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Final integration and evaluation report

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Publishable Summary

Microbiology In Nuclear waste Disposal (MIND) is a multidisciplinary project with the goal of addressing key microbiology technical issues that must be tackled to support the implementation of planned waste disposal across the EU. The project addresses the influence of microbial processes on organic waste forms and their behaviour (WP1), and on the technical and long-term performance of repository components (WP2).

This report is a synthesis of the outcome of the experimental and modelling research activities in WP1 and WP2, performed over the entire project. The scope of the report is broad, covering all scientific projects contained within MIND. The emphasis in this report is on the description of the degradation processes that can be expected and the environmental boundary conditions under which they can occur. For each work package, a summary of conclusions is provided focussed on the relevance of the research findings to microbiological uncertainties concerning the implementation of nuclear waste disposal. In addition, the outcomes and key findings of the MIND project to date are related to issues concerning nuclear waste disposal identified by the project Implementers Review Board (IRB).

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1 General introduction

1.1 The MIND project

Microbiology In Nuclear waste Disposal (MIND) is a multidisciplinary project with the goal of addressing key microbiology technical issues that must be tackled to support the implementation of planned waste disposal across the EU. Whilst it is widely acknowledged that microorganisms are capable of transforming materials within nuclear waste and under the conditions of waste disposal, our understanding of what affects these processes will have *in situ* remain tenuous. The project is designed to tackle a number of topics identified in the Implementing Geological Disposal of Radioactive Waste Technology Platform (IGD-TP) Strategic Research Agenda (SRA) as "high urgency" and "high importance". Specifically, the topics cover the influence of microbial processes on waste forms and their behaviour, and the technical feasibility and long-term performance of repository components. The project comprises 15 European groups from across the EU, and the experimental and modelling activities that are reported in this document are divided between two project 'work packages':

Work package 1 (WP1) addresses Key Topic 2 in the SRA (Waste forms and their behaviour): remaining key issues concerning intermediate level waste (ILW), including the combined effects of radiolysis and biodegradation of anthropogenic and natural organic polymers on (i) radionuclide speciation and release, and (ii) the potential to fuel microbial processes in ILW and the barrier system.

Work package 2 (WP2) addresses Key Topic 3 in the SRA (Technical feasibility and long-term performance of repository components): remaining key technical and scientific issues regarding the effects of microbial activity on the waste containers, buffer, backfill and seals of spent fuel and high-level waste (HLW) repositories.

A third work package (WP3) is responsible for the integration, communication and dissemination of the experimental and modelling research from WP1 and WP2, with the view towards the conceptualization and performance assessment of geological repositories for the safe disposal of nuclear waste.

1.2 Scope of report

This report is the final evaluation report on experiments regarding harmonizing molecular techniques, experimental and modelling research activities in WP1 and WP2 (Deliverable D3.7). Research activities carried out regarding harmonizing molecular techniques are discussed in Chapter 2, reasearch activities carried out as part of WP1 are covered in Chapter 3, and those that fall within WP2 are covered in Chapter 4. Each subchapter focuses on a subproject within that work package, and all partners involved provided a summary of their work. The most important processes and findings within both work packages are summarized in tables provided as appendices to this document. This format could be relevant for integrating the knowledge gained in MIND in the construction of a set of Performance Assessment calculations. In addition, these tables indicate the links to the high priority issues defined by the implementers review board.

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2 Harmonizing of molecular techniques and tools

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 (Kebschull and Zador, 2015, Costea *et al.*, 2017, Karst *et al.*, 2018), hence care should be taken in comparing results obtained using different methods. Within the MIND community, DNA extraction procedures, sequencing processes and bioformatics pipelines are highly diverse among the different partners. Therefore, within WP3, we evaluated the impact of the different strategies on the identified microbial community via experiments comprising a commercial microbial community standard.

As first step in this evaluation was the preparation of a thorough overview of the different DNA extraction procedures and bioinformatics pipelines used by the different partners. What type of samples, how the sampling is performed, DNA extraction & quantification method, PCR conditions, primers, sequencing technology and bioinformatics pipelines were questions that all partners answered.

In a next step, the sequencing process and bioinformatics pipelines were compared and evaluated. To this end, the DNA of a commercial microbial community standard of ZymoBiomics, consisting of eight different bacterial species was send around to eight different microbiology laboratories involved in the MIND project. Important to note is that all data were analyzed blind i.e. involved partners did not know the composition of the mock community. The different laboratories processed these samples with their own sequencing and data analysis tools. In total, five laboratories had sent in their sequencing data. Two different sequencing technologies were used namely Illumina MiSeq and IonTorrent, and the V3-V4 or V4 region of the 16S rRNA gene was selected as amplicon. If different labs analyzed their own data and predicted the mock community, variation was observed in the amount of Operational Taxonomic Units (OTUs) between the different research groups, ranging between 10 and more than thousand OTUs. However, the majority of this variation could be explained by the bioinformatics pipeline used, rather than the amplicon region, the quality of the sequencing data or the type of sequencing technology (Illumina MiSeq versus IonTorrent). This could be confirmed by applying the same 16S rRNA gene amplicon sequencing analysis pipelines on the five different datasets as comparable results were obtained from the different datasets. It was also observed that longer amplicons (e.g. V3-V4) returned a higher sequencing error rate than the approaches focusing on one region (e.g. V4). Additionally, most artefacts in the sequencing data originated from the presence of chimeric sequences, even for the best performing bioinformatics pipeline. Overall, it was clear that the different state-off-the-art sequencing and data analysis approaches used by the different partners in the MIND community are adequate and comparable, providing high quality scientific data. Chimera removal should be a point of attention whenever analyzing sequencing data. Knowledge and experience on how to address and process such samples and data was shared among the partners.

In a next step, an experiment was carried out to compare different DNA extraction protocols to recover DNA from a clay-rich environment. This matrix – although hampers the efficiency of DNA extraction – was selected as it is essential to have robust DNA extraction techniques from relevant host rock formations to assess the microbial impact on the safety of deep geological radioactive waste repositories. Seven laboratories received a 10 g aliquot of Opalinus Clay rock collected from the Mont Terri Underground Rock Laboratory in St. Ursanne, Switzerland spiked with the Zymobomics cell mock. Every participating laboratory extracted DNA using their own method. Afterwards, DNA amplification (16S rRNA gene V4 region) was performed by a single laboratory and

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Illumina MiSeq sequencing was carried out by a single commercial laboratory. In addition, the same bioinformatics pipeline was used to process and compare the DNA sequences obtained. Highly different results were obtained with the different DNA extraction methods. The amount of extracted DNA ranged from 0.66 ng to 418 ng, while in theory ~ 4.5 µg DNA could be obtained. In addition, there was one method outstanding in reassembling the mock community, while for other methods, an over-and underrepresentation of different OTUs was observed. The results of this study will be elaborated further on in future work. Nevertheless, these results indicate that care should be taken with comparison of data obtained with different methods and that testing the efficiency of the used DNA extraction protocol is essential.

3 WP1 – Waste containing organics

WP1 lead – NNL

The overall aim of WP1 is to reduce uncertainty of safety-relevant microbial processes controlling radionuclide and gas release from long-lived intermediate level wastes (ILW) containing organics. To this end, the following objectives are stated:

- To identify key organic chemical species resulting from organic ILW alkaline hydrolysis, radiolysis and biodegradation, and their effects on radionuclide speciation and mobility.
- To quantify the combined rates of alkaline hydrolysis, radiolysis and biodegradation of cellulose and anthropogenic organic polymers (such as bitumen, PVC and resins), present in ILW under disposal conditions.
- To establish the *in situ* chemical and physical conditions that may limit microbial activity in EU repository concepts for ILW utilising cementitious materials within a neutral pH host rock.
- To examine the microbial generation and consumption of H_2 and CH_4 under ILW repository conditions.
- To understand the effect of ILW heterogeneity on bioprocess pathways, pH and redox conditions, barrier degradation and radionuclide release.

3.1 Bituminised waste

Contributing partners: SCK•CEN

3.1.1 Problem statement & current knowledge

At least five European countries have to deal with significant bituminised long-lived low- and intermediate level (LILW-LL) radioactive waste (Abrahamsen *et al.*, 2015). During the MIND project, the Belgian disposal concept, with Boom Clay as the reference host rock and Eurobitum as the reference bituminised waste, were selected to study in more detail. Eurobitum contains 60 % bitumen Mexphalt R85/40 and 40 % waste containing soluble and insoluble salts. Overall, bituminised waste contains 20 - 30 wt % $NaNO_3$, 4 - 6 wt % $CaSO_4$, and small amounts of CaF_2, $Ca_3(PO_4)_2$, $Ni_2[(Fe,Mn)(CN)_6]$, and (hydr)oxides of Fe, Zr, and Al. The residual amount of water present is between 0.5 - 1 %. The total radionuclides inventory (U and Pu isotopes, ^{241}Am, ^{244}Cm, ^{60}Co, ^{90}Sr, ^{137}Cs, ...) counts for 0.4 wt % for drums with an average activity but can be up to 1.15 wt % for drums with higher activities (Stankovskiy 2011).

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To ensure safe geological disposal of the waste, a good understanding of the long-term behaviour of bituminised waste and its influence on the host rock is of paramount importance. In this study, the Belgian disposal concept using cementitious waste packages and Boom Clay as the host rock was considered as a reference concept. In the present design, the Eurobitum drums would be grouped in cement-based disposal waste packages, with the void space between the drums and the concrete disposal waste package to be filled with a cement-based material, thus forming a 'monolith'. These monoliths would then be placed in concrete-lined disposal galleries, and the voids between the monolith and the gallery liner would be filled with a cement-based material, probably a mortar. As a whole, these packages and the cement-based materials form the engineered barrier system (EBS). After closure, the eventual infiltration of Boom Clay pore water into the EBS will result in the saturation of the cement-based materials. Due to the large quantities of cement materials in a disposal gallery, the slow equilibration of the Boom Clay pore water with the cementitious materials will result in a highly alkaline solution (Weetjens *et al.* 2010). This hyperalkaline solution will eventually enter the drums and its uptake by the hygroscopic salts embedded in the waste will result in the swelling of the bitumen. This may lead to an increase of the pressure in and around the waste (Valcke *et al.* 2009). Water uptake by the bituminized waste results in the leaching of large amounts of $NaNO_3$ and other soluble salts in the Boom Clay. Scoping calculations indicate that, close to the waste drums the $NaNO_3$ concentration can reach up to a few molar and that during the first ~300 to ~1400 years the $NaNO_3$ concentration within the monolith will remain above 1 M and 0.5 M, respectively (Weetjens, Valcke and Mariën 2010), resulting in an increase of the ionic strength. Maximum NO_3^- concentrations are predicted to reach between 0.5 and 1 M at the gallery interface, decreasing rapidly with distance into the host rock (e.g. 0.1 M at a distance of 5 m in the Boom Clay) (Weetjens, Valcke and Mariën 2010). Moreover, bituminised waste is prone to chemical and radiolytical degradation triggering the production of hydrogen, nitrite from nitrate and water-soluble organics from Mexphalt. Bitumen degradation studies have been done in wet conditions, where bitumen was brought in contact with synthetic Boom Clay water (SBCW) at pH 8.5 or clay-cement water (CCW) at pH 12.5 and irradiated in anoxic conditions (Valcke *et al.* 2001). In the MIND project deliverables 1.1 (Abrahamsen *et al.* 2015) and 1.3, a detailed overview of the degradation products is described. The extent of some of these processes will be affected by the continuous evolution of the rheological properties of the bitumen. This so-called ageing results in harder bitumen, which tends to lose its binding capacity and becomes increasingly brittle.

The leaching products ($NaNO_3$ and organics) released from the bituminised waste can have a significant impact on the disposal facility, which is dependent on multiple abiotic (chemical) processes. The leached sodium nitrate plume could result in an oxidation of the redox-active Boom Clay components (e.g. pyrite), thereby decreasing its reducing capacity and increasing the mobility of redox-sensitive radionuclides (Se, Tc, U, Np, Pu) (Bruggeman *et al.* 2010, De Cannière *et al.* 2010). A direct chemical reaction between nitrate and redox-sensitive components of the Boom Clay host rock (dissolved organic matter, pyrite, kerogen) is not expected to occur or characterized by very slow kinetics (Marien *et al.* 2011). However, radiolysis of nitrate can result in formation of nitrite, and for nitrite, it has been observed that it can chemically oxidize pyrite and possibly dissolved organic matter from the Boom Clay host rock (Bleyen *et al.* 2017a). However, again it is assumed that the impact will be limited due to the slow reaction kinetics and the expected low concentrations of nitrite leaching from the bituminised waste (Bleyen *et al.* 2017a). However, there can be a direct chemical reaction of nitrate with the steel and/or H_2 present in the disposal facility. H_2 will be present, produced by the anaerobic corrosion of steel drums and from radiolysis of water and bituminised waste. The steel and/or H_2 can act as electron donors, with the steel (or even pyrite) possibly serving as catalytic surface for abiotic nitrate reduction (Bertron *et al.* 2013, Truche *et al.*

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2013, Truche *et al.* 2013). These reactions lead to the production of ammonium, which can sorb onto clay minerals and therefore compete with some radionuclides for sorption (e.g. Ni, Pd, Cs) (Staunton *et al.* 2002, Van Loon and Hummel 1999). Some of the leached water-soluble organics (e.g. oxalate) could act as RN-complexing agents and thereby modify the behaviour of radionuclides in the disposal facility and the Boom Clay.

The fate and thus final impact of the leaching products ($NaNO_3$ and organics) released from the bituminised waste on the disposal facility will also be influenced by microbial processes. Microorganisms will be present in the repository either as indiginous species that are already present in the host formation (although no consensus has been reached yet) or as species brought into the repository during its construction and operation or putatively via the waste monolith. In anoxic conditions, nitrate is the most favourable electron acceptor for microbial metabolism. Consequently, after oxygen is consumed and when nitrate becomes available, the microbial population near the repository of bituminised waste will likely evolve to nitrate and/or nitrite reducing prokaryotes which could augment nitrate reduction in the disposal facility. The first step in dissimilative nitrate reduction is the production of nitrite. Either this could not be further reduced, resulting in an accumulation of nitrite. As already mentioned above, nitrite accumulation can lead to the oxidation of clay, potentially decreasing the reducing capacity of the clay formation (Bleyen, *et al*. 2017a). Or nitrite can be further biologically reduced to to gaseous nitrogen compounds (NO, N_2O, N_2) by denitrification. Denitrification could lead to a net increase or decrease of gaseous compounds, depending on the electron donor used in the denitrification process. If the concentration of the produced nitrogen gases would exceed the solubility limit of the gases (depending on the interplay between production rate and transport rate), a separate gas phase could be formed, which, in case it persists, ultimately could cause local fissuring of the host rock, hence resulting in the formation of preferential pathways for radionuclide migration (Harrington *et al.* 2012). Nitrogen fixating microbial species can reduce the N_2 to ammonia and then assimilate this into organic forms, such as amino acids and nucleotides. On the other hand, nitrite can undergo dissimilatory reduction to ammonium (DNRA) (Madigan 2012). As already mentioned above, ammonium can sorb onto clay minerals and therefore compete with some radionuclides for sorption (e.g. Ni, Pd, Cs) (Missana *et al.* 2004, Staunton, Dumat and Zsolnay 2002, Van Loon and Hummel 1999).

It is expected that dissimilative nitrate reduction to nitrite and denitrification to gaseous nitrogen compounds (NO, N_2O, N_2) will be the preferred processes in geological disposals conditions. The use of hydrogen as the electron donor in the denitrification process results in a net gas consumption, preventing a gas pressure build-up in the disposal facility and consequently gas-related perturbation of the clay could be circumvented (Bleyen *et al.* 2017b).

Thus, several microbial processes can potentially influence the fate of the nitrate in the host rock, and thus the final impact of nitrate on the performance and safety of the disposal. However, *in situ*, several environmental parameters may stimulate or repress such microbial processes. The supressing factors for microbial activity in a disposal facility with bituminised waste can be high salinity (caused by Na^+ from $NaNO_3$), high pH (caused by the $Ca(OH)_2$ leaching from the cement) and space restrictions (due to high consolidation and small poresize) in undisturbed Boom Clay.

3.1.2 Summary of experimental & computational results

The efficiency of the Boom Clay borehole water microbial community to reduce nitrate leaching from thermally aged inactive Eurobitum in the presence or absence of known bitumen degradation products was investigated with different series of anoxic batch experiments. It was shown that

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acetate is the most preferred electron donor for microbial nitrate reduction and the highest nitrate reduction rates were observed in the presence of acetate. Formate also seemed to be an easily accessible electron donor, but in terms of the kinetics of the reaction, not as efficient as acetate in removing nitrate. Oxalate was the least preferred electron donor for nitrate reduction as it was only completely degraded in one of three replicates. However, calcium oxalate crystals were formed, including in sterile conditions, indicating that if oxalate is present, it will probably be less bioavailable compared to other organic compounds. On the other hand, it seems that oxalate enhanced the leaching of sodium nitrate from Eurobitum, although the mechanism remains unclear. In addition, TIC/TOC measurements indicated that besides the added acetate, formate and oxalate, the microbial community was able to use organics that leached from the Eurobitum blocks as electron donors to carry out nitrate reduction. Moreover, a clear biofilm formation was observed in all conditions, suggesting that microorganisms could enhance the degradation of Eurobitum. However, first indications suggest that this can be negligible in relevant conditions prevailing in a repository as no biofilm was observed on Eurobitum when the community was exposed to pH 12.5 for 110 days. In general, pH 12.5 seems a boundary condition for microbial nitrate reduction for both the microbial community of the Boom Clay borehole water and the pH-adapted Harpur Hill sediment. However, stress evoked by this high pH was not enough to completely eliminate the microbial community as intact cells were present after resuscitation in slightly alkaline pH. In addition, the Harpur Hill sediment revealed an initial increase in cell numbers, similar to that observed in the other conditions. This study confirmed that the microbial community present in the Harpur Hill sediment is adapted to high pH conditions as twice as much nitrate was reduced at pH 10.5 compared to pH 9. On the other hand, whilst the Boom Clay microbial population was able to carry out nitrate reduction at pH 10.5, the rates were lower compared to pH 9. It seems that phosphate can quickly become a limiting nutrient in some experimental conditions, hence to distinguish between what factor is most limiting, further research is necessary. Nevertheless, it is not expected to be a limiting nutrient *in situ,* as phosphate is bioavailable in Boom Clay via mineral fractions such as apatite. Beta diversity assessment of both communities based on flow cytometry profiles indicate that high pH can induce a change in the microbial community, which is different depending on the microbial community.

3.1.3 Conclusions

Eurobitum bituminised radioactive waste is subject to chemical and radiolytical degradation, which leads to ageing of the bitumen waste matrix, the generation of radiolysis gases and leaching of soluble organics. Moreover, a nitrate plume arises in the clay water, causing a geochemical perturbation of the surrounding clay. This could affect the redox conditions, have ionic strength effects and affect cation exchange processes, potentially leading to an increased mobility of radionuclides through the host rock. However, next to some abiotic reactions, nitrate reduction is a well-known microbial process. This study demonstrated that the nitrate leaching from Eurobitum rapidly stimulated microbial nitrate reduction. Different rates were observed depending on the organic compounds that were used as the electron donor to fuel the nitrate reduction process. Microbial nitrate reduction resulted in a significant production of nitrite, shown to be able to chemically oxidise pyrite and possibly dissolved organic matter from the Boom Clay host rock. Oxidation of these Boom Clay components means that its reducing capacity will be decreased and the mobility of certain radionuclides can be increased. In addition, conversion of nitrate to gaseous nitrogen compounds (NO, N_2O, N_2) by microbial denitrification could lead to a net gas production. For the Belgian concept in Boom Clay, supporting calculations have been performed to assess the risk of gas pressure development due to such processes (Weetjens 2015). Similar assessments can be done for other concepts. In this study, ammonium was not monitored, however conversion of nitrate to

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NH_4^+ abiotically via the interaction with steel or biotically by DNRA can compete with some radionuclides for sorption on the clay (e.g. Ni and Pd). For the Belgian concept in Boom Clay, current performance assessment considered a variant case with a four orders of magnitude higher solubility, yet without any effect on the long-term radiological impact (Weetjens, 2015). This study demonstrated that microbial nitrate reduction was possible up to pH 10.5, reflecting conditions at the interface of the Boom Clay and the concrete liner of the disposal gallery. However, the more alkaline conditions that are expected in the disposal gallery itself seem to inhibit microbial nitrate reduction. Nevertheless, the presence of microbes can be expected under these conditions, and if niches arise with local pH decreases, microbial nitrate reduction can be induced.

3.2 Cellulose containing waste

Contributing partners: UNIMAN, HZDR & UGR

3.2.1 Problem statement & current knowledge

A major constituent of organic matter in LLW and ILW that will be stored in a geological disposal facility (GDF) will be cellulose based material, including for example wood derivatives (paper and cardboard), and clothing and other cotton derivatives (Leschine, 1995; Abrahamsen *et al,* 2015). Under high pH conditions (between 12.5 and 13.3), similar to those expected in a cementitious GDF, cellulose undergoes chemical hydrolysis (van Loon *et al.*, 1999; Knill and Kennedy, 2003), to produce mainly isosaccharinic acid (ISA) (Glaus *et al.*, 1999). ISA forms water soluble, alkali stable complexes with various metalloids, metals and radionuclides (for example Ca, Ni, U, Np, Th, Am, and Eu) relevant to an ILW-GDF (Warwick *et al.*, 2003, 2004, 2006; Rai *et al.*, 1998, 2003; Vercammen *et al.*, 2001; Wieland *et al.*, 2002; Tits *et al.*, 2005).

The rate of chemical cellulose hydrolysis by alkali, and therefore the rate and amount of ISA produced, along with the solubility and stability of the ISA-radionuclide complexes will affect the rate and extent of radionuclide escape from the GDF. The latest model proposed that the complete hydrolysis of cellulose would require between 1,000 and 5,000 years (Glaus & van Loon, 2008), and the concentration of ISA in the ILW-GDF is estimated to be about 44 mM (van Loon *et al.*, 1999b). This is under the assumtion that the GDF will be anaerobic, dominated by hyperalkaline pH (pH value of 12.5) and a Ca^{2+} concentration of about 20 mM (Berner, 1992),

These models did not include the effect of radiological degradation or biological degradation. To our knowledge, there are no studies on the effect of irradiation on the abiotic alkaline hydrolysis of cellulose and the production of ISA, and nothing on the biodegradation of irradiated cellulose under hyperalkaline conditions. This study addressed these aspects.

Irradiation and biodegradation may enhance the rate of alkaline hydrolysis of cellulose, and therefore lead to faster and increased production of water-soluble organic compounds into the GDF, affecting amongst others the chemical behaviour and mobility of radionuclides. Acetate (AcO-) and α-Isosaccharinic acid (ISA) can be a biotic or abiotic degradation product of cellulose present in nuclear waste (Glaus *et al*., 1999). These degradation products can act as complexing agents for radionuclides (Glaus *et al*., 1999). In the worst case, radionuclide complexation with organic cellulose degradation products will lead to an increased mobility and a decreased retention of radionuclides in the barriers of a nuclear waste disposal facility. In addition to complexation with soluble organic species, radionuclides can be sorbed on and associated with microbial biomass. Therefore, the characterization of radionuclide complexes with soluble degradation products and microbial cells, as

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done in this study, is necessary for the assessment of the safety and the long-term performance of a nuclear waste repository.

3.2.2 Summary of experimental & computational results

Contributing partners: UNIMAN

Aims:

- Study the effect of irradiation on the abiotic hydrolysis of cellulose at alkaline pH.
- Study the effect of irradiation on the biodegradation of cellulose at hyperalkaline pH.

Experimental setup:

- Kimwipes were irradiated with 1 MGy of γ-radiation, at alkaline pH (pH 12.7)
- Batch sacrificial experiments containing irradiated and unirradiated cellulose were inoculated with sediment slurry from a hyperalkaline contaminated site.
- Samples were incubated at 20˚C for 1.5 years.

Results:

- Irradiation enhances the rate of cellulose hydrolysis at hyperalkaline pH (pH 12.7), with the concentration of ISA being around 4 times higher after 5 months reaction.
- The product of the abiotic hydrolysis of cellulose by hyperalkaline (pH 12.7) is predominantly ISA, and its production rate is significantly enhanced after irradiation of the cellulose.
- Irradiation at hyperalkaline pH (pH 12.7) increases the bioavailability of (crystalline) cellulose for microbial degradation.
- The products of the microbial degradation of irradiated cellulose at hyperalkaline conditions (pH 12.5 – 12.7) are H_2, acetate, and presumably CO_2. Methane was not detected at these hyperalkaline conditions.
- Microbial community responsible for degradation of irradiated cellulose at hyperalkaline conditions (pH 12.5 – 12.7) has been characterised (Bassil and Lloyd, 2017; 2018).
- Microbial fermentation of cellulosic material at initial pH > 12 lowers pH (Bassil *et al*. 2015a), possible resulting from microbial activity in low pH niches within microcosm experiments and
- ISA degradation at alkaline pH (pH 10) in aerobic, nitrate reducing and Fe(III) reducing conditions has been demonstrated previously (Bassil *et al*. 2015b, Rout *et al* 2015).
- Studies of Ni interaction and immobilisation with biogeochemical processes associated with the metabolism of cellulose degradation products have been published (Kuippers *et al*, 2018).

3.2.3 Conclusions

Irradiation of cellulose under hyperalkaline conditions (pH 12.7) enhances the rate of the abiotic hydrolysis of cellulose by alkali. This resulted in the production of significantly higher concentrations of the radionuclide complexant ISA, compared to the unirradiated controls. Irradiation also led to an increase in the concentration of dissolved organic carbon, including ISA, and made the cellulose more bioavailable for microbial degradation at a starting pH value of 12.5. Fermentation of the cellulose degradation products led to the production of H_2, and acetate, while CH_4 was not detected.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

In summary, chemical alkaline and radiolytic, and microbial degradation of cellulosic waste will cause leaching of ISA and other water-soluble organics (such as acetate), which will be the subject of abiotic and biotic processes and can have the following impacts:

- ISA forms water soluble, alkali stable complexes with various metalloids, metals and radionuclides; and thus can have a significant impact on the mobility of some radionuclides, i.e. when bound to water soluble ISA, radionuclide mobility in water is enhanced. This has been taken into account in performance assessment. However, microorganisms can degrade ISA, and thus potentially reduce the ISA concentration and subsequently the ISA-radionuclides mobility problem.
- Other soluble organics are produced, which can also from complexes with radionuclides but it is so far unclear what impact they have on the final mobility of radionuclides. The fact that part of the more complex soluble organics can be converted to acetate, and presumably CO_2, by microbial fermentation, could potentially lead to a decrease in complexant concentration, and thus could have beneficial effects. This should be explored further.

3.3 PVC containing waste

Contributing partners: UNIMAN

3.3.1 Problem statement & current knowledge

Plastics represent a significant volumetric contribution of organic material the inventory of ILW and LLW in a number of countries throughout Europe (Abrahamsen *et al.*, 2015). In the UK, halogenated plastics constitute the largest component of the organic-containing waste inventory (NDA/DECC, 2014). Much of this waste arises from the miscellaneous use of these plastics in maintenance and decommissioning operations at nuclear power plants, as well as from reprocessing plants and laboratories. Polyvinyl chloride (PVC) is widely used to manufacture glove box posting bags, protective suits and in tenting operations. The bulk of the PVC in the UK National Inventory is expected to be flexible films and sheets of PVC from these activities (Smith *et al*., 2013).

Owing to strong intermolecular forces between polymer chains, PVC in its pure form is a rigid, mainly amorphous material with little flexibility. As such, PVC must be rendered flexible in order to be of use in the nuclear industry, which necessitates the addition of a variety of additives, including plasticizers, heat stabilizers, fillers, pigments, flame retardants, UV absorbers, colorants and anti-oxidants (Coaker, 2003). Of these, plasticizers are typically present in the largest quantities, accounting for between 30% and 50% by volume. Plasticizers are not covalently bonded to the PVC polymer, rather they sit between and serve to lubricate otherwise rigid polymer layers. There is concern that these additives may diffuse out of the PVC material under the conditions of a geological disposal facility (GDF), which may have implications for microbial activity and for radionuclide mobility (Dawson, 2013). Indeed, phthalic acid, the degradation product of various phthalate ester plasticizers under high pH and irradiating conditions, is known to complex with uranium and other radionuclides (Vazquez *et al*., 2008, 2009; Zhou *et al*., 2004; Panak *et al*., 1995).

To the best of our knowledge, there are no studies on the bioavailability of PVC at the high pH conditions anticipated in a GDF. Whilst the effects of irradiation on PVC at high pH have been previously investigated (Dawson, 2013), the effect of ionizing radiation on the biodegradation of PVC and its additives has yet to be addressed. It is essential to address these knowledge gaps in order to better constrain the fate of PVC and its additives, and the corresponding implications for the safe disposal of nuclear waste.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

3.3.2 Summary of experimental & computational results

Aims:

- Study the bioavailability of plasticised and pure PVC materials for microbial nitrate reduction at high pH
- Study the effect of ionising radiation on PVC chemical degradation and its bioavailability for microbial degradation

Experiments:

- Pure PVC in powder form, and plasticised PVC sheet, were subject to 1 MGy gamma irradiation and/or submergence in hyperalkaline solution (saturated calcium hydroxide, pH 12.7; Figure 3-1)
- Batch microcosm experiments were set up to test the ability of a high pH-adapted community to use these materials as the sole source of carbon and electron donors for nitrate reduction at pH 10:
    - Non-irradiated PVC powder
    - Irradiated PVC powder
    - Non-irradiated PVC sheet
    - Irradiated PVC sheet
- Parallel microcosm experiments were initiated with two of the additives identified in the PVC sheet; triphenyl phosphate (TPP) and phthalic acid
- All experiments were amended with nitrate as the terminal electron acceptor, inoculated with 1% v/v sediment slurry from a high pH environment (Harpur Hill, UK), and incubated at 20°C for a period of 97 and 117 days for PVC and additive experiments, respectively.

Figure 3-1: The effect of radiation on the appearance of PVC materials. Imaged are flame-sealed vials containing saturated $Ca(OH)_2$ and (from left to right) non-irradiated PVC powder; irradiated PVC powder; non-irradiated PVC sheet; irradiated PVC sheet. Note the change in colour of the PVC powder from white to black, and of the PVC sheet from clear translucent to light brown opaque.

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Results:

- Irradiation led to slightly enhanced liberation of phthalate and phosphate additives from PVC sheet. Cracking and coarsening of the surface of PVC sheet was observed at the microscopic level. Irradiation of PVC under hyperalkaline conditions liberated hydrochloric acid, detected by a reduction in pH to near-neutral (PVC sheet) or acidic (PVC powder)
- Microbial nitrate reduction was supported by PVC sheet, whether irradiated or not. Less nitrate reduction was observed with irradiated compared with non-irradiated sheet
- PVC powder only supported minor amounts of microbial nitrate reduction, and only in the case of the irradiated materials; no microbial nitrate reduction was observed with non-irradiated PVC powder
- Concentrations of dissolved organic carbon increased throughout the PVC experiments, whether PVC powder or film, irradiated vs non-irradiated, or sterile vs live, indicative of alkaline hydrolysis of all materials at pH 10
- Compared with the start of the microcosms, the microbial communities in the end-points were characterised by much lower diversity, and enrichment in common soil lineages such as the *Pseudomonadaceae* and *Comamonadaceae* families
- No nitrate reduction was observed in microcosms with phthalate, and minor nitrate reduction (~2 mM of 24 mM reduced in 117 days) was observed with TPP, indicating that additives in the PVC sheet other than those tested here are responsible for the nitrate reduction observed.

3.3.3 Conclusions

The results from this work demonstrate that PVC additives (present in the PVC sheet but not in PVC powder) are able to fuel microbial metabolism at the high pH conditions (pH 10) of an ILW repository. Irradiation of plasticised PVC renders the material less bioavailable at pH 10, but microbial metabolism appears to be supported still. Phthalate, the breakdown product of phthalate esters under irradiating and high pH conditions, was not found to support the metabolism tested.

In summary, chemical alkaline and radiolytic degradation of PVC waste will cause leaching of phthalic acid, triphenyl phosphate (TPP) and other organics, which will be subject of abiotic and biotic processes. From earlier studies, phthalate is known to complex with radionuclides (e.g. Vazquez *et al*., 2009), and the results of this study indicate that microbial activity is unlikely to reduce this risk.

3.4 Ion exchange resins waste

Contributing partners: EPFL, TUL, CV Rez

3.4.1 Problem statement & current knowledge

Ion exchange resins form a significant component of the organic inventory of LLW and ILW in EU member states (Abrahamsen et al, 2015). Resins represent the largest single component of the organic material in the inventory of ILW and LLW in Switzerland and The Czech Republic. Within the MIND project, studies are being undertaken with material from Switzerland and the Czech Republic.

Resins are used in nuclear power plants to remove radionuclides from water. The resin inventory consists of cation exchange resins and anion exchange resins that share a common polystyrene

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polymer chain cross-linked by divinyl benzene. The functional groups with ion-binding capacity are sulfonate groups (cation exchange) or amine groups (anion exchange). Because these resins bind radionuclides, it is expected that they will be exposed to significant gamma radiation, potentially resulting in their radiolytic degradation. Chemical compounds released from the radiolytic degradation of resins could serve as electron donors, electron acceptors, or carbon sources for microorganisms in the repository. Therefore, it is important to have a better understanding of their composition. Evaluating this process is the first phase of the proposed work.

The radiolytic degradation of resins has been investigated in previous work. However, there are several limitations to the current state of knowledge. First, much of the work has been carried out under oxic conditions (Van Loon *et al*, 1995) and it is expected that the repository will transition rapidly to anoxic conditions. Additionally, the range of analytical tools applied to characterize the products of resin irradiation was limited (Rebufa *et al*, 2015) and it is very likely to have overlooked major groups of compounds.

Another aspect of the work that will be evaluated is the potential of microorganisms indigenous to the deep underground water surrounding repository or present within EBS to further degrade (i.e., biodegrade) the radiolytic degradation products. Because these compounds could produce chelating agents for radionuclides, could fuel sulphate reduction or methanogenesis.

One of the key radionuclides present in spent ion exchangers from nuclear power plants is Caesium (Cs). Much attention has been given especially to the radioisotope Cs^{137} due to its long half-life, high solubility of Cs salts in water and its similarity to Potassium (K), a metabolically important chemical element (Ivshina *et al.*, 2002). Environment within ILW and HLW repositories will not be sterile and present microorganisms can uptake Cs^+ either through incorporation via the K^+ transport system thanks to its chemical similarity to K^+ (Kato *et al.*, 2016), or it can be adsorbed on the surface of cell walls (Lan *et al.*, 2014). Such uptake of Cs ions into bacterial cells can influence the mobility of this radionuclide in the environment and hence can affect the safe performance of the repository. High concentration of Cs^+, generally above the mM order, exhibits toxic effects on organisms; however several recent studies showed also the existence of bacterial strains that can tolerate high amounts of Cs^+ in the environment (Dekker *et al.*, 2014, Kato *et al.*, 2016). Most of the studies focusing on the toxicity of Cs ions worked with single bacterial strains. Therefore, we decided to work with natural water from Josef URL containing a diverse bacterial community and espetially SRB.

3.4.2 Summary of experimental & computational results

EPFL

Identical resins to the ones used in the nuclear power plants in Switzerland were obtained. More specifically, a mixture of cation and anion bead resins (Lewatit M 800 KR and Lewatit S 200 KR) as well as a mixture of anion and cation powder resins (Powdex PAO and Powdex PCH) were doped with a mixture of cations and anions (Li^+, BO_3^{3-}, SiO_3^{2}, SO_4^{2-}, Cl^-, Na^+, Mg^{2+}, K^+, Ca^{2+},F^-, SO_4^{2-},Br^-) to mimic the chemical state of the spent resins that will require disposal. The doped resins were placed in Opalinus Clay porewater with pH 8 but also alkaline conditions (pH 12.5) were investigated. Resins were irradiated at a dose of 50 kGy using an electron linear accelerator (Alienor Titan Beta Inc.). It should be noted that an electron accelerator delivers beta radiation rather than the gamma radiation expected from the radionuclides associated with spent resin. However, it has been suggested previously that the radiolytic degradation products of organic liquids was comparable when gamma radiation (from a gamma Cs^{137} source cell) was used or when beta radiation (electron accelerator) was used (Ortiz, *et al*., 2015). The irradiation was carried out after degassing with argon, in order to

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ensure anoxic conditions. The ampules used for the irradiation allow gas sampling, hence allowing analysis of the gas phase formed by gas chromatography (Agilent GC-MS with a quadrupole mass analyser). Additionally, analyses of H_2, CH_4, CO_2, and CO were performed with a µ-GC. Furthermore, unirradiated resin that was otherwise treated identically was also analysed.

Comparison of the irradiated and unirradiated bead resins revealed the production of H_2 and a number of chlorinated aliphatics, such as bromomethane, chloromethane, dichloromethane, and chloroethane, as well as aromatic compounds, such as benzene and toluene, in the gas phase. This is the first time that these compounds have been identified as by-products of resin irradiation. The major reason we think this result was obtained is that these compounds are highly volatile and tend to partition into the gas phase. Previous work that focused on the aqueous phase may have overlooked these compounds.

