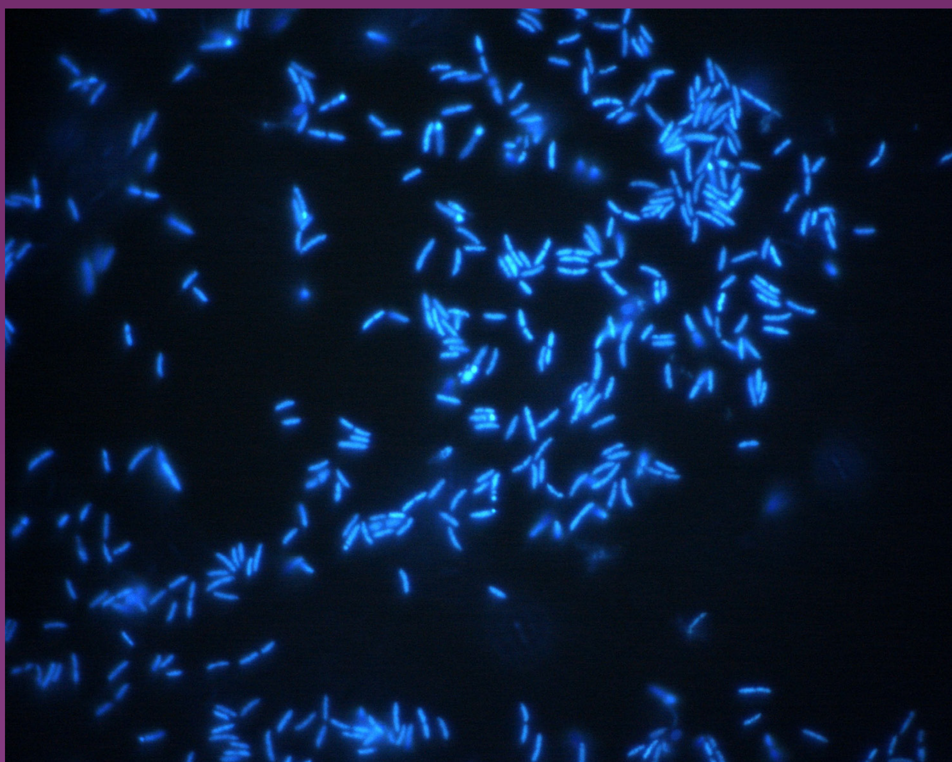
Microbiology In Nuclear waste Disposal

MIND Project Annual Meeting 2
Short Abstracts

Prague May 3–5, 2017



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.

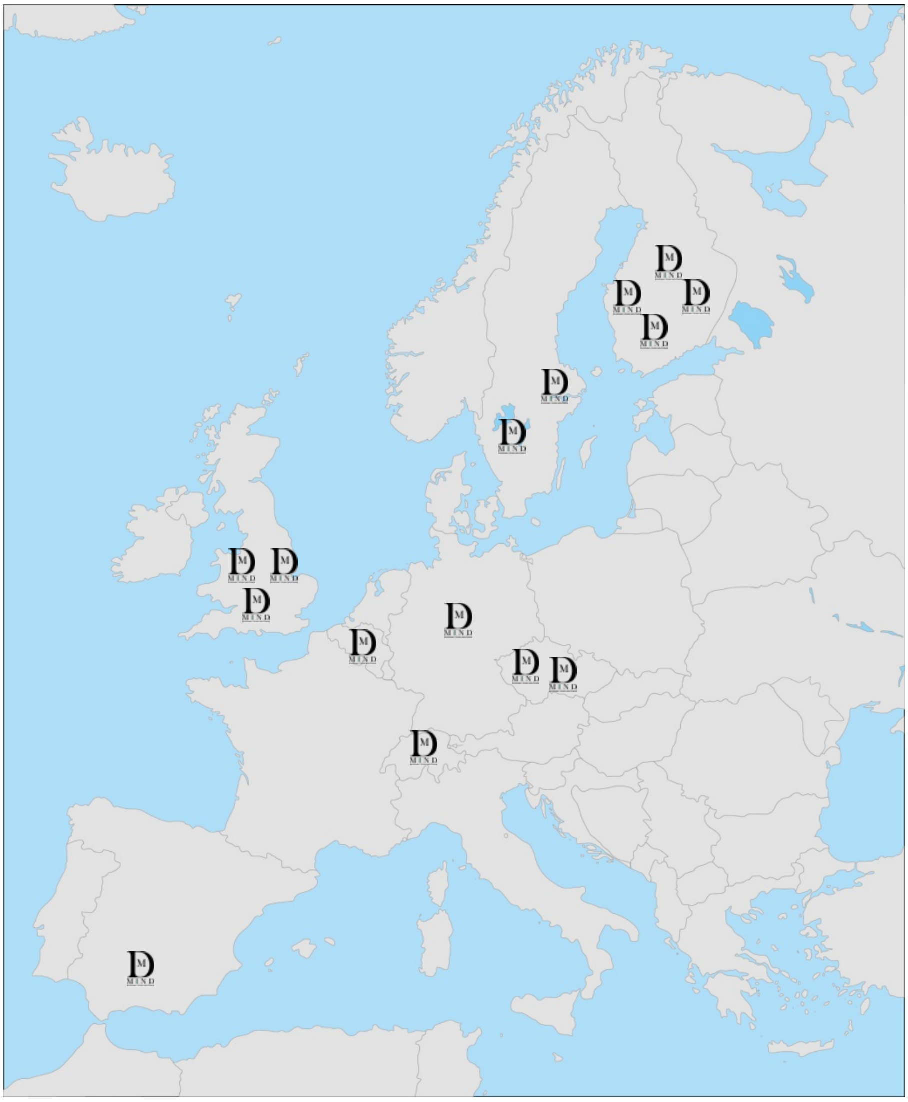


Figure 1: Fifteen organisations from eight countries.

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 and performance assessment under repository-relevant conditions, thus altering the current view of microbes in repositories and leading to significant refinements of safety case and performance assessment 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. Johan Andersson (SKB) has accepted to chair this group and yearly meetings in connection to the PAM will be held. The first meeting was held in Prague during PAM2. 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 and IRSN.

The second Project Annual Meeting (PAM) took place May 3 –5, 2017 and was hosted by the TUL and CVRez.
The meeting started with a pre-meeting workshop organized by Micans on May 3rd. The workshop was divided into two sets of parallel sessions:

- High pH and methanogenesis with focus on low- and intermediate level waste repositories. Workshop leader *Joe Small, NNL* (morning).
- Microbial effects on radionuclide migration. Workshop leaders *Henry Moll and Thuro Arnold, HZDR* (morning).
- Bioinformatics pipelines and mock communities. Workshop leader *Pieter Monsieurs, SCK•CEN* (afternoon).
- Microbial life and effects on clays in natural and engineered barriers. Workshop leader *Karsten Pedersen, MICANS* (afternoon).

Invited speakers Achim Albrecht (ANDRA) and Patrik Sellin (SKB) initiated the morning and afternoon session respectively.
The pre-meeting workshop sessions, included informal presentations and group tasks to analyse and discuss Performance Assessment of microbially mediated processes, some key scientific uncertainties and microbiological techniques. Some further topics for discussion at forthcoming meetings were proposed.
The first day of the PAM started with a welcome by the coordinator and a summary of the pre-workshop discussion by Micans. The morning session started with an introduction by the WP1-leader followed by Klas Källström who gave a brief summary about the SFR (the Swedish repository for low and intermediate level waste). WP1 handles questions regarding *Improving the safety case knowledge of organic long-lived intermediate level waste (ILW)*. Ten presentations were given by, UNIMAN, SCK•CEN, CRC/TUL, NNL, EPFL, VTT, HZDR and UGR.

In the afternoon, the WP2-leader gave an introduction of WP2. WP2 concerns *Improving the safety case knowledge base about the influence of microbial processes on high level waste (HLW) and spent fuel geological disposal.* Nine presentations were given by GTK, VTT, CRC/TUL, EPFL, NERC, HZDR, UNIMAN, Micans andUGR.

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

The day ended with a poster session with eleven posters from TUL, CVREZ, EPFL, UGR, MICANS, SCK•CEN, HZDR andVTT.

The second day started with an introduction by the WP3-leader. WP3 focusses on *integration, communication and dissemination of results*. There were four presentations in total. Natalie Leys (SCK•CEN) gave an introduction on dissemination activities and education followed by Michiel Van Oudheusden SCK•CEN who talked about social aspects and networks between the different actors involved in geological disposal and geomicrobiology research. The WP-leaders of the two experimental packages then gave a synthesis of the progress and outcome of their activities so for. The PAM ended by a summary of the meeting given by the Implementers Review Board.

The PAM was closed by the project coordinator, where special thanks were given to Alena Sevcu, Petr Polivka and their colleagues at TUL and CVRez for their excellent organisation of the workshop and meeting.

A visit to the CVRez laboratory was made in the afternoon on Friday. The attendants got the opportunity to either see the laboratory or the research reactor.

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

Follow us on Twitter @MINDH2020

The coordination team would like to thank all participants for their contribution to our second annual meeting! See you all in Lausanne, May 2018.

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

Birgitta Kalinowski (SKB) technical coordinator

Petra Christensen (SKB) administrative coordinator

Joe Small (NNL), WP1-leader

Karsten Pedersen (MICANS), WP2-leader

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

CONTENTS

Information and Final Agenda

The Mind project would hereby like to welcome you and your colleagues to the second **MIND Project Annual Meeting** and **Project Executive Committee Meeting** (PEC) which are scheduled to take place in **Prague on the 3rd to 5th of May.**

The complete MIND 2017 Workshop will be hosted by the Technical University of Liberec (TUL) and Research Centre Rez (CVRez) thanks to Alena Sevcu (TUL) and Petr Polivka (CVRez). Detailed maps will be distributed together with the final agenda to all participants.

The meeting venue for all meetings:
Czech Association of Scientific and Technical Societies/
Český svaz vědeckotechnických společností z.s.
Novotného lávka 5
110 00 Praha 1
3rd floor, **Křižík hall (Křižíkův sál) – number 319** and **lecture room 318**

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 first week of May is very busy in Prague. Recommended hotels located close to the meeting venue include:

1. Charlesbridgepalace (close to the meeting venue)
2. Pachtuvpalace (close to the Metro station Staromestska)

Travel
The Václav Havel Airport Prague is located 10 km from the city centre. A public transport bus is traveling every 10 minutes from the airport to the nearest metro station (about 1.5 Euros). Taxi rates are about 25 Euros.

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.

1. MIND-project pre-meeting workshops

The MIND-project will on May 3rd host an open for all pre-meeting workshop focused on four of our key subjects:

May 3rd

09:00	Stakeholder's views on microbial issues in MIND – includes time for questions and discussion, *Achim Albrecht, ANDRA.*
09:45	**Coffee**
10:00	Break up in two parallel workshops: • High pH and methanogenesis with focus on low- and intermediate level waste repositories. Workshop leader is *Joe Small, NNL.* • Microbial effects on radionuclide migration. Workshop leaders are *Henry Moll and Thuro Arnold, HZDR*.
12:30	**Lunch**
14:00	Stakeholder's views on microbial issues in MIND – includes time for questions and discussion, *Patrik Sellin, SKB*.
14:45	**Coffee**
15:00	Break up in two parallel workshops: • Bioinformatic work in MIND, mock sample and bioinformatic work. Workshop leader is *Pieter Monsieurs, SCK•CEN*. • Microbial life and effects on clays in natural and engineered barriers. Workshop leader is *Karsten Pedersen, MICANS*.
17:30	**End of day**

The workshop will be organized by MICANS and the MIND-project.

