calculate from pore water acetate concentrations [38] that acetoclastic methanogenesis in CLB sediments proceeds at very similar levels, ranging from $\Delta G_{MP(acetate)} = -12.8$ kJ·mol^{-1} (in the summer) to -10.5 kJ·mol^{-1} (in the winter).

3.3. The Critical Free Energy

The very low energy yields observed for methanogens in CLB sediments, relative to organisms in culture, are likely the result of adaptation by an intact microbial community to long-term substrate limitation. In CLB sediments, the quantity of reactive organic matter (and thus, the rate of fermentative H_2 production) decreases in roughly exponential fashion with depth, corresponding to age of the sediment [26]. Therefore, after colonizing the sediment to an extent that is commensurate with the initial amount of organic matter, bacteria are faced with an ever-decreasing supply of substrate. The community as a whole must adapt for years afterwards to starvation-level conditions. This is reflected in each of the down-core free energy profiles (Fig. 4B and 4D) by initially more negative values of ΔG_{SR} and ΔG_{MP} that only approach asypmtotic levels at depths representing many months of sediment accumulation.

Optimization of energy conservation efficiency, to allow organisms to exploit low energy substrates, would depend on variability in the parameters which ultimately determine the biological energy quantum, ΔG_{min} [39]:

$$\Delta G_{min} = \frac{\Delta G_{ADP \rightarrow ATP}}{n \cdot f} \qquad (6)$$

where n, $\Delta G_{ADP \rightarrow ATP}$, and f are defined as follows:

1. n is the stoichiometry of ions translocated per ATP formed in chemiosmotic energy conservation. Most commonly, $n = 3$ ions per ATP, however recent work suggests that some methanogens may utilize systems with $n = 4$ or 5 (see review in [10]).

2. $\Delta G_{ADP \rightarrow ATP}$ is the energy required for phosphorylation of ADP *in vivo*. This quantity depends directly on the intracellular ratio of ATP to ADP, which is about 10:1 in actively growing cells [6]. However, if the free energy available from the energy-harvesting metabolism (e.g., sulfate reduction or methanogenesis) is insufficient to catalyze ADP phosphorylation, ADP will quickly accumulate as cellular metabolism continues to hydrolyze ATP. This would lower the ATP:ADP ratio until ADP phosphorylation again becomes favorable. Every factor of 10 decrease in the ATP:ADP ratio decreases the overall energy requirement by about 5 $kJ \cdot mol^{-1}$, potentially allowing organisms to capitalize on lower *in situ* free energy changes simply by maintaining a lower "energy charge". This effect must of course be limited to an energy charge not lower than the minimum needed to drive ATP-coupled biochemistry.

3. f, the thermodynamic efficiency factor, is the fraction of total available free energy that is actually conserved. Energy-harvesting metabolic processes frequently occur via multiple reaction steps, not all of which are coupled to energy-conserving mechanisms (e.g., ion gradient formation). Any of these non-coupled reactions which operate at a disequilibrium (as is required for a reaction to proceed in a net forward direction) have an associated free energy change, $\Delta G < 0$, which is lost from the system as heat. In actively growing anaerobic cultures, up to 60% of the available free energy may be lost in this way [6]. However, when the availability of "conservable" free energy becomes limiting, ATP synthesis will become the rate-limiting step in the overall energy-harvesting metabolism. Rate limitation at this final step would cause accumulation of the products, and decrease in forward reaction rate,

of each of the non-coupled reactions, bringing them ever closer to equilibrium (ΔG = 0). This process would continue until the energy-coupled reactions garnered a sufficient fraction of the overall energy yield to drive ATP synthesis or the system reached a value of f = 1 (signifying complete equilibrium in the non-coupled reactions, where metabolism would cease to proceed in a net forward direction). Utilizing values of n = 3, $\Delta G_{ADP \rightarrow ATP}$ = + 50 kJ·mol^{-1} (ATP:ADP = 10), and $f \cong 0.8$, as measured for cells in *actively growing* cultures, gives an energy quantum of about -20 kJ·mol^{-1} [1]. But the population for which we calculate an *in situ* ΔG_{min} is almost certainly *not* actively growing; rather, it is likely functioning at the bare minimum required to sustain metabolic turnover of H_2. The difference between the culture-based and *in situ* ΔG_{min} values might thus reflect the potential for optimization of energy-conserving efficiency, through the mechanisms described above, in the microbial populations of CLB sediments.

