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Dissimilatory bioreduction of iron(III) oxides by *Shewanella loihica* under marine sediment conditions

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ABSTRACT

Shewanella is a genus of marine bacteria capable of dissimilatory iron reduction (DIR). In the context of deep-sea mining activities or submarine mine tailings disposal, dissimilatory iron reducing bacteria may play an important role in biogeochemical reactions concerning iron oxides placed on the sea bed. In this study, batch experiments were performed to evaluate the capacity of *Shewanella loihica* PV-4 to bioreduce different iron oxides (ferrihydrite, magnetite, goethite and hematite) under conditions similar to those in anaerobic sea sediments. Results showed that bioreduction of structural Fe(III) via oxidation of labile organic matter occurred in all these iron oxides. Based on the aqueous Fe (II) released, derived Fe(II)/acetate ratios and bioreduction coefficients seem to be only up to about 4% of the theoretical ones, considering the ideal stoichiometry of the reaction. A loss of aqueous Fe (II) was caused by adsorption and mineral transformation processes. Scanning electron microscope images showed that *Shewanella lohica* was attached to the Fe(III)-oxide surfaces during bioreduction. Our findings suggest that DIR of Fe(III) oxides from mine waste placed in marine environments could result in adverse ecological impacts such as liberation of trace metals in the environment.

1. Introduction

Iron is one of the most important elements on Earth due to its involvement in key biological processes, such as photosynthesis. However, the low solubility of Fe makes it not much bioavailable in most environments (Raiswell and Canfield, 2012). Iron is one of the controlling elements in many ecosystems, especially in marine environments (Field et al., 1998; Morel and Price, 2003). Some studies have shown that iron stimulates the growth of phytoplankton in high-nitrate, low-chlorophyll waters, which account for 25% of the ocean (De Baar et al., 2005). Furthermore, iron participates in important biological processes such as atmospheric carbon dioxide consumption, dimethyl sulfide (DMS) production and organic matter (OM) degradation in sediments (Boyd and Ellwood, 2010). Bioavailability of iron in the sea also played a crucial role in the modulation of carbon dioxide concentration in the atmosphere in the geological past (Martin et al., 1990).

A marine sediment is an aphotic nutrient-rich and low-production zone where most microorganisms are heterotrophic (Fenchel, 1969). In anoxic reduced zones of the sediment, there are OM-degrading anaerobic microorganisms that use inorganic compounds other than oxygen as terminal electron acceptors (TEAs) for the electron transport respiratory chain (Lovley, 1991). Dissimilatory iron reduction mediated by microorganisms uses Fe(III) as TEA to produce Fe(II) species. This process is coupled to the degradation of simple OM and is carried out by different genera of bacteria, like *Geobacter* or *Shewanella* (Lovley and Phillips, 1986). In marine sediments, metabolic products of degradation serve as electron donors for the terminal oxidizing bacteria, which use inorganic TEAs for a complete oxidation of organic matter. Moreover, iron reduction besides sulfate reductors are the most important terminal oxidation processes in the upper anoxic zone (Thamdrup, 2000). For instance, in artic marine sediments lactate (among acetate, propionate and isobutyrate) is degraded in the marine sediment by iron and sulfate reducers (Finke et al., 2007).

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Table 1
Surface area and mineralogical composition (wt. %) of the studied samples.

Sample	Hematite	Goethite	Magnetite	Ferrihydrite	V1	M1	TB
hydroxylapatite ($Ca_5(PO_4)_3(OH)$)						16%	11%
magnetite ($Fe^{2+}Fe_2^{3+}O_4$)			100%		19%	79%	89%
horblende ($Ca_2(Mg, Fe, Al)_5 (Al, Si)_8O_{22}(OH)_2$)					41%		
hematite (Fe_2O_3, α-Fe_2O_3)	100%				40%		
ferro-actinolite ($Ca_2(Mg_{2.5\text{-}0.0}Fe^{2+}_{2.5\text{-}5.0})Si_8O_{22}(OH)_2$)						5%	
goethite (α-FeO(OH))		100%					
ferrihydrite ($(Fe^{3+})_2O_3{\cdot}0.5H_2O$)				100%			

The *Shewanella* genus is well known for its presence in marine sediments and for its metabolic capacity (Hau and Gralnick, 2007). *Shewanella* can use oxygen, nitrate and heavy metals as TEAs. Some strains may even degrade recalcitrant organic compounds, such as chlorinated solvents, providing the genus with the potential to be applied in bioremediation studies (Roh et al., 2006). *Shewanella loihica* is a species from the genus *Shewanella* isolated from a submarine volcano in Loihi, Hawaii (Gao et al., 2006). The metabolic versatility and ubiquitous presence in the marine environment make *Shewanella loihica* a suitable candidate for bioreduction studies. Earlier studies on the capacity and mechanisms of *Shewanella* to bioreduce ferric iron in fresh water have shown that (i) it is able to reduce not only soluble Fe (III) compounds but also (Fe) (III)-bearing minerals such as magnetite (Fe_3O_4) through polysaccharide attachment (Dong et al., 2000) and that (ii) biotic iron reduction coupled to OM degradation requires a direct contact between the microorganisms and the poorly soluble mineral surface (Tugel et al., 1986). However, bioreduction of magnetite and other iron oxides and hydroxides (ferrihydrite, goethite and hematite) under marine conditions has not yet been studied.

