Anaerobic Fe(II)-oxidizing bacteria show As resistance and immobilize As during iron(III) mineral precipitation

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SUPPORTING INFORMATION

Number of tables in supporting information: 1
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Table S1: Ratios of As/Fe for As(III) and As(V) sorbed and/or co-precipitated to/by Fe(III) (hydr)oxides formed during microbial Fe(II) oxidation.

As/Fe ratios were calculated on a per mol basis and are based on the differences in concentrations of dissolved arsenic (As removed) and Fe(II) (Fe(II) oxidized) at different time-points. As(removed) was calculated by subtracting the remaining dissolved As concentration at $t_{removed}$ from the initial As concentration after inoculation (at t_0). Time point t_0 = directly after inoculation and $t_{removed}$ = time point at which no dissolved Fe(II) was detectable anymore (after complete Fe(II) oxidation). Time point t_x represents the time point after As addition before inoculation.

Strain	Initial As(V) concentration before inoculation (t_x) [μM]	As(V) concentration directly after inoculation (t_0) [μM]	Remaining dissolved As after Fe(II) oxidation (at $t_{removed}$) [μM]	Fe(II) oxidized (at $t_{removed}$) [mM]*	As removed (at $t_{removed}$) [μM]	Molar ratio [As(removed)/ Fe(oxidized)] in precipitates
BoFeN1	15.7	8.3	0.09	2.8	8.2	0.003
	35.7	24.4	0.18	2.8	24.2	0.009
KS	18.3	7.2	0.07	2.9	7.1	0.002
SW2	13.9	3.1	0.08	3.2	3.0	0.001
	40.8	12.2	0.04	3.1	12.1	0.004

Strain	Initial As(III) Concentration before inoculation [μM]	As(III) Concentration directly after inoculation (t_0) [μM]	Remaining dissolved As after Fe(II) oxidation (at $t_{removed}$) [μM]	Fe(II) oxidized (at $t_{removed}$) [mM]*	As removed (at $t_{removed}$) [μM]	Molar ratio [As(removed)/ Fe(oxidized)] in precipitates
BoFeN1	13.7[§]	14.9[§]	0.44	2.8	14.5	0.005
	42.9	40.0	1.52	2.9	38.5	0.013
KS	20.4	18.9	0.08	2.9	18.9	0.007
SW2	17.3[§]	18.0[§]	0.06	3.1	17.9	0.006
	43.5	39.3	0.08	2.3	39.3	0.017

* Fe(II) oxidized was quantified by the ferrozine assay as described in [1]
[§] Differences in concentrations are due to analytical error (concentration after inoculation should be lower or equal but not higher than concentrations before inoculation).

S2: Calculation of adsorption of arsenic to Fe(III) minerals that are added by inoculation of Fe(II)-oxidizing cultures.

Stock cultures of Fe(II)-oxidizing bacteria grown with 10 mM Fe(II) in the medium were used in the exponential growth phase for inoculation of fresh Fe(II)-oxidizing (and As-co-precipitating) cultures. After complete oxidation of 10 mM Fe(II) in the stock cultures, the maximum amount of possibly transferred Fe(III) minerals from a stock-culture bottle into an experiment bottle can be calculated as follows: injecting 1.25 ml inoculate from a stock-culture containing 10 mM Fe(III) minerals into 25 ml fresh medium would yield a concentration in the experiment-bottle of about 0.5 mM Fe(III) minerals. Using the experimentally determined sorption-ratios of As per Fe(III) of 0.004 and 0.001 (see Table 1 and Table S1) determined from the SW2 immobilization experiments (for the setups with initial As target concentrations of 50 and 20 µM), 0.5 mM Fe(III) could approximately sorb 2 and 0.5 µM As(V), respectively. Since only about 50% of the Fe was oxidized at the time of cell harvest and inoculation, we estimate that about 1 and 0.3 µM of the initially present As(V) in the medium can be bound to the Fe(III) minerals transferred via inoculation in the experiments with initial As target concentrations of 50 and 20 µM.

This shows that even if Fe(II) oxidation in the stock cultures would have been completed by the time of inoculation, the calculated amounts of possibly sorbed arsenic to inoculation-Fe(III) minerals would still not explain the drop in As(V) concentration of 28 µM (40.8 µM minus 12.2 µM, see Table S1) as observed in our immobilization experiments with strain SW2 (in the bottles with an initial As target concentration of 50 µM As(V) that contained a measured concentration of only 40 µM As(V)) and of 11 µM (13.9 minus 3.1 µM) in the bottle with an initial As target concentration of 20 µM that contained a measured concentration of 13.9 µM As(V). As(V) sorption to transferred Fe(III) minerals could therefore only partially explain the initial drop in As(V).