It is important to point out that optimization of f and $\Delta G_{ADP \rightarrow ATP}$ in the fashion described above represents a trend towards a metabolism that is at equilibrium with respect to ATP formation. This is a dramatic departure from conditions of active growth, in which large quantities of energy are sacrificed in order to assure unidirectional metabolism. In an environmental context, both metabolic states are important. Clearly, estimates of growth energy requirements are needed to address the potential for microbial colonization of oligotrophic environments. However, it seems likely that "steady-state" activity accounts for a much greater proportion of microbially-catalyzed chemical cycling on Earth than does actual cell growth, so that a knowledge of maintenance energy requirements is also critical for understanding environmental biogeochemistry.

This point is illustrated by anaerobic methane oxidation (AMO). This process is widespread in the marine environment and significant in global geochemistry [40], but the responsible microbial agents have not been successfully cultured. Most recent evidence implicates a two-member bacterial consortium consisting of an archaeal (methanogenic?) and a sulfate-reducing partner [23, 41-45]. The total energy available from the process under most environmental conditions would generally be insufficient to support two organisms with a -20 $kJ \cdot mol^{-1}$ growth energy requirement. However, sustained biological catalysis of AMO by an existing consortium would carry a significantly lower energy requirement. For example, AMO actively occurs in CLB sediments at H_2 partial pressures that translate to energy yields of -13.5 ± 1.0 $kJ \cdot mol^{-1}$ for the methanogenic partner [23] and -18.8 ± 1.2 $kJ \cdot mol^{-1}$ for the sulfate-reducing partner – meeting the apparent *in situ* free energy requirements of both organisms.

How many other environmentally relevant processes might function in the region between the energy quantum observed in actively growing cultures and the apparent critical free energies required for intact communities *in situ*? The answer to this question gains added significance when considering that the function of >99% of the microorganisms on Earth remains uncharacterized from the standpoint of culture-based studies [46]. Establishing the magnitude of the gap, if any, between critical growth energies and critical survival energies may be an important step in understanding the distribution and nature of microbial life in oligotrophic environments.

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Figure Captions:

Figure 1. Response of H_2 partial pressure and free energy yield to variation in temperature. **(A)** H_2 partial pressure versus temperature under sulfate-reducing (●) or methanogenic (○) conditions. Error bars represent ± one standard deviation about the mean of n = 4 - 5 replicate sediment samples. **(B)** ΔG_{SR} (●) and ΔG_{MP} (○) (under sulfate-reducing and methanogenic conditions, respectively) versus temperature. Error bars reflect the standard errors of measurement from (A), propagated through the ΔG calculation. Solid lines represent the average free energy yield that was maintained across the range of temperatures: ΔG_{SR} = - 24.7 ± 0.6 kJ·mol^{-1} and ΔG_{MP} = -15.8 ± 0.5 kJ·mol^{-1}. Dashed lines represent the temperature-based change in ΔG that would have occurred if the H_2 partial pressure did not change from the levels measured at the initial sediment temperature of 22°C.

