

# Methane Production by Microbial Mats Under Low Sulfate Concentrations

Brad M. Bebout<sup>1</sup>, Tori M. Hoehler<sup>1</sup>, Bo Thamdrup<sup>2</sup>, Dan Albert<sup>3</sup>, Steven P. Carpenter<sup>1</sup>, Mary Hogan<sup>4</sup>, Kendra Turk<sup>4</sup>, David J. Des Marais<sup>1</sup>

<sup>1</sup>*Exobiology Branch, NASA Ames Research Center;* <sup>2</sup>*Institute of Biology, University of Southern Denmark,* <sup>3</sup>*Department of Marine Sciences, University of North Carolina at Chapel Hill,* <sup>4</sup>*University of California, Santa Cruz*

Corresponding Author:

Brad Bebout  
NASA Ames Research Center  
Mail Stop 239-4, Building N239 Room 338  
Moffett Field, CA 94035-1000  
[bbebout@mail.arc.nasa.gov](mailto:bbebout@mail.arc.nasa.gov)  
(650) 604-3227 (office)  
(650) 604-1088 (fax)

## Abstract

Cyanobacterial mats collected in hypersaline salterns were incubated in a greenhouse under low sulfate concentrations ( $[\text{SO}_4^-]$ ) and examined for their primary productivity and emissions of methane and other major carbon species. Atmospheric greenhouse warming by gases such as carbon dioxide and methane must have been greater during the Archean than today in order to account for a record of moderate to warm paleoclimates, despite a less luminous early sun. It has been suggested that decreased levels of oxygen and sulfate in Archean oceans could have significantly stimulated microbial methanogenesis relative to present marine rates, with a resultant increase in the relative importance of methane in maintaining the early greenhouse. We maintained modern microbial mats, models of ancient coastal marine communities, in artificial brine mixtures containing both modern  $[\text{SO}_4^-]$  (ca. 70 mM) and "Archean"  $[\text{SO}_4^-]$  ( $< 0.2$  mM). At low  $[\text{SO}_4^-]$ , primary production in the mats was essentially unaffected, while rates of sulfate reduction decreased by a factor of three, and methane fluxes increased by up to ten-fold. However, remineralization by methanogenesis still amounted to less than 0.4 % of the total carbon released by the mats. The relatively low efficiency of conversion of photosynthate to methane is suggested to reflect the particular geometry and chemical microenvironment of hypersaline cyanobacterial mats. Therefore, such mats were probably relatively weak net sources of methane throughout their 3.5 Ga history, even during periods of low environmental levels oxygen and sulfate.

## Introduction

Decreased solar luminosity during Earth's early history would have resulted in near global glaciation given our present atmospheric composition, but widespread geologic evidence indicates considerably warmer conditions (Gough, 1981; Gilliland, 1989), giving rise to the so-called "faint young sun problem" (Owen et al., 1979; Walker et al., 1981; Sagan and Chyba, 1997). Carbon dioxide could have provided some (Owen et al., 1979; Kasting, 1993), but not all (Rye et al., 1995; Rye and Holland, 1998), of the needed greenhouse effect. One-dimensional atmospheric mixing models predict that atmospheric methane concentrations in the Archean and Proterozoic must have been 100-300 fold higher than present atmospheric levels (PAL) in order to counteract the effects of the faint young sun (Pavlov et al., 2000; Kasting et al., 2001; Pavlov et al., 2003). Geological sources of methane are very small relative to biogenic sources (Reeburgh, 1996), and, due to a relatively constant mantle oxidation state, have likely not changed greatly over Geologic time (Delano, 2001). Hence, increased rates of biological methane production, or decreased rates of biological methane consumption, are necessary to produce the high atmospheric methane concentrations. It was recently argued (Pavlov et al., 2003) that an increased Archean biogenic methane flux from an ocean containing very little  $\text{O}_2$ , would supply the higher atmospheric methane concentrations necessary to prevent global glaciation. The potential sources of that methane, though, have not been identified. None of the overwhelmingly dominant sources of biogenic methane on the modern Earth - terrestrial plant-dominated wetlands, cultivated fields, symbioses involving higher organisms (e.g., termites, cows) or human activities (Reeburgh, 1996) -

existed in the Precambrian. Biogenic methane is produced uniquely by Archaea, microorganisms which, as evidenced by their deep divergence in the "tree of life", were undoubtedly present in the early Archean (Pace, 1997). However, in the absence of land plants, biological productivity (and therefore sources of organic carbon substrates for methanogenesis) during the Archean and Proterozoic would have been largely dominated by marine sources. Any increase in Proterozoic biogenic methane flux would therefore need to originate from a relatively restricted number of habitats, namely the marine environment, shallow epicontinental seas, and from methanogenic bacteria associated with geochemical sources of hydrogen and carbon dioxide (e.g. vents and seeps).