The fate of iron oxides in seafloor sediments has a major interest for potential sea water contamination caused by deep-sea mining activities or marine disposal of mine tailings, which were practices widely spread worldwide (Ellis and Ellis, 1994) although currently banned in most of the countries (Dold, 2014). For instance, marine contamination associated with continuous tailings disposal has been reported in. Chañaral Bay in northern coast of Chile (Dold, 2006; Medina et al., 2005) and Portman Bay in the south-east coast of Spain (Manteca et al., 2014). In mine tailings originated from sulfide-rich ores the contained Fe(III)-oxides incorporate Mn, Al, Cr, Co, Ni, Zn, V, Pb and As (Knipping et al., 2015; Nadoll et al., 2014). Valence II and III metal cations can be absorbed on the iron oxides or isomorphously substitute iron in the crystalline oxide structure (Cornell and Schwertmann, 1996). Offshore disposal of these mine tailings may result in adverse ecological impacts as bioreductive dissolution of Fe(III) oxides releases aqueous Fe(II) together with trace metals and metalloids co-precipitated or structurally incorporated in Fe (III) oxides (Zachara et al., 2001). Thus, an undesired bioaccumulation of metals and metalloids in sea sediments, in secondary plumes and in the water column and an increase in trophic transfer of metals could occur (Morello et al., 2016; Ramirez-Llodra et al., 2015). A better understanding of the interaction between Fe(III) oxides and microorganisms capable to bioreduce Fe(III) sheds new light on the bioavailability of iron in the ocean and on potential environmental consequences of sea mining activities and marine disposal of mine tailings.

To this end, Fe(III) oxides (synthetic ferrihydrite, commercial goethite, magnetite and hematite and field specimens with different contents of iron oxides and other minerals) were reacted in the laboratory in the presence of *Shewanella loihica* strain PV-4, whose ecophysiology makes it optimal for Fe(III)-bioreduction under marine sediment conditions. Two different experiments were performed to elucidate the kinetics of Fe(III) bioreduction and to examine the bacteria-mineral surface interaction.

2. Materials and methods

2.1. Sample characterization

The iron oxide samples used in this study have three different sources: three samples were commercial powders of magnetite, hematite and goethite purchased from Sigma Aldrich; one sample of 2L ferrihydrite was synthesized in the laboratory following the procedure described by Cornel and Shwertmann (1991); and three samples were field specimens with different contents of magnetite (V1 from Distrito Algarrobo, Chile, TB from Lago Sur, Chile, and M1 from Malmberget, Sweden). Sample V1 also contained hematite. Powder X-ray diffraction (XRD) analysis and Rietveld refinement (Young, 1995), using a Bruker D8 A25 Advance X-ray diffractometer θ- θ with CuKα1 radiation, showed that the commercial and synthesized samples were composed of the respective iron oxides and no accessory minerals were identified. Rietveld analysis confirmed that no impurities were present in the samples. As for the field samples, magnetite was present in all of them (ranging from 19 to 89 wt % for V1 and M1, respectively), hematite was only present in V1 (40 wt %) and goethite was not detected (Table 1). Other minerals identified were silicates (hornblende, and Fe-actinolite) and phosphates (hydroxyapatite) (Table 1).

The size fraction of the commercial powders was about 5 μm. Synthesized 2L ferrihydrite was ground using an agate mortar and pestle and sieved to a size fraction of 5–60 μm. Fragments of field samples were similarly ground and sieved to a size fraction of 60–100 μm. These powdered samples were used in batch experiments to study the Fe(III) bioreduction reaction. The specific surface area of all these samples was determined by the Brunauer-Emmett-Teller (BET) method (Brunauer et al., 1938) using a Gemini 2370 surface area analyzer and 5-point N_2 adsorption isotherms. Sample degassing with nitrogen lasted for 2 h at 137 °C. Data uncertainty was around 10%. Synthesized ferrihydrite showed the largest value (181 $m^2\,g^{-1}$) and M1 and TB the lowest ones (0.6 and 0.2 $m^2\,g^{-1}$, respectively; Table 2).

2.2. Bacterial culture

Shewanella loihica strain PV-4 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ 17748). To obtain a bacterial suspension for the starting inoculum, cells were cultivated in M1 medium (Gao et al., 2006) supplemented with 10 mM of sodium lactate as electron donor and carbon source and 10 mM of Fe (III) citrate as electron acceptor. Cultures were incubated anaerobically for 24 h at 30 °C and then harvested by centrifugation (5000 rpm for 10 min). The pellet was re-suspended in synthetic seawater prepared previously following the standard protocol D1141-98 (ATSM International). Centrifugation and pellet resuspension were repeated three times as a washing step.

A medium simulating seawater (hereafter referred to as marine medium) was developed for the experiments. A basal medium of synthetic seawater (ASTM D1141-98) was amended with sodium lactate (10 mM) as an electron donor and carbon source, ammonium chloride (1.87 mM) as a source of nitrogen, and TRIS-HCl (10 mM) as a pH-buffer. The pH of the medium was adjusted to 8.2 with 0.1 N NaOH

Table 2
Microbial bioreduction activity coefficients calculated from measured acetate and ferrous iron concentrations.

sample	Specific (BET) surface area ($m^2\ g^{-1}$)	$k_{biored\text{-}Fe}$ (μmol g_{oxide}^{-1} d^{-1})	$k_{biored\text{-}Ac}$ (μmol g_{oxide}^{-1} d^{-1})	$k_{biored\text{-}Fe}$ (μmol m^{-2} d^{-1})	$k_{biored\text{-}Ac}$ (μmol m^{-2} d^{-1})	$Fe(II)_{aq}$/acetate (k-Fe/k-Ac)[a]
Hematite	5.4	0.033	0.61	0.006	0.114	0.0530
Goethite	12.3	0.026	0.95	0.002	0.077	0.0276
Magnetite	6.9	0.232	1.72	0.034	0.249	0.1349
Ferrihydrite	181	5.490	32.03	0.030	0.177	0.1714
V1	1.8	0.316	13.06	0.297	0.59	0.5073
TB	0.6	0.057	0.57	0.080	0.80	0.0995
M1	0.2	0.073	1.26	0.309	5.31	0.0583

[a] Bio-reduction stoichiometry is given by the Fe (II)aqueous/acetate ratio (theoretical value = 4).

solution. The final medium was sterilized by autoclave (121 °C for 20 min).

2.3. Batch experiments with powdered samples

In all batch experiments, 0.25 ± 0.01 g of powdered sample were placed in 25 mL glass vials capped with Teflon plugs and then sterilized by autoclave (121 °C for 20 min). Previous studies (Das et al., 2010; Mazzetti and Thistlethwaite, 2002) showed a thermal transformation of ferrihydrite to hematite could occur. A test performed with ferrihydrite showed that XRD analyses after the sterilization revealed no mineral changes in this stage.

