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1 **Formation of single domain magnetite by green rust oxidation promoted by microbial**
2 **anaerobic nitrate-dependent iron oxidation**

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ABSTRACT

Biom mineralization of magnetite is a central geomicrobiological process that might have played a primordial role over Earth's history, possibly leaving traces of life in the geological record or controlling trace metal(loid)s and organic pollutants mobility in modern environments. Magnetite biom mineralization has been attributed to two main microbial pathways to date (namely magnetotactic bacteria and dissimilatory iron-reducing bacteria). Here, we uncover a new route of magnetite biom mineralization involving the anaerobic nitrate-reducing iron(II) oxidizing bacterium *Acidovorax* sp. strain BoFeN1. Using transmission electron microscopy, scanning transmission X-ray microscopy, transmission Mössbauer spectroscopy and rock magnetic analyses, this strain is shown to promote the transformation of hydroxylchloride green rust in equilibrium with dissolved Fe(II) to (1) periplasmic lepidocrocite (γ -FeOOH) and (2) extracellular magnetite, thus leading to strong redox heterogeneities at the nanometer scale. On the one hand, lepidocrocite was associated with protein moieties and exhibited an anisotropic texture, with the elongated axis parallel to the cell wall. On the other hand, magnetite crystals exhibited grain sizes and magnetic properties consistent with stable single domain particles. By comparison, abiotic controls led to a very slow (4 months vs. 2 days in BoFeN1 cultures) and incomplete oxidation of hydroxylchloride green rust towards magnetite. As this abiotic magnetite exhibited the same size and magnetic properties (stable single domain particles) as magnetite produced in BoFeN1 cultures, only the co-occurrence of textured Fe(III)-oxides and magnetite, associated with the persistence of organic carbon molecules, might constitute valuable biosignatures to be looked for in the geological record. Our results furthermore contribute to a more complex picture of Fe redox cycling in the environment, providing an additional process of Fe(II)-bearing phase biom mineralization that is not specific of Fe bio-reduction, but can also result from Fe bio-oxidation.

Keywords : biom mineralization, magnetite, green rust, anaerobic iron oxidation, denitrification, biosignature.

1. INTRODUCTION

The evolution of iron-oxidizing and iron-reducing bacteria is intimately associated with changes in Fe redox cycling and bioavailability over geological timescales (Konhauser et al., 2011; Ilbert and Bonnefoy, 2013). Biominerals produced by these bacteria are thus widely used to reconstruct the evolution of paleo-environments and in particular the deep changes that occurred around the Great Oxidation Event (GOE) some 2.3 Ga ago (Anbar et al., 2007; Kump, 2008; Rasmussen et al., 2008; Konhauser et al., 2009; Frei et al., 2009; Scott et al., 2011; Czaja et al., 2012). Deciphering the characteristics and mineralogy of the phases that are produced by metabolisms relevant to this period of Earth's history is thus crucial in order to assess the mechanisms and environmental conditions that prevailed at the time of iron-bearing phase deposition in the past.

Magnetite (Fe_3O_4) is a common mineral in the geological record as well as in modern environments. For example, huge sedimentary iron deposits known as Banded Iron Formation (BIF) that have been deposited during the Precambrian contain a diversity of Fe-bearing minerals, among which magnetite (Posth et al., 2013; Li YL et al., 2013; Konhauser et al., 2002). Its genesis has been interpreted as the result of a combination of abiotic and biologically-driven Fe(II)-oxidation and Fe(III)-reduction reactions, occurring before and during diagenesis (Morris, 1993; Klein, 2005; Pecoits et al., 2009; Heimann et al., 2010; Papineau et al., 2010; Li et al., 2011, Li YL et al., 2013). In another context, magnetite is the main carrier of remanent magnetization in soils, commonly used for paleoenvironmental reconstructions (e.g. Chang et al., 2012, Roberts et al., 2011; Yamazaki and Shimono, 2013). Finally, magnetite also takes part in the control of pollutant mobility in modern environments (e.g. Wang et al., 2011; Morin et al. 2009; Morin et Calas, 2006).

Biomineralization of magnetite is commonly attributed to two types of bacteria, namely magnetotactic bacteria (MTB) and dissimilatory iron reducing bacteria (DIRB) (Li JH et al., 2013). The former produce intracellular magnetite with finely controlled size and morphology (Blakemore, 1975; Isambert et al., 2007; Li et al., 2010; Komeili, 2012), whereas the latter produce exclusively extracellular magnetite, usually exhibiting more widely distributed sizes. In addition, magnetic properties strongly differ between magnetites produced by (1) MTB that are stable single domain (SD), hence contributing to natural remanence magnetization (NRM) in soils, sediments and sedimentary rocks and (2) DIRB that are usually superparamagnetic (SP), i.e. of smaller size and with non-permanent

1 magnetization (Jimenez-Lopez et al., 2010). Extracellular SD or even larger magnetite has
2 only been described in cultures of DIRB under specific conditions, for instance *Geobacter*
3 *metallireducens* GS-15 grown in the absence of CO₂ in the cell culture headspace (Vali,
4 2004), and *Thermoanaerobacter* spp. strain TOR39 at elevated temperatures (e.g., 65°C)
5 (Zhang et al., 1998) and/or after prolonged incubation (e.g., 65°C and 2 years) (Li YL, 2012).

6 The formation of magnetite by Fe(II)-oxidizing bacteria and their crystallographic and
7 magnetic properties have never been clearly assessed. One study tentatively suggested that
8 this phase might have been produced among mixtures of Fe-oxides and phosphates in cultures
9 of a nitrate-reducing bacterium based on XRD data (Chaudhuri et al., 2001). However, as
10 already underlined (e.g. Pantke et al., 2012), some of the main XRD peaks of magnetite were
11 absent and the relative intensity of the other peaks not consistent with this mineral. (Jiao et al.,
12 2005) suggested that the photoautotrophic Fe(II)-oxidizing bacterium *Rhodospseudomonas*
13 *palustris*, strain TIE-1 biomineralized magnetite at pH > 7.2. However, this previous study
14 did not provide any detailed mineralogical nor magnetic characterization of biomineralized
15 magnetite. Furthermore, the spatial relationship between minerals and bacteria remain
16 unknown.

17 Apart from biological pathways, different abiotic routes lead to the formation of
18 magnetite. Among them, magnetite formation can result from the oxidation of green rust
19 (GR). GRs are Fe(II)-Fe(III)-hydroxides with the general formula $[\text{Fe(II)}_{1-x}\text{Fe(III)}_x(\text{OH})_2]^{x+}$
20 $[(x/n)\text{A}^{n-}(\text{m/n})\text{H}_2\text{O}]^{x-}$, composed of brucite-like hydroxide layers alternating with anions (e.g.
21 carbonate, chloride, formate or sulphite in GR type 1 referred to as GR1 or sulfate, selenate
22 in GR type 2 referred to as GR2) and water molecules in the interlayer space. Abiotic green
23 rust oxidation is kinetically controlled and leads to either lepidocrocite, goethite, magnetite or
24 ferric hydroxycarbonate depending on pH conditions and the nature and concentration of the
25 oxidant (e.g. Hansen et al., 1996; Ruby et al., 2010). In the present study, we use the
26 anaerobic nitrate-reducing Fe(II)-oxidizing bacteria *Acidovorax* sp. strain BoFeN1. This strain
27 has been shown to biomineralize goethite (Kappler et al., 2005; Schädler et al., 2009),
28 lepidocrocite (Larese-Casanova et al., 2010), Fe(III) phosphates (Miot et al., 2009a) as well as
29 some mixed Fe(II)-Fe(III) phases, such as hydroxycarbonate green rust (Pantke et al., 2012)
30 and Fe(II)-Fe(III) phosphates with various Fe(II)/Fe(III) ratios (Miot et al., 2009b) depending
31 on culture conditions. Recent findings show that Fe oxidation by this strain and other nitrate-
32 reducing Fe(II)-oxidizing bacteria is highly dependent on the release of nitrite as an
33 intermediate of nitrate reduction (Klueglein and Kappler, 2013; Kopf et al., 2013; Carlson et

1 al., 2013, Etique et al. 2014), in agreement with previous observations of the periplasmic
2 localization of Fe(III) minerals upon Fe(II) oxidation (Miot et al., 2011).

3 Here, we report the oxidation of hydroxychloride green rust to a mixture of
4 lepidocrocite and SD magnetite in cultures of the anaerobic nitrate reducing iron-oxidizing
5 bacteria *Acidovorax* sp, strain BoFeN1 and discuss what these results involve for the search of
6 biosignatures in geological samples.

8 2. MATERIALS AND METHODS

10 2.1. Culture conditions and sample preparation

11
12 All solutions were prepared with sterile milli-Q water degassed under Ar and
13 manipulations were conducted in an anaerobic chamber under N₂ or N₂/H₂ (95/5%)
14 atmosphere (p(O₂) < 5 Pa). The nitrate-reducing Fe(II)-oxidizing bacteria *Acidovorax* sp.
15 strain BoFeN1 were pre-cultured under strictly anoxic conditions (N₂/CO₂ 80/20%) in rich
16 freshwater mineral medium devoid of Fe prepared after (Ehrenreich and Widdel, 1994) up to
17 an optical density at 600 nm around 0.2 (corresponding to a number of viable cells, estimated
18 by culture on agar plates, of around 10⁵ colony-forming units / mL (CFU/mL)). Phosphate
19 was provided as KH₂PO₄ at a concentration of 4.3 mM. Acetate (5 mM sodium acetate) and
20 nitrate (10 mM sodium nitrate) were provided as a carbon source and as a terminal electron
21 acceptor, respectively. After 2 days, bacteria were harvested by centrifugation (5000 g, 15
22 min) and rinsed twice in 0.6 g.L⁻¹ NaCl.

23 For biomineralization experiments, a specific medium was prepared composed of
24 NaCl (11.4 mM), Na-acetate (5 mM) and FeCl₂ (10 mM), supplemented with vitamins, trace
25 elements and selenite solutions prepared after (Ehrenreich and Widdel, 1994). This medium
26 did not contain phosphate and was thus not designed for bacterial growth. The pH was
27 adjusted at 7.6 with NaOH. NaNO₃ (10 mM) was then added leading to the precipitation of a
28 green phase that was shown by X-ray diffraction (XRD) to consist of green rust (Fig. 1). Pre-
29 cultured rinsed bacteria were inoculated at 50% (v/v) in this biomineralization medium
30 (leading to an initial optical density at 600 nm of 0.109). Cultures prepared in 1L-flasks,
31 closed under N₂ atmosphere with a butyl rubber stopper and crimped, were incubated at 30°C
32 in the dark. Abiotic controls were prepared exactly the same way, unless addition of bacteria
33 was omitted.

