Simulating Martian Conditions: Methanogen Survivability During Freeze-Thaw Cycles. S. Djordjevic[1,2], R.L. Mickol[1], T.A. Kral[3], [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, Arkansas, rmickol@email.uark.edu [2]University of Illinois at Urbana-Champaign, Champaign, Illinois, djordje2@illinois.edu, [3]Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, tkral@uark.edu

Introduction: Methanogens are obligate anaerobes that use molecular hydrogen as an energy source and carbon dioxide as a carbon source to produce methane. They are classified as Archaea and are found in many extreme environments, including hydrothermal vents, volcanoes, and even the human microflora. Thus, it is proposed that these Archaea are able to survive and grow in martian conditions. The current martian atmosphere is low in pressure, very dry (hyper-arid), and high in radiation, and thus the surface is not suitable for life. However, the subsurface contains permafrost, liquid water [1][2][3][4][5], and trace amounts of methane [6][7]. According to data obtained from NASA's Mars Science Laboratory, between August 2012 and late February 2013, the maximum and minimum temperatures on Mars have ranged from +10°C to -90°C. These conditions might be suitable for methanogenic growth. The goals of this experiment are to measure methanogen growth by gas chromatography and analyze temperature constraints on survivability and growth.

Methods: Methanogen growth media were prepared according to Kendrick and Kral [8]. The strains used included Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum, and Methanococcus maripaludis. The media were inoculated with 0.5 mL of each respective methanogen and grown in their optimal temperature at ambient pressure: M. wolfeii at 55°C, M. barkeri/M. formicicum at 37°C, and M. maripaludis at room temperature (22°C).

The freeze-thaw experiment tested the survivability and growth at 4°C, -20°C, and -80°C, reminiscent of the frigid climate found on Mars (Fig. 1), using various freezers found in the laboratory for 7 days at each time point.

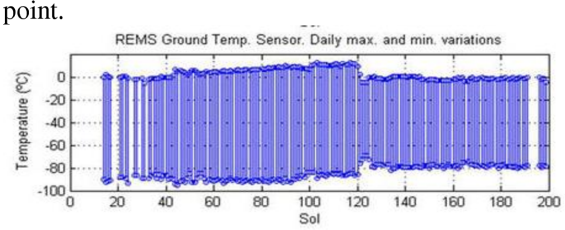

Figure 1. Ground temperature data from the Rover Environmental Monitoring System [9].

Results: Methane concentrations over 5% were identified as viable (Fig. 1). M. barkeri/M. maripaludis were not viable during experiment 2 (Fig. 2). Media were removed if the oxygen indicator, resazurin, turned pink, which was indicative of high molecular oxygen levels in the test tubes.

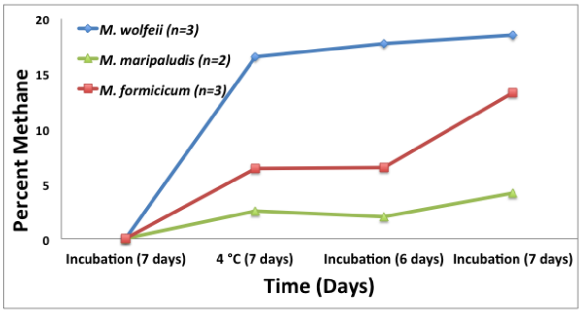

Figure 2. Percent methane concentrations in media inoculations following 7 day incubation period and 4°C freeze-thaw cycle (*M. barkeri not viable).

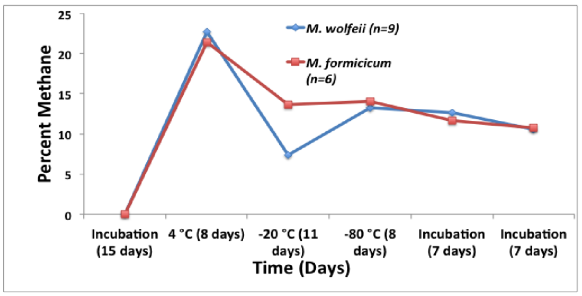

Figure 3. Percent methane concentrations in media inoculations following 7 day incubation period and various freeze-thaw cycles (*M. barkeri/M. maripaludis not viable).

Discussion: Four different species of methanogens were used to analyze the effect of low temperature freeze-thaw cycles on the growth and survivability of these Archaea.

M. wolfeii showed the greatest survival (n=12 replicates) and had the largest percent methane concentrations following the freeze-thaw experiment at 4°C and at lower temperatures of -20°C and -80°C. The 4°C experiment showed constant growth before and after the freeze-thaw cycle, whereas the varying temperature experiment showed a spike only after the -20°C cycle followed by a constant percentage after the -80°C cycle. This might be a temperature constraint for this species.

