

Effects of growth conditions on nickel isotope fractionation in methanogenic and acetogenic microorganisms

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Nickel (Ni) is essential for key enzymes in microbial methanogenesis and acetogenesis. Its bioavailability may play a crucial role in regulating microbial activity and carbon cycling in energy-limited anoxic environments, such as deep seafloor sediments. Biological fractionation of Ni stable isotopes may help to assess its bioavailability in such environments [1]. However, the variability and controlling factors of Ni isotope fractionation during the growth of methanogenic and acetogenic microorganisms remain poorly understood. We examined how metabolic pathway, growth rate, and Ni concentration affect Ni isotope fractionation during the growth of the model methanogen *Methanosarcina barkeri* and the acetogen *Moorella thermoacetica*. We first cultured the methanogen with different substrates (methanol, acetate, or hydrogen and carbon dioxide) and observed similar degrees of light Ni isotope enrichment in methanogen cells relative to growth media for all tested substrates. This suggests that the Ni isotope fractionation factor is independent of the methanogenic pathway, despite differences in the Ni enzymes involved. Similar Ni isotope fractionation was observed in acetogen cells cultured with methanol. However, the fractionation factor was significantly smaller in glucose-amended acetogen cultures, suggesting a minor importance of Ni-dependent metabolism (the Wood–Ljungdahl pathway). Second, we cultured the methanogen at different temperatures to examine the effect of the growth rate. The Ni isotope fractionation factor decreased slightly with lower growth or Ni uptake rates, indicating a reduced kinetic isotope effect. Finally, changes in the Ni isotope composition of growth media and methanogen cells were examined under varying initial Ni concentrations in the media. The isotopic composition of growth media followed Rayleigh-type fractionation, resulting in a fractionation of ~2‰ relative to the initial medium at $[Ni] = 120 \text{ nM}$. The sorption of Ni onto the cell surface, as well as its intracellular uptake, likely plays a significant role in such a large isotopic fractionation. Our results offer valuable insights into Ni isotope behavior during microbial activity in growth-limited natural environments, which is potentially useful for evaluating microbial activities in natural systems using Ni isotopes.

[1]Cameron, Vance, Archer & House (2009), *PNAS* 106, 10944–10948.