Goldschmidt 2022 Abstract
https://doi.org/10.46427/gold2022.11145

Experimental investigations and a pathway thermodynamics model for methane isotopologue fractionations during microbial methanogenesis under energy-limited conditions

SHUHEI ONO[1], JEEMIN H RHIM[2] AND ERIC C RYBERG[3]

[1]Massachusetts Institute of Technology
[2]Dartmouth College
[3]Woods Hole Oceanographic Institution

Presenting Author: sono@mit.edu

Microbial methanogenesis produces a range of isotope ($^{13}C/^{12}C$ and D/H) and isotopologue ($^{13}CH_3D$ and $^{12}CH_2D_2$) fractionations. A quantitative model for methane isotopologue fractionations will help us quantify sources and sinks of methane in the environment.

We investigated isotopologue fractionations by hydrogenotrophic methanogens under energy-limited conditions using 1) electrochemical systems and 2) fed-batch reactors. We then apply our combined pathway thermodynamics-isotopologue flow network model to quantitatively describe our (and previous) observations for the isotopologue fractionations during hydrogenotrophic methanogenesis.

We used a bioelectrochemical system consisting of three-electrode potentiostat, in which cathodic methanogenesis reaction ($CO_2 + 8H^+ + 8e^-$ â†' $CH_4 + 2H_2O$, $E^{0'}$ = -244mV standard hydrogen electrode, SHE) is coupled to anodic oxidation of a redox mediator, anthraquinone-2,6-disulfonate ($E^{0'}$ = -186mV SHE). When the cathodic potential was varied from -0.7 to -0.4V SHE, $^{13}C/^{12}C$ fractionation increased from 50 to 60‰ first and decreased to 50‰, while D/H fractionations (against water) decreased by 40‰ from 280 to 240‰. The systematics was corroborated by fed-batch reactor experiments that showed that fractionation of $^{13}C/^{12}C$ increased and D/H decreased as pH_2 decreased from 10,000 to 40Pa.

Our combined pathway thermodynamics-isotopologue flow network model consists of the 10 enzymatic reactions involved in the methanogenesis pathway and tracks the mass balances of isotopologues by taking into account the reaction symmetries of singly- and doubly-deuterated isotopologues. The model estimates the reversibility of 8 reactions from predicted *in vivo* concentrations of 17 metabolites and cofactors by a linear optimization algorithm.

The model quantitatively describes our observations by the differential reversibility of enzymatic reactions. Here, methane is produced from three near-equilibrium H in methyl-coenzyme M with the addition of one kinetic D-depleted H during the last step of methanogenesis. The magnitude of C and H kinetic fractionations for the last H addition varies according to H_2 concentrations. Accordingly, δD and $\delta^{13}C$ values of methane approach equilibrium values under energy-limited conditions. Our model can predict isotopologue compositions of methane and help quantify methane cycles in the environment.