# Reduction of ferric green rust by Shewanella putrefaciens

F. Jorand, A. Zegeye, F. Landry, C. Ruby

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

# Reduction of ferric green rust by *Shewanella putrefaciens*

F. Jorand, A. Zegeye, F. Landry and C. Ruby

Laboratoire de Chimie Physique et Microbiologie pour l'Environnement (LCPME), UMR 7564 CNRS-UHP, rue de Vandœuvre, Villers-lès-Nancy, France

**Keywords**
biomineralization, green rust, iron reduction, *Shewanella*.

**Correspondence**
Frédéric Jorand, Laboratoire de Chimie Physique et Microbiologie pour l'Environnement (LCPME), UMR 7564 CNRS-UHP, 405, rue de Vandœuvre, 54600 Villers-lès-Nancy, France.
E-mail: jorand@pharma.uhp-nancy.fr

2006/0196: received 8 February 2007, revised and accepted 22 June 2007

doi:10.1111/j.1472-765X.2007.02225.x

## Abstract

**Aims:** To reduce carbonated ferric green rust (GR*) using an iron respiring bacterium and obtain its reduced homologue, the mixed $Fe^{II}$–$Fe^{III}$ carbonated green rust (GR).

**Methods and Results:** The GR* was chemically synthesized by oxidation of the GR and was incubated with *Shewanella putrefaciens* cells at a defined [$Fe^{III}$]/[cell] ratio. Sodium methanoate served as the sole electron donor. The GR* was quickly transformed in GR (iron reducing rate = 8·7 mmol $l^{-1}$ $h^{-1}$).

**Conclusions:** Ferric green rust is available for *S. putrefaciens* respiration as an electron acceptor. The reversibility of the GR redox state can be driven by bacterial activity.

**Significance and Impact of the Study:** This work suggests that GRs would act as an electronic balance in presence of bacteria. It provides also new perspectives for using iron reducing bacterial activity to regenerate the reactive form of GR during soil or water decontamination processes.

## Introduction

The mixed valence compound fougerite (IMA 2003-057), a layered $Fe^{II}$–$Fe^{III}$ hydroxysalt called green rust, is found in transitionally oxic and anoxic environments like hydromorphic soils (Trolard *et al.* 1996; Génin *et al.* 1998). Green rusts are constituted of $Fe^{II}$–$Fe^{III}$ hydroxide sheets separated by interlayers of anions and water molecules balancing the cation layer charge. The formula $Fe^{II}_4$ $Fe^{III}_2(OH)_{12}CO_3 \bullet 3H_2O$ has been proposed for the stoichiometric mixed $Fe^{II}$–$Fe^{III}$ carbonated green rust (GR) (Hansen 2001). More recently, it was shown that oxidation of GR, by air or $H_2O_2$, leads to a new compound called 'ferric green rust' (GR*) (Refait *et al.* 2003; Legrand *et al.* 2004). The variation of the [$Fe^{III}$]/[$Fe_{total}$] ratio of the fougerite would correspond to different oxidation states of the green rust compounds (Génin *et al.* 2005; Ruby *et al.* 2006) where the fully oxidized state corresponds to the GR*. GR can be the by-products of iron respiring bacteria when two-line ferrihydrite or lepidocrocite ($\gamma$-FeOOH) are the starting $Fe^{III}$ substrate (Parmar *et al.* 2001; Glasauer *et al.* 2002; Ona-Nguema *et al.* 2002; Zachara *et al.* 2002; Zegeye *et al.* 2005).

By coupling the organic carbon oxidation to the iron oxide reduction, the iron respiring bacteria are well-known to make the connection between the biogeochemical cycles of carbon and iron. Thus, such bacteria contribute to bioremediation of contaminated soils by removing the hydrocarbons in anoxic environment for example (Anderson *et al.* 1998). But these bacteria can also contribute to decontamination in other ways, i.e. by producing reactive $Fe^{II}$ species during the $Fe^{III}$ respiration. As GRs are considered to be the most reactive $Fe^{II}$-bearing compounds in reaction with organic and inorganic pollutants (Myneni *et al.* 1997; Erbs *et al.* 1999; Loyaux-Lawniczak *et al.* 2000; Refait *et al.* 2000; O'Loughlin *et al.* 2003; Elsner *et al.* 2004), it is therefore relevant to control their biotic synthesis for future prospects in soil or water remediation applications. Currently, GR formation from $\gamma$-FeOOH bioreduction by *Shewanella putrefaciens* can be controlled by adjusting the bacterial inoculum size. In particular, the formation of the thermodynamically stable compound magnetite was avoided (Zegeye *et al.* 2007). GR* represents also a potential ferric substrate for iron reducing bacteria and GR formation. However, its bacterial reducibility and the resulting products have not yet

 

been examined. One may expect that the formation of GR from GR* will occur at a high reduction rate due to the similarity between their crystalline structure.