We used an experimental approach to examine biogenic methane production in photosynthetic microbial mats, ecosystems which likely dominated primary productivity in coastal waters in the Archean and Proterozoic (1984; 1994; Des Marais, 1995) and thus were a likely source of organic substrate for methanogens. In modern marine microbial mats, primary production is remineralized dominantly by aerobic respiration and by the activities of sulfate reducing bacteria (Canfield and Des Marais, 1993); methanogenesis is quantitatively unimportant (Oremland and King, 1989; Conrad et al., 1995). The vastly different environmental conditions which likely existed in Archean oceans, including little to no dissolved free oxygen (Canfield and Teske, 1996; Pavlov and Kasting, 2002) and sulfate concentrations ( $[\text{SO}_4^-]$ ) likely  $< 200 \mu\text{M}$  (Habicht et al., 2002; Hurtgen et al., 2002) (the modern value being ca. 30 mM), have given rise to the speculation that fluxes of methane from oceanic environments may have far exceeded those measured from the same environments today (Pavlov et al., 2003). Low  $\text{O}_2$  levels and  $[\text{SO}_4^-]$  favor the activities of methanogenic Archaea, which are obligate anaerobes (Oremland, 1988; Ferry, 1992), and are largely out-competed by sulfate reducing microbes when  $[\text{SO}_4^-]$  attains modern oceanic levels (Oremland and Taylor, 1978; Lovley et al., 1982; Lovley and Klug, 1983). Using an experimental greenhouse facility capable of maintaining *in situ* rates of biogeochemical cycling and microbial community composition in microbial mats for a period of time greater than one year (Bebout et al., 2002), we followed the effects of experimentally lowered  $[\text{SO}_4^-]$  on the relative rates of the production and remineralization (through both sulfate reduction and methanogenesis pathways) of organic matter.

## Materials and Methods

### *Field site*

Microbial mats were collected in salterns managed for the production of salt by the company Exportadora de Sal S.A. de C.V. The salt works, which cover an area  $> 300 \text{ km}^2$  and the microbial mats growing in them, have been previously described (Des Marais, 1995). Briefly, seawater is pumped from the Ojo de Liebre lagoon into the system, and subsequently flows through a series of concentrating areas slowly enough to result in increased salinity due to evaporation. At the distal end of the series concentrating areas, salt is harvested in crystallization ponds. Well-developed, laminated microbial mats occur at salinities of ca. 50 to 100 parts per thousand (‰), corresponding to concentration areas Area 4 through Area 7. The mats for this study were collected in June of 2001 in Area 4, near the dike that separates Area 5 and Area 4. A total of 18

sections of mat, each 20 cm x 25 cm x 5 cm deep, were cut and removed from the bottom of the concentrating area and immediately placed into tight fitting black acrylic trays. In this way, exposure of the deeper anaerobic layers of the mats to air and light was minimized. Mats were covered with relatively high salinity water (180 ‰) overnight to slow overall metabolic rates during transport. The trays containing the mats were then transported by vans back to our laboratory in larger plastic trays covered by tight fitting plastic film. In this way, the mats were kept moist but not covered with water, and were exposed to some natural light over the ca. 48 hours required for the relocation. An additional set of six mats was collected in October of 2001 in order to serve as a replicate of the first set of mats.

#### *Maintenance of mats:*

Upon arrival at our institute, the mats were transferred to a greenhouse modified for these experiments by replacing the original glass with ultraviolet transparent OP-4 acrylic (transmission in the UV-B, UV-A, and visible ca. 90% in the greenhouse). This greenhouse has been previously described in detail (Bebout et al., 2002). Briefly, the mats were incubated in a set of six flow boxes, (150 cm x 22 cm), each flow box holding three trays of mat. Mats were incubated in a brine solution having the same ionic composition as water from the field site but with ("NORMAL Sulfate" treatment) and without ("LOW Sulfate" treatment) the addition of sodium sulfate to *in situ* (ca. 70 mM) concentrations. Water was recirculated from a single reservoir holding 60 liters of brine through the three interconnected flow boxes that constituted each of the two sulfate treatments. In March, 2001, nine months after the start of the experiment, six mats that had been collected the previous October and maintained in separate flow boxes were transferred into the experiment, one mat into each of the six flow boxes. This served as an independent replication of the first sulfate manipulation. At that time, the six mats that were removed from the system to make room for the mats collected in October were placed into two additional flow boxes, three mats per flow box. The mats in these flow boxes are referred to as the "LOW Sulfate Extended" and "NORMAL Sulfate Extended" to indicate that the environmental conditions experienced by the mats in the "Extended" experiment were not exactly the same as those experienced by mats in the main experiment (temperature and solar radiation may have varied by as much as 10-20% from the previous time period). The LOW Sulfate Extended mats were also shielded from the increase in sulfate caused by the addition of the mats collected in October. After the conclusion of the main experiment in August 2002, the "Extended" mats were maintained under similar experimental conditions and were assayed for their methane flux eight months later.

#### *Flux measurements*

Net fluxes of oxygen, dissolved inorganic carbon, and methane were determined using glass flux chambers placed over the mats for periods of time up to 12 hours using a previously described experimental protocol (Canfield and Des Marais, 1993; Bebout et al., 2002). The chamber design and operation follows that of Canfield and Des Marais (1993). Briefly, each glass chamber (covering an area of mat ca. 0.019 or 0.012 m<sup>2</sup>) was fitted with a central stirring paddle and two sampling ports with septa. The glass paddle rotated at a constant rate of 4.5 revolutions per minute. Samples of gas and/or water were

removed from the chamber at intervals and a linear regression of analyte versus time was created to calculate fluxes.