The latest round of irradiation experiments with a cobalt gamma source at 200 kGy revealed that as well as H_2 and CO_2, chloromethane and benzene were measured again in the gas phase but not the other compounds. Additionally, based on the volatility of these compounds, we calculated that the detection limit afforded by the tool used was too low to detect these compounds in the aqueous phase. Hence, gas phase analysis is the only reliable way to measure these volatile compounds. Radiolytic yields have been quantified for benzene and chloromethane in the gas phase by Sophie Le Caer at CEA, which show that only very low concentrations exist in the gas phase (approximately 10^{-8} M). Identification and quantification of organic compounds in the aqueous phase using GC-MS and ion chromatography (IC) have detected ammonia and trimethylamine.

Using the four organic compounds (benzene, chloromethane, ammonia and trimethylamine), experiments are now ongoing to evaluate the potential for biodegradation of these radiolytic resin degradation products under conditions relevant to the repository. Previous research suggests that all four organic compounds can be oxidised by microorganisms under varying redox conditions, including sulphate-reducing, nitrate-reducing and iron-reducing (Lovley et al., 2000; Doronina et al., 1996; W Yang et al., 2012; Z Yang et al., 2009). Sulphate reduction is likely to be the main metabolic process in the repository in Opalinus clay due to the presence of sulphate as well as H_2 from anoxic steel corrosion and resin irradiation (Bagnoud *et al*., 2016).

TUL/CV Rez

Two independent experiments focused on ILW were conducted as a part of MIND project at TUL and CVR.

Resins radiolysis and biodegradation

The effect of radiation on polystyrene resins and subsequent biodegradation under simulated repository conditions was studied at CV Rez and TUL. The typical representative of polystyrene ion-exchange resins that serve for capturing radionuclides outgoing from a pressurized water reactor in Czech nuclear power plant technology was used for this experiment.

The catex, annex and 2:1 mixture of catex and annex were irradiated by the average dose rate ~2.04 kGy/h up to the the total dose of 1.028 MGy under anaerobic condition. Non-irradiated control resin samples were also included in experimental design. Firstly we analyses the irradiated catex and annex by means of total leachable organic content analysis (LOC) and gas (GS/MS) and liquid (LC/MS) chromatography to detect the presence of radiolysis products. Further, study on microbial degradation of this ionex mixture was carried out. Both irradiated and non-irradiated control resins of final concentrations 0.2, 2 and 20 and g/l were inoculated with anoxic underground water (=VITA)

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from Josef Underground Research Laboratory (URL) (Czech Republic) rich in microorganisms to see the effect of resins and its degradation products on microbial abundance. Batch experiments were performed under anaerobic conditions for 18 months and sampled after 3 days, 1, 6, 12 and 18 months. GS/MS chromatography was performed in samples to detect present volatile radiolytic products and we used molecular methods (qPCR and 16s RDNA amplicon sequencing) to study the changes in microbial abundance and composition during the course of experiment.

Our analyses showed that irradiation highly increased the leachable organic carbon in both catex and anex, compared to non-irradiated ionexes. GS/MS detected the presence of several organic radiolytic products such as trimethylamine, dimethylamin, acetatenitrile, aceton (2-propanon) a 2-butanon originating from anex and benzen originating from catex (Figure 3-2). LC/MS on the other hand detected presence several larger organic degradation products expetially in catex sample (Figure 3-3), the composition of annex sample was rather similar as in non-irradiated annex.

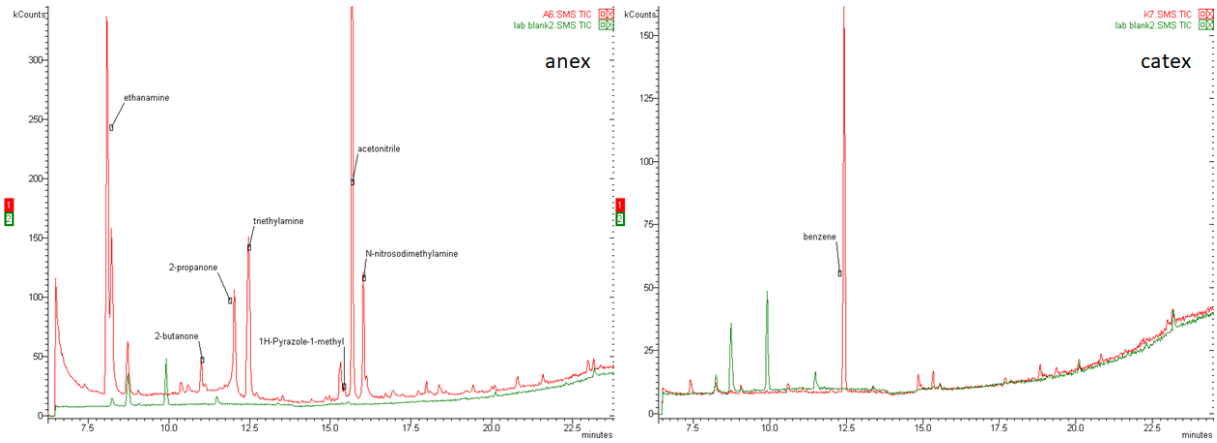

Figure 3-2: GS/MS chromatograms of irradiated catex and annex.

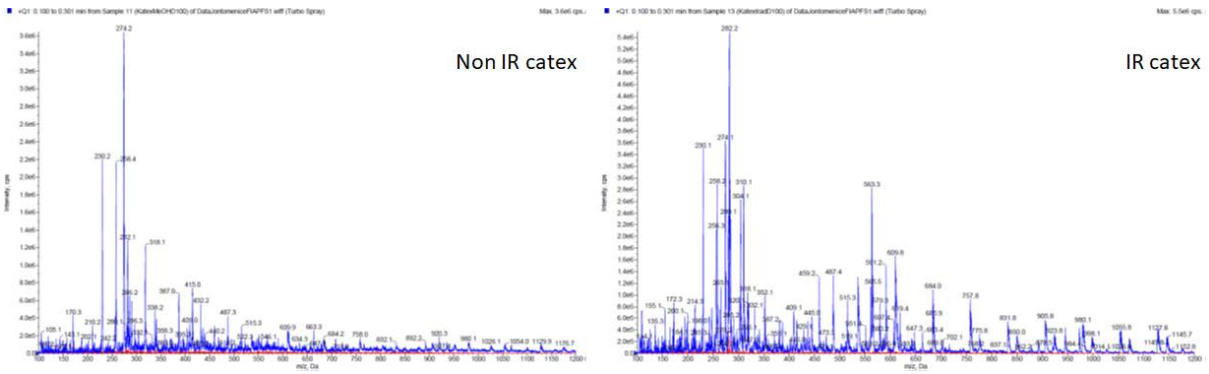

Figure 3-3: LC/MS chromatograms of irradiated and non-irradiated catex.

Although we clearly detected the radiolytic products in irradiated ionexes, we were not able to reliable detect the radiolysis products in water samples from study on microbiological degradation. We found possible presence of carbon disulphide or benzene by GS/MS in some of the samples, but their concentration was below the detection level and thus could not be realibly distinguished from the natural laboratory contaminants. The volatile compounds were either too diluted, consumed by microorganisms or lost during the sample handling.

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On the other hand, the results of qPCR implies possible presence of radiolitic products in samples with irradiated ionexes, where we detected highest increase in relative microbial abundance (at the concentrations 0.2 and 2 g/l) compared to other samples. In non-irradiated resin samples of the same concentrations the increase in relative microbial abundance was lower (Figure 3-4). However, irradiated resins at the concentration of 20 g/l proved to be lethal for the present microorganisms on the contrary to non-irradited resins of the same concentration. We ascribe detected lethal effect of higher concentrations of irradiated ionexes to the the low pH (min detected pH = 4.764 compared to other samples with pH 7-9) resulting from the irradiation and exchange processes. The microbial abundance in control VITA water sample gradually decreased during the experiment (Figure 3-4).

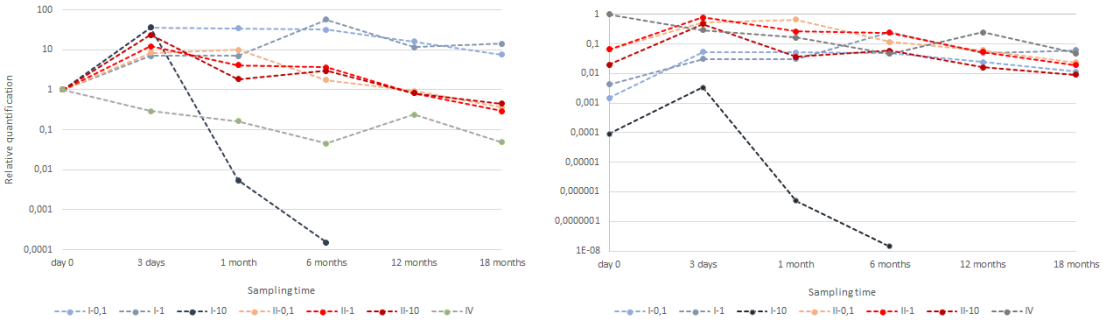

Figure 3-4: Relative quantification of 16S rRNA gene copies (measure of total microbial biomass) in studied samples. On the left changes in relative quantification in each sample during the experiment, on the right relative quantification recalculated to the original Cq value of VITA water (sample IV), which better illustrates the original differences in relative abundances.

The microbial composition in samples detected by NGS was clearly determined by the experimental set-up. The PCoA analysis (Figure 3-5) showed, that the samples are divided firstly by the time factor (zero point samples have different microbial composition from others) and secondly by the presence of irradiated resin. Interestingly, the microbial composition of samples with the highest concentration of irradiated resins clearly does not differ from other samples with irradiated resins indicating the biomass was reduced evenly in most of the detected taxa.

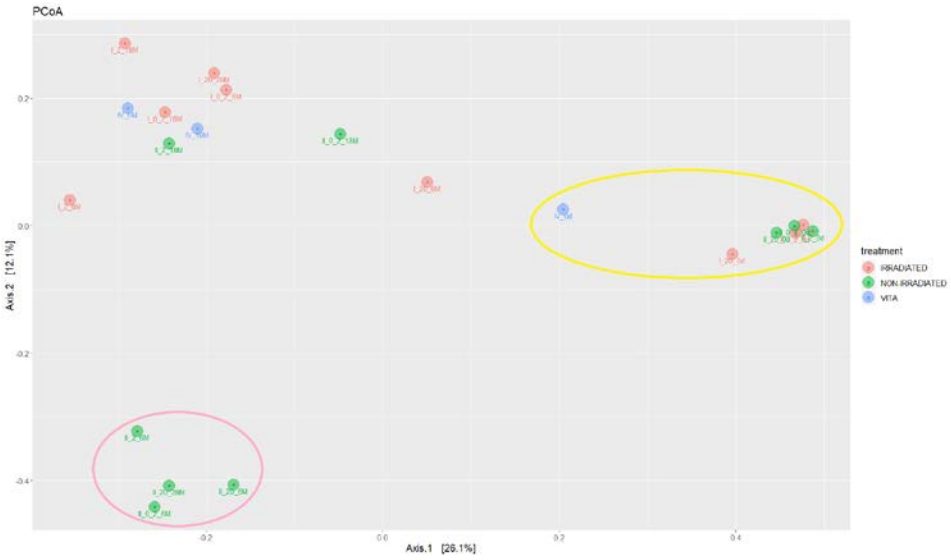

Figure 3-5: PCoA analysis based on detected OTUs in the studied samples. I – irradiated resins, II – non-irradiated resins, IV – VITA water. 0_2, 2, 20 – concentration of ionexes (g/l). Yellow ellipse surrounds all the zero-point samples, pink ellipse groups most of the samples containing non-irradiated resins.

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The effect of Cesium presence on microbial activity

The influence of Cs ions on microbial activity was assessed to study their role in ion exchange resins. Different concentrations of CsCl (0.5, 1 and 5 mM) were added to natural anoxic underground water (VITA) from Josef URL rich in SRB. The effect of Cs^+ ions on microbial abundance and composition was determined using molecular-biological methods (qPCR, amplicon 16S rDNA sequencing) and fluorescent and transmission electron microscopy. The sub-samples were collected after 0, 1, 3, 6, 8 and 23 days. The experiment was performed under anaerobic conditions in an anaerobic glove box. Lower Cs^+ concentration (0.5 mM) led to the proliferation of bacteria, specifically the nitrate reducers. Higher Cs^+ concentrations (1 and 5 mM) were toxic to bacteria in terms of decrease in bacterial biomass. This was observed by both qPCR and Live/Dead cell staining. 16S rDNA amplicon sequencing revealed that the structure of the microbial community changed in the condition without Cs^+ and in the presence of 0.5 mM of Cs^+. Interestingly, OTU composition of the samples with higher Cs^+ concentrations (1 and 5 mM) strongly resembled the species composition of the original microbial inoculum, suggesting the amplification of DNA from dead cells. Nonetheless, *Brevundimonas* spp. might tolerate higher concentrations (1 and 5 mM) of Cs^+ because an increase in their abundance was detected by amplicon sequencing. To conclude, no toxic effect of 0.5 mM of Cs^+ and toxic effect of higher concentrations (1 and 5 mM) of Cs^+ was observed.

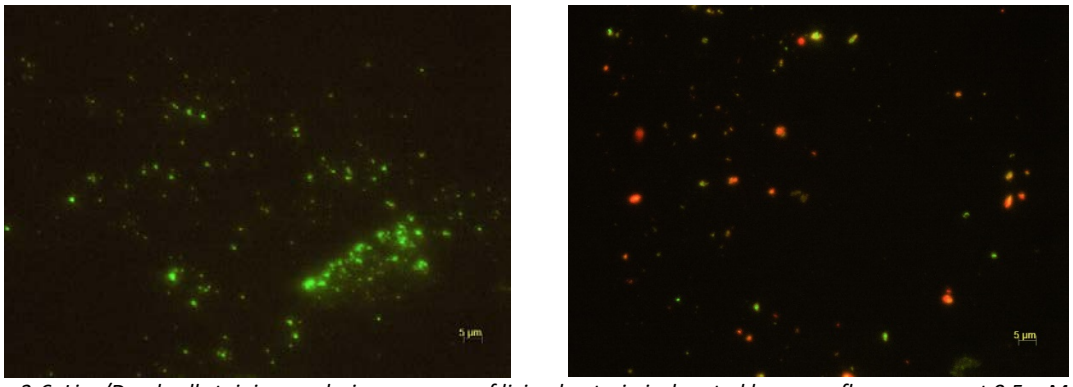

Figure 3-6: Live/Dead cell staining analysis - presence of living bacteria is denoted by green fluorescence at 0.5 mM Cs^+ concentration (left) and dead bacteria are marked with red fluorescence at 5 mM Cs^+ concentration after eight days.

EPFL

Here, we have shown that the complexity of the composition of resin irradiation products is significantly greater than previously thought. This finding suggests that resins are likely to provide potential energy sources for microbial metabolic activity, although some compounds, such as aromatics may not be biodegradable by the microorganisms found *in situ* at Mont Terri URL. Further results from the biodegradation microcosm studies will reveal if this microbial community is capable of utilising these compounds.

TUL/CV Rez

The experiment with irradiated polystyrene ion exchangers revealed that degradation products caused by resin radiolysis might serve as nutrients for bacteria under anaerobic conditions. However, only low concentrations (0.2 and 2 g/l) of the exchangers supported bacterial proliferation. Concentration of 20 g/l was lethal in samples with irradiated resins, but not in non-irradiated resins indicating that radiolysis products (probably due to low pH) probably causes the toxicity.

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Increasing concentrations of Cs^+ ions (1 and 5 mM) that are commonly present in spent ion exchangers showed increasing negative effect on anaerobic microbial consortium from the natural underground water from Josef URL. On the other side, lower Cs^+ concentrations (0.5 mM) promoted bacterial proliferation, especially total biomass and nitrate-reducing bacteria.

3.5 Radionuclide complexation

Contributing partners: HZDR & UGR

3.5.1 Problem statement & current knowledge

As described in the sections above, various water-soluble organic degradation products from organic waste (such as ISA, acetate, etc.) can act as complexing agent for radionuclides (Glaus *et al*., 1999). Radionuclide complexation with such organic degradation products will lead to an increased mobility and a decreased retention of radionuclides in the barriers of a nuclear waste disposal. Therefore the characterization of radionuclide complexes with soluble degradation products was included in this MIND study, and the obtained experimental data are integrated with the respective organic degradations studies, reported above.

In addition to complexation with soluble organic species, radionuclides can also be sorbed on and associated with microbial biomass. When sorbed to microbes in attached biofilms, the radionuclides are removed from the water and the retention of radionuclides in the barriers of a nuclear waste disposal is enhanced, although depending on the sorption mechanism this effect could be transitory. The characterization of radionuclide complexes (U, Se, Eu) with microbial cells, as done in this study, is necessary for the assessment of the safety and the long-term performance of a nuclear waste repository.

Organic polymers (e.g. cellulose, PVC, bitumen) present in low and intermediate level wastes (LILW) are exposed to ionizing radiation, alkaline pH, and organic degrading microorganisms. This may lead to the formation of smaller, water-soluble organic compounds, affecting amongst others the chemical behaviour and mobility of radionuclides. In the worst case, complexation will lead to an increased mobility and a decreased retention of radionuclides in the engineered barriers of a nuclear waste disposal facility. Therefore, the characterization of radionuclide complexes with degradation products is necessary for the assessment of the safety and the long-term performance of a nuclear waste repository.

Acetate (AcO^-) can be a biotic or abiotic degradation product of organic material (e.g. cellulose) present in nuclear waste, while α-Isosaccharinic acid (ISA) is formed by alkaline hydrolysis of cellulose, typically at pH >12.5. These degradation products can act as complexing agent for radionuclides (Glaus *et al*, 1999). This may affect the mobility as well as the sorption behaviour adversely.

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3.5.2 Summary of experimental & computational results

Contributing partners: HZDR & UGR

HZDR

Radionuclide speciation studies with organic degradation products:

- Synthesis of $Ca(ISA)_2$ based on a protocol from UNIMAN. Based on $Ca(ISA)_2$ the Na-form, NaISA, was prepared for the speciation studies. The purity of both salts was verified by ^{13}C- and ^{1}H-NMR. NaISA is the starting compound for subsequent speciation analyses with U(VI).
- Application of spectroscopic methods (e.g. UV-vis, TRLFS, ATR-FT-IR, XAS), NMR and ESI-MS in combination with theoretical calculations for U(VI)-species characterization in the acetate and ISA systems. Our focus was on the U(VI)-ISA system.
- Outlook: The influence of ISA on the retention of UO_2^{2+} on bentonite under alkaline conditions will be elucidated.

A long-term tissue paper biodegradation experiment under hyperalkaline conditions and in the presence of uranium was prepared in cooperation with UNIMAN in August 2016. Ongoing sampling and analysis of uranium and degradation products may give first insights into the effect of uranium on the biotic degradation of cellulosic material and into the fate of uranium in the presence of such degradation products. The experiment was stopped at the beginning of 2019 (after running for approximately 2.5 years) and afterwards will be evaluated.

<u>U(VI) – acetate system (acidic pH range)</u>

The interaction of the uranyl-ion (UO_2^{2+}) with acetate seemed to be a well-characterized system, with the general assumption that three complexes were formed under acidic conditions. It was the initial aim of the present study to fill the gap of missing absorption and luminescence data for all proposed complexes, serving as reference data for further studies. However, the findings were contradictory to the general assumption, since the spectroscopic data could consistently be interpreted with the formation of only two UO_2^{2+}-acetate complexes.

For instance, Figure 3-7 shows the luminescence spectra of test series 2 at -120°C. A similar pattern for the room temperature spectra was found. The congruence of the spectra with pH from 1.1 to 2.2 strengthens again the conclusion that only one species was formed during that phase. In phase 2 a significant band shift as well as an increase in luminescence intensity occurs with increasing pH at both temperatures. The congruence of the normalized spectra of phase 2 again reveals that the changes were caused by the formation of a single species. Consequently the determined band positions of Species 3 can be assigned to the second UO_2^{2+}-acetate complex $[UO_2(AcO)_2]$ (1:2-complex).

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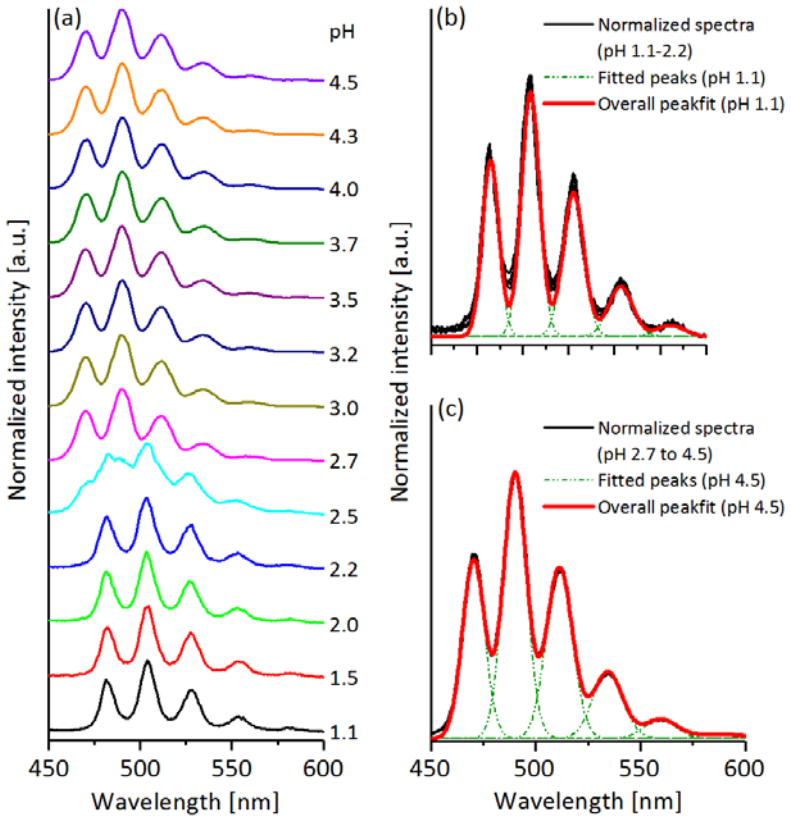

Figure 3-7: Normalized luminescence spectra of test series 2 (50 µM UO_2^{2+} and 0.1 M Acetate) at -120°C (a) and the related normalized spectra with fitted peaks of phase 1 and 2 (b and c).

Hence, former studies, focusing on the determination of the number of formed complexes, were critically reviewed, showing indeed uncertainties with respect to the third complex. This in combination with the experimental findings resulted in a new approach to interpret the uranyl-acetate system, providing single component absorption and luminescence spectra for both uranyl-acetate complexes.

U(VI) – ISA system (Brinkmann et al. 2019)

Different spectroscopic techniques (NMR, ATR-FTIR, UV-Vis, ESI-MS, EXAFS) in combination with DFT calculations led to a process understanding on a molecular level in the U(VI)-ISA system. Apart from the carboxylic group, the involvement of the α- and β-hydroxy groups in complex formation was identified. This indicates the formation of 5- and 6-membered rings as dominant binding motifs, which coexist in solution. Furthermore, two different concentration-dependent complex formation mechanisms were identified, involving either mono- ($[UO_2(ISA)(H_2O)_3]^+$) or binuclear ($[(UO_2)_2(ISA)(H_2O)_6]^{3+}$) species. In this context, the interaction of UO_2^{2+} with the protonated form of ISA was demonstrated, promoting the lactone formation. This qualitative description of the UO_2^{2+}-α-isosaccharinic acid system substantially contributes to a better understanding concerning the structural behavior of ISA during complex formation and provides valuable information for the determination of reliable thermodynamic data.

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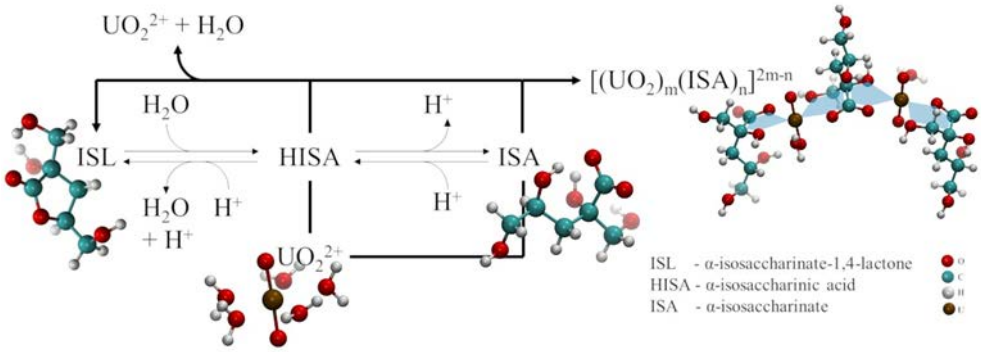

Figure 3-8: Scheme describing the mutual influence of the interaction of ISA with UO_2^{2+} on their speciation, based on spectroscopic techniques (Brinkmann et al. 2019).

UGR

Studies examining Se and Eu interaction with microbial cells were performed with the bacterium *Stenotrophomonas bentonitica* isolated from Spanish bentonites. STEM revealed the presence of insoluble Eu accumulates surrounding the cell surface, with extracellular and intracellular accumulates also observed (Figure 3-9). Time-resolved Laser-induced fluorescence microscopy (TRLFS) studies suggest that phosphoryl and carboxyl groups on bacterial cell envelopes have an important role in the Eu(III) complexation. *S. bentonitica* is able to tolerate mobile Se(IV) by reducing it to immobile Se(0) under aerobic, anaerobic and alkaline (up to pH 10) conditions as revealed by microscopic, spectroscopic and microbiological techniques. Thus, we have demonstrated by means of different microscopic techniques that this strain is able to reduce Se(IV) to Se(0) forming initially amorphous Se(0) (a-Se) nanospheres, which subsequently transform to one-dimensional (1D) trigonal selenium (t-Se) nanostructures with diverse crystallinity, morphology and size. Due to the low solubility of t-Se nanostructures comparing to that of a-Se nanopsheres and Se(IV), the mobility of selenium in the environment may be significantly reduced, which in turn will help to support the implementation of planned repositories (Ruiz-Fresneda *et al*. 2018). These Se nanostructures of different morphology (hexagonal, nanotubes, etc.) are associated with sulphur, indicating S containing organic compounds are involved in the reduction of this oxyanion.

This bacterial strain is able also to reduce Se(VI) to Se(0) forming intracellular monoclinic and trigonal Se nanotubes with low solubility as in the case of the Se(IV) reduction products mentioned below. In addition, the cells are able to tolerate up to 200 mM of selenate.

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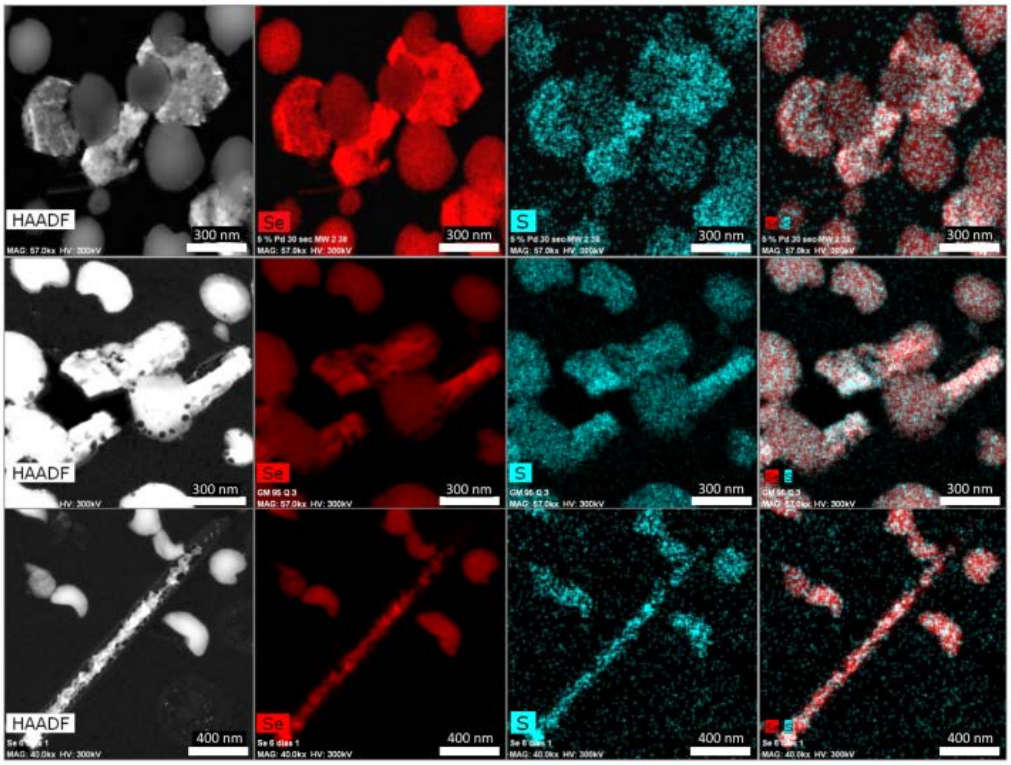

Figure 3-9: HAADF-STEM micrographs of a thin section showing the different morphologies (spherical, hexagonal, polygonal, and nanowires) of SeNPs produced by S. bentonitica after 144 h of incubation. EDX element-distribution maps confirmed that they are mainly composed of Se and S. (Ruiz-Fresneda et al. 2018)

3.5.3 Conclusions

HZDR

For the first three years of the MIND project, our main conclusions drawn from the experimental studies are summarized in our Deliverable D1.4 (A Report of Uranium(VI) Speciation Associated with Cellulose Degradation Products).

U(VI)-acetate system in the acidic pH range: Taken everything into account two main conclusions can be drawn from the new experiments in the uranium-acetate system. The UO_2^{2+}-acetate system can be consistently described with the formation of two complexes, underpinning the hypothesis that the third complex cannot be determined spectroscopically or even does not exist under given conditions. The new approach of interpreting the UO_2^{2+}-acetate system as well as the new spectroscopic reference data might improve the general understanding of the interaction of UO_2^{2+} with organic ligands. Finally, this study clearly shows how important it is to critically review studies before relating it to own results. It is in this context essential to designate uncertainties or concerns, which is also a substantial contribution to make progress in good science.

The U(VI)-ISA study substantially contributes to a better understanding concerning the structural behavior of isosaccharinic acid during complex formation with UO_2^{2+}(Brinkmann et al. 2019). On the one hand, differences are revealed with respect to the coordination of spherical metal centers and the sterically demanding UO_2^{2+} entity. On the other hand, the ability of isosaccharinic acid to form polynuclear complexes is elucidated. This is a valuable finding with respect to the interpretation of

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experimental data from solubility and sorption experiments, where a crucial step is the determination of the complex stoichiometry. Reasonable non-integer values for n, the number of coordinated ligand molecules, should therefore be considered. The identification of two different complex formation mechanisms is particularly important. In excess of U(VI) to ISA (2:1) the formation of the 2:1 complex could be confirmed. If equimolar cocncentrations were used finaly polynuclear chains were detected. In excess of ISA a binuclear complex (2:3) which is the shortest version of a polynuclear chain was formed. At a high ISA excess 1:2 complexes dominated. In this context, the knowledge concerning the different species is one benefit, which supports the interpretation of experimental data. Furthermore, the understanding of the processes on a molecular level, revealing a concentration dependency of the mechanisms, allows the explicit setting of experimental conditions to determine reliable thermodynamic constants for all occurring reactions. Additionally, this information is also important with respect to the migration of RN away from a nuclear waste repository. High local concentrations of ISA might prevail at the places, where cellulosic materials will be degraded. But there will be a gradient to much lower ISA concentrations, going to other compartments or even to the far field of the repository. Therefore, different concentration scenarios and the corresponding reactions should be considered.

UGR

Novel mechanisms of radionuclide (Se) interactions with bacteria isolated from bentonites, forming water insoluble complexes associated with the cells may have the effect of limiting radionuclide mobility in groundwater. The Se(0) precipitates display a variety of morphologies that evolve with time and may be expected to have a range of solubilities as the particles recrystallize to more stable forms according to Ostwald's step rule. However, such beneficial effects are currently neglected in performance assessments, but perhaps could/should be evaluated.

3.6 *In situ* processes: hydrogen consumption

Contributing partners: EPFL

3.6.1 Problem statement & current knowledge

The accumulation of gases in the repository is a potential source of concern for waste management organizations. If the concentration of the produced gases would exceed the solubility limit of the gases in the water, a separate gas phase could be formed, putatively causing fissuring of the host rock, hence resulting in the formation of preferential pathways for radionuclide migration and release**.** In particular, H_2 accumulation is expected in both HLW as well as in L/ILW, due to the anoxic chemical corrosion of steel canisters. In L/ILW, the radiolytic degradation of organic compounds may also lead to an increased H_2 production.

Microbial processes that consume H_2 could be highly beneficial to the safety case because it would result in decreased gas pressures. Microbial hydrogen consumption in Opalinus Clay repository conditions has been evidenced in previous work (Bagnoud *et al*., 2016a). So far, it has been clearly shown that in Opalinus Clay porewater, H_2 oxidation coupled to sulphate reduction is a readily achievable process due to the presence of autotrophic sulphate-reducing bacteria. However, the results were obtained somewhat artificially by maintaining the concentration of sulphate in the porewater. Hence, the remaining question is whether diffusion limitations may result in the depletion of sulphate from porewater. If that occurs, methanogenesis (utilization of CO_2 as an electron acceptor instead of sulphate) may be a viable microbial metabolism and would also consume H_2 but with a concomitant production of CH_4. Nevertheless, this would still result in a net

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decrease of the gas volume according to the following reaction stoichiometry: $4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$. Thus, an experiment aiming at evidencing the potential for methanogenesis *in situ* in Opalinus clay porewater was devised.

3.6.2 Summary of experimental & computational results

The investigation centred on the repeated amendment of an *in situ* borehole in Opalinus clay with H_2 and the monitoring of this system with a diversity of geochemical and microbial ecology tools. In addition, the experiment entailed the repeated replacement of the borehole water with artificial Opalinus clay porewater devoid of sulphate. This was performed to accelerate the transition to methanogenic conditions. For over ~350 days of weekly amendment with H_2, the microbial community was monitored and was found to be dominated by sulphate-reducing bacteria. Geochemical data such as $\delta^{34}S$-sulphate and $\delta^{13}C$-methane confirmed the biological reduction of sulphate and the lack of methanogenesis. The sulphate concentration decreased to ~ 4 mM but no transition to the next metabolic regime was observed. Subsequently, thermodynamic calculations revealed that the bicarbonate concentration in the system was likely too low to support methanogenesis.

In parallel to *in situ* experiments, microcosm experiments with artificial Opalinus Clay porewater depleted in sulphate and Opalinus Clay were set-up and monitored over time after amendment with H_2. Experiments also contained borehole water from Mont Terri URL as inoculum. In the microcosm experiments, no methanogenesis was observed, but there was evidence of sulphate reduction by sulphate-reducing bacteria and the release of iron(II) from Opalinus Clay. The latter process is thought to be abiotic and due to the dissolution of an iron(II) containing mineral as it was found in both inoculated and non-inoculated microcosms. X-ray absorption spectroscopy (XAS) analysis of the clay revealed the presence of FeS in the inoculated samples but its absence in the non-inoculated control. Pyrite is the dominant species in the control, reflecting the known presence of this mineral in Opalinus Clay. This result suggests that sulphide produced by the sulphate-reducing bacteria reacted with Fe^{2+} to produce FeS within the Opalinus Clay. Extended X-ray absorption fine structure (EXAFS) analysis suggests that iron is mainly found in pyrite, siderite and within clay minerals including illite. PHREEQC modelling has been used to identify possible mineral dissolution within the experimental system (siderite is hypothesized to be the iron(II) source). There was no evidence from this study that sulphate-reducing bacteria were supplied from the solid clay phase.

Methanogens have been found dominating the microbial community during an *in situ* experiment with a porewater saturated porous medium (80% sand: 20% bentonite, w/w) and H_2 addition. Within nine months, the biofilm was dominated by methanogenic archaea including *Methanosarcina* and *Methanoculleus*. Further *in situ* tests are being complemented by laboratory experiments with sulphate-depleted porewater from Mont Terri URL. The laboratory experiments will focus on the importance of trace metals and nutrient availability to the onset and dominance of methanogenic archaea.

Deliverable D1.7 provides a more detailed description and discussion of attempts to develop methanogenesis under *in situ* conditions in the Opalinus Clay through the addition of H_2.

3.6.3 Conclusions

Here, we have investigated the potential for methanogenesis as hydrogen consuming microbial process in Opalinus Clay porewater, results so far have successfully evidenced this type of metabolism *in-situ* in the presence of sand:bentonite porous medium. Batch microcosm experiments

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are ongoing to determine the geochemical conditions necessary for this metabolism to occur, hence, further conditions will be tested including the addition of trace metals to the incubation experiments, as well as further *in-situ* tests at Mont Terri URL.

3.7 *In situ* processes: methane generation from organic waste

Contributing partners: NNL, VTT & TVO

3.7.1 Problem statement & current knowledge

As noted above in the Section 3.6, gas generation and consumption processes are important concerns for ILW repositories regarding physical processes resulting from gas overpressure. Besides hydrogen production, also methane production is of major concern. Methane generation can currently solely be explained by microbial degradation of organic (e.g. cellulose) wastes (there is no low temperature chemical process or radiolytic process identified) and can lead to a gas volume/pressure increase. Methanogenesis is of additional concern due to the potential transport and release of ^{14}C in the form of $^{14}CH_4$.

