Iron oxidation under continuous flow conditions

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Iron is an essential nutrient to almost all known organisms. In the environment it is cycled between the two common oxidation states: Fe(II) and Fe(III). Microorganisms play an important role during this cycling, either by oxidizing Fe(II) or reducing Fe(III). In laboratories, Fe(II) oxidation by microbes is commonly studied in closed containers, so called batch experiments. Here, concentrations of Fe as electron donor usually exceed typical values found in the environment. We established a method for cultivation of Fe(II)-oxidizing bacteria in a continuous system, a chemostat. This system allows to investigate iron oxidation over a longer period of time and gradually change growth conditions. We cultivated the autotrophic, nitrate-dependent iron-oxidizing microbial co-culture KS in the chemostat for durations of up to a month.

To gain deeper insights into Fe(II) oxidation and nitrate reduction, we traced Fe and N speciation by wet chemistry methods, allowing determination of Fe(II) oxidation rates and significant nitrite concentrations. We utilized fluorescence microscopy and scanning electron microscopy to investigate the cell-mineral interactions from micro- to nanoscale. Mineral samples were analyzed to identify initial, intermediate, and final speciation and composition by XRD and Mössbauer spectroscopy. Control batch experiments were performed in order i) to confirm the comparability of chemostat to batch and ii) to show that there are no upscaling effects.

Results show that our system is comparable to the common batch experiment. Therefore we propose that cultivation at lower concentration can now representatively be done in continuous conditions, and thus be considered environmentally relevant. Our results also suggest that culture KS encrusts during or after enzymatic oxidation of Fe(II), and reveal that nitrite is accumulated. Nitrite is highly reactive and abiotically oxidizes Fe(II). This previously overlooked nitrite enrichment might hence change the understanding of nitrate dependent iron oxidation. These findings will help develop new knowledge of often overlooked, or cryptic, processes which occur during nitrate dependent iron oxidation and reveal new and fundamental understanding to solve the puzzle of iron cycling in the environment.