# Fractionation of $^{13}CH_3D$ during microbial methanogenesis

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The majority of atmospheric methane is reported to be microbial, generated by microbial methanogenesis via the acetoclastic or hydrogenotrophic pathways. Conventional carbon and hydrogen stable isotope ratios have been widely used to apportion the relative contribution of microbial methanogenesis in the environment and to track metabolic shifts in response to climate change. However, two physiologically distinct types of methanogens and three different metabolic pathways often produce methane with a wide range of overlapping $\delta^{13}C$ and $\delta D$ values. Thus, conventional stable isotope measurements do not always unambiguously identify the methane source.

It has been suggested that the "clumped isotope" ($^{13}CH_3D$) composition of methane, can reveal the temperature at which the methane was formed. In microbial culture experiments and environmental samples, however, we found that most microbial methane corresponds to inferred growth temperatures that are significantly higher than the actual growth temperatures. To examine the main factors controlling the $^{13}CH_3D$ abundance of microbial methane, we conducted pure culture experiments of methanogens grown on $H_2/CO_2$, acetate, and methanol. The source of H in methane was also investigated by culturing methanogens with isotopically-labeled water and substrates. Our results demonstrate a general trend between thermophilic and mesophilic methanogens, and between hydrogenotrophic and acetoclastic pathways in combined bulk $\delta^{13}C$, $\delta D$, and $\Delta^{13}CH_3D$ systematics. We compare these results to methane from natural environments including swamps, gas hydrates, and the bovine rumen to gain a first order understanding of $^{13}CH_3D$ systematics in nature.