The TVO gas generation experiment (GGE, see Figure 3-10) has been in operation for nearly 20 years studying methanogenic gas generation from cellulose and steel containing LLW from the operation of the Olkiluoto power plants. Previous work (Small *et al*, 2008) has described the GGE and presented data and modelling of the first 9 years of its operation. Key finding from this previous work have highlighted the effect of heterogeneity in the LLW is establishing niches for microbial activity to flourish.

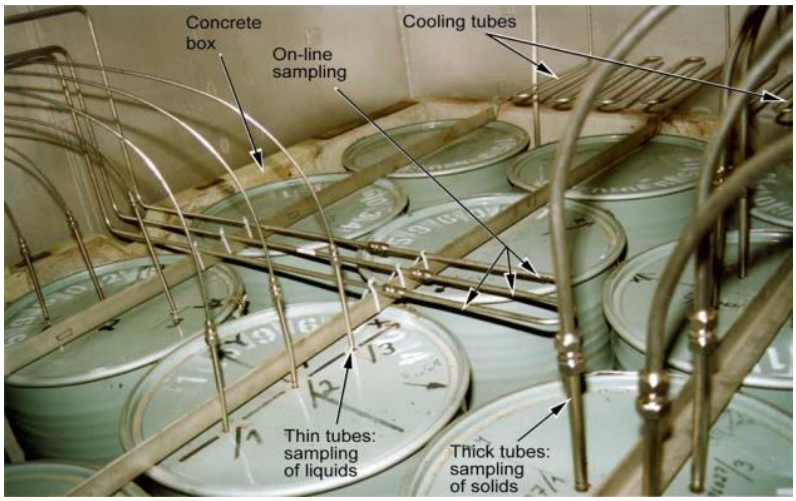

Figure 3-10: View inside the Gas Generation Experiment before filling with water, showing waste drums containing LLW, sampling tubes within waste drums and in the tank water and the concrete box as used to emplace waste drums within the VLJ repository (Photograph courtesy of TVO).

3.7.2 Summary of experimental & computational results

The MIND project has enabled further interpretation and modelling of an 18 year dataset of chemical and gas measurements and further microbiological studies have been undertaken of stored and new water samples. The data interpretation and modelling work has been published (Small *et al*., 2017) and discusses how the alkaline cellulose degradation processes occurring in waste drums has neutralised the initial alkaline pH conditions (pH 10-11) of tank water buffered by concrete. Other

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effects evident in the 18 year chemical dataset include an initial process of microbial sulphate reduction occurring over the first 2 years, which resulted in peak concentrations of sulphide of ~ 0.2 mM, which have slowly declined to micromole concentrations that are saturated with respect to the Fe sulphide phase mackinawite. Bicarbonate concentration has increased progressively up to a concentration of 14 mM, while dissolved organic carbon initially rose to around 8 mM but since 2006 declined. This latter effect is coincident with an increase in the rate of methane gas generation to around 1 m^3 per year from the whole experiment. This is consistent with methanogenesis occurring throughout the GGE and in tank water once the pH had declined to below pH 9. It is also possible that methanogenesis was slowed, but not completely inhibited by sulphide present initially and the increased rate of methanogenesis is a consequence of the decline in aqueous sulphide concentration to very low values ($< 3 \times 10^{-6}$ M). The interpretation of the chemical data also indicates that over the 18 years, carbonate equilibrium redox processes appear to attain a steady state. Methane gas generation is expected to continue until the cellulose and metal inventory is exhausted.

A model of the GGE has been developed (Small *et al*, 2017) using the NNL Generalised Repository Model (GRM) that represents the kinetic microbial processes along with corrosion, equilibrium chemical reaction and diffusive transport. The GRM model has been updated from that first developed (Small *et al*, 2008) and can represent the observed pH neutralisation behaviour considering diffusion of alkalis from concrete and the generation of acidity by chemical alkaline cellulose degradation processes. A good fit to the gas generation rate data has also been obtained.

Microbiological activity and diversity in the GGE has been studied concentrating especially microbial groups involved in methane generation and corrosion. Samples have been taken from various compartments of the GGE as a function of time, and analysed microscopically and by quantitative PCR and 16S rRNA amplicon sequencing. In addition, mass spectrometry and capillary electrophoresis have been used to measure microbial metabolites. Heterogenic chemical conditions in the beginning of the GGE had a big influence on the microbial activity and resulted in the initiation of gas generation already after one year of operation. Microbial activity has reduced the pH by producing microbial metabolites during operation of the GGE. The microbiological results demonstrated that cellulose- and hemicellulose-based LLW is converted to methane and carbon dioxide as a successive action of complex microbial consortia. Microbial groups with the potential to hydrolyse cellulose and hemicellulose, metabolize formed saccharides to acetate, hydrogen and volatile fatty acids were detected in GGE. According to the quantitative qPCR, hydrogenotrophic methanogens dominated in the GGE after one year of operation, which is related to the utilisation of hydrogen generated by the anaerobic corrosion of steel. Acetoclastic methanogens were detected for the first time in 2005, which coincides with an increase in the gas generation rate. Several factors influencing the gas generation were identified including the occurrence of competing microbial groups, environmental conditions (especially pH) and high concentrations of inhibitive substances like volatile fatty acids and sulphide. Methanogens compete with other microbial groups for electron acceptors and especially sulphate reducing bacteria (SRB) can influence gas generation. The relative ratio of SRBs compared to methanogens was shown to decrease considerably during the operation of the GGE. The activity of SRBs is linked to sulphate, which can be leached from the waste materials or enter the repository with groundwater. Experiments with labelled sulphate and water samples from the GGE showed that sulphate reduction could initiate quite rapidly, if sulphate-containing ground water flows to the repository. This could reduce gas generation at least in short term. Microbiological findings were consistent with the NNL Generalised Repository Model (GRM). These microbiological studies are reported in Deliverable D1.6 and in a manuscript submitted to Applied Geochemistry (Vikman *et al*, 2019).

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3.7.3 Conclusions

The GGE continues to provide a valuable insight into gas generation processes from organic containing LLW. The experiment shows how heterogeneities in the initial chemical (pH) conditions have permitted the development of microbial processes that led to overall increased gas generation rate. The results have relevance to other LLW/ILW repositories, with increased proportions of concrete materials, but the processes make take place over longer timescales. The model developed may have use to such extrapolation.

3.8 Modelling of microbial processes

Contributing partners: NNL

3.8.1 Problem statement & current knowledge

Models are required to assist in the interpretation of experiments studying microbial processes and coupled to chemical and transport processes. Such models may also be utilised in performance assessment studies to examine the significance of specific microbial processes. Modelling of microbial processes relevant to geological disposal is in its infancy, reflecting the previous less detailed consideration of microbial processes prior to the MIND project. Nevertheless kinetic models of microbial processes relevant to geological disposal have previously been included in the PHREEQC geochemical model (Parkhurst and Appelo, 2013) e.g. Tournassat *et al*, 2011; Maia *et al*, 2016. In the case of LLW disposal in the UK, a biogeochemical model (The Generalised Repository Model, GRM) has been developed to model the redox evolution and microbial gas generation processes (Small *et al*, 2010) and which has been validated using long term experimental data from the Finnish gas generation experiment (GGE; Small *et al*, 2008). Other performance assessment models include the T2GGM devloped to examine microbial gas generation in the Canadian deep geological repository (Suckling *et al*, 2015). Recently, NAGRA have developed models to assess the sources and sinks of gas generation (Leupin *et al*, 2016).

Within this task of the MIND project, PHREEQC and GRM are being used to assist in the interpretation of microbial experiments and to form the basis of new modelling tools and approaches for end users.

3.8.2 Summary of experimental & computational results

PHREEQC models of processes in borehole experiments at the Mont Terri underground rock laboratory are being developed and link to studies within Task 1.3 (studies under *in situ* conditions). The Bitumen-Nitrate-Clay (BN) experiment (Bleyen *et al*, 2017b) examines the interaction of nitrate with Opalinus Clay. The BN experiment has examined denitrification driven by electron donors present in the clay together with added acetate or hydrogen, representing hydrolysis and radiolysis products of bituminised waste. A PHREEQC model of a pulsed hydrogen equilibration experiment (see Bleyen *et al*, 2017b for experiment details) has been developed and is described in MIND deliverable D1.8. The PHREEQC approach to represent radial diffusion (see Tournassat *et al*, 2011) has been adopted and the model is discretised into a series of concentric cells to represent the borehole, a filter screen and void space as well as the Opalinus Clay (Figure 3-11). In the experiment, nitrate was first injected into the borehole attaining a concentration of 15 mM after initial circulation. The circulating fluid passes through the borehole, passed a UV spectrophotometer and through a gas equilibration unit, which initially contained argon. The nitrate concentration was then monitored for a period of 54 days, where the concentration declined mainly due to diffusion into the

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Opalinus Clay. After 54 days, the gas phase was changed to hydrogen and then after a further 24 days the gas was replaced by argon, giving a pulsed equilibration of the nitrate containing borehole fluid with hydrogen.

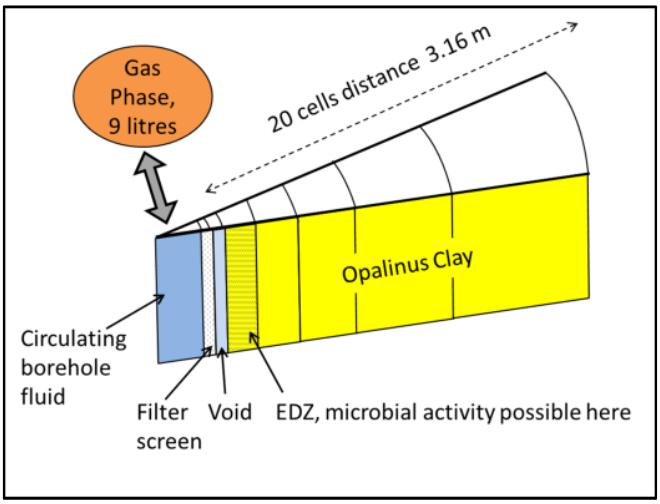

Figure 3-11: Schematic of the PHREEQC model of the BN experiment

The denitrification process was represented as a two stage reaction forming nitrite (NO_2^-) as an intermediate in nitrate (NO_3^-) reduction by hydrogen (H_2):

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

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

In the PHREEQC model these reactions are implemented as being kinetic processes defined by a Monod type expression that also simulates the growth of microbial biomass. The initial mainly diffusive phase was modelled considering diffusion coefficients determined from bromide tracer tests of the BN borehole and accounting for a small amount of denitrification resulting from reaction with the Opalinus Clay estimated from previous GRM modelling (Bleyen *et al*, 2017b). The nitrate and nitrite concentration during the H_2 pulse were fitted by varying the Monod kinetic parameters for Equation 1 and 2. A good fit to the nitrate concentration data was obtained and the formation of nitrite was also quite well represented (Figure 3-12).

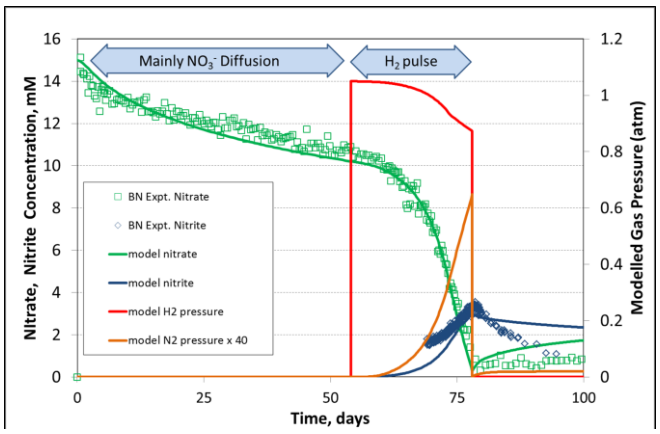

Figure 3-12: Measured and modelled nitrate and nitrite concentration and modelled gas pressures during the pulsed hydrogen injection of the BN experiment.

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PHREEQC modelling of sulphate reduction process in the MA experiment in Opalinus clay (see above Section 3.6) has also been undertaken, with initial results presented in MIND deliverable D1.8. The model is based on the PHREEQC model of sulphate reduction by hydrogen developed by Maia *et al*, (2016), with coupling to a radial diffusion model, as in the model of the BN experiment, to represent the supply of sulphate from the Opalinus clay. The model has been parameterised to fit the observations from the initial data collected from the MA experiment (Bagnoud *et al*, 2016a). A two stage model has been developed to represent an initial autotrophic process, stimulated by H_2 injection whereby organic carbon is fixed for a second heterotrophic sulphate reduction process. Further work is required to represent the changing conditions of the experiment (H_2 injection phases, etc.) within the model. Further refinement of initial autotrophic phase to include sulphate reduction is also requited. Of particular interest is the potential effect of sulphide arising from sulphate reduction acting as an inhibitor for certain microbial populations. This can be accounted for in the model by a rate inhibition factor and by considering the precipitation of low-solubility iron sulphide phases, such as mackinawite (FeS).

Within Task 1.3 (Section 3.7, Small et al, 2017), The GRM code has been used to model the suite of cellulose degradation processes leading to methanogenesis in the GGE. Building on previous modelling (Small et al, 2008), the GRM code has been further developed to consider diffusive transport processes that control the mixing of the initially heterogeneous chemical conditions present at the start of the experiment and the release of alkalis from the concrete present. The modelling showed that the rate of cellulose hydrolysis (representing an enzymatic type process, rather than alkaline hydrolysis) influences both the rate of gas generation (Figure 3-13) and the pH neutralisation (Figure 3-14). In the latter the rate of cellulose hydrolysis is the rate limiting step in the formation of organic acids and carbon dioxide, which neutralise the initial alkaline tank water. By varying the cellulose hydrolysis rate a fit has been obtained to the rate of gas generation (Figure 3-13) and the pH data (Figure 3-14). The model simulates that the increase in methane gas generation rate observed after 2004 is a result of neutralisation of the initially alkaline tank water, which permits the utilisation of dissolved organic carbon in the tank water to form methane, as indicated by the growth of organic consuming methanogens in the water region of the model (Figure 3-15, Small et al, 2017). The development of the GRM model of the gas generation experiment is further discussed in MIND deliverable D1.8.

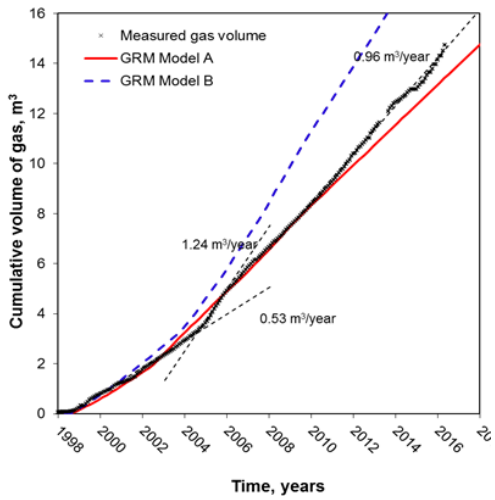

Figure 3-13: Rate of gas generation measured by the TVO Gas Generation Experiment and modelled rate of gas generation (Small, et al 2017).

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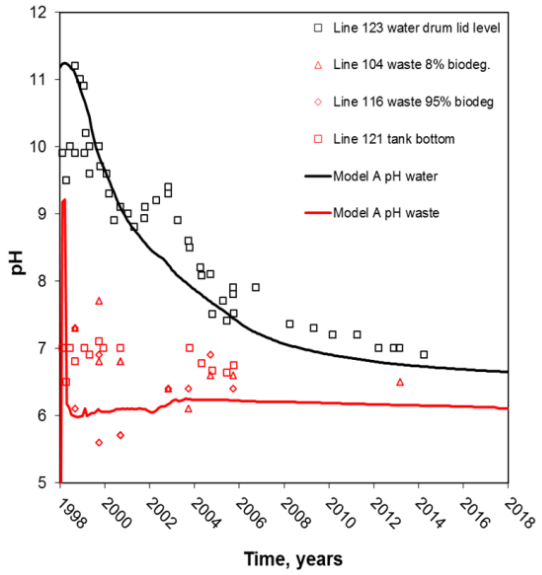

Figure 3-14: pH measurements from the waste drums and tank water regions of the TVO Gas Generation Experiment and modelled pH of the water and waste regions of the mode (Small, et al 2017).

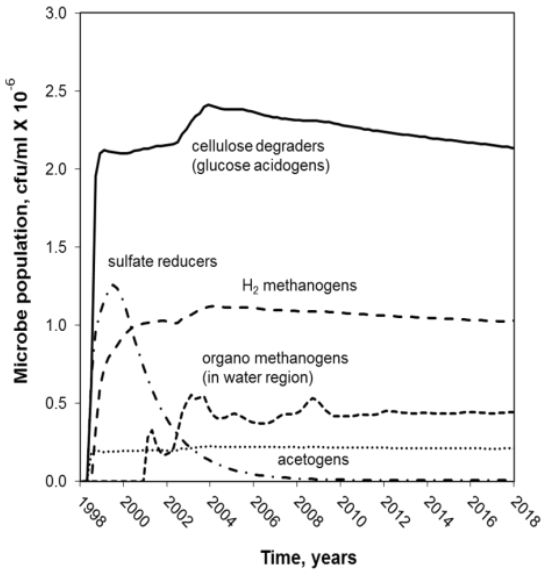

Figure 3-15: Modelled biomass concentration of the main anaerobic microbial processes simulated for the TVO Gas Generation Experiment (Small, et al 2017).

MIND deliverable D1.8 provides a description of the representation of Monod microbial growth, including the growth of microbial biomass by hetertrotrophic and autotrophic processes. The main reactions required to be considered in modelling the degradation of cellulose containing LLW and ILW, including denitrification, iron (III) reduction, sulphate reduction, fermentation and methanogenesis are described. An approach to couple these microbial kinetic processes with chemical speciation is described, with examples implemented with PHREEQC (see above). The report discusses how such models can be used to support the safety case for the disposal of LLW/ILW such as in quantifying rates of gas generation and in underpinning the understanding of near field

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biogeochemical processes. The modelling approaches described are also applicable to study processes of sulphate reduction of particular relevance to issues of microbially induced corrosion, considered by MIND WP2.

3.8.3 Conclusions

Biogeochemical modelling has been used to assist in the interpretation of data from *in situ* experiments, including an 18 year study of microbial gas generation from cellulose- containing LLW. Models have been developed with the PHREEQC code to represent anaerobic microbial processes stimulated by H_2 injection in borehole experiments. The modelling methodology has been documented in detail in D1.8, and has potential for wider development by end users to represent the effects of microbial processes laboratory and *in situ* experiments and in wider nuclear waste disposal scenarios.

3.9 WP1 – summary and conclusions

The overall objective of MIND WP1 was to reduce the uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from long-lived ILW containing organics. The outcome of MIND WP 1 research was presented in 9 deliverable reports. In addition 11 peer reviewed open access journal papers have been published to date. Summaries of the WP research are presented above organised on a task basis. Mind deliverable D1.9 (Small, 2019) provides a synthesis of the WP1 research summarising results and discussing the following key topics and processes:

1. Organic polymer degradation
2. Gas generation / consumption
3. Radionuclide interactions
4. Limits of microbial life in an ILW repository

Organic polymer degradation

The MIND project has examined the combined effects of irradiation and biodegradation of; cellulose, polyvinylchloride (PVC), bitumen and polystyrene based ion exchange resins under alkaline conditions. In this first study of this type the organic materials were mainly irradiated with maximal doses of gamma radiation (1 M Gy, ^{60}Co), in most cases in pH 12.5 $Ca(OH)_2$ solution. The biodegradation of irradiated and hydrolysed materials was then examined. In the case of cellulose, dissolved organic carbon (DOC) and isosaccharinic acid (ISA) generation was enhanced by irradiation but this stimulated higher levels of microbial activity (fermentation), which led to a decrease in pH, to 11, which would be expected to reduce the rate of formation of ISA by alkaline hydrolysis. The MIND project has thus provided further evidence of the biodegradation of ISA under strongly alkaline conditions.

Pure PVC polymer was found to be not biodegradable under anaerobic alkaline (pH 10) conditions, but some additives (plasticisers and fire retardants) present in PVC sheet were biodegradable and capable of nitrate reduction at pH 10. Irradiation enables minor biodegradation of the polymer, but the biodegradation of additives is reduced slightly. Pure phthalate additives, which have potential to form complexes with radionuclides, were not found to be biodegradable under the pH 10 conditions. The rather limited overall biodegradation of such PVC materials suggests that these organic materials should not provide a significant source of microbial (CH_4) gas generation.

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Study of nitrate containing bituminised wastes examined the ability of known organic bitumen degradation products to consume nitrate leaching from aged bitumen samples under Boom Clay and alkaline, cement buffered pH conditions. Acetate was found to be the most effective electron donor followed by formate and oxalate. Microbes were also observed to grow on the surface of the bitumen, possibly using it as a nutrient and energy source. In the case of a microbial inoculum adpated to alkaline conditions nitrate reduction was found to be most efficient at pH 10.5.

Studies of ion exchange resin degradation indicate that microorganisms are capable of utilising ion exchange resin radiolysis products (amines, benzene, ketones), especially by coupling their oxidation to reduction of sulfate, which itself will be readily sourced from cationic resins. Ion exchange resins thus have the potential to undergo biodegradation without addition of electron acceptors. Breakdown of the resin by radiolysis is an important step in the overall degradation process liberating soluble organic carbon. At high doses (1 M Gy) the resins develop strong acidity, which may prevent subsequent biodegradation unless the waste resins are conditioned or radiolysis products are diluted or dispersed. At lower doses (100 kGy) amines released from anionic resins have been shown to be able to fuel sulfate reduction processes. Oxidation of the amine functional groups by such biodegradation may eliminate any radionuclide complexation effects of released amines. Hydrogen and CO_2 are the main gaseous radiolysis products detected, but CH_4 gas formation has not been observed in the current microcosm experiments undertaken due to the presence of sulfate.

Gas generation / consumption

Microbiological and modelling studies of a long term (18 y) Gas Generation Experiment (GGE) has provided further understanding of processes of CH_4 generation from cellulose and steel containing low level waste (LLW). The GGE illustrates how microbial processes can neutralise pH initially buffered by concrete by fermentation of organic waste. The GGE thus provides an insight into how LLW/ILW repositories with higher proportions of concrete may be neutralised over longer periods of time. A doubling of gas generation rate occuring after 8 years is attributed to neutralisation and homogenisation of pH and the growth of organic consuming methanogens in initial alkaline regions of the experiment. Initially, CH_4 generation mainly occured in neutral pH waste drums by methanogens utilising H_2 generated by anaerobic corrosion. Sulfate reduction occurs in parrallel to methanogenesis, but at a low level limited by its slow release from solid wastes. High concentrations of sulfide at the initial stages of the experiment may also have inhibited the initial rates of gas generation in the GGE. Higher rates of sulfate input, such as through groundwater would be expected to slow the rate of methanogenesis by competition for electron donors.

In the case of *in situ* H_2 injection in borehole experiments at the Mont Terri URL, reaction of H_2 with nitrate and sulfate electron acceptors has shown to be rapid. In the case of sulfate reduction it has been impossible to fully exhaust the supply of sulfate provided by diffusion from the Opalinus Clay. It is apparent, from microcosm experiments and also from observations from the GGE that sulfate concentration must be lowered to very low levels (<1 µM) before methanogenesis can be established. The configuration of a sand:bentonite column experiment allows a much greater proportion of H_2 to react with sulfate for the growth of methanogens to be observed. Reactive-transport modelling approaches developed through the MIND project have been used to interpret both the GGE and H_2 injection experiments.

Radionuclide interactions

Studies of radionuclide-microbe interactions were not a major component of the MIND project, nevertheless a number of scientific observations have been made that are relevant to the discussion

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of microbiology and nuclear was disposal. For example, interpretation of geochemical data from the GGE is consistent with attainment of redox equilibrium of iron and sulfur species during degradation of steel and cellulose containing LLW after a period of 18 years. This observation supports the assumption made in performance assessment studies that redox processes, including multivalent radionuclide speciation (e.g. Se, Tc, U, Np, Pu), attain equilibrium over the timescales of nuclear waste disposal. In the case of the GGE microbial processes have mediated the relatively rapid attainment of redox equilibrium.

Microbes have an important role in the biodegradation of organic complexants, which can affect radionuclide solubility and sorption. In particular, this is highlighted by further study within MIND of the formation and biodegradation of the cellulose degradation product ISA. In addition, MIND research has relevance to the consideration of potential radionuclide complexants released from PVC, bitumen and ion exchange resins.

Other MIND studies have examined the speciation and reaction of radioelements and their chemical analogues. Spectroscopic techniques have been developed to examine the complexation of uranium with cellulose degradation products. Studies of the fate of Ni and U complexed with the cellulose degradation product ISA during biodegradation reveal how such contaminants may be sequestrated in secondary sulfide and other mineral phases that are formed by biodegradation of ISA and other organics released from ILW. Studies of direct interactions of radionuclides with bacteria isolated from bentonite illustrate mechanisms of bioprecipitation by the bacteria to control toxicity of Se and processes of biosorption were Eu is associated with phosphate groups present on the bacterial cell surface.

Limits of microbial life in an ILW repository

The principal uncertainty concerning microbial processes and the disposal of ILW is the ability of microbes to grow and metabolise under the high pH conditions that prevail in cementitious ILW wastepackages and engineered barriers. Physical limitations, such as the requirement for space to grow and availability of water will also be relevant, but are likely less important than in the case of bentonite backfilled engineered barrier system for HLW considered by MIND WP2.

The ability of microbes to grow in high pH environments is linked to the amount of chemical energy available for metabolism. Under anaerobic conditions relevant to most disposal scenarios nitrate reduction provides the most energy. MIND project studies indicate that pH in the range 10-11 represents a realistic upper limit for microbial nitrate reduction process, including the consumption of ISA and gluconate and organics present in plasticised PVC and bitumen. Faster rates of nitrate reduction were observed for an inoculum collected from a former lime kiln site that that has adapted to higher pH conditions than from a Boom Clay inoculum. The high pH adapted inoculum also showed some evidence of survival of pH 12.5 conditions.

Methanogenesis is the lowest energy yielding process of relevance to ILW disposal and is expected to have the lowest pH tolerance. Studies of the GGE imply that pH 9 represents a likely upper limit for active methanogenesis, although other toxicity effects and competition with sulfate reduction and fermentation processes may further reduce this threshold to pH 8. It is also evident from the GGE studies and other smaller scale high pH microcosm experiments that heterogeneity of pH and the occurrence of low pH micro-niches, where microbes may thrive, are important to defining such pH thresholds.

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The MIND project studies also indicate that a number of other chemotoxic effects may result from the contaminants presented in ILW (e.g. Se, Cs) and that microbial processes may generate toxic species such as H_2S, which may exert a self-limiting effect on the rate of microbial activity.

Outlook

Understanding of CH_4 generation and H_2 consumption processes has benefitted from use of *in situ* experiments within the MIND project. Further study of the competition between sulfate reduction and methanogenesis is critical to developing predictive models of these processes. MIND has also revealed new insights into the degradation processes of organic wastes present in ILW. Further study of these processes under *in situ* conditions is warranted, including processes within wasteforms and using borehole experiments to study host rock interactions. Such studies should develop the microbiological techniques used within MIND, alongside geochemical, mineralogical characterisation; examination of radionuclide complexation, and modelling. pH thresholds for the activity of nitrate reduction and methanogenic processes have been defined through MIND WP1, but there is scope to define limits for sulfate reduction processes, which has wider relevance to MIND WP2. Study of the spatial scale and magnitude of pH heterogeneity in ILW wasteforms and engineered barriers and the occurrence of niches for microbial activity is however required to fully define such pH thresholds.

4 WP2 – Engineered barrier

WP2 lead: MICANS

The objectives of WP2 are to:

- Quantify the contribution of microbially produced sulphide in buffers and in backfill and in the geosphere to the overall rate of sulfidic canister corrosion (Tasks 1 and 2).
- Gain systematic information on the effectiveness of specific bentonite buffers and their properties (density, pH) in inhibiting microbial activity. (Task 3).
- Characterize 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. (Tasks 4 and 5).

In this synthesis report, the results are grouped and reported per objective, rather than per task.

4.1 The availability of sulphide and its effects on canister corrosion

Contributing partners: TUL, VTT, CVR, GTK, EPFL, MICANS

4.1.1 Problem statement & current knowledge

Metallic canisters will be used to ascertain the long-term isolation of spent nuclear fuel and other highly radioactive wastes in geological repositories. Iron (steel) and copper metals are in most cases considered as the canister materials. Complete containment and longevity with respect to corrosion are the performance targets of the metallic waste canisters. Geosphere around the repository forms the outer boundary of the near field, in which the backfill-buffer system has the target to protect the waste containers from corrosion.

In simple terms the microbial generation of sulphide can be represented as Equation 1, and where the sulphide can subsequently react with metallic copper or iron.

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Microbial sulphate reduction	$4\ H_2 + SO_4^{2-} + 2\ H^+ \rightarrow H_2S + 4\ H_20$	Equation 1
Copper corrosion	$2\ Cu + H_2S \rightarrow Cu_2S + H_2$	Equation 2
Iron corrosion	$Fe + H_2S \rightarrow FeS + H_2$	Equation 3

Other organic electron donors could also drive sulphate reduction, including methane and organic contaminants present in bentonite. It is useful to note that hydrogen is cycled through Equation 1, 2 and 3. It should also be noted that Equation 2 and 3 occur on the surface of the canister but Equation 1 could occur at different locations in the EBS and thus transport of sulphide through the EBS could limit the overall reaction. Equation 1 will be subject to requirements for space, water for microbial activity and could occur at the rock/EBS interface (Pedersen, 2010, Smart *et al*., 2017, Huttunen-Saarivirta *et al*., 2017 Smart *et al* 2014).

Both iron and copper have strong affinities to form sulphide compounds in geological environment. Sulphidic corrosion of canisters is an important process to be evaluated in performance and safety assessment. Consequently, a thorough understanding of the entire biogeochemical sulphur system and the electrochemical (redox) and hydrogeological constraints of mobile sulphur system must be assessed in detail to constitute a firm basis for the safety case.

Geochemical redox processes of sulphur are strongly bound to microbial catalysis. Microbial sulphide concentration strongly depends on the geochemical conditions in the ground water at the disposal depth. Dissolved sulphide (as HS^-, aq) can be present in deep anoxic waters, but the concentration typically remains low because of the limited solubility of iron, copper and other metal sulphides in these conditions. These sulphide minerals exist in small amounts as primary minerals in most rock types. In the presence of oxidants (electron acceptors) oxidative dissolution of these minerals is strongly catalysed by iron oxidizing and sulphide oxidizing microbes, and metals dissolve in the acidic sulphate solution thus formed. In changing hydrogeological conditions, sulphate-bearing waters may again enter anoxic conditions facilitating sulphate reduction to sulphide with the aid of sulphate reducing microbes. Relatively high sulphide concentration may develop in this transient sulphate reduction front, at depths and locations devoid of iron and other dissolved metals as preferential electron acceptors.

Microorganisms can affect the rate of copper and steel corrosion in two related ways. First, they could produce water-soluble sulphide species that could diffuse to the surface of the canister and enhance corrosion. Second, they could form a biofilm on the surface of the canister and enhance corrosion via the precipitation of iron sulphide minerals directly on the metal surface and the consumption of H_2 produced by to the anoxic chemical corrosion of steel or copper. Sulphate-reducing bacteria play a key role in steel corrosion processes (Venzlaff *et al*. 2013). This group of microorganisms is able to take away electrons from the steel (Fe^0) and/or the H_2 and reduce sulphates to sulphides (S^{2-}) or HS^-, which can create corrosive agents such as H_2S, FeS *etc*. However, microbial enhanced corrosion of steel canisters is still a hotly debated point within the community. Microbial activity on the metal surface can accelerate the corrosion rate but can also act protectively. Careful design may harness the ability of microorganisms to consume H_2 rapidly, reducing an overpressure that is otherwise expected in the repository, while relegating sulphate reduction to an iron-rich porous medium that would sequester sulphide. For instance, by combining a barrier of bentonite, a swelling clay, around the canister with a higher permeability zone between the host rock and the bentonite, SRB may grow in the higher permeability zone where sulphide would precipitate, while relying on H_2 diffusing from the canister surface across the bentonite. This mechanism can maintain the integrity of the host-rock as well as minimize the impact of sulphide on canister

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corrosion (Bagnoud *et al.,* 2016a). Moreover, it remains unclear to what extent does microbial activity, particularly sulphate-reducing activity, impact the rate of corrosion of steel within the constraints of the repository conditions, including constraints in the bentonite barrier. There is much evidence that microorganisms survive in bentonite (Jalique *et al*., 2016; Stroes-Gascoyne *et al*. 2011) but their impact on corrosion rates is less obvious.

Key processes to be studied are:

- Microbial dissolution of sulphide mineral as a source of aqueous sulphide
- Sulphate sources and fluxes in the bedrock, as a source for sulphate-reducing bacteria
- Electron donors (e.g. hydrogen) facilitating microbial sulphate reduction
- Availability of nutrients (N, P) and limiting factors for microbial growth (e.g. in bentonite buffer)

4.1.2 Summary of experimental & computational results

GTK

GTK has compiled hydrogeochemical data from about 100 deep drill holes (depth range 400 –2500 m) from different localities in Finland, including published data from 26 drill holes of Posiva site selection and Olkiluoto site program. About 240 sulphate analyses are available as a function of depth, but sulphide analyses are available only from a few newest samplings (about 25). As a rule, sulphate concentrations show decreasing trend with depth, in some cases distinct sulphate reduction fronts can be observed. Twenty different gas sampling sites in Finland have been identified based on a database survey and literature review (D2.1). More detailed information on the mode of occurrence of bioavailable sulphur compounds in crystalline rock as a function of depth, lithology and groundwater composition was included in the Interim report on deep gases and sulphur compounds in crystalline rock, which was completed in June 2017 (D2.3). Thermodynamic calculations based on the collected data have begun, and first results were presented in the Goldschmidt conference in Paris, France, in August 2017. Background and conceptualization of the sulphide production and concentration model in the bedrock have been prepared, with a special reference to the biogeochemical processes of the iron-sulphur system. All hydrogeochemical data are compiled to an Excel database, which is continuously updated and available for MIND project partners on request.

Comparison of the sulphide concentrations and the results of geochemical modelling of sulphur-iron system indicates a solubility control by a solid iron sulphide phase, mackinawite. Anomalously high sulphide concentrations sometimes associated with a sulphate reduction front in some deep samples are evidently due to the limited availability of iron. With time, mackinawite will undergo a sequence of irreversible reactions leading to the formation of pyrite, which can only be dissolved by strong oxidants.

Sulphate reduction requires effective electron donors, e.g. dissolved hydrogen or organic molecules. Geochemical modelling indicates that methane is a relatively weak electron donor for sulphate reduction, which is in accordance with their observed co-existence in some water types. Sulphate concentrations of several thousands of ppm's were detected at a depth of 600 m together with methane.

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MICANS

Gases can migrate in pores of rocks. It was, therefore deemed important to study the composition of gases in porewater. Drill cores from two different drill holes were supplied in kind from Posiva Oy. The total quantities of H_2 and CH_4 in these cores were analysed. These gas "quantities" are needed to resolve the key issue on long-term sulphide production rates in the geosphere. While the concentrations of these gases are reasonably well documented in groundwater, the amount in the host rock matrix was, previously, unknown. Methods are described in D2.1 and the testtubes are shown in Figure 4-1. The amount of gases emitted from the cores were calculated as mL gas per L of pore water assuming a porewater volume of 0.5%. There were on average 18 mL/L H_2 and 31 mL/L CH_4 in the porewater of the ONK-KR17 cores and the amount of H_2 and CH_4 increased with increasing depth in OL-KR58. While the concentration of CH_4 was within the range of what is found in deep groundwater, H_2 was much higher. H_2 is rapidly used by SRB, acetogens and methanogens in deep groundwater (Pedersen 2012). Therefore, any H_2 that leaves porewater to groundwater by diffusion has a great chance to be metabolised by bacteria down to concentrations below Km for H_2 (approximately 1 µM).