M. formicicum was the second most survivable species (n=9 replicates). The 4°C experiment showed a delayed onset of methane production and the varying temperature experiment showed a constant percentage during the -20°C cycle

followed by a decline after the -80°C cycle. The temperature range between -20°C and -80°C might be a temperature constraint for this species.

M. maripaludis was only marginally survivable (n=2 replicates) during the 4°C experiment. The percent methane concentrations were below 5%, indicating only slight survivability throughout the experiment.

M. barkeri was not viable throughout the course of the experiment and was difficult to culture at any temperature.

The decrease in methane following incubation might be due to the reduced partial pressure of methane following each measurement, meaning that no new methane was produced. The increase in methane following incubation might be due to methane being released from water or soil in addition to new methane production.

Conclusion: Some of the problems associated with this study are that it is very difficult to replicate the martian atmosphere and subsurface as we know it. What we can do is manipulate one variable at a time to get baseline measurements in terms of temperature, pressure, rock/sand composition, and different species of methanogens, among others. This experiment is preliminary data for the four species of methanogens and the survivability/growth in low-temperature freeze-thaw cycles. This study has shown that some, but not all, of the strains of methanogens used in this study can survive prolonged low-temperature conditions, in a state of suspended animation, for at least a week at a time. Further studies will continue to analyze the temperature constraints for these Archaea in an attempt to understand the life implications found in martian conditions.

References: [1] PR Christensen. (2004) Science 306,1733–1739. [2] KE Herkenhoff. (2004) Science 306, 1727–1730. [3] G Klingelhofer et al. (2004) Science 306, 1740–1745. [4] SR Rieder et al. (2004) Science 306, 1746–1749. [5] SW Squyres et al. (2006) Science 306, 1709–1714. [6] V Formisano et al. (2004) Science 306, 1758–1761. [7] VA Krasnopolsky et al. (2004) Icarus 172, 537–547. [8] Kendrick, M.G. and Kral, T.A. (2006) Astrobiology, 6, 546–551. [9] NASA Jet Propulsion Laboratory.

Acknowledgements: Special thanks to Dr. Julia Kennefick and NSF Grant #1157002.

Modulating the Pegasus Chamber: An Anoxic Approach. S. Djordjevic[1,2], R.L. Mickol[1], T.A. Kral[3], [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, Arkansas, rmickol@email.uark.edu [2]University of Illinois at Urbana-Champaign, Champaign, Illinois, djordje2@illinois.edu, [3]Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, tkral@uark.edu

Introduction: The Pegasus Chamber at the Arkansas Center for Space and Planetary Sciences at the University of Arkansas is a vacuum chamber used to simulate martian conditions. However, due to the sub-atmospheric pressure in the chamber, oxygen will inevitably leak in any way that it can. As a result, previous attempts at growing methanogens, which are obligate anaerobes, have been unsuccessful. The purpose of this experiment is to modulate the Pegasus Chamber so as to limit the amount of oxygen seepage into the chamber. Resazurin, an oxygen indicator with a high dichromatic index, will be used as a qualitative test for oxygen levels in various media.

Methods: In order to simulate the martian subsurface conditions (Fig. 1), methanogen growth media were prepared according to Kendrick and Kral (2006). The four species include Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum, and Methanococcus maripaludis. After growing the methanogens in their respective growth temperatures, they were transferred to the Pegasus Chamber for 12 days to grow between 120 and 125 Torr. After 3 days inside of the chamber, the test tubes were punctured with a 22G syringe to allow the ambient pressure inside of the chamber to acclimate with the pressure inside of the tubes.

Duct seal putty was obtained from Rainbow Technologies. The putty was placed around the opening to the chamber, and around any other modified parts with a quarter-inch thickness to inhibit oxygen entry. A palladium catalyst was also inserted during the experiment to react with any trace amounts of oxygen.

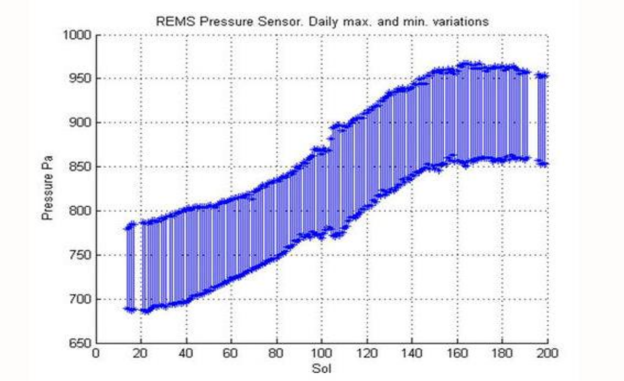

Figure 1. Pressure variation data obtained from Mars Science Laboratory between August 2012 and late February 2013 (700 Pa = 5.25 Torr) [2].