2. MIND Project Annual Meeting

The MIND meeting will be hosted from the evening of May 3rd when we start with an optional icebreaker and registration. The actual Project Annual Meeting will start on the morning of May 4th and continue until lunch-time on May 5th.

May 3rd

18:00 – 20:00	Icebreaker and registration (The same venue as the meetings)

May 4th

08:30	Welcome and Introduction to the MIND-project, *Birgitta Kalinowski, Petra Christensen, SKB* Summary of the pre-meeting workshop, *Karsten Pedersen, MICANS*
09:00	**Theme session I (WP1: ILW):** Introduction
09.10	Microbial degradation of cellulose and its alkali hydrolysis products under hyperalkaline conditions, *Naji Bassil and Jonathan Lloyd, UNIMAN*
09.25	PVC degradation, *Sophie Nixon et al, UNIMAN*
09.40	Microbial degradation of organics and nitrate leaching from bituminised radioactive waste, *Kristel Mijnendonckx, SCK•CEN*
09.55	Effect of microbial consortium naturally containing SRB on degradation products of gamma irradiated organic radioactive wastes, *Petr Polivka, Jana Steinova, Hana Vanova, Tomas Cernousek, Rojina Shrestha, Alena Sevcu, RCR/TUL*
10.10	**Coffee**
10.30	Speciation of uranium (VI) in the presence of cellulose degradation products, *Hannes Brinkmann, Henry Moll, HZDR*
10.45	The speciation of selenium and europium associated with bacteria isolated from Spanish bentonites, *Miguel Angel Ruiz Fresneda et al, UGR*
11.00	Ion exchange resins as sources of organic compounds in repositories, *Rizlan Bernier-Latmani, EPFL*
11.15	Modelling sulphate reduction in an in situ borehole experiment, *Liam Abrahamsen-Mills, Joe Small, NNL*
11.25	Microbiological degradation of LLW under in situ conditions, *Minna Vikman et al, VTT*
11.40	Modelling of microbial gas generation processes after 18 years experimental study of LLW degradation, *Joe Small, Mikko Nykyri, Minna Vikman, Merja Itävaara, Liisa Heikinheimo, NNL/VTT/TVO*
11.50	Summary discussion and close of session
12:00	**Lunch**

May 4th

13:30	**Theme session II (WP2: HLW):** Introduction
13.40	Gases and Sulphur compounds in deep bedrock groundwater across Finland, *Riikka Kietäväinen, GTK*
13.55	Activation of deep subsurface ground water microorganism by electron acceptors and donors, *Hanna Miettinen, Minna Vikman, Merja Itävaara, VTT*
14.10	Corrosion of carbon steel in presence/absence of microorganisms under anaerobic conditions, *Tomas Cernousek, Hana Vanova, Jana Steinova, Rojina Shrestha, Alena Sevcu, Petr Polivka, TUL & CV-REZ*
14.25	In situ iron corrosion in bentonite and first results on microbial investigations of Opalinus clay rock, *Niels Burzan, EPFL*
14.40	Initial finding from experiments into microbial effects on the behaviour of compacted bentonite, *Simon Gregory, NERC*
14.55	**Coffee**
15.15	Microbial influence on transformation of Bavarian bentonites transformation, *Nicole Matschiavelli, HZDR*
15.30	Response of Bentonite Microbial Communities to Stresses Relevant to Radioactive Waste Disposal, *Haydn Haynes, UNIMAN*
15.15	Viability, cultivability and activity of acetate- and sulphide-producing bacteria in compacted, water saturated clays, *Karsten Pedersen, MICANS*
16.00	The effect of acetate on the microbial diversity, mineralogy of compacted Spanish bentonites at different density, *Cristina Povedano-Priego, Fadwa Jroundi, Javier Huertas, Mohamed Merroun, UGR*
16.15	Summary discussion and close of session
16:30	**Poster session** (until 18:00)
18:30	**Guided Tour Prague**
20:00	**Conference dinner**

May 5th

08:30	**Theme session III (WP3: Integration):** Introduction
08:40	Mind dissemination & education activities, *Natalie Leys, SCK•CEN*
09:00	How microbes network (and are networked) in geological disposal research: a sociological perspective, *Michiel Van Oudheusden, SCK•CEN*
09:25	**Coffee**
09:40	Synthesis of WP1 progress and outcome and its translation towards performance assessment, *Joe Small, NLL* (30 min presentation, 15 min discussion with IRB)
10:15	Synthesis of WP2 progress and outcome and its translation towards performance assessment, *Karsten Pedersen, MICANS*
11:15	Summary (Implementers Review Board, IRB)
	Closing of meeting
12:30	**Lunch**
13:30–16:15	**Visit to CVRez**

3. MIND Project Executive Committee meeting

The final part of the 2017 Mind Project Annual Meeting will be the MIND **Project Executive Committee meeting** which is held on the afternoon of May 4th (16:30–17:30). This meeting is only open for **PEC** members who will in due time receive a call and agenda for the meeting.

Poster session

1. Microbial influence on transformation of Bavarian bentonites transformation, *Andrea Cherkouk, Nicole Matschiavelli, HZDR.*

2. Possible impact of microbial processes on cementitious materials in *in situ* conditions, *Kristel Mijnendonckx, SCK•CEN*.

3. Biofilm formation on surface of carbon steel under anaerobic conditions and its microbial composition*, Jana Steinova, Hana Vanova, Rojina Shrestha, Alena Sevcu, Petr Polivka, Tomas Cernousek, TUL*.

4. Long-term experiment on corrosion of carbon steel in artificial bentonite pore water inoculated with natural consortium of SRB, *Tomas Cernousek, Hana Vanova, Jana Steinova, Rojina Shrestha, Alena Sevcu, Petr Polivka, CV Rez*.

5. Long-term lab-scale microbial bentonite storage experiment, *Hanna Miettinen, Minna Vikman, Michał Matusewicz and Merja Itävaara, VTT.*

6. The effect of uranium on microbial diversity and mineralogy of bentonites microcosms, *Cristina Povedano-Priego, Fadwa Jroundi, Javier Huertas, Mohamed Merroun, UGR*.

7. Determination of low-molecular weight Natural Organic Matter (NOM) in Bentonite clays*, Trevor Taborowski, Anders Blom, Alexandra Chukharkina, Andreas Bengtsson, Karsten Pedersen, MICANS.*

8. Analysis of bacterial beta-diversity combining datasets from different sequencing projects, *Johanna Edlund, MICANS*, *Hanna Miettinen, VTT, Malin Bomberg, VTT, Karsten Pedersen*, *MICANS.*

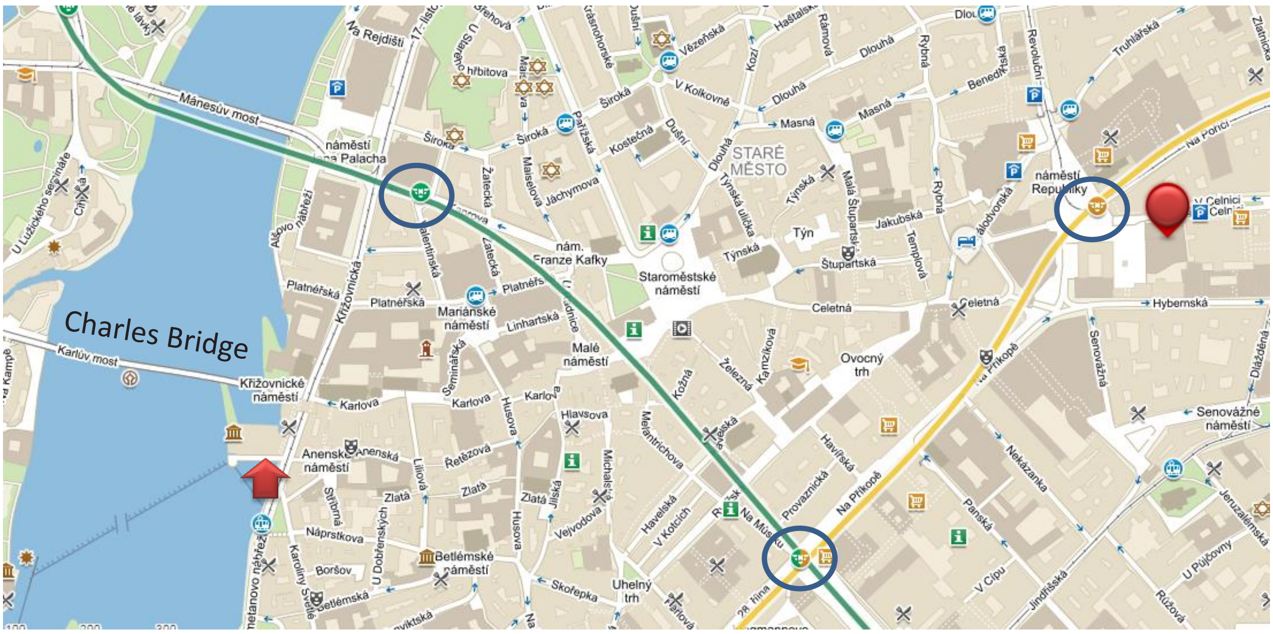
9. The speciation of selenium and europium associated with bacteria isolated from Spanish bentonites, *Miguel Angel Ruiz Fresneda et al. UGR.*

10. The COSMOS experimental setup: An infrastructure able to closely mimic geo-mechanic and -hydrological *in situ* conditions on lab scale dimensions, *Hugo Moors, SCK•CEN*

11. The Bitumen-Nitrate-clay interaction (BN) experiment: an *in situ* experiment to study the impact of nitrate and bitumen degradation product on the residing microbial community, *Hugo Moors, SCK•CEN.*

Registered Name:	Organisation:
Liam Abrahamsen	National Nuclear Laboratory (NNL)
Lasse Ahonen	Geological Survey of Finland (GTK)
Achim Albrecht	Andra
Alena Ševců	TUL
Thuro Arnold	Helmholtz-Zentrum Dresden-Rossendorf
Benny de Blochouse	ONDRAF/NIRAS
Andrea Cherkouk	Helmholtz-Zentrum Dresden-Rossendorf
Petra Christensen	SKB
Cristina Povedano-Priego	University of Granada
Fadwa Jroundi	University of Granada
Simon Gregory	BGS
Haydn Haynes	University of Manchester
Jana Steinová	TUL
Jennifer McKelvie	Canadian Institute for Advanced Research (on behalf of Nuclear Waste Management Organization)
Johan Andersson	SKB
Karsten Pedersen	Microbial Analytics Sweden AB
Birgitta Kalinowski	SKB
Riikka Kietäväinen	Geological Survey of Finland
Klas Källström	Swedish Nuclear Fuel and Waste management Company (SKB)
Tiina Lamminmäki	Posiva Oy
Rizlan Bernier-Latmani	EPFL
Nicole Matschiavelli	HZDR
Miroslav Cernik	TUL
Tomáš Černoušek	Research Centre Rez
Mohamed Larbi Merroun	Universidad de Granada
Hanna Miettinen	VTT
Kristel Mijnendonckx	SCK-CEN
Dr. Moll, Henry	Helmholtz-Zentrum Dresden-Rossendorf (HZDR)
Miguel Angel Ruiz-Fresneda	University of Granada
Sophie Nixon	UNIMAN
Michiel Van Oudheusden	SCK-CEN
Petr Polivka	Research Centre Rez
Patrik Sellin	SKB
Rojina Shrestha	Technical university of Liberec
Jantine Schröder	SCK-CEN
Joe Small	NNL
Tereza Sázavská	TUL
Daniel Svensson	SKB
Hana Vanova	Research Centre Rez
Minna Vikman	VTT
Kirsi Weckman	TVO
Dr. Irina Gaus	Nagra
Satoru SUZUKI	Nuclear Waste Management Organization of Japan

Registered Name:	Organisation:
Don Reed	Repository and Science Operations (RSO) Los Alamos National Laboratory
Naji Milad Bassil	The University of Manchester
Jon Lloyd	University of Manchester
Carla Perez Mon	EPFL
Niels Burzan	EPFL
Charles Wittebroodt	IRSN
Natalie Leys	SCK CEN
Trevor Taborowski	Micans
Johanna Edlund	Microbial analytics Sweden AB MICANS
Lucie Hausmannova	SURAO
Raymond Kowe	RWM
Hugo Moors	SCK•CEN
Ilona Pospiskova	SURAO/RAWRA
Pieter Monsieurs	Belgian Nuclear Research Center (SCK•CEN)
Premysl Moucka	Research Centre Rez
David Dobrev	ÚJV Řež, a. s.



