Experimental Study of Methane Isotopologue Fractionation during Microbial Methanogenesis

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Carbon (δ13C) and hydrogen (δD) stable isotope ratios have been widely used to identify the source of CH4 as well as to admeasure the relative contribution of different microbial methanogenic pathways. However, owing to the significant overlaps in the δ13C 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 batherodescens was grown in an open (to gas) system flow reactor. Methane isotopologue ratios (among 12CH4, 13CH4, 12CH3D and 13CH3D) were measured using tunable laser direct absorption spectroscopy, and the 13C/12C ratio of CO2 and the D/H ratios of H2 and H2O were measured by conventional isotope ratio mass spectrometry.

Our results confirm the previous observation of increased 13C/12C fractionation during stationary phase, compared to exponential growth phase, approaching close to a range expected for the thermodynamic equilibrium for CO2 and CH4 [1]. In contrast, D/H fractionation is larger than that expected at equilibrium with respect to H2O, and ∆13CH3D values (a metric that quantifies the deviation of 13CH3D abundance from the expected statistical value [2]) are negative. These observations indicate kinetic control on the fractionation of 12CH3D and 13CH3D isotopologues. In particular, negative ∆13CH3D values yield no apparent clumped isotopologue temperature.

The observed decoupling among 13CH4, 12CH3D and 13CH3D 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.