MIND Project Annual Meeting 3
Short Abstracts

Lausanne, May 7–9, 2018

PREFACE

The idea of the MIND (Microbiology In Nuclear waste Disposal) project was born at the IGD-TP EF4 in Prague 2013, where the opportunity was provided to gather a working group for microbiology. Representatives from Waste Management Organisations (WMOs), academic institutions, research institutes, consulting companies and one regulator (FANC) attended. The participants came from 8 European countries. Although there are differences between the different repository concepts, there are many unresolved issues in common between the different WMOs. The MIND working group identified a number of specific microbial processes and effects that are of significance to "high urgency" and "high importance" topics highlighted in the most recent IGD-TP Strategic Research Agenda, namely; processes in waste forms, and the technical feasibility and long-term performance of repository components.

It was therefore decided to write a proposal to Horizon2020 focussing on the above mentioned key issues. The project MIND officially started June 1, 2015 and will end May 31, 2019.

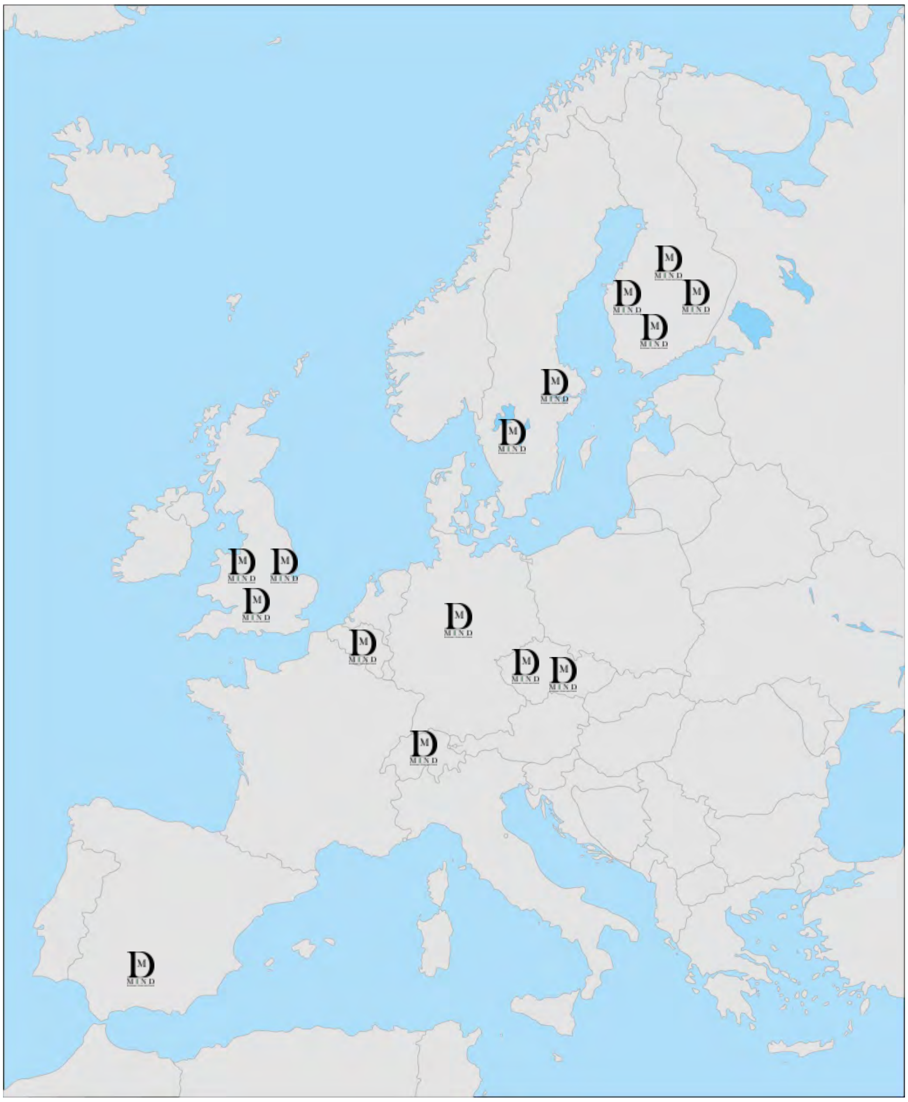

Figure 1: Fifteen organisations from eight countries.

Fifteen European groups are currently working on the impact of microbial processes on the safety cases for geological repositories across Europe, focusing on key questions posed by waste management organisations (WMOs). The emphasis is to quantify specific measureable impacts of microbial activity on safety cases under repository-relevant conditions, thus altering the current view of microbes in repositories and leading to significant refinements of safety case models currently being implemented to evaluate the long-term evolution of radioactive waste repositories. Representatives from academic institutions, research institutes, consulting companies, national laboratories and eight WMOs contributed to formulate the application. In order to make sure that the objectives of the project are followed and make sure that the project focusses on the issues that were addressed in the application a review group (Implementers' review board) has been put together. The following end users have accepted to be part of the review process of the project: SKB, Posiva, TVO, Andra NWMO, Nagra, RWM, ONDRAF/NIRAS, NUMO, LANL, SURAO, CNSC and IRSN. Johan Andersson (SKB) is the chairman of this group. The first IRB meeting was held in Prague during the second Project Annual Meeting (PAM2) where a draft of a Gap-list, which identifies microbiological-related research topics relevant to radioactive waste disposal, was created. The list has then been circulated among the WMOs in order to add and rate issues. The list has then been forwarded to the WP-leaders and been compared with what was said in the Grant Agreement that the MIND project should do.

The third Project Annual Meeting (PAM3) took place May 7-9, 2018 and was hosted by the EPFL. The meeting started at noon with a brief introductory talk by the coordinators who welcomed all to the meeting. This was followed by a much appreciated plenary talk by Satoru Suzuki from NUMO that initiated the pre-workshop. The GAP-workshop was organized by Johan Andersson focussing on the high-priority issues (priority 1-3). List follow below:

- 1a. Organic waste, degradation, oxidation; (radiolysis: RWM-NDA)
- 1b. Isosaccharinic acid (ISA) degradation (oxidation): SKB
- 1d. Ion exchange resins (styrene) Radiolysis/hydrolysis: Nagra
- 1e. Dissolved or solid OM present in bentonite. Degradation, i.e. acetogenesis: Nagra
- 1f. Gas. Pressure build up and chemical reaction. C-14 speciation: Nagra, RWM-NDA
- 1g. Oxyanions (i.e. nitrate, sulphate, carbonate) and organic substances. A variety of chemical reactions affecting redox potentials and associated overall geochemistry (sorption, speciation): Andra, ONDRAF/NIRAS.
- 1h. Radionuclides. Speciation and mobility: Andra, RWM-NDA
- 1j. Steel / copper (canister). Sulphate reduction and other redox reactions: Nagra, NWMO
- 1k. Bentonite. Redox reactions and clay stability: Posiva and SKB
- 2a. Ionic strength. Metabolic activities in salt solutions (salt from host rock or waste component): Andra
- 2b. Water/space availability. Metabolic activity in low porosity environments (i.e. bentonite, host rock): Posiva
- 2c. pH. Only in the presence of high energy electron acceptors (O_2, NO_3^-) is activity beyond pH 10 possible; the general upper limited ~ pH 12: SKB, ONDRAF/NIRAS
- 2g. Alteration. Very localised conditions could 'sustain' microbial life even though the general conditions do not. This microbial activity could cause alteration of the conditions which may spread: SKB
- 2j. Saturation. Some repository scenarios may have long unsaturated phases. NWMO

The workshop ended with a poster session that was initiated with 2 minutes presentations by all authors. Sixteen posters from TUL, CVREZ, EPFL, UGR, MICANS, SCK•CEN, HZDR, VTT, CNSC and CRIEPI.

The morning session of the second day started with an introduction by the WP1-leader Joe Small. WP1 handles questions regarding *Improving the safety case knowledge of organic long-lived*

intermediate level waste (ILW). Eight presentations were given by, UNIMAN, SCK•CEN, TUL, NNL, EPFL, VTT, HZDR and UGR.

In the afternoon, the WP2-leader Karsten Pedersen welcomed all to the WP2 session. WP2 concerns *Improving the safety case knowledge base about the influence of microbial processes on high level waste (HLW) and spent fuel geological disposal.* Eight presentations were given by GTK, VTT, CVREZ, EPFL, NERC, HZDR, UNIMAN, Micans and UGR.

Valuable input was given by the end users both in the discussion during the meeting and during the summary of the whole meeting the last day. In addition, the interest in the MIND project shown by the regulators IRSN and CNSC and by colleagues from outside the EU such as the US (LANL), Canada (NWMO and CNSC) and Japan (NUMO and CRIEPI) were very much appreciated.

The last day started with an introduction by the WP3-leader Natalie Leys. WP3 focusses on *integration, communication and dissemination of results*. There were six presentations in total. Natalie Leys (SCK•CEN) gave an introduction on dissemination activities and the advanced MIND course that will be given at SCK•CEN October 8-11 this year. The PAM ended by a summary of the progress of the MIND project so far given by the Implementers Review Board.

The PAM was closed by the project coordinator. A special thanks goes to Rizlan Bernier-Latmani and Maria Fernandes Coelho at EPFL for their excellent organization the meeting in Lausanne.

For more information about the project please visit www.mind15.eu

The coordination team would like to thank all participants for their contribution to our third annual meeting! See you all in Stockholm, May 2019.

The abstracts of this volume have not been peer viewed and should be regarded as minutes from the meeting.

Birgitta Kalinowski (SKB) project coordinator

Petra Christensen (SKB) administrative coordinator

Joe Small (NNL), WP1-leader

Karsten Pedersen (MICANS), WP2-leader

Natalie Leys/ Kristel Mijnendonckx (SCK•CEN), WP3-leader

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Information and Final Agenda

The MIND project would hereby like to welcome you and your colleagues to the third **MIND Project Annual Meeting (PAM), Implementers Review Board (IRB)** and **Project Executive Committee Meeting (PEC)** which are scheduled to take place in **Lausanne on the 7th to 9th of May** with a planned city tour.

The complete MIND 2018 PAM will be hosted by the University of Lausanne thanks to Rizlan Bernier-Latmani (EPFL) and Maria Fernandes Coelho (EPFL).

The meeting venue for all meetings:
The Aquatis Hotel
Route de Berne 148
1010 Lausanne
Vaud – Suisse
+41 (0)21 654 24 24

How to reach the venue:
By train: from Lausanne train station, take the underpass and take the metro M2 (direction Croisettes) - get off at "Vennes". The journey takes about 10 minutes.

Hotel Bookings
Each participant is responsible for managing their own travel arrangements and hotel bookings. We recommend that you book your hotel room as soon as possible as the beginning of May is very busy in Lausanne.

Reservation code: **EPFL MIND18** (in order to benefit from EPFL special rates, participants need to book one month prior to the meeting. We have requested pre-reservations that will be cancelled without fees one month before the meeting).
Recommended hotels located close to the meeting venue include:

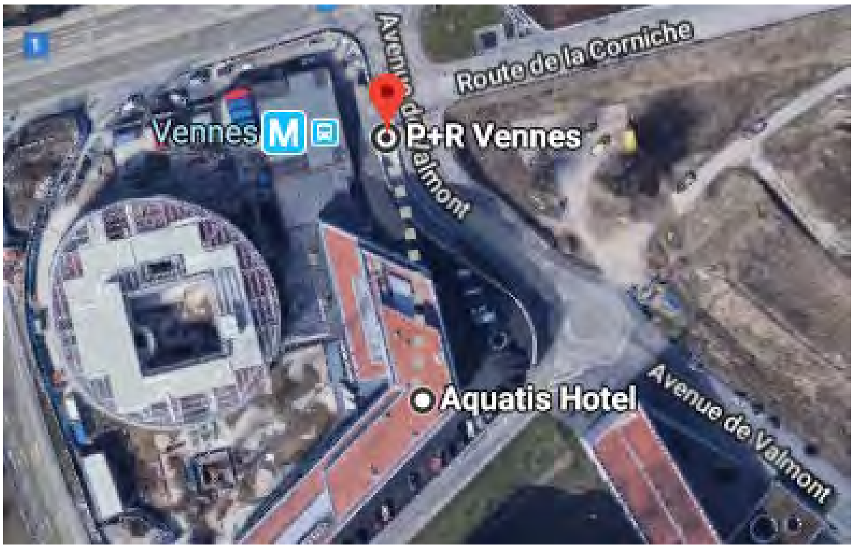
1. Hotel Regina***
   Rue Grand-St-Jean 18, CH - 1003 Lausanne (+41) 21 320 24 41.
   Double room, single use: CHF 130
   (walk 5 min. + Public transportation M2 - 16 min.) Double room, 2 people: CHF 170 Breakfast, Wifi included, city tax extra CHF 3.10/night and person. Travel card bus/metro provided for free.
2. Hotel Cristal***
   Rue Chaucrau 5, 1003 Lausanne (+41) 21 317 03 03.
   Single room: CHF 134 (walk 4 min. + Public transportation M2 - 13 min.) Double: CHF 184
   Breakfast and Wifi included, city tax extra CHF 3.10/night and person

The MIND project brings together 15 European groups working on the impact of microbial processes on safety cases for geological repositories, focusing on key questions posed by waste management organisations throughout Europe.
This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No. 661880.

3. The Aquatis Hotel (the same as the meeting venue)
   Route de Berne 148, 1010 Lausanne (+41) 21 654 24 24.
   Standard single room at CHF 160.- per room and per night breakfast included. City tax extra CHF 3.10/night and person
4. Au Lac*** (very nice, good value if you like to stay by the water)
   Place de la Navigation 4, 1006 Lausanne (+41) 21 613 15 00
   Single room: CHF 140. Double room: CHF 190. Breakfast not included, public transport card and WIFI included. Taxe extra CHF 3.10/day and person

Travel

- Geneva Airport is the nearest airport (45 minutes by train. There is a train station located in the airport building. Turn left when you come out of baggage claim. Continue down the hallway following the sign for 'CFF' and buy a train ticket to Lausanne at the machines. Price from 28CHF).
- Zurich airport (2h30min.) with very good connections to Lausanne. Zurich airport is located 10 min. away from Zurich main station. Train ticket Zurich-Lausanne (from 79CHF).
- Train CFF, Metro 2 is traveling every 6 or 7 minutes (prices from 3.70CHF). Most hotels offer a complimentary transportation pass for Lausanne. Aquatis hotel is located next to Vennes metro stop (1 minute walk).



1. MIND-Project Registration and Pre-meeting workshop

The MIND Project Annual Meeting will be hosted from noon on May 7th when we start with registration and workshop. The focus of the workshop will be on the achievements in accordance with the GAP-list that has been compiled by the Implementers Review Board. The meeting will end at lunch-time on May 9th.

12:00- Registration
12:30-12.40 Welcome to PAM (Birgitta Kalinowski, Petra Christensen, SKB)
12:40-13:00 R&D plan for microbiologically induced corrosion in Japan and expectations to MIND (Satoru Suzuki, NUMO)

13:00-16.30 GAP- workshop (Johan Andersson, SKB and Achim Albrecht, Andra)
14.30-15.00 Coffee

Assessment of the high-priority (priority 1-3) issues;
i) Short introduction by designated IRB member(s) (see below) and if this is an area where the agencies invest now or will in the future.
ii) Feedback from MIND WP leaders – is the area handled in MIND,
iii) Short assessment (including possibilities of resolution within MIND). The following issues are to be discussed:

- 1a. Organic waste, degradation, oxidation; (radiolysis: RWM-NDA,
- 1b. Isosaccharinic acid (ISA) degradation (oxidation): SKB
- 1d. Ion exchange resins (styrene) Radiolysis/hydrolysis: Nagra
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- 1g. Oxyanions (i.e. nitrate, sulphate, carbonate) and organic substances. A variety of chemical reactions affecting redox potentials and associated overall geochemistry (sorption, speciation): Andra, ONDRAF/NIRAS.
- 1h. Radionuclides. Speciation and mobility: Andra, RWM-NDA
- 1j. Steel / copper (canister). Sulphate reduction and other redox reactions: Nagra, NWMO
- 1k. Bentonite. Redox reactions and clay stability: Posiva and SKB
- 2a. Ionic strength. Metabolic activities in salt solutions (salt from host rock or waste component): Andra
- 2b. Water/space availability. Metabolic activity in low porosity environments (i.e. bentonite, host rock): Posiva
- 2c. pH. Only in the presence of high energy el- acceptors (O2, NO3-) is activity beyond pH 10 possible; the general upper limited ~ pH 12: SKB, ONDRAF/NIRAS
- 2g. Alteration. Very localised conditions could 'sustain' microbial life even though the general conditions do not. This microbial activity could cause alteration of the conditions which may spread: SKB
- 2j. Saturation. Some repository scenarios may have long unsaturated phases. NWMO

Overall assessment – and is anything missing on the GAP-list. What type of young engineers are mostly in demand in the future?

2. Poster session, Icebreaker, IRB and Sequencing meeting

16:30-17:00 Oral Poster presentations (invitation to poster, 2 minutes maximum)
17:00-18:00 IRB meeting / Sequencing meeting (EPFL)
18:00-20.00 Icebreaker / Poster session

1. Aislinn Boylan, EPFL, “Characterisation of organic compounds released from irradiated resins”

2. Minna Vikman et al, VTT, “Gas generation experiment with LLW - Corrosion of metal samples”

3. Gina Kuippers, UNIMAN “Cellulose degradation / complexation” - 2 posters

4. Miguel Angel Ruiz Fresneda, UGR – “Reduction of selenite to amorphous and crystalline selenium nanostructures by *Stenotrophomonas bentonitica*: impact on selenium mobility within the concept of radioactive waste repositories”

5. Liam Abrahamsen and Joe Small, NNL, “Modelling of microbial processes with PHREEQC”

6. Hana Kovarova, CVREZ, “Impact of high pressure and temperature on the microbial activity in BaM bentonite suspension”

7. Nicole Matschiavilli, HZDR, “Bentonite – a natural source of sulphate-reducing bacteria”

8. Andrea Cherkouk presented by Nicole Matchiavelli, HZDR, “Results from the FEBEX samples”

9. Kristel Mijnendonckx, SCK-CEN “Possible impact of microbial processes on cementitious materials used during the geological disposal of radioactive waste”

10. Hanna Miettinen, VTT, “Comparison of three DNA-extraction methods from MX-80 bentonite”

11. Rikka Kietäväinen Lasse Ahonen, GTK, “Gases in Bedrock Groundwater: Geochemical Potential for Sustaining Deep Life”

12. Niels Burzan, EPFL, “In situ hydrogen-fuelled sulfate reduction in an engineered gas consumption system at the Mont Terri URL”

13. Trevor Taborowski and Karsten Pedersen, MICANS, “Microbial activity in a bentonite-concrete interface”

14. Trevor Taborowski, Andreas Bengtsson and Karsten Pedersen, MICANS, “Transport and reactivity of sulphide from bacterial activity in compacted bentonite clays”

15. Richard R. Goulet et al. CNSC, ”Microbial Gas Generation From Low Level Radioactive Waste”

16. Toru Nagaoka, Criepi, ”Microbiological research on nuclear waste disposal in CRIEPI”

3. MIND-Project Annual Meeting

May 8th

08:15-08.20 Welcome

Session I (Work Package 1):

08:20-08.30 Introduction, Joe Small, NNL

08.30-08.50 Microbial degradation of organics and nitrate leaching from bituminized radioactive waste, Kristel Mijnendonckx, SCK-CEN

08.50-09.10 Overview of irradiation and biodegradation studies on cellulose and PVC, Jon Lloyd, UNIMAN

09.10-09.30 The mutual influence of UO_2^{2+} and isosaccharinic acid on their speciation: A spectroscopic study under acidic conditions, Hannes Brinkmann, HZDR

09.30-09.50 Reduction of selenite to amorphous and crystalline selenium nanostructures by stenotrophomonas bentonitica: impact on selenium mobility within the concept of radioactive waste repositories, Miguel Angel Ruiz Fresneda, UGR

09.50-10.10 Coffee

10.10-10.30 The effect of caesium ions on a natural anaerobic microbial community, Alena Sevcu, TUL

10.30-10.50 H_2 fuelled microbial metabolism in Opalinus Clay, Aislinn Boylan, EPFL

10.50-11.10 Large-scale gas generation experiment with low-level radioactive waste, Minna Vikman, VTT

11.10-11.30 WP1 synthesis and GAP cross-reference, Joe Small, NNL

11.30-11.50 Discussion

11.50-12.50 End of session/Lunch

Session II (Work Package 2):

12.50-13.10 Microbial Viability in Clay Barriers: From Lab Study to Field Scale, Haydn Haynes, UNIMAN

13.10-13.30 Microbially induced corrosion of carbon steel in a natural groundwater and a synthetic bentonite pore water, Tomáš Černoušek, RCR

13.30-13.50 Experiments on the impact of microbes on clay swelling and steel corrosion in compacted bentonite flow experiments, Simon Gregory, BGS

13.50-14.10 Bentonite characteristics after one year storage with indigenous bentonite and water microbes, Hanna Miettinen, VTT

14.10-14.30 Bentonite; a natural source of sulphate-reducing bacteria, Nicole Matschiavilli, HZDR

14.30 Coffee

14.50-15.10 Geochemical constraints of sulphide production and concentration, Lasse Ahonen, GTK

15.10-15.30 In situ corrosion of steel embedded in bentonite: enumeration and characterization of the associated microbial community, Niels Burzan, EPFL

15.30-15.50 Microbial activity in bentonite buffers, Karsten Pedersen, MICANS

15.50-16.10 WP2 synthesis and GAP cross-reference, Karsten Pedersen, MICANS

16.10 PEC-meeting

17.00 City tour, sponsored by the EPFL and the SKB and Dinner

May 9th

Session III (Work Package 3):

08:30-08.45 Overview of goals and status, Natalie Leys, SCK-CEN

08.45-09.10 Assessment of DNA extraction protocols for Opalinus Clay rock, Niels Burzan, EPFL

09.10-09.20 Personal experience of the MIND exchange program, Hanna Miettinen, VTT

09.20-09.30 Advanced course in geomicrobiology, Kristel Mijnendonckx, SCK-CEN

09.30-09.45 Coffee

09.45-10.15 The inclusion of microbiological processes and their effects in Performance Assessment, Joan Govaerts, SCK-CEN

10.15-10.30 Update on Social Science Research within MIND, Jantine Schröder, SCK-CEN

10.30-11.00 The EU Joint Programming on Radioactive Waste Management, and microbiology

11.00-11.30 Comments & recommendations of the Implementers Review Board (IRB)

12.00 Closing of meeting/Lunch

MIND Project Executive Committee Meeting

The MIND **Project Executive Committee meeting** is held on the afternoon of May 8th (16:10–17:00). This meeting is only open for **PEC** members who will in due time receive a call and agenda for the meeting.

MIND Implementers Review Board Meeting

The MIND **Implementers Review Board meeting** is held on the afternoon of May 7th (17:00–18:00). This meeting is only open for **IRB** members who will in due time receive a call and agenda for the meeting.

MIND Sequencing Meeting

The MIND **Sequencing meeting** is held on the afternoon of May 7th (17:00–18:00). This meeting is intended to discuss the results of phase 1 of the Round-Robin experiment aiming at comparing DNA extraction and analysis tools for clays. The next phase will be focused on DNA extraction from Opalinus Clay rock and will be the second topic of discussion.

PAM Granada 2016

PAM Prague 2017

Attending the meeting

Name	Organization
Achim Albrecht	ANDRA
Aislinn Boylan	EPFL
Alena Ševců	Technical University of Liberec
Benny de Blochouse	ONDRAF/NIRAS
Birgitta Kalinowski	SKB
Charles Wittebroodh	IRSN
Cristina Povedano-Priego	University of Granada
Ekaterina Markelova	Amphos21 Consulting Ltd.
Fadwa Jroundi	University of Granada
Gina Kuippers	University of Manchester
Hannes Brinkmann	Helmholtz-Zentrum Dresden-Rossendorf
Haydn Haynes	University of Manchester
Henry Moll	Helmholtz-Zentrum Dresden-Rossendorf
Irina Gaus	Nagra
Jakub Kokinda	Centrum vyzkumu Rez, s.r.o. (CVREZ)
Jana Steinova	Technical University of Liberec
Jantine Schröder	SCK-CEN
Jennifer McKelvie	NWMO
Joan Govaerts	SCK-CEN
Joe Small	NNL
Johan Andersson	SKB
Jon Lloyd	University of Manchester
Karsten Pedersen	Microbial Analytics Sweden AB
Kirsi Weckman	Teollisuuden Voima oyj (TVO)
Kristel Mijnendonckx	SCK-CEN
Lasse Ahonen	GTK, Geological Survey of Finland
Lorraine Field	British Geological Survey
Matthew Bailey	Radioactive Waste Management (RWM) UK
Miguel Angel Ruiz-Fresneda	University of Granada (UGR)
Minna Wikman	Technical Research Centre of Finland, VTT
Mohamed Larbi Merroun	Universidad de Granada
Natalie Leys	SCK-CEN
Nicole Matschiavelli	HZDR
Niels Burzan	EPFL – EML
Olivier Leupin	Nagra
Patrik Sellin	SKB
Petr Polívka	Centrum vyzkumu Rez, s.r.o. (CVREZ)
Petra Christensen	SKB

Petteri Pitkänen	Posiva Oy
Richard Goulet	Canadian Nuclear Safety Commission
Riikka Kietäväinen	GTK (Geological Survey of Finland)
Rizlan Bernier-Latmani	EPFL
Satoru Suzuki	Nuclear Waste Management Organization of Japan
Simon Gregory	British Geological Survey
Tiina Lamminmäki	Posiva
Tomáš Černoušek	Centrum výzkumu Rez, s.r.o. (CVREZ)
Toru Nagaoka	CRIEPI, Central Research Institute of Electric Power Industry

R&D PLAN FOR MICROBIOLOGICALLY INDUCED CORROSION IN JAPAN AND EXPECTATIONS TO MIND

SATORU SUZUKI

Nuclear Waste Management Organization of Japan, Tokyo, Japan

NUMO is an implementer of geological disposal of high level vitrified waste and a part of low-level waste which contains relatively higher amount of radionuclide generated from the reprocessing of spent fuels and mixed-oxide fuel fabrications in Japan. These radioactive wastes are planned to be disposed into deep underground below the surface level <-300 m with the multiple engineered barriers system (called as EBS hereafter). The EBS consists of the metal container and the bentonite buffer which is designed to make the radionuclide contained and retarded to release to geosphere.

The study of microbes in geological disposal in Japan was initiated by PNC (currently JAEA) in 1990's and they discussed the viability of microbes in intact rocks at deep underground, and microbiologically induced corrosion of carbon steel (MIC, hereafter). At the beginning of 2000, the influence of microbes on radionuclide migration was actively studied, such as scenario and development of quantitative models.