Red arrow – Novotného Lávka 5, workshop and PAM
Red circle – PAM dinner: V Celnici 1031/4, http://www.kolkovna.cz/en/kolkovna-celnice-13
Blue circles: metro stations nearby

Location of the workshop, icebreaker and PAM: Novotného lávka 5, 3rd floor
The entrance is on the left side (marked with arrow)

Křižík hall is equiped with data projector and microphone

Room 318 is equiped with data projector

I. High pH and methanogenesis with focus on low- and intermediate level waste repositories

Workshop leader: Joe Small, NNL

This topical workshop has close relevance to MIND Workpackage 1 and was facilitated by a series of informal presentations with active discussion from participants including MIND partners, regulators and waste management organisation representatives from the Implementers Review Board, Japan and the U.S.A.

Joe Small (NNL) introduced the workshop setting, the background and its relevance to processes affecting C-14 release from low and intermediate level waste (LILW). Evidence from high pH natural analogues was considered alongside data from the TVO large scale gas generation experiment (GGE) that highlights the effects of chemical heterogeneity in LILW. The limitation of the GGE, with its relatively low proportion of concrete to organic (cellulose waste), representative of the Finnish VLJ Repository, compared to other repositories (SFR, Sweden; Centre Aube, France) where higher pH conditions will prevail, that may limit methanogenesis for long periods, was highlighted in the discussion. Further discussion here noted that in the case of the U.S. (WIPP repository) methanogenesis was viewed as having a positive effect by reducing the generation of CO_2. But, as we will see later, it is unlikely that carbonate reduction will occur in the presence of high sulphate concentrations present in brines.

The importance of the competing effect of sulfate was further discussed by Rizlan Bernier-Latmani (EPFL) in her presentation focussing on H_2 driven methanogenesis in *in-situ* experiments at the Mont Terri underground rock laboratory. Here, sulphate reduction is seen to be beneficial in acting as a sink for H_2 generated by anaerobic corrosion. Sulfate reducing bacteria, present in the Opalinus Clay experimental chambers, effectively outcompete methanogenic bacteria, likely due to the very low energy yield obtained of the latter reaction. The effect of carbonate on exerting a further thermodynamic control on the autotrophic (H_2 + CO_2) route to methanogenesis was also discussed.

Thermodynamic and physiological controls on microbial activity at high pH were further discussed by Jon Lloyd (Manchester) in his talk focussed on research using a high pH adapted microbial inoculum from a former lime kiln site. Here, the discussion developed into the fate of organic hydrolysis products (including the complexant isosaccharinic acid, ISA) in such high pH systems which can be fermented or oxidised, the latter in the presence of high energy electron acceptors (nitrate) up to a pH of 12 or in the presence of sulphate at reduced pH (< 10-11). The behaviour of metals and radionuclides (Fe, U, Np, Tc) in such complex systems has been discussed. Development of methanogenesis at this pH is unclear, but can be demonstrated at near neutral pH corresponding to far-field (host rock) conditions. The workshop discussion returned to the question of the role of methanogenesis (and more broadly microbial activity) in safety assessment; “as far as actinide chemistry is concerned, microbial activity is generally a good thing in metal reduction and breakdown of organics complexants”. However, “microbial activity cannot form the core of the safety case but clearly is important”. “What is important in all these cases is the effect they lead to. For actinide chemistry this is clearly good, but for other parameters (C-14 release) it might be different.”

Finally, Achim Albrecht (Andra) discussed the wider context and uncertainties relevant to deep and near surface LILW facilities. If complex carbon-involving redox reactions (i.e. fermentation, methanogenesis) are occurring in modern repositories (i.e. SFR, Centre Aube, where significant concrete is present), these reactions will be even more likely in older repositories (LLWR trenches, UK; Centre Manche, France), where less concrete buffers the high pH environment. The importance of water content was discussed and processes in wastes prior to disposal. In particular the complicated processes involving radiolysis of organic wastes, dose rates and presence of absence of water led to an extended discussion, which was proposed as a workshop topic at the next PAM.

II. Microbial effects on radionuclide migration

Workshop leaders: Henry Moll and Thuro Arnold, HZDR

The participants of the workshop intensively discussed microbial effects on radionuclide migration and how they can be included in the performance assessment (PA) for nuclear waste repositories. Microorganisms are present in all types of host rocks as well as barrier materials relevant for nuclear waste disposal in a deep geological formation. The geochemical conditions in such a repository can be influenced by processes such as microbial induced corrosion, microbial mediated transformation of bentonites as well as microbial influence on transport processes via e.g. biosorption, biomineralization, bioreduction, or biotransformation. The question is: "How can we quantify the influence of microorganisms on transport processes and implement them into the PA?" The workshop was introduced by Thuro Arnold and Henry Moll from the Helmholtz-Zentrum Dresden-Rossendorf. After that Liam Abrahamsen-Mills of the National Nuclear Laboratory (NNL) from the UK gave a talk about "Performing speciation and diffusion modelling, including microbial reactions". Then each participant briefly presented his microbiological results and stated what kind of influence this will have on PA. In this regard the following questions were answered: What is your microbial topic? What is your main result? Is this negligible/small/significant? Do you quantify the microbial influence? How can your results eventually be included in currently used PA models and tools? The consensus of the discussion was that microbial processes, such as the bioreduction of multivalent radionuclides, should be represented in PA, in some way, and that initially a simple model according to the concept from NNL is a good starting point for such implementation.

III. Bioinformatics pipelines and mock communities.

Workshop leader is Pieter Monsieurs, SCK•CEN.

In order to evaluate the sequencing process and the bioinformatics pipelines used by the different partners within MIND, the DNA of a mock community consisting of 8 bacterial species (mock community of ZymoBiomics) was sent around to 8 different microbiology laboratories involved in the MIND project. The different laboratories processed these samples with their own sequencing and data analysis tools, and the outcome was discussed on the 'bioinformatics' workshop preceding the at the Project Annual Meeting, held in Prague, in May 2017. In total, five laboratories had sent in their sequencing data (University of Manchester, École polytechnique fédérale de Lausanne, Technical University of Liberec, Technical Research Centre of Finland and Belgian Nuclear Research Center). Two different sequencing technologies were used i.e. Illumina MiSeq and IonTorrent, and the V3-V4 or V4 region of the 16S rRNA gene was selected as amplicon.

The workshop basically consisted of two parts: a first part where the different labs reported on their own sequencing data and predict the composition of the mock community, and a second part where the sequencing data were analyzed in a standardized way. For the first part, variation was observed in the amount of Operational Taxonomic Units between the different research groups, ranging between 10 and more than thousand OTUs. However, the majority of this variation could be explained by the bioinformatics pipeline used, rather than the amplicon region, the quality of the sequencing data or the type of sequencing technology (Illumina MiSeq versus IonTorrent). This could be confirmed in the second part of the workshop. By applying the same 16S rRNA gene amplicon sequencing analysis pipelines on the five different datasets, comparable results were obtained from the different datasets. It was also observed that longer amplicons (e.g. V3-V4) returned a higher sequencing error rate than the approaches focusing on one region (e.g. V4). Additionally, most artefacts in the sequencing data originated from the presence of chimeric sequences, even for the best performing bioinformatics pipeline.

Knowledge and experience on how to address and process such samples and data was shared among the partners. Overall, it was clear that the different state-off-the-art sequencing and data analysis approaches used by the different partnes in the MIND community are adequate and comparable, providing high quality scientific data. Chimera removal should be a point of attention whenever analyzing sequencing data.

As a next step, it could be of interested to evaluate, discuss and share among the partners also more in detail the upstream steps of in the sequencing pipeline, including DNA extraction, internal standards (mocks) and PCR procedures (e.g. to avoid chimera prodution).

As final remark it was also suggested to keep an eye on the new sequencing technologies coming up, more specifically the so-called third generation sequencing technologies such as NanoPore and PacBio, which could bring some advantages for the MIND research because as they allow a more accurate taxonomic classification in less studied environments such as deep geological layers.

IV. Microbial life and effects on clays in natural and engineered barriers

Workshop leader: Karsten Pedersen

The participants of the workshop discussed microbial life in bentonite clay which will be used as a buffer material in engineered barrier systems (EBS). The EBS will contain, protect and surround nuclear waste canisters in geological disposal concepts. The concepts rely on the swelling capabilities of the bentonite clay when it becomes water saturated as one of the main protective features. To reach the desired swelling pressure of >5 MPa a clay dry density of >1600 kg m^{-3} is generally required. Depending on the minerology of the specific bentonite type and the groundwater composition, where salinity is an important factor, different swelling pressures are produced at the same wet density. A high density is believed to have an inhibiting influence on bacterial activity of the natural bacterial populations in the bentonite clays, meaning that growth will stop and metabolic activity will cease, but present bacteria will not necessarily die. 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 ATP 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. The workshop was introduced by Karsten Pedersen on terminology and conditions for life in compacted bentonite, as briefly described above. Thereafter the participants split up in groups and discussion a set of performance assessment questions with microbiology in focus: What can go wrong? (What if?) How likely is it? Consequences, negligible, small or significant? The workshop ended with brief presentations from each group.

MICROBIAL DEGRADATION OF CELLULOSE AND ITS ALKALI HYDROLYSIS PRODUCTS UNDER HYPERALKALINE CONDITIONS

NAJI M. BASSIL*, JONATHAN R. LLOYD

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Intermediate-level radioactive waste from the nuclear fuel cycle, which is expected to contain cellulosic material encapsulated in cement, will be disposed of in a cementitious deep geological disposal facility (GDF). Under the hyperalkaline conditions imposed by the resaturation of cement with groundwater, cellulose will be chemically hydrolysed to short chain organic acids. The most abundant hydrolysis product is isosaccharinic acid (ISA), which has been shown to bind to and mobilise various radionuclides, thereby increasing the probability of their release from the GDF. However, alkaliphilic microorganisms may survive in such extreme environments potentially using these organics as a carbon and energy source.

Microcosms poised at pH 12.5, and inoculated using sediments from a legacy lime-kiln, showed biodegradation of the added cellulose and fermentation of the degradation products into acetate, while halting ISA production from the abiotic alkaline cellulose hydrolysis [1]. Irradiation enhanced the rate of the abiotic cellulose hydrolysis by alkali, and further made the cellulose fibres more available for biodegradation and fermentation of the degradation products, which led to the production of H_2 followed by its utilisation. Methanogenic microorganisms and methane have not been detected in these systems yet [1].