1 Precipitates were collected from bacterial cultures and abiotic controls at t0 (i.e. before
2 inoculation of bacteria) and after 3 hours to 4 months by centrifugation (6500 g, 15 min),
3 rinsed twice with milli-Q water (with pH adjusted to 7.6 by addition of NaOH/HCl 0.1 M)
4 and vacuum-dried inside the glovebox.

5 All samples studied were collected from the same batch, unless for the following analyses: (a)
6 the 5-day old cultures used for magnetic measurements, (b) samples used for TEM imaging of
7 thin sections, and (c) 2-day old cultures used for STXM analyses, were collected from
8 separate cultures. All samplings were performed under anoxic conditions in a glovebox.

10 **2.2. Analytical methods**

11
12 The bulk Fe(II)/Fe(III) ratio was determined in the solid phase. Precipitates were
13 harvested by centrifugation (6500 g, 15 min), rinsed twice in degassed milli-Q water (pH
14 adjusted to 7.6) and vacuum-dried in the anoxic chamber. The solid was then dissolved in 6M
15 HCl and iron contents were determined spectrophotometrically at 562 nm using the ferrozine
16 assay (Viollier et al., 2000), either after dilution in 1 M HCl (for determining Fe(II) content)
17 or using hydroxylamine-HCl as a reducing agent (for the determination of Fe(II)+Fe(III)
18 content).

20 **2.3. Mineral characterization by X-ray diffraction**

21
22 The bulk mineralogy was determined by X-ray diffraction (XRD) of the solid phases
23 collected by centrifugation (5500 g, 15 min) at different stages of the experiments. Samples
24 were rinsed twice with degassed milli-Q water (pH adjusted to 7.6) and vacuum dried. The
25 powder was ground in an agate mortar, re-suspended in a small volume of degassed milli-Q
26 water and placed in an anoxic cell equipped either with a Kapton or a beryllium window in a
27 glovebox under N₂/H₂ (95% / 5%) or Ar atmosphere. This preparation guaranteed strictly
28 anoxic conditions over the duration of XRD measurements.

29 XRD measurements were performed with Co K α radiation ($\lambda_1 = 1.78897\text{\AA}$, $\lambda_2 =$
30 1.79285\AA) on either a Panalytical X'Pert Pro MPD diffractometer equipped with an
31 X'celerator® detector mounted in Bragg-Bertano configuration or on a Bruker D8 Advance
32 diffractometer equipped with a Vantec detector. A continuous collection mode was applied
33 over the 10-60° 2 θ range with a 0.033° 2 θ step and a total counting time of 1h30.

1

2 **2.4. Transmission Mössbauer spectroscopy**

3

4 ^{57}Fe Transmission Mössbauer spectra were measured with a source of ^{57}Co in rhodium
5 metal. The measurements were performed at room temperature (both the source and the
6 absorber at 294 K). For all measurements the spectrometer was operated with a triangular
7 velocity waveform, and a NaI scintillation detector was used for the detection of gamma rays.
8 The spectra were fitted with Lorentzian lines using the PC-Mos II computer program (Grosse,
9 1993). The absorbers were prepared in an Ar filled glovebox by mixing 20 mg of the powder
10 with 80 mg of boron nitride as a binder and packed in sealed coffee-bags to avoid any contact
11 with the ambient atmosphere during spectra acquisition. The isomer shift values are given
12 relative to αFe standard at room temperature.

13

14 **2.5. Microscopy analyses**

15

16 Two types of samples were prepared for TEM analyses.

17 First, ultrathin sections were prepared by ultramicrotomy. Cells were fixed for 2 h in 1%
18 glutaraldehyde at 4°C, centrifuged (5000 g – 10 min), rinsed three times in 20 mM HEPES
19 buffer (pH 7.5) for 18 h at 4°C. They were then post-fixed for 90 min in 1% OsO_4 in the same
20 buffer, rinsed three times in distilled water, dehydrated in graded ethanol and propylene
21 oxide-1, 2 and progressively embedded in epoxy resin (Epoxy, Sigma). Ultrathin sections (40-
22 nm thick) were cut with a LEICA ultramicrotome (EM-UC6). After deposition on copper
23 grids, they were stained with uranyl acetate (2% w/v).

24 Secondly, for scanning transmission electron microscopy (STEM), TEM and high-
25 resolution TEM (HRTEM), whole cells were deposited on a carbon-coated 200-mesh copper
26 grid after 2 rinses in degassed milli-Q water and stored under N_2 before analysis. STEM,
27 TEM and HRTEM observations were performed with a field emission gun (FEG)
28 JEOL2100F microscope operating at 200 kV. STEM observations were performed in high-
29 angle annular dark field (HAADF) mode. Selected-area electron diffraction (SAED) patterns
30 were obtained on areas of interest. Magnetite particle sizes were measured on at least 100
31 particles on TEM images.

32 For scanning electron microscopy (SEM) observations, samples were prepared by
33 depositing a drop of the rinsed culture (or abiotic sample) on a stub. Samples were carbon
34 coated before analysis. SEM pictures were recorded using a Zeiss Ultra 55 SEM equipped

1 with a field emission gun in secondary electron or back-scattered electron modes at 5 and 15
2 kV respectively and at working distances of 3 and 7.5 mm respectively.

4 **2.6. Scanning Transmission X-Ray Microscopy**

6 Samples were prepared and stored under anoxic conditions before analysis as described
7 earlier (Miot et al., 2009a). Briefly, rinsed samples were deposited on silicon nitride window
8 fixed on an aluminum sample holder, and covered with a second Si₃N₄ window sealed to the
9 first one with araldite. Samples were transferred to the microscope under N₂ flow and the
10 microscope chamber was then flushed with helium.

11 Part of the STXM analyses at the Fe L_{2,3} edges were performed at the Advanced Light
12 Source (Lawrence Berkeley National Laboratory, Berkeley, USA). Other analyses at the Fe
13 L_{2,3} edges as well as analyses at the C K-edge were performed at the 10ID-1 beamline at the
14 Canadian Light Source (CLS, Saskatoon). More details about beamline at the ALS can be
15 found in (Bluhm et al. 2006) and about beamline 10ID-1 at the CLS in (Kaznatcheev et al.,
16 2007). Both beamlines have an energy resolving power $E/\Delta E > 3000$. The energy scales for
17 this study were calibrated using the well-resolved 3p Rydberg peak of gaseous CO₂ for the C
18 K-edge and the major peak of hematite at 708.5 eV for the Fe L₃-edge.

19 Data acquisition was performed at the C K-edge and at the Fe L_{2,3}-edges as described
20 in (Miot et al., 2009a). Data (stacks and Near-Edge X-ray Absorption Fine Structure
21 (NEXAFS) spectra) were processed using the aXis2000 software-package (Hitchcock, 2001)
22 as described in (Miot et al., 2009a). More recent analytical details on STXM data processing
23 for Fe redox state investigation can be found in (Bourdelle et al., 2013). A review on the use
24 of STXM for characterizing biomineralizing systems and details on the type of information
25 that can be retrieved is provided by Cosmidis and Benzerara (2014).

27 **2.7. Magnetic measurements**

29 Samples collected from bacterial cultures and the abiotic control were rinsed twice in
30 degassed milli-Q water, dried under vacuum and stored under N₂ in sealed aluminum pockets
31 before analyses. To avoid oxidation, the cell sample preparation was conducted within an
32 anoxic chamber.

33 Rock magnetic measurements were performed as described in Li JH et al. (2012).
34 Briefly, room-temperature magnetic measurements were performed using a Model 3900

1 vibrating sample magnetometer (Princeton Measurements Corporation VSM3900, sensitivity
2 = 0.5×10^{-9} Am²). FORCs (Pike et al., 1999; Roberts et al., 2000) were measured following
3 the protocol as described by Roberts et al. (2000). For each sample, a total of 120 FORCs
4 were measured with a positive saturation field of 1000 mT, an increasing field step (δH) of
5 1.87 mT, and an averaging time of 200 ms. The FORC diagrams were calculated using the
6 FORCinel version 1.22 software with a smoothing factor of 3 (Harrison and Feinberg, 2008).
7 The FORC characteristic coercivity ($B_{c, \text{FORC}}$) is given by the median coercivity of the
8 marginal coercivity distribution (Harrison and Feinberg, 2008). Compared with other rock
9 magnetism methods such as conventional hysteresis loop, the FORC method simultaneously
10 incorporates information regarding magnetostatic interaction, microcoercivity and domain state
11 of particles (e.g., Li JH et al., 2012; Li JH et al., 2013).

12 Low-temperature magnetic measurements were performed with a Quantum Design
13 Magnetic Property Measurement System (MPMS XP-5, sensitivity = 5.0×10^{-10} Am²) under
14 vacuum. ZFC and FC curves were obtained by cooling samples from 300 K to 10 K in zero
15 field and in a 2.5-T field, respectively, followed by imparting a saturation isothermal
16 remanent magnetization SIRM in a 2.5-T field (hereafter termed as SIRM_{10K_2.5T}), and then
17 measuring the remanence in zero field during warming to 300 K.

18

19

3. RESULTS

20

3.1. Evolution of the mineralogy upon green rust oxidation in BoFeN1 cultures

21

22
23 Adjusting the pH of the biomineralization medium to 7.6 in the presence of 10 mM nitrate
24 led to massive precipitation of a dark green phase, identified by X-ray diffraction (XRD) (Fig.
25 1). The prominent peaks of this solid phase with d -spacings at $d_{003} = 7.92$ Å and
26 $d_{006} = 3.97$ Å correspond to those of hydroxylchloride green rust, GR(Cl), in accordance with
27 the trigonal symmetry $R\bar{3}m$ and computed unit cell parameters (hexagonal axes) $a = 0.319$
28 nm and $c = 2.385$ nm reported by Refait et al. (1998).

29 In biomineralization experiments with BoFeN1, bacterial growth was very limited (optical
30 density at 600 nm evolved from 0.109 up to 0.131 over one week), which can be attributed to
31 the composition of the biomineralization medium (absence of phosphate) that was not
32 designed to not promote bacterial growth. The starting precipitate turned into a mixture of
33 green and orange phases within the first day, which evolved after 2 days towards a black

1 precipitate that adhered to a magnetic rod. This is consistent with the mineralogy obtained
2 from XRD analyses indicating a mixture of lepidocrocite (γFeOOH) and magnetite (Fe_3O_4) in
3 the 4-day old BoFeN1 culture (Fig. 1). Accordingly, the $\text{Fe(II)}/(\text{Fe(II)}+\text{Fe(III)})$ ratio in the
4 solid phase rapidly dropped from 60 to 20% (i.e. bulk Fe redox state = $\text{Fe}^{2.8+}$) as indicated by
5 ferrozine analyses.

