TESTING METHANOGEN GROWTH AT LOW PRESSURE. J. M. González-Medina[1, 2], R. L. Mickol[2], T. A. Kral[2, 3], [1]Dept. Chemical Engineering, University of Puerto Rico, Mayagüez, Puerto Rico, 00681, USA, [2]Arkansas Center for Space and Planetary Sciences, 202 Old Museum Building, University of Arkansas, Fayetteville, Arkansas 72701, USA, [3]Dept. 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. On Earth, about 90% of methane comes from a biological source; therefore, it has been proposed that there could be a biological source of methane on Mars [2]. In past studies, methanogens have been proposed as a life form on Mars [3, 4, 5, 6]. Methanogens are anaerobic microorganisms from the domain Archaea that produce methane [7].

Methods: Methanogen growth medium was prepared following the protocol proposed by Kendrick and Kral [6]. The medium was prepared at three different concentrations: normal concentration, half concentration, and quarter concentration. Around 10 mL of medium were distributed into each of nine anaerobic culture tubes per each concentration group. Each tube was sealed with a butyl rubber stopper and crimped with an aluminum cap, following the method of Boone et al. [8]. All tubes were autoclaved for sterilization.

Sterile 2.5% sodium sulfide was added to each tube as described by Boone et al. [8]. Within each concentration group (normal, half, quarter), two tubes contained around 250 µL (1X) of 2.5% sodium sulfide, two tubes contained 2X sodium sulfide, and two tubes contained 4X sodium sulfide. One tube per concentration group did not have any sodium sulfide. Two tubes with 250 µL of sodium sulfide per concentration group remained outside of the chamber as controls. Around five drops of medium containing *Methanobacterium formicicum* was inoculated into each tube. Each tube was pressurized with H_2 to 200 kPa.

Syringe needles were inserted into the stoppers of the experimental tubes, and these tubes were placed into the Pegasus Planetary Simulation Chamber [9] with a palladium catalyst box and a desiccant. The chamber went through five cycles in which it was evacuated to around 200 mbar, and then filled with a gas mixture of 80% H_2 / 20% CO_2 to 600 mbar, in order to remove atmospheric oxygen. During the last cycle, the pressure of the chamber was 130 mbar. The chamber was maintained at room temperature between 100 mbar and 200 mbar for two weeks. After two weeks, all tubes were analyzed for methane production using gas chromatography.

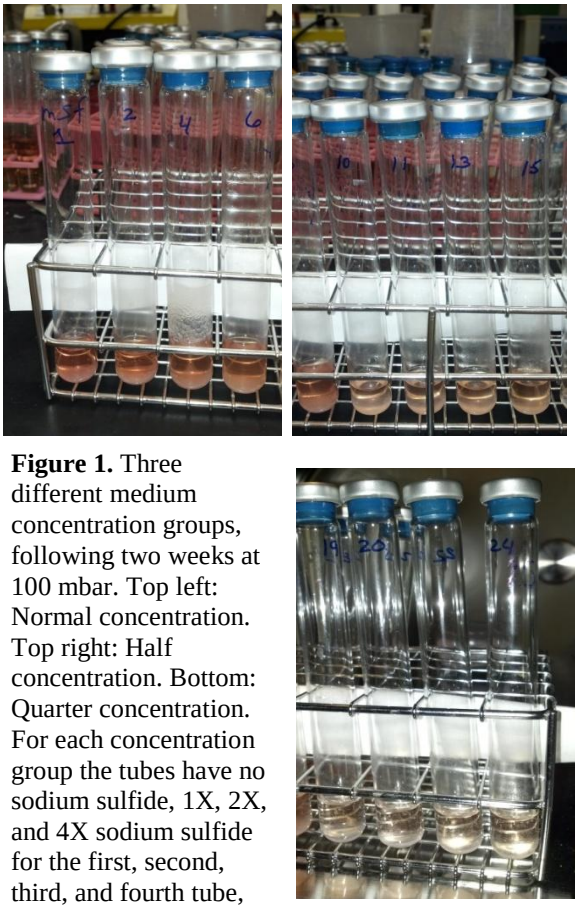

Figure 1. Three different medium concentration groups, following two weeks at 100 mbar. Top left: Normal concentration. Top right: Half concentration. Bottom: Quarter concentration. For each concentration group the tubes have no sodium sulfide, 1X, 2X, and 4X sodium sulfide for the first, second, third, and fourth tube, respectively.

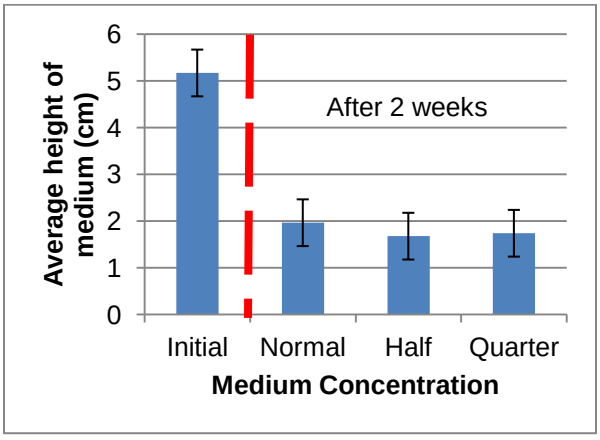

Figure 2. Height of methanogen growth medium at different concentrations, following two weeks at room temperature and 130 mbar.

Results and Discussion: The pink color in the medium indicates the presence of oxygen within the Pegasus Planetary Simulation Chamber. Since methanogens are anaerobic microorganisms, aerobic conditions are lethal, which explains the lack of methane production. In addition, the same color intensity per medium concentration group suggests that increasing the amount of sodium sulfide in the medium had no significant effect on the reduction of oxygen (Figure 1). Moreover, medium concentration did not affect evaporation rate (Figure 2).

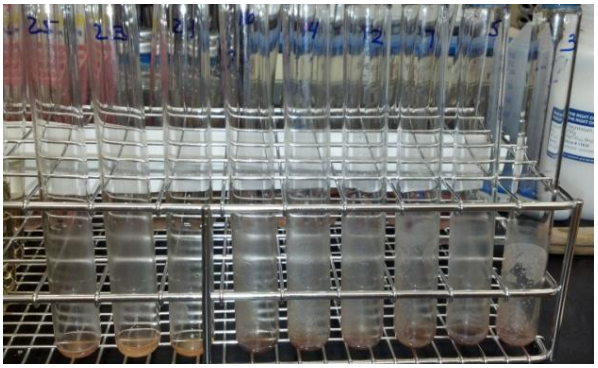

Figure 3. Evaporation of different concentrations of medium. Duplicates in back row are completely evaporated.

Conclusion: Atmospheric oxygen within the vacuum chamber created an aerobic environment that prevented methanogen growth. In addition, increasing the amount of sodium sulfide in the medium did not provide sufficient protection against oxygen exposure. Varying the medium concentration also did not affect low-pressure evaporation of the medium.

Evaporation of medium at low pressures is a problem that requires attention (Figure 3). Further experiments with varying amounts of agar (0.0%-0.5%) show no affect of agar concentration on evaporation under low pressure (R. L. Mickol et al., LPSC XLIV, this conference).

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