#### *Gross Oxygenic Photosynthesis Measurements*

Rates of gross oxygenic photosynthesis were measured using oxygen microelectrodes. We used Clark-type microelectrodes incorporating guard cathodes (Diamond General 737-GC, Diamond General Development Corporation, Ann Arbor, Michigan, USA). Positioning was controlled by, and data acquired with, custom software written in the LabVIEW programming environment (National Instruments Corporation, Austin, Texas, USA). Gross oxygenic photosynthesis was determined using the dark shift method, (Revsbech et al., 1981). Small cores of the mats in the flow boxes were removed to the laboratory and placed into a small flow box (Lorenzen et al., 1995) in recirculating, temperature controlled brine collected from the flow boxes. Artificial illumination was provided, using a quartz tungsten halogen light source, liquid light guide, and collimating optics, at five irradiance levels (40, 140, 260, 600, and 1120  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The photosynthesis irradiance relationship was fitted with a hyperbolic tangent function (Jassby and Platt, 1976), which does not include provisions for photoinhibition, in order to obtain the photophysiological parameters  $P_{\text{max}}$  (light saturated rate of photosynthesis) and  $\alpha$  (slope of the light limited portion of the photosynthesis irradiance relationship). Gross photosynthesis and oxygen concentration measurements were generally obtained at 200  $\mu\text{m}$  intervals. Calibration of the electrodes was achieved by a two-point calibration; the current output of the electrodes was determined in the air-saturated water over the mats and in the deeper layers of the mat. The concentration of dissolved oxygen in the air-saturated water was calculated on the basis of its temperature and salinity using standard tables (Sherwood et al., 1991), and the oxygen concentration was assumed to be zero in the deeper layers of the mat. The assumption of total anoxia at this depth is valid; an asymptotic minimum of current was achieved, and the actual current output of the electrodes was only a few picoamperes, the typical dark current of these microelectrodes.

#### *Sulfate reduction rate measurements*

Rates of bacterial sulfate reduction were determined using the  $^{35}\text{SO}_4^{2-}$  tracer technique (Jørgensen, 1978) with similar modifications as previously applied to mats from the same saltern (Canfield and Des Marais, 1993). Mats were cored vertically with 1.4 cm i.d. open-end plastic syringes, and cores were immediately injected with radiotracer from the top in three vertical traces (per core 10  $\mu\text{l}$  of 100 % NaCl solution containing 0.5  $\mu\text{Ci}\ \mu\text{l}^{-1}$  carrier-free  $^{35}\text{SO}_4^{2-}$ ). Incubations were terminated after 30 – 40 minutes by sectioning the cores into 20 wt.-% zinc acetate followed by freezing. Core sections were obtained for the following depth intervals: 0.25 cm to 1 cm, 0.5 cm to 2 cm, and 1 cm to the bottom of the core. Reduced radiolabeled sulfur was recovered by distillation with boiling acidic chromous chloride solution (Zhabina and Volkov, 1978) using zinc sulfide as carrier and 2 wt.-% zinc acetate for trapping. Radiolabeled sulfate and reduced sulfur were quantified by liquid scintillation counting. Interstitial water for determination of sulfate concentrations was obtained by filtration through glass fiber filters (Whatman GF/F) forced by centrifugation. For this, mat sections were handled and centrifuged under  $\text{N}_2$  and centrifugate was collected in 1 wt.-% zinc acetate to avoid the oxidation of hydrogen sulfide.

### *Analytical methods*

Sulfate concentrations in the brine were determined by ion chromatography, using a Dionex DX-120. Water samples collected for organic acid analysis were immediately filtered using Acrodisc® 0.45 µm syringe filters (Pall Corporation, Ann Arbor, Michigan, USA) and frozen. Samples were later derivatized and processed via HPLC according to previously published methods (Albert and S., 1997). Organic acid flux calculations have been blank corrected for small amounts (<5 µM) of lactate, acetate and formate originating from the derivatization reagents and filters. Concentrations of dissolved oxygen in the chambers were determined using mini-electrodes (Diamond General Model 768) that exhibit a linear response within the range of oxygen concentrations measured. These mini-electrodes are housed in hypodermic needles, and were placed into the water phase within the chambers through rubber septa in the sampling ports. Calibration of the mini-electrodes was accomplished with a two-point calibration; the current output of the electrodes was measured in solutions of brine sparged with air (air-saturated brine) or nitrogen (anoxic brine). The actual oxygen concentrations of the air-saturated brine was calculated for a given temperature or salinity using published equations (Sherwood et al., 1991), the oxygen content of the nitrogen-sparged brine was assumed to be zero. Oxygen in the headspace of the chamber was measured using a SRI model 8610 gas chromatograph with a thermal conductivity detector and fitted with a 2 m Molsieve 5A column held at 30 °C. Concentrations of dissolved inorganic carbon were determined using a flow injection analyzer (Hall and Aller, 1992), and concentrations of methane were determined by gas chromatography using a Shimadzu GC-14A (flame ionization detector) having a 2 m Porapak N column held at 40 °C. The stable carbon isotopic composition of the biogenic methane that accumulated in the headspace of the flux chambers was determined using a Finnigan Delta Plus gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS) interfaced with a cold trap assembly (Scientific Instrument Services, Inc.).

## **Results**

### *Sulfate depletion in mats*

The  $[\text{SO}_4^-]$  values in the water overlying the experimental mats decreased as sulfate diffused out of the porewaters of the mats, and was removed from the system through repeated water changes (Figure 1a). About 5 months after mat collection, and three water changes, the  $[\text{SO}_4^-]$  in the overlying water was lower than 200 µM (simulating Archaeal oceanic levels). The  $[\text{SO}_4^-]$  decreased further with depth in the mat (data not shown), consistent with active scavenging by sulfate reducing bacteria. The values of  $[\text{SO}_4^-]$  in the water were maintained at < 200 µM for about 4 months, after which they increased to values near 1 mM with the addition of a set of new mats (collected in October 2001) to the experimental system.

