

## **Mechanisms of Fe(II) oxidation coupled to nitrate reduction in a freshwater enrichment culture: a metagenome-based approach**

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Nitrate reduction coupled to Fe(II) oxidation can be microbially-mediated or can occur abiotically via reactive N-species. Neutrophilic nitrate-reducing Fe(II)-oxidizing (NRFeOx) bacteria can survive under different anoxic growth conditions: autotrophic, with Fe(II) as sole energy source; mixotrophic, with Fe(II) and additional organic carbon as energy and carbon source; and heterotrophic, with organic carbon as sole energy and carbon source. Most NRFeOx require an additional organic carbon source for growth, i.e. they are mixotrophic. However, the NRFeOx enrichment culture KS (two parallel cultures are located at University of Wisconsin–Madison and University of Tuebingen), as well as a new enrichment culture from a freshwater pond in Northern Germany (Bremen Pond, BP) are examples of communities growing under autotrophic and anoxic conditions, which can survive with Fe(II) as sole energy source. The physiology and metabolic mechanisms including potential survival strategies of microorganisms contributing to NRFeOx under autotrophic conditions are still largely unknown. For instance, a microaerophilic Fe(II)-oxidizer is dominant in enrichment cultures KS and BP, yet it is surviving and growing under anoxic conditions indicating the potential for internal oxygen production.

In this study, metagenomic analysis was applied to determine the mechanisms employed by NRFeOx bacteria in the new enrichment culture BP and investigate the potential for internal oxygen production. The *in situ* microbial community composition at the culture BP sampling site, the Max Plank Institute in Bremen, Germany, was analyzed by 16S rRNA amplicon sequencing to investigate the abundance and diversity of culture BP-related organisms in the ecosystem.