SUPPORTING INFORMATION

Identifying and quantifying the intermediate processes during nitrate dependent iron(II) oxidation

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Table S1: Thermodynamic Gibbs free energies for potential enzymatic NDFO where nitrate reduction is coupled to Fe oxidation. Bolded reactions are those used in this study for calculating the thermodynamic kinetic term $F_{T.}$

$10Fe^{2+} + 2\,NO_3^- + 24\,H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18\,H^+$	1
$30Fe^{2+} + 5HCO_3^- + 2NO_3^- + 54\,H_2O \rightarrow 5Fe_4^{II}Fe_2^{III}(OH)_{12}CO_3 + N_2 + 53\,H^+$	2
$\mathbf{2\,Fe^{2+} + NO_3^- + 5H_2O \rightarrow 2\,Fe(OH)_3 + NO_2^- + 4\,H^+}$	**3**
$\mathbf{6Fe^{2+} + HCO_3^- + NO_3^- + 11\,H_2O \rightarrow Fe_4^{II}Fe_2^{III}(OH)_{12}CO_3 + NO_2^- + 11\,H^+}$	**4**
$8\,Fe^{2+} + 2\,NO_3^- + 19H_2O \rightarrow 8\,Fe(OH)_3 + N_2O + 14\,H^+$	5
$24Fe^{2+} + 4HCO_3^- + 2NO_3^- + 43\,H_2O \rightarrow 4Fe_4^{II}Fe_2^{III}(OH)_{12}CO_3 + N_2O + 42\,H^+$	6

$^1\Delta G^{o\prime} = -100.4\frac{kJ}{mol}$, $^2\Delta G^{o\prime} = -50.47\frac{kJ}{mol}$, $^3\Delta G^{o\prime} = -69.9\frac{kJ}{mol}$, $^4\Delta G^{o\prime} = -40$

$53\ kJ/mol$, $^5\Delta G^{o\prime} = -85.8\ kJ/mol$, $^6\Delta G^{o\prime} = -45.8\ kJ/mol$

Table S2: Theoretical substrate conversion and growth stoichiometry for *Acidovorax* sp. strain BoFeN1 and *Paracoccus denitrificans* strain Pd 1222 from literature data sets used in this study for development of biogeochemical model. Chemodenitrification is ignored giving a conservative estimate of the electrons available for nitrate reduction. Molar concentration of biomass growth are calculated values taken from models that assume a biomass yield coefficient of 13.92 g per mol of acetate, a representative cell formula of $C_5H_7O_2N$ and a cell mass of 0.1 pg/cell.

Electron Balance (Supplied/Consumed)	**Experiments with varying acetate for BoFeN1**				***Paracoccus denitrificans***
Acetate(mM)	0	2	5	5	5
Electrons Supplied					
Cell Mass[#] (mM)		2.7E-04	6.4E-04	6.2E-04	3.63E-04
Acetate assimilated (mM)[*]		0.67	1.60	1.54	0.91
Acetate Oxidized (mM)		1.33	3.40	3.46	4.09
Acetate (electrons)		10.63	27.22	27.70	32.74
FeSO4 (electrons)		2.00	8.00	6.50	6.50
Total Electrons Supplied		12.6	35.2	34.2	39.2
Electrons Consumed					
Nitrate Consumed (mM)		5	10	10	10
Nitrite formed (mM)		1	0.9	3.5	1.13
Nitrate - N_2 (electrons)		20	45.5	32.5	44.35
Nitrate-Nitrite (electrons)		2	1.8	7	2.26
Total Electrons Consumed		22	47	40	47
Electrons Recovered (%)		174	134	116	119

[*] $5\,CH_3COO^- + 2NH_4^+ + 3\,CO2 \rightarrow 2\,C_5H_7O_2N + 3\,H_2O + 3\,HCO_3^-$

[#]Taken from model input where not stipulated in original source material.

Table S3: Overall stoichiometric reactions for denitrification for all bacteria species investigated in simulation S1a. Full stoichiometric reactions were derived using the principles and protocol from Rittmann and McCarty (2001).

