Potential role of nitrite for abiotic Fe(II) oxidation and cell encrustation during nitrate reduction by denitrifying bacteria

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SUPPLEMENTAL INFORMATION

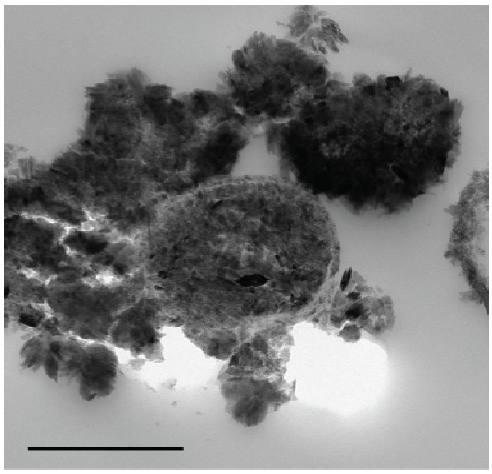

Figure S1: Resin section (TEM images) of cryofixed and freeze-substituted *Acidovorax* strain BoFeN1 grown in the presence of 10 mM nitrate, 5 mM acetate and ~8 mM Fe(II). Cells are not stained. Scale bar is 500 nm.

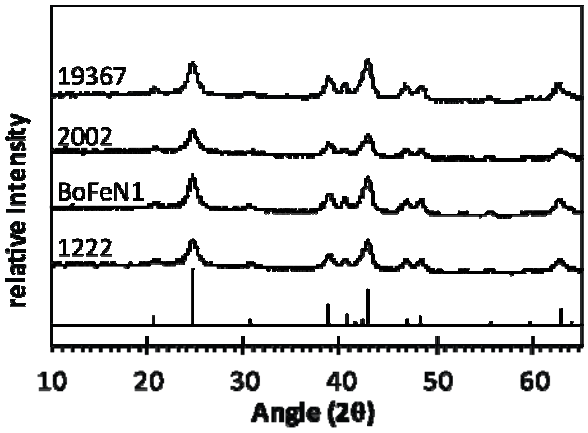

Figure S2: X-ray diffractogram of the mineral products formed within 11 days in the presence of ~8 mM Fe(II), 10 mM nitrate and 5 mM acetate by *Paracoccus denitrificans* ATCC 19367, *Pseudogulbenkiania* strain 2002, *Acidovorax* strain BoFeN1, and *Paracoccus denitrificans* Pd 1222. For comparison the specific reflections of goethite are shown at the bottom.

