CRC 1253 CAMPOS — Catchments as Reactors

EBERHARD KARLS UNIVERSITÄT TÜBINGEN

Insights into mechanisms of nitrate-dependent pyrite oxidation by an autotrophic enrichment culture isolated from a pyrite-rich limestone aquifer

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Introduction

Microbial pyrite oxidation coupled to denitrification is regarded as a natural nitrate attenuation process in limestone aquifer belonging to Ammer catchment. However, the poor solubility of pyrite at low temperature raises questions regarding the bioavailability and mechanism of pyrite oxidation.

Goal

Here we investigate whether the presence of more bioavailable Fe(II)-bearing minerals – which, when processed through microbial nitrate-reducing Fe(II) oxidation, act as a source of Fe(III) as oxidant – can further drive indirect oxidation of pyrite and influence overall rates of denitrification **(Fig 1.).**

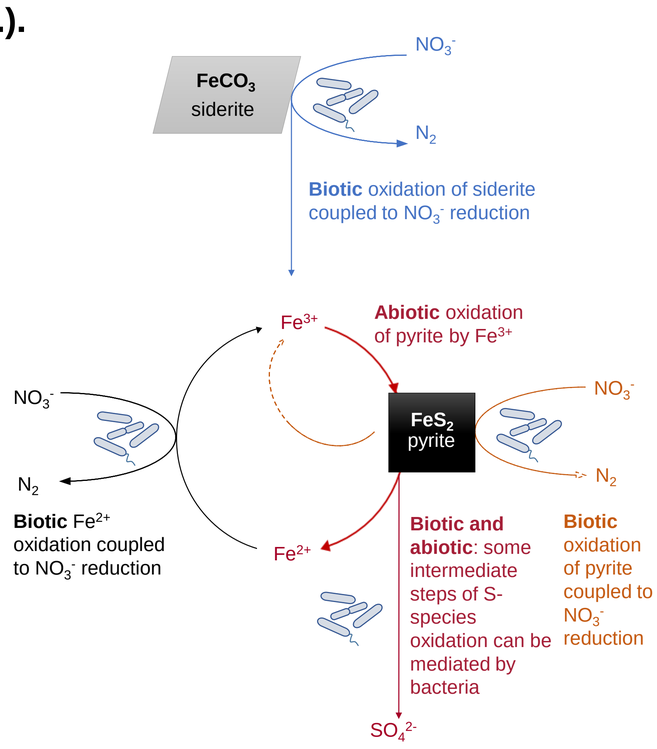

Fig 1. *Potential processes governing microbially driven oxidation of pyrite in the aquifer.*

Methods

Autotrophic nitrate-dependent Fe(II)-oxidizing (NRFeOx) culture enriched from the aquifer **(Fig 2.)** was incubated in medium containing nitrate, pyrite (91.7% of ^{56}Fe(II)) and ^{57}Fe-labeled siderite **(Fig 3.).**

