

MIND Project Annual Meeting 4 Short Abstracts

Stockholm May 7–9, 2019



PREFACE

The idea of the MIND (Microbiology In Nuclear waste Disposal) project was brought up at the IGD-TP EF4 meeting in Prague, 2013. An opportunity to set up a working group for microbiology actualized and gathered attendees from eight European countries representing Waste Management Organisations (WMOs), academic institutions, research institutes, consulting companies and one regulator (FANC). Although there are differences between the different repository concepts, there are many common unresolved issues among the various WMOs to be solved. The MIND working group therefore 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.

As a result of this it was decided to write a proposal to the Horizon2020, EU Research and Innovation programme, focussing on the above mentioned key issues. The project MIND officially started June 1, 2015 and ended May 31, 2019.

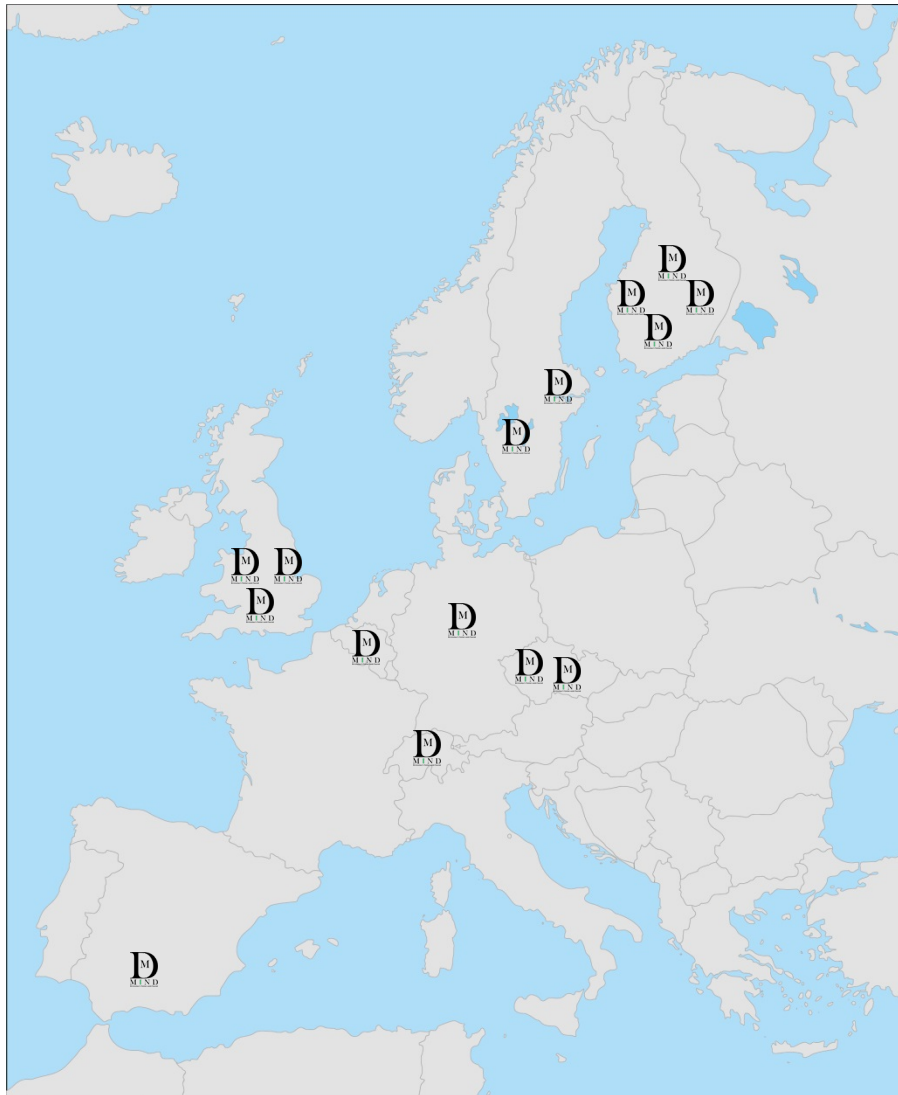


Figure 1: Fifteen organisations from eight countries.

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

Another workshop, with parts of the IRB and all WP-leaders, was held in Brussel on October 12, 2018 where the focus was on the evaluation report. The final meeting was held on May 6th, 2019 in connection to the final project annual meeting in Stockholm. The following WMOs were represented: SKB, Posiva, TVO, Andra, Nagra, RWM, NWMO and NUMO.

The fourth and final Project annual meeting was held in Stockholm May 7-9, 2019.

The meeting was opened by the coordinators and followed by a presentation by the EC project officer Athanasios Petridis whom emphasised the importance of maintaining such an invaluable network as the MIND consortium.

Olivier Leupin from Nagra had a keynote talk about *the relevance of microbial processes in Nagra's safety case*. After overviews by the WP-leaders Tanja Perko from SCK-CEN had a talk about *effective communication*. The oral poster presentations were followed by a poster session. Eleven posters were presented.

Theme session 1: *Microbes and radionuclide interactions* started with an introduction by Joe Small. Four presentations were given by UNIMAN, HZDR and UGR. Theme session 2: *Organics and gases* was also introduced by Joe Small. Six presentations were given by EPFL, VTT, SCK-CEN and NNL.

Theme session 3: *Microbiology and engineered barriers* was introduced and chaired by Karsten Pedersen. Eight presentations by HZDR, BGS, CVREZ, TUL, GTK and VTT were held. Johannes Johansson (SKB) held a keynote presentation about *Iron and copper corrosion morphologies related to MIC?*

Mehran Behazin (NWMO) and Julie Swanson from Los Alamos National Laboratory (LANL) held presentations about *NWMO microbiology R&D program* and *LANL Microbiology and performance assessment for waste isolation pilot plant* respectively. The final talk was given by Joan Govaerts at SCK-CEN. The meeting ended with a discussion about how to continue and how to preserve the network.

The PAM was closed by the project coordinators. The coordinators would like to thank Maria Berg Estrada for her flawless organization of lunches, coffee and handling the contacts with the restaurant Modis, Anders Lindblom for help with graphical design, putting together abstract volumes, name tags, Inger Nordholm, Erica Wallin and Malin Gustafsson are thanked for hosting the post-meeting excursion to SFR in Forsmark.

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

All participants are thanked for their contribution to our final annual meeting, the WP-leaders for their engaged work to keep their workpackages together and finally Johan Andersson and the IRB for their interest and contribution to the MIND project.

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

The coordinators of the project:

Birgitta Kalinowski (SKB)

Petra Christensen (SKB)

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Information and final agenda

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

The complete MIND 2019 PAM will be hosted by SKB.

The meeting venue for the meetings:

Vattenfall building
Vattenfallet
Evenemangsgatan 13
169 03 Solna

+46 (0)46 76 70 45
+46 (0)46 459 85 82

How to reach the venue:

By train: Commuter train from Arlanda to Solna Station. Exit Arenastaden.
The journey takes about 45 minutes.

Hotel Bookings

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

Recommended hotels located close to the meeting venue include:

1. [Hotel Friends Rasta Strandväg 1, Solna](#) (close to the meeting)
2. [Grand Central by Scandic, Kungsgatan 70, 111 40 Stockholm](#) City center (150 m from the Central Station, two stops by commuter trains to the meeting place in Solna)
3. [Ibis Styles Stockholm Odenplan](#) (one stop by commuter trains to the meeting place in Solna)
4. [Scandic Kontinental Vasagatan 2, Stockholm City Center](#) (150 m from the Central Station, two stops by commuter trains to the meeting place in Solna)

MIND Project Annual Meeting 2019

Information and Final Agenda

Travel

- [Arlanda Airport](#) is the nearest airport (45 minutes by commuter train). There is a train station located in the airport building.



Commuter train station: Solna. Exit Arenastaden.



Vattenfall building, entrance, Evenemangsgatan 13

MIND Implementers Review Board Workshop

May 6th The IRB workshop will be organized by Johan Andersson and take place at Arenastaden, Moorborg on the 3rd floor. A separate agenda will be distributed to those that are expected to participate.

1. MIND-Project Meeting Registration

The MIND Project Annual Meeting will be hosted from May 7th and will end on May 9th. Registration will be open from 8:30 on May 7th. The meeting will be held in conference hall Vattenfallet on the 2nd floor.

2. MIND-Project Annual Meeting

May 7th

- 08:45-10:00 Registration and coffee outside conference hall "Vattenfallet".
- 10:00-10:20 Opening of meeting by the coordinators *Birgitta Kalinowski, Petra Christensen, SKB*
- 10:20-10:40 Project Officer: *Athanasios Petridis, European Commission*
- 10:40-11:40 Keynote: The relevance of microbial processes in Nagra's safety case. *Olivier Leupin, Nagra*
- 11:40-12:00 Summary of IRB-meeting on May 6th, *Johan Andersson, SKB*
- 12:00-13:00 *Lunch*
- 13:00-14:30 Overview by WP-leaders (30 min per WP)
 - WP1: The behaviour of organic containing long-lived intermediate level wastes. *Joe Small, NNL*
 - WP2: The influence of microbial processes on high level waste and spent fuel geological disposal. *Karsten Pedersen, Micans*
 - WP3: Integration, Communication and Dissemination. *Natalie Leys, SCK-CEN*
- 14:30-15:00 *Coffee*
- 15:00-16:00 Effective communication about microbes in radioactive waste management through improved understanding of expert and lay persons' conceptualizations. *Tanja Perko, SCK-CEN*
- 16:00-16:36 Oral poster presentations (3 minutes/ poster)
- 16:45-18:45 Poster session with refreshments (Modis)

May 8th Theme sessions

Theme session 1: Microbes and radionuclide interactions (chair: Joe Small)

- 09:00-09:05 Introduction. *Joe Small, NNL*
- 09:05-09:25 Impact on *Stenotrophomonas bentonitica* BII-R7 on the mobility of Se, Eu and U within the deep geological repository system of radioactive waste. *Miguel Angel Ruiz-Fresneda et al., UGR*
- 09:25-09:45 Microbial controls on the mobility of uranium and other radionuclides in nuclear waste disposal in the presence of ISA and gluconate. *Gina Kuippers, UniMan*
- 09:45-10:05 The fate of polycarboxylic organic decontamination agents in high pH Low Level Waste disposal. *Natalie Byrd, UniMan*
- 10:05-10:30 Coffee
- 10:30-10:50 Radionuclide interaction with organic compounds: characterization on a molecular level. *Hannes Brinkmann et al., HZDR*
- 10:50-11:10 General discussion on microbes and radionuclide interactions. *All*

Theme session 2: Organics and gases (chair: Joe Small)

- 11:10-11:15 Introduction. *Joe Small, NNL*
- 11:15-11:35 Investigating the biodegradation of organic compounds released from irradiated ion exchange resins. *Aislinn Boylan et al., EPFL*
- 11:35-11:55 Biodegradation of maintenance waste and gas generation. *Minna Vikman et al., VTT*
- 11:55-12:15 Methane generation under repository relevant conditions, *Niels Burzan et al., EPFL*
- 12:15-13:15 Lunch
- 13:15-13:35 An active microbial community in boom clay pore water collected from piezometers impedes validating predictive modelling of ongoing geochemical processes. *Kristel Mijnenonckx et al., SCK-CEN*
- 13:35-13:55 Acetate is an important carbon source of the Fennoscandian deep biosphere. *Malin Bomberg et al., VTT*
- 13:55-14:15 Monod kinetic modelling of microbial processes in borehole experiments. *Liam Abrahamsen-Mills and Joe Small, NNL*
- 14:15-14:35 General discussion on organics and gases. *All*
- 14:35-15:00 Coffee

Theme sessions 3: Microbiology and engineered barriers (chair: Karsten Pedersen)

- 15:00-15:05 Introduction. *Karsten Pedersen, Micans*
- 15:05-15:25 Natural occurring spore-forming sulfate reducers and their influence on the bentonite barrier. *Nicole Matschiavelli et al. HZDR*
- 15:25-15:45 Biofilm - influenced anaerobic corrosion of stainless steel and carbon steel. *Tomáš Černoušek et al. CVREZ*

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- 15:45-16:05 The effects of microbial activity on the physical, chemical and transport properties of bentonite on steel corrosion experiments. *Simon Gregory et al. BGS*
- 16:05-16:25 Temporal evolution of the Microbial Community associated with bentonite *in situ*. *Niels Burzan et al., EPFL*
- 16:25-16:45 Bentonite characteristics after two years of storage with indigenous bentonite and water microbes. *Hanna Miettinen et al., VTT*
- 18:00-22:00 Joint walk to the conference dinner at Fjärilshuset in Hagaparken

May 9th

Theme sessions 3 (cont.)

