Supplementary information of:

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3	Pseudo-first-order reaction of chemically and biologically formed green
4	rusts with Hg ^{II} and C ₁₅ H ₁₅ N ₃ O ₂ : effects of pH and stabilizing agents
5	(phosphate, silicate, polyacrylic acid, and bacterial cells)
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23 <u>**Table S1**</u>: Total mercury concentration in each compound used for experimentation with mercury test. *S.p. = Shewanella putrefaciens*

Hg (nnh) 0.482 0.760 0.370 1.872 0.315 0.574 0.519 104.514 ND		Pure water	SnCl ₂	GR1 _{bio}	GR1 _{ab+P}	GR2 _{ab}	<i>S.p</i> .	Fe ^{II} aq.	Hg ^{II}	GR1 _{ab}
115(ppb) 0.102 0.700 0.570 1.072 0.515 0.571 0.517 101.511 11D	Hg (ppb)	0.482	0.760	0.370	1.872	0.315	0.574	0.519	104.514	ND

24

25 <u>**Table S2a**</u>: d_{hkl} (nm) parameters from XRD analysis for chemically synthesized and biogenic carbonate green rust from the present study 26 (GR1_{ab+P}, GR1_{ab}, and GR1_{bio}) and from the literature ^a(Drissi *et al.*, 1995), ^b(Zegeye *et al.*, 2007).

27 n.d. : no data

Relative	hkl	$d_{hkl} GR1_{ab+P}$	$d_{hkl} GR1_{ab}$	$d_{hkl} GR1_{bio}$	Ref ^a abiotic	Ref ^b biotic
intensity						
100	003	0.759	0.758	0.759	0.751	0.751
32	006	0.377	0.379	0.378	0.373	0.375
1	101	0.274	-	0.274	0.273	n.d.
15	012	0.268	-	0.268	0.265	0.266
3	104	0.247	-	0.248	0.247	n.d.
12	015	0.235	0.235	0.235	0.233	0.233
1	107	0.209	-	0.209	0.208	n.d.
9	018	0.198	0.197	0.197	0.196	0.196
1	0012	0.189	0.189	0.188	0.188	n.d.
2	1010	0.175	-	0.174	0.173	n.d.

- $\frac{\text{$ **Table S2b** $:}}{\text{from the literature }^{a}(\text{Simon et al., 1997}), ^{b}(\text{Hansen et al., 1994}).}$
- 33 n.d. : no data

Relative intensity	hkl	d _{hkl} GR2 _{ab}	Ref ^a	Ref ⁶
100	001	1.091	1.09	1.10
80	002	0.547	0.545	0.549
60	003	0.365	0.362	0.366
20	004/100	0.276	0.275	0.275
30	101	0.67	0.266	0.226
30	102	0.246	0.245	n.d.
30	005/103	0.220	0.219	0.2195

38 Table S3. Mössbauer hyperfine parameters of spectra measured at 7, 150 and 12 K for the GR1_{bio}, GR1_{ab+P} and GR2_{ab} synthesized according to Zegeye et al. (2007), Bocher et al. 39 (2004), and Ruby *et al.* (2006), respectively. The $GR1_{bio}$ Mössbauer spectrum indicates the presence of 20 % of a Fe^{II}-Fe^{III} phase assumed to be magnetite in the sample (four sextets). 40 41 However, no trace of magnetite was detected with XRD under anoxic conditions, and we 42 43 cannot exclude that the presence of magnetite could be due to an oxidation of GR1_{bio} exposed to ambient air before the Mössbauer analysis. $GR1_{ab}$ was not characterized by TMS, and its ratio Fe^{II}/Fe^{III} was determined by colorimetry (Ferrozine) on the solid phase. 44 45

46

		<i>C. S.</i>	Δ or 2e	Н	RA	-
		(0.11 ± 4)	$(mm \ s^{-1})$	(kOe)	(%)	
		$mm s^{-1}$)				
	$D_1 (Fe^{II}_{GR})$	1.25	3.13		22.6 %	
$GR1_{bio}$	D_2 (Fe ^{II} _{GR})	1.24	2.64		26.1 %	
	D_3 (Fe ^{III} _{GR})	0.42	0.51		30.4 %	
					$\mathrm{Fe}^{\mathrm{II}}/\mathrm{Fe}^{\mathrm{III}} = 1.6$	
	\mathbf{S} (Eq. \mathbf{O} 2)	0.60	0.10	572		
	$S_1 (Fe_3O_4?)$	0.09	-0.19	323		
	$S_2 (Fe_3O_4?)$	0.24	0.23	493		
	S_3 (Fe ₃ O ₄ ?)	1.72	0.21	383		
	S_4 (Fe ₃ O ₄ ?)	0.33	0.56	267		
	D_1 (Fe ^{II} _{GR})	1.10	2.93		28 %	
$GR1_{ab+P}$	D_2 (Fe ^{III} _{GR})	0.25	0.36		45 %	
	D_3 (Fe ^{II} _{GR})	1.10	2.57		27 %	
					$\mathrm{Fe}^{\mathrm{II}}/\mathrm{Fe}^{\mathrm{III}} = 1.2$	
GR2.	D_1 (Fe ^{II} _{CP})	1 31	2 91		66 %	
	$D_1 (Fe^{III}_{GR})$	0.49	0.47		34 %	
	\mathbf{D}_2 (i \mathbf{C}_{GR})	0.72	0.77		$\mathrm{Fe^{II}/Fe^{III}} = 2.0$	
GRI _{ab}		ND	ND	ND	2.2*	

