

## **Using He-Ion microscopy to map the growth and mineralization of twisted stalks produced by microaerophilic Fe(II)-oxidizing bacteria**

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Neutrophilic, microaerophilic Fe(II)-oxidizing bacteria (e.g. *Gallionella ferruginea* or *Mariprofundus ferrooxydans*) gain energy by oxidizing Fe(II) coupled to the reduction of oxygen. However, due to the fact that abiotic Fe(II) oxidation is so fast, the microbial activity is limited to micro-oxic ( $O_2 < 50 \mu M$ ) conditions where the bacteria can outcompete abiotic oxidation. Some microaerophilic Fe(II)-oxidizers produce long twisted stalk features, consisting of organic material and Fe(III) minerals, which appear to be loosely bound to the bacterial cells. These appendages are thought to be essential for eliminating Fe(III) waste produced during Fe(II) oxidation by sorbing Fe(III) and transporting it away from the cell. They may also play an important role in the sorption of transition metals, organic compounds and nutrients.

Recently we isolated a microaerophilic Fe(II)-oxidizer from brackish sediments in the Bay of Aarhus, Denmark, where the Fe(II) concentration is 70-100  $\mu M$ , representative of marine environments. We used He Ion Microscopy (HIM) to image the development of the stalks over a period of 4 weeks to probe mineralogical and structural changes. HIM has several advantages over conventional scanning electron microscopy (SEM) due to its higher spatial resolution and the fact that samples can be imaged without coating (e.g. with Pt/Au). The HIM images were then correlated with Energy Dispersive X-ray (EDX) maps in order to observe changes in the elemental composition over time, combined with mineralogical analysis using Mössbauer spectroscopy.

The results showed the initial formation of long, Fe-free stalks which, after just 2 days, became encrusted with spikey lepidocrocite crystals. After 1 month, the characteristic twisted stalk shape was almost indiscernible and instead appeared heavily mineralised. These results could have important implications for our ability to distinguish potential biomarkers from microaerophilic Fe(II)-oxidizing bacteria in the rock record.