Therefore, the purpose of this work was to demonstrate that GR* can serve as an electron acceptor for iron reducing bacteria and be transformed into a mixed $Fe^{II}$–$Fe^{III}$ green rust. A fast reduction of the GR* by *S. putrefaciens* and a subsequent transformation into GR was effectively observed, suggesting that green rust compounds could act as an electronic balance in the environment or during diverse water or soil remediation processes. These findings suggest that GR* is a very good substrate for the GR bio-(re)generation.

## Materials and methods

### Preparation of the oxyhydroxycarbonate green rust

The fully GR* has been prepared by fast oxidation of a GR by using a hydrogen peroxide solution (Legrand *et al.* 2004; Ruby *et al.* 2006). The GR has been prepared by the co-precipitation of $Fe^{II}$ and $Fe^{III}$ species in a basic solution containing NaOH and $Na_2CO_3$ (Ruby *et al.* 2003).

The GR* suspension was centrifuged (10 000 ***g***, 10 min) and washed with purified water (MilliQ+/Helix40; Millipore, Billerica, MA, USA). The pellet was dried at ambient temperature and then crushed in a mortar to obtain a fine and homogenous powder. Finally, the powder was added to the culture medium and treated with ultrasound (1 min, 40 W, with a probe of 13-mm diameter) in aliquots of 25 ml. As green rust compounds are not stable at high temperature (80°C), only the culture medium was heat sterilized prior to the mineral addition.

### Culture conditions and cell preparation

*Shewanella putrefaciens* CIP80·40 (Collection of Institut Pasteur, Paris, France) suspension was prepared according to Zegeye *et al.* (2007). After 24 h of aerobic growth in tryptic soy broth (30 g $l^{-1}$, 51019; BioMérieux, Marcy l'Étoile, France) the cells, exhibiting a typical salmon colour, were washed (centrifugation 10 000 ***g***, 10 min, 20°C) and resuspended in NaCl solution (7 g $l^{-1}$ in purified water). The cells ($1{\cdot}1 \times 10^{10}$ cell $ml^{-1}$, final concentration) were added to serum bottles (sealed with thick butyl rubber stoppers, Bellco Glass Inc., Vineland, NJ, USA) containing the anaerobic incubation medium ($O_2$ free, flushed with 100% $N_2$ gas) composed of the following constituents (per litre of basal mineral medium described previously, Zegeye *et al.* 2007) : GR* [$Fe_6O_4(OH)_8{\cdot}CO_3{\bullet}3H_2O$], 30 g (i.e. 277 mmol $Fe^{III}$ $l^{-1}$); sodium methanoate (as electron donor), 10·9 g (i.e. 160 mmol $l^{-1}$); anthraquinone disulfonate (as electron shuttle), 100 $\mu$mol $l^{-1}$. All incubations were at 30°C in the dark and conducted in triplicate.

### Analytical techniques

$Fe^{III}$ reduction was monitored by measuring the $Fe^{II}$ accumulation over time. The amount of $Fe^{II}$ extracted by HCl 1 mol $l^{-1}$ after 1 week, was determined with ortho-phenanthroline (Fadrus and Maly 1975). As previously described (Zegeye *et al.* 2007), the initial rate of reduction was computed from the first derivative of a nonlinear curve fit for $Fe^{II}$ *vs* time data to the following equation: $Fe^{II}{}_t = Fe^{II}{}_{max}\ [1 - \exp(-k_{obs}t)]$, where $Fe^{II}{}_t$ is the concentration of total $Fe^{II}$ produced at time $t$, $Fe^{II}{}_{max}$ is the maximum $Fe^{II}$ concentration observed at the end of the reduction period, and $k_{obs}$ is the pseudo-first order constant.

The soluble fraction of $Fe^{II}$ was performed on the solution filtered through 0·22 $\mu$m filter before extraction by HCl. The cell numbers were determined by the epifluorescence microscopy technique (Hobbie *et al.* 1977) modified by Saby *et al.* (1997).

