	Experiment title: Monitoring Dissimilatory iron reduction by live procaryotes under pressure and temperature conditions of deep sea hydrothermal vents	**Experiment number**: 30-02-869
Beamline: BM30b	**Date of experiment**: from: 16/04/2008 to: 22/04/2008	**Date of report**: August 22, 2008
Shifts: 18	**Local contact(s)**: Jean-Louis Hazemann	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Aude Picard*, Isabelle Daniel*, Philippe Oger*: Laboratoire de Sciences de la Terre – UMR 5570, CNRS – ENS Lyon – UCB Lyon1, 46 allée d'Italie, F-69364 Lyon cedex

Jean-Louis Hazmann*: Institut Néel, CNRS – UJF Grenoble

Report:

Choice of the strain: *Shewanella oneidensis* MR-1 was selected on several grounds. The reduction of Se(IV) in Se(0) by MR-1 was already successfully investigated in a pioneering experiment to 150 MPa carried out at ID22 and BM30b. It demonstrated a metabolic activity to ca. 160 MPa (Picard et al., submitted), and that the maximum pressure for metabolic activity depends on cell concentration.
The strain MR-1 is versatile in terms of electron acceptor, among which Fe(III). It was thus of prime importance to investigate whether the pressure limit for metabolism is dependent on the strain only or on the exact metabolic pathway of a microorganism.
Shewanella oneidensis MR-1 was thus the ideal candidate. During those **6 days**, **9 experiments** were performed to measure the metabolic reduction of Fe by *S. oneidensis* MR-1 for two cell concentrations corresponding to DOs of 0.5 and 0.05, respectively.

Choice of the culture medium:
Two culture media were tested on the ground of the metabolic requirements of MR-1.

Culture medium 1 : Luria Bertani diluted 10 times (LB/10) + sodium lactate 20 mM + HEPES 100 mM + ferric citrate 5 mM. Sodium lactate as the preferred electron donor of MR-1 reduces instantaneously the Fe(III) into Fe(II). The use of that medium was discarded.

Culture medium 2 : LB/10 + HEPES 100 mM + ferric citrate 5 mM, as a minimum culture medium was thus selected, with the electron acceptor included in the LB medium. It also abiotically reduced Fe(III) into Fe(II), but at an acceptable level.

Conditions of acquisition:
The spectra were taken in 101 points of 1s each, hence minimizing the irradiation of the bacteria (Oger et al., 2008). Given the low energy of Fe, the low concentration in Fe considering the high-pressure environment, and the novelty of the experiment, the X-ray detection was optimized at the expense of the maximum pressure. The autoclave was equipped with thin Be windows. In such a configuration, the maximum pressure was limited to rather low pressure.

Reference kinetic experiments at ambient pressure and 30°C:

- 1 experiment with culture medium 1, and a DO of 0.5. This first experiment discarded the use of culture medium 1.
- 3 experiments with culture medium 2 at different DOs

With a DO of 0.5, the reaction is complete within 4 hours (Fig. 1a). With a DO of 0.05 the a kinetic extends over ca 24 h, and the transformation is still uncomplete (Fig. 1b). This is very important to build the model for the evolution of the metabolic yield as a function of pressure and cell concentration.

