Supplemental Material

Bioreduction of biotite and chlorite by a *Shewanella* species

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Experimental details

Culturing bacteria

Shewanella oneidensis MR-1, subcultured from colonies cultivated on LB agar plates, was grown in 50 ml aerobic *Shewanella* Minimal Medium (SMM, (von Canstein et al., 2008)) in 250 ml conical flask at a temperature of 30°C and shaken at 130 rpm on an orbital shaker. At late log phase, the cells were transferred to 100 ml bottles containing anaerobic SMM (with lactate as the electron donor and fumarate as the electron acceptor), to a final OD_{600} of ~0.2, and incubated at 30°C.

The cultures were harvested via centrifugation using a Sigma 6K15 centrifuge at 5000 g relative centrifugal force (RCF) and 4°C for 20 minutes. The cells were washed twice with and re-suspended in 30 mM $NaHCO_3$ buffer under an atmosphere of N_2.

Fluorescence microscopy and cell enumeration

For fluorescence microscopy, three flakes of the biotite per experiment were extracted from the experimental bottles and secured on glass slides using a 1% agarose solution. They were stained with a 1:200 dilution of 0.1% solution of the fluorescent DNA dye Acridine orange in double-deionised water (Wilkins et al., 2007). After three minutes of staining, the excess solution was washed off with double-deionised water. The slides were viewed with a green fluorescence filter on a Zeiss microscope. Photographs of at least five different areas per flake were obtained for further analysis. Each photographed view-area was exposed to the light for less than two minutes to minimise any light-related bleaching of the dye. The photographs of microscope views were overlain with a 10 x 10 µm grid. The number of bacteria found in each square along the sixth column and row of squares on each photograph were counted.

