Supplementary Methods

LC-MS/MS Methods

Trypsin-digested proteins were analyzed using a quadrupole time-of-flight mass spectrometer (Q-tof Premier, Waters, Milford, MA) that was connected in-line with an ultraperformance liquid chromatograph (nanoAcquity UPLC, Waters). The UPLC was equipped with C_{18} trapping (20 mm × 180 µm, 5 µm particles, Waters Symmetry) and analytical (100 mm × 100 µm, 1.7 µm particles, Waters BEH130) columns and a 10 µL sample loop. Solvents A and B were 99.9% water/0.1% formic acid and 99.9% acetonitrile/0.1% formic acid (v/v), respectively. Following sample injection, trapping was performed for 3 min with 100% A at a flow rate of 15 µL/min. The injection needle was washed with 500 µL of solvent A and 200 µL of solvent B. The elution program consisted of a linear gradient from 5% to 30% B over 80 min, a linear gradient to 95% B over 0.33 min, isocratic conditions at 95% B for 3.67 min, a linear gradient to 1% B over 0.33 min, and isocratic conditions at 1% B for 12.67 min, at a flow rate of 500 nL/min. The analytical column and sample compartment were maintained at 35 ºC and 8 ºC, respectively.

The UPLC column exit was connected to a nanoelectrospray ionization (nanoESI) emitter that was mounted in the ion source of the mass spectrometer. External mass calibration was performed immediately prior to analysis using a solution of sodium formate. Survey scans were acquired in the positive ion mode over the range m/z = 350-1500 using a 0.45 s scan integration and a 0.05 s interscan delay. In the data-dependent mode, up to 7 precursor ions exceeding an intensity threshold of 30 counts per second (cps) were selected from each survey scan for tandem mass spectrometry (MS/MS) analysis. Real-time deisotoping and charge state recognition were used to select 1+ through 6+ charge state precursor ions for MS/MS. Collision energies for collision-induced dissociation were automatically selected based on the mass and charge state of a given precursor ion. MS/MS spectra were acquired over the range m/z = 100-2000 using a 0.15 s scan integration and a 0.05 s interscan delay. Ions were fragmented to achieve a minimum total ion current (TIC) of 50,000 cps in the

cumulative MS/MS spectrum for a maximum of 1.5 s. Real-time dynamic exclusion was used to preclude re-selection of previously analyzed precursor ions (exclusion width of ±0.3 *m*/*z* unit).

Data were searched against the *Acidovorax ebreus* (formerly *Diaphorobacter sp.*) strain TPSY protein database (Microbial Genomics Program, Joint Genome Institute, U.S. Department of Energy) using ProteinLynx Global Server software (version 2.3, Waters) with the following criteria: precursor ion mass tolerance 100 ppm, fragment ion mass tolerance 0.25 Da, digest reagent trypsin, allowing for up to three missed cleavages by trypsin, carbamidomethylcysteine as a fixed modification and methionine oxidation as a variable modification. The measurement of at least three consecutive fragment ions from the same series, i.e., b or y-type fragment ions, was required to validate assignment of a peptide to an MS/MS spectrum.

UV-Vis Spectroscopy

Difference absorbance spectra of whole cell suspensions treated with Fe(II)-NTA or NTA followed by NO_3^- were collected on a Varian Cary 50 Bio spectrophotometer according to previously published methods (1, 2). Anoxic cell suspensions from organotrophic growth cultures were resuspended in anoxic basal bicarbonate buffered media. Fe(II)-NTA, NTA and sodium nitrate were added from anoxic sterile stock solutions to final concentrations of 10 mM. UV-Visible absorbance spectra were recorded (1) 30 minutes after NTA or Fe(II)-NTA addition and (2) 30 minutes after subsequent sodium nitrate addition. The second spectrum was subtracted from the first to observe *c*-type cytochromes which were reduced by Fe(II)-NTA, then re-oxidized by nitrate.

