Environmental constraints that limit methanogenesis.

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Summary

Methanogens are active in many different ecosystems, including habitats with biologically-derived organic matter as substrates such as aquatic sediments, wetlands, agricultural or natural soils subject to inundation, sewage digesters, and the anoxic portions of animal digestive tracts. Methanogens are also present in habitats with geochemically-supplied substrates such as hot springs, hydrothermal vents, volcanically-influenced habitats, and, potentially, the deep crustal subsurface. Methanogens as a group tolerate a broad range of physicochemical conditions, including temperatures from -2 to 122˚C, pH values of 3.0 to 10.2, salinities up to halite saturation, and pressures of at least 75 MPa. Globally, variations in methane emissions can be explained to a large degree by variations in temperature and water availability. The distribution and activity of methanogens are constrained by ecological interactions that can be stimulatory or competitive, and by physicochemical factors that act at the biochemical or bioenergetic levels. In addition to the constraints placed on methanogens by physicochemical extremes, methanogen distribution and activity are constrained by the availability of energy and nutrients, the presence of inhibitory molecules (most notably oxygen), and the seawater anion, sulfate, due to competitive ecological interactions. Although methanogen tolerances to individual extremes are documented in culture, and the corresponding biochemical adaptations are understood to varying degrees, the natural environment frequently presents combinations of extreme conditions and energy limitations that may limit methanogen distribution to less than the optimally tolerated range of a single parameter. Little is understood about the compound effects of such extremes, nor the

commonalities among them that will ultimately form the basis for predictive models of environmental methanogen population distribution. Future work that targets these questions, through a combination of culture work, "omic" analyses, *in situ* studies, and conceptual and quantitative models, will be needed to better understand the physiological ecology of methanogens.

Introduction

Biological production of methane, termed methanogenesis, is a quantitatively important component of the global carbon cycle on the modern Earth (Hedderich and Whitman, 2006) and has likely been so since the origin of microbial methanogenesis over 3.5 billion years (Ueno et al., 2006). Approximately 1 to 2% of the net photosynthetic carbon produced annually is ultimately processed through methanogenesis (Hedderich and Whitman, 2006). About 1,100 Tg of biogenic methane is produced annually, of which about 450 Tg enters the atmosphere (Dlugokencky et al., 2011; Reeburgh, 2007). The remainder of the methane is consumed through aerobic and anaerobic microbial processes. Of concern is the increase in the rate of methane efflux since 2006, possibly due to an increase in Artic methane emissions due to global warming (Dlugokencky et al., 2011). Newly recognized sources of biogenic methane emissions include methane production by aerobic bacteria from the cleavage of methyl phosphonate (Karl et al., 2008) and by terrestrial plants as a result of ultraviolet irradiation (Bloom et al., 2010a; Keppler et al., 2006). This chapter will focus on the environmental constraints that limit methane production by methanogenic Archaea (Zinder, 1993).

Although the abundance of methane in the modern atmosphere is less than one percent of the abundance of CO_2, it is considerably more efficient, on a per-molecule basis, as a "greenhouse" gas (Ramanathan et al., 1985). Emissions of methane from wetlands and marine methane hydrates have been implicated as strong contributors to global warming historically (Nisbet and Chappellaz, 2009). Because methane is an important component of the Earth's radiation budget, the global methane budget and its associated microbial cycles are essential to understand and quantify. The perspective of this chapter will be to provide mechanistic understanding on how physicochemical changes in the environment will affect methanogenesis in different ecosystems.

The methanogen biosphere encompasses a diverse array of ecosystem types (Zinder, 1993; Liu and Whitman, 2008) and a broad range of physicochemical conditions. The best known and the most common methanogenic niches are in oxygen-free, aqueous systems that contain organic matter, which is degraded by a complex array of microorganisms to methanogenic substrates such as H_2, formate, acetate, and various methylated compounds (Table 1). Such systems include marine, lacustrine, and riverine/estuarine sediments; wetlands, such as swamps, bogs, and periodically flooded forest soils; agricultural soils subject to inundation, such as rice paddies; sewage digesters; and the anoxic portions of animal digestive tracts (Chaban et al., 2006; Hedderich and Whitman, 2006; Liu and Whitman, 2008). Additionally, because H_2 is produced through the interaction of crustal rocks and water (Hoehler, 2005), the potential exists for a methanogen biosphere that is supported by geochemical energy sources rather

than by photosynthetic activity. Methanogenic activity is known to occur in environments with temperatures from -2 °C to 122 °C, at pH from 3.0 to 10.2, at salt concentrations from near 0 to > 5 M NaCl, and at pressures greater than 75 MPa (Table 2).

The prevalence of methanogens in ecosystems with a wide range of physiochemical "extremes" may arise from a combination of factors, including: (i) the availability of methanogenic substrates in a wide variety of settings, including from geochemical sources; (ii) a relatively simple biochemical machinery where a smaller genome, fewer core enzymes, and less complexity in general may foster tolerance to a broader range of physicochemical conditions and/or more rapid adaptation to new conditions; and (iii) more than three billion years in which to adapt and evolve a variety of phenotypes around a simple core metabolism. Together, these factors have presented methanogens with the impetus, potential, and time to evolve and occupy a broad range of ecological niches. Despite this ecological plasticity, the distribution of methanogens in nature is quite limited in comparison with the distribution/availability of potential methanogenic substrates. In general, methanogen distribution is constrained by ecological interactions or physicochemical environmental factors that breach biochemical or bioenergetic limits. This chapter considers these limitations and the resulting major environmental controls on methanogenesis. The interested reader is also directed to (Zinder, 1993; Liu and Whitman, 2008; Thauer et al., 2008) for a thorough consideration of the physiological ecology of methanogens.

Biochemical and Bioenergetic Considerations

Biochemical limitations are encountered principally through physical or chemical disruption of core metabolic molecules, structures, networks, or processes. Examples include the thermal destabilization of enzyme tertiary structure, enhanced chemical hydrolysis of biopolymer linkages, chemical inactivation of enzyme binding sites, or the inherent limitation of enzymes to take up and process substrates at levels needed to compete effectively or support metabolism. Specific biochemical effects on methanogens are considered below.

Bioenergetic constraints on environmental habitability arise from life's fundamental need to harness energy from the surroundings and use the energy to maintain what is, ultimately, a disequilibrium state – that is, the maintenance of complex biological molecules and structures that are thermodynamically unstable with respect to the general environment. For an environment to be habitable from an energetic standpoint, the provision of energy by that environment and the organism's capability to access and use that energy must balance or exceed the organism's demand for energy (Hoehler, 2004, 2007; Shock and Holland, 2007; Hoehler and Jørgensen, 2013).