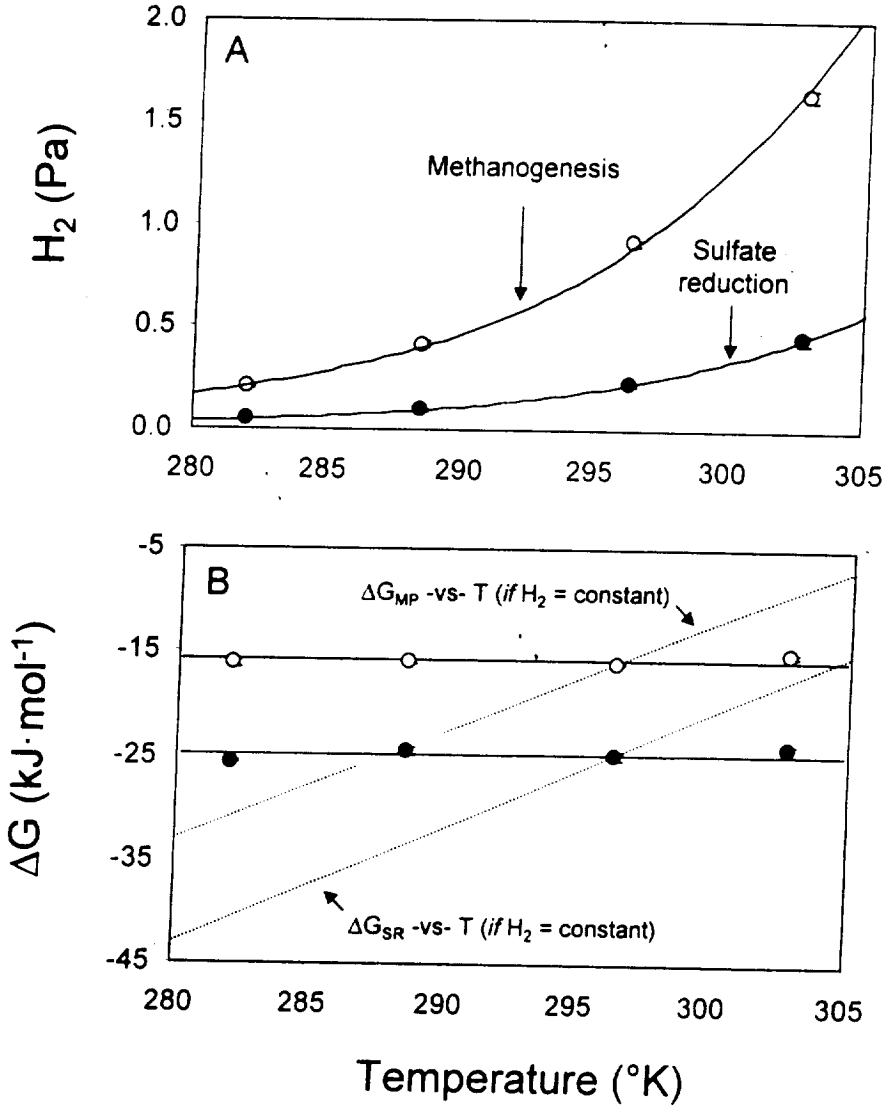
Figure 2. Response of H_2 partial pressure and free energy yield to variation in pore water sulfate concentration. **(A)** H_2 partial pressure versus sulfate concentration in sediments dominated by sulfate-reducing bacteria. Error bars represent one standard deviation about the mean of n = 3 replicate sediment samples. For the 105 mM sulfate sample, no replicates were measured. The solid curve represents a power function fit to the data. Compare the observed exponential dependence of H_2 on sulfate, –0.26 ± 0.01 (r^2 = 0.993), to the value of –0.25 theoretically required to maintain a constant free energy yield. **(B)** ΔG_{SR} versus sulfate concentration. Error bars reflect the standard errors of

