LOW-PRESSURE DESICCATION EFFECTS ON METHANE PRODUCTION BY METHANOGENS. T. S. Altheide[1] and T. A. Kral[1,2], [1]W. M. Keck Laboratory of Space Simulation, Arkansas Center for Space and Planeatry Sciences, MUSE 202, University of Arkansas, Fayetteville, AR, 72701, talthei@uark.edu. [2]Department of Biological Sciences, Science and Engineering 601, University of Arkansas, Fayetteville, AR, 72701.

Introduction: Due to the environmental conditions and the recent detection of methane on Mars, organisms known as methanogens have become a plausible analogue for putative life there [1,2]. Methanogens take in molecular hydrogen and carbon dioxide, and release methane as a waste product. On Earth, these Archaeal organisms can be found in a variety of extreme conditions, including in permafrost, within underground rocks, and in desert soils [3,4]. Such versatility makes methanogens great models for extraterrestrial studies.

Recent work has shown that methanogens from martian analogue permafrost may survive freezing and thawing cycles under simulated martian conditions [5]. In addition, it has also been demonstrated that methanogens may survive on JSC Mars-1 soil simulant by obtaining all their required nutrients directly from the soil, and that they may survive periods of prolonged desiccation as well [6,7]. These and other experiments have reinforced the idea that methanogenic microbes are an ideal model for potential martian life.

However, in assessing the possibility of microbial life at the surface of Mars, the effect of low pressure desiccation would factor in greatly. Currently, there is little information on the effect of low pressure exposure for many organisms, including methanogens. Here, we present early results on the production of methane from methanogens subjected to 6 mbar of pressure for 60 days.

Methods and Materials: Four methanogenic species were tested: *Methanococcus maripaludis*, *Methanobacterium formicicum*, *Methanosarcina barkeri*, and *Methanothermobacter wolfeii*. Organisms were inoculated in tubes under two conditions: in tubes containing just cells with no soil (naked), and in tubes containing 5 mL of growth media and 0.5 g of JSC Mars-1 soil simulant. Once the tubes were inoculated with the appropriate organisms and sealed with rubber stoppers, small syringe needles were placed through the stoppers and the tubes were placed into the Pegasus simulation chamber (Figure 1). Placing needles through the stoppers allowed the inside of each tube to reach low pressure once the chamber was pumped down. Pressure was maintained at approximately 6 mbar for 60 days by using a pressure control system. After 60 days, the tubes were removed and nutrient media added to each tube of "naked" cells, while sterile buffer was added to tubes with JSC Mars-1. Starting two days after their removal from the chamber, methane production in each tube was monitored using a Varian CP-4900 Gas Chromatograph.

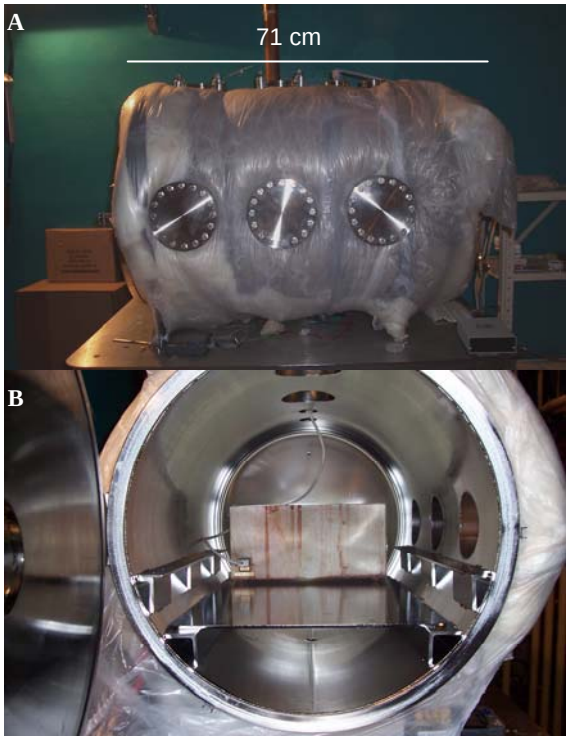

Figure 1. The custom built low-pressure simulation Pegasus Chamber. An Alcatel ACP28 vacuum pump is located beneath, along with the pressure control system. **A)** Side view of chamber. The length of the chamber is approximately 71 cm from front to back. Three ports can be seen, while three more are located at top along with a viewing window. **B)** View inside chamber. The diameter across is 50 cm. An oxygen scrubber is attached in the back, along with an inlet for gas injection. The chamber can easily allow testing of more than 100 tubes at once.

Results: Methane production was monitored for 36 days after 60 days of exposure to 6 mbar of pressure for each of the four organisms (Figures 2-5). Some interesting results can be seen in Figures 2-5. All four organisms were able to produce methane after 60 days of exposure to 6 mbar of pressure, yet this was only for the naked cells – those without soil present. Only *M. barkeri* showed significant production of methane from tubes containing the Mars JSC-1 soil simulant. It was originally hypothesized that the presence of soil in the tubes would confer some protection for the cells during low pressure exposure.

