



**(GRANT AGREEMENT: 661880)**

## **DELIVERABLE 3.5**

# **Year three synthesis report**

Editors:

K. Mijndendonckx (SCK-CEN)  
J. Small (NNL)  
L. Abrahamsen-Mills (NNL)  
K. Pedersen (Micans)  
J. Govaerts (SCK-CEN)  
N. Leys (SCK-CEN)

Date of issue of this report: 01.11.2018

Report number of pages: 87

Start date of project: 01/06/2015

Duration: 48 Months

This project has received funding from the Euratom research and training programme 2014-2018 under Grant Agreement no. 661880		
Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the partners of the MIND project	
CO	Confidential, only for partners of the MIND project	

## **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 first 3 years of the project. The scope of the report is broad, covering all completed scientific and 'in progress' 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).

# Contents

Publishable Summary .....	1
1 General introduction .....	5
1.1 The MIND project .....	5
1.2 Scope of report.....	5
2 WP1 – Waste containing organics.....	6
2.1 Bituminised waste .....	6
2.1.1 Problem statement & current knowledge.....	6
2.1.2 Summary of experimental & computational results .....	8
2.1.3 Conclusions.....	9
2.2 Cellulose containing waste .....	10
2.2.1 Problem statement & current knowledge.....	10
2.2.2 Summary of experimental & computational results .....	10
2.2.3 Conclusions.....	11
2.3 PVC containing waste .....	12
2.3.1 Problem statement & current knowledge.....	12
2.3.2 Summary of experimental & computational results .....	12
2.3.3 Conclusions.....	14
2.4 Ion exchange resins waste.....	14
2.4.1 Problem statement & current knowledge.....	14
2.4.2 Summary of experimental & computational results .....	15
2.4.3 Conclusions.....	17
2.5 Radionuclide complexation .....	18
2.5.1 Problem statement & current knowledge.....	18
2.5.2 Summary of experimental & computational results .....	19
2.5.3 Conclusions.....	24
2.6 <i>In situ</i> processes: hydrogen release from organic waste .....	25
2.6.1 Problem statement & current knowledge.....	25
2.6.2 Summary of experimental & computational results .....	26
2.6.3 Conclusions.....	26
2.7 <i>In situ</i> processes: methane release from organic waste .....	27
2.7.1 Problem statement & current knowledge.....	27

2.7.2	Summary of experimental & computational results .....	27
2.7.3	Conclusions.....	28
2.8	Modelling of microbial processes.....	29
2.8.1	Problem statement & current knowledge.....	29
2.8.2	Summary of experimental & computational results .....	29
2.8.3	Conclusions.....	33
2.9	WP1 - current status and intermediate conclusions .....	33
3	WP2 – Engineered barrier .....	37
3.1	The availability of sulphide and its effects on canister corrosion .....	37
3.1.1	Problem statement & current knowledge.....	37
3.1.2	Summary of experimental & computational results .....	38
3.1.3	Conclusions.....	42
3.2	Microbial activity in and degradation of bentonite buffers .....	43
3.2.1	Problem statement & current knowledge: .....	43
3.2.2	Summary of experimental & computational results .....	46
3.2.3	Conclusions.....	52
3.3	Microbial activity in backfill and influence on plugs and seals .....	52
3.3.1	Problem statement & current knowledge.....	52
3.3.2	Summary of experimental & computational results .....	54
3.3.3	Conclusions.....	55
3.4	WP2 current status and intermediate conclusions .....	55
4	General conclusions and cross reference to the IRB review .....	60
4.1	Swelling of bentonite .....	60
4.2	Gas generation and consumption .....	61
4.3	Organic waste degradation .....	62
4.4	Effect on radionuclide transport .....	63
4.5	Limitations on microbial activity in repositories .....	64
5	References.....	65
6	Acknowledgement.....	74
7	Appendix.....	75
WP 1	.....	75
	Bitumen (SCK-CEN) .....	75
	Cellulose (UNIMAN).....	77

PVC (UNIMAN).....	78
Ion exchange resins (EPFL, TUL/RCR) .....	79
Radionuclide complexation (HZDR, UGR).....	80
CASE studies: <i>In-situ</i> processes (NNL, VTT, EPFL).....	81
WP2 – Engineered barriers.....	82
The availability of sulphide and its effects on metal canister corrosion (VTT) .....	82
Canister corrosion & Hydrogen production .....	83
Microbial activity in bentonite buffers.....	84
Degradation of Bentonite buffers .....	85
Degradation of cementitious materials, as used in seals & plugs.....	86

# 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 impact 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 year 3 evaluation report on experimental and modelling research activities in WP1 and WP2 (Deliverable D3.5). Research activities carried out as part of WP1 are covered in Chapter 2, and those that fall within WP2 are covered in Chapter 3. Each subchapter focuses on a subproject within that work package, and all partners involved provided a summary of their progress. 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.

## 2 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<sub>2</sub> and CH<sub>4</sub> under ILW repository conditions.
- To understand the effect of ILW heterogeneity on bioprocess pathways, pH and redox conditions, barrier degradation and radionuclide release.

### 2.1 Bituminised waste

Contributing partners: SCK•CEN

#### 2.1.1 Problem statement & current knowledge

In Europe, at least five 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<sub>3</sub>, 4 - 6 wt % CaSO<sub>4</sub>, and small amounts of CaF<sub>2</sub>, Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, Ni<sub>2</sub>[(Fe,Mn)(CN)<sub>6</sub>], 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, <sup>241</sup>Am, <sup>244</sup>Cm, <sup>60</sup>Co, <sup>90</sup>Sr, <sup>137</sup>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).

