Scientific report for an exchange visit grant

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Influence of bacterial activity on the mineralogical ageing of the iron ore and his micromechanical properties within the abandoned mines in Lorraine (France)

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Special thanks to the Professor Andreas Kappler who accept me in the Geomicrobiology laboratory at Tübingen, and who took time to discuss with me when he crossed me in the lab!

Special mention for Emily!! You learnt me a lot of things during my stay, and your sympathy and your patience (important with me !) allow me to make progress in Science, cultivation techniques and to improve my English !! I hope I will have the opportunity to work with you again.

Thanks Annika for the little talks around the bunch when the Ferrozine assay made me feel crazy, and your amazing talent for sampling chores! ;-)

And of course thanks to all the team geomicrobiology team for your help and your time!

Context of the thesis

The context of my PhD project is related to the mining collapses which currently occur in Lorraine (France) above abandoned room-and-pillar iron mines. Some of these pillars are abandoned for one hundred years. In these areas of partial extraction, mining collapses have occurred several times, usually owing to the failure of abandoned pillars. The end of mining caused the stoppage of mine drainage pumps and then the phreatic surface rise. Some collapses occurred during this phase. So, an important research program entitled GISOS (research Group for the Impact and Safety of Underground Works) was set up in order to explain the mechanisms involved in these mine collapses. This PhD thesis is related to this research group.

Short state of the art

Usually, iron ore consists of a large fraction of joined oolites, constituted of concentric layers of goethite (α-FeOOH). Calcite ($CaCO_3$), siderite ($FeCO_3$) and berthierine (very ferriferous species of phyllosillicate), with varied proportions and compositions, cement these oolites (Grgic *et al.*, 2001, 2002; Dagallier *et al.*, 2002). The most common facies of iron ore is a ferri-arenite with homogeneous grain size.

The mineralogical ageing of iron ore constituting pillars and exposed to the mining atmosphere, has been characterised thanks to several experimental techniques (SEM, X-ray diffraction, Mössbauer spectroscopy)(Figure 1 and Table 1). This ageing corresponds to a significant oxidation of iron carbonates (siderite) of the inter-oolitic cement, then to an iron oxyhydroxides neoformation (hematite). These neoformed iron oxides (i.e. ‘‘rust’’) correspond to the residue of the siderite alteration and they cover the oolites and give to the altered iron ore a red colour. Considering the field observations (*in situ* experimental sites of the cities of Tressange and Angevilliers), we can suppose that the mineralogical ageing is very effective when iron ore is exposed to aerobic environmental conditions over a long period (more than 70 years).

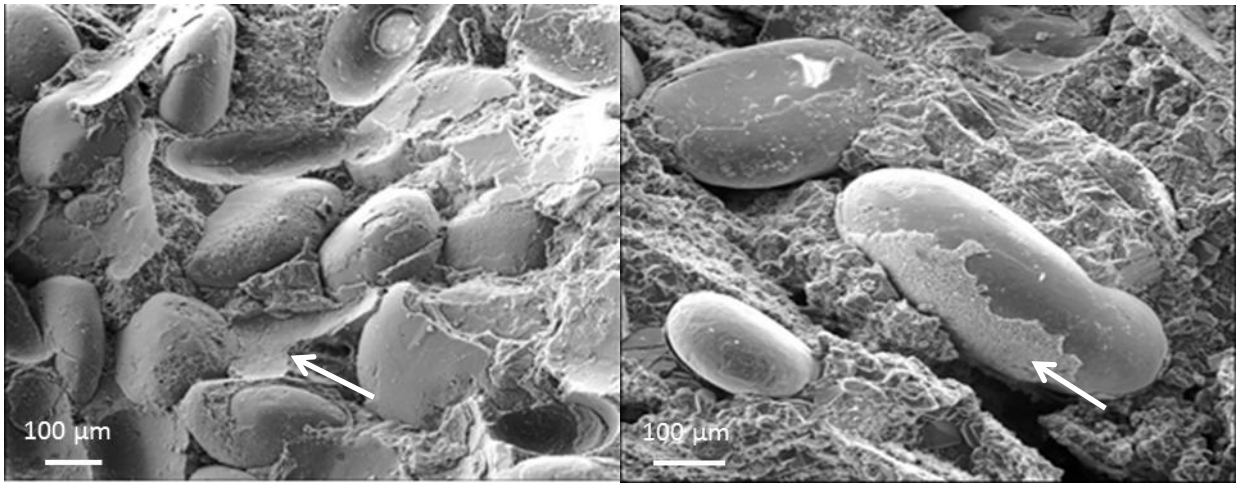
The ageing is characterized by the loss of the cement between the oolites (Figure 1). This cement is composed by siderite and replaced by crust of iron(III) oxides in the old ore (see white arrow in Figure 1b). That was confirmed by Mössbauer analysis in a previous preliminary study (Table 1) (Jorand, 2004, unpublished data). It was hypothesized that this alteration would be promoted or be the result of bacterial activities.

