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Reversibility controls on extreme methane clumped isotope signatures from anaerobic oxidation of methane

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Microbial anaerobic oxidation of methane (AOM) significantly mitigates atmospheric methane emissions on Earth and represents a thermodynamically favorable metabolic strategy for astrobiological targets where methane has been detected. Some of the most extreme methane clumped isotope signatures can be attributed to AOM, but to date the mechanistic and environmental controls on those signatures have been lacking. Here we measured methane clumped isotope ratios of residual methane in AOM-active microbial incubations using sediment slurry and/or fracture fluid from Svalbard methane seeps, Santa Barbara Channel methane seeps, Nankai Trough, and Beatrix Gold Mine. We also analyzed methane isotopologue abundances in sub-seafloor fluids from a Mariana mud volcano where AOM occurs. Extremely high $\delta^{13}CH_3D$ and $\delta^{12}CH_2D_2$ values are found in the Svalbard slurry incubation and Mariana natural fluids where minimal reversibility of AOM intracellular reactions results in kinetic fractionation of clumped isotopologues. Significantly, experiments with methyl-coenzyme M reductase (Mcr) confirm the conditions under which methane isotopologues approach intramolecular quasi-equilibrium, similar to deep biosphere incubations with a high degree of reversibility. We outline future lines of investigation of isotopologue ratios as interplanetary biosignatures through characterization of environmental controls on enzymatic activity in intracellular pathways and their reversibility, or lack thereof, of AOM.