Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Complexation by cysteine and iron mineral adsorption limit cadmium mobility during metabolic activity of *Geobacter sulfurreducens*

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Supplementary information

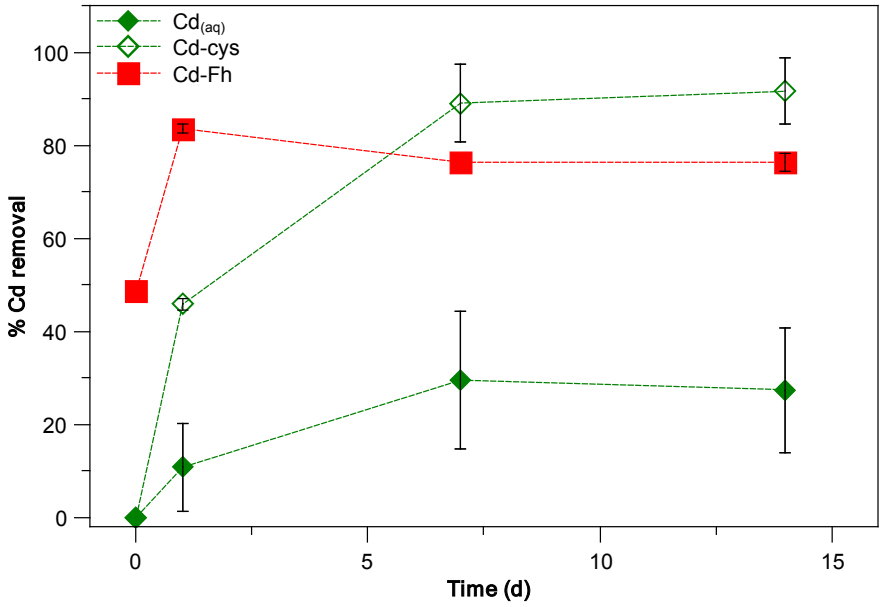

Figure S1. Cd geochemistry from microcosms used in CLSM experiments. Cd was quantified using MP-AES and all data shown is from reactors containing cells. $Cd_{(aq)}$ (◆) represents microcosms where 11 mg/L Cd was initially a free aqueous cation, Cd-cys (❋) represents microcosms where 11 mg/L Cd was initially complexed with cysteine and Cd-Fh (■) represents microcosms where 11 mg/L Cd was initally primarily adsorbed to Fh. Error bars are the standard deviation of duplicate reactors.

