Supporting Information

Adsorption and Incorporation of Arsenic to Biogenic Lepidocrocite Formed in the Presence of Ferrous Iron during Denitrification by *Paracoccus denitrificans*

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Analytical methods. For quantification of Fe(II), the modified ferrozine assay was used for the nitrite-containing samples developed by Kluglein and Kappler.[1] Briefly, 100 μL of culture suspension was withdrawn using a syringe and dissolved in 900 μL of 40 mM sulfamic acid for 1 h at room temperature. After 1-hour reaction, 10 μL of the extracts were transferred to 2 mL of ferrozine solution (1 g of $C_{20}H_{13}N_4NaO_6S_2 \cdot xH_2O$ in 1 L of 50 mM HEPES buffer at pH 7) to make a ferrous complex. The Fe(II)-ferrozine complex was quantified at 562 nm using a UV/Vis spectrometer. A separate calibration curve was prepared for the revised ferrozine assay, using ferrous ethylenediammonium sulfate ($C_2H_{10}FeN_2O_8S_2$) that was dissolved in 40 mM sulfamic acid. Nitrate and nitrite were determined by ion chromatography (Dionex, CA) equipped with IonPac AG14 guard and AS14 analytical columns (Thermo Fisher Scientific, Sunnyvale, CA). Acetate was quantified by a high-performance liquid chromatography (HPLC) equipped with a photodiode array (PDA) detector (Varian, Walnut Creek, CA) and an Aminex HPX-87H ion-exclusion column (Bio-Rad, Hercules, CA). The mobile phase was 5 mM sulfuric acid with a flow rate of 0.6 mL/ min for 20 min. Ten microliters of each sample was injected into the HPLC, and UV detection was performed at 210 nm. Concentrations of total As and As(III) were analyzed using inductively coupled plasma mass spectrometry (ICP−MS, 7500ce, Agilent Technologies, Palo Alto, CA), after separating As(III) from the total aqueous As by solid-phase extraction (SPE) using a commercial cartridge (Supelclean LC-SAX SPE, Supelco Inc., PA). For analysis of As(V), the solution retrieved from the culture medium was filtered by the SPE method and As(V) retained in the cartridge was eluted by 1 N HCl. The liquid phase (0.5 mL) of the culture medium was periodically sampled without disturbing the settled precipitates, to determine the concentration of aqueous As. Following filtration with a syringe filter unit (0.2 μm, Advantec MFS, Dublin, CA), the sample was diluted with 2% HNO_3

solution for ICP-MS analysis. All processes for sample preparation were carried out in the glovebox.

Characterization of minerals. Transmission electron microscopic (TEM, JEOL JEM-2100, Tokyo, Japan) analysis was performed with prepared samples to observe their mineralogical morphologies. Samples were prepared as described previously by Schadler et al.[2] To prepare the specimen, the precipitates in the bacterial culture medium were collected and washed three times with anoxic DI water. The washed 10 μL of the samples were fixed with 3% glutaraldehyde for 2 h at 4°C, centrifuged for a few minutes at 3,637 ×*g*, and subsequently washed in distilled water. The fixed samples were placed onto carbon-coated 200-mesh copper grids. The cross-sections were prepared by ultramicrotomy. Cross-sections with a nominal thickness of 70 nm were prepared with an EM-UC6 ultramicrotome (Leica, Wetzlar, Germany) using a diamond knife. After deposition on a copper grid, they were stained with uranyl acetate [2% (w/v)] and lead citrate (2 g/ L). For mineralogical analysis by X-ray diffraction (XRD), the precipitates from 7-d incubated bacterial cultures were collected by centrifugation (5415D, Eppendorf, Hamburg, Germany) at 2,300 ×*g* for 10 min and washed three times with N_2-purged Milli-Q water to remove medium components. The washed minerals were dried under anoxic conditions in the glovebox. XRD analysis was performed on the BL6D beamline of a Pohang light source (PLS-II). The X-rays were monochromated (wavelength, $\lambda = 0.6213$ Å) using a Si(111) double-crystal monochromator and focused using a K-B type mirror system to be ca. 350 μm (H) × 30 μm (V) (full-width-at-half-maximum) at the sample position. For clarity, the diffraction angles were finally converted to those of Cu Kα radiation ($\lambda = 1.5418$ Å) after a calibration process with NIST standard LaB_6 (SRM 660b).