- 09:00-09:05 Good morning and welcome. *Karsten Pedersen, Micans*
- 09:05-10:05 Keynote: Iron and copper corrosion morphologies related to MIC? *Johannes Johansson, SKB*
- 10:05-10:25 Survival of microorganisms in bentonite subjected to gamma radiation and the effect of anaerobic conditions on the microbial ecosystem evolution in bentonite. *Kateřina Černá, TUL*
- 10:25-10:55 Coffee
- 10:55-11:15 Microbial effects from the point of view of bentonite safety. *Markus Olin, VTT*
- 11:15-11:35 Geochemical patterns and constraints of microbial sulphate reduction in the geosphere. *Riikka Kietäväinen et al., GTK*
- 11:20-11:40 General discussion on microbiology and engineered barriers. *All*
- 11:40-12:00 NWMO microbiology research and development program. *Mehran Behazin, NWMO*
- 12:00-13:00 Lunch
- 13:00-13:20 How Microbiology Fits into the Performance Assessment for the Waste Isolation Pilot Plant. *Julie Swanson, LANL*
- 13:20-13:50 Performance Assessment. *Joan Govaerts, SCK-CEN*
- 13:50-13:55 Poster presentation: Alexey Safonov
- 14:00-14:30 Coffee
- 14:30-16:00 IRB/ IGDTP/MIND2 WMO perspective and discussion
MIND2 (internal view – *Andrea Cherkouk HZDR and Rizlan Bernier- Latmani, EPFL*)
MIND2 (IRB/ WMO view – *Johan Andersson + all*)
- 16:00-16:10 Closing of meeting *Birgitta Kalinowski, Petra Christensen*

Poster session

The MIND **Poster Session** is held on May 7th (17.00 – 19.00). This session is open for all and will include mingle and refreshments.

1. Lorraine Field, BGS, “Petrological and mineralogical findings from experiments into microbial effects on steel and compacted
2. Pablo Martínez-Rodríguez, UGR, “Phosphates speciation influences BE9 bacterial strain uranium bioremediation potential”
3. Mark Dopson, Linnaeus University, “Shining a light on sulfur cycling in the deep biosphere”
4. Katie Gilmour, Newcastle University, “Growth and activity of the microbial community of MX80 bentonite in the presence of iron”
5. Alena Ševců, TUL, “Effect of concrete on microbial community under repository relevant conditions”
6. Kateřina Černá, TUL, “Detection of living bacteria in bentonite – cell staining”
7. Susanna Maanoja, Tampere University, “Compacted clay as a potential source of substrates for groundwater and clay microbes”
8. Toru Nagaoka, Criepi, “Microbial influenced corrosion of carbon steel in compacted buffer material composed of Kunigel V1 and sand mixture”
9. Tom Rogiers/Kristel Mijndendonckx, SCK-CEN, “Dynamic genetic adaptation in the bacterium *Cupriavidus metallidurans* in response to uranium”
10. Sarah Butterworth, Manchester University, “Identifying the limits and impacts of microbial metabolism in geodisposal scenarios”
11. Takahiro Goto, Nuclear Waste Management Organization of Japan, “Investigation of bentonite products in Japan for alternative buffer materials”
12. Alexey Safonov, Russian Academy of Science, “Microbial corrosion of carbon and stainless steel by thermophilic microbiota in contact with bentonite for crystalline rock RW repository in Russia”

Project Executive Committee Meeting

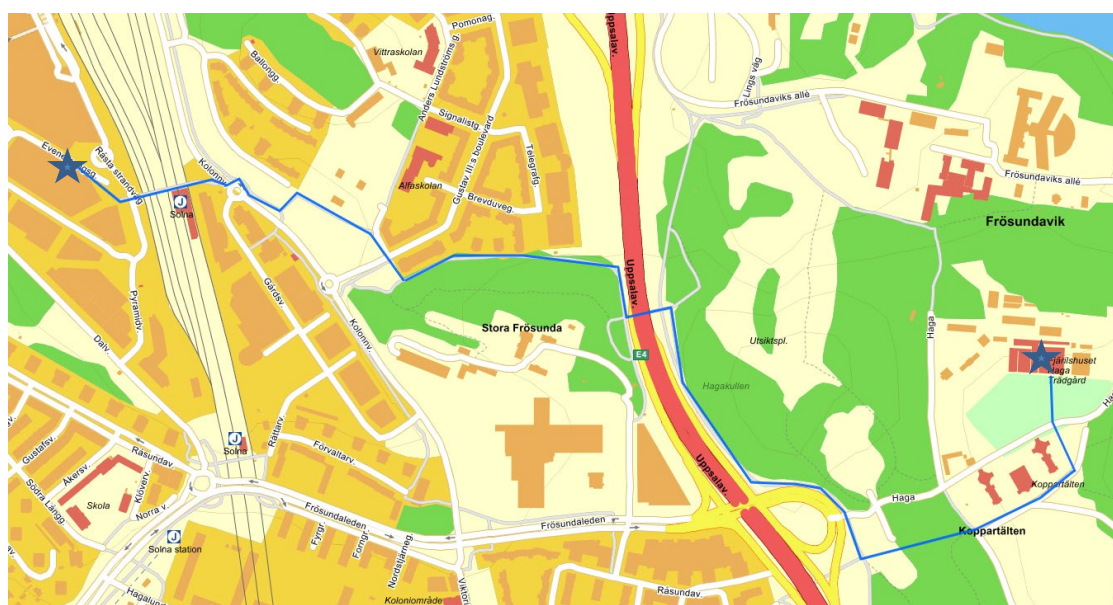
The MIND Project Executive Committee meeting is held on the afternoon of May 8th (16:45–17:45). This meeting is only open for PEC members who will in due time receive a call and agenda for the meeting.

Project Dinner

The MIND Project Dinner is held on May 8th (18:15-22:00). We will meet up outside the Vattenfall building for a joint walk to Fjärilshuset (*Butterfly House*) in Hagaparken (approx. 30 minutes' walk). Fjärilshuset is situated behind the Copper tents.



The Copper tents in Hagaparken.



Directions to Fjärilshuset from the Vattenfall building. The distance is about 2 km.

3. Post-meeting excursion to SFR, Forsmark

May 10th

- 09:00 The bus departs from Evenemangsgatan 13, outside the Vattenfall building
- 11:15 *Arriving at the Forsmark Inn for lunch*
- 12.30 Leaving for The final repository for L/ILW, SFR
- 13.00-15.00 Visit to SFR
 - 13:00 Presentation of SKB, Forsmark
 - 13:45 Check-in at SFR: passport and security control.
 - 14:15 Guided tour underground.
- 15.30 Transportation back. There will be two stops: First Arlanda Airport 17:15 and then Stockholm Central station 18:00.



SFR

MIND Project Annual Meeting 2019

Information and Final Agenda



PAM1, Granada 2016



PAM2, Prague 2017



PAM3, Lausanne 2018

THE RELEVANCE OF MICROBIAL PROCESSES IN NAGRA'S SAFETY CASE

OLIVIER X. LEUPIN (1), IRINA GAUS (1), RIZLAN BERNIER-LATMANI (2),

(1) Nagra, Wettingen, Switzerland, (2) EPFL, Lausanne, Switzerland

As implementing organization in Switzerland, Nagra plans geological repositories for both low- and intermediate waste (ILW) and high-level waste (HLW). The current concept for the ILW foresees to emplace the waste in large caverns backfilled with a high porosity mortar. In the repository for HLW, the vitrified glass and the spent fuel is placed within a steel canister which is then embedded in highly compacted bentonite. Both repositories will be constructed in Opalinus Clay.

In the context of repository-induced effects in the subsurface, processes and phenomena related to microorganisms are being evaluated alongside those related to thermal effects, to gas or chemistry and/or to rock mechanics. More specifically, the focus is on effects on barrier integrity (e.g., microbial induced corrosion (MIC)), on pathways for radionuclides or gases (e.g., metabolisms of ^{14}C under changing environmental conditions), and on the evolution of the repository (e.g., microbial consumption or production of gas and its effect on the repository re-saturation).

Over the years, Nagra and EPFL have conducted a number of joint microbial studies. Experiments were designed and conducted in a multi-disciplinary environment, allowing a clearer picture of the extent to which microorganisms could impact the safety of repository to emerge. As a result, microbiological processes and phenomena are part and parcel in Nagra's safety case. It could be shown that: (i) the microbial-induced corrosion in compacted granular bentonite is inhibited; (ii) microbial gas consumption in a repository can be meaningful; (iii) methanogenesis in a repository built in an Opalinus Clay hostrock should be considered; and (iv) microorganisms had no significant effect on the diffusion of radionuclides in an in-situ test. In addition, methods to characterize microbial communities in compacted clay systems were tested and validated.

For the general licence application, currently planned in 2024, Nagra prepares an update of its safety case. This safety case will include consideration of microbial aspects in both repositories. From today's perspective, Nagra will seek the support of microbiologists for several broad topics. In this presentation, I will summarize the major findings related to microbial processes in the Swiss context.

MIND WP1 OVERVIEW: THE BEHAVIOUR OF ORGANIC CONTAINING LONG-LIVED INTERMEDIATE LEVEL WASTES

JOE S. SMALL

National Nuclear Laboratory, Chadwick House, Birchwood Park, Warrington, WA3 6AE, United Kingdom

Microbiology in Nuclear waste Disposal (MIND) is an interdisciplinary project consisting of two experimental work packages focusing on the influence of microbial processes on waste forms and their behaviour and long-term performance of repository components. Work package 1 (WP 1) concerns microbial processes affecting organic containing long-lived intermediate level wastes (ILW). WP 1 research has been undertaken by 9 European partner organisations using a variety of advanced microbiological, spectroscopy, microscopy and modelling techniques. WP 1 has included further study of a long-term (18 year) gas generation experiment (VLJ Repository, Olkiluoto, Finland) and borehole experiments at the Mont Terri underground rock laboratory (URL), Switzerland.

The main safety issues concerning radionuclide, chemical and gas release from ILW that are addressed by the work package are summarised as:

1. The degradation of anthropogenic polymers (bitumen, polyvinylchloride (PVC) and ion exchange resins) and hence their contribution to gas generation and release of soluble organic species.
2. The biodegradation of cellulose hydrolysis products (e.g. isosaccharinic acid; ISA) and the overall effect on radionuclide complexation and transport.
3. Microbial gas generation (CH_4) and consumption (H_2) studied under *in situ* conditions.
4. The effect of high pH on limiting the above microbial processes and effects in cementitious ILW.

The presentation will discuss the background rationale for the work package and summarise the key findings from laboratory and *in situ* experimental systems that are reported in WP1 deliverables (D1.1-D1.8) and in several peer-reviewed journal publications. Overall, MIND WP1 research has reduced the uncertainty concerning the effect of microbial processes in organic-containing ILW, such as; concerning the limited extent of degradation of man-made organic polymers; the potential for biodegradation of the radionuclide complexant ISA; the limitation of CH_4 generation to near-neutral pH conditions, in cellulose-rich systems; and the dominance of sulfate reduction processes in H_2 injection experiments, which outcompetes and further limits methanogenesis. MIND WP 1 research examining interactions with radionuclides has yielded new understanding of complexation behaviour, toxicity effects and insights into microbial interactions that have the potential to sequester specific radionuclides.

These outcomes of WP1 research are viewed as having a predominantly positive effect on the safety case for geological disposal for ILW and should provide justification for less pessimistic consideration of processes of gas generation and organic (e.g. ISA) complexation of radionuclides. The inclusion of long-term and *in situ* URL experiments has proved invaluable in examining processes of CH_4 generation and H_2 consumption. Further advances in the understanding of the role of microbes in the disposal of organic containing ILW, such as examining degradation processes under *in situ* conditions, are expected through such URL experiments.

THE INFLUENCE OF MICROBIAL PROCESSES ON HIGH LEVEL WASTE AND SPENT FUEL GEOLOGICAL DISPOSAL

KARSTEN PEDERSEN

Microbial Analytics Sweden AB, Mölnlycke, Sweden

Microbiology in Nuclear waste Disposal (MIND) is an interdisciplinary project consisting of two experimental work packages focusing on the influence of microbial processes on waste forms and their behaviour and long-term performance of repository components. Work package 2 investigates microbial processes affecting engineered barriers such as canisters, bentonite buffers and backfill and concrete plugs and other concrete constructions. WP2 research has been undertaken by 11 European partner organisations using a variety of microbiological, technical, analytical techniques. Partners have taken advantage of access to underground research laboratories (URL) such as Josef in the Czech Republic and Mont Terrie rock laboratory in Switzerland.