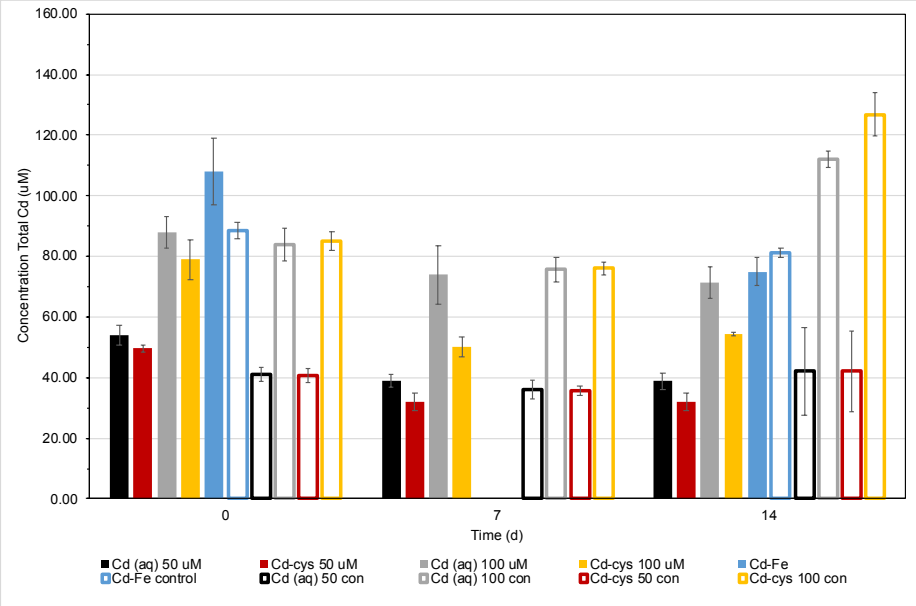

Figure S2. Total Cd was quantified by measuring Cd in samples taken from microcosms without separation of aqueous and solid phases. Samples were taken with a needle and syringe through a butyl stopper under anoxic conditions. Slurry samples were dissolved in 6 M HNO_3 and further diluted into 2% b.v. HNO_3 for quantification with the MP-AES.

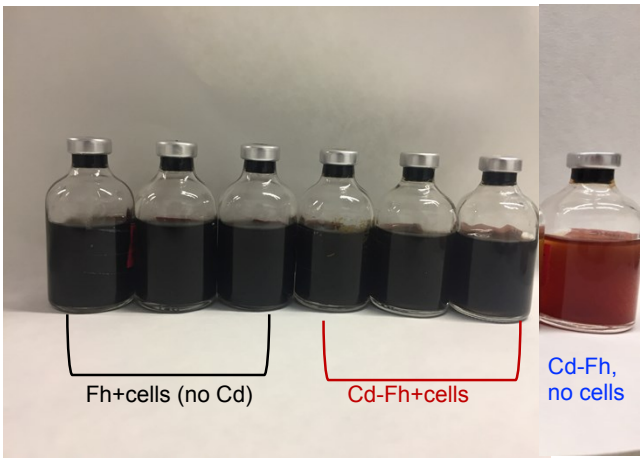

Figure S3. Fe microcosm reactors from 5 days of incubation, containing Fh+cells with no Cd (left side), Cd-Fh+cells (middle) and an example of an abiotic control reactor (right side).

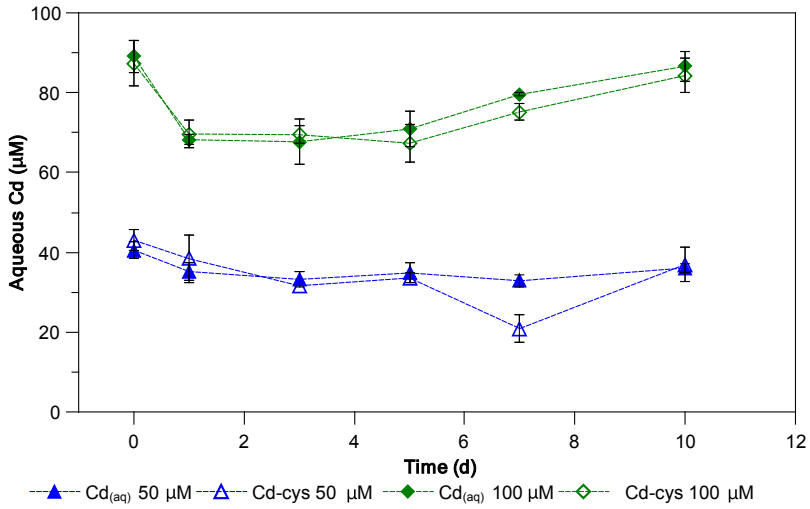

Figure S4. Abiotic Cd geochemistry controls from fumarate microcosms over 10 days. Aqueous Cd was measured over time in from microcosms containing only media, acetate and fumarate. Cd was initially present as an aqueous cation (□,◆) or complexed with cysteine (❐,❋). Error bars represent standard deviation from triplicate reactors.