Goals of the thesis

The aim of this thesis is to validate the hypothesis of ore alteration from iron-oxidising bacteria or eventually other metabolisms (e.g.: iron reducing bacteria, siderophore effects…). From an applied point of view, this project could provide data to improve the management of abandoned mines. From a fundamental point of view, this project aims to answer to the scientist question how cells, especially iron oxidising bacteria, could use iron solid species (e.g., Fe^{II} from inter-oolithic siderite) which are supposed to be barely available and accessible.

Purpose of the visit

In order to carry out the PhD project we need to improve the culture conditions of the iron oxidising bacteria. The laboratory of Pr. Kappler (University of Tübingen, dept. of Geomicrobiology) has an excellent expertise on iron oxidising bacteria (e.g. : Kappler *et al.*, 2002, Kappler & Newman, 2003, Miot *et al.*, 2009).



(a) (b)

Figure 1 : (a) Typical crack surface of ''healthy'' oolitic iron ore. Oolites are well cemented by an assembly of siderite and ferriferous phyllosilicate (white arrow). This ore was exploited in 1990. (b) Typical crack surface of ''old'' oolitic iron ore. Neoformation of a crust of iron oxides at the surface of oolites (white arrow). Iron ore sample exploited towards 1900-1930 (SEM observations).

Table 1 : Distribution of the different iron-bearing phases in iron ore samples (1990 and 1930 iron ore), obtained from Mössbauer spectrometry (Jorand, 2004).

	1990	***1930***
Total Phases Fe(II)	**14 %**	**4 %**
berthierine	7 %	4 %
sidérite	7 %	nd
Total Phases Fe(III)	**86 %**	**96 %**
goethite	81 %	88 %
berthierine	nd	3 %
unidentified	5 %	nd
hematite	nd	5 %

nd : undetected

Description of the work carried out during the visit

Few tasks have been done, during this visit:

- Learning of the cultivation techniques for anaerobic iron oxidizing / reducing bacteria:
  - media synthesis for iron(II) oxidizing bacteria (BoFeN1) and photo-autotrophe (TIE-1),
  - Incubation of BoFeN1 and TIE-1with different iron sources (iron ore, $Fe^{II}Fe^{III}$ minerals, …),
- To improve knowledges for the ferrozine assay ($Fe^{II}Fe^{III}$ measures),
- To improve optical microscopy lectures and experiments (for cell counts, use of new fluorochrome and software)

1- Strains

BoFeN1 is a nitrate-dependent Fe(II)-oxidizing strain BoFeN1 was isolated from Lake Constance littoral sediments (Kappler *et al*., 2005). It is closely related to *Acidovorax* sp.
TIE-1 is phototrophic Fe(II)-oxidizing bacterium which finds in an iron-rich mat from School Street Marsh in Woods Hole, MA (Jiao *et al*., 2005*).* It is closely related to *Rhodopseudomonas palustris*.