Supplementary Figures:

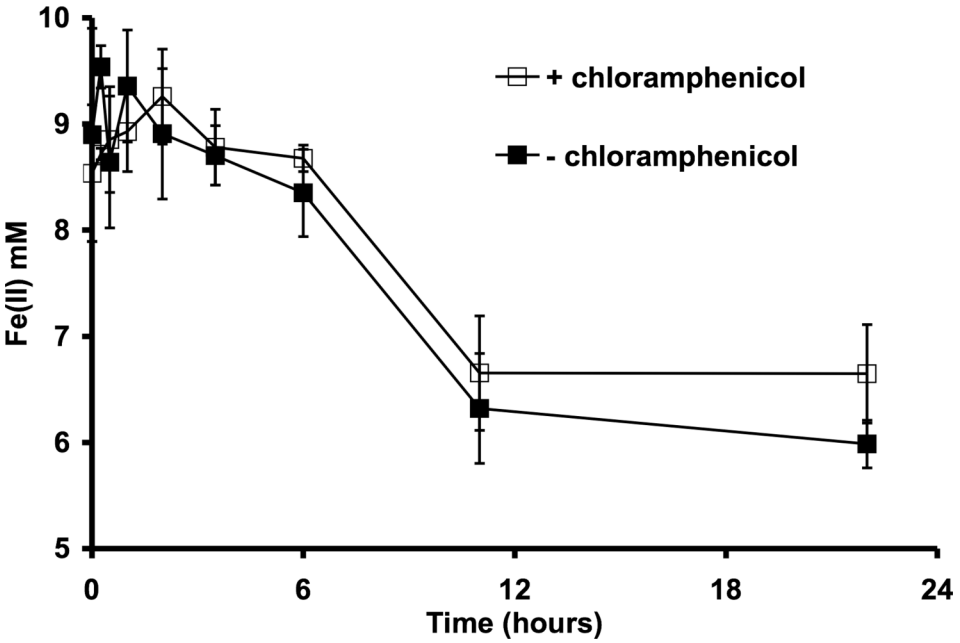

Fig. S1. Concentration of total Fe(II) (sum of soluble and insoluble) in washed NDFO cell suspensions (10 mM Fe(II), 10 mM nitrate) containing identical numbers of *Acidovorax ebreus* cells in the presence or in the absence of chloramphenicol.

