Isotopologue Fractionation During Microbial Methanogenesis in a Bioelectrochemical System

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While the stable carbon and hydrogen isotope ratios as well as the clumped isotopologue (e.g., $^{13}CH_3D$) abundances help us identify the sources of methane, the effect of environmental and physiological conditions during microbial methanogenesis on methane clumped isotopologue fractionation is not well understood. For example, microbial methane from marine environments often indicates external equilibria with substrates (e.g., CO_2 and H_2O) and internal equilibria among methane isotopologues. In contrast, microbial methane from laboratory experiments is often characterized by strong kinetic isotope signals [1,2,3]. This apparent discrepancy has been attributed to the differences in hydrogen (H_2) availability [2,4], which is closely linked to the redox environment. So far, laboratory conditions explored in fed-batch reactors or co-cultures have not yielded equilibrium fractionation in D/H ratios or clumped isotopologue abundances [4,5].

We present a new method to address this problem by culturing methanogens in a bioelectrical system (BES). In a BES, hydrogenotrophic methanogens can use H_2 produced via the electrolysis of water at low applied potentials or directly use electrons or reduced electron mediators to produce methane. Either case provides a means to precisely control the supply rate of H_2 or reducing equivalents. We demonstrate the growth of methanogens in a BES, both as pure cultures and in enrichments. A pure culture of *Methanosarcina barkeri* was grown in a BES with a poised cathodic potential at –0.6 V vs. Ag/AgCl and the presence of electron mediators (anthraquinone-2,6-disulfonate). Preliminary data show a correlation between methane production rates and current measurements. Anaerobic sediment incubations in a BES resulted in methane production. The results of scanning electron microscopy and 16S rRNA analysis suggested the enrichment of distinct microbial communities on different electrodes with a dominance of methanogens on the cathode. Finally, we will present the $^{13}CH_3D$ abundance of methane collected from these BES cultures and discuss the effect of redox environments on isotope fractionation during microbial methanogenesis.

[1] Stolper *et al*. (2014) *Science* **126,** 169–191; [2] Wang *et al*. (2015) *Science* **348,** 428–431; [3] Young *et al*. (2017) *GCA* **203,** 235–264; [4] Valentine *et al*. (2004) *GCA* **68,** 1571–1590; [5] Okumura *et al*. (2016) *Prog. Earth Planet. Sci*. **3,** 1–19.