

SOM Table 3 Iron and manganese respiration by the anammox bacterium *Kuenenia stuttgartiensis*

The presence of multiple divergent paralogues of respiratory complexes in the genome assembly of *K. stuttgartiensis* triggered the experimental investigation of manganese and iron respiration in this anammox bacterium.

Single cells of *K. stuttgartiensis* were incubated with different electron donors and acceptors [1]. Fe(II) or Mn(II) oxidation rates were measured with nitrate as electron acceptor and Mn(IV) and Fe(III) reduction rates were measured using freshly synthesized δ -MnO₂ [2], amorphous iron oxyhydroxide (FeOOH; [3] or Fe(III) citrate in combination with formate as electron donor. The conversion rates were calculated based on Mn(II) and Fe(II) consumption or production in time [4,5]. Negative controls with heat-killed cells and without electron donor were performed. In addition, further negative controls were performed with *Escherichia coli* (a dissimilative nitrate reducer) and *Paracoccus denitrificans* (a denitrifier). As a positive control, the Mn(IV) and Fe(III) reduction rates of *Shewanella putrefaciens*, a known Mn(IV) and Fe(III) reducer [6], were measured.

Organism	Mn (IV) reduction rate ^a	Fe (III) reduction rate ^b		Fe (II) oxidation rate ^b
		FeOOH	Fe citrate	
<i>K.stuttgartiensis</i>	0.40	0.2	0.9	4.8
<i>S. putrefaciens</i>	1.21	1.0	5.6	ND
<i>E. coli</i>	0.10	0.05	ND	ND
<i>P. denitrificans</i>	0.13	0.04	ND	ND

SOM Table 3. Mn and Fe reduction rates and Fe oxidation rates of *K. stuttgartiensis*, *S. putrefaciens* (positive control), *E. coli* (negative control) and *P. denitrificans* (negative control). ^anmol Mn(II) (mg protein)⁻¹ min⁻¹, ^bnmol Fe(II) (mg protein)⁻¹ min⁻¹. ND, not determined

The single cells of *K. stuttgartiensis* oxidized Fe(II) at a rate of 4.8 nmol Fe(II) (mg protein)⁻¹ min⁻¹ but not Mn(II) with nitrate as electron donor. The Mn(IV) and Fe(III) reduction rates obtained with *K. stuttgartiensis* single cells and formate as electron donor were significantly higher than the rates obtained for the negative controls with *E. coli*, *P. denitrificans* or heat-killed cells (**SOM Table 3**). *K. stuttgartiensis* did not convert Mn(IV) or Fe(III) with ammonium as electron donor, and *S. putrefaciens* was not able to oxidize ammonium with nitrite as electron acceptor. Under the conditions tested *K. stuttgartiensis* can oxidize ammonium anaerobically to N₂ with nitrite as electron donor, at a rate up to 25 nmol ammonium (mg protein)⁻¹ min⁻¹.

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