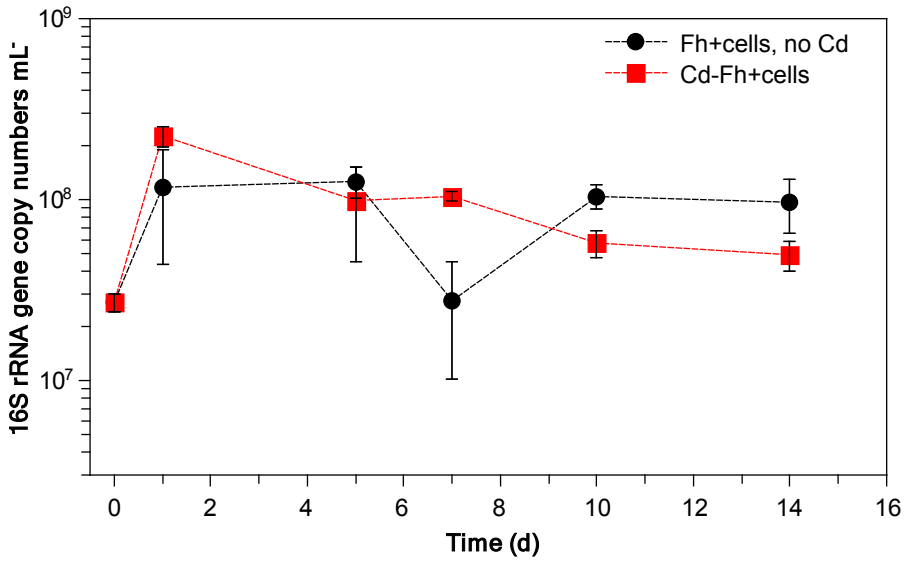

Figure S5. Gene copy numbers from Fe microcosm experiments. DNA was extracted using a DNeasy Power Soil Kit and purity was verified using Qubit® 2.0. The 16S rRNA gene copy numbers of *Geobacter* were amplified and quantified with qPCR using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) on a iQ5 Real Time PCR system (BioRad, Hercules, CA, USA). Fh+cells, no Cd (●) represents microcosms with *Geobacter sulfurreducens* and Fh but no Cd. Cd-Fh+cells (■) represents microcosms where 11 mg/L Cd was initally primarily adsorbed to Fh and *Geobacter sulfurreducens* was added. Error bars are representative of standard deviation from triplicate reactors.