The solids were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM). Briefly, the suspension was filtered under $N_2$ atmosphere, mixed with glycerol to avoid oxidation and poured on a sample carrier to be analysed by XRD. For TEM observation, one drop of the suspension was laid on an amorphous carbon-coated grid and the sample was loaded into the microscope (CM20/STEM; Philips Electronics, Eindhoven, Netherlands). For observation, using optical microscopy (BX-51; Olympus, Tokyo, Japan) a droplet of the suspension was deposited on a glass slide, covered with a cover slip and observed under natural light using phase contrast.

## Results

### Characterization of the starting material

Optical microscopy observation of the chemically synthesized GR* shows brown solids of different size up to 20 $\mu$m, but the resolution of the microscope is too low to allow the crystal shape to be distinguished (Fig. 1a). The GR* observed by TEM displays crystal length around 100 nm and exhibits a typical hexagonal geometry (Fig. 1b). Electron diffraction and XRD indicate $d_{hkl}$ parameters specific of a GR* (Table 1, Fig. 1b,c).

### Ferric green rust reduction

Total $Fe^{II}$ concentration measurement (i.e. both in the solid and aqueous phase) showed a rapid increase as soon

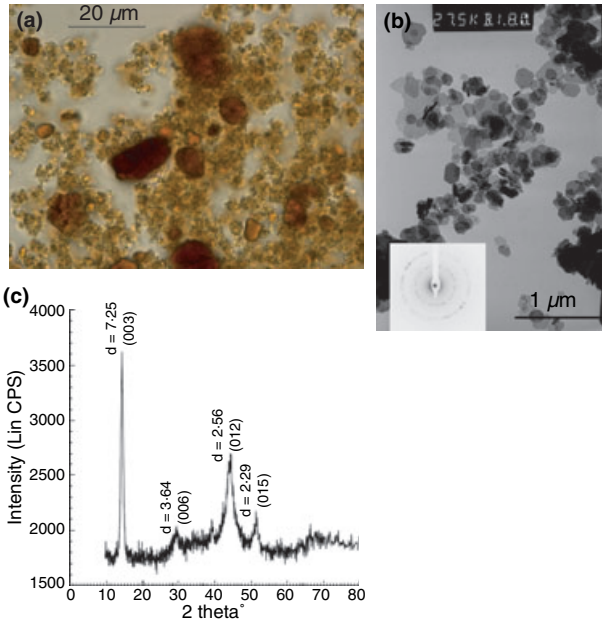


**Figure 1** Characterization and observation of the ferric carbonated green rust by (a) optic microscopy in natural light; (b) transmission electronic microscopy; and (c) X-ray diffraction. The $d_{hkl}$ values are indicated on each peak of the diffractogramm, these values are characteristic of the structure of the solid and here are specific of the GR* compounds. The inset image represents the electron diffraction of the crystals, the $d_{khl}$ values have been calculated by using the radius of the diffraction pattern (Table 1).

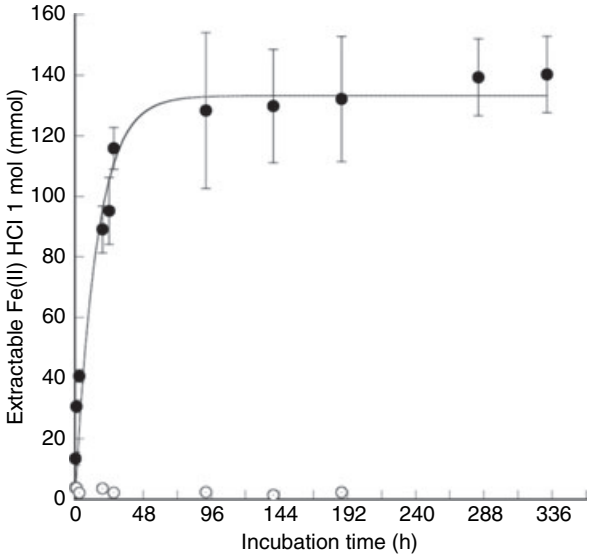


**Figure 2** Production of Fe(II) (both in the soluble and solid phases) during incubation of 46 mmol $l^{-1}$ of the GR* (277 equivalent millimolar iron concentration) with the *Shewanella* cells and with 160 mmol $l^{-1}$ of methanoate as the electron donor (closed circle). The blank (open circle) was performed without cells. The initial pH was comprised between 6·7 and 7·2. Data correspond to mean values of triplicate experiments ± SD.

as the bacterial cells were added to the mineral suspension (Fig. 2). The initial rate of reduction was 8·7 mmol $l^{-1}$ $h^{-1}$ (SD = 5%). To our knowledge, this is the fastest reduction rate for iron bioreducing systems leading to $Fe^{II}$–$Fe^{III}$ GR formation.