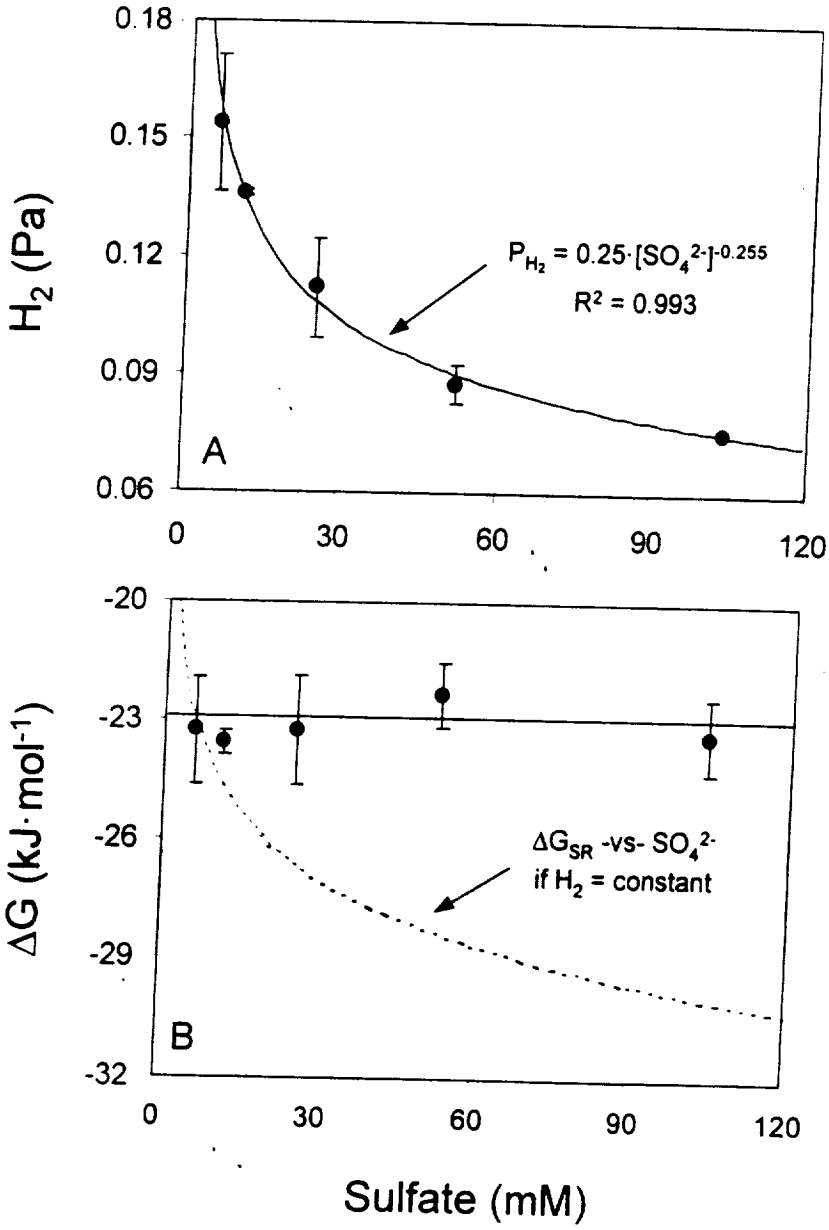
measurement from (A), propagated through the ΔG calculation. For the 105 mM point, which had only 1 replicate, an error of ± 7% for H_2 partial pressure (representing the average measurement error for the other four sulfate concentrations) was assumed for the error propagation calculations. The solid line represents the average free energy yield that was maintained across the range of sulfate concentrations: $\Delta G_{SR} = -22.8 \pm 0.8$ kJ·mol^{-1}. The dashed line represent the sulfate-based change in ΔG_{SR} that would have occurred if the H_2 partial pressure did not change from the level measured at the initial pore water sulfate concentration of 5.9 mM.