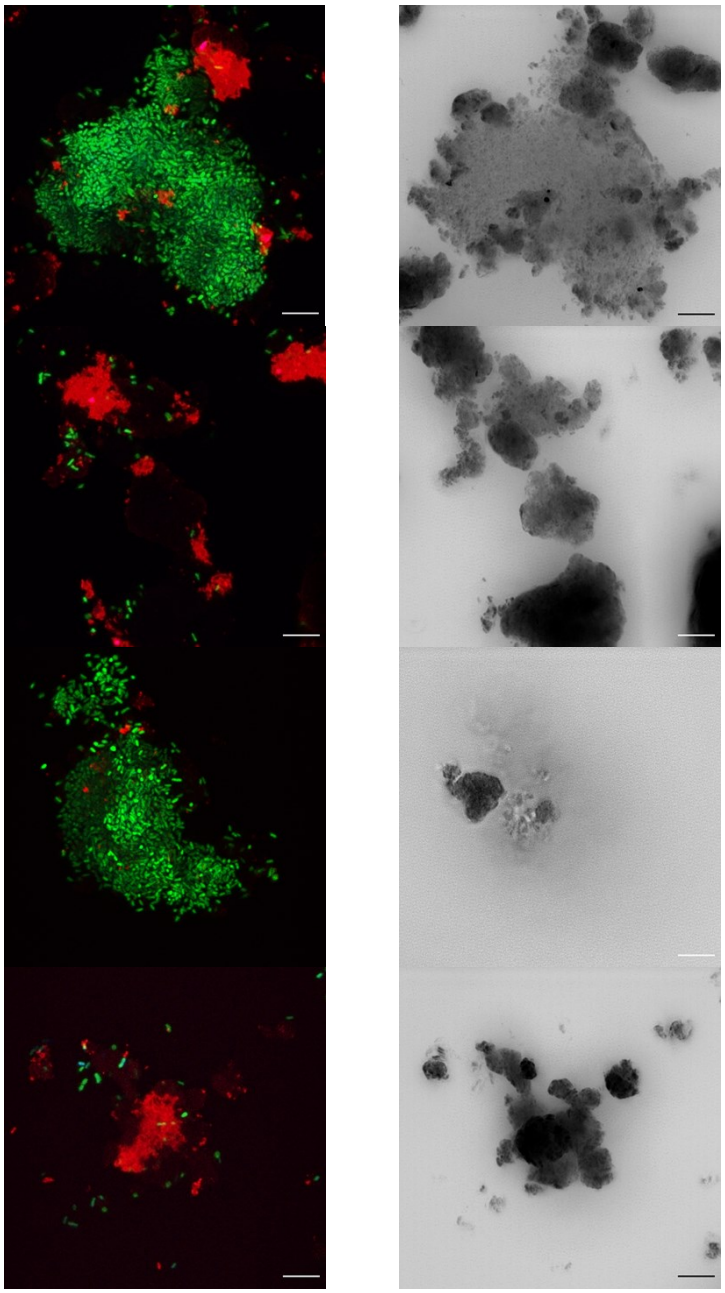

Figure S6. Confocal laser scanning micrographs from control (no Cd) Fe microcosms. Colored images represent fluorescence channels, with the sum of ConA and WGA lectins in red, the Syto 40 stain in green and the Cd Heliosense stain in blue. Black and white images are transmission data of the same area wherein the contrast is dominated by the absorbance of the Fe-minerals. Scale bars are 5 μm.

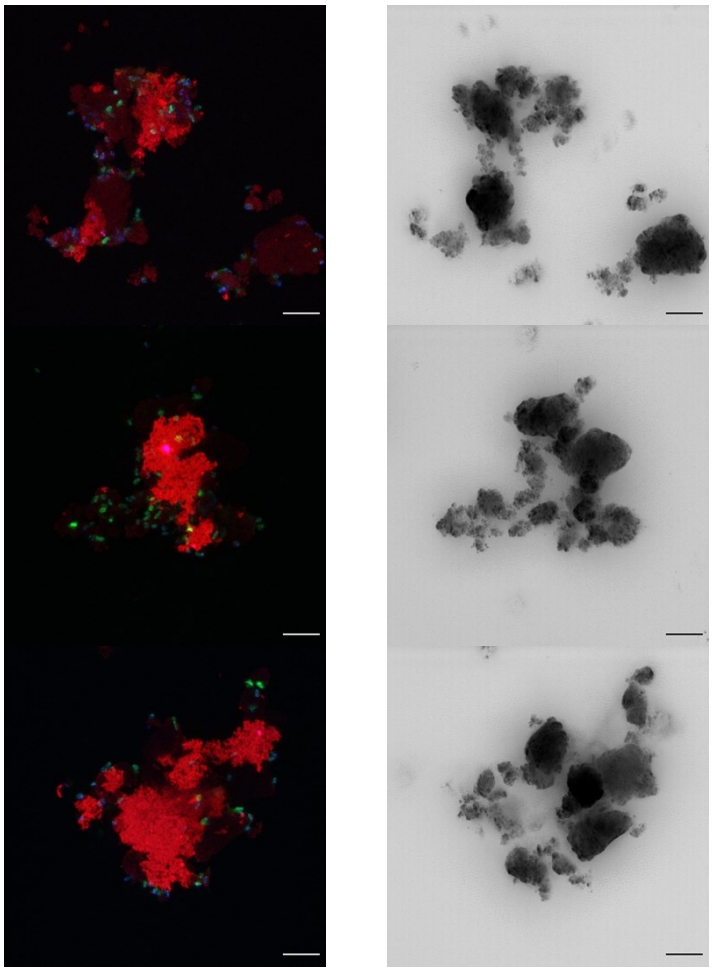

Figure S7. Confocal laser scanning micrographs from Cd-Fe microcosms. Colored images represent fluorescence channels, with the sum of ConA and WGA lectins in red, the Syto 40 stain in green and the Cd Heliosense stain in blue. Black and white images are transmission data of the same area wherein the contrast is dominated by the absorbance of the Fe-minerals. Scale bars are 5 μm.

