Supplementary information for:

ABIOTIC PROCESS FOR Fe(II) OXIDATION AND GREEN RUST MINERALIZATION DRIVEN BY A HETEROTROPHIC NITRATE REDUCING BACTERIA (*KLEBSIELLA MOBILIS*)

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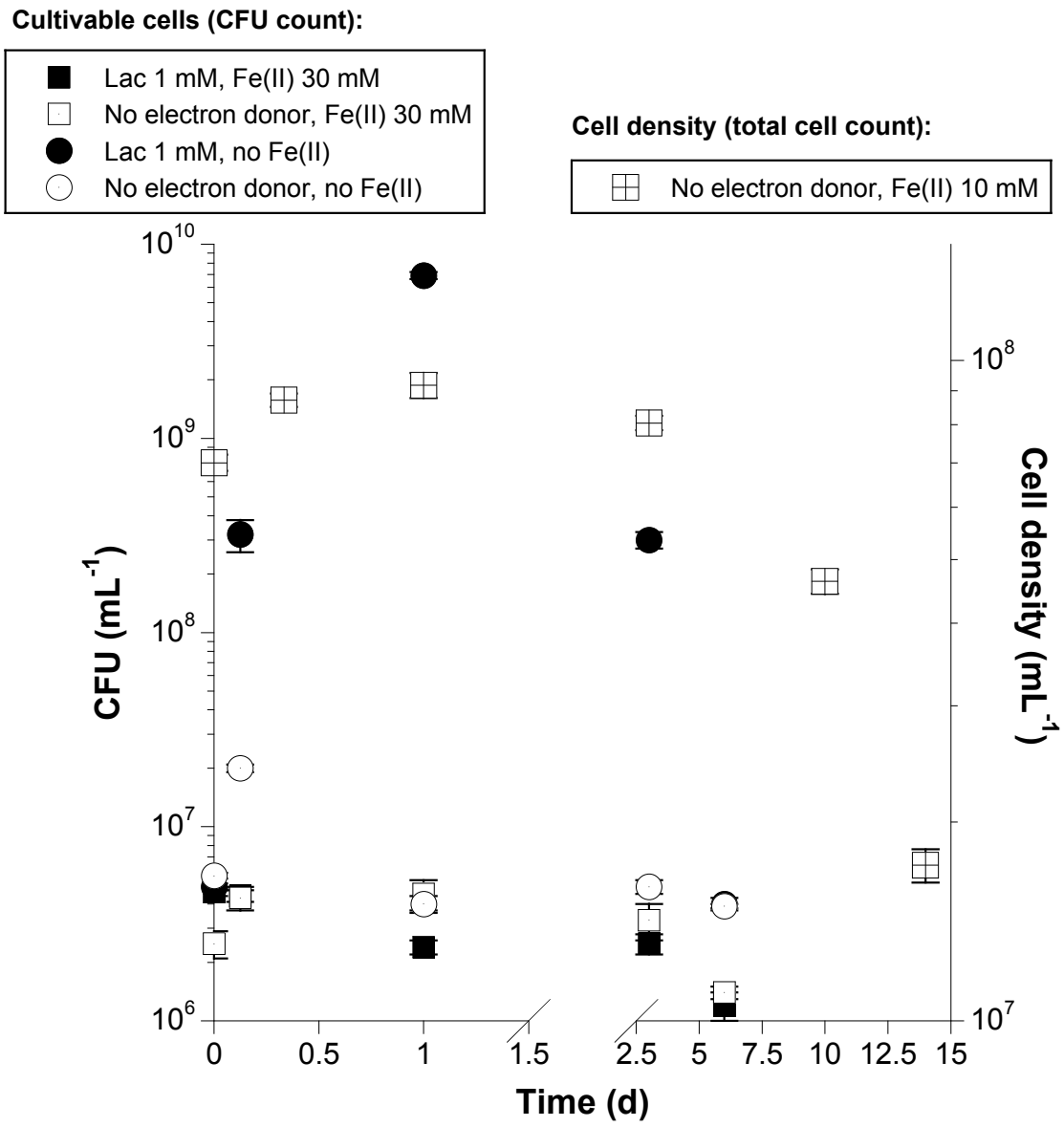
This file contains 8 pages (including title page), 5 figures, 1 table and 1 explanation text.

Table S1. Initial and final concentrations for nitrate, Fe(II) and ammonium of the experiments #1 - #7. n.a. stands for not applicable and n.d. stands for non determined. Nitrite was detected but non quantifiable in the initial and final medium. All data are means of duplicate experiments (except #6, n = 4).