The main safety issues concerning engineered barriers, longevity of canisters with respect to microbially influenced corrosion, microbial effects on buffers, backfill and concrete structures, can be summarized as:

- The contribution of microbially produced sulphide in buffers and in backfill and in the geosphere to the overall rate of sulfidic canister corrosion.
- The effectiveness of specific bentonite buffers and their properties (swelling pressure, density, pH, organic content) in inhibiting or promoting microbial activity.
- The impact of microbial activity the long-term performance of bentonites and cementitious materials used in European geological disposal concepts, i.e. microbial degradation of bentonites and cementitious materials.

The presentation will discuss the background rationale for the work package and summarise the key findings from laboratory and in situ experimental systems that are reported in WP2 deliverables (D2.1-D2.18) and in several peer-reviewed journal publications. Overall, MIND WP2 research has increased our understanding about boundary conditions for the availability of sulphide in deep geological disposal conditions which is constrained by the sulphate source and the availability of possible electron donors. Further, data has been collected regarding anaerobic microbial corrosion of canister material and rates of corrosion of carbon steel in bentonite under biotic and abiotic conditions. The diversity of microbes in aged bentonite has been studied as has the role of bentonite density on the rate of corrosion of carbon steel. Research on the degree of survival of microorganisms in bentonite subjected to different levels of irradiation and swelling pressure has delivered insight in survival mechanisms of microorganisms under extreme conditions. Investigations of diversity, mobility, activity and survival of microorganisms in bentonite environments has increased our understanding of to what extent microorganisms can influence characteristics of bentonite. The effect of alkalinity from concrete on microbial activity and survival, and opposite, has been studied as well.

MIND WP3 OVERVIEW

NATALIE LEYS (1)

(1) *Belgian Nuclear Research Centre SCK-CEN, Boeretang 200, 2400 Mol - Belgium*

Microbiology in Nuclear waste Disposal (MIND) is an interdisciplinary project consisting of two experimental work packages and a third work package that focuses on integration, communication and dissemination. This work package includes 3 main tasks:

1. Synthesis, evaluation, abstraction and integration of experimental and computational output
2. The impact of the inclusion of microbiology on expert conceptualization and public perception of geological disposal
3. Knowledge and information exchange

This presentation gives an overview of the motivation for these tasks and most important results.

Overall, all experimental work obtained within WP1 and WP2 was synthesized on a yearly basis (D3.4 and D3.5). The first year, an evaluation of the initial state of the art knowledge base was made (D3.1). In addition, it is known that the analysis of microbial communities via next generation sequencing based on the 16S rRNA amplicon can be biased due to the DNA extraction protocol, primer design, PCR amplification, sequencing artefacts and bioinformatics analysis procedures. Also within MIND, DNA extraction procedures, sequencing processes and bioinformatics pipelines are highly diverse among the different partners. The impact of the different strategies on the identified microbial community was evaluated with experiments comprising a commercial microbial community standard.

In order to understand how MIND experts (MIND researchers & members of the MIND Implementer Review Board) and experts outside the project (e.g. geologists, waste management regulators, policy makers) conceptualize the role of microbes in radioactive waste disposal, social scientists conducted semi-structured interviews with MIND members, participated as observers and presenters at MIND meetings, and analyzed guiding RWM documents within the MIND project. Elicited findings served as inputs for communication with project participants and civil society. To this end, group discussions, interviews and an interactive workshop with implementers, project participants and civil society were organized.

Recurrent issues concerned the possibility and scope of interdisciplinary collaboration within and outside the MIND research community, the importance of public communication (particularly the communication of uncertainty), and the institutional recognition for microbiology in radioactive waste governance at large. In a concluding interactive workshop, members of civil society reproduced and reiterated these issues, while also shedding new light on them, by stressing that communicating uncertainty can generate (rather than erode) public trust in science, and by probing the relationship between MIND research and public safety.

Education and training activities were initiated (D3.3) to cultivate awareness of the relevance of microbial issues in otherwise typically abiotic fields of expertise, and to dissipate the knowledge gained in the MIND project beyond the known geomicrobiology expert circles. To this end, two courses related to microbiology in nuclear waste disposal were organized during the project, an exchange program for Master and PhD students has been developed and focused communication on these initiatives has been undertaken.

EFFECTIVE COMMUNICATION ABOUT MICROBES IN RADIOACTIVE WASTE MANAGEMENT THROUGH IMPROVED UNDERSTANDING OF EXPERT AND LAY PERSONS' CONCEPTUALIZATIONS

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In order to understand how MIND experts, MIND researchers, members of the MIND Implementer Review Board, and experts outside the project (e.g. geologists, waste management regulators, policy makers) conceptualize the role of microbes in radioactive waste disposal, social scientists conducted semi-structured interviews with MIND members, participated as observers and presenters at MIND meetings, and analyzed guiding RWM documents within the MIND project. Elicited findings served as inputs for communication with project participants and civil society.

To study the impact of the inclusion of microbiology on expert conceptualization and public perception of geological disposal, group discussions, interviews and an interactive workshop were organized with implementers, project participants and civil society. How do different communication stimuli affect the perception of the role of microbes in RWM among different target groups, is investigated by using an embedded experiment in a survey. In order to improve scientific outreach and communication about microbes in RWM the MIND experts have been challenged with a role-play, discussion and brainstorming on the workshop “Speaking for, with and about microbes”. This presentation illustrates how the results of these combined activities contribute to the MIND communication guidelines and effective communication between experts and lay people.

Acknowledgements

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IMPACT OF *STENOTROPHOMONAS BENTONITICA* BII-R7 ON THE MOBILITY OF SELENIUM, CURIUM, EUROPIUM, AND URANIUM WITHIN THE DEEP GEOLOGICAL REPOSITORY SYSTEM OF RADIOACTIVE WASTE

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The University of Granada, as collaborator on the Work Package 1 of the MIND project, contributes by studying the impact of the bacterium *Stenotrophomonas bentonitica* on the speciation and mobility of selenium (Se), curium (Cm), europium (Eu), and uranium (U) under relevant conditions for the deep geological repository (DGR) of radioactive wastes. This bacterium, isolated from Spanish bentonite formations [1], has been recently described as a novel species as indicated molecular and phenotypic analyses [2]. The genome analysis of this species revealed the presence of enzymes described for being involved in the interaction with elements such as Se, and U.

S. bentonitica is able to reduce Se^{IV} to Se^0 under aerobic, anaerobic, and alkaline conditions (up to pH 10) forming Se nanostructures with different shapes (spherical, hexagonal, polygonal, and nanowires) and crystallographic properties (amorphous and trigonal Se) [3]. A time-dependent transformation process from unstable amorphous Se nanospheres to different stable trigonal Se nanostructures has been proposed, in which proteins could be involved. The bioproduction of volatile methylated Se species was also detected by means of GC-MS. *S. bentonitica* cells can also efficiently interact with Cm^{III} and Eu^{III} , as inactive analogue of Cm^{III} , mainly through biosorption process. Thus, Eu^{III} form bidentate bridging complexes with carboxyl groups probably arising from acetate in addition to phosphate moieties as was revealed by TRLFS, FTIR and XPS. The obtained Eu/bacteria complexes are located mainly at the cell wall and at less extent at the extracellular space as was demonstrated by STEM/HAADF. Similar local coordination of Cm^{III} and Eu^{III} within the cells of this bacterial strain was observed. Finally, recent results demonstrated the ability of this strain to precipitate U as U phosphate phases due to the activity of phosphatase leading to the immobilization of U.

The present work provides new insights on the impact of microbial processes in the security of future geological disposal of radioactive wastes.

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MICROBIAL CONTROLS ON THE MOBILITY OF URANIUM AND OTHER RADIONUCLIDES IN NUCLEAR WASTE DISPOSAL IN THE PRESENCE OF ISA AND GLUCONATE

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Cementitious Low-level (LLW) and Intermediate-level (ILW) radioactive waste repositories contain, in addition to radioactive elements, a heterogeneous mix of organic materials, including plastics, cellulose and rubber, which are challenging for the disposal of radioactive waste. Cellulosic items, such as cloth, tissue, filters, paper and wood, are known to be degraded via alkaline hydrolysis to mainly isosaccharinic acid (ISA), a strong chelating agent. Other organics can be also added to the cement, such as plasticisers, which is used to improve physical and rheological properties of the cement (e.g. gluconic acid (GLU)). It is expected that a range of water-soluble complexes with radionuclides, including Ni(II), Am(III), Eu(III), Np(IV), Th(IV), U(VI) and U(IV), will form due to the presence of ligands. Complexes with uranium are considered particularly problematic due to its long half-life and the high abundance in nuclear waste.

As such, the impact of microbial degradation of ISA and GLU on the mobility of uranium was investigated, to help determine the role of microorganisms in moderating the transport of uranium from a multi-barrier Geological Disposal Facility (GDF). Sediment incubations were used to explore the biodegradation of ISA and GLU under GDF-relevant conditions, underpinned by cross-disciplinary analyses. Within this approach we have demonstrated the ability of bacteria to degrade ISA over a wide range of biogeochemical conditions. Furthermore, U(VI) was precipitated from the groundwater system during ISA biodegradation and was also observed to be reduced to less soluble U(IV) mineral phases. This study highlights the potential for microbial activity to help remove chelating agents from groundwaters surrounding a GDF for ILW and prevent the transport of U(VI). Thus, GDF safety cases that do not include microbial processes may be overly conservative, over-estimating the impact of ISA on radionuclide transport and thus the introduction of a new barrier, the “bio-barrier” is suggested.

THE FATE OF POLYCARBOXYLIC ORGANIC DECONTAMINATION AGENTS IN HIGH PH LOW LEVEL WASTE DISPOSAL

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The Low Level Waste Repository (LLWR) in Cumbria is the UK's principal facility for disposal of low level radioactive waste (LLW). It is engineered to minimise waste contaminant mobility by generating an anoxic, high pH (~11), reducing environment once the facility is sealed (Small, et al., 2011; Roch, 2015). Post closure the waste will remain in place indefinitely and is likely to be colonized by microbial communities. Indeed, previous work has already shown that microbes can proliferate under conditions relevant to a cementitious repository (Rizoulis et al., 2012). Anaerobes present within the LLWR will derive energy either by fermenting the organics directly in the waste, or oxidising them concurrent with the reduction of inorganic species. These biogeochemical interactions may have significant implications for near-field geochemistry and contaminant fate. Furthering understanding of biogeochemistry is necessary to enhance the accuracy of model predictions regarding contaminant transport and manage associated risks to sensitive receptors.

In the context of LLW disposal, information regarding the high pH behaviour and degradation of many organic electron donors is scant (with the notable exception of cellulose and its degradation products). Organic compounds are of interest due to their ability to act as complexing agents, potentially enhancing subsurface mobility of contaminants. The principal sources of organic complexing agents within the UK's LLW inventory are wastes from the chemical decontamination of material originating from nuclear sites (Randall, et al., 2011). Organic decontaminants can fall under the umbrella of either polycarboxylic (e.g. citric and oxalic acids) or aminopolycarboxylic acids (e.g. NTA, EDTA and DTPA). The former are of keen interest as: (a) the LLWR has no capacity assigned for disposal of these compounds within the facility (b) the model that underpins the sites Environmental Safety Case (ESC) relies on assumptions regarding their biodegradability at high pH and experimental evidence would improve the validity of outputs. Therefore, this work aims to explore the biogeochemical fate of polycarboxylic acids under high pH conditions relevant to LLW disposal, using citric acid as a model compound. Furthermore, high pH microbial metabolism of citrate in the presence of cement is also examined.

Two sets of anaerobic microcosm experiments were set up under conditions intended to simulate typical low level radioactive waste disposal, using a high pH adapted sediment inoculum from a legacy lime works. In the first set, microcosms were poised at pH 10, 11 and 12 and citrate was supplied as the sole electron donor and carbon source, under fermentative, denitrifying, Fe(III)-reducing and SO_4^{2-} -reducing conditions. The second set, were buffered to pH 11 using a 3 g cement pellet made according to the grout formulation used in the LLWR. Findings indicate that citrate is readily oxidised using nitrate or Fe(III) as electron acceptors at pH > 11. The upper pH limit for nitrate reduction, including in the presence of cement, was around 11.2. In the absence of cement, Fe(III)-reducing microcosms have shown Fe(II) ingrowth commencing at pH values as high as 11.7; there is some evidence to suggest that citrate may enhance high pH Fe(III)-reduction. There have also been some sporadic signs of SO_4^{2-} reduction at pH 10. However, the presence of cement in these systems appears to inhibit microbial Fe(III) and SO_4^{2-} reduction.