6 The end-products in the BoFeN1 4-day old culture (mixture of lepidocrocite and
7 magnetite) were further characterized by transmission Mössbauer spectroscopy (TMS) at
8 room temperature (RT) (Fig. 2, Table 1). The Mössbauer spectrum exhibited two sextets S_A
9 and S_B with hyperfine magnetic fields H of 48.7 T and 45.4 T corresponding to magnetite
10 (90%) (McCammon, 1995; Vandenberghe et al., 2000) and a doublet $D\gamma$ with hyperfine
11 parameters ($\delta = 0.37 \text{ mm.s}^{-1}$, $\Delta = 0.61 \text{ mm.s}^{-1}$) similar to those reported for lepidocrocite (10%)
12 (Ona-Nguema et al., 2002; Mitov et al., 2002; McCammon, 1995). Based on the best fit of
13 this spectrum, there was a deficit of Fe^{2+} ions in the octahedral sites of magnetite with 39% of
14 Fe^{3+} ions in the tetrahedral site (defined by $\delta = 0.30 \text{ mm.s}^{-1}$ and $\varepsilon = -0.02 \text{ mm.s}^{-1}$) vs. 51 %
15 ($\text{Fe}^{2+} + \text{Fe}^{3+}$) ions in the octahedral site (defined by $\delta = 0.63 \text{ mm.s}^{-1}$ and $\varepsilon = 0.02 \text{ mm.s}^{-1}$),
16 consistent with non-stoichiometric magnetite. The $\text{Fe(II)}/\text{Fe(III)}$ ratio in magnetite was thus
17 estimated to be 0.4 from TMS data. Moreover, the flat baseline of the TMS signal supports
18 the absence of small super-paramagnetic iron oxides. Given the relative proportions of
19 lepidocrocite and magnetite in this sample (10/90) deduced from Mössbauer data, the bulk Fe
20 redox state in the solid phase was thus estimated to be $\text{Fe}^{2.8+}$ from TMS data, which is similar
21 to the value deduced from acid-extraction Fe(II) measurements (ferrozine assay).

22 23 **3.2. Evolution of mineralogy and Fe redox state down to the nm-scale in BoFeN1** 24 **cultures**

25
26 The evolution of the mineralogy was followed at the sub-micrometer scale by (HR)TEM
27 in cultures of BoFeN1. As shown in Fig. 3, GR particles of a few tens of nanometers were
28 still present in the culture medium after 15 hours as determined by SAED of single crystals.
29 Their morphology exhibited however defects and was never perfectly octahedral.
30 Intermediate stages of GR transformation into lath-shaped lepidocrocite were observed in the
31 vicinity of the cells in 15-hour old samples (Fig. 3B). In contrast, only lepidocrocite was
32 identified by SAED directly at the cell contact from 15 hours on. This phase persisted over
33 the duration of the experiments (4 months, Fig. 3F to 3I), and was localized at the periphery

1 of BoFeN1 cells as observed in ultramicrotomy sections (Fig. 3F to H). This lepidocrocite
2 layer exhibited a thickness of 19 ± 10 nm, consistent with the thickness of the periplasm. In
3 addition, lepidocrocite particles exhibited a crystallographic orientation within the cell wall,
4 the crystals being parallel to the direction of the membranes (Fig. 3G to I). In 38-hour old
5 cultures, some magnetite crystals started to form at the contact of the GR particles (Fig. 3C,
6 3D) at distance from the bacteria (i.e. not at the cell surface). After 2 days, single crystals of
7 magnetite were observed by TEM and SAED (Fig. 3E). The mean size of the magnetite
8 particles increased slightly from 48 ± 17 nm to 55 ± 15 nm between 15 days and 4 months,
9 with a shape factor (width/length) around 0.8-1.0 (Fig. 4A and 4B), thus ranging within the
10 SD range (Butler and Banerjee, 1975; Muxworthy and Williams, 2009). Finally, in 4-month
11 old samples, magnetite particles consisted of single crystals (Fig. 3J to 3L and Fig. 5A and
12 5B) with some defects or aggregation, as deduced from SEM, HRTEM and SAED analyses.

13 The iron oxidation state of particles in 3-hour to 4-month old BoFeN1 samples was
14 further studied at the sub-micrometer scale using STXM at the Fe- $L_{2,3}$ edges (Fig. 6).
15 NEXAFS spectra measured on the starting green rust ($t = 0$) exhibited main absorption peaks
16 at 708 eV and 710.1 eV, consistently with a mixed valence Fe phase, as well as special
17 features previously described at the Fe L_2 edge for GR (shoulders at 719.5 and 723.3 eV and
18 double peak centered around 721 eV) (Pantke et al., 2012).

19 After 3 hours, the spectrum measured at the Fe $L_{2,3}$ edges on the bacterium was well fitted
20 with a mixture of lepidocrocite (17 %) and green rust (83 %) (Fig. 6A-G). This can be
21 interpreted either as the coexistence of GR and lepidocrocite on the bacteria or as the presence
22 of an intermediate oxidized GR phase or as the persistence of the initial Fe(III) (hydr)oxide
23 evidenced by TMS in the starting material. After 1 day, NEXAFS spectra recorded on the
24 bacteria exhibited a main absorption peak at 710.1 eV, consistent with lepidocrocite, in
25 agreement with TEM data. After 15 days, the culture consisted of a mixture of magnetite and
26 lepidocrocite, with lepidocrocite being localized exclusively at the cell contact and magnetite
27 at some distance from the cells (Fig. 6D-F). This mineralogical pattern persisted in 4-month
28 old samples.

29 STXM analyses at the C K-edge showed the association of lepidocrocite with organic
30 carbon, dominated by proteic moieties (Fig. 7). The map obtained by subtracting the image at
31 280 eV from the image at 288.2 eV thus corresponds mainly to the signature of amide
32 carbonyl groups (peptidic bonds) in proteins (Benzerara et al. 2004b, Fig. 7). Based on
33 spectroscopy at the C K-edge, no extracellular organic C signature (e.g. indicative of EPS)
34 could be clearly detected in these samples.

3.3. Evolution of mineralogy and Fe redox state down to the nm-scale in abiotic control

In the abiotic control, no change in the color of the precipitate was detected after 3 days, in agreement with the persistence of GR as the sole XRD-crystalline phase (Fig. 1) and by the absence of significant further oxidation of solid Fe(II) as deduced from acid-extracted Fe analyses of the solid phases. The kinetics of abiotic transformation of the initial precipitate into magnetite were many orders slower than in BoFeN1 cultures, with the first detection of magnetite observed by XRD only after 4 months (Fig. 1). This transformation might be attributed to slow diffusion of the XRD-amorphous Fe(III)-(hydr)oxide within the solution and subsequent reaction with GR(Cl).

Single crystals of magnetite obtained after 4 months exhibited a rather narrow size distribution, with a mean length of 94 ± 18 nm and a shape factor of 0.8-1.0 (Fig. 4 C), consistent with the range size of SD magnetite (Butler and Banerjee, 1975; Muxworthy and Williams, 2009). These crystals laid at the surface of a poorly electron-dense phase (Fig. 3M to O). The nature of this phase was further elucidated by STXM.

STXM data at the Fe $L_{2,3}$ edges showed the coexistence of magnetite with an Fe(II)-rich mixed valence phase, the NEXAFS spectrum of which exhibited the typical features of GR (Fig. 6H to 5K). These results suggested the persistence of a GR-like phase, which was not detected by XRD, most probably because of too low concentration.

3.4. Magnetic properties of Fe-bearing phases

Magnetic properties of Fe-bearing phases were examined over the course of the BoFeN1 cultures and in the abiotic control using FORC diagrams (Fig. 8). Consistently with TEM observations, the FORC diagrams showed a clear evolution of magnetite crystals from SP to SD particles with time (Carvallo et al., 2009; Li et al. 2009), consistently with the grain sizes measured by TEM (Fig. 4). The FORC diagram of 3-h old BoFeN1 culture is characterized by a small horizontal distribution (< 10 mT) and intersection with B_b axis with wide vertical distribution. This indicates that the magnetite is dominated by SP particles, along with a small fraction of particles with grain size around the threshold of SP/SD boundary. The FORC diagram of the 4-day old sample exhibits close concentric contours around a central peak, which is typical of SD particles (Pike et al., 1999; Roberts et al., 2000). Some contours still intersect with B_b axis, indicating co-occurrence of a small volume of SP particles. The wide

1 vertical distribution (e.g., close to ~20 mT) in the FORC diagram indicates strong magnetic
2 interactions within the samples (e.g., Pike et al., 1999; Roberts et al., 2000; Li JH et al.,
3 2012), consistent with TEM observations of aggregates of extracellular magnetite. The
4 calculated characteristic $B_{c, \text{FORC}}$, which was recently demonstrated to be equal to remanence
5 coercivity (e.g., Li JH et al., 2013b), is 2.0 mT and 15.6 mT for the 3-h and 4-day old
6 samples, respectively. Low-temperature magnetic measurements (i.e., ZFC and FC curves)
7 were performed to further characterize the magnetic anisotropy and chemical composition
8 (i.e., stoichiometry) of BoFeN1 magnetite (e.g., Moskowitz et al., 1993; Kakol & Honig,
9 1989; Li JH et al., 2012). Fig. 8c shows the thermal demagnetization curves of
10 SIRM10K_2.5T for 4-day old BoFeN1 culture. Both ZFC and FC warming curves exhibit
11 steep SIRM decay and intensity difference at temperatures lower than 30 K, which results
12 from unblocking behavior of SP particles and magnetic interactions within samples (e.g.,
13 Moskowitz et al., 1993; Li JH et al., 2012). Above 30 K, ZFC shows identical behavior of
14 thermal demagnetization to FC, and no obvious Verwey transition around 100-120 K is
15 observed in both curves. The absence of Verwey transition could be explained by (1) the
16 dominance of non-stoichiometric magnetite crystals within the 4-day old BoFeN1 culture, in
17 accordance with TMS and FORC analyses (Fig. 2 and 8) and (2) the co-existence of SP
18 particles (Muxworthy and McClelland, 2000; Wang and Lovlie, 2008). Moreover, the ZFC-
19 and FC-SIRM_{10K_2.5T} warming curves obtained for BoFeN1 magnetites differ from that
20 reported for magnetite produced by magnetotactic bacteria, which are generally controlled by
21 shape anisotropy (Moskowitz et al., 1993; Li JH et al., 2012; Li JH et al., 2013a). In contrast,
22 magnetic analyses of BoFeN1 magnetites indicate the absence of particles chains or of
23 elongated crystals (magnetocrystalline anisotropy), in accordance with TEM observations of
24 randomly aggregated cubic or spherical crystals (Fig. 3). The 4-month old BoFeN1 culture
25 displays no obvious difference in magnetic behavior from the 4-day old sample (data not
26 shown).

