METHANOGENESIS AND STABLE CARBON ISOTOPE FRACTIONATION BY METHANOGENS GROWING ON DIFFERENT MARS REGOLITH ANALOGS Navita Sinha[1], T. A. Kral[1,2] [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, Arkansas, 72701, USA, [nxs017l@uark.edu], [2]Dept. of Biological Sciences, SCEN 632, University of Arkansas, Fayetteville, Arkansas, 72701, USA.

Introduction: Methanogenic archaea are anaerobic chemoautotrophs, which mostly consume CO_2 and H_2, and produce methane, and they have been considered models for possible life forms on Mars for a long time. Isotopic fractionation is one of the methods to differentiate between biogenic and abiogenic sources of methane in the martian atmosphere (1).

Here, we characterize the carbon isotope fractionation pattern of methane produced by three different strains of methanogens, *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, and *Methanobacterium formicicum*, growing on four different Mars regolith analogues, namely JSC Mars-1 (2), JSC Mars-2 (a mixture of 45% smectite, 45% basalt, and 10% hematite; 3), montmorillonite, and Mojave Mars Simulant (MMS; 4).

Methods: A total of thirty-six 150 mL serum bottles containing 3g of the regolith analog and 60 mL of bicarbonate buffer for three different strains of methanogens and four different Mars simulants were used. For positive controls, three bottles of each containing 60 mL of MM, MS, and MSF media for *M. wolfeii*, *M. barkeri*, and *M. formicicum* were also prepared. The centrifuged and washed methanogenic cells were inoculated into their respective bottles. All bottles were pressurized with 200 kPa of H_2. *M. wolfeii* was incubated at its optimum growth temperature, 55^0C, while *M. barkeri*, and *M. formicicum* were incubated at 37^0C.

Headspace gas was analyzed periodically for methane concentration and stable carbon isotopic fractionation using a Varian CP-4900 Micro-GC, and a Cavity Ringdown Spectrometer G2201-1 isotopic CO_2/CH_4 (University of Arkansas Stable Isotope Laboratory) respectively.

Results: The carbon isotope fractionation, $\delta^{13}C$, was calculated using the following equation:

$$\boldsymbol{\delta}^{13}\mathbf{C}_{Sample} = \left\{ \frac{\left(\frac{^{13}\mathbf{C}}{^{12}\mathbf{C}}\right)_{Sample}}{\left(\frac{^{13}\mathbf{C}}{^{12}\mathbf{C}}\right)_{Reference}} \mathbf{-1} \right\} \mathbf{*1000}$$

The reference isotopic standard for $\delta^{13}C$ is PDB (Pee Dee Belemnite). The δ is measured in terms of parts per thousand, or "per mil," and is expressed as ‰.

M. wolfeii, *M. barkeri*, and *M. formicicum* demonstrated substantial headspace methane concentration on JSC Mars-1 and montmorillonite, as well as in their respective media control, but relatively less methane in the headspace gas samples when they were grown on JSC Mars-2 and MMS (Figs. 1A, 1C, and 1E). All methanogens except *M. formicicum* (Fig. 1F) demonstrated relatively depleted ^{13}C when they were cultured on JSC Mars-1, JSC Mars-2, montmorillonite, and MMS, compared to their respective growth media controls (Figs. 1B and 1D).

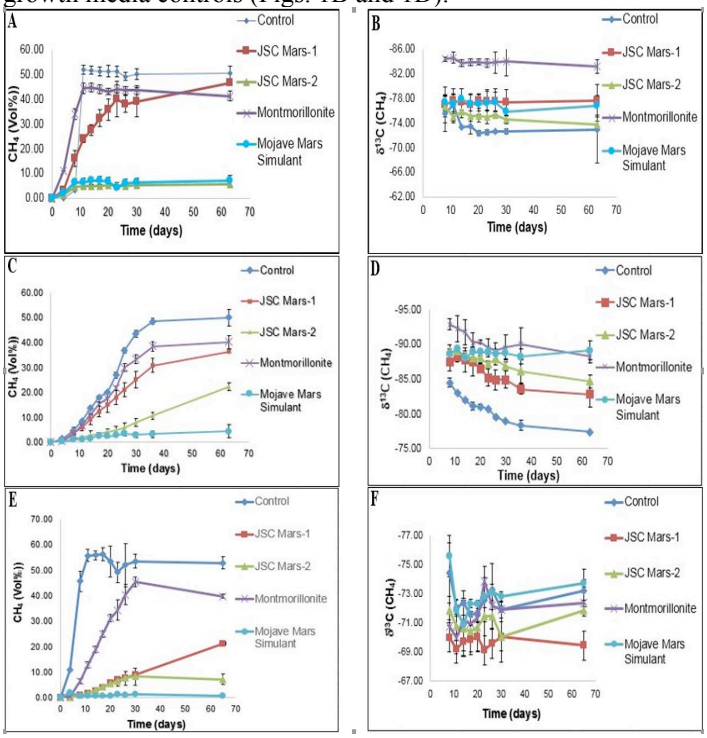

Figure 1: Methane concentration and carbon isotope fractionation values of methane produced during methanogenesis (A) and (B) for *M. wolfeii*, (C) and (D) for *M. barkeri,* and (E) and (F) for *M. formicicum.*

Discussion and Conclusions: The various factors responsible for the extent of fractionation of carbon depend on the species of methanogen and their growth rate, substrate concentration, isotope composition and availability of substrates, environmental factors, isotopic effects of enzymes involved in biosynthetic pathways, and use of different metabolic pathways by methanogens (5-7).

Overall, the characterization of carbon isotope fractionation content during methanogenesis on different kinds of Mars analogs represents a step forward toward understanding the ambiguous sources of methane on Mars.

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