Sequential chemical extraction of As from biogenic As-containing minerals. The sequential extraction procedure was slightly modified from Keon et al.[3] All extractants were prepared in Milli-Q water and deoxygenated by purging with 100% N_2. Table S5 summarizes the sequential extraction procedure in the present experiment, and the solid phase targeted by each extractant. The extractants were adjusted to pH values listed in Table S5 by using either HCl or NaOH. Following 7 d of bacterial incubation, all the remaining precipitates in the bacterial medium were collected by centrifuge (8,655×*g* for 20 min) and subsequently washed three times with N_2-purged Milli-Q water to remove medium components. The precipitates, which were formed in the bacterial medium in the presence of both As(III) and As(V), were dried in the glove box for 24 h, resulting in wet weights of 0.055±0.001 g and 0.052±0.003 g for biogenic As(III)-containing minerals and As(V)-containing minerals, respectively. After treating each of the extractants, the suspensions were centrifuged, the supernatants were then filtered to 0.2 um and analyzed by ICP-MS, and the solid pellets were treated with the next extractant. The dried As-containing minerals formed in the bacterial medium were also prepared for XAFS analysis.

Remobilization of As from As-bearing biogenic goethite by Fe(III)-reducing bacteria. In order to demonstrate remobilization of arsenic from preformed biogenic As-bearing lepidocrocite, the As-bearing lepidocrocite mineral precipitates produced by *P. denitrificans* (ATCC 17741) were centrifuged at 15,700 x*g* for 10 min and washed tree times with DI water. The washed biogenic As-bearing lepidocrocite (ca. 23 mg) were added to 100 ml of the basal medium. Dissimilatory Fe(III)-reducing bacterium, *Shewanella oneidensis* MR-1 was inoculated into the medium containing either biogenic As(III)- or As(V)-bearing lepidocrocite. The *S. oneidensis* MR-1 was pre-grown aerobically on Luria-Bertani (LB) broth at 30^{o}C with

shaking at 200 rpm for 12 h. Cells were centrifuged (9000 x g for 10 min), washed with HEPES buffer (10 mM, pH 7.0), and resuspended in HEPES buffer. The cells were subsequently inoculated into medium containing As-bearing biogenic lepidocrocite to achieve an optical density (OD) of 0.1 at a wavelength of 600 nm of the medium. Sodium lactate (0.2 μm filter-sterilized) was added as the electron donor with the final concentration of 10 mM in the medium. Concentrations of total As and As(III) were analyzed using inductively coupled plasma mass spectrometry (ICP−MS, 7500ce, Agilent Technologies, Palo Alto, CA), after separating As(III) from the total aqueous As by solid phase extraction (SPE) using the commercial cartridge (Supelclean LC-SAX SPE, Supelco Inc., PA). For analysis of As(V), the retrieved solution from culture medium was filtered by the SPE method and the retained As(V) in the cartridge was eluted by 1 N HCl. The liquid phase (0.5 mL) of culture medium was sampled without disturbing the settled precipitates to determine the concentration of aqueous As. Following filtration with a syringe filter unit (0.2 μm, Advantec MFS, Dublin, CA), the sample was diluted with 2% HNO_3 solution for ICP-MS analysis.

Fe and As K-edge XAFS measurement. Fe and As K-edge XAFS were recorded on the BL10C (multipole wiggler) beamline of a Pohang light source (PLS-II) with a ring current of 350 mA at 3.0 GeV. A Si(111) double crystal monochromator was employed to monochromatize the X- ray photon energy. The Fe and As K-edge XAFS data were collected in transmission and fluorescence mode with N_2 gas-filled ionization chambers and passivated implanted planar silicon (PIPS) as detectors, respectively. Higher-order harmonic contaminations was eliminated by detuning to reduce the incident X-ray intensity by approximately 30%. Energy calibrations were simultaneously carried out for each measurement, with reference Fe metal foil and elemental As powder placed in front of the

third ion chamber. The data reductions of the experimental spectra to normalized XANES and Fourier-transformed radial distribution functions (RDFs) were performed through the standard XAFS procedure.

