HYDROGEN CONSUMPTION BY METHANOGENS ON THE EARLY EARTH

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Abstract. It is possible that the first autotroph used chemical energy rather than light. This could have been the main source of primary production after the initial inventory of abiotic organic material had been depleted. The electron acceptor most readily available for use by this first chemoautotroph would have been CO_2. The most abundant electron donor may have been H_2 that would have been outgassing from volcanoes at a rate estimated to be as large as 10^{12} moles yr^{-1}, as well as from photo-oxidation of Fe^{+2}. We report here that certain methanogens will consume H_2 down to partial pressures as low as 4 Pa (4×10^{-5} atm) with CO_2 as the sole carbon source at a rate of 0.7 ng H_2 min^{-1} μg^{-1} cell protein. The lower limit of pH_2 for growth of methanogens can be understood on the basis that the pH_2 needs to be high enough for one ATP to be synthesized per CO_2 reduced. The pH_2 values needed for growth measured here are consistent with those measured by Stevens and McKinley for growth of methanogens in deep basalt aquifers. H_2-consuming autotrophs are likely to have had a profound effect on the chemistry of the early atmosphere and to have been a dominant sink for H_2 on the early Earth after life began rather than escape from the Earth's atmosphere to space.

1. Introduction

The standard theory for the origin of life postulates that life arose from an abiotically produced soup of organic material (e.g., Miller, 1953; Miller, 1992). The first organism would have therefore been a heterotroph deriving energy from this existing pool of nutrients. This theory for the origin of life is not without competitors (for a review of theories for the origins of life see Davis and McKay, 1996), but has received considerable support from laboratory experiments in which it has been demonstrated that biologically relevant organic materials can be easily synthesized from mildly reducing mixtures of gases (e.g., Chang *et al.*, 1983). The discovery of organics in comets (e.g., Kissel and Kruger, 1987), on Titan (e.g., Sagan *et al.*, 1984), elsewhere in the outer solar system (e.g., Encrenaz, 1986), as well as in the interstellar medium (e.g., Irvine and Knacke, 1989) has further strengthened the notion that organic material was abundant prior to the origin of life.

A key question in this scenario is how life survived after this initial feedstock of organics had been depleted due to unchecked microbial growth. It would have been necessary for life to develop autotrophic pathways. Margulis (1970) and others

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(Wächtershäuser, 1990; Walker, 1977) have suggested that the first autotrophs used chemical energy rather than photosynthesis. Walker (1977) suggested one possible pathway that is expressed in methanogens:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O\,.$$

The early atmosphere of the Earth is thought by some researchers, but not all, to have contained high levels of CO_2, from 0.1 to 10 bars, perhaps enough to account for warm surface temperatures despite solar luminosities lower by about 25% (Kasting *et al.*, 1993; Walker, 1977). Volcanic gases would have provided a source of H_2 estimated to be as large as 1.6×10^{12} moles yr^{-1} (Holland, 1978). Additional smaller fluxes of H_2 would have arisen from photo-oxidation of aqueous Fe^{+2} (Braterman *et al.*, 1983) and diagenesis of organic compounds.

The atmospheric effects of microbial consumption of H_2 could have been profound. By converting H_2 and CO_2 to CH_4, and limiting H_2 levels in the atmosphere, microorganisms would have altered the chemical and radiative properties of the atmosphere. Previous studies have shown that methanogens can consume H_2 down to levels of 22 ppm (6.5 Pa) when provided with a source of organic material (Lovley, 1985). For the application to the early Earth we must extend these results to purely chemoautotrophic growth.

To determine the lower limit at which chemoautotrophic methanogens can consume H_2 we have conducted tests on several methanogens. In particular we report results for *Methanobacterium wolfei, Methanobacterium formicicum, Methanococcus maripaludis* and *Methanosarcina barkeri*. The *Methanobacterium* species are commonly found in anaerobic digesters and in anaerobic sediments from freshwater environments. *Methanococcus* species may be found in anoxic salt-marsh, marine or estuarine environments. *M. barkeri* is found in marine and freshwater mud as well as anaerobic digesters (Stanley, 1989).