2- Cultivation techniques

For BoFeN1, a low phosphate medium (LPM) was used. The mineral base is composed by KH_2PO_4, 1 mM; NH_4Cl, 5 mM; $MgSO_4,H_2O$, 2 mM; NaCl, 3 mM; and $CaCl_2,2H_2O$, 0.7 mM (Kappler *et al*., 2005). Vitamins, trace elements, and buffer ($NaHCO_3$, 50 mM) have been added separately, alike for sodium acetate, 0.5 mM; $FeCl_2$, 10 mM and $NaNO_3$, 2 mM. The distribution of the medium has been done by Widdel flask (Figure 2). Using this flask, we can degas an important amount of medium, add specific additives which have to be sterilized separately, and easily distribute the medium in bottles.

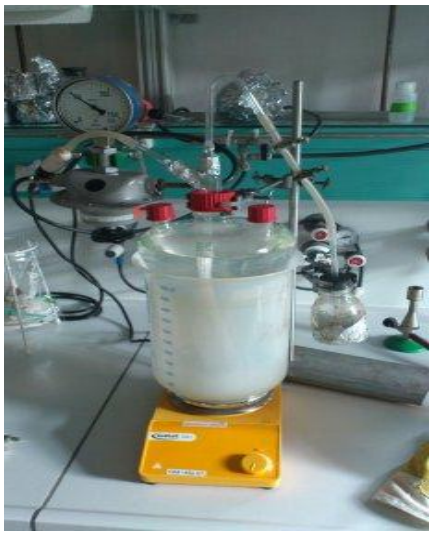


Figure 2: Photography of the widdel flask used during the internship

After autoclave of the minimal salt medium in the Widdel flask, this one is placed under a N_2/CO_2 pressure to degas the medium and exchange O_2 by N_2/CO_2. Vitamins, trace elements and buffer are added. Then the medium is distributed in 100 mL sterile serum bottles. The headspace of these bottles is gazed by N_2/CO_2. Finally the bottles are closed by butyls septum.

When the iron(II) is added in the medium, the equilibrate is made during two days. Some green precipitates appear. Thus the medium is filtrated (PEJ-membrane, 0.2 µm, sterilcup, Millipore) in an anoxic chamber. The pH must be in the range 7.05 and 7.1. The final concentration of iron(II) is 6 mM. At the time of the inoculation, acetate, nitrate and bacteria are added at the same time.

Ratio between Iron (II), acetate and nitrate is defined to respect the equation (1) (Straub *et al*., 1996).

$$10Fe^{2+} + 2NO_3^- + 24H_2O \rightarrow 10Fe(OH)_3 + N_2 + 18H^+ \qquad (1)$$

For TIE-1 the same pattern was used. Only the composition of the medium is different: NH_4Cl (5 mM), KH_2PO_4 (5 mM), $CaCl_2$,$2H_2O$ 0.7 mM and $MgSO_4$,H_2O (2 mM). Neither acetate nor nitrate has been added. The final concentration of iron(II) is 4.5 mM. The pH must be in the range 7.1 and 7.2.

3- Iron extraction

In order to estimate the iron (II and III) concentrations in the samples (1930 and 1990 iron ore and the $Fe^{II}Fe^{III}$ model), an extraction with 6 M HCl has been done. This experiment allowed us to put the same amount of iron(II) for each iron source in each incubation. The iron concentration has been measured by the Ferrozine method.

4- Ferrozine assay

This method has been described in Hegler *et al* (2008). Shortly, in a microplate, to 20 µL of sample 80µL of HCl 1 M and 100 µL of ferrozine solution (50% (w/v) ammonium acetate, 0.1% (w/v) ferrozine) are added to quantify iron(II). For total iron (II and III), to 20 µL of sample, have been add 80µL of hydroxylamine hydrochloride which is a powerful reducing agent (10% w/v in 1M HCl). After 30 min in the dark, the ferrozine solution has been added. This solution has to react during 5 min in the dark.