The vials were filled with 25 mL of marine medium, keeping a 1% solid/liquid ratio (g/mL), and inoculated with *Shewanella loihica* to an approximate final number of $1{\cdot}10^7$ colony-forming units (cfu) mL^{-1}, measured by agar culture (LB). The vials were sealed with screw caps, leaving a minimal head space (small air bubble) to prevent overpressure, and statically immersed in a thermostatic water bath at 10 ± 1 °C in the dark. These conditions with the use of the marine medium mimicked the suboxic zone in marine sediments (Jørgensen and Kasten, 2006; Rosselló-Mora et al., 1999). Abiotic controls without inoculum of *Shewanella loihica* were also prepared under the same conditions as biotic experiments.

For each solid sample, a single-point batch experiment was carried out. Five vials were prepared as replicates, and each one was sacrificed at different time spans (13, 27, 47, 70 and 111 days). Sampling was performed in a glove box with N_2 atmosphere to maintain the anoxic conditions. The vials were shaken just before sampling and then the medium from the vial was totally recovered, sampled and filtered using a sterile syringe and syringe filters (0.22 μm pore size). Sample aliquots were used for pH/Eh measurements and for chemical analyses of cations and anions. To evaluate the carbon and energy source consumption, lactate and acetate, being the latter the oxidation end product under anaerobic conditions, were measured. For ion analysis a volume of 10 mL was preserved at pH < 2 by adding 100 μL of 60% (v/v) HNO_3 solution. For Fe(II)/Fe(III) measurements by Phenanthroline colorimetry (Stucki, 1981), an additional volume of 10 mL was preserved with the addition of 100 μL of 6 M HCl solution. Thereafter, all samples were stored at 4 °C in the dark until analysis.

2.3.1. Chemical analyses

Measurements of pH (± 0.02 pH units) and Eh (± 10 mV) were performed in the glove box using pH and Eh electrodes (Crison and SenTix ORP, Ag/AgCl, WTW, respectively). Oxidation-reduction potential readings were converted to standard Eh values by correcting for the electrode potential of the reference hydrogen electrode. Total iron was analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin- Elmer 3000). Owing to the high dissolved iron concentrations in the experiments with ferrihydrite, iron measurements were performed using ICP–Optical Emission Spectroscopy (ICP-OES). The uncertainty of the ICP-MS (and ICP-OES) measurements was better than ± 5%. Total iron measured was checked to be Fe (II) with a modified protocol of the Phenanthroline method. Lactate and acetate concentrations were determined by high performance liquid chromatography (HPLC). The equipment used consisted of a Waters 600 HPLC pump controller equipped with an Aminex HPX-87H column (300 × 7.8 mm), BioRad, and a Waters 717 plus autoinjector. Triplicates were performed for iron, lactate and acetate measurements.

2.4. Fe (II)-ferrihydrite adsorption experiments

Fe(II) adsorption on powdered ferrihydrite in marine medium (0.5 g of ferrihydrite and 50 mL of solution, 1% w/v) was determined in gently mixed batch experiments at room temperature (23 ± 2 °C). Different amounts of $FeCl_2$ were added to distinct vials, from 0.4 to 40 mM, in order to get a wide range of initial Fe(II) aqueous concentration in the experiment. Samples were collected after reaching equilibrium at 24 h (Dzombak and Morel, 1990) to measure total and ferrous iron by the phenanthroline method. At the end of the experiments the solid fractions were retrieved, freeze dried and preserved under nitrogen atmosphere until analysis. Subsequently, XRD-Rietveld analyses and measurement of BET specific surface areas were performed. The concentration of adsorbed Fe(II) was determined by subtracting the aqueous ferrous iron concentration after equilibration from the initial concentration according to (1):

$$C_{Fe-ads} = (C_{Fe-i} - C_{Fe-eq}) \cdot \frac{V}{M} \quad (1)$$

where $C_{Fe\text{-}ads}$ is the amount of adsorbed iron per gram of ferrihydrite, $C_{Fe\text{-}i}$ and $C_{Fe\text{-}eq}$ are the initial and equilibrium aqueous concentrations of Fe(II), respectively, V is the volume of solution and M is the mass of ferrihydrite.

2.5. Experiments with microbial cells and field samples

Surface mineral-bacteria interaction was investigated by scanning electron microscope (SEM). Fragments of field samples (M1 and TB) were cut down to small rectangular pieces (surface of ≈10 mm^2 and ≈3 mm thick) in order to fit into sample holders used for the critical point drying technique. These pieces were used to study the interaction between *Shewanella* and the surface of the iron oxides. Top surfaces were polished by conventional metallographic polishing to improve the observation of the surface mineral-bacteria interaction by SEM. The M1 and TB pieces were placed in 200 mL bottles filled with marine medium (synthetic sea water) without head space and incubated with $1{\cdot}10^7$ cfu mL^{-1} of *Shewanella loihica*. Experiments were conducted in the N_2-atmosphere glove box in the dark for 115 days at 25 °C.

At the end of the experiments, the pieces incubated with *Shewanella* were retrieved and treated for 2 h with a glutaraldehyde 2.5% w/v in 0.1 M phosphate buffered saline (PBS) cell-fixation solution. Several washes with PBS (10 min each) were done, and post-fixation of the mineral pieces was carried out using 1% osmium tetraoxide and 0.8% potassium ferricyanide in 0.1 M PBS for up to 2 h in darkness. To evaluate potential effects of the dehydration process on the bacteria

