Experimental Study of Methane Isotopologue Fractionation During Microbial Methanogenesis

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The advent of CH_4 clumped isotopologue (e.g., $^{13}CH_3D$) analysis has enabled a more comprehensive investigation on the isotopic composition of microbial CH_4. Laboratory cultures produce CH_4 with strong kinetic signals in the $^{13}CH_3D$ abundance, whereas environmental samples carry much less kinetic signals [1,2]. Previous studies have suggested that enzyme-mediated metabolic processes and environmental factors such as pH_2 can affect isotope fractionation during microbial methanogenesis [1,3]. However, it is unclear how fractionation mechanisms in bulk isotope and clumped isotopologue systematics are correlated.

We performed a series of culturing experiments to trace the origin of kinetic isotope effects during hydrogenotrophic methanogenesis. A hyperthermophilic methanogen *Methanocaldococcus bathoardescens* was grown in a fed-batch flow reactor. The isotopologue ratios among $^{12}CH_4$, $^{13}CH_4$, $^{12}CH_3D$ and $^{13}CH_3D$ were measured by tunable laser direct absorption spectroscopy, and the bulk isotope ratios of CO_2, H_2 and H_2O were measured by isotope ratio mass spectrometry.

Our results confirm the previous observation of metabolic state-dependent patterns in $^{13}C/^{12}C$ fractionation, approaching thermodynamic equilibrium during stationary phase [4]. D/H fractionation, however, shows convergence to the same degree of disequilibrium during stationary phase, apparently independent of temperature and pH_2. $\Delta^{13}CH_3D$ values (the deviation of $^{13}CH_3D$ abundance from the expected statistical value) carry varying degrees of kinetic signals under all tested conditions, including a pH_2 range conceivable in the natural habitat of this organism [5].

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] Wang *et al.* (2015) *Science* **348,** 428-431. [2] Young *et al.* (2017) *GCA* **203,** 235-264. [3] Valentine *et al.* (2004) *GCA* **68,** 1571-1590. [4] Botz *et al.* (1996) *Org. Geochem.* **25,** 255-262. [5] Ver Eecke *et al.* (2012) *PNAS* **109,** 13674-13679.