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  $\text{NaNO}_3$  and other soluble salts in the Boom Clay. Scoping calculations indicate that, close to the waste drums the  $\text{NaNO}_3$  concentration can reach up to a few molar and that during the first ~300 to ~1400 years the  $\text{NaNO}_3$  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  $\text{NO}_3^-$  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 ( $\text{NaNO}_3$  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  $\text{H}_2$  present in the disposal facility.  $\text{H}_2$  will be present, produced by the anaerobic corrosion of steel drums and from radiolysis of water and bituminised waste. The steel and/or  $\text{H}_2$  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.* 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 ( $\text{NaNO}_3$  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 indigenous 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 gaseous nitrogen compounds ( $\text{NO}$ ,  $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) 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  $\text{N}_2$  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 ( $\text{NO}$ ,  $\text{N}_2\text{O}$ ,  $\text{N}_2$ ) 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 suppressing factors for microbial activity in a disposal facility with bituminised waste can be high salinity (caused by  $\text{Na}^+$  from  $\text{NaNO}_3$ ), high pH (caused by the  $\text{Ca}(\text{OH})_2$  leaching from the cement) and space restrictions (due to high consolidation and small poresize) in undisturbed Boom Clay.

### 2.1.2 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 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.

### 2.1.3 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<sub>2</sub>O, N<sub>2</sub>) 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 NH<sub>4</sub><sup>+</sup> 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.

## 2.2 Cellulose containing waste

Contributing partners: UNIMAN, HZDR & UGR

### 2.2.1 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. Assuming that the GDF will be anaerobic and dominated by hyperalkaline pH (pH value of 12.5) and a  $\text{Ca}^{2+}$  concentration of about 20 mM (Berner, 1992), 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).

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. These aspects are addressed in this study.

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 ( $\text{AcO}^-$ ) and  $\alpha$ -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 done in this study, is necessary for the assessment of the safety and the long-term performance of a nuclear waste repository.

### 2.2.2 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  $\gamma$ -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 so far:

- 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<sub>2</sub>, acetate, and presumably CO<sub>2</sub>. 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).

### 2.2.3 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<sub>2</sub>, and acetate, while CH<sub>4</sub> was not detected.

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 form 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<sub>2</sub>, by microbial fermentation, could potentially lead to a decrease in complexant concentration, and thus could have beneficial effects. This should be explored further.

## 2.3 PVC containing waste

Contributing partners: UNIMAN

### 2.3.1 Problem statement & current knowledge

Plastics represent a significant volumetric contribution to 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.

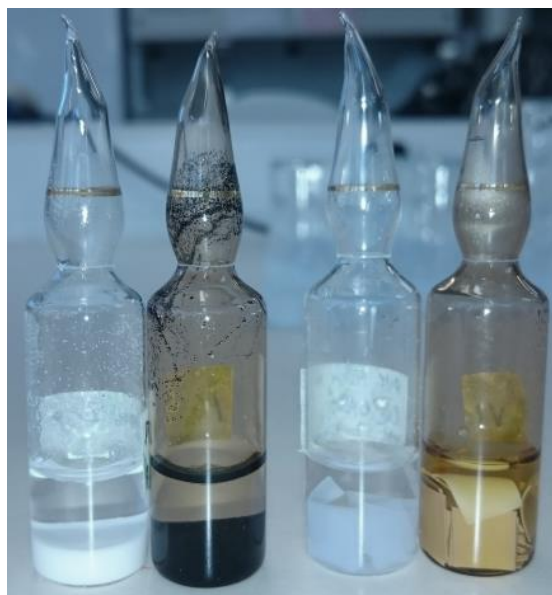
### 2.3.2 Summary of experimental & computational results

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 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 1: The effect of radiation on the appearance of PVC materials. Imaged are flame-sealed vials containing saturated  $\text{Ca}(\text{OH})_2$  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.*

#### 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.

### 2.3.3 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.

## 2.4 Ion exchange resins waste

Contributing partners: EPFL, TUL, CV Rez

### 2.4.1 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 (40%) of the organic material in the inventory of ILW and LLW in Switzerland. Within the MIND project, studies are being undertaken with material from Switzerland and the Czech Republic.

#### EPFL

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 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, hence it is important to have a better understanding of their composition. Evaluating this process is the first phase of the proposed work.

Previous work has been carried out to investigate the radiolytic degradation of resins. 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.

Additionally, the potential of microorganisms to further degrade (i.e., biodegrade) the radiolytic degradation products has not been evaluated. These compounds, if biodegradable by the microbial community that best represents repository conditions in Switzerland (i.e., that present in the Mont Terri underground rock laboratory), could produce chelating agents for radionuclides, could fuel sulphate reduction or methanogenesis. This potential is another aspect of the work that will be evaluated.

### **TUL/CV Rez**

Radioactive ILW and LLW include different types of organic compounds. Organic ion exchange resins, which are present in significant amounts in the national inventories, were selected as an appropriate study material for the MIND project. The effect of radiation of polystyrene resins under simulated repository conditions was studied in batch experiments at CV Rez and TUL. Microbial degradation of radioactive waste was carried out in batches inoculated with underground water collected in the Josef URL from a depth of approximately 100 m.

Ion exchangers are an important part of radioactive waste representing a significant amount originated from pressurized water reactor (PWR). One of the key radionuclides present in spent ion exchangers is Caesium (Cs). Therefore much attention has been given to the radioisotope  $\text{Cs}^{137}$  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). Microorganisms can take up  $\text{Cs}^+$  either through incorporation via the  $\text{K}^+$  transport system thanks to its chemical similarity to  $\text{K}^+$  (Kato *et al.*, 2016), or it can be adsorbed on the surface of cell walls (Lan *et al.*, 2014). High-level radioactive waste is planned to be disposed in deep, stable, geological structures, usually by encapsulating in a metal canister surrounded by bentonite buffer and embedding it in the host rock. The deep geosphere is not a sterile environment, however, microorganisms showing a diverse metabolic activity may be present in both the buffer material and the host rock (Masurat *et al.*, 2010).

