

Near-equilibrium multiple-sulfur isotope and methane isotopologue ratios in the slow and deep biosphere

SHUHEI ONO*, DAVID T. WANG, AND JEEMIN H. RHIM

Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA. (*e-mail: sono@mit.edu)

Microbial sulfate reduction and methanogenesis are two key metabolisms that operate in the deep biosphere. Experimental, theoretical, and field studies of isotope and isotopologue fractionation, e.g., $^{13}\text{-}^{12}\text{C}$ between CH_4 and CO_2 during microbial methanogenesis/methanotrophy (1, 2), and $^{32}\text{-}^{33}\text{-}^{34}\text{-}^{36}\text{S}$ of SO_4^{2-} and HS^- during sulfate reduction (3), indicate that slow microbial processes are characterized by relatively large isotope fractionations that approach thermodynamic equilibrium. This is a predictable consequence of increased reversibility during enzyme-catalyzed reactions under energy limitation (4, 5). Here, we explore the insights derived from, and capabilities and limitations of this model to illuminate the deep biosphere.

Secondary sulfide minerals associated with some altered rocks in oceanic crust indicate $^{32}\text{-}^{33}\text{-}^{34}\text{S}$ systematics consistent with sulfate reduction at ca. 65°C (6). Methane recovered from coal bed horizons 2.5km below seafloor carry apparent equilibrium temperatures of $70\pm 10^\circ\text{C}$ for the clumped isotopologue disproportionation reaction ($^{12}\text{CH}_4 + ^{13}\text{CH}_3\text{D} \rightleftharpoons ^{13}\text{CH}_4 + ^{12}\text{CH}_3\text{D}$) (7). Because uncatalyzed isotope exchange among $\text{CH}_4\text{-CO}_2\text{-H}_2\text{O}$ and $\text{SO}_4^{2-}\text{-HS}^-$ occurs sluggishly below ca. 200°C , these isotope signals indicate slow and energy-limited microbial activity in the deep biosphere.

One caveat to this approach to estimate true temperatures associated with microbial processes is related to the difficulty in determining accurate equilibrium fractionation factors at low temperatures. In particular, theoretical calculations often assume gas-phase harmonic oscillator, and accurate estimates of the effect of hydration, pressure, and salt are often challenging. We will discuss several ways to approach this problem via the combination of field studies, laboratory experiments, and theoretical calculations.

References:

1. Yoshinaga *et al.*, *Nature Geoscience* **7**, 190 (2014).
2. Botz *et al.*, *Organic Geochemistry* **25**, 255 (1996).
3. Sim *et al.*, *Science* **333**, 74 (2011).
4. Wing & Halevy, *PNAS* **111**, 18116 (2014).
5. Valentine *et al.*, *Geochim. Cosmochim. Acta* **68**, 1571 (2004).
6. Ono *et al.*, *Geochim. Cosmochim. Acta* **87**, 323 (2012).
7. Inagaki *et al.*, *Science* **349**, 420 (2015).