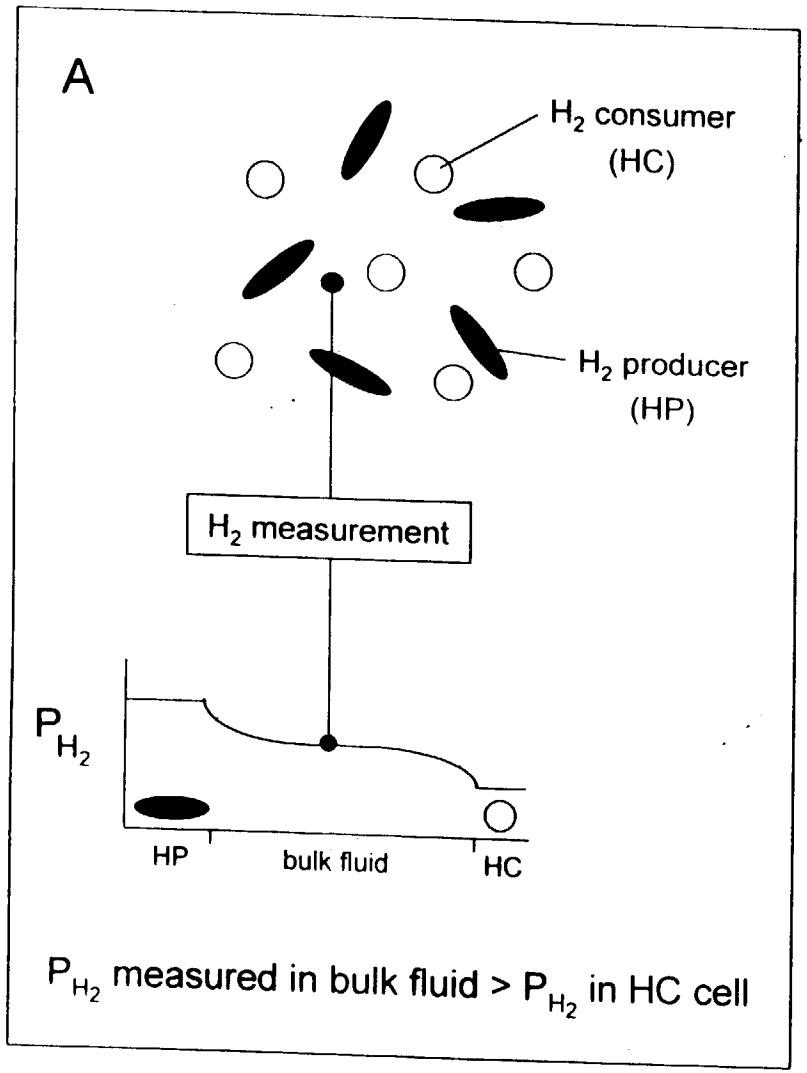
Figure 3. Dependence of measured H_2 partial pressure on spatial organization of H_2 consumer cells. **(A)** Random arrangement of H_2 consumer cells in relation to H_2 sources. To maintain mass transport of H_2, consumer cells maintain a gradient in P_{H_2} between the bulk extracellular fluid and the intracellular medium. H_2 measurements, which sample the bulk extracellular fluid, overestimate the intracellular partial pressure. **(B)** H_2 consumer cells arranged in a network around individual sources of H_2. An efficient network would continue to remove H_2 down to the minimum biologically useful level, with the remainder escaping to the bulk extracellular fluid. H_2 measurements in this fluid would thus reflect exactly the H_2 partial pressure in the intracellular medium of the last "shell" of H_2-consuming organisms.