The adsorption of Cs ions on the surface of bacterial cells or the incorporation 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  $\text{Cs}^+$ , 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  $\text{Cs}^+$  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.

### **2.4.2 Summary of experimental & computational results**

#### **EPFL**

Resins were obtained that are identical to the ones used in the nuclear power plants in Switzerland. 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 ( $\text{Li}^+$ ,  $\text{BO}_3^{3-}$ ,  $\text{SiO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{F}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{Br}^-$ ) 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  $\text{Cs}^{137}$  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 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  $\text{H}_2$ ,  $\text{CH}_4$ ,  $\text{CO}_2$ , and  $\text{CO}$  were performed with a  $\mu$ -GC. Furthermore, unirradiated resin that was otherwise treated identically was also analysed.

Comparison of the irradiated and unirradiated bead resins revealed the production of  $\text{H}_2$  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. The liquid phase is not yet fully analysed. 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 probably overlooked these compounds.

The latest round of irradiation experiments with a cobalt gamma source at 200 kGy revealed that as well as  $\text{H}_2$  and  $\text{CO}_2$ , chloromethane and benzene were again measured 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 is being attempted using reverse-phase column separation prior to GC analysis to detect any dissolved species.

No experiments have yet been carried out to evaluate the biodegradation of these radiolytic resin degradation products under conditions relevant to the repository. However, benzene and toluene degradation by sulphate-reducing bacteria has been documented by several authors (Vogt *et al.*, 2011; Beller *et al.*, 1997). 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  $\text{H}_2$  from anoxic steel corrosion and resin irradiation (Bagnoud *et al.*, 2016).

## **TUL/CV Rez**

Work in the Czech Republic examines irradiated cationic and anionic bead resins and the subsequent biodegradation of the irradiated resin materials with indigenous bacteria from a near-surface well containing SRB. In the Czech Republic, cationic and anionic resins have been subject to gamma irradiation at the Prazdroj facility at a total dose of 1.028 MGy. Irradiations have been undertaken at a range of resin/water concentrations. Degradation products of polystyrene resins detected in the liquid fraction mainly consisted of trimethylamine and dimethylaminacetatenitrile. Subsequent microbial microcosm experiments showed that the lowest concentration of irradiated anion exchangers (0.2 g/l) caused the highest relative increase in bacterial abundance (14 fold increase). Cation-exchanger caused a total decline in bacterial biomass in the two highest concentrations

studied (20 and 60 g/l), probably because of the extremely low pH (pH 3.36 and pH 3.15) resulting from the irradiation and exchange processes. Lower concentrations studied (0.2 and 2 g/l) were comparable to a control.

The study focused on the typical representative of polystyrene ion-exchange resins that serve for capturing radionuclides outgoing from a pressurized water reactor in nuclear power plant technology. The sulfonic (cation exchange) or amine (anion exchange) resins were irradiated at a total dose of ~1 MGy, which corresponds to the expected dose during the first 1,000 years in the repository. The average dose rate ~2.04 kGy/h was high to achieve a shorter time of irradiation. Irradiated resins of concentrations 0.2, 2, 20 and 60 g/l were inoculated with natural underground water (VITA) from Josef URL. Batch experiments were performed under anaerobic conditions. The changes in microbial abundance were monitored by qPCR. Irradiation products of polystyrene resins mainly consisted of trimethylamine and dimethylaminacetatenitrile. The increase in bacterial biomass was observed at lower concentrations of resins. Higher content of resins (>20 g/l) caused a rapid decrease in the bacterial biomass.

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 underground water (VITA) from Josef URL. The effect of Cs<sup>+</sup> ions was determined using molecular-biological methods (qPCR, amplicon 16S rDNA sequencing) 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<sup>+</sup> concentration (0.5 mM) led to the proliferation of bacteria, specifically the nitrate reducers. Higher Cs<sup>+</sup> 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<sup>+</sup> and in the presence of 0.5 mM of Cs<sup>+</sup>. Interestingly, OTU composition of the samples with higher Cs<sup>+</sup> 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<sup>+</sup> because an increase in their abundance was detected by amplicon sequencing. To conclude, no toxic effect of 0.5 mM of Cs<sup>+</sup> and toxic effect of higher concentrations (1 and 5 mM) of Cs<sup>+</sup> was observed.

### **2.4.3 Conclusions**

#### **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 some potential energy sources for microbial metabolic activity, although some compounds, such as aromatics may not be biodegradable. More results on the aqueous phase composition will reveal whether further compounds are released and metabolised.

The potential of indigenous communities from the host rock borehole waters, to degrade organics released from the irradiated anion resin materials has begun to be shown, if the acidity generated by resin degradation is not extreme.

#### **TUL/CV Rez**

The experiment with irradiated polystyrene ion exchangers revealed that the degradation products may serve as nutrients for bacteria; while only low concentrations (0.2 g/l) of the exchangers

supported bacterial proliferation (14 fold increase). Two higher concentrations (20 and 60 g/l) displayed a toxic effect on bacteria, probably due to the low pH values in media. Increasing concentrations of Cs<sup>+</sup> ions (1 and 5 mM) that are 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<sup>+</sup> concentrations (0.5 mM) promoted bacterial proliferation, especially total biomass and nitrate-reducing bacteria.

## 2.5 Radionuclide complexation

Contributing partners: HZDR & UGR

### 2.5.1 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<sup>-</sup>) can be a biotic or abiotic degradation product of organic material (e.g. cellulose) present in nuclear waste, while  $\alpha$ -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.

