Growth by *Methanobacterium formicicum* at Pressures Down to 50 mbar. R. L. Mickol[1] and T. A. Kral[1,2], [1]Arkansas Center for Space and Planetary Sciences, 332 N. Arkansas Ave, U. of Arkansas, Fayetteville, AR, 72701 [rmickol@email.uark.edu]; [2]Dept. of Biological Sciences, SCEN 601, U. of Arkansas, Fayetteville, AR, 72701.

Introduction: Few experiments consider the biocidal nature of the low pressure atmosphere of Mars [1, 2]. Schuerger et al. (2013) have proposed a potential "25 mbar limit" to growth at low pressure [1]. Four species of methanogens (*Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanothermobacter wolfeii*, *Methanococcus maripaludis*) were tested for their ability to actively grow (increase in methane production/optical density) under 50 mbar or 100 mbar.

Methods: Methanogens were initially grown in their respective anaerobic growth media [3]. Media were prepared anaerobically, and 10 mL were dispensed into each of ten anaerobic test tubes (ten tubes for each of four methanogen species). The tubes were sealed, then autoclaved. Next, each test tube was inoculated with 0.5 mL from a pre-existing culture (two tubes out of each set of ten were not inoculated so as to serve as blanks) and the tubes were pressurized with 185 kPa hydrogen (H_2) gas. The tubes were incubated at the methanogens' respective growth temperatures for 7 days. After the initial incubation period, each tube was tested for both methane production and optical density to determine initial growth. Next, the tubes were placed inside the Pegasus Planetary Simulation Chamber [3]. For each species, five tubes were punctured and exposed to low pressure, and five tubes remained sealed within the chamber to serve as controls [4]. The experimental tubes that had been punctured were allowed to equilibrate with the chamber atmosphere for 30 min. The needles were then removed. Following the designated period of time, the tubes were removed from the chamber and all tubes were tested for methane production and optical density. The tubes were then re-pressurized with 185-200 kPa H_2, placed back at the organisms' respective growth temperatures and monitored for growth over time.

Results: For all three experiments, *M. formicicum* was the only organism to demonstrate both an increase in methane and an increase in optical density during the low pressure exposure period (Figs. 1, 2 and 3). In Expts. 11 and 12, *M. barkeri* showed an increase in optical density during the low pressure exposure period, but a decrease in methane abundance. During incubation, following exposure to low pressure, cultures resumed methane production (data not shown).

Discussion/Conclusions: Low pressure may not be a biocidal factor for certain methanogen species, with growth possible under low pressure. Results show that low pressure exposure may just be inhibitory during the exposure itself, and metabolism may resume following incubation under more ideal conditions. Further work is needed to address growth/survival under more Mars-relevant pressures and temperatures.

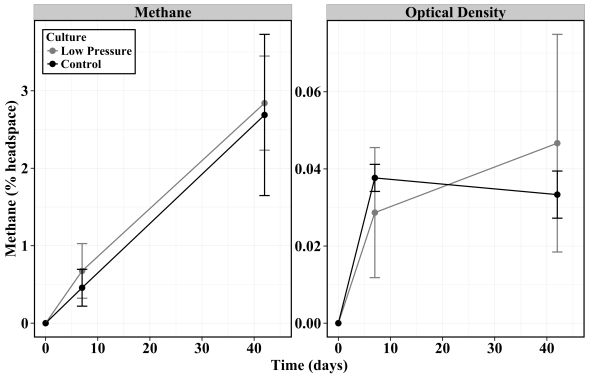

Fig. 1. Methane production (left) and optical density (right) of cultures of *M. formicicum* before and during exposure to 50 mbar (gray circles).

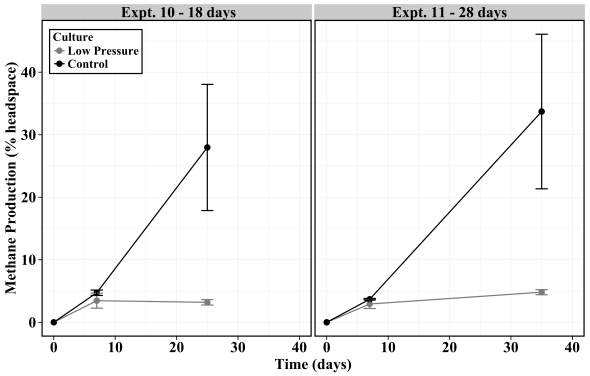

Fig. 2. Methane production by *M. formicicum* before and during exposure to 100 mbar (gray circles).

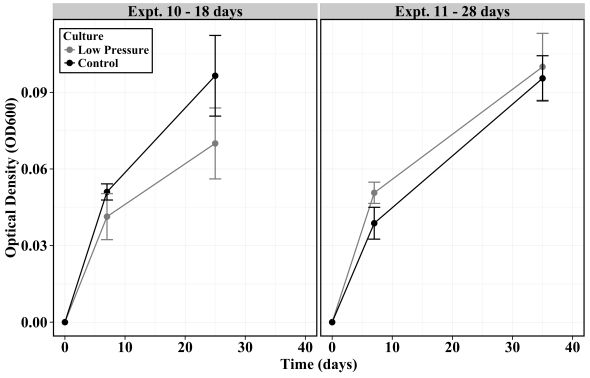

Fig. 3. Optical density measurements for cultures of *M. formicicum* before and after exposure to 100 mbar (gray circles).

References: [1] Schuerger, A. C. et al. (2013) *Astrobiology*, 13(2), 115-131. [2] Nicholson, W. L., et al. (2012) *PNAS*, 110(2), 666-671. [3] Kral, et al. (2011) PSS, 59, 264-270. [4] Mickol, R. L. and T. A. Kral (2016) *Origins Life Evo. Biosp*., 1-22.