Exp. nb			Initial concentrations (0 day)			Final concentrations (18 d)	
		Cells mL^{-1} $\times 10^8$	NO_3^- (mM)	Fe^{II} (mM)	NH_4^+ (mM)	NO_3^- (mM)	Fe^{II} (mM)
#1	Unstarved	13.7 ± 0.3	38.1 ± 0.8	27.6 ± 0.1	1.70 ± 0.09	37.6 ± 0.3	24.1 ± 0.1
#2	Starved	8.6 ± 0.3	40.4 ± 0.1	26.9 ± 0.9	1.68 ± 0.02	39.2 ± 0.2	18.7 ± 0.4
#3	Unstarved	13.7 ± 0.3	4.2 ± 0.9	28.3 ± 0.3	1.78 ± 0.02	3.7 ± 0.1	24.5 ± 0.3
#4	Starved	8.6 ± 0.3	4.1 ± 0.2	29 ± 0.4	1.71 ± 0.05	2.7 ± 0.3	18.2 ± 0.2
#5	Unstarved	2.8 ± 0.1	38.0 ± 0.2	27.7 ± 0.5	1.75 ± 0.04	37.7 ± 0.2	25.9 ± 0.1
#6	Unstarved	0.7 ± 0.1	9.4 ± 0.5	5.7 ± 0.3	1.68 ± 0.02	9.1 ± 0.7	3.6 ± 0.4
#7	Unstarved	2.8	24	30	1.70	n.d.	n.d.
Control	No cells	/	40.1 ± 0.2	28.1 ± 0.2	1.72 ± 0.05	37.9 ± 0.5	27.8 ± 0.3

Figure S2. Time course of the population of *Klebsiella mobilis* (non starved, CFU or cell density) with/without 30 mM Fe(II), with/without 1 mM lactate, and with 8 mM nitrate.



Explanation S3. Detailed explanation for calcul of potential endogenous electron donor available by *K. mobilis*.

From Fig. 2 the amount of nitrite produced is 0.2 mM, since the reduction of nitrate in nitrite needs 2 e^- mol^{-1} (equation (3)), we can assume that the amount of equivalent electrons presumed to be stored by the cell is equivalent to 0.4 mM e^- equiv. Reported to organic matter (presume to exhibit a general formula $(CH_2O)n$, we could estimate the cells have stored 0.1 mmol L^{-1} (CH_2O) (taken into account that $4e^-$ are involved in the oxidation of 1 mole of CH_2O in CO_2). For 7×10^{10} cells l^{-1}, the stored TOC could be 1.43×10^{-15} mol/cell. The total amount of carbon associated to cells can be estimated as follow: if the cell weight is 0.1 pg/cell, then 7×10^{10} cells l^{-1} count for 0.007 g of DSS l^{-1}, the cell mass counts for ~ 50 % in carbon, then it counts for ~ 0.0035 g C / L or ~ 0.3 mmol l^{-1}. The potential amount of glycogen stored (20-30% of the dry mass, Potts, 1969) is then ~ 0.1 – 0.2 mM equivalent CH_2O which is relatively closed to the amount of electron donor used to reduce nitrate in nitrite. In addition, the bacteria could use a part of the ~ 50 mg l^{-1} of DOC content in the suspension medium.

Ref.

Potts J.M. (1969) *Survival of microorganisms under conditions of total starvation* PhD Thesis, The University of British Columbia, Canada, pp. 166.

Figure S4. Change of nitrate, ammonium and Fe(II) in control experiment (abiotic condition: sterile medium without *Klebsiella mobilis*). Symbols were voluntarily reduced to exhibit the bar errors.

