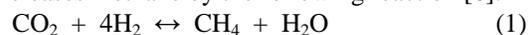


OXIDATION OF IRON AND METABOLIZATION OF CARBONATE THROUGH METHANOGENESIS: IMPLICATIONS FOR THE EARLY TERRESTRIAL ENVIRONMENT. T. Altheide¹, V. Chevrier¹, and T. Kral², ¹W.M. Keck Laboratory for Space Simulations, Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, and ²Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72701, USA <talthei@uark.edu>.

Introduction: The early atmospheric conditions of Earth were anoxic and very reducing in nature [1,2]. However, with the appearance of photosynthetic life around 3.5 billion years ago, Earth's environment would drastically change over the course of the next 1 billion years [3,4]. This oxidation process was ultimately driven by the simultaneous loss of H⁺, generally believed to be due to thermal escape. Hydrogen loss would have also been accomplished through its acquisition by organisms requiring an energy source [5]. Such organisms would include the Archeal methanogens, or life quite similar.

These methane producing microorganisms carry out the process of methanogenesis, which usually requires the intake of carbon dioxide and hydrogen gas, and releases methane by the following reaction [6]:



Molecular analysis has revealed methanogens to be some of the oldest organisms on Earth [7,8]. Their ancient origins are also reflected by their preference for an anoxic environment, like that which dominated early Earth. The possibility of a large scale, maybe even global, community of methane-producing microbes, in the form of mats, seems quite likely [5,9]. It is therefore reasonable to believe that these organisms might have had an active role in altering the local environment, and maybe even contributing to atmospheric changes as well.

Siderite is stable in hydrogen-rich, non-oxidizing environments, implying its presence on early Earth, in surface and subsurface deposits [10,11]. Its presence also reflects an environment richer in CO₂ than present day values [12]. In such an environment, the carbonate component would provide an excellent carbon source, in an aqueous solution, for organisms that could harness it, such as methanogens. In the process, these organisms could be responsible for the subsequent alteration of this material, thereby contributing to its disappearance.

Methods: Two methanogen species were used; *M. wolfei* and *M. formicicum*. Each organism was tested in duplicate under four specific gas conditions, along with a set of control tubes with no methanogens present (Table 1). All tubes used, both experimental and control, contained 0.5 g of siderite, along with 10 mL of media made with deionized water instead of sodium buffer; MM media for *M. wolfei* and MSF for *M. formicicum*.

	H ₂	CO ₂	H ₂ + CO ₂	Ar	O ₂
<i>M. wolfei</i>	1,2	3,4	5,6	7,8	—
<i>M. formicicum</i>	9,10	11,12	13,14	15,16	—
controls	17,18	19,20	21,22	23,24	25

Table 1. Atmospheric gas contents of all tubes used in the experiment, with the numbers corresponding to the number of the tube. Each gas condition was tested in duplicate for each organism and for the controls.

Media preparation was accomplished in an anaerobic chamber to ensure no oxygen contamination. Siderite was added to each tube along with the media. The appropriate gas was then bubbled through each tube, for a duration of 15 minutes to remove any excess unwanted gas. All tubes were then sealed, clamped, and autoclaved for complete sterilization. After autoclaving, cysteine was added to all tubes in order to remove any oxygen present in solution. At this point, the organisms were inoculated into the tubes, then each tube pressurized with the corresponding gas.

Tubes containing just hydrogen gas tested for the ability of methanogens to metabolize carbon from the carbonate. Tubes pressurized with just carbon dioxide tested for the ability of methanogens to acquire free hydrogen gas from the interaction of water and siderite. The tubes with the normal hydrogen and carbon dioxide mixture were essentially positive controls for methanogen growth and/or metabolization. To test for both oxidation and metabolization, tubes were pressurized with just argon, an inert gas not involved with the process. Finally, the effect of outside oxygen contamination was tested by intentional injecting oxygen into one of the control tubes (tube 25).

Methane production, and hence methanogen growth and/or metabolization, was monitored through gas chromatograph readings, taken every week for five weeks. Pictures were taken during the course of the experiment to document any precipitate formation and color change to the siderite. Complete characterization of the siderite from both the experimental and control tubes will be accomplished through X-ray diffraction, scanning electron microscopy, and magnetic analysis. This will enable identification of any mineral alternations and products that may have formed throughout, and should help describe what processes may have occurred.

Results and discussion: Tables 2 and 3 show GC methane readings, for *M. wolfei* and *M. formicum*, respectively. GC readings of the control tubes were also taken, all of which did not demonstrate the presence of methane, thereby confirming the methanogens as the sole source of the methane in the experiment. The presence of methane in the experimental tubes confirms that the organisms were at least able to metabolize under the specific conditions in the tubes, enough to stay alive.