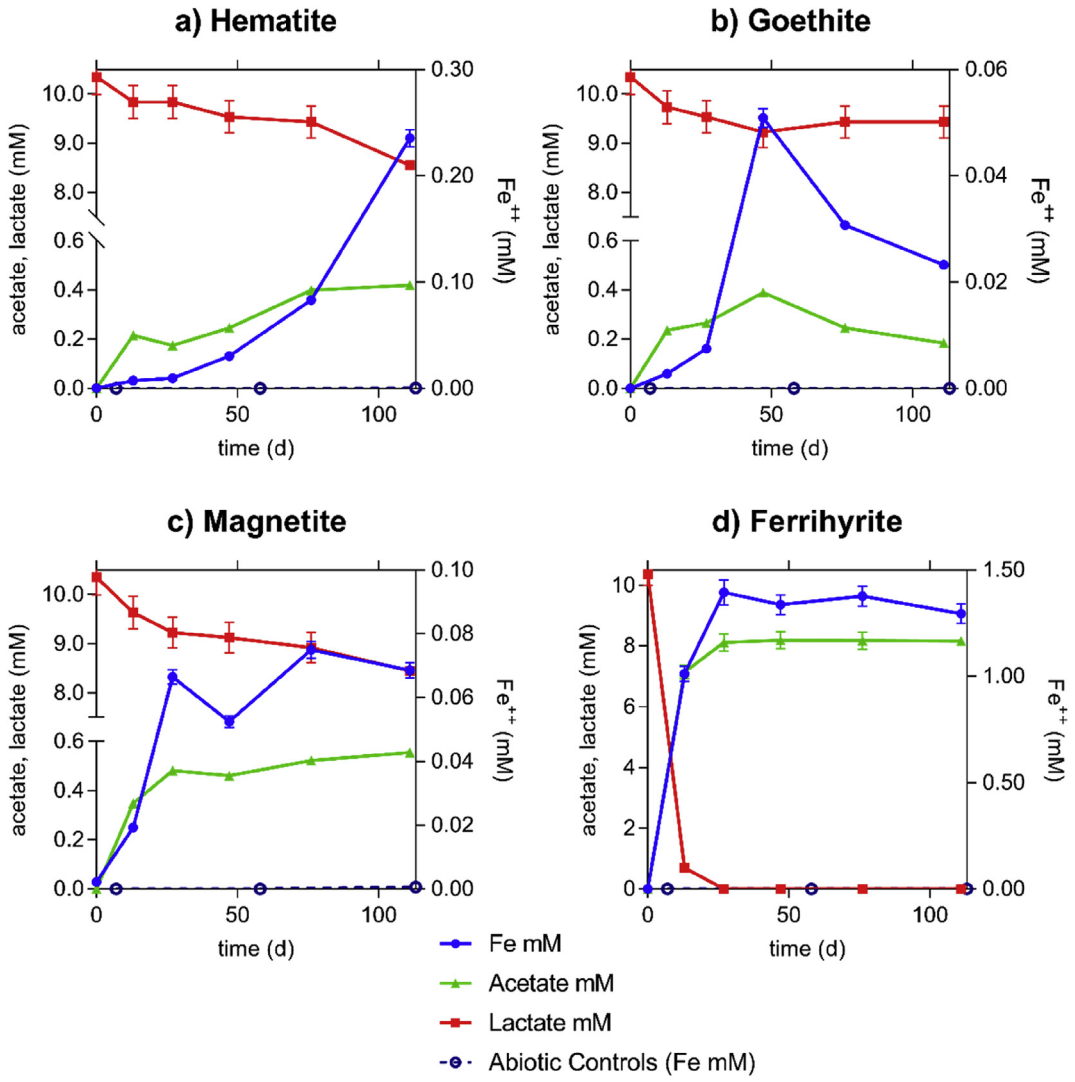

Fig. 1. Variation in concentration of lactate, acetate and total aqueous Fe over time in the bioreductive dissolution experiments with synthetic and commercial iron oxide samples: a. Hematite, b. Goethite, c. Magnetite, d. Ferryhydrite. Key: (□) Lactate; (▲) Acetate; (●) Total dissolved iron and (o) Abiotic controls. Error bars correspond to the analytical uncertainty (SD).

structure, two different dehydration methods were carried out. In one dehydration method the critical point drying technique was performed by replacing water in the samples with increasing concentrations of ethanol (50–100%) (Anderson, 1951). In the other method sample dehydration was performed using a hexamethyldisilazane (HMDS) solution (Nation, 1983). Thereafter, all samples were coated with carbon before SEM observation (Hitachi H-4100FE instrument under a 15–20 kV potential in a high vacuum) using the backscattered electron detector (BSD) in Field Emission (FE) and an energy-dispersive spectrometer (EDS).

3. Results

3.1. Bioreductive dissolution of Fe-oxides

Dissolution of the iron oxide minerals and production of aqueous Fe (II) did not take place in the abiotic control experiments. In contrast, bioreductive dissolution occurred in all experiments inoculated by *Shewanella loihica*. Fig. 1 shows the variation of total aqueous iron concentration over time for the experiments with synthetic and commercial samples. Measured total aqueous iron in all the experiments was confirmed to be Fe (II) by the phenanthroline method. Aqueous iron concentration increased over time in the experiment with hematite (Fig. 1a), initially increased and then decreased in the case of goethite (Fig. 1b) and increased and levelled off in the experiments with magnetite and ferrihydrite (Fig. 1c and d). The highest Fe(II) concentration (1.3 mM) was reached in the ferrihydrite experiment. In all experiments, the change in aqueous ferrous iron concentration was accompanied by consumption of lactate and production of acetate. Only in the case of ferrihydrite experiment lactate was totally consumed. For the experiments prepared with field samples, reductive dissolution of the iron oxides showed similar trends (Fig. 2), in which iron increased in different steps (Fig. 2a,c) or gradually (Fig. 2b). The concentrations of released iron were lower than those of the synthetic and commercial samples (< 0.03 mM). As observed for the experiments with synthetic and commercial samples, consumption of lactate and production of acetate accompanied the ferrous iron release.