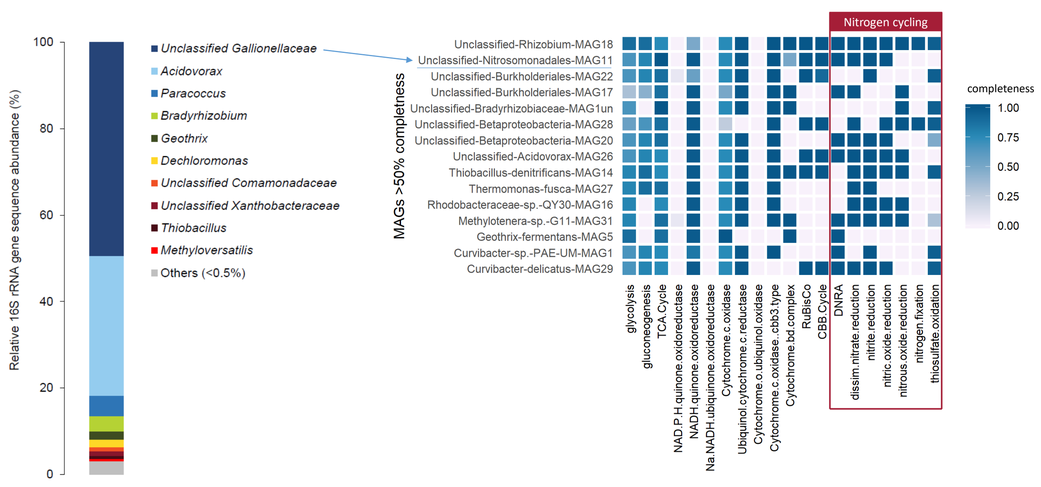

Fig 2. *Microbial community composition of NRFeOx culture enriched from the aquifer used in this study **(A)** and a heatmap of KEGG-annotated genes **(B)** in MAGs from the culture showing metabolic potential of enriched microbes to perform i.a. CO_2-fixation (autotrophy), denitrification and thiosulfate oxidation.*

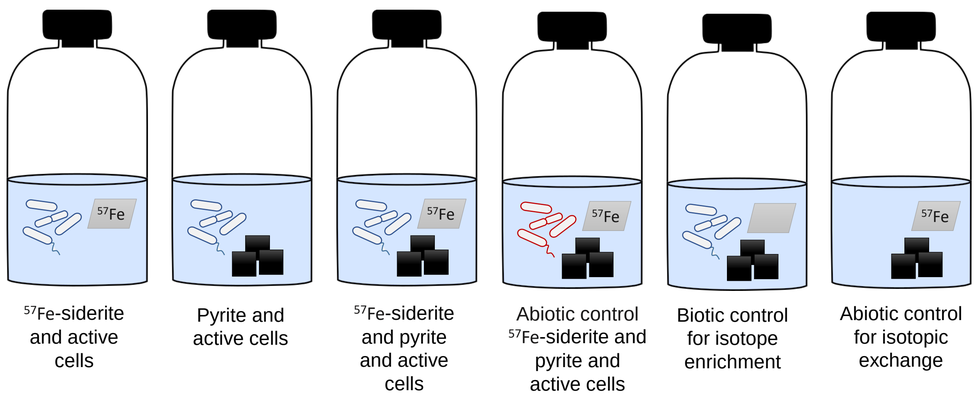

Fig 3. *Experimental setup and controls.*

Results

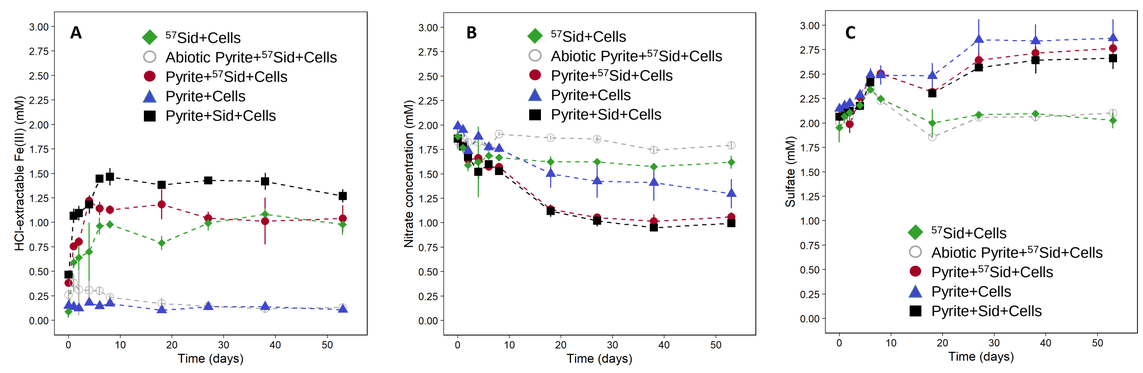

Fig 4. *Concentration of HCl-extracted Fe(III) **(A)**, nitrate **(B)** and sulfate **(C)** over time of the experiment.*