The biological demand for energy is manifest in two requirements, which are analogous to the voltage and power requirements of an electrical device. The analog to voltage (energy per unit of energy carrier) is the biological energy quantum (BEQ), which is defined as the smallest Gibbs free energy change (ΔG) of a metabolic reaction that can

still be used to drive ATP synthesis, which is needed to sustain metabolic activity (Schink, 1997; Schink and Stams, 2006). The analog to power (energy per unit time) is the maintenance energy (ME), which is the flux of energy needed to support a unit of biomass in a steady state at a net zero growth rate (kJ • mol^{-1}• hr^{-1}) (Harder, 1997; Tijhuis et al., 1993). Each requirement is characterized by a minimum value, below which sustained metabolism is not possible, and both requirements must be met in order for a given environment to be habitable by a given organism (Hoehler, 2004; Hoehler 2007) (Figure 1).

The magnitudes of the BEQ and, in particular, the ME requirements are set in part by the biochemical and physiological characteristics of the organism in question and in part by the environment in which it lives. Nominally, the magnitude of the BEQ has been estimated at about -20 kJ·mol^{-1} for actively growing organisms (Schink, 1997) and -12 to -15 kJ·mol^{-1} for organisms operating under energy-limiting conditions (Schink and Stams, 2006). Measurements of energy yields associated with methanogenesis in various environments suggest that methanogens may be able to capitalize on energy yields as small as -10 kJ·mol^{-1} (Hoehler et al., 1998 and 2001). The magnitude of the ME requirement may vary more substantially across different organisms, and is considerably less well constrained than that of the BEQ (Hoehler and Jørgensen, 2013). Estimates of ME derived from culture-based and environmental studies, and from growing versus non-growing organisms vary over orders of magnitude (Morita, 2000; Price and Sowers, 2004; Tijhuis et al., 1993). Environmental deviations from biologically optimal physiochemical conditions may increase an organism's energy demands significantly.

Indeed, natural systems can present multiple physicochemical extremes such as high temperature and low pH that may have compound effects in increasing cellular energy demands. For environments offering only limited fluxes of methanogenic substrates, energy demands due to physiochemical stresses may ultimately exceed the environmental energy supply, and thereby render the environment uninhabitable (Hoehler, 2007). Thus, in natural systems, bioenergetic factors may limit habitability before biochemical limitations are encountered.

While the bioenergetic considerations outlined above are applicable for all organisms, they are especially relevant for metabolisms with low energy yields where the Gibbs free energy change of the catabolic reactions under typical environmental conditions is close to the BEQ. Methanogenesis exemplifies this situation, and many aspects of the environmental distribution/limitation on methanogen activity are attributable to bioenergetic effects.

Ecological Interactions

Methanogens as an overall metabolic group are capable of using H_2/CO_2, formate, acetate, CO, and several methylated compounds as substrates for methane production (Table 1), although individual taxa may use only a subset (Zinder, 1993; Liu and Whitman, 2008). While any of these may conceivably predominate in a given environment, the overall most quantitatively important methanogenic substrates are H_2/CO_2 (and/or formate) and acetate. Both occupy a central role in carbon and electron flow in anaerobic microbiology, and in the metabolic processes of a variety of

197 microorganisms. As such, they present a basis for interactions, both stimulatory and
198 inhibitory, between methanogens and other organisms.
199
200 *Syntrophic Interactions*
201 The ability of methanogens to grow autotrophically with H_2 as an electron donor
202 potentially allows them to directly access geochemical sources of energy such as the H_2
203 produced by water-rock reactions. However, most of the known methanogenic
204 ecosystems are instead fueled by the decomposition of complex organic matter (Chaban
205 et al., 2006; Hedderich and Whitman, 2006; Liu and Whitman, 2008). In the absence of
206 oxygen, the complete decomposition of complex organics requires the collective
207 activities of a diversity of anaerobic microorganisms, each catalyzing individual steps in
208 the overall process (Schink, 1997). Only in the final steps of this process are the
209 methanogenic substrates H_2, formate and acetate made. For this reason, methanogen
210 activity in anaerobic environments fueled by the decomposition of complex organic
211 matter is dependent on the activity of syntrophic partner organisms. The production of
212 H_2 from NADH and $FADH_2$ generated during fermentative metabolism is unfavorable
213 when the pressure of H_2 is high (> 200 Pa) (Schink, 1997). Hydrogen-using methanogens
214 rapidly use H_2 produced by fermentative bacteria, thereby maintaining low H_2
215 concentrations low, which makes hydrogen production by fermentative bacteria
216 favorable. The interaction between hydrogen-producing fermentative bacteria and
217 hydrogen-using methanogens is called “interspecies electron transfer.” Because of the
218 strict interdependence between H_2-producing fermentative microorganisms and H_2-using
219 methanogens, any environmental or ecological factor that influences one of the partners

220 can also influence the overall rate and extent of methanogenesis in that ecosystem. Thus,
221 the environmental distribution of methanogenesis may be limited by community-level
222 effects that disrupt syntrophic interactions before the absolute physicochemical tolerances
223 of methanogens are exceeded. This is principally a bioenergetic limitation on
224 methanogen distribution, by virtue of disruption of substrate flow to methanogens at
225 levels or rates needed to meet cellular energy demands.
226
227 *Competitive Interactions*
228 Acetate and H_2 are utilized in a wide range of microbial metabolisms, so that methanogen
229 distribution may be limited by competition for these substrates. In anoxic systems driven
230 by organic matter decomposition, the principal competitors for acetate and/or H_2 are
231 organisms that oxidize these substrates using inorganic electron acceptors such as nitrate,
232 Mn^{4+}, Fe^{3+}, and sulfate (Zehnder and Stumm, 1988). Other organic and inorganic
233 oxidants can serve the same function and may be important in specific environmental
234 settings. Observations of anoxic sediments show that organic matter decomposition often
235 proceeds via successive oxidants, in the order indicated above, with one oxidant (e.g.,
236 sulfate) being completely exhausted before another one (e.g., CO_2, in methanogenesis) is
237 utilized. The order in which oxidants are used reflects the magnitude of the standard
238 Gibbs free energy yield associated with oxidation of hydrogen or acetate by that oxidant,
239 with the reduction of nitrate to N_2 by H_2 yielding the largest standard Gibbs free energy
240 change ($\Delta G^{o'}$ = - 560 kJ • mol^{-1} of oxidant) and the reduction of CO_2 to methane by H_2
241 yielding the smallest standard Gibbs free energy change ($\Delta G^{o'}$ = - 135 kJ • mol^{-1} of
242 oxidant) (Zehnder and Stumm, 1988).