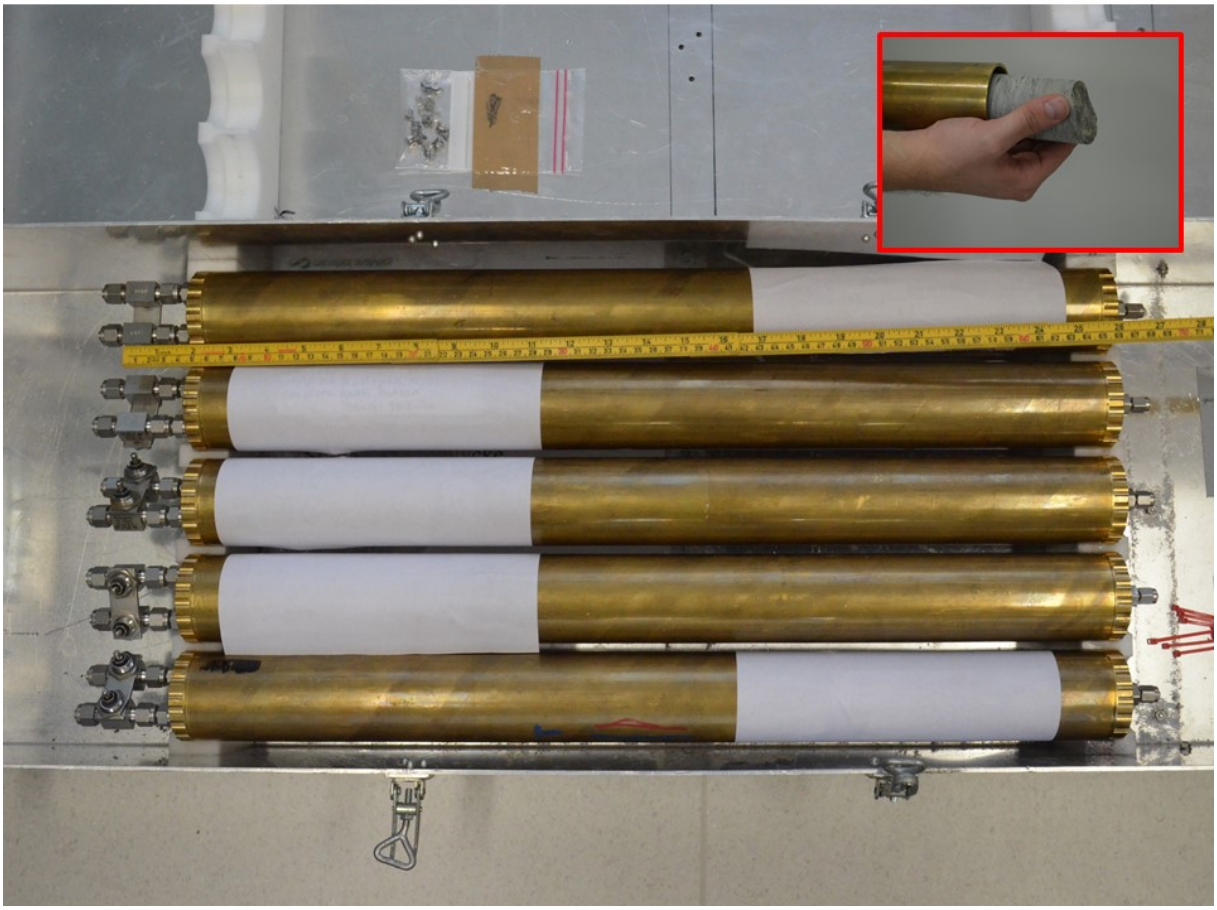

Figure 4-1. Brass tubes used for inoculation and analysis of gases emitted from porewater in drillcores.

TUL/CV Rez

The main scope of our experiments was to study the microbial influenced corrosion of the carbon steel 12020. In total, three laboratory experiments were performed under sterile and non-sterile

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conditions (VITA water from Josef URL) and under laboratory and elevated temperature (20 and 35 °C). All experiments were undertaken in the anaerobic glove boxes (O_2 < 1 ppm).

The first experiment was performed at laboratory temperature and was inoculated with the underground water VITA (obtained from Josef URL) in which SRB are the commonest bacterial group. After 240 days, high increase in SRB in both biofilm and water samples were observed by qPCR and 16S rRNA amplicon sequencing. These findings coincide with corrosion data of the Electrochemical Impedance Spectroscopy (EIS) analysis. EIS described the evolution of three time constants indicating two-layer of biofilm on the surface under non-sterile conditions without the presence of local attack; and scanning electron microscopy confirmed that the carbon steel surface was covered with a two-layer biofilm. Our observations support the SRB ability to enhance the carbon steel corrosion.

The second experiment was set up at temperature of 35°C in order to compare how the microbial consortium reacts on the higher temperature and to study how the increased temperature influences the corrosion of carbon steel. EIS results showed different corrosion behaviour at increased temperature compared to the previous corrosion experiment, because only a single biofilm layer was observed under non-sterile conditions. After 293 days, the biofilm reduced the corrosion rate and it seemed to have a protective character. The observed corrosion rate under sterile conditions was in a good agreement with Arrhenius law in the area where the carbon steel was in the steady passive state when temperature increased from laboratory temperature (~ 20°C) to 35°C. We did not observe this relationship under non-sterile conditions. No corrosion marks in the form of the local attack was observed.

Relative quantification qPCR analysis of both corrosion experiments (laboratory temperature and 35°C) showed that the total relative abundance of bacterial biomass detected by universal 16S rRNA gene primers was more or less similar throughout the experiment**.** The results of the amplicon sequencing showed that biofilm, as well as water samples, were dominated by two SRB: *Desulfomicrobium* and *Desulfovibrio* spp. under laboratory temperature, whereas only *Desulfovibrio* spp. was able to cope with the elevated temperature. Although different corrosion behaviour was observed for ~20°C and for 35°C, this did not result in significantly different corrosion rates.

In the third experiment conducted in room temperature, Czech synthetic bentonite pore water (SBPW) was used in room temperature as the corrosion environment in order to simulate conditions in which the carbon steel container will be surrounded in compacted bentonite. VITA water from Josef URL was used as a microbial inoculum. The experiment was running for 26 months and the corrosion rate was determined by the weight loss method. The corrosion rate in synthetic bentonite pore water under sterile condition after 26 months exposure was 0,4 µm/y and exhibit a tendency to decrease with time during experiment. The detected corrosion rate was much reduced after 18 and 26 months. In the case of non-sterile environment, the highest corrosion rate was 5,4 µm/y after 6 months and after 26 months the corrosion rate was 3,5 µm/y. The biofilm present after 26 months was characterized by the presence of extensive local attacks (Figure 4-2) with macroscopic filamentous heterogeneity.

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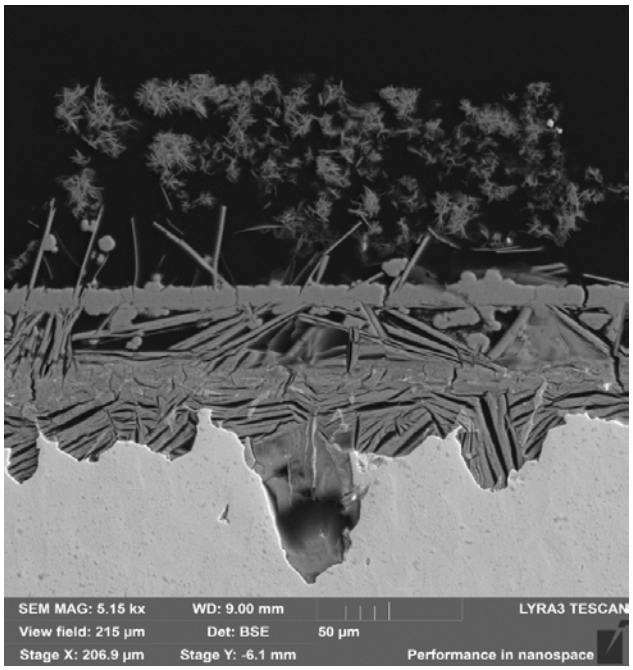

Figure 4-2: SEM analysis - the biofilm on carbon steel in the inoculated synthetic bentonite pore water (SBPW) after 26 months.Relative quantification qPCR analysis revealed that the relative abundance of total bacterial biomass and NRB dramatically increased after 3 months in samples with carbon steel and but the increase in relative abundance of both groups was much lower in samples without carbon steel. However, by the end of experiment the relative abundance of both groups decreased in carbon steel containing samples indication depletion of available nutrients or physiochemical changes in the solution.

Next-generation 16S rDNA amplicon sequencing showed the difference in community composition in both biofilm and water compared to the previous experiments in VITA water. The nitrate reducing microorganisms such as Pseudomonas, Brevundimonas and Methyloversatilis dominated the samples. This shift in microbial community structure can be explained by the chemical composition of SBPW, which is rich in nitrates and offers to nitrate reducing bacteria (NRB) thermodynamically favourable terminal electron acceptor. Furthermore, the Principal coordinates analysis (PCoA) based on detected OTUs showed existence of space dstribution of samples defined mostly by the sampling time (Figure 4-3). VITA water and SBPW sample without presence of carbon steel are clearly different from the carbon containing samples. PCoA analysis confirmed the existence of gradual shift in microbial composition in samples containing carbon steel during the experiment.

Although NRB dominated the samples in SBPW unlike the previous two experiments dominated by SRB, we observed a presence of massive corrosion in the samples. This result shows that corrosion processes can be significatnt also in the environments dominating by NRB.

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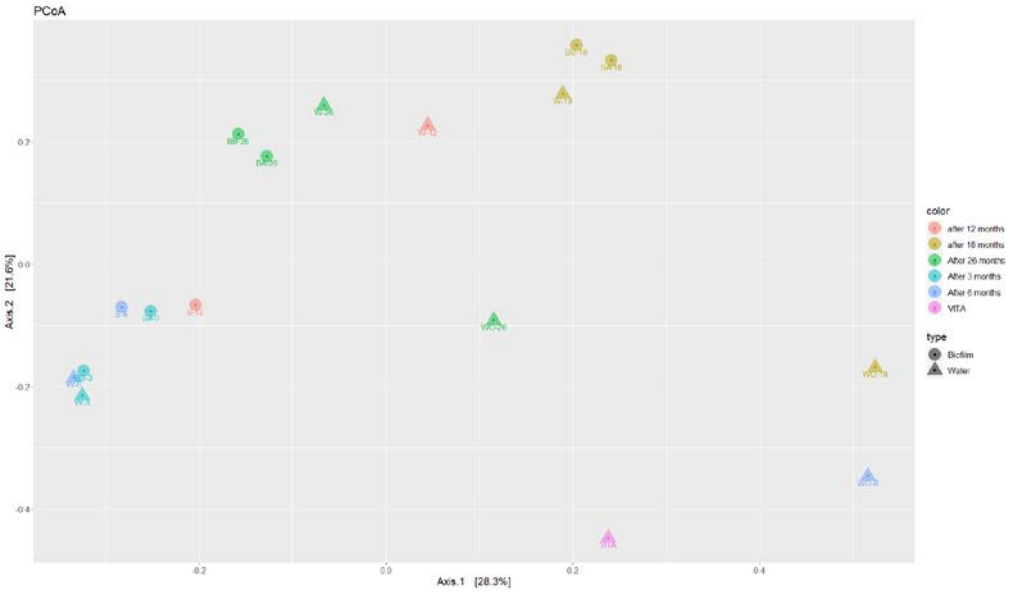

Figure 4-3: PCoA analysis of corrosion experiment 3 with bentonite pore water (SBPW) inoculated by underground VITA water consorcium based on detected OTUs in the samples. Samples of water (W) and biofilm (BA and BB) were taken after 3,6,12,18 and 26 months. WO – SBPW + VITA without carbon steel.

VTT

Deep subsurface microbial communities in steady-state do not usually cause unidentified risks. However, when different groundwater types including sulphate rich waters mix with methane and hydrogen containing waters, metabolic activations of the resident microorganisms take place. Because of such activations, corrosive sulphide accumulation can occur, for example in the final disposal site for nuclear waste in Olkiluoto, Finland (Posiva, 2013). The aim here was to study and simulate the deep groundwater mixing and infiltration, which introduces previously limited electron donors and acceptors for microbial communities and generate activation of microbial communities. Activation of deep subsurface microbial populations detected with fluorescent redox dyes was an efficient screening method to study induced microbial metabolism. Redox dyes used (CTC and RSG) were incorporated into cells by metabolically active microbes and reduced by the electron transport system after which they turned fluorescent. However, the used redox dyes stained a large part of the studied groundwater populations already before activation. For this reason, not all activations induced by electron acceptor and donor amendments were possible to detect. Another tested approach to study microbial community activation, the reverse transcriptase PCR (RT-PCR) was not as successful and either the tested electron donors and acceptors did not activate the groundwater community during the 3 hour activation period or the studied groundwater was contaminated with oxygen during sampling, which inactivated sensitive anaerobic microbes.

The two deep groundwaters studied with redox sensing fluorescent dye CTC were very different from each other. OL-KR6 groundwater from Olkiluoto, Finland, was a sulphate rich groundwater with high bacterial and archaeal diversity and clear activation with several electron donors and acceptors was detected. Relatively rapid sulphate reduction and sulphide formation started in this water after acetate addition, detected with labelled sulphate ($^{35}SO_4$) whereas no sulphate reduction could be detected without acetate addition. OL-KR15 groundwater on the other hand, had lower bacterial and archaeal diversity than OL-KR6 water and was activated with only acetate and acetate together with sulphate. Hydrogen, sulphide, nitrate or methane were not as good activators as acetate in OL-KR6

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water and even inhibited microbial activity in OL-KR15 groundwater. Overall, acetate was the most efficient activator of the studied microbial communities, which indicates acetate's important role as an electron donor for different Olkiluoto deep subsurface groundwater communities (Miettinen *et al*., 2018). However, part of the sulphate reducers also produce acetate as do also acetogens detected in Olkiluoto. These studies are reported in D2.5.

EPFL

Previous work has shown that the water in the boreholes in Opalinus Clay in Mont Terri harbour a microbial community that is able to rapidly and continuously reduce sulphate, while consuming H_2 (Bagnoud *et al*., 2016a; Bagnoud *et al*., 2016b; Bagnoud *et al*., 2016c). Primary producers in this system were identified as an autotrophic sulphate-reducing bacterium and a sulphide-producing organism. Necromass from these primary producers was then found to be degraded by fermenting organisms that produced low molecular weight organic acids such as acetate. Heterotrophic sulphate-reducing organisms then utilized these organic acids as electron donors for further sulphate reduction. While we clearly showed sulphate reduction in Opalinus borehole water, and Bagnoud *et al*. (2016c) were able to quantify rates in borehole water, the experimental design did not allow assessment of the rate of sulphate reduction (and hence H_2 consumption) in porous media where sulphate-reducing bacteria might establish biofilms. This could occur at interfaces in the repository or within engineered gas consumption areas of higher porosity than the bentonite buffer.

In the MIND project, two new *in situ* experiments were set-up in Opalinus clay, (1) to investigate the rate of microbial sulphate reduction (catalysed by H_2 oxidation) and sulphide production and (2) to evaluate the impact of microorganisms on steel corrosion and hydrogen production/consumption when packed in compacted bentonite; under relevant geochemical conditions for the Swiss disposal concept.

The first experiment (MA-A) utilizes porewater directly from a dedicated borehole to run flow-through reactors with a sand and bentonite mixture. The system is entirely located within an anoxic chamber to minimize exposure to oxygen. The system monitors sulphate consumption and sulphide production as well as H_2 consumption. The flow-through cells were designed to allow a gradient of sulphate and H_2 to establish in such a way as to localize the microbial growth within a narrow region of the reactor. In doing so, it will be possible to collect the biomass and analyse its composition as well as to identify the concentrations of sulphate and H_2 at which the growth is maximized. Finally, because the effluent is monitored, it will be possible to provide a rate of sulphate reduction, in combination with biological H_2 oxidation.

The second experiment (IC-A) entails the deployment of modules containing steel coupons embedded in bentonite into a borehole that was drilled under anoxic conditions and maintained anoxic for the entire duration of the experiment. These modules were retrieved after 1, 1.5, 2.5 and 5.5 years of incubation in the borehole and the microbial community was characterized by cultivation techniques. It was observed that the viable microbial biomass of anaerobic heterotrophs increases until after 1.5 years post-deployment time but decreases between 1.5 and 2.5 years, reaching levels observed for the initial bentonite used to start the experiment. Surprisingly, the viability in modules deployed for 5.5 years was observed to be higher than after 2.5 years. Counts of aerobic heterotrophs tend increase until 1.5 years despite the anoxic conditions within the borehole, and remain viable until the latest timepoint, 5.5 years. Since their numbers are greater than the ones obtained from the cultivation of anaerobes, an exclusive contribution of facultative anaerobes can be ruled out. Therefore, we propose that molecular oxygen is adsorbed to bentonite and stays bioavailable over the investigated experimental duration. Generally, the results suggest microbial

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growth in the early experimental stages, when the bentonite in the modules is not fully saturated, enabling microbial activity with pore space and water, independently from dry density. Additionally, we found that the borehole had developed a sulphate-reducing microbial community since the initial drilling. However, this microbial community does not appear to colonize the bentonite as there is no gradient of viable counts when considering the bentonite's proximity to porewater. Detailed analysis of the microbial community in the borehole using DNA-based tools provided a view of the change in the microbial community composition as a function of time with a clear increase in complex organic matter degraders and a decrease in sulphate-reducing organisms after 1.5 years of experiment. This suggests that electron donors from the rock formation may transiently fuel sulphate reduction but that these sources of energy are rapidly depleted. After 5.5 years, the increase in complex organic matter degraders was reversed and the relative abundance of autotrophic sulphate-reducing primary producers increased again, showing a more diverse community than initially observed. Further durations of deployment, e.g. 7 years deployment will help provide support for this data interpretation.

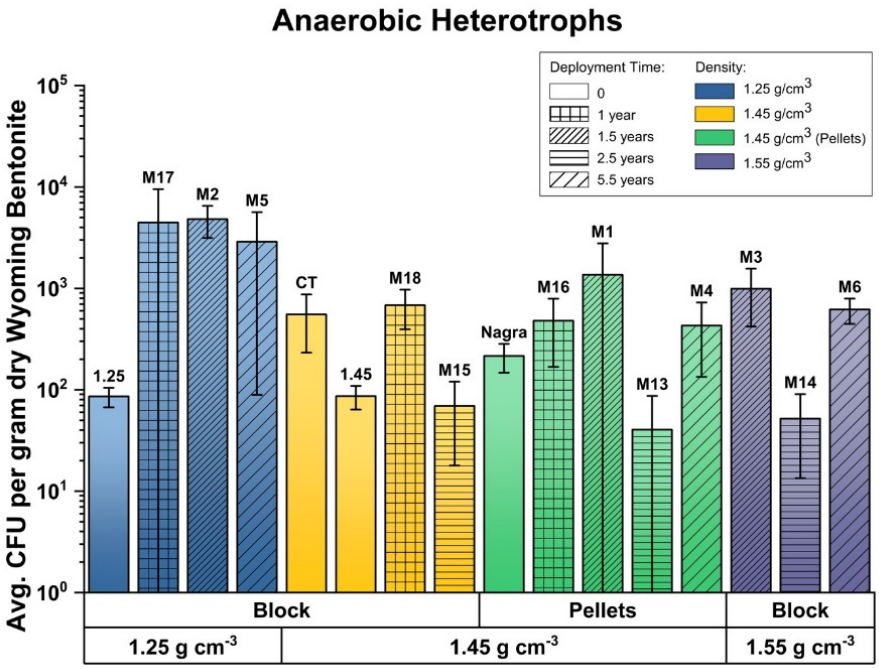

Figure 4-4: Average number of viable anaerobic heterotrophs as a function of time and the targeted dry density The number of viable anaerobic heterotrophs was averaged over all layers per module, error bars represents standard deviation amongst layers. CT pellets were used to produce the different blocks, Nagra denotes the time zero sample of pellets provided by Nagra for the pellet modules (green).

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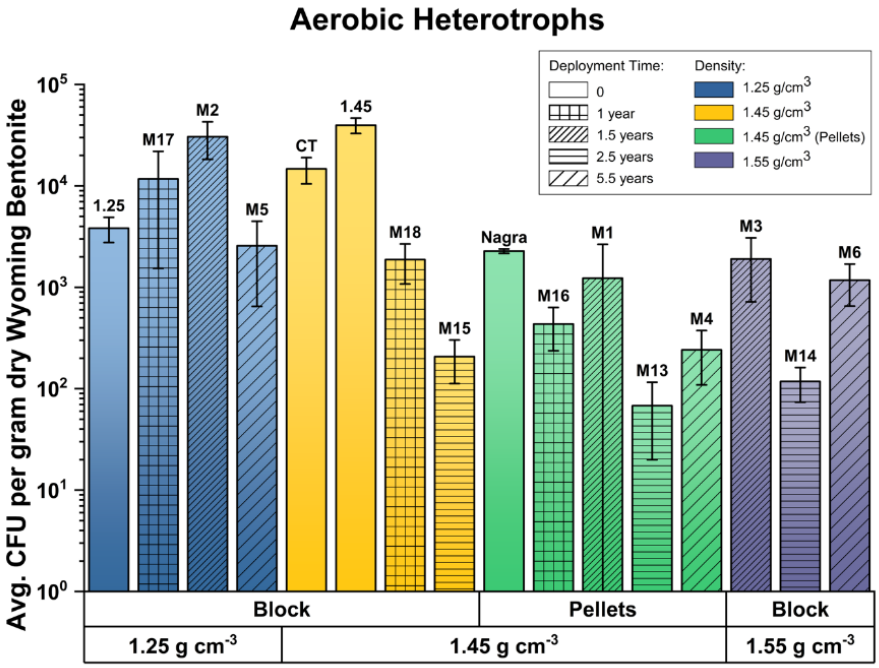

Figure 4-5: Average number of viable aerobic heterotrophs as a function of time and the targeted dry density. The number of viable aerobic heterotrophs was averaged over all layers per module, error bars represents standard deviation amongst layers.

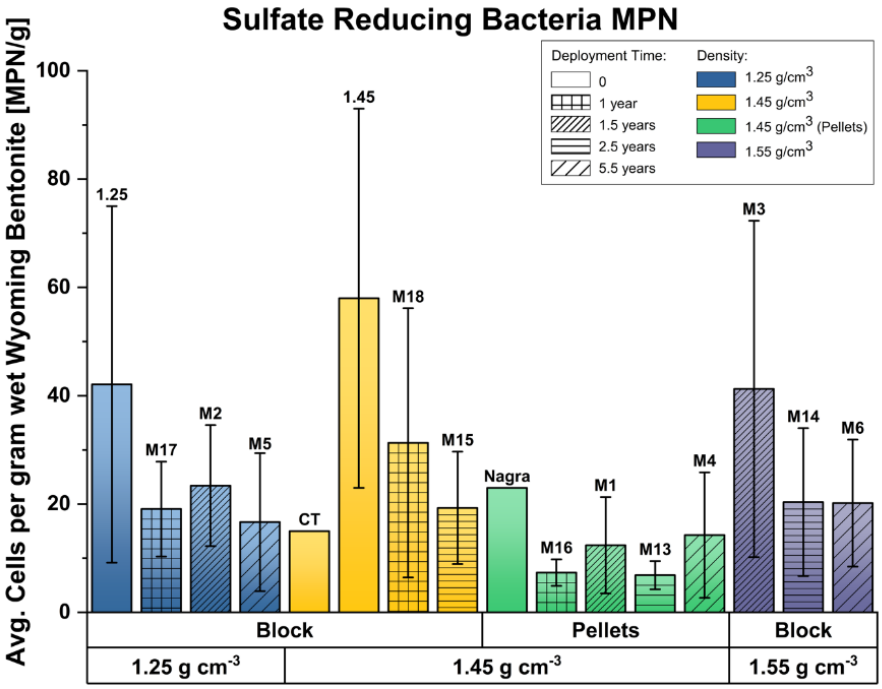

Figure 4-6: Average number of viable sulfate-reducing bacteria (SRB) as a function of time and the targeted dry density. Number of viable SRB is averaged over all layers for each module and error bars represent standard deviation amongst layers. Modules from initial state are included (no pattern) and represented by the blocks from which the modules were made. The highest SRB counts are observed for the initial samples (1.25 and 1.45). The longer the exposure time to Opalinus clay porewater, the lower the counts of viable SRB. There is no observed trend correlating dry density to SRB number

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4.1.3 Conclusions

GTK

Factors controlling microbial sulphide production in the geosphere around the repository (Task 2.1, D2.17) are the availability of suphur compounds, electron donors facilitating microbial sulphide formation, and nutrients required by the micro-organisms. Lack of sulphur sources can hardly be considered as a limiting factor, even though sulphur-bearing minerals are not always common in all rock types, as for example in granitic rocks. Aqueous sulphate is not solubility-limited, it is a common constituent of near-surface waters and provides the source for microbial sulphide formation, if coming in contact with deep anoxic zone.

Redox conditions of the groundwater-bedrock system are considered as a qualitative measure of the sulphide production capacity of the system. Very low redox values indicate the abundance of electron donors, whereas high redox potentials indicate the presence of oxygen or other strong electron acceptors suppressing the sulphate reduction to sulphide. Presence of electron donors is one of the most important controlling factors for microbial sulphide formation. Dissolved molecular hydrogen is the most effective electron donor, whereas methane is the most common one. Currently, more attention has been paid to the role of organic C-2 compounds, especially acetate.

Nutrient limitation is not considered as an important limiting factor, because the bedrock environment provides an adequate source. In deep biosphere, recycling of essential elements and syntrophic interactions play key roles in sustaining microbial life.

Solubility limitation provides the general abiotic constraint on the concentration of sulphide in the groundwater-bedrock system. Relatively high sulphide concentrations occasionally observed in deep groundwaters are evidently due to active microbial sulphate reduction fronts, but transient in space and time.

Transport limitations, based on advection and diffusion, are easily quantified ways to estimate long-term sulphide production rates. Ideal conditions in terms of all other factors required by the microbial sulphide production would evidently lead to limited supply by the limited transport rate.

Energetic calculations demonstrate that life deep in the bedrock has a plausible energy source supplied by the surrounding chemical energy. The life-sustaining energy flux, i.e. power, remains however low, and cannot be multiplied by many orders of magnitude in a sustainable way.

Micans

Gases can migrate in pores of rocks. It was, therefore deemed important to study the composition of gases in porewater. Drill cores from two different drillholes were supplied in kind from Posiva Oy. The budgets of H_2 and CH_4 in these cores were analysed. A budget of core gas inventory is needed to resolve the key issue on long-term sulphide production rates in the geosphere. While the concentrations of these gases are reasonably well documented in groundwater, the amount in the host rock matrix was, previously, unknown. However, the concentrations will certainly vary from sit to site, over depth and over rock types which merites inclusion of core gas analysis at repository sites.

TUL/CV Rez

We demonstrated that the microbial activity highly influences carbon steel corrosion under anaerobic conditions with SRB and NRB being the major corosion causing agents under tested conditions. The biofilm formation differed depending on the temperature (~20 and 35 °C) in anoxic

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underground VITA water environment rich in SRB. While in 20°C the biofilm increased the corrosion rate, in 35°C the biofilm reduced the corrosion rate and it seemed to have a protective character. In the presence of synthetic bentonite pore water (SBPW), with composition based on Czech BaM bentonite material, we observed rapid development of NRB consortia capable to increase the corrosion rate, similarly to SRB. SBPW is rich in nitrates, and thus represents a suitable environment for the NRB enrichment. Further study is needed to to investigate the possible role of NRB in canister corrosion in more detail.

VTT

Activation experiments of microbial metabolism were performed with two deep groundwaters from the final repository site of Olkiluoto, Finland. The waters were different from each other in terms of chemical parameters and microbial diversity. Acetate was overall the most efficient activator whereas hydrogen, sulphide, nitrate or methane were not as good activators or even inhibited microbial activity. Acetate was also able to activate sulphate reduction and sulphide formation in the other water, which was not detected without acetate addition. This highlights the importance of acetate as an electron donor for different Olkiluoto deep subsurface groundwater communities in relation to microbial activity and sulphide formation.

EPFL

The current results obtained from *in situ* experiments in the Opalinus clay in Switzerland offer a partial view on microbial activity, and thus the potential impact of microbial processes on canister corrosion, under repository relevant conditions. Continued monitoring of the ongoing experiments may provide further evidence of the role of microbes in sulphide production and steel corrosion. The results obtained lead to the conclusion that microbial activity in bentonite is governed by the degree of saturation and is less influenced by the dry density than anticipated. However, after full saturation has occurred, microbial viability is shown to persist along all investigated microbial groups (aerobic and anaerobic heterotrophs, sulphate-reducing bacteria). A review of bentonite block producing processes is recommended, as the highest counts of viable sulphate-reducing bacteria was found for the initial block samples, at around 3 times higher than in the bentonite material used to produce these blocks. For the Swiss repository design, bentonite blocks of 1.55 g/cm^3 are considered for pedestal material for spent fuels and high-level waste canisters, thus the introduction of SRB might have considerable consequences for microbial induced corrosion at times when the bentonite is not yet fully saturated. Because of the slowly decreasing heat production of spent fuel, the time of partial bentonite saturation might remain long enough to let microbes thrive close to the canister surface.

4.2 Microbial activity in and degradation of bentonite buffers

Contributing partners: MICANS, EPFL, UNIMAN, TUL & RCR, VTT, BGS/NERC

4.2.1 Problem statement & current knowledge:

Bentonites rich in swelling montmorillonite clay are used to construct engineered barriers, as buffer, backfill, plugs and seals, to absorb radionuclides and retard their migration as well as to reduce microbial mediated processes in geological repositories for low- intermediate- and soon also high-level radioactive wastes. While there are several low- and intermediate-level repositories in operation around the world, high-level repositories are still in planning or under construction. There all still a number of unresolved questions related to the microbial survival and activity in such bentonites.

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The survival of microorganisms has been demonstrated in compacted bentonite samples (Pedersen 2010; Pedersen *et al*. 2000a, b; Stroes-Gascoyne *et al*. 2011; Stroes-Gascoyne *et al*. 1997), however further work is required to fully understand the activity of microbes in compressed bentonite. Previous laboratory and full-scale experiments considering microbial survival in compacted bentonite have found that microbial activity is correlated with bentonite density, swelling pressure and its resulting water activity (Pedersen 2010; Pedersen *et al*. 2000a, b; Stroes-Gascoyne *et al*. 2011; Stroes-Gascoyne *et al*. 1997). Individual commercial bentonites have been shown to display varying effectiveness in mitigating microbial activity at similar densities. This variability may be due to sulphate or organic matter content in the bentonites or it may be due to intrinsic differences in the swelling pressures and pore spaces obtained. An important remaining key issue is to identify if there are conditions (including buffer density) under which relevant bentonites inhibit microbial activity. Because of the risk for loss of density and swelling capacity due to buffer erosion and illitization, a key issue is to document the lower density/pressure limit for microbial activity in bentonite buffer and host clays. Parameters important to investigate are shown in Figure 4-7.

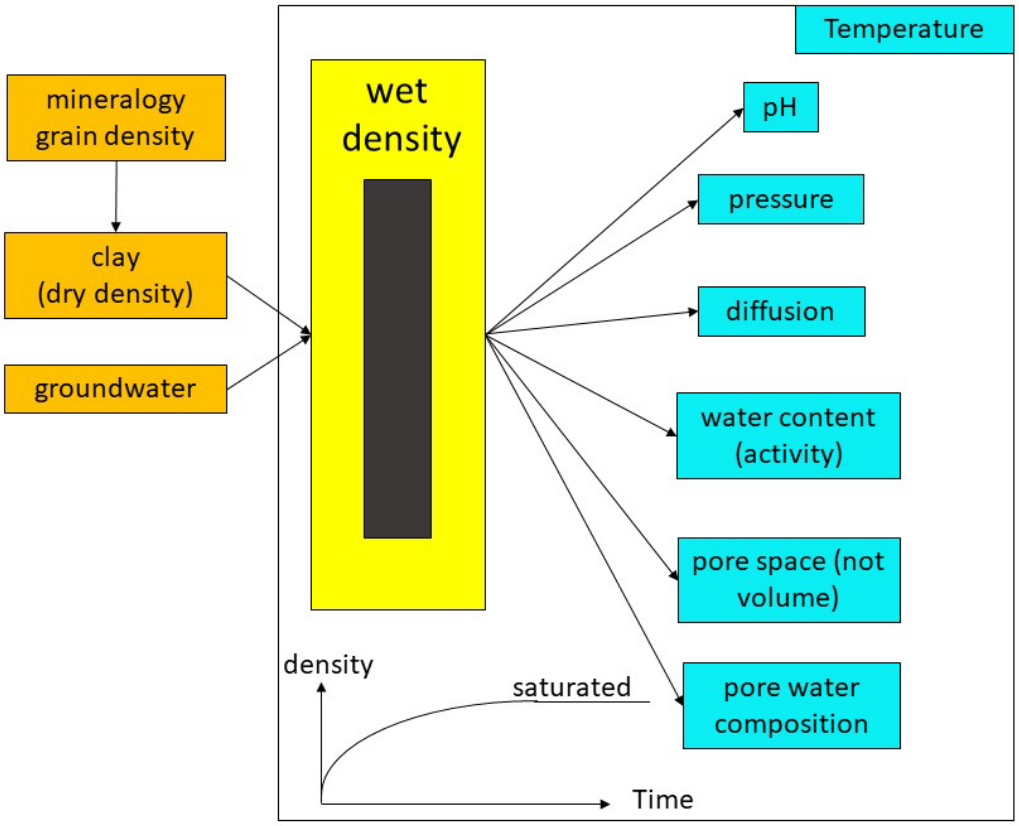

Figure 4-7: Variables influencing wet density and swelling pressure that upon water saturation give rise to suggested factors influencing bacterial viability, cultivability and activity (turquoise) except temperature that is not coupled to wet density. Bentonite clays of varying types will be saturated with groundwater over time and reach a final saturated wet density. The environmental conditions for microbial activity will be set by type of clay, groundwater composition final wet density and concurrent swelling pressure.

Microbial activity in bentonite can however have an important impact on canister corrosion and buffer functionality. First, as reported above, metal containers, made of iron or copper will be used to encapsulate the wastes; but they will be vulnerable to sulphide corrosion, and safety cases for

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radioactive waste disposal must, therefore, evaluate the risks involved with sulphide corrosion (King *et al*. 2011). The inorganic reduction of sulphate to sulphide is kinetically hindered at normal pressure and temperature (Cross *et al*. 2004). The main source of sulphide in geological repository environments is, therefore, past and present microbial reduction of sulphate, sulphur and thiosulphate to sulphide. Sulphide-producing bacteria (SPB) have been found in most commercially available bentonites (Masurat *et al*. 2010; Svensson *et al*. 2011) and they frequently occur in deep geological formations and deep groundwater (Moser *et al*. 2005; Pedersen *et al*. 2014). It is thus of interest to investigate conditions preventing or reducing microbial activity and sulphide production in bentonite buffers.

Second, microbial iron-reducing bacteria have been shown to reduce ferric iron in nontronite to ferrous iron, thereby destroying the swelling properties of such clays (Kim *et al*. 2004; Liu *et al*. 2012). The presence of similar processes, i.e. microbial mineralogical alterations of bentonite is not well investigated. If iron-reducing bacteria can reduce the ferric iron component of bentonite buffers the swelling capacity of the clay may concomitantly be reduced and thereby open up for microbial activity inside the buffer, to increase diffusion of sulphide and possibly allow the discharge of radionuclides. A remaining key issue is therefore to study whether bacteria can degrade bentonite based buffers via iron reducing processes.

Third, this task addresses the possibility that microbial processes that may degrade buffers one way or another. Several different groups of bacteria produce H_2S in their dissimilatory metabolism. It was, consequently, important to investigate the effect of sulphide on bentonite clays. If sulphide reacts with ferric iron in the clay, it may have implications to the stability of the montmorillonite and the transport properties of sulphide through bentonite buffers and back-fill. Bentonite clays are known to have a significant capacity for adsorption of H_2S gas. This fact is utilised to produce industrial filters for removal of H_2S in low concentration gas streams. The absorbance capacity can be increased by the addition of iron to bentonite (Nguyen-Thanh *et al*. 2005; Stepova *et al*. 2009). The sulphide scrubbing mechanism is explained by reactions between sulphide and ferric iron. The reduction of ferric iron by sulphide from SPB was recently demonstrated for freshwater sediments (Hansel *et al*. 2015). The authors showed that sulphide was oxidized to sulphur concomitant with the reduction of a range of different ferric iron oxides to ferrous iron. They suggested that this was an abiotic oxidation-reduction process. A similar process may occur in bentonites, where sulphide reduces ferric iron to ferrous iron concomitant with the oxidation of sulphide to elemental sulphur (Eq 2). The ferrous iron could react with free sulphide and form FeS (Eq 3). The generalised sum of these processes is given in Eq 4.

$$HS^- + 2\,Fe^{3+} \rightarrow 2\,Fe^{2+} + S^0 + H^+ \qquad \text{(Eq. 2)}$$

$$HS^- + Fe^{2+} \rightarrow FeS + H^+ \qquad \text{(Eq. 3)}$$

$$2\,HS^- + 2\,Fe^{3+} \rightarrow Fe^{2+} + S^0 + FeS + 2H^+ \qquad \text{(Eq. 4)}$$

The dissociation constant for HS^-/H_2S is $10^{-6.98}$ (Richard and Luther 2007). The pH of groundwater in repository environments, and in clay pore water is buffered above 7, which means that sulphide mainly will be present as HS^- when pH approaches 8 or higher.

These processes will cause an S immobilisation effect that can reduce the mass of sulphide that reacts with metal canisters over repository life times that may influence the longevity of metal canisters. However, the concomitant reduction of structural ferric iron may be problematic due to

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the destabilizing effect of ferrous iron on dioctahedral smectites (Bradbury *et al*. 2014; Lantenois *et al*. 2005; Soltermann 2014).

Mobility of bacteria in bentonite buffer and back-fill

Although a good understanding of the issue of microbial mobility in compacted bentonite is crucial for the safety of nuclear waste repositories, several questions remained unanswered. The size distribution of pores and pore throats is an important factor controlling microbial activity in soil and most probably in bentonite. It is influenced both by the compactness of bentonite and by its composition. It was shown, that bacteria are motile in channels where the width is 30% greater than cell diameters. Furthermore, bacteria can penetrate channels twice as narrow than their diameters by growing through the pore space (Männik *et al*., 2009). Natural bentonite is typified by its compact structure and small pore sizes, with gradual decrease of pore sizes with its compactness (Appelo, 2013). However, the presence of microorganisms belonging to a wide range of species and metabolic groups has repeatedly been reported from various clay types under different environmental and laboratory conditions (Jalique *et al*., 2016; Lopez-Fernandez *et al*., 2015).

