Microbial community structure and dynamics during radioactive waste disposal

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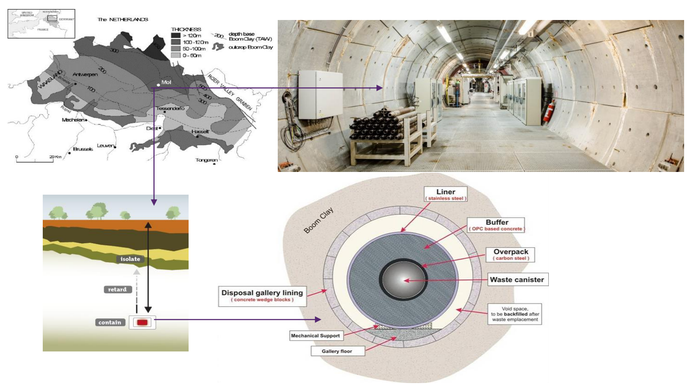
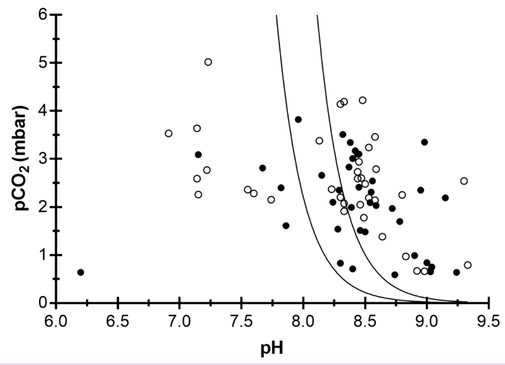
Introduction

Geological Disposal of radioactive waste



Detailed understanding of the pore water composition is essential, as its composition determines among others, the speciation and solubility of radionuclides. The real mechanisms controlling pCO_2 and pH, the two most important parameters, are not completely understood.

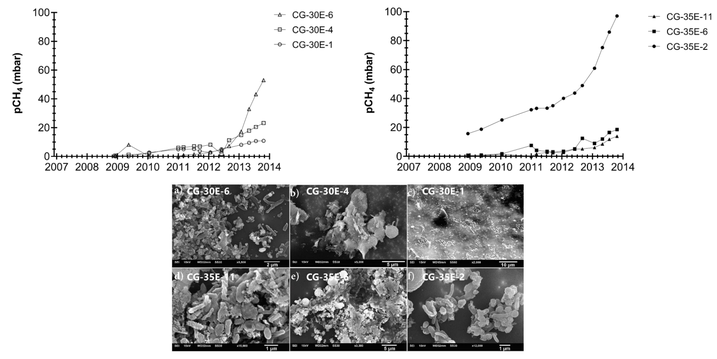
Experimental pCO_2 and pH data do not fit the model



Solid lines delineate lower and upper boundaries of pH-pCO_2 values calculated from measured Ca^{2+} and HCO_3^- values as input parameters for inverse modelling using the code Geochemist's workbench and the BRGM database. Circles are experimental pH – pCO_2 values, which are mostly outside the model.

Mijnendonckx *et al.* 2019, *Applied Geochemistry*

Importance of microbial communities in pore water variations

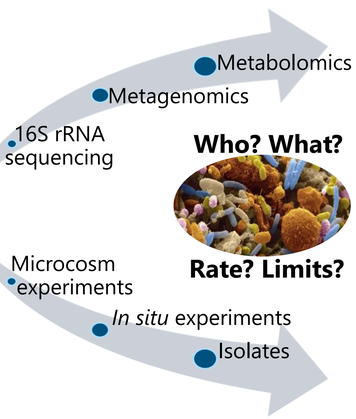


Methane was measured in all samples, showing that a complex methanogenic community is present in the Boom Clay pore water obtained via piezometers. This community affects the geochemistry of the porewater. However, the precise ongoing microbial processes are not known.

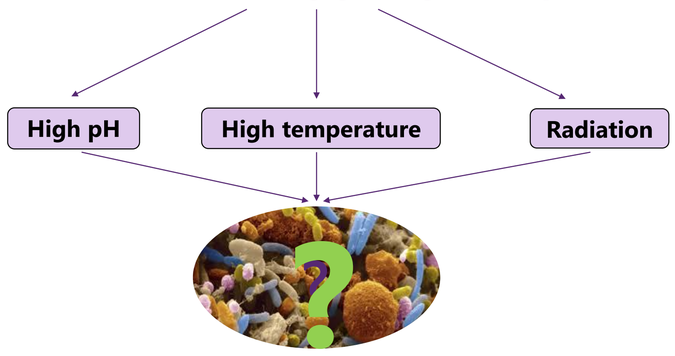
Mijnendonckx *et al.* 2019, *Applied Geochemistry*