2. Materials and Methods

With some modifications, our experimental procedure follows that of Lovley (1985). Cultures were obtained from David R. Boone, Oregon Graduate Institute, Beaverton, OR. *M. wolfei, M. formicicum, and M. barkeri* were grown in a standard medium (MS medium) for methanogens as described by Boone *et al.* (1989) or the same medium without any organic material (Xun *et al.*, 1988). *M. maripaludis* was grown in MSH medium (MS medium plus additional amounts of NaCl, $MgCl_2$, and KCl; Ni and Boone, 1991) with or without organics. All media were prepared in a Coy Anaerobic Environmental Chamber. Media were autoclaved at 121 °C for 20 min. Sodium sulfide (2.5%) was added 1 hr before inoculation from sterile, oxygen-free stock solutions (Boone *et al.*, 1989). Cultures (10 mL) were grown under 300 kPa total pressure of hydrogen-carbon dioxide (75:25) in anaerobic pressure tubes (Hungate, 1969), followed by horizontal incubation in

environmental shakers at 55 °C for *M. wolfei*, 37 °C for *M. formicicum* and *M. barkeri*, and 25 °C for *M. maripaludis*. Culture optical densities were measured at 660 nm in a Bausch & Lomb Spectronic 20. When each culture reached an optical density of approximately 0.1 (*M. barkeri* only reached 0.04), the headspace was flushed and replaced with pure CO_2 (300 kPa total pressure). Hydrogen was then added to provide 10 000 to 20 000 ppm in the headspace. To prove the reproducibility of the result, we reinjected H_2 after 48 hr and observed again the return to the lower limit of uptake.

Headspace gas samples (1 mL) were removed at time intervals and analyzed by a Hewlett-Packard model 5890 gas chromatograph with a thermal conductivity detector at an oven temperature of 40 °C using argon as the carrier gas.

For H_2-utilization rate studies, cells were subjected to sodium dodecyl sulfate (1% final conc.) for two hr at 80 °C. Cell protein was determined using a BCA Protein Assay Reagent Kit (Pierce).

3. Results

The results for growth on inorganic medium are shown in Figure 1. For all four methanogens considered there is a clear and reproducible limit on the levels of H_2 where uptake occurs. These levels vary among the organisms tested with the lowest value, 15 ± 7 ppm (equivalent to 4.5 Pa in the 300,000 Pa total pressure in our system), corresponding to *M. formicicum*. The digital resolution of the gas chromatograph was 10 ppm. For *M. formicicum* only we observed values that were above and below 10 ppm (Figure 1, panel c) and we assume that values below the resolution are near 10 ppm. For this reason any values below 10 ppm were recorded as 10 ppm. The highest value of the H_2 uptake level on inorganic medium was 157 ± 21 ppm for *M. barkeri*.

In Figure 2 we present the results for growth on organic-supplemented medium. The results are comparable to those obtained on the organic-free medium.

4. Discussion

Our results for heterotrophic growth (Figure 2) can be compared to those of Lovley and colleagues (Lovley, 1985; Lovley and Ferry, 1985; Lovley and Goodwin, 1988). For example, they found that *M. formicicum* in pure culture would take up H_2 to a partial pressure of 6.5 Pa (Lovley and Ferry, 1985; equivalent to 22 ppm in our experiments). Studies on whole sediments (Lovley and Goodwin, 1988) also show uptake of H_2 down to levels of 7–10 nM in solution (equivalent to 3–5 ppm in our experiments).

The lower limit of pH_2 for growth of methanogens can be understood in terms of the Gibbs free energy available from the reduction of CO_2.