### *Oxygenic photosynthesis net oxygen flux, and rates of sulfate reduction.*

At  $[\text{SO}_4^-] < 200 \mu\text{M}$ , integrated gross oxygenic photosynthesis, as determined by microelectrode measurements, was slightly enhanced at low light intensities in the low  $[\text{SO}_4^-]$  mats (Figure 2), probably due to relaxation of hydrogen sulfide inhibition of

oxygenic photosynthesis (Miller and Bebout, in press). Over the full range of light intensities encountered by the mats on a daily basis, however, integrated gross oxygenic photosynthesis was not different in low and normal  $[\text{SO}_4^-]$  mats (Table 1). The net flux of carbon into the mats during the daytime and out of the mats at night was likewise not affected by variations in  $[\text{SO}_4^-]$  (Table 1), even under the anoxic conditions typical (Miller and Bebout, in prep.) of the natural environment of these mats at night. In contrast (Table 1), sulfate reduction rates were significantly reduced (by 3-fold) in the low- versus normal- $[\text{SO}_4^-]$  treatment, according to radiotracer experiments (Figure 3), and consistent with diffusion-reaction calculations based on measured porewater  $[\text{SO}_4^-]$  and hydrogen sulfide concentration profiles (not shown).

#### *Net methane flux*

Given equivalent rates of gross and net photosynthetic carbon fixation in the two treatments, the reduction of sulfate-reducing activity in the low  $[\text{SO}_4^-]$  treatment should correspond directly to an increase in the amount of substrate that is potentially available for methanogenesis. Indeed, methane flux from the mats increased at lowered  $[\text{SO}_4^-]$ , reaching values ten times higher than those in the normal sulfate treatment (Figure 1b). Transfer of a second set of mats into the low sulfate experimental manipulation produced a similar increase in net methane flux with lowered  $[\text{SO}_4^-]$  (Figure 1c). Because we measured the net flux of methane from the mat, we are unable to distinguish whether the increased flux results from an increase in production or a decrease in consumption, but corollary observations suggest that consumption was probably an unimportant term in these mats. Specifically, the lack of diel variation in methane flux (under alternately oxic and anoxic conditions, Bebout, in prep.) argues that aerobic methane oxidation was probably not significant, in agreement with other studies of methane fluxes from microbial mats (Conrad et al., 1995). Similarly, the presence of  $\text{O}_2$  during the day and highly reducing conditions at night (Hoehler et al., 2001) create an environment inappropriate for anaerobic methane oxidation, given the electron-transferring consortium mechanism currently hypothesized for this process. Hence, the trend we observe likely represents an increase in methane production, but only at a small fraction of the rate that is made possible by the decrease in sulfate reduction.

#### *Methane carbon isotopic composition*

The stable carbon isotopic composition of the methane produced by the mats was dramatically different in the two  $[\text{SO}_4^-]$  treatments (Figure 34). Mats maintained under normal  $[\text{SO}_4^-]$  produced methane markedly depleted in  $^{13}\text{C}$  ( $\delta^{13}\text{CH}_4$  ca. -85 ‰); mats maintained under low  $[\text{SO}_4^-]$  produced methane having a  $\delta^{13}\text{CH}_4$  of ca. -50 ‰. Methane produced by freshly collected mats, in flux experiments identical to those conducted in the experiment, had an isotopic composition indistinguishable from the NORMAL  $[\text{SO}_4^-]$  treatment ( $\delta^{13}\text{CH}_4$  ca. -85 ‰).

#### *Carbon mass balance*

Less than 0.4% of the total carbon efflux was as methane, even in the low-sulfate treatment. Instead, the bulk of fixed carbon not consumed by sulfate reduction appears to have been lost from the mat via low molecular weight carbon compounds (Table 1). The compounds are presumably derived from fermentation processes within the mat matrix.

At the time sulfate reduction rates were measured (3 January 2002), and at the next sampling date, March 2002, the measured fluxes of organic acids were approximately two-fold higher in the low  $[\text{SO}_4^-]$  treatment, sufficient to account for the loss in sulfate-reducing activity. Over the course of the rest of the experiment, however, the difference between the treatments diminished. In fact, at the end of the "extended" portion of the experiment, the trend had reversed, and organic acids made up a far greater percentage of the total carbon loss in the NORMAL  $[\text{SO}_4^-]$  treatment, (almost seven fold higher than in the LOW  $[\text{SO}_4^-]$  treatment). Interestingly, the largest differences between the treatments in both daytime DIC uptake, and nighttime DIC release were also observed on this sampling date.

## Discussion

In aquatic environments having  $[\text{SO}_4^-]$  as low as those reached in our experimental manipulation, methanogenesis frequently accounts for a large proportion of carbon remineralization. It is therefore somewhat surprising that methane production accounted for the remineralization of less than 0.4% of the organic carbon production in these mats. Relatively low rates of methanogenesis have been previously reported for microbial mats growing in a variety of environments, including hypersaline (Oremland et al., 1982a; Oremland and King, 1989), normal marine (King, 1988; King, 1990), and even environments containing relatively low concentrations of sulfate (Ward, 1978). We suggest here that a fundamental characteristic of all microbial mats, namely the small spatial scales over which microbial mat production and consumption of organic matter take place, prohibits high rates of methanogenesis, even under low  $[\text{SO}_4^-]$ . These small spatial scales are created and maintained by the limited extent to which light penetrates these densely packed assemblages of photosynthetic organisms [Jørgensen, 1988 #378; (Lassen et al., 1992b; Garcia-Pichel and Bebout, 1996) resulting in the limitation of photosynthetic activity to within a few millimeters of the mat surface. Greater than 98% of the labile organic carbon becomes available and is remineralized in this narrow region (Canfield and Des Marais, 1991; Canfield and Des Marais, 1993), with two important consequences for methane production. First, as strict anaerobes (Oremland, 1988; Ferry, 1992) methanogens are likely excluded from the zone of active photosynthesis (which contains abundant free  $\text{O}_2$  during every daylight period), and therefore also from the zone of active carbon cycling. This is consistent with the observed constancy of the methane flux during the 24-hour daily cycle, indicating that methanogenesis occurs virtually exclusively within the deeper, permanently anoxic regions in the mat. Additionally, at the small spatial scales of the photosynthetic zone, diffusion is a highly efficient transport mechanism. This means that sulfate can be rapidly re-supplied to the zone of active carbon cycling even when the flux-driving  $[\text{SO}_4^-]$  in the overlying water is relatively low. As a result, the capacity for sulfate reduction may not be decreased as much as would be at depth in sedimentary systems. In typical sedimentary deposits in deeper waters, the delivery of organic matter is primarily as more complex and refractory particulate matter from exogenous sources. The longer time scale for degradation of these complex molecules allows a greater fraction of the organic matter to escape remineralization during its burial transit of the sulfate-containing zone, and therefore leaves a greater fraction of the carbon available for remineralization by methanogenesis.