As suggested by the ordering of oxidants based on free energy yield, the competitive exclusion of methanogenesis is hypothesized to have a thermodynamic basis. Differences in standard Gibbs free energy yields by themselves, however, do not provide a mechanism for exclusion of one organism by another. Rather, a larger free energy yield *potentially* enables one organism to compete more effectively for a common substrate, such as H_2 or acetate, because it should be able utilize the substrate to a lower concentration than an organism that uses a less energetic oxidant and still extract a Gibbs free energy yield that meets the BEQ requirement. Complete inhibition of one metabolism by another will occur if one organism can actuate this potential and consume a common substrate down to a concentration that does not meet the BEQ or thermodynamic favorability requirement of its competitor. Complete inhibition of H_2-consuming methanogenesis by H_2-consuming sulfate reduction via this mechanism has been hypothesized or demonstrated in a variety of systems (Cord-Ruwisch et al., 1988; Lovley and Goodwin, 1988; Hoehler et al, 1998) and is illustrated in Figure 2. Whether or not the same potential is actuated in other microbial interactions that involve another electron donor or different oxidants depends on the energetically-advantaged organism having (i) enzyme kinetics that allow it take up the substrate at a concentration and rate that excludes its competitor and (ii) a supply rate of oxidant that exceeds the supply rate of the electron donor.

Enzyme kinetic considerations may limit the potential for competitive exclusion in the case of acetate. This is because the change in acetate concentrations required to actuate

266 an energetic advantage is very large, and would require enzymes with capabilities to take
267 up substrate at extremely low levels. For example, to actuate the >15 $kJ \cdot mol^{-1}$ advantage
268 that sulfate reducers appear to exercise over methanogens in the marine sediments
269 presented in Figure 2 would only require a > 4.5-fold decrease in H_2 concentrations. Such
270 a decrease in H_2 concentrations was observed in Cape Lookout Bight sediments shown in
271 Figure 2 (Hoehler et al., 2001). However, more than a 430-fold decrease in acetate
272 concentrations would be needed for acetate-using sulfate reducers to exclude acetate-
273 using methanogens. In practice, acetate concentrations are only a few-fold lower in
274 sulfate-reducing sediments compared to methanogenic sediments; hence, it is not clear
275 that the same bioenergetic basis for competitive exclusion exists for acetate as in the case
276 of H_2. Nonetheless, methanogenesis including that from acetate is often completely
277 excluded by sulfate reduction. These seemingly discrepant observations can be
278 reconciled if methanogenic metabolism of acetate is influenced by H_2 concentrations in
279 the system and therefore by the actuated energetic advantage of sulfate reducers. Indeed,
280 Finke et al. (2007) showed that methylotrophic methanogens can covert >95% of the
281 substrate methyl carbon to CO_2 and H_2 rather than to methane when porewater H_2
282 concentrations are held at low levels by sulfate reducers. This hypothesis could explain
283 the apparent competitive exclusion of acetate-based methane production from sulfate-
284 containing sediments, despite the *superficial* lack of a thermodynamic basis, while also
285 associating a potentially energy-yielding metabolic activity (Finke et al., 2007) with
286 methylotrophic methanogens that appear to be present in some such sediments (Kendall
287 et al., 2007).
288

289 By virtue of competition for common substrates, the presence of any of the oxidants
290 mentioned above (e.g., NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}) has potential to completely exclude
291 methanogenesis. In practice, the presence or absence of sulfate, a major anion in
292 seawater (~28 mM), is by far the most important among these oxidants in limiting the
293 environmental distribution of methanogenesis. Specifically, in the sediments of marine,
294 estuarine, and hypersaline environments (which globally comprise a large repository of
295 potential methanogenic fuel), methanogenic activity is largely restricted to deeper
296 sediment layers where sulfate has been fully consumed by sulfate reduction or, in cases
297 where sulfate persists throughout the sediment column, methanogenesis may be
298 completely absent. Limitations in the supply of the oxidant may serve to limit the
299 effectiveness of terminal electron-consuming processes other than sulfate reduction in
300 excluding methanogenesis by competition for common electron donors. In many natural
301 systems, nitrate is rapidly used and not readily replenished, so the potential for nitrate
302 reducers to exclude other competitors for common electron donors is limited. However,
303 in systems high in nitrate and largely lacking in sulfate such as fertilized agricultural soils
304 or wetlands affected by agricultural run-off, nitrate may be an important agent for
305 exclusion of methanogenesis. Mn^{4+} and $Fe^{3+}$are present as insoluble, particulate oxides,
306 and, thus, may be in short supply if the organisms that utilize them depend on diffusive
307 supply of the dissolved form. As a result, the capability of Mn^{4+}- and Fe^{3+}-respiring
308 organisms to lower the concentrations of common substrates in accordance with their
309 energetic advantage may be limited. Lovley and Goodwin (1988) and Achtnich et al.
310 (1995) observed decreased H_2 concentrations in the presence of these metal oxides, but
311 this effect may be highly concentration dependent (Hoehler et al., 1998). Indeed, metal

312 reduction in sediments is frequently accompanied by co-occurring sulfate reduction or
313 methanogenesis, except at high metal oxide concentrations (Thamdrup, 2000).
314 Although bioenergetic considerations appear to underlie much of the competitive
315 exclusion of methanogenesis as illustrated by the competitive effects of sulfate reduction,
316 other mechanisms may also be important. In particular, it has been suggested that low
317 temperatures may favor autotrophic acetogenesis (production of acetate from CO_2 to H_2)
318 over hydrogentrophic methanogenesis in some systems (Conrad, 1999; Kotsyurbenko,
319 2005; Nozhevnikova et al., 2007), even though methanogenesis is the more
320 thermodynamically favored of the two processes under most environmental conditions. It
321 is suggested that homoacetogens outcompete methanogens on the basis of having a
322 greater maximum rate of hydrogen use at low temperatures than methanogens, rather than
323 on a bioenergetic basis (Kotsyurbenko et al., 2001).
324
325
326 *Substrate preferences*
327 The use of a non-competitive substrate such as trimethylamine (Table 1) (King, 1983)
328 may allow methanogenesis to co-occur with sulfate reduction in marine sediments where
329 complete inhibition of H_2-consuming methanogenesis by H_2-consuming sulfate reduction
330 would be predicted based on the bioenergetics model described above. Recently,
331 methanogens with substrate utilization patterns different from those of cultured
332 methanogens have been described, which would also change the bioenergetics
333 considerations used for habitability analysis. *Candidatus* "Methanofastidiosum
334 methylthiophilus" lacks pathways for CO_2-reducing and acetoclastic methanogenesis, but

has enzymes for reducing dimethylsulfide to methane (Nobu et al., 2016). At 10 Pa H_2, methanogenesis from H_2 and dimethylsulfide ($\Delta G' = -140$ kJ mol^{-1}) is much more favorable than that from H_2 and CO_2 ($\Delta G' = -18$ kJ mol^{-1}) (Nobu et al., 2016). Similar bioenergetic considerations hold for members of the order Methanomassiliicoccales (Dridi et al. 2012; Borrel et al. 2013 and 2014) and the archaea phylum, Verstraetearchaeota (Vanwonterghem et al., 2016), whose members are known to require both H_2 and methylated compounds for methanogenesis. Two genomes in the archaeal phylum *Bathyarchaeota* indicate that methanogen members of this phylum may be capable of carbohydrate fermentation (Evans et al. 2015). Another example of novel substrate for methanogenesis is the use of methoxylated aromatic compounds present in coal by *Methermicoccus shengliensis* (Mayumi et al., 2016). The diversification of substrate use by some methanogens would allow them to occupy habitats that would exclude hydrogenotrophic or acetoclastic methanogens.

