SUPPLEMENTAL INFORMATION Chakraborty and Picardal 2012





PIPES-buffered AGW medium with **no cells**, 5 mM nitrate, 2.5 mM nitrite, 1.5 mM acetate and 5 mM Fe(II)-EDTA. Incubated in dark at 30 ^oC.





PIPES-buffered AGW medium with **no cells**, 5 mM nitrate, 2 mM nitrite, 1.5 mM acetate and 5.2 mM Fe(II)-NTA. Incubated in dark at 30 ^oC.

Supplemental Figure S1: Abiotic reaction of NO_x^- with (A) Fe(II)-EDTA or (B) Fe(II)-NTA. Since live NDFO reactors can contain both NO_3^- and NO_2^- , replicates contained both NO_2^- and NO_3^- to simulate conditions when both compounds are present. Data show the mean of 3 replicates and error bars represent the standard deviation.

| Speciation of Fe and EDTA | Medium B (mM) | Medium C (mM) |
|--|------------------------|-------------------------|
| EDTA ⁴⁻ | 1.1 x 10 ⁻⁹ | 1.5 x 10 ⁻¹¹ |
| Fe^{2+} | 1 x 10 ⁻² | |
| Fe ^(II) EDTA ²⁻ | 4.93 | |
| Fe ^(II) OHEDTA ³⁻ | 5×10^{-2} | |
| Fe ³⁺ | | 6×10^{-12} |
| Fe ^(III) EDTA ⁻ | | 3.1 |
| $\operatorname{Fe}^{(\mathrm{III})}_{2}(\mathrm{OH})_{2}(\mathrm{EDTA})_{2}^{4}$ | | 4.9 x 10 ⁻¹ |
| Fe ^(III) OHEDTA ²⁻ | | 9.4 x 10 ⁻¹ |
| CaEDTA ²⁻ | 2×10^{-2} | 2.6 x 10 ⁻⁴ |
| MgEDTA ²⁻ | 8.3 x 10 ⁻⁴ | 1.2 x 10 ⁻⁵ |
| NaEDTA ³⁻ | 5.9 x 10 ⁻⁹ | 8.4 x 10 ⁻¹¹ |

Supplemental Table S1: Tabular representation of the concentrations (mM) of predominant Fe and EDTA species in media B and C at 25 °C and at pH 7, determined by the Visual MINTEQ version 3.0 software. Media B and C were amended with 5 mM Fe(II)-EDTA and 5 mM Fe(III)-EDTA, respectively.

Effect of different CAP concentrations on growth and oxygen utilization of *Acidovorax* sp. 2AN in aerobic medium

Batch growth experiments were performed in duplicate to examine the effects of a range of chloramphenicol (CAP) concentrations (15-100 μ M) on aerobic growth of strain 2AN. The basal medium used was a PIPES-buffered, aerobic medium with 10 mM acetate. Since the stock solution of CAP was prepared in 95% ethanol, we used suitable controls to evaluate the effects of ethanol. Three lowest concentrations of CAP (30, 35 and 40 μ M) that greatly retarded or prevented growth were further examined for their effects on rate of oxygen utilization during aerobic growth of both strains (Fig. S1a). Finally, one CAP concentration (30 μ M) that showed the least effect on the oxygen utilization rate of the strains was chosen for use in the cell suspension assay (Fig. S1b). Similar results were obtained for *Dechloromonas* sp. UWNR4 (data not shown).



Supplemental Figure S2: (a) Effect of different CAP concentrations on aerobic growth of *Acidovorax* sp. 2AN in duplicate batch cultures. Two sets of controls (C1, C2) were used. C1 contained no ethanol and no CAP. C2 contained 4.12 mM ethanol and no CAP. All other replicates contained 4.12 mM ethanol. (b) Effect of 30, 35 and 40 μ M CAP on oxygen utilization of strain 2AN in duplicate batch reactors. Data were normalized by dividing the % O₂ values by the OD₆₀₀ of the sample used. When not shown, error bars are smaller than symbol size.





Supplemental Figure S3: Fe(II)-EDTA oxidation in cell suspensions of resting cells of (a) *Acidovorax* sp. 2AN and (b) *Dechloromonas* sp. UWNR4. Figures show Fe(II) oxidation in reactors containing cells that were previously incubated with Fe(II)-EDTA (closed symbols, medium B inoculum) or with Fe(III)-EDTA (open symbols, medium C inoculum). The cell suspensions contained neither CAP nor ethanol. Via comparison with data in figures 3c and 3d which lacked CAP but contained 1.2 mM ethanol, these data were used to evaluate the effects of 1.2 mM ethanol used as a solvent in the CAP stock solution. Data are presented as mean \pm standard deviations (n=3). When not shown, error bars are smaller than the symbol size.

| Medium | CAP | Strain | Fe(II) oxidation rate | R ² values | Time period (h) |
|--------|-------|--------|-----------------------|-----------------------|-----------------|
| | (µM) | | (mM/hr) | | |
| В | 0 | 2AN | 0.83 ± 0.05 | 0.9427 | 1 - 4 |
| С | 0 | 2AN | 0.58 ± 0.03 | 0.9431 | 1 - 6 |
| В | 30 | 2AN | 0.47 ± 0.02 | 0.9158 | 0.5 - 6 |
| В | 0 | UWNR4 | 0.67 ± 0.02 | 0.9573 | 0.5 - 6 |
| С | 0 | UWNR4 | 0.43 ± 0.02 | 0.9475 | 2 - 8 |
| В | 30 | UWNR4 | 0.69 ± 0.07 | 0.8503 | 2 - 5 |

Supplemental Table S2: Tabular representation of mean Fe(II)-EDTA oxidation rates (mM/hr) determined by linear regression analysis of the changes in Fe(II)-EDTA concentrations over time. The regression was done using 3 to 6 hours of data (time period shown) for linear portions of curves depicted in Figures 3 and S3. Media B and C were amended with 5 mM Fe(II)-EDTA and 5 mM Fe(III)-EDTA, respectively.