The information about the motility of bacteria in compacted bentonite is rather scarce. The conducted laboratory experiments almost identically reported existence of bacterial motility in compacted bentonite but in a very limited form. Mobility of introduced *Pseudomonas stutzeri* was studied in the MX-80 bentonite compacted to the densities of 1.68 and 1.80 g/ cm^3 (Stroes-Gascoyne and West, 1997b). Results showed no evidence for *P. stutzeri* in the plugs except for the first 0.5 cm of the plugs in a 16-week period of the experiment. Similarly, Pedersen *et al*., 2000 studied the process of bacterial migration into compacted MX-80 bentonite clay (1.9 kg/m^3 dry density) during the swelling phase. Bentonite infested with aerobic and anaerobic bacteria was sampled for the presence and activity of bacteria at three depth zones 0-1, 1-3 and 3-6 mm in four times - 8 h, 2, 12 and 28 weeks. In general, fewer cultivable bacteria were detected with increasing depth and experimental duration. The highest numbers of cultivable bacteria were observed after 8 h and the aerobic bacteria were detected in higher depths, than anaerobes regardless of time. Some bacterial species remained cultivable at all sampled depths during the whole course of the experiment at lower temperatures, but their numbers were significantly decreased. None of the species remained cultivable after 28 weeks in 80°C.

The most relevant study concerning microbial mobility *in situ* conditions was performed by Fru and Athar, 2008. These authors studied, how indigenous groundwater bacteria interact with MX-80 bentonite buffer materials in the long term HLW repository simulating experiments conducted in the aquifers. They concluded that the repository simulating conditions appeared to retard the overall success of the predominantly gram-negative mesophilic groundwater microorganisms. The spore-forming gram-positive bacteria commonly found in the clay predominated, suggesting that the non-spore-forming microorganisms and gram-negative microorganisms predominant in granitic groundwaters might not form a prominent portion of the bacterial community that will develop in bentonite under repository conditions. Furthermore, the complete absence of the gram-negative microorganisms in the bentonite samples that were incubated at ≥ 67°C in contrast to their presence in the lower temperature blocks implied that their survival was most likely controlled by temperature and not by compaction density or the porosity of the bentonite.

Most of the studies above were performed on highly compacted MX-80 bentonite, which is Na-bentonite of very specific features. On the other hand, there is no information concerning bacterial mobility in BaM bentonite, which moreover belongs to Ca-Mg bentonites. Choi and Oscarson demonstrated that used Ca-bentonite developed more large pores than Na-bentonite at the same

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density and the diffusion coefficients of Ca-bentonite was thus higher than detected in Na-bentonite (Choi and Oscarson, 1996). Both of these results can be ascribed to the generally larger quasicrystal size of the Ca-bentonite clay. This findings agree with the results of Fukunaga *et al*., who showed that bacteria freely diffused through the buffer material compacted to 1800 kg/m^3 when the proportion of Na-bentonite was 20 wt % or that of Ca-bentonite was 50 wt % (Fukunaga *et al*., 2000). Bacterial movement was less than 5 mm in three weeks when the proportion of Na-bentonite was 70-100 wt % or that of Ca-bentonite was 100 wt %. Further experiment of the bacterial mobility in compacted Ca-bentonites of high density are thus needed.

Effect of irradiation on microbial survival in bentonite

Microbial response to radioactivity is very important question for the HLW repository concepts and their long-term safety. Swedish KBS-3 HLW repository concept expects the maximum dose outside the canister to be less than 0,5 Gy/h (Svensk Kärnbränslehantering AB, 2006). The gamma and neutron radiation are expected to be significant during approximately 1,000 years past deposition (Svensk Kärnbränslehantering AB, 2006) and it will take 100,000 years for the radioactivity to decrease to similar levels to those of natural uranium (Lopez-Fernandez et al., 2018).

Ionizing radiation causes various changes in cells due to reactive oxygen species production from water radiolysis and subsequent chemical cascades generating additional cell-damaging molecules (Azzam et al., 2012; Reisz et al., 2014; Riley, 1994). The radiation resistance of a microorganism is commonly measured by the decimal reduction dose (D_{10}), which is defined as the radiation dose (kGy) required to reduce the number of that microorganism by one log. The average D_{10} dose for most vegetative microorganisms was calculated to be 0.420 kGy (van Gerwen et al., 1999), but the radiation sensitivity among bacteria is highly variable. Radiation highly resistant bacteria such as *Deinococcus radiodurans* have the average D_{10} 10,4 kGy (van Gerwen et al., 1999). Their radiation resistance is probably a consequence of microbial adaptation to desiccation (Mattimore and Battista, 1996). On the other hand, dissimilatory metal-reducing bacterium *Shewanella oneidensis* is extremely sensitive to radiation although it encodes more complex DNA repair systems that one in *D. radiodurans* (Daly, 2004). Direct DNA damage is thus not the major lethal effect of irradiation in this case and there are other factors, such as oxidative stress, protein damage, and prophage-mediated cell lysis, that cause the lethal effect of irradiation (Qiu et al., 2006).

Survival data from pure culture studies may not be fully applicable to relatively nutrient limited environment of proposed HLW, where different species of the community will be competing, and where radiation is highly probably not the only selective stress. There are three major ways, which could help us to understand the effect of irradiation on repository environment:

a) <u>Irradiation studies on environmental samples of soils, rocks or sediments</u>
Based on literature review (McNamara et al., 2003) stated that Gama irradiation of the soil samples generally eliminates actinomycetes, fungi and invertebrates at the 10 kGy dose, the majority of soil bacteria are eliminated by 20 kGy, but a dose higher than 70 kGy may be required to kill certain radio-resistant bacteria. The native microbiota seems to be capable of surviving long-term low dose Gama irradiation and under proper conditions retains its metabolic activity (Brown et al., 2015; Pitonzo et al., 1999b). On the other hand, the experimental evidence also suggests the existence of threshold in total absorbed radiation dose, which effectively eliminate the bacterial presence in various soil materials (Bunzl and Schimmack, 1988; McNamara et al., 2003).

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b) Studies on environmental samples from radioactive contaminated sites

Short-term exposure of microbes to physical or chemical deleterious agents can lead to the survival and selection of resistant mutants with an enhanced capacity to synthesize DNA-repair enzymes (Ahmad, 1996; Wright and Hill, 1968) and similar or even more pronounced effect can be expected under chronic low-dose irradiation, which will be more comparable to conditions expected in HLW repositories. Several microbiological studies were performed on various radioactive waste storage places e.g. (Bruhn et al., 1999; Chapon et al., 2012; Fredrickson et al., 2004; Theodorakopoulos et al., 2017) and all of them showed that bacteria, with clear domination of Gram-positive strains, indigenous to various long-term radioactivity contaminated sites show increased resistance to extreme physical and chemical stress associated with radioactive waste presence.

c) Irradiation studies specifically targeted to bentonite environments.

Studies on radiation effect in bentonite are surprisingly rare considering the importance of bentonite as one of the key factors limiting metabolic activity of bacteria in future repositories. It was shown, that long-term irradiation under repository condition probably will not have significant direct effect on the bentonite properties, although the total absorbed doses over time will be high. Compacted MX-80 bentonite heated to 90-130°C and irradiated for one year with a total radiation dose of 3.27×10^7 Gy showed no significant change in the montmorillonite quality and quantity in either of the samples (Pusch et al., 1992). Similarly, (Galamboš et al., 2012) detected no changes in structure and quality of Slovak bentonites with absorbed doses of 10^4 and 10^5 Gy and only insignificant changes with absorbed doses of 1 MGy of Gama radiation.

(Stroes-Gascoyne et al., 1995) studied the radiation sensitivity of naturally occurring microorganisms in MX-80 bentonite. The D_{10} dose at dose rate 100 Gy/min was between 0.65 and 1.68 kGy for natural microbiota depending on bentonite type and the radiation survival responses decreased linearly with increasing total dose. Using these values authors estimated, that it would take 9-33 days (depending on the canister type) after emplacement to completely sterilize the outer container surface. Formation of biofilm and resulting MIC of canister surface will thus be rather improbable at the early repository phase. However, sterilization of 25cm thick buffer barrier by low radiation doses outside the canister is not likely and the repopulation of canister surface during the repository lifetime is thus real possibility. In subsequent experiment (Lucht and Stroes-Gascoyne, 1996) detected no general effect of dose rate on the radiation sensitivity, the total dose was the most important factor with the values similar to previous experiment. However, they detected group of highly radiation resistant indigenous microorganisms, which showed increase in radiation resistance with increasing temperature possibly due to synergic desiccation effect of both factors.

Published evidence thus implies the possible natural presence of radiation resistant bacteria in bentonite buffer systems, however, their behavior and especially evolution under long-term low-level irradiation remains completely unknown. Long-term experiments under repository conditions including the effect of irradiation are thus highly needed.

4.2.2 Summary of experimental & computational results

MICANS

Bacterial activity is generally measured by the turn-over of one or several metabolic products such as the production of acetate or sulphide. Bacterial viability (or presence) on the other hand does not imply that the bacteria must be active *in vivo* or *in situ*, it only states that they are able to become activated when a suitable environment presents itself. It has been hypothesised that cut-off bentonite density thresholds exist above which all bacterial activity stops or is inhibited to such a

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level that it can be regarded as negligible. The deliverable D2.4 discusses if that hypothesis can be considered true or if more variables other than clay density determine bacterial activity in bentonites. The clays analysed and discussed in the deliverable have been investigated previously (Bengtsson et al. 2016; Bengtsson et al. 2017a; Bengtsson et al. 2017b; Bengtsson et al. 2015; Bengtsson and Pedersen 2017). This deliverable summarizes previously performed work and new work performed within MIND, briefly summarized here.

Six different bentonites of interest for geological disposal concepts have been studied: Wyoming Volclay MX-80 (USA), Asha (India), Calcigel (Germany), GMZ (Gaomiaozi, China, Rokle (Czech Republic) and FEBEX clay (Switzerland) (Bengtsson et al. 2017). In addition, Boom Clay (Belgium) has been studied as a reference to a non-swelling clay (Bengtsson and Pedersen 2016). These clays had a varying element and mineral composition. In particular, the amount of montmorillonite varied from 66 % to 88 % and the iron content, analysed as Fe_2O_3 varied from 3.3 % to 13.4 %. A new method for the analysis of natural organic matter in the clays showed a great diversity of organic compounds in the clays. Alcohols, esters, ketones, aldehydes, fatty acids, alkanes, and more were detected. These compounds can serve as electron donors and sources of carbon for growth of bacteria in the clays.

Sulphide-producing bacteria could be cultivated from all clay samples and the numbers decreased over wet density for some but not all tested clays. The range of saturated wet densities studied was from 1400 to 2000 kg/m^3. Experiments analysing bacterial sulphide-production showed that there were intervals where the measurable accumulation of copper sulphide for each clay changes from significant to below detection within the experimental timeframe of 3-4 months. For two clays, these density ranges are yet to be determined, sulphide was produced at all studied densities. It is therefore concluded that density alone does not control bacterial activity in clays. Other variables must be studied as well.

The relation between water saturated clays at varying wet density and bacterial sulphide-producing activity is well studied. However, wet density is just a value of the total amount of clay and water. That value does not reflect the growth conditions for bacteria in a compacted clay where several variables of importance for bacterial life can be of importance, such as clay type, pH, temperature, transport conditions, water content, pressure, pore space and pore water composition (Figure 4-7). These variables need further attention for a full understanding of what conditions influence bacterial activity in compacted bentonite clays.

The effect of swelling pressure on micobial activity can be summarised as follows. Analysed sulphide production versus measured pressure shows a boundary value at approximately 1000 kPa above which sulphide production could not be detected, or, was very close to the detection limit (Figure 4-8.). The swelling pressure in the bentonite originates from separating flocs in the bentonite. This means that a mechanical pressure arises between the separating flocs, approximately equal to the swelling pressure. Even in low-density bentonites (1500 kg m^{-3}), a pore size in the nm range would theoretically not allow for bacterial existence unless the bacteria could withstand the mechanical pressure from the separating flocs (90 kPa at 1500 kg m^{-3}). Prokaryotic cells can compensate for the mechanical pressure in compacted bentonite by turgor pressure. Published data on turgor pressure in prokaryotic cells mention pressures between 80 kPa and 2000 kPa (Potts, 1994). An upper limit of 2000 kPa turgor pressure would mean that cell integrity is possible, though limited, at bentonite swelling pressures below 2000 kPa. Present results suggest a limit at approximately 1000 kPa (Figure 4-8.). However, endospores can survive a much higher pressure. Bacteria are very small and if there are pores or other inhomogeneities in buffer and backfill with lower than planned pressures, local microbial activity will be possible. In addition, there will be interfaces between rock engineered

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disturbed zone and bentonite and between bentonite and canisters at which pressures may differ from the bulk of buffer and backfill. New results from several partners in the MIND project show that sulphide-producing bacteria can form local colonies in compacted clays, likely in positions were impurities of the clay offer enough large pore space for bacterial life.

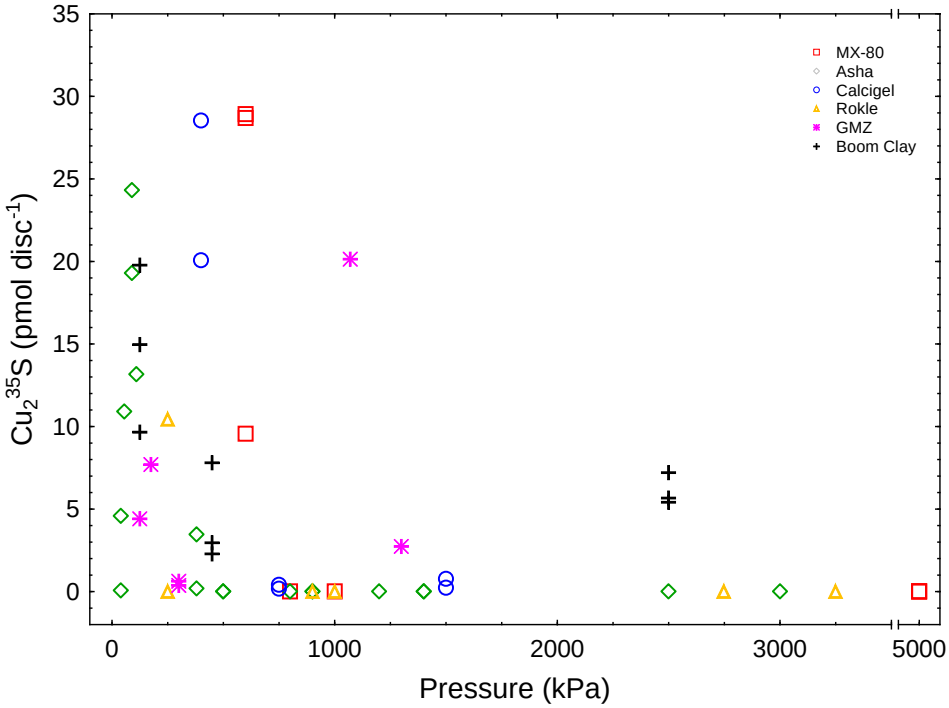

Figure 4-8. Amount of ^{35}S copper sulphide on copper discs versus swelling pressure Data collected from Bengtsson et al. 2016; Bengtsson et al. 2017a; Bengtsson et al. 2017b; Bengtsson et al. 2015; Bengtsson and Pedersen 2016, Bengtsson and Pedersen 2017.

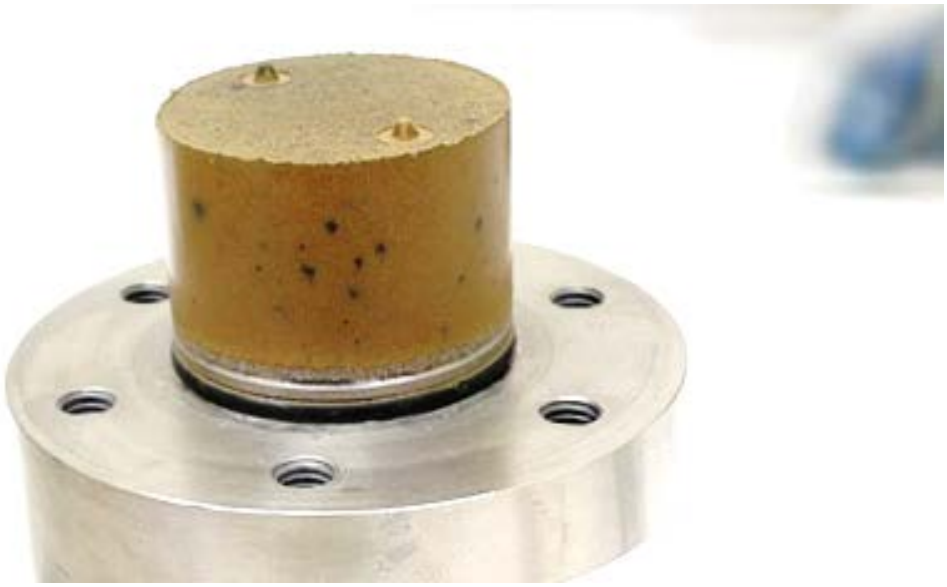

Figure 4-9. Black colonies of iron sulphide produced by sulphide producing bacteria in Asha bentonite at 1750 kg/m3 wet density and with addition of lactate and sulphide producing bacteria.

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In a recent MIND-study, investigating the effect of sulphide on bentonites, sulphide was found to reduce ferric iron in bentonites denoted Asha, MX-80 and Calcigel under the formation of elemental sulphur, ferrous iron and iron sulphide (Pedersen et al. 2017). These reactions rendered an immobilisation capacity of the clays that was 40 µmole sulphide (g clay)$^{-1}$ or more, depending on the load of sulphide, and type of clay. In addition, using a diffusion method developed for Boom clay (Bengtsson and Pedersen 2016), the effective diffusion coefficients for sulphide ($^{35}S^{-2}$) in Asha bentonite, compacted to saturated wet densities of 1750 kg m^{-3} and 2000 kg m^{-3}, were determined to 2.74×10^{-11} m^2 s^{-1} and 6.60×10^{-12}m^2 s^{-1}, respectively. In opposite, when the diffusion experiment was repeated with Calcigel, the added $^{35}S^{-2}$ did not come through the clay. Likely, Calcigel immobilised all added $^{35}S^{-2}$. In tests with addition of sulphide on Volclay MX80, Asha and Calcigel clays, blackening due to FeS formation clearly demonstrated how the majority of the sulphide moved in a front until exhausted. The immobilisation effect is visual in Figure 4-10..

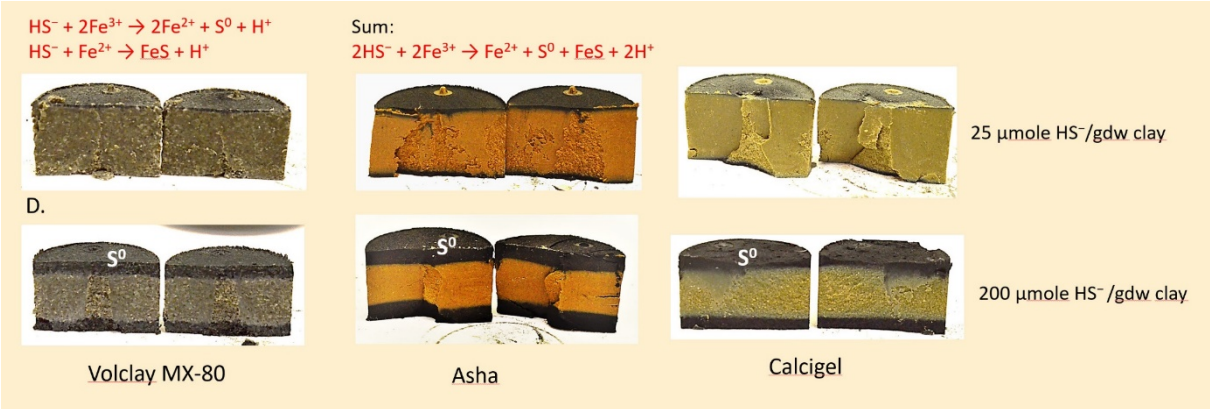

Figure 4-10. transport of 2 different amounts of sulphide added to bentonite clays after 90 days of water saturation to 2000 kgm^{-3} wet density. There were significant amounts of S^0 in the black layers.

These processes will cause an S immobilisation effect that can reduce the mass of sulphide that reacts with metal canisters over repository life times that may influence the longevity of metal canisters. However, the concomitant reduction of structural ferric iron may be problematic due to the destabilizing effect of ferrous iron on dioctahedral smectites (Bradbury and Baeyens 2003; Lantenois et al. 2005; Soltermann 2014).

Previous experiments have utilised the addition of lactate and sulphide-producing bacteria to bentonite for a conservative experimental approach (Bengtsson et al. 2016; Bengtsson et al. 2017a; Bengtsson et al. 2017b; Bengtsson et al. 2015; Bengtsson and Pedersen 2016, Bengtsson and Pedersen 2017). New experiments tested if growth could occur without addition of sulphide-producing bacteria, salts in the saturation medium and lactate. The experiments confirmed previous data showing best growth at 1750 kg/m^3 wet density with all additions (Figure 4-11.). Absence of added sulphide-producing bacteria still resulted in vivid growth showing that sulphide-producing bacteria were present in the tested bentonite. Increasing the wet density to 2000 kg/m^3 reduced growth somewhat but not to nil. Finally, there was growth also in test cells without addition of lactate, bacteria and salts. This shows that there is enough space and energy available in the tested Asha bentonite at 2000 kg/m^3 wet density to allow growth of sulphide-producing bacteria without conservative additions of bacteria and energy sources. Likely, there were enough organic carbon in the clay, as shown in D2.4, to support growth,. The exposure time was 250 days, which is short in a repository perspective.

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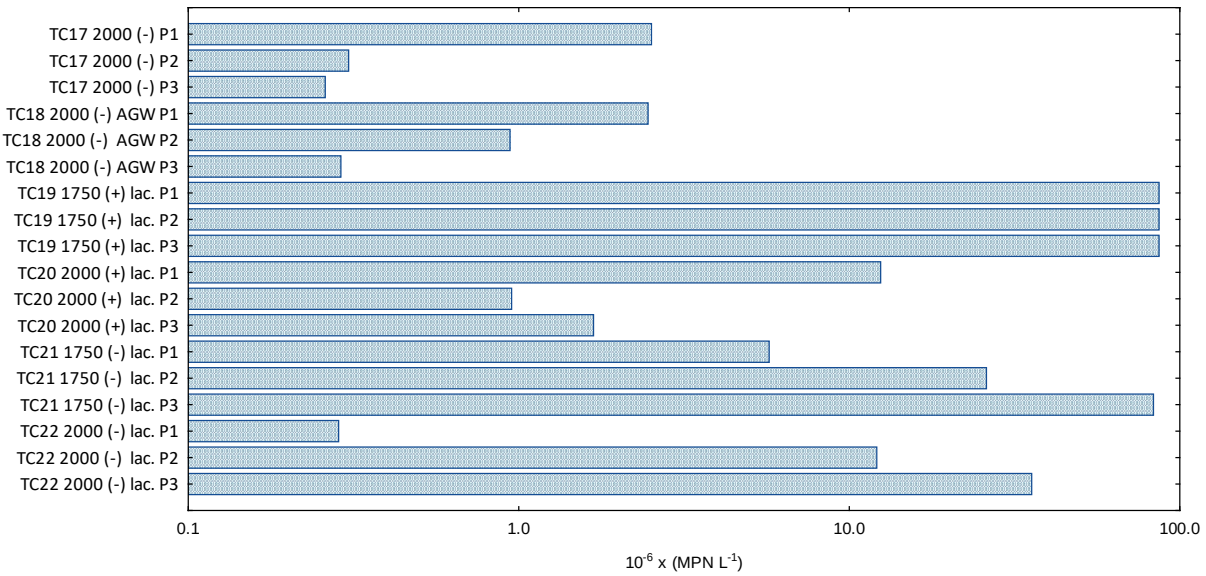

Figure 4-11. Most probable numbers of sulphide producing bacteria in pore water at three positions in bentonite cores of each test cell, P1 at water inlet, P2, 7 mm and P3, 14 mm from water inlet, respectively. The names indicate test cell (TC) number, bentonite wet density (kg/m^3), addition of bacteria (+/-), Analytical grade water without salts as saturation medium (AGW), addition of lactate (lac.) to the saturation medium and position (P). The experiment was run for 250 days.

EPFL

Bioreactors were filled with a 80:20 % (wt.) sand:bentonite mixture to investigate growth in porous medium. This experimental set-up will provide insights into the hydrogen oxidation and sulphate reduction rates in a sessile microbial community under repository-relevant conditions. Porewater from Opalinus Clay rock is flowing at a rate of approx. 20 mL/day through the bioreactors and microbial activity is facilitated by the daily addition of hydrogen gas. Throughout the course of a pre-test with two bioreactors, two different conditions were created. The first condition facilitated the growth of a community dominated by sulphate-reducing bacteria and fermenting bacteria while the second condition led to the community dominated by methanogenic archaea. In both cases, the precipitation of a dark mineral (potentially iron-sulphide) is likely associated with sulphate reduction. Additionally, a brownish mineral (potentially iron-oxide) was observed. It is hypothesized that residual oxygen, adsorbed to bentonite, may persist for longer time periods than expected. Further bioreactor experiments will be carried out to characterize the geochemical conditions necessary for a microbial community to transition from a sulphate reduction-dominated to a methanogenesis-dominated metabolism.

UNIMAN

UNIMAN had three aims for the study of bacteria and bentonites:

- Impact of temperature and evolving resaturation on microbial populations within the bentonite buffer, along with their *in situ* interactions with the clay, through the use of FEBEX samples from the Grimsel Test Site.
- Distribution and control of anaerobes, especially Fe(III)-reducing bacteria in experimental systems with bentonite, and the impact of these organisms on the bentonite clay structure.

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- Influence of radiation treatment on mineralogy of the bentonite clay, and the effect of radiation, porosity and other key parameters on the microbial activity.

Progress towards these objectives initially focused on the quantification, and identification of Fe(III)-reducing and sulphate-reducing microorganisms in a range of bentonites, as well as investigating structural changes in the clay minerals, after microbial treatment. This was described in a manuscript published in 2018 (Haynes *et al.*, 2018), and focused on the impact of geodisposal-relevant parameters, including heat, pressure and radiation treatments, on indigenous Fe(III)-reducing and sulphate-reducing bacteria. Follow-up papers are in advanced preparation that document the changes in clay mineralogical structure linked to microbial Fe(III) reduction in bentonites; a wide range of imaging and spectroscopy techniques were used to support this work. Attempts to extract DNA from the FEBEX core samples also proved successful, and the extracted DNA was sequenced using an Illumina platform and the data is analysed (and suggested limited microbial colonisation close to the FEBEX heating unit). Pressure cell systems were also constructed and high-pressure studies on Fe(III)-reduction in bentonites were completed, and similar survival/proliferation data to those obtained by other MIND workers focusing on SRBs were obtained. Finally data were also collected that quantified the impact of microbial metabolism on radionuclide fate in bentonite-rich systems. Haydn Haynes, the student completing this MIND-affiliated, RWM-funded PhD project successfully defended his thesis covering this work in 2019, and we hope that this thesis will deliver at least three published manuscripts describing the work undertaken.

BGS/UKRI

Two temperature controlled constant volume axial-flow cells were designed and constructed to carry out experiments to investigate microbial survival and interaction with steel and bentonite under realistic conditions, and to identify the influence of microbial activity on the physical, chemical and transport properties bentonite. These bespoke vessels used in experiments were made from grade two titanium, each was fitted with 3 load cells (2 axial and 3 radial) to continuously monitor changes in stress. This allowed real time monitoring of flow experiments using artificial groundwater flowing through compressed bentonite samples containing steel chips. (Figure 4-12). Filters at either end of the sample allowed water to be injected and water to be collected. Injection and backflow HPLC pumps controlled the pressure and flow, allowing hydration of the sample and the application of a hydraulic gradient across the sample to create flow. Paired experiments used gamma-irradiated FEBEX bentonite and compared the response of a non-inoculated “sterile” sample to a sample inoculated with a sulphate reducing bacteria enrichment from the unirradiated bentonite. For the first two pairs of tests, the core was prepared to a dry density of 1400 kg/m^3. The bentonite in the final pair of tests was prepared to a dry density of 1200 kg/m^3. All tests contained 5 g unalloyed steel chips.

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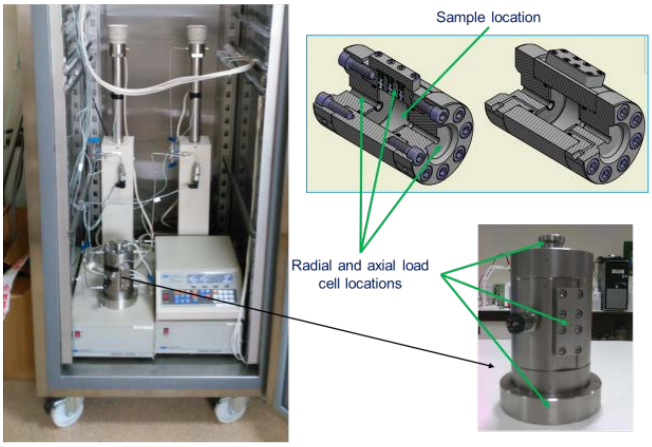

Figure 4-12: Images showing the experimental apparatus used for monitoring the impact of microbes on the physical properties of compacted bentonite samples.

The gamma irradiation was successful in eliminating viable microbial cells from the bentonite, but there was evidence of minimal contamination of the non-inoculated tests (which was likely due to contamination during the lengthy sample preparation or due to difficulties fully sterilising the whole CVAF apparatus between tests). The results confirmed previous reports that microbial survival is higher in lower density bentonite samples, and suggest that bacterial survival in FEBEX bentonite is at the upper range of quoted dry densities for other bentonites.

There was evidence of steel corrosion in all experiments. XRD data showed an increase in the basal spacing of smectites in the zone immediately surrounding the steel, compared to the starting material and material taken from further away from the steel, indicating replacement of monovalent cations with divalent ones. The degree of replacement of monovalent cations with divalent cations was reduced in later tests. Proximity to the steel, but not the presence of inoculated microorganisms, affected this cation displacement. At large scales such changes could reduce clay swelling capacity.

SEM examination of the bentonite around the corroded steel identified calcium enrichment along the edge of fractures in the bentonite which probably opened up as the steel corrosion consumed water from the bentonite was associated. Iron enrichment was also observed in the bentonite around the steel in most tests. The first two pairs of tests showed particular iron phases (with a "fibrous" appearance) and the development of aragonite crystals in fractures around steel, were associated with inoculated tests. However, later experiments lower density experiments (1200 kg m^{-3}) did not show this pattern. Precipitation of iron sulphide was only seen in one of the low-density, non-inoculated tests.

No differences in the evolution of stress and swelling pressure could be attributed to inoculation of the sample. However, in all of the tests, permeability was lower in the inoculated sample. The differences were typically small, but in the low-density tests (1200 kg m^{-3}) a 20% reduction in permeability was observed in inoculated samples.

Although survival of bacteria in compacted FEBEX bentonite has been demonstrated, the extent to which microbial activity alters the hydraulic behaviour of bentonite remains unanswered. Other than a possible reduction in permeability of inoculated samples, there was no consistent pattern associated with the presence or absence of microorganisms. However, the possibility that corrosion influences the mineralogical and petrological character of the bentonite has been highlighted. These changes could affect its performance as a barrier material and further studies need to be undertaken

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to confirm the role of microorganisms in steel corrosion and its impact on bentonite behaviour. Therefore these interactions will be the focus of future tests involving higher iron:bentonite ratio and longer test durations.

TUL+CVR

At TUL and CV Rez two different experiments about limitations to microbial activity in bentonites were conducted. Firstly, we tested the ability of bacteria to survive and migrate through compacted bentonite. Secondly, we tested the effect of Gama radiation and elevated pressures on indigenous microbial ecosystems in bentonite suspensions.

a) Microbial mobility in compacted bentonite

Previous tudies showed only very limited ability of microorganisms to penetrate through compacted MX-80 bentonite but only limited information is know about the behaviour of microorganisms in Ca-bentonites. The evidence sugests, that Ca-bentonites tend to be more easily penetrable than Na bentonites (Choi and Oscarson, 1996)(Fukunaga *et al*., 2000). The important question for Czech disposal concept is the mobility of microorganisms in BaM bentonite, which belongs to Ca-Mg bentonites.

A set of experimental migration cells with necessary accessories were designed, prepared and the functionality was verified. A method of bentonite compaction to required density was developed. Two cells containing Czech BaM bentonite compacted to 1400 kg/m^3 and 1600 kg/m^3 were saturated by underground water VITA using high pressure pump (up to 2 MPa). Saturation took place in the glove box under anaerobic conditions ($C_{(O2)}$ < 1 ppm) and full saturation of both compacted bentonite samples was reached after 11 days in the case of 1400 kg/m^3 sample and after 55 days in case of 1600 kg/m^3 sample.

Bentonite samples of both densities were sliced and 14 samples across each bentonite plug were taken. The detection of microorganism was performed by a novel extraction protocol and aerobic and anaerobic cultivations. Our novel protocol for bacterial detection combines bacterial extraction from bentonite by density gradient centrifugation with Percoll and a subsequent Live/Dead staining of extracted bacteria.

The presence of both alive and dead bacteria was detected in all samples of both bentonite densities (Figure 4-13, Figure 4-14). This finding indicates that bacteria are able to survive in compacted bentonite of relatively high dry density (1600 kg/m^3). Although we were not able to explicitelly count the detected bacteria due to technical problems, there were general patterns: Firtsly, the overall microbial density was higher in 1400 kg/m^3 bentonite than in 1400 kg/m^3 one and the cells in the higher density samples tended to be noticeably smaller. Secondly, there were fewer cells visible as we got deeper inside the compacted bentonite samples.

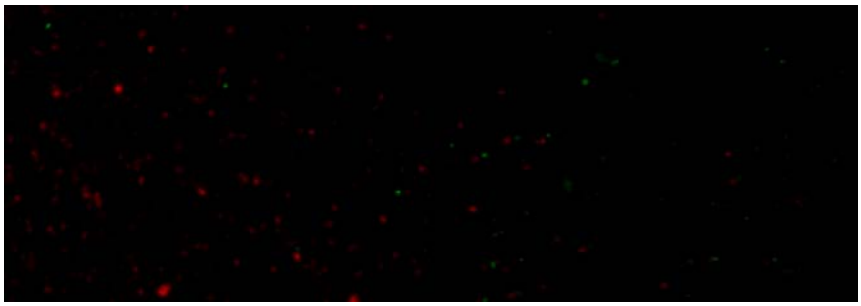

Figure 4-13: Viable (green fluorescence) and dead (red fluorescence) cells detected in the 1400 kg/m3 bentonite.

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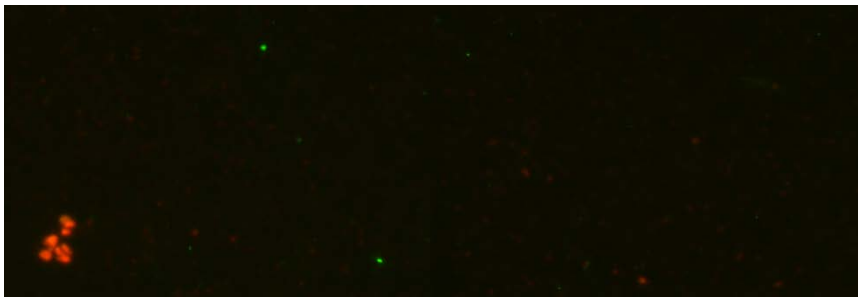

Figure 4-14: Viable (green fluorescence) and dead (red fluorescence) cells detected in the 1600 kg/m³ bentonite.

Dry density of 1600 kg/m^3 equals to the wet density of over 2000 kg/m^3 and swelling pressure exceeding 5 MPa after full saturation, which are values recommended as cut-off in bentonite density in various waste disposal concepts (Pedersen, 2017). It was however demonstrated that similar density enables the presence and activity of bacteria (Pedersen, 2017). Furthermore, BaM bentonite belongs to Ca-bentonites that might generally have better microbial permeability, due to their composition, than Na-bentonites, where only very limited bacterial mobility was reported. However, further experiments are needed to prove this hypothesis. Both aerobic and anaerobic cultivations further confirmed the microscopic results and we detected the presence of anaerobic and aerobic cultivable bacteria in all samples of both densities.

Because bentonite plugs were sliced into 14 parts, each of the analysed samples was too small for parallel DNA extraction. For this reason we did not perform NGS 16S rDNA amplicon sequencing to show, whether detected microorganisms belong to indigenous BaM bentonite microflora or originate from VITA water. The sequenation data from other experiments indicate, that the observed bacteria were probably indigenous to BaM bentonite, because most of the bacteria from VITA water are usually lost within a few days in bentonite environment. This question needs to be properly answered I the future experiment. We are also further continuing in extraction protocol optimization and new studies will be performed to verify our first results.

b) Survival of microorganisms in bentonite subjected to Gama radiation or increased pressure

In the repository, negative effects of high pressure, elevated temperature or low-level ionizing radiation on the bacteria can be expected. Nowadays, we have reasonable good understanding about the effect of each particular variable separately, however their synergic effect and especially the effect of radiation and incredible long time frame remains rather unclear. Our study aims to increase the knowledge about the effect of Gama radiation and pressure on the indigenous microbial community in bentonite.

