

## **Microaerophilic iron(II) oxidation: An experimental approach to quantify microbial iron(II) oxidation rates**

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In the oxic regime of environmental systems the oxidation of iron(II) (Fe(II)) is stimulated by chemical and/or microbially mediated reactions. The lower the O<sub>2</sub> concentrations, the more competitive microbial Fe(II)-oxidizing bacteria are. Although optimal O<sub>2</sub> concentrations for successful competition with the abiotic process have been detected to be in the low micromolar range, the rates for microaerophilic Fe(II) oxidation still remain difficult to assess. This is due to (i) interference of microbial oxidation rates with the metabolic production of ferric minerals that lead to rapid abiotic surface-catalyzed Fe(II) oxidation, and (ii) the maintenance of stable low O<sub>2</sub> concentrations in experimental setups. The quantification of rates of microbial Fe(II) oxidation is a necessary step to fully evaluate its contribution in the environmental biogeochemical iron cycle.

In order to master this challenge we have cultivated several microaerophilic Fe(II)-oxidizing strains that were isolated from different environments in gradient tubes. These tubes feed the culture with Fe(II) and O<sub>2</sub> in opposing gradients. Oxygen microsensor measurements revealed that optimal growths occurs at 20-25 micromolar O<sub>2</sub> and that microaerophilic Fe(II) oxidation is outcompeted by the chemical reaction after 2-3 days. Based on these findings, miniaturized microcosms were set up at optimal geochemical growth conditions. The oxidation of Fe(II) was followed over time and compared to inactivated setups. We could show that at such low O<sub>2</sub> concentrations the microbial Fe(II) oxidation rate was approx. 30% higher compared to the inactivated setup. A thermodynamic and kinetic modelling approach demonstrated that the measured geochemical conditions provide optimal energetic benefit for microbial Fe(II) oxidation and highlighted the impact of ferric mineral production on the substrate competitiveness of microaerophilic Fe(II)-oxidizing bacteria. This study provides a reliable approach to assess microbial Fe(II) oxidation rates.