	NO_3^- reduced (mM)	Fe(III) formed (mM)	SO_4^{2-} formed (mM)	NO_3^-/SO_4^{2-} Ratio	NO_3^- /Fe(III) ratio
^{57}Sid+Cells	0.22 ± 0.17	0.88 ± 0.13	0.07 ± 0.17	-	0.25
Pyrite+Cells	0.80 ± 0.14	0.00 ± 0.02	0.72 ± 0.23	1.11	-
Pyrite+^{57}Sid+Cells	0.80 ± 0.03	0.66 ± 0.13	0.70 ± 0.09	1.14	1.2
Pyrite+Sid+Cells	0.89 ± 0.05	0.80 ± 0.04	0.60 ± 0.12	1.49	1.1
Abiotic+Pyrite+^{57}Sid+Cells	0.04 ± 0.05	0.00 ± 0.06	0.09 ± 0.07	-	-

Fig 5. *Decrease of NO_3^- and formation of HCl-extractable Fe(III) and SO_4^{2-}, stoichiometries of NO_3^- reduction and SO_4^{2-} formation, and NO_3^- reduction.*

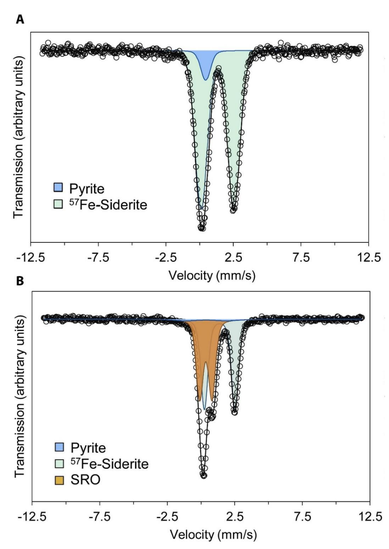

Fig 6. *Mössbauer spectra of 'Pyrite+57Sid+Cells' setup at the beginning of the experiment **(A)** and after 8 days **(B)** showing decrease of pyrite and siderite abundance and formation of short-range ordered (SRO) Fe(III)-phases as a result of oxidation.*

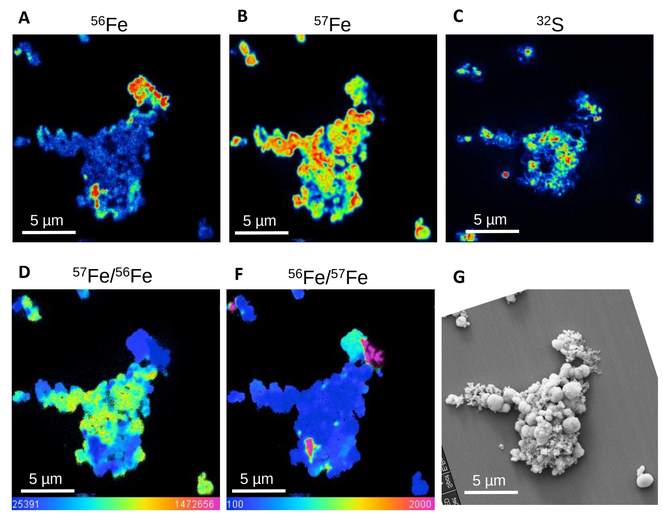

Fig 7. *NanoSIMS high spatial resolution analysis of the reaction product of 'Pyrite+57Sid+Cells' setup at the 8th day of incubation; the spatial distribution of two isotopes of iron ^{56}Fe **(A)** and ^{57}Fe **(B)** along with ^{32}S**(C)** distribution in combination with SEM **(F)** imagining. <u>High ^{56}Fe/^{57}Fe **(D, E)** ratio correlated with the distribution of sulfur indicates pyrite while the absence of sulfur indicates products of pyrite oxidation.</u>*

Conclusions

We conclude that in anoxic aquifers, where multiple sources of Fe(II)-bearing minerals are present, microbially driven lithoautotrophic oxidation of bioavailable Fe(II) coupled to denitrification can lead to the production of Fe(III), which drives a positive feedback loop causing more indirect pyrite oxidation and increasing denitrification. However, the extent of this process is limited to the bioavailability of Fe(II).

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