The present report represents an attempt to constrain geochemical arguments about the importance of methanogenesis (arguments based primarily on numerical models) using experimental manipulations of extant ecosystems, namely living, intact, communities of microorganisms similar to those that existed during the Archean Era. The microbial mats used for these manipulations were collected from a moderately hypersaline environment. Our basic assumption, common to all such research, is that the ecophysiologicals of modern and ancient communities are similar under similar environmental conditions. It has previously been reported that energy requirements for osmoregulation may limit the growth of methanogens, and thereby rates of methanogenesis, in highly hypersaline environments (Oren, 2001). It has also been previously reported that methanogenesis in hypersaline environments is supported by the de-methylation of so-called "non competitive" substrates, which are not available to sulfate reducing bacteria (King, 1984; King, 1988; Oremland and King, 1989). If the methane produced by our experimental mats were produced exclusively from these non-competitive substrates, one would predict that a lowering of  $[\text{SO}_4^-]$  would have no effect on the amounts of methane produced. The ca. 12 fold increase in net methane flux we observed in response to sulfate depletion argues that 1) potential bioenergetic limitations are not prohibitive of methanogenesis in these mats, and 2) methanogens and sulfate reducers did, in fact, compete for substrate in our experiment.

Non-competitive substrates are likely to be responsible for some fraction of the methane produced in these mats, as evidenced by a number of observations. Firstly, the methane produced by mats under normal  $[\text{SO}_4^-]$  and *in situ* was profoundly depleted in  $^{13}\text{C}$  (Figure 4). Similar, extremely light values of methane have been previously reported from a culture of methanogens utilizing the non-competitive substrate trimethylamine (Summons et al., 1998). Secondly, the potential to utilize non-competitive substrates in these mats certainly exists; we have observed several fold increases in net methane production upon addition of a variety of non-competitive substrates (Bebout, in prep.). Lastly, the nearly 20‰ difference in the isotopic composition of the methane produced by the mats under normal and low  $[\text{SO}_4^-]$  conditions (Figure 4) provides evidence that the substrate used to support methanogenesis in the low  $[\text{SO}_4^-]$  treatment is different from both the *in situ* substrate and from the substrate being utilized in mats maintained under normal  $[\text{SO}_4^-]$  concentrations. The most likely explanation for this difference would seem to be a decrease in the relative proportions of non-competitive substrates utilized by mats under lowered  $[\text{SO}_4^-]$ . Further work is currently underway to clarify the effects of the substrates on the isotopic composition of the methane produced by these mats.

As a collective result of factors owing to the particular construction of microbial mats, only a small fraction of the fixed carbon that derives from photosynthetic activity within the mat is ultimately channeled through methanogenesis, even under low  $[\text{SO}_4^-]$ . A limited number of studies of methanogenesis in photosynthetic microbial mats in environments that have naturally low  $[\text{SO}_4^-]$ , e.g., hot springs, have also reported relatively low rates of methanogenesis (Ward and Olson, 1980). The factors controlling rates of microbial mat methanogenesis thus contrast with those of better studied aquatic environments, with important implications to assessing their importance in generating

methane over geologic time. Constraints on the activities of methanogenic Archaea limit their quantitative importance in modern day microbial mats and, due to their physicochemical nature, these constraints seem likely to have operated over geologic time. The quantitatively more important loss of carbon through small molecular weight organic acids also seems likely to represent an historical feature of microbial mat systems; the possibility for predominantly O<sub>2</sub>-free water columns in the Archean ocean would serve only to stimulate the fermentation process that generates these organic acids (Hoehler et al., 2001), and thereby exacerbate the loss of fixed carbon from the system. In view of the relatively modest contributions of the marine environment (in general), and of microbial mats (in particular), to modern global methane fluxes, *in situ* microbial mat primary production seems an unlikely source of an increased net methane flux necessary to support an atmosphere containing 100-300 ppm methane, (certainly far short of the proposed value of 50% of net primary production (Pavlov et al., 2003)) even in a low sulfate Archean ocean. It would seem prudent to explore more fully the abundance, areal extent, and methane producing potential of water column methanogenesis and/or ancient marine sedimentary basins, where organic matter remineralization could proceed under sulfate concentrations lowered even further than the Archean oceanic mean concentrations near 200  $\mu$ M.