The GR* reduction ceased when approx. 50% of the $Fe^{III}$ (277 mmol $l^{-1}$ $Fe^{III}$ were initially added) have been reduced, i.e. after 92 h (around 4 days) of incubation when a plateau was reached at 140 mmol $l^{-1}$of $Fe^{II}$. The soluble fraction of $Fe^{II}$ at the end of the incubation time indicated that only 70 ± 17 μmol $l^{-1}$ remained in the solution confirming that $Fe^{II}$ species were essentially present in the solid state. A blank incubated in the same conditions without *Shewanella* cells, did not exhibited any $Fe^{II}$ increase during the time of the experiment (Fig. 2), the soluble fraction of $Fe^{II}$ measured was below the detection limit (<20 μmol $l^{-1}$).

### Secondary mineral by-product characterization

The solid phase was sampled after 6 days of incubation and characterized by XRD and TEM. XRD gave typical diffractogram of GR($CO_3^{2-}$) with a very intense peak at $d$ = 0·7553 nm (Fig. 3b). TEM observation indicates a mixed population of large and small hexagonal crystals,

**Table 1** $d_{hkl}$ and a (mesh) parameters of the ferric GR (GR*) and of the stoichiometric GR from this study and from literature

| Mineral | (*hkl*) | $d_{hkl}$ calculated (nm) from electronic diffractogram data | Cell parameter *a* (nm) calculated from $d_{hkl}$ | Cell parameter *a* (nm) from literature |
|---|---|---|---|---|
| GR*($CO_3^{2-}$) | (100) | 0·251 (0·006) | 0·290 (0·007) | 0·301* |
| | (120) | 0·147 (0·002) | 0·295 (0·003) | |
| GR($CO_3^{2-}$) | (100) | 0·264 (0·007) | 0·305 (0·008) | 0·3175788† |
| | (120) | 0·154 (0·002) | 0·308 (0·003) | |

$d_{hkl}$ is the interplanar distance between the crystallographical plans named '(*hkl*)'.
Values in parenthesis denote standard error.
*Génin *et al.* (2006).
†Aissa *et al.* (2006).

