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Anaerobic pyrite oxidation by two autotrophic nitrate-reducing iron(II)-oxidizing enrichment cultures at neutral pH

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The oxidation of pyrite (FeS_2) under nitrate-reducing, anoxic conditions has been studied for years, yet its fundamental mechanisms are still debated. While most research has focused on nitrate-reducing sulfur-oxidizing microorganisms, particularly *Thiobacillus denitrificans*, the role of neutrophilic and anaerobic Fe(II)-oxidizing microorganisms in pyrite oxidation and dissolution remains largely unexplored.

Enrichment cultures KS and BP are autotrophic nitrate-reducing Fe(II)-oxidizing (NRFeOx) microbial communities that couple Fe(II) oxidation to nitrate (NO_3^-) reduction using carbon dioxide (CO_2) as carbon source. Each culture is dominated by novel candidate species of the genus *Ferrigenium*, the main Fe(II)-oxidizers. Interestingly, autotrophic NRFeOx relies on microbial collaboration, as *Ferrigenium* spp. require members of the flanking community for growth under anoxic nitrate-reducing conditions. Notably, the flanking microbial species of each culture differ significantly, with culture BP containing Fe(III)-reducers and sulfur-oxidizers, albeit in low abundance.

To investigate their ability to use pyrite as an electron donor, cultures KS and BP were grown and maintained over several transfers under anoxic conditions with pyrite as the sole electron and energy source, while NO_3^- and CO_2 were provided as electron acceptor and carbon source. When incubated with pyrite, we found that 0.72 mM and 0.98 mM of Fe was solubilized in cultures KS and BP after 50 days, respectively, while 0.24 mM and 0.63 mM of NO_3^- were reduced. The dissolved S:Fe ratio was 2.5 in culture KS and 1.85 in culture BP, indicating pyrite dissolution in both microcosms. Negligible pyrite dissolution was observed in abiotic controls. Importantly, most cells were attached to the pyrite surface (74% of cells in culture KS and 67% in culture BP), suggesting that cell-mineral interactions played a crucial role in pyrite oxidation under autotrophic NRFeOx conditions.

These results show that pyrite can be used as an electron and energy source for NRFeOx communities, leading to pyrite dissolution. Differences between cultures KS and BP suggest that the identity and activity of the flanking microorganisms influence the extent of pyrite oxidation, likely by modulating Fe and S cycling. This study highlights the importance of NRFeOx-driven pyrite oxidation when evaluating Fe and N cycling in anoxic subsurface environments.