Fe and As K-edge EXAFS data analysis for biogenic lepidocrocites. Using AUTOBK module in UWXAFS package,[4] the k^3-weighted Fe and As K-edge EXAFS spectra, $k^3\chi(k)$, have been obtained through background removal and normalization processes. In order to present effective radial distribution functions (RDF) for each sample, the $k^3\chi(k)$ spectra have been Fourier-transformed (FT) in the k range between 2.5 and 12.0 $Å^{-1}$. The experimental Fourier-filtered $k^3\chi(k)$ spectra have been inversely Fourier-transformed with the *hanning* window function in the r space range between 1.0 and 3.5 Å. To determine the structural parameters for each bond pair, the curve-fitting process has been carried out by using the model structures with layered lepidocrocite [γ-FeOOH] for Fe. Theoretical scattering paths have been calculated with FEFF8 code.[5, 6] In the EXAFS curve fitting process with FEFFIT module, total amplitude reduction factor, S_i^2, were fixed to 0.85 for the central Fe and As atoms. The EXAFS structural parameters, energy shift (ΔE), bond distance (R) and coordination numbers (N), have been determined with allowed R-factor value which is quality of the fit determined with $\sum\{Re\Delta\chi(\mathrm{k})^2 + Im\Delta\chi(\mathrm{k})^2\}/\sum\{Re(\chi(\mathrm{k})_{\mathrm{data}})^2 + Im(\chi(\mathrm{k})_{\mathrm{data}})^2\}$, where χ(k) is EXAFS-function) and $\Delta\chi(\mathrm{k})$ means $\chi(\mathrm{k})_{\mathrm{data}} - \chi(\mathrm{k})_{\mathrm{best\text{-}fitted}}$.

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Table S1. Arsenic-resistant *ars* genes in genome sequence of *Paracoccus denitrificans* PD1222

Arsenic resistant gene	***Function***	***Accession number***
arsR	Transcriptional regulator, ArsR family	ABL71416.1 ABL71217.1 ABL70965.1 ABL68331.1 ABL68282.1 ABL72762.1 ABL72582.1 ABL72210.1 ABL69319.1 ABL72873.1 (plasmid)
arsB	Arsenical-resistance protein; Arsenite efflux pump	ABL 71414.1 ABL71215.1 ABL68328.1 ABL68305.1
***ars*C**	Arsenate reductase	ABL70363.1 ABL71216.1 ABL71415.1 ABL72107.1
arsH	NADPH-dependent FMN reductase	ABL71413.1 ABL71214.1

Table S2. Concentrations of arsenic species and ferrous iron in aqueous phases of bacterial medium containing 5 mM acetate and 10 mM nitrate before and after 7 d incubation with *P. denitrificans* (ATCC 17741)

Sample	$[As]_{initial}$ (µM)	$[As]_{final}$ (µM)	$[As]_{immobilized}$ (%)	$[Fe(II)]_{initial}$* (mM)	$[Fe(II)]_{final}$ (mM)	Molar ratio [As/Fe] in precipitates
As(III) 500 µM without Fe(II)	491.8±5.4	493.8±4.8	-0.4 ±0.1	-	-	-
As(V) 500 µM without Fe(II)	498.5±5.9	494.4±4.3	0.8±0.3	-	-	-
As(III) 500 µM with Fe(II)	489.6±4.2	10.5±7.4	97.9±1.5	7.97±0.36	0.0	0.06±0.003
As(V) 500 µM with Fe(II)	493.7±9.5	0.4±0.5	99.9±0.1	7.88±0.21	0.0	0.063 ±0.02

Table S3. Sequential chemical extraction procedure for As-bearing solid phase (modified from Keon et al., 2001)

Step	Extractant	Target phase	Possible mechanism
$MgCl_2$	1 M $MgCl_2$, pH 8, 2 h, 25°C, two repetitions + one water wash	Ionically bound As	Anion exchange of Cl for As; possible Mg-As complex formed
NaH_2PO_4	1 M NaH_2PO_4, pH 5, 16 and 24 h, 25°C, one repetition of each time duration + one water wash	Strongly adsorbed As	Anion exchange of PO_4 for AsO_4 and AsO_3
HCl	1 N HCl, 1 h, 25°C, one repetition + one water wash	As coprecipitated with very amorphous Fe-oxyhydroxides	Proton dissolution; Fe-Cl complexation
OX	0.2 M ammonium oxalate/oxalic acid, pH 3, 2 h, 25°C in dark (wrapped in Al foil), one repetition + one water wash	As coprecipitated with amorphous Fe-oxyhydroxides	Ligand-promoted dissolution
HF	10 M HF, 1 and 24 h, 25°C added boric acid at 16 h, one repetition of each time duration + one boiling water wash	As oxides and As coprecipitated with crystalline Fe oxyhydroxides	Total dissolution of crystalline Fe oxyhydroxides by strong acid