$$CO_2(g) + 4H_2(g) \rightarrow CH_4(g) + 2H_2O(l)$$

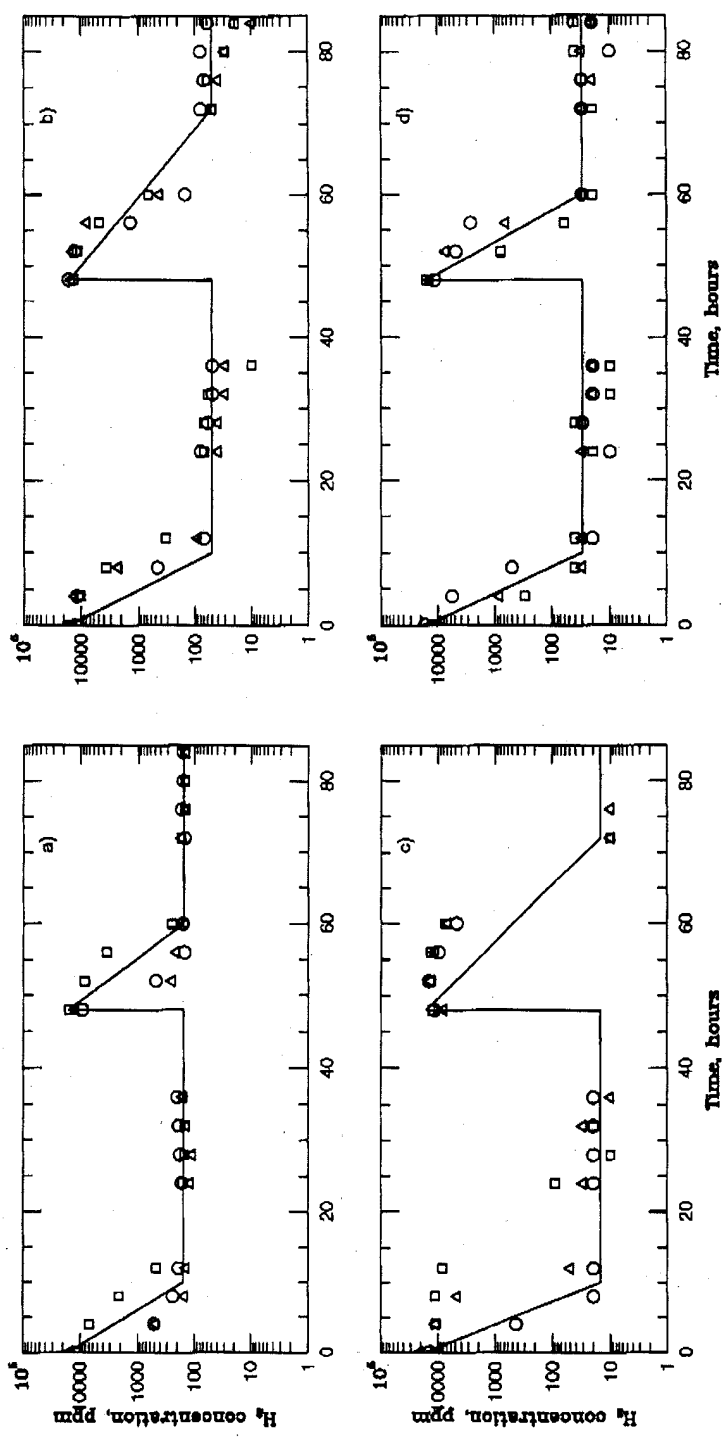

Figure 1. Time course for H_2 utilization by methanogens in three atmospheres of CO_2. At the start of the time course, and again at 48 hr, H_2 was added to the cultures. In all cases the organisms rapidly depleted H_2 levels down to a minimum value of 157±21 ppm for *M. barkeri* (panel a); 51±22 ppm for *M. wolfei* (panel b); 15±7 ppm for *M. formicicum* (panel c); 30±12 ppm for *M. maripaludis* (panel d). Three time courses are shown for each organism using different symbols.