We prepared closed microcosms with bentonite suspension consisting of 3 parts by weight of BaM bentonite powder and 10 parts of underground VITA water from Josef URC under anaerobic conditions. In irradiation experiment, the prepared suspension was irradiated at constant dose rate 13 Gy/h for up to 9 weeks reaching 19,656 Gy total absorbed dose. The experiment was sampled regularly. We included both aerobic and anaerobic non-irradiated controls in this experiment to se the baviour of closed microcosm in aerobic and anaerobic condition without effect of radiation. Moreover, half of the anaerobic control microcosms were enriched in nutrients (nitrate, sulphate, acetate) to see the effect of nutrient availability on microbial composition in the closed microcosms. In pressure experiment, the prepared suspension was pressurized by 2 and 5 MPa in laboratory autoclaves for one month, non-pressurized control suspension was included in this experiment.

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We extracted DNA from all the samples, performed NGS of 16S ribosomal RNA gene amplicons to detect the microbial diversity in the samples. Detected microorganisms were metabolically characterized and were assigned to particular metabolic groups. Further, we performed quantitative PCR with several primers to quantify the total microbial biomass as well as the amount of nitrate reducers, iron reducers and sulphur reducers in the samples.

The composition of microbial communities within the closed bentonite suspension samples was changing continuously during the experiment. At the beginning, indigenous microorganisms present in the underground VITA water and BAM bentonite went through bottleneck during adaptation to specific environment of bentonite suspension. Most of the bacterial genera occurring in the VITA water disappeared and both total biomass and species richness was 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 (*Figure 4-15*, Figure 4-16). In the samples influenced by the presence of oxygen the community changes were based on the available electron donors evolving from heterotrophs (chemorganotrophs) to autotrophs (chemolitotrophs) with the decrease of available organic material. In anaerobic conditions limitation by both available electron acceptors and donors was detected. The communities evolved from heterotrophic facultative anaerobic nitrate reducers to autotrophic 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. Notably, overall microbial densities and IRB and SRB abundances were markedly higher under anaerobic conditions than in the presence of oxygen, which indicates, that anaerobic conditions are much more favourable for indigenous microorganisms in BaM bentonite.

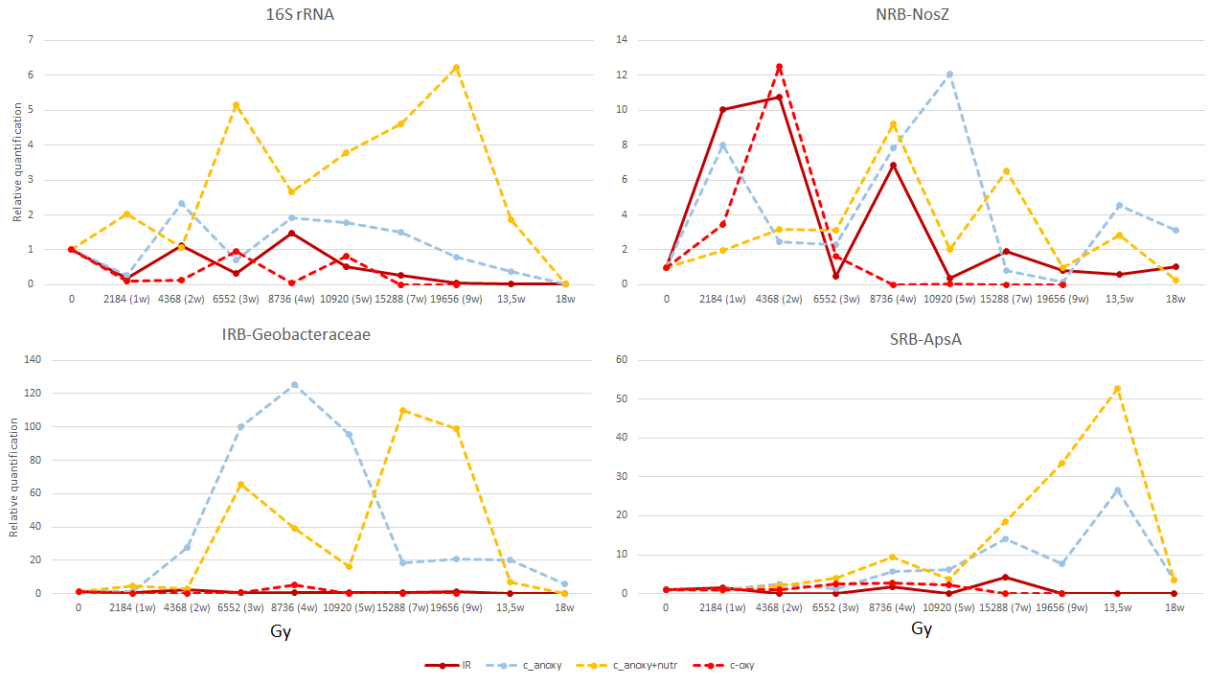

Figure 4-15: Relative quantification of changes in microbial abundance in irradiated (full line) and control (dashed lines) samples. IR – irradiated sample, c_anoxy – non-irradiated anaerobic control, c_anoxy+nutr – non irradiated anaerobic control enriched in nutritions, c-oxy – non-irradiated aerobic control. w – week.

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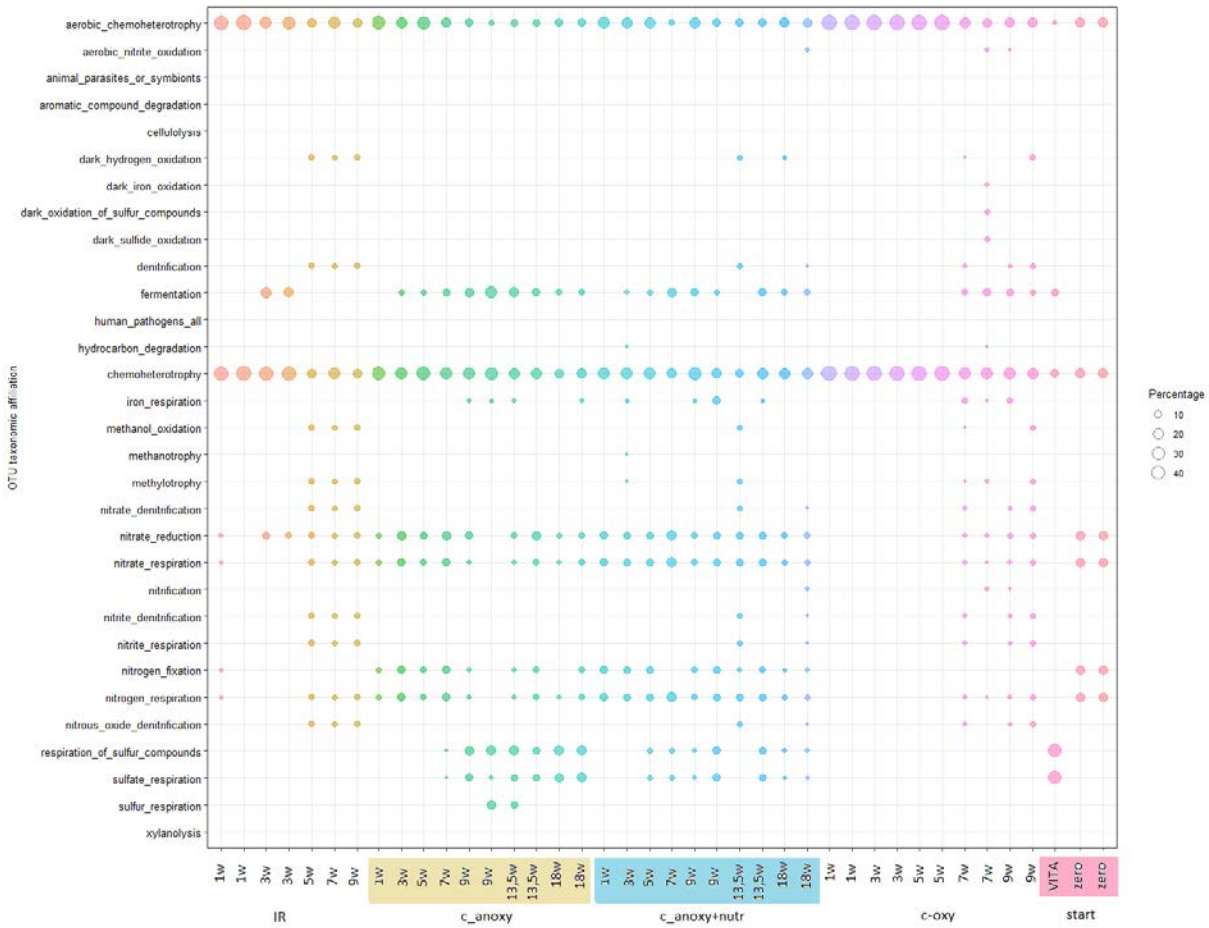

Figure 4-16: FAPROTAX analysis of detected OTUs showing the frequency of each of the features on y axis among detected OTUs in studied samples. About 25% of detected OTUs was succesfully used for the analysis. 1w – sampling after one week. VITA – original composition of underground water. Zero – composition of zero point bentonite suspension.

Only microorganisms which were able to adapt and survive under harsh conditions specific for the bentonite suspension environment underwent a selection caused by the effect of Gama radiation. In the aerobic bentonite suspension samples, 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. We observed decline in total microbial biomass in time in both the irradiated samples and aerobic control samples. The irradiated and control samples differed slightly in microbial composition. Gram-negative non-spore-forming microorganisms dominated the aerobic irradiated samples as well as aerobic controls although spore-formers are generally more radiation resistant. We ascribed this unexpected pattern to the presence of oxygen. As anaerobic control samples were dominated by Gram-positive spore-forming bacteria generally more resistant to radiation, we can expect that even higher absorbed doses of ionizing radiation would be needed to eliminate microbial activity under anaerobic conditions.

The results of irradiation experiment further showed a key role of iron reduction in the microbial processes occurring in the bentonite under anaerobic conditions. Ferric ions naturally occur in the bentonite molecular structure and their reduction might cause decrease of bentonite swelling ability and thus influence the performance and safety of the repository (Kim *et al*. 2004). To better estimate the effect of irradiation on microbial community changes in bentonite under repository conditions,

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subsequent irradiation experiments performed under strictly anaerobic condition and exposed to higher total absorbed doses are needed.

Exposure to 2 and 5 MPa pressure did not markedly reduce the total microbial biomass nor did substantially change the composition of the microbial community present in the bentonite suspension. Markedly higher pressure is probably needed to influence the microbial composition.

HZDR

HZDR compared two Bavarian bentonites – B25 (industrial, processed) and N01 (natural) – in microcosm-experiments. In these experiments, bentonite samples were supplied with synthetic, anaerobic Opalinus-clay pore water solution and filled up in serum vials, containing an N_2/CO_2-gas-atmosphere (in the ratio of [80:20]). For stimulating microbial activity and potential geochemical effects substrates were added (acetate, lactate or H_2). The set-ups were incubated in the dark, without shaking at 30°C and 60°C for one year. During this year bio-geochemical parameters (pH, redox potential, organic and inorganic metabolites) were monitored and the microbial diversity was analysed. Regarding these analyses, the B25 setups, incubated at 30 °C supplemented with lactate or hydrogen gas, respectively showed the most striking effects. Here we observed the formation of black and grey precipitates as well as the formation of fractures and cavities, very likely formed due to gas generation (figure 3-9). Additionally, in lactate-containing batches, the lactate was consumed as well as the sulphate. At the same time acetate was formed and the concentration of ferrous iron increased in the respective batches. Microcosms containing the N01 bentonite and/or were incubated at 60° C, respectively, did not show significant effects when lactate or hydrogen were present (data not shown)

Samples from selected B25 microcosms were analysed regarding their mineralogy with x-ray diffraction (XRD) and SEM, at the University of Greifswald. The SEM mapping analysis indicated the formation of iron-sulphur accumulations in hydrogen- and lactate-containing B25-samples (data not shown). These accumulations could not have been found within the raw material or samples without the addition of an external electron donor. Again, microcosms that were incubated at 60 °C and/or containing the N01 bentonite, did not show significant effects on the here addressed mineralogical parameters over the tested period of one year (data not shown). Thus, microbial activity under ideal conditions (30° C, supplemented with lactate or hydrogen) led to an alteration of minerals.

Regarding the analysed microbial diversity, we found that the diversity within B25 bentonite microcosms that incubated at 30° C changed dramatically compared to the raw material or control batches. The respective lactate- and hydrogen-containing batches revealed the presence and clear dominance (from 65 % up to 81 %) of sulphate-reducing and spore-forming organisms from the order Desulfosporosinus (data not shown). Species belonging to this order can use the here supplied lactate or hydrogen for the reduction of sulphite. As a result, the lactate- and sulphate-concentrations decreased whereas acetate, carbon dioxide and hydrogen sulphide were formed, which may potentially leads to the formation of the here observed fractures and cavities. The formed hydrogen sulphide can furthermore reduce ferric iron to ferrous iron, thereby potentially forming iron-sulphide species. This could explain the here observed increase of ferrous iron and concomitant decrease of ferric iron in the respective B25 microcosms (Figure 3-9).

A manuscript on the above described results is submitted to Environmental Science and Technology and is currently under review.

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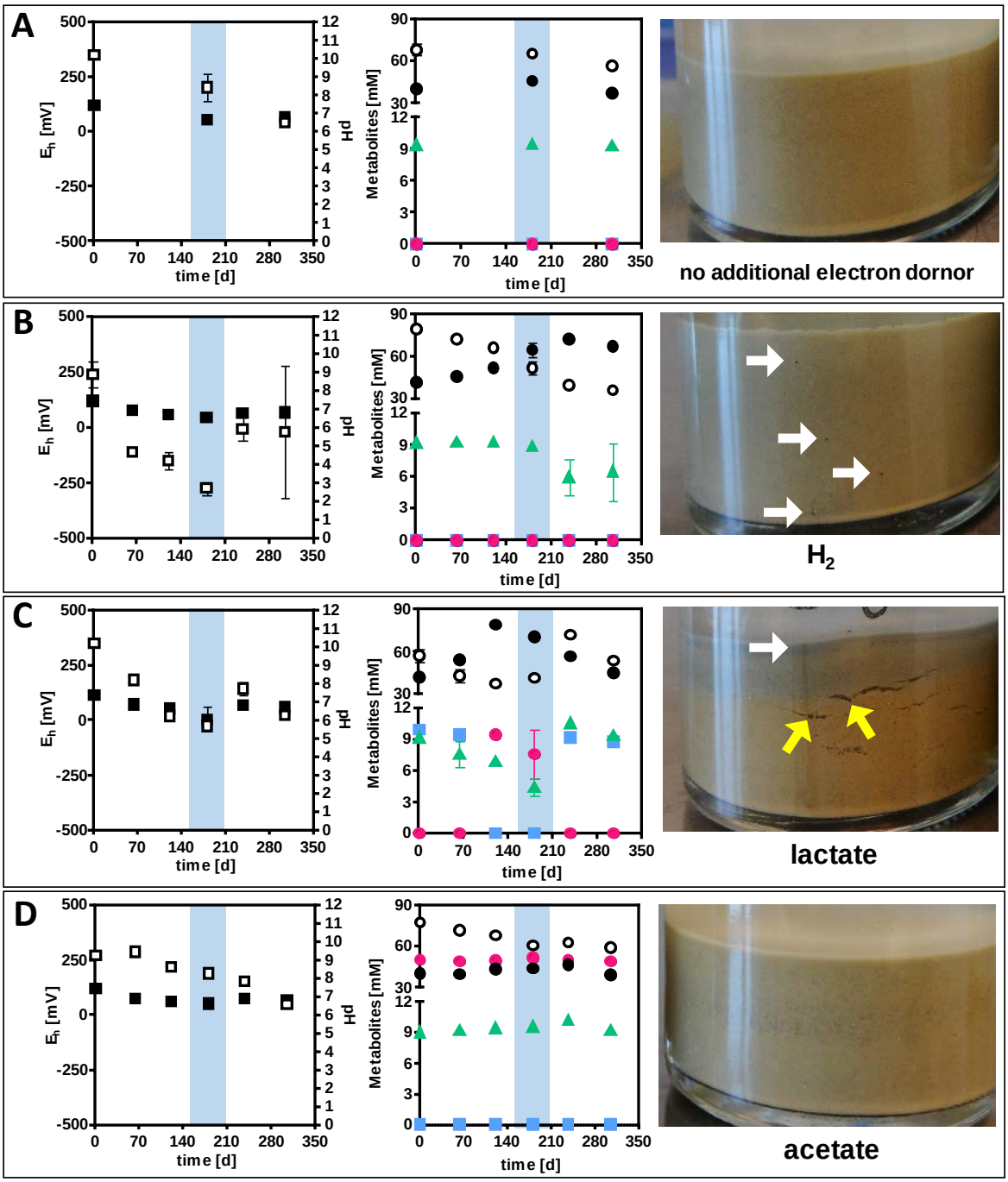

Figure 3-9: Evolution of geochemical parameters and metabolites in microcosms containing the commercial B25 bentonite incubated at 30 °C for 305 days. Shown are results from microcosms containing no substrate (A), 50 kPa H_2 (B) or 10 mM lactate (C). The left panel describes the evolution of redox potential (E_h;□) and pH (■). The right panel depicts the concentration of lactate (■), acetate (●), sulfate (▲), HCl-extractable ferric iron (○) and HCl-extractable ferrous iron (●). Shown are mean values with standard deviations from at least two independent microcosms. The photographic images show representative microcosms after 181 days incubation (blue bar in the left and right panels). White arrows indicate the formation of black spots and grey precipitates. Yellow arrows indicate the formation of fractures/cavities due to gas-generation.

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VTT

VTT long-term compacted bentonite experiment, which began in 1997 and was dismantled 2013, was further studied for microbiology in the MIND project. In the experiment Na-bentonite was compacted inside a copper cylinder, which was incubated in groundwater simulant both in oxic and anoxic conditions. Microbiological analyses included microscopic visualization and evaluation of bacterial and fungal communities by 16S rRNA and ITS amplicon sequencing. In addition, changes of chemical, mineralogical and microstructural parameters of bentonite were analysed. The results have been recently published by Vikman *et al.* (2018). The presence of living microbes on bentonite and on copper surface could not be demonstrated but microscopy studies revealed living microbial cells in the external water surrounding copper cylinders. According to the IonTorrent sequencing, SRB and iron reducing bacteria (IRB) were detected in bentonite matrix, water and copper surface. Fungal conidia and hyphae were detected by SEM in water and several groups of Ascomycetes and Basidiomycetes were identified from bentonite samples. Many of the fungal genera detected are able to produce organic or inorganic acids that help fungi in solubilisation of minerals from rock substrates (reviewed by Sterflinger, 2000).

VTT started a new long-term bentonite experiment in spring 2016 in order to simulate bentonite behaviour in circumstances that can take place in the interfaces of bentonite, host rock fractures and water flow in nuclear waste geological disposal. The bentonite was studied as a slurry in which water, gases, nutrients and microbes were able to move freely at the temperature hospitable for microbes that originated from bentonite and disposal site water and rock. The objective was to find out if microorganisms and the metabolites they produce were able to change the bentonite structure in hospitable conditions and if these changes could be significant for the bentonite stability in long-term. MX-80 bentonite microcosms after one-year of storage initiated both at oxic and anoxic conditions showed no essential changes in bentonite mineralogy compared to the experiment beginning (HAADF; HR-TEM). However, clear microbial activity in terms of ongoing sulphate reduction and sulphide formation as well as high number of sulphite reductase genes (*dsr*B) were detected in anoxic samples. Microbial activity also affected bentonite water phase chemistry and bentonite cation exchange capacity (CEC) already after a year of storage. These effects were not detected in abiotic controls demonstrating the microbial origin of these changes. In oxic microcosms oxygen was used steadily and after half a year only a trace of oxygen was left (D2.8). After two years of storage there were no significant changes detected in the bentonite samples compared to one year-samples analysed with atomic force microscopy (AFM) or CEC. The number 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 anoxic conditions than from the samples started with oxygen. The amounts of microorganisms remained at the same level or their amount decreased slightly as a function of storage time. Overall, microbial activity was lower in oxic than in anoxic microcosms and no sulphate reduction and only occasionally sulphite reductase genes were detected in the oxic microcosms during the two-year storage. 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 bentonite samples still remaining, have been amended with acetate and formate to accelerate the microbial activity. Only a week after the nutrient addition, the anaerobic samples showed visible black precipitates, not detected in abiotic control microcosms (Fig 3-17). This indicates activation of sulphate reducers and concomitant precipitaton of formed sulphide with iron. The experiment is planned to continue for some years further as potential changes in bentonite mineralogy caused by microbial activity happen slowly in the studied conditions.

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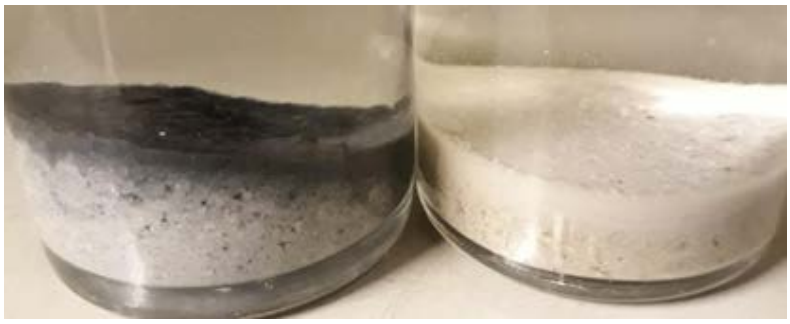

Figure 4-17. Precipitated iron sulphide (FeS) on top of a bentonite sample (left) after acetate and formate addition compared to an abiotic control bentonite sample with the same addition.

4.2.3 Conclusions

Significant acetate formation from natural organic matter present in the clays was detected in some of the studied bentonites. This production occurred at all wet densities and suggests that bacterial activity, *per see*, was possible also at densities where sulphide-production could not be detected. Acetate is known to induce stress-corrosion cracking of copper and other metals and the possible formation of acetate should, therefore, be further investigated. Analysis of mobility and reactivity of sulphide with the clays showed an immobilisation effect that can reduce the mass of sulphide that corrode metal canisters over repository life times (Pedersen, *et al*, 2017). However, the concomitant reduction of ferric iron may be problematic due to the destabilizing effect of ferrous iron on dioctahedral smectites such as montmorillonites.

Migration of natural bacteria from Josef URL was studied in compacted BaM bentonite from Czech Republic. Compaction to densities of 1400 and 1600 kg/m^3 did not prevent bacterial mobility in BaM bentonite. This finding is based on both fluorescence microscopy observation of live and dead cells and cultivation of anaerobic and aerobic bacteria from different sections of the saturated bentonites. BaM bentonite belongs to Ca-bentonites that might generally have better microbial permeability, than Na-bentonites, where only very limited bacterial mobility was reported. Further studies will be performed to verify our first results.

The study on effect of ionizing radiation on microorganisms in bentonite showed, that total absorbed doses of 19,656 Gy at 13Gy/h dose rate was not sufficient to eradicate the present microorganisms under aerobic conditions and probably much higher needs to be applied to sterilize bentonite. We further demonstrated, that microorganisms from underground water are probably very effectively selected by the bentonite environment and indigenous bentonite microflora thus represent the most important microbial group for the evaluation of repository safety. Indigenost bentonite microflora reaches higher abundances under anaerobic conditions than in aerobic conditions and further research on the effect of irradiation under strictly anaerobic conditions is thus needed.

4.3 Microbial activity in backfill and influence on plugs and seals

Contributing partners: SCK•CEN, MICANS

4.3.1 Problem statement & current knowledge

SCK-CEN

Various parts of the engineered barrier in the Belgian disposal system consists of cementitious materials. Cement is one of the matrices used for the immobilization of low- and intermediate level

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long-lived (LL-LILW) radioactive waste. In addition, the primary waste packages will be placed in a prefabricated concrete container after which the voids are filled with mortar and the container will be closed with a concrete lid (ONDRAF/NIRAS, 2013). For the disposal of high-level radioactive waste (HLW), the 'supercontainer' is selected as reference design in Belgium. In this concept, primary waste packages are surrounded by a carbon steel overpack which will be placed in a massive ordinary Portland cement based concrete buffer and, if needed, a stainless steel envelope (ONDRAF/NIRAS, 2013). The waste will be placed in a disposal gallery lined with concrete wedge blocks and after waste emplacement, all voids will be filled probably with mortar (ONDRAF/NIRAS, 2013). To avoid direct contact between the disposal waste package and the Boom Clay, a concrete end plug is foreseen at the end of the disposal galleries. Disposal galleries will be sealed, but it is not yet defined whether a bentonite or a concrete seal or a combination of both is preferable (ONDRAF/NIRAS, 2013).

The waste monolith will be designed such that it serves as permanent shielding for the workers during the operational phase, and the concrete will slow down radionuclide release. As such the concrete waste monolith provides a first safety barrier (Weetjens *et al.*, 2010, ONDRAF/NIRAS, 2013). Similarly, the supercontainer will form a first safety barrier by providing a permanent shielding for the workers during the operational phase. Additionally, in this case, the integrity of the engineered barrier system has to be ensured at least for the duration of the thermal phase of the waste after emplacement in the repository (several hundreds of years for vitrified HLW and a few thousands of years for spent nuclear fuel) (ONDRAF/NIRAS, 2013). In addition, in both cases, the high pH originating from the cementitious materials results in the passivation of the steel, hence reduces the uniform anaerobic corrosion rate (i.e. the main corrosion mechanism) (ONDRAF/NIRAS, 2013).

Several geochemical processes can affect the chemical evolution of concrete buffer such as chloride ingress, sulphate attack, alkali-silica reactions, carbonation and Ca^{2+} leaching, which are mostly followed by alteration of the microstructure what might change the physical properties (e.g. transport and mechanical properties) of the concrete (Phung *et al.*, 2017). Moreover, these processes can result in a local decrease in pH (Phung *et al.*, 2017), leading to niches where growth of microorganisms can be possible. In addition, at the interface of Boom Clay with the disposal gallery, the pH will be maximally pH 10.5 (Wang *et al.*, 2007). This pH is not expected to be limited for microbial activity (shown in MIND deliverables), thus, microbial activity might be present at the Boom Clay - disposal gallery interface of the repository. Furthermore, concrete has a low capacity for deformation under tensile stress, leading to the formation of (micro)cracks through which microorganisms are able to migrate and at the surfaces of cracks, they are able to form a biofilm (Kunin *et al.*, 2010, Zhu and Dittrich, 2016). Microbial degradation of cementitious materials in a wide variety of conditions is commonly known (Wei *et al.*, 2013), and can result in a loss of alkalinity, erosion, spalling of the concrete skin, corrosion of reinforcing bars, loss of water- or airtightness, and collapse (Bertron, 2014). In addition, microorganisms can produce organic and inorganic acids, which can be corrosive towards cementitious materials and can lead to a decrease in pH. On the other hand, microbial metabolic activity can lead to the formation of calcium carbonate (Zhu and Dittrich, 2016), which can result in partially clogging of the fractures, as the molar volume of calcite is larger than that of portlandite (Phung *et al.*, 2017). On the other hand, carbonation results in a decrease in pH and thus increased rate of rebars (Phung *et al.*, 2017). Consequently, the interactions and the evolution of cementitious materials with other repository materials, with the host rock and its ground water and with microorganisms need to be assessed.

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The backfill of many repositories will consist of bentonite and crushed rock in various combinations. They will constitute a source of electron acceptor and donors that can be utilized by microorganisms. Microorganisms are often active at interfaces and may therefore be active at the interfaces between cement plugs and seals and backfill too. A key issue is consequently how microbial activity in backfill will influence the long-term behaviour and integrity of plug systems and seals. Within different disposal concepts, cementitious materials can be an essential part of the plug and seal systems. The integrity of the cementitious materials should therefore be ensured over a long period of time. Microbial activity, although expected to be inhibited by high pH, might affect the performance of cement on the long term, mostly by either (i) the production of biogenic acids, thereby lowering pH and/or enhancing calcium leaching (a detrimental effect), (ii) the enhancement of carbonation, thereby clogging the cement pores (a desirable effect) or (iii) minor processes, like biologically induced sulphate release, triggering the production of the voluminous ettringite (also detrimental). Previous studies showed that the alkaline solution leached from high pH concrete induces dissolution and precipitation (Sánchez *et al.*, 2006). The cementation by secondary minerals such as zeolites may lead to degradation of the desirable properties of the buffer (Savage *et al.*, 2007). However, the significant chemically changes in mineralogy and physical performance occur only within a few centimetres at the concrete/clay surface and there are no changes at larger distances (Pusch *et al.*, 2003).

4.3.2 Summary of experimental & computational results

SCK-CEN

The objective of this study was the investigation whether microorganisms could affect the long-term evolution of the cementitious materials present in the engineered barriers of a geological repository for radioactive waste, under relevant *in situ* conditions. Should this be possible, it will be assessed if this impact is positive or negative. To fulfil this task, two different anoxic batch experiments were be performed in which microbial presence and activity and biofilm development on Ordinary Portlandite (OPC) CEM I was examined. In first instance, OPC CEM I was hardened for ca. 1 month and afterwards, Boom Clay borehole water (either sterile or harbouring a microbial community) supplied with nitrate and formate was added. After 49 days, samples were supplemented with acetate. In another series of experiments, sulphate was added as electron acceptor and these samples were supplemented with lactate as electron donor.

In the presence of OPC CEM I, the pH of the Boom Clay borehole water rapidly increased and after 14 days, in all conditions a pH > 12 was observed (initial pH ~ 9.5). In conditions with nitrate as electron acceptor, the pH was > 12 in all samples up to 89 days. In the presence of sulphate and OPC CEM I, the pH remained > 12 up to 94 days in all samples, except for one replicate containing the Boom Clay borehole water microbial community, where the pH dropped to pH 10 after 41 days and remained 10 up to 94 days. In addition, in another sample with the microbial community, the pH dropped to pH 10.44 after 94 days. These high pH conditions imposed by the OPC CEM I inhibit microbial nitrate reduction of the Boom Clay borehole water community. However, SEM analysis indicated the presence of intact cells in the supernatans and putative biofilm structures on the cement. This suggest that the high pH environment does not completely eliminate the microbial population. Similarly, in sulphate reducing conditions, the high pH environment seem to inhibit microbial activity but does not eliminate the microbial community. Interestingly, flow cytometry indicated the presence of a microbial community in the supernatans of one of the replicates in sulphate reducing

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conditions. Sulphate concentrations decreased in all conditions, including the negative control. Lactate was supplemented as calcium lactate and in the presence of high Ca^{2+} concentrations sulphate can built in the OPC CEM I (Pan *et al.*, 2016), hence this might explain the observed decrease in sulphate concentration. Interestingly, this decrease seemed much slower in the sample harbouring the microbial community in the supernatans. The extracellular polymeric substances (EPS) produced by the microbial community has been shown to interact with Ca^{2+} and result in the precipitation of $CaCO_3$ (Braissant *et al.*, 2007), thereby indirectly resulting in more sulphate in solution. In addition, carbonation might explain the observed decrease in pH from pH > 12 to pH 10 after 41 days (Phung *et al.*, 2017). However, more detailed analysis is planned to elucidate the precise ongoing mechanism. In addition, the present microbial community will be identified and the structure of the OPC CEM I will be investigated.

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MICANS performed laboratory experiments with compacted MX-80 bentonite on top of a concrete plug in titanium test cells. Additionally, glucose was added to the bentonite to induce microbial fermentation. For saturation of the clay an oxygen-free salt saturation solution was used. After incubation, the bentonite clay was analysed in profile for pH and microbial activity at four positions. Position 1 was the closed to the concrete plug and 4 the furthest. The analysis of the control test cell, which did not have a concrete plug inside, showed a constant pH of 9.4 at the four analysed positions. However, in the test cells with added glucose and a concrete plug the pH decreased from position 1 to 4. At position 1 the pH was on average 10 and at position 4 the pH was on average 8. In an additional test cell without glucose but concrete the pH stayed constant at 10 at all four positions.

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

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

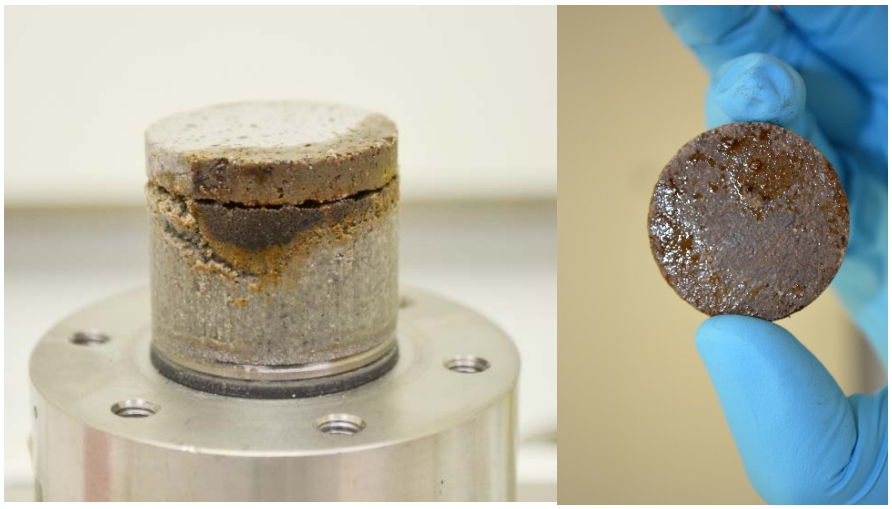

Figure 4-18. Image of compacted, water saturated and spiked MX-80 of test cell 15 after incubation (left) and the concrete plug surface which was in contact with the bentonite clay (right).

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4.3.3 Conclusions

SCK-CEN

The high pH environment imposed by the OPC CEM I likely inhibits nitrate reduction, however it does not completely eliminate microbial presence. Therefore, if niches arise with lower pH values, nitrate reduction can be induced. In addition, at the interface between the Boom Clay – gallery liner, the pH is not expected to be higher than 10.5, hence microbial activity likely can be present. On the other hand, in sulphate reducing conditions, the microbial community might be able to lower the pH of the environment, possibly creating more optimal conditions for other species within the microbial community. However, the precise mechanism needs to be elucidated with further analysis.

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The results of the microbial activity experiments indicated that alkaline pH of 10 caused by the alkalic concrete likely will inhibit the growth of bacteria.

4.4 WP2 – summary and conclusions

Work in WP2 addressed the behaviour and technical feasibility and long-term performance of repository components. Key issues for the geological disposal of high-level waste concern the factors controlling sulphide production in the geosphere, including to what extent bacteria can accelerate canister corrosion in the near-field either by H_2 scavenging or by sulphide and/or acetate production. Further, it is important to identify conditions under which relevant bentonites inhibit bacterial activity, and to understand whether bacteria can accelerate degradation of bentonite-based buffers and influence the long-term behaviour of plug systems and seals.

Microbial production of sulphide in the geosphere

Two deliverables summarized knowledge on occurrence of sulphur compounds in groundwaters of Finnish crystalline bedrock. The potential role of rock matrix fluids and gases for sulphide production were identified as important issues. One deliverable describes results from simulations of deep groundwater mixing and infiltration, which introduce limiting electron donors and acceptors for microbial communities and their activation. The understanding of the limits of the deep life in the geosphere was expanded. Presence of electron acceptors in sufficient amounts was found to be one of the most important controlling factors for sulphide formation. Consequetly, the MIND project has identified factors of importance for sulphide production in the geosphere.

Microbial induced corrosion of canisters

Work describing bacterial sulphide-production in bentonites compacted to various densities and swelling pressures was reported previously. The sulphide produced in the clay was found to corrode copper specimens. When bulk bentonite near the corrosion steel coupons was studied, there was no evidence of abundant sulphate-reducing bacteria. In contrast, on the surface of a canister, and on the surface of the bentonite, there were black spots, in which sulphate-reducing bacteria clearly were present and growing. With respect to microbially influenced corrosion, four deliverables report that sulphide-producing bacteria can be active and produce sulphide in buffer and backfill. They also identify constraining factors including time. The corrosion related processes observed are slow and several deliverables identified the need for longer-term experiments to be able to conclude on the extent and rates of MIC under repository conditions. These may vary from repository concept to concept and also between clays and canister materials.

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Microbial activity in bentonite buffers

Several partners report that cultivable sulphide-producing bacteria (SPB) were present in all investigated clays, irrespective of density and swelling pressure. Large bacterial and fungal diversities in bentonites were demonstrated using 16S rDNA sequencing and fungal internal transcribed spacer (ITS) gene markers, respectively. One partner specifically reports representatives of genera *Nocardia*, *Pseudomonas*, *Pseudonocardia*, *Saccharopolyspora* and *Streptomyces*. Another found the spore-forming SPB *Desulfosporosinus* to be common in the studied clay. Generally, analysis of DNA sequences in libraries of DNA extracted from various bentonites showed a large diversity of bacteria including SPB, and there is commonly a large proportion of spore-forming bacteria, a survival form well adapted to dry clays. Visualisation methods for cells in clays have been developed and tested on bentonite buffer from the full scale FEBEX. They both rely on a density gradient centrifugation step. The MIND project thoroughly confirms previously published and reported data that have shown microbes to be present in commercial clays as well as in compacted clay in laboratory and in field scale experiments simulating buffer and backfill conditions in future radioactive waste repositories.