Physicochemical Environment

Physicochemical "extremes" can impact methanogens at both biochemical and bioenergetic levels, either directly or by affecting their ecological interactions with other organisms.

Oxygen

The ambient concentration of oxygen is an important determinant of the environmental distribution of methanogenesis. Methanogens are strict anaerobes and, in culture, will

not grow or produce methane in the presence of even trace levels of oxygen (Zinder, 1993). Given the high abundance of oxygen in Earth's atmosphere, this sensitivity has the potential to severely limit methanogenesis by methanogenic archaea, although not by aerobic, methyl phosphonate-cleaving bacteria (Karl et al., 2008). Two factors serve to mitigate oxygen inhibition of methanogenesis to some degree. First, while methanogens do not grow or metabolize in the presence of atmospheric levels of oxygen, they do exhibit some tolerance to oxygen exposure (Zinder, 1993). This suggests that core methanogen enzymes may not be irreversibly damaged or, at least, can be reactivated, following oxygen exposure. Oxygen tolerance may allow methanogens to persist in environments that may fluctuate between oxic and anoxic conditions. Second, aqueous habitats containing particulate organic matter tend to endure limited permeation by oxygen because: (i) the solubility of oxygen in water is relatively low, which effectively reduces the potential mass flux of oxygen from an overlying gas phase to methanogens inhabiting the aqueous phase; (ii) organic-containing sediments provide a physical matrix that limits oxygen mass transfer to molecular diffusion, which is slow over spatial scale of more than a few millimeters; and (iii) sediments contain active microbes or reactive chemicals that reduce oxygen quickly. For ecosystems with large amounts of organic matter, the slow diffusion and rapid consumption restricts oxygen penetration to a narrow surface zone that may range from microns to a few centimeters. Methanogenic decomposition of organic matter can then occur below the zone of oxygen penetration.

Temperature

Methanogens are represented across most of the known biologically-tolerated range of

temperature from -2 ºC to 122 ºC (Table 2). The rates of methanogenesis increase more steeply with temperature than do other important biological processes such as heterotrophic respiration or photosynthesis (Yvon-Durocher et al., 2014). The sensitivity of the rates of methanogenesis to temperature demonstrates the importance of temperature in controlling methane flux globally. However, large site-to-site variations in methane emission exist showing that other variables such as water saturation and vegetation are also important (Turetsky et al., 2014). For example, methane emissions from wetlands are best explained when variations in surface temperature and water-table depth are both considered (Bloom et al., 2010b).

Psychrophily. Methanogens are found in many low temperature habitats. Methanogenic low temperature habitats include high latitude wetlands such as boreal fens, tundras, and bogs, marine and freshwater sediments underlying deep waters, and sediments in arctic and antarctic regions (Cavicchioli, 2006). Collectively, these habitats also have significant differences in pH, salinity, pressure, and energy availability. Temperature ranges for cell growth in psychrophilic and psychrotolerant methanogens generally span from -2 to 54˚C (Table 2). The lower limit can extend below 0˚C when cells possess means to suppress ice formation (Cavicchioli, 2006). Cell doubling times for *Methanococciodes burtonii* and *Methanogenium frigidum* are considerably below that described for the extreme thermophiles, and may be in the range of 0.1 to 0.3 generations per day (Cavicchioli, 2006).

The vast proportion of Earth's surface, including most or all of the deep ocean basins,

404 high latitude lakes and ocean shelves, and high latitude wetlands and soils, is subjected to
405 periodically or permanently low temperatures. The presence of the significant reserves of
406 organic carbon, which are sequestered in periodically or permanently cold environments,
407 suggest that low temperature environments represent a vast potential habitat for
408 psychrophilic methanogens. Nonetheless, relatively little is understood about the
409 environmental diversity of the psychrohilic and psychrotolerant methanogens, or the
410 mechanisms that allow them to adapt to low temperature niches, but environmental
411 genomic analyses are beginning to yield insights. Metagenomic analyses revealed that
412 partially thawed, Arctic permafrost samples were dominated by a single archaeal
413 phylotype, *Candidatus* 'Methanoflorens stordalenmirensis', which belongs to the
414 uncultivated lineage 'Rice Cluster II' (*Candidatus* 'Methanoflorentaceae') (Mondav et
415 al., 2014). Members of *Ca.* 'Methanoflorentaceae' are globally distributed in habitats
416 with diverse physiochemical conditions. Such metagenomic analyses are important tools
417 for identifying methanogens present in cold environments and characterizing their
418 potential metabolic properties, given that the typical very slow growth of psychrophiles
419 makes laboratory studies challenging.
420
421 *Thermophily.* Thermophilic and hyperthermophilic methanogens are found in fluid
422 outflows from marine and fresh water volcanic seeps, hot springs, thermal mud pools,
423 and solfataric fields (Huber et al, 2000). These habitats are typically rich in H_2 and
424 minerals, low in organics, and may vary significantly in pH and salinity (from fresh to
425 marine). The chemoautotrophic methanogens *Methanotorris igneus (Methanococcus*
426 *igneus)* and *Methanothermus fervidus* were isolated from a shallow offshore submarine

vent and a thermal terrestrial waterhole in the mountains of Iceland, respectively. *Methanopyrus kandleri*, currently the high temperature "record holder" among cultured methanogens, was isolated from a deep hydrothermal "black smoker" vent at 2000 meters in the Gulf of California (Kurr et al., 1991; Takai et al., 2008). It has a growth optimum of 105 ^{o}C at 40 MPa and is capable of growth at 122 ^{o}C (Table 2). By virtue of the general enhancement of metabolic rate by increasing temperature, cell doubling times for these thermophilic methanogens can be less than one per hour (Jeanthon et al., 1998; Takai et al., 2004).