5- Experiments and main results

Two series of incubation has been done with four iron(II) sources: soluble Fe(II), suspension of mestastable FeII-bearing mineral (carbonated green rust), suspension of 1930 and 1990 iron ores with the two strains BoFeN1 and TIE-1.

Data of the total iron and iron(II) are given in Table 2. From these data, an equivalent amount of iron(II) (extractible by HCl 6M) for each iron source has put in the cultivation media.

Table 2 : Iron(II) and iron(III) concentrations after 6M HCl extraction

NAME	Fe(II) mM	SD*	Fe(III) mM	SD
LPM Medium	6.2044	0.3521	0.9316	0.7587
$Fe^{II}Fe^{III}$ mineral**	3.3747	0.2213	1.3498	0.086
1930 iron ore	12.3164	0.2859	4.3428	0.018
1990 iron ore	20.7532	0.6981	8.2612	0.7534
TIE-1 Medium	4.4882	0.8693	0.5221	0.8678

*SD = standard deviation, **here we used the Green Rust (GR), a metastable Fe(II) components.

So, for BoFeN1 culture, ≈ 6 mM of iron(II) has been used as electron donor in each sample (≈ 1.5 mM for GR sample). Nitrate (2 mM) and acetate (0.5 mM) have been added in the same time of 5 mL of pre-culture. For TIE-1 culture, the iron(II) concentration is ≈ 4.5 mM (≈ 1.3 mM for GR sample). Light and CO_2 from the bicarbonate buffer are used to oxidize Fe(II). Biotic and abiotic experiments have been lead in duplicate.

5-1- BoFeN1

Five sampling have been done at 0, 2, 4, 6 and 9 days after the inoculation. The iron (II, III and Total) have been followed for each iron source. The iron in the dissolved phase and the total phase has been quantified by ferrozine assay. Figure 3 show the followed protocol for sampling.

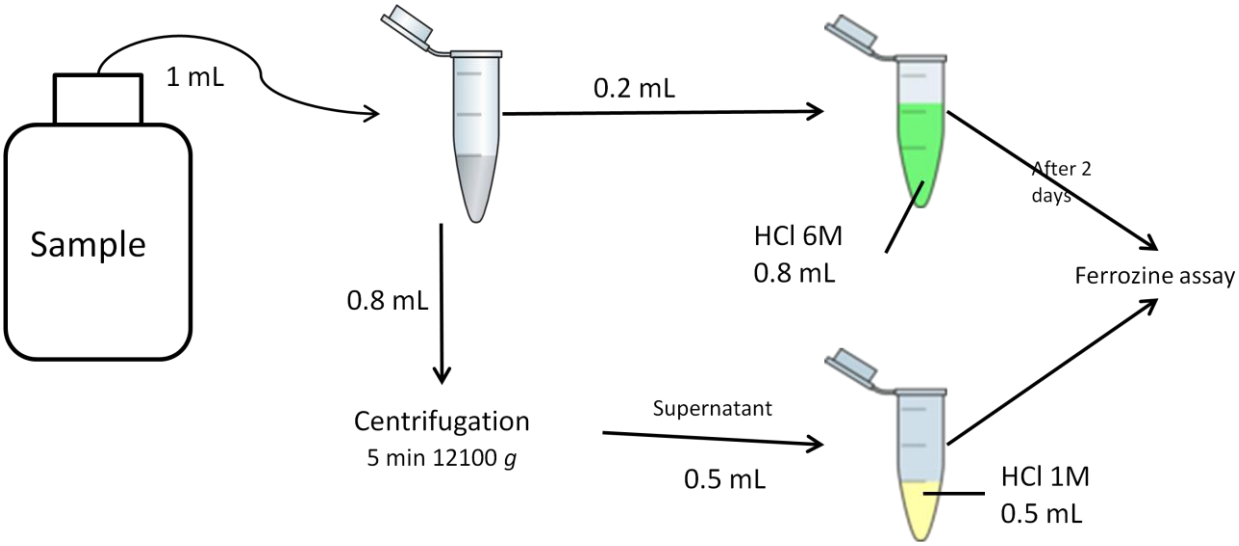


Figure 3 : Schema of sampling for iron measurement during BoFeN1 and TIE-1 culture.