The prevention of MIC of the metal overpack by increasing the density of bentonite buffer is key design requirement of bentonite buffer. Recently, JAEA obtained the results on the microbial activity at the dry density of 1.6 Mg/m^3 mixed with 70 wt.% of bentonite and 30 wt. % of silica sand which is a reference specification of the bentonite buffer. We, thus, need to reconsider the specification of bentonite buffer. The four different materials of Kunigel V1® which is a sodium-type bentonite as a reference material in Japan, two alternative domestic bentonites and MX-80® bentonite will be examined. We plan to determine the critical density of bentonite materials in which MIC can be prevented by combining the three different experiments of microbial viability test, corrosion test and radioactive tracer test. Pros and cons of each experiment will be discussed in the presentation.

The number of researchers of microbes in geological disposal decreases year by year in Japan in the last decade. We therefore desire the discussions and sharing the knowledge and experiences together with members of MIND.

MICROBIAL DEGRADATION OF NITRATE LEACHING FROM BITUMINISED RADIOACTIVE WASTE AT HIGH PH

KRISTEL MIJNENDONCKX*[(1)], ILSE CONINX[(1)], AXEL VAN GOMPEL[(1)], NELE BLEYEN (1), & NATALIE LEYS[(1)]

[(1)] Belgian Nuclear Research Centre (SCK•CEN), Boeretang 200, 2400 Mol, Belgium

In Belgium, an important fraction of the current stored volume of long-lived intermediate level radioactive waste is immobilised in a bituminous matrix as Eurobitum, which contains besides bitumen and radionuclides, large amounts of soluble salts with sodium nitrate as the most dominant. Geological disposal of this waste in a water-saturated sedimentary formation such as Boom Clay will induce water uptake by the hygroscopic salts present in the bituminized waste. The dissolution of these salts will result further osmosis-induced water uptake through the semi-permeable bitumen membrane and thus in swelling of the Eurobitum. Additionally, sodium nitrate will slowly leach from the waste. The nitrate plume in the clay water could cause a geochemical perturbation of the surrounding clay, possibly affecting the redox conditions, causing ionic strength effects and cation exchange processes, which might result in an increase in the mobility of the radionuclides through the host rock. However, it is known that nitrate can also be removed inside the disposal gallery or in the near-field by various processes. Abiotically, the reduction of nitrate can occur with H_2 (produced during radiolysis of bitumen or water and during corrosion of steel) and/or steel acting as electron donors and/or with the steel or even pyrite in the clay possibly serving as catalytic surface. These abiotic reactions would lead to the production of ammonium, which can sorb onto clay minerals and would therefore compete with some radionuclides for sorption. Biotically, nitrate can be consumed as electron acceptor by microorganisms, if proper growth conditions are provided. Respiratory microbial consumption of nitrate leads to the intermediate production of nitrite, and finally to nitrogenous gases. Most of the other leachates from bituminized waste (e.g. acetate, H_2) are biodegradable and can be used as electron donor, to fuel the microbial nitrate reduction. Depending on the electron donor used in this denitrification process, the final overall result could lead be a gas pressure decrease or increase. During disposal conditions, the microbial population will be exposed to hyperalkaline conditions originating from the pore water from the concrete lining of the waste monolith and the backfill material, which could. affect the denitrification potential of the microbial population.

Batch experiments were performed in anoxic Boom Clay pore water to investigate the potential microbial reduction of nitrate leaching from thermally aged inactive bituminized waste at different pH (9 – 10.5 – 12.5). Boom Clay borehole water or the Harpur Hill sediment (natural analogue to a cementitious geological disposal facility) were used as inoculum and sodium acetate was added as electron donor and C source. At pH 9, the microbial community of the Boom Clay borehole water reduced ~3.8 mM nitrate per day during the first 5 days, which is twice as much as the microbial community from the Harpur Hill sediment. Nevertheless, after 110 days, a similar amount of nitrate was reduced by both communities. On the other hand, when the communities were exposed to pH 10.5, the nitrate reduction rates were almost 30 times lower during the first 5 days for the Boom Clay borehole water microbial community, while those of the Harpur Hill sediments were comparable with reduction rates observed at pH 9. In addition, at pH 10.5, the Harpur Hill community reduced almost 2.5 times more nitrate after 110 days compared to the Boom Clay borehole water community and oxidized all 15 mM acetate, while acetate concentrations only dropped by 7 mM when Boom Clay borehole water was used as inoculum. It seems that the Harpur Hill microbial community reduced nitrate to nitrogen gas (or ammonium), while the microbial community of the Boom Clay borehole water produced more nitrite. At pH 12.5, no nitrate or acetate was removed by neither communities. However, in the samples at pH 12.5 the total microbial cell counts of the Harpur Hill community increased in time, while that of the Boom Clay borehole water decreased. Based on flow cytometry community profiles, principal component analysis shows that flow cytometry profiles at pH 12.5 differ from those at pH 9 and pH 10.5, which are quite similar. In addition, at pH 12.5 flow cytometry profiles for the Harpur Hill community clearly differ from that of the Boom Clay borehole water community. Both communities were able to form a biofilm after 110 days on the inactive bituminized waste product at pH 9 and pH 10.5, but at pH 12.5 no biofilm was observed. Altogether, our data indicate that, although flow cytometry profiles are similar at pH 9 and 10.5, nitrate reduction rates differ between both microbial communities. Although exposure to pH 12.5 inhibits nitrate reduction, the Harpur Hill community was still viable at this high pH as total cell counts increased in time. Hence, it seems that high pH alone as stress factor will not eliminate all microbial presence in a geological repository, though it seems to provide enough stress to cause a significant shift in the microbial population and reduce its nitrate reducing activity.

Acknowledgements

This project is part of the MIND project and has received funding from the Euratom research and training programme 2014 - 2018 under grant agreement No. 661880.

OVERVIEW OF IRRADIATION AND BIODEGRADATION STUDIES ON CELLULOSE AND PVC

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University of Manchester MIND experiments have focused on the potential impact of microbial metabolism on high pH systems analogues to cementitious nuclear waste. Experiments are ongoing in three key areas; (i) microbial metabolism of cellulose and cellulose degradation products (such as isosaccharinic acid, ISA), (ii) microbial degradation of PVC and (iii) the fate of metals and radionuclides during ISA biodegradation. A multidisciplinary approach is being applied, including multiomics techniques (genomic sequencing, transcriptomics, proteomics and metabolomics) to understand the fundamental microbiology of these systems, alongside state of the art imaging and spectroscopy. Results from a range of experimental systems will be presented, encompassing both pure culture and microcosm model systems, both with and without irradiation treatments. Collectively these data show that microbes have the potential to colonise the alkali-disturbed zone surrounding a geodisposal facility, and in these cases their metabolism can offer beneficial roles to the safety case, degrading organic chelates and immobilising priority radionuclides.

[1] Bassil, N.J. and Lloyd, J.R. (2017) Draft genome sequences of four alkaliphilic bacteria belonging to the *Anaerobacillus* genus. Genome Announcements 5 (3), e01493-16

[2] Bassil, N. and Lloyd, J.R. (2018) *Anaerobacillus alkaliisosaccharinicus* sp. nov., an alkaliphilic bacterium which degrades isosaccharinic acid. International Journal of Systematic and Evolutionary Microbiology DOI 10.1099/ijsem.0.002721

[3] Kuippers, G., Boothman, C., Bagshaw, H., Ward,, M., Beard, R, Bryan, N. and Lloyd, J.R (in revision). The biogeochemical fate of nickel during microbial ISA degradation; implications for nuclear waste disposal. Scientific Reports

[4] Nixon, S.L, van Dongen, B.E., Boothman, C., Small, J.S. and Lloyd, J.R. (submitted) PVC additives fuel microbial nitrate reduction at high pH: Implications for nuclear waste disposal. Front Env Sci submitted.

THE MUTUAL INFLUENCE OF UO_2^{2+} AND ISOSACCHARINIC ACID ON THEIR SPECIATION: A SPECTROSCOPIC STUDY UNDER ACIDIC CONDITIONS

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The large amounts of cellulosic materials, being present in low and intermediate level waste, in combination with the use of cement-based materials as stabilization, construction and backfilling materials cause great concerns in the context of nuclear waste disposal. The alkaline degradation of cellulose will lead to the formation of small water-soluble organic compounds, which can interact with radionuclides (RN) and can affect their mobility as well as sorption behavior adversely. The main product of alkaline cellulose degradation is isosaccharinic acid (ISA). It has been shown, that the complexation of ISA with different RN led to a dramatic increase of the solubility. [1] However, in the particular case of uranium the number of studies is very limited and therefore reliable thermodynamic data are missing.

This study focuses on the structural characterization of the formed complexes under acidic conditions and provides valuable information as well as reference data for further investigations to generate thermodynamic data. It is furthermore the basis for ongoing investigations under neutral and alkaline investigations. To elucidate the structure of UO_2^{2+}-αISA-complexes, different spectroscopic techniques (UV-vis, luminescence, ATR-FTIR, EXAFS) were combined with quantum chemical calculations. The formation of three different complexes was observed, having a five-membered ring as main binding motive. In addition it was observed that the presence of UO_2^{2+} has an effect on the equilibrium between the lactone and the protonated form of ISA (ISL and HISA).

ISL HISA α-ISA

[1] Van Loon, L. R., *et al*., Radiochimica Acta, 1999, Vol. 86, https://doi.org/10.1524/ract.1999.86.34.183

REDUCTION OF SELENITE TO AMORPHOUS AND CRYSTALLINE SELENIUM NANOSTRUCTURES BY *STENOTROPHOMONAS BENTONITICA*: IMPACT ON SELENIUM MOBILITY WITHIN THE CONCEPT OF RADIOACTIVE WASTE REPOSITORIES

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The University of Granada contributes with the study of microbial processes controlling radionuclide speciation within the Work Package 1 of the MIND project. Specifically, we are focused on the study of the molecular speciation of uranium (U), europium (Eu) and selenium (Se) under disposal relevant conditions after their contact with the new bacterial species *Stenotrophomonas bentonitica*, isolated from Spanish bentonites [1]. Se^{79} isotope produced by nuclear fission reactions is considered a critical radionuclide present in radioactive wastes [2].

We have previously reported that *S. bentonitica* is able to reduce Se^{IV} to Se^{0} under alkaline environments (up to pH 10) and different physiological conditions forming selenium nanoparticles (SeNPs). Recent results obtained by using STEM/HAADF showed the production of selenium nanostructures with different shapes (spheres, hexagonal-shaped, and nanowires) when the cells grow aerobically from 144 h of incubation under 2 mM Se^{IV} stress. Selected-area electron diffraction (SAED) confirmed the amorphous nature of the Se nanospheres. High-resolution scanning transmission electron microscopy (HRSTEM) combined with Fast Fourier Transform (FFT) revealed the crystalline structure (trigonal phase) of Se hexagonal-shaped and nanowires. Amorphous Se (*a*-Se) nanospheres were produced intracellularly in the beginning (24 h of incubation) and then start to aggregate within the extracellular space after period of 48 and 72 h. From 144 h of incubation, *a*-Se nanospheres and trigonal Se (*t*-Se) nanostructures (*e.g.* hexagonal-shaped and nanowires) were observed. A time-dependent transformation from *a*-Se nanospheres to different *t*-Se nanostructures has been proposed. Trigonal Se nanorods have been previously reported for their high settleability [3]. For this reason, the mobility of Se in future radioactive waste repositories would be considerably reduced. *S. bentonitica* and their extracellular flagella-like proteins were proposed to play an important role in the transformation process as suggested microscopic analysis carried out by Field Emission Gun Environmental Scanning Electron Microscopy (FEG-ESEM).

The results obtained in the present work provide new insights into the impact of microbial processes in the speciation and hence the transport of Se in future radioactive repositories.

References

[1] Sanchez-Castro I., Ruiz-Fresneda M.A., Bakkali M., Kämpfer P., Glaeser S.P., Busse H.J., et al. (2017) *Stenotrophomonas bentonitica* sp. nov., isolated from bentonite formations. Int J Syst Evol Microbiol, 67(8):2779–86.

[2] Breynaert E., Scheinost A.C., Dom D., Rossberg A., Vancluysen J., Gobechiya E., Kirschhock C. and Maes A. (2010). Reduction of Se (IV) in Boom Clay : XAS Solid Phase Speciation. Environ Sci Technol, 44: 6649-6655.

[3] Jain R, Jordan N, Tsushima S, Hübner R, Weiss S, Lens PNL. Shape change of biogenic elemental selenium nanomaterials from nanospheres to nanorods decreases their colloidal stability. Environ Sci Nano. 2017;4(5):1054–63.

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THE EFFECT OF CAESIUM IONS ON A NATURAL ANAEROBIC MICROBIAL COMMUNITY

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Radioactive isotopes and fission products represent a serious danger to humans and other living organisms. The radioisotope Caesium (Cs)-137 has received much attention due to its long half-life, high solubility in water and similarity to potassium, a metabolically important chemical element. Interestingly, some groups of microorganisms can accumulate or adsorb Cs and, consequently, have been suggested as an excellent Cs bioremediators. Here we studied the effect of different concentrations of non-radioactive Cs^+ ions on the survivability of natural anaerobic bacteria using qPCR analysis, 16S rDNA amplicon sequencing, fluorescence microscopy (LIVE/DEAD cell staining), and transmission and scanning electron microscopy. The experiment lasted 23 days and was performed under anaerobic conditions. The results of microbiological analyses revealed that lower Cs concentration (0.5 mM) caused an increase in the total bacterial biomass and the nitrate-reducing bacteria, while higher concentrations (1 mM) limited bacterial growth, and 5 mM caused a strong decline in bacterial biomass. These findings were supported by electron and fluorescence microscopy. Our results suggest that higher Cs^+ concentration might have an inhibitory effect on the anaerobic microorganisms in radioactive waste repository regardless radioactivity, while the growth of some bacterial groups (e. g., nitrate-reducing bacteria) might be induced by lower Cs^+ concentrations. The qualitative changes within anaerobic microbial consortium determined by amplicon sequencing will be shortly discussed.

H_2 FUELLED MICROBIAL METABOLISM IN OPALINUS CLAY

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In Switzerland, the Opalinus Clay formation is considered the most likely host rock for a deep geological repository of nuclear waste, with H_2 expected to be the primary gas phase formed from metal corrosion and organic waste degradation. H_2-driven sulfate reduction was evidenced in borehole water in Opalinus Clay [1] but there is little known about the interactions between the sulfide generated by this microbial process and the host rock. The key question is whether sulfide produced in the Opalinus Clay is scavenged, potentially limiting the impact on canister corrosion.

Microcosm experiments were conducted with Opalinus Clay and artificial porewater using porewater from the Mont Terri underground research laboratory as an inoculum. Control microcosms were established without Mont Terri porewater to investigate abiotic processes.

Results show an initial release of sulfate into solution when clay is present. In the inoculated experiments, this decreases over time, however there is no evidence of associated hydrogen sulfide production in the aqueous phase. There is an increase in aqueous Fe^{2+} concentrations in all experiments with clay with the highest concentrations found in non-inoculated experiments. After the incubation period, the microbial community is dominated by the *Desulfobulbaceae* family as found previously [1] suggesting active sulfate reduction is occurring. Sulfur speciation in the Opalinus Clay shows a larger proportion of elemental sulfur when exposed to microbial activity (37%) compared to non-inoculated (10%) and a lower percentage of pyrite (55% and 88%, respectively).

The study suggests that microbially-mediated sulfate reduction can lead to the production of elemental sulfur within the clay rock. This mechanism removes hydrogen sulfide from solution and potentially reduces the corrosion rate of waste-containing canisters. These findings enhance knowledge of the fate of microbial metabolites in the repository. In particular, this works contributes to addressing questions relating to the impact of hydrogen sulfide (Posiva priority 4 and NWMO priority 1).

[1] Bagnoud A., (2016) *Nat. Comm.*, 7, 12770.

LARGE-SCALE GAS GENERATION EXPERIMENT WITH LOW-LEVEL RADIOACTIVE WASTE

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The Gas Generation Experiment (GGE) has been established in 1997 in TVO's final disposal repository for LLW and ILW in Olkiluoto, Finland, to examine gas generation from LLW. LLW contains miscellaneous maintenance waste produced during operation of nuclear power plants. It includes scrap metals, plastics and considerable amounts of cellulosic materials like paper sheets, cardboard and cotton gloves. In GGE carbon steel drums with the volume of 200 L were filled with LLW, the GGE was filled with river water and maintained at 8-11°C. The GGE has been monitored for volume and composition of generated gas, water chemistry and microbiology [1,2,3]. The aim of our study was to examine relevant microbial groups influencing the gas generation and corrosion of steel using molecular technologies including quantitative PCR and high-throughput sequencing.

Our studies [3] has shown that LLW is converted to methane and carbon dioxide as a successive action of complex microbial consortia including cellulose degrading bacteria, acetogenic bacteria and methanogens. Extensive degradation of cellulosed-based material and the formation of volatile fatty acids seemed to reduce the activity of methanogens and gas formation. Methanogens also compete with SRBs for electron donors like H_2 which can influence the gas generation rate. SRBs are a diverse group of anaerobic bacteria and archaea that can use sulphate as a terminal electron acceptor and release hydrogen sulphide as a metabolic by-product. The relative ratio of sulphate reducers compared to methanogens has decreased considerably during the operation of GGE and the amount of sulphate has been close to the detection limit since 2000. In 2017 ^{35}S tracer method was used to analyze microbial sulphate reduction in GGE. Sulphate reduction rate in GGE was very low but was rapidly increased when sulphate or sulphate-containing groundwater was added to the system. This indicates that groundwater flow to the LLW repository can influence the gas generation rate.

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[1] Small, J., Nykyri, M., Helin, M., Hovi, U., Sarlin, T., Itävaara, M. 2008. Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. Appl. Geochem. 23, 1383–1418.

[2] Small, J., Nykyri, M., Vikman, M., Itävaara, M., Heikinheimo, L. 2017. The biogeochemistry of gas generation from low and intermediate level nuclear waste: modelling after18 years study under in situ conditions. Appl. Geochem. 84, 360-372.

[3] Vikman, M., Marjamaa, K.,Itävaara, M. 2016. Microbial degradation of LLW under repository conditions- case TVO. MIND Project Annual Meeting 1 - Extended Abstracts, 2 - 4 May 2016, Granada, Spain, Book of Abstracts. European Union, 39-42.

WP1 SYNTHESIS AND GAP CROSS-REFERENCE

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This summary presentation will provide a synthesis of current work within WP1 of the MIND project; Improving the geological safety case knowledge of the behaviour of organic containing long-lived intermediate level wastes (ILW). The presentation will discuss project deliverables and external peer-reviewed publications produced to date. The Gap list produced by the Implementers Review Board will be discussed and cross-referenced against the results obtained so far and anticipated by the MIND project.

Microbial Viability in Clay Barriers: From Lab Study to Field Scale

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Since the 1940's the UK has been generating nuclear waste. High heat generating wastes (HHGW) consisting mostly of spent fuel (SF), and high level waste (HLW) from SF reprocessing are being considered for disposal in a geological disposal facility (GDF)[1]. In many HHGW disposal concepts, the waste canister will be protected by a bentonite clay barrier that delivers long-term low permeability and a high swelling pressure to restrict microbial activity, limit waste container corrosion and radionuclide transport[2]. However, microbes have been found to exist in natural bentonite strata, as well as after barrier material fabrication and under simulated conditions[3]. If active under in-situ conditions, Fe(III)-reducing bacteria may contribute to the dissolution of the engineered barrier[4], and sulphate-reducing bacteria (SRB) can induce microbially-induced corrosion (MIC) of the waste canisters[2].

This study looked at the influence of pelletisation, temperature, and irradiation on the culturable number of bacteria in Fe(III)-reducing, and SRB enrichments. Cores from the Full-scale Engineered Barriers Experiment (FEBEX)[5], a 20 year long full-scale experiment were also investigated using the latest molecular ecology techniques.

Our results show that Fe(III)-reducing, and sulphate-reducing (SRB) bacteria are present in the bentonites, and persist through the treatments in reduced quantities. Microbes were also isolated from the FEBEX cores, and may have been active during the operation of the experiment.

Keywords bentonite, microbiology, FEBEX-DP,

References

[1] NDA. (2010). Geological Disposal: An overview of the generic Disposal System Safety Case

[2] J. Wilson. (2011). Bentonite: A Review of key properties, processes and issues for consideration in the UK context

[3] K. Pedersen. (2010). Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. *Journal of Applied Microbiology*, **108** 1094-1104.

[4] J. Kim. (2004). Role of microbes in the smectite-to-illite reaction. *Science*, **303** 830-832.

[5] F. Huertas. (2000). Full-scale engineered barriers experiment for a deep geological repository for high-level radioactive waste in crystalline host rock.

MICROBIALLY INDUCED CORROSION OF CARBON STEEL IN A NATURAL GROUNDWATER AND A SYNTHETIC BENTONITE PORE WATER

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Microbially induced corrosion of carbon steel in two different environments was studied in long-term experiments. First, a natural granitic underground water (so called Vita water) containing microbial community dominated by sulphate-reducing bacteria, and second, the synthetic bentonite pore water inoculated with the same Vita water (in the ratio 9:1) were used for the experiments. The first experiment inoculated with Vita water was performed both at room temperature and at 35 °C. The experiments were performed under anaerobic conditions in parallel arrangements including sterile controls, and no nutrients were added. The corrosion behaviour of carbon steel was investigated by electrochemical impedance spectroscopy, mass loss and potentiodynamic polarization measurements. The morphology of the carbon steel surface after exposure was investigated using SEM-EDS and characterized by Raman spectroscopy. In order to determine the proliferation of relevant bacterial groups in water as well as biofilm samples, the molecular-biological approach was used (specifically quantitative PCR and 16S rDNA amplicon sequencing). Synthetic bentonite pore water which is rich in nitrates and sulphates represents an environment which enhances bacterial proliferation (especially of nitrate reducers). Biofilm formation has been observed in both Vita water and synthetic bentonite pore water inoculated with Vita water. In the case of Vita water, biofilm decreased the corrosion rate at the temperature 35 °C, in contrast to samples incubated at room temperature.

INITIAL FINDINGS FROM EXPERIMENTS INTO MICROBIAL EFFECTS ON STEEL AND COMPACTED BENTONITE.

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Experiments are underway to address microbially induced corrosion of canister materials (WP2.2) and microbial degradation of bentonite buffers (WP2.4) in flow tests using FE bentonite and steel chips. Two sets of experimental apparatus have been set up, each comprising a titanium pressure vessel fitted with three radial and two axial load cells which constantly monitor changes in stress within the clay sample. Compacted bentonite samples containing steel chips near the inlet were prepared to a dry density of 1400 kg m^{-3} and inserted into the pressure vessel. The pressure and flow is controlled by an injection and backflow HPLC pump that allow hydration of the sample with an anoxic artifical groundwater based on a Grimsel groundwater recipe. The HPLC pumps are set to create a hydraulic gradient across the sample allowing measurement of permeability and to bath system in nutrients.

Two paired test have been run and post-experimental analysis is ongoing. Each pair comprised one sample of sterile (irradiated) bentonite without microbial inoculum and one sterile (irradiated) bentonite inoculated with a mixed microbial culture enriched for sulphate reducers from uniradiated bentonite samples. In both tests an artificial goundwater based on Grimsel groundwater was prepared, but in the second pair this was ammended by the addition of sodium lactate to act as a carbon source for the microbes. During the experiments, in/out flow was being monitored along with total stress to see if the corrosion of steel has a direct effect on swelling behaviour of the samples. Tests were run for approximately 3 months. Microbial analysis so far has been limited to culture based methods. These reveal very few microbes in the uninoculated sample and higher microbial counts in the inoculated samples, especially associated with the portion of the sample containing the steel. SEM analysis identified euhedral aragonite and fibrous iron in the inoculated samples but not the inoculated samples. XRD data shows alteration to the clay around the steel in all samples analysed so far. The evolution of the stress in the clay was monitored throughout the experiment, but it appears that any effect of microbial activity on the clay in these experiments is too small to detect using our method. A third pair of experiments have been set up at a dry density of 1200 kg m^{-3}. We expect greater microbial growth in this experiment which will provide some insight into whether greater microbial activity (as might be expected over longer timescales) has any effect on the evolution of the stress regime idicating a loss of swelling capacity.

BENTONITE CHARACTERISTICS AFTER ONE YEAR OF STORAGE WITH INDIGENOUS BENTONITE AND WATER MICROBES

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The aim of this laboratory scale bentonite storage experiment was to simulate conditions that can take place in some interfaces of the nuclear waste final repository. The bentonite was studied as a slurry in which water, gases, nutrients and microorganisms were able to move freely at temperature hospitable for microorganisms. The objective was to find out if microorganisms and metabolites they produce in favourable conditions are able to change the bentonite structure and if these changes could be significant for the bentonite stability in long-term scale.

After one year of storage no noticeable changes were observed in bentonite-water pH or conductivity in microcosms initiated in oxic or anoxic conditions. In general, concentrations of aluminium, silicon and iron increased in the water phase of the experiments during one year. The sulphate concentration profiles were different in oxic and anoxic microcosms. In anoxic conditions sulphate amount decreased compared to sterilized control microcosms whereas in oxic conditions sulphate concentration slightly increased or remained stable. The decrease of sulphate concentration in anoxic microcosms may be explained by microbial sulphate reduction which was observed in anoxic microcosms using labelled $^{35}SO_4$ –method as labelled sulphide was formed. In addition, cation exchange capacity was slightly increased in anoxic microcosms compared to controls and oxic microcosms as well as the original bentonite. Atomic force microscopy (AFM) showed that the bentonite particle size had decreased overall and also the roundness increased during one year of bentonite storage. With HAADF-STEM microscopy (High-angel annular dark-field scanning transmission electron microscopy) no significant changes were detected between oxic and anoxic bentonite microcosms or original bentonite and bentonites after one year of microbial storage.

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Bentonite – a natural source for sulfate-reducing bacteria

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In order to analyze the potential influence of natural occuring microorganisms within the bentonite on the properties of the bentonite barrier, we set up microcosm-experiments. Two different Bavarian bentonites (a natural and an industrial one) were supplied with an anaerobic, synthetic Opalinus-clay pore water solution under an N_2/CO_2-atmosphere and were incubated for one year at 30 °C and 60 °C. To some set ups organics (acetate or lactate) or H_2 were supplemented. During the incubation time samples were analyzed for several biogeochemical parameters and the evolution of microbial community.

Our results clearly demonstrate, that natural occuring microbes affect geochemical parameters. Set ups containing the industrial bentonite supplemented with lactate or H_2 show the most striking effects. The respective batches were dominated (up to 81 %) by *Desulfosporosinus* spp. after 6 months – spore-forming, strictly anaerobic, sulfate-reducing organisms, able to survive under very harsh conditions. Concomitantly, an increase of ferrous iron and a simoultaneous decrease of ferric iron was observed as well as a decrease in sulfate – alterations that could effect different properties of and reactions within the barrier system of an HLW.

GEOCHEMICAL CONSTRAINTS OF SULPHIDE PRODUCTION AND CONCENTRATION

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Prerequisites of the reactions between metals (copper, iron) and dissolved sulphide in groundwater have to be understood and quantified in assessing the performance of the copper and iron canisters in nuclear waste disposal. As a rule, dissolved sulphide concentration in groundwaters is low because of the strong tendency of metal sulphide precipitation. Sometimes, however, groundwater analyses seems to indicate higher sulphide concentrations than a simplified solubility-control model predicts. An evident reason is the relatively rapid microbial sulphate reduction in the absence of sulphide-precipitating metals, mainly dissolved iron. Consequently, a sulphate reduction front associated with a high-sulphide “peak” may form.