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## Figure Captions

Figure 1: Sulfate concentrations (A) and net fluxes of methane from mats collected in June 2001 (B) and October 2001 (C) and maintained in brine containing either no sulfate (open symbols) or concentrations of sulfate normal for the field site (closed symbols). The effect of water changes in lowering the water column sulfate concentrations and diffusion of sulfate from the mats in elevating them can be seen in the saw tooth pattern of sulfate concentrations over time. Net methane flux from the mats collected in October of 2001 (C) are plotted using the same horizontal date scale in order to facilitate comparison with the more complete data set collected for the mats collected in June 2001 (B).

Figure 2: Depth integrated rates of oxygenic photosynthesis, as measured using the dark shift method, in mats incubated in lowered (open symbols) and normal (closed symbols) concentrations of sulfate at a range of irradiances, approximating the range of irradiances experienced by the mats *in situ*. Data have been fitted with a hyperbolic tangent function in order to yield the photophysiological parameters  $P_{\max}$  (light saturated rate of photosynthesis) and  $\alpha$  (slope of the light limited portion of the photosynthesis irradiance relationship).

Figure 3. Sulfate reduction rates in mats incubated at lowered (open symbols) and normal (filled symbols)  $[\text{SO}_4^-]$ . Measurements were performed on 3 January 2002. Error bars represent the range of duplicate measurements.

Figure 4: Carbon isotopic composition of the methane produced by microbial mats maintained in either low (open symbols) or normal (closed symbols) sulfate concentrations.