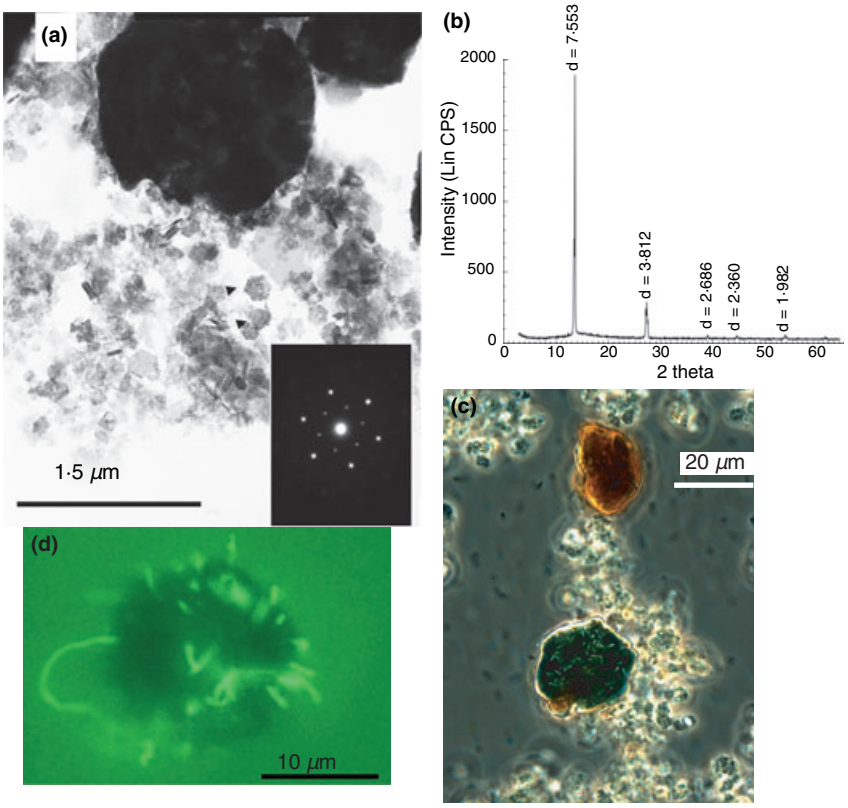


**Figure 3** Characterization and observation of the $Fe^{II}$–$Fe^{III}$ carbonated green rust by (a) transmission electronic microscopy, arrows indicate the lower sized hexagonal crystals, the inset image represents the electron diffraction of the larger crystals at the top of the picture; (b) X-ray diffraction diagram, each annotated peak is specific of GR; (c) picture from optical microscopy in natural light (phase contrast); and (d) picture of bacteria adhered on a GR crystal, observed by optical microscopy under epifluorescence (bacteria were marked by SYTO9®; Molecular Probes).

some crystals appeared fractured in particular the smaller ones, and others appeared notched especially the larger ones as also illustrated by optical microscopy (Fig. 3a,c). No other crystal shape was found. The electronic diffraction of the large hexagonal crystals confirms the nature of the GR (Fig. 3a). The GR cell parameter *a* is higher than the one measured for GR* (Table 1) because the deprotonation induces a global compaction of the structure (Génin *et al.* 2006). The small sized crystals were mainly associated in aggregates and were constituted of perfectly hexagonal crystals, similar in shape to the starting GR* (Fig. 1), and by fractured crystals. Electronic diffraction of these crystal aggregates gives a series of rings corresponding to both GR and GR* (not shown). In addition, a Mössbauer spectroscopy analysis was performed (at ambient temperature) on the GR* bioreduction products, it confirms the presence of both $Fe^{II}$ and $Fe^{III}$ oxidation states in the solid product (not shown).

A physical association was revealed between bacteria and the green hexagonal crystals, which was assumed to be the $Fe^{II}$–$Fe^{III}$ GR. As it can be seen on optical microscopy, especially when bacteria have been coloured by SYTO9 (S34854, Molecular Probes, Invitrogen Corp., Eugene, OR, USA), the GR crystals are partially covered by rod-shaped bacteria attached end-on to the crystals by their apical side (Fig. 3d).

## Discussion

We demonstrate in the present study that the ferric oxyhydroxide GR* can serve as an electron acceptor for *Shewanella* cells. In addition, the GR* is the third ferric crystalline solid, after lepidocrocite and ferrihydrite, being transformed into GR by bacterial activity (Parmar *et al.* 2001; Ona-Nguema *et al.* 2002). The reduction proceeds at a particularly high reduction rate (near 9 mmol $l^{-1}$ $h^{-1}$). In comparison, the well crystallized ferric oxyhydroxide lepidocrocite ($\gamma$-FeOOH), common in intermittently anaerobic soils (Cornell and Schwertmann 2003), is reduced at a much lower rate (<1 mmol $l^{-1}$) under the same experimental conditions (Zegeye 2006; Zegeye *et al.* 2007). As the solubility of iron oxides is a key factor in determining their reduction rate (Bonneville *et al.* 2004), the high reduction rate observed could be explained by the relatively high solubility of the GR (Bourrié *et al.* 1999).

Of significance is that the starting and the final products present a similar crystalline structure as indicated by XRD and TEM. Specifically, the morphology of the crystal is hexagonal and the diffraction lines are conserved, for example the 003 line (Figs 1c and 3b). This suggests that the bacteria reduce the solid without any dissolution-recrystallization processes and that such a direct


 

transformation could be energetically more favourable, and could also explained the high reduction rate.

The question of how electrons are transferred inside the mineral arises. The bacteria are systematically localized at the surface of the GR crystals by their apical region, indicating a close association between cells and GR crystal during its reduction. The same behaviour is usually observed when lepidocrocite was the starting material (Zegeye *et al.* 2006), indicating that the *de novo* GR formation, or GR* reduction into GR in that particular case, occurs closely to the bacterial cells. This contrasts to what has been previously suggested with 2-line ferrihydrite as the $Fe^{III}$ substrate (Glasauer *et al.* 2002).