Sulphate rich waters are not common in deep anoxic groundwater conditions, sharp sulphate reduction boundary can often be discerned at the depth range 300 – 500 m in crystalline rock. High sulphate concentrations often indicate sea water intrusions, sometimes associated with glacial events. Representativeness of the sulphate analyses must be carefully examined because of the effective microbial metal sulphide oxidation even at slightly oxidative conditions, as often observed in sulphide mines. Abundance of sulphate can also be linked with the lack of electron donors, of which hydrogen and simple carbon molecules (e.g. acetate, lactate) are very effective. Methane is kinetically less reactive, and the energy yield of methane as a sulphate reducer is low.Geochemistry of gases plays an important role in the safety assessment of geological disposal of nuclear wastes. Within fractured bedrock, the fluid phase can both mobilize and disperse potentially hazardous or corrosive compounds, such as ^{14}C or sulphide, as well as provide energy and nutrients to deep-dwelling microorganisms. Indeed, some of the most important electron donors in anoxic deep biosphere, including H_2 and CH_4, are gases.

This research is a part of the Horizon2020 project MIND, which has received funding from the Euratom research and training programme 2014-2018 under grant agreement No. 661880.

IN SITU CORROSION OF STEEL EMBEDDED IN BENTONITE: ENUMERATION AND CHARACTERIZATION OF THE ASSOCIATED MICROBIAL COMMUNITY

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An important step to evaluate the potential impact of biogeochemical processes on the safety of deep geological radioactive waste repositories is the quantification of corrosion rates of proposed metal alloys. In this context, microbial induced corrosion is of particular interest. One of the functions of the bentonite backfill proposed in several concepts, including the Swiss concept, is to minimize microbial activity in the vicinity of the waste in order to preclude microbially-enhanced corrosion of the canisters.

Here, we present the results of an ongoing *in situ* small scale test in Opalinus Clay rock in the Mont Terri Underground Rock Laboratory in St. Ursanne, Switzerland. We simulated the engineered barrier systems with retrievable Wyoming bentonite MX-80-filled modules, in which steel and copper coupons of several relevant alloys were embedded. Both pellets and preformed blocks of bentonite of varying dry densities were tested. These modules were placed in the BIC-A1 borehole and exposed to pore water under native conditions for 20 months (phase 1) or 33 months (phase 2), respectively. The initial microbial community in the borehole was characterized, as was the community at the first and second sampling time point (20 & 33 months). Additionally, cultivable microorganisms from the bentonite were enumerated at both time points. Results show that viable microorganisms are present within MX-80 and that the number of cells decrease with higher dry density. The obtained dry densities deviate from the desired ones, indicating heterogeneous swelling. The diversity of the BIC-A1 borehole pore water microbial community decreased over the 20 months.

This is an ongoing investigation and a new set of six modules are in preparation with the aim to distinguish the impact of microorganisms (a) within Wyoming bentonite MX-80 and (b) in the porewater on the corrosion rate of steel and copper.

MICROBIAL ACTIVITY IN BENTONITE BUFFERS

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Bentonite clay will be used as a buffer material in engineered barrier systems (EBS) which will contain, protect and surround nuclear waste canisters in geological disposal concepts. The dissimilatory reduction of sulphate, thiosulphate and sulphur to sulphide by sulphide-producing bacteria (SPB) is a main concern for the safety case of a geological disposal since sulphide is a corrosive agent for metal waste canisters. Bacterial activity is generally measured by the turn-over of one or several metabolic products such as acetate or in the SPB case, the production of sulphide. Bacterial viability (or presence) on the other hand does not imply that the bacteria must be active *in vivo* or *in situ*, it only states that they are able to become activated when a suitable environment presents itself. It has been hypothesised that a cut-off bentonite density threshold exists above which all bacterial activity stops or is inhibited to a such a level that it can be regarded as negligible. This presentation discusses if that hypothesis can be considered true or if more variables other than clay density determine bacterial activity in bentonites. Six different bentonites have been studied: Wyoming Volclay MX-80 (USA), Asha (India), Calcigel (Germany), GMZ (Gaomiaozi, China, Rokle (Czech Republic) and FEBEX clay (Switzerland). In addition, Boom Clay (Belgium) has been studied as a reference to a non-swelling clay. These clays had a varying element and mineral composition. In particular, the amount of montmorillonite varied from 66% to 88% and the iron content, analysed as Fe_2O_3 varied from 3.3% to 13.4%.

Sulphide-producing bacteria could be cultivated from bentonite clay samples and the numbers decreased over wet density for some but not all tested clays. The range of saturated wet densities studied was from 1400 to 2000 kg m^{-3}. Experiments analysing bacterial sulphide-production showed that there were intervals where the measurable accumulation of copper sulphide for each clay changes from significant to below detection within the experimental timeframe of 3 to 4 months. For two clays, these density ranges are yet to be determined, sulphide was produced at all studied densities. It is, therefore, concluded that density does not control bacterial activity in clays *per see*. This is a reasonable conclusion because there is nothing in biology that can explain a density effect. Wet density is just a value of the total amount of clay and water in a defined volume. Density alone does not reflect the growth conditions for bacteria in a compacted clay where multiple variables of importance for bacterial life should be considered, such as clay type, pH, temperature, transport conditions, water content/water activity, pressure, pore space and pore water composition. The presentation reviews mainly swelling pressure and water activity.

COMPARISON OF DNA EXTRACTION PROTOCOLS FOR OPALINUS CLAY ROCK

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In order to assess the microbial impact on the safety of deep geological radioactive waste repositories, robust techniques to extract microbial DNA from the relevant host rock formation are needed.
Here, we present the results of a test of various DNA extraction protocols involving seven laboratories. Each laboratory received an 10 g aliquot of Opalinus Clay rock collected from the Mont Terri Underground Rock Laboratory in St. Ursanne, Switzerland and amended with a microbial community of known compostion. Every participating laboratory extracted DNA, resulting in DNA isolates in the range of 0.66 ng – 418 ng total DNA. DNA amplification (16S rRNA gene V4 region) was performed by a single laboratory and Illumina MiSeq sequencing was carried out by a single commercial laboratory. The same bioinformatic pipeline was used to process and compare the DNA sequences obtained.

THE INCLUSION OF MICROBIOLOGICAL PROCESSES AND THEIR EFFECTS IN PERFORMANCE ASSESSMENT

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In the near field of a radioactive waste repository, there may be opportunities for microbial life during the operational phase and for some time after repository closure. Nevertheless, substantial uncertainties remain concerning the persistence and potential of microorganisms in repository conditions. An overview and examples of how these processes are dealt with in a Performance & Safety Assessment considering disposal in Boom Clay in Belgium will be given.
The most straightforward approach is to apply conservative hypotheses in performance assessment in order to to deal with these uncertainties and to treat the direct or indirect impact of these processes on radionuclide release or mobility. More detailed process level models can be included to increase the understanding of the system, to increase the level of realism in system level models or reduce conservatism in the safety assesment models. Depending on the application, different levels of complexity can be introduced. This presentation aims at dissipating this knowledge within the MIND consortium and to bridge the gap between microbiological research communtity and safety assessors. Finally, a proposal for integrating the knowledge gained in MIND into a format which is the most usuable in the contruction of a set of Safety & Performance Assesment calculations is given.

SOCIAL SCIENCE RESEARCH WITHIN MIND

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Geological disposal is often presented as a passive, a-biotic isolation strategy for long term radioactive waste management, while a microbially influenced environment can be considered as lively and continuously dynamic on a range of scales and time spans. What is the impact of microbes on our understanding of geological disposal? Are microbes and microbiologists part of the knowledge production and evaluation process? Are they part of the safety case? Can experts and non-experts understand each other in these matters? How are uncertainties dealt with and communicated? This presentation focuses on the social science research methods that were, and will be, used to respond to these questions, which are qualitative in nature and involve both experts and non-experts. It gives an overview of the social science activities done in MIND so far and outlines the activities for the coming period: a Public Value Mapping workshop with MIND scientists and interested laypeople and a workshop on Risk Perception and Communication. These activities serve (a) to create an enhanced reflexive awareness among scientists and nonscientists about the opportunities and pitfalls of integrating microbiology into nuclear waste management; (b) elicit joint assessment of the possible risks, safety issues, and uncertainties inherent in designing, implementing, and communicating nuclear waste management strategies and technologies.

CHARACTERISATION OF ORGANIC COMPOUNDS RELEASED FROM IRRADIATED RESINS

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Ion exchange resins represent a significant proportion of the solid waste inventory for intermediate level waste in Switzerland. They are used to bind radioactive ions in nuclear power plants and thus are subject to high radiation doses over long timescales. Under these conditions, it is conceivable that solutes and gases will be released from the degradation of irradiated resins into the aqueous phase. However, there is little knowledge on the types of solutes and gases that could be released under repository relevant conditions (e.g., anoxic) and the potential role of these compounds in fueling microbial growth. The results of a series of irradiation experiments primarily using a cobalt gamma source subjected two resin types to high irradiation doses (200 kGy). The gas phase was analysed immediately using gas chromatography (GC) with the aqueous phase analysed using GC-mass spectrometry following removal of sodium chloride present in the porewater representative of the repository.

Results of the gas phase analysis suggest that the main compounds produced are H_2 and CO_2. Benzene and chloromethane are produced as well as, however the concentrations of these compounds are very low. Current work is focused on determining the concentration of organic compounds in the aqueous phase. Compounds released due to the irradiation will then be used in microcosm experiments investigating whether these compounds could be used by microbial communities which exist within the Opalinus Clay and what impact any utilization of the compounds may have on a repository environment.

GAS GENERATION EXPERIMENT WITH LLW - CORROSION OF METAL SAMPLES

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The Gas Generation Experiment (GGE) has been established in 1997 in TVO's final disposal repository for low (LLW) and intermediate level waste (ILW) in Olkiluoto, Finland. The aim of the GGE was to examine gas generation from LLW which is produced during operation of nuclear power plants and in Finland includes considerable amounts of cellulose-based materials like paper sheets, cardboard and cotton gloves. The microbiological degradation of LLW in anoxic conditions results in gas generation and can also accelerate corrosion of steel. In GGE carbon steel drums with the volume of 200 L were filled with LLW and the GGE was filled with river water. The amount of cellulose-based materials inside the drums varied from 5 to 95 w-%. The GGE has been monitored for volume and composition of generated gas, water chemistry and microbiology [1,2,3]. In addition, capsules containing a piece of the drum steel and LLW were loaded to the experiment. They were located inside waste drums and in tank water. Capsules have been removed at certain intervals and used to study corrosion rate of steel, corrosion products and microbial biofilms attached on steel plates and waste materials.

The corrosion rate of steel was highest inside the drum containing 95 w-% of cellulose-based LLW. In addition, the high concentration of soluble ferrous iron Fe^{2+} and total iron also indicated dissolution of the carbon steel during the GGE. Enhanced microbial activity inside the drum containing highest amount of cellulose can be related to the more rapid corrosion rate of steel. On the other hand, possible reasons related to the differences in chemical conditions (e.g. pH) between the drums cannot be totally excluded. The main corrosion product in steel plates in all GGE compartments was siderite $FeCO_3$, which is generally formed under methanogenic and acetogenic conditions.

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[1] Small, J., Nykyri, M., Helin, M., Hovi, U., Sarlin, T., Itävaara, M. 2008. Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. Appl. Geochem. 23, 1383–1418.

[2] Small, J., Nykyri, M., Vikman, M., Itävaara, M., Heikinheimo, L. 2017. The biogeochemistry of gas generation from low and intermediate level nuclear waste: modelling after18 years study under in situ conditions. Appl. Geochem. 84, 360-372.

[3] Vikman, M., Marjamaa, K.,Itävaara, M. 2016. Microbial degradation of LLW under repository conditions- case TVO. MIND Project Annual Meeting 1 - Extended Abstracts, 2 - 4 May 2016, Granada, Spain, Book of Abstracts. European Union, 39-42.

THE GEOMICROBIAL FATE OF THE RADIONUCLIDE COMPLEXING AGENT ISOSACCHARINIC ACID (ISA); RELEVANCE TO NUCLEAR WASTE GEODISPOSAL

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The nuclear waste inventory of the UK comprises large quantities of intermediate level wastes (ILW), which will be immobilised by encapsulation within a cementitious grout in stainless steel containers, followed by disposal in a deep engineered geological disposal facility (GDF) within a suitable geological formation. These wastes contain, in addition to radioactive elements, a heterogeneous mix of organic materials, including plastics, cellulose and rubber. Cellulosic items, such as cloth, tissue, filters, paper and wood, are known to be susceptible to degradation under alkaline conditions, forming small chain organic acids. One of the main degradation products is expected to be isosaccharinic acid (ISA) is considered particularly problematic, because it has the ability under alkaline conditions to complex metals and radionuclides, including Ni(II), Am(III), Eu(III), Np(IV), Th(IV), and U(IV). As a result, the presence of ISA could affect the migration behaviour of these elements, by increasing their solubility and reducing their sorption, thus enhancing their mobility into the near and far field surrounding a GDF.

During site operation and then after closure of a GDF, microbial communities have the potential to colonise steep biogeochemical gradients, that will propagate from the highly alkaline GDF “near field” to the circumneutral pH conditions in the surrounding geosphere. Within these steep pH gradients microbial processes may control the fate of organic compounds, such as ISA, and can therefore be considered as an effective self-attenuating mechanism to remove ISA (and chelated radionuclides) from the groundwater.

This study aimed to improve our understanding of microbial processes that can potentially use ISA as a carbon source and electron donor, removing it from solution, and thus having a positive impact on radionuclide mobility under GDF-relevant conditions. A microbial enrichment approach was used to explore the biodegradation of a Ni-ISA complex under GDF-relevant conditions, underpinned by cross-disciplinary analyses. Within this approach we have demonstrated the ability of bacteria to degrade ISA over a wide range of biogeochemical conditions. Furthermore, key radionuclides (and their non-active analogues), including Ni(II) and U(VI), were precipitated from the groundwater system during ISA biodegradation. Nickel was precipitated as a Ni-sulfide, potentially associated with Fe. Microbial community screening with 16S rRNA revealed microorganisms associated with Fe(III)- and sulfate-reduction, relevant to the precipitation of Ni. This study highlights the potential for microbial activity to help remove chelating agents from groundwaters surrounding an ILW GDF, and suggests that safety cases that do not include microbial processes may be overly conservative, over-estimating the impact of ISA on radionuclide transport.

MODELLING OF MICROBIAL PROCESSES WITH PHREEQC

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In order to evaluate the significance of microbial processes, such as the mediation of slow redox reactions between oxidised and reduced major element and trace radionuclide species in radioactive waste repositories, it will be necessary to represent the kinetic microbial processes in reactive transport models. Such models need to be developed and tested using data from bench and underground rock laboratory (URL) experiments and may assist in the interpretation of complicated URL experiments. Ultimately, once such models are validated they should be able to be up-scaled both spatially and temporally and incorporated in Thermal, Hydraulic, Mechanical, Chemical (THMC) models of geological repositories.

In this presentation an example model of denitrification processes is developed using the PHREEQC geochemical speciation and reaction-path code [1]. A two stage Monod kinetic model is developed to represent the denitrification reaction where nitrate is first reduced to nitrite and then nitrite is further reduced to nitrogen gas. The microbial model represents the growth of biomass and can examine both heterotrophic and autotrophic processes.

The model is applied to examine denitrification processes in the Bitumen-Nitrate-clay (BN) interaction experiment at the Mont Terri URL using data from an experiment that studied hydrogen gas as an electron donor for denitrification processes under in-situ conditions. In this experiment nitrate was injected in a borehole drilled in the Opalinus Clay and subsequently reacted with a pulse of hydrogen that was equilibrated with fluid in the borehole and where the reactions were monitored by an online chemical analysis system [2]. The PHREEQC model of the BN experiment includes a radial diffusion model to represent the diffusion of nitrate and other species in the Opalinus Clay and a range of equilibrium mineral and gas phase reactions.

The modelling results obtained to date provide a good representation of the complicated set of biogeochemical and diffusion processes occurring in the BN experiment that has aided the interpretation of the hydrogen injection experiment. The study builds confidence in the ability to develop models of microbial processes that can be usefully applied to geological repositories.

[1] Parkhurst, D.L. and Appelo, C.A.J., (2013). Description of Input and Examples for PHREEQC Version 3—A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations: U.S. Geological Survey Water-Resources Investigations. Chapter 43 of Section A, Groundwater Book 6, Modeling Techniques: Techniques and Methods 6–A43, U.S. Department of the Interior, U.S. Geological Survey, pp 437.

[2] Bleyen, N., Smets, S., Small, J., Moors, H., Leys, N., Albrecht, A., De Cannière, P., Schwyn, B., Wittebroodt, C., Valcke, E. (2017) Impact of the electron donor on in situ microbial nitrate reduction in Opalinus Clay: results from the Mont Terri rock laboratory (Switzerland). Swiss Journal of Geosciences.110(1), pp. 355-374.

MICROBIAL DIVERSITY AND ACTIVITY IN BENTONITE SAMPLES FROM THE FEBEX PROJECT

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Bentonite samples from the Full-scale Engineered Barrier Experiment (FEBEX) – Dismantling Project (DP) were provided to study the microbial diversity. A combination of culture-independent and culture-dependent approaches was used to the study the microbial diversity. DNA was extracted from 20 g of bentonite following the analysis of the 16S rRNA genes. The culture-dependent approach included the enrichment of microbes in selected media for iron-reducers, sulphate-reducers and H_2 consumers. High-molecular DNA was extracted from bentonite samples of the section 54 (back of heater) and 60 (far back of heater). The microbial community in these two samples was dominated by Actinobacteria and Proteobacteria. However, *Desulfitobacterium* spp. dominated the enrichment for iron-reducers, whereas *Desulfotomaculum* spp. were highly abundant in the enrichment for sulphate reducers. In media addressing hydrogenotrophic organisms, *Clostridium* spp. and *Bacillus* spp. were mainly identified. Some of the respective bacteria (*Desulfitobacterium* sp., *Clostridium* sp. and *Desulfosporosinus* sp.) were isolated. The enriched and isolated bacteria are known to form spores. Furthermore, DNA from spores is difficult to extract, which could be the reason that respective species were only detected in low abundance *via* the culture-independent approach. The suitable conditions in the enrichment-media lead to the germination of present spores, resulting in metabolically active cells.

POSSIBLE IMPACT OF MICROBIAL PROCESSES ON CEMENTITIOUS MATERIALS USED DURING THE GEOLOGICAL DISPOSAL OF RADIOACTIVE WASTE

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Cementitious materials have been used for centuries in many construction and engineering applications because of their long-term durability. Also for the geological disposal of radioactive waste, cementitious materials are used in many parts of the engineered barrier. Consequently, the interactions and the evolution of these materials with other repository materials, the host rock and its ground water, need to be assessed [1,2]. Organic acids (e.g. acetate), carbon dioxide and sulphur compounds, originating from the waste and host rock or produced by microbes in the repository, can be corrosive towards cementitious materials resulting in Ca^{2+} leaching and a decrease in the original high alkaline pH. The latter will give rise to lower pH niches on the concrete were microbial activity will be enhanced and which in turn can have a possible impact on the mineralogy and chemistry of the cementitious materials. Interestingly, microbial processes can either have a detrimental effect on or be beneficial for the functional performance of the cementitious materials used within a geological disposal [3].

The objective of this study is to investigate whether microorganisms could affect, in a positive or negative way, the long-term evolution of the cementitious materials present in the engineered barriers of a geological repository for radioactive waste, under relevant *in situ* conditions. To fulfil this task, batch experiments are set-up where a microbial community collected from Boom Clay borehole water is grown in the presence of a layer of Portland cement (CEM-1). Nitrate or sulphate is added as electron acceptor and respectively acetate and lactate is added as electron donor. Data regarding microbial activity in the liquid phase are gathered and biofilm formation on the cementitious surface is investigated. In addition, at the end of the experiment, cement integrity will be studied. Here, the preliminary results of these tests will be presented.

Ultimately, our findings will be evaluated towards their use for the assessment of the long-term performance of the cementitious engineered barrier system in geological disposal of radioactive waste.

[1] ONDRAF/NIRAS, Research, Development and Demonstration (RD&D) Plan for the geological disposal of high-level and/or long-lived radioactive waste including irradiated fuel if considered as waste. 2013, NIROND-TR-2013-12 E.

[2] ONDRAF/NIRAS, The Category B Waste Monolith Design Basis Report, SFC1 level 5 report: first full draft. 2013, NIROND-TR2013-03E

[3] Bertron, A., Understanding interactions between cementitious materials and microorganisms: a key to sustainable and safe concrete structures in various contexts. Materials and Structures, 2014. 47(11): p. 1787-1806.

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COMPARISON OF THREE DNA EXTRATION METHODS FOR MX-80 BENTONITE

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The ability to measure microbial growth, behaviour and metabolism in bentonite is an important step in assessing the performance of clay barriers in nuclear waste repository conditions. Clay minerals are challenging targets for DNA extraction as nucleic acids tend to bind tightly to clay particles. In addition, different bentonites vary in terms of exchangeable cations and mineralogical compositions, which impact the DNA extraction efficiency. The aim of this study was to compare the applicability of three different methods for DNA extraction from MX-80 bentonite.

The tested protocols were modified from methods already used for some bentonites or soil materials. The first method consisted of a modified version of Lever et al [1] that included lysis of microbial cells with a lysis buffer containing phenol-chloroform-isoamyl alcohol, lysozyme and proteinase K with a beat beating step followed by purification with chloroform-isoamyl alcohol and PEG-6000 precipitation of DNA. The second method was modified from Selenska-Pobell [2] and it was combined with NucleoBond (Macherey-Nagel) DNA purification. The third method was modified from a method that will be published by Povedano-Priego et al. and it included lysis with buffer containing lysozyme and proteinase K, bead beating, DNA precipitation with PEG-6000 and purification with NucleoBond columns.

DNA was successfully extracted from MX-80 bentonite with all three tested methods. The DNA amount was highest with the first method, in average 140 ng DNA g^{-1} room dry bentonite. The other two methods were less efficient yielding around 30 ng DNA g^{-1} bentonite. The bentonite studied was amended with 2.5 x 10^8 cells g^{-1} bentonite with mixture of *Esherichia coli* and an anaerobic mixed culture including sulphate reducers. Estimated theoretical DNA extraction yields varied between 21% and 0.5% of the added DNA amounts. Bacterial 16S rRNA gene and dissimilatory sulphite reductase *dsr*B gene copy numbers analysed with qPCR correlated ($r>0.95$) significantly ($p<0.01$) with the obtained DNA yields. However, *dsr*B genes were not detected from DNA extracted with the third method.

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The research has been supported by the Horizon 2020 project MIND through funding from the Euratom research and training programme 2014-2018 under Grant Agreement no. 661880. The research was also funded by the KYT, the Finnish Research Program on Nuclear Waste Management and VTT.

[1] Lever, M.A., Torti., A., Eickenbusch, P., Michaud, A.B., Šantl-Temkiv, T., Jørgensen, B.B. 2015. A modular method for the extraction of DNA and RNA, and the separation of DNA pools from diverse environmental sample types. Frontiers in Microbiology, 6:476. doi: 10.3389/fmicb.2015.00476

[2] Selenska-Pobell, S. 1995. Direct and simultaneous extraction of DNA and RNA from soil. Molecular Microbial Ecology Manual. 1.5.1:1-17.

GASES IN BEDROCK GROUNDWATER: GEOCHEMICAL POTENTIAL FOR SUSTAINING DEEP LIFE

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Geochemistry of gases plays an important role in the safety assessment of geological disposal of nuclear wastes. Within fractured bedrock, the fluid phase can both mobilize and disperse potentially hazardous or corrosive compounds, such as ^{14}C or sulphide, as well as provide energy and nutrients to deep-dwelling microorganisms. Indeed, some of the most important electron donors in anoxic deep biosphere, including H_2 and CH_4, are gases.

In order to provide data needed to address the question on geochemical constraints of biological activity at nuclear waste repository depths, we made a literature and database survey and collected geochemical data from deep drill holes and mines in Finland. Gas data were found from 20 separate localities, of which, the absolute concentrations of gases were available from 11 locations. The sites include both drill holes and deep mines in central and southern Finland with the deepest samples from 2480 m below surface.

Based on the dissolved gas composition, deep groundwaters were divided into CH_4-dominated and N_2-dominated types. Other commonly detected gases included H_2, He, Ar and occasionally CO_2, although significant variation existed between different sites and with depth.

At least partly the variation in the gas phase could be related to differences in lithology, which has been found to correlate also with microbial community structure [1], [2]. Another controlling factor is the residence time of water within the bedrock, which expands to tens of millions of years [3]. Methane-dominated groundwaters also had higher gas/water ratios clearly indicative of accumulation of gas after groundwater recharge in the crust.

Site to site as well as depth dependent variation should be taken into account and can be used to predict changes related to, for example, different rock types and will give valuable information to be used in the assessment of biogeochemically induced risks at nuclear waste repository sites.

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[1] Kietäväinen R., Ahonen L., Kukkonen I.T., Hendriksson N., Nyyssönen M., Itävaara M. (2013). Characterisation and isotopic evolution of saline waters of the Outokumpu Deep Drill Hole, Finland – Implications for water origin and deep terrestrial biosphere. Applied Geochemistry 32, 37-51.

[2] Kietäväinen R., Ahonen L., Niinikoski P., Nykänen H., Kukkonen I.T. (2017). Abiotic and biotic controls on methane formation down to 2.5 km depth within the Precambrian Fennoscandian Shield. Geochimica et Cosmochimica Acta 202, 124-145.

[3] Kietäväinen R., Ahonen L., Kukkonen I.T., Niedermann S., Wiersberg T. (2014). Noble gas residence times of saline waters within crystalline bedrock, Outokumpu Deep Drill Hole, Finland. Geochimica et Cosmochimica Acta 145, 159-174.

RATES OF HYDROGEN AND SULFATE COMSUMPTION BY MICROBIAL BIOFILMS IN RADIOACTIVE WASTE REPOSITORIES

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An important step to evaluate the potential impact of biogeochemical processes on the safety of deep geological radioactive waste repositories is the identification and quantification of the metabolic reaction of the extant microbiota. Due to waste degradation and anoxic metal corrosion, amounts of hydrogen will be produced and potentially lead to a pressure build-up which may potentially harm the integrity of the host rock. Indicated by reactive transport modelling results microbial metabolic activity could reduce a pressure build-up by consumption of hydrogen. For this purpose, operational tunnels could be backfilled with materials engineered to provide sufficient pore space for growth of microbial biofilms, which may allow for the biological consumption of excess H_2 via sulfate reduction.

Here, we present the concept of an ongoing *in situ* small scale test in Opalinus Clay rock in the Mont Terri Underground Rock Laboratory in St. Ursanne, Switzerland. We stimulate the formation of a hydrogen-fuelled biofilm community under repository relevant condition in a porous medium consisting of a 80:20% (w/w) sand-Wyoming bentonite mixture. The porous medium is provided with anoxic Opalinus Clay pore water from a borehole drilled at the transition between shaly to sandy facies. The system is designed to deliver H_2 at varying rates and to measure the resulting sulfate consumption and sulfide production rates. The resulting solid-associated microbial community will be characterized and will be representative of the community expected to grow under repository conditions if H_2 is present.