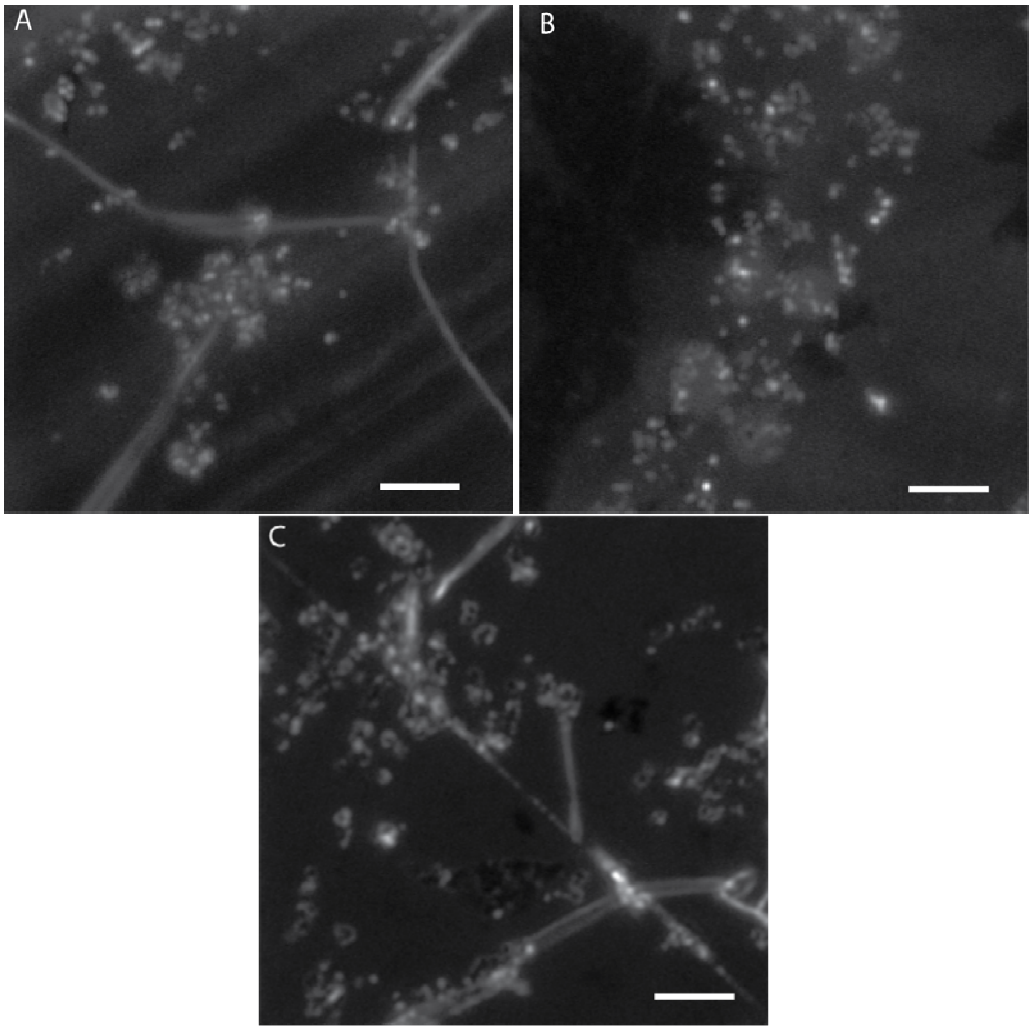

Figure S3: Fluorescence image of (A) *Acidovorax* strain BoFeN1, (B) *Pseudogulbenkiania* strain 2002 and (C) *Paracoccus denitrificans* ATCC 19367 grown in the presence of Fe(II). EPS in resin-sections was stained with WGA-Alexa Fluor® 633 conjugate directly on TEM grids. Bright color indicates the fluorescing EPS-shells. Scale bar is 10 µm.