Results: M. wolfeii was difficult to culture and thus was not viable throughout the course of the experiment. Prior to adding media to the chamber, any media that turned pink were removed from the experiment, which was indicative of high molecular oxygen levels in the test tubes. The tubes that remained yellow for the duration of the experiment indicated that molecular oxygen had not entered the chamber. The pressure of the chamber was constant between 120 and 125 Torr, or 16 kPA to 16.5 kPa. The temperature of the chamber was constant between 80 and 85 °F. Lastly, after puncture, the relative humidity began a steady increase to near 45% humidity over the course of 9 days (Fig. 2).

After evacuation of the chamber, the media turned a slight pink, and therefore sodium sulfide was added to react with the molecular oxygen inside of the tubes, and the media returned to the yellow color. The media was measured for methane concentrations three consecutive times (Fig. 3).

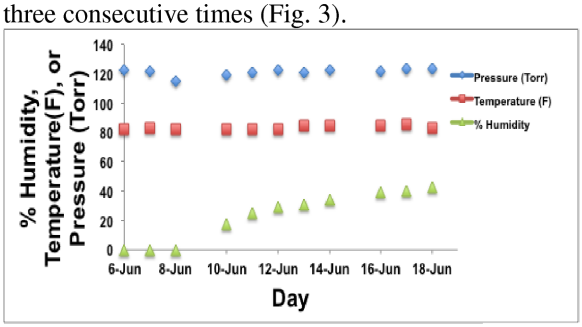

Figure 2. Pressure (Torr), Temperature (°F), and Percent Relative Humidity in the Pegasus Chamber during a 12-day cycle.

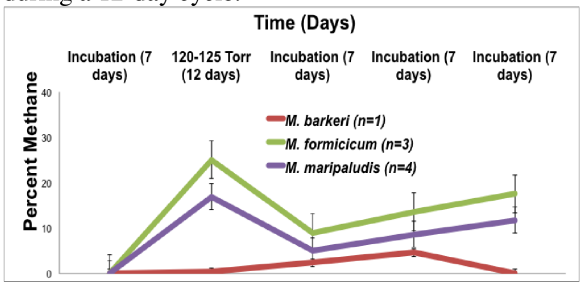

Figure 3. Percent methane concentrations in media inoculations following 7-day incubation period and 7-day low-pressure cycle. (*M. wolfeii not viable)

Discussion: M. maripaludis showed the greatest survival with n=4 replicates. M. formicicum survived with n=3 replicates. M. barkeri had only 1 replicate survive (out of 3 replicates), and M. wolfeii was not viable throughout the experiment. This was most likely due to the media preparation, however

these results might also be indicative of the media survivability in low-pressure conditions.

Three of the tubes (2 M. formicicum and 1 M. maripaludis) might not have been fully punctured the whole time (they were punctured for at least three days), suggesting that the puncture apparatus might not have been installed properly.

The steadily increased percent relative humidity might have been due to atmospheric leakage, however the more likely cause was the evaporation of the media, since the color of the tubes did not change throughout the experiment.

Although the temperature was extremely high for martian conditions, the temperature was maintained between 80-85 degrees Celsius, and was not the purpose of the experiment.

Lastly, upon evacuation of the chamber, the puncture apparatus was crooked and the media quickly turned pink after removal. This might be due to the gauge size of the syringes (22G) and the permeability of the rubber caps that were used.

Conclusion: The Pegasus Chamber puncture apparatus must be re-aligned so as to allow for an even puncture and un-puncture procedure throughout the experiment. A smaller gauge syringe might be more practical. Also, a camera inside of the chamber would allow for a better visual representation during incubation, which could record the color of the tubes during the experiment.

Continuing studies will be conducted to more accurately simulate martian conditions. This includes a lower temperature inside of the Pegasus Chamber, various regolith compositions, lower pressures (4-6 Torr), other strains of methanogens, and a way to remove any vapor that may appear (Mars is hyper-arid [3][4]). This will allow for a more pertinent, as well as accurate gauge, of low-pressure implications for life on Mars.

References: [1] MG Kendrick and TA Kral (2006) Astrobiology, 6, 546–551. [2] NASA Jet Propulsion Laboratory. [3] V Formisano et al. (2004) Science 306, 1758–1761. [4] VA Krasnopolsky et al. (2004) Icarus 172, 537–547.

Acknowledgements: Special thanks to Dr. Julia Kennefick, Walter Graupner, and NSF Grant #1157002.