27 We also measured the FORC diagram of the 4-month old abiotic sample where
28 magnetite crystals formed through slow oxidation of GR. As shown in Fig. 8d, abiotic
29 magnetite exhibits similar magnetic properties as 4-day and 4-month old BoFeN1 magnetites,
30 i.e., dominated by strongly interacting SD particles. The calculated $B_{c, \text{FORC}}$ value of ~16.4 mT
31 indicates comparable crystal size distribution, crystallinity, and/or particle-particle
32 interactions.

33

34

4. DISCUSSION

4.1. Biomineralization of extracellular magnetite induced by nitrate-dependent iron oxidation.

Biomagnetite production by magnetotactic bacteria and Fe(III)-reducing bacteria has been extensively studied (see Li JH et al., 2013a for a review). In contrast, whether magnetite can be formed by Fe(II)-oxidizing bacteria remained still unclear. Here, we experimentally evidence that the nitrate-reducing Fe(II)-oxidizing strain BoFeN1 can promote the formation of stable single domain magnetite.

This strain can form a diversity of Fe-bearing minerals depending on culture conditions: lepidocrocite is obtained at neutral pH (Larese-Casanova et al., 2010; Miot et al., 2014), whereas increasing pH, phosphate or carbonate concentrations, as well as adding humic acids promote the formation of goethite (Kappler et al., 2005; Larese-Casanova et al., 2010). In addition, hydroxycarbonate green rust was shown to form as an intermediate product on the way to goethite biomineralization (Pantke et al., 2012). Eventually, in a medium rich in dissolved phosphate or in the presence of the solid Fe(II)-phosphate vivianite, amorphous Fe-phosphates, exhibiting varying $\text{Fe}^{\text{III}}/(\text{Fe}^{\text{II}}+\text{Fe}^{\text{III}})$ ratios are obtained (Miot et al., 2009a; Miot et al., 2009b). Moreover, growth of magnetite crystals in cultures of BoFeN1 initially seeded with magnetite particles had also been reported (Dippon et al., 2012). In the present study, magnetite precipitation is induced by the activity of BoFeN1 under non-growth conditions, without the need for initial magnetite particles serving as nucleation sites. Our results not only add to the list of iron-bearing phases known to form in cultures of this bacterial strain, but also contribute to a more complex picture of Fe redox cycling, involving Fe(II)-oxidizing bacteria in the formation of mixed valence Fe-bearing minerals.

The mechanisms of Fe(II) oxidation by nitrate-reducers is (at least partly) linked to the production of reactive intermediate nitrite molecules (Miot et al., 2011; Klueglein and Kappler, 2013; Kopf et al., 2013; Carlson et al., 2013; Etique et al., 2014), thus potentially extending the number of anaerobic strains able to promote Fe-bearing mineral formation to all nitrite-producing bacteria. Interestingly, magnetite biomineralization dependent upon the periplasmic nitrate reductase Nap has been recently evidenced in the magnetotactic bacterium *Magnetospirillum gryphiswaldense* MSR-1 (Li YJ et al., 2012), suggesting an intrinsic link between nitrate reduction and magnetite biomineralization in some magnetotactic strains. Future investigations using diverse nitrate-reducing bacteria would potentially provide further

1 insights about the extent of the processes of magnetite biomineralization associated with
2 microbial nitrate reduction.

4 **4.2. Green rust transformation to magnetite and lepidocrocite promoted by BoFeN1**

6 Usually, GR oxidation proceeds either by dissolution reprecipitation or by *in situ*
7 deprotonation (Ruby et al., 2010). At low redox potential, GR can directly transform to
8 magnetite, with a concomitant release of dissolved Fe(II), whereas at higher redox potential,
9 GR oxidation is complete, leading to the formation of Fe(III)-oxyhydroxides, e.g. ferrihydrite.
10 With carbonate green-rust, both reactions have been shown to occur through dissolution-
11 reprecipitation processes (Ruby et al., 2010). In addition, dissolved Fe(II) can further
12 reductively transform Fe(III)-oxyhydroxide to magnetite (Usman et al., 2012). All these
13 considerations can be reconciled with our observations in the following scenario (Fig. 9): GR
14 transformation depends on local oxidant concentration (i.e. redox potential) in cultures of
15 BoFeN1. (1) At low oxidant concentration, GR(Cl) directly transforms to magnetite,
16 involving a net release of dissolved Fe(II). (2) In parallel, at higher oxidant concentration,
17 GR(Cl) transforms to Fe(III)-oxyhydroxides (lepidocrocite), that further react with dissolved
18 Fe(II) to transform to magnetite (Misawa et al., 1974). This last step is enhanced by the
19 release of dissolved Fe(II) from the first reaction. In addition, periplasmic oxidation of
20 dissolved Fe(II) by BoFeN1 (through reaction with nitrite, (Klueglein and Kappler, 2013,
21 Kopf et al., 2013; Carlson et al., 2013) leads to periplasmic encrustation by lepidocrocite,
22 enhancing the dissolution of GR by equilibrium displacement, hence promoting an increased
23 extracellular dissolved Fe(II) concentration. Assessing the mechanisms of each of these steps
24 will require a complete chemical mass balance (including N species tracking) as well as
25 monitoring the redox potential over the course of the culture. In the end, heterogeneities in the
26 mineralogy of this system may reflect heterogeneities in redox conditions induced by bacterial
27 nitrate-reducing activity.

28 There are differences in the formation of magnetite between the abiotic control and
29 BoFeN1 cultures: (1) the kinetics of the reaction are many orders more rapid in BoFeN1
30 cultures than in abiotic controls (3 days vs. 4 months) and (2) the transformation of GR is
31 complete in the presence of BoFeN1 (no remaining GR could be detected by any of the
32 methods used in this study) whereas it was incomplete in the abiotic sample as suggested by
33 the persistence of an electron-light phase (Fig. 3) having a NEXAFS spectrum similar to GR
34 at the Fe L_{2,3}-edge (Fig. 6). The very slow transformation of GR to magnetite in the abiotic

1 control might be related to the slow diffusion of an amorphous Fe(III) (hydr)oxide phase
2 reacting with green rust (Fig. 4, Table 1) and/or to nitrate reduction (Hansen et al., 1996),
3 whereas nitrate might be much more rapidly reduced through bacterial activity in BoFeN1
4 cultures and thus unavailable for GR oxidation.

6 **4.3. Patterns of iron biomineralization**

8 TEM observations evidence the presence of a lepidocrocite layer at the periphery of the
9 bacteria, exhibiting a thickness consistent with that of the periplasm. Such a periplasmic
10 encrustation of BoFeN1 cells by Fe-bearing phases is also consistent with previous reports
11 showing periplasmic encrustation of this strain by either Fe-phosphates (e.g. Miot et al.,
12 2009a) or Fe-oxyhydroxides (e.g. Miot et al., 2014). These observations also add to the
13 diversity of bacteria that were shown to localize biomineralization within the periplasm,
14 leading to the formation of cells encrusted with phosphates (Benzerara et al., 2004a; Goulhen
15 et al., 2005; Dunham-Cheatham et al., 2011; Cosmidis et al., 2013), oxides (Gloter et al.,
16 2004; Benzerara et al., 2008) or sulfides (Donald and Southam, 1999).

17 Moreover, in the present study, periplasmic lepidocrocite crystals are strongly anisotropic,
18 elongated parallel to the cell wall (Fig. 3). Such a crystallographic orientation within bacterial
19 cell walls has also been reported for phosphates within the periplasm of *Ramlibacter* sp.
20 (Benzerara et al., 2004a) and for hematite ($\alpha\text{Fe}_2\text{O}_3$) obtained after heating encrusted BoFeN1
21 cells (Miot et al., 2014). Hence, the periplasm seems to control the crystallographic
22 orientation of these biominerals.

23 We observe that periplasmic lepidocrocite persists even after a few months, whereas one
24 may expect conversion to magnetite by partial reduction by dissolved Fe(II). This might be
25 explained either by an active control of Fe(II) traffic towards the periplasm, a protective role
26 played by organic matter (e.g. Jones et al., 2009) or the trapping of dissolved Fe(II) through
27 instantaneous reaction with extracellular Fe(III)-oxyhydroxides, i.e. before dissolved Fe(II)
28 reaches the cell.

29 Magnetites produced in BoFeN1 cultures are dominantly controlled by magnetocrystalline
30 anisotropy (i.e., cubic morphology) (Fig. 8), which is distinct from MTB magnetite usually
31 controlled by shape anisotropy (particle elongation or/and chain structure) (Li JH et al. 2013a,
32 Li JH et al., 2010). Moreover, the bulk Fe(II)/Fe(III) of magnetite in the BoFeN1 system is
33 0.4 as deduced from TMS data, i.e. magnetite is non-stoichiometric, slightly oversaturated
34 with Fe(III). This differs strongly from what is known in other magnetite biomineralization

1 pathways. Indeed, the obvious presence of Verwey transition behavior in magnetites produced
2 by magnetotactic bacteria suggests that they are close to stoichiometry, although all
3 magnetites from magnetotactic bacteria discovered thus far have reduced Verwey transition
4 temperature (i.e., ~100-110 K) compared to ~120-125 K for chemically synthesized
5 magnetites (e.g. Moskowitz et al., 1993; Li JH et al. 2012). In contrast, nanomagnetite
6 produced by dissimilatory Fe(III)-reduction (cultures of *Shewanella* sp.) has been shown to be
7 oversaturated with Fe(II) compared with abiotic magnetite (Carvallo et al., 2008; Kukkadapu
8 et al., 2005; Coker et al., 2007).

9 Here, the Fe(II)/Fe(III) ratio estimated at the nm-scale in cultures of BoFeN1 reflects
10 redox microenvironments controlled by bacterial Fe(II) oxidation and nitrate reduction, with
11 lepidocrocite in the cell wall and magnetite at distance from the cells. Heterogeneous
12 mineralization patterns reflecting redox microenvironments have been observed in other
13 systems, e.g. in cultures of dissimilatory iron-reducing bacteria (Coker et al. 2012) or in
14 cultures of photoferrotrophs (Miot et al., 2009c). However, in these previous studies, the
15 mineralogy at the nm-scale was very different with (1) the coexistence of magnetite at the cell
16 contact and maghemite-like phases at distance from the cells in cultures of the dissimilatory
17 Fe(III)-reducing bacteria *Shewanella oneidensis* (Coker et al. 2012) and (2) the presence of
18 nano-goethite exhibiting Fe(II)/Fe(III) gradients along lipopolysaccharidic fibers in cultures
19 of *Rhodobacter* sp. strain SW2 (Miot et al. 2009c). Thus, the biomineralization patterns
20 observed here at the nm-scale exhibit very specific features.