The measured ferrous iron and acetate concentrations throughout the experiments were used to estimate initial bioreduction coefficients based on the initial release of iron and acetate associated with the microbial activity. These coefficients were calculated by linear regression of the first two sampling points for ferrihydrite and the first three ones for the other oxide experiments using the following expressions (2,3):

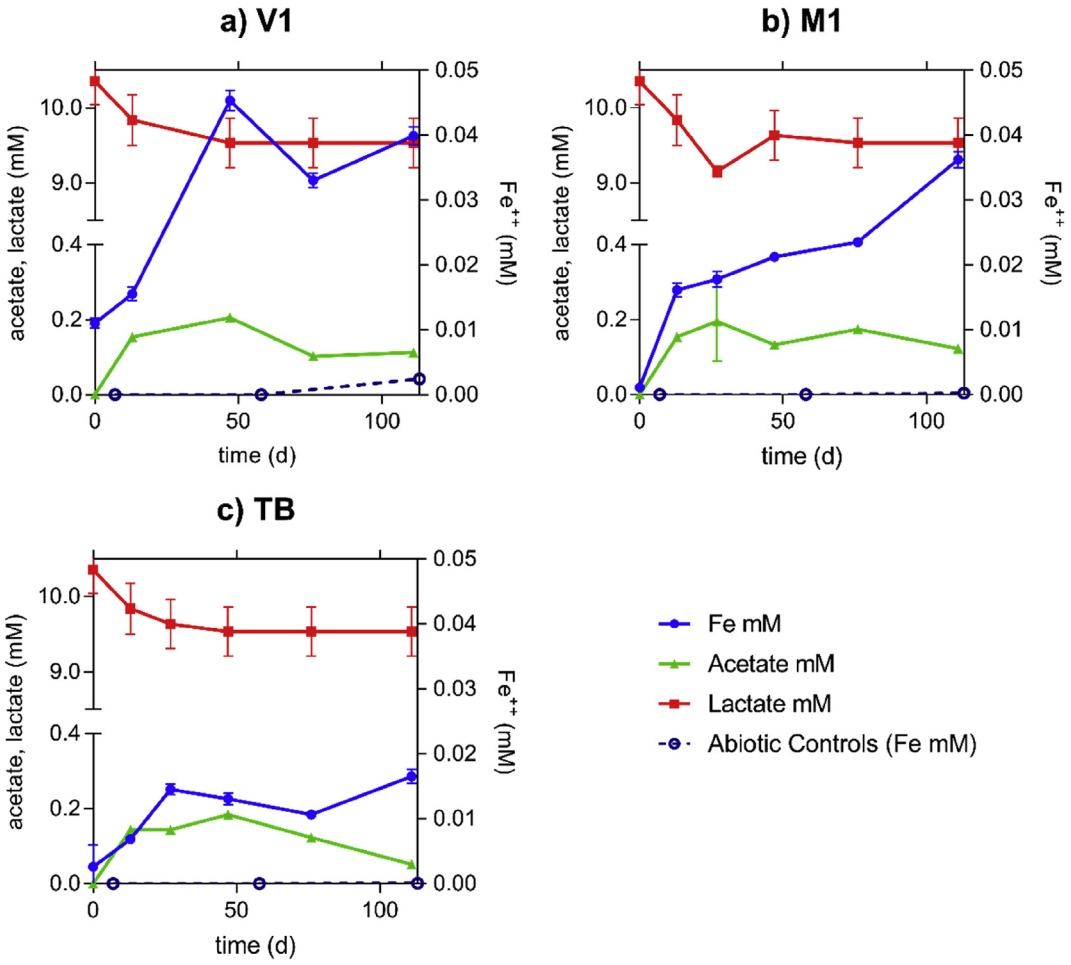

Fig. 2. Variation in concentration of lactate, acetate and total aqueous Fe over time in the bioreductive dissolution experiments with field powdered samples. a. Sample V1 (Distrito Algarrobo, Chile); b. Sample M1 (Malmberget, Sweden); c. Sample TB (Lago Sur, Chile). Key: (□) Lactate; (▲) Acetate; (●) Total dissolved iron and (o) Abiotic controls. Error bars correspond to the analytical uncertainty (SD).

$$k_{biored-Fe} = \frac{C_{Fe(II)} \cdot V}{\Delta t \cdot M} \quad (2)$$

$$k_{biored-Ac} = \frac{C_{acetate} \cdot V}{\Delta t \cdot M} \quad (3)$$

where $C_{Fe(II)}$ and $C_{acetate}$ are the measured iron and acetate concentrations (μM), V is the solution volume (L), M is the Fe(III)-oxide mass (g) and t is time (d). Linear regressions showed R^2 values between 0.8 and 0.99. The values of the iron and acetate bioreduction coefficients are listed in Table 2. Fig. 3a shows that the bioreduction coefficient (μmol g_{oxide}^{-1} d^{-1}) for the ferrihydrite experiment is much larger than those of the other samples. However, when the coefficients are normalized with the specific BET surface area (μmol m^{-2} d^{-1}) the coefficients of magnetite are higher (Fig. 3b).

In all experiments, pH slightly decreased from 8.2 to an average pH of 7.8 (Fig. S1) whereas Eh significantly decreased from 300 mV to an average value of 18.5 mV (Fig. S1).

3.2. Fe(II)-ferrihydrite adsorption

Due to the high specific surface area of ferrihydrite (181 $m^2 g^{-1}$) compared to the other iron oxides investigated (Table 1), this phase was used to evaluate Fe(II) adsorption on Fe(III)-oxides in the marine medium. Fig. 4 shows the measured adsorption of Fe(II) on powdered ferrihydrite. The amount of adsorbed Fe (II) increased with Fe (II) aqueous concentration, exceeding the theoretical adsorption capacity of ferryhydrite (0.6 mmol g^{-1}) (Hiemstra, 2013). The XRD patterns and Rietveld semi-quantitave analysis of the retrieved ferrihydrite allowed us to elucidate the mineralogical change at the end of the experiment and showed the presence of both ferrihydrite (≈10 wt%) and magnetite (≈90 wt%) (Fig. 5).

3.3. Bacteria and Fe-oxide surfaces

Independently of the dehydration technique used to preserve the bacteria structure, SEM images of the reacted field samples showed the presence of bacteria (*S. loihica*) attached on the iron-oxide surfaces (Fig. 6). Bacteria cells colonized the iron-oxide surfaces, either as individual cells or forming clusters. Most of the cells were attached preferably on the iron-oxide surfaces rather than on the surfaces of the other minerals present in the field samples (Fig. 6a). Extracellular structures by *S. loihica* have been observed suggesting that bacteria connect with the mineral surface and with other cells (Fig. 6b and c).