Enrichment cultures prepared at pH 10 and inoculated with sediments from the same legacy lime-kiln showed that alkaliphilic bacteria degrade ISA under aerobic and anaerobic conditions [2]. An ISA-oxidising obligate alkaliphile belonging to the genus *Anaerobacillus* was isolated from these cultures, and was found to be the only species of this genus capable of ISA utilisation [3]. Further studies showed that the growth of this bacterium led to the precipitation of radionuclides from solution, including soluble U(VI). The genomes of these bacteria were sequenced [4], and comparative genome and transcriptome analysis, coupled to transmission electron microscopy and X-ray absorption spectroscopy are helping identify the mechanisms of ISA degradation and radionuclide precipitation by this novel bacterium. Taken together, these results highlight the role that microorganisms may play in stabilising radioactive waste in the subsurface, and help reduce uncertainties in the long-term performance assessment of the GDF for radioactive wastes.

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PVC ADDITIVES FUEL MICROBIAL NITRATE REDUCTION AT HIGH PH

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The safe disposal of nuclear waste relies on a thorough understanding of the role microorganisms play on the fate of these wastes. This is particularly important for organic-containing waste, such as polyvinly chloride (PVC) plastic, which represents a significant volume of intermediate level and low level wastes. However, little is known of its fate in a geological disposal scenario in which high pH conditions are expected to dominate. To address this gap in our understanding, we initiated microcosms experiments at pH 10 to assess the potential of PVC in pure (powder) and plasticised (sheet) forms to fuel nitrate reduction by a high pH-adapted microbial community. To assess the effect of gamma radiation on bioavailability, samples of PVC powder and sheet were irradiated in saturated calcium hydroxide (pH 12.4) to a cumulative dose of 1 MGy. Both forms of PVC, irradiated or not, were supplied as the sole source of carbon and electron donors to nitrate-containing microcosms, and inoculated with sediment from an alkaline environment known to harbour denitrifying bacteria.

Results show that nitrate reduction was fuelled by plasticised PVC sheet, whether irradiated or not, though the extent of nitrate reduction was less with irradiated material. Irradiated PVC powder was found to fuel minor nitrate reduction, however no nitrate reduction was observed with non-irradiated PVC powder. Analysis of dissolved organic carbon (DOC) concentrations indicate that PVC materials were degraded abiotically via alkaline hydrolysis throughout the experiments. No major differences to the major organic composition of the PVC materials was observed after radiation, but scanning electron micrographs show physical damage to the surface of PVC sheets. Analysis of bacterial 16S rRNA gene sequences from the start and end-points of the microcosm experiments highlight the enrichment of common soil lineages not typically associated with high pH conditions. The results suggest that the additives used to make PVC flexible can fuel microbial processes under nuclear waste geological disposal conditions. Future work should focus on constraining the implications of PVC additive biodegradation on radionuclide mobility to better constrain the safety case for nuclear waste disposal.

MICROBIAL DEGRADATION OF ORGANICS AND NITRATE LEACHING FROM BITUMINISED RADIOACTIVE WASTE

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Deep geological disposal of radioactive waste has been considered in many countries as a potential safe long-term solution. In Belgium, Boom Clay has suitable physico-chemical characteristics to serve as natural barrier for the disposal of low- and intermediate level long-lived radioactive (LILW-LL) waste. Part of this LILW-LL radioactive waste has been immobilized in a bituminous matrix and typically contains also a large amount of soluble salts with sodium nitrate as the most dominant. The steel waste drums containing this bituminised waste will further be encapsulated in concrete monoliths and as such inserted in the subsurface repository. Over time, water from the surrounding clay host rock will infiltrate into the waste monoliths and the waste drums resulting in osmotically driven water uptake by the waste, followed by a progressive dissolution of the salts. These dissolved salts will leach slowly from the waste, together with organic bitumen degradation products (e.g. carboxylic acids such as formate, acetate and oxalate). In the repository, gas (mainly H_2) will be produced as well originating from radiolysis of water and bitumen and from the anaerobic corrosion of steel. The subsequent 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 from the clay water by various processes. Abiotically, the reduction of nitrate can occur with the produced H_2 and/or steel acting as electron donors and with the steel or even pyrite possibly serving as catalytic surface [1, 2]. These reactions lead to the production of ammonium which can sorb onto clay minerals and therefore compete with some radionuclides for sorption [3, 4]. In addition, nitrate is a known electron acceptor for microorganisms and most of the other leachates from bituminized waste are degradable by microorganisms as well, if proper growth conditions are provided. Microbial consumption of nitrate is leading to the intermediate production of nitrite, and finally to nitrogen gases. Depending on the electron donor used in the denitrification process, it could lead to a pressure decrease or increase. However, scoping calculations indicate that, close to the waste drums the $NaNO_3$ concentration can reach up to a few molar and that during the first ~300 to ~1400 years the $NaNO_3$ concentration within the monolith will remain above 1 M and 0.5 M, respectively. Maximum NO_3^- concentrations reach between 0.5 and 1 M at the gallery interface, and decrease also fast within the host formation (e.g. 0.1 M at a distance of 5 m in the Boom Clay) [5]. Consequently, within the waste monolith, microorganisms will be exposed to high pH and high salt (sodium) concentrations, putatively inhibiting their activity. The latter was investigated in this study.

Batch experiments were performed in hermetically closed and anoxic culture bottles to investigate more in detail the potential of microbes to reduce nitrate leaching from non-aged and thermally aged inactive bituminized waste in the presence or absence of relevant organic electron donors. In addition, batch experiments were performed without inactive bituminized waste as source of nitrate, but in which known concentrations of sodium nitrate or sodium nitrite were added. Borehole water samples from different layers within the Boom Clay, which have been shown to comprise a highly diverse bacterial community of which a large fraction is active [6], were collected and used as water environment and inoculum for these experiments. As additional electron donors, formate or acetate, known organic leaching products from bitumen, were tested. No steel, concrete or clay were added in the test at this stage. Experiments were performed at 30°C.

Without an additional electron donor, no nitrate reduction was observed after 50 days demonstrating that the organic material present in Boom Clay borehole water was a poor electron donor. In the presence of formate, 20% of the nitrate leached from the inactive bituminized waste was reduced. In the presence of acetate, reduction increased up to 50% of the nitrate leached from the inactive bituminized waste, demonstrating that acetate is more favourable as electron donor compared to formate or the organic material present in Boom Clay borehole water. 16S DNA metagenomics showed that after 17 days, the microbial population was dominated by *Pseudomonas* when no additional electron donor or acetate was present, while *Acidovorax* was the most dominant genus with formate as electron donor. In addition, boundary conditions regarding the high salt (sodium nitrate) concentrations were investigated. Concentrations up to 4 M $NaNO_3$ were not lethal for the microbial community as the viable cell count, determined with flow cytometry, remained stable up over the full 80 days of

testing. Nevertheless, concentrations of 1 M of $NaNO_3$ or $NaNO_2$, or higher, probably inhibited microbial activity.
In conclusion, nitrate can be removed by nitrate reducing microorganisms present in Boom Clay borehole water, with increasing rates in the presence of formate or acetate but very high concentrations of $NaNO_3$ or $NaNO_2$ are likely to inhibit microbial activity.

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EFFECT OF MICROBIAL CONSORTIUM NATURALLY CONTAINING SRB ON DEGRADATION PRODUCTS OF GAMMA IRRADIATED ORGANIC RADIOACTIVE WASTE

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Radioactive organic material of different origin belongs to important component of intermediate level waste (ILW) and requires specific storage and disposal conditions. One of important ILW representative consists of ion exchange resins used to clean the water circulating through the pressurized water reactor (PWR).
We studied the effect of natural bacterial consortium on gamma irradiated ion exchangers in order to describe possible scenarios in geological repository conditions. Prepared ion exchangers (cation and anion exchangers) were first gamma irradiated at total dose around 1 MGy that corresponds to expected dose during first 1,000 years in the repository. Samples, positioned in the irradiation facility "PRAZDROJ", got an average dose rate 2.04 kGy/h. Aqueous and acetone extracts of irradiated samples were analysed using FTIR–Raman spectroscopy, TOC and gas chromatography (GC/MS) techniques. Irradiation caused a remarkable increase in TOC in both materials (was below detection limit before irradiation), a higher TOC concentration being detected in irradiated anion exchanger. Moreover, a significant proportion of trimethylamine and dimethylaminacetatenitrile was detected.
The irradiated ion exchangers were then inoculated with water containing SRB. The concentration of ion exchangers in batches are 0.2; 2; 20 and 60 g/l. Changes of bacterial abundancies were studied by real-time quantitative PCR (qPCR) analysis targeting 16S rDNA gene. The first results of experiment indicate that the lowest concentration of irradiated anion exchangers (0.2 g/l) caused the highest relative change in bacterial abundance (14 fold increase) whereas the other studied concentrations caused almost no effect. Cation-exchanger, on the other hand caused total decline in bacterial biomass in two highest concentrations studied (60 g/l and 20g/l) caused probably by extremely low pH. Results of lower concentrations studied (2 g/l and 0.2 g/l) were comparable to control.

SPECIATION OF URANIUM(VI) IN THE PRESENCE OF CELLULOSE DEGRADATION PRODUCTS

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The HZDR-IRE activities within MIND Work Package 1 lies on radionuclide (RN) speciation studies with organic degradation products as a result of both abiotic and microbial activities (e.g. degradation of cellulose). Low and intermediate level waste (LILW) contain cellulosic material in considerable amounts. In a repository nuclear waste is often surrounded by cementitious backfill material. If water ingresses this results in a hyperalkaline environment. Since cellulose will be relatively fast degraded under alkaline conditions, there is a high risk that small organic, water soluble molecules will be formed. These molecules can act as complexing agents for radionuclides and thereby affecting their sorption behavior and solubility adversely. Therefore the focus of current investigations is on the interaction of uranium(VI) with Isosaccharinic acid (ISA) as main degradation product of cellulose. The progress and results of these studies will be presented.
Based on a protocol provided by UNIMAN, we could successfully synthesize the compound $Ca(ISA)_2$. Since this substance is only sparingly soluble in water, we prepared via cation exchange the corresponding sodium salt as starting material for U(VI) speciation studies. Spectroscopic studies regarding the interaction of ISA with UO_2^{2+} are scarce. Hence, we performed UV-vis (see Figure 1), TRLFS, ATR-FT-IR, and XAS experiments combined with theoretical modelling (DFT calculations) to explore the U(VI) speciation in the presence of ISA in the acidic pH region.

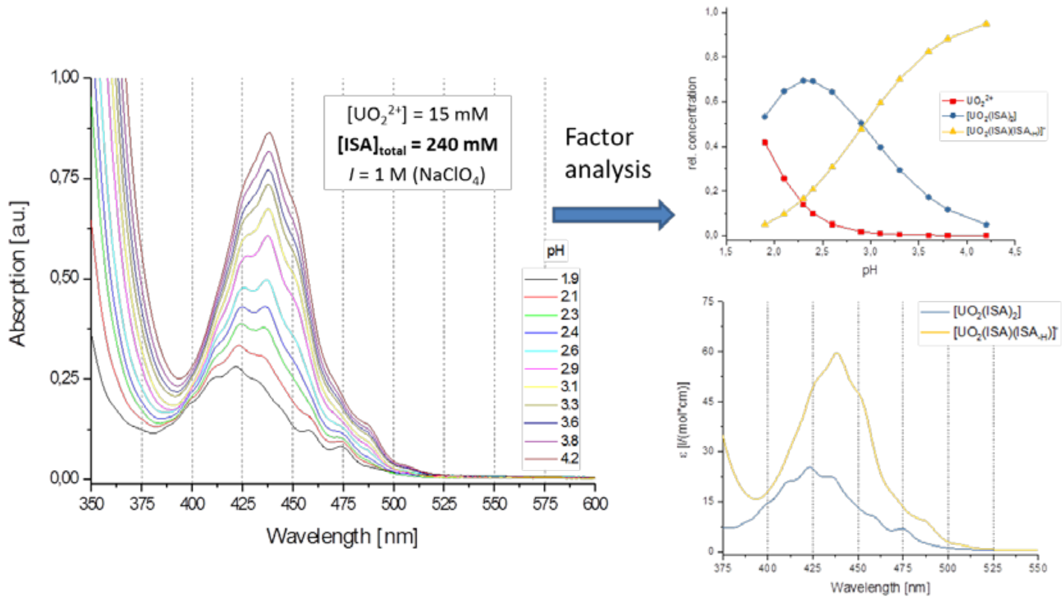

Figure 1: Speciation analysis in the U(VI)-ISA system based on UV-vis data.