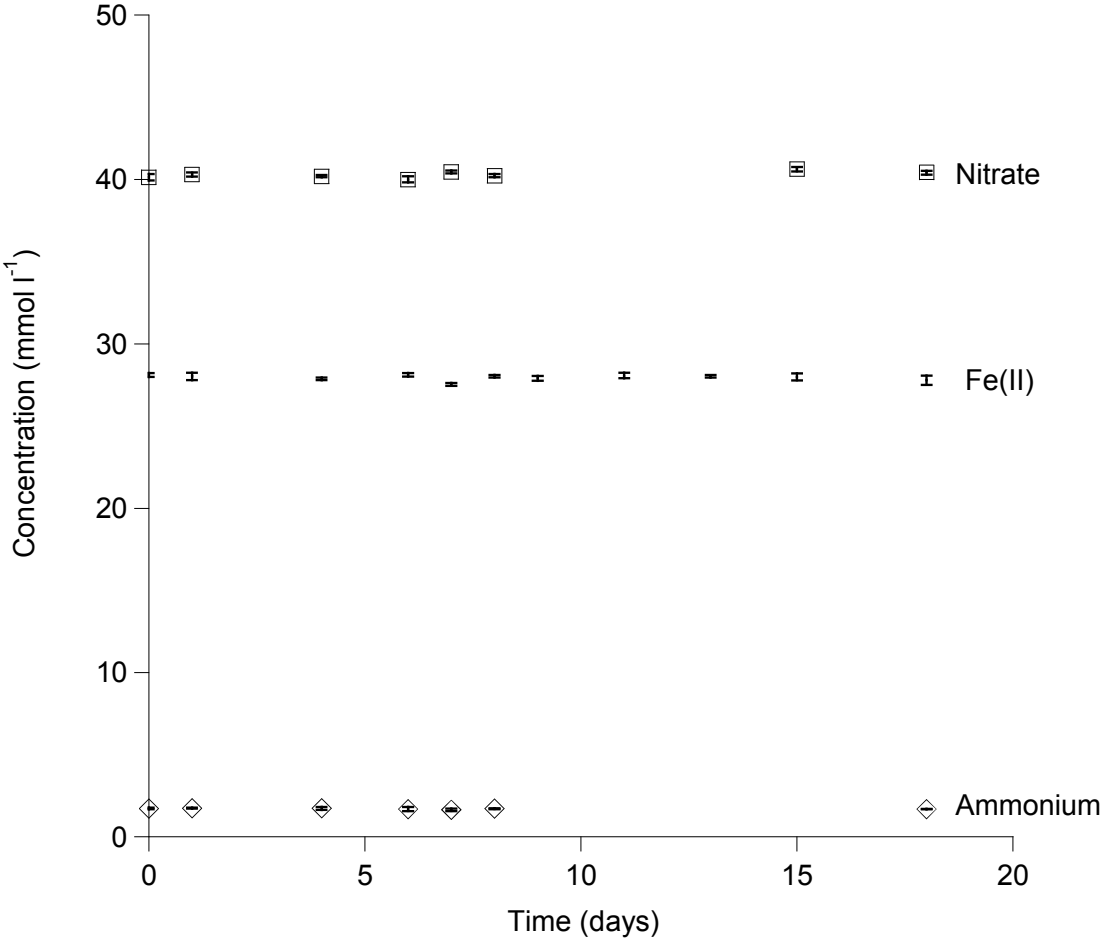


Figure S5. Time courses of total (dissolved plus particular) Fe(II) (circle), nitrate (square), ammonium (diamond) and 1.50 mM lactate (inverted triangle) in anoxic incubation medium with a resting cell suspension of *Klebsiella mobilis*. Error bars are based on duplicate experiments.