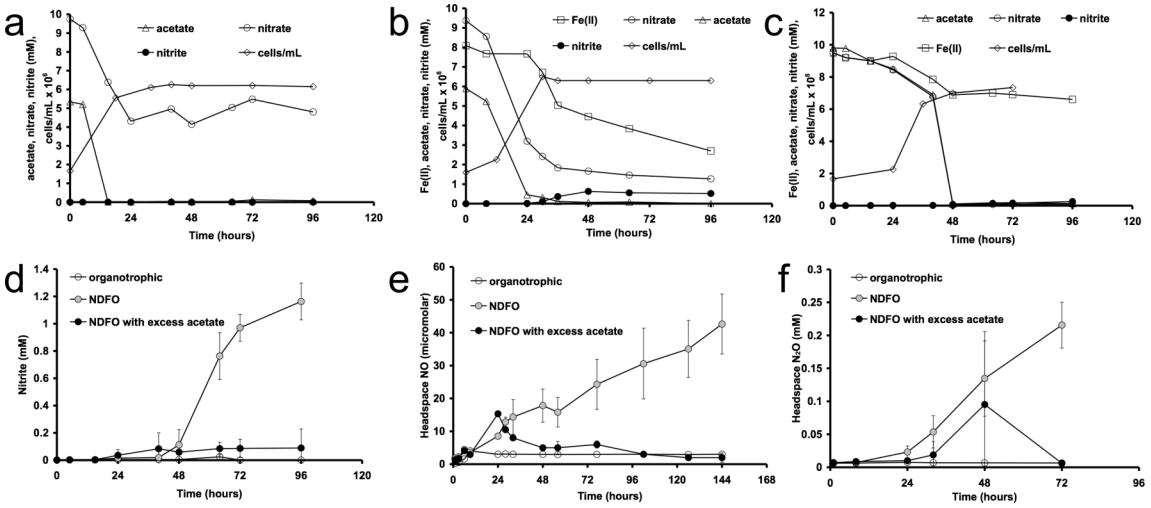

Fig. S2. **a-c**. Concentrations of acetate, nitrate, nitrite, cells, and, when present, total Fe(II) in representative growth cultures of *Acidovorax ebreus* **a.** organotrophic (5 mM acetate, 10 mM nitrate) **b.** NDFO with excess nitrate (5 mM acetate, 10 mM nitrate, 10 mM Fe(II)). **c.** NDFO with excess acetate (10 mM acetate, 10 mM nitrate and 10 mM Fe(II)). **d.** nitrite, **e.** headspace nitric oxide and **f.** nitrous oxide concentrations in organotrophic, NDFO with excess nitrate and NDFO with excess acetate growth cultures. Points represent averages and error bars represent the standard deviations of triplicates.

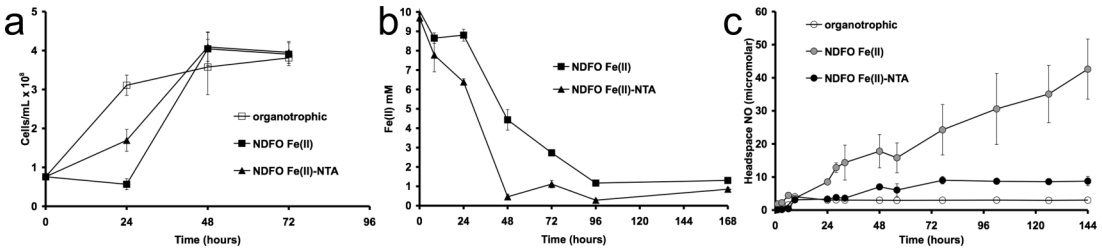

Fig. S3. **a.** Growth of *Acidovorax ebreus* expressed as cells/mL in organotrophic (5 mM acetate, 10 mM nitrate), NDFO (5 mM acetate, 10 mM nitrate, 10 mM Fe(II) as soluble and vivianite), and NDFO Fe(II)-NTA (5 mM acetate, 10 mM nitrate, 10 mM Fe(II)-NTA) growth cultures. **b.** Total Fe(II) concentrations over time in NDFO growth cultures of *A. ebreus* containing 10 mM Fe(II) or 10 mM Fe(II)-NTA. **c.** Concentration of nitric oxide in growth cultures of *Acidovorax ebreus* under organotrophic (5 mM acetate, 10 mM nitrate) and NDFO (10 mM Fe(II), 5 mM acetate, 10 mM nitrate) Fe(II) or Fe(II)-NTA growth conditions. Points represent averages and error bars represent the standard deviations of triplicates.