Acidovorax spp. strain BoFeN
$CH3COO^- + 3.23\ NO_3^- + 0.08\ NH_4^+ \rightarrow 3.23\ NO_2^- + 0.92\ H_2O + 0.08\ C_5H_7O_2N + 0.92\ HCO_3^- + 0.69\ CO_2$ (Reaction 1)
$CH3COO^- + 1.89\ NO_2^- + 1.89\ H^+ + 0.12\ NH_4^+ \rightarrow 0.94\ N_2 + 1.83\ H_2O + 0.12\ C_5H_7O_2N + 0.88\ HCO_3^- + 0.53\ CO_2$ (Reaction 2)
Acidovorax spp. strain TSPY
$CH3COO^- + 3.60\ NO_3^- + 0.04\ NH_4^+ \rightarrow 3.60\ NO_2^- + 0.96\ H_2O + 0.04\ C_5H_7O_2N + 0.96\ HCO_3^- + 0.84\ CO_2$ (Reaction 3)
$CH3COO^- + 2.23\ NO_2^- + 2.23\ H^+ + 0.07\ NH_4^+ \rightarrow 1.12\ N_2 + 2.05\ H_2O + 0.07\ C_5H_7O_2N + 0.93\ HCO_3^- + 0.74\ CO_2$ (Reaction 4)
Acidovorax spp. strain 2AN
$CH3COO^- + 3.19\ NO_3^- + 0.09\ NH_4^+ \rightarrow 3.19\ NO_2^- + 0.91\ H_2O + 0.09\ C_5H_7O_2N + 0.91\ HCO_3^- + 0.65\ CO_2$ (Reaction 5)
$CH3COO^- + 1.79\ NO_2^- + 1.79\ H^+ + 0.13\ NH_4^+ \rightarrow 0.89\ N_2 + 1.76\ H_2O + 0.13\ C_5H_7O_2N + 0.87\ HCO_3^- + 0.47\ CO_2$ (Reaction 6)
Paracoccus denitrificans strain Pd 1222
$CH3COO^- + 3.42\ NO_3^- + 0.06\ NH_4^+ \rightarrow 3.42\ NO_2^- + 0.94\ H_2O + 0.06\ C_5H_7O_2N + 0.94\ HCO_3^- + 0.77\ CO_2$ (Reaction 7)
$CH3COO^- + 2.06\ NO_2^- + 2.06\ H^+ + 0.09\ NH_4^+ \rightarrow 1.03\ N_2 + 1.94\ H_2O + 0.09\ C_5H_7O_2N + 0.90\ HCO_3^- + 0.64\ CO_2$ (Reaction 8)
Pseudogulbenkiania strain *2002*
$CH3COO^- + 3.36\ NO_3^- + 0.06\ NH_4^+ \rightarrow 3.36\ NO_2^- + 0.94\ H_2O + 0.06\ C_5H_7O_2N + 0.94\ HCO_3^- + 0.74\ CO_2$ (Reaction 9)
$CH3COO^- + 2.00\ NO_2^- + 2.00\ H^+ + 0.10\ NH_4^+ \rightarrow 1.00\ N_2 + 1.90\ H_2O + 0.10\ C_5H_7O_2N + 0.90\ HCO_3^- + 0.60\ CO_2$ (Reaction 10)
(Biomass is represented as $C_5H_7O_2N$)

Table S4: All model parameters estimated by PEST/PSO for model variant S1a simulations