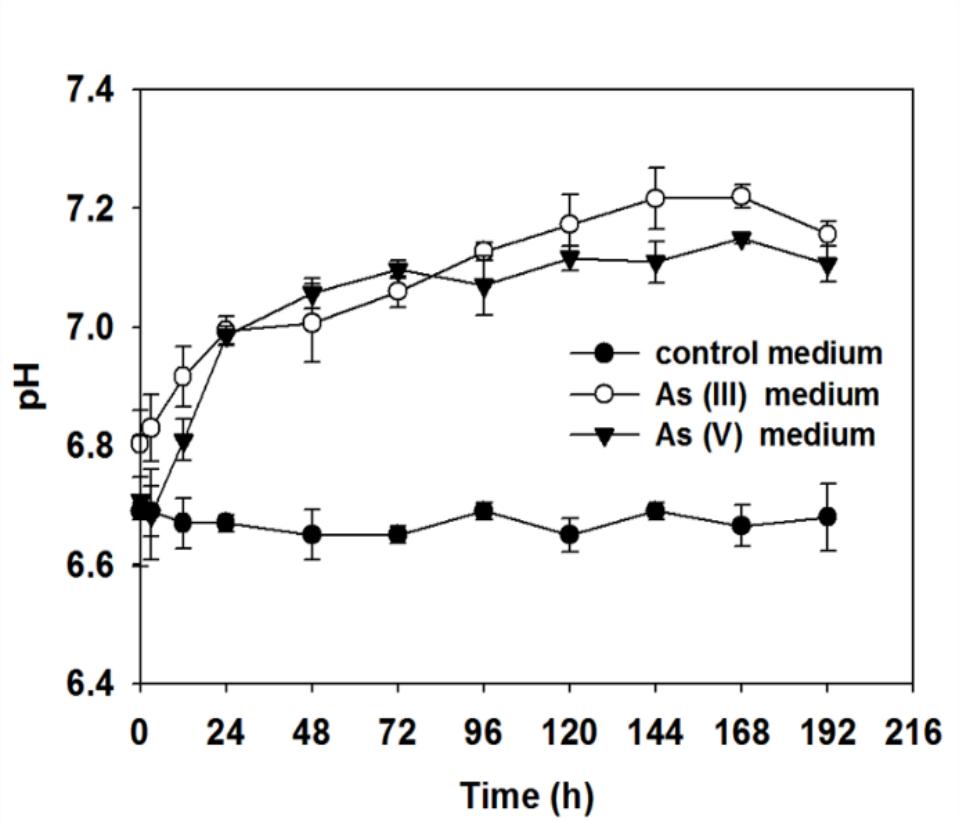


Figure S1. pH changes of bacterial medium containing acetate, nitrate, Fe(II) in the presence of As(III) (○) and As(V) (▼). Data of pH values was determined in an independent experiment with an initial arsenic concentration of 500 μM. The pH of medium was adjusted to 6.7-6.8 at the medium preparation stage.