TRANSPORT AND REACTIVITY OF SULPHIDE FROM BACTERIAL ACTIVITY IN COMPACTED BENTONITE CLAYS

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Bentonite clays will be used as barriers in geological repositories for radioactive wastes. Anoxic conditions will prevail in such repositories, and the presence of sulphide-producing bacteria (SPB) in commercial bentonites and deep groundwater environments is well established. The inorganic reduction of sulphate to sulphide is kinetically hindered at normal pressure and temperature. The main source of sulphide in geological repository environments is, therefore, past and present microbial reduction of sulphate, sulphur and thiosulphate to sulphide. Sulphide-producing bacteria (SPB) have been found in most commercially available bentonites (Masurat et al. 2010; Svensson et al. 2011), and they frequently occur in deep geological formations and deep groundwater (Moser et al. 2005; Pedersen et al. 2014). The average value for D_e of sulphide in water saturated bentonite with a density between 2000 – 2100 kg m^{-3} has been estimated to $5.9(\pm3.6) \times 10^{-12}$ m^2 s^{-1} and 1.54 $(\pm1.1) \times 10^{-11}$ m^2 s^{-1} for 1750 kg m^{-3} bentonite (Pedersen et al. 2017). The D_e consequently decreased with increasing degree of compaction with $0.19(\pm0.11) \times 10^{-11}$ m^2 s^{-1} per 50 kg m^{-3} water saturated clay.

Bentonite clays have a significant capacity for adsorption of H_2S gas. This fact is utilised to produce industrial filters for removal of low concentrations of H_2S in gas streams. The absorbance capacity can be increased by the addition of iron to bentonite. The sulphide scrubbing mechanism is explained by reactions between sulphide and ferric iron. The reduction of ferric iron by sulphide from SPB was recently demonstrated for freshwater sediments (Hansel et al. 2015). Similar reduction has been found for bentonites (Pedersen et al. 2017). The poster presents and overview of results regarding sulphide formation, transport and reactions in clays.

Hansel, C.M., Lentini, C.J., Tang, Y., Johnston, D.T., Wankel, S.D., Jardine, P.M., 2015. Dominance of sulfur-fueled iron oxide reduction in low-sulfate freshwater sediments. ISME J 9, 2400-2412.

Masurat, P., Eriksson, S., Pedersen, K., 2010. Evidence of indigenous sulphate-reducing bacteria in commercial Wyoming bentonite MX-80. Applied Clay Science 47, 51-57.

Moser, D.P., Gihring, T.M., Brockman, F.J., Fredrickson, J.K., Balkwill, D.L., Dollhopf, M.E., Lollar, B.S., Pratt, L.M., Boice, E., Southam, G., Wanger, G., Baker, B.J., Pfiffner, S.M., Lin, L.H., Onstott, T.C., 2005. *Desulfotomaculum* and *Methanobacterium* spp. dominate a 4- to 5-kilometer-deep fault. Appl. Environ. Microbiol. 71, 8773-8783.

Pedersen, K., Bengtsson, A., Blom, A., Johansson, L., Taborowski, T., 2017. Mobility and reactivity of sulphide in bentonite clays – Implications for engineered bentonite barriers in geological repositories for radioactive wastes. Applied Clay Science 146, 495-502.

Pedersen, K., Bengtsson, A., Edlund, J., Eriksson, L., 2014. Sulphate-controlled diversity of subterranean microbial communities over depth in deep groundwater with opposing gradients of sulphate and methane. Geomicrobiol. J. 31, 617-631.

Svensson, D., Dueck, A., Nilsson, U., Olsson, S., Sandén, T., Lydmark, S., Jägewall, S., Pedersen, K., Hansen, S., 2011. Alternative buffer material. Status of the ongoing laboratory investigation of reference materials and test package 1. SKB Technical Report TR-11-06. Swedish Nuclear Fuel & Waste Management Co, Stockholm, Sweden, 1-146.

MICROBIAL ACTIVITY IN A CONCRETE-BENTONITE CLAY SURFACE

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The backfill of many repositories will consist of bentonite and crushed rock in various combinations. They will constitute a source of electron acceptor and donors that can be utilized by microorganisms. Microorganisms are often active at interfaces and may therefore be active at the interfaces between cement plugs and seals and backfill too. A key issue is consequently how microbial activity in backfill will influence the long-term behaviour and integrity of plug systems and seals. Within different disposal concepts, cementitious materials can be an essential part of the plug and seal systems. The integrity of the cementitious materials should therefore be ensured over a long period of time. Microbial activity, although expected to be inhibited by high pH, might affect the performance of cement on the long term, mostly by either (i) the production of biogenic acids, thereby lowering pH and/or enhancing calcium leaching (a detrimental effect), (ii) the enhancement of carbonation, thereby clogging the cement pores (a desirable effect) or (iii) minor processes, like biologically induced sulphate release, triggering the production of the voluminous ettringite (also detrimental). Previous studies showed that the alkaline solution leached from high pH concrete induces dissolution and precipitation. [1] The cementation by secondary minerals such as zeolites may lead to degradation of the desirable properties of the buffer. [2] However, the significant chemically changes in mineralogy and physical performance occur only within a few centimetres at the concrete/clay surface and there are no changes at larger distances. [3]

MICANS performed laboratory experiments with compacted MX-80 bentonite on top of a concrete plug in titanium test cells. Additionally, glucose was added to the bentonite to induce microbial fermentation. For saturation of the clay an oxygen-free salt saturation solution was used. After incubation the bentonite clay was analysed in profile for pH and microbial activity at four positions. Position 1 was the closed to the concrete plug and 4 the furthest.

The analysis of the control test cell which did not have a concrete plug inside, showed a constant pH of 9.4 at the four analysed positions. However, in the test cells with added glucose and a concrete plug the pH decreased from position 1 to 4. At position 1 the pH was on average 10 and at position 4 the pH was on average 8. In an additional test cell without glucose but concrete the pH stayed constant at 10 at all four positions.

Microbial activity was analysed trough CHAB cultivation and measuring of ATP. The control test cell showed the same order of magnitude ATP (1 x 10^5 amol mL^{-1}) at position 1 to 3 and one order of magnitude lower at position 4. The test cells with added glucose and concrete showed low amounts of ATP (0 to 10^3 amol mL^{-1}) at position 1-3 but higher amounts (10^3 to 10^5) at position 4.

For the control test cell CHAB were detected at position 4. However, the agar plates of position 1 to 3 could not be analysed because of mould growth. CHAB were also detected at position 4 for test cell with added glucose and concrete. The test cell without added glucose but concrete showed no microbial activity.

The results of the microbial activity indicate that the alkaline pH of 10 at position 1 – 3 might have inhibited the growth of bacteria. However, the test control cell had a pH of 9 at all four positions, contained no concrete but showed high ATP values and CHAB growth at position 4. Another reason for the lack of microbial activity in test cells with concrete, at position 1 to 3, might be that the saturation leaked an alkalic solution into the clay and inhibited microbial growth.

[1] Sánchez, L., J. Cuevas, S. Ramírez, D. R. De León, R. Fernández, R. V. D. Villa & S. Leguey, 2006. Reaction kinetics of FEBEX bentonite in hyperalkaline conditions resembling the cement–bentonite interface. Applied Clay Science 33(2):125-141.

[2] Savage, D., C. Walker, R. Arthur, C. Rochelle, C. Oda & H. Takase, 2007. Alteration of bentonite by hyperalkaline fluids: A review of the role of secondary minerals. Physics and Chemistry of the Earth, Parts A/B/C 32(1):287-297.

[3] Pusch, R., H. Zwahr, R. Gerber & J. Schomburg, 2003. Interaction of cement and smectitic clay—theory and practice. Applied Clay Science 23(1):203-210.

GAS GENERATION FROM ORGANIC WASTE OVER A 7 YEARS PERIOD: IMPLICATION FOR THE MANAGEMENT OF LOW AND INTERMEDIATE LEVEL RADIOACTIVE WASTE

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Deep geological repositories have been adopted in many countries for the permanent disposal of low and intermediate level radioactive waste (LILW). One of the primary focus when assessing long residence times of LILW in geologic facilities are: how gas pressure affects re-saturation with meteoric water of underground cavities and how these two forces affect the transport of aqueous radionuclides by groundwater and gaseous radionuclide migration through rock fractures or shaft seals; and how microbial processes affect both the waste and the speciation and transport of radionuclides present in the waste. Over long time scales, *in situ* anaerobic biodegradation of the non-radiological components of the waste is expected to produce:

1. gas and volatile compounds which could result in pressure build-up reducing the re-saturation rate of an underground cavity and delaying migration of soluble radionuclides and

2. acids which can affect the initial integrity of the host bedrock and shaft seals that isolate and contain the radioactive waste.

Hydrogen, carbon dioxide, methane and other volatile compounds are the gasses expected to be generated by the biodegradable organic components of the waste. We monitored the gas pressure evolution, headspace gas composition, and microbiology of candidate organic waste spanning over a seven year period. The gas pressure evolution and changes in gas composition are interpreted according to the fungal, bacterial and archaeal composition of the candidate waste and in terms of the functional genes for methane and acetate formation, which are both processes that consume hydrogen and carbon dioxide, reducing pressure build-up in an underground cavity. In our experiment, methane formation appeared low while acetate formation was prevalent. In such scenario, acetogens could produce acidity and locally impact barriers, which may need to be considered in the design of shaft seals. Long-term safety cases of repositories typically have sufficient margins of safety to account for the presence of acetogens. However, including acetogenesis in safety assessments of repositories will reduce uncertainty.

CRIEPI'S MICROBIAL RESEARCH ON NUCLEAR WASTE DISPOSAL

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We introduce our research activities regarding Microbiology In Nuclear waste Disposal (MIND).

Microbial impacts on radionuclide migration: To demonstrate microbially degraded migration enhancement of carbon-14 organic compounds released from low-level radioactive waste repository, the natural-gradient tracer test using ^{13}C-labeled acetate under anaerobic condition was carried out in a tunnel at about 100 m below ground in a Neogene pumice tuff rock aquifer, Rokkasho, JAPAN. Three boreholes were drilled: one for injecting a tracer and two for collecting the tracer. The distances between the boreholes for injection and recovery of tracer were 120 and 150 mm, respectively. After tracer injection (^{13}C-labeled sodium acetate [1-^{13}C] + KBr), the ground-water effluents from the boreholes were collected at specific intervals of time, and the concentrations of dissolved ions (acetate, bromide, and ^{13}C-bicarbonate), total cell count, viable count, and microbial community using molecular microbiological technique were analysed. After 30 days from tracer injection, ^{13}C-bicarbonate ions were detected along with bromide ions as a tracer of groundwater flow, which were faster than the acetate ions. These breakthrough curves show that acetate was degraded to bicarbonate by anaerobes, possibly sulfate-reducers and methanogens, during transport in the aquifer, thus causing acceleration of the migration rate for radioactive carbon released from the repository [1].

Microbially mediated redox changes in nuclear waste disposal: In order to assess the microbial impacts on the geochemical processes around the nuclear waste repository, the laboratory jar experiments were conducted using the deep sedimentary rock and groundwater in the Horonobe URL, Japan. In the experiments, pulverized rock and groundwater were suspended in the jar and the redox changes were induced by aeration and discontinuation to sediment slurry, which simulated the redox process occurring during operation of nuclear waste repositories. During the experiments, redox potential, pH and dissolved oxygen in the slurry were monitored, and also the concentrations of dissolved ions and head space gasses in the jar were analysed. In addition, microbial DNA was extracted from the slurry, and analysed the response of microbial communities toward the geochemical changes. As a results, after discontinuation of air exposure with lactate and acetate amendments as an electron donor, redox potentials decreased from ca. +100 mV (vs. Ag/AgCl) to -600 m V for lactate and -300mV for acetate, and the microbial communities were changed with the redox potentials of the slurry, and also the sequential terminal electron-accepting process (TEAPs) such as aerobic respiration, iron reduction was observed. These results indicated that the microbial activities would affect the geochemical changes around nuclear waste repositories [2].

MIC of carbon steel in compacted buffer materials: To determine the dry density of buffer materials to suppress microbial activities in compacted buffer materials, we carried out the MIC experiments during 12 months using buffer materials (Kunigel V1:Silica sand=7:3) at different dry densities, 1.0, 1.3 and 1.6 Mg/m^3. The increase of dry density of buffer materials reduced microbial activities and little corrosion occurred, except for at the density of 1.0Mg/m^3. Even at 1.6 Mg/m^3, however, the increase of gene copies were detected by quantitative PCR using bacterial 16S rRNA primer set [3].

[1] Nagaoka T. et al., (2010). Field-scale tracer test of ^{13}C-labelled acetate in a pumice tuff aquifer: Implication for microbially enhanced migration, CRIEPI report, V09029 (in Japanese)
[2] Nagaoka T. et al., (2014). Laboratory simulation of geochemical disturbance in the sedimentary subsurface environment. ISSM2014.
[3] Hirano S. et al., Bio-corrosion of carbon steel in compacted buffer materials, in preparation

Gas Generation Experiment with LLW – Corrosion of Metal Samples

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INTRODUCTION

In Finland low-level radioactive waste (LLW) produced by nuclear power plants (activity < 1 MBq/kg) includes scrap metals and considerable amounts of paper products, cardboard, cotton gloves, natural rubber and plastics. Compressible LLW is compacted in carbon steel drums and disposed in repositories situated inside the bedrock in the plant sites at a depth of 60–110 meters. The biodegradation of cellulose-based LLW in anoxic conditions can result in gas generation and accelerate corrosion, and enhance the mobility of radionuclides from the repository to the surrounding environment.

GAS GENERATION EXPERIMENT

In 1997 the Gas Generation Experiment (GGE) was constructed to simulate the amount of gases generated from LLW to estimate the risks for the final disposal (Fig. 1). In GGE carbon steel drums with the volume of 200 L were filled with LLW and the GGE was filled with river water. The amount of cellulose-based materials inside the drums varied from 5 to 95 w-%. The GGE has been monitored for volume and composition of generated gas, water chemistry and microbiology [1,2,3]. Microbiological samples have been taken from the water at different locations in the tank.

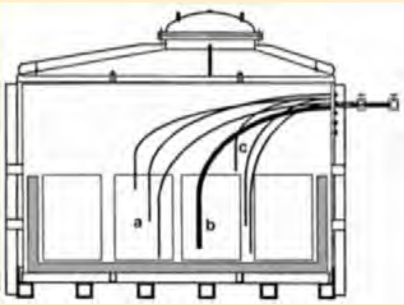
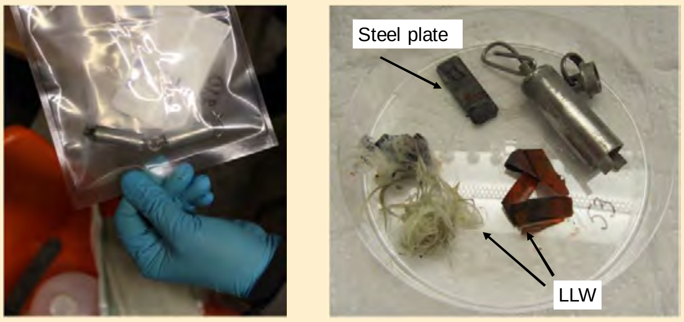


Figure 1. The gas generation experiment GGE with waste drums containing LLW waste and sampling lines for water samples and capsules. Before closing the GGE was filled with local river water and has been maintained at temperature of 8-11°C. *(Small et al., 2008).*

CAPSULES

Capsules containing a piece of drum steel and LLW were loaded to the experiment (Figs. 2 and 3). They were located in within waste drums and in tank water. Capsules have been removed at certain intervals and used to study the corrosion rate of steel (ISO 8407) and corrosion products with an X-ray diffraction spectrometer (XRD).



Figures 2 and 3. Capsules containing LLW and a piece of drum steel have been loaded to the GGE tank in 2001 and removed at certain time points (2013,2015 and 2017) to study corrosion and microbiology.

RESULTS AND DISCUSSION

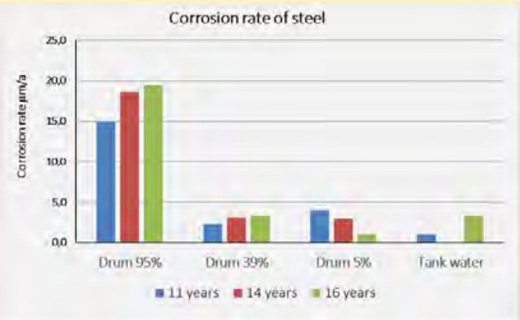


Figure. 4. Corrosion rate of steel in different compartments of GGE (tank water and drum containing various amounts of cellulose) after 11, 14 and 16 years.

Table 1. The amount of methanogens (mrcA gene copies), pH, dissolved organic carbon (DOC) and concentration of Fe(II) in water samples taken from different compartments of GGE in 2002 and 2013. The amount of *mrcA* gene copies is measured by quantitative PCR. nd=not determined

	Tank water Drum lid level		Drum with 95% of LLW		Drum with 5% of LLW	
	2002	2013	2002	2013	2002	2013
pH	9.4	7.0	6.4	nd	6.4	6.5
Fe (II) (mg/L)	1.2	53	1600	nd	420	420
DOC (mg/L)	78	25	3800	nd	1200	600
***mrcA* (copies/mL)**	4 x10^3	3 x 10^5	nd	8 x 10^6	nd	2 x10^5

- The corrosion rate of steel was highest inside the drum containing 95 w-% of cellulose-based LLW (Fig. 4).
- The concentration of soluble ferrous iron Fe^{2+} was also high in water sample taken from this drum indicating dissolution of the carbon steel (Table 1).
- Alkaline conditions in the tank water in the beginning of this study could have protected the steel from corrosion. The pH conditions inside the drums have been close to neutral during the whole experiment.
- The amount of DOC was three times higher in the drum with 95 w-% of cellulose compared to the drum with 5% of cellulose.
- One possible reason for more rapid corrosion rate can be the increased microbial activity. The amount of methanogens was one logaritmic unit higher in the drum with 95% of LLW.
- The main corrosion product in steel plates in all GGE compartments was siderite $FeCO_3$, which is generally formed under methanogenic and acetogenic conditions.

REFERENCES

[1] Small, J., Nykyri, M., Helin, M., Hovi, U., Sarlin, T., Itävaara, M. 2008. Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. Appl. Geochem. 23, 1383–1418.

[2] Small, J., Nykyri, M., Vikman, M., Itävaara, M., Heikinheimo, L. 2017. The biogeochemistry of gas generation from low and intermediate level nuclear waste: modelling after18 years study under in situ conditions. Appl. Geochem. 84, 360-372.

[3] Vikman, M., Marjamaa, K.,Itävaara, M. 2016. Microbial degradation of LLW under repository conditions- case TVO. MIND Project Annual Meeting 1 - Extended Abstracts, 2 - 4 May 2016, Granada, Spain, Book of Abstracts. European Union, 39-42.

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The fate of nickel during isosaccharinic acid (ISA) biodegradation relevant to nuclear waste disposal

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Background:

- Intermediate-level radioactive wastes (ILW), which contain cellulosic wastes, will be encapsulated & disposed in a cementitious geological disposal facility (GDF)
- Cellulosic items are expected to undergo alkaline degradation in the wastes, under evolving hyperalkaline conditions (pH ~12), forming isosaccharinic acid (ISA)
- ISA is a stable, water-soluble, low molecular weight compound [1] that forms strong complexes with radionuclides, such as Ni-59 and Ni-63 [2]
- Microorganisms in the pH-neutral geosphere of a GDF can metabolize ISA as carbon source & immobilize radionuclides as bio-precipitates [3]

Aims & objectives:

- Study microbial enrichment cultures prepared from a lime workings site in Buxton, UK [4] grown on an ISA-Ni-complex with Fe(III) as terminal electron acceptor in
- Analyze fate of nickel during biodegradation and identify mineralogical end products as well as key microorganisms facilitating ISA degradation

Material & Methods:

Incubations prepared in minimal medium [5] with 1% microbial inoculum from a lime workings site in Buxton, UK [6], with 2 mM $Ca(ISA)_2$, 25 mM Fe(III) oxyhydroxide and spiked with 0, 0.1 or 1mM Ni.

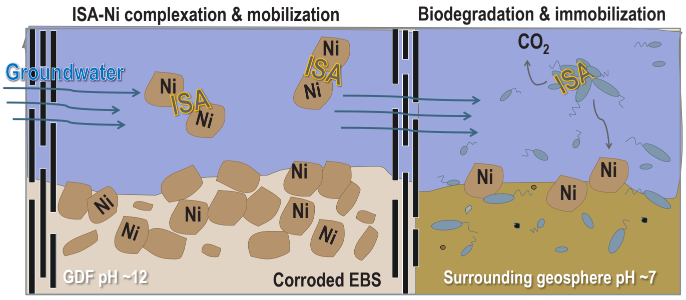


Figure 1: Schematic of Ni mobilization from a GDF (pH ~12; left) & transport into geosphere (pH ~7; right).

Results:

Geochemistry:

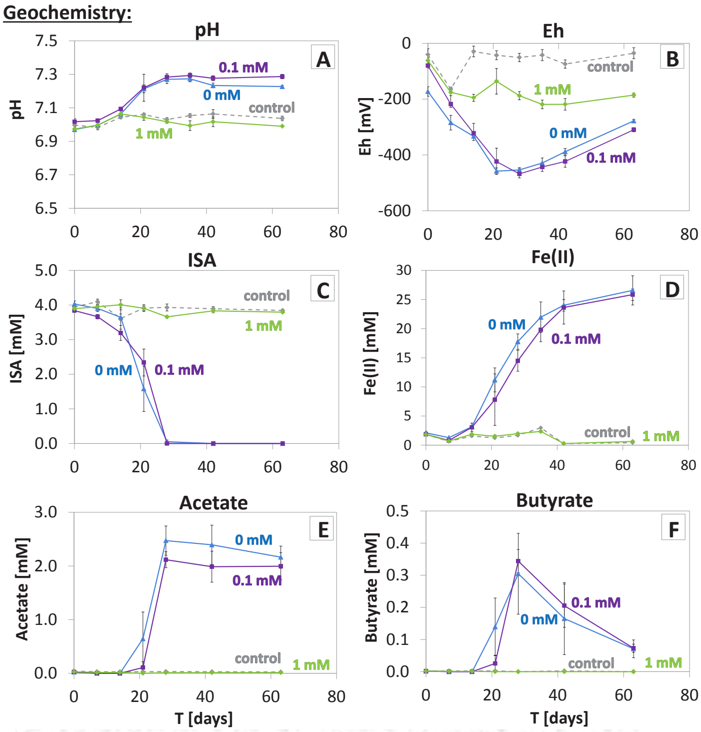


Figure 2. Geochemistry in incubations, showing A) pH; B) Eh; C) ISA; D) Fe(II); E) acetate and F) butyrate with time. Treatments are: Sterile control with 1 mM Ni (•), and microbially active experiments at 0 mM Ni (•), 0.1 mM Ni (•) and 1 mM Ni (•).

Microbial community analysis:

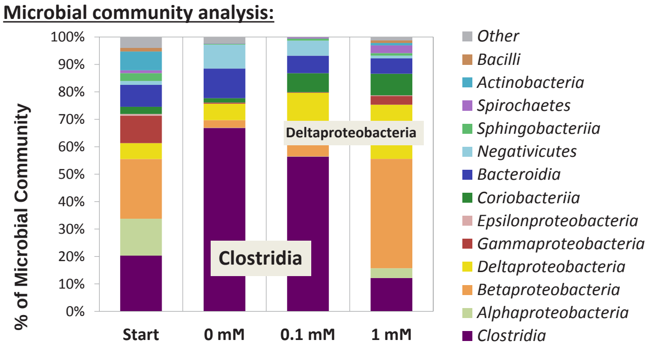
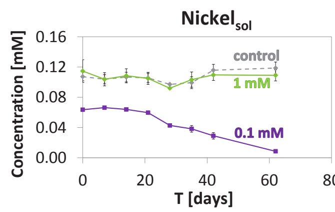


Figure 3. 16S rRNA gene sequencing analysis of microbial phylogenetic classes before & after enrichment on ISA-Ni complex.



Fate of nickel:

Figure 4. ICP-AES analysis of Ni [mM] after ISA-Ni biodegradation in sterile control with 1 mM Ni (•), and microbially active experiments at 0.1 mM Ni (•) and 1 mM Ni (•).

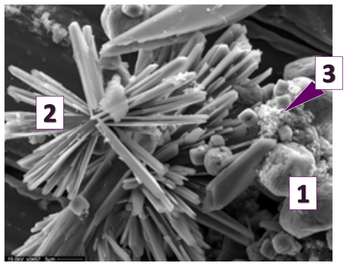
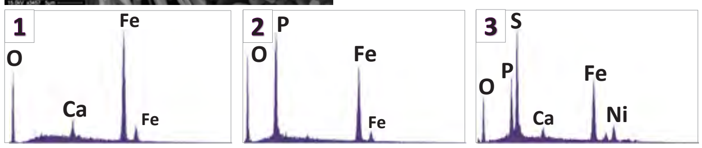


Figure 5. ESEM image after incubation of ISA-Ni experiment at 0.1 mM Ni. Three mineralogical phases (identified by XRD):

1) crystalline Fe(II)carbonate ($FeCO_3$, siderite)
2) crystalline Fe(II)phosphate ($Fe_3(PO_4)_2*8H_2O$, vivianite)
3) amorphous phase containing mainly S, Ni & some Fe (millerite, mackinawite)



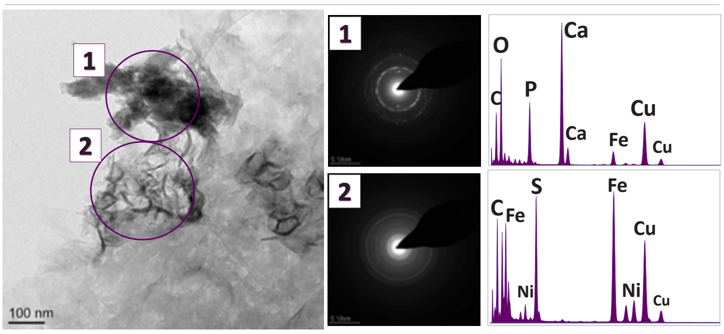


Figure 6. TEM image after ISA biodegradation at 0.1 mM Ni concentration showing 1) crystalline area with Ca-P-O-Fe and 2) amorphous area with Fe-S-Ni.