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RADIONUCLIDE INTERACTION WITH ORGANIC COMPOUNDS: CHARACTERIZATION ON A MOLECULAR LEVEL

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Organic compounds can have different origins within the context of nuclear waste disposal. They can be, amongst others, parts of nuclear waste as well as their degradation products, cement additives, metabolic products of microorganisms and natural occurring matter. Studying their interaction with radionuclides (RN) is essential for the safety assessment of a nuclear waste repository, since this might have adverse effects on RN sorption and solubility. Isosaccharinic acid (ISA) is the main product of alkaline cellulose degradation and therefore a problematic organic ligand within the context of nuclear waste disposal.[1] This polyhydroxy carboxylic acid forms complexes with certain RN, leading to an increased solubility or decreased sorption of certain RN. Two studies will be presented concerning the effect of ISA on the fate of UO_2^{2+} under alkaline conditions. First, results from an ongoing long-term tissue degradation experiment under alkaline conditions in the absence and presence of microorganisms will be discussed. Second, the influence of ISA on the retention of UO_2^{2+} on bentonite under alkaline conditions will be elucidated. In both studies, spectroscopic techniques were used to identify the species, being responsible for the increased UO_2^{2+} -solubility.

Furthermore, an approach to characterize RN-organic ligand interactions, concerning structural as well as mechanistical aspects, will be elucidated based on the UO_2^{2+} -ISA system. Therefore, different spectroscopic techniques (NMR, ATR-FTIR, EXAFS, UV-Vis, ESI-MS) in combination with DFT-calculations will be complementarily discussed, leading to an understanding on a molecular level.

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INVESTIGATING THE BIODEGRADATION OF ORGANIC COMPOUNDS RELEASED FROM IRRADIATED ION EXCHANGE RESINS

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Ion exchange resins are used extensively in the capture of radioactive material at nuclear power plants, accounting for approximately 40% of the organic nuclear waste inventory in Switzerland [1] and as such are expected to contribute significantly to the total intermediate level waste. These wastes will be stored in deep geological repositories and subject to radiation doses over very long timescales. Under these conditions, it is considered likely that the exchange resins will release organic compounds into the aqueous phase within the repository. A series of anoxic irradiation experiments subjected two resin types (bead and powder) to high irradiation doses (200 kGy) using a cobalt gamma source. The results show that several organic compounds are formed. In the gas phase, the primary constituents are H₂ and CO₂ with contributions of benzene and chloromethane detected at concentrations of 10⁻⁸M. In the aqueous phase, ammonia (~10⁻⁴M) and trimethylamine were detected.

To study the biodegradation of the identified organic compounds, a series of microcosms were initiated in 200 mL serum bottles using either benzene (¹³C-6 labelled), chloromethane, trimethylamine or ammonium chloride. Artificial porewater (180 mL) was amended with 20 g/L of Opalinus Clay from the Mont Terri Underground Research Laboratory, Switzerland. Porewater from Mont Terri was added as an inoculum. Controls were established without inoculum. Geochemical, microbiological and spectroscopic analyses were undertaken to determine the extent of biodegradation of these compounds by the deep subsurface microorganisms present in Opalinus Clay porewater and to consider the implications of these processes occurring in a repository environment.

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BIODEGRADATION OF MAINTENANCE WASTE AND GAS GENERATION

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The microbiological degradation of organic waste in anoxic conditions is a multi-step process, which can lead to the gas generation in repository conditions. Generated gas can influence mobility of gaseous radionuclides, cause pressurisation of the repository and deteriorate the multi-barrier systems. In Finland, low level radioactive waste (LLW) including maintenance waste is packed into carbon steel drums and concrete boxes which are then disposed in silos or repositories inside the bedrock. LLW contains considerable amount of organic cellulose and hemicellulose-based waste and it is therefore easily biodegradable.

In order to study the gas generation from LLW, microcosm experiments were initiated with microcrystalline cellulose and simulated maintenance waste. Microcosms were filled with groundwater obtained from the repository and they contained piece of carbon steel corresponding steel drums. The results obtained from the microcosm experiments were compared to the results from *in-situ* Gas generation experiment (GGE) ongoing in TVO's final repository for LLW and ILW in Olkiluoto, Finland [1]. After two years, biodegradation of organic waste in the microcosm experiment was demonstrated by formation of volatile fatty acids and carbon dioxide. Hydrogen formed as a result of steel corrosion and degradation of organic waste, was detected in gas phase only in the beginning of experiments. This indicates that hydrogen as an electron donor is probably rapidly consumed by the microbes present in the microcosms. While methane generation in the GGE started approximately after one year of operation, no methane has been detected in the microcosm experiments. The number of methanogens in the microcosms was below detection limit of qPCR analysis but methanogens were detected by amplicon sequencing showing potential for the initiation of methane generation. A possible reason for the absence of methane generation in the microcosms is the competition with the sulphate reducers as sulphate reduction is ongoing. The formation of microbial metabolites reduced pH both in the microcosm experiments and in the GGE, which could enhance microbial activity and gas generation. The obtained results also demonstrate that the simulation of the heterogeneous repository conditions in the laboratory scale is challenging.

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METHANE GENERATION UNDER REPOSITORY-RELEVANT CONDITIONS

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Methanogenesis is one of the potential microbial process in deep geological repositories after closure. It could have several impacts on the repository environment due to the consumption and production of gases and induced changes to pH, which can affect the surrounding geochemistry. It is potentially less harmful than sulfate reduction in a repository environment as it does not produce any corrosive substances and can consume hydrogen gas.

An *In-situ* experiment at Mont Terri URL has shown that there is a diverse microbial community within borehole water which is capable of utilizing hydrogen gas. Methanogens have been identified in this community but only at very low abundance (<0.1%), and there no evidence for active methanogenesis with no increase in the methane concentration over time.

However, during *in situ* experiments in which a porewater saturated porous medium (80% sand: 20% bentonite, w/w) was supplied with hydrogen gas, we observed the growth of a biofilm in which the microbial community was dominated by methanogenic archaea from the genera *Methanosarcina* and *Methanoculleus*. The contribution of methanogens was only dominant when sulfate was depleted. The biofilm also included families that contain bacteria able to ferment such as Pseudomonadaceae, Gracilibacteraceae and Ruminococcaceae but also sulfate-reducing bacteria such as *Desulfosporosinus* and *Defultomaculum*. These results provide evidence for the hypothesis that methanogenesis will prevail upon depletion of sulfate from the host rock.

Further *in situ* experiments of porewater saturated porous medium bioreactors are deployed to obtain better insights into the evolution of a biofilm under sulfate-depleted conditions and to characterize the necessary environmental conditions for the transition from sulfate reduction to methanogenesis in a system mimicking the repository backfill.

AN ACTIVE MICROBIAL COMMUNITY IN BOOM CLAY PORE WATER COLLECTED FROM PIEZOMETERS IMPEDES VALIDATING PREDICTIVE MODELLING OF ONGOING GEOCHEMICAL PROCESSES

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Safe geological disposal of radioactive waste requires a detailed understanding of the geochemical conditions present in the host formation. Consequently, analysis of pore water is essential, as its composition determines among others, the speciation and solubility of radionuclides. In Belgium, Boom Clay is considered a potential host formation. Although the elemental composition of Boom Clay pore water is relatively well known, the real mechanisms controlling the $p\text{CO}_2$ (g) and the pH, the two most important parameters, are not completely understood. Currently, these parameters are under investigation based only on inorganic chemistry. Borehole waters of different Underground Research Facilities (URF) harbour an active microbial community; however, their possible impact on the geochemistry of Boom Clay pore water extracted from piezometers is not yet examined. The present study discusses the evolution of the geochemistry and the microbial community in the pore water from the piezometers around the PRACLAY gallery of the HADES URF during 7 years after installation of the piezometers. Overall, the elemental composition seemed to vary during the first 4 years, while afterwards it remained quite stable. However, the $p\text{CO}_2$ values varied substantially over time, while the $p\text{CH}_4$ increased in all filters. The presence of an active microbial community in the piezometers, could explain why experimental $p\text{CO}_2$ – pH data do not correspond to the data obtained by predictive modelling, hampering validation of current predictive models of the ongoing geochemical processes. Moreover, the nature of the sampling equipment and the sampling procedure possibly stimulated the present microbial community, resulting in increased methane production rates. To improve predictive modelling, microbial processes are needed to be taken into account together with inorganic geochemistry considered at the current stage, which necessitates detailed microbial and geochemical monitoring in future studies.

ACETATE IS AN IMPORTANT CARBON SOURCE FOR THE FENNOSCANDIAN DEEP BIOSPHERE

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Acetate is a preferred carbon and energy source in the Fennoscandian deep continental subsurface. It has been shown to rapidly activate otherwise dormant/inactive microbial communities¹ and function as a preferred carbon and energy source in the deep biosphere^{2,3}. Acetate is produced by both autotrophic and heterotrophic acetogenesis, by incomplete oxidation of electron donors during sulphate reduction, and through fermentation. Here, we aimed to identify acetate cycling microbial metabolic pathways in the deep continental subsurface of Olkiluoto, Finland, by metagenomic analysis of groundwater samples from 100 m and 276 m depth. We also aimed to get a more detailed glimpse into novel microbial lineages by identifying metagenomic bins and identifying their metabolic characteristic. We identified altogether 9 bins with over 75% completeness. All bins had genes for acetate consumption (ACSS or ackA), but only 3 had genes for acetate production (ptc or acyP). The most commonly identified carbon fixation pathway was the 3-Hydroxypropionate bi-cycle, especially in the bins from 100 m depth. The Wood-Ljungdahl pathway was also detected in 3 out of 9 bins. Parts of the reductive TCA cycle was detected from all bins, but was well covered in 3 out of 9 bins. We did not manage to capture any methanogens in the bins, although methanogenesis especially from acetate and CO₂-H₂ was detected in the metagenomes. Bin 6 (Betaproteobacterium) from the depth of 100 m was well supplied with genes for dissimilatory nitrate reduction and denitrification as well as nitrogen fixation. In the metagenomes, genes for nitrogen fixation had 10 times higher relative abundance in the sample from 276 m, compared to the genes for denitrification and nitrate. reduction. In addition, nitrogen metabolism appeared also to be more common at 276 m depth than at 100 m depth.

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MONOD KINETIC MODELLING OF MICROBIAL PROCESSES IN BOREHOLE EXPERIMENTS

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Numerical models can be constructed to elucidate complex microbial processes and to integrate them with the wider geochemistry of experimental systems and the repository concept [1]. The Monod kinetics approach [2] represents microbial processes as kinetically-controlled reactions defined in terms of the availability of chemical substrates, biomass populations, growth yield factors and rate constants. By incorporating the kinetic microbial processes within the chemical speciation code PHREEQC [3], it is possible to examine potential gas exchange, mineral reactions (such as precipitation of sulfide phases) and the geochemical speciation of the systems.

Within MIND, two case studies have used PHREEQC to model biogeochemical processes occurring in borehole experiments at the Mont Terri underground rock laboratory that study the fate of hydrogen injected into Opalinus Clay. The model of the Bitumen Nitrate Clay (BN) experiment [4] represents the radial diffusion of nitrate into the Opalinus Clay and its reaction with a pulsed equilibration of hydrogen. The model represents the formation of nitrite as an intermediate in the overall denitrification process to form N_2 gas and the overall drop in gas pressure as H_2 gas is consumed (Figure 1).

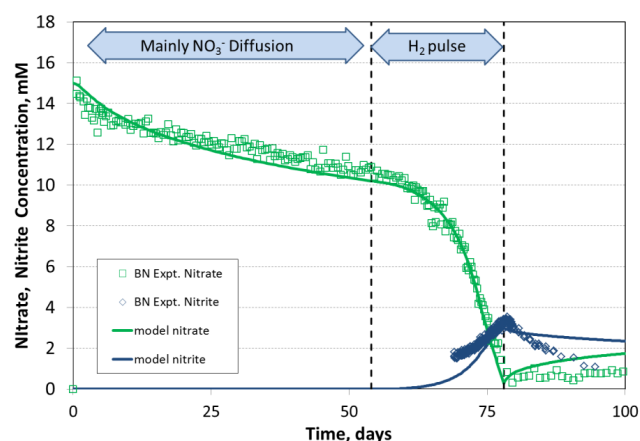


Figure 1. Model representation of nitrate and nitrite concentration during a pulsed hydrogen experiment

The second case study is of the Microbial Analysis (MA) experiment undertaken in the BRC-3 bioreactor, from which there is evidence that hydrogen injection firstly stimulates an autotrophic process that fixes inorganic carbon that is used as a carbon source for heterotrophic sulfate reduction [5]. The acetogenic process has been represented in the PHREEQC model of the experiment, which generates acetate that is used by the sulfate reduction process.

