Nontronite and Montmorillonite as Nutrient Sources for Life on Mars. P. I. Craig[1], R. L. Mickol[2], P. D. Archer[3], T. A. Kral[2,4], [1]Lunar and Planetary Institute, 3600 Bay Area Blvd, Houston TX 77058 (craig@lpi.usra.edu), [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR 72701, [3]Jacobs at NASA Johnson Space Center, Houston, TX 77058, [4]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR, 72701.

Introduction: Clay minerals have been identified on Mars' oldest (Noachian) terrain and their presence suggests long-term water-rock interactions. The most commonly identified clay minerals on Mars to date are nontronite (Fe-smectite) and montmorillonite (Al-smectite) [1], both of which contain variable amounts of water both adsorbed on their surface and within their structural layers. Over Mars' history, these clay mineral-water assemblages may have served as nutrient sources for microbial life.

Methods: Two methanogen species, *Methanobacterium formicicum* and *Methanosarcina barkeri*, were tested for their ability to grow in the presence of nontronite, montmorillonite, or a mixture of both, without the use of additional nutrients. In the first set of experiments, two grams of each mineral were added to each of five test tubes. In the second set, test tubes consisted of a mixture of one gram of each mineral. The tubes were placed into a Coy Anaerobic Chamber to deoxygenate overnight. Ten milliliters of bicarbonate buffer were added to each tube. The tubes were sealed with crimps, then sterilized via autoclave. Before being inoculated into the sterilized clay solutions, methanogens were aerobically washed [2]. Next, 0.5 mL cells+buffer were added to each tube. The tubes were pressurized with 170 kPa H_2, incubated at 37°C, and monitored over time for methane production. Each set also included negative controls (clay mineral only, no microbes) that underwent identical experimental conditions as those tubes with microbes.

After 70 days, the minerals were removed from the tubes and analyzed for the presence of possible biosignatures in the form of mineralogical changes using X-ray diffraction (XRD), chemical changes using scanning electron microscopy with energy dispersive spectroscopy (SEM/EDS), and volatile and organic content with evolved gas analysis (EGA).

Results: *M. barkeri* failed to produce significant methane in any of the 2 g nontronite or 2 g montmorillonite sets but did show slight growth after ~30 days in the mixture set. *M. formicicum* produced the most methane on montmorillonite, but was unsuccessful with nontronite (Fig. 1).

No mineralogical changes were observed via XRD in any of the reacted clay minerals [3]. However, SEM/EDS analysis showed micro-scale textural changes in montmorillonite with *M. formicicum* and elemental depletions in the new textures. Figure 2 shows a sample of montmorillonite after being altered by *M. formicicum*. The bright, "fluffy" texture is chemically similar to the montmorillonite from the negative control group. However, there is also a darker, smoother texture that is depleted in Fe, Na, Mg, and Al relative to the control.

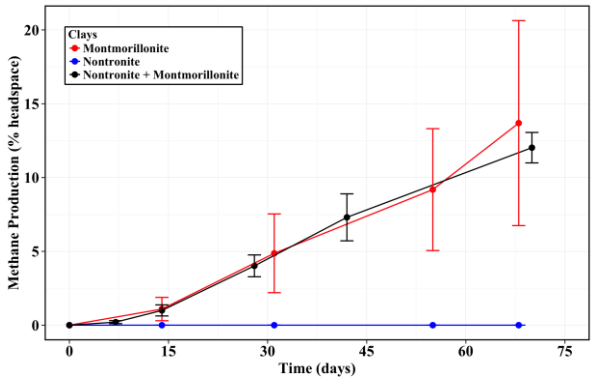

Figure 1. Methane production by *M. formicicum* in media containing solely bicarbonate buffer and clay mineral.

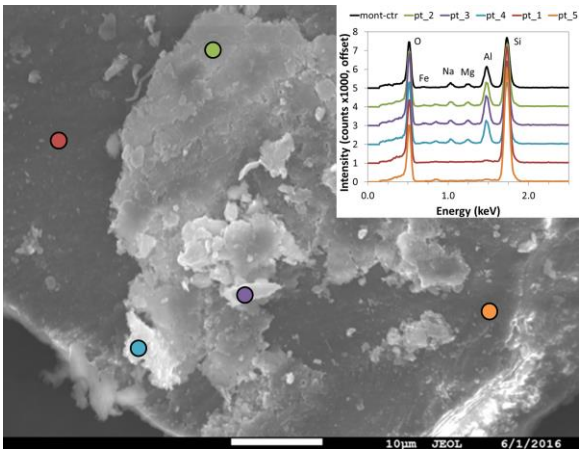

Figure 2. SEM/EDS analysis showing new texture and elemental depletion of montmorillonite with *M. formicicum*.

Montmorillonite altered by *M. formicicum* showed another textural difference from the control sample of montmorillonite. This texture is depleted in Fe and Mg but appears enriched in Na relative to the control montmorillonite sample. This could be a relative enhancement of Na due to a depletion of other elements.

