Secular variation of multiple sulfur isotopic compositions of sulfate and sulfide during long term incubation of *Desulfovibrio desulfuricans*

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Incubation experiments of sulfate reducing microbes have been carried out since 1950s to quantify sulfur isotope fractionation. Sulfur isotope fractionation models are constructed from the results of these experiments. These models are applicable only when steady state is accomplished in the cells; thus, most of incubation experiments targeted cells exponential growth phase. We aimed to quantify the isotope fractionation in stationary to death phase of the cells and carried out two series of incubation experiment of sulfate reducing bacteria, *Desulfovibrio desulfuricans* (DSM-642), until c.a. 2000 hours. In addition, we used glucose as electron donor to make cell specific sulfate reduction rate low. Cells grew linearly rather than exponentially until cell concentration became c.a. 2×10^7 cells/mL in the early stage of incubation. Calculated sulfur isotope fractionation increased from 13.5±3.7‰ at the earliest stage of linear growth phase to 70.5±21.0‰ at the middle of linear growth phase. The cell specific sulfate reduction rate was 1.0±0.3 fmol/cell/day when sulfur isotope fractionation became 70.5±21.0‰, consistent with the results of a previous study. In the stationary to death phase, sulfide concentration started decreasing on the contrary to our expectation. Besides, $\delta^{34}S_{sulfide}$ value increased concomitant with the decrease of $\Delta^{33}S'_{sulfide}$ value. The $\delta^{34}S'_{sulfide}$ and $\Delta^{33}S'_{sulfide}$ have good linear correlation, and that indicate a sulfide originated reaction in a closed system with a unique fractionation factor dominates sulfur cycle during stationary to death phase. The calculated enrichment factors of the reaction in the two series experiments are 10.5‰ and 30‰. These enrichment factors are large compared to those of biotic or abiotic sulfide oxidation experiments of previous studies. We checked the reaction occur without cells and infer that the reaction is caused from sulfide oxidation by oxidants containing in the medium or sulfurization of organic matter containing in the medium.

Keywords: sulfate reducing bacteria, sulfur isotope fractionation, long term incubation

