Evaluation of microbial nitrate-reducing Fe(II) oxidation by *Pseudogulbenkiania* sp. strain 2002 using dual nitrogen-oxygen isotope fractionation

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The microbially mediated nitrate-reducing Fe(II) oxidation (NRFO) plays an key role in iron and nitrogen interaction under neutral-anoxic conditions. Nitrogen (N) and oxygen (O) isotope fractionation analysis is a promising approach for revealing the mechanism of nitrate reduction during NRFO. Here, we investigated the magnitude of nitrogen and oxygen isotope fractionation during nitrate reduction by *Pseudogulbenkiania* sp. strain 2002 in the presence / absence of Fe(II). The cells were encrusted with Fe(III)-rich minerals and nitrate reduction rate significantly decreased in the presence of Fe(II). However, the N isotope enrichment factors (ε) were identical within uncertainty (22.0 ± 2.1‰ and 22.2 ± 1.4‰) with or without the addition of Fe(II). This result implies that the process of Fe(II) oxidation had little impact on the extent of N isotope fractionation and the formation of cell encrustation was unlikely to cause additional mass transfer limitations. In addition, the ratios of O isotope composition to N isotope composition ($^{18}\varepsilon$: $^{15}\varepsilon$) has been proven to be a powerful tool to identify the type of nitrate reductases and nitrate reduction positon. The values of $^{18}\varepsilon$: $^{15}\varepsilon$ were near 1 for strains with membrane-bound nitrate reductases (Nar) and was around 0.5 to 0.6 for strains with periplasmic nitrate reductase (Nap). The values of $^{18}\varepsilon$: $^{15}\varepsilon$ were 0.69 ± 0.11 and 0.47 ± 0.05 with or without the addition of Fe(II), respectively, indicating that Nap was involved during NRFO by strain 2002. This study confirms that the dual N-O isotope fractionation analysis was a powerful tool for investigating the mechanism of microbial nitrate reduction during NRFO, and would be helpful for interpreting nitrogen biogeochemical cycling in the anoxic iron-bearing environments.

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