The modelling case studies show that microbial models can be used to interpret in-situ experimental systems, where several sensitive chemical parameters are monitored and can be accurately represented. Such models are useful to represent metabolic and growth processes inferred by DNA sequencing studies. Representation of kinetic microbial within geochemical speciation codes, such as PHREEQC, enables a consistent understanding of the chemical effects of microbial processes to be developed.

Forward modelling of repository scenarios is possible. However, the data requirements for multiple Monod kinetic models representing the synergistic and competing processes that are likely to occur in repository systems may be limiting.

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NATURAL OCCURRING SPORE-FORMING SULFATE REDUCERS AND THEIR INFLUENCE ON THE BENTONITE BARRIER

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To investigate the potential influence of natural occurring microorganisms within the bentonite on its mineralogical properties, we prepared anaerobic microcosm-experiments containing bentonite and a synthetic Opalinus Clay pore-water solution. Two different Bavarian bentonites (a natural and an industrial one) were incubated for one year at 30 °C and 60 °C and analyzed for bio-geochemical parameters and microbial diversity. For stimulation of microbial activity, some set ups were supplied with organics (acetate or lactate) or H₂.

Only microcosms containing the industrial bentonite show striking effects. The presence of supplemented lactate or H₂ led to the dominance (up to 81 %) of spore-forming *Desulfosporosinus* spp. – strictly anaerobic, sulfate-reducing microorganisms. The respective microcosms show an increase of ferrous iron and a simultaneous decrease of ferric iron as well as a decrease in sulfate-concentration. Concomitantly, the redoxpotential dropped and hydrogen-sulfide was formed – leading very likely to the formation of the observed fractures and iron-sulfur precipitations. Furthermore, lactate-containing microcosms show the formation of acetate in the same amount as lactate was consumed. The here mentioned, microbial formed metabolites could affect the dissolution behavior of minerals and ions within the bentonite and, thus, potentially change the sealant and adsorbent properties of the bentonite barrier.

BIOFILM-INFLUENCED ANAEROBIC CORROSION OF STAINLESS STEEL AND CARBON STEEL

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The deep geological repository (DGR) was selected for safe disposal of spent fuel and high-level radioactive waste in metal canisters. Microbially influenced corrosion (MIC) may considerably limit the lifetime of nuclear waste canisters, because microorganisms are able to change the electrochemical conditions at the interface between metal and electrolyte solution by biofilm formation. Interestingly, the biofilm may accelerate the corrosion, but it could, in certain conditions, inhibit the corrosion process. Sulfate-reducing bacteria (SRB) are the most important players considered responsible for the MIC. The anaerobic MIC of canister material (steel and stainless steel) was studied in mesocosm environment containing natural microbial community dominated by SRB to improve our understanding of the effect of microbiological processes related to the repository. Natural anaerobic granite groundwater with SRB and synthetic bentonite pore water mimicking Czech bentonite were used in the experiments held under anaerobic conditions at laboratory temperature or 35°C. MIC was determined by electrochemical measurement (EIS, OCP and polarization curves), weight loss method, surface analysis, and microbiological analysis. The unstable passivity and MIC inhibition of stainless steel was observed in the granite groundwater at laboratory temperature. The microbial consortium attached onto the stainless steel specimen did not produce a continuous biofilm even after 111 days. Notably, biofilm was verified after only 23 days on carbon steel in natural granite water at laboratory temperature. We could distinguish three-step biofilm life cycle, leading to formation of the second layer of biofilm. The biofilm increased the corrosion rate by a factor of two after 240 days. Molecular biological analysis of the water and biofilm showed dominance of *Desulfomicrobium* with *Desulfovibrio* sp. On the contrary, by increasing the temperature to 35°C, biofilm was observed by the EIS on the carbon steel after 27 days and inhibited the corrosion rate. Molecular biological analysis of the water and biofilm showed only dominance of *Desulfovibrio* sp. whereas the other SRB that were initially present (*Desulfobacula*, *Desulfomicrobium*, *Desulfovibrio*) declined over time. Weight loss measurement of carbon steel in synthetic bentonite pore water inoculated with granitic water with the sampling after 3, 6, 12 and 26 months showed the presence of several local attacks in non-sterile environment. Microbial presence was confirmed by SEM and EDS analysis in accordance to qPCR analysis in non-sterile conditions. Members of nitrate-reducing bacteria (NRB) were found to highly proliferate among which *Pseudomonas*, *Methyloversatilis* and *Brevundimonas* were the most abundant. Clearly the nitrate-rich synthetic bentonite pore water favored the growth of bacteria that could reduce nitrate. Consequently, iron oxidation coupled to nitrate reduction provided energy for NRB respiration that could lead to increased rate of MIC. In the case of sterile conditions, uniform corrosion of carbon steel was observed and the corrosion rate decreased with increasing exposure time. Raman analysis of the carbon steel in natural granite water at 35°C and in synthetic bentonite pore water showed that the surface contained magnetite in the end of the experiment. However, local depressions were filled with calcite or aragonite. Moreover, in the case of non-sterile conditions, the mackinawite was detected on the carbon steel as a proof of presence SRB.

THE EFFECTS OF MICROBIAL ACTIVITY ON THE PHYSICAL, CHEMICAL AND TRANSPORT PROPERTIES OF BENTONITE IN STEEL CORROSION EXPERIMENTS.

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To address microbially induced corrosion of canister materials (WP2.2) and microbial degradation of bentonite buffers (WP2.4) under realistic GDF conditions, a series of flow experiments using compacted FE bentonite and steel chips have been completed. Bespoke high pressure experimental apparatus has been used, comprising a titanium pressure vessel fitted with three radial and two axial load cells which constantly monitor changes in stress within the clay sample. Compacted bentonite samples containing steel chips were prepared to dry densities of 1200 kg m^{-3} and 1400 kg m^{-3} and inserted into the pressure vessel. Samples were hydrated with an anoxic artificial groundwater based on a Grimsel groundwater (amended with sodium lactate in some tests) recipe using HPLC pumps. A hydraulic gradient of 2500 kPa to 1000 kPa was applied to allow measurement of flow and permeability.

Four paired tests (each of approximately 3 months) have been run and post experimental material has been analysed. Each pair comprised one sample of sterile (irradiated) bentonite without microbial inoculum and one sterile (irradiated) bentonite inoculated with a mixed microbial culture enriched for sulphate reducers from unirradiated bentonite samples. During the experiments, in/out flow was monitored along with total stress to see if the corrosion of steel has a direct effect on swelling behaviour of the samples, and whether this effect was enhanced by the presence of microbes. Microbial analysis of samples revealed very few microbes in the uninoculated samples and higher microbial counts in the inoculated samples, especially associated with the portion of the sample containing the steel. SEM analysis identified calcium enrichment and sometimes euhedral aragonite and fibrous iron in the inoculated samples. Generally this appear to a greater extent in inoculated experiments. XRD data shows alteration to the clay around the steel in all samples analysed. Although the evolution of the stress in the clay was monitored throughout the experiment no loss of swelling capacity was detected, though this could be due to the sensitivity of the monitoring equipment or the relatively short duration of the experiments. There was considerable alteration of the steel and bentonite observed within the samples. However, because this varied from sample to sample there was no definitive evidence for microbial activity affecting the physical, chemical or transport properties of the bentonite buffer.

TEMPORAL EVOLUTION OF THE MICROBIAL COMMUNITY ASSOCIATED WITH BENTONITE *IN SITU*.

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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, microbially-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 any microbially-derived enhancement of the rate of canister corrosion.

Here, we present the results of an ongoing *in situ* small scale test in Opalinus Clay rock in the Mont Terri Underground Rock Laboratory in St. Ursanne, Switzerland. We simulated the engineered barrier systems with retrievable Wyoming bentonite MX-80-filled modules, in which steel and copper coupons of several relevant alloys were embedded. Both pellets and pre-formed blocks of bentonite of varying dry densities were tested. These modules were placed in the BIC-A1 borehole and exposed to porewater under native conditions for 12, 20, 33, and 66 months. The initial microbial community in the borehole was characterized, as was the community at the first, second and third sampling time point (i.e., 0, 20, 33 and 66 months). Additionally, cultivable microorganisms from the bentonite were enumerated for the four bentonite time points. Results show that viable microorganisms are present within MX-80 over all observed time-frames. After an initial increase in number, they appear to remain viable for longer times. There was no evidence of a correlation between number of viable organisms and bentonite dry densities.

The diversity of the BIC-A1 borehole pore water microbial community decreased over the first 20 months but increased for the 33 and 66 months timepoints. Especially the observed decrease in the relative abundance of OTUs related to sulfate-reducing bacteria was reversed at the latest time point (66 months).

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

BENTONITE CHARACTERISTICS AFTER TWO YEARS OF STORAGE WITH INDIGENOUS BENTONITE AND WATER MICROBES

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The aim of this laboratory scale bentonite storage experiment was to assess the influence of microorganisms naturally occurring in the deep subsurface groundwater of the Finnish final disposal site and in the studied bentonite (Wyoming type Na-bentonite) on the structure of bentonite. The experiments were planned to simulate the microbial effects in so-called “worst case” conditions, which can occur locally in the interfaces of the final disposal site bedrock, water and bentonite, where the bentonite is not yet operational. The bentonite was studied as a slurry in which water, gases, nutrients and microorganisms were able to move freely at temperature hospitable for microorganisms. The objective was to find out if microorganisms and metabolites they produce in favourable conditions are able to change the bentonite structure and if these changes could be significant for the bentonite stability in long-term scale.

After a year of storage, there was no significant structural changes detectable in the bentonites (HR-TEM). Microbial activity consumed oxygen in few months from the bentonite bottles started from oxic conditions. In the anaerobic bentonite bottles, hydrogen gas was consumed in few weeks from the start. In addition, there were clear changes in the chemistry and in the microbiology of the bentonite samples after one year of storage. Cation exchange capacity (CEC) increased slightly in the microbiological anaerobic bentonites and microbiological sulphate reduction was ongoing in the bentonite bottles started from the anaerobic conditions, whereas it was not detected in the control samples or in the samples started from the aerobic conditions. After two years of storage, there was no significant changes detected in the bentonite samples compared to one-year samples analysed with atomic force microscopy and CEC. The amounts of bacteria, archaea, fungi and sulphate reducers were quantified with quantitative PCR (qPCR). More bacteria, archaea and especially sulphate reducers were detected in the bentonite samples started from the anaerobic conditions than from the bentonite samples started with oxygen. The amounts of microorganisms remained at the same level or their amount decreased a little as a function of storage time. Based on the results it is likely that a shortage of energy sources in the samples with liquid volume of only 80 mL, started to limit the microbiological activity after one-year of storage. The results from the bentonite experiments do not provide enough information so far to assess the effects of the microbial activity, and more research is needed from the on-going experiments after nutrient amendments.

Acknowledgements

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IRON AND COPPER CORROSION MORPHOLOGIES RELATED TO MIC?

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The KBS-3 canister will experience different types of sulphidic phases during the development of the repository.¹ In some locations in the repository unsaturated buffer conditions will prevail for a long time and hydrogen sulphide transport from saturated locations could then occur through the gas-phase. During the long-term reducing phase, the majority of deposition holes will be fully water saturated and sulphide is transported via diffusion through the dense clay. In some locations, the advective flow of water can cause erosion of the bentonite clay on longer time scales, which could eventually lead to advective conditions at the canister surface. What is known about the corrosion process during these different phases, for example regarding the tendency to localise? SKB has conducted a number of experiments in which the KBS-3 canister materials copper and iron have been exposed to various types of sulfide environments. Ranging from laboratory tests with high degree of parameter control to complex environments like the field experiment MiniCan conducted at the Äspö HRL (Figure 1).²⁻⁴ In this talk it will be discussed what has been learned from these experiments regarding localised corrosion under repository conditions, some of the challenges in studying corrosion morphology of copper will be elaborated on and it will be discussed how (and if?) localised corrosion due to microbial influence can be distinguished from abiotic processes.^{5,6}

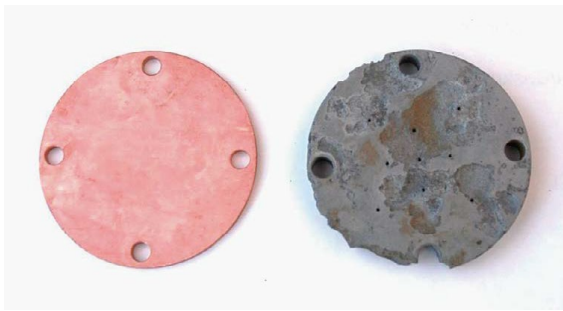


Figure 1. Copper and iron plates after nine years of exposure in the Äspö HRL and removal of corrosion products.