*Shewanella oneidensis* MR1 can transfer electrons to iron oxides through pili-like appendages serving as electron-wires (Gorby *et al.* 2006). It is probable that *S. putrefaciens* CIP80.40 can proceed similarly. However, in our experimental conditions, we did not identify any such appendages, probably because our culture conditions were not the same as those used for such pili-like expression, or simply because cell suspension preparation (centrifugation, agitation, washing step) broke down these structures (Gorby *et al.* 2006). In this study, the transfer of electrons probably proceeds through direct contact between the cell surface and the solid and/or via the electron shuttle (AQDS) as previously suggested (Myers and Myers 1997; Lovley *et al.* 1998). However, both bacteria and AQDS are too large to diffuse between the hydroxide sheets, suggesting that an electron transfer occurred inside the solid as it was recently suggested in regards to pollutants reduction by structural $Fe^{2+}$ of GR (Williams and Scherer 2004). On the other hand, a *de novo* GR formation from a dissolution-recrystallization mechanism should not be discarded. In that case, the GR would be formed by co-precipitation of $Fe^{III}$ and $Fe^{II}$ as shown by Géhin *et al.* (2002).

The GR* could be the best substrate to produce fougerite or its GR homologous. Firstly because it has crystalline structure closely related to the $Fe^{II}$–$Fe^{III}$ GR (Génin *et al.* 2005). Secondly, it was previously shown that the oxidation of GR can directly yield to GR* (Ruby *et al.* 2006). Therefore, the green rusts would play the role of an electron balance sometimes having the function of oxidant, sometimes that of reductant. As the transformation from one redox state to another occurs inside a unique crystalline structure as illustrated in the Fig. 4, reactions with fast kinetics are expected. In that way, the reducing power of the very reactive $Fe^{II}$-bearing mineral green rust (Elsner *et al.* 2004) would be quickly restored.

The results presented here provide the first step to an applied research aiming to bacterially drive the formation/regeneration of these very reactive and versatile minerals, which are able to alternatively serve as an electron donor for redox reactions with oxidized pollutants and an electron acceptor for iron reducing bacterial activity. As GR activity is known to occur in permeable reactive barriers for soil remediation (e.g. Gaber *et al.* 2002), the bacterially driven transformation described here could aid greatly in a better understanding the mechanisms of such GR-related processes.

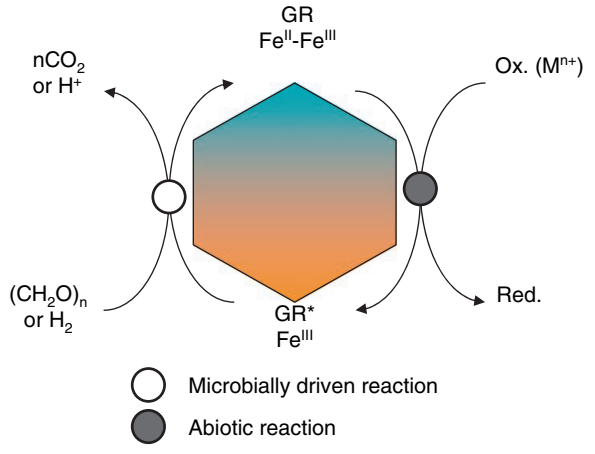


**Figure 4** A possible mechanism of a nondestructive oxidation of the green rust by oxidants (Ox-$Mn^+$) (Ruby *et al.* 2006) and its reduction (i.e. recovery of its reactivity) by bacterial reduction (this study). The oxidative green rust is GR* (in brown) and the reduced form is GR (in green).

## Acknowledgements

The authors would like to thank Dr Jaafar Ghanbaja (Université Henri Poincaré, Nancy, France), and Dr Isabelle Bihannic (LEM UMR 7569, CNRS-INPL, Nancy, France) for TEM and DRX analysis respectively.

## References

Aissa, R., Francois, M., Ruby, C., Fauth, F., Medjahdi, G., Abdelmoula, M. and Génin, J.M. (2006) Formation and crystallographical structure of hydroxysulphate and hydroxycarbonate green rusts synthetised by coprecipitation. *J Phys Chem Solids* **67**, 1016–1019.

Anderson, R.T., Rooney-Varga, J.N., Gaw, C.V. and Lovley, D.R. (1998) Anaerobic benzene oxidation in the Fe(III) reduction zone of petroleum-contaminated aquifers. *Environ Sci Technol* **32**, 1222–1229.

Bonneville, S., Van Cappellen, Ph. and Behrends, T. (2004) Microbial reduction of iron(III) oxyhydroxides: effects of mineral solubility and availability. *Chem Geol* **212**, 255–268.