## 2.5.2 Summary of experimental & computational results

Contributing partners: HZDR & UGR

### HZDR

Radionuclide speciation studies with organic degradation products:

Systems: U(VI)-acetate, U(VI)-ISA

- Starting conditions: acidic pH range, investigations in the neutral and alkaline pH region have started.
- Synthesis of  $\text{Ca}(\text{ISA})_2$  based on a protocol from UNIMAN. Based on  $\text{Ca}(\text{ISA})_2$  the Na-form,  $\text{NaISA}$ , was prepared for the speciation studies. The purity of both salts was verified by  $^{13}\text{C}$ - and  $^1\text{H}$ -NMR.
- Application of spectroscopic methods (e.g. UV-vis, TRLFS, ATR-FT-IR, XAS) in combination with theoretical calculations for U(VI)-species characterization.
- Ongoing investigations involve the synthesis of crystalline  $\text{NaUO}_2(\text{AcO})_3$ , having the third  $[\text{UO}_2(\text{AcO})_3]^-$ -complex as central unit. SC-XRD, ATR-FTIR and TRLFS (and probably EXAFS) will be applied to characterize the solid compound and will be compared to the results of the aqueous system. This includes also the synthesis of crystalline U(VI)-ISA complexes.
- Eu(III)-ISA investigations are currently running. On the one hand Eu(III) is an analogue for trivalent actinides and on the other hand we want to know, whether the “-yl”-oxygens of the  $\text{UO}_2^{2+}$ -entity have an effect on the binding properties of ISA.
- Outlook: evaluation of experiments in the neutral and alkaline pH region.

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.

U(VI) – acetate system (acidic pH range)

The interaction of the uranyl-ion ( $\text{UO}_2^{2+}$ ) 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  $\text{UO}_2^{2+}$ -acetate complexes.

For instance, Figure 2 shows the luminescence spectra of test series 2 at  $-120^\circ\text{C}$ . 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  $\text{UO}_2^{2+}$ -acetate complex  $[\text{UO}_2(\text{AcO})_2]$  (1:2-complex).

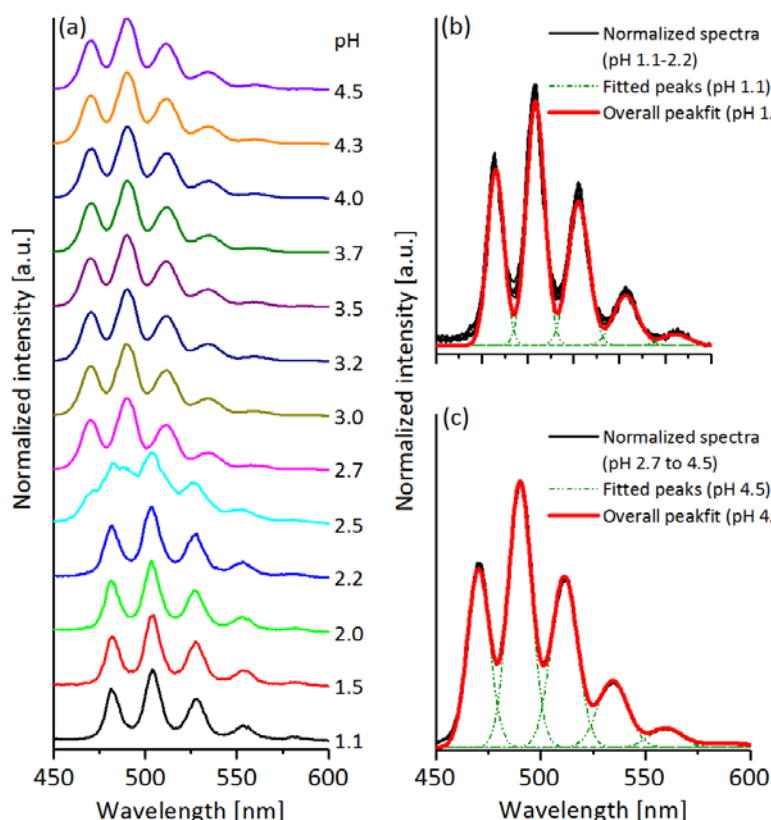


Figure 2: Normalized luminescence spectra of test series 2 (50  $\mu\text{M}$   $\text{UO}_2^{2+}$  and 0.1 M Acetate) at  $-120^\circ\text{C}$  (a) and the related normalized spectra with fitted peaks of phase 1 and 2 (b and c).

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 (acidic pH range)

In order to characterize complexes formed in the  $\text{UO}_2^{2+}$ -ISA system by NMR- and ATR-FTIR-spectroscopy, it was necessary to provide reference data of the pure ligand. Therefore, NaISA-solutions were characterized with these two methods in the absence of  $\text{UO}_2^{2+}$ . The purity of the synthesized NaISA stock solution can be estimated as  $>95\%$  by NMR-spectroscopy. Whereas several studies reported NMR-data for ISA and its conformations, no references are available for IR-data. To determine IR-reference data for ISA and its conformations ATR-FTIR-spectra of 90 mM NaISA-solutions at different pH values were measured and ITFA<sup>2</sup> was applied to calculate the single component vibrational spectra of ISL ( $\alpha$ -D-Isosaccharino-1,4-lactone), HISA (Isosaccharinic acid) and ISA (Isosaccharinate) (see Figure 3). Based on literature values for (hydroxy-)carboxylic acids and lactones, the most important and prominent bands were assigned to the vibrations of related functional groups.