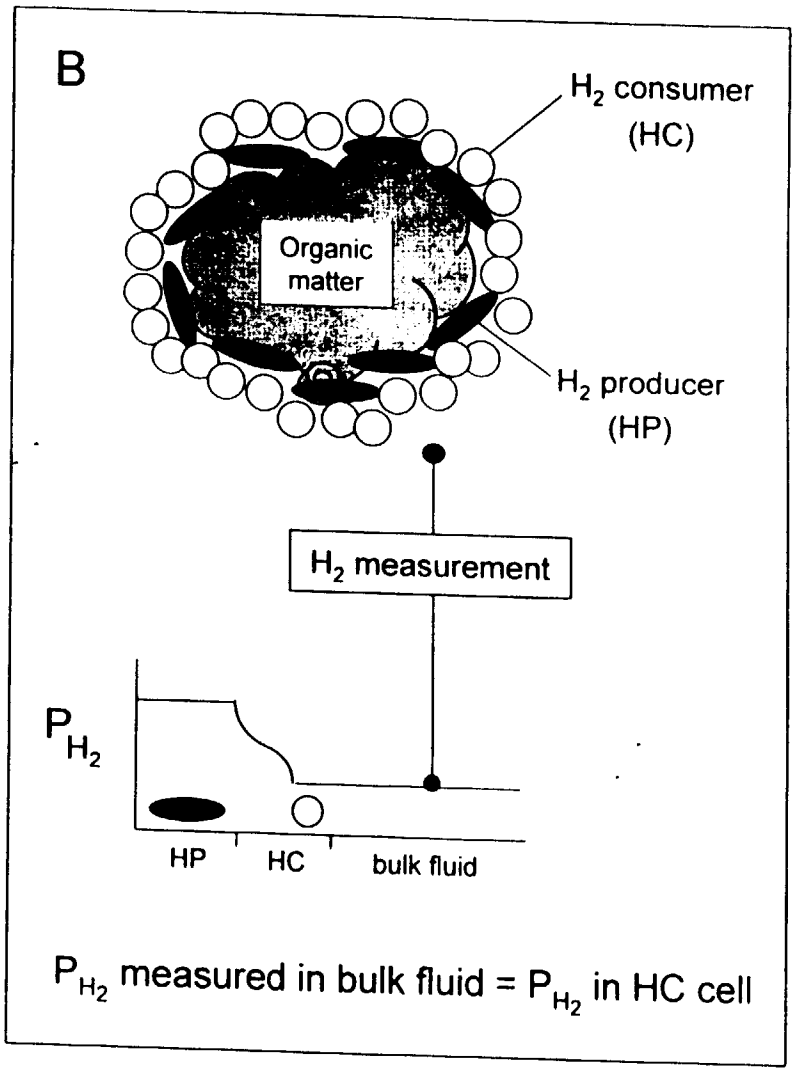
Figure 4. Down-core profiles of *in situ* concentrations and free energy yields in sediment cores from Cape Lookout Bight. **(A)** and **(C)**: Concentrations of H_2 (○) and sulfate (●) in a sediment cores taken in August (27.0°C) and November (14.5°C), respectively. Error

