EFFECT OF HIGH PRESSURE, HIGH TEMPERATURE, AND LOW PH ON THE GROWTH AND SURVIVABILITY OF METHANOGENIC ARCHAEA: IMPLICATIONS FOR SUBSURFACE LIFE ON MARS Navita Sinha[1], Sudip Nepal[2], T. A. Kral[3,1], Pradeep Kumar[2,1] [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, Arkansas, 72701, USA, [2]Department of Physics, University of Arkansas, Fayetteville AR 72701, [3]Dept. of Biological Sciences, SCEN 632, University of Arkansas, Fayetteville, Arkansas,72701,USA [nxs017l@uark.edu]

Introduction: The surface condition of Mars is hostile for life. However, the subterranean environments, where relatively higher pressure-temperature might exist, could provide a suitable setting for a biosphere. Also, it has been suggested that subsurface oases may exist today on Mars (1). On Earth, the hydrothermal environments, which usually sustain life have pH lower than physiological pH (2). Methanogenic archaea are chemolithoautotrphs, which mostly consume CO_2 and H_2 and produce CH_4 as their metabolic byproduct. These methanogens have been considered as model Martian life-forms for a long time (3).

In this research, we examine growth and survivability of a methanogenic archaea, *Methanothermobacter wolfeii*, at four different pressures--1atm, 400atm, 800atm, and 1200atm; four different temperatures--45°C, 55°C, 65°C, and 75°C; and four different pHs--4, 5, 6, and 7.

Methods: A quartz cuvette (Spectracell) was filled with 1ml of fresh liquid culture of *M. wolfeii* in an anaerobic chamber and was placed into a high hydrostatic pressure-temperature chamber (ISS, Illinois) for 15 hours at desired pressure and temperature. A high pressure piston was used to pressurize the fluid (water) and the pressure was measured with a pressure gauge attached to the piston (4,5). To control the temperature of the chamber, a circulating water bath was used.

Exposed cells were then transferred into the anaerobic tubes containing sterilized MM growth media, pressurized with 200kPa hydrogen gas and incubated at their optimum growth temperature, 55°C and 1 atm pressure. Methane concentration of the headspace gas of each of the samples was measured periodically by a gas chromatograph. Phase contrast images of the cells before and after exposure to high pressure-temperature were acquired using a Nikon microscope with 40x objective. To asses the growth and survivability of methanogens at optimal and non-optimal temperatures, methanogenic cells were incubated at 45°C, 55°C, 65°C, and 75°C at 1 atm and headspace gas samples were analysed. Also, the growth and survivability of *M. wolfeii*, in different pHs--4, 5, 6, and 7 at 65°C were examined.

Results and Discussion: For all the pressures, temperatures, and pHs studied here, *M. wofeii* exhibited methanogenesis (Figures1 and 2). However, the lag phase of *M. wolfeii* increases with the decreasing temperature and pH (Figure 2). The growth rate (γ) of the cells treated to high pressures does not change significantly until 400 atm, but γ increases as the pressure is increased beyond 400 atm at 55°C (Figure 1b).

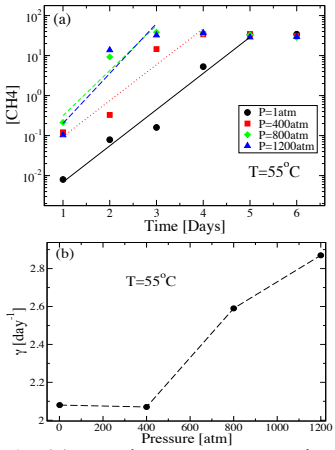

Figure 1. (a) Methane concentration of cells exposed to different pressures as a function of time at T=55°C. (b) Growth rate of exposed cells as a function of exposed pressure obtained from (a).

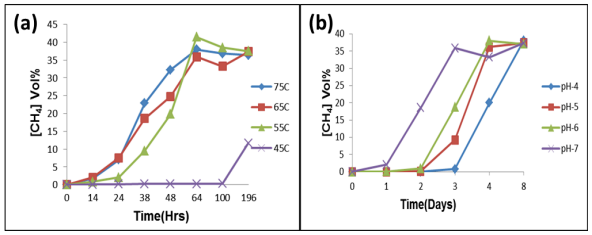

Figure 2. (a) Methane concentration as a function of time at different temperatures. (b) Methane concentration as a function of time, produced at different pHs.

Images of exposed cells were also analyzed to see morphological changes due to the effect of high pressures. We observed the increased number of elongated cells at higher pressures suggesting a lack of cell division [4].

Conclusions: In the light of recent evidence of water and methane on Mars, our results suggest that methanogens could be a plausible life form that can survive and thrive at subsurface conditions.

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