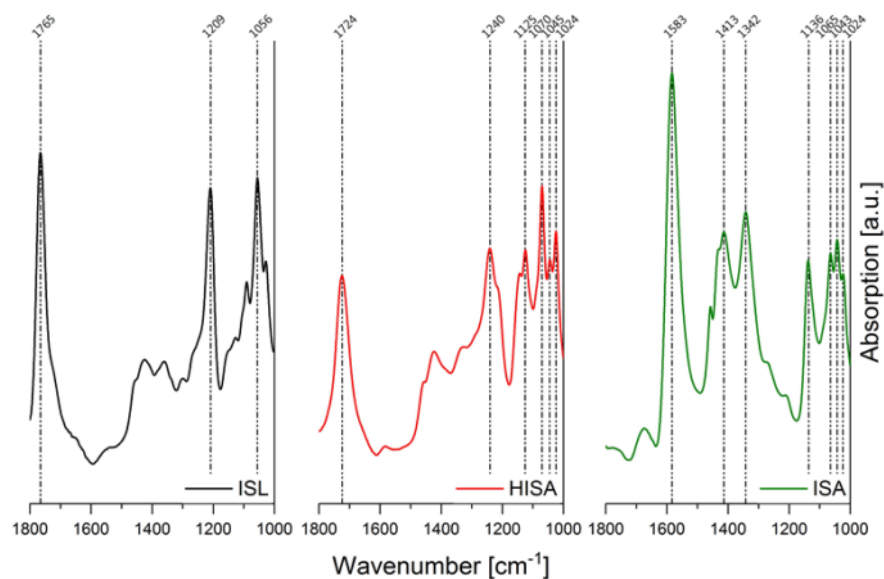


Figure 3: Single component ATR-FTIR-spectra of ISL, HISA and ISA

Absorption spectroscopy (UV-vis) was used to determine the number of formed complexes between  $\text{UO}_2^{2+}$  and ISA. Changes of the absorption spectra with changing pH, at different metal to ligand ratios were measured (see Figure 4).

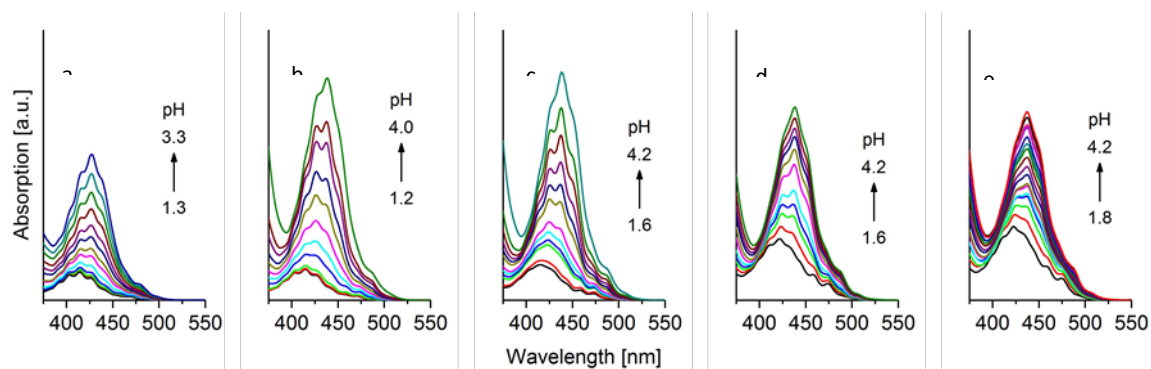


Figure 4: UV-vis-spectra of pH-series at different  $\text{UO}_2^{2+}$  (15 mM) to ISA ratios: 2:1 (a), 1:1 (b), 1:2 (c), 1:6 (d) and 1:16 (e).

As a result of ITFA (Roßberg *et al*, 2003) the spectra in Figure 4 could be fairly good reproduced with four components (species) (see Figure 5). Component 1 (species 1) can be assigned to  $\text{UO}_2^{2+}$  meaning that 3 different U(VI)-ISA complexes could be identified. Several observations have to be emphasized. Species 4 became more dominant, the higher the excess of ISA was. In contrast to that was the amount of Species 3 lowered, at higher ISA concentrations. Furthermore, the formation of that component was suppressed by the formation of Species 2, which was present at lower pH values and became more dominant the higher the excess of ISA was. Species 2 was always dominant at lower pH values indicating an unexpected interaction between  $\text{UO}_2^{2+}$  and HISA.

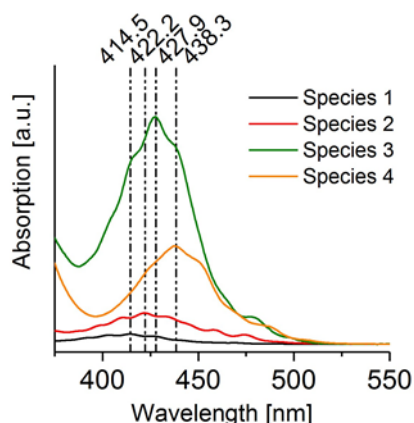


Figure 5: ITFA derived single component spectra of species in the  $\text{UO}_2^{2+}$ -ISA system.

These single component spectra are very valuable for continuing experiments, since UV-vis-spectroscopy could now be used as reference method. The conclusions drawn from absorption spectroscopy could be successfully confirmed by luminescence spectroscopy.

In order to estimate the limiting metal to ligand ratio in the formed complexes, the method of continuous variation was applied (Renny *et al*, 2013), which is commonly known as Job Plot. Therefore, absorption spectra were recorded at pH 4 and different metal to ligand ratios, ranging from 1:0 to 1:3.7. The absorption at 438 nm, the maximum of Species 4, was then plotted against the mole fraction of  $\text{UO}_2^{2+}$ . The observed maximum at 0.33 is indicative for a metal to ligand ratio of 1:2. But it has to be mentioned that a complex with a ratio of 2:4 or higher is also possible, since this cannot be distinguished with this method.

A comparison of the superimposed  $^{13}\text{C}$ -NMR spectra of free ISA and a sample containing  $\text{UO}_2^{2+}$  and ISA in a ratio of 1 to 2 at pH 4.2 showed clearly that changes occur in the chemical shifts of certain carbons. Based on these findings, a chelate binding of  $\text{UO}_2^{2+}$  via the carboxylic group ( $\text{C}_1$ ) and either the  $\text{C}_2$ -(3°)- or the  $\text{C}_6$ -(1°)-alcohol, resulting in a 5- or 6-membered ring, can be expected.