The results from the total extraction are showed in Figure 4. Only for the LPM soluble Fe(II), we can see a decrease of the iron (II) (rate: 673 µM/day) in the biotic test (Figure 4A) (abiotic is stable (Figure 4B)). For $Fe^{II}Fe^{III}$ mineral, 1930, 1990 no significant change was observed: biotic and abiotic systems are quite similar (data not show). Although iron (II) is present in these minerals, it seems to be rather not available (at the considered time) for anaerobic iron oxidizing bacteria, even if the Fe(II) from the GR which is metastable . So the experiment needs to be conducted on a longer period of time. The variation of the total iron is due to the sampling method which is not accurate.

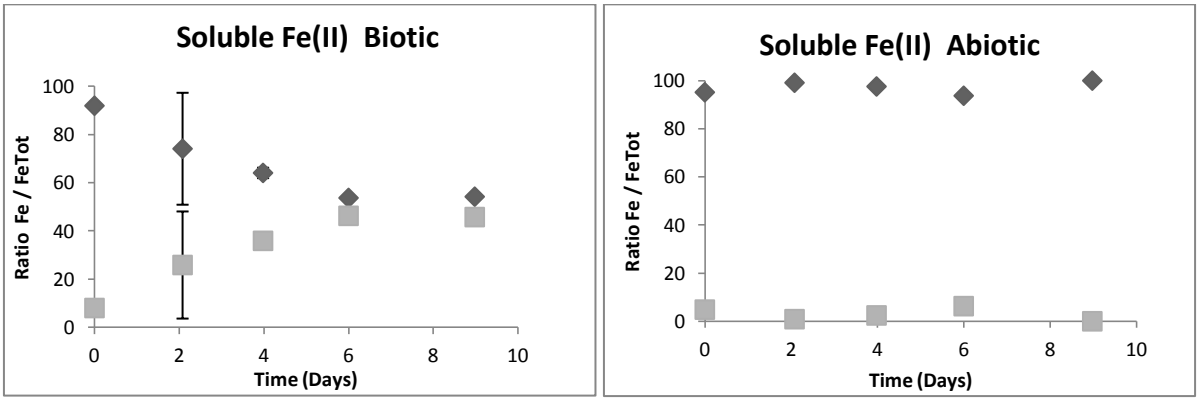


Figure 4 : Concentration of iron, in the total extraction phase, for BoFeN1 sample with soluble Fe(II) (A: biotic, B: abiotic). Ratio Fe(III)/FeTot (■), Fe(II)/FeTot (◆). Error bares are hidden by the symbols.

5-2- TIE-1

The same process has been used to sampling TIE-1 (Figure 3). As for BoFeN1, no significant change was observed for 1930 and 1990 iron ore (data not show). On the other hand, soluble iron(II) and GR has been oxidized (Figure 5, Figure 6). The oxidation of soluble iron(II) by photoautotrophic bacteria is well known since several decades and the hypothesis is that these photoautotrophes are involved in the banded iron formation (Jiao *et al*., 2005). Even if the abiotic middle is slightly oxidized (photo-oxidation?), the oxidation is higher in the biotic medium. Then, the Fe(II) of the GR is available for TIE-1. Sampling along the time will continue and the characterization of the solid at the end of the incubation will be performed (Mössbauer analysis, Raman spectroscopy and TEM).

