# Nitrogen and oxygen isotopic fractionation during nitrate-reducing Fe(II) oxidation mediated by *Acidovorax* sp. strain BoFeN1

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Microbially mediated nitrate-reducing Fe(II) oxidation (NRFO) is widespread in the environmental biogeochemical cycles of iron and nitrogen under anoxic conditions, but the underlying mechanisms are largely unknown due to the co-occurence of biotic and chemical reactions. Fortunately, isotope fractionation is regarded as a potential technique for investigating the complex NRFO processes. *Acidovorax* sp. strain BoFeN1, as a mixotrophic NRFO bacterium, could reduce nitrate with or without Fe(II). Nitrite, the intermediate of nitrate reduction, can be biologically reduced by strain BoFeN1 and chemically reduced by Fe(II), so the different processes of nitrate and nitrite reduction resulted in different nitrogen and oxygen isotope fractionation. The nitrogen and oxygen isotope fractionation of nitrate and nitrite would therefore potentially allow to distinguish the biotic and chemical reactions in the NRFO system.

Cell encrustation in Fe(III) minerals caused by Fe(II) oxidation with nitrate and strain BoFeN1 had no significant effect on the kinetics of nitrate reduction, but affected the diffusion of nitrate from the outside to cytoplasm, and resulted in different isotope values ($^{15}\varepsilon$-N and $^{18}\varepsilon$-O) of nitrate. Furthermore, $^{18}\varepsilon$ and $^{15}\varepsilon$ showed a 1:1 relationship, which resulted from the nitrate reductases (Nar and Nas) of strain BoFeN1. The faster rate of biological nitrite reduction by strain BoFeN1 than chemical reduction by Fe(II) resulted in lower discrimination between light and heavy nitrogen isotopes ($^{15}\varepsilon_{\text{chmical}} > {}^{15}\varepsilon_{\text{biological}}$). Specifically, the biological process enriched light oxygen isotopes in nitrite, which was supposed to be related to the exchange of oxygen atoms between nitrite and water, the diffusion process of nitrite, and nitrite reductases (Fe-NIR) of strain BoFeN1. These findings provide a quantitative understanding of the chemical and biological processes in NRFO systems.

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