

Stable Carbon Isotope Fractionation by Methanogens

Navita Sinha¹ and Timothy A. Kral^{1,2}

¹Arkansas Center for Space and Planetary Sciences, University of Arkansas

²Department of Biological Sciences, University of Arkansas

nx017@uark.edu



Introduction:

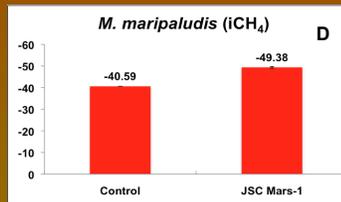
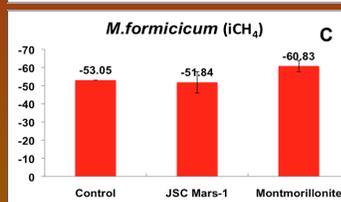
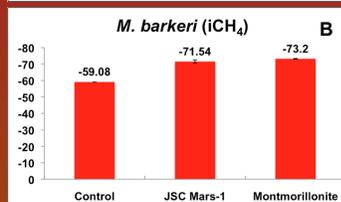
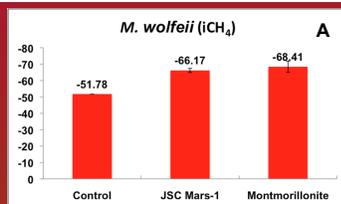
Methanogenic archaea, which consume CO₂ and H₂, have been considered models for possible life forms on Mars for a long time, even before the discovery of methane in the Martian atmosphere (1-8). Several intensive experimental studies have been conducted to investigate survivability of methanogens under conditions that approach those found on Mars (2, 3, 9). Various potential sources of methane in the martian atmosphere have been suggested such as volcanic, meteoritic, cometary, hydrogeochemical, and biogenic sources (11). Isotopic fractionation is one of the methods to differentiate between biogenic and abiogenic organic compounds (10).

Objective:

Our goal was to investigate the nature of carbon isotope fractionation of methane by four different species, *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum*, and *Methanococcus maripaludis*. All were grown on the same H₂/CO₂ substrates, but in different microenvironments such as growth-supporting media and two Mars regolith analogues, JSC Mars-1 and montmorillonite, a smectite clay.

Methods:

- Anaerobic stock cultures in growth-supporting media were prepared (12):
 - MM for *M. wolfeii*, MS for *M. barkeri*, MSF for *M. formicicum*, and MSH for *M. maripaludis*
- Subcultured every two weeks
- Three experimental sets were prepared:
 - Controls:** Four 160 mL serum bottles containing 60 mL of MM, MS, MSF or MSH
 - JSC Mars-1:** Twelve 160 mL serum bottles each containing 3g of JSC Mars-1 regolith
 - Montmorillonite:** Twelve 160 mL serum bottles, each containing 3g of montmorillonite
- There were triplicate samples for each of four different methanogens
- Bottles were left unsealed overnight in a Coy Laboratories anaerobic chamber for deoxygenation
- 30 mL and 60 mL of bicarbonate buffer were added to the anaerobic JSC Mars-1 and montmorillonite bottles, respectively, in the anaerobic chamber.
- Sealed, crimped, and autoclaved
- Centrifugation:** 10 mL of each methanogenic culture were centrifuged for 20 min. at 5000 rpm and washed two times with reduced sterile bicarbonate buffer (4)
- Resuspended in 5 mL reduced sterile bicarbonate buffer
- JSC Mars-1, montmorillonite and control bottles received 1 mL of each methanogenic cell suspension
- Pressurized with 200kPa of H₂
- Incubated at their optimum growth temperatures: *M. wolfeii* 55°C, *M. maripaludis* 25°C, *M. barkeri* and *M. formicicum* 37°C
- Measured methane concentration periodically by gas chromatograph
- To reduce the presence of any residual micronutrients, cultures were transferred into new sterile anaerobic microenvironment bottles containing their respective analogues and reduced sterile bicarbonate buffer
- 1 mL of each sample was transferred into the second transfer bottles
- Pressurized with 200kPa of H₂ and incubated
- Methane analyzed periodically by gas chromatograph
- Stable carbon isotope fractionation of methane in the headspace gas was measured by a Picarro Cavity Ringdown Spectrometer G2201-i isotopic CO₂/CH₄ at the University of Arkansas Isotope Lab



Figures A, B, C, and D: Carbon isotope fractionation of methane by *M. wolfeii*, *M. barkeri*, *M. formicicum*, and *M. maripaludis* growing on growth media MM, MS, MSF, and MSH respectively, and Mars regolith analogues JSC Mars-1 or montmorillonite

Results:

- M. maripaludis* did not grow on montmorillonite in the first transfer
- δ¹³C_{CH₄} for *M. wolfeii* in growth media was -51.78 ‰, but more negative fractionation on JSC Mars-1 and montmorillonite, of -66.17 ‰ and -68.41 ‰, respectively
- Similar trend found in *M. barkeri* and *M. maripaludis*
- δ¹³C_{CH₄} value in growth media of *M. barkeri* was -59.08 ‰, but more negative when grown on JSC Mars-1 and montmorillonite of -71.54 ‰ and -73.2 ‰, respectively. δ¹³C_{CH₄} in *M. maripaludis* showed -40.59 ‰ in media and -49.38 ‰ on montmorillonite
- M. formicicum* demonstrated little or no difference in isotopic values in media and JSC Mars-1, which are -53.05 ‰ and -51.84 ‰, respectively. But on montmorillonite, it demonstrated depleted δ¹³C_{CH₄} data of -60.83 ‰.

Discussion and Conclusion:

- The δ¹³C_{CH₄} is calculated as:

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

Where (13C/12C)_{standard} = 0.0112372 for the reference material called Pee Dee Belemnite (PDB)

- Several factors are responsible for the magnitude of fractionation such as the species of methanogens, hydrogen supply, growth phase, temperature, substrate level, and the isotopic effects of enzymes involved in biosynthetic pathways of methane production (13, 14)
- Microenvironments play an important role on the isotopic signatures of methane produced by methanogens
- All four species of methanogens showed more depleted values of δ¹³C_{CH₄} when grown on JSC Mars-1 and montmorillonite than on their respective growth media except *M. formicicum*, which showed comparable fractionation in growth media as well as on JSC Mars-1

Future work:

Future experiments will examine carbon isotope fractionation of methane by methanogens growing on other Mars regolith analogues, such as basalt and JSC Mars-2

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