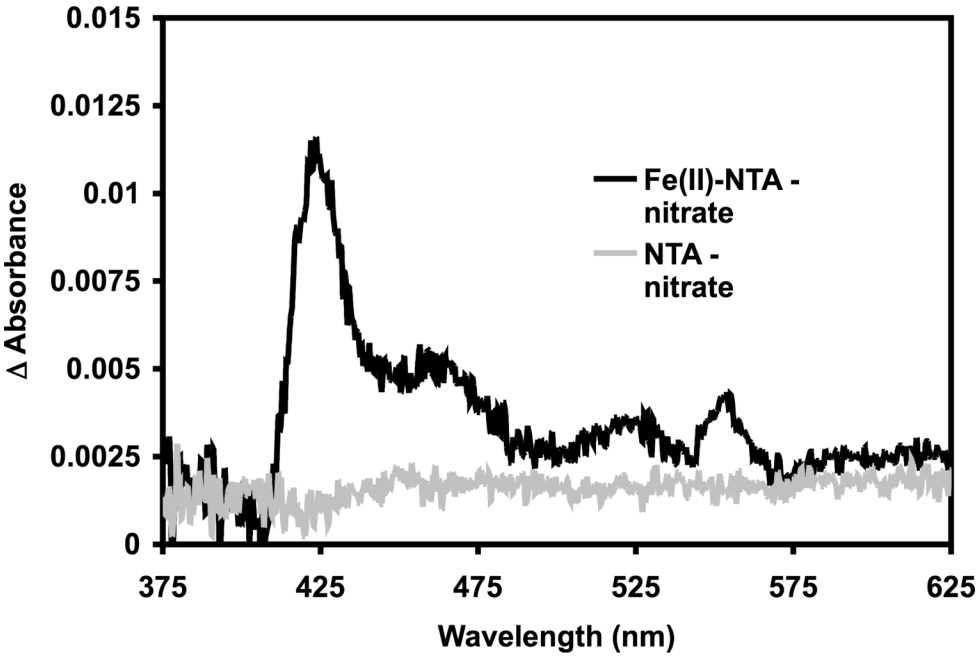

Fig. S4. Whole-cell oxidized-minus-reduced absorbance difference spectra of *A. ebreus*. Gray trace, difference spectrum of cells treated with 10 mM NTA for 30 minutes and a spectrum recorded followed by 10 mM NO_3^- for 30 minutes and a spectrum recorded. Black trace, difference spectrum of cells treated with 10 mM Fe(II)-NTA for 30 minutes and a spectrum recorded then 10 mM NO_3^- for 30 minutes and a spectrum recorded. Absorbance maxima at 424, 526, and 553 nm are indicative of *c*-type cytochromes.