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SURVIVAL OF MICROORGANISMS IN BENTONITE SUBJECTED TO GAMA RADIATION AND THE EFFECT OF ANAEROBIC CONDITIONS ON THE MICROBIAL ECOSYSTEM EVOLUTION IN BENTONITE

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Indigenous microorganisms in bentonite can negatively influence long term safety and performance of deep geological repositories. Our study aims to increase the knowledge about the effect of Gama radiation on these microorganisms.

Microcosms consisting of BaM bentonite powder and deep underground water (3:10 w/w) were used for the experiments. In irradiation experiment performed under aerobic conditions, the microcosms were irradiated at constant dose rate 13 Gy/h for up to 9 weeks reaching 19,656 Gy total absorbed dose. Both irradiated samples and non-irradiated controls were sampled regularly. Furthermore, we run series of anaerobic controls, where half of the control microcosms were enriched in nutrients to compare the evolution of similar microcosm in aerobic and anaerobic conditions and to see the effect of nutrient availability on microbial composition in the closed microcosms. We performed qPCR by various markers and NGS of 16S ribosomal RNA gene amplicons to detect the microbial abundance and diversity in all the samples. We used ion chromatography to detect nitrate, sulphate and acetate within anaerobic control samples.

The composition of microbial communities within the bentonite suspension samples was changing continuously during the experiments. Most indigenous microorganisms from underground water disappeared at the very beginning of experiment and both total biomass and species richness markedly decreased in bentonite suspension samples at this phase. Subsequently, gradual changes in microbial community composition were observed mirroring the prevailing conditions in the samples.

In the aerobic irradiated and control samples the community changes were based on the available electron donors evolving from chemorganotrophs to chemolitotrophs with the decrease of available organic material. Only microorganisms surviving initial bottleneck underwent a selection caused by the effect of Gama radiation. Application of 19,656 Gy absorbed dose of Gama radiation at the constant dose rate 13 Gy/h did not manage to completely eradicate present bacteria. However, it caused the decline in total microbial biomass in time (but not species richness) and slight changes in the microbial community structure.

In anaerobic control microcosms limitation by both available electron acceptors and donors was detected. The anaerobic communities evolved from heterotrophic facultative anaerobic nitrate reducers to chemolitotrophic anaerobic iron or sulphur reducers and fermenting microorganisms. Community changes were not reflected in detected species richness, which remained rather constant after the initial bottleneck. These results showed the better suitability of anaerobic conditions for the indigenous microorganisms than aerobic conditions. They also clearly demonstrate a key role of iron reduction in the microbial processes occurring in the bentonite under anaerobic conditions which might have implication for the repository safety. To better estimate the effect of irradiation on microbial community changes in bentonite under repository conditions, subsequent irradiation experiments performed under strictly anaerobic condition and exposed to higher total absorbed doses are needed.

MICROBIAL EFFECTS FROM THE POINT OF VIEW OF BENTONITE SAFETY FUNCTIONS

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The roles of buffer between waste canister and fractured rock are contradictory: while it is relatively easy to have low enough water conductivity and diffusional transport, it more demanding to parallel fulfil requirements for rock shear load (dislocation) and microbial activity (e.g. Posiva SKB, 2017). For example, the bentonite chosen as a buffer material for KBS-3 repository, has to have dry density of 1 950 kg/m³ to prevent all microbial activity, but cannot be higher than 2 050 kg/m³ to be ductile enough for rock shear protection. However, scientific investigation of both rock dislocation and microbial effects has appeared not all straightforward and easy task. This presentation discuss only microbial activity.

Microbes can jeopardise the multibarrier system in several ways (Figure 1): 1) enhancing corrosion of waste canister (e.g. sulphide production may corrode copper canister in KBS-3 concept), 2) changing the chemical conditions, 3) enhancing alteration and dissolution/precipitation of montmorillonite and other minerals, 4) changing the (micro)structure, maybe thus enhancing mass transport and increasing porosity and 5) coupled interaction of all these. The completely functioning buffer is able to resist all these reactions and phenomena, but on certain locations (i.e. boundary between buffer and bed rock) and during certain periods of time (buffer not fully saturated and temperature low enough) the conditions may be beneficial for initiating microbial activity, which may then extend to other locations and continue after saturation. Another, novel issue may follow from applying higher temperatures of the buffer to optimise rock space and costs: the buffer may be after these high temperatures either in more difficult (microbes destroyed and saturation taking place at high temperature) or more beneficial (larger pores and lower swelling pressure) state for microbes.

In my presentation I aim to discuss the ways to study above described issues, review recent advances on those studies and propose what to do next with the microbe-bentonite - interaction: scientific and safety case related aims are to be considered both. My work will be based on scientific documents, especially from the MIND project; Waste Management Organisations reports (Posiva, SKB) about microbes in bentonite; and regulator's decisions (STUK).

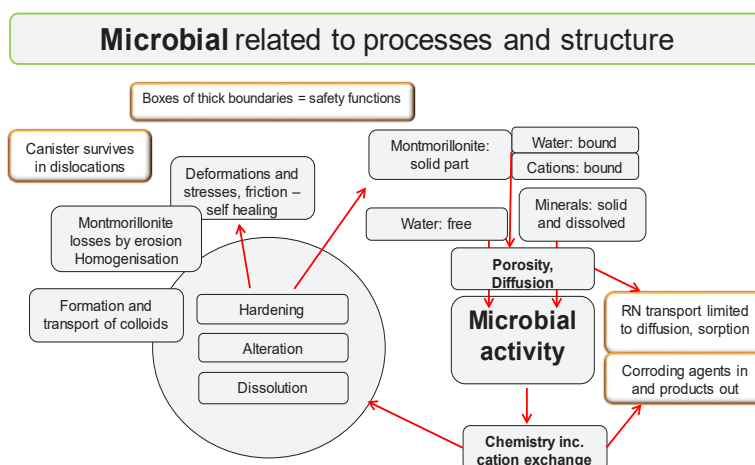


Figure 1: Complexity of couplings between bentonite processes and structures with the possible microbial effects, which here are assumed to appear mainly by changes in chemistry.

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GEOCHEMICAL PATTERNS AND CONSTRAINTS OF MICROBIAL SULPHATE REDUCTION IN THE GEOSPHERE

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Sulphide is the most important corrosive agent with respect to the copper canisters in the KBS-3 disposal concept of spent nuclear fuel that will be applied in Sweden and Finland. In addition to the potential corrosion of copper, sulphide may also affect the buffering capacity of bentonite by precipitation of iron sulphides [1]. A key process producing sulphide in the geosphere is microbial sulphate reduction. Major geological and chemical factors controlling sulphate reduction include concentration of sulphate, the availability of electron donors and temperature [2]. As it is an anaerobic process, absence of oxygen is also a necessary boundary condition for sulphide formation.

In this study we have compiled a database of more than 400 analyses of geochemical parameters from deep groundwaters in Finland. As the majority of the studies did not report sulphide concentrations, we have used two case study sites to demonstrate sulphur systematics in more detail: The Outokumpu Deep Drill Hole in eastern Finland is representative of a highly metamorphosed metasedimentary environment with minor occurrence of ophiolitic, sulphide ore and granitoid related lithologies, whereas the Pyhäsalmi mine in central Finland represents more sulphur rich environment with volcanic massive sulphide (VMS) ore and minor granitic rock types. In both cases the deepest sampling depths expand to ca. 2.5 km below the ground surface. However, due to the low geothermal gradient, maximum temperatures do not exceed 40°C. In previous studies, anoxic conditions suitable for sulphate reducing microbes has been confirmed at the sites [3, 4].

In order to determine geological constraints for microbial sulphate reduction (or sulphide formation) in these environments, both the availability of sulphate and important electron acceptors (CH_4 , H_2) were examined. Thermodynamic calculations were used to predict the most favourable reaction pathways, potential power supply in W as well as energy densities in J/L.

At Outokumpu sulphate peak (up to 0.3 mM) was observed close to the ophiolitic rocks at 1300 m depth and followed downwards by a sulphide peak (up to 28 μM) at 1700 m depth. At Pyhäsalmi the highest concentration of sulphide (18 μM) was detected in the freshly made drill hole R2250 at -1350 m level, whereas sulphate concentrations were highest (2.6 mM) in the drill hole R2227 at -1430 m level. Power available from the reactions considered was 1 - 100 fW (10^{-13} to 10^{-15} W). Typical energy densities were below 0.4 J/L for the $\text{SO}_4 + \text{H}_2$ reaction and below 0.1 J/L for the $\text{SO}_4 + \text{CH}_4$ reaction, except at R2250 where the energy density of the $\text{SO}_4 + \text{CH}_4$ reaction was 0.56 J/L. At Outokumpu sulphide production seemed to be mostly limited by the availability of sulphate, while availability of electron donors was more important control at Pyhäsalmi.

This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No. 661880 and the Finnish Research Programme on Nuclear Waste Management (KYT2018 and KYT2022).

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NWMO MICROBIOLOGY RESEARCH AND DEVELOPMENT PROGRAM

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The use of a deep geological repository (DGR) for the long-term disposal of used nuclear fuel is an approach currently being investigated by several agencies worldwide, including Canada's Nuclear Waste Management Organization (NWMO). Within the DGR, used nuclear fuel will be contained in engineered barrier system (EBS) that involves copper-coated steel containers surrounded by a bentonite clay buffer. NWMO is currently undertaking an extensive site selection process to find a willing and informed community to host Canada's DGR. It is expected that the site will be situated within either a crystalline or a sedimentary environment 500 m below ground surface. While copper is generally thermodynamically stable, corrosion can occur due to the presence of corrosive species produced via microbial activities under anaerobic conditions. While the presence and potential viability of microorganisms, will be, in part, site-specific, the material selection and the repository design and construction will also impact overall microbial activity. As such, NWMO's microbiology research and development (R&D) program aims to characterize microorganisms in the natural and engineered barriers and evaluate their implications on long-term safety of DGR. In this talk, the main objectives of the microbiology R&D program along with a few examples of on-going research work will be presented.

HOW MICROBIOLOGY FITS INTO THE PERFORMANCE ASSESSMENT FOR THE WASTE ISOLATION PILOT PLANT

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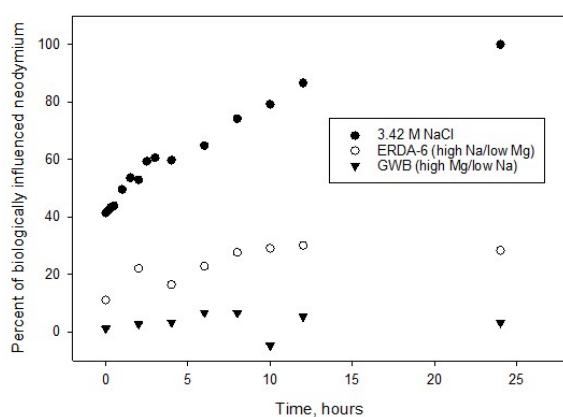
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Microbial influence on the Waste Isolation Pilot Plant (WIPP) is incorporated into the performance assessment/safety case in two main areas: 1) gas generation from the degradation of waste organics and 2) the enhancement of actinide mobility via biocolloid transport. Numerous assumptions were made about these topics, based on prevailing knowledge at the time of the original certification and on a series of experiments conducted specifically for the WIPP. Since that time, several new studies have emerged regarding the types, metabolisms, and behaviors of organisms indigenous to hypersaline systems. The results of these studies will not change the conservatism of the WIPP safety case, but they have been difficult to present to regulators, since they do not quite adhere to the conventional way of thinking in repository microbiology.

The canonical sequence of terminal electron acceptors is the first assumption made at WIPP regarding which organisms will degrade waste and which gases will be generated. WIPP PA follows the thermodynamically favorable nitrate followed by sulfate sequence (metal reduction and methanogenesis have been removed). However, in hypersaline systems, fermentation can play a large role, sulfidogenesis by other means is more likely, and the removal of methanogenesis due to an abundance of sulfate is not entirely accurate. The incorporation of these new findings into the WIPP conceptual model is unlikely to happen in the near future, due to the repeatedly negative results obtained under strict WIPP conditions and the added complexity and uncertainty that these additional reactions will incur.

Regarding biocolloid transport, the PA assumes that all organisms adsorb actinides and that all organisms are mobile. Bioassociation behaviour of actinides, or their analogs, with extremely halophilic archaea differs from that of bacteria [1] and varies significantly depending on the test matrix [2]. In a high sodium/low magnesium WIPP brine, the influence of a haloarchaeon on the concentration of analog in solution was very slow; while, in WIPP brines with high magnesium content, there was little to no biological influence (see figure). This has resulted in extremely low to zero values for input into PA.