The presented results in the U(VI)-ISA system will be the starting point for investigations under neutral and alkaline conditions (including solubility experiments).

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THE SPECIATION OF SELENIUM AND EUROPIUM ASSOCIATED WITH BACTERIA ISOLATED FROM SPANISH BENTONITES

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As a part of the studies undertaken within the Work Package 1 of the Microbiology In Nuclear Disposal (MIND) project, the University of Granada contributes in the study of microbial processes controlling radionuclide migration from long-lived intermediate level wastes (ILW). Specifically, we are focused on the selenium (Se) and europium (Eu) speciation by bacteria isolated from Spanish bentonite formations under disposal relevant conditions. It is well known that the speciation of this kind of elements determines their transport and migration behaviour in the environment. Selenium is a common component of radioactive wastes produced by nuclear fission [1]. It is commonly assumed that europium, as a trivalent lanthanide (Ln(III)), mimic trivalent actinides (e.g. Cm(III) and Am(III)) characteristic of nuclear wastes. Therefore, it can be used as suitable non-radioactive chemical analogous [2].

The aim of this work is to determine the role of the bacterial strain *Stenotrophomonas bentonitica* BII-R7 [3], isolated from bentonite clays of Cabo Gata (Almeria, Spain), in the speciation and migration of Se and Eu. For this purpose, anaerobic, alkaline and other repository relevant conditions were employed. *S. bentonitica* is able to reduce Se(IV) to Se(0) nanoparticles located extracellular and intracellularly under both aerobic and anaerobic conditions. Simulating anaerobic relevant conditions, the selenium reduction can occur up to pH 11. For this study, a combination of microbiological, spectroscopic and microscopic techniques was applied. In addition, cell surfaces of the bacterial strain BII-R7 has the capacity to interact with Eu(III) mainly by a biosorption process as was revealed by transmission electron microscopy (TEM) (Figure 1) and kinetic analysis. In a lesser proportion, extracellular and intracellular Eu(III) accumulates were also observed. Time-Resolved Laser-induced Fluorescence Spectroscopy (TRLFS) suggested that phosphoryl and carboxyl groups seem to have an important role in the Eu complexation under both aerobic and anaerobic conditions.

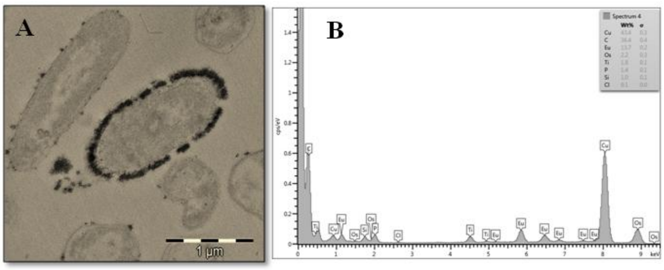

Figure 1. TEM micrograph of thin sections of the cells of *S. bentonitica* treated with Eu showing electron-dense accumulates extracellularly and around the cell surface (A). The dark accumulates are composed mainly of Eu(III) as determined from Energy Dispersive X-ray (EDX) analysis (B).

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PRODUCTS FORMED DURING THE RADIOLYSIS OF ION EXCHANGE RESINS UNDER REPOSITORY-RELEVANT CONDITIONS

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Ion exchange resins (IER) are polystyrene polymers cross-linked with divinylbenzene, to which either sulfonic (cation exchange) or amine (anion exchange) functional groups are added[1]. IER are used in nuclear power plants to remove radionuclides from liquids and they represent the 40% of the organic part of the low and intermediate level waste (L/ILW) in Switzerland[2]. The Swiss concept of deep geological repository (DGR) for L/ILW consists of embedding the waste within cementitious materials and placing it within the Opalinus Clay geological formation (OPA), at ~600 mbgs[3]. The radiolysis of IER under the expected DGR conditions of anoxia and pH > 10[4], can potentially generate organic compounds (e.g., benzene and alkanes) that can be utilized by the resident microbiota. The by-products derived from the biotransformation of these organics might importantly influence the geochemical processes and, ultimately, the integrity of the DGR.

In the present study, we investigate the products resulting from the irradiation of IER loading with representative ions from OPA pore water under anoxic conditions. We tested two resins of varying particle size (powder resin and bead resin) and two pH values for the pore water: pH 8 which corresponds to the natural pH of OPA, and pH 12.5 which corresponds to the pH expected (at least transiently) in the DGR in the vicinity of the cementicious material. Irradiation of samples at 50 kGy and pH 8 evidenced the formation of benzene, bromomethane and chloromethane in the gas phase for both types of resins. In addition, chloropropanone, toluene, ethylchloride and methylchloride were detected in the powder resins. Irradiation of samples at 100 kGy did not show the formation of any product in the liquid phase following analysis by GC-MS. Future experiments will include irradiation at higher doses and further development of the analytical tools to characterize the aqueous phase. After confirmation of the formation of the compounds identified above, their transformations by the OPA microbial community will be investigated.

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MODELLING SULPHATE REDUCTION IN AN IN-SITU BOREHOLE EXPERIMENT

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In order to include microbial processes (oxidation, reduction, gas generation / consumption etc) within modelling codes that consider processes relevant to geological disposal (metal corrosion, gas generation, radioactive decay, sorption, precipitation, transport etc), NNL is exploring two avenues of model development. The first is the development of an Application Programming Interface (API) that implements the existing model approaches of the Generalised Repository Model (GRM) code within more modern, commercial modelling codes. GRM is an NNL-owned, FORTRAN-based code and has been used previously to predict the performance of the Low Level Waste Repository (LLWR) in the UK [1] and validated using the Gas Generation Experiment at the VLJ repository, Olkiluoto, Finland [2]. The GRM microbial model considers a wide range of potential electron donors and acceptors, different microbial communities and is based on Michaelis-Menten kinetics. The API will enable the proven GRM microbial model to be coupled with geochemical and reactive-transport codes. This work is ongoing.

The second approach is to implement a more limited number of microbial processes within the open-source PHREEQC modelling code [3]. Researchers from SKB and Amphos 21 Consulting [4] have developed an approach to simulating time-dependent sulphate reduction within PHREEQC, using both lactate and hydrogen gas as electron donors. The rate equations are based on Michaelis-Menten kinetics and chemical equilibrium is determined for the gas phase and any solid phases predicted to form (eg iron sulphide phases such as mackinawite).

To begin to model the Microbial Activity (MA) borehole experiment at Mont Terri [5] we have taken the SKB model, which considers one model cell only, and introduced a series of additional model cells to represent layers of clay rock surrounding the borehole solution. This approach allows the inclusion of diffusive transport of host rock pore water solution into the borehole solution. In the model this is represented using a 1-D column of cells with exponentially increasing volume. Diffusion is simulated using the approach of Appelo & Wersin [6].

The diffusive model functions correctly, with microbial reactions dependent on the slow diffusion of nutrients into the borehole from the clay rock. Further work is required to include realistic concentrations of species in the borehole and the pore water, as well as estimation or fitting of the kinetic parameters used in the Michaelis-Menten kinetics.

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MICROBIOLOGICAL DEGRADATION OF LLW UNDER *IN SITU* CONDITIONS

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In Finland, low-level radioactive waste (LLW) includes 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. LLW is packed into carbon steel drums and concrete boxes which are then disposed in silos or repositories at depth of 60–110 metres inside the bedrock. The microbiological degradation of cellulosic materials in anoxic conditions results in gas generation and can also accelerate corrosion, deteriorate the performance of multi-barrier systems, and enhance the mobility of gaseous radionuclides from the repository to the surrounding environment.

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 [1]. Waste drums with the volume of 200 L were filled with representative maintenance waste from nuclear power units and were arranged in a concrete box. The GGE was filled with river water and maintained a temperature of 8-11°C. The GGE has been monitored for volume and composition of generated gas, water chemistry and microbiology. 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.

In the beginning of the GGE concrete structures created alkaline environment at the lid level of the tank but pH was close to neutral at the bottom of the tank and inside the waste drums. These heterogeneous chemical conditions in the GGE created optimal niches for microbial action and differences in the microbial abundances in different compartments of GGE were detected. By 2013, chemical conditions in the various compartments in the GGE were stabilized and pH of water was decreased close to neutral as result from the microbial production of CO_2 and other microbial metabolites. LLW was converted to methane and carbon dioxide as a successive action of complex microbial consortia including cellulose degrading bacteria, acetogenic bacteria and methanogens. Hydrogenotrophic methanogens dominated during the first years of GGE operation and acetoclastic methanogens were detected after 2005. The relative ratio of sulphate reducers compared to methanogens decreased considerably both in the lid and bottom level of the tank between 1998 and 2015. In addition to bacteria and archaea, fungi have been detected in GGE but their role in degradation of LLW needs further research. In addition, the results can be used for validation of simulation modelling.

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MODELLING OF MICROBIAL GAS GENERATION PROCESSES AFTER 18 YEARS EXPERIMENTAL STUDY OF LLW DEGRADATION

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Gas generation from low- and intermediate-level nuclear waste (L/ILW) is an important process affecting the safety of near surface and geological nuclear waste repositories. Generation of a gas phase has the potential to transport gaseous radionuclides such as ^{14}C to the biosphere. Furthermore, pressurisation and gas phase formation can affect the engineered barrier system and water flow, which can affect the transport of radionuclides and other soluble contaminants in water. Microbial gas generation is coupled with a wider range of biogeochemical processes that are also relevant to the safety of L/ILW repositories through effects on pH, Eh and organic complexation.

The Gas Generation Experiment (GGE) located at the VLJ Repository for L/ILW at Olkiluoto, Finland records the generation of a CH_4–rich gas from LLW from the operating power plants at Olkiluoto. A description of the GGE that includes 16 200 dm^3 drums of cellulose containing LLW and results of the first 9 years of operation was published in [1]. We now present an 18 year set of geochemical and microbiological data from the GGE that has been interpreted with a biogeochemical model to examine how the chemical conditions in the experiment have evolved as a result of the microbiological processes occurring and where pH, Eh and carbonate have homogenised.

Initially, tank water in the experiment, which surrounds waste drums was buffered at pH 10 to 11 by a concrete overpack container. Neutralisation of the water to below pH 9 by cellulose degradation processes within the waste drums has resulted in a doubling of the rate of gas generation to around $1m^3$ per year. After 18 years the Eh of the tank water measured by a Pt electrode is consistent with that calculated for the S(6)/S(-2) and methanogenesis redox couples suggesting equilibration of redox processes. Dissolved organic carbon attained a peak concentration of 8mM during the early stages of the experiment, but after 9 years declined. Carbonate concentration has increased steadily to around 15mM. After 7 years CO_2 gas and aqueous and solid carbonates were also close to equilibrium.

