	Experiment title: How deep can the model bacteria *Shewanella oneidensis* respire Fe ?	**Experiment number**: 30-02/945
Beamline: BM 30b	**Date of experiment**: from: may 6th to: may 12th 2009	**Date of report**:
Shifts: 18	**Local contact(s)**: Denis Testemale	*Received at ESRF:*

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Report:

This experiment was the continuation of the experiment 30-02-869 performed at BM30b in april 2008. During experiment 30-02-869 we have measured the dissimilatory reduction of 5 mM iron by the bacteria *Shewanella oneidensis* MR-1 to 70 MPa. We stopped at that pressure since we were using thin 1.5 mm Be windows. The goal of the present experiment was to investigate the metabolic activity of MR-1 at higher hydrostatic pressure until it stops.

Culture medium :

We used the workable minimum culture medium defined during exp. 30-02-869 : LB/10 + HEPES 100 mM + ferric citrate 5 mM. The electron acceptor is included in the LB medium. This medium abiotically reduced Fe(III) into Fe(II), at an acceptable level that is quantified during each run.

Conditions of acquisition :

The spectra were taken between 7.06 and 7.50 keV in 147 points of 1s each, hence minimizing the irradiation of the bacteria (Oger et al., 2008). This leads to an survival rate of 105% at ambient pressure (still growing…), when the non-irradiated control display a survival rate of 150%.

Given the good I/σ ratio obtained with 1.5 mm Be windows during exp. 30-02-869, the goal of the present experiment was to expand the pressure range of investigation by using thicker 4.5 mm Be windows. Altough theoretically possible, 5 mM Fe cannot actually be measured through the 4.5 mm Be windows of the

autoclave. The signal is too low, compared to the background induced by the 0.08% Fe impurities in the Be windows. Consequently, the autoclave was switched again to the configuration with 1.5 mm Be windows limited in pressure. Nevertheless, a number of kinetics could be performed at high pressure.

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). The amount of Fe^{3+} converted as a function of time could alwyas be fitted by a first-order kinetic law. The constant of the reaction is always comprised between 0.003 and 0.10 min^{-1}.

Results :

They are summarized in table 1, and displayed in Figure 1 together with those acquired during the previous experiment 30-02-869. The ability of MR1 to reduce Fe^{3+} decreases lineraly with pressure and becomes zero at ca. 100 MPa. This is much lower that the maximum of pressure of 155 MPa observed in our previous study using Se as metabolite for MR1, but at a much higher cell density.

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

P (MPa)	T (°C)	O.D.	$[Fe]_i$ mM	$[Fe^{2+}]_i$ mM	Amount of Fe^{3+} reduced by MR1
0.1	30	0.5	5	3.0	100%, 2 mM
5	32	0.5	5	3.5 mM	100%, 1.5 mM
90	31	0.5	5	0	0%, opened cell when unloading
40	30	0.5	5	1.0	100%, 4 mM
70	30	0.5	5	0	100%, 5 mM, could reduce 8 mM
80	30	0.5	5	2.5	100%, 2.5 mM
87	30	0.5	5	0.7	1.2 mM
92	30	0.5	5	1.8	2.0 mM
100	30	0.5	5	4.0	1.2 mM
55	30	0.5	10	2.0	6.9 mM

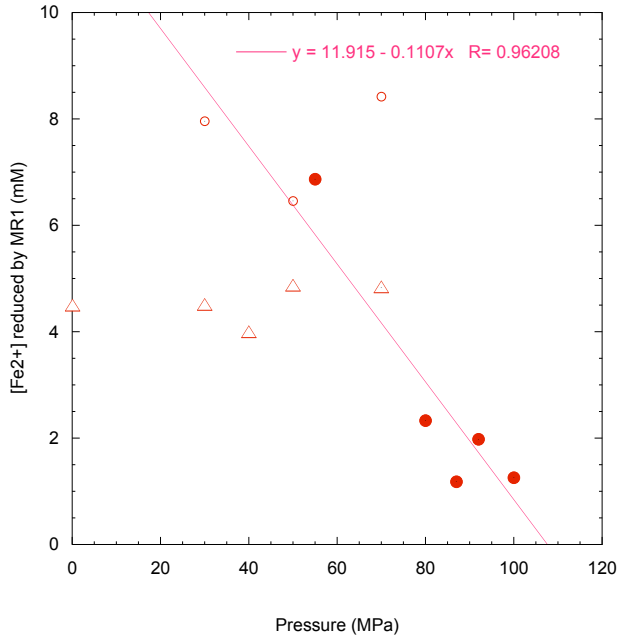

Fig. 1 : Yield of the DMR of Fe^{3+} into Fe^{2+} by MR1. Triangles corresponds to minimum amounts Fe that could be converted. The strain could have used more if available. The empty circles correpond to the maximum it may have used, as obtained from the first order kinetic fit to the data. The filled circles correpond to the exact amount converted, with Fe3+ left in excess at the end of the reaction.