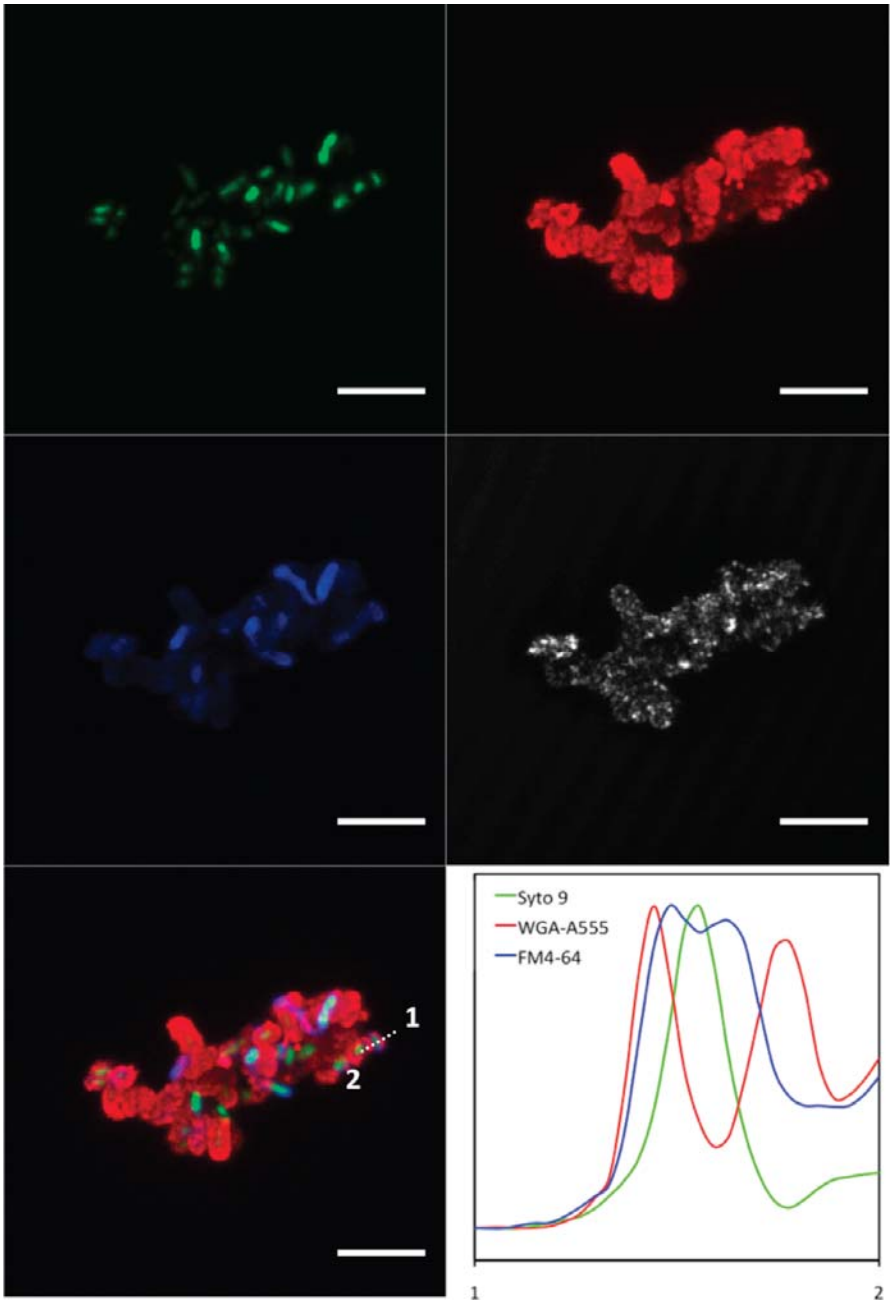

Figure S4: CLSM images of *Acidovorax* strain BoFeN1 incubated for seven days with 10 mM nitrate, 5 mM acetate and ~8 mM Fe(II). DNA was stained with Syto9 (green), EPS with WGA-Alexa Fluor® 555 conjugate (red), lipids with FM4-64 (blue), white is showing the reflection of minerals. The left panel in the bottom row shows the RGB-overlay of the first three channels. The right panel at the bottom shows a cross-section through one cell (marked by 1-2). Scale bars are 5 µm. Profile show relative intensities.

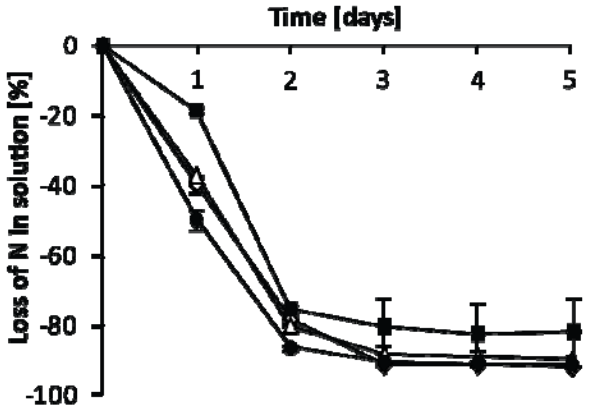

Figure S5: Loss of nitrogen from solution over time given in % and calculated by subtraction of measured nitrate and nitrite concentrations from the initial nitrate concentration. *Acidovorax* strain BoFeN1 (●), *Pseudogulbenkiania* strain 2002 (■), *Paracoccus dentrificans* ATCC 19367 (◇), and *Paracoccus dentrificans* 1222 (△).

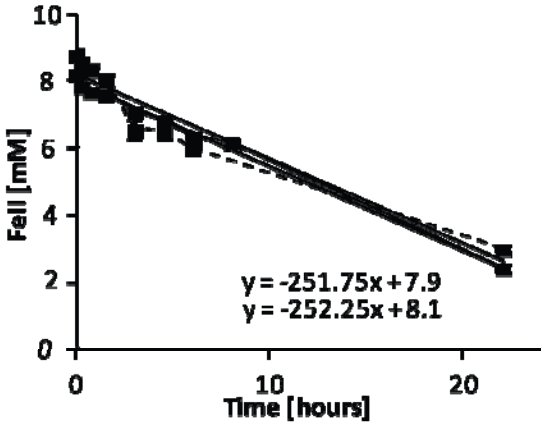

Figure S6: Fe(II) oxidation over time at 40°C in media containing cells of *Acidovorax* strain BoFeN1 (solid line) or sterile filtered media (dashed line). All cultures contained ~ 4 mM accumulated nitrite. The equations of the trend lines are given.

Supplemental information 1: Preparation of samples for the transmission electron microscopy image shown in figure 6 B.

Biofilms were collected off granitic bedrock in the tunnel at 432 m underground at the Äspö Hard Rock Laboratory near Oskarshamn, Sweden. Iron oxide precipitates develop directly on the granite at locations where neutral to slightly alkaline (pH 7.0-8.0) groundwater enters the tunnel through hydraulically conductive fracture zones (see Ferris et al., 1999 for details). The sample was recovered off the hard substrata with sterile scalpels and immediately placed in 5 mL metal-free plastic tubes containing aqueous 2.0% (vol/vol) glutaraldehyde. The biofilm samples were prepared for thin-sectioning by washing in a solution of 0.05 M N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) buffer (Research Organics Inc., Cleveland), pH 7.2, to remove excess glutaraldehyde. After washing, the samples were dehydrated through a graded acetone series and embedded in epoxy resin (Epon 812, CanEM, Guelph), as previously described by Graham and Beveridge (1990). Thin sections, approximately 60 nm in thickness, were obtained with a Reichert-Jung Ultracut E ultramicrotome, and mounted on Formvar and carbon-coated 200-mesh copper grids. To increase the electron contrast of cytoplasmic material inside intact cells, some thin sections were stained with 1% uranyl acetate before imaging in the electron microscope. Grids were viewed with a Philips EM400 Transmission electron microscope. The TEM was operated at 100 keV, with a liquid nitrogen-cooled anticontamination device in place.

References

Ferris, F.G., Konhauser, K.O., Lyvén, B., and Pedersen, K., 1999. Accumulation of metals by bacteriogenic iron oxides in a subterranean environment. Geomicrobiology Journal, 16:181-192.

Graham, L.L., and T.J. Beveridge. 1990. Evaluation of freeze-substitution and conventional embedding protocols for routine electron microscope processing of eubacteria. J. Bacteriol., 172:2141-2149.