

Methane clumped isotope signatures and microbial activity: a case study from two mud volcanos in central Japan

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Analytical techniques of paired mass-18 isotopologues of methane ($^{12}\text{CH}_2\text{D}_2$ and $^{13}\text{CH}_3\text{D}$), also named as methane clumped isotope approach, have been developed and applied during the past decade to understand methane cycle¹⁻³. Yet, some unsolved questions are still existent as more and more observations have been reported. For instance, we have not fully understood the inconsistent microbial methane signatures between natural sources and laboratorial cultivation experiment, which might be controlled by distinct methanogenesis rate and/or the methane consumption processes like AOM⁴⁻⁶. Thus, applying this novel approach in natural environment requires to understand the clumped isotope signatures and their relation to in situ microbial activities.

In this study, we focused on methane collected from multiple locations of two mud volcanos in Niigata Prefecture, Japan, where methane is considered to originate mainly from the thermal-cracking of organics in deep oil/gas reservoirs. The paired methane clumped isotope signatures have suggested different degrees of microbial contributions at various locations of the same area. By combination of multiple approaches, such as bulk carbon and hydrogen isotopes of hydrocarbons, position-specific isotope analysis of propane⁷, microbial density and diversity, we discuss to which extent microbial activity alters the clumped isotope signatures of methane in these mud volcanoes.

References: [1] Stolper et al., 2014, *Geochim. Cosmochim. Acta.*; [2] Stolper et al., 2014, *Science*; [3] Young et al., 2017, *Geochim. Cosmochim. Acta.*; [4] Ash et al., 2019, *Geochem. Perspect. Lett.*; [5] Ono et al., 2021, *Geochim. Cosmochim. Acta.*; [6] Zhang et al., 2021, *Geochim. Cosmochim. Acta.*; [7] Gilbert et al., 2019, *Proc. Natl. Acad. Sci. U.S.A.*