

Cell-mineral associations in the autotrophic nitrate-reducing Fe(II)-oxidizing enrichment culture KS

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Most microbes described as nitrate-reducing Fe(II)-oxidizers require an organic co-substrate, although cell growth in these cultures was also shown to increase in the presence of Fe(II) and even Fe(III). Encrustation of cells in Fe(III) minerals can occur in these mixotrophic nitrate-reducing Fe(II)-oxidizing cultures, whilst this has not been observed among phototrophic and microaerophilic Fe(II)-oxidizers. It has been suggested that nitrite formed during heterotrophic nitrate reduction (denitrification) abiotically oxidizes Fe(II) and that Fe(II) oxidation in these mixotrophic cultures might be, at least to some extent, a by-product of microbial denitrification [1].

The anaerobic enrichment culture KS is one of the few lithoautotrophic, nitrate-reducing Fe(II)-oxidizing cultures described so far [2]. It can be cultured autotrophically with Fe(II) and nitrate continuously without any organic substrate added. The culture is dominated by an organism related to the microaerophilic iron-oxidizer *Sideroxydans lithotrophicus*, but also contains phylotypes related to heterotrophic nitrate-reducers (*Bradyrhizobium*, *Nocardioides* and *Rhodanobacter*) whose role during autotrophic growth with Fe(II) is not yet fully understood.

Our goal is to determine whether the autotrophic Fe(II)-oxidizing bacteria or the heterotrophic nitrate-reducers in the KS culture encrust in Fe(III) minerals and to gain further insights into the interactions of the different culture members.

To this end, we used confocal laser scanning microscopy, scanning electron microscopy and nanoSIMS, to analyze the association of cells, minerals and EPS in the KS culture under both autotrophic and mixotrophic growth conditions.

Furthermore, we also use a chemostat for continuous culturing with lower Fe(II) concentrations compared to the typically used substrate-rich batch systems, to determine whether the encrustation of nitrate-reducing Fe(II)-oxidizers and heterotrophic nitrate-reducers in Fe(III) minerals occurs at environmentally relevant Fe(II) concentrations.

[1] Klueglein et al. (2014) *AEM* **80**(3), 1051-1061. [2] Straub et al. (1996) *AEM* **62**(4), 1458-1460.