To identify the binding motifs, samples with different metal to ligand ratios were measured at pH 3 and 4 by ATR-FTIR-spectroscopy. All gained information indicate the existence of two dominant binding sites, between the carboxylic group and either the 3° ( $\text{C}_1$ ) or the 1° alcohol ( $\text{C}_6$ ), resulting in a 5- or 6-membered ring. The structural schemes as well as the optimized structures of the corresponding 1:1 complexes for each binding motif are depicted in Figure 6.

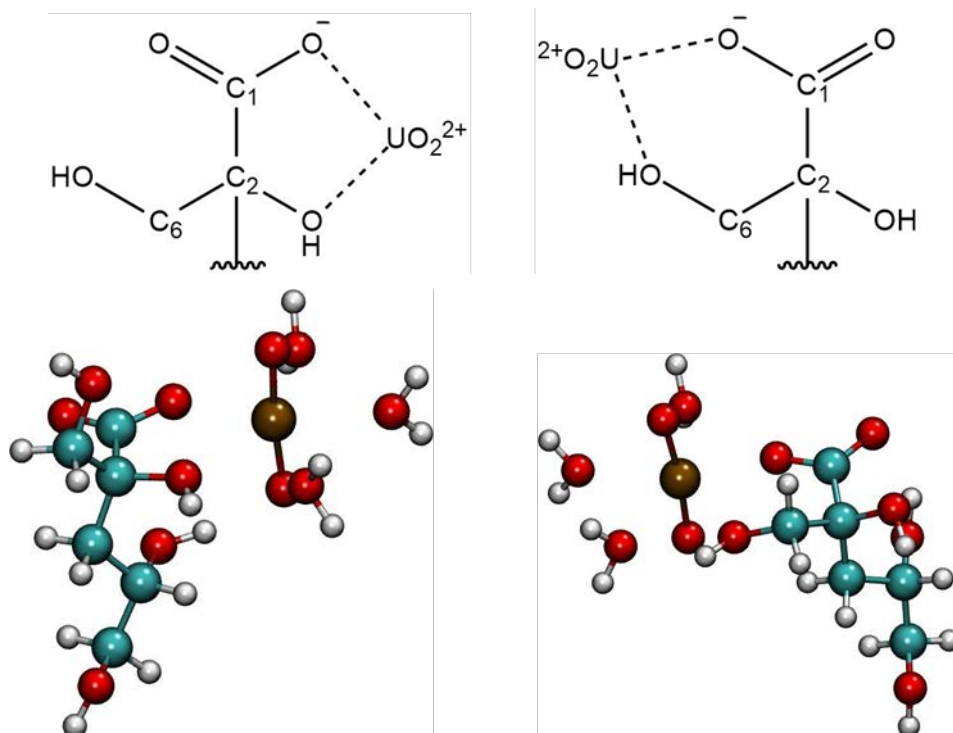


Figure 6: Dominant binding sites in  $\text{UO}_2^{2+}$ -ISA-complexes: general structure of the 5- (a) and 6-membered ring (b); optimized structures of the  $[\text{UO}_2\text{ISA}]^+$ -complex with a 5- (c) and 6-membered ring (d).

Ongoing long-term tissue paper biodegradation experiment under hyperalkaline conditions in cooperation with UNIMAN

Within the first four sampling points increasing U(VI) concentrations in solution (influence of formed ISA and acetate as tissue degradation products),  $0 \rightarrow 1 \mu\text{M}$ , were measured. This experiment is currently running, and the samples will be analysed.

## 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 7). 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 nanospheres 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.

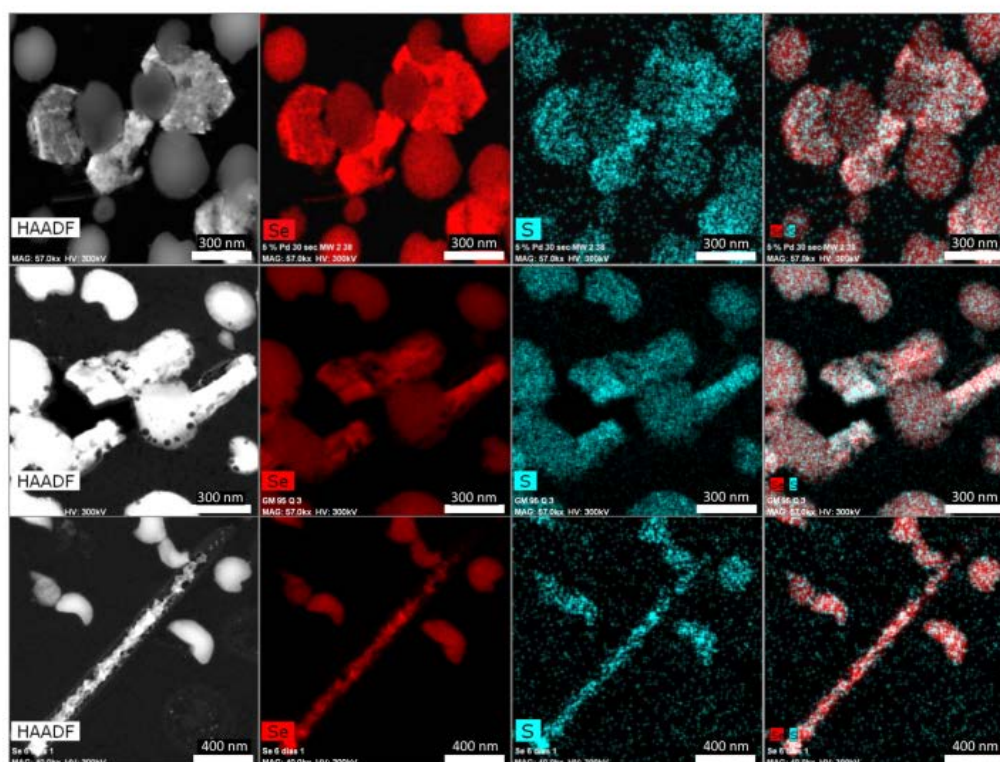


Figure 7: HAADF-STEM micrographs of a thin section showing the different morphologies (spherical, hexagonal, polygonal, and nanowires) of SeNPs produced by *S. bentonitica* after 144 h of incubation. EDX element-distribution maps confirmed that they are mainly composed of Se and S. (Ruiz-Fresneda et al. 2018)

### 2.5.3 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  $\text{UO}_2^{2+}$ -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  $\text{UO}_2^{2+}$ -acetate system as well as the new spectroscopic reference data might improve the general understanding of the interaction of  $\text{UO}_2^{2+}$  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.