Parameter name	Lower limit	Upper limit	Estimated value	Posterior variance	Posterior standard deviation	Estimated value minus two standard deviations*	Estimated value plus two standard deviations*
Efficiency parameter (ε) for calculation of full stoichiometric reactions							
ε_1 (BoFeN1)	1.50E-01	5.00E-01	3.02E-01	1.76E-04	1.33E-02	2.8E-01	3.3E-01
ε_2 (TPSY)	1.50E-01	5.00E-01	2.07E-01	3.42E-04	1.85E-02	1.7E-01	2.4E-01
ε_3 (2AN)	1.50E-01	5.00E-01	3.01E-01	2.16E-04	1.47E-02	2.7E-01	3.3E-01
ε_4 (Pd 1222)	1.50E-01	5.00E-01	2.60E-01	2.21E-04	1.49E-02	2.3E-01	2.9E-01
ε_5 (2002)	1.50E-01	5.00E-01	2.75E-01	2.37E-04	1.54E-02	2.4E-01	3.1E-01
Parameters for microbial respiration of nitrate reduction to nitrite							
K_D	1.00E-07	1.00E-02	2.18E-03	6.52E-01	8.08E-01	1.0E-07	1.0E-02
K_A	1.00E-07	1.00E-03	2.38E-05	2.08E-02	1.44E-01	1.0E-07	1.0E-03
k_1	1.00E-06	1.00E-03	7.99E-05	9.999E-01	9.999E-01	1.0E-06	1.0E-03
Parameters for microbial respiration of nitrite reduction to dinitrogen							
K_D	1.00E-07	1.00E-03	2.25E-05	8.34E-01	9.13E-01	1.0E-07	1.0E-03
K_A	1.00E-07	1.00E-03	3.50E-06	8.11E-04	2.85E-02	1.0E-07	1.0E-03
k_1	1.00E-06	1.00E-03	1.10E-04	4.58E-04	2.14E-02	1.0E-06	1.0E-03
Encrustation Inhibition	1.00E-04	1.00E-02	7.59E-03	7.28E-01	8.53E-01	1.0E-04	1.0E-02

*If the interval goes beyond specified upper or lower limits, the limit value is listed instead.

Figure S1: Enzymatic Fe(II) oxidation rate for all strains investigated were calibrated from batch experiments with solution media containing nitrate but where Fe(II) was added as the sole electron donor. Selected data from these 'chemolithoautotrophic' batch experiments were used as biogenic nitrite from heterotrophic nitrate reduction could be ignored. Results are presented for *Acidovorax* spp. strains BoFeN1 (left), TSPY (middle) and 2AN (right) from Klueglein and Kappler[1], Carlson et al.[2] and Chakraborty et al.[3], respectively. Solid lines represent simulation results while symbols represent observed concentrations for total Fe(II) (red circle), nitrate (blue down triangle) and nitrite (yellow square).

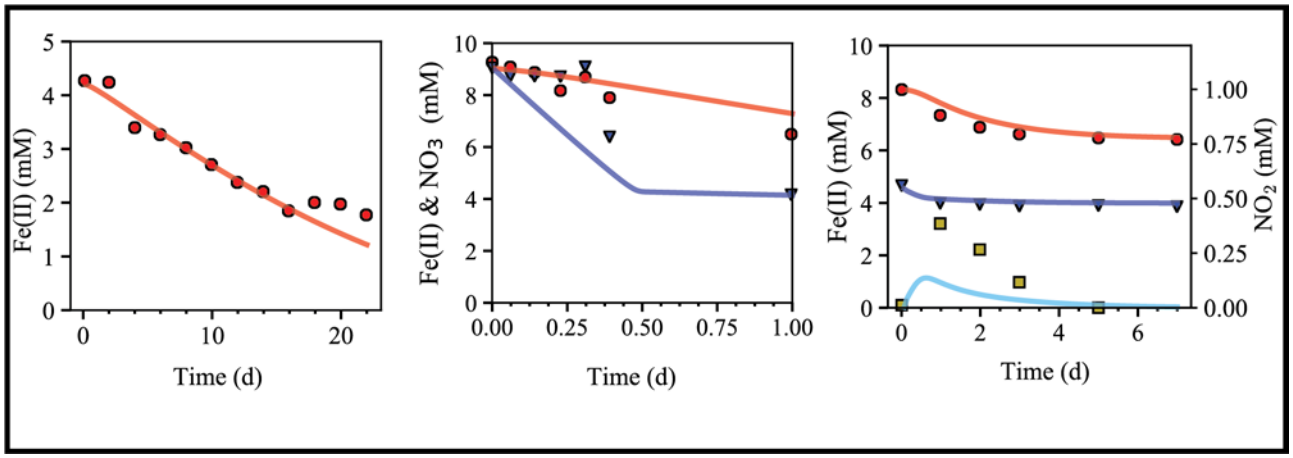


Figure S2: Green rust Fe(II) oxidation rate. Rate constant (k_5) calibrated from data collected by Hansen et al[4].