Figure 1

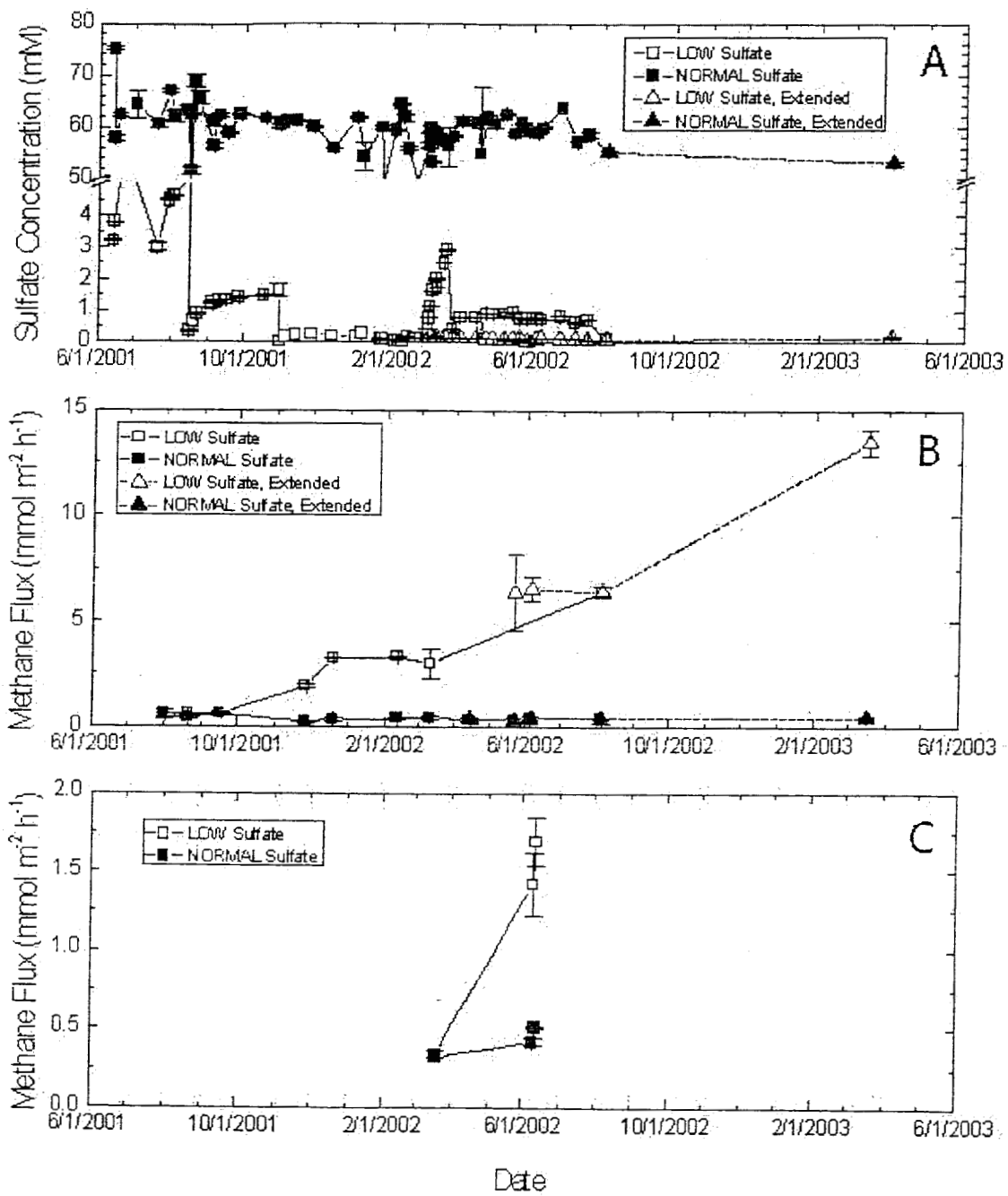


Figure 2

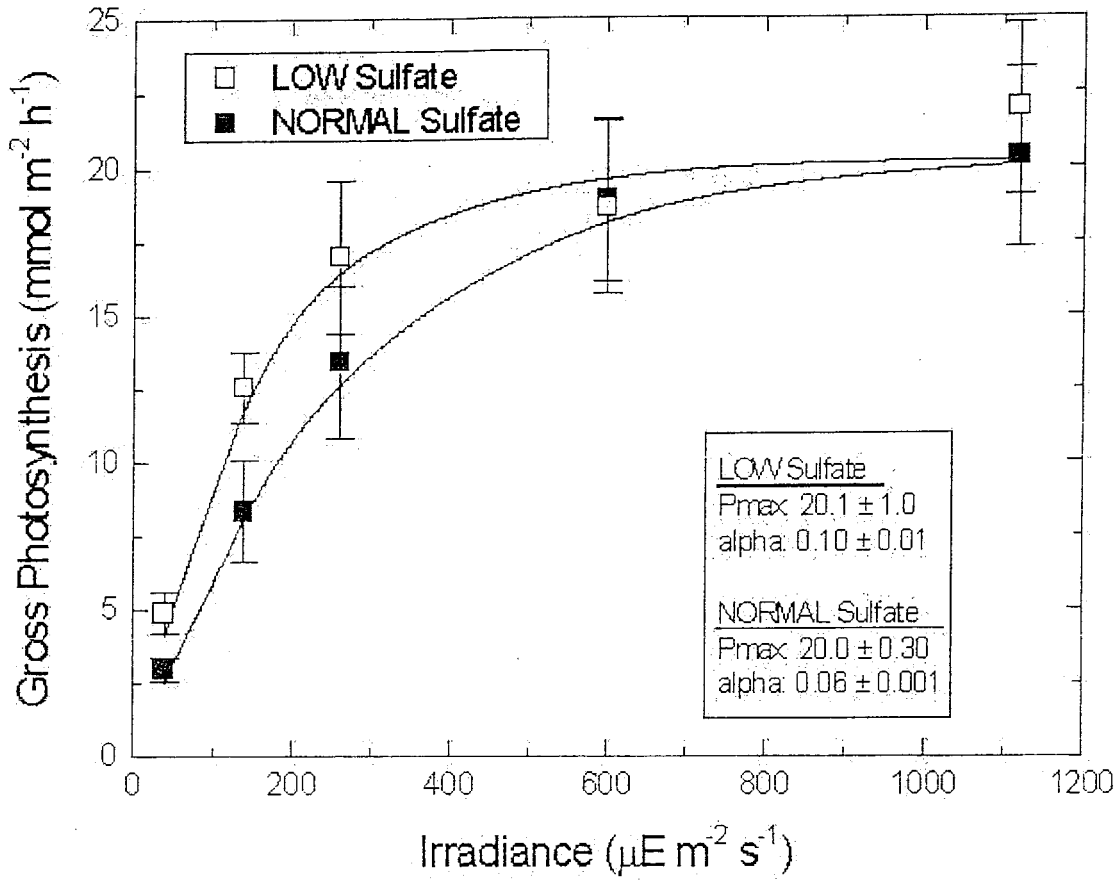




Figure 3

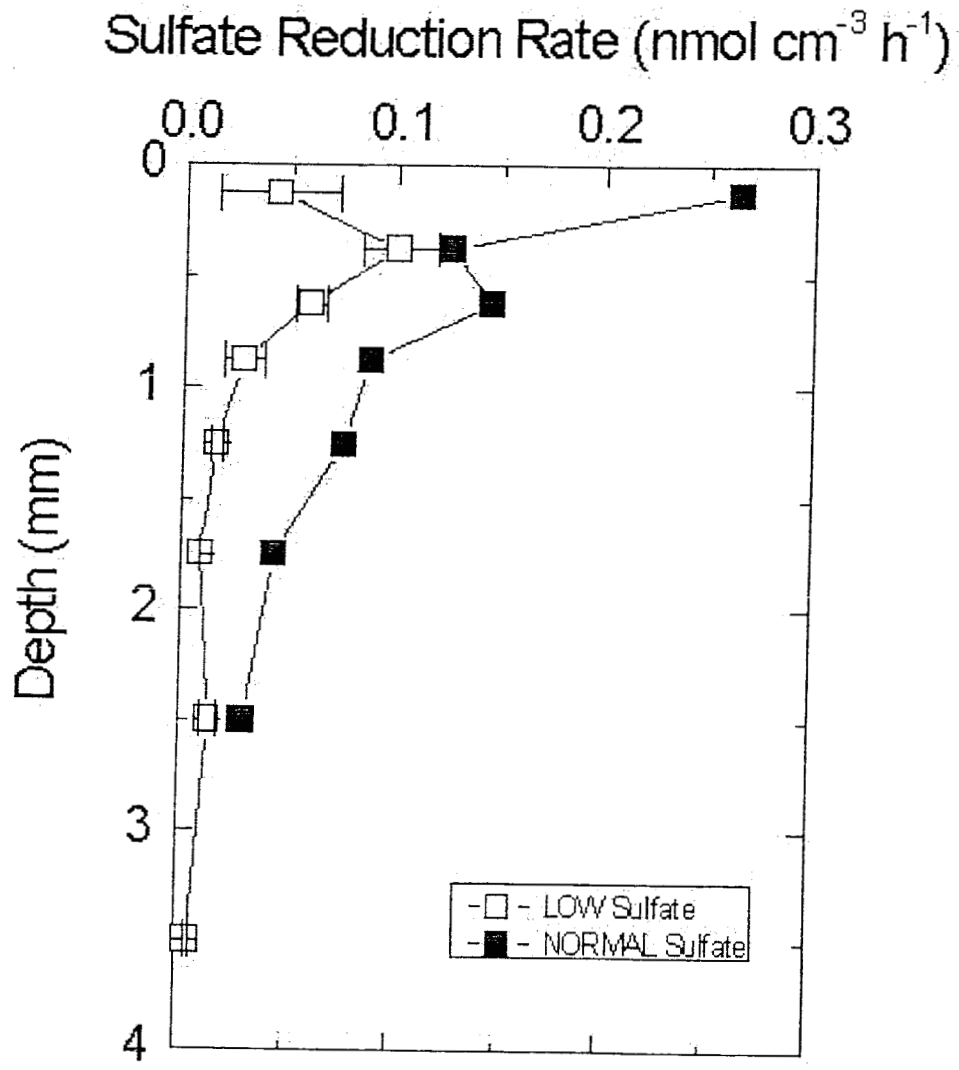
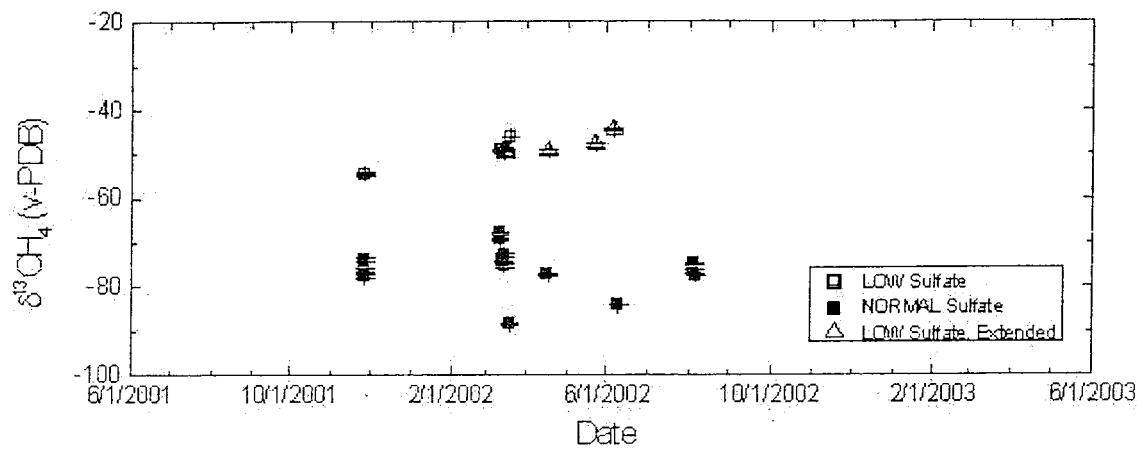