21 22 23 **4.4. Implications for the search of biosignatures in the fossil record**

24
25 Anaerobic Fe(II)-oxidizing bacteria have been proposed to play an important role over
26 Earth's history. On the one hand, anaerobic photosynthetic Fe(II)-oxidizing bacteria are
27 increasingly thought to have played a quantitative role in the Fe redox biogeochemical cycle
28 on the early anoxic Earth, by promoting the precipitation of Fe(III)-(oxyhydr)oxides
29 (Konhauser et al., 2002; Posth et al., 2008; Planavsky et al., 2009; Czaja et al., 2013; Köhler
30 et al., 2013). On the other hand, anaerobic nitrate-reducing iron(II)-oxidizers, whose activity
31 might have been dependent upon nitrate advent in the nitrogen cycle under (at least locally)
32 more oxidizing conditions (e.g. Ilbert and Bonnefoy, 2013; Busigny et al., 2013) can produce
33 miscellaneous Fe(III)-bearing minerals. Here, we uncover the production of stable SD
34 magnetite by such nitrate reducers, whereas SD magnetite biomineralization has been usually

1 attributed to the activity of magnetotactic bacteria (Li JH et al., 2013a). These results have
2 potential implications for the study of geomicrobiological processes occurring in past and
3 modern environments. Indeed, stable SD magnetite is the main carrier of stable remanent
4 magnetization in some sediments and sedimentary rocks (Petersen et al., 1986; Chang, 1989;
5 Roberts et al., 2012). Moreover, this mineral is widespread in the geological record, from the
6 ancient Earth to modern environments. The potential role played by nitrite-producing bacteria
7 should thus be taken into account when evaluating the processes responsible for magnetite
8 biomineralization.

9 Past and modern geochemical systems involving green rust and magnetite are usually
10 interpreted as to result from abiotic processes and/or from the activity of DIRB (Lovley et al.,
11 1987). Our present study shows that microbial anaerobic iron oxidation can also play a role in
12 such systems. Interestingly, the co-occurrence of green rust and magnetite has been recently
13 observed in the meromictic lake Matano considered as an analog of Precambrian oceans
14 (Zegeye et al., 2012).

15 Magnetite is a major component of Banded Iron Formations (BIFs) (Klein, 2005), along
16 with hematite and siderite. Growing evidence suggests that primary iron oxides originated
17 from bacterial anaerobic iron oxidation (namely photoferrotrophy, e.g. Konhauser et al., 2002;
18 Posth et al., 2008), although cyanobacteria-mediated O₂ oxidation has also been proposed.
19 Moreover, as an alternative or in addition to potential diagenetic origins (e.g. Morris, 1993;
20 Pecoits et al., 2009), Fe(II)-bearing phases (e.g. siderite, magnetite) have been proposed to
21 originate from Fe(III)-(hydr)oxides bioreduction driven by DIRB. This biological origin is
22 supported by isotopic compositions of Fe (Johnson et al., 2008; Heimann et al., 2010) and C
23 (Papineau et al., 2010), crystallochemical (Li YL et al., 2011), and experimental data (Li YL
24 et al., 2013). Potential involvement of anaerobic nitrate-reducing Fe(II)-oxidizing bacteria in
25 the formation of magnetite in such past environments would have been dependent upon the
26 availability of nitrate (i.e. upon the advent of atmospheric oxygenation, or locally O₂-rich
27 areas or any other – e.g. microbial – source of nitrate) in the Archean ocean (e.g. Busigny et
28 al., 2013).

29
30 Importantly, our study shows that magnetite exhibiting similar crystallochemical and
31 magnetic properties could be obtained by an abiotic route at low temperature. Hence, none of
32 the properties of magnetite produced by BoFeN1 can be held as a biosignature *per se*.
33 However, the coexistence of (1) stable single domain magnetite with (2) lepidocrocite
34 exhibiting a crystallographic orientation and a thickness consistent with that of a bacterial cell

1 wall and (3) in association with protein moieties (or protein-derived moieties after diagenesis)
2 might represent a very specific feature to be looked for in the geological record. Such redox
3 heterogeneities at the nanometer-scale, associated with organic matter, and reflecting the
4 redox conditions imposed by bacterial activity have been previously reported in cultures of
5 iron-oxidizing bacteria (Miot et al., 2009a; Miot et al., 2009c) and suggested to provide
6 biosignatures of iron oxidizing metabolism. The evolution of such assemblages upon
7 diagenesis and metamorphism has to be evaluated but might preserve primary redox and
8 organic signatures (e.g. Bernard et al., 2007; Koehler et al., 2013). Indeed, heating
9 lepidocrocite mineralized BoFeN1 cells at 700°C in the air led to structures exhibiting an
10 intact bacterial morphology and composed of hematite crystallographically oriented parallel
11 to the cell wall (Miot et al., 2014). Preservation of organic carbon molecules in heated
12 mineralized BoFeN1 cells under anoxic conditions has also been recently observed (Li JH et
13 al. 2013a). Eventually, as shown by (Li YL et al., 2013) diagenetic conditions might induce
14 magnetite crystal growth from a few tens of nm up to a few micrometers.

15

16

17

5. CONCLUSION

18

19 The present study reports a new pathway of magnetite biomineralization through
20 hydroxychloride green rust oxidation promoted by the anaerobic nitrate-reducing iron-
21 oxidizing bacteria *Acidovorax* sp. strain BoFeN1. STXM analyses coupled with TEM
22 observations evidence strong redox heterogeneities. Whereas lepidocrocite is mineralized
23 within the bacterial periplasm and thus associated with protein moieties, stable single domain
24 magnetite is precipitated extracellularly. By comparison, abiotic oxidation of green rust
25 operating at much slower kinetics (4 months vs. 2 days) provides an incomplete
26 transformation of hydroxychloride green rust to stable single domain magnetite, and does not
27 produce lepidocrocite. Hence, the association of redox heterogeneities with the persistence of
28 organic compounds might constitute valuable biosignatures to be looked for in the rock
29 record. In addition, this study uncovers a new pathway for magnetite biomineralization that
30 should be taken into account when looking for the microbial processes involved in magnetite
31 formation in past and modern environments. Eventually, this study stresses the importance of
32 nitrite-producing bacteria in iron biogeochemistry and adds to the complexity of Fe redox
33 cycling in the environment, which might have implications for the processes controlling
34 pollutant mobility.

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2

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Figure captions

Figure 1 - X-ray diffraction patterns of solid phases produced in abiotic control and BoFeN1 cultures.

Figure 2 – Room-temperature Mössbauer spectra of the 4-day old precipitate collected from BoFeN1 cultures (A) and the 4-month old abiotic control (B). Dots: experimental curves; line: global computed curve; coloured lines: elementary components.

Figure 3 – TEM analysis of the mineralogy obtained in cultures of BoFeN1 at pH 7.6 (A to L) and in abiotic control (M to O). (A) After 15 h, bacteria are encrusted with lepidocrocite, as shown by SAED (bottom), whereas green rust is observed in the extracellular medium (corresponding SAED pattern displayed in the top panel). (B) 15-h old sample, showing local transformation of GR to needles. (C) 38-h old sample: magnetite particles at the surface of partly transformed GR (with corresponding SAED pattern). (D) 38-h old sample: magnetite particles (white) at the surface of a GR hexagonal platelet (light grey) observed by STEM. (E) 2-day old sample: extracellular magnetite with corresponding SAED pattern. (F) 15-day old sample observed in thin section showing lepidocrocite precipitated within the periplasm. (G and H) HRTEM analysis of these lepidocrocite particles showing crystallographic orientation with the (020) axis parallel to the cell wall direction. (I) to (L): HRTEM observations of 4-month old BoFeN1 sample, with preserved crystallographic orientation of lepidocrocite within the periplasm (I) and extracellular single domain magnetite crystals (J, K, L). (M) to (O): (HR)TEM observations of single domain magnetite crystals produced in the abiotic control after 4 months. Arrow in (O) indicates an amorphous rim at the surface of the Mt particle.

Figure 4 – Distribution of grain size of magnetite particles in 15-day old (A) and 4-month old BoFeN1 cultures (B) and in the 4-month old abiotic control (C).

Figure 5 - SEM observations of magnetite formed in 4-month old cultures of BoFeN1 (A, B) and in 4-month old abiotic control (C, D).

Figure 6 – STXM analysis at the Fe $L_{2,3}$ -edges of BoFeN1 cultures (A to G) and abiotic control (H to K). (A, B, C): 3-h old BoFeN1 culture, with the map of the GR (A) and oxidized GR (B) respectively as well as the composite overlay map (C). (D, E, F): 15-day old BoFeN1 sample with the maps of magnetite (D) and lepidocrocite (E) and the corresponding overlap (F). (G) NEXAFS spectra collected on starting GR (green), on the bacterium in (C) (orange), on a bacterium from a 1-day old sample (red) and on extracellular magnetite in (F) (black). The NEXAFS spectrum of the 3-h old bacteria was fitted with 83% of the GR component and 17% of the lepidocrocite component. (H, I, J): 4-month old abiotic control, with GR-like (H) and magnetite-like (I) maps and corresponding overlap (J). (K) displays the corresponding NEXAFS spectra.

1 **Figure 7** – STXM analysis of BoFeN1 1-day old sample at the C K-edge and Fe L_{2,3}-edges.
 2 (A): map of organic carbon, mainly showing the contribution of proteins (288.2 – 280 eV).
 3 (B): map of total Fe (710 – 700 eV). (C): Composite map of proteins (C, 288.2 – 280 eV,
 4 blue), lepidocrocite (Lp, 710eV – 708.5 eV, pink) and Fe(II)-bearing phase, i.e. magnetite
 5 (Mt, 708.5 – 700 eV, green). (D) C K-edge NEXAFS spectra of reference albumin and
 6 mineralized 1-day old BoFeN1 cells. Scale bars, 1 μm .

7
 8 **Figure 8** - Magnetic properties of green rust transformed by BoFeN1: FORC diagrams for
 9 BoFeN1 cultures after 3 h (A) and 4 days (B), thermal demagnetization curves of
 10 SIRM10K_2.5T for 4-day old BoFeN1 (C), and FORC diagram obtained for the 4-month old
 11 abiotic control (D).

12
 13 **Figure 9** – Proposed mechanisms for the transformation of GR to periplasmic lepidocrocite
 14 and extracellular magnetite.

15
 16
 17 **Table captions**

18
 19 **Table 1** – Mössbauer hyperfine parameters of spectra from Fig. 2 measured at room
 20 temperature.