Results from repeated experiments with different bentonite types suggested a limit for microbial activity at approximately 1000 kPa in swelling pressure. Exposure to 2000 and 5000 kPa of BaM bentonite did not markedly reduce the total microbial biomass or the community composition. Similar results were obtained with Volclay MX80 during ongoing experiments in Mont Terri Underground Research Laboratory (URL). Experiments in the laboratory also showed a limited effect of pressure (74 000 kPa 30 sec.) on survival of bacteria. Independent investigations repeatedly showed that SPB can form local colonies in compacted clays, likely in positions were impurities of the clay offer enough large pore space for bacterial life and where the intended swelling pressure is mitigated. In short, the MIND project has identified swelling pressure as an important limiting factor for microbial activity, but not for presence and survival. Although microbial colonisation of bentonite barriers may help attenuate the release of priority radionuclides, control of iron and sulphate-reducing bacterial activities will be important to maximise the physical integrity of the bentonite barrier and to prevent corrosion of the metal canisters. Thus, the careful selection of bentonite materials (e.g. with low microbial presence) and achievement of appropriate swelling pressures (to control microbial proliferation) is crucial to bentonite buffer, and canister stability.

Microbial degradation of bentonite buffers

It was tested if microbial activity had an influence on the stress from swelling in clay samples, but effects, if any, were too small for detection of the used system. In addition, it was found that corroding steel influenced the clay in the vicinity of the steel, but the results did not show any obvious effect from samples with SRB compared to sterile samples. The effect from sulphide on bentonite samples has been studied in MIND. It was found that sulphide reacts with ferric iron in the tested clays under the formation of FeS and elemental sulphur (S^0). Thus, the MIND project has found limited impact from microbial activity on bentonites with the exception of sulphide produced by active SPB that reacts with clay components, mainly ferric iron.

Microbial activity in backfill and influence on plugs and seals

On partner found that the high pH conditions imposed by the cement inhibited microbial sulphate and nitrate reduction. However, the presence of intact cells in the suspension on top of cement and putative biofilm structures on the cement was indicated by scanning electron microscopy investigations. Interestingly, in sulphate-reducing conditions, a pH decrease from >12 to pH 10 was observed. It was also concluded by another partner that the alkaline solution leached from the

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concrete inhibited microbial activity due to an increased pH in the bentonite clay. Induced microbial activity in MX-80 with glucose led to a decreased in pH. A reason might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. In conclusion, the MIND project has demonstrated that microbial activity may decrease pH in high alkaline repository barriers.

5 Cross reference to the IRB review

As described in the grant agreement of the MIND project, this synthesis reports lists and explains the key biogeochemical topics and processes that are addressed in the experimental work packages WP1 and WP2. In addition, it gives an overview of the performed experiments and results and their relevance for geological disposal facility performance and safety, as well as the remaining issues to be addressed and knowledge gaps and uncertainties that (still) exist.

Summaries of progress of the technical work undertaken in WP1 and WP2 and conclusions are provided above (Sections 3.9 and 4.4). In parallel, to the technical work undertaken in WP1 and WP2 the Implementers Review Board (IRB) have reviewed the research findings. The IRB have related the outcomes of the MIND research to specific areas:

1. The swelling of bentonite and its role in preventing sulphate reduction, sulphide migration and “microbial induced” corrosion
2. Microbial mediated gas generation including gas consumption and gaseous C-14 containing species
3. Microbial induced organic waste matrix degradation
4. Microbial enhanced processes potentially affecting radionuclide transport / migration
5. Limiting microbial impacts in repositories, including limits of microbial activity and effect of heterogeneity

Conclusions from WP1 and WP2 that relate to these areas is summarised as follows

5.1 Swelling of bentonite

Bacterial life, survival and activity will depend on several different variables in a buffer or backfill that relate to the water saturation and swelling of bentonites compacted to high density. Consequently, it is not density *per see* that controls microbial activity in compacted clays. Rather, other factors that to some extent are related to the density and type of clay, control microbial activity.

- The pH of most bentonite clays is slightly alkaline but still well within the range of what most bacteria can tolerate.

- In radioactive waste repository concepts, the maximum surface temperature of canisters may not exceed 90°C in order to avoid formation of steam when water comes in contact with the canister. It was previously found that heat treatment at 120°C for 15 h or 110°C for 170 h did fail to kill inherent bacteria in the bentonite. Heat was expected to be efficient, but that was still not enough to kill off sulphide producing bacteria in the bentonite because intensive sulphide-producing activity and large numbers of cultivable sulphide producing bacteria were observed in MX-80. Bentonite or rather montmorillonite, has a verified high affinity for water and the cell membrane of bacterial cells is water permeable. If a bacterial cell is surrounded by bentonite, it is possible that the water affinity of montmorillonite will extract water from

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the cell, leaving it in a desiccated state. The phenomenon of drying cells for prolonged disposal is well known and commonly used in microbiology. Slow desiccation can yield higher viability, after prolonged disposal, than can fast and also increased heat resistance and viability for both spores and vegetative cells. Bacteria consequently have several mechanisms to survive prolonged periods of exposure to heat (See D2.4 for details).

- Transport of nutrients to and metabolic products from bacteria will be diffusion limited due to the low porosity of buffers and backfill. Bacterial activity will, consequently be diffusion limited in backfill and buffers when the bentonite buffer and backfill are fully water saturated. The only position not affected by diffusion barriers will be the interface between rock/aquifers/engineered disturbed zone and buffer and backfill.

- The swelling pressure in the bentonite originates from separating flocs in the bentonite. This means that a mechanical pressure arises between the separating flocs, approximately equal to the swelling pressure. Even in low-density bentonites (1500 kg/m^3), a pore size in the nm range would theoretically not allow for bacterial existence unless the bacteria could withstand the mechanical pressure from the separating flocs (0.09 MPa at 1500 kg/m^3). Prokaryotic cells can compensate for the mechanical pressure in compacted bentonite by turgor pressure. Published data on turgor pressure in prokaryotic cells mention pressures between 0.08 MPa and 2 MPa 28. An upper limit of 2 MPa turgor pressure would mean that cell integrity is possible, though limited, at bentonite swelling pressures below 2 MPa. However, endospores can survive a much higher pressure.

- Water is needed for active bacterial life and this water must be present externally because bacteria (except spores) cannot keep water inside their cell membranes that are freely permeable for water. Low water content in clays will inactivate or kill bacteria. However, as was discussed for temperature above, many bacteria survive desiccation and can be activated again when there is enough water.

- Bacteria are very small and if there are pores or other heterogeneities in buffer and backfill with lower than planned pressures, local microbial activity will be possible. In addition, there will be interfaces between rock engineered disturbed zone and bentonite and between bentonite and canisters at which pressures may differ from the bulk of buffer and backfill. New results from several partners show that sulphide-producing bacteria can form local colonies in compacted clays, likely in positions were impurities of the clay offer enough large pore space for bacterial life.

- The pore water composition will vary with the type of bentonite and the composition of the saturating groundwater. Bentonites vary in composition with respect to elements and minerals and the type and content of natural organic matter. The conditions for survival and activity of bacteria may, consequently vary significantly between different bentonite types as inferred by the variation in the highest wet density at which sulphide production can be detected in compacted clays.

5.2 Gas generation and consumption

Gas generation and consumption mainly concerns WP1 where further insight into the process of methanogenesis has been obtained from the TVO gas generation experiment (GGE), Olkiluoto,

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Finland. Here interpretation of the geochemical data collected over 20 years together with biogeochemical modelling (Small *et al*, 2017) and detailed microbial analysis of samples (D1.6, Vikman, 2019) has correlated increasing rates of methane generation to homogenisation of the pH of the experiment to conditions < pH 9. Further observations from the data interpretation also include a decline in aqueous sulphide concentration to below 10^{-6} M related to slow equilibration with FeS (Mackinawite) over 8 years, correlated with a doubling of the rate of methanogenesis. DNA and RNA sequencing has revealed that the increased gas generation relates to the later growth of organic consuming methanogens with H_2 consuming methanogens being developed initially. This conclusion supports the biogeochemical modelling and observations of decreased dissolved organic carbon at later times. The GGE data and modelling confirm that hydrogen gas, formed by anaerobic corrosion of steel, is readily consumed by the microbial processes occurring during the degradation of cellulosic LLW. In addition to methanogenesis, sulphate reduction is likely to consume hydrogen in the GGE.

The MA experiment at the Mont Terri Rock Laboratory has further investigated methanogenesis and the consumption of hydrogen under *in situ* conditions. Building on previous work that has demonstrated rapid hydrogen consumption by sulphate reduction processes (Bagnoud *et al*, 2016a). D1.7 reports that methanogenesis fuelled by hydrogen injection in the borehole could not be established by repeated injection with hydrogen. Methanogenesis is supressed by competition with sulphate reduction processes even where sulphate concentration in the borehole was reduced from 12 mM to 4 mM. It is postulated that under the conditions in Opalinus Clay methanogenesis is limited by low bicarbonate content. However, this could not be confirmed through microcosm experiments, where also no methane was produced. On the other hand, methanogenesics archaea have been found as most dominant species during an *in situ* experiment with a porewater saturated porous medium (80% sand: 20% bentonite, w/w) and H_2 addition. Further laboratory tests are needed to unravel the importance of trace metals and nutrient availability to the onset of methanogenic processes. Consumption of hydrogen under *in situ* conditions at Mont Terri has also been demonstrated in the BN experiment, where hydrogen is used as an electron donor for denitrification processes. Here the MIND project has enabled the development of biogeochemical reactive-transport models of sulphate and nitrate reduction that confirms the likely autotrophic nature of such H_2 injection experiments with associated effects of pH increase and decreasing bicarbonate concentration, which could limit the extent of the H_2 consumption reaction (D1.8).

In summary, the MIND project has provided further insights into the chemical conditions that may limit methanogenesis and hydrogen consumption in LLW/ILW repositories. The research related to H_2 consumption by sulphate reduction processes is also of relevance to microbial induced corrosion of metal canisters (IRB topic 1).

5.3 Organic waste degradation

Microbial effects on organic waste degradation are the focus of Task 1.2 of WP1 and are summarised in Section 2.9. Key points or relevance to safety, (e.g. gas generation and radionuclide complexation) are highlighted as follows:

- Irradiation of cellulose under hyperalkaline conditions (pH 12.7) enhances the rate of the abiotic hydrolysis of cellulose by alkali and increases the production of higher concentrations of the radionuclide complexant ISA. The total dissolved organic carbon content was also increased by irradiation making the cellulose potentially more bioavailable for microbial gas generation.

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- Microbial activity was initiated in microcosms at a starting pH of 12.5, probably in low pH regions of the cellulose. Fermentation of the cellulose degradation products (including ISA) at starting hyperalkaline pH led to the production of H_2, and acetate by fermentation processes and a decrease in pH. Microbial activity was initiated in microcosms at a starting pH of 12.5, probably in low pH regions of the cellulose, and is thus relevant to IRB topic 5, below.
- Additives present in plasticised PVC are able to fuel nitrate reduction at pH 10. Irradiation of plasticised PVC renders it less bioavailable, possibly due to binding of the additives with the polymer. It remains to be seen if such plasticisers could significantly contribute to methane gas generation.
- Biodegradation of pure PVC polymer is negligible at pH 10, but there is some evidence of enhanced biodegradation after irradiation. Overall, given the lack of metabolism in the presence of nitrate, which provides a relatively high energy source, the PVC polymer itself is unlikely to contribute significantly to microbial gas generation.
- A range of organic additives in PVC were characterised that could fuel microbial activity and further work may be required to further refine the types of additive that are biodegradable and which can complex radionuclides. Phthalate, the breakdown product of phthalate ester additive, under irradiating and high pH conditions, was not found to support nitrate reduction at pH 10.
- The biodegradation of bitumen radiolysis products under alkaline conditions has been examined. Nitrate leaching from Eurobitum rapidly stimulated microbial nitrate reduction including under pH 10.5 conditions typical of the interface of the EBS. Formation of nitrite as an intermediate could decrease (spontaneously) the reducing capacity of the Boom Clay and thus has the potential to increase the mobility of certain radionuclides.
- Work concerning the biodegradation of irradiated ion exchange resin has shown a variety of gaseous radiolysis products with potential to support microbial activity. The complexity of the composition of resin irradiation products is significantly greater than previously thought and includes CO_2, H_2 and a number of other metabolisable organic compounds.
- Studies examining the bacterial colonisation of irradiated resins have demonstrated bacterial growth provided that the pH doesn't become too acidic by the resin irradiation.

Overall, the MIND project has increased the knowledge base concerning the range of organic polymers and additives that may contribute to gas generation from LLW/ILW including the combined effects of irradiation and biodegradation under alkaline conditions. Further knowledge has also been obtained regarding the biodegradation of potential radionuclide complexants, including ISA under alkaline conditions.

5.4 Effect on radionuclide transport

This IRB topic area is broad and includes effects on pH/Eh, radionuclide complexation and more direct bioreduction and biosorption type interactions between microbes and radionuclides. Following previous advice from Waste Management Organisations during the definition of the proposal, the scope of the MIND project in these areas is limited.

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Nevertheless, the MIND research has provided several novel cases, which where microbial activity has induced resulted in solid partitioning of radionuclides, which could have significance to their mobility in groundwater:

- Sanchez-Castro *et al*, 2017, 2018; Ruiz-Fresneda *et al*, 2018 and D1.5 discuss the formation of Se(0) nanoparticles by bioreduction of selanate and selenite by a bacterium isolated from Spanish bentonite, apparently as a mechanism to control Se toxicity.
- In further applied experiments examining Ni speciation in cellulose degradation microcosms Kuippers *et al* (2018) discusses complex mechanisms by which Ni may be immobilised by sulphide phases formed as a consequence of sulphate reduction fuelled by cellulose degradation.

D1.4 (Section 3.5.2) presents new data and knowledge concerning uranium complexation with cellulose hydrolysis and biodegradation products, ISA and acetate, which will be an input to the assessment of the mobility of uranium, albeit in the case of more soluble U(VI).

MIND research concerning methanogenesis discussed above (Section 5.2) is relevant to understanding the speciation of C-14, particularly that present in LLW and ILW. Here the transfer of C-14 to the gas phase as $^{14}CH_4$ by microbial processes is of primary importance. Additionally, the fate of ^{14}C labelled soluble organics such as cellulose degradation products and fermentation products will be of significance to migration of C-14 in groundwater as such organic species will be less easily retarded than inorganic carbonate species, which will be prone to precipitation under high pH cementitious conditions. Here, the mechanism of cellulose degradation and transient stability of dissolved organic species in the Finnish gas experiment (GGE) has relevance to the discussion of C-14 release from LLW and ILW.

The GGE is of further relevance to assumptions made in safety cases concerning the Eh equilibrium that is used as a basis to calculate the speciation of multivalent radionuclides such as Tc, U, Np and Pu assuming thermodynamic equilibrium. The MIND interpretation of the GGE geochemical data (Small *et al*, 2017) presents an analysis of the Eh electrode measurements from the GGE that are consistent with the control of Eh by the sulphide/sulphate redox couple that is calculated from measured concentrations of sulphate and sulphide after a period of over 10 years. Such interpretation builds confidence in the assumption of thermodynamic redox equilibrium made in safety assessments.

5.5 Limitations on microbial activity in repositories

This IRB topic is of common relevance to both MIND WP1 and WP2. In the case of WP2 the issue is of key concern as the bentonite buffer is commonly considered to have a safety function of preventing microbial activity in the vicinity of the HLW or spent fuel disposal canister.

To keep consensus of the terminology used to describe degrees of bacterial metabolisim these definitions are important to follow:

- Activity: microorganisms are active and have an ongoing metabolism.
- Presence: microorganisms exists in the sample or at the sampled site and they are present as live or dead cells.
- Viability: microorganisms are alive and active or exists as dormant cells, which have the power to become active when presented with a favourable environment.

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- Cultivability: microorganisms can be cultivated in vessel with liquid media or on agar plates.

While limitations to microbial activity are rather straightforward to define, it is more complicated to evaluate limitations with respect to presence, viability and cultivability. All active microbial life require access to electron donors for energy and acceptors for respiration. For growth activity, they also need a range of different elements of which they are composed. Further, specific sets of environmental conditions such as temperature, pH, radiation and water activity will select for various adaptations, generally reflected in microbial diversity indexes. The typical repository environment will be transport limited, something that is required in the safety to reduce radionuclide migration in cases with canister failure. A conservative approach to the evaluation of limitations of microbial activity in repository environments should encompass transport rates of electron donors and acceptors and elements to, and metabolites from microbial life, all other parameters set as positive.

Similarly, for WP1 concerning microbial activity in cementitious LLW/ILW some safety cases assume that microbial activity will not occur where hyperalkaline pH conditions (pH > 12.5) are maintained. Here, large proportions of concrete materials are used to maintain the high pH condition for the purpose of limiting microbial gas generation (methanogenesis) and the release of gaseous C-14. Such a hyperalkaline safety function is however a less clear cut as for cellulose containing waste as it will promote the generation of ISA; a very strong radionuclide complexant.

MIND WP1 research has provided further evidence that the upper pH limit for microbial activity is dependent on the energy that can be provided for a specific electron accepting process. Considering only anaerobic processes MIND WP1 studies at high pH have considered and observed mainly nitrate reduction at the pH 10-11 range and have readily observed activity for PVC additives (Nixon *et al*, 2018; D1.2) and for known bitumen radiolysis products (D1.3). Moreover, D1.3 showed that although no nitrate reduction was observed at pH 12.5, the microbial community was not completely eliminated and could be revived. Irradiated cellulose microcosm experiments that had a starting pH of 12.5, developed fermentation processes, yielding H_2 acetate and other volatile fatty acids despite the initial high pH and low energy yield of the fermentation reactions (D1.2). It is likely that such processes developed in low pH regions of the cellulose, and the acidity generated neutralised the pH of the calcium hydroxide present. Similar observations are made in the case of the Finnish gas generation experiment (GGE) discussed above (Small *et al*. 2008; 2017) where initially heterogeneous pH conditions, ranging from neutral pH in waste drums to pH 11 in tank water, initially buffered by concrete are neutralised and homogenised over a period of around 10 years.

Heterogeneity in pH occurring at various scales from cellulose microcosm experiments to that of GGE is thus of key importance to establish the upper limit of microbial activity. Where such heterogeneity exists, it probably cannot be ruled out that low energy yielding anaerobic processes such as fermentation and methanogenesis may occur. The extent of such processes and their ability to affect the bulk pH of the system (as in the GGE) will however depend on the proportions of concrete to cellulose materials in the inventory. Most LLW and ILW concepts contain a significantly higher proportion of concrete than that studied by the GGE.

6 General conclusions

The main objective of the MIND project was to increase the understanding of how microbial processes can influence the safety and performance of future geological disposal repositories in the EU by focussing on key topics defined in the most recent version of the IGD-TP strategic agenda (Version July 14, 2011).

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Overall, the MIND project was a first of a kind highly scientific and technical research project gathering a multidisciplinary team including geo-microbiologist, geochemists, geologists, engineers, modelling experts, bio-informaticians, performance assessment experts & social scientists spread over 15 institutes from 8 European countries, including members of far advanced as well as less advanced programs. Moreover, the team was clearly gender inclusive (good gender balance: 49 % female versus 51 % male) and stimulated cross-discipline knowledge exchange. Such a multidisciplinary and dynamic research network can be highly valuable for future projects. Moreover, international cooperation is essential for sharing competences where the national contexts is too small, especially on issues essentially only of interest to the nuclear waste community. MIND has reached far beyond the EU borders, and attracted interest and enabled interactions with non-EU member states on these topics (e.g. Canada, Japan). The clear synergies with the other non-EU programs should be explored further with benefit for both parties involved. In the future, such knowledge management and international cooperation will be even more important. Guidelines and other recommendations issued by the international agencies, on how to deal for example with the microbiological issues in geological waste disposal, will not only be important for programmes being developed but would also serve as a fundamental memory to far advanced programmes, when the experts being authoring such guides now have retired or soon will retire.

The work within the project resulted in a significant advanced scientific knowledge on possible microbial processes in a geological repository and further increased the knowledge on the limits of life as shown in the 42 deliverables and > 20 publications. Moreover, the MIND members presented their findings on several international conferences, organised dedicated discussion sessions with peers, and are preparing a book assembling all the gathered scientific knowhow. Obtained results were presented to and discussed with the IRB on a regularly basis to clarify what issues are of potential importance and how further efforts in the areas should be prioritized. Moreover, gathered knowledge was synthesised and linked to the gaps defined by the waste management organizations. Those discussions with the IRB stimulated and allowed extensive networking on a detailed level directly with a broad range of researchers and other experts.

In addition to the experimental and evaluation work, the MIND project created a unique platform for education and training for the scientific community. Multiple young scientists (Phd and PostDoc) were associated to the project in the different partnering groups, and an internship and exchange visit platform from Master and PhD students and professionals was created. In addition, several workshops, discussion sessions, and joint experiments were organised to compare particular protocols applied in the different teams. Different partners could learn from each other and bring their skills to a higher level. Furthermore, two dedicated courses regarding 'geomicrobiology in nuclear waste disposal' were organised during the project and various contributions to other existing training programs were made. The training material developed in MIND is modular and can be incorporated in existing and future training modules on radioactive waste management ranging from basic level courses to highly advanced courses.

This new knowledge base is the perfect building ground to further define and refine the still existing and newly described knowledge gaps, in the new research and technical programs to follow. Moreover, a future project should further build the bridge from the knowledge data towards actual performance and safety assessments for the different disposal concepts.

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7 References

Abrahamsen, L., T. Arnold, H. Brinkmann, N. Leys, M. Merroun, K. Mijnendonckx, H. Moll, P. Polvika, A. Ševců, J. Small, M. Vikman and K. Wouters (2015). A Review of Anthropogenic Organic Wastes and Their Degradation Behaviour. Deliverable 1.1. MIND.

Ahmad, S.I., 1996. A mutant of *Escherichia coli* hyper-resistant to a number of DNA damaging agents: location of the mutational site. J. Photochem. Photobiol. B 36, 47–53.

Appelo, C.A.J., 2013. A review of porosity and diffusion in bentonite. Posiva Oy.

Azzam, E.I., Jay-Gerin, J.-P., Pain, D., 2012. Ionizing radiation-induced metabolic oxidative stress and prolonged cell injury. Cancer Lett. 327, 48–60.

Bagnoud, A., Chourey, K., Hettich, R.L., de Bruijn, I., Andersson, A.F., Leupin, O.X., Schwyn, B., Bernier-Latmani, R. 2016a Reconstructing a hydrogen-driven microbial metabolic network in Opalinus Clay rock. Nature Communications; 7:12770.

Bagnoud, A., I. De Bruijn, A. F. Andersson, N. Diomidis and O. X. Leupin *et al*. 2016b. A minimalistic microbial food web in an excavated deep subsurface clay rock. FEMS Microbiology Ecology, 92(1), fiv138.

Bagnoud,A., O. Leupin, B. Schwyn and R. Bernier-Latmani. 2016c. Rates of microbial hydrogen oxidation and sulphate reduction in Opalinus Clay rock. Applied Geochemistry, 72, 42-50.

Bassil, N. M., Bewsher, A. D., Thompson, O. R. & Lloyd, J. R. (2015a). Microbial degradation of cellulosic material under intermediate-level waste simulated conditions. Mineral Mag 79, 1–9.

Bassil, N. M., Bryan, N. & Lloyd, J. R. (2015b). Microbial degradation of isosaccharinic acid at high pH. ISME J 9, 310–320. Nature Publishing Group.

Bassil, N and Lloyd, J (2017) Draft Genome Sequences of Four Alkaliphilic Bacteria Belonging to the Anaerobacillus Genus. Genome Announcements, Volume 5, Issue 3, e01493-16 doi: 10.1128/genomeA.01493-16

Bassil, N and Lloyd, J (2018) Anaerobacillus isosaccharinicus sp. nov., an alkaliphilic bacterium which degrades isosaccharinic acid. International Journal of Systematic and Evolutionary Microbiology. https://doi.org/10.1099/ijsem.0.002721

Beller, H.R., Spormann, A.M. Benzylsuccinate formation as a means of ananerobic toluene activation by sulfate-reducing strain PRTOL-1. Applied and Environmental Microbiology 1997; 63(9):3729-3731.

Bengtsson, A., Blom, A., Hallbeck, B., Heed, C., Johansson, L., J., S., Pedersen, K., (2016). Microbial sulphide-producing activity in water saturated MX-80, Asha2012 and Calcigel bentonite at wet densities from 1500 to 2000 kg m−3. SKB report TR 16-09. Swedish Nuclear Fuel and Waste Management Co, Stockholm Sweden, 1-96.

Bengtsson, A., Blom, A., Johansson, L., Taborowski, T., Eriksson, L., K., P., (2017a). Bacterial sulphide- and acetate-producing activity in water saturated Rokle and Gaomiaozi bentonite at wet densities from 1750 to 1950 kg m−3. SKB report TR 17-05. Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden, 1-38.

Bengtsson, A., Blom, A., Johansson, L., Taborowski, T., Lena, E., Pedersen, K., 2017b. Bacterial sulphide- and acetate-producing activity in water saturated Calcigel bentonite cores in a wet density

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

gradient from 1 750 to 2 000 kg m−3. SKB report 17-18. Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden, 1-26.

Bengtsson, A., Edlund, J., Hallbeck, B., Heed, C., Pedersen, K., (2015). Microbial sulphide-producing activity in MX-80 bentonite at 1750 and 2000 kg m–3 wet density. SKB Report R-15-05. Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden, 1-47.

Bengtsson, A., Pedersen, K., (2016). Microbial sulphate-reducing activity over load pressure and density in water saturated Boom Clay. Applied Clay Science 132-133, 542-551.

Bengtsson, A., Pedersen, K., (2017). Microbial sulphide-producing activity in water saturated Wyoming MX-80, Asha and Calcigel bentonites at wet densities from 1500 to 2000kg m−3. Applied Clay Science 137, 203-212.

Bengtsson, A., Blom, A., Taborowski, T., Schippers, A., Edlund, J., Pedersen, K., (2017). FEBEX-DP: Microbiological report. NAB 16-015. National Cooperative for the Disposal of Radioactive Waste, Wettingen, Switzerland.

Berner, U. R. (1992). Evolution of pore water chemistry during degradation of cement in a radioactive waste repository environment. Waste Manag 12, 201–219.

Bertron, A., H. Ranaivomanana, N. Jacquemet, B. Erable, C. Sablayrolles, G. Escadeillas and A. Albrecht (2013). "Physico-chemical interactions at the concrete-bitumen interface of nuclear waste repositories." International Workshop Nucperf 2012: Long-Term Performance of Cementitious Barriers and Reinforced Concrete in Nuclear Power Plant and Radioactive Waste Storage and Disposal (Rilem Event Tc 226-Cnm and Efc Event 351) 56.

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

Bleyen, N., A. Mariën and E. Valcke (2017a). The geochemical perturbation of Boom Clay due to the $NaNO_3$ plume released from Eurobitum bituminised radioactive waste: status 2013. External Report. Mol, Belgium, SCK-CEN. SCK•CEN-ER-221.

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

Bradbury, M.H., Baeyens, B., 2003. Porewater chemistry in compacted re-saturated MX-80 bentonite. J. Contam. Hydrol. 61, 329-338.

Bradbury, M.H., Berner, U., Curti, E., Hummel, W., Kosakowski, G., Thoenen, T., 2014. The long term geochemical evolution of the nearfield of the HLW repository. NAGRA report 12-01. National Cooperative for the Disposal of Radioactive Waste (NAGRA), CH-5430 Wettingen, Switzerland.

Braissant, O., *et al*. (2007). "Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals." Geobiology 5(4): 401-411.

Brown, A.R., Boothman, C., Pimblott, S.M., Lloyd, J.R., 2015. The Impact of Gamma Radiation on sediment microbial processes. Appl. Environ. Microbiol. 81, 4014–4025.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Bruggeman, C., N. Maes, M. Aertsens, J. Govaerts, E. Martens, E. Jacops, M. Van Gompel and L. Van Ravestyn (2010). Technetium retention and migration behaviour in Boom Clay. External Report. Mol, Belgium, SCK•CEN. SCK•CEN-ER-101.

Bruhn, D.F., Breckenridge, C.R., Tsang, M.N., Watkins, C.S., Windes, W.F., Roberto, F.F., Wright, R.N., Pinhero, P.J., Brey, R.F., 1999. Irradiation of Microbes from Spent Nuclear Fuel Storage Pool Environments (No. INEEL/CON--99-00611). Idaho National Engineering and Environmental Lab.

Bunzl, K., Schimmack, W., 1988. Effect of microbial biomass reduction by gamma-irradiation on the sorption of 137Cs, 85Sr, 139Ce, 57Co, 109Cd, 65Zn, 103Ru, 95mTc and131I by soils. Radiat. Environ. Biophys. 27, 165–176.

Coaker AW (2003) Fire and flame retardants for PVC. Journal of Vinyl and Additive Technology, 9:108-115.

Chapon, V., Piette, L., Vesvres, M.-H., Coppin, F., Marrec, C.L., Christen, R., Theodorakopoulos, N., Février, L., Levchuk, S., Martin-Garin, A., Berthomieu, C., Sergeant, C., 2012. Microbial diversity in contaminated soils along the T22 trench of the Chernobyl experimental platform. Appl. Geochem., 25 years after the Chernobyl power plant explosion: Management of nuclear wastes and radionuclide transfer in the environment 27, 1375–1383.

Choi, J.-W., Oscarson, D.W., (1996). Diffusive transport through compacted Na-and Ca-bentonite. J. Contam. Hydrol. 22, 189–202.

Costea, P. I., G. Zeller, S. Sunagawa, E. Pelletier, A. Alberti, F. Levenez, M. Tramontano, M. Driessen, R. Hercog, F.-E. Jung, J. R. Kultima, M. R. Hayward, L. P. Coelho, E. Allen-Vercoe, L. Bertrand, M. Blaut, J. R. M. Brown, T. Carton, S. Cools-Portier, M. Daigneault, M. Derrien, A. Druesne, W. M. de Vos, B. B. Finlay, H. J. Flint, F. Guarner, M. Hattori, H. Heilig, R. A. Luna, J. van Hylckama Vlieg, J. Junick, I. Klymiuk, P. Langella, E. Le Chatelier, V. Mai, C. Manichanh, J. C. Martin, C. Mery, H. Morita, P. W. O'Toole, C. Orvain, K. R. Patil, J. Penders, S. Persson, N. Pons, M. Popova, A. Salonen, D. Saulnier, K. P. Scott, B. Singh, K. Slezak, P. Veiga, J. Versalovic, L. Zhao, E. G. Zoetendal, S. D. Ehrlich, J. Dore and P. Bork (2017). "Towards standards for human fecal sample processing in metagenomic studies." Nat Biotechnol 35.

Cross, M.M., Manning, D.A.C., Bottrell, S.H., Worden, R.H., (2004). Thermochemical sulfate reduction (TSR): experimental determination of reaction kinetics and implications of the observed reaction rates for petroleum reservoirs. Org. Geochem. 35, 393-404.

Daly, M.J., 2004. Accumulation of Mn(II) in *Deinococcus radiodurans* facilitates Gamma-radiation resistance. Science 306, 1025–1028.

Dawson, J., (2013) The potential for non-aqueous phase liquid production from irradiated PVC and vinylesterstyrene (VES). Report to NDA RWMD. AMEC/PPE-1008/001 Issue 02. De Cannière, P., A. Maes, S. Williams, C. Bruggeman, T. Beauwens, N. Maes and M. Cowper (2010). Behaviour of Selenium in Boom Clay. External Report Mol, Belgium, SCK•CEN. SCK•CEN-ER-120.

Dekker, L., *et al*. (2014). "Isolation and identification of cobalt- and caesium-resistant bacteria from a nuclear fuel storage pond." FEMS Microbiology Letters 359(1): 81-84.

Doronina, N.V., Sokolov, A.P. and Trotsenko, Y.A., 1996. Isolation and initial characterization of aerobic chloromethane-utilizing bacteria. *FEMS microbiology letters*, *142*(2-3), 179-183.

Enning D and Garrelfs J. (2014). Corrosion of Iron by Sulfate-Reducing Bacteria: New View of an Old Problem. Applied and Environmental Microbiology. 1226 – 1236.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Fredrickson, J.K., Zachara, J.M., Balkwill, D.L., Kennedy, D., Li, S.W., Kostandarithes, H.M., Daly, M.J., Romine, M.F., Brockman, F.J., 2004. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford Site, Washington State. Appl Env. Microbiol 70, 4230–4241.

Fru, E.C., Athar, R., (2008). In situ bacterial colonization of compacted bentonite under deep geological high-level radioactive waste repository conditions. Appl. Microbiol. Biotechnol. 79, 499–510. https://doi.org/10.1007/s00253-008-1436-z

Fukunaga, Honya, M., Yokoyama, E., Arai, K., Mine, T., Mihara, M., Senju, T., (2000). A Study on Conditions for Microbial Transport through Compacted Buffer Material. MRS Online Proc. Libr. Arch. 663. https://doi.org/10.1557/PROC-663-675

Galamboš, M., Daňo, M., Rosskopfová, O., Šeršeň, F., Kufčáková, J., Adamcová, R., Rajec, P., 2012. Effect of gamma-irradiation on adsorption properties of Slovak bentonites. J. Radioanal. Nucl. Chem. 292, 481–492.

Glaus, M. A., van Loon, L. R., Achatz, S., Chodura, A. & Fischer, K. (1999). Degradation of cellulosic materials under the alkaline conditions of a cementitious repository for low and intermediate level radioactive waste. Part I: Identification of degradation products. Anal Chim Acta 398, 111–122.

Glaus, M. A. & van Loon, L. R. (2008). Degradation of cellulose under alkaline conditions: New insights from a 12 years degradation study. Environ Sci Technol 42, 2906–2911.

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

Harrington, J. F., A. E. Milodowski, C. C. Graham, J. C. Rushton and R. J. Cuss (2012). "Evidence for gas-induced pathways in clay using a nanoparticle injection technique." Mineralogical Magazine 76(8): 3327-3336.

Haynes, H. M., C. I. Pearce, C. Boothman and J. R. Lloyd (2018). "Response of bentonite microbial communities to stresses relevant to geodisposal of radioactive waste." Chemical Geology 501.

Huttunen-Saarivirta, E., P. Rajala, M. Bomberg and L. Carpén (2017). "Corrosion of copper in oxygen-deficient groundwater with and without deep bedrock micro-organisms: Characterisation of microbial communities and surface processes." Applied Surface Science 396, 1044-1057.

Iino, T., *et al*. (2015). "*Prolixibacter denitrificans* sp. nov., an iron-corroding, facultatively aerobic, nitrate-reducing bacterium isolated from crude oil, and emended descriptions of the genus *Prolixibacter* and *Prolixibacter bellariivorans*." International journal of systematic and evolutionary microbiology 65(9): 2865-2869.

Ivshina, I., *et al*. (2002). "Efficient uptake of cesium ions by Rhodococcus cells." Microbiology 71(3): 357-361.

Jalique, D.R., S. Stroes-Gascoyne, C.J. Hamon, D.G. Priyanto, C. Kohle, W.G Evenden, G.M.Wolfaardt, A.A. Grigoryan, J. McKelvie and D.R. Korber. 2016. Culturability and diversity of microorganisms recovered from an eight-year old highly-compacted saturated MX-80 Wyoming bentonite plug. Applied Clay Science 126, 245-250.

Karst, S. M., M. S. Dueholm, S. J. McIlroy, R. H. Kirkegaard, P. H. Nielsen and M. Albertsen (2018). "Retrieval of a million high-quality, full-length microbial 16S and 18S rRNA gene sequences without primer bias." Nat Biotechnol 36(2).

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Kato, S., *et al*. (2016). "Enrichment and isolation of *Flavobacterium* strains with tolerance to high concentrations of cesium ion." Scientific reports 6: 20041.

Kebschull, J. M. and A. M. Zador (2015). "Sources of PCR-induced distortions in high-throughput sequencing data sets." Nucleic Acids Research 43(21).

Kim, J., Dong, H., Seabaugh, J., Newell, S.W., Eberl, D.D., (2004). Role of microbes in the smectite-to-illite reaction. Science 303, 830-832.

King, F., Lilja, C., Pedersen, K., Pitkänen, P., Vähänen, M., (2011). An update of the state-of-the-art report on the corrosion of copper under expected conditions in a deep geologic repository. Posiva Report 2011-01. Posiva Oy, Eurajoki, Finland, 1-252.

Knill, C. J. & Kennedy, J. F. (2003). Degradation of cellulose under alkaline conditions. Carbohydr Polym 51, 281–300.

Kryachko, Y. and S. M. Hemmingsen (2017). "The role of localized acidity generation in microbially influenced corrosion." Current microbiology 74(7): 870-876.

Kuipers, G. Boothman, C., Bagshaw, H., Ward, M., Beard, R., Bryan, N., Lloyd, J.R. (2018) The biogeochemical fate of nickel during microbial ISA degradation; implications for nuclear waste disposal. Scientific Reports Volume 8, Article number: 8753 https://doi.org/10.1038/s41598-018-26963-8.

Kunin, V., *et al*. (2010). "Wrinkles in the rare biosphere: pyrosequencing errors can lead to artificial inflation of diversity estimates." Environ Microbiol 12.