Finally, there are features, events, and processes (FEPS) in the PA that should be readdressed, given the possible increase in Pu inventory for the WIPP. These include the influence of temperature and radiation on gas generation rates and also the formation of biofilms. New data are unlikely to change the screening arguments.

While WIPP PA adequately and conservatively models microbial effects, the justification for its approach and the associated uncertainties can be improved with additional research.

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

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Experiments have been carried out to investigate microbially induced corrosion of canister materials (WP2.2) and microbial degradation of bentonite buffers (WP2.4) in flow tests using FE bentonite and steel chips. Two sets of experimental apparatus were set up comprising compacted bentonite samples containing steel chips near the inlet. The bentonite was prepared to a dry density of 1400 kg m^{-3} or 1200 kg m^{-3} . The system was hydrated with an anoxic artificial groundwater based on a Grimsel groundwater recipe. Experiments were paired comprising one sample of sterile (irradiated) bentonite without microbial inoculum and one sterile (irradiated) bentonite inoculated with a mixed microbial culture enriched for sulphate reducers from unirradiated bentonite samples. The initial pair of tests were run with Grimsel groundwater recipe but subsequent pairs were amended by the addition of sodium lactate to act as a carbon source for the microbes. Tests were run for approximately 3 months.

Following removal from the experimental rigs and immediate sub-sampling it was noted that there was no brown/yellow ferruginous staining around the iron filings. However, photographs of the samples immediately after removal from the vacuum packs for analysis suggest that green rust had initially been present which rapidly oxidised resulting in a strong brown/yellow ferruginous staining around the iron filings a few days later. Samples were analysed using SEM techniques in both thin section and hand sample. Various secondary products were identified including euhedral aragonite and various iron products. Chemical mapping indicated a fibrous iron and sulphur-rich phase was present in only one of the experiments (1200 kg m^{-3} dry density, lactate amended, sterile). XRD data shows alteration to the clay around the steel in all samples analysed so far. Although there is considerable alteration observed within the samples, there is as yet no clear indications as to the extent or role of microbial corrosion, nor the associations with the corrosion products, within this experimental set up. This poster provides a summary of key petrographic and mineralogical observations from the experiments to date.

PHOSPHATES SPECIATION INFLUENCES BE9 BACTERIAL STRAIN URANIUM BIOREMEDIATION POTENTIAL

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Natural attenuation in areas containing high uranium concentrations, mainly due to anthropogenic causes, is a slow process linked to potential environmental and health risks. New remediation approaches based on microbial activities are being increasingly investigated to accelerate the environmental recuperation. Certain microbes may participate in the mobilization or immobilization of determined pollutants (e.g. heavy metals, radionuclides) depending on their metabolic potential and external conditions. In particular, members of *Microbacterium* genus exhibit high uranium tolerance due to multiple detoxication mechanisms (1). The bacterial strain Be9, belonging to this genus, was isolated from mining porewaters in our laboratory (2). In order to evaluate its capacity to interact with uranium, we employed a multidisciplinary approach combining colorimetric methods for measuring inorganic phosphates release, Scanning Transmission Electron Microscopy-High Angle Annular Dark-Field (STEM-HAADF), etc. Our results showed that *Microbacterium* sp. Be9 behaviours differently, depending on the physico-chemical conditions, when contacting with uranium. Tests by using a complex culture medium (LPM, low-phosphate medium) and a model background electrolyte (MOPS buffer) in presence or absence of an organic phosphate source (G2P, glycerol-2-phosphate), showed dissimilar uranium removal rates. Uranium precipitates obtained were localized both intracellularly and extracellularly depending on the incubation conditions. Enzymatic versatility showed by this isolate (2) seems to have a central role in its variable ability for interacting with uranium solved in aqueous solutions. These results show a remarkable behaviour of the microbial strain *Microbacterium* sp. Be9 in presence of uranium under distinct conditions which should be considered before its potential application in bioremediation strategies. However, further experiments should be performed in order to fully understand these processes.

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SHINING A LIGHT ON SULFUR CYCLING IN THE DEEP BIOSPHERE

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Microorganisms are present several kilometres below the surface and the continental ‘deep biosphere’ is estimated to contain 2 to 19% of the earth’s total biomass. However, due to the difficulty of obtaining samples it is one of the least understood ecosystems on the planet. One site that provides access to sub-surface fracture waters is the Äspö Hard Rock Laboratory, a unique facility comprising of a tunnel extending approximately 460 meters below the surface. The studied boreholes contained ‘old saline’ water that has a median dissolved organic carbon concentration of 1.4 mg l^{-1} and a residence time in the range of thousands of years and a ‘modern marine’ water that has a residence time of less than 20 years due to infiltration of organic carbon-rich Baltic Sea water. In this work we utilized high throughput 16S rRNA gene sequencing, metagenomics, and metatranscriptomics to identify the metabolic potential and interrogate the activities of microbial populations able to oxidize and reduce sulfur compounds. All three methods suggested both the presence and activity of a *Thiobacillus denitrificans*-like population in the old saline water as well as several populations that aligned with heterotrophic and autotrophic sulfur and sulfate reducing bacteria including the Deltaproteobacteria family’s Desulfobulbaceae, Desulfobacteraceae, and Syntrophaceae. The modern marine water also contained many SSU rRNA transcripts assigned to sulfur and sulfate reducing Deltaproteobacteria along with RNA transcripts from an Epsilonproteobacteria affiliated with the sulfur compound oxidizing *Sulfurimonas denitrificans*. Finally, metagenome assembled genomes were used to reconstruct the phylogeny and core versus accessory genomes in key sulfur oxidizing and reducing populations. With both active oxidative and reductive sulfur processes, the data suggest the presence of cryptic sulfur cycling in this biome and emphasizes the need to further investigate microbial activities in the deep biosphere.

GROWTH AND ACTIVITY OF THE MICROBIAL COMMUNITY OF MX80 BENTONITE IN THE PRESENCE OF IRON

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MX80 bentonite clay has been selected as the buffer and backfill in the proposed UK method of long-term deep geological storage of nuclear waste. Extensive studies have been carried out on the geomechanical and physical properties of the clay and in relation to the corrosion of steel waste canisters, as may be relevant to a UK repository design. However, fewer studies have considered the effect microbes in this situation and specifically, the effect that iron-reducing bacteria may have on functionality of the clay barrier. Iron-reducing bacteria are particularly of interest in this context because they can reduce Fe(III), (found naturally both in the clay and the canisters) to Fe(II) and some are adapted to high temperatures and low water availability, in keeping with conditions within the repository. Furthermore, in this work, iron-interacting bacteria have been isolated from MX80 SKB compacted bentonite. Experiments were carried out with the indigenous MX80 community and various iron substrates. Direct and indirect interactions were observed through a series of plate and liquid experiments. Fe(II), Fe(Total), and pH were measured throughout the experiment and all substrates were collected and analysed by XRD, SEM and EDX analysis. Significant differences in structure and appearance were seen in all indirect interaction experiments. Etching experiments showed evidence of micropitting and surface colonisation on compacted MX80. The changes observed by indirect interactions could greatly affect the structure and mineralogy of the MX80 and therefore may influence its ability to act as an effective barrier.

EFFECT OF CONCRETE ON MICROBIAL COMMUNITY UNDER REPOSITORY RELEVANT CONDITIONS

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The concept of geological repository of radioactive waste comprises engineered and natural barrier system that work together to isolate the waste from the environment. Wide variety of microbial communities with specific metabolic pathways exists under the deep biosphere and their metabolic activity may influence and compromise the safety performance of the repository. Here, we focus on the effect of cementitious and bentonite materials on anaerobic microorganisms under repository relevant anaerobic condition.

Czech BaM bentonite (Mg-Ca bentonite) from Černý vrch and low-alkaline concrete from Josef Underground Research Centre (URC), Czechia, was inoculated with natural ground water source VITA from Josef URC (1.5:1.5:10 w/w). The experiment was performed under anaerobic conditions. It was divided into two sets, both containing similar indigenous microflora from underground water and bentonite. Set A was carried at 30°C with addition of inoculum of four anaerobic alkaliphilic bacterial strains whereas Set B was carried at room temperature without any additional microbial strains. Controls for both sets did not contain any concrete. Additionally, no culture control NCC (bentonite + concrete + sterile water) and no culture no concrete control NCNCC (bentonite + sterile water) were also included. The experiment lasted for 2 months and was sampled at the beginning, after one week, one month and two months. Molecular-biological approach including qPCR analysis and next-generation 16S rRNA amplicon sequencing was used to determine the diversity and proliferation of relevant bacterial groups.

Indigenous bacteria from bentonite and underground water were negatively affected by the presence of cementitious material. The microbial abundance was much higher in control samples without concrete than in concrete containing samples in the set B. In the set A, most of the inoculated alkaliphilic bacteria did not survived in bentonite environment, although the pH and temperature was optimal for them. Furthermore, elevated temperature in set A probably also inhibited bacterial growth of most indigenous bentonite bacteria compared to set B performed at laboratory temperature. Principal coordinates analysis (PCoA) based on detected OTUs showed four distinct groups – first set A, second set B, third control A and fourth control B similar to NCNCC. By the end of experiment, the most dominating genera in Set A were *Alkaliphilus* and *Dethiobacter*, in controls A and B *Lacunisphaera* and in Set B *Bacillus* and *Dethiobacter*.

Our results demonstrate that presence of concrete has the potential to reduce microbial activity. Further research is necessary to estimate the possible biomineralization or biodegradation activity in cementitious materials by bacteria from bentonite that might be important for the repository performance.

DETECTION OF LIVING BACTERIA IN BENTONITE – CELL STAINING

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The detection of microbial presence (both active and dormant cells) is crucial question for engineered bentonite barrier within geological repository, because microbial metabolic activity can compromise the long term safety of repository. There are several possible attitudes, how to study the bacterial presence, viability and activity in bentonite samples. Cultivation methods using either liquid or solid media are the first option. The major drawback of them is that only a minor fraction of the bacteria present in the bentonite and deep aquifers is generally cultivable [1]. Further, bacterial activity can be measured by the amount and/or turn-over of metabolic products such as acetate, sulphide, oxygen or ATP [2]. However, these methods are not very suitable for precise estimation of bacterial density as it requires the knowledge of the metabolite rate per cell. Moreover, presence of bentonite in the sample hinders the ATP analysis, because clays very effectively binds ATP resulting in ATP recovery less than 10% in high clay samples [3]. 16S rRNA amplicon NGS sequencing is another commonly used method for estimation of microbial presence in bentonite samples. However, its result can be severely biased by the presence of extracellular DNA resulting from cell death [4], which might represent major part of isolated DNA from the sample [5,6]. Furthermore, in case of low-DNA bentonite samples the risk of microbial or DNA contamination is very high and needs to be account for during sequenation [7].

Clearly, the most reliable way how to detect the presence of bacteria in studied sample is direct count by means of epifluorescent microscopy or flow cytometry. There are various commercially available dyes, which indiscriminately dyes all present cells, other dyes selectively stain either live cells or dead (damaged) cells. By combining them we can distinguish between viable cells and dead (damaged) cells in the sample (live/dead staining). Although these dying methods work well in water samples, electronegatively charged bentonite negatively interferes with often electrically charged dyes excluding them from use in bentonite samples [8,9], which is also confirmed by our experience with all the tested dyes (AO, L/D, CFDA-AM, DAPI). There are several protocols for extraction of microbial cell form soils eg. [4,10,11] that are generally based on the physical detaching bacteria from the bentonite matrix using various detergents, vortexing and sonication followed by density gradient separation of cells from bentonite on various density media. Based on these works, we decided to develop protocol for cell extraction from bentonite, which will enable the easy cell staining and counting of extracted cells. At the recent stage we obtained very promising results for nutrient enriched bentonite suspensions rich in microbial cells, subsequently we will focus on the protocol optimization for the suspensions with low density of microbial cells and finally extraction of cells from compacted bentonite, that proves to be most tricky. We will present the actual state of our protocol development in our contribution.

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COMPACTED CLAY AS A POTENTIAL SOURCE OF SUBSTRATES FOR GROUNDWATER AND CLAY MICROBES

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Spent nuclear fuel is planned to be disposed to an underground repository in Olkiluoto, Finland by sealing it in canisters made of cast-iron and copper and isolating them from the surrounding bedrock with swelling clay [1]. Activity of the microbes inhabiting groundwater and rock fractures of the excavation-damaged zone (EDZ) of the repository have been identified as one possible risk for the long-term safety of disposal of spent nuclear fuel [1]. For example, sulfate-reducing bacteria (SRB) produce sulfide, which can corrode copper if it reaches the canister [1,2]. The clay is packed in high density ($\rho_{\text{wet}} > 1800 \text{ kg m}^{-3}$) to prevent activity of SRB and migration of sulfides in the clay, but the density might decrease towards the interface of EDZ [1,2]. In this study, our aim was to study the growth of SRB on organic compounds and sulfate dissolving from clay compacted to wet density of 1861 kg m^{-3} .