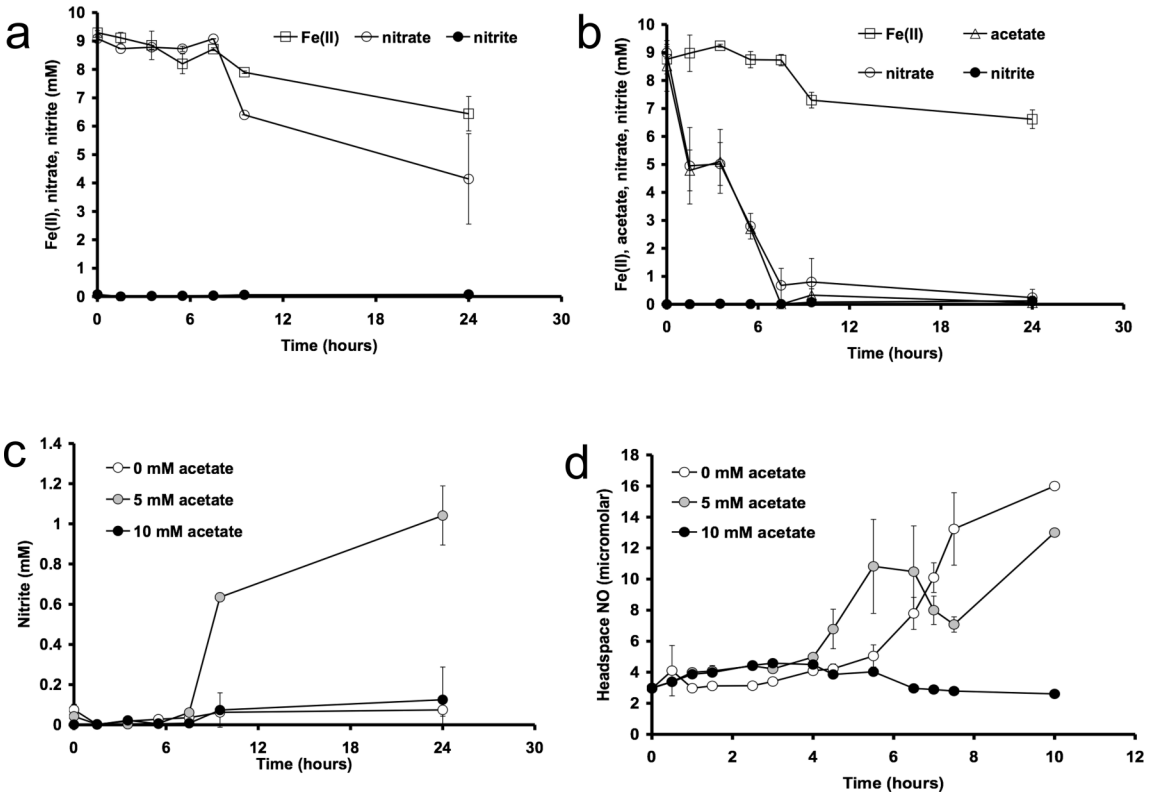

Fig. S5. **a-b**. Concentration of total Fe(II), acetate, nitrate and nitrite in NDFO cell suspensions (10 mM Fe(II), 10 mM nitrate) of *Acidovorax ebreus* in the presence of chloramphenicol and (**a**) 0 mM acetate or (**b**) 10 mM acetate. **c-d**. Concentration of (**c**) nitrite and (**d**) headspace nitric oxide in NDFO cell suspensions of *Acidovorax ebreus* (10 mM Fe(II), 10 mM nitrate) and 0 mM acetate, 5 mM acetate or 10 mM acetate. Points represent averages and error bars represent the standard deviations of triplicates.

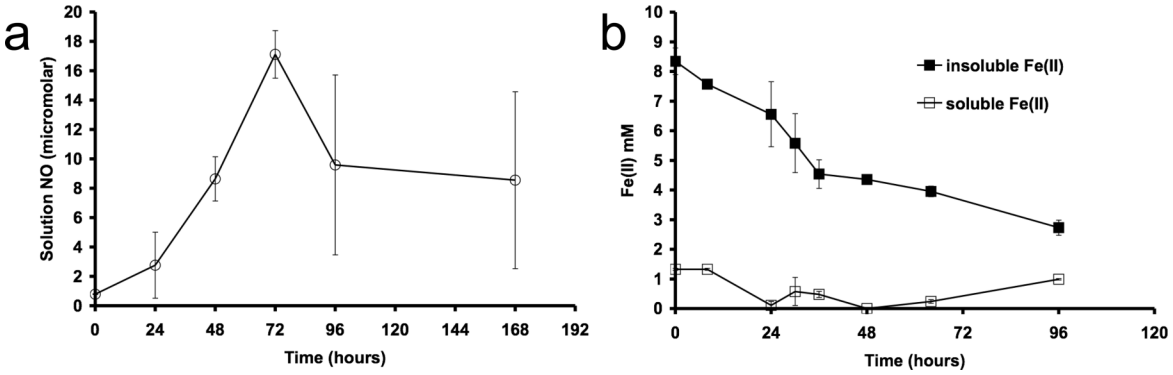

Fig. S6. a. Concentration of solution nitric oxide in NDFO growth cultures (10 mM Fe(II), 5 mM acetate, 10 mM nitrate) of *Acidovorax ebreus*. **b**. Concentration of soluble Fe(II) and insoluble Fe(II) in NDFO growth cultures (10 mM Fe(II), 5 mM acetate, 10 mM nitrate) of *Acidovorax ebreus*. Points represent averages and error bars represent the standard deviations of triplicates.