The model represents the coupled processes of organic waste degradation, pH buffering and microbial gas generation. The model simulates that the majority of the CH_4-rich gas is generated by H_2 and organic consuming methanogenic processes in the waste drums. The increase in CH_4 generation is simulated to be the result of methanogenic processes that consume soluble cellulose degradation products that diffuse into the tank water. This increase in gas generation rate occurred when the pH had fallen below pH 9. It is expected that under the steady state and homogeneous conditions now established, resulting from gas/liquid/solid equilibrium and diffusion processes, that gas generation should continue until the cellulose and steel materials are exhausted.

The study illustrates the complex coupled biogeochemical processes that affect gas generation and pH buffering of cementitious conditioned organic L/ILW, which has further implications for complexation and sorption processes that can affect radionuclide mobility.

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GASES AND SULPHUR COMPOUNDS IN DEEP BEDROCK GROUNDWATERS ACROSS FINLAND

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Biogeochemically induced risks at nuclear waste repository sites are mainly related to changes in release, disperse and mobilisation of radioactive, toxic and corrosive substances. On the other hand biogeochemical processes may also diminish the risks, for example, by maintaining favourable redox-conditions. Thus, interaction between microorganisms and engineered and geological barriers forms an integral part of the safety assessment of nuclear waste disposal.

In order to get an overview, and also to define boundary conditions, of microbial activity in deep crystalline bedrock environment, a literature and database survey was conducted which covered geochemistry of deep groundwater study sites in Finland. The sites include both drill holes and deep mines with deepest samples from 2480 m depth below the ground. The results were combined in one data table for further use in graphical and numerical analysis. As of April 2017 more than 400 samples from 27 separate locations have been included. As many of the biogeochemical risks identified so far, such as corrosion of copper canisters and mobilization of ^{14}C, are related to gases and sulphur compounds, these were targeted in more detail.

Based on dissolved gas composition, groundwaters could be divided into two main types: methane-dominated and nitrogen-dominated. Other commonly detected gases included H_2, He, Ar and occasionally CO_2, although significant variation existed between different sites and with depth. Beyond these results, obtained by the traditional gas chromatographic (GC) method, thermal desorption-GC-MSD analysis was performed for samples from the Outokumpu Deep Drill Hole in order to get more detailed information on volatile organic compounds (VOCs). The results revealed a wide array of compounds, which also included some sulphur containing compounds, e.g. dimethyl sulphide, diethyl sulphide and thiophene (C_4H_4S).

The most abundant sulphur compound in deep groundwaters is sulphate (SO_4). The highest concentration of 4400 mg L^{-1} was detected at Liminka, in the northern west coast of Finland, although concentrations in the order of tens or few hundreds of mg L^{-1} are more common. Sulphide concentrations have been analysed more rarely, and those determined are usually below 0.1 mg L^{-1}, although concentrations as high as 1.9 mg L^{-1} were found from the Pyhäsalmi mine, which is located in a volcanogenic massive sulphide deposit. A discrepancy is sometimes observed between total sulphur content and combined sulphate and sulphide concentrations, which can arise from the presence of other sulphur species, such as those mentioned above.

As a conclusion, the concentration patterns of gases and sulphur compounds are very variable even within Finland. This is probably due to variation in rock types combined with differences in hydrogeological history, including the location relative to sea shore and residence time of groundwater. Furthermore, the geochemistry of sulphur and carbon in deep groundwaters is more complex than previously thought. The results call for detailed site specific determination of boundary conditions which should preferably include both spatial (with depth) and temporal aspects.

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ACTIVATION OF DEEP SUBSURFACE GROUNDWATER MICROORGANISM BY ELECTRON ACCEPTORS AND DONORS

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Deep groundwaters of the final disposal repository area of spent nuclear fuel in Olkiluoto, Finland, were studied to simulate the effects of water mixing and infiltration of previously limited energy sources. The aim was to detect metabolic activation of microbial communities due to increase in different electron acceptors and donors and later on to analyse transcriptomics of activated cells. The activity of two microbial communities from groundwater fracture fluids (OL-KR6/125-130m and OL-KR15/446-460m) originating from the final nuclear waste disposal site area in Olkiluoto was induced by additions of electron acceptors and donors. Microbial activation was studied with two fluorescent dyes, 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) and RedoxSensor^{TM} Green (RSG), and activated fluorescent cells were detected with flow cytometry. The CTC dye is mainly reduced by the electron transport system components or dehydrogenases to red fluorescent formazan and RSG is modified by microbial reductase activity in the electron transport system to give stable green-fluorescent signal. The activation was measured by comparing the number of particles fluorescent with CTC or RSG in relation to particles stained with DAPI (4',6-diamidino-2-phenylindole, a DNA stain).

Prior to the experiment, the fracture fluids were starved for six weeks before performing the anaerobic activations. Regardless of the starvation, the microorganisms in the fluids were relatively active. In the OL-KR6 fracture fluid, 36% and 75% of the cells were found fluorescent with CTC and RSG, respectively. In OL-KR15 water the portion of fluorescent cells was even higher, 54% and 90%. Further tests were carried out with only CTC. Acetate proved to be the best activator of both fracture fluid communities as the portion of active cells increased from 36 to 75% in OL-KR6 and from 54 to 65% in OL-KR15 fracture fluid. In OL-KR6 water, other activating electron donors in the descending order were methane and hydrogen and activating electron acceptors were nitrate and nitrate+methane. Sulphate was not studied as the fracture fluid already contained high amounts of sulphate (4.5 mM). By contrast, OL-KR15 fracture fluid was a low sulphate (0.06 mM) water and so also sulphate as an electron acceptor was studied. In addition to acetate alone, acetate with sulphate activated OL-KR15 microbial cells in the fracture fluid from 54 to 63%. Other tested electron donors and acceptors reduced the amount of active microorganisms. Especially methane, methane+sulphate, methane+nitrate and hydrogen reduced the community activity to less than half of the original.

The microbial communities in the two unstarved fracture fluid samples, characterized by amplicon sequencing of the bacterial and archaeal 16S rRNA genes, differed greatly from each other. However, the most abundant groups detected were typical for Olkiluoto fracture fluids [1]. In OL-KR6 the most abundant genus was sulphur oxidising *Sulfurimonas* (73% of the bacterial 16S rRNA gene sequences) whereas in OL-KR15 alphaproteobacterial Caulobacteraceae (36%) and gammaproteobacterial Pseudomonadaceae (33%) were the biggest groups. Both fracture fluids contained also sulphate reducing bacteria with around 3 to 4% of the bacterial 16S rRNA gene sequences regardless of the original sulphate concentration. ANME-2D archaea were abundant in the archaeal fraction (67% of the archaeal 16S rRNA gene sequences) in OL-KR15 fracture fluid but rare in OL-KR6 (0.6%). Both fracture fluids had also plenty of Thermoplasmata (TMEG, Terrestrial Miscllanoeous Euryarchaeotal group) archaeal sequences.

Acknowledgements
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CORROSION OF CARBON STEEL IN PRESENCE/ABSENCE OF MICROORGANISMS UNDER ANAEROBIC CONDITIONS

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High-level radioactive waste and spent nuclear fuel require a secure and reliable disposal system for tens of thousands of years. The Czech concept of disposal considers a double-walled container with the outer layer of carbon steel and the inner layer of stainless steel. To ensure the maximum protection of disposed material it is necessary to determine carbon steel behaviour in similar conditions that are presumed in the deep geological repository. Therefore, we studied the corrosion resistance of carbon steel 12020 in presence of natural consortia of microorganisms. Two variants of corrosion experiments containing carbon steel 12020 were prepared in: i) natural groundwater „VITA“ (obtained from Josef URC) containing sulphate reducing bacteria (SRB) - the main agents involved in biologically induced corrosion, ii) artificial bentonite pore water (BPW) inoculated with „VITA“ water (ratio 9:1) simulating environment surrounding containers. In order to distinguish between chemical and microbially induced corrosion processes, negative sterile controls were carried out in a parallel arrangement to both corrosion experiments. Experiments were performed in glove boxes under anaerobic conditions. For the in-situ characterization of corrosion behaviour, Electrochemical Impedance Spectroscopy (EIS) and polarisation measurements were performed. Weight losses of carbon steel samples were also determined for the purpose of evaluation of corrosion rates in the long-term BPW experiment. EIS measurement revealed three corrosion stages in "VITA" experiment due to biofilm and corrosion products formation. The calculated corrosion rates from weight losses (BPW experiment) confirmed that carbon steel 12020 had higher corrosion rate in the environment with the presence of bacteria (corrosion rate 4,7 μm/year) than in sterile control (3,0 μm/year). Evaluation of EIS measurement from BWP experiment is still in process. Microbial communities from "VITA" and BPW experiment differed in structure: samples from "VITA" were dominated by sulphate-reducing bacteria whereas nitrate-reducing bacteria were the most abundant group in the BPW experiment. Presence of nitrate-reducers can be explained by the composition of artificial bentonite pore water rich in nitrates.

IN SITU CORROSION OF STEEL IN BENTONITE – ROLE OF MICROBES

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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 first results of an *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 MX-80 bentonite-filled modules, in which steel 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. The initial microbial community in the borehole was characterized, as was the community at the first sampling time point (20 months). Additionally, cultivable microorganisms from the bentonite were enumerated at the 20 months time point. 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 in diversity over the 20 months in the borehole.

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 the MX-80 bentonite and (b) in the porewater on the corrosion rate of steel. In addition, the influence of gamma irradiation on the swelling pressure of MX-80 will be evaluated.

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 unalloyed 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. A compacted bentonite sample containing steel chips near the inlet was 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.

The first two experimental tests are currently running. Test 1 contains sterile (irradiated) bentonite without microbial inoculum and has been running for 80 days. The second test contains sterile (irradiated) bentonite inoculated with a mixed microbial culture enriched for sulphate reducers from uniradiated bentonite samples. During the experiments, in/out flow is being monitored along with total stress to see if the corrosion of steel has a direct effect on swelling behaviour of the samples. Stress and pressure data show that the sample in test 1 hydrated over a period of 26 days reaching a swelling pressure of 1660 kPa. On day 26 a hydraulic gradient was applied which resulted in increase in total stress and divergence of the stress measurements on the five load cells, reflecting the gradient in hydraulic pressure across the sample. Inflow reached asymptote around day 45 and flux remained constant thereafter. Test 2 hydrated over a period of 25 days. Swelling pressure remains to be confirmed due to issues with the data logging requiring recalibration of load cells at the end of the test. A hydraulic gradient was applied on day 25 resulting in an increse in total stress. Inflow reached asymptote at around day 30. Both samples had permeability of $1.8x10^{-20}$ m^2, indicating repeatability of the sample manufacture process. Both tests are ongoing; stress, flow and permeability evolution will be monitored with time. After approximately 90 days both tests will be dismantled, SEM analysis will be carried out on core sample (steel and bentonite) to assess degradation/corrosion, XRD will be used to examine clay mineralogy. Sulphate reducing bacterial numbers will be assessed using MPN and microbial community analysis and microbial activity assays will be carried out on both test samples. Water will be collected (if sufficient volume has passed through the core) for chemical analysis (IC/ICP). The results of the current test will inform the set up of future tests.

MICROBIAL INFLUENCE ON TRANSFORMATION OF BAVARIAN BENTONITES

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For the deep geological disposal of high-level radioactive waste (HLW) a multi-barrier concept is favoured consisting of a container that includes the HLW (technical barrier), which is surrounded by a geotechnical barrier (Bentonite). These packages are placed into the host rock (geological barrier). Bentonites are clay minerals, which are characterized by a high swelling capacity and a low hydraulic conductivity. Could indigenous microorganisms influence these barrier functions by their metabolic activity?

