THE EFFECTS OF DIFFERENT GRANULE GEOMETRIES ON THE SURVIVAL OF METHANOGENIC ARCHAEA AT FREEZING TEMPERATURES. Yuta A. Takagi[1], Rebecca L. Mickol[2], and Dr. Timothy A. Kral[2,3], [1]Oberlin College Dept. of Biology (ytakagi@oberlin.edu), Oberlin, OH 44074, USA, [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR, 72701, USA, [3]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR, 72701, USA.

Introduction: Methanogenic archaea are strict anaerobes that can use molecular hydrogen and carbon dioxide as their sole energy and carbon sources, respectively, making them good analogues for potential life on Mars [1]. Previous studies have examined the growth of methanogens under various parameters at Mars-like conditions including desiccation, low pressure, starvation, freezing temperatures, and exposure to Mars regolith analogues [2, 3]. Temperatures on Mars diurnally fluctuate between -80 ºC and 20 ºC [4]. It has been shown that regolith and dust could play an important role in drastically modifying the immediate environmental parameters of the martian surface at micrometer scales [5]. In order to examine whether regolith grain size and geometry had any mitigating effects on freezing temperatures, we examined methanogen growth and survival at martian temperatures, in the presence of different types of glass granules.

Methods: Four species of methanogen were used: *Methanothermobacter wolfeii* (55ºC, MM growth medium), *Methanosarcina barkeri* (37ºC, MS growth medium), *Methanobacterium formicicum* (37ºC, MSF growth medium), *Methanococcus maripaludis* (25ºC, MSH growth medium)**.** Cultures were grown in anaerobic test tubes in 10 mL of media under an atmosphere (headspace) of H_2. The headspace was initially pressurized to 180 kPa. Tubes were inoculated with 0.5 mL of media from a stock culture. Gas chromatography (GC) was used to determine the methane concentration of the headspace which was used as a proxy for metabolism/growth. Three tubes each of each species were prepared with media including no glass, glass wool, glass beads (2 mm diameter), and crushed glass shards/dust. Cultures were grown at incubation temperatures for 6 to 14 days, then subjected to -15 ºC for 1 day. Then they were returned to incubation temperatures. GC measurements were taken regularly before and after the freeze event.

Results: The inclusion of glass reduced growth rates for *M. wolfeii* and *M. barkeri*, and had no discernible effect on survivability in freezing temperatures. Glass beads aided in the growth of *M. formicicum* and both beads and crushed glass aided in the growth of *M. maripaludis.* These glass types also increased the recovery rate after freezing, and may have aided in survival of freezing conditions.

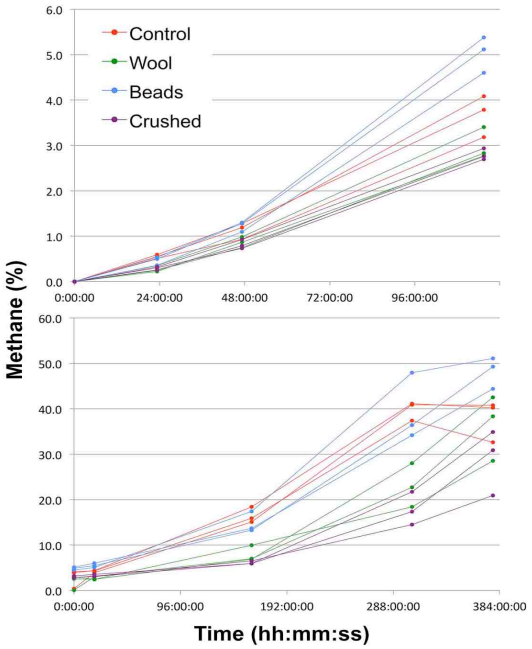

Figure 1. Methane production by *M. formicicum* in media containing glass following exposure to freezing conditions. Three replicates per glass-type per species. Top: data before freeze-event. Bottom data after freeze event.

Discussion/Conclusions: Exposure to different glass particles has an effect on growth rate and possibly survivability of freezing. However, the results are unclear, organisms may have entered a state of metabolic maintenance (but not active growth) following exposure to freezing temperatures, obfuscating the validity of our exponential growth model. We can still conclude that regolith geometries may change environmental parameters and extend the viable temperature range for life.

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