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Methane doubly-substituted isotopologue signatures ($\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$) of aerobic methane oxidation by *Methylosinus trichosporium*

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Methane produced by biogenic, thermogenic or abiotic sources can diffuse into aerobic environments where it can be consumed by microbial aerobic oxidation of methane (MOx). MOx has been detected ubiquitously in terrestrial soils, wetlands, freshwater lakes and in marine systems and is facilitated by aerobic methane-oxidizing bacteria. Recent technological advancements permit detection of rare methane doubly-substituted isotopologues that are useful tools for identifying methane sources and sinks. Here we present preliminary and ongoing research on methane doubly-substituted isotopologue signatures ($\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$) of MOx by a type (II) aerobic methane-oxidizing bacterium, *Methylosinus trichosporium*. Our study comprises two timeseries incubations where pure cultures of *M. trichosporium* were grown on agar plates, within a sterilized gas tight 3L flange flask, flushed with sterile air and supplemented with 1.2L of sterile ultra-high pure methane. The $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ values along the timeseries were obtained using the Panorama Mass Spectrometer at the University of California, Los Angeles. Preliminary results show both methane and oxygen concentrations decreasing with time, suggesting methane is being consumed aerobically. Bulk $\delta^{13}C$ and δD values for residual methane become more positive during the timeseries, with shifts of up to -30 and 21 ‰, respectively indicating a preferential uptake of isotopically lighter methane molecules by MOx. Both $\Delta^{13}CH_3D$ and $\Delta^{12}CH_2D_2$ values became more negative "anti-clumped" during the timeseries, in keeping with expectations for the shifts in bulk isotope ratios. Fractionation factors for $^{13}CH_4/CH_4$, $^{12}CH_3D/CH_4$, $^{13}CH_3D/CH_4$ and $^{12}CH_2D_2/CH_4$ obtained from the time series define the ratio of the fractionation factor to the rule of the geometric-mean (RGM)-values for $^{13}CH_3D$ and $^{12}CH_2D_2$ (sometimes referred to as γ). The γ values suggests that while $^{13}CH_3D$ adheres to the RGM, $^{12}CH_2D_2$ exhibits significant departures from the RGM. Results suggest that for closed-system oxidation, markedly low $\Delta^{12}CH_2D_2$ values could result in signatures that resemble microbial methanogenesis, while open-system flow of methane through a region of MOx will result in positive shifts in $\Delta^{12}CH_2D_2$ that would be a tell-tale signature of oxidation. These results have consequences for interpretations in systems where methane is both produced and oxidized by MOx.