Tube #	Week 1	Week 2	Week 3	Week 4	Week 5
1	0.00	0.02	0.01	0.00	0.00
2	0.00	0.02	0.00	0.05	0.00
3	0.01	0.02	0.02	0.01	0.01
4	0.00	0.01	0.01	0.01	0.01
5	0.01	0.01	0.01	0.00	0.01
6	0.00	0.01	0.04	0.00	0.00
7	0.02	0.01	0.00	0.00	0.01
8	0.00	0.01	0.01	0.00	0.00

Table 2. Methane readings of *M. wolfei* tubes.

Tube #	Week 1	Week 2	Week 3	Week 4	Week 5
9	0.18	2.22	2.21	1.30	2.02
10	0.05	0.42	0.81	1.38	2.21
11	0.04	0.10	0.07	0.10	0.13
12	0.03	0.01	0.00	0.00	0.00
13	0.31	0.11	0.10	0.27	0.35
14	0.14	0.06	0.00	0.38	0.31
15	0.03	0.00	0.84	-----	-----
16	0.06	0.70	1.20	2.22	1.72

Table 3. Methane readings of *M. formicum* tubes. The results from the tubes containing higher methane production are highlighted. Tube 15 was removed after the third week of readings in order to analyze the dark precipitate that formed.

Pictures of tubes demonstrating the presence of precipitates and/or color changes to the siderite were taken (Fig. 1). Two tubes are displayed, with the one on the left displaying a noticeable pink color, indicating the presence of oxygen. The tube on the right displays possible alternation to the original siderite. The appearance of dark material is clearly seen. Tubes 9, 10, 15 and 16, all containing *M. formicum*, developed a black color to the siderite. Consequently, all four tubes also displayed the highest percentage of methane detected, highlighting a possible connect between the two conditions.

In light of the current results, we present a possible geochemical model for such a mechanism, involving five general reactions. Siderite first loses its carbonate component upon its interaction with water:

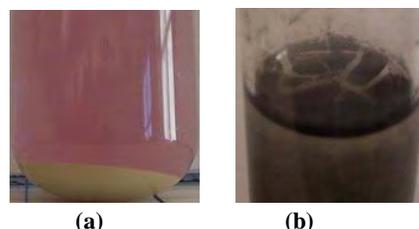
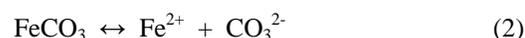
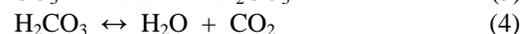


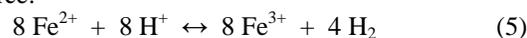
Figure 1. Photographs of three tubes used in the experiment. (a) Control tube number 25, showing the appearance of oxygen contamination (pink color). (b) Experimental tube 15 (argon), demonstrating presence of black material.



Carbonate first forms bicarbonate, which quickly dissociates to form water and carbon dioxide in an aqueous solution:



Iron is subsequently oxidized from the 2^+ to 3^+ state, while hydrogen gas becomes an available energy source:



The final step is the process of methanogenesis itself – equation (1) – which is able to harness the free hydrogen and carbon dioxide in solution.

Thus, a plausible mechanism exists for the oxidation of iron, driven by the sequestering of hydrogen and carbon, derived from carbonate, by methanogenic species. Such a biotic-driven process may have greatly contributed to the direct alteration of localized terrestrial environments, possibly even on a larger scale.

Conclusions: The methane readings suggest that *M. formicum* responds more favourably under the various conditions tested in the experiment. This might be due to changes in the pH level of the solution. The appearance of black material in four of the experimental tubes offers some interesting possibilities that may be occurring. However, the direct chemical analysis of the siderite is required before anything can be concluded, significant or not.

References: [1] Kasting J. (1993) *Science*, 259, 920-926. [2] Tian F. et al. (2005) *Science*, 308, 1014-1017. [3] Kasting J. (2002) *Science*, 296, 1066-1068. [4] Catling D. (2005) *Earth Planet. Sci. Lett.*, 237, 1-20. [5] Catling D. et al. (2001) *Science*, 293, 839-843. [6] Thauer R. (1998) *Microbiology*, 144, 2377-2406. [7] Woese C. et al. (1978) *J. Mol. Evol.*, 11, 245-252. [8] Jones W. et al. (1987) *Micro. Reviews*, 51, 135-177. [9] Bebout B. et al. (2004) *Geobiology*, 2, 87-96. [10] Hessler A. et al. (2004) *Nature*, 428, 736-738. [11] Ohmoto H. et al. (2004) *Nature*, 429, 395-399. [12] Liu L. (2004) *Earth Planet. Sci. Lett.*, 227, 179-184.