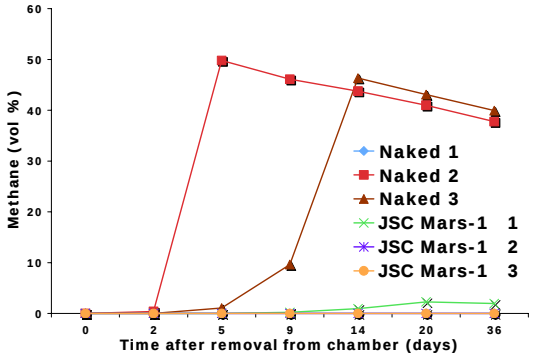

Figure 2. Percent methane production of *M. maripaludis* following exposure to 6 mbar of pressure for 60 days.

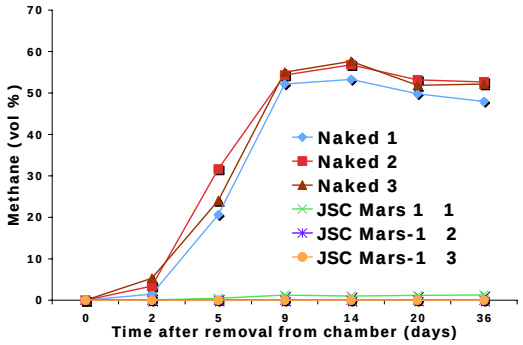

Figure 3. Percent methane production of *M. formicicum* following exposure to 6 mbar of pressure for 60 days.

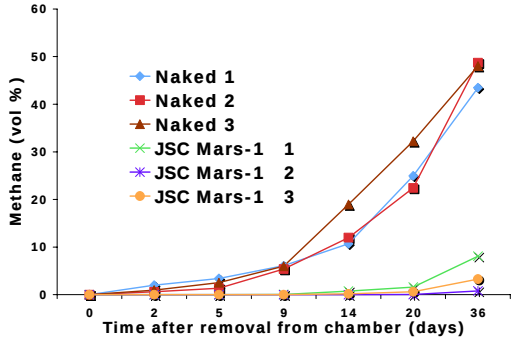

Figure 4. Percent methane production of *M. barkeri* following exposure to 6 mbar of pressure for 60 days.

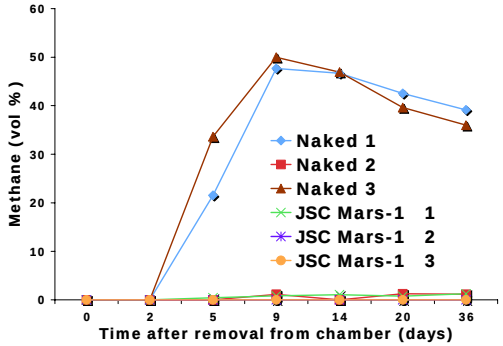

Figure 5. Percent methane production of *M. wolfeii* following exposure to 6 mbar of pressure for 60 days.

Discussion: Since it has been shown previously that methanogens can be sustained on Mars JSC-1 soil under their normal growth conditions, the results here are intriguing. They suggest a few possibilities as to the trend we see. First, because the low pressure desiccation process was observed to cause turbulent mixing of the soil and nutrient media in the tubes, cells might have been exposed to higher levels of iron and/or other ions from the soil. Some of these ions would have been initially dissolved in the medium, but after desiccation, they would have become more concentrated in the remaining liquid. Thus, cells might have been exposed to higher than normal levels of iron from the soil and/or concentrated salt from the medium. A second possibility is that the soil itself may become reactive in such a way that inhibits detection of methane, if the methanogens have become thoroughly mixed in the soil. The presence of any reactive iron oxides, including nano-sized particles, may provide a mechanism for sequestering methane.

At this point, its mere speculation until further experimentation is completed. However, these data are potentially significant in light of the Viking life detection results, and may provide knowledge which could be beneficial when considering future biological experiments at the surface of Mars.

Conclusions: Experiments are currently in progress which should provide better understanding of what is happening under these conditions. First, cells both naked and with soil will be exposed to 6 mbar for 90 days, since all organisms were able to survive 60 days of exposure. Second, tubes placed into the chamber will have all liquid removed first, to both limit exposure to oxygen during the transition to the chamber and to potentially limit concentration of detrimental ions. And third, different soil substrates will be used to determine the role of soil reactivity during low-pressure exposure. This should help clarifiy the effects of low-pressure desiccation under different evaporative conditions.

References: [1] Onstott, T.C. et al. *Astrobiology 6*, 377-395. [2] Reid, I.N. et al. *Inter. J. Astrobiology 5*, 89-97. [3] Tung, H.C. et al. *PNAS 102*, 18292-18296. [4] Moran, M. et al. *Icarus 178*, 2005. [5] Morozova, D. et al. *OLEB 37*, 189-200. [6] Kral, T.A. et al. *OLEB 34*, 615-626. [7] Kendrick, M.G. and Kral, T.A. *Astrobiology 6*, 546-551.