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	$\delta(\text{mm/s})$	$\Delta\text{or } \varepsilon(\text{mm/s})$	HF (T)	RA (%)	Attribution
4-day old BoFeN1 culture					
S_A	0.30 ± 0.01	-0.02 ± 0.01	48.7 ± 0.1	39 ± 1	Magnetite (Td)
S_B	0.63 ± 0.01	0.02 ± 0.01	45.4 ± 0.1	51 ± 1	Magnetite (Oh)
D_γ	0.37 ± 0.01	0.61 ± 0.02		10 ± 1	Lepidocrocite
4-month old abiotic control					
S_A	0.29 ± 0.01	-0.02 ± 0.02	48.7 ± 0.1	34 ± 3	Magnetite (Td)
S_B	0.64 ± 0.02	0.02 ± 0.03	45.4 ± 0.1	58 ± 4	Magnetite (Oh)
D_γ	0.18 ± 0.08	0.66 ± 0.12		8 ± 7	Fe(III) in XRD-amorphous Fe ^{III} oxide

25 δ , isomer shift taking iron as reference at room temperature; $\Delta\text{or } \varepsilon$ quadrupole splitting; RA ,
 26 relative abundance.

27
 28 **Table 1**

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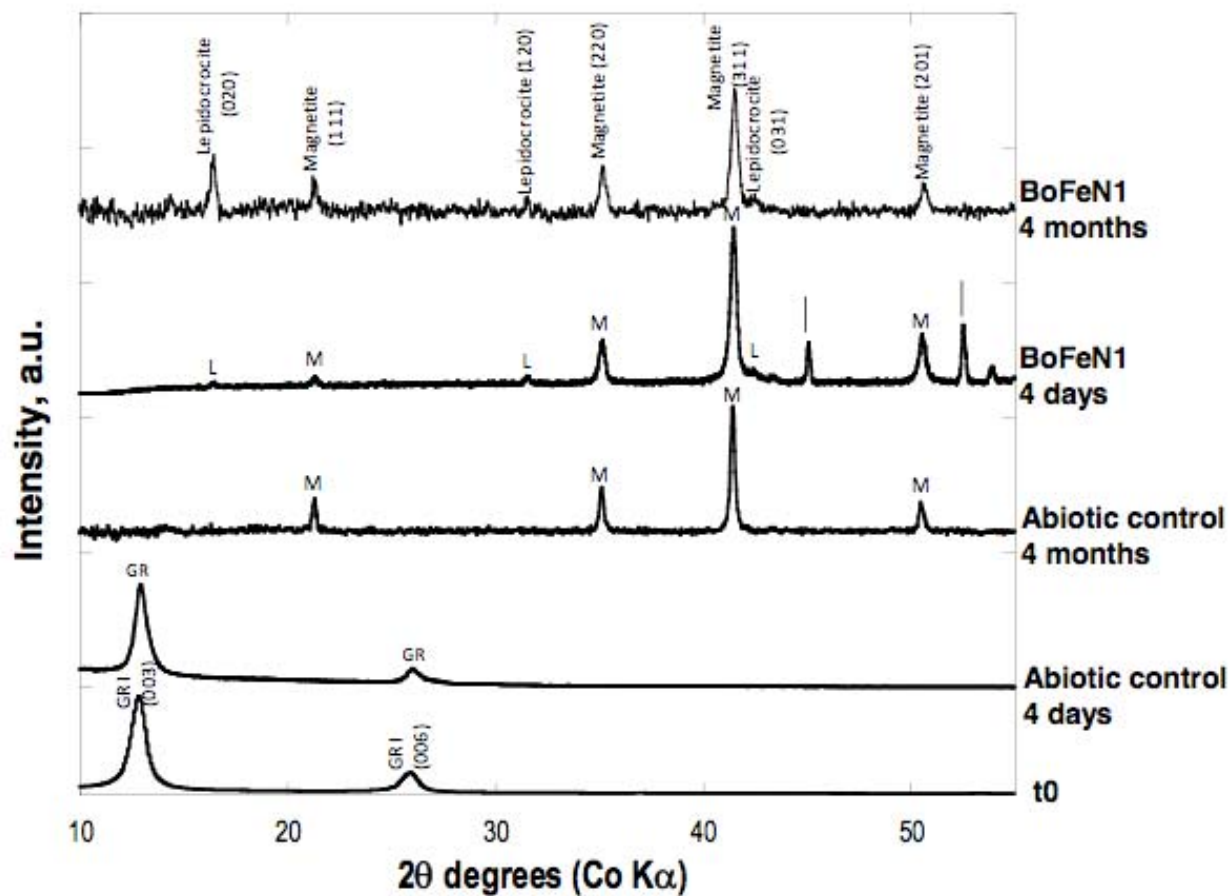