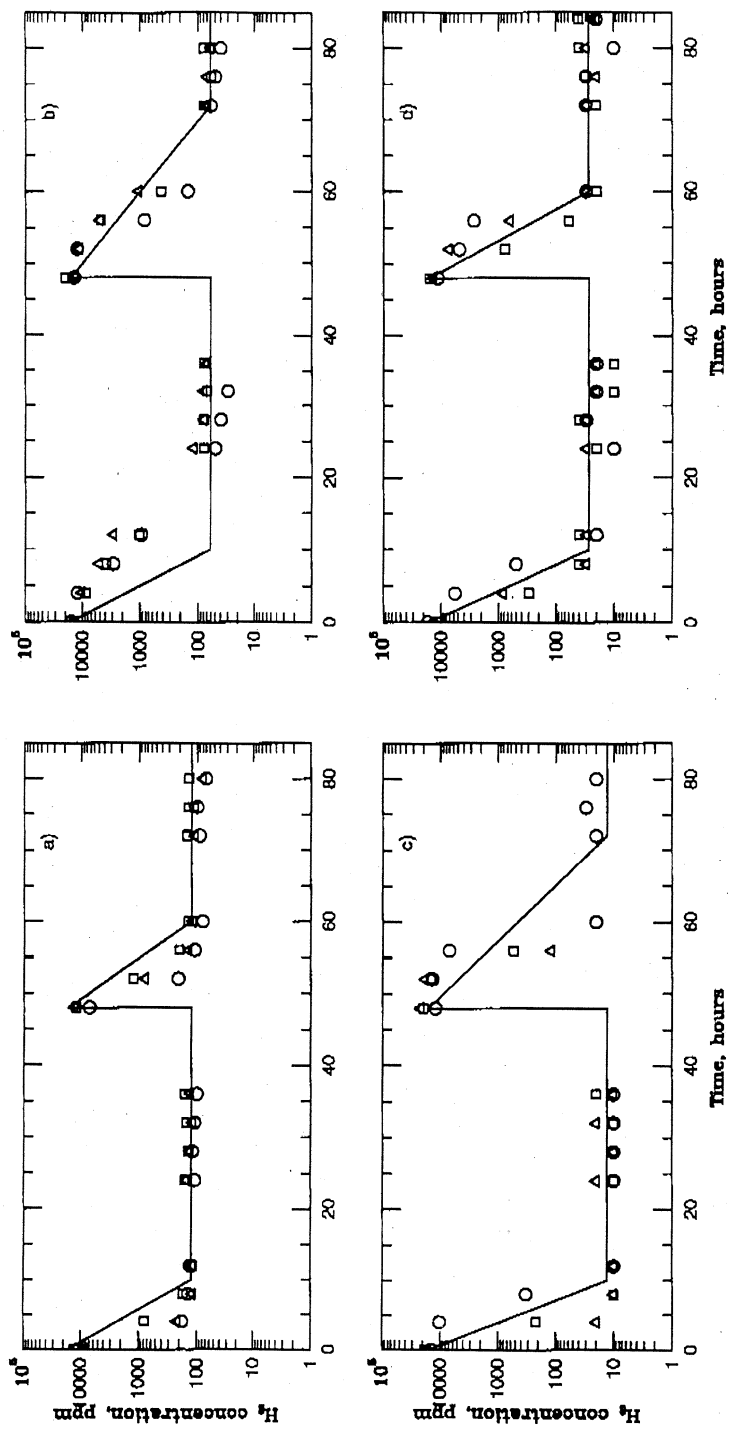

Figure 2. Same as Figure 1 but with organic medium. Minimum values for H_2 uptake were 124±34 ppm for *M. barkeri* (panel a); 61±19 ppm for *M. wolfei* (panel b); 13±5 ppm for *M. formicicum* (panel c); 27±11 for *M. maripaludis* (panel d).

Using the free energies of formation (Stull *et al.*, 1969; Miller and Smith-Magowan, 1990) gives

$$G^{\circ}_{react} = -31.27 \text{ kcal mole}^{-1} \ (25\ ^{\circ}\text{C}) \text{ and } H^{\circ}_{react} = -60.48 \text{ kcal mole}^{-1}$$

$$\log K_{eq} = -21.405 + 13,217/T$$

$$G^{\circ}_{react}(\text{kcal mole}^{-1}) = -60.48 + 0.09795T\ .$$

The free energy change is very temperature dependent with

$$G^{\circ}_{react}(37\ ^{\circ}\text{C}) = -30.12 \text{ kcal mole}^{-1}$$

and

$$G^{\circ}_{react}(55\ ^{\circ}\text{C}) = -28.37 \text{ kcal mole}^{-1}\ .$$

The free energy change at pressures different than the standard 1 atm is given by

$$G_{react} = G^{\circ}_{react} + RT\ln Q$$

where

$$Q = (pCH_4)(a_{H_2O})^2/(pCO_2)(pH_2)^4\ .$$

The free energy values and H_2 utilization rates for the four methanogens are seen in Table I. Since the ΔG° of hydrolysis of ATP is –8.5 kcal mole^{-1}, this is enough to synthesize just one ATP, if it is assumed that the overall reduction of CO_2 needs to be irreversible, as is the case for most biosynthetic pathways. Within the errors of our analysis, the minimum energies listed in Table I are consistent with one ATP production per reaction.

