Clumped isotopologue fractionation during anaerobic oxidation of methane

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Microbial methane from marine environments is often characterized by near-equilibrium isotope (D/H and $^{13}C/^{12}C$ among H_2O, CO_2 and CH_4) as well as isotopolgoue (among $^{12}CH_4$, $^{13}CH_4$, $^{12}CH_3D$ and $^{13}CH_3D$) fractionations [1-3]. Laboratory cultures of methanogens, however, so far yielded strong disequilibrium D/H isotope and isotopologue signals [1-4]. Thus, the mechanism of methane isotopologue equilibration remains ambiguous.

Here we investigated sediment-free enrichment cultures of anaerobic oxidation of methane (AOM) obtained from hydrothermally heated gas-rich sediments of the Guaymas Basin to examine the role of AOM in methane isotopologue equilibration. We incubated these AOM cultures dominated by consortia of ANME-1 and partner bacteria using sulfate as terminal electron acceptor at 37°C, and measured the relative abundance of four isotopologues, $^{12}CH_4$, $^{13}CH_4$, $^{12}CH_3D$ and $^{13}CH_3D$, of the remaining methane. As oxidation progressed, $\Delta^{13}CH_3D$ (a measure of excess $^{13}CH_3D$ relative to stochastic abundance) increased toward and beyond the value expected for thermodynamic equilibrium. This contrasts from the results from our previous study of aerobic methane oxidation, where $\Delta^{13}CH_3D$ value of remaining methane decreased as oxidation progressed [5].

We will present a model of microbial methane oxidation that links the reversibility of enzymatic reactions and isotopologue fractionations to explain the different isotopologue systematics duiring aerobic versus anaerobic oxidation of methane. Our observation suggests that AOM may contribute to, although is not necessarily required for, the production of near-equilibrium isotopologue signals observed in marine environments.

Reference: [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] Okumura *et al*. (2016) *Prog. Earth Planet. Sci*. **3,** 1–19; [5] Wang et al. (2017) *GCA* **192**,186-202