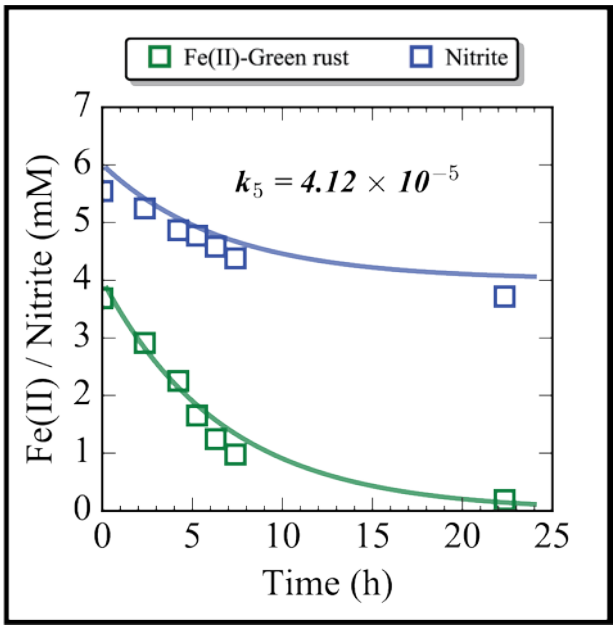


Figure S3: Simulation of Fe(II) concentrations (solid lines) during NDFO in *Acidovorax* sp. strain BoFeN1 (bottom panel) and TPSY (top panel) growth incubations at different acetate (Ace) concentrations. Symbols represent observed concentrations of Fe(II) from experiments within Kappler et al.[5] and Carlson et al.[2]