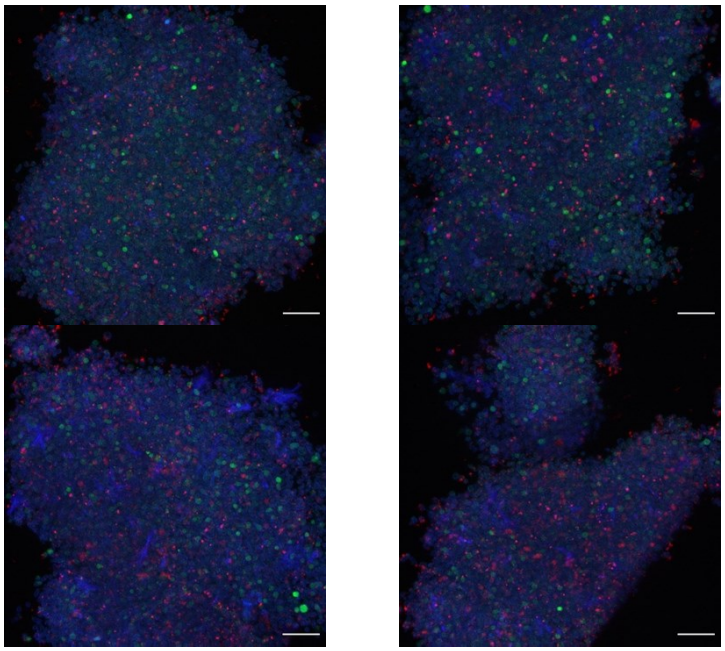

Figure S8. Confocal laser scanning micrographs from Cd aqueous microcosms. The sum of ConA and WGA lectin stains are shown in red, the Syto 40 stain is shown in green and the Cd Heliosense stain is shown in blue. Scale bars are 5 μm.

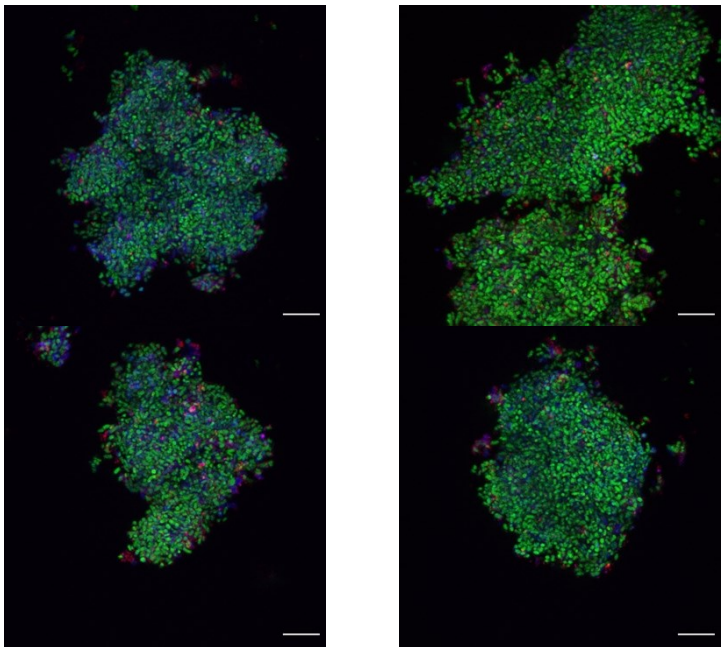

Figure S9. Confocal laser scanning micrographs from Cd-cysteine microcosms. The sum of ConA and WGA lectin stains are shown in red, the Syto 40 stain is shown in green and the Cd Heliosense stain is shown in blue. Scale bars are 5 μm.