The mechanisms of high temperature limitation of methanogens can be biochemical, bioenergetic, and/or ecological. While some large organisms can maintain internal temperatures significantly above or below ambient, individual microbes, or microbes in small clusters, cannot. Biologically meaningful temperature gradients cannot be maintained at the scale of microbial cells, so the environmental temperature is, effectively, the intracellular temperature. Thus, temperature effects can act directly upon the biochemical machinery of the cell. The deleterious effects of high temperatures relate principally to the thermal destabilization of core biomolecules, with resulting impacts on functionality. For example, high temperatures disrupt the tertiary structure necessary for enzyme function as well as lipid membrane stability. The biochemical impacts of high temperature for organisms in general, which are applicable to methanogens, are thoroughly reviewed by Jaenicke and Sterner (2002). The impact of high temperature may set the ultimate upper limit on methanogen growth and metabolism in habitats where energy is in abundance and other growth parameters are optimal.

Temperature can also influence methanogen metabolism by factors other than biochemical mechanisms. As noted earlier, for systems driven principally by organic matter decomposition, methanogen activity is ultimately dependent on the collective function of a broader community of organic matter-degrading organisms, and is therefore subject to the physicochemical limitations of critical organisms within that population. Thus, elevated temperatures may limit methanogen distribution by inhibiting partner organisms before the biochemical temperature limits of methanogens are encountered. Consistent with this notion, the cultured methanogens representing the upper end of the tolerated temperature range are generally derived from environments in which the substrate, principally H_2, is provided by geochemical sources, rather than by community-enabled organic matter decomposition. The maximum growth temperature so far for a cultured syntrophic metabolizer is about 75°C (Table 2).

Lastly, temperature has a strong effect on cellular maintenance energy, and may thereby serve to limit methanogenic activity via bioenergetic inhibition. The effect of temperature on maintenance energy has been quantified experimentally (Tijhuis et al., 1993) and conforms to an Arrhenius-type relationship (Harder, 1997) (equation 1):

$$ME = A \cdot e^{-Ea/RT}$$

where A is a positive constant, E_a is the activation energy ($kJ \bullet mol^{-1}$), R is the universal gas constant, and T is temperature in °K. According to this relationship, the energy required to support a unit of biomass increases exponentially with temperature. Empirically determined values for E_a (Tijhuis et al., 1993; Harder, 1997) predict that

maintenance energy increases more than three-thousand fold as temperature increases from 0 to 100 ºC. Thus, in environments offering limited substrate fluxes, increasing temperatures may rapidly lead to bioenergetic limitation of methanogen growth and maintenance before absolute biochemical limits are reached.

pH

Methanogenesis is common in marine and freshwater boreal fens, tundras, and bogs, where accumulation of plant tannins and organic acids can lower pH to values ranging from weakly acidic to 3.5 or less (Zinder, 1993). Methane formation has been observed in peat samples at pH values as low as 3, although higher values were needed for optimal rates of methanogenesis (Williams and Crawford, 1984; Bräuer et al., 2006; Cadillo-Quiroz et al., 2008). These habitats are frequently characterized by low temperatures, and potentially represent multiple "extremes" to microbial inhabitants. As a group, acidiphilic methanogens are relatively little studied. However, several acidiphilic methanogens have been isolated that grow and produce methane at pH values as low as 4.3 although optimal growth occurs at pH $\geq$ 5.0 (Table 2).

Some methanogens are also capable of growth or metabolism under alkaline conditions. Most of the studied alkaliphilic methanogens listed in Table 2 are associated with evaporitic basins such as Mono Lake, California, the Dead Sea, the Rift Valley Lakes of East Africa, or desert soda lakes and streams. In addition, methanogenic activity has been inferred in alkaline seeps where serpentinization yields waters with elevated pH (Kelley

et al., 2005). Cultured representatives are moderately alkaliphilic (Table 2), with *Methanocalculus natronophilus* and *Methanosalsum natronophilum* having a pH growth optimum of 9.0-9.5 and a growth limit of 10.2 (Mathrani et al., 1988). Alkaline environments may present multiple extremes for methanogen activity. Alkalinity associated with evaporitic settings is frequently accompanied by concentrated salts. Mono Lake, for example, has sodium concentration of 1.3 M and carbonate concentration of 0.4 M (Oremland et al., 1993). Thus, some alkaliphilic methanogens are also halophilic. *M. natronophilus* and *M. natronophilum*, for example, are capable of growth at salinities up to 3.3-3.5 M NaCl, or about 5-6 times seawater salinity (Mathrani et al., 1988). Alkaline environments also contain the requisite syntrophic partners needed for organic matter decomposition (Table 2). Alkalinity may also be associated with high temperatures, as in alkaline hot springs or, in particular, hydrothermal settings associated with serpentinizing host rocks. For example, some venting fluids at the Lost City hydrothermal field, which is located about 15 km away from of the Mid-Atlantic Ridge, reach pH values of 10-11 at temperatures of 70˚C (Kelley et al., 2005).

Significant deviations from neutral pH have the potential to adversely affect cellular biochemistry at a variety of levels, so that only modest variations in intracellular pH can be tolerated. However, habitation of environments with higher or lower than biochemically tolerable pH values is nonetheless feasible because the lipid bilayer membrane is an effective barrier to ionic species like H^+, OH^- or CO_3^{2-}. Thus, it is possible to maintain intracellular pH at more moderate levels than in the extracellular medium, through active regulation. The mechanisms associated with such regulation are

519 understood biochemically (Krulwich, 1995, 2000; Krulwich et al., 1996) and it is clear
520 that actuation of these strategies such as active transport of protons must increase cellular
521 maintenance energies. The effect of pH on maintenance energy of methanogens has not
522 been quantified directly. However, the minimal energetic cost of pH regulation is
523 determined by the rate of proton pumping necessary to maintain the appropriate internal
524 pH. The internal pH, in turn, depends on the leakiness of the membrane, the presence of
525 weak acids and bases that may diffuse across the membrane, and the energy required to
526 pump a unit quantity of protons (Krulwich, 2000). All of these factors can be expected to
527 increase monotonically with increasing or decreasing extracellular pH, so that
528 maintenance energy should increase as the environmental pH deviates from the
529 biochemical optimum. Thus, bioenergetic effects may factor prominently in setting the
530 practical environmental pH limits.
531
532 A secondary effect that may significantly constrain the habitability of alkaline or acidic
533 environments with respect to methanogenesis is the speciation of methanogenic
534 substrates in response to pH. Specifically, the conversion of methanogenic substrates
535 into predominantly ionic forms that cannot diffuse across the cell membrane will require
536 either energy expenditure for the active transport of these substrates or an increase in
537 membrane permeability. The latter would be problematic as it would increase pH leakage
538 and require higher rates of proton pumping to maintain the appropriate internal pH. Such
539 effects are probably most important in limiting methanogenesis in alkaline environments,
540 due to the deprotonation of acetic and carbonic acid, but could also conceivably be

important for the methanogenic consumption of methylamines due to its protonation in acidic environments.