Conclusions:

- Stimulated microbial community dominated by *Clostridia* sp. (48%) and *Geobacter* sp. (13%), sulfate reducers *Desulfovibrio* sp. also detected (1.5%) by 16S rRNA gene sequences
- Nickel removal only noted during ISA metabolism at 0.1 mM Ni , but removal inhibited at 1 mM Ni due to toxicity
- >90% nickel removal via amorphous nickel-sulfides at 0.1 mM Ni
- SRB key microbes for Ni precipitation

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References

[1] Whistler, R.L. & BeMiller, J.N. (1958) Adv. Carbohydr. Chem., 13, 289-329. [2] Greenfield, B.F., et al. (1992) Mater. Res. Soc. Symp. Proc., 257, 299-306. [3] Bassil, N.M., et al. (2014) ISME J., 1-11. [4] Williamson, A.J., et al. (2013) Appl. Environ. Microbiol., 79(11). [5] Lovley, D.R., et al. (1984) Appl. Environ. Microbiol, 48, 81-87. [6] Williamson, A.J., et al. (2013) Appl. Environ. Microbiol., 79(11).

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Can microbes help stabilise radioactive waste in the subsurface

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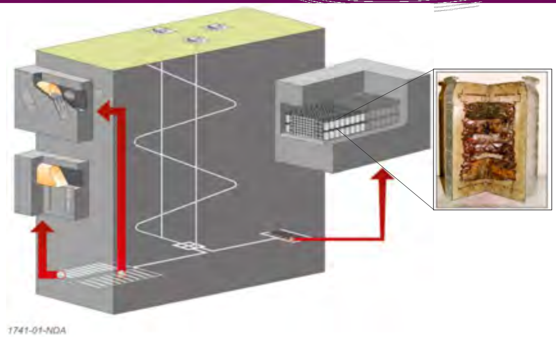
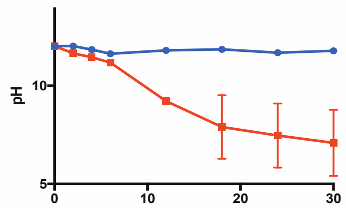
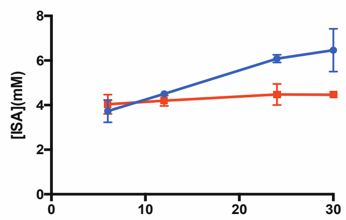
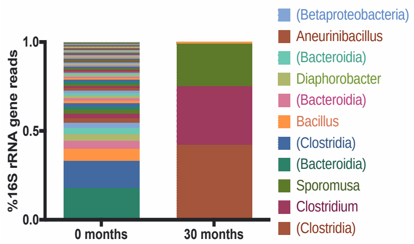
- **Intermediate-level radioactive waste, which contain cellulosic materials, will be encapsulated in concrete and disposed of in a geological disposal facility (GDF)**
- **Cellulose is abiotically degraded under the anoxic, high pH (pH >12) conditions that are expected in a GDF, to produce Isosaccharinic acid (ISA)**
- **ISA can bind to and mobilise some radionuclides like:**
  - **Eu(III), Am(III), Th(IV), Np(IV), U(IV)**
- **Increased radionuclide mobility can potentially lead to biosphere contamination**



Microbial degradation of cellulose under hyperalkaline conditions

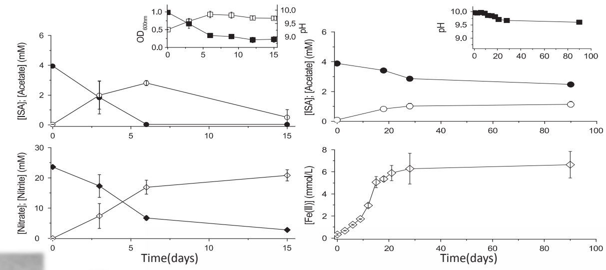


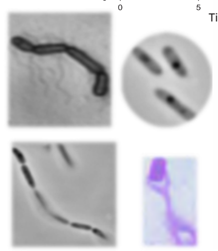


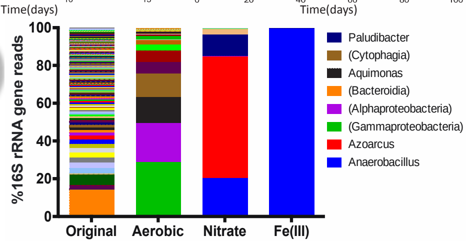


Bacteria present in hyperalkaline samples from a legacy lime-kiln in Harpur Hill, Buxton, degraded tissue paper at a starting pH of 12.2, which was accompanied with a cessation of abiotic ISA production. These samples were dominated by Clostridia.

Microbial degradation of ISA at high pH

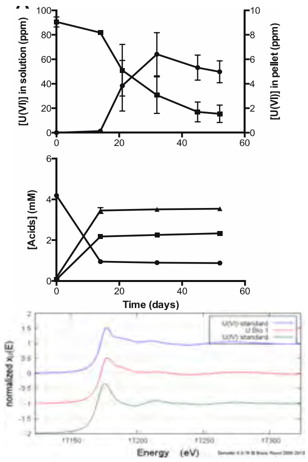


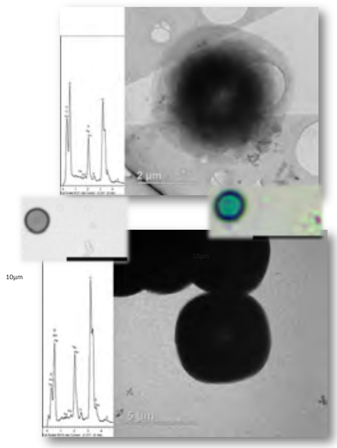




Alkaliphilic bacteria growing in minimal media at pH 10, degraded ISA (which was added as the only electron donor) under a number of biogeochemical conditions. Bacterial diversity dropped under anaerobic conditions.

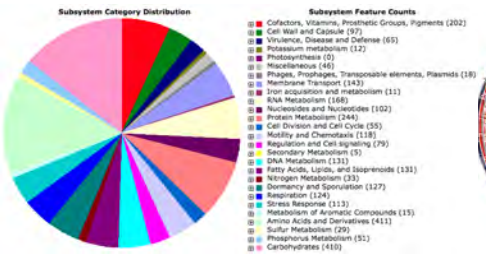
Bacillus sp. nov., an alkaliphilic bacterium which can immobilise U(VI)

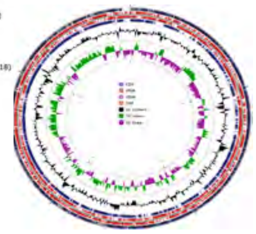




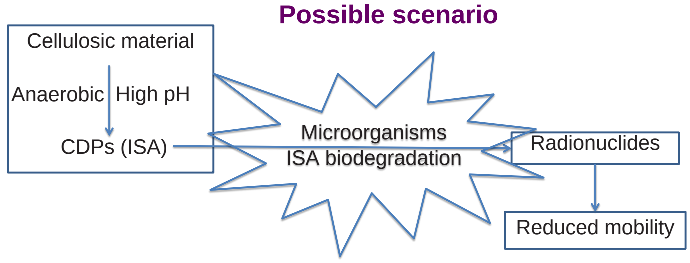
In the presence of U(VI) in solution, growth of the novel obligate alkaliphilic bacterium *B. sp.* led to the precipitation of U(VI) out of solution, with very little recovery after treatment with 10 mM HCl. TEM and EDS analysis show uranium associated with organic structures resembling bacterial spores.

Bacillus sp. genome





Possible scenario



Conclusions

- **Collectively, these results indicate that bacteria can potentially reduce radionuclide transport in the subsurface through 1) prevention of the production of organic complexants (like ISA), 2) degradation of these complexants, or 3) through direct radionuclide immobilisation.**
- **Ongoing work includes further studies on the biosorption/bioaccumulation of U(VI) by this bacterium, accompanied with transcriptome analysis.**

References

Bassil et al. 2015. *Mineralogical Magazine* **79**:1433-1441. doi: 10.1180/minmag.2015.079.6.18

Bassil et al. 2015. *The ISME Journal* **9**:310-320. doi: 10.1038/ismej.2014.125.

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Acknowledgement

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REDUCTION OF Se(IV) TO AMORPHOUS AND CRYSTALLINE Se NANOSTRUCTURES BY *STENOTROPHOMONAS BENTONITICA*: IMPACT ON Se MOBILITY WITHIN THE CONCEPT OF RADIOACTIVE WASTE REPOSITORIES

Ruiz-Fresneda M.A. [1*], Gomez-Bolivar J.[1], Delgado-Martin J. [1], Fernandez-Cantos M.V. [1], Merroun M.L.[1]

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BACKGROUND

Deep geological repositories (DGR) have been proposed as one of the best option for the disposal of radioactive wastes in the near future. These repositories consist of the encapsulation of radioactive wastes in metal containers surrounded by natural and artificial barriers like bentonite clay formations. Specifically Spanish bentonites have been selected for their possible use as an engineered barrier in DGR because of their well-characterized mineralogical and geochemical properties. The recently described new species *Stenotrophomonas bentonitica*, isolated from Spanish bentonites, interact efficiently with elements relevant for DGR like selenium (Se). This strain is able to reduce selenite [Se(IV)] to less toxic forms [Se(0)] producing nanoparticles under aerobic conditions. Scanning Transmission Electron Microscopy (STEM) equipped with High-Angle Annular Dark Field (HAADF) detector combined with Fast Fourier Transform (FFT) demonstrated the production of SeNPs with different morphologies (*e.g.* spheres, hexagons, and nanowires) and distinct crystallographic properties (amorphous (*a*-Se) and trigonal selenium (*t*-Se)). In addition, EDX (Energy Dispersive X-ray) along with elemental mapping confirmed the presence of selenium and sulphur in the nanoparticles. A time-dependent transformation from *a*-Se nanospheres to different *t*-Se nanostructures has been proposed. The production of crystalline Se by *S. bentonitica* provide promising results within the DGR concept due to their high settling efficiency and low solubility. These results demonstrate the potential impact of microorganisms isolated from bentonites on the long-term safety of the DGR system.

METHODS

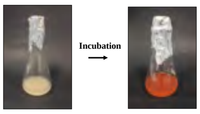
STEM/HAADF
Scanning Transmission Electron Microscope associated with a High Angle Annular Field Detector

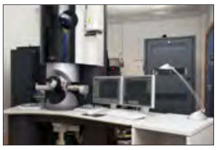
FFT
Fast Fourier Transform (FFT)

SAED combined with HRSTEM
Selected Area Electron Diffraction (SAED) and High-Resolution Scanning Transmission Electron Microscopy (HRSTEM)

EDX
Energy Dispersive X-ray analysis

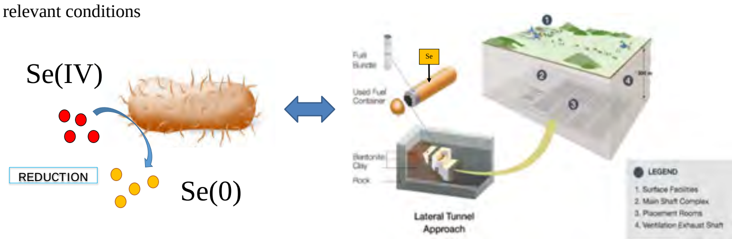
***S. bentonitica* cells + Se(IV)**





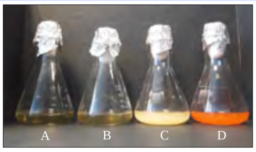
OBJECTIVES

To study the chemical speciation of Se associated by the bacterial strain *Stenotrophomonas bentonitica*, isolated from bentonite formations of Cabo Gata (Almería, Spain), under disposal relevant conditions



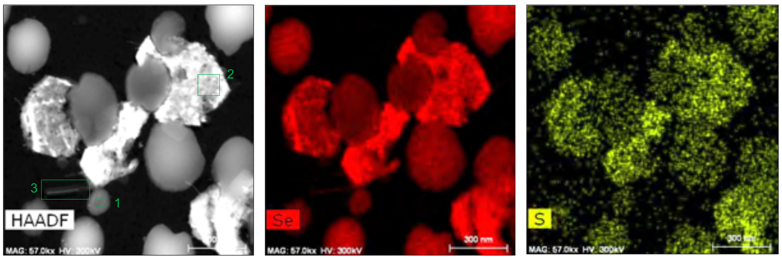
RESULTS

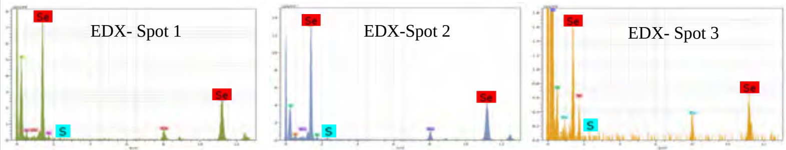
Biological selenium reduction



- No colour change were observed in abiotic (A) and dead cells (B) samples amended with Se(IV) as well as untreated cultures (C)
- Se(IV)-treated cultures turned into an intense red colour after 24 h (D)
- The Se(IV) reduction is mediated by a biological process

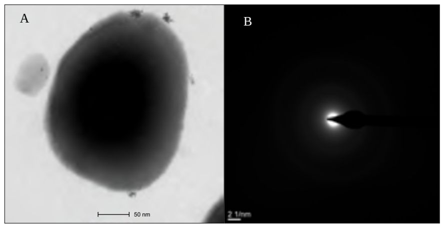
HAADF/STEM: elemental composition of reduced Se nanostructures



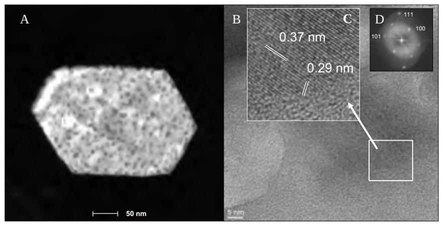


- HAADF/STEM pictures showed the presence of different Se nanostructures: i) nanospheres (spot 1); ii) hexagons (spot 2) and iii) nanowires (spot 3) when *S. bentonitica* cells were grown for 144 h in the presence of Se(IV)
- EDX element-distribution maps derived from these nanostructures confirmed their Se and S composition

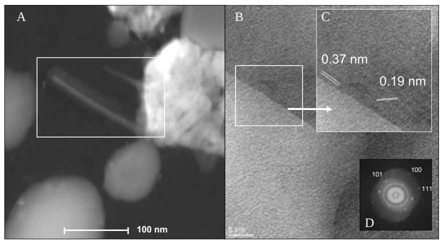
HAADF/STEM: structural characterization



i) Selenium nanospheres (A) presented an amorphous nature as revealed Selected Area Electron Diffraction (SAED) (B)

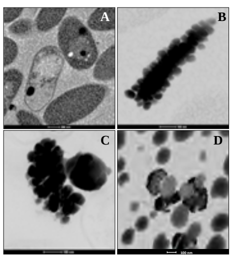


ii) The HRTEM image from an hexagonal-shaped nanoparticle (A, B and C) showed 2 distinct lattice spacings of 0.37 and 0.29 nm corresponding to (100) and (101) planes of trigonal (*t*-Se), respectively. The lattice spacing values obtained for all the diffraction rings from the FFT (D) of the image are in agreement with the trigonal phase of Se



iii) The HRTEM image from an individual nanowire (A, B and C) showed 2 distinct lattice spacings of 0.37 and 0.19 nm corresponding to (100) and (111) planes of *t*-Se. The FFT of the image further confirms the trigonal phase of Se(D)

Biotransformations of amorphous Se nanospheres to trigonal Se nanostructures

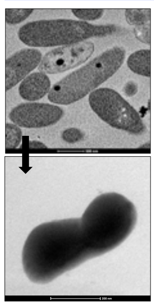


The production of different Se nanostructure shapes by the cells is a time dependent process

- After 24 h, individual amorphous Se (*a*-Se) nanospheres were synthesized (A)
- The Se nanospheres start to coalesce forming aggregates after 48 h (B) and 72 h (C)
- One-dimensional (1D) trigonal Se (*t*-Se) nanostructures (nanowires, hexagons, etc.) and *a*-Se nanospheres were observed after 144 h (D)

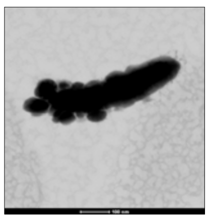
The mobility of Se within the DGR system may be significantly reduced due to the high settling efficiency and low solubility of crystalline Se

Proposed transformation mechanism

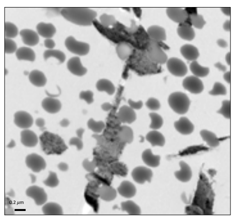


Intracellular accumulation of *a*-Se nanospheres (after 24 h)

Extracellular release of *a*-Se nanospheres and beginning of their aggregation (after 48 h)



Growth of *a*-Se nanospheres aggregates probably on the axis of flagella-like proteins (after 72 h)



Formation of 1D *t*-Se nanostructures with different shapes after 144 h

CONCLUSIONS

- *S. bentonitica* is able to produce crystalline Se(0) nanostructures (*t*-Se hexagons and nanowires) with a high settling efficiency and low solubility from toxic Se oxyanions such as Se(IV). For this reason, the mobility of produced selenium in the environment may be significantly reduced
- *S. bentonitica* has a potential impact on the long-term safety of the DGR by changing the speciation of Se through its reduction and formation of crystalline SeNPs

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Centro de Instrumentación Científica

Modelling of microbial processes with PHREEQC

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1. Background

In order to evaluate the significance of microbial processes, such as the mediation of slow redox reactions between oxidised and reduced major element and trace radionuclide species in radioactive waste repositories, it will be necessary to represent the kinetic microbial processes in reactive-transport models.

- Such models need to be developed and tested using data from bench and underground rock laboratory (URL) experiments and may assist in the interpretation of complicated URL experiments.
- Ultimately, once such models are validated they should be able to be up-scaled both spatially and temporally and incorporated in models of geological repositories.

Here we present a PHREEQC [1] biogeochemical-diffusive transport model of nitrate evolution in a clay environment in the presence and absence of hydrogen gas (H_2) in the Mont Terri BN experiment [2]. The effect of H_2 was studied as this gas will be formed as a result of, radiolysis of bitumen and water or, the anaerobic corrosion of metals.

2. BN pulsed H_2 experiment

In this specific experiment nitrate (NO_3^-) was injected in Interval 1 of the BN experiment at an initial concentration of 15 mM in Opalinus Clay artificial pore water and a pulse of H_2 was applied.

- Initially the bore hole fluid was circulated through a gas exchange vessel (Fig 1, HEU) containing argon and a UV analytical system that monitored the slow attenuation of NO_3^-, mainly by diffusion into the Opalinus Clay.
- After 54 days the argon gas in the HEU was replaced by H_2, which after a lag phase, stimulated denitrification and the formation of nitrite (NO_2^-) and nitrogen (N_2) gas.
- After a further 24 days, when the NO_3^- concentration had declined to around 0.5mM, the gas phase was changed back to argon.

4. Spatial representation and diffusion modelling

The well mixed fluid circulation system of the BN experiment (Fig 1) is represented in PHREEQC as a single cell, that has an associated gas phase to represent the HEU. The volumes of the water and gas phase cells are those of the experiment [2]. 22 "Stagnant" cells are also associated with the main cell to represent radial diffusion of NO_3^- and other species with through the filter screen, void space and the Opalinus Clay (Fig 2).

- Microbial activity is assumed to occur in the circulating fluid, filter screen, void space and the first cell of the Opalinus Clay representing an excavation disturbed zone (EDZ).
- In the remaining cells only chemical speciation, equilibrium reactions and diffusive transport are assumed to occur.

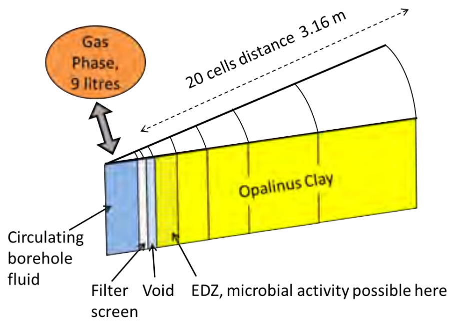


Fig 2. Schematic of the PHREEQC model of the BN experiment.

The diffusion model, has been parameterised from initial tests of the BN experiment that monitored the decline of injected Br^- concentration as a ratio to its initial concentration (C/C_0) [2] (Fig 3). Br^- is a non-reactive anionic tracer with similar diffusion behaviour to NO_3^- and NO_2^-.

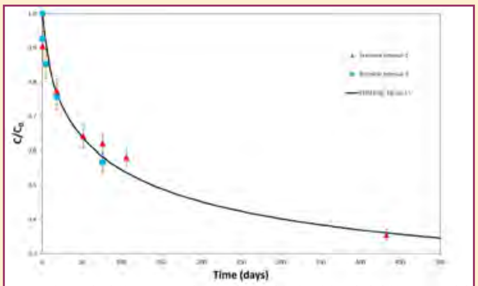
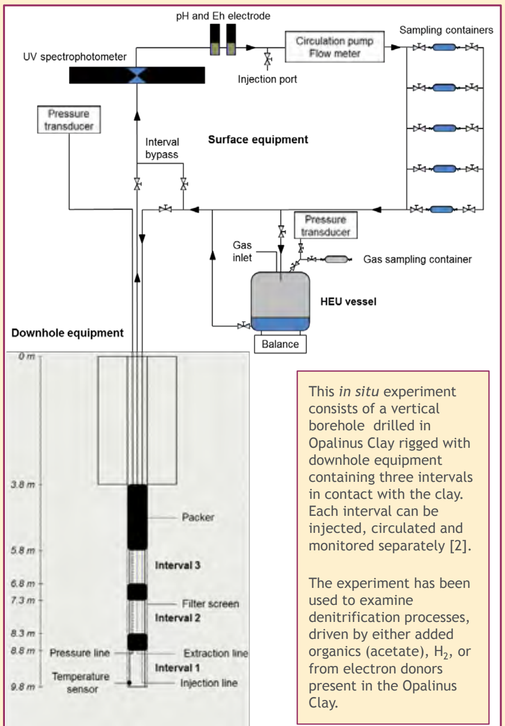


Fig 3. Bromide diffusion test data and fitted PHREEQC radial diffusion model

3. The Bitumen – Nitrate-Clay (BN) experiment

The BN experiment at the Mont Terri rock laboratory has been designed to examine possible geochemical and/or gas-related perturbations induced in the near field of a geological repository for the disposal of nitrate-containing bituminised waste [2].



This *in situ* experiment consists of a vertical borehole drilled in Opalinus Clay rigged with downhole equipment containing three intervals in contact with the clay. Each interval can be injected, circulated and monitored separately [2].

The experiment has been used to examine denitrification processes, driven by either added organics (acetate), H_2, or from electron donors present in the Opalinus Clay.

Fig 1. Schematic of the BN experiment, showing downhole intervals, fluid circulation and analysis system and the hydrogen equilibration unit (HEU), Bleyen et al, (2017) [2].

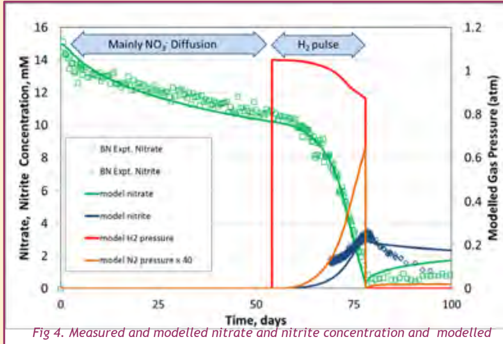


Fig 4. Measured and modelled nitrate and nitrite concentration and modelled gas pressures

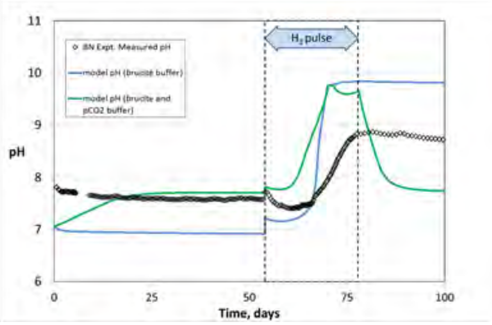


Fig 5. Measured pH during H2 pulsed denitrification and modelled pH considering different buffer constraints

4. Modelling H_2 driven denitrification

Denitrification is a complicated multiple step reduction process that can lead to either N_2 gas or ammonia (NH_3) end products [2]. Nitrite (NO_2^-) is observed as an intermediate to either N_2 or NH_3 formation, while gaseous NO and N_2O can form in the pathway to N_2 gas. In consideration of the results of the BN experiment the following main reactions are considered that involve the formation of NO_2^- as an intermediate in the overall reduction of NO_3^- by H_2,

$$H_2 + NO_3^- \rightarrow NO_2^- + H_2O \qquad \text{Eq 1}$$

$$3\,H_2 + 2\,NO_2^- + 2\,H^+ \rightarrow N_2 + 4\,H_2O \qquad \text{Eq 2}$$

In the PHREEQC model these reactions are implemented as being kinetic processes defined by a Monod type expression, the rate of NO_3^- reduction being,

$$\frac{d[NO_3^-]}{dt} = -[X] \cdot k_{max} \cdot \frac{[H_2]}{K_{H_2} + [H_2]} \cdot \frac{[NO_3^-]}{K_{NO_3} + [NO_3^-]} \qquad \text{Eq 3}$$

Where [NO_3^-] and [H_2] are molal concentrations k_{max} is the maximum substrate removal rate and K_{H2} and K_{NO3} are half saturation constants. [X] is the concentration of biomass, which is assumed to have the general formula $C_5H_7O_2N$. The PHREEQC model can be easily configured to represent different sources of carbon and nitrogen for biomass growth. In this instance the processes are assumed to be autotrophic utilising HCO_3^- and ammonia, which are both present in Opalinus Clay pore water,

$$5HCO_3^- + 10\,H_2 + 5\,H^+ + NH_3 \xrightarrow{\text{Biomass}} C_5H_7O_2N + 13\,H_2O \qquad \text{Eq 4}$$

Growth of biomass [X] is defined by a yield coefficient (Y), the rate of nitrate reduction and a microbe death rate (D).

$$\frac{d[X]}{dt} = -Y.\frac{d[NO_3^-]}{dt} - D[X] \qquad \text{Eq 5}$$

5. Experimental and modelling results

During the 54 days prior to the H_2 pulse, NO_3^- concentration declines by ~4 mM mainly as a result of its diffusion into the Opalinus Clay. Rapid denitrification is associated with the H_2 pulse forming NO_2^-, which slowly declines (Fig 4.)

The experimental results are quite well represented by the model.

- The initial mainly diffusive phase, is represented, using the diffusion model fitted to the Br- tracer tests and including a small amount of denitrification by Opalinus Clay, quantified by previous modelling [2].
- A good fit to the rapid decrease in NO_3^- and increase in NO_2^- concentrations associated with the H_2 pulse is obtained; mainly by adjusting the k_{max} parameters for the NO_3^- and NO_2^- reduction reactions (Eq 1 and 2).
- The model simulates a decrease in H_2 pressure and a small increase in N_2 pressure, which is also observed in the BN experiment [2].

After the H_2 pulse the model simulates increasing NO_3^- concentration, which results from diffusion of NO_3^- back out of the Opalinus Clay once the chemical gradient is reversed. This effect is not seen in the BN experimental data and may indicate that denitrification is occurring within the Opalinus Clay by reaction with organic matter or reduced mineral phases. Similarly, NO_2^- may be affected by reactions with the clay, which are not currently represented in this model.

Fig 5. illustrates the significant increase in pH measured in the BN experiment associated with the H_2 pulse and autotrophic denitrification reactions (Eq 1, 2 and 4), which consume H^+. The model represents the general pH trend but further refinement of the model is required.

