Effects of Nickel Limitation on Carbon and Hydrogen Isotope Fractionation by Methylotrophic Methanogens

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Bioavailablity of Ni has been invoked as a key factor in methanogen productivity over geologic time. As Ni-dependent microoganisms, methanogens have developed mechanisms for efficiently scavenging and utilizing Ni from their surroundings. The isotopic composition of methane generated in the environment and pure cultures is commonly used to understand methane formation pathways. Pure culture experiments used to measure isotope fractionations generally use concentrated trace metal solutions for high growth yields, but do not mimic the low-Ni concentrations in most natural environments. We propose that the cellular responses to Ni limitation could impart distinctive isotope fractionation factors for C, H, and Ni itself, and thus stable isotopes could provide unique insights into methanogen populations.

We conducted growth experiments with pure cultures of methylotrophic methanogens to test how Ni availability affects the C and H isotope fractionation of produced CH_4. Our model organism, *Methanosarcina acetivorans* strain C2A, is a marine methanogen with minimal ability to substitute Ni with other trace metals (e.g., Fe). Aqueous Ni in the medium broth was decreased from optimal growth conditions (8μM) to examine growth under Ni-limited conditions (<100 nM) and the attendant $\delta^{13}C$ and δD of the headspace CH_4. Growth curves based on time-series measurements of both [CH_4] and optical density were employed to determine when cultures became Ni-limited. Using mass balance constraints, we report both the C and H isotope fractionation factors between the initial medium and produced CH_4 as a function of aqueous Ni concentrations. This study will improve our understanding of how Ni availability impacts the resultant stable isotope signatures produced by methanogenic archaea.