Salinity

Methanogens are found in environments with salinities ranging from that of freshwater to halite-saturated (> 5M NaCl). Halophilic and extremely halophilic methanogens are most commonly associated with hypersaline environments that include dead seas, solar salterns and halite crystallizing ponds, and alkali lakes. As mentioned above, these environments often have high concentrations of sodium chloride, magnesium chloride, sulfate, carbonate and other salts (Oren, 2002; Ollivier et al., 1994). Notably, the conditions responsible for generating hypersaline conditions may also enhance alkalinity, and are the result of elevated temperatures, so that methanogenic inhabitants of hypersaline environments most likely face multiple extremes. Described halophilic methanogens include moderate halophiles such as *Methanohalophius mahii, Methanohalophilus halophilus*, and *Methanosalsus zhilinae* (Ollivier et al., 1994), and extreme halophiles, including *Halomethanococcus doii* and *Methanohalobium evestigatum* (Table 2). The former have optimal growth with 1-2.5 M NaCl, while the latter are able to grow in halite-saturated brines (over 5 M).

Elevated intracellular salt concentrations would directly and significantly impact cellular biochemistry. Some organisms, e.g., *Halobacteria,* employ a "salt-in" strategy in which biochemical accommodations are made to high intracellular salt concentrations (Oren, 2001). However, all of the known methanogens are "salt out" strategists (Oren, 2001)

564 where intracellular salt concentrations are held below environmental levels by virtue of
565 the barrier presented to ionic species by the lipid bilayer membrane, and through active
566 transport of salts across the membrane. To compensate for the resulting differential in
567 osmotic pressure, methanogens produce and concentrate intracellular osmolites such as
568 glycine betaine, β-glutamate, β-glutamine, and *N*ε-acetyl-β-lysine (Lai and Gunsalus,
569 1992; Lai et al., 1991). Production of these compounds at the rates and levels needed to
570 compensate for high salinity, along with active regulation of ion transport across the cell
571 membrane, constitutes significant and ongoing energy expenditure. Indeed, the energy
572 expenditure to maintain osmotic balance and regulate intracellular salt concentrations can
573 be expected to increase monotonically with the salinity of the environment. Thus,
574 salinity-based limitations on methanogenic metabolism may act principally at a
575 bioenergetic level (Oren, 1999, 2001). Typically, methanogens that grow at high
576 salinities are methylotrophic rather than H_2- or acetate-utilizing methanogens, and likely
577 due to the abundance of methyl group-containing osmolytes like betaine. Oren (2001)
578 noted that the standard Gibbs free energy change per substrate is greater for di- and tri-
579 methylamines than H_2 or acetate (Table 1). The larger energy yields may serve to balance
580 the higher rates of energy expenditure required for life at higher salinities. While higher
581 overall rates of metabolic energy production will certainly serve to balance higher
582 cellular maintenance energies, some caution is warranted in this interpretation.
583 Specifically, larger standard Gibbs free energy yields are, by themselves, only one
584 determinant of the total metabolic energy yield, and the rate of substrate
585 flux/consumption must also be considered. For example, a methanogen consuming
586 acetate with a 5-fold lower Gibbs free energy yield than a methanogen consuming

methylamine will nonetheless have a higher overall rate of metabolic energy production if it receives a 10-fold higher flux of substrate. Importantly, however, methylotrophic substrates – some of which are among the breakdown products of osmoregulants – appear to be proportionately more abundant as methanogenic substrates in hypersaline settings. The high substrate concentration in combination with the larger free energy yields associated with methylotrophic methanogenesis may account for the higher salinity tolerance of methylotrophic methanogens compared to their H_2- or acetate-utilizing counterparts. Regardless, energy balance appears to be a critical determinant of the methanogenic habitability of hypersaline environments.

Pressure

Elevated pressure is a characteristic of sediments underlying the deep ocean basins, and methanogen activity is documented to pressures of 75 MPa, equivalent to >7000 m water depth (Miller et al., 1988). However, the difficulties associated with conducting physiological studies at very high pressures have limited our direct understanding of the tolerance and adaptations of methanogens to high pressures. Because biochemical and metabolic reactions occur in aqueous solution, and because the partial molar volume changes associated with aqueous reactions are typically quite low, high pressures have only minimal effects on the thermodynamics of methane production. However, high pressures are known to affect key biochemical functions (Abe, 2007). Pressures of 10 to 20 MPa can impair important cellular processes in mesophilic bacteria such as motility, cell division, nutrient uptake, and membrane protein function. Replication, transcription and protein synthesis are affected at pressures of 50 MPa and above. Thus, if

610 methanogens respond to high pressure in a manner similar to that of bacteria, piezophilic
611 (barophilic) methanogens must have evolved adaptive mechanisms to survive and thrive
612 at high pressure. Elevated pressures also enhance the solubility of gaseous substrates and
613 products of metabolism. The transport of hydrogen can thus be significantly greater at
614 high pressure, which would allow for higher maximum rates of substrate conversion
615 (energy production) by hydrogen-using methanogens. However, high pressure can impair
616 nutrient uptake, which may increase maintenance energy requirements.
617
618 **Research needs**
619 Culture-based microbiology has given us a snapshot of the methanogen tolerance to
620 environmental extremes, but it is not clear how to translate these observations into a
621 realistic predictor of their population distribution and activity in the natural world, where
622 these organisms may function in the context of syntrophic and competitive interactions,
623 and may face energy limitation and multiple physicochemical extremes. Advances in a
624 variety of areas will ultimately help to address this question.
625
626 The study of methanogens in laboratory cultures has greatly enhanced our understanding
627 of the physiological ecology of methanogenesis. Traditionally, however, culture work
628 optimizes energy availability and other growth factors, while seeking to isolate individual
629 physicochemical variables for study. To begin to probe the question of survival in
630 complex environments, these studies must begin to incorporate constraints – for example,
631 energy limitation or combinations of physicochemical extremes – that realistically mimic
632 the natural environment. Key areas requiring further study are how microbial energy

633 metabolism, in particular, maintenance energy, responds to non-optimal growth
634 conditions and what biochemical and regulatory mechanisms are used to adapt to extreme
635 growth conditions. Such studies will also benefit from continued attempts to sample the
636 full diversity of methanogens in environments presenting individual and combinations of
637 extremes (including energy limitation), to ensure that the full range of environmental
638 tolerances and mechanisms of adaptation are reflected in cultured organisms.
639
640 Beyond the petri dish, advances in characterizing microbial ecology and physiology *in*
641 *situ* will significantly enhance our predictive capability regarding the environmental
642 distribution of methanogenic activity vis-à-vis a variety of extremes. Key areas for
643 advancement include accurate *in situ* rate measurements, especially in cases of low
644 metabolic activity (e.g., in cold or low energy settings); methods for discerning and
645 discriminating metabolic status (e.g., active growth vs. simple maintenance) and for
646 obtaining accurate cell counts at low numbers; methods for linking geochemical function
647 with genetic identity; and means for better resolving complex ecological interactions and
648 associations involving the syntrophic partnerships and competitive interactions.
649
650 Combining genomic and transcriptomic analyses with comprehensive and quantitative *in*
651 *situ* analyses will provide a much clearer picture of the phylogenetic and metabolic
652 diversity of methanogens and how various physiochemical factors affect their activity.
653 Improved bioinformatics tools are needed to provide a more complete understanding of
654 the physiological properties of uncultured methanogens and the signaling and regulatory
655 systems that they use to respond to changing environmental conditions. The combination

of metagenomic, metatranscriptomic, metabolomic, bioinformatic and computational approaches is needed to understand the factors that govern interspecies interactions between methanogens and their syntrophic partners and how these organisms orchestrate their metabolisms into a coordinated catalytic unit.

