Evolution of microbial populations under the influence of increasing temperature in the Callovian-Oxfordian clay-rich rock

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Introduction

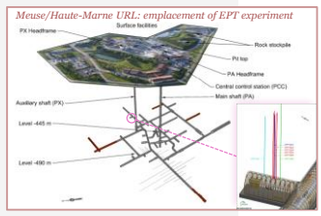
Context: Callovian-Oxfordian (COx) clay-rich rock

An experiment aiming at characterizing the evolution of the COx pore water composition from ambient temperature to 80 °C is running since 2012 in Andra's URL.

In situ experiment provides access to information on:

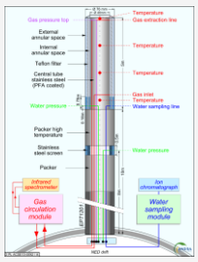
- bacterial populations likely to grow under such conditions
- potential influence of their metabolisms on water composition

This poster presents the main results of the microbiological monitoring carried out since the beginning of the EPT experiment.



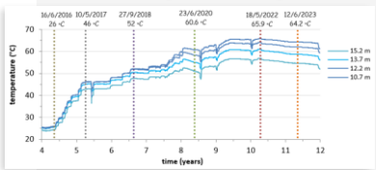
Experimental principle

- ✓ Gas circulation and water sampling in a 5-m long test interval open to the rock →
- ✓ Drilling: 0.2 µm filtered argon & tools cleaned (chlorinated water & alcohol)
- ✓ Borehole and surface equipment were disinfected
- ✓ Progressive T increase by means of 4 heating boreholes (more details in [1])

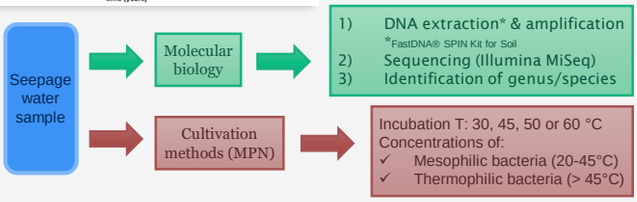


Microbiological analyses

- ✓ 3 rock samples tested for the presence of viable bacteria
- ✓ Regular sampling of seepage water into sterile Hungate tubes or nitrogen-inerted glass bottles



← *Temperatures measured at different depth in the test interval and indication of microbiological water sampling since the start of the temperature increase.*



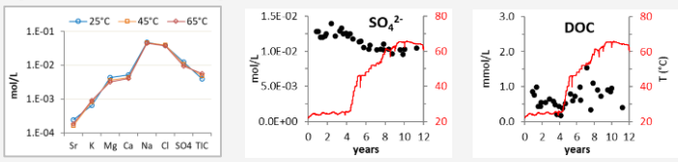
Results

1) Core samples

no viable bacteria were found

- ✓ Previous studies [2, 3] have shown that microbial activity is very limited within such clay rocks, due to space and water restrictions
- ✓ This apparent sterility validates the precautions taken during drilling & installation of equipments

2) Pore water composition



In the test interval: favourable conditions for bacterial growth (water + space + OM)

3) Bacterial populations in the seepage water

An interpretation is proposed by combining (i) the results obtained in MPN, (ii) detailed analysis of the important molecular biology results obtained using the MiSeq technique and (iii) the metabolic pathways obtained using the cultivable species identified.

A drastic evolution of the biodiversity towards an adaptation to high temperature

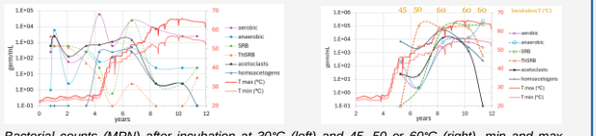
A clear break in biodiversity is observed between 50 and 60°C:

- ➢ disappearance of many species that are only mesophilic or thermotolerant
- ➢ in favour of true thermophilic species
  - ❖ A sharp increase in *Thermoanaerobacterales*, an order representative of many thermophilic species, is observed at 45°C and above
  - ❖ Sulfurogen type active metabolism remain active such as *Desulfofundulus thermocisternus* where sulfate and thiosulfate were used as electron acceptors and 62 °C as the optimum conditions for growth on lactate and sulfate [4, 5]. Moreover, in the absence of an electron acceptor, the organism grew syntrophically on propionate together with a hydrogenothrophic methanogen [4]

The vast majority of identified thermophilic species are representative of non-anthropic (deep or oligotrophic) environments

Cultivation methods confirm that:

- ✓ Thermophilic bacteria become >> mesophilic bacteria
- ✓ Sulfurogen type bacteria (SRB, ThRB) remain notably present



Bacterial counts (MPN) after incubation at 30°C (left) and 45, 50 or 60°C (right), min and max temperature measured at top and bottom of the test interval.

Conclusion and perspectives

- ➢ At temperatures intentionally maintained above 50 to 60 °C, a drastic evolution of the biodiversity towards a rapid adaptation to high temperature is observed: the rise in temperature is coupled with the transient progress of many resistant species and with the regression of many mesophilic species.
- ➢ What is planned: continuing rise in temperatures around the test interval (currently at 66 °C) until it reaches 80 °C
- ➢ A subsequent phase of the experiment could study the influence of a decrease in temperature on porewater composition and bacterial populations.

As most of the species of fully characterized bacteria issued from non-thermophilic environments are known to be unable to grow when temperature is upper than 80°C, it would then be interesting to study the potential adaptation of the bacterial populations to a subsequent drop in temperature, as expected in a geological repository.

References

[1] M. Lundy, B. Garitte, Y. Lettry and A. Vinsot, "Experimental design for in situ characterization of the Callovo-Oxfordian pore water composition at 85°C," Procedia Earth and Planetary Science 7, 533-536, 2013.
[2] S. Poulain *et al*, "Microbial investigations in Opalinus Clay, an argillaceous formation under evaluation as a potential host rock for a radioactive waste repository," Geomicrobiol J, 25:5, 240-249, 2008, doi: 10.1080/01490450802153314.
[3] B. Mayeux, "Ecologie microbienne et métabolisme associé : étude de l'eau interstitielle et de la roche argileuse du Callovo-Oxfordien dans le Laboratoire de Recherche Souterrain de l'Andra (Meuse/Haute-Marne)," PhD thesis, Aix-Marseille Université, 2012.
[4] R. K. Nilsen, T. Torsvik and T. Lien, "*Desulfotomaculum thermocisternum* sp. nov., a sulfate reducer isolated from a hot North Sea oil reservoir," *Int. J. Syst. Bacteriol.*, 46, 397-402, 1996, doi: 10.1099/00207713-46-2-397.
[5] M. Watanabe, H. Kojima, M. Fukui, "Review of *Desulfotomaculum* species and proposal of the genera *Desulfallas* gen. nov., *Desulfofundulus* gen. nov., *Desulfofarcimen* gen. nov. and *Desulfohalotomaculum* gen. nov," Int J Syst Evol Microbiol, 68:2891-2899, 2018, doi: 10.1099/ijsem.0.002915.

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