- The model overestimates the maximum pH, approaching pH 10, buffered by brucite ($Mg(OH)_2$) precipitation).
- Imposing a fixed partial pressure of CO_2 (log pCO_2 = -3) also has a buffering effect in the model, buffering at around pH 7.8. However, this underestimates the measured pH after the H_2 pulse (pH 8.7 to 8.9).
- It is possible that aluminosilicate phases may buffer the pH below that of brucite and also pH buffering by such minerals or CO_2 may be subject to kinetic effects over the rapid timescale of the H_2 pulsed stimulation of denitrification in the BN experiment.

6. Conclusions

- H_2 driven denitrification in the BN experiment has been represented using a Monod type microbial growth model, coupled to diffusion and equilibrium chemical speciation.
- Models of this type have potential to be applied in performance assessment of geological repositories.

Acknowledgements

This project has received funding from the Euratom research and training programme 2014 - 2018 under Grant Agreement no. 661880

The BN experiment is co-funded by the Mont Terri consortium, in particular currently by Andra, IRSN, SCK•CEN and FANC.

Achim Albrecht (Andra), Pierre De Cannière (FANC), Charles Wittebroodt (IRSN) , Hugo Moors and Natalie Leys (SCK•CEN), are thanked for their technical discussion of the hydrogen pulsed experiment.

References

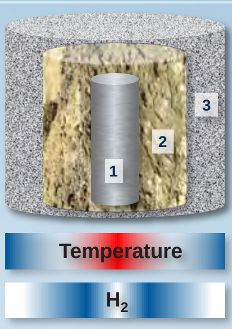
1. Parkhurst, D.L. and Appelo, C.A.J., (2013). Description of Input and Examples for PHREEQC Version 3—A Computer Program for Speciation, Batch-Reaction, One-Dimensional Transport, and Inverse Geochemical Calculations: U.S. Geological Survey Water-Resources Investigations. Chapter 43 of Section A, Groundwater Book 6, Modeling Techniques: Techniques and Methods 6-A43, U.S. Department of the Interior, U.S. Geological Survey, pp 437.
2. Bleyen, N., Smets, S., Small, J., Moors, H., Leys, N., Albrecht, A., De Cannière, P., Schwyn, B., Wittebroodt, C., Valcke, E. (2017) Impact of the electron donor on in situ microbial nitrate reduction in Opalinus Clay: results from the Mont Terri rock laboratory (Switzerland). Swiss Journal of Geosciences.110(1), pp. 355-374.

Bentonite – a natural source for sulfate-reducing bacteria

Nicole Matschiavelli, Sindy Kluge, Andrea Cherkouk

HZDR
HELMHOLTZ ZENTRUM DRESDEN ROSSENDORF

STORAGE OF HIGH-LEVEL RADIOACTIVE WASTE (HLW)



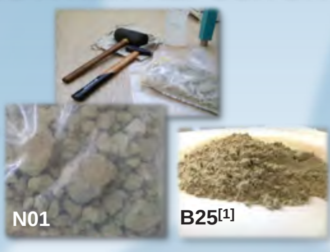
formation of gradients

For the deep geological disposal of HLW a multi-barrier concept is favoured consisting of a container that includes the HLW (technical barrier (1)), which is surrounded by a geotechnical barrier (Bentonite (2)). These packages are placed into the host rock (geological barrier (3)).

Bentonites are clay minerals which are characterized by a high swelling capacity and a low hydraulic conductivity. Could indigenous microorganisms influence these properties by their metabolic activity?

MIMICING A HLW-REPOSITORY ENVIRONMENT

sampling site (N01): pit mining „Bergschapfl“ (IMERYS, Landshut, Germany)



two Bentonites are analyzed

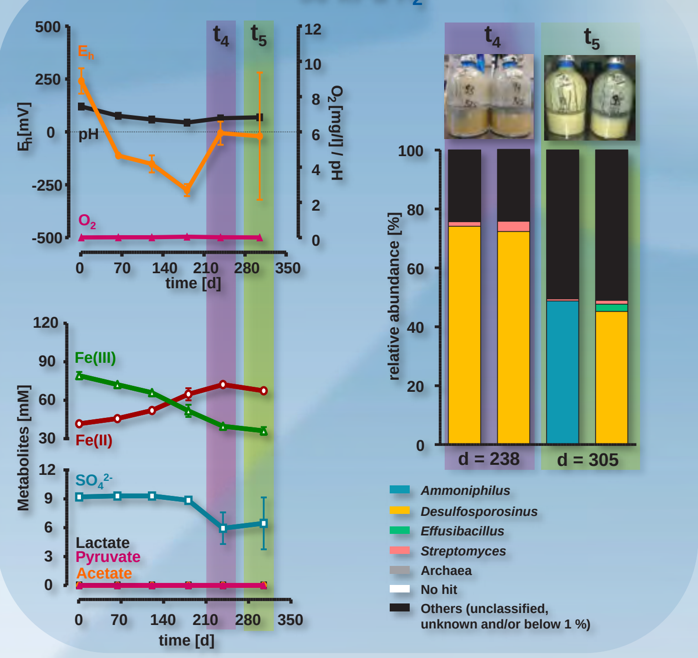
anaerobic, synthetic pore water solution

+ Lactate
+ Acetate
+ H_2

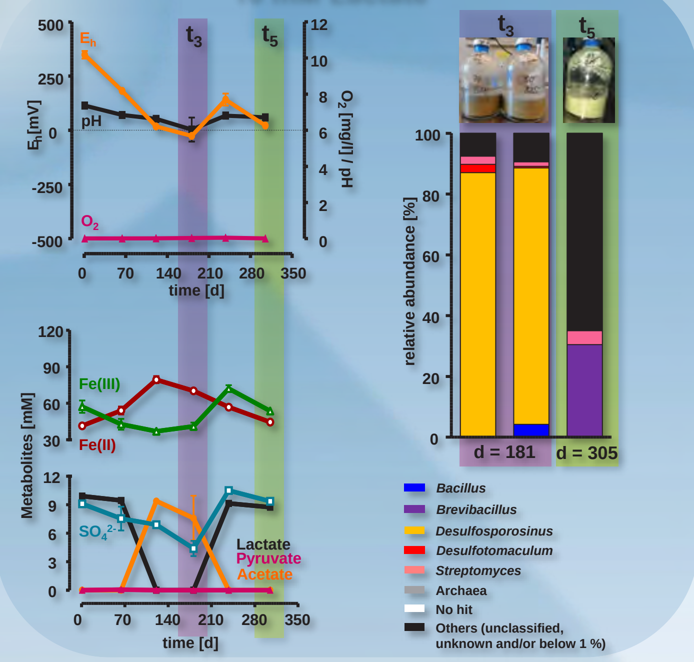
incubation of set up microcosms at 30°C and 60°C

MICROBIAL ACTIVITY & DIVERSITY OF BENTONITE B25 AT 30° C

50 kPa H_2



10 mM Lactate



DNA of Bentonites was isolated by using a modified protocol of Selenska-Pobell[2]. 16S rRNA genes were amplified by using complementary oligonucleotides against the V4-region[3]. Gene fragments were sequenced with MiSeq Illumina (RTL Genomics).

Sulfate-reducing bacteria are dominant and active – potentially influencing the properties of Bentonite!

ACKNOWLEDGEMENTS AND REFERENCES

This work is funded by the Euratom research and training programme 2014-2018 under grant agreement No. 661880.

[1]: Bentonite B25 (processed, industrial; Bavaria) was provided by Stephan Kaufhold (BGR, Hannover, Germany)
[2]: Selenska-Pobell (1995), Direct and simultaneous extraction of DNA and RNA from soil. *Molecular Microbial Ecology Manual* 1.5.1, 1-17
[3]: Caporaso *et al.* (2010), Global patterns of 16S rRNA diversity at depth of millions of sequences per sample. *PNAS* 108:4516-4522

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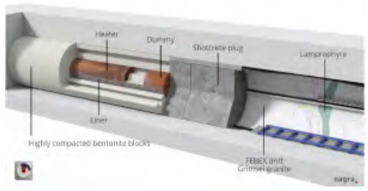
MICROBIAL DIVERSITY AND ACTIVITY IN BENTONITE SAMPLES FROM THE FEBEX PROJECT

Jennifer Drozdowski, Sindy Kluge, Andrea Cherkouk

Storage of high-level radioactive waste – Bentonite as a geotechnical barrier

Multi-barrier concept for the storage of high-level radioactive waste (HLW).
1) technical barrier (container including HLW)
2) geotechnical barrier (bentonite);
3) geological barrier (host rock)

- For the storage of highly radioactive waste in a deep geological repository a multi-barrier concept (Fig. 1) is favoured, which combines a technical barrier (container including the high-level radioactive waste), a geotechnical barrier (e.g. bentonite) and the geological barrier (host rock).
- Bentonite fulfils in this system a sealing and buffering function (geotechnical barrier) due to its properties, namely a high swelling capacity and a low hydraulic conductivity.
- One objective of the Full-scale Engineered Barrier Experiment (FEBEX) is to demonstrate the feasibility of the emplacement of the engineered barrier system (EBS) for high-level radioactive waste disposal



A part of the Full-scale Engineered Barrier Experiment (FEBEX) – Dismantling Project (DP) overview video [1]

The aim of our work is to study the microbial diversity and activity in bentonite samples from the FEBEX – Dismantling Project (DP)

Samples: Bentonite samples from the Full-scale Engineered Barrier Experiment (FEBEX) – Dismantling Project (DP):

Samples B-C-42-8 and B-C-42-14 from section 42 (front of heater),
Samples B-C-47-13 and B-C-47-15 from section 47 (middle of heater)
Samples B-C-54-14 and B-C-54-17 rom section 54 (back of heater)
and samples B-C-60-7 and B-C-60-9 from section 60 (far back)

Experimental setup: Culture-independent approach

- 20 g bentonite for DNA extraction *via* method from Selenska-Pobell [2]
- Amplification of the 16S rRNA genes with primers 519F and 806R [3]
- Send to RTL Genomics for MiSeq Illumina sequencing

Bentonite sample

20 g bentonite for DNA extraction

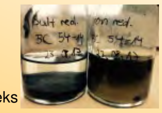
Purification of extracted DNA

Experimental setup: Culture-dependent approach

- Enrichments: 5 g of bentonite (samples B-C-54-14 and B-C-60-9) were added to each 50 ml Medium DSM63 (*Desulfovibrio* medium = S) and Medium DSM579 (*Geobacter* medium = G) and incubated for 24 h at room temperature with shaking
- Transfer from enrichments to anaerobic culture tubes containing 10 ml of media, incubation at 30°C
- DNA extraction of 500 µl inoculated media with PowerWater® DNA Isolation Kit from MoBio
- Amplification of 16S rRNA genes with primers 519F and 806R [3] ⇨ RTL Genomics for sequencing
- Inoculation on plates ⇨ selection and transfer of formed colonies ⇨ phylogenetic identification *via* 16S rRNA gene sequencing

Enrichments: medium for sulphate- and iron-reducers after inoculation

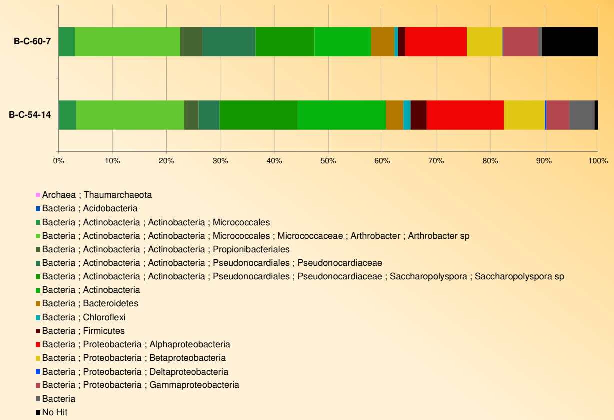
after 10 weeks



Enrichments: medium for sulphate- and iron-reducers after inoculation and incubation for 10 weeks

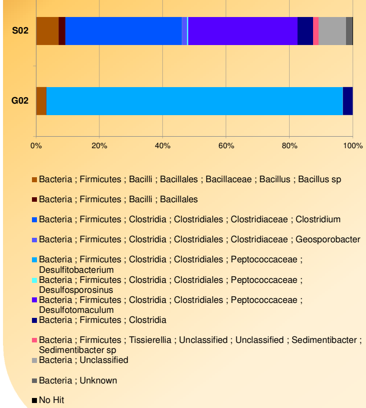
Results: Culture-independent approach

Microbial diversity analysis of samples B-C-54-14 (back of the heater) and B-C-60-7 (far back of the heater)

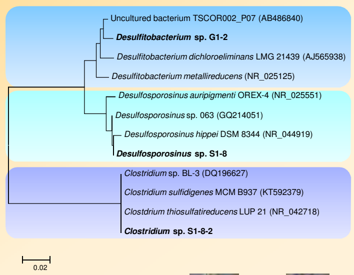


Results: Culture-dependent approach

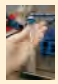
Microbial diversity in enrichments of sample B-C-60-9

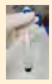


Isolates recovered from sample B-C-60-9



Sulphate-reducer Medium S





Iron-reducer Medium G

Conclusions

- Microbial community in bentonite samples was dominated by Actinobacteria and Proteobacteria *via* culture-independent approach
- Sulphate-reducing and iron-reducing microorganisms could be enriched in selected media
- *Desulfitobacterium* sp., *Desulfosporosinus* sp. and *Clostridium* sp. were isolated from the enrichments
- The enriched and isolated bacteria are known to form spores ⇨ DNA from spores is difficult to extract ⇨ reason why respective species were only detected in low abundance *via* culture-independent approach
- The suitable conditions in the enrichment-media lead to the germination of present spores, which result in metabolically active cells.

Acknowledgements
We thank Karsten Pedersen and FEBEX-DP project partner for providing the samples. Partly funding was received from the Euratom research and training programme 2014 - 2018 under grant agreement No. 661880.

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References
[1] http://www.grimsel.com/gts-phase-vi/febex-dp/febex-dp-introduction
[2] Selenska-Pobell 1995, Molecular Microbial Ecology Manual 1.5.1: 1-17
[3] Caporaso *et al.*, 2011, PNAS 140: 4516–4522.

Possible impact of microbial processes on cementitious materials used during the geological disposal of radioactive waste

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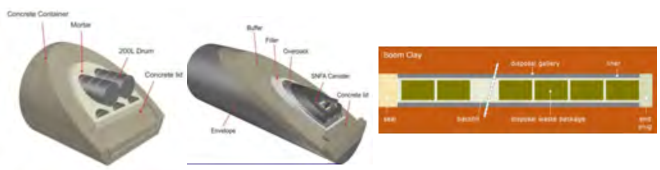
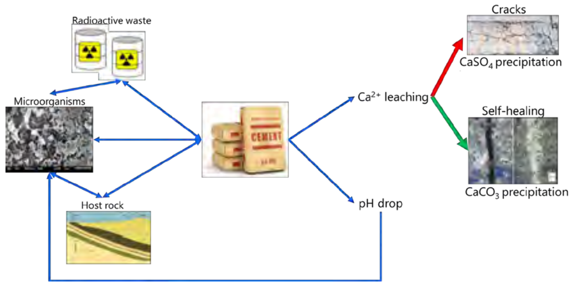
Introduction

During the geological disposal of radioactive waste, cementitious materials are used as part of the engineered barrier, in plugs and seals and in construction or lining of the disposal drift. Consequently, the interactions with and the evolution of these materials with other repository materials, the host rock and its ground water, needs to be assessed.



Cross sections through category B waste monoliths and a schematic longitudinal cross-section of a disposal gallery in the Belgian concept (NIRAS/ONDRAF)

Microbial activity is known to affect the mineralogy, chemistry and structure of cementitious materials, which can be either deleterious or beneficial for the cementitious and its durability.



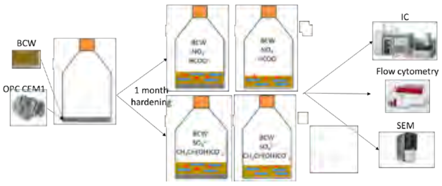
Possible reactions and impact on cementitious materials in which microorganisms can be directly or indirectly involved

Objective

The objective of this study is to investigate whether microorganisms could affect, in a positive or negative way, the long-term evolution of the cementitious materials present in the engineered barriers of a geological repository for radioactive waste in conditions representative for geological disposal.

Experimental set-up

Anoxic batch experiments are running where the Boom Clay microbial population is exposed to Ordinary Portlandite (OPC) CEM I or grown without OPC CEM I.



10 g of OPC CEM I cement was hardened by adding 5 ml sterile Boom Clay pore water. After 1 month, Boom Clay pore water harbouring a microbial community was added and the medium was supplemented with different electron donors and acceptors. The supernatans was monitored by ion chromatography, flow cytometry and SEM. Everything was performed in triplicate in anoxic conditions.

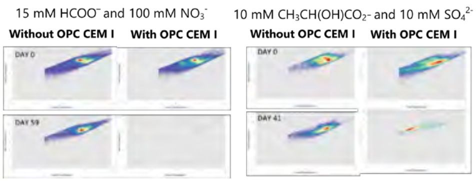
Results

IC of supernatans

IC measurements of the supernatans show that the microbial community is unable to perform nitrate or sulphate reduction in the presence of OPC CEM I but without OPC CEM I, formate is quickly oxidized to CO_2 and lactate to acetate. The pH of conditions exposed to OPC CEM I increased from pH 9,5 to pH 12,5, while in the other conditions, ~pH 10,5 was obtained.

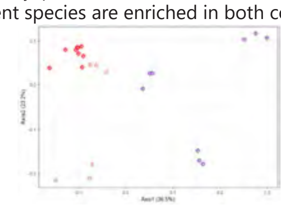
Flow cytometry

The microbial community was monitored by flow cytometry. In the supernatans of conditions exposed to OPC CEM I, no clear viable microbial community was observed.



Flow cytometric analysis visualized by their normalized log-transformed signals on the green (530/30 nm) and red (> 670 nm) fluorescence channels.

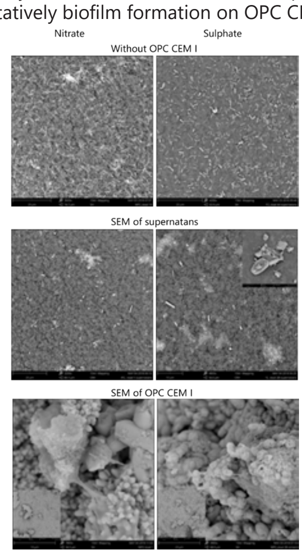
Principal coordinate analysis based on flow cytometry profiles show that without OPC CEM I, different species are enriched in both conditions.



Community beta diversity assessment of Boom Clay Borehole water in the presence of $HCOO^-$ and NO_3^- (orange & red) and NO_3^- (red) and $CH_3CH(OH)CO_2^-$ and SO_4^{2-} (purple),

SEM analysis

SEM analysis indicates intact cells in supernatans and putatively biofilm formation on OPC CEM I



SEM analysis of the Boom Clay microbial community inoculated in the absence of cement (top); supernatans of conditions exposed to OPC CEM I (middle); OPC CEM I (bottom). Left are samples supplemented with $HCOO^-$ as electron donor and NO_3^- as electron acceptor. Right are conditions supplemented with $CH_3CH(OH)CO_2^-$ and SO_4^{2-}.

Conclusions

In conditions with OPC CEM I, the pH of the Boom Clay pore water quickly increased to from pH 9,5 to pH 12,5, while in the absence of OPC CEM I an increase up to ~pH 10,5 was observed. pH 12,5 hampers the microbial community of Boom Clay pore water as no formate dependent nitrate reduction or lactate dependent sulphate reduction is observed. Nevertheless, SEM analysis show intact cells in the supernatans and putatively biofilm structures on the salt crystals present on the cement and in the supernatans, suggesting that the microbial population is not eliminated. More detailed analysis on the microbial community and of the structure of the OPC CEM I is planned.

References

Bertron, A., Understanding interactions between cementitious materials and microorganisms: a key to sustainable and safe concrete structures in various contexts. Materials and Structures, 2014. 47(11): p. 1787-1806.

ONDRAF/NIRAS, Research, Development and Demonstration (RD&D) Plan for the geological disposal of high-level and/or long-lived radioactive waste including irradiated fuel if considered as waste. 2013, NIROND-TR-2013-12 E.

This project has received funding from the Euratom research and training programme 2014 - 2018 under grant agreement No. 661880

Methanogenic archaea residing in Boom Clay borehole water

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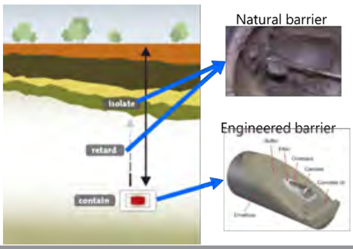
Introduction: deep geological disposal of heat-emitting radioactive waste

Concept: multi-barrier system



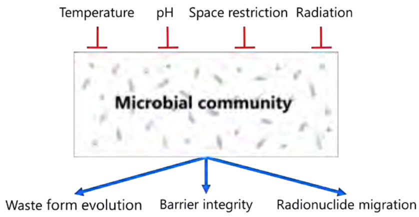
During geological disposal, radioactive waste will be put deep underground in a stable environment. Placing multiple barriers (i.e engineered & natural barriers) between the waste and the biosphere protects both people and the environment from the harmful effects of this waste.

Reference host formation: Boom Clay

- low permeability
- chemical buffering capacity
- self-sealing
- high sorption capacity

Top: Occurrence, thickness and examples of suitable physico-chemical characteristics of the Boom Clay formation in Belgium. The location of the HADES underground research laboratory (225 m) is indicated in white. Bottom: Schematic view of the HADES URL. The PRACLAY gallery is circled in red.

Microbial presence and activity



Microorganisms will be present in a radioactive waste repository, and could, if sufficient active, affect waste form evolution *in situ*, multi-barrier integrity, the geochemical environment and ultimately radionuclide migration. However it remains unclear whether they can be active as the conditions are far from optimal.

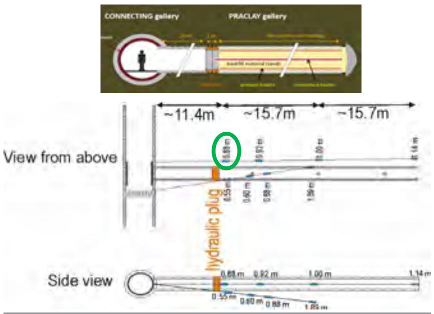
Objectives

To investigate the effect of temperature on the microbial population residing in piezometers installed in the PRACLAY gallery.

Methods & Results

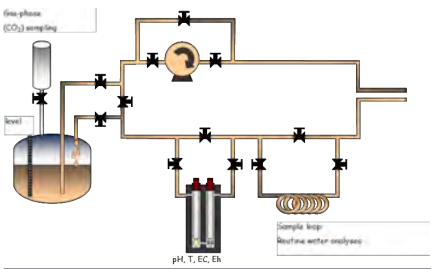
Sampling site

The PRACLAY Heater test simulates a full scale (except time) disposal gallery through all phases. For at least ten years, the test gallery will be heated until 80°C to demonstrate that the thermal load generated by the heat-emitting radioactive waste will not jeopardize the safety functions of the host rock.



Schematic view of the position of piezometers and filters around the PRACLAY gallery. The filter that is monitored – CG35-E11 – is located at 0,88 m circled in green.

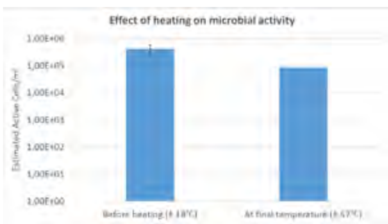
Sampling system



Schematic drawing of the *in situ* circulation system for the pore water sampling, sampling of the dissolved gasses, and the measurement of the pH.

Microbial presence and activity

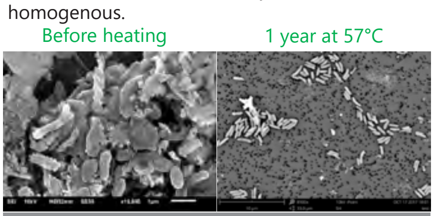
The number of microorganisms present was estimated measuring the amount of intracellular ATP. Heating of the gallery reduced microbial activity from 4.5 x 10^5 to 8.6 x 10^4 cells/ml.



The number of estimated active cells/ml based on intracellular ATP measurements showing the average number and standard deviation of during a period of 2 years before the heating of the PRACLAY gallery and the estimated active cells/ml from one time point after 1 at the final temperature.

Scanning electron microscopy

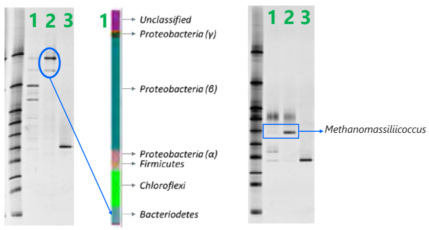
The microbial community present in the samples was visualized by scanning electron microscopy. With respect to there appearance, a highly diverse microbial community seems to be present before heating, while after the heating, the microbial community seems more homogenous.



Scanning electron microscopy picture showing the microbial community present in filter CG35-E11 before heating the gallery (left) and after 1 year at 57°C (right). The scale of the pictures is shown in the black bar.

Denaturing gradient gel electrophoresis

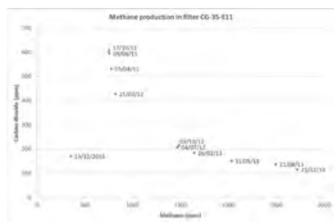
Denaturing gradient gel electrophoresis profiles based on 16S rRNA showed that heating the gallery induced a clear shift in the bacterial and archaeal population.



DGGE profiles based on the 16S rRNA amplicon showing the composition of a) the bacterial community 1) before and 2) after the heating; 3) *Cupriavidus metallidurans* CH34 as positive control; b) the archael community 1) before and; 2) after the heating; 3) *Sulfolobus tokodaii* as positive control.

Methanogenic activity

In situ methanogenic activity was confirmed before heating as CO_2 levels decreased in time and a clear correlation with CH_4 was observed. After heating, methanogenic archaea were still present and CH_4 was still measured, although at lower amounts indicating that methanogenic activity is still possible at 57°C.



CO_2 reduction to CH_4 in time before heating of the gallery (blue) and at the final temperature (orange).

Conclusions

- This study indicates that Boom Clay borehole water is hosting methanogenic archaea and that methane production was induced after installation of a piezometer.
- After 1 year at 57°C, microbial activity decreased and is accompanied with a clear change in the microbial community.

Acknowledgements: This work is partly performed in close cooperation with, and with the financial support of ONDRAF/NIRAS, the Belgian Agency for Radioactive Waste and Fissile Materials, as part of the programme on geological disposal/surface disposal that is carried out by ONDRAF/NIRAS.