4. Discussion

4.1. Aqueous chemistry

The capacity of *Shewanella* to reduce soluble (e.g. iron citrate) or structural (e.g. biogenic magnetite) ferric iron has been studied (Kostka and Nealson, 1995), but its capacity to reduce magnetite and other iron-oxide minerals under marine conditions remained unknown. Our study demonstrates that *S. loihica* was able to bioreduce not only magnetite, but also hematite, goethite and ferrihydrite in conditions similar to

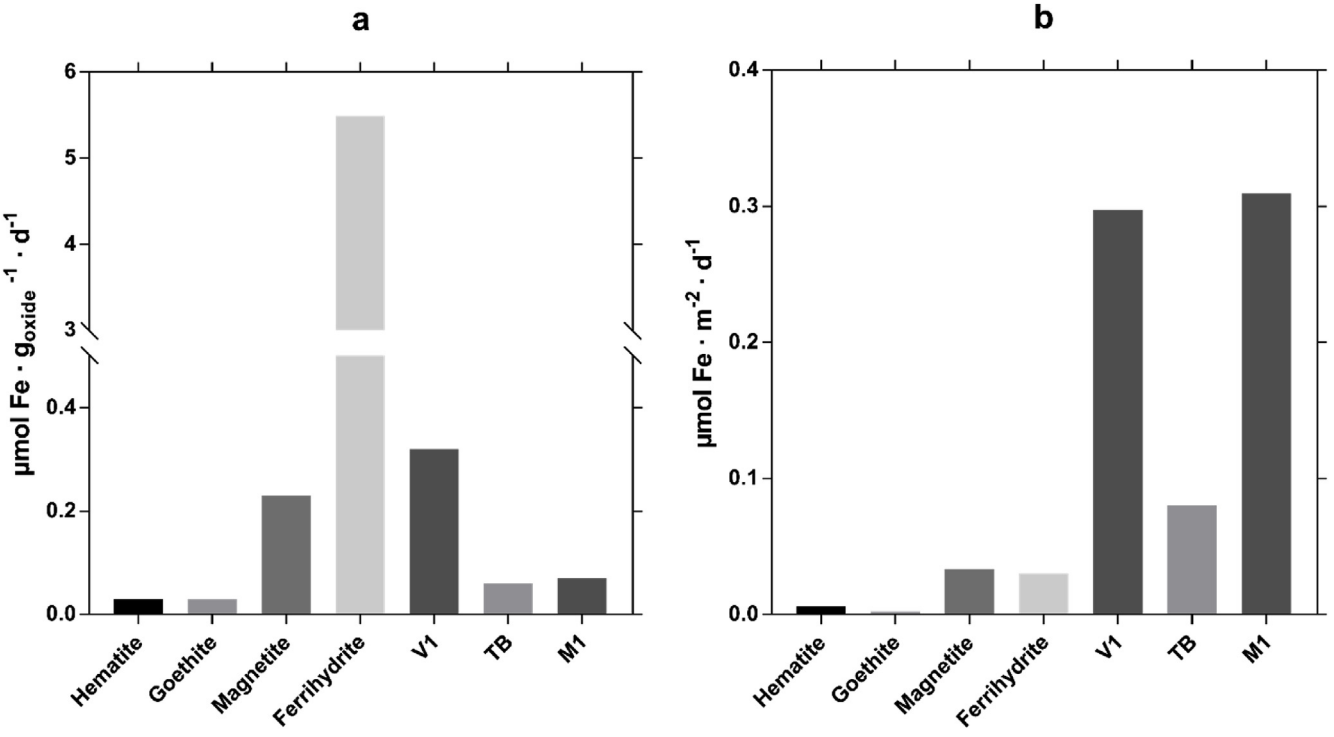

Fig. 3. Iron bioreduction coefficients of the iron oxides mediated by *S. loihica*: a) values normalized to mass and b) normalized to surface area.

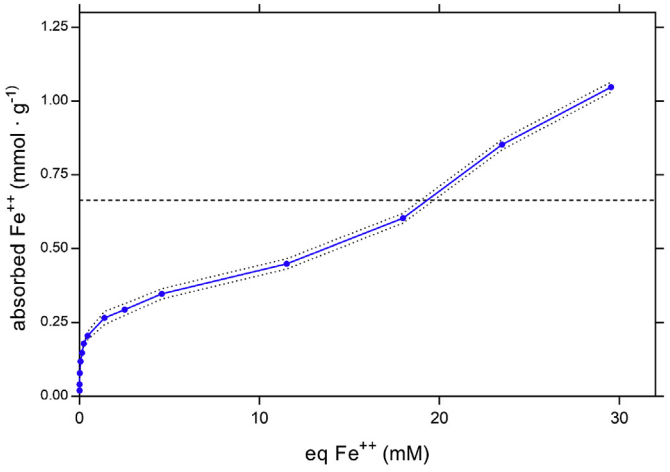

Fig. 4. Ferrous iron adsorption isotherm onto ferrihydrite in the marine medium. Dashed line indicates the saturation point based on the calculated number of sorption sites for ferrihydrite. Dotted lines indicate the SD of the experiment.