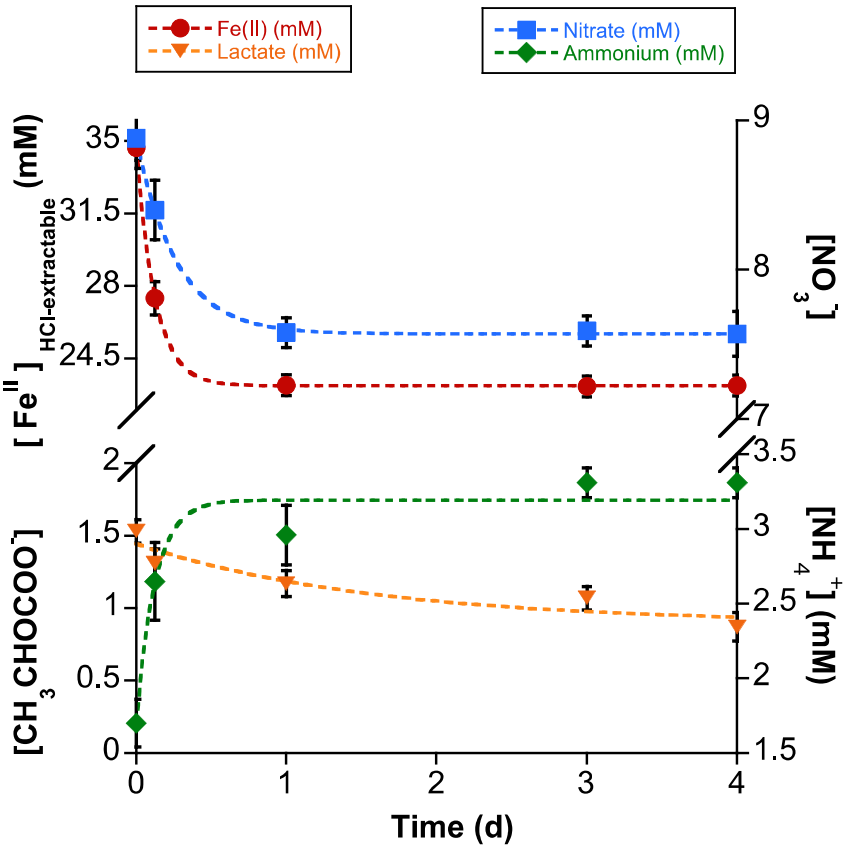


Figure S6. Abiotic reaction of ferrous iron (40 mM) in the mineral medium (a) and nitrite (1 mM) after 10 min (b) and 15 days (c). The Raman spectrum confirmed the formation of green rust as the end-product (d).

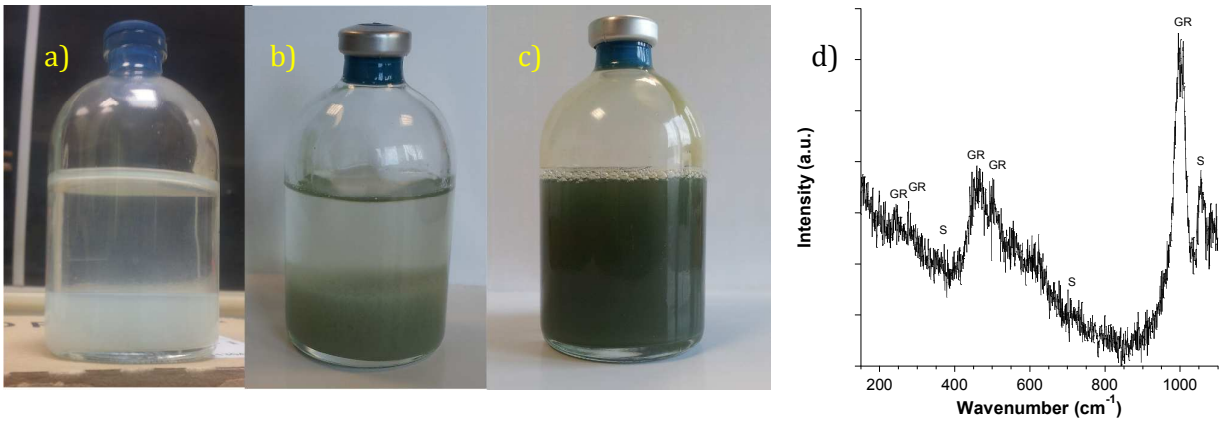
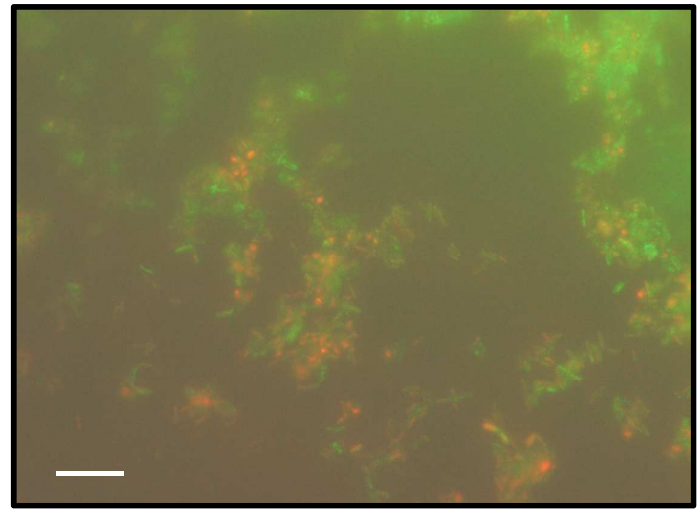


Figure S7. Epifluorescence pictures of cells colored by propidium iodide (PI, in red, assumed to be cell membrane damaged) and by Syto 9 (in green, assumed to be undamaged cells). Percentage of damaged cells count for 10 ± 2 % (n=20) initially to the incubation time (1), and for 59 ± 9 % (n = 20) at 18 days (2). The scale bar corresponds to 1 µm



(1)

(2)