Using stable isotopes to investigate microbial H_2 and N_2O production

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Stable isotopes can provide important insights into complex biological and environmental processes. In particular, hydrogen, carbon, nitrogen, and oxygen isotopes can help dissect enzymatic reaction mechanisms and can be employed as tracers to follow metabolic pathways. In particular, isotope-ratio mass spectrometry has been instrumental in our ability to improve our understanding of microbial H_2 and N_2O metabolism.

Biological hydrogen production is mainly mediated by hydrogenase enzymes, which combine protons from water with electrons to generate H_2. Because each hydrogenase discriminates against ^2H slightly differently, each will generate H_2 with a unique isotopic signature that can be used to monitor individual enzyme activity. We purified a number of hydrogenases and, as predicted, each produces H_2 with a distinct isotopic signature and the fractionation factors for specific hydrogenases cluster with hydrogneases of the same family. Furthermore, we used this data to ascertain the relative contributions of the [FeFe]-hydrogenase and the [NiFe]-hydrogenase in *Shewanella oneidensis* H_2 metabolism.

We are employing a similar approach to study the microbial production of the potent greenhouse gas nitrous oxide (N_2O). Depending on its source, N_2O has a unique site preference, i.e. difference in the abundance of ^{15}N in the central atom versus the outer atom of the linear N_2O molecule. However, fractionation factors are generally determined in cell cultures or field settings in which the observed fractionation is a function of many steps (e.g. diffusion, nitrate reduction to nitrite, etc.). Here we define the unique isotopic signature of different N_2O producing enzymes, which ultimately will lead to an improved understanding of the atmospheric N_2O flux as well as insight into the molecular mechanism of N_2O biosynthesis.