In order to mimic an environment within the repository, we set up microcosm experiments. For this purpose 20 g Bentonite (two Bavarian Bentonites, a processed one (B25) and a natural one (N01)) and 40 ml of synthetic, anaerobic Opalinus clay pore water solution were mixed and an N_2/CO_2-gas-atmosphere was established. To stimulate microbial growth and potential associated geochemical and biological effects we added different substrates (lactate, acetate and H_2). The microcosms incubate at 30°C and 60°C in the dark without shaking for approximately one year. Regularly samples are taken in order to analyse different geochemical and biological parameters.
Until now, an anaerobic atmosphere was created within all our microcosms. Batches with Bentonite N01 at 60°C show no significant changes in pH, E_h and sulphate-concentration after 200 days of incubation. However, low amounts of pyruvate were formed after 60 days of incubation in microcosms containing lactate, indicating microbial activity within these batches. Similar results were obtained for Bentonite B25. Furthermore in these batches, E_h was decreasing from 300 mM to -170 mM within the first 140 days of incubation at 60°C when H_2 was present. The decrease of E_h could potentially support growth of further strictly anaerobic microorganisms like sulphate reducers and methanogenic archaea. First results from batches at 30°C indicate again a strong decrease of E_h in the microcosms containing B25 when H_2 was supplemented as electron donor. So far, no significant changes in the Fe(II)- and Fe(III)-concentration have been detected, in none of the set ups. DNA was successfully extracted from the raw or original material of N01 and B25 and amplified 16S rDNA gene fragments were sequenced with MiSeq Illumina. The results show the presence of aerobic and anaerobic bacteria within the raw material, providing the potential to survive under these harsh conditions.

RESPONSE OF BENTONITE MICROBIAL COMMUNITIES TO STRESSES RELEVANT TO RADIOACTIVE WASTE DISPOSAL

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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, a chemical environment limiting the corrosion of the canister, mitigates radionuclide transport, and minimises pore space which is believed to physically inhibit microbial activity[2]. However, active microbes have been found in natural bentonite strata, as well as after barrier material fabrication, and under simulated conditions[3]. 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) in 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.

Our results show that Fe(III)-reducing, and sulphate-reducing (SRB) bacteria are present in the bentonites, and remained active after the treatments, in reduced quantities.

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File must be named as follows: Work Package X (1, 2 or 3) and author.

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VIABILITY, CULTIVABILITY AND ACTIVITY OF ACETATE- AND SULPHIDE-PRODUCING BACTERIA IN COMPACTED, WATER SATURATED CLAYS

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Bentonites rich in swelling montmorillonite clay are used to construct engineered barriers in geological repositories for low- intermediate- and high-level radioactive wastes. While there are several low- and intermediate-level repositories in operation around the world, high-level repositories are still in planning or under construction. Various types of metal containers, made of iron or copper, are, or will be used to encapsulate the wastes, often together with concrete or bitumen. Sulphide is in general very corrosive to these metals and safety cases for radioactive waste disposal must, therefore, evaluate the risks involved with sulphide corrosion. The main source of sulphide in geological repository environments is past and present bacterial reduction of sulphate, sulphur and thiosulphate to sulphide. Acetate is listed as a potential stress corrosion cracking agent and bacterial production of acetate in compacted clays is not well studied. Sulphide- and acetate producing bacteria (SPB) have been found in most commercially available bentonites [2, 4] and in groundwater [1, 3]. This work investigated the influence of 5 clay types and wet densities from 1500 to 2000 kg m^{-3} on cultivability of sulphate-reducing bacteria and sulphide- and acetate-producing activity of sulphate-reducing bacteria and acetogens.

The following results were obtained:

- Cultivability of sulphate-reducing bacteria decreased with increasing wet density for most tested bentonites.
- Sulphide production decreased to values at or below detection at the highest tested wet densities in 4 of a total of 5 tested clays. Acetate production was ongoing at all tested wet densities.
- Sulphide production and acetogenesis decreased with increasing content of iron at similar wet densities (variance in available natural organic matter between clays may have had an influence as well).
- Cultivability, viability and activity of bacteria in compacted bentonite appeared to depend on a combined effect from wet density and iron content.
- Other factors that can influence cultivability, viability and activity of bacteria in compacted bentonite can be water activity, pressure, pore space and temperature.

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EFFECT OF ACETATE ON THE MICROBIAL DIVERSITYAND MINERALOGY OF COMPACTED SPANISH BENTONITES AT DIFFERENT DENSITIES

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Compacted bentonite-based materials are often considered as suitable sealing materials for deep geological disposal (DGR) of radioactive wastes, due to their low permeability, high radionuclide retardation capacity and high swelling ability [1]. Indeed several studies have been carried out for the selection and characterization of such clays, an in-depth understanding of the compacted bentonite and their appropriate physical properties (e.g. density, pore size, etc.) after placement in a repository site is still required, including the impact of microorganisms under DGR relevant conditions.
In Spain, bentonite formations located in "El Cortijo de Archidona" (Cabo de Gata, Almería) have been well characterized [2] due to their possible use as natural analogue of the bentonite-engineered barrier in the future Spanish repository. Studying potential effects of the microbial activity on the different DGR barriers is also of high relevance. These studies must cover, among other topics, the presence of indigenous microorganisms and their survival and mobility in the compacted bentonite under these relevant conditions. The study of microbial community of the Spanish bentonite is the key point to determine whether indigenous microbes are capable to produce metal corrosion, to transform bentonite Fe-containing minerals and/or to affect the mobility and migration of radionuclides from repositories to the biosphere [3, 4].
In this study, samples of Spanish bentonite (control and acetate-treated samples) were compacted at two different densities (1.5 and 1.7 g/cm^3), and then incubated, under anaerobic conditions, for six months at room temperature. To stimulate the growth of iron-reducing bacteria, acetate was used as an electron donor at the concentration of 30 mM. Next Generation Sequencing (NGS) based on Illumina system was used to determine the structure and composition of bacterial community before and after anaerobic incubation. Using this technique, the bacterial diversity analyses of both samples before incubation revealed similar results, showing the presence of bacterial phyla such as *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Verrucomicrobia* and *Planctomycetes*, among others. These groups have been classified in 560 different genera which include bacteria described for their role in biogeochemical cycle of iron such as *Acidiferrobacter*, *Ferrimicrobium*, *Ferrithrix* and *Aciditerrimonas*. Some organisms are nitrite-oxidizing bacteria such as *Nitrospira*, nitrate reducing bacteria such as *Conexibacter* and *Ramlibacter*, and sulfur-oxidizing bacteria such as *Thiobacillus* and *Thioalbus*. Furthermore, some of the found bacteria are being used in other studies for metal/radionuclide bioremediation purposes such as *Variovorax*, *Sphingomonas*, *Stenotrophomonas*, among others. Finally, data obtained from mineralogical (X-Ray Diffraction) and microscopic (Scanning Electron Microscopy) studies will be linked to the microbial diversity results, and the effects of the compaction and the acetate addition on the bentonite mineralogy and on the indigenous microbiota will be discussed.

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How microbes network, and are networked, in geological disposal research: a sociological perspective

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Abstract

In MIND subtask 3.2, we ascertain whether and how microbes, microbiologists, and microbiology are enrolled into research on the geological disposal of radioactive waste. We adopt a social studies of science (actor-network theory) perspective that observes how actors within MIND (e.g. MIND researchers, members of the MIND Implementer Review Board) and outside the project (e.g. geologists, waste management regulators, policy makers) process data and generate insights on the role of microbes in waste disposal, and how these processes elicit challenges and opportunities for enrolment. Examples of the latter include mixed appreciations of the impact of microbial activity on artificial barriers (e.g. negligible vs. substantial), the scope for, and appeal of, institutional uptake of microbiology research (e.g. through the coupling of microbiology research to "the safety case"), and the (re)drawing of disciplinary boundaries (e.g. between microbiology and geology). Drawing on these and related examples, we explore how through projects like MIND, microbes network, and are networked, differently for the joint purpose of radioactive waste disposal.

Keywords: Geological Disposal, Microbiology, Network, Nuclear Waste, Social Studies of Science.

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 during the geological disposal of radioactive waste, cementitious materials are used in many parts of the engineered barrier. In Belgium, cement is one of the matrices used for the immobilization of low- and intermediate level long-lived (LL-LILW) radioactive waste. In addition, the primary waste packages will be placed in a prefabricated concrete container after which the voids are filled with mortar and the container will be closed with a concrete lid. These concrete waste monoliths with be placed in horizontal disposal galleries lined with concrete wedge blocks and again all voids will be backfilled with cementitious materials. Finally, plugs and seals that may include cementitious components are used to close the disposal gallery. 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 niches were microbial activity will be enhanced and consequently its possible impact on the mineralogy and chemistry of the cementitious materials should be studied. 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 the investigation whether microorganisms could affect 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. Should this be possible, it will be assessed if this impact is positive or negative.

To fulfill this task, several batch experiments will be performed in which microbial activity, biofilm development and overall shifts in microbial communities will be investigated. In addition, cement integrity will be monitored and chemical analysis of the liquid phase will be executed. During and/or after incubation of the experimental set-ups, microbial, molecular, chemical and microscopy analysis will provide an insight in the mechanisms affecting either deterioration or enhancement of cement integrity.

Complex metabolic pathways are expected, of which intermediate and end-products will be elucidated and boundary conditions for their occurrence will be determined. These results will be correlated to microbial community patterns and shifts, dominant microbial species and surface colonization. Ultimately, these results will be evaluated towards their use for the assessment of the long-term performance of the cementitious engineered barrier system.

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BIOFILM FORMATION ON SURFACE OF CARBON STEEL UNDER ANAEROBIC CONDITIONS AND ITS MICROBIAL COMPOSITION

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Many studies have already shown the ability of some microorganisms to survive and propagate in the environment similar to high-level radioactive waste repositories. These, mainly anaerobic, microorganisms with diverse types of metabolism may, consequently, influence and compromise the long term safety performance of the repository. Presence and metabolic activity of bacteria in the repository may lead, besides other possible negative effects, to biofilm formation and later to deterioration of metal canisters resulting in possible failure of disposal safety system. In order to simulate this situation and estimate potential risks, we set up a long term corrosion experiment on carbon steel 12020 in natural underground water "VITA" (obtained from Josef URC) containing autochthonous microbial consortium with sulphate-reducing bacteria (SRB). Negative sterile control prepared in the same arrangement was monitored in order to distinguish chemical and microbially induced corrosion processes. The experiment lasted 240 days.

Electrochemical impedance spectroscopy (EIS) was used for in-situ monitoring of carbon steel 12020 corrosion behaviour. EIS study was performed by Gamry electrochemical multisystem in anaerobic glove boxes in the three-electrode arrangement. EIS measurement revealed three corrosion phases in the non-sterile environment. In the initial phase only single time constant, which represents uniform corrosion, was observed. After 23 days, the second time constant was detected in the EIS spectrum. This can be probably attributed to the formation of biofilm and corrosion products layers (that were not stratified yet). The third time constant was firstly observed after approximately 120 days and it indicates the presence of two already stratified layers - biofilm and corrosion products. In contrast, only single time constant was recorded throughout duration of the experiment in the sterile environment.

The exposed surfaces of carbon steel samples were analysed by SEM (Scanning Electron Microscopy), EDS (Energy Dispersive Spectroscopy) and Raman microspectroscopy. A layer of biofilm was observed by SEM on the carbon steel surface exposed to microorganisms. EDS mapping showed the presence of sulphur which corresponds well to the Raman-detected FeS, the final product of microbially induced corrosion.

