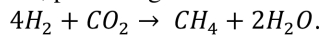


Testing Methanogen Growth at Low Pressure. J.M. González-Medina^{1,2}, R.L. Mickol², and T.A. Kral^{2,3}, ¹Dept. Chemical Engineering, University of Puerto Rico, Mayagüez, Puerto Rico, 00681, USA, ²Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, University of Arkansas, Fayetteville, Arkansas 72701, USA, ³Dept. Biological Sciences, SCEN-632, University of Arkansas, Fayetteville, Arkansas, 72701, USA.

Introduction: In 2004 methane was found in the martian atmosphere at an abundance of 10 ± 3 ppb [1]. There is no known mechanism to maintain methane in the martian atmosphere at this abundance, and further studies have also detected methane more recently [1,2]. On Earth, about 90% of methane comes from a biological source, indicating the possibility of a biological source on Mars [2]. Past studies have proposed methanogens as a life form on Mars subsurface [3,4,5,6]. Methanogens are anaerobic microorganisms from the domain Archaea that produce methane [7]. Methanogens can consume hydrogen for an energy source and carbon dioxide for a carbon source, producing methane as a waste product:



However, in order for methanogens to grow in the martian subsurface, they need to adapt to Mars pressures. The pressure on Mars' surface is around 6 mbar [8]. As you increase in depth under the surface, the pressure increases. The pressure used for this experiment was around 100 mbar, simulating Mars pressure at the near subsurface. This study aims to increase our knowledge of microorganism metabolic activities at pressures below Earth ambient (1 bar), which is scarce [9].

Methods: MSF medium was prepared following the protocol proposed by Kendrick and Kral [6]. For this research, the MSF medium was prepared at three different concentrations: normal concentration, half concentration, and quarter concentration. Each concentration group included nine anaerobic culture tubes, each with 10 mL of medium. Each tube was sealed with a butyl rubber stopper and crimped with an aluminum cap, following the method by Boone et al. [10]. All tubes were autoclaved for sterilization.

Following sterilization, sterile 2.5% sodium sulfide solution was added to each tube as described by Boone et al. [10]. Each concentration group contained four tubes with a normal amount of sodium sulfide (around 125 μ L of the 2.5% stock), two tubes with 2X sodium sulfide, and another two tubes with 4X sodium sulfide. One tube per concentration group did not have sodium sulfide. Around five drops of medium containing *Methanobacterium formicicum* were inoculated to each tube. Each tube was pressurized with H_2 to 200 kPa.

Two tubes with a normal amount of sodium sulfide per concentration group remained outside of the chamber as controls. Syringe needles were inserted into the stoppers of the rest of the tubes and the tubes

were placed into the Pegasus Martian Simulation chamber with a palladium catalyst box and a desiccant. The chamber went through five cycles to ensure a sufficient vacuum was maintained. At each cycle, the pressure of the chamber was lowered and a gas mixture of 80% H_2 /20% CO_2 was added to increase the pressure. At the last cycle, the pressure was lowered to 27 inHg (around 100 mbar). After two weeks at room temperature, all tubes were analyzed for methane production by gas chromatograph and measured for height loss due to evaporation.

Results and Discussion: The pink color in the medium indicates the presence of oxygen in the Pegasus Martian Simulation chamber. Since methanogens are anaerobic microorganisms, aerobic conditions are lethal to methanogens. This explains the lack of methane production in the experiment. In addition, the same color intensity per medium