Bourrié, G., Trolard, F., Génin, J.M.R., Jaffrezic, A., Maitre, V. and Abdelmoula, M. (1999) Iron control by equilibria

between hydroxy-green rusts and solutions in hydromorphic soils. *Geochim Cosmochim Acta* **63**, 3417–3427.

Cornell, R.M. and Schwertmann, U. (2003) The iron oxides. Weinheim, Germany: Wiley VCH.

Elsner, M., Schwarzenbach, R. and Haderlein, S.B. (2004) Reactivity of iron(II) bearing minerals towards reductive transformation of organic contaminants. *Environ Sci Technol* **38**, 799–807.

Erbs, M., Hansen, H.C.B. and Olsen, C.E. (1999) Reductive dechlorination of carbon tetrachloride using iron(II) iron(III) hydroxide sulfate (green rust). *Environ Sci Technol* **33**, 307–311.

Fadrus, H. and Maly, J. (1975) Suppression of iron(III) interference in the determination of iron(II) in water by the 1,10-phenanthroline method. *Analyst* **100**, 549–554.

Gaber, H.M., Comfort, S.D., Shea, P.J. and Machacek, T.A. (2002) Metolachlor dechlorination by zerovalent iron during unsaturated transport. *J Environ Qual* **31**, 962–969.

Géhin, A., Ruby, C., Abdelmoula, M., Benali, O., Ghanbaja, J., Refait, P. and Génin, J.M.R. (2002) Synthesis of Fe(II-III) hydroxysulphate green rust by coprecipitation. *Solid State Sci* **4**, 61–66.

Génin, J.M.R., Bourrié, G., Trolard, F., Abdelmoula, M., Jaffrezic, A., Refait, P., Maitre, V. and Humbert, B. (1998) Thermodynamic equilibria in aqueous suspensions of synthetic and natural Fe(II) - Fe(III) green rusts; occurences of the mineral in hydromorphic soils. *Environ Sci Technol* **32**, 1058–1068.

Génin, J.M.R., Aissa, R., Gehin, A., Abdelmoula, M., Benali, O., Ernsten, V., Ona-Nguema, G. and Upadhyay, C. (2005) Fougerite and Fe(II-III) hydroxycarbonate green rust : ordering deprotonation and/or cation substitution; structure of hydrotalcite-like compounds and mythic hydroxide Fe(OH) (2+x). *Solid State Sci* **7**, 545–572.

Génin, J.M.R., Ruby, C. and Upadhyay, C. (2006) Structure and thermodynamics of ferrous, stoichiometric and ferric oxyhydroxycarbonate green rusts; redox flexibility and fougerite mineral. *Solid State Sci* **8**, 1330–1343.

Glasauer, S., Langley, S. and Beveridge, T.J. (2002) Intracellular iron minerals in a dissimilatory iron-reducing bacterium. *Appl Environ Microbiol* **67**, 5544–5550.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., Beveridge, T.J. and Chang, I.S. (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc Natl Acad Sci USA* **103**, 11358–11363.

Hansen, H.C.B. (2001) Environmental chemistry of iron(II)-iron(III) LDHs. In *Layered Double Hydroxides* ed. Rives, V. pp. 413–434. Huntington, NY: Nova Sci. Publ.

Hobbie, J.E., Daley, R.J. and Jasper, S. (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* **33**, 1225–1228.

Legrand, L., Mazerolles, L. and Chaussé, A. (2004) The oxidation of carbonated green-rust into ferric phases: solid state reaction or transformation via solution. *Geochim Cosmochim Acta* **68**, 3497–3507.

Lovley, D.R., Fraga, J.L., Blunt-Harris, E.L., Hayes, L.A., Phillips, E.J.P. and Coates, J.D. (1998) Humic substances as a mediator for microbially catalyzed metal reduction. *Acta Hydrochim Hydrobiol* **26**, 152–157.

Loyaux-Lawniczak, S., Refait, P., Ehrhardt, J.J., Lecomte, P. and Génin, J.M.R. (2000) Trapping of Cr by formation of ferrihydrite during the reduction of chromate ions by Iron (II)-Iron(III) hydroxysalt green rusts. *Environ Sci Technol* **34**, 438–443.

Myers, C.R. and Myers, J.M. (1997) Outer membrane cytochromes of *Shewanella putrefaciens* MR-1: spectral analysis, and purification of the 83-kDa c-type cytochrome. *Biochim Biophys Acta* **1326**, 307–318.