Applying the same calculation for As(III) with sorption ratios of 0.017 for experiments with higher initial As(III) concentration (initial target concentration of 50 µM As(III); measured concentration of 43.5 µM) and 0.006 for experiments in bottles with lower initial As(III) concentration (initial target concentration of 20 µM As(III); measured concentration of 17.3 µM), yields a possible sorption of 8.5 µM and 3 µM of As(III) to 0.5 mM inoculated Fe(III)-minerals in the culture.

In all immobilization experiments with As(III), we observed no drop in As(III) concentration. This confirms (as stated in the main text of the paper) that the drop in As(V) is due to binding to cell-surface functional groups via Fe(II) bridged ternary complexes (cell surface-Fe(II)-As(V)) rather than to binding to minerals transferred during inoculation.

S3: Moessbauer spectrum of mineral products formed by the nitrate-reducing Fe(II)-oxidizer strain BoFeN1
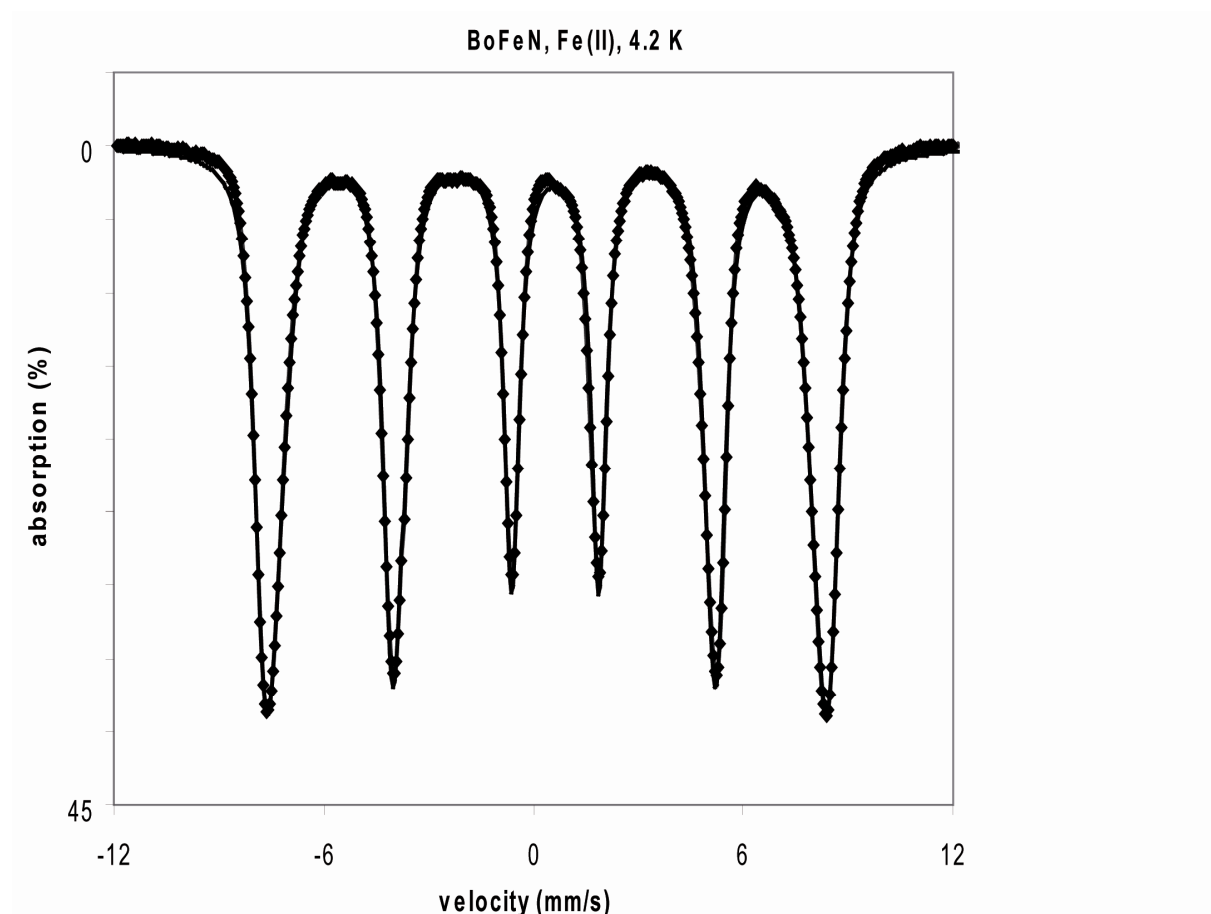

Figure S3: Moessbauer spectrum at 4.2 K of the mineral products formed by the nitrate-reducing Fe(II)-oxidizer strain BoFeN1. The sextet was modeled by a distribution of hyperfine fields with <H> with greatest probability 49.0 T, assigned to goethite. Centershift was 0.50 mm/s, quadrupole shift -0.12 mm/s.