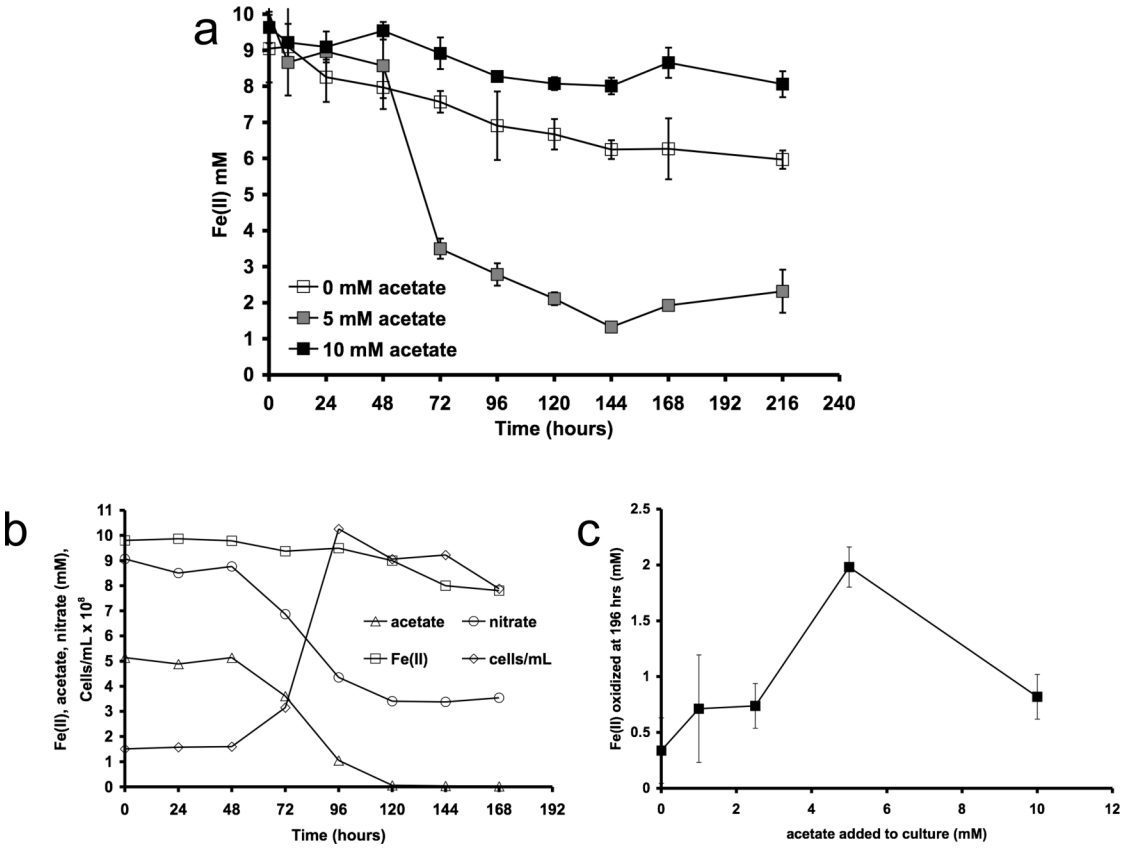

Fig. S7. a. Concentration of total Fe(II) in growth cultures of *Acidovorax ebreus* with 10 mM Fe(II), 10 mM nitrate and either 0 mM acetate, 5 mM acetate or 10 mM acetate. Points represent averages and error bars represent the standard deviations of triplicates. **b**. Representative growth data expressed as cells/mL and concentrations over time of acetate, nitrate and Fe(II) in NDFO growth cultures (10 mM Fe(II), 5 mM acetate, 10 mM nitrate) of *Azospira suillum.* **c.** Fe(II) oxidized after 196 hrs in NDFO growth cultures (10 mM Fe(II), 10 mM nitrate) of *Azospira suillum* for different initial concentrations of acetate (0-10 mM) added.