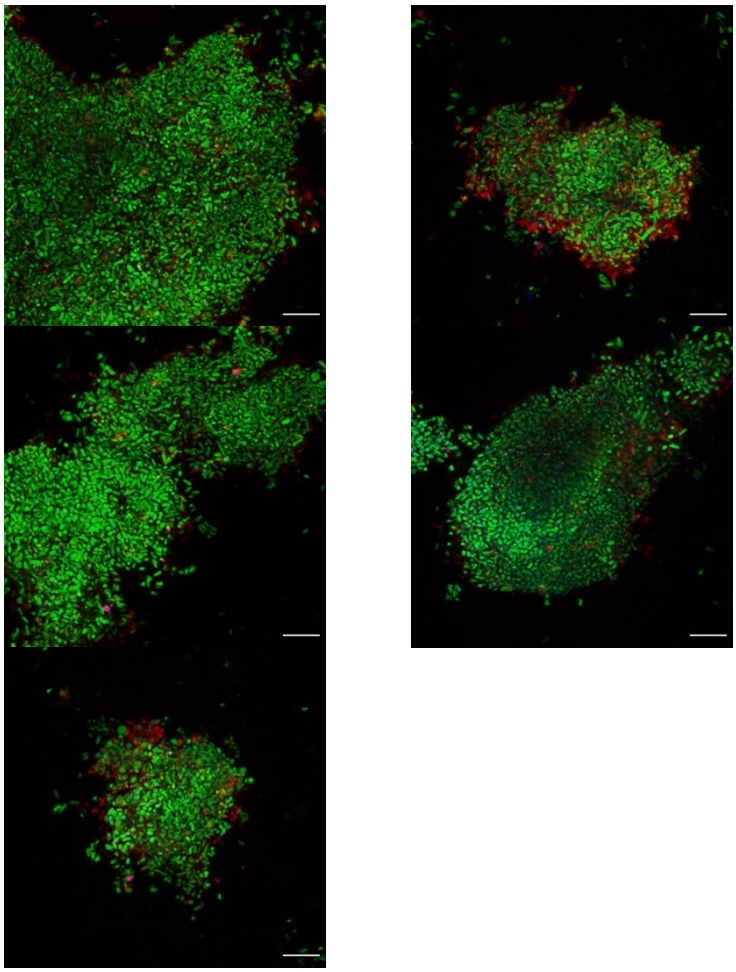

Figure S10. Confocal laser scanning micrographs from fumarate control (no Cd) microcosms. The sum of ConA and WGA lectin stains are shown in red, the Syto 40 stain is shown in green and the Cd Heliosense stain in blue. Scale bars are 5 μm.