The experiment was carried out in two anaerobic laboratory setups, which contained saturated, compacted Na-bentonite, and a section mimicking EDZ separated from the clay by a sinter. The clay was not sterilised to mimic the conditions in real situation. The EDZ section of the other setup was inoculated with SRB to create the worst-case scenario of abundant SRB population, while the other setup was not inoculated with microbes. The experiment lasted for 270 days and the liquid of the EDZ section was sampled every three weeks. In the end of the experiment, the EDZ section and clay were dismantled and sampled.

During the experiment, sulfate dissolved from clay to the liquid phase of the setups, but the increase in the concentration of the inoculated setup was slower ($41.6 \text{ mg SO}_4^{2-} \text{ L}^{-1} \text{ d}^{-1}$) than in the uninoculated setup ($45.0 \text{ mg SO}_4^{2-} \text{ L}^{-1} \text{ d}^{-1}$), suggesting microbial consumption of sulfate in the inoculated setup. Sulfate concentration decreased in the clay below the EDZ section of both setups, but the decrease reached deeper in the clay of the inoculated setup than that of the uninoculated setup. There were more bacterial cells found from the liquid phase of the EDZ section of the inoculated setup ($10.1 \cdot 10^8 \text{ cell L}^{-1}$) than from the uninoculated setup ($3.7 \cdot 10^8 \text{ cell L}^{-1}$), but both samples showed similar sulfate-reducing activity when incubated in a growth medium optimised for SRB. The consumption of sulfate required longer time to initiate in the samples collected from the uninoculated setup.

The results showed that clay has a potential to act both as a source of substrates and as a source of bacteria including SRB. Sulfate was mainly consumed in the inoculated setup, but also the uninoculated setup contained microbial cells that were capable of reducing sulfate in favourable conditions. The results of sulfate analysis also showed that microbial consumption of dissolved sulfate caused mobilisation of sulfate from deeper of the clay phase and not just from the interface. This could have been resulting from sulfate concentration shifting towards equilibrium in the liquid phase, or from microbial activity within the clay. The threshold density of microbial activity depends on the clay type [2] and 1861 kg m^{-3} might not have been high enough for this Na-bentonite.

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MICROBIAL INFLUENCED CORROSION OF CARBON STEEL IN COMPACTED BUFFER MATERIAL COMPOSED OF KUNIGEL V1 AND SAND MIXTURE

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Spent nuclear fuel resulted from nuclear energy production will be stored in metallic container, such as carbon steel. The lifetime of metallic containment is strongly related to the corrosion rate of carbon steel in compacted buffer material. Corrosion of carbon steel buried in compacted buffer material was investigated in lab experiments using corrosive microbial consortium. The buffer material was composed of 70% bentonite (Kunigel V1) and 30% silicate sand, compacted to dry densities of 1.0, 1.3, and 1.6 g/cm³ in test cells. Compacted buffer materials were incubated in nutrient medium with or without inoculation by an enriched microbial community for one year at 30°C and 50°C.

Corrosion rates were suppressed (9.4 – 12.9 mg/cm²/year) in samples with compacted densities of 1.3 and 1.6 g/cm³ at 50°C. In contrast, a high corrosion rate was observed in the inoculated 1.0 g/cm³ compacted bentonite buffer materials at 30°C. Although the corrosion rate was low until the sixth month (3.2 mg/cm²/year), it significantly increased to 52.0 mg/cm²/year after one year. It is demonstrated that a sufficiently high dry density is one of the important key factors to suppress microbial activity in buffer material surrounding metal containers, because of the physical characteristics such as small pores, low water activity, less nutrient supply caused by low hydraulic conductivity.

Microbial growth and diversity changes were analyzed by molecular techniques during the incubation experiments. Quantification analysis demonstrated that 1.0 g/cm³ compacted buffer materials contained the highest microbial abundance, and microbial abundance increased with high dry density of buffer materials. Microbial diversity analysis showed the relative ratio of sulfate-reducing bacteria, such as *Desulfovibrio* sp., and they had increased only in 1.0 g/cm³ compacted buffer materials. These data suggested that dry density at 1.0 g/cm³ allowed movement and growth of corrosive bacteria. In contrast, compacted buffer materials with dry density of 1.3 and 1.6 g/cm³ suppressed movement of corrosion-promoting microbes from outside compacted buffer materials, while small growth of indigenous microbes in the buffer material were observed.

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DYNAMIC GENETIC ADAPTATION IN THE BACTERIUM *CUPRIAVIDUS METALLIDURANS* IN RESPONSE TO URANIUM

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Environmental contamination of radionuclides due to human activities has become a worldwide problem. Radionuclides have been introduced in nature by e.g. nuclear releases (weapons, testings & accidents) as well as nuclear industries. The enhancement of naturally occurring radioactive material (NORM industry) is also contributing to the potential for exposure from elevated environmental radionuclide concentrations. Examples of NORM industry are mining and processing of ores, phosphate industries, and production of natural gas or oil. Microorganisms are often found in radionuclide-contaminated sites where they can influence radionuclide mobility, toxicity and distribution. Key processes are reduction, uptake and accumulation by cells, biosorption and complexation with proteins, polysaccharides and microbial biomolecules, and biomineralization with phosphates and carbonates. In turn, long-lived radionuclides can exert a permanent pressure on the prevailing microbial population. Consequently, fundamental understanding of the interaction between microorganisms and radionuclides is essential to assess correctly the microbial impact on the long-term behaviour of radionuclides in contaminated environments. Although the interaction of microorganisms with uranium is extensively studied, there is far less information about the cellular response of microorganisms to uranium exposure.

The bacterium *Cupriavidus metallidurans* is a well-known model organism for metal resistance and is mostly isolated from industrial sites linked to mining, metallurgic and chemical industries. The interaction of *C. metallidurans* with uranium (U^{238}) and americium (Am^{241}) has been demonstrated, however, neither the genes or proteins involved nor the precise mechanism is known. A laboratory evolution experiment resulted in a spontaneous *C. metallidurans* NA4 mutant (NA4U), able to resist five times higher concentrations than the parental strain. Growth of NA4U in the presence $UO_2(NO_3)_2$ gave preliminary evidence that NA4U is using an active mechanism to retain uranium from the medium. Furthermore, whole genome sequencing revealed a 999 bp deletion in *czcS2*, coding for a sensor histidine kinase of the two-component system *czcR2S2*. Deletion of *czcS2* in the parental strain *C. metallidurans* NA4 resulted in an increased uranium resistance, confirming the key role of the *czcS2* deletion in the observed phenotype. This mutation is probably causing the constitutive over-expression of the response regulator (*CzcR2*) in NA4U compared to the parental strain, which was confirmed with whole-genome expression profiling in non-selective growth conditions. On its turn, *CzcR2* can cross-regulate the expression of other genes, causing the uranium resistance phenotype.

This study underscores the rapid evolution of *C. metallidurans* towards significantly increased uranium resistance and the first steps in unravelling its cellular response to uranium were taken. Investigating the uranium resistance mechanisms of *C. metallidurans* NA4U can provide further insights in the microbial impact on the long-term behaviour of radionuclides.

IDENTIFYING THE LIMITS AND IMPACTS OF MICROBIAL METABOLISM IN GEODISPOSAL SCENARIOS

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Despite radioactive waste geological disposal facilities (GDFs) being extreme environments for microbial life, microorganisms have been observed to persist through the unfavourable conditions. This work aims to further constrain the physical and chemical limits on microbial metabolism within cement (high pH) and clay (high pressure) environments, using geochemical, omic and microcalorimetry techniques.

Extensive work at The University of Manchester on analogues of cementitious intermediate level waste^[1] has led to the discovery of the alkaliphile *Anaerobacillus isosaccharinicus*. At high pH, *A. isosaccharinicus* is able to metabolise isosaccharinic acid (ISA), the main product anticipated from the alkaline degradation of cellulose in a cement-based GDF^[2]. This area of work focuses on the use of ¹³C stable isotope probing and metabolomics in order to identify and understand the ISA metabolic degradation pathway.

For many high level waste (HLW) GDF concepts, clay has been selected as an engineered barrier due to its low permeability and high swelling pressure. These properties are not only expected to increase mechanical integrity and reduce radionuclide transport, but also to reduce sulphate-reducing bacteria (SRB) activity that could have the potential to corrode metal waste canisters^[3]. This second area of work focuses on further understanding the relationship between groundwater chemistry, pressure and SRB activity, an important topic as the UK continues its search for a suitable GDF site.

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INVESTIGATION OF BENTONITE PRODUCTS IN JAPAN FOR ALTERNATIVE BUFFER MATERIALS

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Although Kunigel V1 has been well studied as a bentonite buffer or backfill for geological disposal in Japan, it might be better to seek the other candidates of bentonite products to ensure the procurement if the supplement of Kunigel V1 bentonite might be lack in future. NUMO therefore started to investigate the properties of bentonite products commercially supplied or to be supplied in Japan. Firstly, 130 bentonite products were selected and narrowed down to 22 products based on the literature survey of the fundamental properties and amount of reserves. 6 bentonite products were selected from 22 products by comparing the properties of bentonite with the requirements of cost, swelling power, plastic limit, cation exchange capacity, chemical conditioning, reserve of bentonite and locality. Now the detailed properties such as mechanical and hydraulic were measured for them. Simultaneously, the microbial activities in compacted bentonite are now being studied as a function of bentonite density, solution, and bentonite product to determine the critical density to prohibit the microbially induced corrosion of steel or copper.

MICROBIAL CORROSION OF CARBON AND STAINLESS STEEL BY THERMOPHILIC MICROBIOTA IN CONTACT WITH BENTONITE FOR CRYSTALLINE ROCK RW REPOSITORY IN RUSSIA

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The first Russian underground repository for both high- and intermediate level radioactive waste is being built in the crystalline Archean granite gneisses at Yeniseisky site (Krasnoyarsk region, Siberia). The geological features of the Yeniseisky site have much in common with the sites at Forsmark (Sweden) (see Hedin and Olsson 2016 this issue) and at Olkiluoto (Finland). HLW will be disposed in the form of an aluminophosphate glass and ILW (with long-lived radionuclides) will be cemented [1]. Nowadays we are at the step of studies working out, including field experiments for complex safety modelling. The plan of microbiological researches is one of the clue tasks. The radioactive decay of HLW is well known to lead to containers warming up and as a consequence to activation of the thermophilic microflora at an adjacent zone of storage too. Actually, the thermophilic bacteria are the first organisms that is able to influence on the barrier after cooling below 90 °C.

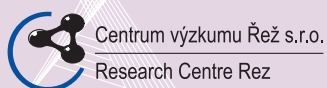
Apparently, carbon steel is one of the main barrier constituents along with bentonite clays. It is well known that typical steel corrosion according to its' essence is accompanied by both bacterial oxidation and bacteria mediated processes. At the same time, either radiolytic hydrogen or the organic matter which is contained in clay can perform as the electrons donor.

In the present study, the microbial corrosion of AISI 304 stainless steel and A570-36 carbon steel was investigated. Tests were carried out in anaerobic conditions in lack of chemical oxidizers with the use of deep bacterial communities isolated from Tau Tona mine, depth~ 1km (South Africa Republic) [2]. The natural temperature of life of these bacteria is 50 °C. The second bacterial community was isolated from surface hot spring in Tuva (Russia) [3] with natural temperature near to 45 °C. Khakass bentonite was chosen as the main component of the clay barrier and as a source of indigenous microflora. Water solution in the experiments contained 5 mmol/l Uranyl nitrate as an electron acceptor, acetate ion and trehalose were used as an electron donor. The tests temperature was 45°C, the experiment lasted for 8 days.

Finally, the corrosion nature of the chosen carbon steel samples in these test conditions was revealed. It is subjected to general corrosion with a relatively uniform film of corrosion products. The increase of corrosion rate by 220% was established on the samples, that were treated by the bacteria. Tau Tona cultures containing trihalose revealed the maximum corrosion rate ((48 µm/year). Microbial consortium isolated from bentonite lead to the rate corrosion 19 µm/year. Besides, the stainless steel samples during the corrosion tests showed the formation of local corrosion damage, which was identified as pitting corrosion, while the remaining surface of the steel stayed passive. The formation of pittings is likely promoted by bacteria due to the testing media contained no depassivation ions.

As a result, the complex model of carbon steel corrosion is worked out. It possesses not only temperature dependence but also blocking of a surface by the newly formed corrosion products.

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