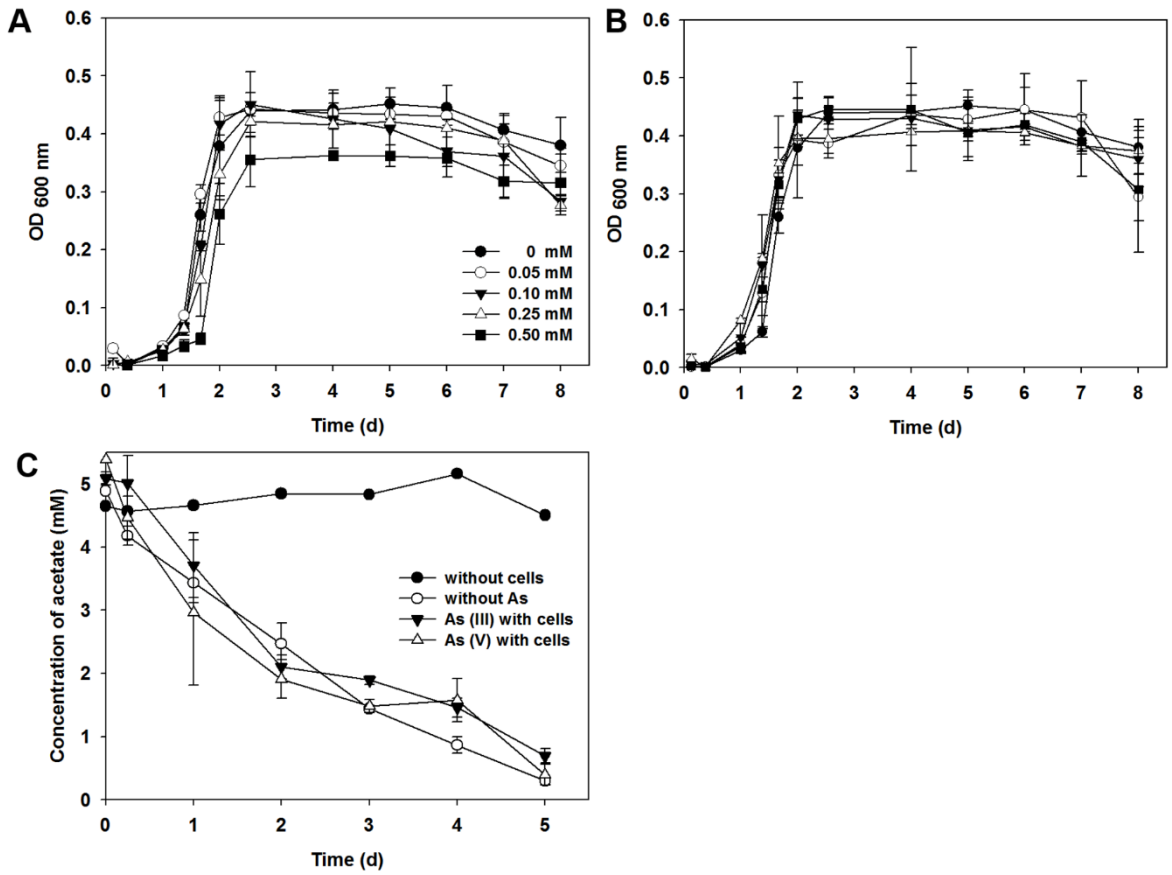


Figure S2. Growth of *P. denitrificans* (ATCC 17741) in the presence of As(III) (A) or As(V) (B), and the concentration of acetate consumed by the strain in the presence of 0.5 mM of As(III) or As(V) (C).

