ESRF	**Experiment title:** How deep does a subsurface bacterium respire Fe ?	**Experiment number**: EC-668
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Report:

We have previously developed a protocol for the *in situ* monitoring of microbial metal redox transformations under pressure using X-ray absorption spectroscopy[1]. We have successfully used this protocol for the study of pressure effects on dissimilatory iron reduction (DIR) by the piezosensitive bacterium *Shewanella oneidensis*[2]. Following those previous successful experiments, the goal of experiment EC-668 was to investigate the effects of high pressure on DIR by a piezophilic (i.e. adapted to pressure) bacterium related to *Shewanella oneidensis* MR-1: the deep-sea piezophilic bacterium *Shewanella profunda* LT13a[3]. During the 6 days of experiment, kinetics of DIR by strain LT13a were measured at 0.1, twice at 10, 50, 60 and 80 MPa (30°C) using 3 mM Fe(III) and a cell concentration of ca. 10^9 cells/ml.

Choice of the bacterial strain :

Shewanella profunda LT13a was selected because it has the same pressure range for growth than strain MR-1 (0-50 MPa) but an optimal pressure for growth shifted at 10 MPa (vs 0.1 MPa for strain MR-1)[3]. Both strains have the same temperature optimum of 30°C. Indeed, one fundamental question is to know if the pressure limit for metabolic activity is dependent on the pressure optimum for growth. We used the M1 culture medium composed of a mineral solution, 100 mM HEPES buffer, 0.2 g/l yeast extract, 2 g/l peptone and ferric citrate at 3 mM (terminal electron acceptor). Strain LT13a was grown in rich medium, harvested in stationary phase and used to prepare cell suspensions at ca. 10^9 cells/ml in the M1 medium.

Conditions of measurements :

Acquisition of XANES spectra at the Fe K-edge was performed similarly to previous experiments (experimental reports 30-02-869 and 30-02-945). The spectra were acquired between 7.06 and 7.50 keV in 147 points of 1s each, hence minimizing the irradiation of the bacteria[4]. A culture irradiated during the acquisition of 20 XANES spectra shows a survival rate of 83.5 % at ambient pressure when the non-irradiated control displays a survival rate of 91.8 %.

Pressure range explored during the experiment :

The pressure and temperature conditions were obtained by using the same autoclave as before.[1,2,5] We used 1.5 mm-Be windows in the autoclave. This setup limited the pressure to 80 MPa. But it is necessary since the thicker 4.5-mm Be windows allowing higher pressure prevent the measurement of 3 mM Fe because of the 0.08% Fe(0) impurities they contain. There is an ongoing experimental development to design really pure windows for the autoclave that would allow measurements with very low iron concentrations.

Temperature control in the autoclave :

We observed at ambient pressure in the autoclave slower iron reduction rate than in the control experiments performed in the laboratory. This indicated *a posteriori* a possible discrepancy between the regulated temperature (30°C) and the actual temperature in the autoclave (24-25°C). The precision of temperature regulation is critical for microbial cultures since they respond very severely to small changes of temperature. In the next experiment, we will give special attention to this problem.

Data analysis :

The XANES spectra were processed using the interactive graphical utility ATHENA (Ravel B. & Newville M., 2005, J. Synchrotron Rad., 12:4, 537-541) and its linear combination module. Experimental spectra could always be fitted by a combination of soluble Fe^{2+} and Fe^{3+} standard spectra (measured during the experimental run as well).

Results :

They are summarized in table 1, and displayed in Figure 1. The ability of LT13a to reduce Fe^{3+} decreases linearly with pressure and is estimated (linear extrapolation) to stop at ca. 180 MPa. The decrease in Fe(III) reduction extent is correlated with a decrease in survival. The first part of the experiment was successful despite some problems with the regulation of temperature and the limitation of pressure.

Table 1 : Series of run carried out, and preliminary results

P (MPa)	Time of incubation (h)	[Fe] (mM)	Reduced $[Fe^{3+}]_i$ (mM)	Survival of culture (%)
0.1	16	3	1 (underestimated)	47
0.1	3	3	n.d. (Irradiation test)	83.5
10	15.5	3	2.4	70.2
10	18	3	2.4	68.2
50	16.25	3	0.7 (underestimated)	21.2
60	21.75	3	1.6	0.8
80	8.5	3	1.4	0.06

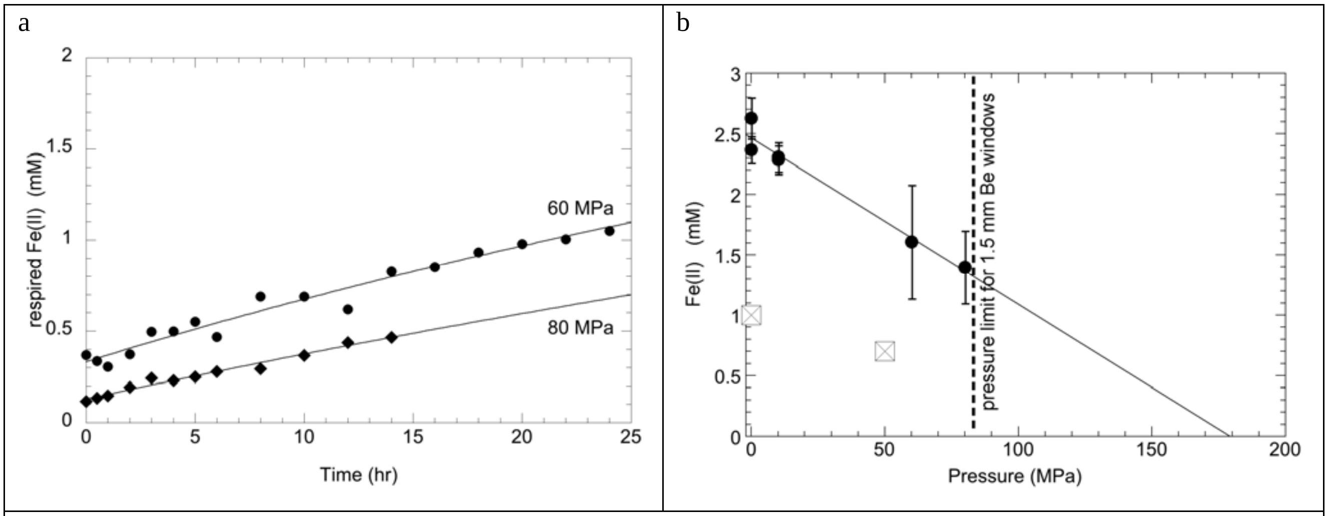

Fig. 1: Dissimilatory iron reduction by *Shewanella profunda* LT13a. (a) Kinetics of Fe(III) reduced as a function of time at 60 MPa and 80 MPa, as determined by linear combination of experimental XANES spectra (b) Final amount of Fe(III) reduced as a function of pressure. Crossed squares represent experiments during which we encountered problems regulating the temperature. Black squares at atmospheric pressure represent ex situ experiments with Fe2+ measured spectrophotometrically.

Perspectives:

- go to higher pressures to determine pressure limit in DIR by *Shewanella Profunda*.
- improve the reliability of temperature regulation.
- **compare the results obtained on the piezosensitive *Shewanella oneidensis* MR-1 and the piezophilic *Shewanella Profunda* LT13a to derive a more general model.**

References:

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