Figure 1

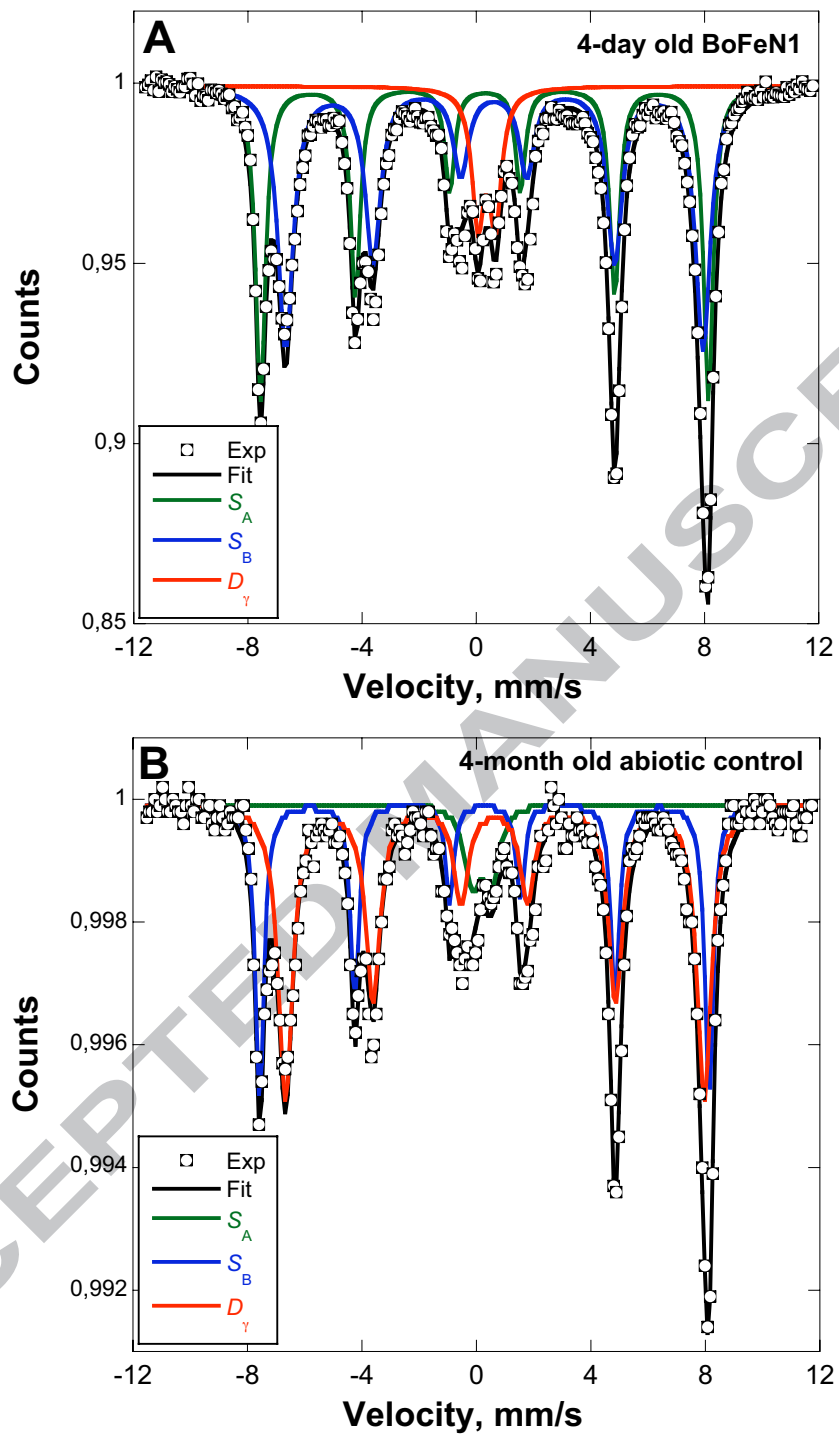


Figure 2

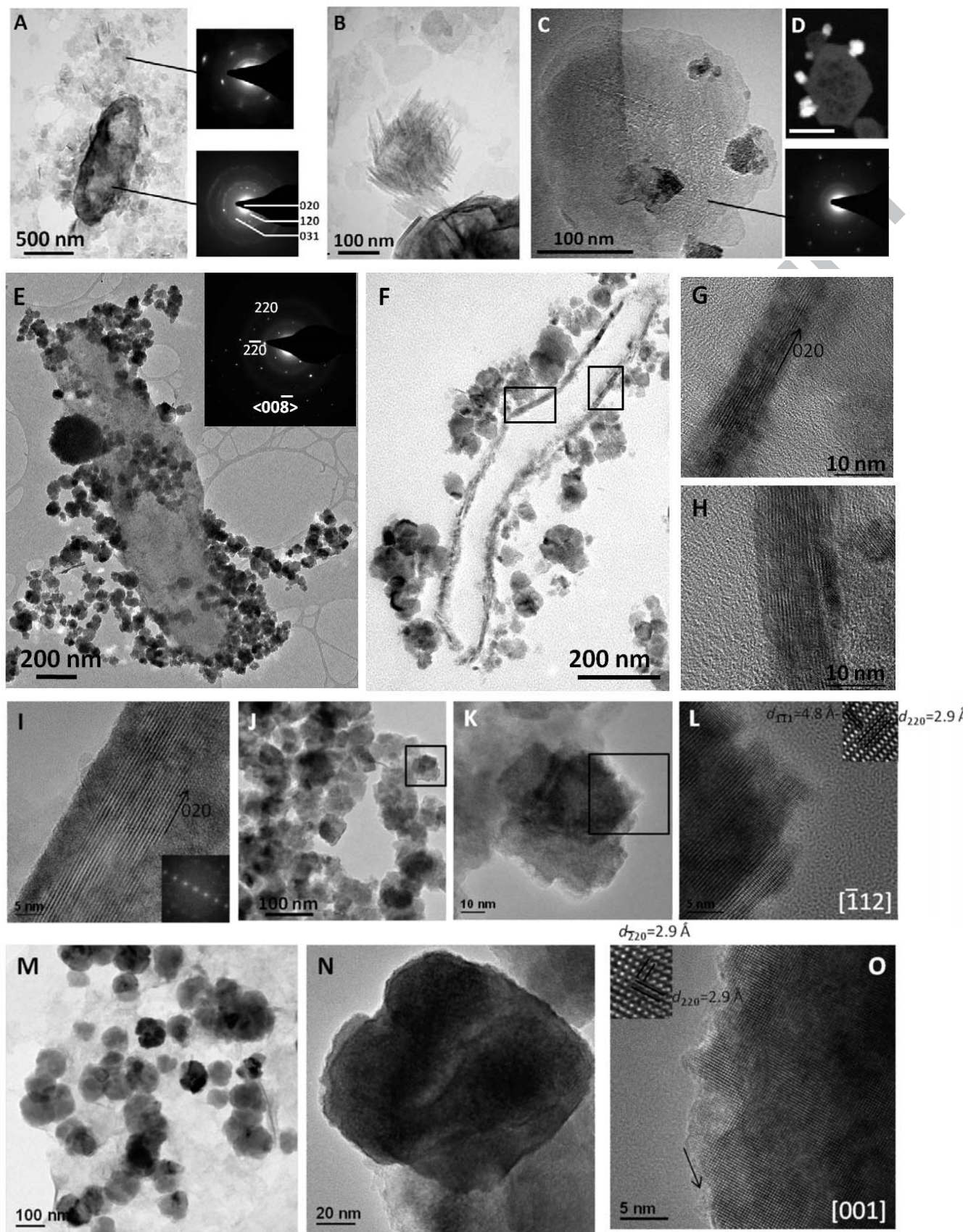


Figure 3

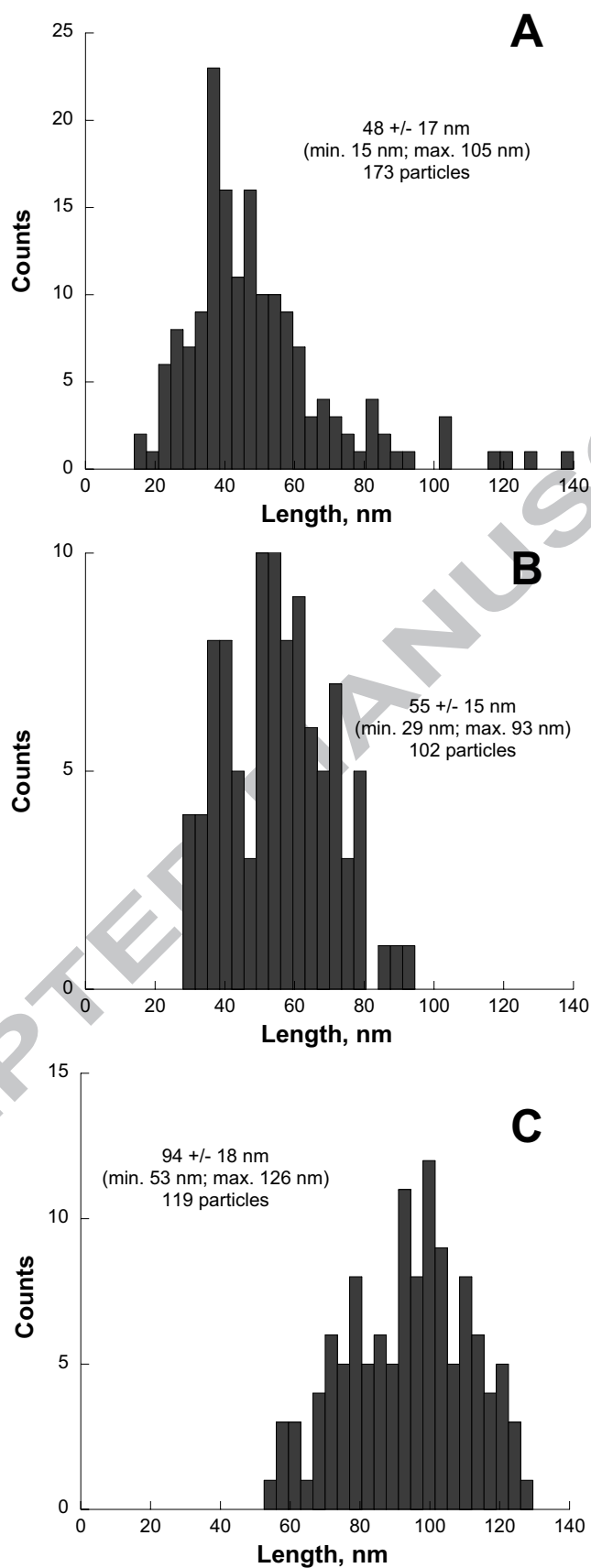


Figure 4

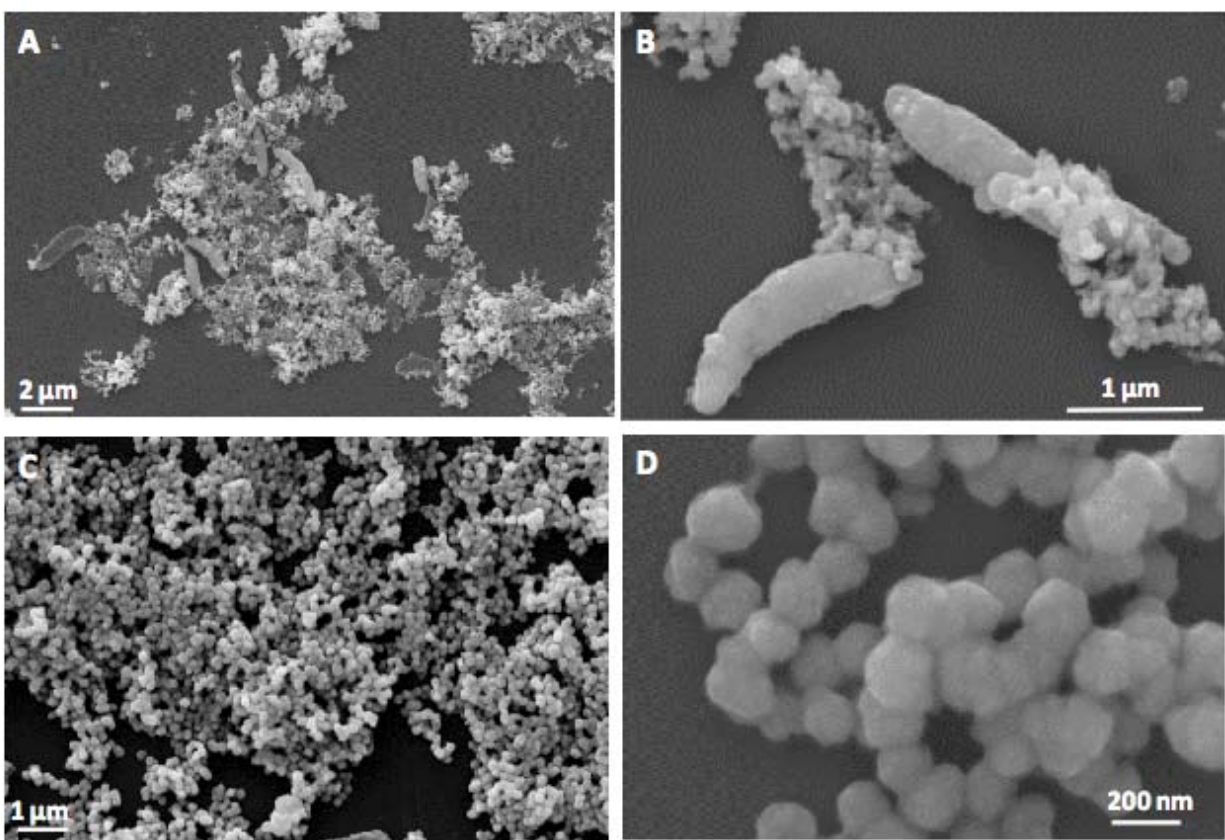


Figure 5

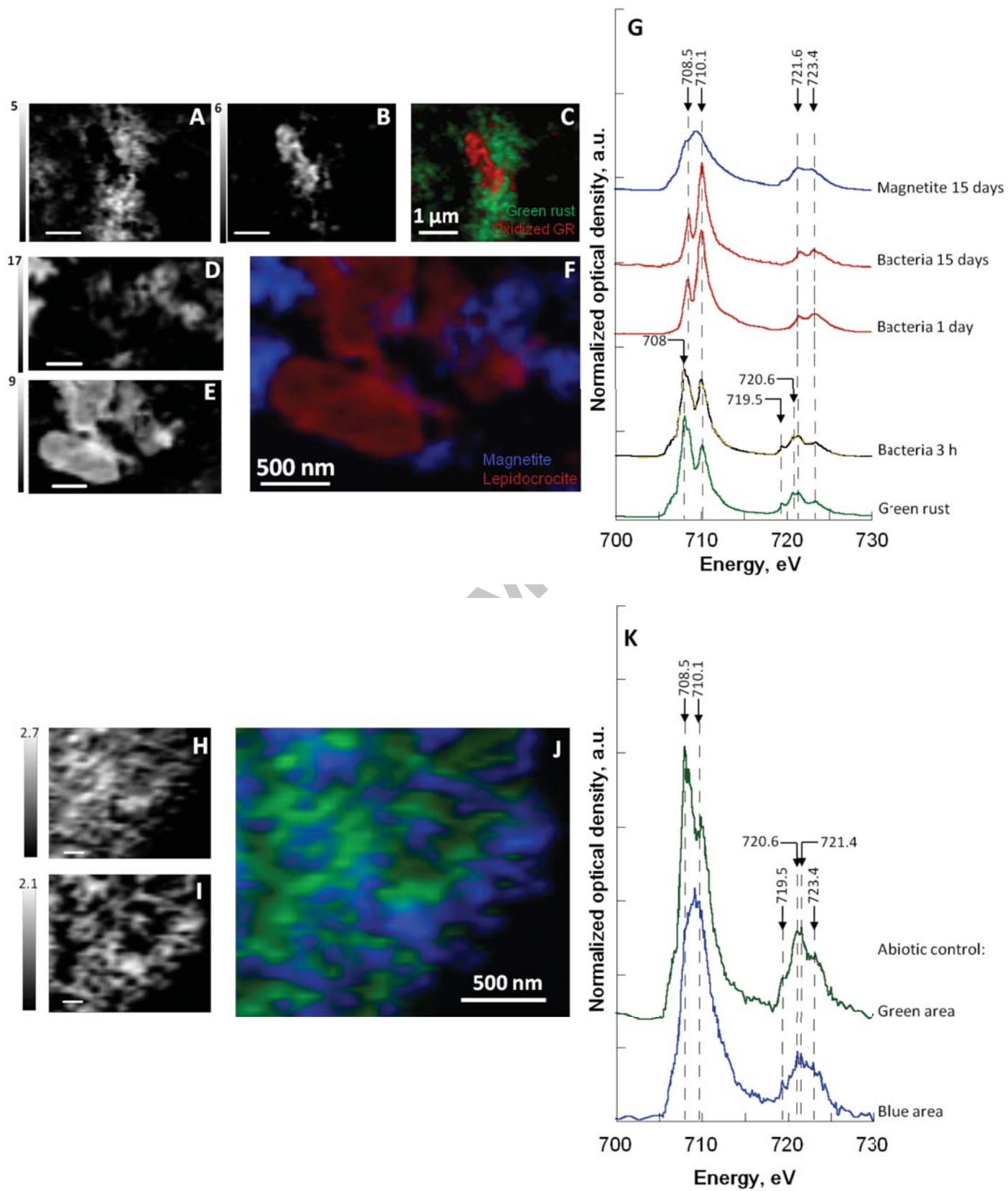


Figure 6

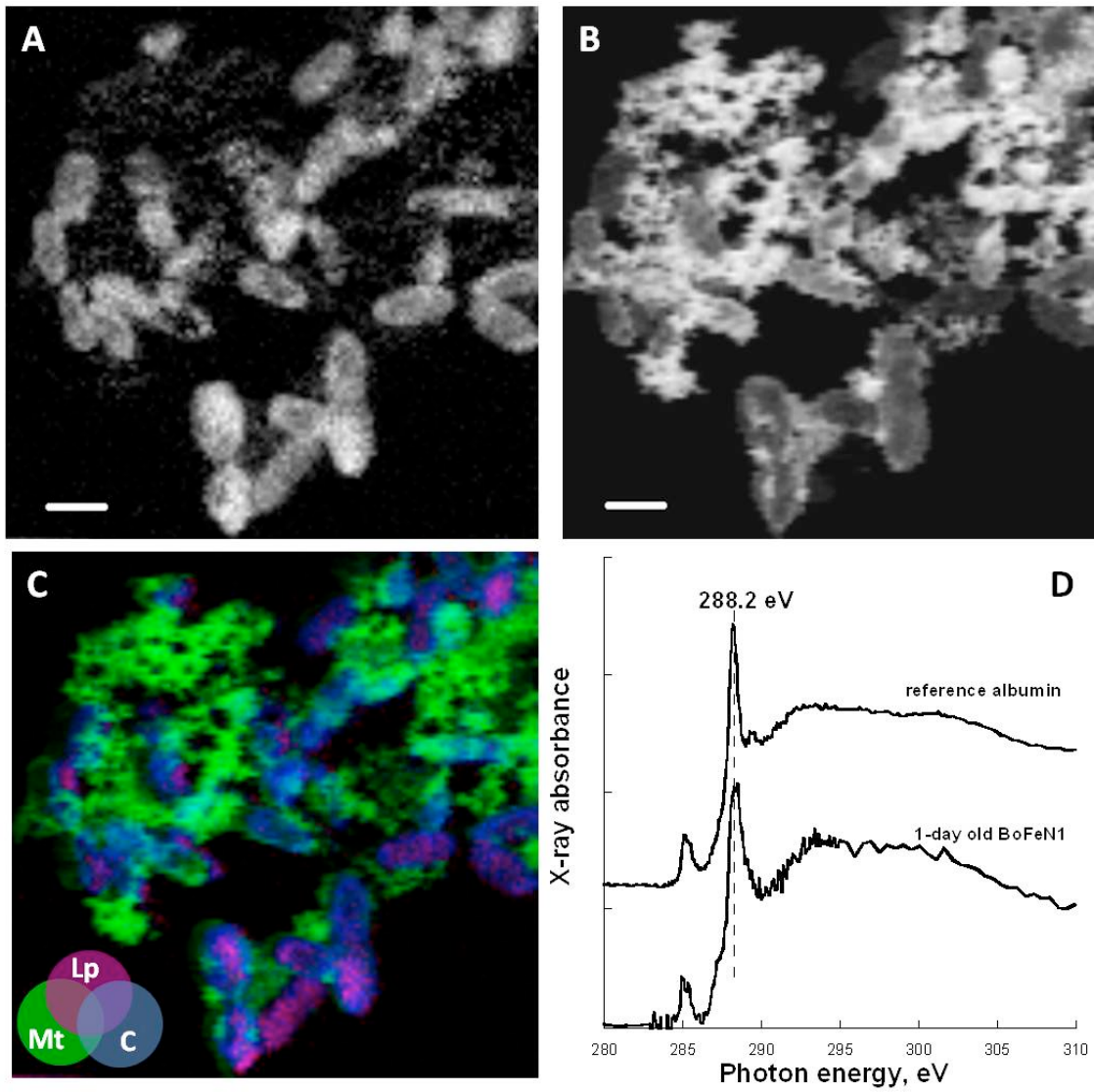


Figure 7

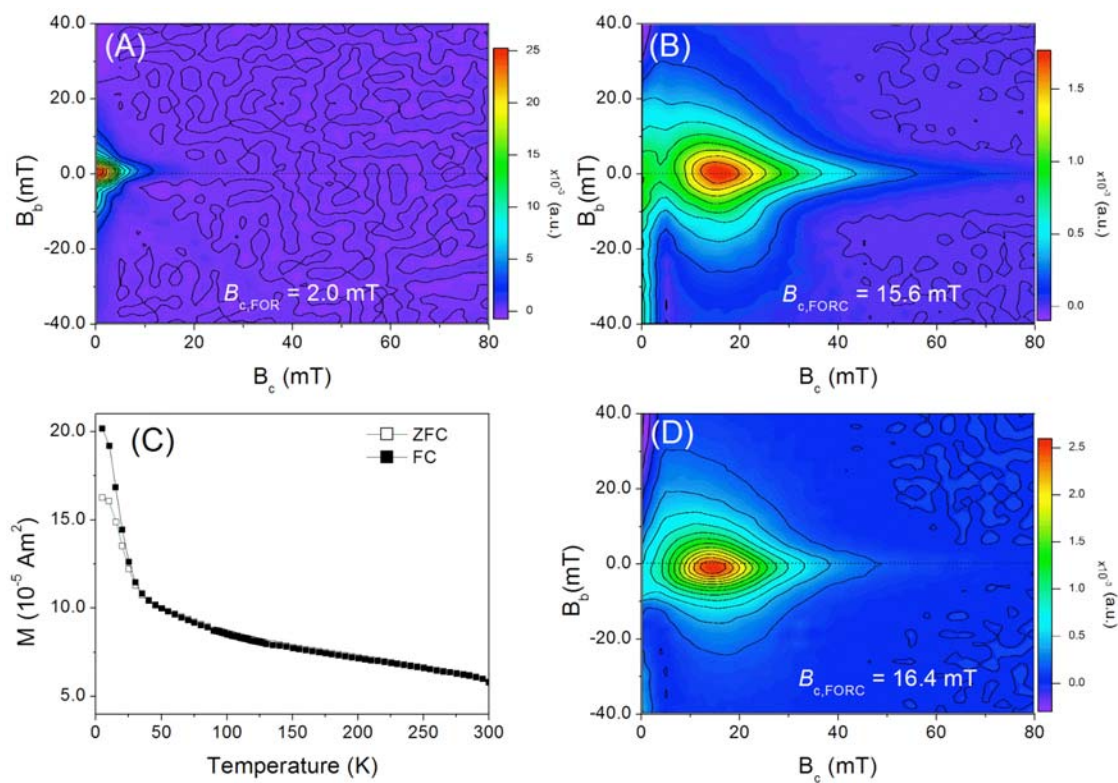


Figure 8

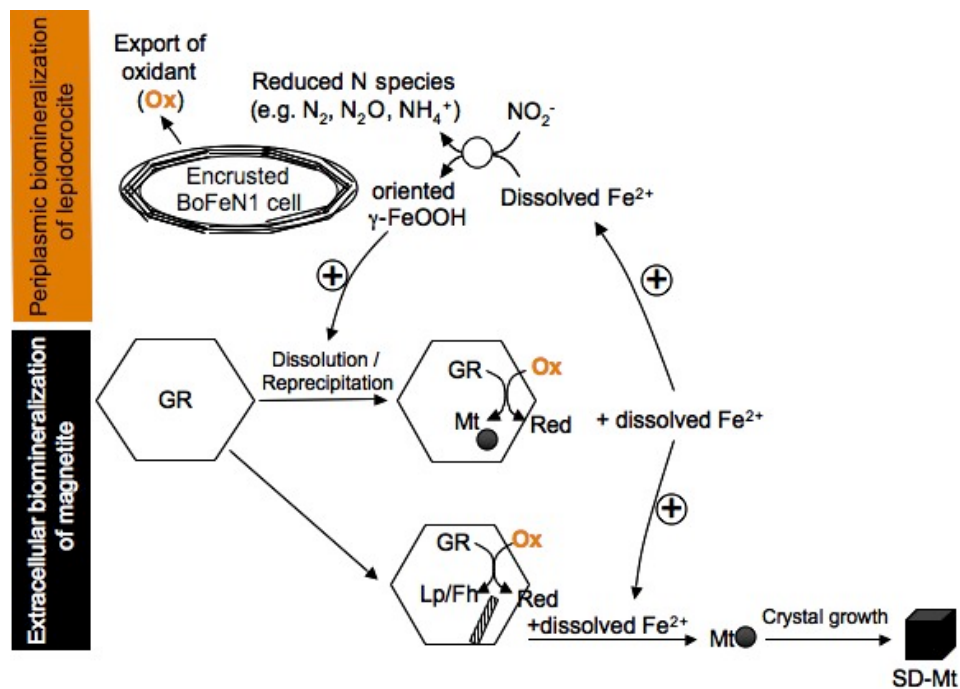


Figure 9

	δ (mm/s)	Δ or ε (mm/s)	HF (T)	RA (%)	Attribution
4-day old BoFeN1 culture					
S_A	0.30 ± 0.01	-0.02 ± 0.01	48.7 ± 0.1	39 ± 1	Magnetite (Td)
S_B	0.63 ± 0.01	0.02 ± 0.01	45.4 ± 0.1	51 ± 1	Magnetite (Oh)
D_γ	0.37 ± 0.01	0.61 ± 0.02		10 ± 1	Lepidocrocite
4-month old abiotic control					
S_A	0.29 ± 0.01	-0.02 ± 0.02	48.7 ± 0.1	34 ± 3	Magnetite (Td)
S_B	0.64 ± 0.02	0.02 ± 0.03	45.4 ± 0.1	58 ± 4	Magnetite (Oh)
D_γ	0.18 ± 0.08	0.66 ± 0.12		8 ± 7	Fe(III) in XRD-amorphous Fe ^{III} oxide

δ , isomer shift taking α -iron as reference at room temperature; Δ or ε , quadrupole splitting; RA , relative abundance.

Table 1