C. S. (mm s⁻¹) isomer shift with respect to metallic alpha-iron at room temperature; Δ or 2ε (mm s⁻¹) quadrupole 47

splitting; H (kOe) hyperfine field; RA, relative abundance. 48

49 50 *determined by ferrozine measurement

- <u>**Table S4.</u>** Values of pH for batch experiments (n = 2) before (initial pH) and after 60 min of incubation (final pH) with Hg^{II}.</u> 52

Batch experiment	Initial pH	Final pH
SnCl ₂	2.4	2.4
GR2 _{ab}	7.0 ± 0.2	6.6 ± 0.1
	8.0 ± 0.1	7.2 ± 0.1
	9.5 ± 0.1	8.9 ± 0.3
GR2 _{ab+cells}	7.0 ± 0.1	6.5 ± 0.1
GR1 _{bio}	7.0 ± 0.1	7.2 ± 0.3
	8.1	8.0
	9.5 ± 0.1	9.0 ± 0.6
GR1 _{ab}	8.2 ± 0.1	9.0 ± 0.1
$GR1_{ab+P}$	7.0 ± 0.1	7.6 ± 0.3
	8.0 ± 0.1	8.0 ± 0.2
	9.5 ± 0.1	8.8 ± 0.3
Fe^{II} (2 mM)	3.3 ± 0.1	3.5 ± 0.1

56 **Note S1.** Calculation of structural Fe^{II}

57
$$[Fe^{total}] = [Fe^{II}] + [Fe^{III}] = [GR] \times 6$$

- 58 $[Fe^{II}]/[Fe^{III}] = M$ össbauer ratio
- 59 *e.g.* GR1_{ab+P}
- $60 \qquad [Fe^{II}] = 1.2 \times [Fe^{III}]$
- $61 \qquad 1.2 \times [Fe^{III}] + [Fe^{III}] = [Fe^{total}]$
- 62 $[Fe^{III}] = [Fe^{total}]/2.2$
- 63 $[Fe^{II}] = [Fe^{total}] [Fe^{total}]/2.2 = 1.63 \text{ mM}$
- 64 [Fe^{II}]GR1_{ab+P} = 1.63×10^{-3} M
- 65 [Fe^{II}]GR1_{bio} = 1.5×10^{-3} M
- 66 [Fe^{II}]GR2_{ab} = 2×10^{-3} M
- 67 [Fe^{II}]GR1_{ab} = 2×10^{-3} M
- 68
- 69 <u>Note S2</u>. Calculation of k_{FeII} (L mol⁻¹ min⁻¹) = k_{obs} normalized to the structural Fe^{II} 70 concentration.
- 71 $k_{\text{FeII}} = k_{\text{obs}} / [\text{Fe}^{\text{II}}] \text{GR}$
- 72 Where
- 73 [Fe^{II}]GR is the structural Fe^{II} concentration (mol L^{-1})
- 74
- 75 <u>Note S3</u>. Calculation of k_s (L mmol⁻¹ min⁻¹) = k_{obs} normalized to the surface Fe^{II} sites (μ M) 76 (see manuscript)
- 77 $k_{\rm s} = k_{\rm obs} / ({\rm surface Fe^{II} sites})$
- 78
- 79
- 80



Figure S1. UV-Vis spectra of initial MR solution (+) and the same solution after 300 min of 83 incubation with 500 μ M GR2_{ab} (×).



86 **Figure S2.** X-ray diffractograms of biologically synthesized hydroxycarbonate green rust

- 87 (GR1_{bio}) before addition of Hg^{II} at the concentration of 5 μ M (green line, t = 0 h), and 24 h
- after (black line, t = 24 h). The initial concentration of $GR1_{bio}$ was 400 μ M. The lattice planes
- 89 of green rust (GR) are written in brackets. The intensity is expressed in arbitrary unit (a. u.).

Figure S3. Mössbauer spectra of a) $GR1_{bio}$, b) $GR1_{ab+P}$, and c) $GR2_{ab}$.





