

APPROACHING MARTIAN CONDITIONS: METHANOGEN SURVIVAL AT LOW PRESSURE. R. L. Mickol¹ and T. A. Kral^{1,2} Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, University of Arkansas, Fayetteville, Arkansas, 72701, USA, [rmickol@uark.edu], ²Dept. of Biological Sciences, SCEN 632, University of Arkansas, Fayetteville, Arkansas, 72701, USA.

Introduction: Of the various conditions on Mars that contribute to its inhospitability, the effect of the low atmospheric pressure on organism growth and metabolism has not been widely explored [1, 2]. The average surface pressure of Mars is 7 mbar, a little over 1/1000th that of Earth. The effects of low pressure on the growth and cell structure of organisms remain unknown.

Methanogens are ideal organisms for life on Mars because they are anaerobic, do not require organic nutrients, and are non-photosynthetic (they can exist in sub-surface environments). Four separate strains of methanogen (*Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum*, *Methanococcus maripaludis*) were tested for their ability to survive prolonged periods of time under low pressure conditions. These four species were chosen as representatives of the Archaea domain.

Methods: Four separate types of methanogen growth media (MM, MS, MSF, MSH) were prepared following the procedure of Kendrick and Kral [3]. Ten milliliters of each of the four media were added to each of 5 test tubes, for a total of 20 test tubes. A sterile solution (~125 μ L) of 2.5% Na₂S was added to the media following sterilization via autoclave. Each test tube was inoculated with 0.5 mL of the corresponding methanogen (MM: *M. wolfeii*; MS: *M. barkeri*; MSF: *M. formicicum*; MSH: *M. maripaludis*). The tubes were pressurized with 200 kPa of H₂ gas and placed at their respective incubation temperatures (37°C for *M. barkeri* and *M. formicicum*, 55°C for *M. wolfeii*, 25°C for *M. maripaludis*).

Each tube was tested for methane production on a gas chromatograph to confirm growth. The tubes were placed into the Pegasus Planetary Simulation Chamber [4] with a palladium catalyst box to remove residual oxygen. The chamber was evacuated to 1 mbar, filled with 80% H₂/ 20% CO₂ gas to 100 mbar, and evacuated again to 1 mbar. This cycle was repeated three times to remove the atmosphere. At the third cycle, gas was added to the chamber while under vacuum for a total of three minutes in order to ensure removal of the atmosphere.

Experiment 1: The pressure of the chamber after the cycles was 67 mbar. The chamber was maintained between 67 mbar and 73 mbar at 30°C for 13 days. Two days after the tubes were placed in the chamber, the tubes were punctured with a specialized device

containing 22-gauge syringe needles. On the 13th day of the experiment, the chamber was filled to atmospheric pressure with CO₂ gas. On the 16th day of the experiment the tubes were unpunctured.

Experiment 2: The pressure of the chamber after the cycles was 33 mbar. The chamber was maintained between 33 mbar and 40 mbar at 30°C for 8 days. Two days after the tubes were placed in the chamber, the tubes were punctured with a specialized device containing 22-gauge syringe needles. The chamber was filled to atmospheric pressure with CO₂ gas on the 8th day of the experiment. On the 9th day of the experiment the tubes were unpunctured.

Following removal, additional 2.5% sodium sulfide solution was added to each test tube and the tubes were pressurized with 200 kPa of H₂ gas. A second set of sterile methanogen growth media was prepared as above (5 test tubes for each of the 4 types of media). Each of these 20 tubes was inoculated with 0.5 mL of methanogen media from one of the original tubes (e.g. 0.5 mL from original tube #1 was used to inoculate transfer tube #1). Both the original and transfer sets were kept at the organisms' respective incubation temperatures, and were monitored over time for methane production via gas chromatography.

Results: **Experiment 1:** The tubes were exposed to pressures between 67 and 73 mbar for 11 days. During the experiment, the humidity in the Pegasus Planetary Simulation Chamber rose from 0% to 46%.

Experiment 2: The tubes were exposed to pressures between 33 and 40 mbar for 6 days. The humidity in the chamber rose from 0% to 90% during the experiment. Significant evaporation was also witnessed in each of the exposed test tubes (Fig. 3).

For each of the four methanogen strains, living cells were successfully transferred to new media following the exposure to low pressure. In the transfer sets for both experiments, each of five replicates for each strain of methanogen showed methane production at least 37 days after transfer (Figs. 1, 2).

Discussion: The methane production for each of the four methanogen strains was generally similar between the original and transfer tubes in both Experiment 1 and 2 (Figs. 1, 2). In both experiments, the transfer tubes produced greater amounts of methane than the original tubes, except for *M. wolfeii* in Experiment 1 and *M. barkeri* in Experiment 2. While the original tubes contained a greater abundance of organ-

isms, the nutrients required for organism growth were diminished in these tubes, compared to the transfer tubes, which may contribute to the greater methane production seen in the transfer tubes.