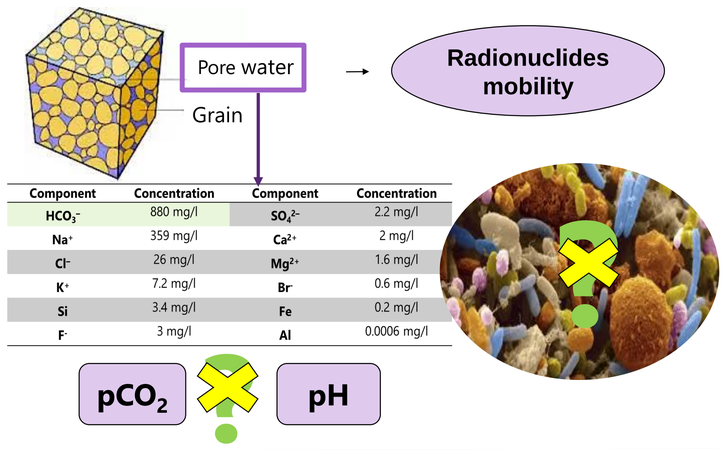
Objectives

De Craen et al., 2019.



Conditions during geological disposal





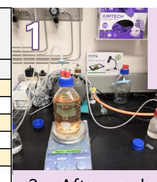
Results

1. Isolation of methanogenic archaea

Methanomassiliicoccus luminyensis was selected as control strain and also different samples from HADES were selected to grow in SAB medium, a versatile methanogenic medium.

SAB medium composition

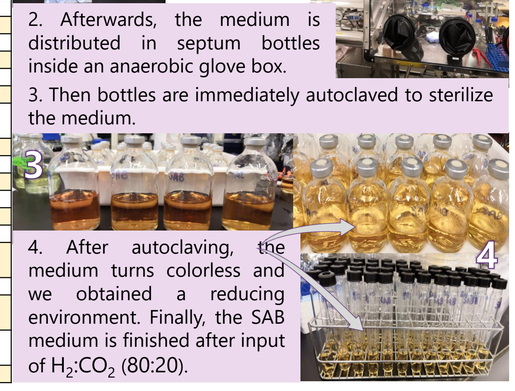
Anoxic conditions and Eh= -240 mV	
Compound	Quantity
$NiCl_2$ x 6 H_2O	1.5 mg
$FeSO_4$ x 7 H_2O	0.5 mg
$MgSO_4$ x 7 H_2O	0.8 mg
K_2HPO_4	0.5 mg
KCl	0.05 mg
$CaCl_2$	0.05 mg
NaCl	1.5 mg
NH_4Cl	1 g
Na-Acetate	1 g
Yeast extract	5 g
Trypcase peptone	5 g
Selenite/tungstate solution	5 ml
Resazurin solution	1 ml
Na_2S (2%)	0.1 ml
L-Cysteine	0.1 ml
$NaHCO_3$ (10%)	0.1 ml
Na-Formate 4M	0.1 ml
Methanol 4M	0.1 ml
Volatile fatty acid solution	0.1 ml
Vitamin solution	0.1 ml
Trace element solution Widdel	1 ml
Trace element solution Balch	5 ml
Distilled water	1,000 ml



1. After dissolving all the compounds, a gas mixture Ar:CO_2 (80:20) is added to make the medium anoxic.

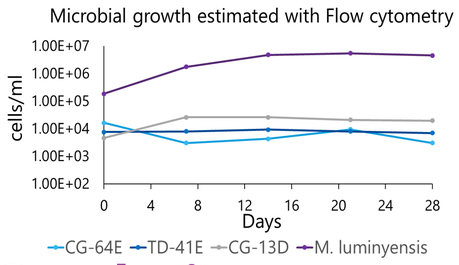
2. Afterwards, the medium is distributed in septum bottles inside an anaerobic glove box.

3. Then bottles are immediately autoclaved to sterilize the medium.



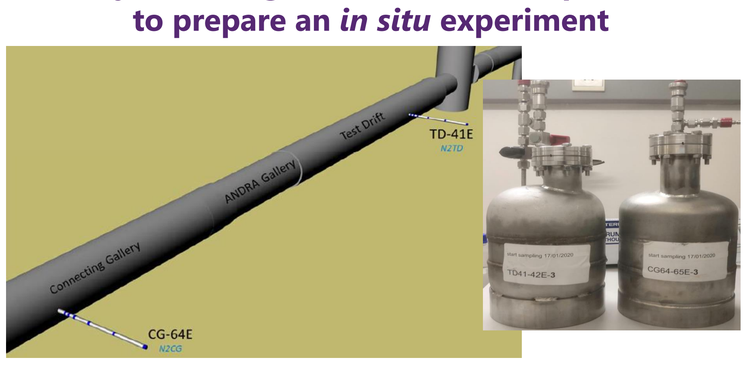
4. After autoclaving, the medium turns colorless and we obtained a reducing environment. Finally, the SAB medium is finished after input of H_2:CO_2 (80:20).