Figure 4



	February 2002			March 2002			May 2002			March 2003		
	Low [SO <sub>4</sub> <sup>2-</sup> ]	Normal [SO <sub>4</sub> <sup>2-</sup> ]		Low [SO <sub>4</sub> <sup>2-</sup> ]	Normal [SO <sub>4</sub> <sup>2-</sup> ]		Low [SO <sub>4</sub> <sup>2-</sup> ]	Normal [SO <sub>4</sub> <sup>2-</sup> ]		Low [SO <sub>4</sub> <sup>2-</sup> ]	Normal [SO <sub>4</sub> <sup>2-</sup> ]	
Daytime Gross Photosynthetic Oxygen Production (mmol·m <sup>-2</sup> ·d <sup>-1</sup> ) <sup>1</sup>	N/A	N/A		138 ± 29.5	116 ± 31.8		N/A	N/A		N/A	N/A	
Daytime Dissolved Inorganic Carbon (DIC) Uptake (mmol·m <sup>-2</sup> ·d <sup>-1</sup> ) <sup>2</sup>	-58 ± 2	-53 ± 3		-52 ± 3	-50 ± 2		N/A	N/A		-44 ± 4	-59 ± 1	
Total Nighttime Carbon Released (as DIC, Organic Acids and Methane) (mmol·m <sup>-2</sup> ·12h <sup>-1</sup> ) <sup>2</sup>	+46 ± 2	+57 ± 0.5		+28 ± 3	+37 ± 3		+80 ± 1	+80 ± 3		+68 ± 5	+49 ± 3	
Carbon Released at Night as DIC (percent)	54 %	80 %		7.4 %	56 %		25 %	52 %		90 %	32 %	
Carbon Released at Night as Organic Acids (percent)	46 %	20 %		93 %	44 %		75 %	48 %		10 %	68 %	
Carbon Released as Methane (percent)	0.11 %	0.01 %		0.15 %	0.01 %		0.10 %	0.01 %		0.33 %	0.01 %	
Carbon Release at Night Attributable to Sulfate Reduction (percent) <sup>3</sup>	47 %	117 %		77 %	177 %		27	83		31	135	

N/A = not available

<sup>1</sup> Rates of Gross Photosynthesis were calculated using data from the time period during which sulfate concentrations were below 200 μmol, ca. 1 January 2002 to 1 March, 2002. Means and standard deviations are shown for averages for 50 days calculated using light meter recordings and the photosynthesis versus irradiance relationship shown in Figure 2.

<sup>2</sup> Means and standard deviation for duplicate flux measurements on each occasion are shown. Flux measurements at night were made under anaerobic conditions. By convention, a negative number denotes a flux into the mat, a positive number a flux out of the mat.

<sup>3</sup> Measured rates of sulfate reduction were used to estimate the amount of dissolved inorganic carbon that would be liberated by that process, assuming a stoichiometry of 2 mol carbon released for every mol of sulfate reduced. Sulfate reduction measurements were performed only once, on 3 January 2002.