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Impact of the electron donor on *in situ* microbial nitrate reduction processes in Opalinus Clay: Results from the BN experiment (Mont Terri rock laboratory)

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ANDRA IRSN FANC AFCN

Mont Terri Project

Introduction

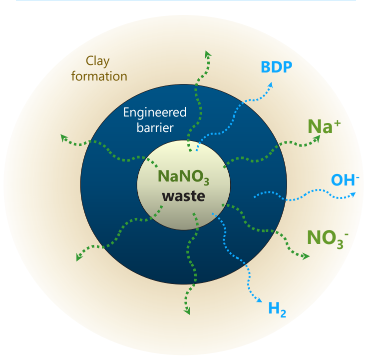
Disposal of nitrate-containing bituminised intermediate-level radioactive waste in a deep clay formation will result in

- leaching of large amounts of $NaNO_3$ and minor organic bitumen degradation products (BDP, *e.g.* acetate)
- production of H_2 due to anaerobic steel corrosion and radiolysis of the bitumen matrix and water.

The release of nitrate could initiate several geochemical and biochemical processes in the near field, potentially affecting the geochemical and mineralogical properties of the host rock.

Bitumen-Nitrate-Clay interaction (BN) experiment

Disposal of nitrate-containing bituminised radioactive waste in clay formation



Possible perturbations due to nitrate leaching from the waste

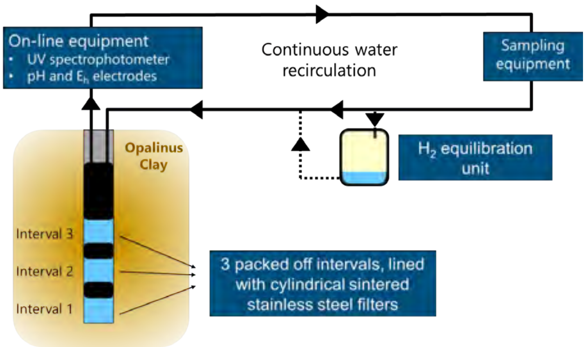
(Bio)chemical perturbation

- **Oxidation of clay components**
  → Reducing capacity of clay ↓
  → Effect on radionuclide (RN) speciation?
  - ? Effect of contaminants from
    - waste (*e.g.* acetate, H_2)?
    - EBS (H_2, alkaline plume)?
- **Production of reduced N species**
  → Sorption on clay, more reactivity, gas production
- Effect nitrate on **microbial reduction of RN** (*e.g.* selenate)?

Microbial perturbation

- **Stimulation of growth** of certain microbial species → Shifts in community
- **Enhanced microbial activity**

Materials and methods



Set-up injection tests

- Injection and circulation of nitrate-containing artificial pore water (APW) solutions (circumneutral pH):

	Injected with	Additional electron donor
Interval 2	APW + ~25 mM $NaNO_3$	Pulse injection of acetate after 70 days (initial concentration 4 mM)
Interval 1	APW + ~15 mM $NaNO_3$	Addition of 2.3 mM dissolved H_2 after 55 days

- H_2 dissolved in solution by flow through H_2 equilibration unit with pure H_2 gas phase
- Continuous monitoring of water pressure, temperature and flow rate
- Follow-up of chemical composition of interval solutions by chemical and/or gas analyses and by on-line analyses of pH, E_h, NO_3^- and NO_2^- (by UV spectrophotometry)
- Microbiological analyses (performed at SCK•CEN and HZDR)
- Biogeochemical modelling by NNL

Results

In the absence of additional electron donors

- **Slow decrease** in nitrate concentration, mainly (~90%) due to **diffusion**, but also some denitrification and nitrite production
- Several clay e^- donors [*e.g.* pyrite, (dissolved) organic matter] could have been used
- Important organotrophic and nitrate-reducing community

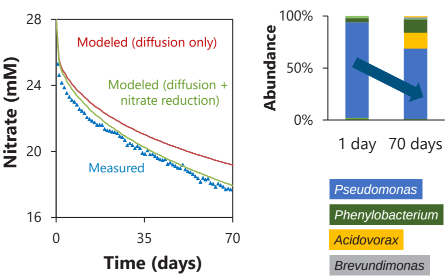


Figure 1: Left: comparison of measured and modeled concentrations of nitrate after injection in Interval 2 with nitrate (before pulse with acetate). Modeled results were obtained using (1) the pore diffusion coefficient of Br^-, derived from a tracer diffusion test carried out in BN borehole and (2) the fitted microbial nitrate reduction rate (~20 µM / day). **Right**: Evolution of the microbial community during the first 70 days after injecting Interval 2 with nitrate. Only the most important genera are shown.

When acetate is available

- **Fast microbial nitrate reduction, mainly to nitrite** (~90% of total nitrate decrease) but also some denitrification
- **Preferential use of acetate** as electron donor
- Fast pH decrease during acetate oxidation
- Shift towards species able to grow on acetate

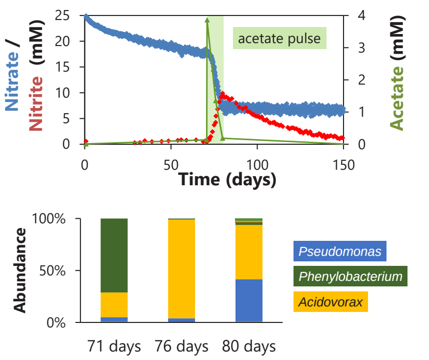


Figure 2: Top: Time evolution of NO_3^-, NO_2^- and acetate concentrations in Interval 2 after injection with nitrate. The pulse of acetate is indicated in green. Addition of acetate causes a 50-fold increase in reaction rate. **Bottom:** Evolution of the microbial community during acetate pulse in Interval 2. Only the most important genera are shown.

When H_2 is available

- After 1 week lag time (a): **fast microbial nitrate reduction** (b) to **nitrite** (~40% of total nitrate decrease), but also to **ammonium** and **denitrification**
- **Preferential use of H_2 as electron donor**
- Fast pH increase during H_2 oxidation
- Presence of hydrogenotrophic species

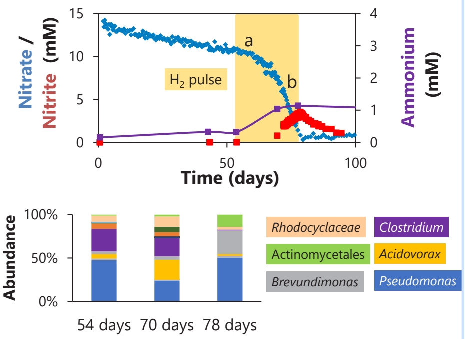


Figure 3: Top: Time evolution of NO_3^-, NO_2^- and NH_4^+ concentrations in Interval 1 after injection with nitrate. The pulse of H_2 is indicated in yellow. Addition of H_2 causes a 35-fold increase in reaction rate. **Bottom:** Evolution of the microbial community during H_2 pulse in Interval 1. Only the most important genera are shown.

Conclusions

- The system can readily switch from natural electron donors and both organic and inorganic waste derived electron donors
- Preferential use of energetically more favourable electron donors, otherwise redox-active clay components are used during microbial nitrate reduction
- The microbial nitrate reduction rate is not dependent on the nitrate concentration, but rather on (bio)availability and energy content of the electron donor

Acknowledgements: This work is undertaken in close co-operation with Swisstopo, the operator and the project management team at the Mont Terri Rock Laboratory, in particular Christophe Nussbaum and Thierry Theurillat. Financial support was provided by the Mont Terri Consortium. Technical assistance of Wim Verwimp and Patrick Boven (SCK•CEN) is also greatly appreciated.

Comparison of three DNA-extraction methods for MX-80 bentonite

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Introduction

The ability to measure microbial growth, behaviour and metabolism in bentonite is an important step in assessing the performance of clay barriers in nuclear waste repository conditions. Clay minerals are challenging targets for DNA extraction as nucleic acids tend to bind tightly to clay particles, which reduces the DNA extraction yield (Goyer and Dandie, 2012). In addition, different bentonites vary in terms of exchangeable cations and mineralogical compositions, which impact the DNA extraction efficiency. Commercial kits would help in reducing extraction method variability and save time, however they are only poorly applicable for DNA extraction from different bentonite types. A custom designed DNA extraction method optimized separately for each bentonite type is needed. The aim of this study was to compare the applicability of three different methods for DNA extraction from MX-80 bentonite. The tested protocols were modified from methods already used for some bentonites or soil materials.

Materials and Methods

The studied bentonite (MX-80, Colloid Environmental Technologies Co.) is a Wyoming Na-bentonite containing some amounts of exchangeable Ca and Mg (Kiviranta and Kumpulainen, 2011). Bentonite was saturated with deep groundwater from Olkiluoto, Finland and mixed well. The bentonite slurry was inoculated with equal amounts of live *Escherichia coli* and an anaerobic mixed culture containing deltaproteobacterial sulphate reducers around 6% of the total community (including *Desulfovibrio* sp. and *Desulfobulbus* sp.) mixed and incubated over night at 20°C. On the next day the bentonite slurry was thoroughly mixed and portioned into 10 mL aliquots, which were frozen at -80°C. Each aliquot contained in average 5 x 10^8 added cells and 2 g of room dry bentonite. Before DNA extraction bentonite samples were thawed, centrifuged for 30 min at 10 000 x g, supernatant was decanted and the samples were mixed with 1 volume of Na_2HPO_4 pH 8. DNA was extracted according to Table 1. The concentration of the extracted DNA was determined with the Qubit 2.0 (Life Technologies). The numbers of bacterial 16S rRNA and *dsr*B genes were determined with quantitative PCR (qPCR) (Purkamo et al., 2017). All qPCR reactions were conducted in duplicate and a negative template control was included in each run.

Table 1. DNA-extraction methods tested for MX-80 bentonite samples.

Method/Reference	Sample	Weight (g)	Method description
M1 Modified from Lever et al., 2015	A	2	2.5x volume of lysis buffer pH 10 (EDTA, Tris-HCl, Guanidium hydrochloride, Triton X-100) and 2.5x volume of Phenol-chloroform-isoamyl alchol 25:24:1, Lysozyme and Proteinase K added. Beat beating twice for 45 s. Incubated for 30 min at 37°C and for 60 min at 60°C. Supernatant collected after centrifugation. Lysis, beat beating and incubation repeated. One volume of Chloroform-isoamyl alcohol 24:1 added to supernatants and centrifuged. Washing of water layer for 2 times. Precipitation of DNA with NaCl and PEG-6000. Precipitated DNA centrifuged and washed with EtOH.
	B	5	
M2 Modified from Selenska-Pobell, 1995	A	2	1/5 volume of 5% SDS added. Mixed gently for 60 min at 70°C. Supernatant collected after centrifugation. Repeated twice and the supernatants combined. Centrifuged for 30 min at 8 000 x g at 4°C. Precipitation of DNA with NaCl and PEG-6000. DNA precipitate centrifuged and purified with NucleoBond AXG (Macherey-Nagel) columns according to the manufacturer.
	B	5	
M3 To be published by Povedano Priego et al., here modified from that	A	2	1.5x volume of lysis buffer pH 8.0 (Tris-HCl, EDTA, NaCl, Polyvinylpyrrolidone, SDS), Lysozyme and Proteinase K added. Beat beating twice for 45 s. Incubated for 30 min at 37°C and for 60 min at 60°C. Supernatant collected after centrifugation. Lysis, beat beating and incubation repeated. Combined supernatants precipitated with NaCl and PEG-6000. DNA precipitate centrifuged and purified then with NucleoBond AXG columns according to the manufacturer.
	B	5	

Results and Conclusions

DNA was successfully extracted from all bentonite samples with all three tested methods (Table 2). The DNA amount was highest with the M1, on average 140 ng DNA g^{-1} room dry bentonite. The other two methods were less efficient yielding around 30 ng DNA g^{-1} bentonite. The added amount of bacteria with estimated average genome size of 3 Mb (*E. coli* 5 Mb) results in around 750 ng of added DNA g^{-1} bentonite. Estimated theoretical yields varied between 21% and 0.5% of the added DNA amounts. Bacterial 16S rRNA gene and dissimilatory sulphite reductase *dsr*B gene copy numbers analysed with qPCR (Figure 1) correlated (r>0.95) significantly (p<0.01) with the obtained DNA yields. However, 16S rRNA genes were not detected from the smaller M3 extracted sample (M3A) and *dsr*B gene was not detected from either M3 sample. In general, the number of 16S rRNA genes was steadily around two logarithmic units higher than that of the *dsr*B genes.

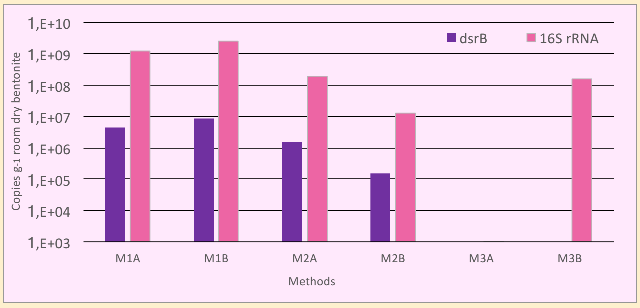

Figure 1. Copy numbers of sulphate reducers (dsrB) and bacterial 16S rRNA genes from DNA extracted from MX-80 bentonite with three (M1, M2, M3) different methods.

Table 2. DNA-yields of three DNA-extraction methods from MX-80 bentonite.

Method	Sample	DNA ng g^{-1} bentonite	Theoretical yield (%)	Comment
M1	A	120	16	Laborious, includes hazardous chemicals
	B	160	21	
M2	A	37	5	In sample B, SDS disturbed purification, longer centrifugation needed for SDS removal
	B	4	0.5	
M3	A	16	2	Laborious, has been further developed
	B	43	6	

- DNA extraction needs to be optimised for each bentonite type
- There were significant differences in the DNA extraction efficiency between the tested extraction methods, efficiency varying between 0.5% to 21% of the estimated theoretical DNA amount in the samples
- Extracted DNA amounts correlated well with the obtained copy numbers of bacterial 16S rRNA gene and dissimilatory sulphite reductase *dsr*B gene determined with qPCR

References

Goyer C., and Dandie, C.E. (2012) In Fillon, M. (Ed.) Quantitative real-time PCR in applied microbiology. Caister Academic Press, UK.

Kiviranta, L., and Kumpulainen, S. (2011). Posiva Working Report 2011-84.

Lever, M., Torti, A, Eickenbusch P., Michaud, A., Šantl-Temkiv, T., and Jørgensen, B.B. 2015. Frontiers in Microbiology. 6:476. doi: 10.3389/fmicb.2015.00476

Purkamo, L., Bomberg, M., Nyyssönen, M., Ahonen, L., Kukkonen, I., and Itävaara, M. 2017. Forntiers in Microbiology. 8:232. doi: 10.3389/fmicb.2017.00232

Selenska-Pobell, S. (1995). Molecular Microbial Ecology Manual. 1.5.1:1-17.

Acknowledgement

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Gases in Bedrock Groundwater: Geochemical Potential for Sustaining Deep Life

Riikka Kietäväinen & Lasse Ahonen

Geological Survey of Finland (GTK)

BACKGROUND

Geochemistry of gases plays an important role in the safety assessment of geological disposal of nuclear wastes. Within fractured bedrock, the fluid phase can both mobilize and disperse potentially hazardous or corrosive compounds, such as ^{14}C or sulphide, as well as provide energy and nutrients to deep-dwelling microorganisms.

Some of the most important electron donors in anoxic deep biosphere, including H_2 and CH_4, are gases.

COLLECTION OF DATA

In order to provide data needed to address the question on geochemical constraints of biological activity at nuclear waste repository depths, we made a literature and database survey and collected geochemical data from deep drill holes and mines in Finland.

Gas data were collected from 20 separate localities (Fig. 1), of which, the absolute concentrations of gases were available from 11 locations.

The sites include both drill holes and deep mines in central and southern Finland with the deepest samples from 2480 m below surface.

Fig. 1 Sites included in the deep gas database of the Geological Survey of Finland

GENERAL FEATURES

Based on the dissolved gas composition, deep groundwaters can be divided into CH_4-dominated and N_2-dominated types (Fig. 2A), of which the CH_4-type is common in metasedimentary regions [1]. Other commonly detected gases include H_2, He, Ar and occasionally CO_2, although significant variation exists between different sites and with depth.

Methane-dominated groundwaters have higher gas/water ratios clearly indicative of accumulation of gas after groundwater recharge in the crust.

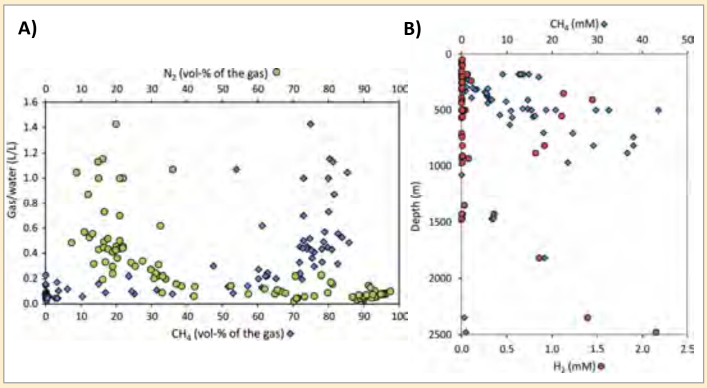

Fig. 2 Examples of diagrams created from the deep gas database. A) Major division between N_2- and CH_4-rich groundwaters, B) Concentrations of CH_4 and H_2 with depth.

CASE STUDY – OUTOKUMPU DEEP DRILL HOLE

As a case study, data from the Outokumpu Deep Drill Hole (2516 m), Eastern Finland (Fig. 1) was used to study geochemical potential of CH_4 and H_2 to sustain deep life. A set of reactions were selected which are thought to be important in anoxic, reductive conditions of the deep biosphere.

In order to determine directions in which these reactions are likely to proceed in the prevailing conditions, Gibbs free energies were determined (Fig. 3A). Furthermore, energy densities (in J/L) were calculated taking into account concentration of the limiting nutrient (Fig. 3B).

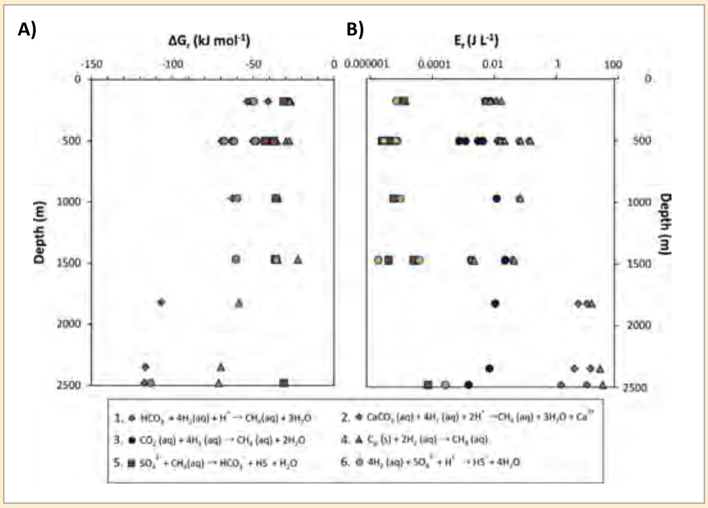

Fig. 3 Thermodynamic constraints of deep life at the Outokumpu Deep Drill Hole. A) Gibbs free energies for six key reactions involving CH_4 and/or H_2 (note that ΔG_r for the reactions 1.-3. is the same). B) Energy densities (note logarithmic scale).

Based on these calculations, it can be seen that methanogenesis from dissolved inorganic carbon or graphite and H_2 is a plausible mechanism, whereas, in this case, sulphate reduction combined with methane oxidation probably will not produce enough energy to sustain anaerobic methanotrophic archaea (ANMEs). Neither does sulphate reduction with H_2 seem favourable, despite the clearly negative ΔG_r. However, if sulphide is rapidly removed from the system, e.g. by precipitation of iron sulphides, these two pathways will become more energetically feasible.

DISCUSSION AND CONCLUSIONS

Formation of gases in the upper crust is affected by both geological and biological factors. The observed variation in the composition of the gas phase is probably related to differences in lithology, which has been found to correlate also with microbial community structure [1,2]. Furthermore, residence time of water, which can be on the order of tens of millions of years [3] is likely to affect gas accumulation and energetics of the deep biosphere.

Site to site as well as depth dependent variation in gas compositions and concentrations should be taken into account.

Compilation of hydrogeochemical and gas data from the same samples, combined with information on temperature, pH, and redox potential *in situ*, enables thermodynamic methods to be applied, as showcased at the Outokumpu Deep Drill Hole.

When carefully examined, geochemical data will give valuable information on potential life sustaining reactions to be used in the assessment of biogeochemically induced risks, for example, at nuclear waste repository sites.

REFERENCES

[1] Kietäväinen et al. (2017) GCA 202, 124-145. [2] Kietäväinen et al. (2013) Appl Geochem 32, 37-51. [3] Kietäväinen et al. (2014) GCA 145, 159-174.

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ACKNOWLEDGEMENTS

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Rates of H_2 and SO_4^{2-} Consumption by Microbes in a Radioactive Waste Repository

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1. Background – Low / Intermediate Level Waste Repository

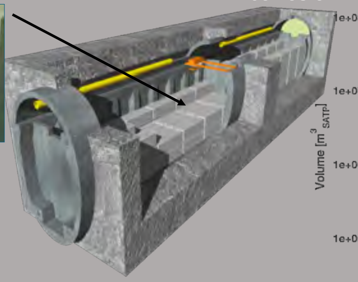
Deep geological repository – most likely in Opalinus Clay

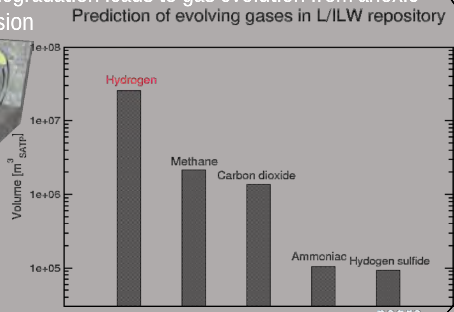
400-800 meters below the surface, three sites in Northwest Switzerland under consideration

1. Main facility L/IWL
2. Pilot facility
3. Test zones
4. Access tunnel
5. Ventilation and construction shafts

Image: Infel AG, Claudio Koeppel

Low / Intermediate Level Waste storage: Waste degradation leads to gas evolution from anoxic corrosion

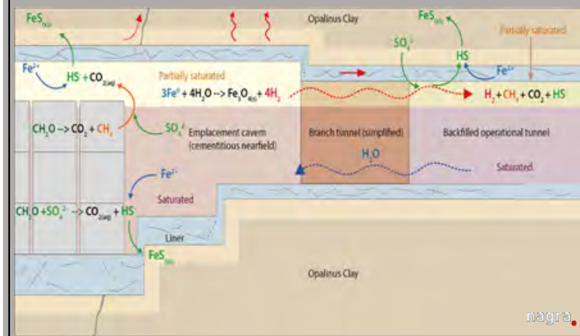




Diomidis, N. et al. Production, consumption and transport of gases in deep geological repositories according to the Swiss disposal concept - Technical Report 16-03. (2016).

2. Taking microbial activity into account

Microbial activity influences the near-field geochemistry: Rock-barrier-waste-biosphere interaction



Evolving gases may be consumed by microbial activity in backfilled operational tunnels. Gas transport systems provide H_2 from anoxic metal waste degradation, SO_4^{2-} rich porewater is provided from the host rock, leading to sulfate reduction as the most likely microbial metabolism to influence the gas balance of a radioactive waste repository in Switzerland.

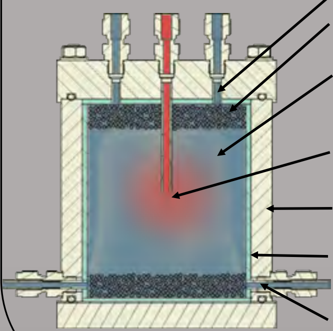
Investigation of a repository design including microbes, potentially enhancing the safety of the repository. Open questions are: Which bacterial community thrives there? What kind of metabolism is active? At which *in situ* rate?

Leupin, O. X. et al. An assessment of the possible fate of gas generated in a repository for low- and intermediate-level waste - Technical Report 16-05. (2016)

3. Bioreactor design

Simulate repository relevant conditions: Sand-Bentonite, SO_4^{2-} rich porewater, H_2 gas

Microbial activity is simulated using a bioreactor placed in an anoxic chamber and supplied with porewater and hydrogen gas to facilitate the growth of microbial biofilms under repository relevant conditions.



Porewater outlet
Coarse sand layer
Saturated sand-bentonite mix 80:20% (w:w)
Hydrogen gas injection
Stainless steel cylindrical case
Inner Plexiglas cylinder
Opalinus Clay rock porewater injection

Opalinus Clay rock porewater chemistry
BMA-A1 more acidic, higher $NH4^+$, SO_4^{2-} and $HCO3^-$, lower Na^+, Ca^{2+} and Cl^- concentrations than average OPA porewater*.

[mM]	OPA porewater*	BMA-A1 porewater	Seawater*
pH	7.96	7.18±0.57	8.1
Na^+	245	184.4±18.8	470
K^+	1.1	1.6±0.2	10.1
Ca^{2+}	15.2	12.5±0.1	10.3
Mg^{2+}	17.1	13.9±0.1	53.8
NH_4^+	0.57	3.25±1.89	-
Cl^-	287	240±5.5	546
SO_4^{2-}	13.7	15.1±0.4	28.2
HCO_3^-	0.8	1.18±0.39	2.25
DOC	1.1	0.23±0.05	0.04

*Pearson, F. J. et al. Mont Terri Project – Geochemistry of Water in the Opalinus Clay Formation at the Mont Terri Rock Laboratory – Reports of the FOWG, Geology Series. (2003)

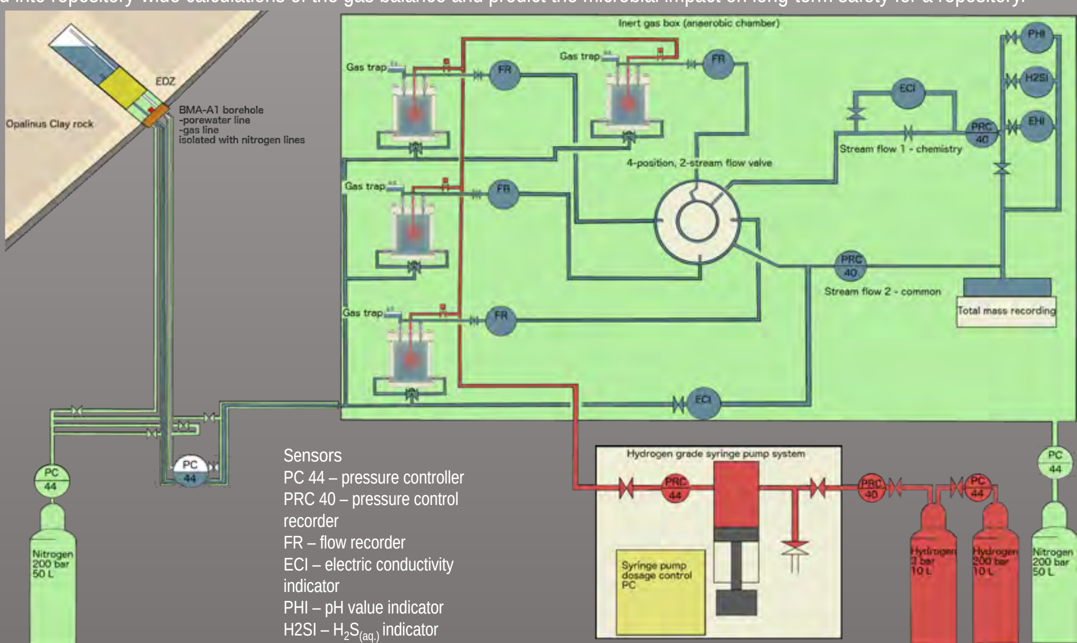
4. Experimental setup – *in situ* rates of H_2 and SO_4^{2-} consumption

Overview: experimental setup

BMA-A1 borehole, 47 degree inclination into the ceiling of gallery, V_{tot}=52.08 L, drilled in May 2015 using disinfected drilling equipment. Lines provide anoxic porewater to an anaerobic chamber where bioreactor experiments are conducted. Bioreactors will be provided with H_2 gas from a syringe pump and continuous flow of porewater from the borehole. Planktonic microbes from the borehole will settle among indigenous Wyoming bentonite and quartz sand microbes within the bioreactors. The microbiota, fueled by H_2, will be characterized using molecular biology tools and their *in situ* consumption rates of H_2 and SO_4^{2-} will be determined. These rates can than be incorporated into repository-wide calculations of the gas balance and predict the microbial impact on long-term safety for a repository.