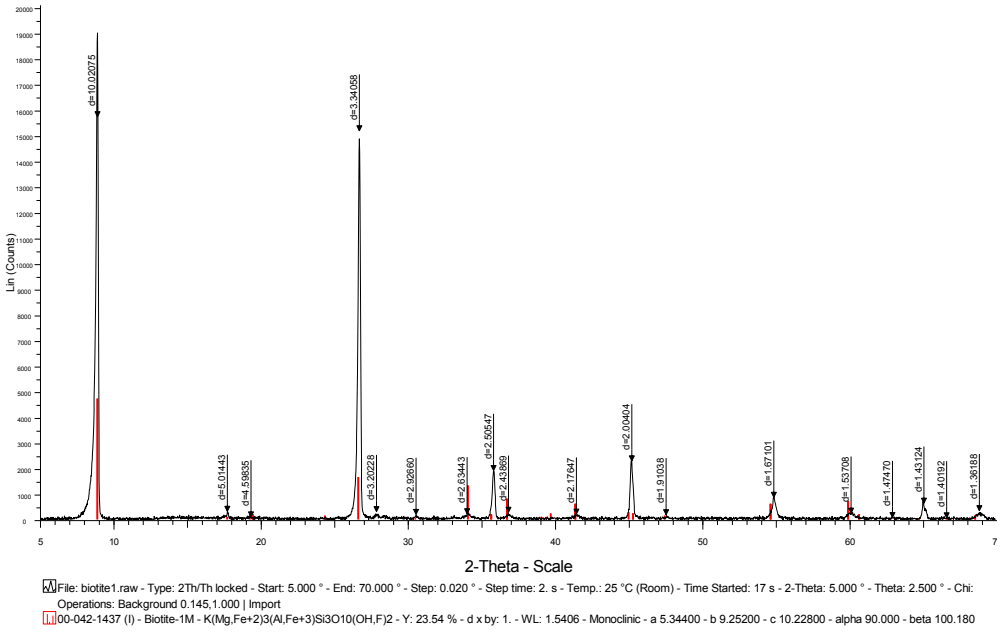

Figure S1 – XRD spectrum of powdered unaltered biotite

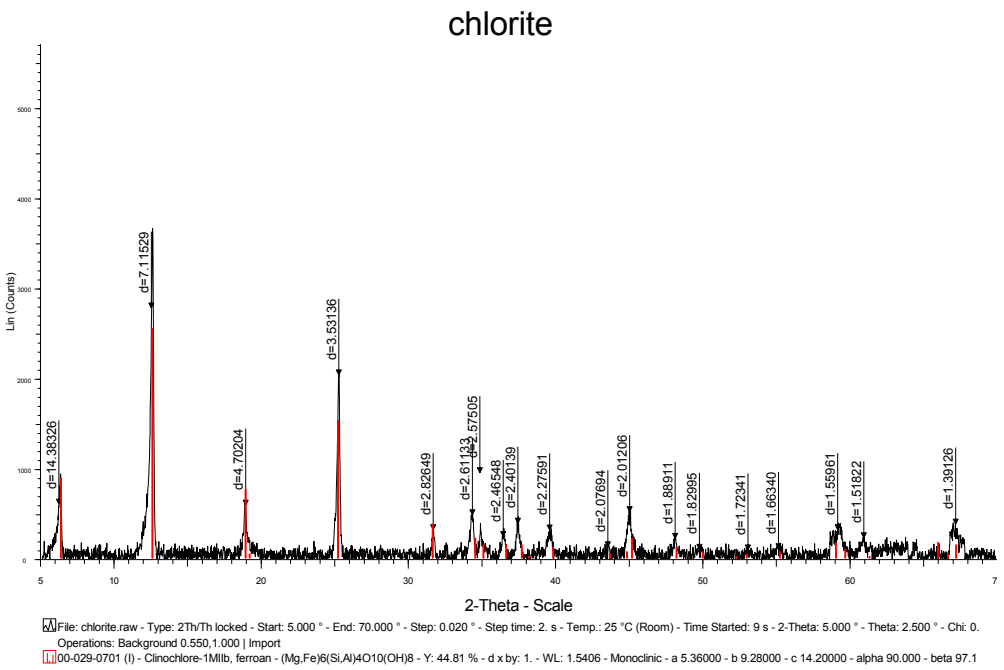

Figure S2 – XRD spectrum of powdered unaltered chlorite

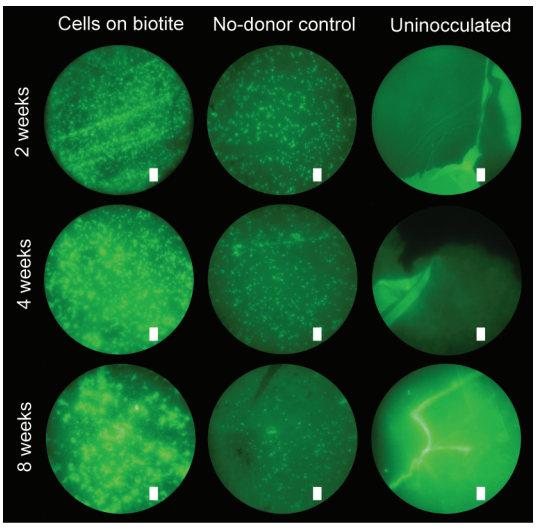

FIGURE S3. Fluorescence microscopy images of *S. oneidensis* MR-1 cells stained with acridine orange on biotite flakes. The figure shows cells grown with Fe(II) in biotite as the sole electron acceptor (left) at 2 weeks (top), 4 weeks (middle) and 8 weeks (bottom) after inoculation. No increase in cell numbers was observed in no-donor (middle) or abiotic (left) controls. White scale bar in bottom right of each image is 10 µM.

References:

von Canstein, H., Ogawa, J., Shimizu, S., and Lloyd, J.R. (2008) Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. Applied and Environmental Microbiology, 74(3), 615-623.

Wilkins, M.J., Wincott, P.L., Vaughan, D.J., Livens, F.R., and Lloyd, J.R. (2007) Growth of *Geobacter sulfurreducens* on poorly crystalline Fe(III) oxyhydroxide coatings. Geomicrobiology Journal, 24(3-4), 199-204.