Microbial growth estimated with Flow cytometry



Gas Chromatography showed methane in all samples

2. Analysis of long-term undisturbed piezometer to prepare an *in situ* experiment



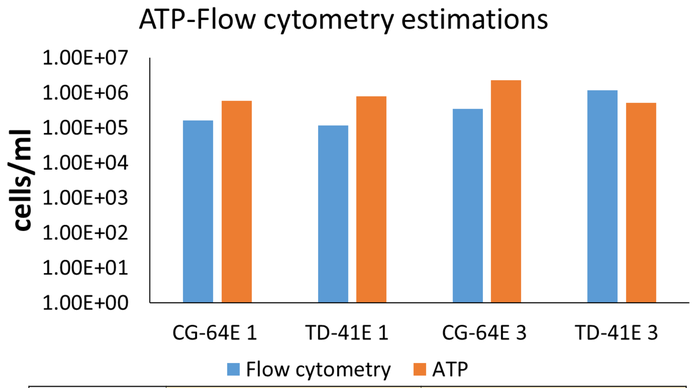
De Craen et al., 2019.

Piezometers TD-41E and CG-64E, which are undisturbed for >10 years are sampled to investigate the presence of methane. In addition, the microbial community will be studied in detail.

Two sampling campaigns were already finished:

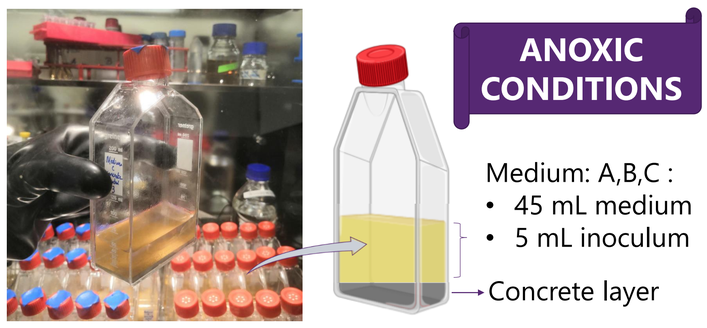
1. Sampling of filter CG-64E1 and TD-41E1 started in Mid-January 2020 and finished end June 2020.
2. Sampling of filter CG-64E3 and filter TD-41E3 started end June 2020 and finished end October 2020

ATP-Flow cytometry estimations



	18.06.2020 filter 1			23.10.2020 filter 3		
Sample	**pH**	**Eh (mV)**	**CH_4**	**pH**	**Eh (mV)**	**CH_4**
CG-64E	8.86	-153	✓	8.928	5.7	✓
TD-41E	8.64	-181	✓	8.717	104.9	✓

3. Conditions during geological disposal: high pH



Microcosm experiment was started to investigate microbial presence & activity in a cementitious environment. Different microbial metabolisms are studied.

- Boom Clay water filtrated and autoclaved used as medium
- Medium A: Sodium Sulphate 10 mM + Calcium lactate 10 mM
- Medium B: Sodium Nitrate 100 mM + Sodium acetate 15 mM
- Medium C: Boom Clay water
- 5 ml of Boom Clay water added as inoculum
- Sterile control samples were included by adding 5 ml sterilized Boom Clay water
- All conditions are performed in triplicate

We will monitor:

- pH & E_h
- Number of cells (Flow Cytometry & ATP)
- Ion Chromatography & spectroscopy
  - -Nitrate (NO_3^-)
  - -Nitrite (NO_2^-)
  - -Sulphate (SO_4^{2-})
  - -Lactate ($C_3H_6O_3$)
  - -Acetate ($C_2H_3O_2^-$)

Conclusions

Growth of a control strain in a versatile methanogenic medium was successful. This medium will be used to further enrich and isolate methanogenic archaea from HADES samples. Methane could be detected in long-term undisturbed piezometers. Finally, microcosm experiments are running to investigate microbial activity in high pH.

References

- De Craen, M., Moors, H. & Verstricht, J.2019. Description of the HADES piezometers used for the study of *in situ* Boom Clay pore water chemistry. SCK CEN/12861301
- Mijnendonckx, K., et al., "An active microbial community in Boom Clay pore water collected from piezometers impedes validating predictive modelling of ongoing geochemical processes," Appl. Geochemistry, 106,2019, doi: 10.1016/j.apgeochem.2019.05.009.

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