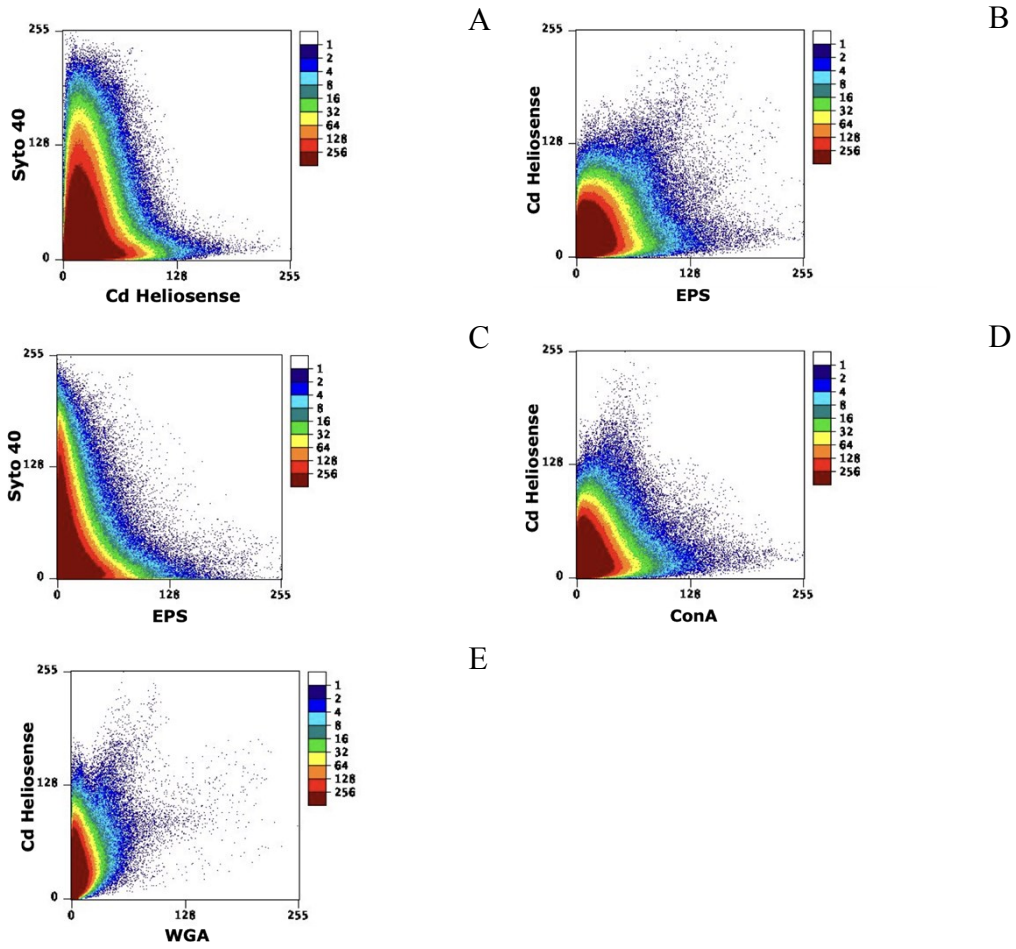

Figure S11. Example correlation analysis plots for the following fluorescence channels: a) Cd Heliosense vs. Syto 40, used to correlate Cd and cells, b) EPS vs. Cd Heliosense, used to correlate EPS and Cd, c) EPS vs. Syto 40, used to correlate EPS and cells d) ConA vs. Cd Heliosense, used to correlate specific glycoconjugates and Cd and e) WGA vs. Cd Heliosense, used to correlate specific glycoconjugates and Cd. The EPS channel was created by combining ConA and WGA channels. Scatterplots show the fluorescence intensity of individual pixels for the described dyes. Colors represent the frequency of occurrence of pixels with a certain intensity. Scatterplots shown here are from analysis performed on the micrograph in Figure 6b.

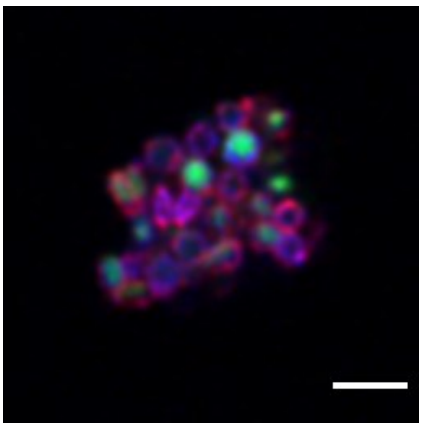

Figure S12. CLSM experiment in which the FM-4-64 stain (red) was used to dye lipid membranes and demonstrate the association of Cd (Cd Heliosense; blue) with cells (Syto 40; green). This is an example from the aqueous Cd set up. Distinct halos of Cd can be observed around cell structures and are highly associated with lipid membranes. The scale bar is 2 μm.