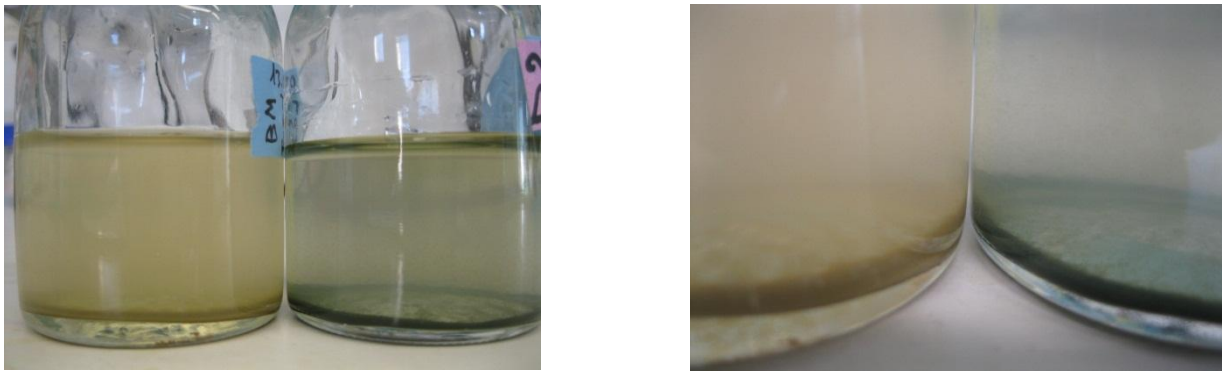


Figure 5 : State of the medium after 4 days of incubation: with bacteria (left one) and without it (right one). On the left one the characterized greenish color of the GR has disappeared.