We can compare our results in pure cultures with corresponding results from natural systems. Stevens and McKinley (1995) report that the growth of methanogens in deep basalt aquifers stops at $[H_2]$ <0.02 μ molar. From the solubility of H_2 at 25 °C (Wilhelm *et al.*, 1977), this corresponds to $pH_2 = 2.6 \times 10^{-5}$ atm. The free energy change cannot be calculated because the pCH_4 is not given for each pH_2 measurement, but these ΔG values are approximately in agreement with those we obtained from growth in culture.

Considering again the early Earth, our results for growth on organic-free medium suggest that consumption of H_2 by methanogens could have been an important sink for H_2 and source for CH_4. Methanogens can use CO_2 at pH_2 levels predicted by photochemical models for the early Earth (Walker, 1977; Kasting *et al.*, 1993). These levels are significantly in excess of the threshold for consumption by methanogens. The major non-biological net sink of H_2 is escape to space (Walker, 1977). The rate of escape of total hydrogen depends on the mixing ratio of all

Table I
Free energy values and H_2 utilization rates

	Free energy ΔG_{react} (kcal mole^{-1})	H_2-utilization rate (ng H_2 min^{-1} μg^{-1} cell protein)
M. formicicum (inorganic)	–9	0.7
M. formicicum (organic)	–8	1.1
M. maripaludis (inorganic)	–9	1.6
M. maripaludis (organic)	–9	2.5
M. wolfei (inorganic)	–12	0.7
M. wolfei (organic)	–12	0.5
M. barkeri (inorganic)	–14	56.9
M. barkeri (organic)	–15	60.0

hydrogen-containing species. However, since H_2 is not significantly produced photochemically in the atmosphere – its source is outgassing (Kasting *et al.*, 1993) – its upward flux is determined by its own mixing ratio. Thus, as methanogens convert H_2 and CO_2 to CH_4, the consumption of H_2 by microorganisms becomes the only important net sink for H_2. If methanogens represented an efficient and dominant sink for H_2 on the early Earth, then the H_2 concentration in the atmosphere would be about 4 Pa, approximately the lower limit at which we find uptake is possible.

Methanogens produce CH_4 as a byproduct. Methane is an important greenhouse gas but because it is rapidly photolyzed, a continuous source is required for it to have played an effective role in maintaining above freezing temperatures on the early Earth against the lower luminosity of the early sun (Caldeira and Kasting, 1992). Conversion of H_2 and CO_2 to CH_4 by methanogens could be such a source. To adequately address these issues requires that photochemical and greenhouse models of the early Earth explicitly include the effects of microbial consumption of H_2 and production of CH_4. Our results for the rate of H_2 uptake can be used to compute the microbial sink of H_2 and source of CH_4 in numerical models.

Methanogenic chemosynthesis can have implications beyond the early evolution of life and can be the basis for microbial ecosystems on Earth and Mars. Recently, Stevens and McKinley (1995) have reported on a microbial ecosystem deep within basaltic sediments. Primary productivity is based on methanogens consuming H_2 from solution at levels between 0.05 and 60 uM. The H_2 may come

from water reacting with basaltic rocks. This system represents an extant model for methanogenic primary production such as we have hypothesized might have existed on the early Earth.

It has also been suggested that a microbial ecosystem based on H_2 and CO_2 could be the basis for subsurface life on Mars where surface conditions are inhospitable to life (Boston *et al*., 1992). Subsurface volcanic activity could provide liquid water by melting ground ice and could also be a source of reduced gases such as H_2S and H_2. Alternatively H_2 could be produced by water reacting with basalt – abundant on Mars – as in the terrestrial example.

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