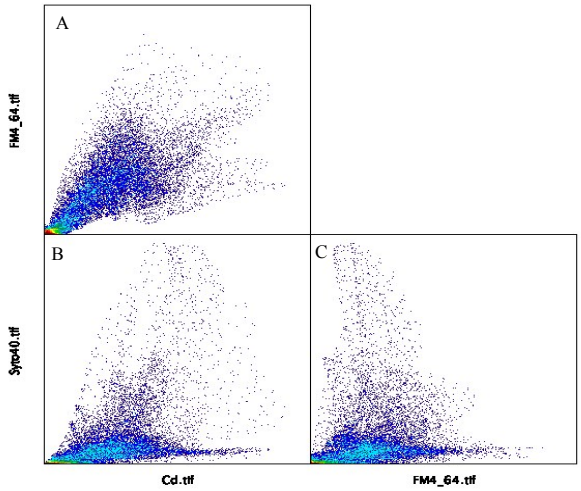

Figure S13. Correlation analyses between Cd and lipid membranes (A), Cd and cells (B) and lipid membranes and cells (C), corresponding to the image in figure S11. The heliosense Cd dye was used to stain Cd, FM-4-64 dye was used to stain lipid membranes and Syto 40 was used to stain cells.

Table S1. Pearson's coefficients for correlation analyses. Values shown are averages from 5-6 images and error is representative of standard deviation.

Condition	Cd vs. cells	EPS vs. Cd	EPS vs. cells	ConA vs. Cd	WGA vs. Cd
Fe control (no Cd)	N/A	N/A	-0.0007 ± 0.009	N/A	N/A
Fe-Cd	0.43 ± 0.06	0.31 ± 0.08	0.04 ± 0.03	0.27 ± 0.1	0.26 ± 0.2
Cd aqueous	0.72 ± 0.02	0.51 ± 0.04	0.35 ± 0.04	0.49 ± 0.03	0.19 ± 0.02
Cd cysteine	0.51 ± 0.05	0.56 ± 0.05	0.25 ± 0.03	0.48 ± 0.05	0.43 ± 0.04
Fumarate control (no Cd)	N/A	N/A	0.09 ± 0.06	N/A	N/A

Supplemental methods

Total DNA was extracted from 0.25 g of triplicate frozen samples and using the DNeasy Soil Power DNA Isolation Kit (QIAGEN, MD, USA). The quality and quantity of DNA were verified on 1% (w/v) ethidium-bromide-died agarose gels and fluorometric quantification with Qubit® 2.0. The 16S rRNA gene copy numbers of *Geobacter* were amplified and quantified with qPCR using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) on a iQ5 Real Time PCR system (BioRad, Hercules, CA, USA). As a standard, a 16S rRNA gene fragment from Geobacter spp. was used according to Muehe et al., 2013. In a 10 µL of reaction volume, 1 µL of 100-fold diluted DNA extract or a tenfold dilution series of the standard plasmid DNA were used with 1× SsoAdvanced Universal SYBR Green Supermix, 350 nM DSMO, 125nM of each primer (Geo577-F (5'-GCGTGTAGGCGGTTTSTTAA-3') and Geo822-R (5'-TACCCGCRACACCTAGTACT-3')). The qPCR program ran with 3 min at 98 °C, 40 cycles of 15 s at 98 °C and 30 s at 60 °C, and followed by melting curve analysis. The data analysis was performed using the StepOneTM 2.3 software. Each of three independent DNA extractions were measured in triplicates.

References

E. M. Muehe, M. Obst, A. Hitchcock, T. Tyliszczak, S. Behrens, C. Schröder, J. M. Byrne, F. M. Michel, U. Krämer and A. Kappler, *Environ. Sci. Technol.*, 2013, **47**, 14099–14109.