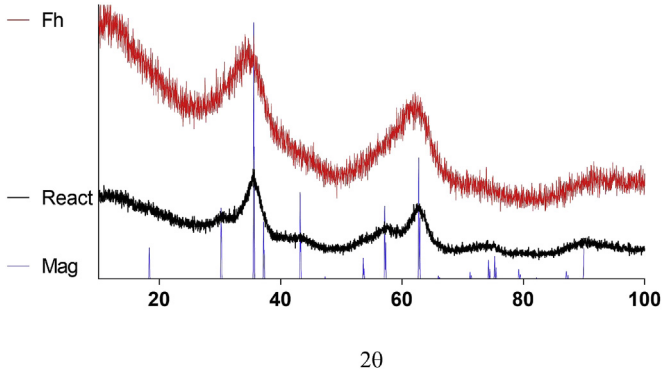

Fig. 5. XRD patterns of non-reacted pure ferrihydrite (red) and reacted ferrihydrite after absorption experiments (black). Blue lines indicate the position of the main XRD peaks of pure crystalline magnetite (blue) obtained from Ruff database. Most of the reacted ferrihydrite transformed to magnetite after surface adsorption of iron (II). Rietveld analysis of the reacted sample indicates that 10% of the sample is ferrihydrite and 90% is magnetite with nanocrystalline morphology. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

those found in anoxic marine environments, such as in seafloor sediments and in offshore mine-tailings disposal sites (Ramirez-Llodra et al., 2015). It appeared that *S. loihica* used the structural ferric iron of the iron oxides as an electron acceptor in the respiratory chain (Tugel et al., 1986). In the experiments, a simultaneous consumption of light organic matter (lactate) to produce acetate was observed along with an increase in aqueous Fe(II). Production of acetate was attributed to the anaerobic metabolism of the bacteria during ferric iron reduction. This finding is in agreement with previous studies showing that the metabolism of *S. loihica* was sustained by the production of acetate from lactate, which acts as electron donor (Scott and Nealson, 1994; Tang et al., 2007).

Bioreduction may be expressed in a simple form as (Lovley, 1991) (4):

$$Lactate^- + 4Fe^{3+} + 2H_2O \rightarrow acetate^- + HCO_3^- + 4Fe^{2+} + 5H^+ \quad (4)$$

Where one and 4 mol of acetate and ferrous iron are respectively produced (i.e., Fe(II)/acetate ratio = 4). Experiments with high lactate consumption and acetate formation correlated well with those having high aqueous ferrous iron concentration. In the ferrihydrite experiment, however, bioreduction was halted by total exhaustion of lactate (Fig. 1d). Mass balance between lactate consumption and acetate production showed carbon deficit in all experiments (lactate consumed > acetate produced). In a first stage ($\approx < 12$ h) of bioreduction experiments, *Shewanella loihica* consumed all the remaining oxygen in solution in the full oxidation of lactate to CO_2 via the aerobic metabolic pathway. The observed carbon mismatch (reaching up to ≈20%) was attributed to both carbon assimilation in biomass formation during

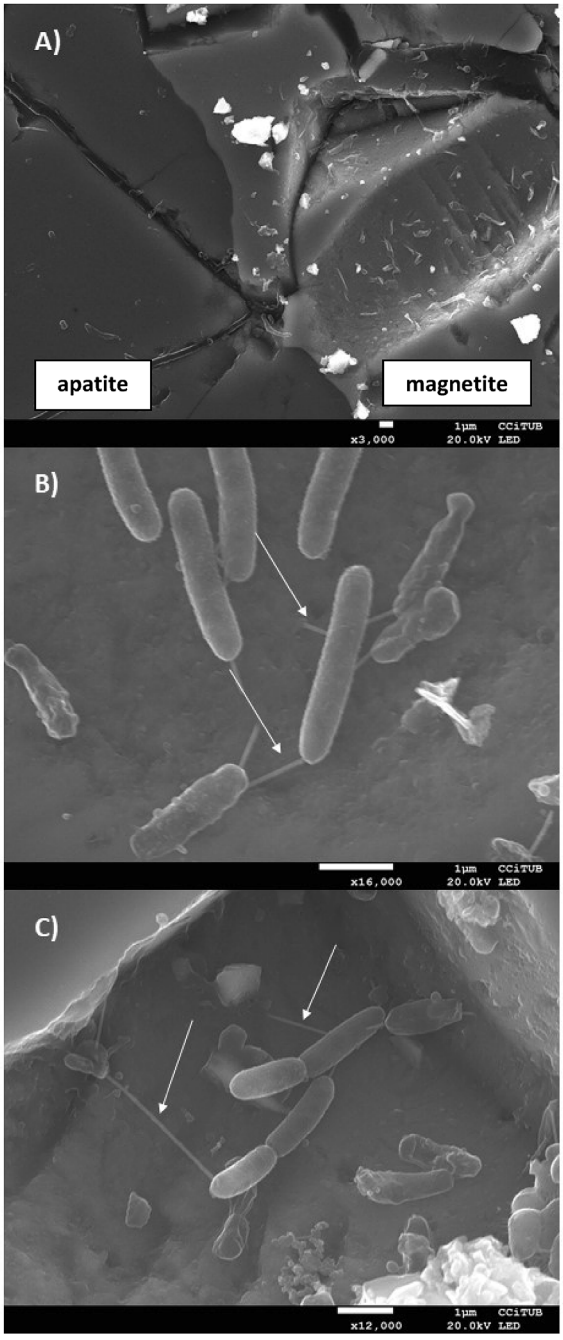

Fig. 6. SEM images of *S. loihica* cells colonizing an iron-oxide surface: a) Bacteria growing preferably on the oxide surfaces (magnetite); b,c) Bacteria growing on the surface of magnetite (M1) with developed extracellular structures (see arrows) interconnecting cells and/or connecting cells with the mineral surface.

microbial growth and the use of the aerobic metabolic pathway in oxygen consumption by *Shewanella loihica*.

According to the bioreduction reaction (Eq. (4)), a significant deficit of ferrous iron, based on measured aqueous Fe(II) relative to acetate, was found in all experiments. The Fe(II)/acetate ratios range between 0.03 and 0.17, which is between 0.7% and 4.3% of the stoichiometric ratio (Table 2). Several previous studies (Benner et al., 2002; Bonneville et al., 2004; Roden et al., 2000) reported a similar Fe(II) deficit, suggesting that (1) the apparent extent of bioreduction based on measured aqueous Fe(II) reached only about 3% solubilization of initial Fe(III) and (2) Fe(III) reduction could then be largely underestimated due to adsorption of Fe(II) on the dissolving iron oxides and/or formation of secondary mineral phases containing structural Fe(II).