Microbial composition of both biofilm and water samples (collected after 240 days) was studied using advanced molecular biological tools – next generation sequencing (NGS) and quantitative PCR (qPCR). Biofilm as well as water samples were dominated by sulphate-reducing bacteria (*Desulfomicrobium* sp. and *Desulfovibrio* sp.). More details on NGS results will be presented on the poster.

LONG-TERM EXPERIMENT ON CORROSION OF CARBON STEEL IN ARTIFICIAL BENTONITE PORE WATER INOCULATED WITH NATURAL CONSORTIUM OF SRB

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Here we present the first outcomes of long term experiment on microbially induced corrosion (MIC) of carbon steel in an anaerobic environment. The current strategy for the disposal of high-level radioactive waste is based on its confinement in metallic containers that might be susceptible to MIC. Although the disposal system consists of engineered and natural barrier system, the metal canister represents the only absolute barrier in the system. MIC may thus influence and compromise the safety performance of the repository by alteration of metal canisters, possibly leading to release of radionuclides. The Czech Radioactive Waste Repository Authority, SÚRAO, has selected carbon steel as a canister material and bentonite as a buffer material in the deep geological disposal concept. Therefore, we established a long term study (24 months) to describe the corrosion processes of carbon steel 12020 in artificial bentonite pore water inoculated with natural underground water "VITA" (Josef URC) containing sulphate-reducing bacteria. The experiment is maintained under anaerobic conditions and at laboratory temperature. Negative sterile control was prepared in order to distinguish chemical and microbially induced corrosion processes. Estimated duration of the ongoing experiment is 24 months with planned sampling points after 3, 6, 12, 18 and 24 months. Corrosion of carbon steel was characterised by weight loss and electrochemical methods (Electrochemical Impedance Spectroscopy – EIS, polarisation measurements) that allow monitoring of corrosion processes in-situ. Weight loss method proved higher corrosion rate of carbon steel after the first sampling time in the presence of bacteria (4,7 µm/year) compared to the sterile environment (3,0 µm/year). Additionally, biofilm and water samples were characterised using molecular biological analysis (real time qPCR and next generation sequencing) after three months. Real time qPCR revealed a rapid increase of nitrate reducing bacteria (detected by markers nirS, nirK and nos Z) which was confirmed by 16S rDNA amplicon NGS sequencing. This phenomenon can be explained by the composition of artificial bentonite pore water being rich in nitrates, an attractive terminal electron acceptor. High nitrate concentration in artificial bentonite pore water reflect leachate of real bentonite sample, but it is expected to decrease over time. To conclude, both biofilm formation and faster corrosion rate was found in the sample inoculated with natural undeground water containing SRB.

LONG-TERM LAB-SCALE MICROBIAL BENTONITE STORAGE EXPERIMENT

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The final disposal of high-level radioactive waste in Finland is planned to start in 2020s. The waste will be encapsulated in copper canisters and be surrounded by compacted bentonite barrier in the geological repository at the depth of 400 m, in Olkiluoto, Finland. The relationship between microbes and minerals like clay, and the complex nature of reactions occurring between them are not well characterised at present. The possible deterioration of bentonite structure due to microbial activity is a risk that needs to be studied to ensure the bentonite's ability to function as planned in long-term.

The aim of this laboratory scale bentonite storage experiment was to simulate worst case scenario conditions that could happen in real life at least in some parts of the repository. In the experiment bentonite was not compacted and water, gases, nutrients and microorganisms were able to move along easily at temperature hospitable for microorganisms. The objective is to find out if microorganisms and the produced metabolites are able to change the bentonite structure in favourable conditions and if these changes are significant for the bentonite stability.

The experiment was initiated both in aerobic and in anaerobic conditions in the spring 2016. The bentonite used was MX-80 (CETCO). The changes in bentonite will be studied with microbiological microscopical and chemical methods. In addition, the gas the changes in composition in the head-space is followed with the gas chromatography. Storage experiment may continue several years.

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EFFECT OF URANIUM ON MICROBIAL DIVERSITY AND MINERALOGY OF BENTONITE MICROCOSMS

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Deep geological repositories (DGR) are the preferred option for treatment and management of nuclear wastes. In this concept, radioactive wastes are placed in corrosion-resistant containers, surrounded by a bentonite-engineered barrier and emplaced at a depth of 500-1000 m in a low permeability host-rock formation. An in-depth understanding of host rock processes relevant to safety is thus required, including the impact of microorganisms under repository relevant conditions [1].

Spanish bentonites from "El Cortijo de Archidona" (Cabo de Gata, Almería) have been well characterized from mineralogical, geochemical and technological points of view [2]. Thus, these clay formations were selected as a reference material for bentonite-engineered barriers for future Spanish DGR. Indigenous microbes may have the ability to produce metal corrosion, Fe-containing minerals transformation and/or to affect the mobility and migration of radionuclides from repository to the biosphere [3]. For this reason, the study of microbial diversity is the key point to estimate these implications.

In this study, bentonite microcosms were prepared in triplicate and treated with uranium (1.26 mM) and uranium along with glycerol-2-phosphate (G2P; 10 mM) and incubated under aerobic conditions at room temperature for six months. Also, microcosms were treated with distilled water, G2P and sodium nitrate (with and without G2P) as controls. G2P was select to act as an electron donor and organic phosphate source in order to stimulate uranium phosphate precipitation. After incubation, DNA were extracted from all samples and the bacterial diversity analyses were carried out using Next Generation Sequencing based on Illumina technology, in order to determine the structure and composition of bacterial community present in these samples. Our results showed the presence of bacterial phyla such as *Actinobacteria*, *Proteobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Acidobacteria*, *Verrucomicrobia*, *Planctomycetes*, among others; and one phylum of Archaea (*Euryarchaeota*). These groups have been classified in 558 different genera, some of which were previously described for their role in metal/radionuclide bioremediation purposes such as *Variovorax*, *Sphingomonas*, *Stenotrophomonas*, among others. They may be involved in the precipitation of uranium phosphates by biomineralization process. The genus *Rhizobium* was represented in all G2P-containing microcosms while *Azotobacter* was abundant in the microcosms treated with only G2P. In G2P-uranium microcosms and G2P-nitrate microcosms, *Streptomyces* was abundant reaching 11% of the total community, while in the rest of treatments it was represented by only 1%. The outputs of this study would help to understand biogeochemical processes within the bentonite barrier under repository relevant conditions as well as to develop appropriate waste treatment, remediation and long-term management strategies.

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DETERMINATION OF LOW-MOLECULAR WEIGHT NATURAL ORGANIC MATTER (NOM) IN BENTONITE CLAYS

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Various bentonite clays have small but significant amounts of natural and introduced (during mining) natural organic matter (NOM) than may be utilized by sulphide- and acetate-producing bacteria. Such NOM may also decrease or increase the mobility of radionuclides. There is a need in the long-term safety case for geological disposal radioactive wastes to explore if NOM in such buffers can promote activity of bacteria and influence radionuclide migration[1] A new method has been developed for the high-resolution determination and identification of low-molecular weight NOM in bentonite clays using solvent extraction and analysis by ion-trap mass spectrometry (MS) coupled with gas chromatography (GC).

According to a recent review[1] there is considerable lack of knowledge on the exact nature of NOM in bentonite clays. Current analysis methods have focussed on determination of the total organic content and not on speciation. Furthermore, the analysis methods used for determination of total NOM involve heating steps that could possibly affect detection of more low-molecular NOM that could be lost by evaporation.

This poster presents a new high-resolution analysis of extractable NOM from bentonite. The method has been tested on six different clays and the results show that a considerable amount of total NOM exists as relatively low-molecular molecules containing functional groups as carboxylic acids, ketones, aldehydes and alcohols, among others. This kind of NOM is easily accessible as carbon source for microbiological growth and earlier presumptions that the NOM would be present as hardly-digested larger molecules and even graphite carbon will have to be reconsidered. More tests on the clays are needed for a certain evaluation of the fraction of NOM that is easily accessible versus determinations of total NOM. The main goal has been to set up a useful analytical method for this kind of analysis.

The method development has involved selection of extraction solvent to improve recovery and induce swelling of the clay, extraction conditions necessary for quantitative extraction, methods for pre-treating of the clay and concentration of the extract that not causes loss of NOM, optimisation of the actual injection on the GC to avoid thermal decomposition of larger molecules and methods for evaluating obtained data. The last step involving the actual identification of different substances is carried out using spectral deconvolution using the AMDIS software (NIST institute, USA) and library search against a large, validated spectral library with more than 400 000 different substances (NIST 14, NIST institute, USA).

With an existing high-resolution method for identification of NOM accessible for microorganisms it is possible to evaluate different bentonite clays as a possible carbon source for microbial growth. Preliminary results indicate that differences between the composition of NOM in the clays exist, that some clays are contaminated by substances originating from the excavation as explosives and hydraulic fluids and finally that the heating of clay necessary for some methods determining total NOM by for instance combustion could result in loss of low-molecular NOM from the dry clay.

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ANALYSIS OF BACTERIAL B-DIVERSITY COMBINING DATASETS FROM DIFFERENT SEQUENCING PROJECTS

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Amplicon sequencing of bacterial 16S rRNA genes is a standard approach to describe and compare bacterial composition and population dynamics in environmental samples. However, the methods used to generate data vary considerably regarding sampling approach, primer regions, library construction, sequencing platforms as well as bioinformatic pipelines. Different research groups depend on preferred approaches and availability of sequencing platforms and utilities at respective laboratories. How these differences affect results are often not known. Still many research questions may be answered by combining data from different studies. Therefore, concerns have been raised on how to best handle differences to end up with meaningfully comparable and unbiased data when interpreting diversity [1] [2].

The study presented on this poster describes a data merging procedure starting with different sampling approach, DNA isolation, primer regions and PCR amplification of bacterial 16S rRNA genes. Followed by sequencing on either 454 or Illumina platforms ending with high throughput sequencing data processed in an uniform bioinformatic workflow. Subsequently, an evaluation of reliability and biases of performed analyses of combined datasets from different projects at beta diversity level is performed [3] [4] [5] [6]. Weighted UniFrac distance measures were calculated for the beta diversity analysis and Principal Coordinate analysis (pCoA) was chosen to emphasize variation and bring out strong patterns of correlation to metadata in the merged dataset [7]. When examining the pCoA plots for metadata categories biome (attached, planktonic) and primer regions (v1v3, v4v6, v6). None of the categories themselves drive the diversity patterns, indicating that the weighted UniFrac measures performed well even if there were differences that could not be excluded in experimental protocols across studies. However, when comparing taxonomy composition at genus level for datasets processed on two different OTU clustering algorithms prior to, or after merging of datasets some samples in dataset correlate very well while some samples did not. Hence, results show stable patterns in microbial communities for some samples and for some samples it remains to investigate how to find ways to correct for bias by differences such as sampling approach, primer region, library preparation protocol or sequencing platform etc. between merged studies. Quantitative Insights Into Microbial Ecology (QIIME) was used for all data processing and Emperor software implemented in QIIME was used for visualization of pCoA analysis [8].

This comparative analysis procedure generally performed well considering the beta diversity estimates and correlation to metadata, with insignificant effects of variation between individual samples. However, it remains to conclude on differences in taxonomy composition. Therefore, our data indicates that datasets can be combined if a standardized pipeline is applied with sufficient correction for biases to be used for describing patterns in beta diversity and in near time hopefully also for taxonomic composition.

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