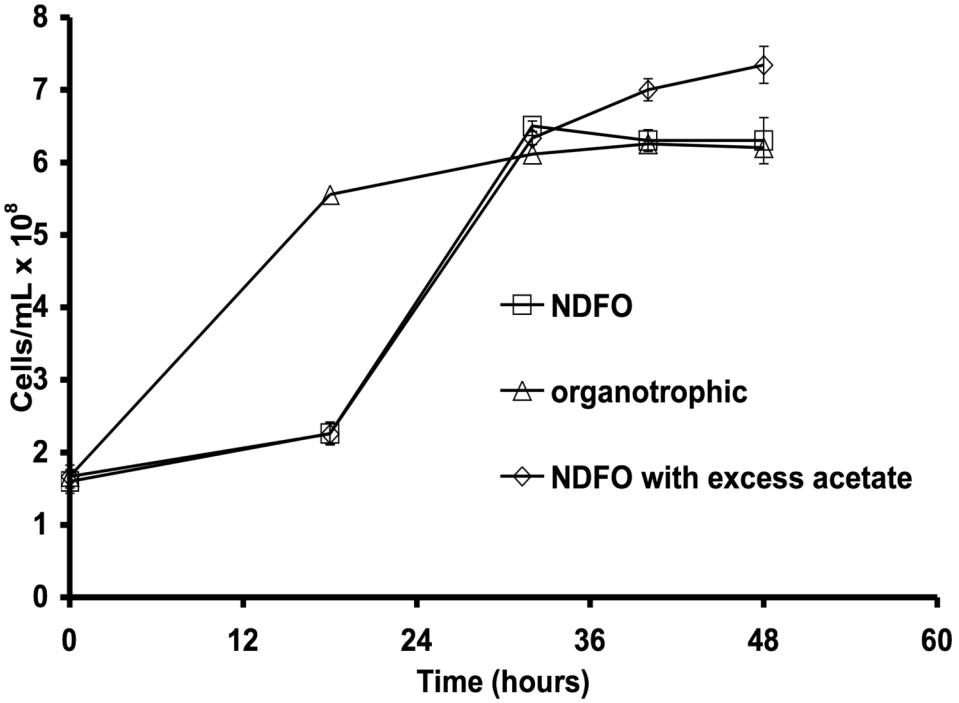

Fig. S8. Representative growth data expressed as cells/mL for *Acidovorax ebreus* in NDFO, organotrophic and NDFO with excess acetate cultures. Organotrophic (5 mM acetate, 10 mM nitrate), NDFO (10 mM Fe(II), 5 mM acetate, 10 mM nitrate), NDFO with excess acetate (10 mM Fe(II), 10 mM acetate, 10 mM nitrate).

Dataset S1. Tab A. Proteomics data sorted by accession #. Tab B. Proteomics data sorted by p-value. Tab C. Proteomics data sorted by NDFO to organotrophic ratio. Tab D. The primary proteomic change in NDFO cultures is a stress response. *Acidovorax ebreus* proteins displaying the greatest increase in peptide counts in NDFO growth cultures (10 mM Fe(II), 5 mM acetate, 10 mM nitrate) compared to organotrophic growth cultures (5 mM acetate, 10 mM nitrate) when all normalized peptide counts, after combining all timepoints, are compared. Proteins in the table had at least 7 peptides observed across the experiment and greater than 2.5 fold more peptides observed in a condition. Total number of peptides observed in all samples for a given peptide, ratio of normalized peptides observed in NDFO cultures versus organotrophic cultures and p-values from Student's t-test are reported.

Tab E. Extracytoplasmic proteins upregulated in NDFO cultures. Extracytoplasmic proteins for which more peptides were observed in NDFO cultures (10 mM Fe(II), 5 mM acetate, 10 mM nitrate) compared to organotrophic cultures (5 mM acetate, 10 mM nitrate). Proteins were predicted to be extracytoplasmic based on Joint Genome Institute (JGI) annotation, and the presence of export signals was confirmed using the SignalP 4.0 server (http://www.cbs.dtu.dk/services/SignalP/). Alongside the annotation provided by the JGI as part of the genome sequencing project, a putative function is assigned to each protein based on BLAST searches and homology to proteins of known function.

References Cited

1. Chaudhuri SK, Lack JG, & Coates JD (2001) Biogenic magnetite formation through anaerobic biooxidation of Fe(II). *Appl Environ Microbiol* **67,** 2844-2848.
2. Bruce RA, Achenbach LA, & Coates JD (1999) Reduction of (per)chlorate by a novel organism isolated from paper mill waste. *Environ Microbiol* **1,** 319-329.