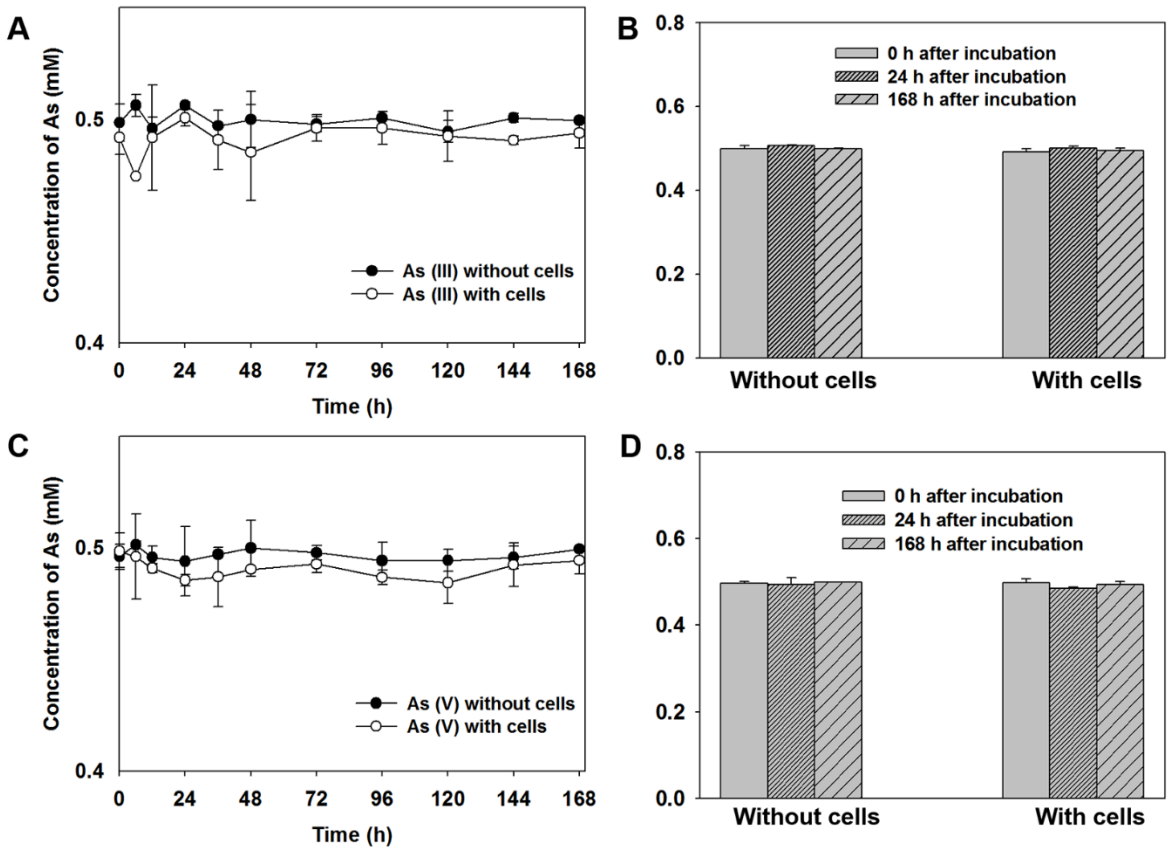
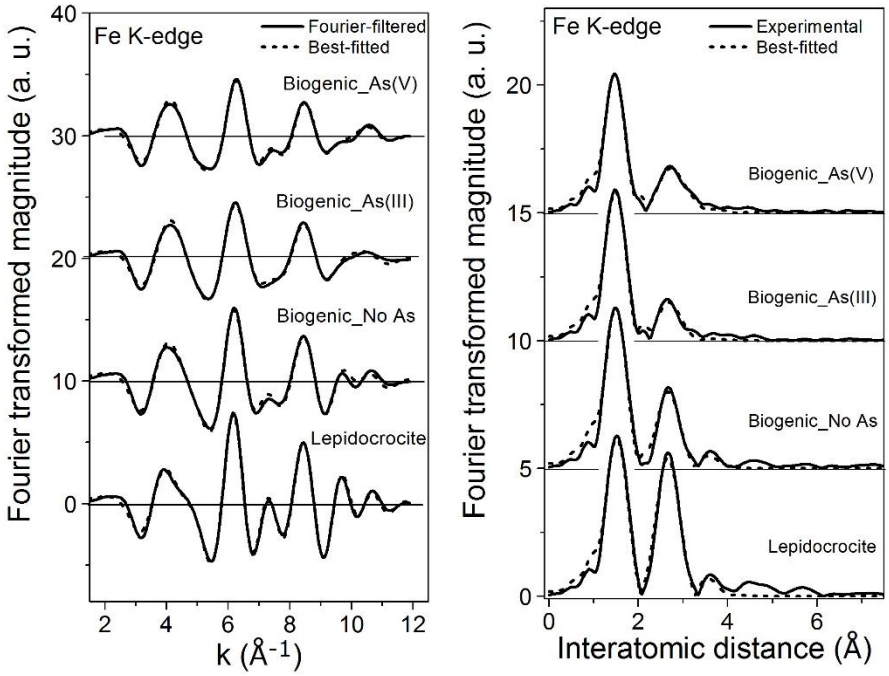


Figure S3. Concentrations of As(III) (A) and As(V) (B) in the aqueous phases of media in the absence of Fe(II) as a function of incubation time. The medium contains 10 mM acetate and nitrate as an electron donor and acceptor, respectively.