Myneni, S.C.B., Tokunaga, T.K. and Brown, G.E. Jr (1997) Abiotic Se redox chemistry in the presence of Fe(II, III)-oxides. *Science* **278**, 1106–1109.

O'Loughlin, E.J., Kelly, S.D., Kemner, K.M., Csencsits, R. and Cook, R.E. (2003) Reduction of $Ag^{I}$, $Au^{III}$, $Cu^{II}$, and $Hg^{II}$ by $Fe^{II}/Fe^{III}$ hydroxysulfate green rust. *Chemosphere* **53**, 437–446.

Ona-Nguema, G., Abdelmoula, M., Jorand, F., Benali, O., Géhin, A., Block, J.C. and Génin, J.M.R. (2002) Iron (II,III) hydroxycarbonate green rust formation and stabilization from lepidocrocite bioreduction. *Environ Sci Technol* **36**, 16–20.

Parmar, N., Gorby, Y.A., Beveridge, T.J. and Ferris, F.G. (2001) Formation of green rust and immobilization of nickel in response to bacterial reduction of hydrous ferric oxide. *Geomicrobiol J* **18**, 375–385.

Refait, P., Simon, L. and Génin, J.M.R. (2000) Reduction of $SeO_4^{2-}$ anions and anoxic formation of iron(II)-iron(III) hydroxy selenate green rust. *Environ Sci Technol* **34**, 819–825.

Refait, P., Benali, O., Abdelmoula, M. and Génin, J.M.R. (2003) Formation of ferric green rust and/or ferrihydrite by fast oxidation of iron(II-III) hydroxychloride green rust. *Corros Sci* **45**, 2435–2449.

Ruby, C., Gehin, A., Abdelmoula, M., Génin, J.M.R. and Jolivet, J.P. (2003) Coprecipitation of Fe(II) and Fe(III) cations in sulphated aqueous medium and formation of hydroxysulphate green rust. *Solid State Sci* **5**, 1055–1062.

Ruby, C., Upadhyay, C., Géhin, A., Ona-Nguema, G. and Génin, J.M.R. (2006) In situ redox flexibility of FeII-III oxyhydroxycarbonate green-rust and fougerite. *Environ Sci Technol* **40**, 4696–4702.

Saby, S., Sibille, I., Mathieu, L., Paquin, J.L. and Block, J.C. (1997) Influence of water chlorination on the counting of bacteria with DAPI (4′,6-diamidino-2-phenylindole). *Appl Environ Microbiol* **63**, 1564–1569.

Trolard, F., Abdelmoula, M., Bourrié, G., Humbert, B. and Génin, J.M.R. (1996) Evidence of the occurrence of a

"Green Rust" component in hydromorphic soils. Proposition of the existence of a new mineral: "fougerite". *C R Acad Sci Paris* **323**, 1015–1022.

Williams, A.G.B. and Scherer, M.M. (2004) Spectroscopic evidence for Fe(II)-Fe(III) electron transfer at the iron oxide-water interface. *Environ Sci Technol* **38**, 4782–4790.

Zachara, J.M., Kukkadapu, R.K., Fredrickson, J.K., Gorby, Y.A. and Smith, S.C. (2002) Biomineralization of poorly crystalline Fe(III) oxides by dissimilatory metal reducing bacteria (DMRB). *Geomicrobiol J* **19**, 179–207.

Zegeye, A. (2006) Formation et stabilité des hydroxysels Fe(II – III) de type rouille verte en cultures bactériennes. PhD Thesis, Université Henri Poincaré, Nancy 1, Nancy, France.

Zegeye, A., Mustin, C., Ruby, C. and Jorand, F. (2006) Factor controlling the biomineralization pathway of $\gamma$–FeOOH into green rust *versus* magnetite. *Proceedings of 19th General Meeting of the International Mineralogical Association*, July 23–28th 2006, Kobe, Japan.

Zegeye, A., Ona-Nguema, G., Carteret, C., Huguet, L., Abdelmoula, M. and Jorand, F. (2005) Formation of hydroxysulphate green rust 2 as a single iron (II-III) mineral in microbial culture. *Geomicrobiol J* **22**, 389–399.

Zegeye, A., Ruby, C. and Jorand, F. (2007) Kinetic and thermodynamic analysis during dissimilatory $\gamma$-FeOOH reduction: formation of green rust 1 and magnetite. *Geomicrobiol J* **24**, 1–14.