U(VI)-ISA system in the acidic pH-range: Fundamental information about the interaction of  $\text{UO}_2^{2+}$  with ISA were gathered by a variety of spectroscopic techniques. Undoubtedly is the following

information essential to completely describe this system: (a) three dominant  $\text{UO}_2^{2+}$ -ISA-complexes were discovered; (b) two of the three complexes are probably polynuclear; (c) HISA has to be considered as binding partner up to pH 3; (d) 5- and 6-membered rings are the dominant binding motifs; and (e) the limiting metal to ligand ratio is 1:2.

Since the indications for the 5- and 6-membered rings in the ATR-FTIR- and NMR-spectra occur simultaneously it is so far not possible to say if the 1:1 complexes depicted in Figure 5 coexisted together or if complexes were present, having both binding sites. Based on the identified three building blocks, an almost endless number of conceivable complex-structures are possible. The formation of chain-like structures as well as bridged complexes seem to be realistic. An approach to identify the formed complexes was to compare the experimentally determined relative shift of the asymmetric stretching of the  $\text{UO}_2^{2+}$ -entity with theoretically calculated shifts. Unfortunately no calculated relative shift does perfectly fit to the experimentally observed shifts, while fulfilling the spectroscopically determined properties. Instead of trying to find the ideal complexes based on speculative suggestions, other approaches are necessary to extend the knowledge derived so far from spectroscopy. For this purpose, experiments are running, with the goal to synthesize crystalline  $\text{UO}_2^{2+}$ -ISA-compounds. Then these materials will be characterized by single-crystal-XRD. A second approach is to apply MS to obtain information concerning the mass of the formed complexes, which could dramatically decrease the number of conceivable complex structures. Finally, the ultimate objective is to generate reliable complex-formation constants. But to do this properly it is necessary to clarify the speciation in advance.

## 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.

## 2.6 *In situ* processes: hydrogen release from organic waste

Contributing partners: EPFL

### 2.6.1 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,  $\text{H}_2$  accumulation is expected in both HLW as well in L/ILW, due to the anoxic chemical corrosion of steel canisters and in L/ILW, also the radiolytic degradation of organic compounds may lead to an increased  $\text{H}_2$  production.

Microbial processes that consume  $\text{H}_2$  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,  $\text{H}_2$  oxidation coupled to sulphate reduction is a readily achievable process due to the presence of autotrophic sulphate-reducing bacteria. However, the

results were obtained by somewhat artificially 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<sub>2</sub> as an electron acceptor instead of sulphate) may be a viable microbial metabolism and would also consume H<sub>2</sub> but with a concomitant production of CH<sub>4</sub>. Nevertheless, this would still result in a net decrease of the gas volume according to the following reaction stoichiometry:  $4 \text{ H}_2 + \text{CO}_2 \rightarrow \text{CH}_4 + 2 \text{ H}_2\text{O}$ . Thus, an experiment aiming at evidencing the potential for methanogenesis *in situ* in Opalinus clay porewater was devised.

### 2.6.2 Summary of experimental & computational results

The investigation centred on the repeated amendment of an *in situ* borehole in Opalinus clay with H<sub>2</sub> 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<sub>2</sub>, the microbial community was monitored and was found to be dominated by sulphate-reducing bacteria. Geochemical data such as δ<sup>34</sup>S-sulphate and δ<sup>13</sup>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<sub>2</sub>. 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 elemental sulphur 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 an unknown form of iron(III) to produce elemental sulphur 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. At present, the only possible source of iron(III) that was detected was illite. PHREEQC modelling is also being used to identify possible mineral dissolution within the experimental system (siderite is hypothesized to be the iron(II) source).

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<sub>2</sub>.

### 2.6.3 Conclusions

Here, we have investigated the potential for methanogenesis as hydrogen consuming microbial process in Opalinus Clay porewater. The results so far have not successfully evidenced this type of metabolism but thermodynamic considerations have identified potential limitations in the experimental conditions. Hence, further conditions will be tested to attempt to show this process *in situ* and in microcosm (batch) settings.

## 2.7 *In situ* processes: methane release from organic waste

Contributing partners: NNL, VTT & TVO

### 2.7.1 Problem statement & current knowledge

As noted above in the Section 2.6, 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  $^{14}\text{C}$  in the form of  $^{14}\text{CH}_4$ .

The TVO gas generation experiment (GGE, see Figure 8) 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.

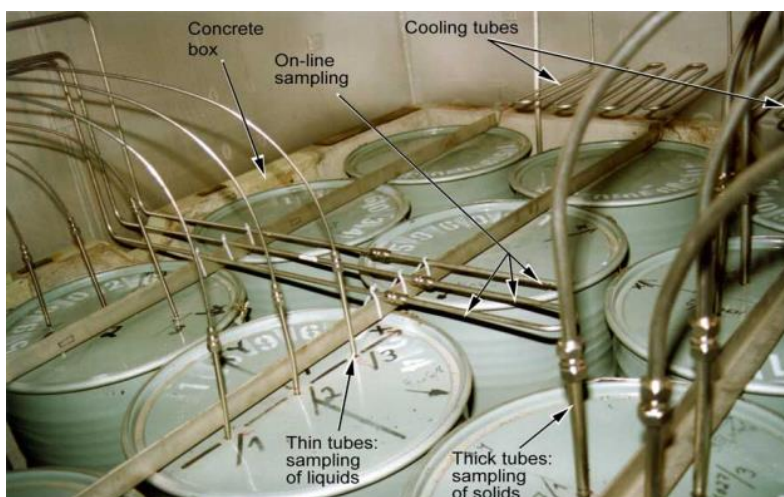


Figure 8: View inside the Gas Generation Experiment before filling with water, showing waste drums containing LLW, sampling tubes within waste drums and in the tank water and the concrete box as used to emplace waste drums within the VLJ repository (Photograph courtesy of TVO).