Lan T, Feng Y, Liao J, Li X, Ding C, Zhang D, Yang J, Zeng J, Yang Y, Tang J (2014): Biosorption behavior and mechanism of cesium-137 on *Rhodosporidium fluviale* strain UA2 isolated from cesium solution. Journal of environmental radioactivity 134, 6-13

Lantenois, B., Lanson, B., Muller, F., Bauer, A., Jullien, M., Plançon, A., 2005. Experimental study of smectite interaction with metal Fe at low temperature: 1. Smectite destabilization. Clays Clay Miner. 53, 597-612

Leschine, S. B. (1995). Cellulose degradation in anaerobic environments. Annu Rev Microbiol 49, 399–426.

Leupin, O.X., Zeyer, J., Cloet, V., Smith, P., Bernier-Latmani, P. Marschall, P. Papafotiou, A. Schwyn, B. Stroes-Gascoyne, S. (2016) An assessment of the possible fate of gas generated in a repository for low- and intermediate-level waste. Nagra Technical Report 16-05.

Liu, D., Dong, H., Bishop, M.E., Zhang, J., Wang, H., Xie, S., Wang, S., Huang, L., Eberl, D.D., 2012. Microbial reduction of structural iron in interstratified illite-smectite minerals by a sulphate-reducing bacterium. Geobiology 10, 150-162.

Lopez-Fernandez, M., A. Cherkouk, R. Vilchez-Vargas, R. Jauregui, D. Pieper, N. Boon, I. Sanchez-Castro and M. L. Merroun (2015). "Bacterial Diversity in Bentonites, Engineered Barrier for Deep Geological Disposal of Radioactive Wastes." Microb Ecol 70, 922-935

Lopez-Fernandez, M., Vilchez-Vargas, R., Jroundi, F., Boon, N., Pieper, D., Merroun, M.L., 2018. Microbial community changes induced by uranyl nitrate in bentonite clay microcosms. Appl. Clay Sci., ACS - SI ICC 2017 XVI International Clay Conference – Clays, from the oceans to space 160, 206–216.

Lovley, D.R., 2000. Anaerobic benzene degradation. *Biodegradation*, *11*(2-3), pp.107-116.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Lucht, L.M., Stroes-Gascoyne, S., 1996. Characterization of the radiation and heat resistance of the natural microbial population in buffer materials and selected pure cultures (Technical Report No. TR-744). AECL EACL.

Madigan, M. T. (2012). Brock biology of microorganisms. San Francisco, Benjamin Cummings.

Maia, F., Puigdomenech, I., Molinero, J. (2016) Modelling rates of bacterial sulfide production using lactate and hydrogen as energy sources. SKB TR-16-05.

Männik, J., Driessen, R., Galajda, P., Keymer, J.E., Dekker, C., (2009). Bacterial growth and motility in sub-micron constrictions. Proc. Natl. Acad. Sci. 106, 14861–14866.

Marien, A., N. Bleyen, S. Aerts and E. Valcke (2011). "The study of abiotic reduction of nitrate and nitrite in Boom Clay." Physics and Chemistry of the Earth 36(17-18): 1639-1647.

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

Mattimore, V., Battista, J.R., 1996. Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J. Bacteriol. 178, 633–637.

McNamara, N.P., Black, H.I.J., Beresford, N.A., Parekh, N.R., 2003. Effects of acute gamma irradiation on chemical, physical and biological properties of soils. Appl. Soil Ecol. 24, 117–132.

Miettinen, H., Bomberg, M., Vikman, M., (2018). Acetate activates deep subsurface fracture fluid microbial communities in Olkiluoto, Finland. Geosciences 8, 399. doi:10.3390/geosciences8110399

Missana, T., M. Garcia-Gutierrez and U. Alonso (2004). "Kinetics and irreversibility of cesium and uranium sorption onto bentonite colloids in a deep granitic environment." Applied Clay Science 26(1-4): 137-150.

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

NDA/DECC (2014) Radioactive Wastes in the UK: A Summary of the 2013 Inventory. URADIONUCLIDE14D039; NDA/ST/STY (14) 0006; February 2014.

Nguyen-Thanh, D., Block, K., Bandosz, T.J., 2005. Adsorption of hydrogen sulfide on montmorillonites modified with iron. Chemosphere 59, 343-353.

Nixon, S.L., van Dongen, B.E., Boothman, C., Small, J.S., and Lloyd, J.R. (2018) Additives in Plasticised Polyvinyl Chloride Fuel Microbial Nitrate Reduction at High pH: Implications for Nuclear Waste Disposal. Frontiers in Environmental Science. https://doi.org/10.3389/fenvs.2018.00097

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

ONDRAF/NIRAS (2013). The Category B Waste Monolith Design Basis Report, SFC1 level 5 report: first full draft, NIROND-TR-2013-03E.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Ortiz, D., Steinmetz, V., Durand, D., Legand, S., Dauvois, V., Matire, P., Le Caer, S. Radiolysis as a solution for accelerated ageing studies of electrolytes in lithium-ion batteries, Nature Communications 2015; 6:6950.

Pan, F., *et al*. (2016). "Factors Influencing the Stability of AFm and AFt in the Ca–Al–S–O–H System at 25°C." Journal of the American Ceramic Society 99(3): 1031-1041.

Panak P, Klenze R, Kim JI, Wimmer H. (1995). A study of intramolecular energy transfer in Cm(III) complexes with aromatic ligands by time-resolved laser fluorescence spectroscopy. Journal of Alloys and Compounds, 225:261–266.

Parkhurst, D.L., Appelo, C.A.J. (2013). Description of input and examples for PHREEQC version 3--A computer program for speciation, batch- reaction, one-dimensional transport, and inverse geochemical calculations: U.S. Geological Survey Techniques and Methods, book 6, chap. A43, 497.

Pedersen, K., Motamedi, M., Karnland, O., Sandén, T., (2000a). Cultivability of microorganisms introduced into a compacted bentonite clay buffer under high-level radioactive waste repository conditions. Engineering Geology 58, 149-161.

Pedersen, K., Motamedi, M., Karnland, O., Sandén, T.,(2000b). Mixing and sulfate-reducing activity of bacteria in swelling, compacted bentonite clay under high-level radioactive waste repository conditions. J. Appl. Microbiol. 89, 1038-1047.

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

Pedersen, K., (2012) Subterranean microbial populations metabolize hydrogen and acetate under in situ conditions in granitic groundwater at 450 m depth in the Aspo Hard Rock Laboratory, Sweden. FEMS Microbiology Ecology, 81, 217-229.

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

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

Pedersen, K., (2017). Bacterial activity in compacted bentonites. Micans, MIND.

Phung, Q. T., *et al*. (2017). Current concerns on durability of concrete used in nuclear power plants and radioactive waste repositories. CIGOS 2017: Proceedings of the 4th Congrès International de Géotechnique - Ouvrages -Structures, Springer.

Pitonzo, B.J., Amy, P.S., Rudin, M., 1999. Resuscitation of Microorganisms after Gamma Irradiation. Radiat. Res. 152, 71.

Posiva: Olkiluoto site description 2011, POSIVA 2011-02, 2013. www.posiva.fi/tietopankki/olkiluoto_site_description_2011.1793.xhtml#.Wwz2Uqm-l0s

Potts, M., (1994). Desiccation tolerance of prokaryotes. Microbiol. Mol. Biol. Rev. 58:755-805.

Pusch, R., *et al*. (2003). "Interaction of cement and smectitic clay—theory and practice." Applied Clay Science 23(1): 203-210.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Pusch, R., Karnland, O., Lajudie, A., Decarreau, A., 1992. MX 80 clay exposed to high temperatures and gamma radiation (Technical Record No. SKB-TR--93-03). Swedish Nuclear Fuel and Waste Management Co.

Qiu, X., Daly, M.J., Vasilenko, A., Omelchenko, M.V., Gaidamakova, E.K., Wu, L., Zhou, J., Sundin, G.W., Tiedje, J.M., 2006. Transcriptome analysis applied to survival of *Shewanella oneidensis* MR-1 exposed to ionizing radiation. J. Bacteriol. 188, 1199–1204.

Rai, D., Hess, N. J., Xia, Y., Rao, L., Cho, H. M., Moore, R. C. & Van Loon, L. R. (2003). Comprehensive thermodynamic model applicable to highly acidic to basic conditions for isosaccharinate reactions with Ca(II) and Np(IV). J Solution Chem 32, 665–689.

Rai, D., Rao, L. & Xia, Y. (1998). Solubility of crystalline calcium isosaccharinate. J Solution Chem 27, 1109–1122.

Rebufa, C., Traboulsi, A., Labed, V., Dupuy, N., Sergent, M. Experimental design approach for identification of the factors influencing the γ-radiolysis of ion exchange resins. Radiation physics and chemistry 2015, 106:223-234.

Reisz, J.A., Bansal, N., Qian, J., Zhao, W., Furdui, C.M., 2014. Effects of ionizing radiation on biological molecules — mechanisms of damage and emerging methods of detection. Antioxid. Redox Signal. 21, 260–292.

Renny, J. S.; Tomasevich, L. L.; Tallmadge, E. H.; Collum, D. B. (2013) Method of Continuous Variations: Applications of Job Plots to the Study of Molecular Associations in Organometallic Chemistry. Angew. Chemie Int. Ed. 2013, 52 (46), 11998–12013.

Richard, D., Luther, G.W., 2007. Chemistry of Iron sulfides. Chem. Rev. 107, 514-562.

Riley, P.A., 1994. Free Radicals in Biology: Oxidative stress and the effects of ionizing radiation. Int. J. Radiat. Biol. 65, 27–33. Rizk, T. Y. (2015). "Nitrate Treatment—Effect on Corrosion and Implementation Guidelines."Saudi-Arammco Journal of Technology”

Roßberg, A.; Reich, T.; Bernhard, G. Complexation of Uranium(VI) with Protocatechuic Acid. Application of Iterative Transformation Factor Analysis to EXAFS Spectroscopy. Anal. Bioanal. Chem. 2003, 376 (5), 631–638.

Ruiz-Fresneda, M.A., J. D. Martín, J. G. Bolívar, M. V. Fernández Cantos, G. Bosch-Estévez, M. F. Martínez Moreno and M. L. Merroun* . (2018) Green synthesis and Biotransformation of amorphous Se nanospheres to trigonal 1D Se nanostructures: impact on Se mobility within the concept of radioactive wastes disposal. Environmental Science: Nano. https://doi.org/10.1039/c8en00221e.

Rutting, T., P. Boeckx, C. Muller and L. Klemedtsson (2011). "Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle." Biogeosciences 8(7): 1779-1791.

Sánchez, L., *et al*. (2006). "Reaction kinetics of FEBEX bentonite in hyperalkaline conditions resembling the cement–bentonite interface." Applied Clay Science 33(2): 125-141.

Sanchez-Castro, I., Bakkali, M., Merroun, M.L (2017) Draft Genome Sequence of *Stenotrophomonas bentonitica* BII-R7T, a Selenite-Reducing Bacterium Isolated from Spanish Bentonites. Genome Announcements, Volume 5, Issue 31, American Society for Microbiology, 2017, e00719-17. https://doi.org/10.1128/genomeA.00719-17

The MIND-project has received funding from the European Union’s Euratom research and training program (Horizon2020) under grant agreement 661880.

Sanchez-Castro, I., M. A. Ruiz-Fresneda, M. Bakkali, P. Kämpfer, S. P. Glaeser, H. J. Busse, M. López-Fernández, P. Martínez-Rodríguez, M.L. Merroun (2018) *Stenotrophomonas bentonitica* sp. nov., isolated from bentonite formations. International Journal of Systematic and Evolutionary Microbiology 67:2779–2786. https://doi.org/10.1099/ijsem.0.002016

Savage, D., *et al*. (2007). "Alteration of bentonite by hyperalkaline fluids: A review of the role of secondary minerals." Physics and Chemistry of the Earth, Parts A/B/C 32(1): 287-297.

Scrivener, K. and J. Young (1997). Mechanisms of chemical degradation of cement-based systems

Shrestha, R. (2018) The effect of caesium ions on a natural anaerobic microbial community. Waste Forum, Vol 2, CEMC, Prague, 2018.

Small, J., Nykyri, M., Helin, M., Hovi, U., Sarlin, T., Itävaara, M. (2008). Experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste. Applied Geochemistry, 23 (6), 1383-1418.

Small, J.S., Nykyri, M., Vikman, M., Itävaara, M. and Heikinheimo, L. (2017) The biogeochemistry of gas generation from low-level nuclear waste: modelling after 18 years study under in situ conditions. Applied Geochemistry, 84, 360-372.

Small, J. (2019) Final synthesis report for WP1. MIND project deliverable D1.9.

Smart, N. R., A. P. Rance, B. Reddy, L. Hallbeck, K. Pedersen and A. J. Johansson (2014). "*In situ* evaluation of model copper-cast iron canisters for spent nuclear fuel: a case of microbiologically influenced corrosion (MIC)." Corrosion Engineering, Science and Technology 49 548-553.

Smart, N. R., B. Reddy, A. P. Rance, D. J. Nixon, M. Frutschi, R. Bernier-Latmani and N. Diomidis (2017). "The anaerobic corrosion of carbon steel in compacted bentonite exposed to natural Opalinus Clay porewater containing native microbial populations." Corrosion Engineering, Science and Technology 52 101-112.Smith V, Magalhaes S, Schneider S, (2013) The role of PVC additives in the potential formation of NAPLs. Report to NDA RWMD. AMEC/PPE-2834/001.

Soltermann, D., 2014. Ferrous iron uptake mechanisms at the montmorillonite-water interface under anoxic and electrochemically reduced conditions, ETH Zurich.

Stankovskiy, A. (2011). Calculation of decay power release, activity and absorbed dose in Eurobitum intermediate level waste, SCK-CEN-ER-181.

Staunton, S., C. Dumat and A. Zsolnay (2002). "Possible role of organic matter in radiocaesium adsorption in soils." J Environ Radioact 58(2-3): 163-173.

Stepova, K., Maquarrie, D., Krip, I., 2009. Modified bentonites as adsorbents of hydrogen sulfide gases. Applied Clay Science 42, 625-628.

Sterflinger, K. 2000. Fungi as geologic agents. Geomicrobiol. J. 17, 97-124.

Stroes-Gascoyne, S., Haveman, S.A., Hamon, C.J., Delaney, T., Pedersen, K., Arlinger, J., Ekendahl, S., Hallbeck, L., Jahromi, N., Dekeyser, K., Daumas, S., 1997. Occurrence and identification of microorganisms in compacted clay-based buffer material designed for use in a nuclear fuel waste disposal vault. Can. J. Microbiol. 43, 1133-1146.

Stroes-Gascoyne, S., West, J.M., 1997b. Microbial studies in the Canadian nuclear fuel waste management program. FEMS Microbiol. Rev. 20, 573–590. https://doi.org/10.1111/j.1574-6976.1997.tb00339.x

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Stroes-Gascoyne, S., Hamon, C.J., Maak, P., 2011. Limits to the use of highly compacted bentonite as a deterrent for microbiologically influenced corrosion in a nuclear fuel waste repository. Physics and Chemistry of the Earth, Parts A/B/C 36, 1630-1638.

Suckling, P., Avis J., Calder, N., Nasir, O., Humphreys, P., King, F. and Walsh, R. (2015) T2GGM Version 3.2: Gas Generation and Transport Code NWMO-TR-2015-13.

Svensk Kärnbränslehantering AB, 2006. Buffer and backfill process report for the safety assessment SR-Can (No. TR-06-18). Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden.

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

Theodorakopoulos, N., Février, L., Barakat, M., Ortet, P., Christen, R., Piette, L., Levchuk, S., Beaugelin-Seiller, K., Sergeant, C., Berthomieu, C., Chapon, V., 2017. Soil prokaryotic communities in Chernobyl waste disposal trench T22 are modulated by organic matter and radionuclide contamination. FEMS Microbiol. Ecol. 93.

Tits, J., Wieland, E. & Bradbury, M. H. (2005). The effect of isosaccharinic acid and gluconic acid on the retention of Eu(III), Am(III) and Th(IV) by calcite. Appl Geochemistry 20, 2082–2096.

Tournassat, C.,-Epping P., Gaucher, E.C., Gimmi, T., Leupin, O.X., Wersin, P. (2011) Biogeochemical processes in a clay formation in situ experiment: Part F – Reactive transport modelling. Applied Geochemistry 26, 1009–1022.

Truche, L., G. Berger, A. Albrecht and L. Domergue (2013). "Abiotic nitrate reduction induced by carbon steel and hydrogen: Implications for environmental processes in waste repositories." Applied Geochemistry 28: 155-163.

Truche, L., G. Berger, A. Albrecht and L. Domergue (2013). "Engineered materials as potential geocatalysts in deep geological nuclear waste repositories: A case study of the stainless steel catalytic effect on nitrate reduction by hydrogen." Applied Geochemistry 35: 279-288.

Valcke, E., A. Sneyers and P. Van Iseghem (2001). "The effect of radiolytic degradation products of eurobitum on the solubility and sorption of Pu and Am in boom clay." Scientific Basis for Nuclear Waste Management Xxiv 663: 141-149.

Valcke, E., F. Rorif and S. Smets (2009). "Ageing of EUROBITUM bituminised radioactive waste: An ATR-FTIR spectroscopy study." Journal of Nuclear Materials 393(1): 175-185.

Valencia-Cantero E and Pena-Cabriales JJ.2014.Effect of Iron-Reducing Bacteria on Carbon Steel Corrosion Induced by Thermophilic Sulfate –Reducing Consortia.

Van Gerwen, S.J.C., Rombouts, F.M., Riet, K. van't, Zwietering, M.H., 1999. A data analysis of the irradiation parameter D_{10} for bacteria and spores under various conditions. J. Food Prot. 62, 1024–1032.

Van Loon, L.R., Hummel, W. The radiolytic and chemical degradation of organic ion exchange resisns under alkaline conditions: effect on radionuclide speciation. 1995. NAGRA Technical report 95-08.

Van Loon, L. and W. Hummel (1999). The Degradation of Strong Basic Anion Exchange Resins and Mixed-Bed Ion-Exchange Resins: Effect of Degradation Products on Radionuclide Speciation. 128: 388-401.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Van Loon, L. R., Glaus, M. A., Laube, A. & Stallone, S. (1999a). Degradation of cellulosic materials under the alkaline conditions of a cementitious repository for low- and intermediate-level radioactive waste. II. Degradation kinetics. J Polym Environ 7, 41–51.

Van Loon, L. R., Glaus, M. A. & Vercammen, K. (1999b). Solubility products of calcium isosaccharinate and calcium gluconate. Acta Chem Scand 53, 235–240.

Vazquez GJ, Dodge CJ, Francis AJ, (2008). Interaction of Uranium(VI) with Phthalic Acid. Inorganic Chemistry 47:10739–10743.

Vazquez GJ, Dodge CJ, Francis AJ, (2009). Bioreduction of U(VI)-phthalate to a polymeric U(IV)-phthalate colloid. Inorganic Chemistry, 48:9485–9490.

Venzlaff H, Enning D, Srinivasan J, Mayrhofer K.J.J., Hassel A W, Widdel F and Stratmann M. 2013. Accelerated cathodic reaction in microbial corrosion of iron due to direct electron uptake by sulfate-reducing bacteria. Corrosion Science. 88-96

Vercammen, K., Glaus, M. A. & van Loon, L. R. (2001). Complexation of Th(IV) and Eu(III) by α-isosaccharinic acid under alkaline conditions. Radiochim Acta 89, 393–401.

Vik, E. A., *et al*. (2007). Nitrate Based Souring Mitigation of Produced Water-Side Effects and Challenges from the Draugen Pproduced Water re-Injection Pilot. International Symposium on Oilfield Chemistry, Society of Petroleum Engineers.

Vikman, M., Matusewicz, M., Sohlberg, E., Miettinen, M., Tiljander, M., Järvinen, J., Aku Itälä, Rajala, P., Raulio, M., Itävaara, M., Arto Muurinen, A., Olin, M. 2018. Long-term experiment with compacted bentonite, VTT Technology 332.

Vikman, M. Marjamaa, K., Nykyri, M., Small, J.S., Miettinen, H. Heikinheimo, L. Haavisto, T. and Itavaara, M. 2019. The biogeochemistry of gas generation from low-level nuclear waste: Microbiological characterization during 18 years study under *in situ* conditions. Applied Geochemistry, accepted for publication.

Vogt, C., Kleinsteuber, S., Richnow, H. Anaerobic benzene degradation by bacteria. Microbial biotechnology, 2011; 4(6): 710-734.

Wang, L., *et al*. (2007). Effects of an alkaline plume on the Boom Clay as a potential host formation for geological disposal of radioactive waste SCK-CEN-ER-28.

Warwick, P., Evans, N., Hall, T. & Vines, S. (2003). Complexation of Ni(II) by α-isosaccharinic acid and gluconic acid from pH 7 to pH 13. Radiochim Acta 91, 233–240.

Warwick, P., Evans, N., Hall, T. & Vines, S. (2004). Stability constants of uranium(IV)-α-isosaccharinic acid and gluconic acid complexes. Radiochim Acta 92, 897–902.

Warwick, P., Evans, N. & Vines, S. (2006). Studies on some divalent metal α-isosaccharinic acid complexes. Radiochim Acta 94, 363–368.

Weetjens, E., E. Valcke and A. Mariën (2010). Sodium nitrate release from Eurobitum bituminised radioactive waste - Scoping calculations, SCK•CEN-ER-146.

Weetjens, E. (2015). Safety Assessment calculations for geological disposal of Eurobitum bituminised waste in Boom Clay, SCK•CEN - Studiecentrum voor Kernenergie/Centre d'Etude de l'Energie Nucléaire.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Wei, S., Z. Jiang, H. Liu, D. Zhou and M. Sanchez-Silva (2013). "Microbiologically induced deterioration of concrete--a review." Braz J Microbiol 44(4): 1001-1007.

West, J. M., I. G. McKinley and S. Stroes-Gascoyne (2002). "Chapter 9 Microbial effects on waste repository materials." Radioactivity in the Environment 2: 255-277.

Wieland, E., Tits, J., Dobler, J. P. & Spieler, P. (2002). The effect of α-isosaccharinic acid on the stability of and Th(IV) uptake by hardened cement paste. Radiochim Acta 90, 683–688.

Wouters, K., H. Moors, P. Boven and N. Leys (2013). "Evidence and characteristics of a diverse and metabolically active microbial community in deep subsurface clay borehole water." Fems Microbiology Ecology 86(3): 458-473.

Wright, S.J.L., Hill, E.C., 1968. The development of radiation-resistant cultures of *Escherichia coli* I by a process of 'Growth-irradiation Cycles.' Microbiology 51, 97–106.

Xu D, Li Y, Song F, Gu T (2013): Laboratory investigation of microbiologically influenced corrosion of C1018 carbon steel by nitrate reducing bacterium *Bacillus licheniformis*. Corrosion Science 77, 385-390

Yang, W.H., Weber, K.A. and Silver, W.L., 2012. Nitrogen loss from soil through anaerobic ammonium oxidation coupled to iron reduction. *Nature Geoscience*, *5*(8), 538.

Yang, Z., Zhou, S. and Sun, Y., 2009. Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor. *Journal of Hazardous Materials*, *169*(1-3), 113-118.

Zhou Z, Shu W, Ruan J, Huang B, Liu Y, (2004). Synthesis, characteristics, and fluorescence properties of complexes of europium with benzoic acid and its derivatives. Rare Metals 23:306-310.

Zhu, T. and M. Dittrich (2016). "Carbonate Precipitation through Microbial Activities in Natural Environment, and Their Potential in Biotechnology: A Review." Front Bioeng Biotechnol 4.

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9 Appendix

9.1 WP 1

9.1.1 Bitumen (SCK-CEN)

Table 1: Possible microbial processes involved in the degradation of bituminised waste

Chemical/Radiolytic end products	Microbial Process	Biological end products	Effect	Boundary conditions (pH, temp, rad,...)	Possible impact on PA	References	IRB Gap reference
Hydrogen & Nitrate/nitrite	Hydrogenotrophy nitrate/nitrite reduction	H^+	H_2 gas removal; Gas volume decrease		Gas pressure decrease	Bleyen *et al.* 2017a, b	
	Nitrate reduction to nitrite	Nitrite	(intermediate) production of nitrite	pH limit < 12.5?	(-) Redox impact on clay host rock, reducing RN sorption capacity of clay host rock	Bleyen *et al.* 2017a, b	
	Nitrate/nitrite reduction to nitrogen gas	N_2	Gas volume increase	pH limit < 12.5?	Gas pressure increase	Bleyen *et al.* 2017a, b	**1f**
	Nitrate/nitrite reduction to ammonium	NH_4^+					
Small soluble organics & Nitrate or nitrite	Organotrophic Nitrate/nitrite reduction	CO_2	Removal small soluble organics & less complexation of RN with organics; biofilm on bitumen	Oxalate only degraded in 1 replicate; pH < 12.5 for acetate degradation (others pH effect not tested)	(+) Less complexation of RN with organics & less impact on RN mobility	This study	**1a**
	Nitrate reduction to nitrite	Nitrite	(intermediate) production of nitrite	pH limit < 12.5	(-) Redox impact on clay host rock, reducing RN sorption capacity of clay host rock	This study	**1g, 1c**
	Nitrate/nitrite	N_2	Gas volume increase	pH limit	(-) Gas pressure increase	This study	**1f**

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	reduction to nitrogen gas			< 12.5; Electron donor, C/N ratio			
	Nitrate/nitrite reduction to ammonium	NH_4^+				Bleyen *et al.* 2017a, b	**1g, 1c**
Hydrogen & Sulphate	Hydrogenotrophic Sulphate reduction	S^{2-}	Generation of corrosion-aggressive species	If nitrate as electron acceptor is depleted $E^{0'}$= -217 mV	Corrosion	Not this study	**1j**
Hydrogen & CO_2	Hydrogenotrophic Methanogenesis	CH_4	Gas volume decrease	If nitrate and sulphate as electron acceptor are depleted $E^{0'}$= -238 mV	Gas pressure decrease	Not this study	**1f**

Table 2: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	**Process investigated**	**Organisms studied**	**End products**	**Boundary conditions**
Anoxic batch experiment	Biofilm formation on Eurobitum	Boom Clay borehole water	NO_2^-, N_2, TIC, +?	Only tested at pH ~9.5,
Anoxic batch experiment	Organotrophic nitrate reduction rates of nitrate leaching from Eurobitum in the presence of different electron donors	Boom Clay borehole water	NO_2^-, N_2, TIC, +?	Oxalate is not preferred as electron donor, Phosphate can become limiting
Anoxic batch experiments	Organotrophic nitrate reduction rates of nitrate leaching from Eurobitum at high pH	Boom Clay borehole water	NO_2^-, N_2, +?	pH 12.5 & Phosphate can become limiting

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9.1.2 Cellulose (UNIMAN)

Table 3: Possible microbial degradation processes in waste containing cellulose

Chemical end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible impact on PA	References	IRB Gap reference
Cellulose (& ISA)	Hydrolysis Fermentation	H_2	Reduce the extent of ISA production and degrade the produced ISA; Increased biomass	Temperature Salinity	Potential production of radioactive gasses that may affect pressurisation or may be released to the biosphere	This study	**2f**
Cellulose (& ISA)	Acetogenic fermentation	Acetate	Reduce the mass of cellulose in the GDF, reduction of pH	pH < 13	Reduce the amount of radionuclide complexants in the GDF	Bassil 2015a and b; and this study	**2f**
Cellulose (& ISA)	Fermentation and respiration	CO_2	Potentially drop the pH in the GDF. Potentially Encapsulate or sorb radionuclides	Chemical toxins Radioactivity		Bassil *et al.*, 2015a, b; and this study	**2f**

Table 4: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	**Process investigated**	**Organisms studied**	**End products**	**Boundary conditions**
Batch sacrificial experiments	Microbial degradation of irradiated cellulose under hyperalkaline conditions	Sediment slurry from a hyperalkaline contaminated site	H_2, CO_2, Acetate	pH

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9.1.3 PVC (UNIMAN)

Table 5: Possible microbial degradation processes in waste containing PVC

Chemical end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible impact on PA	References	IRB Gap reference
PVC	PVC degradation	Unknown	Reduced mass of PVC in the GDF Liberation of PVC additives	pH ≤10	Drop in pH, influencing radionuclide solubility and microbial processes	This study	**1a**
Phthalate ester	Phthalate ester degradation	Unknown	Reduced phthalate ester concentration (phthalate anion only tested in this study, but phthalate esters may be bioavailable); Increased biomass	pH ≤10	Reduce the risk of NAPL generation Reduce the amount of phthalate-radionuclide complexants in the GDF	This study	**1a**
Phosphate ester	Phosphate ester degradation	Unknown	Reduced phosphate ester concentration	pH ≤10	unknown	This study	**1a**

Table 6: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	Process investigated	Organisms studied	End products	Boundary conditions
Batch microcosm experiments	The degradation of irradiated PVC under alkaline conditions	Sediment slurry from a hyperalkaline environment	Nitrite	pH
Batch microcosm experiments	The degradation of irradiated two representative PVC plasticisers under alkaline conditions	Sediment slurry from a hyperalkaline environment	Nitrite	pH

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9.1.4 Ion exchange resins (EPFL, TUL/RCR)

Table 7: Possible microbial degradation processes in waste containing ion exchange resins

Chemical end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, IS, rad)	Possible impact on PA	References	IRB Gap reference
H_2	Sulfate reduction	HS^-	Consumption of H_2 and SO_4^{2-}; Establishment of sulfate-reducing community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	This study	
H_2 and CO_2	Hydrogenotrophic methanogenesis	CH_4	Consumption of H_2 and CO_2; Shift in microbial community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	This study	**1f**

Table 8: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	**Process investigated**	**Organisms studied**	**End products**	**Boundary conditions (pH, temp, IS, chemical, rad)**
ongoing				

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9.1.5 Radionuclide complexation (HZDR, UGR)

Table 9: Possible microbial interactions with radionuclides

Radionuclide	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible impact on PA	References	IRB Gap reference
U(VI) complexed with cellulose & ISA	Biodegradation and fermentation of the organics	uncomplexed U(VI) ?	Effect of U(VI) on the biotic degradation of tissue paper, fate of U(VI) in the presence of degradation products	[U(VI)] 0.05 mM, pH_{Start} 12.2, [$Ca(OH)_2$] 1g/L, [tissue paper] 2.5 % (w/v), [bacterial inoculum] 2.5 % (v/v), T 20 °C	Mobilization of U(VI) due to organic degradation products		**1a**
Se and Spanish bentonite bacterial isolate	Se(VI) and Se(IV) reduction to and Se(0) nanostructure formation	Monoclicnic and trigonal Se(0) nanostructures	Increased insolubility of Se oxyanions		Positive impact by the Immobilization of Se oxyanions	Ruiz-Fresneda et al. 2018)	**1h**
Eu and Spanish bentonite bacterial isolate	Eu(III) biosorption/ biomineralization	Eu(III) phosphates/carboxylate complexes	Decrease solubility of Eu		Positive impact by immobilization of Eu(III)		**1h**

Table 10: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	Radionuclide investigated	Organisms studied	End products	Boundary conditions

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

9.1.6 CASE studies*: In-situ* processes (NNL, VTT, EPFL)

Table 11: Possible important in-situ microbial processes – MA experiment, Mont-Terri (EPFL)

Chemical/Radiolytic end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible impact on PA	References	IRB Gap reference
H_2 and SO_4^{2-}	Sulfate reduction	HS^-	Consumption of H_2 and SO_4^{2-}; Establishment of sulfate-reducing community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	Bagnoud et al., 2016	**1f**
H_2 and CO_2	Hydrogenotrophic methanogenesis	CH_4	Consumption of H_2 and CO_2; Shift in microbial community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	This study	**1f**
	Methanogenesis	CH_4	Gas formation	pH >8, high concentration of volatile fatty acids and sulphide, occurrence of sulphate (activation of SRBs)	Pressure build up, transport of radionuclides from repository	Small et al. (2017)	
	Sulphate reduction	HS^-	Formation of corrosive sulphides, consumption of H_2	Availability of sulphate	Reduction in gas generation, enhancement of corrosion		

Table 12: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	**Process investigated**	**Organisms studied**	**End products**	**Boundary conditions**
In situ	methanogenesis	Microbial community	HS-	pH 8, *in situ* conditions
batch	methanogenesis	Microbial community	Fe(II)	pH 8, *in situ* conditions
TVO gas generation experiment, batch, *in situ*, modelled	Gas generation, methanogensis, sulphate reduction	Methanogens, sulphate reducers, bacterial and archaeal community	Methane	

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9.2 WP2 – Engineered barriers

9.2.1 The availability of sulphide and its effects on metal canister corrosion

Table 13: Possible microbial processes involved in canister corrosion

Chemical/Radiolytic end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible possible impact on PA	References	IRB Gap reference
Sulphate	Sulphate reduction	S^{2-}	Production of corrosive agents	Availability of energy source and electron donor. pH > 6	General/local corrosion		**1j**
Sulphate	Metallic Iron oxidation	S^{2-}	Increases the corrosion rate		Iron corrosion	Enning and Garrelfs, 2014	**1j**
	Sulphide oxidation	sulphate	mobilization of sulphate				

Table 14: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	Process investigated	Organisms studied	End products	Boundary conditions
Batch experiment with carbon steel in BPW inoculated with groundwater VITA	Biofilm formation, corrosion	Nitrate reducing bacteria SRB *Geobacteraceae*	N_2 S^{2-} Fe(II)	O_2 < 1 ppm pH <8.6; 9.1>
Batch experiment with carbon steel in groundwater VITA	Biofilm formation, corrosion	SRB *Geobacteraceae*	S^{2-} Fe(II)	O_2 < 1 ppm pH <7.9; 9.0>

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9.2.2 Canister corrosion & Hydrogen production

Table 15: Possible microbial processes involved in canister corrosion

Chemical end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible impact on PA	References	IRB Gap reference
H_2 and SO_4^{2-}	Hydrogenotrophic Sulfate reduction	HS^-	Removal of gas H_2	pH 8 and Opalinus Clay conditions; presence of sulfate-reducing bacteria	Positive impact by consuming gases	Bagnoud et al., 2016	**1f**
			Production of corrosive agent HS^-				
H_2 and CO_2	Hydrogenotrophic methanogenesis	CH_4	Removal of H_2 and CO_2	pH 8 and Opalinus Clay conditions; presence of methanogenic archea	Positive impact by consuming gases	This study	**1f**

Table 16: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	Process investigated	Organisms studied	End products	Boundary conditions
In situ	methanogenesis	Microbial community	HS-	pH 8, in situ conditions
batch	methanogenesis	Microbial community	Fe(II)	pH 8, in situ conditions

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9.2.3 Microbial activity in bentonite buffers

Table 17: Possible microbial processes involved in long-term performance of bentonites

Chemical end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible possible impact on PA	References	IRB Gap reference
Sulphate and H_2 or organic matter	sulphate reduction	sulphide	Sulphide production;	Density, swelling pressure, water activity	Canister corrosion;	MIND studies	**1k**
Organic matter of bentonite	Acetogenic fermentation (acidogenesis)	acetate	Stress corrosion cracking of metals	Under investigation	Stress corrosion of metals; no impact on bentonite buffer capacity	MIND studies	

Table 18: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	Process investigated	Organisms studied	End products	Boundary conditions
Long-term experiment with compacted bentonite, batch, aerobic/anaerobic	changes in bentonite structure and mineralogy	SRBs, iron reducing bacteria, fungi		

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9.2.4 Degradation of Bentonite buffers

Table 19: Possible microbial processes involved in long-term performance of bentonites

Chemical end products	**Process**	**Biological end products**	**Effect**	**Boundary conditions (pH, temp, IS, rad)**	**Possible impact on PA**	**References**	**IRB Gap reference**
Sulphate and H_2 or organic matter	sulphate reduction	sulphide	Sulphide production; Reduces ferric iron; Elemental Sulphur formation	Density, swelling pressure, water activity	Bentonite alteration; reduction in swelling pressure?	Pedersen, K., et al. (2017).	**1k**

Table 20: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	**Process investigated**	**Organisms studied**	**End products**	**Boundary conditions (pH, temp, IS, chemical, rad)**
'Worst-case scenario experiment', batch, aerobic, slurry, nutrients	degradation of bentonite: sulfate and iron reduction, mineralogy	indigenous including SRBs, iron reducers, archaea, fungi	FeS	
'Worst-case scenario experiment', batch, anaerobic, slurry, nutrients	degradation of bentonite: sulfate and iron reduction, mineralogy	indigenous including SRBs, iron reducers,archaea, fungi	FeS	

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9.2.5 Degradation of cementitious materials, as used in seals & plugs

Table 21: Possible microbial processes involved in long-term performance of cementitious materials, as seals & plugs

Chemical end products	Process	Biological end products	Effect	Boundary conditions (pH, temp, rad)	Possible possible impact on PA	References	IRB Gap reference
Ca^{2+}, CO_2	Carbonation	$CaCO_3$	Decrease in pH, Decreases transport properties & refines pore structure	Not yet defined	Increased corrosion rate rebars	Phung, 2017	**1i**
Organic matter	Microbial metabolism	Acids	Decrease in pH	Not yet defined	Increased corrosion rate rebars	Phung, 2017	
SO_4^{2-}, H_2 or organic matter	Sulphate reduction	Sulphide	Lowering external SO_4^{2-} attack, but generation corrosion aggressive species	Not yet defined	Increased corrosion rate rebars	Phung, 2017	

Table 22: Results of microbial experiments

Test set-up (batch, *in situ*, computationally predicted, …)	Process investigated	Organisms studied	End products	Boundary conditions
Anoxic batch experiments	Interaction with OPC CEM I	Boom Clay borehole water	?	In denitrification conditions pH >12

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