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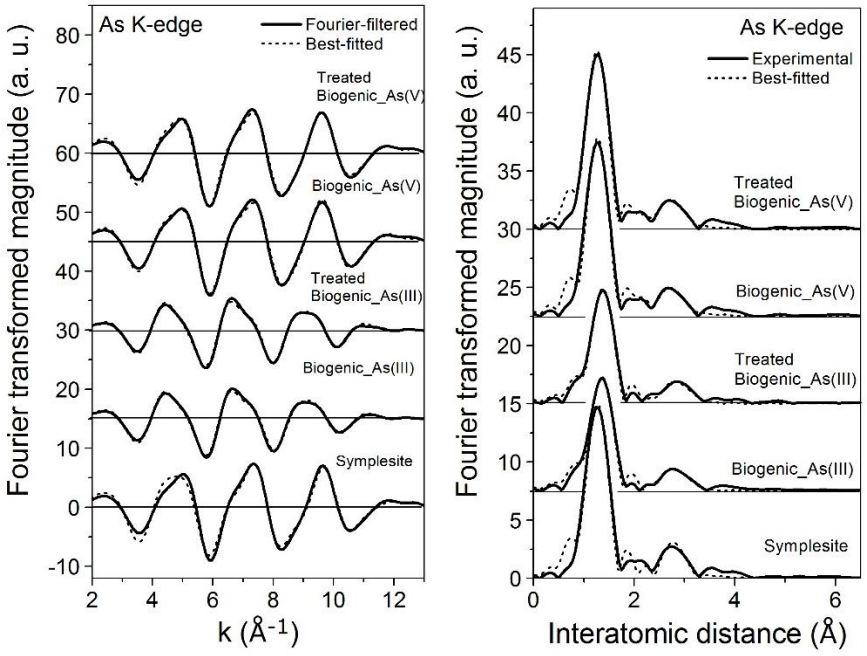

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25 **Figure S4.** Experimental and fitted spectral results of Fe (top) and As (bottom) K-edge k^3-
26 weighted EXAFS spectra (left) and corresponding radial distribution functions (right) for
27 biogenic lepidocrocites obtained in the presence of arsenic species under nitrate-reducing and
28 Fe(II)-oxidizing conditions by *Paracoccus denitrificans* (ATCC 17741).

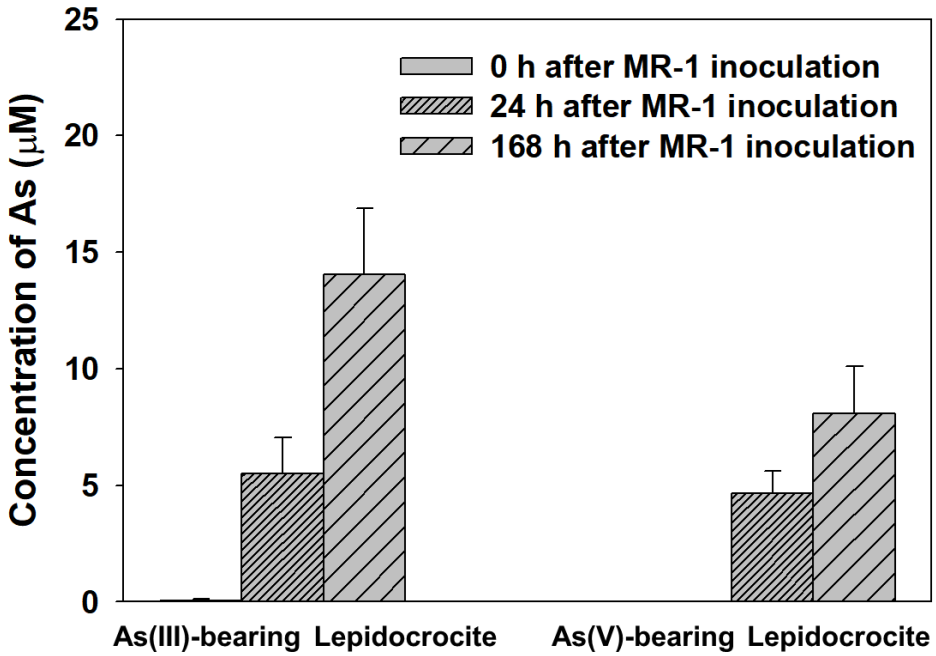

Figure S5. Concentration of total As in the aqueous phases released from As-bearing lepidocrocite by reduction of *S. oneidensis* MR-1 as a function of incubation time under anaerobic conditions. The medium contains As-bearing biogenic lepidocrocite preformed by *P. denitrificans* (ATCC 17741), 10 mM of lactate, and *S. oneidensis* MR-1. The total arsenic was analyzed for the remained arsenic in lepidocrocite formed after 7 d incubation of *P. denitrificans* with Fe(II), nitrate, and As, and also 24 h and 168 h after strain MR-1 inoculation.