Finally, theoretical work on microbial energy metabolism may aid in developing a quantitative framework in which to understand and predict the effects of multiple environmental forcing factors. The approach would combine with culture-based and environmental studies to assess the biochemical and bioenergetic impacts and adaptations associated with environmental extremes. Identifying common denominators such as the effects of diverse physicochemical extremes on cellular maintenance energies will lead to an improved ability to quantify and predict multifactorial effects on methanogen metabolism. Numerical models that include substrate transport and reaction at single-cell scales are needed to simulate and predict biological processes at spatial or temporal scales that are not currently accessible by experimental or observational means.

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1018 Table 1. Gibbs free energy changes of methanogenic reactions.[a]

Reaction	ΔG°' (kJ mol^{-1} of carobon substrate)	ΔG°' (kJ mol^{-1} of methane)
Methanogenesis		
$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	-135.6	-135.6
4 HCOO- (Formic acid) $+ H_2O + H^+ \rightarrow CH_4 + 3\ HCO_3^-$	-32.5	-130.1
4 CH_3OH (Methanol) $\rightarrow 3\ CH_4 + HCO_3^- + H_2O + H^+$	-78.6	-104.8
$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	-112.5	-112.5
2 $(CH_3)_2$-S (Dimethylsulfide) + 3 $H_2O \rightarrow 3\ CH_4 + HCO_3^- + 2\ HS^- + 3\ H^+$	-60.6	-40.4
$(CH_3)_2$-S + $H_2 \rightarrow CH_4 + CH_3SH$	-161	-161
4 CH_3-NH_2 (Methylamine) + 3 $H_2O + H^+ \rightarrow 3\ CH_4 + HCO_3^- + 4\ NH_4^+$	-91.9	-122.6
2 $(CH_3)_2$-NH (Dimethylamine) + 3 $H_2O + H^+ \rightarrow 3\ CH_4 + HCO_3^- + 2\ NH_4^+$	-143.1	-95.4

4 $(CH_3)_3$-N (Trimethylamine) + 9 H_2O + H^+ → 9 CH_4 + 3 HCO_3^- + 4 NH_4^+	-190.9	-84.8
CH_3COOH (Acetic acid) → CH_4 + CO_2	-31	-31

[a]Calculated from the Gibbs free energy of formation from Thauer et al. (1977) and Wagman et al. (1968) except for the reaction involving dimethylsulfide and hydrogen, which was from Nobu et al. (2016).

1022 Table 2. Examples of extreme methanogens and syntrophic metabolizers.

Physiological type	Organism	Environmental Condition		Reference
		Optimum	Range	
Hyperthermophilic Methangens	*Methanocaldococcus spp.*	82-90 C	50-92 C	Jeanthon et al., 1998; Jeanthon et al., 1999; L'Haridon et al., 2003; Mehta and Baross, 2006: Ver Eecke et al., 2012
	Methanopyrus kandleri	98 C 105 C at 40 MPa	84-122 C	Kurr et al., 1991; Takai et al., 2008
	Methanothermus fervidus	83 C	65-97 C	Stetter et al., 1981
	Methanothermus sociabilis	88 C	55-97 C	Lauerer et al., 1986
	Methanotorris igneus	88 C	45-91 C	Burggraf et al., 1990
Psychrophilic methanogens	*Methanococcoides burtonii*	23 C	1.7-30 C	Fransmann et al., 1992
	Methanogenium frigidum	15 C	0-18 C	Fransmann et al., 1997
	Methanogenium marinum	25 C	5-25 C	Chong et al., 2002
	Methanolobus psychrophilus	18 C	0-25 C	Zhang et al., 2008
	Methanomethylovorans hollandica	25-35 C	1-35 C	Simankova et al., 2003
	Methanosarcina baltica	21 C	-2-28 C	Singh et al., 2005; von Klein et al., 2002
	Methanosarcina lacustris	25 C	1–35 C	Simankova et al., 2001
	Methanosarcina soligelidi	28 C	0-54 C	Wagner et al., 2013
	Methanospirillum psychrodurum	25 C	4-32 C	Zhou et al., 2014

	Methanospirillum stamsii	20-30 C	5-37 C	Parshina et al., 2014
	*Ca. "*Methanoflorens stordalenmirensis"	NA[a]	NA	Mondav et al., 2014
Acidiphilic methanogens	*Methanobacterium espanolae*	pH 5.6-6.2	pH 4.7	Patel et al., 1990
	Methanococcus aeolicus	NR[b]	pH 4.3-7.5-7	Kendall et al., 2006b
	Methanoregula boonei	pH 5.1	pH 4.5-5.5	Bräuer et al., 2011
	Methanosphaerula palustris	pH 5.7	pH 4.8-6.4	Cadillo-Quiroz et al., 2009
Halophilic methanogens	*Halomethanococcus doii*	3.0 M NaCl	>1.8 M NaCl	Yu and Kawamura, 1987
	Methanohalobium evestigatum	4.3 M NaCl	2.6–5.1 M NaCl	Zhilina and Zavarzin, 1987
	Methanohalophilus halophilus	1.2–1.5 M NaCl	0.3–2.6 M NaCl	Zhilina, 1983
	Methanohalophilus mahii	1.0–2.5 M NaCl	0.5–3.5 M NaCl	Paterek and Smith, 1988
	Methanohalophilus portucalensis	0.6–2.1 M NaCl	>1.4 M NaCl	Boone et al, 1993
Akalophilic methanogens	*Methanocalculus natronophilus*	pH 9-9.5 Na^{+c} 1.4-1.9 M	pH 8-10.2 Na^{+} 0.9-3.3 M	Zhilina et al., 2013
	Methanohalophilus zhilinae	pH 9.2 0.7 M NaCl	pH 8.0–10 0.2–2.1 M NaCl	Mathrani et al. 1988
	Methanolobus oregonensis	pH 8.6	pH 8.2–9.2	Liu et al., 1990
	Methanolobus taylorii	pH 8	pH 5.5–9.2	Oremland and Boone, 1994