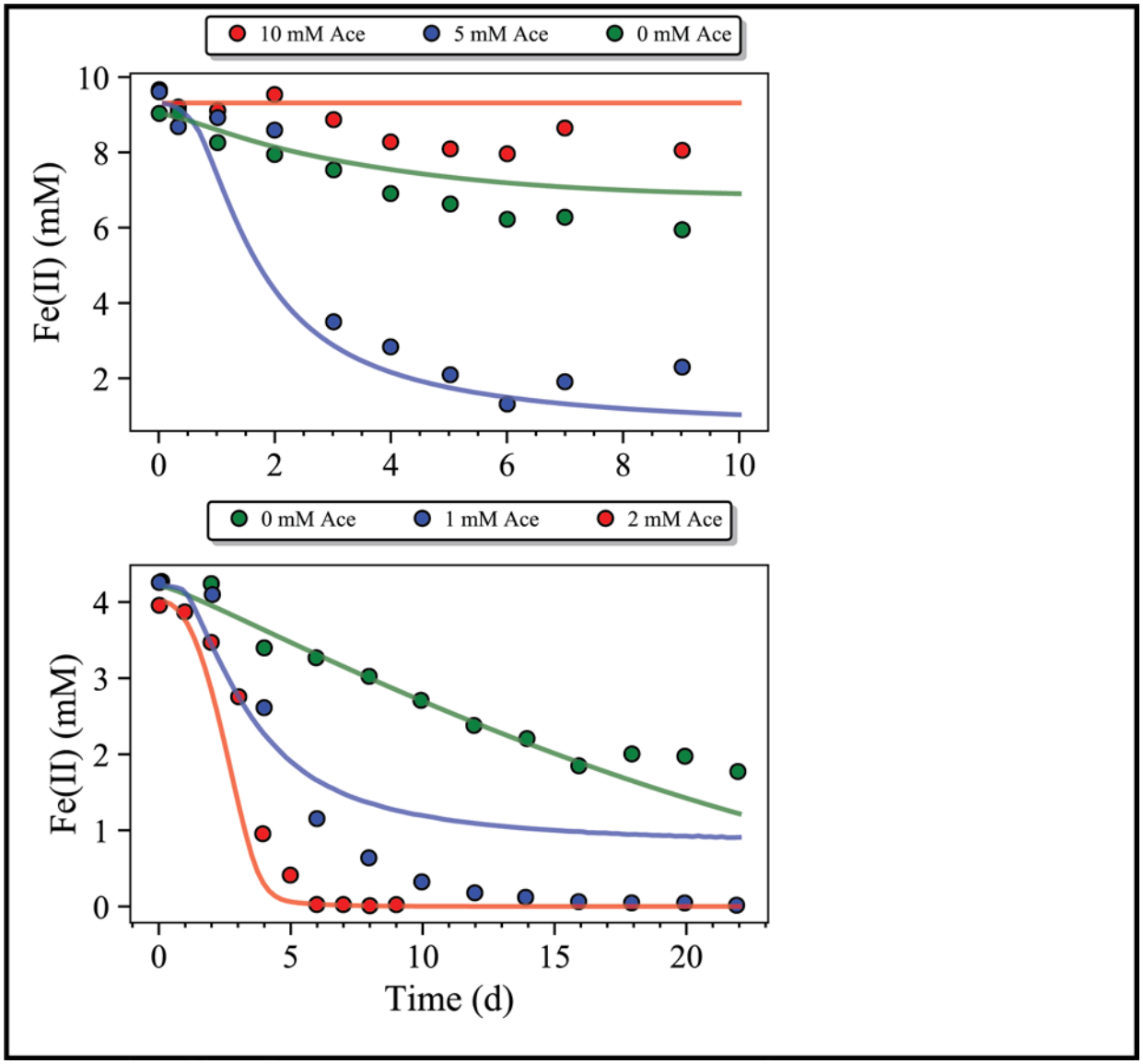
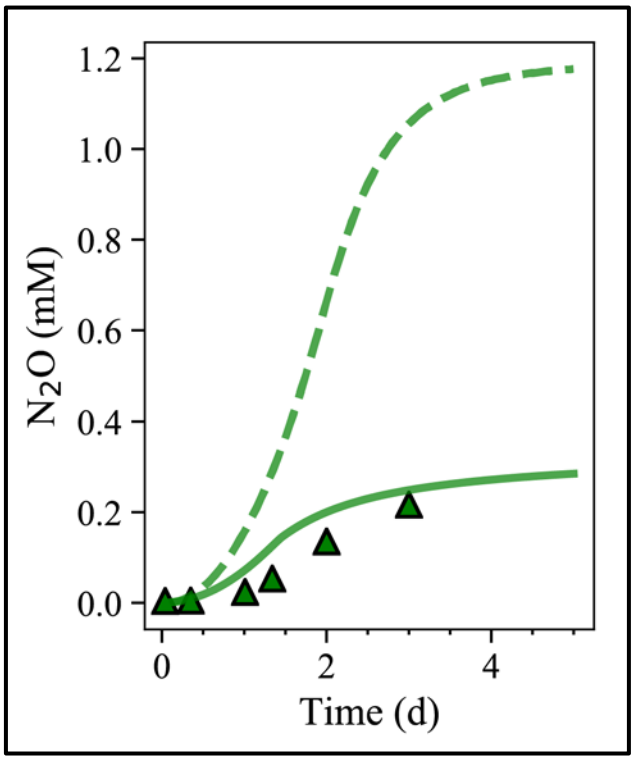


Figure S4: S1a (solid line) and S2 (dashed line) model simulation results for nitrous oxide for strain *Acidovorax sp.* strain TPSY. Symbols represent observed concentrations of nitrous oxide from experiments within Carlson et al.[2]



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