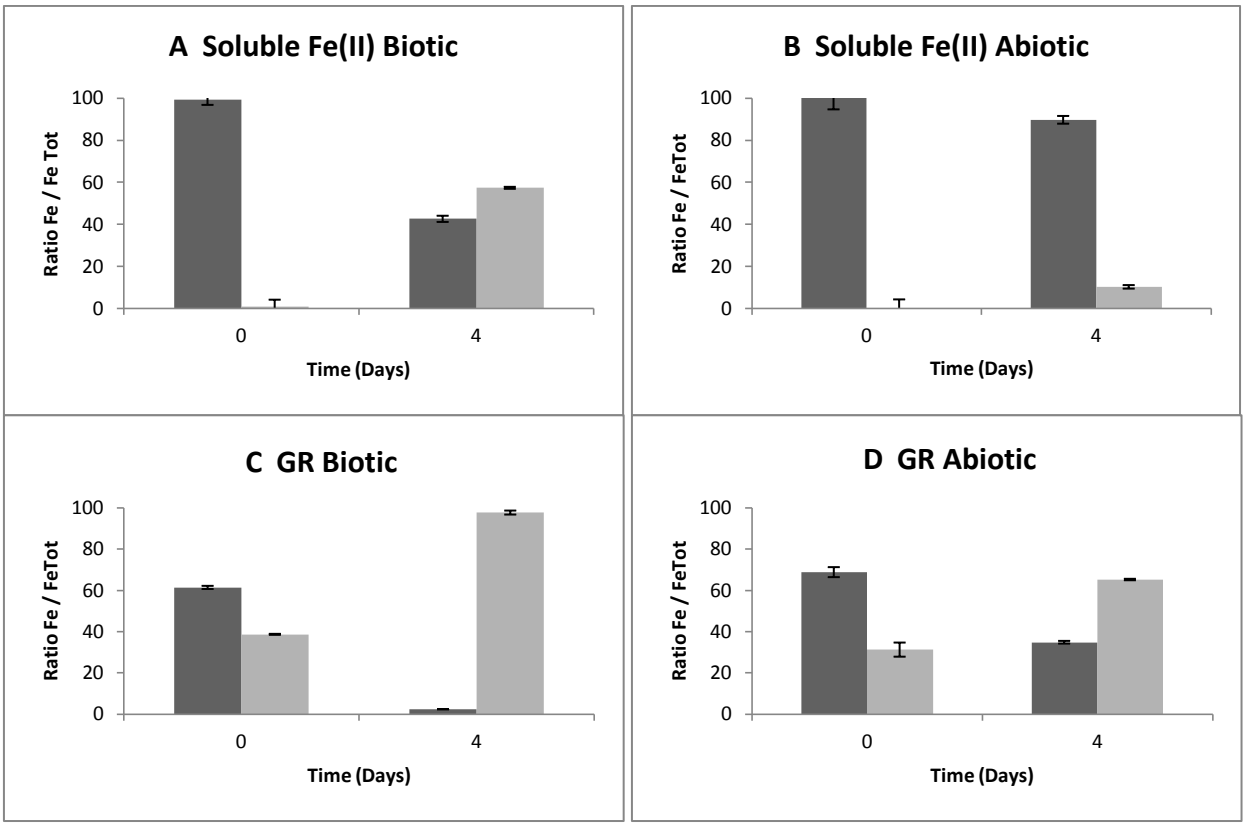


Figure 6 : Concentration of iron, in the total extraction phase, for TIE-1 sample with soluble Fe(II) (A: biotic, B: abiotic) and GR (C: biotic, D: abiotic) as iron(II) source. Ratio Fe(III)/FeTot (▒), Fe(II)/FeTot (■).

Conclusions and perspectives

During this internship, two main tasks have been done: how to prepare anoxic media for iron(II) oxidizing bacteria and photoautotrophes, and the ferrozine assay on two incubation series. The results showed that both BoFeN1 and TIE-1 can use soluble Fe(II) as electron donor source, but only the phototrophic bacteria are able to use solid form of Fe(II) from the GR. That is not the case for the Fe(II) from the 1930 and 1990 iron ore in the time of the experimental study. Then this experiment need to be lead on a longer period to observe some changes. The solid analysis will be done.

Other tasks have been done like staining for microscopy (with Dapi and Cytox), sediment chore sampling, and some little tricks which will help me for improve the quality of the experiments (there is no data for this, just some protocols or advices). Unfortunately, the microaerophilic cultures haven't been done because of a lack of time and an incompatibility of schedule. However it could be planned for another visit. Moreover, it was a good opportunity to share knowledge with people working in various field of the geomicrobiology.

The collaboration will continue between our two laboratories, on these two incubation series. If the results are interesting, a publication will be writing. In this framework, another exchange grant could be asked to FIMIN to pursue this project.

Bibliography

Dagallier G., Grgic D., Homand F. 2002. Mineralogical and microtextural characterisation of the anthropic-origin ageing of iron ore in Lorraine (France).C. R. Geoscience 334: 455–462

Grgic, D. 2001. Modélisation du comportement à court et à long terme des roches de la formation ferrifère lorraine. Thèse de doctorat, INPL, Nancy, 169p.

Hegler F,. Posth N R, Jiang J and Kappler A. **2008.** Physiologyof phototrophic iron(II)-oxidizing bacteria: implications for modern and ancient environments. FEMS Microbiol Ecol 66 : 250-260.

Jiao Y, Kappler A, Croal L R, and Newman D K. 2005. Isolation and Characterization of a Genetically Tractable Photoautotrophic Fe(II)-Oxidizing Bacterium, *Rhodopseudomonas palustris* Strain TIE-1. Appl. Environ. Microbiol., 71: 4487-4496.

Jorand, F. 2004. Influence de l'activité bactérienne sur le vieillissement du minerai de fer dans les mines abandonnées de Lorraine. Internal Report (in French) LCPME UMR 7564 CNRS-UHP, Nancy 1, Faculté de Pharmacie, 29 p.

Kappler, A and Newman, K. 2003. Formation of Fe(III)-minerals by Fe(II)-oxidizing photoautotrophic bacteria. Geochimica et Cosmochimica Acta, 68: 1217–1226.

Miot J, Benzerara K, Morin G., Bernard S, Beyssac O., Larquet E., Kappler A. and Guyot F. 2009. Transformation of vivianite by anaerobic nitrate-reducing iron-oxidizing bacteria. Geobiology, 7:373-384.

Straub, K. L., Benz M., Schink B. and Widdel F. 1996. Anaerobic, nitrate-dependent microbial oxidation of ferrous iron. Appl. Environ. Microbiol. 62: 1458-1460.