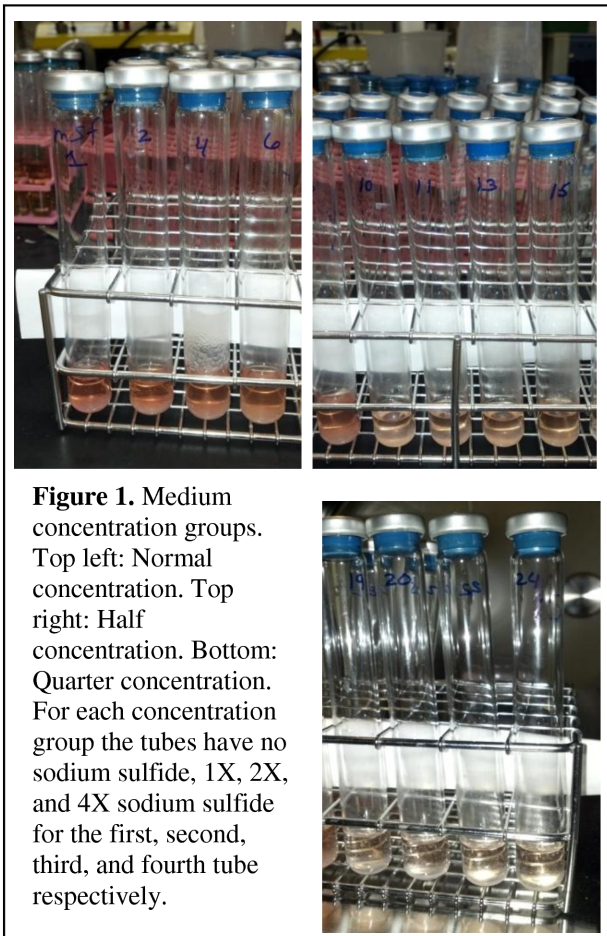


Figure 1. Medium concentration groups. Top left: Normal concentration. Top right: Half concentration. Bottom: Quarter concentration. For each concentration group the tubes have no sodium sulfide, 1X, 2X, and 4X sodium sulfide for the first, second, third, and fourth tube respectively.

concentration group suggests that there is no significant effect of increasing sodium sulfide amount (Figure 1). Moreover, altering the medium concentration did not affect the evaporation rate of the medium (Figure 2). Evaporation at low pressures is a problem that requires attention due to the complete loss of medium in the experiments over two weeks long (Figure 3).

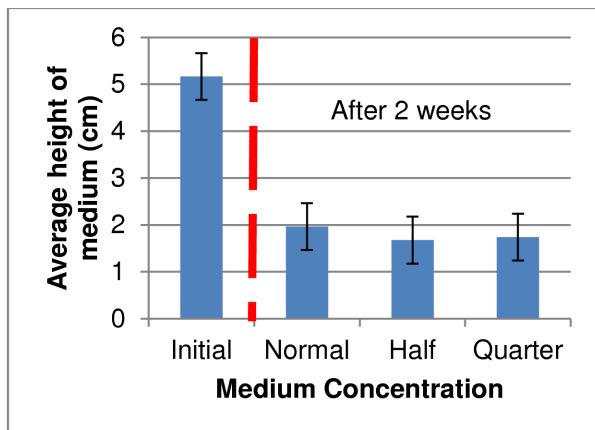


Figure 2. Decrease in medium height following two weeks at 100 mbar.

Conclusion: Atmospheric oxygen leaked into the vacuum chamber causing an aerobic environment that prevented methanogen growth. In addition, increasing sodium sulfide did not provide sufficient protection against oxygen accumulation. Varying medium concentration also did not affect evaporation of the medium. However, current work with varying amounts of agar (0.0-0.5%) shows reduced evaporation after one week. Future work will focus on oxygen reduction in the Pegasus Martian Simulation chamber to ensure the best anaerobic environment for the methanogens.

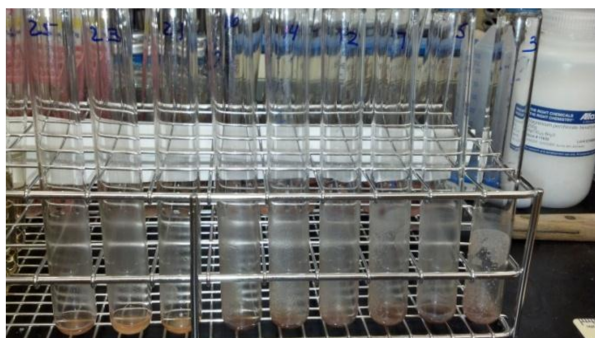


Figure 3. Evaporation of different concentration of medium. Duplicates in back row completely evaporated.

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