Observables

Microbial activity in bioreactors to be determined by (a) H_2S evolution (H_2 and SO_4^{2-} consumption rates) and by biomass quantification (total DNA content); (b) Identification of microbial community (DNA sequencing); (c) Water chemistry (cations, anions, DIC, DOC); (d) Micro-computer tomography and scanning electron microscopy for porosity and biofilm imaging; (d) XRD mapping to image bio-precipitates



Sensors
PC 44 – pressure controller
PRC 40 – pressure control recorder
FR – flow recorder
ECI – electric conductivity indicator
PHI – pH value indicator
H2SI – $H_2S_{(aq.)}$ indicator

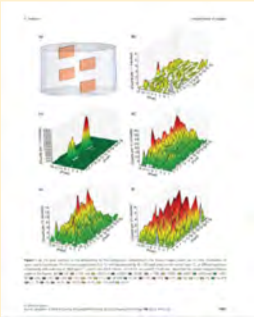
This project has received funding from the Euratom research and training programme 2014 - 2018 under grant agreement No. 661880

Transport and reactivity of sulphide from bacterial activity in compacted bentonite clays

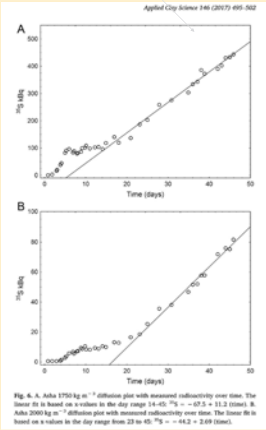
Trevor Taborowski, Andreas Bengtsson and Karsten Pedersen
Microbial Analytics Sweden AB

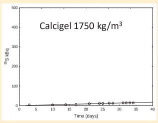
Sulphide production in EDZ* and in groundwater

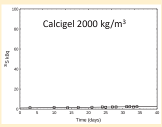
A. From: Pedersen, K., 2010. Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. J. Appl. Microbiol. 108, 1094-1104



B. From Pedersen, K., Bengtsson, A., Blom, A., Johansson, L., Taborowski, T., 2017. Mobility and reactivity of sulphide in bentonite clays – Implications for engineered bentonite barriers in geological repositories for radioactive wastes. Applied Clay Science 146, 495-502







Diffusion and reaction

C.

$HS^- + 2Fe^{3+} \rightarrow 2Fe^{2+} + S^0 + H^+$

$HS^- + Fe^{2+} \rightarrow FeS + H^+$

Sum:

$2HS^- + 2Fe^{3+} \rightarrow Fe^{2+} + S^0 + FeS + 2H^+$

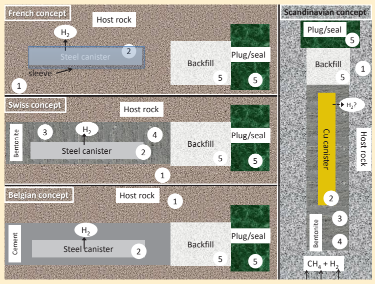
D.

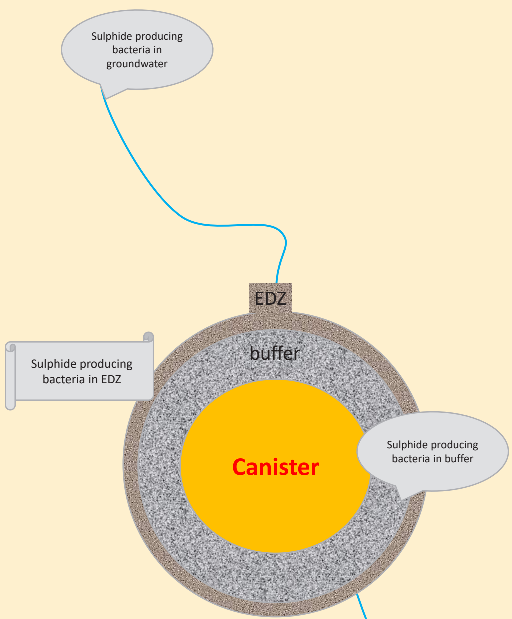
25 µmole HS⁻/gdw clay

200 µmole HS⁻ /gdw clay

Volclay MX-80 | Asha | Calcigel

Representation of European high level waste disposal concepts (not to scale). Microbial processes are possible at the numbered points, which correspond to task numbers. 1. Microbial generation of sulphide in the geosphere. 2. Microbially induced corrosion of canisters. 3. Microbial activity in bentonite buffer. 4. Microbial degradation of bentonite buffer. 5. microbial activity in backfill and plug/seals

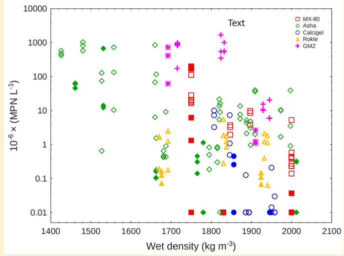




* EDZ = Excavation Damaged Zone

Sulphide production in buffer

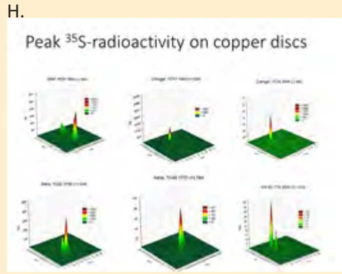
E. From Deliverable D2.4: Figure 5-5. Number of cultivable cells (MPN) over wet density. Note that the y-axis is logarithmic. Control samples without bacterial addition are marked with filled symbols for each clay according to legend.



F. From Deliverable D2.4: Figure 5-1. Left: A schematic cross section of a test cell. Right: An assembled test cell, spacers are not mounted.

G. From Deliverable D2.4: Figure 5-6. Black spots observed during sampling of test cell with GMZ bentonite.

H.



A. Previously, sulphide from sulphide producing bacteria was found to diffuse through bentonite and sulphide was also observed to be immobilised by the clay (MX-80). **B.** Later, we could establish the diffusion coefficient for sulphide in Asha bentonite but break-through did not occur with Calcigel using a similar configuration. **C.** It was found that sulphide reacts with ferric iron in the clays under the formation of elemental sulphur, ferrous iron and FeS. **D.** When compacted bentonite was exposed to varying amounts of sulphide, we found a that sulphide altered the clay in a redox front appearance.

In conclusion:

Sulphide reacts with ferric iron in clays. These clays then act as sinks for sulphide produced in groundwater and EDZ. Most of the sulphide that reach the tested clays will be immobilised as S^0 and FeS although some will escape reaction with ferric iron and diffuse towards the cannister (except for Calcigel?).

E. Bentonite clays commonly contain sulphide-producing bacteria (SPB) that, just like added SPB, survive over a large range of wet densities. **F.** A method utilising ^{35}S as a tracer for sulphide-production by SPB in compacted clays has been developed and used for a long time. Sulphide production is registered as formation of $Cu^{35}S$ om copper discs. **G.** Spots stained black by FeS have been observed in laboratory experiments (and also in field experiments, reports in press). **H.** Such spots agree with observations of peak ^{35}S-radioactivity on copper discs indicating local SPB-activity in compacted clays.

In conclusion:

Sulphide-producing bacteria can survive and locally produce sulphide in compacted clays, likely in positions where inhomogeneities and a concomitant low swelling pressure allow microbial activity. The processes described in **A. – D.** can proceed in such spots.

References

Pedersen, K., 2010. Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. J. Appl. Microbiol. 108, 1094-1104.

Pedersen, K., Bengtsson, A., Blom, A., Johansson, L., Taborowski, T., 2017. Mobility and reactivity of sulphide in bentonite clays – Implications for engineered bentonite barriers in geological repositories for radioactive wastes. Applied Clay Science 146, 495-502

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Bacterial viability in a concrete-bentonite clay interface

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Microbial Analytics Sweden AB

Objectives

- Investigate saturated compacted MX-80 in contact with concrete
- Analyse in profile pH and bacterial viability

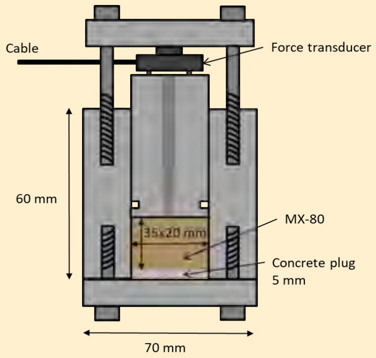

Figure 1. Experimental scheme

Material & Methods

- MX-80 spiked with three different species of SRB
  - *Desulfovibrio aespoeensis* (DSM 10631), *Desulfotomaculum nigrificans* (DSM 574) and *Desulfosporosinus orientis* (DSM 765)
- Glucose was added to induce fermentation
- Conducted in titanium test cells (Figure 1)
- Saturated with an oxygen-free salt saturation solution for 180 days
- After saturation sampled at four position where position 1 was closest to concrete plug
- Samples analysed for pH, ATP and Cultivable heterotrophic Aerobic Bacteria (CHAB)

Table 1. Experimental parameters

Name	Aimed density	Glucose	Concrete plug
Test cell 11	1750 kg m^{-3}	No	No
Test cell 12	1750 kg m^{-3}	No	Yes
Test cell 15	1750 kg m^{-3}	Yes	Yes
Test cell 16	1750 kg m^{-3}	Yes	Yes
Test cell 23	1750 kg m^{-3}	Yes	Yes
Test cell 24	1750 kg m^{-3}	Yes	Yes

Results

- Decrease of pH from position 1 to 4 in clays with concrete and glucose (Table 2)
- Control test cell (11) showed bacterial viability at all positions at pH 9.4
- Test cells with concrete and glucose showed bacterial viability at position 4
  - Low ATP at position 1 to 3
- Test cell (12) with concrete and without added glucose showed no bacterial viability, this cell had the highest average pH.

Table 2. Analysed pH for each test cell

Position	Test cell 11	Test cell 12	Test cell 15	Test cell 16	Test cell 23	Test cell 24
1	9.4	10.2	10.6	10.8	10.6	10.7
2	9.4	10.1	9.6	9.6	9.5	10.1
3	9.4	10	8.9	8.7	8.5	8.9
4	9.4	9.8	8.1	7.4	7.9	7.9

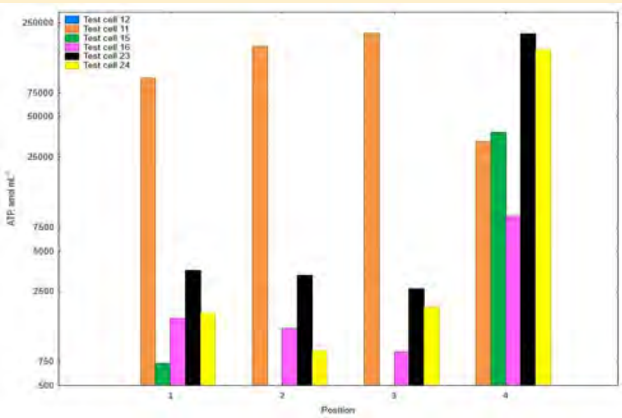

Figure 2. Analysed ATP at four positions, test cells according to symbol description.

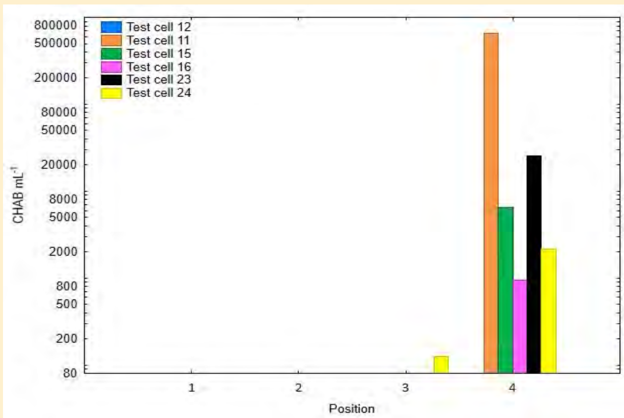

Figure 3. Analysed CHAB at four positions, test cells according to symbol description.

Conclusions

The results of the bacterial viability indicate that the alkaline pH of 10 at position 1 – 3 might have inhibited the viability of bacteria in test cells with concrete and glucose. The control test cell ,without concrete, had a pH of 9 throughout the clay and showed high ATP values and CHAB growth at position 4. The difference in bacterial viability indicates that pH 10 might be a threshold for bacterial viability, and thereby activity, in this experiment. The saturation of the clay possibly leaked an alkalic solution continuously into the clay in test cells with concrete. This could have inhibited bacterial viability at position 1 to 3.

The data from the pH measurement and bacterial viability indicates the decrease of pH might be due the fermentation of the glucose trough bacterial activity.

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Acknowledgements

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Microbial Gas Generation in Low-Level Radioactive Waste

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Canada's Nuclear Regulator

Introduction.

Microbial degradation of organic substrate in low- and intermediate-level waste produces H_2, CO_2 and CH_4.

Reaction	Description	Reaction #
Cellulose → soluble carbohydrate (CH_2O)	Hydrolysis of solid cellulose	Reaction 1
CH_2O → organic acids + CO_2 + H_2	Hydrolysis of carbohydrate and fermentation	Reaction 2
$4 H_2 + CO_2 \rightarrow CH_4 + 2 H_2O$	Hydrogenotrophic methanogenesis	Reaction 3

Microbial gas generation leads to:

- gas pressure buildup in cavity chambers delay resaturation of cavity chambers with water which delays soluble radionuclide transport and favour C-14 transport
- methanogens produce acidity which can lead to localized dissolution of host rock and shaft seals

Study objectives

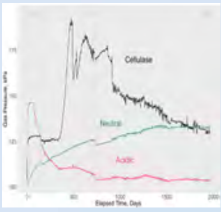
1. Measure methane production and gas pressure buildup over long time periods in surrogate low-level waste with different levels of organic material and at pHs likely to be encountered in a repository
2. Quantify the importance of methane production and its implication for repository safety cases

Experimental set-up

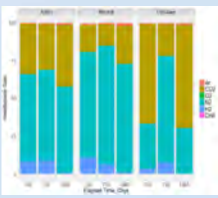
1. Gas pressure monitored over 464 days and 1,965 days
2. Gas composition measured at 464 days, and at 150,730 and 1,965 days

Results

1. 1,965-day experiment

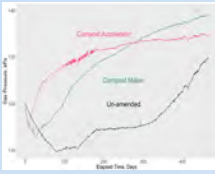


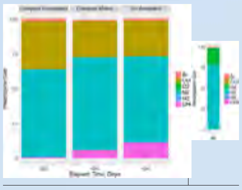
- Acidic condition kept gas pressure low, cellulase increased pressure initially and pressure returned to normal
- Enzymatic activity and pH are important



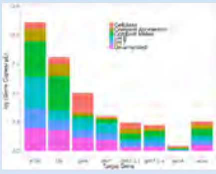
- H_2 (0.1-11%), CO_2 (14-70%) and N_2 (30-80%) were the main gases produced; neither CH_4 O_2 nor H_2S detected
- Production of H_2 and CO_2 without CH_4 suggests acetogenesis and higher pressure buildup

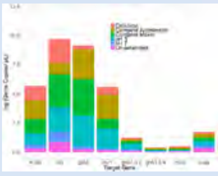
2. 464-day experiment





- Addition of compost-accelerated gas pressure onset and rise compared to waste without compost
- Gas pressure was increasing at a higher rate after 300 days in waste without compost
- N_2 (62-67%), CO_2 (25-35%), H_2 (~0.24%) and CH_4 (0.6 to 12%) were generated
- Low H_2 combined with CH_4 suggests methanogens established, or possible aceticlastic methanogenesis?





- The archaea (A16s) were dominant on the solid waste; bacteria and fungi in the leachate
- Fungal (18s) higher than bacterial (glnA) diversity; only fungi degrading cellulose detected (gh 61.3.2 and gh 61.5.4), as well as fungal cellulolytic cellulase responsible for H_2 and CO_2 production
- Methanogens (mcrA) were minor
- Acetogens (acas) were detected in higher abundance than methanogens for both solids and leachate

Implication for repositories

Likely microbial activity scenario

Reaction	Description	Reaction #
Cellulose → soluble carbohydrate (CH_2O)	Hydrolysis of solid cellulose	Reaction 1
CH_2O → organic acids + CO_2 + H_2	Hydrolysis of carbohydrate and fermentation	Reaction 2
$4 H_2 + 2 CO_2 \rightarrow CH_3COOH + 2 H_2O$	Acetogenesis	Reaction 3
$CH_3COOH \rightarrow CH_4 + CO_2$	Aceticlastic methanogenesis	Reaction 4
$4 H_2 + CO_2 \rightarrow CH_4 + 2 H_2O$	Hydrogenotrophic methanogenesis	Reaction 5

The possible presence of acetogens and fermenters promoting acidity can dissolve carbonate minerals in the host rock and cement. Hence, barriers could be locally affected, which may need to be considered in the design of repositories.

Conclusions

While repositories are designed with sufficient margins of safety, including acetogenesis in safety assessment will reduce uncertainty.

nuclearsafety.gc.ca

CRIEPI'S MICROBIAL RESEARCH ON NUCLEAR WASTE DISPOSAL

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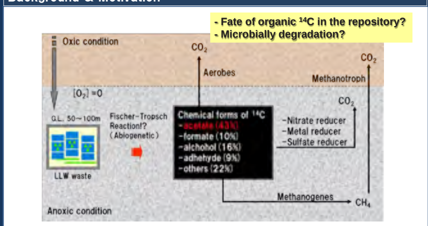
2 Geological Isolation Research and Development Directorate, Japan Atomic Energy Agency, Tokai, Ibaraki, Japan

Microbial impacts on radionuclide migration

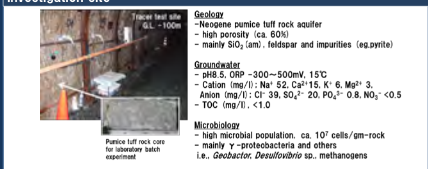
To demonstrate microbially degraded migration enhancement of carbon-14 organic compounds released from low-level radioactive waste repository, the natural-gradient tracer test using ^{13}C-labeled acetate under anaerobic condition was carried out in a tunnel at about 100 m below ground in a Neogene pumice tuff rock aquifer, Rokkasho, JAPAN. Three boreholes were drilled: one for injecting a tracer and two for collecting the tracer. The distances between the boreholes for injection and recovery of tracer were 120 and 150 mm, respectively. After tracer injection (^{13}C-labeled sodium acetate [1-^{13}C] + KBr), the ground-water effluents from the boreholes were collected at specific intervals of time, and the concentrations of dissolved ions (acetate, bromide, and ^{13}C-bicarbonate), total cell count, viable count, and microbial community using molecular microbiological technique were analysed. After 30 days from tracer injection, ^{13}C-bicarbonate ions were detected along with bromide ions as a tracer of groundwater flow, which were faster than the acetate ions. These breakthrough curves show that acetate was degraded to bicarbonate by anaerobes, possibly sulfate-reducers and methanogens, during transport in the aquifer, thus causing acceleration of the migration rate for radioactive carbon released from the repository.

Nagaoka T. et al., (2010). Field-scale tracer test of ^{13}C-labelled acetate in a pumice tuff aquifer: Implication for microbially enhanced migration, CRIEPI report, V09029 (in Japanese)
Nagaoka T. et al., (2008). Field-scale tracer test of carbon-14 organic compounds in a pumice tuff aquifer: Evidence for microbially enhanced migration. 7th International Symposium for Subsurface Microbiology (ISSM2008). Shizuoka Japan 2008.11

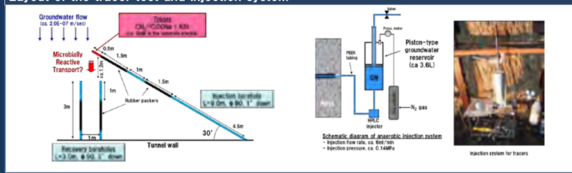
Background & Motivation



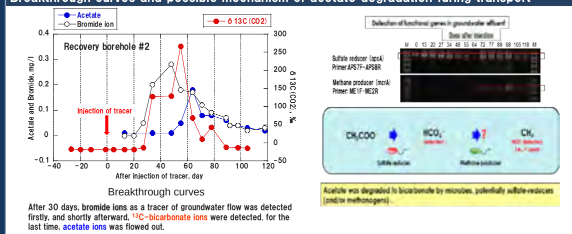
Investigation site



Layout of the tracer test and injection system



Breakthrough curves and possible mechanism of acetate degradation furing transport

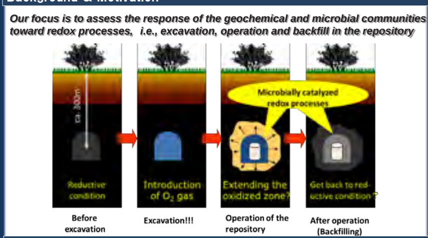


Microbially mediated redox changes in nuclear waste disposal

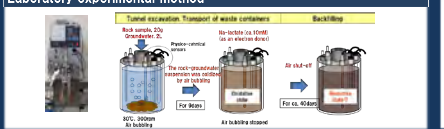
In order to assess the microbial impacts on the geochemical processes around the nuclear waste repository, the laboratory jar experiments were conducted using the deep sedimentary rock and groundwater in the Horonobe URL, Japan. In the experiments, pulverized rock and groundwater were suspended in the jar and the redox changes were induced by aeration and discontinuation to sediment slurry, which simulated the redox process occurring during operation of nuclear waste repositories. During the experiments, redox potential, pH and dissolved oxygen in the slurry were monitored, and also the concentrations of dissolved ions and head space gasses in the jar were analyzed. In addition, microbial DNA was extracted from the slurry, and analyzed the response of microbial communities toward the geochemical changes. As a results, after discontinuation of air exposure with lactate and acetate amendments as an electron donor, redox potentials decreased from ca. +100 mV (vs. Ag/AgCl) to -600 m V for lactate and -300mV for acetate, and the microbial communities were changed with the redox potentials of the slurry, and also the sequential terminal electron-accepting process (TEAPs) such as aerobic respiration, iron reduction was observed. These results indicated that the microbial activities would affect the geochemical changes around nuclear waste repositories.

Nagaoka T. et al., (2014). Laboratory simulation of geochemical disturbance in the sedimentary subsurface environment. Ninth International Symposium on Subsurface Microbiology, Pacific Grove, CA. 2014.10
Nagaoka T. et al., (2009). Microbial impacts on the geochemistry evolution in a nuclear waste repository - Laboratory experiment of microbially mediated redox changes with lake sediment- CRIEPI report V08034 (in Japanese)

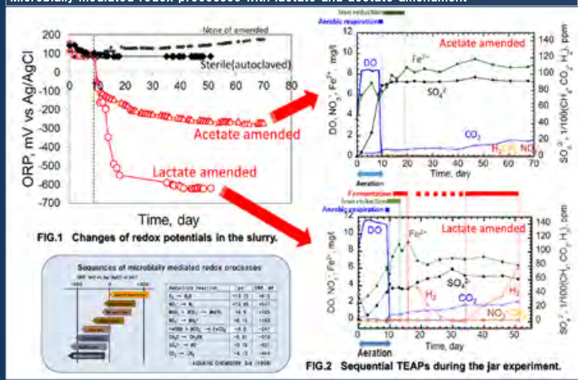
Background & Motivation



Laboratory experimental method



Microbially mediated redox processes with lactate and acetate amendment

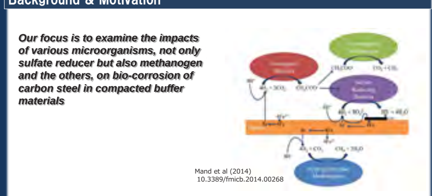


MIC of carbon steel in compacted buffer materials

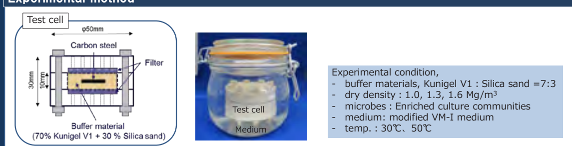
Recently, it is reported that various microorganisms influence iron corrosion processes, such as acetate producer, nitrate reducer, methanogen and sulfate reducer. We enriched the iron corrosive microbial communities from lake sediments, and carried out the MIC experiments during 12 months using buffer materials (Kunigel V1:Silica sand=7:3) at different dry densities, 1.0, 1.3 and 1.6 Mg/m^3 in order to determine the dry density of buffer materials to suppress microbial activities in compacted buffer materials. As a result, the increase of dry density of buffer materials reduced microbial activities and little corrosion occurred, except for at the density of 1.0Mg/m^3. Even at 1.6 Mg/m^3, however, the increase of gene copies were detected by quantitative PCR using bacterial 16S rRNA primer set. This highlights that the dry density at 1.6Mg/m^3 would not be good enough to suppress microbial activities in compacted buffer materials.

Hirano S. et al., Bio-corrosion of carbon steel in compacted buffer materials, in preparation
Hirano S. et al., (2015). Carbon steel Corrosion Induced by Microbial Community in Soil Environment and Its Analysis, Zairyo-to-Kankyo, 64, 535-539 (in Japanese)

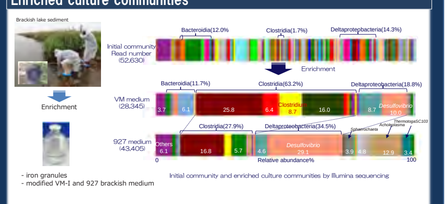
Background & Motivation



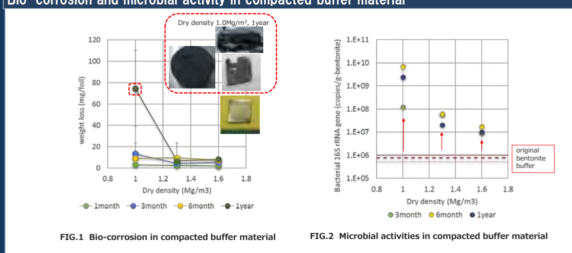
Experimental method



Enriched culture communities



Bio-corrosion and microbial activity in compacted buffer material



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