Samples of *M. formicicum* in montmorillonite and mixed montmorillonite/nontronite that showed the highest production of methane were subjected to EGA on instruments that have been configured to reproduce the instrument operating conditions of the Sample Analysis at Mars (SAM) instrument on the Mars Science Laboratory (MSL) *Curiosity* rover [4]. Samples were heated to ~1000°C at 35°C/minute in a 30 mbar atmosphere using He as a carrier gas at a flow rate of ~0.8 sccm.

Samples incubated with microbes show clear differences from control samples, particularly for m/z (mass/charge) 44, which is likely CO_2 evolution (Fig. 3). Additional work is needed to determine if this increased CO_2 released around 150°C is the result of the decomposition of the microbial biomass itself or due to biologically-mediated changes to the original clay minerals. Other masses, such as m/z 18 (H_2O), also show differences between the control samples and samples with microbes.

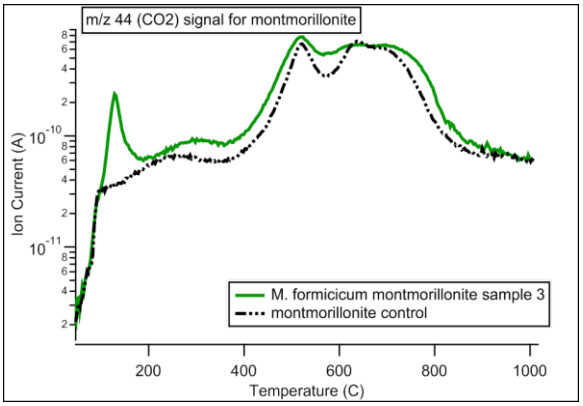

Figure 3: EGA curves for the control montmorillonite and montmorillonite with *M. formicicum.*

Discussion: Our experiments were specifically designed so that the methanogens would be deprived of the supplements they use to thrive and force them to utilize nutrients from the clay mineral substrate. Of the two species and three substrates tested, *M. formicicum* fared the best on montmorillonite. Although no mineralogical changes were detected via XRD, SEM analysis showed two new textures different from the control montmorillonite sample. Not only were new textures created as a result of biological alteration, these new textures were of a different chemical composition from the control montmorillonite. We view this as proof that the methanogens were forced to draw their nutrients from the clay minerals substrate. This suggests that this sort of nutrient utilization could have taken place, or could still be taking place, on Mars.

Implications for Mars: An Al-phyllosilicate akin to montmorillonite is among the most commonly identified clay minerals in the martian Noachian terrain. The presence of clay minerals suggests eras of long-standing, near-neutral, liquid water on the surface. These conditions create a setting for the development and sustainability of microbial life.

If methanogens did once occupy the surface or subsurface of Mars, they would have relied on their surroundings for nutrients. We have shown that at least one species of methanogen can indeed survive using only montmorillonite as a source of nutrients.

If methanogens do occupy the current martian subsurface (it has been theorized that the radiation environment would not support methanogens on the surface), the microbes could still be utilizing nutrients from clay mineral substrates. The results of this utilization is the production of methane, which could be the source of recently observed methane plumes on Mars. Short-lived increases of atmospheric methane have been detected both from orbiters over Nili Fossae [5] and in Gale Crater by the SAM instrument on the MSL *Curiosity* rover [6].

Conclusions: We have shown that methanogens can utilize nutrients from montmorillonite without supplemental media. Not only can methanogens utilize montmorillonite for their nutrients, we have identified potential biosignatures in the form of textural and chemical changes in the minerals. Although mineralogical changes were not identified, given more time or increasing the ratio of methanogens to minerals could increase the amount of altered clay minerals above the detection limits of XRD. We will compare our laboratory data to observations of Mars in order to identify potential biosignatures on Mars.

Future Work: The promising results of our experiments have shown that methanogens can utilize clay minerals as nutrient source without supplemental media. However, there is much more to explore regarding the potential for methanogenesis on Mars. In future experiments, we will alter the methanogen/clay mineral ratio and run the experiments longer (e.g. up to 150 days). We hypothesize that this would allow the methanogens to alter more of the clay mineral in the tubes making the altered minerals detectable via XRD. Additionally, we will continue experiments with other methanogen species and other Mars-relevant clay minerals.

References: [1] Carter, J., et al., (2015) *Icarus* 248, 373-382. [2] McAllister, S.A., and Kral, T.A., (2006) *Astrobio* 6, 819-823. [3] Mickol et al., (2016) *Biosig. Workshop*, #2035. [4] Archer, P.D. Jr, et al., (2014) *JGR* 119, 237-254. [5] Mumma, M., et al., (2009) *Science* 323, 1041-1045. [6] Webster, C., et al., (2015) *Science* 347, 415-417.