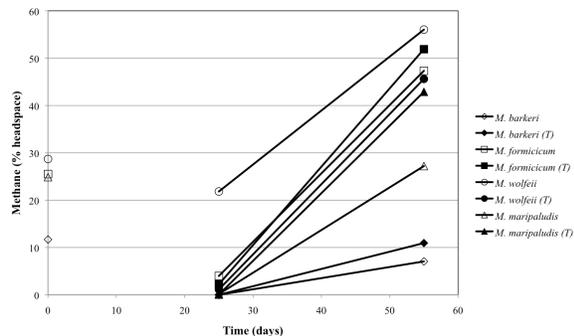


Figure 1. Experiment 1, original and transfer sets. Average methane produced (percent headspace) for each of four methanogen strains (*M. barkeri*, *M. formicicum*, *M. wolfeii*, *M. maripaludis*). Day 0 refers to the day the original tubes were placed in the Pegasus Planetary Simulation Chamber. Original tubes were exposed to 67 mbar for 11 days between Days 2 and 13. The transfer set was inoculated on Day 18. Open symbols indicate the original set; closed symbols indicate the transfer set. Error bars are omitted for clarity.

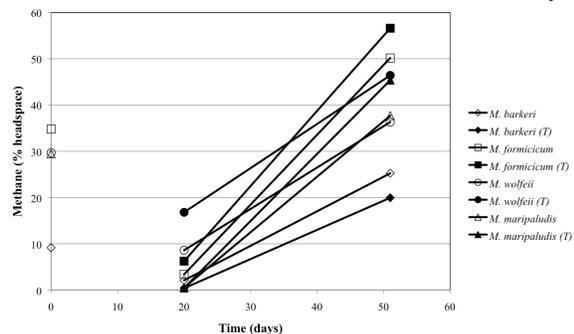


Figure 2. Experiment 2, original and transfer sets. Average methane produced (percent headspace) for each of four methanogen strains (*M. barkeri*, *M. formicicum*, *M. wolfeii*, *M. maripaludis*). Day 0 refers to the day the original tubes were placed in the Pegasus Planetary Simulation Chamber. Original tubes were exposed to 33 mbar for 6 days between Days 2 and 8. The transfer set was inoculated on Day 11. Open symbols indicate the original set; closed symbols indicate the transfer set. Error bars are omitted for clarity.

The methane production for each of the four methanogen strains is also similar between Experiment 1 and 2. The initial methane readings (taken on Day 0) for the Experiment 2 tubes were slightly higher than those for Experiment 1. These higher initial readings may account for the higher methane amounts during

the second reading in Experiment 2. However, *M. wolfeii* does not follow this trend. Additionally, the initial methane reading for *M. barkeri* was higher in Experiment 1 than Experiment 2.

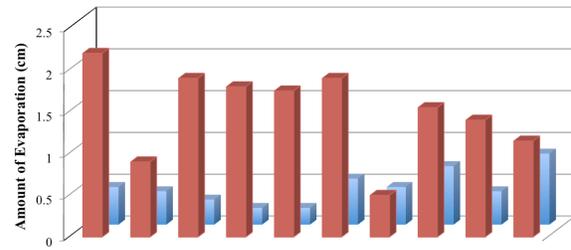


Figure 3. Total evaporation (in centimeters) for each of 20 test tubes exposed for 6 days to 33 mbar.

Previous experiments have demonstrated the varying rates of evaporation witnessed within the Pegasus Planetary Simulation Chamber [5, 6]. No noticeable evaporation occurred during Experiment 1 at 67 mbar. Varying rates of evaporation were witnessed during Experiment 2 at 33 mbar (Fig. 3). The discrepancy in evaporation between the front row and back row of test tubes may be due to the air flow through the chamber as a result of the fan within the palladium catalyst apparatus. The limiting factor in the length of experiments is the how quickly the liquid medium evaporates. Experiments currently in progress are testing diffusion barriers such as cotton and different regoliths, including JSC Mars-1.

Conclusions: In both experiments, each of the five replicates for each of the four methanogen strains retained living cells following exposure to low pressure at both 67 mbar (Experiment 1) and 33 mbar (Experiment 2). Generally, the exposure to low pressure did not hinder the growth (in terms of methane production) of any of the four methanogen strains.

Future experiments will be conducted at lower pressures more characteristic of the Martian surface (6 – 10 mbar). These experiments will also continue to test diffusion barriers to slow evaporation, as well as analyze air flow in the chamber.

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References: [1] Schuerger, A. W., et al. (2013) *Astrobiology*, 13, 115-131. [2] Nicholson, W. L. et al. (2013) *PNAS*, 110, 666-671. [3] Kendrick, M.G. and Kral, T. A. (2006) *Astrobiology*, 6, 546-551. [4] Kral T. A., et al. (2011) *Planetary and Space Science*, 59, 264-270. [5] Mickol, R. L., et al. (2013) LPSC XLIV, abs. #1782. [6] Gonzalez-Medina, J. M., et al. (2013) LPSC XLIV, abs. #1353.