Experimental Study of Methane Isotopologue Fractionation during Microbial Methanogenesis

JEEMIN H. RHIM[1*] AND SHUHEI ONO[1]

[1]Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139, USA (*correspondence: jrhim@mit.edu)

Carbon ($\delta^{13}C$) and hydrogen (δD) stable isotope ratios have been widely used to identify the source of CH_4 as well as to admeasure the relative contribution of different microbial methanogenic pathways. However, owing to the significant overlaps in the $\delta^{13}C$ and δD values of methane from different origins, a clear source identification remains challenging.

To better understand what factors control isotope fractionation during microbial methanogenesis, we performed a series of culturing experiments during which a hyperthermophilic, hydrogenotrophic methanogen species *Methanocaldococcus bathoardescens* was grown in an open (to gas) system flow reactor. Methane isotopologue ratios (among $^{12}CH_4$, $^{13}CH_4$, $^{12}CH_3D$ and $^{13}CH_3D$) were measured using tunable laser direct absorption spectroscopy, and the $^{13}C/^{12}C$ ratio of CO_2 and the D/H ratios of H_2 and H_2O were measured by conventional isotope ratio mass spectrometry.

Our results confirm the previous observation of increased $^{13}C/^{12}C$ fractionation during stationary phase, compared to exponential growth phase, approaching close to a range expected for the thermodynamic equilibrium for CO_2 and CH_4 [1]. In contrast, D/H fractionation is larger than that expected at equilibrium with respect to H_2O, and $\Delta^{13}CH_3D$ values (a metric that quantifies the deviation of $^{13}CH_3D$ abundance from the expected statistical value [2]) are negative. These observations indicate kinetic control on the fractionation of $^{12}CH_3D$ and $^{13}CH_3D$ isotopologues. In particular, negative $\Delta^{13}CH_3D$ values yield no apparent clumped isotopologue temperature.

The observed decoupling among $^{13}CH_4$, $^{12}CH_3D$ and $^{13}CH_3D$ fractionations can be used to locate the isotope sensitive and rate limiting steps in the hydrogenotrophic methanogenesis pathway. This model can be used to correlate various environmental parameters with isotopic signatures observed in nature.

[1] Botz *et al*. (1996) *Org. Geochem.* **25**, 255-262. [2] Wang *et al*. (2015) *Science* **348**, 428-431.