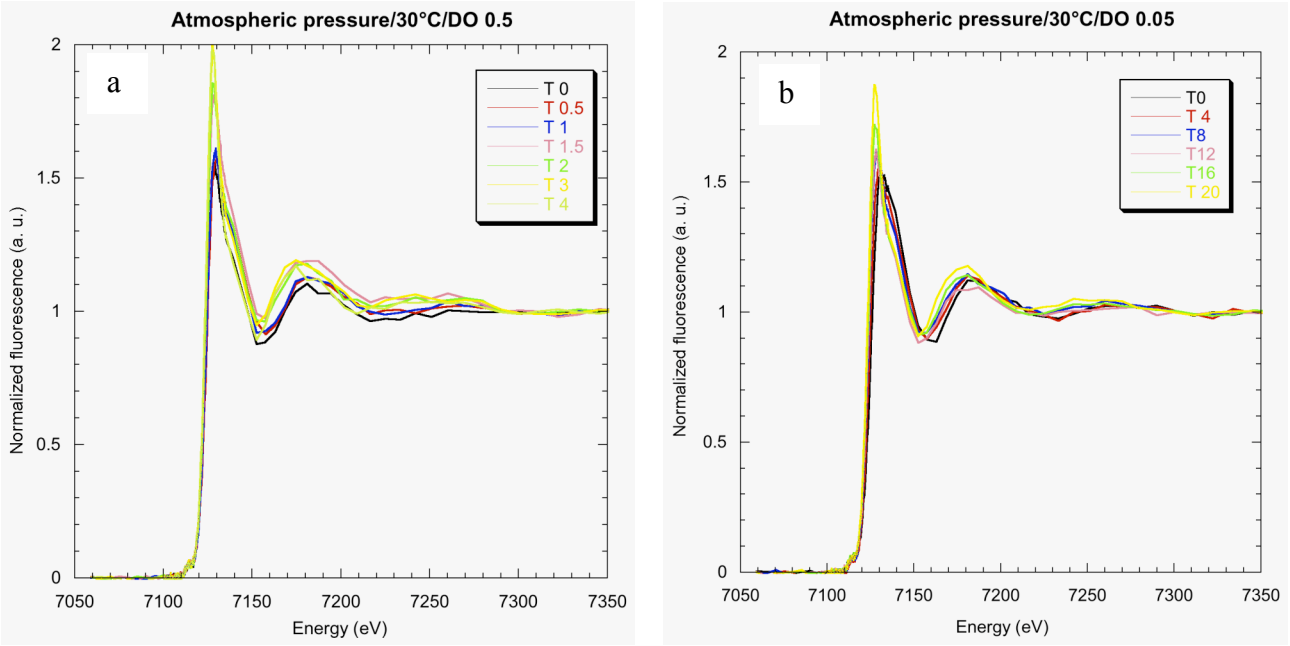
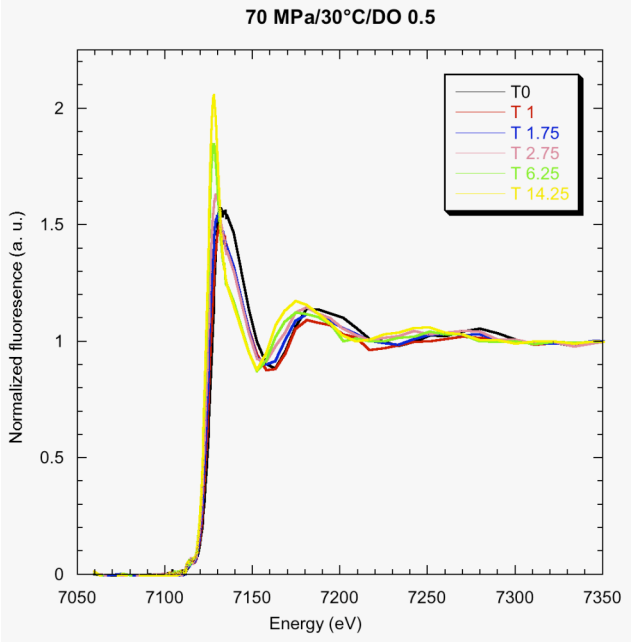


Fig. 1: evolution of the XANES spectrum of Fe as a function of time (T in hours), that show a progressive reduction of Fe(III) into Fe(II) by S. oneidensis MR-1 at ambient pressure and 30°C. (a) The reduction is complete within 4h only with a cell concentration corresponding to a DO of 0.5, whereas (b) with a lower cell concentration – DO = 0.05, the metabolic reduction of Fe extends over 24 h.

Experiments under high pressure , 30°C:

Once we had establish the exact protocole for Fe as a new metabolite, we had only time for 5 successful experiments under high hydrostatic pressure at 30°C.

- 2 experiments at 30 MPa (DO 0.5 and 0.05)
- 1 experiment at 50 MPa (DO 0.5)
- 2 experiments at 70 MPa (DO 0.5 and 0.05)



At 30 MPa, the ambient yield of the metabolic reaction is retained.
At 50 MPa, the metabolic activity is still on the plateau.
At 70 MPa, it has slightly decreased, slowed down, but the strain is still metabolically active…

Fig. 2: series of Fe XANES spectra at 70 MPa and 30°C, showing the reduction of Fe(III) into Fe(II) by S. oneidensis MR-1

The first part of the experiment was very successful. We now need 12 shifts (4 pressures & 2 cells concentrations) to continue the experiment at higher pressure, until complete disappearance of the metabolic reduction of Fe (III) into Fe(II) by S. *oneidensis* MR-1.