97 Fig. S3 b) GR1_{ab+P}

c)



- 101 Fig. S3 c) GR2_{ab}



Figure S4. Removal of mercury and oxidation of ferrous iron content of chemically

synthesized green rust (GR1_{ab}, a) and biologically formed green rust (GR1_{bio}, b) over the time at pH 8.2. The initial concentration of Hg^{II} species was 5 μ M and the initial concentrations of

green rusts used were 4 μ M, 40 μ M, 400 μ M for GR1_{bio}, and 50 μ M, 500 μ M for GR1_{ab}. The

Hg^{II}/GR ratios were 1/0.8, 1/8, 1/80 and 1/10, 1/100 respectively. Error bars were drawn for

two independent assays.



Figure S5. (a): Removal of mercury over time in the presence of 50 µM Sn^{II} (SnCl₂) (pH 115 3.0 ± 0.5), and 0.2 mM aqueous Fe^{II} (pH 7.0 ± 0.1) or 6×10^5 cells mL⁻¹ of Shewanella 116 *putrefaciens* (pH 7.0 ± 0.1) (cells), a cell density that is equivalent to that used for the 117 synthesis of GR_{bio} and that is expected to remain with GR1_{bio}. The changes in pH after a 60 118 119 min incubation time period are reported in Table S7. Bars are the errors of two independent 120 experiments. The stannic solution (SnCl₂, 50 μ M pH = 3) was prepared from a stock solution (22.5 mM in HCl 1.5 M) (SnCl₂ salt, 98% anhydrous, 196981000, Acros). It was used as an 121 indicator for Hg^{II} reduction, since Sn^{II} is known to be a powerful reducer (Zheng and 122 Hintelmann, 2010). In order to avoid Sn^{II} precipitation, which can occur along with increasing 123 pH, Hg^{II} reduction by aqueous Sn^{II} were only performed at pH 3. 124

125 (b): Removal of methyl red (MR) over time in the presence of 0.2 mM aqueous Fe^{II} (pH 7.0),

126 6×10^5 cells mL⁻¹ of a fresh *S. putrefaciens* suspension (cells) or heat inactivated cells (dead

127 Sp cells), or a solution of MR in pure water.

128





130 **Figure S6**. X-ray diffractograms of biologically synthesized hydroxycarbonate green rust

- 131 (GR1_{bio}) before addition of Hg^{II} at the concentration of 5 μ M (green line, t = 0 h), and 24 h
- 132 after (black line, t = 24 h). The initial concentration of $GR1_{bio}$ was 4 μ M. The green rust (GR)
- 133 was oxidized into magnetite (M) during the reduction of mercury. The lattice planes are
- 134 written in brackets. The intensity is expressed in arbitrary unit (a. u.).

- 136
- 137 <u>References for supplementary data:</u>
- Bocher, F., Géhin, A., Ruby, C., Ghanbaja, J., Abdelmoula, M., Génin J-M.R. 2004.
 Coprecipitation of Fe(II–III) hydroxycarbonate green rust stabilized by phosphate
- adsorption. Solid State Sciences, 6 (1), 117–124.
- Drissi, SH, Refait, P, Abdelmoula, M, Génin, J.-M.R. 1995. The preparation and
 thermodynamic properties of iron(II)-iron(III) hydroxide-carbonate (green rust); Pourbaix
 diagram of iron in carbonate-containing aqueous media. Corrosion Science 37, 2025–
 2041.
- Hansen H. C. B., Borgaard O. K., Sorensen J. 1994. Evaluation of the free energy of
 formation of Fe(II)-Fe(III) hydroxide-sulphate (green rust) and its reduction of nitrite.
 Geochimica et Cosmochimica Acta 58, 2599-2068.
- Ruby, C., Haissa, R., Géhin, A., Abdelmoula, M., Génin, J.-M.R., 2006. Chemical stability of
 hydroxysulphate green rust synthesized in the presence of foreign anions: carbonate,
 phosphate and silicate. Hyperfine Interactions, 167: 803-807.
- Simon L., Francois M., Refait P., Renaudin G., Lelaurain M., and Génin J.-M. R., 2003.
 Structure of the Fe(II-III) layered double hydroxysulphate green rust two from Rietveld analysis. Solid State Sciences 5, 327–334.
- Zegeye, A., Ruby, C., Jorand, F., 2007. Kinetic and thermodynamic analysis during
 dissimilatory γ-FeOOH reduction: formation of green rust 1 and magnetite.
 Geomicrobiology Journal 24, 51-64.
- Zheng, W., Hintelmann, H., 2010. Nuclear field shift effect in isotope fractionation of
 mercury during abiotic reduction in the absence of light. The Journal of Physical
 Chemistry A 114 (12), 4238-4245.