	Methanosalsum natronophilum	pH 9.5 Na^+ 1.5 M	pH 8.2-10.2 Na^+ 0.5-3.5 M	Sorokin et al., 2015
Piezophiles (Barophiles)	*Methanocaldococcus jannaschii*	75 MPa	<75 MPa	Miller et al., 1998
	Methanococcus thermolithotrophicus	50 MPa		Bernhardt et al., 1988
Thermophilic syntrophic metabolizers	*Desulfotomaculum thermocisternum*	62 C	41-75 C	Nilsen et al., 1996
	Pelotomaculum thermopropionicum	55 C	37-70 C	Imachi et al., 2002
	Syntrophothermus lipocalidus	55 C	45-60 C	Sekiguchi et al., 2000
	Thermosyntropha lipolytica	60-66 C pH 8.1-8.9	52-70 C pH 7.5-9.5	Svetlitshnyi et al., 1996
Psychrophilic syntrophic metabolizer	*Algorimarina butyrica*	15 C	10-25 C	Kendall et al., 2006a
Akalophilic syntrophic metabolizers	*"Ca.* Syntrophonatronum acetioxidans"	pH 8.9 Na^+ 0.3-1.0	pH 8.9-10.2 Na^+ 0.5-3.0	Sorokin et al., 2016
	"Ca. Syntrophocurvum alkaliphilum"	pH 9.0 Na^+ 1.0	8.5-10 1-3	
	Tindallia spp.	pH 10 Na^+ 0.6-1	8-10.4 0.3-3.75	
	"Ca. Desulfonatronobulbus propionicus*"*	pH 10 Na^+ 1.0	8.5-10.3 0.3-4	Sorokin and Chernyh, 2016

[a] NA, not applicable as information derived from metagenomic analysis only.

[b] NR, not reported.

[c] Na^+ indicates the sodium concentration with anions other than chloride.

Figure

Figure 1. The importance of energy yield and energy flux in determining the habitability of an environment (after Hoehler, 2007). The free energy of the catabolic reaction (ΔG_{rxn}) must be more favorable (e.g., more negative ΔG) than the minimum amount of energy needed to make ATP (biological energy quantum, BEQ). The flux of energy must be larger than the minimum amount to maintain cellular functions and viability (maintenance energy minimum, ME_{min}). Growth will occur when the energy flux is large enough to supply sufficient energy for biosynthesis. If either requirement is not met by the energy available in the local environment, the system is uninhabitable (unshaded region). Note that the magnitude of both BEQ and particularly maintenance energy are highly dependent on the physicochemical environment and may, in the case of environmental extremes, rapidly exceed levels that can be sustained within a given system.

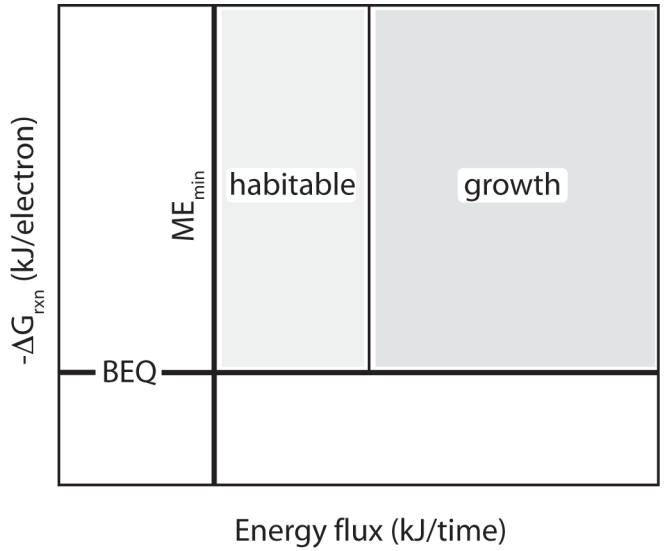
habitable
growth
ME_{min}
BEQ
$-\Delta G_{rxn}$ (kJ/electron)
Energy flux (kJ/time)

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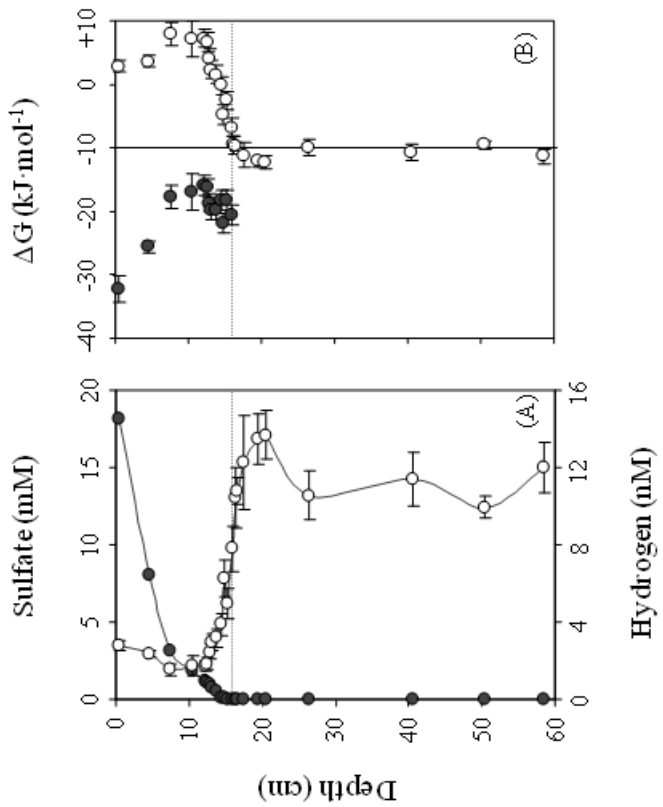
Figure 2. Thermodynamic-based competitive exclusion of methanogenesis by sulfate reduction in a marine sediment (Cape Lookout Bight, North Carolina, USA). (A) Depth profiles of concentrations of sulfate (filled circles) and hydrogen (open circles). Note that hydrogen concentrations are maintained, by the activity of sulfate reducers, at 5- to 10-fold lower levels within the sulfate-containing zone. (B) Depth profiles of Gibbs free energies of reaction for H_2-based sulfate reduction (per mole sulfate; filled circles) and methanogenesis (per mole methane; open circles). By virtue of their control over H_2 concentrations, sulfate reducers limit methanogenic energy yields to values below the minimum bioenergetic requirement and, for the upper thirteen centimeters of the sediment column, at thermodynamically unfavorable levels (right of the solid vertical line at $\Delta G=0$). Below the depth of sulfate depletion (dashed horizontal line), methanogenesis yields about -10 $kJ \cdot mol^{-1}$, consistent with lower-end estimates of the BEQ. In both (A) and (B), error bars represent the standard deviation about the mean of triplicate samples. (Figure modified from Hoehler et al., 2001.)

Fig. 2



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