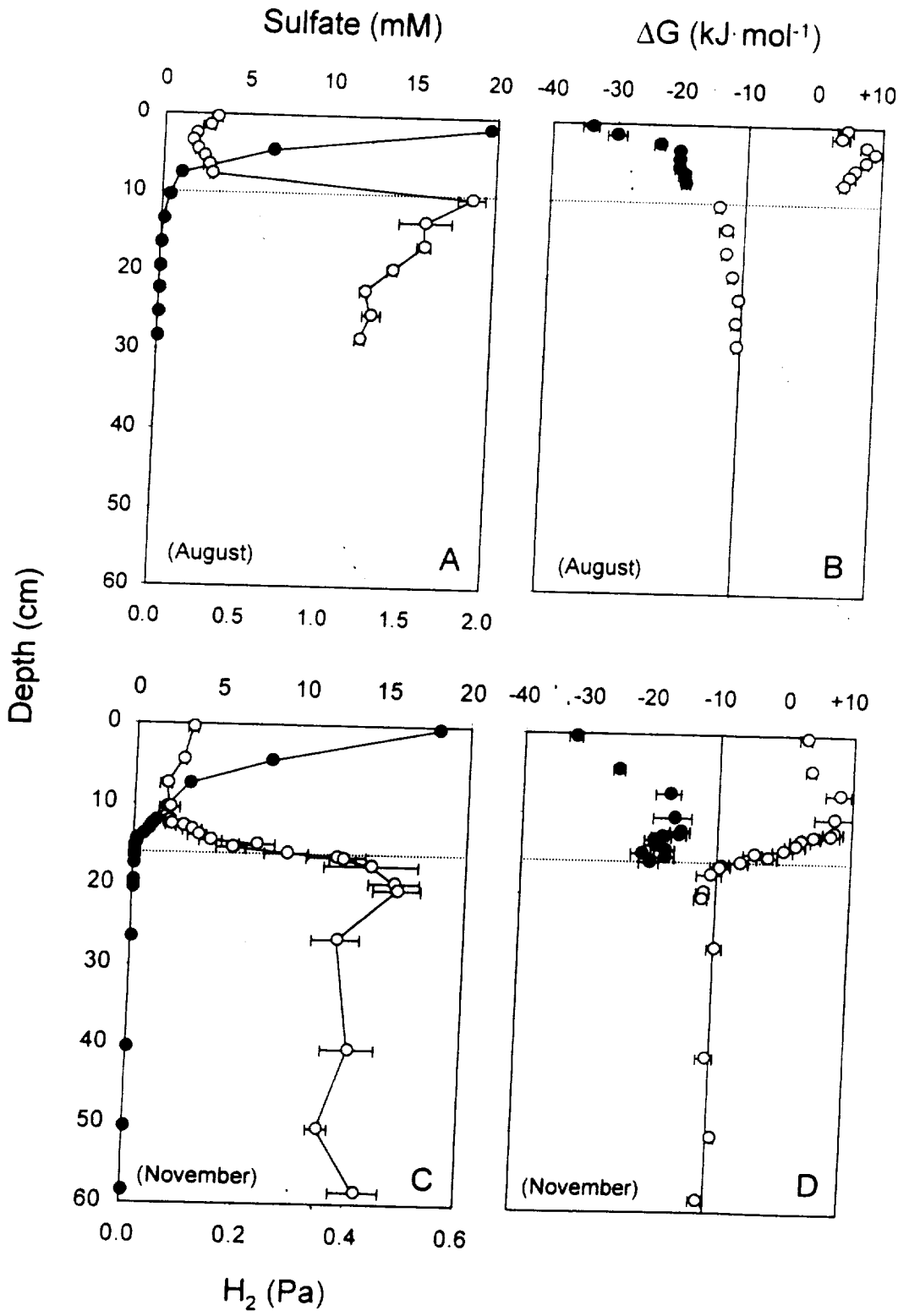
bars represent one standard deviation about the mean of n = 3 replicate sediment samples. **(B)** and **(D):** ΔG_{MP} (○) and ΔG_{SR} (●) in August and November, respectively, calculated from the concentrations in (A) and (C), along with measured concentrations of CH_4 and estimated concentrations of ΣH_2S and ΣCO_2 (see methods). Error bars reflect the standard errors of measurement from (A) and (C), along with estimated errors for ΣH_2S and ΣCO_2 (see methods), propagated through the ΔG calculation. The horizontal dashed lines in each profile represent the approximate depth of transition from sulfate-reducing to methanogenic conditions.


A
2.0
1.5
1.0
0.5
0.0
H_2 (Pa)
Methanogenesis
Sulfate reduction
280
285
290
295
300
305
B
-5
-15
-25
-35
-45
ΔG ($kJ \cdot mol^{-1}$)
ΔG_{MP} -vs- T (*if* H_2 = constant)
ΔG_{SR} -vs- T (*if* H_2 = constant)
280
285
290
295
300
305
Temperature (°K)


0.18
0.15
0.12
0.09
0.06
H2 (Pa)
PH2 = 0.25·[SO4 2-]-0.255
R2 = 0.993
A
0
30
60
90
120
-20
-23
-26
-29
-32
ΔG (kJ·mol-1)
ΔGSR -vs- SO4 2-
if H2 = constant
B
0
30
60
90
120
Sulfate (mM)


A
H_2 consumer
(HC)
H_2 producer
(HP)
H_2 measurement
P_{H_2}
HP
bulk fluid
HC
P_{H_2} measured in bulk fluid > P_{H_2} in HC cell


B
H_2 consumer
(HC)
Organic
matter
H_2 producer
(HP)
H_2 measurement
P_{H_2}
HP
HC
bulk fluid
P_{H_2} measured in bulk fluid = P_{H_2} in HC cell


Sulfate (mM)
ΔG (kJ·mol⁻¹)
Depth (cm)
(August)
A
(August)
B
(November)
C
(November)
D
H2 (Pa)