In the current study, the measurement of adsorption of Fe(II) on powdered ferryhidrite in marine medium (Fig. 4) indicated a probable adsorption of solubilized ferrous iron, as it has also been found for other Fe(III)-oxides in previous studies using aqueous solutions with a composition different than in our study (Larese-Casanova and Scherer, 2007; Rajput et al., 2016). Considering the adsorption capacity of ferryhidrite (0.6 mmol g^{-1}) and the released acetate in the ferrihydrite bioreduction experiment (Fig. 1d), a Fe(II)/acetate ratio of 3.25 would be obtained if only adsorption of ferrous iron had occurred. Therefore, the marked Fe deficit observed (Fe(II)/acetate = 0.17; Table 2) cannot be explained by adsorption of ferrous iron alone. Moreover, the Fe(II)-ferrihydrite adsorption experiments showed that the adsorbed Fe(II) exceeded the maximum capacity (0.6 mmol g^{-1}; Fig. 4). This indicated that an additional process, such as ferrihydrite transformation to magnetite, could be responsible for the extra Fe(II) uptake. A comparison between the XRD patterns of non-reacted and reacted ferrihydrite samples showed the presence of magnetite, a more crystalline phase (Fig. 5), confirming the occurrence of magnetite formation. Previous studies suggested that the Fe(III)-oxide-magnetite transformation is driven by an electron transfer between the absorbed Fe(II) and Fe(III) in iron oxides, resulting in the formation of nano-crystalline, stoichiometric magnetite (Byrne et al., 2011; Williams and Scherer, 2004). This mineralogical transformation may be expressed as (5):

$$2Fe(OH)_3 + Fe^{2+} \leftrightarrow Fe_3O_4 + 2H_2O + 2H^+ \quad (5)$$

It is suggested that in alkaline environments magnetite is the most stable iron oxide phase (Tronc et al., 1992), in contrast to lepidocrocite and goethite in neutral environments (Boland et al., 2014). Simultaneous Fe(II) adsorption and mineral transformation could therefore explain the systematically high deficit of aqueous ferrous iron. These two processes, which act as a sink for dissolved biogenic ferrous iron, were also observed in *Geobacter* mediated ferrihydrite bioreduction (Chen et al., 2018).

The estimated initial bioreduction coefficients differed between the different oxides studied (Table 2). Bioreduction kinetics is dependent on the reactive surface area of the iron oxides, which plays a key role in the process (Burdige et al., 1992). Ferrihydrite has the largest surface area (Table 1), up to three orders of magnitude higher than that of the other minerals. As a result, the bioreduction coefficient of ferrihydrite shows the highest value (5.49 µmol of Fe(II) g_{oxide}^{-1} d^{-1};Fig. 3a). In contrast, the lowest bioreduction coefficient corresponds to goethite (Table 2), commercial powder (0.026 µmol of Fe(II) g_{oxide}^{-1} d^{-1}; Fig. 3a). Nevertheless, when microbial bioreduction coefficients were normalized with the specific surface area of the oxides, magnetite, either synthetic or natural (Magnetite and M1) shows the highest bioreduction coefficient (0.034 µmol Fe(II) m^{-2} d^{-1} for Magnetite and 0.309 µmol Fe(II) m^{-2} d^{-1} for M1; Fig. 3b). According to the normalized acetate coefficients, both commercial and field magnetite samples showed the highest acetate production compared to other commercial and synthetic samples (0.249 µmol acetate m^{-2} d^{-1} and 5.31 µmol acetate m^{-2} d^{-1}, respectively). These phases therefore show high preference for *Shewanella*'s bioreduction. Yet, the high values of the magnetite bioreduction coefficient could be also associated with an extra release of ferrous iron from the lattice of the magnetite (Kostka and Nealson, 1995). Furthermore, if Fe(III) of the magnetite lattice is reduced to Fe(II), an increase in crystal radius (with IV coordination) from 63 to 77 p.m. might destabilize magnetite structure, yielding high coefficient values (Shannon, 1976). The variability in the bioreduction coefficients of the studied iron oxides could be attributed to the differences in the intrinsic mineral properties, such as the degree of crystallinity, grain size and impurity content (Li et al., 2012; O'Loughlin et al., 2010).

4.2. Shewanella loihica and Fe-oxide surfaces

Bacteria use several strategies to perform bioreduction process. A well-known strategy is the contact of bacteria with mineral surfaces, allowing the electron transport (Burdige et al., 1992). SEM images showed *Shewanella loihica* cells colonizing the iron-oxide surfaces (Fig. 6a), either as individual cells or forming clusters. In addition, the SEM images revealed the presence of extracellular structures apparently connecting single cells with the mineral surface and/or with other cells (Fig. 6b and c). In fact, previous studies have shown that the genera *Shewanella* is able to develop extracellular structures to perform the electron exchange in the bioreduction process (i.e. nanowires) (Gorby et al., 2006; Pirbadian et al., 2014; Shi et al., 2016). A similar morphology between the extracellular structures in our study and those reported in previous studies exist. Nevertheless, to fully prove the electron-exchange capacity of the extracellular structures observed in this work, further studies are necessary.

5. Conclusions

Shewanella loihica is able to dissolve Fe (III) oxides via dissimilatory iron reduction under conditions of anoxic marine sediments. The deficit of aqueous ferrous iron relative to acetate produced during bioreduction was explained by adsorption of Fe(II) on the dissolving iron oxides and transformation of the iron oxides into stoichiometric magnetite. Hence, calculated bioreduction coefficients based on measured aqueous Fe(II) account for only up to about 4% of the actual reaction, considering the theoretical release of Fe (II) and acetate productions. During bioreduction, *Shewanella loihica* colonizes the surface of the iron oxides.

Results indicate a potential unfavorable role of iron-oxide bioreduction in deep-sea mining activities or coastal mine-tailings disposal, where release of trace and toxic metals represents an environmental threat. Furthermore, the iron released from metal mine tailings disposed offshore could affect primary production, jeopardizing the resilience of offshore ecosystems. However, a positive implication of iron-oxide bioreduction is found for biotechnology as iron-oxide bioleaching could potentially be used for recovery of iron and trace elements in metallurgical treatments.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2019.104782.

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