### 2.7.2 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 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  $\sim 0.2$  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<sup>3</sup> 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. 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*, submitted).

### 2.7.3 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.

## **2.8 Modelling of microbial processes**

Contributing partners:>NNL

### **2.8.1 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 developed 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.

### **2.8.2 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. 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 9). 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 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.

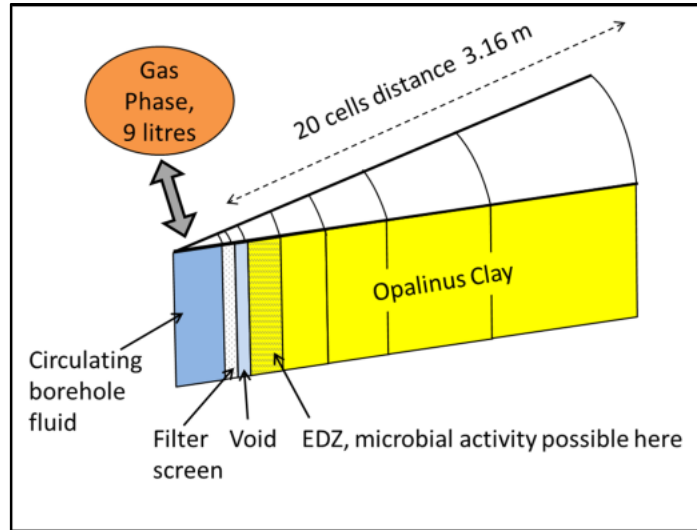
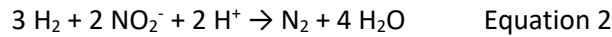


Figure 9: Schematic of the PHREEQC model of the BN experiment

The denitrification process was represented as a two stage reaction forming nitrite ( $\text{NO}_2^-$ ) as an intermediate in nitrate ( $\text{NO}_3^-$ ) reduction by hydrogen ( $\text{H}_2$ ):



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  $\text{H}_2$  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 10).

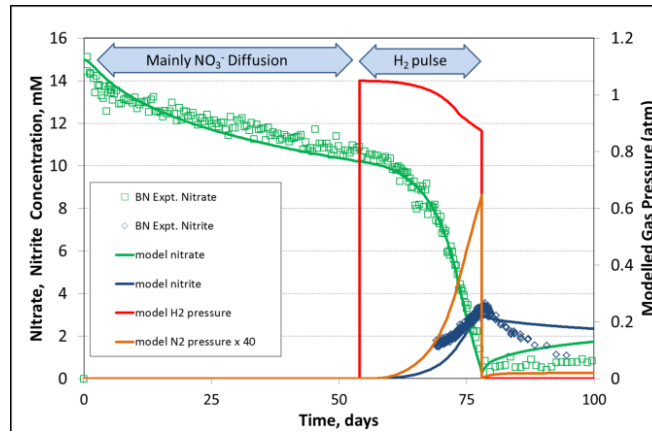


Figure 10: Measured and modelled nitrate and nitrite concentration and modelled gas pressures during the pulsed hydrogen injection of the BN experiment.



PHREEQC modelling of sulphate reduction process in the MA experiment in Opalinus clay (see above Section 2.6) is in progress. 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. Currently the model is being parameterised to fit the observations from the initial data collected from the MA (Bagnoud *et al*, 2016a). Further work is required to represent the changing conditions of the experiment ( $H_2$  injection phases, etc.) within the model. Also, the estimation or fitting of the kinetic parameters used in the Michaelis-Menten equations is required. Of particular interest is the potential effect of sulphide arising from sulphate reduction acting as an inhibitor for certain microbial populations. This is accounted for in the model by a rate inhibition factor and could be the reason for the incomplete reduction of sulphate observed in the MA experiment. However, it is possible that the effect is mitigated by the precipitation of low-solubility iron sulphide phases, such as mackinawite (FeS).

Within Task 1.3 (Section 2.7, 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 11) and the pH neutralisation (Figure 12). 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 11) and the pH data (Figure 12). 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 13, Small *et al*, 2017).

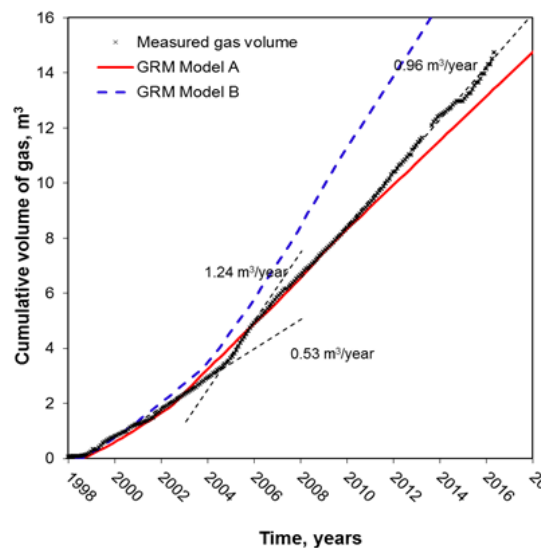


Figure 11: Rate of gas generation measured by the TVO Gas Generation Experiment and modelled rate of gas generation (Small, *et al* 2017).



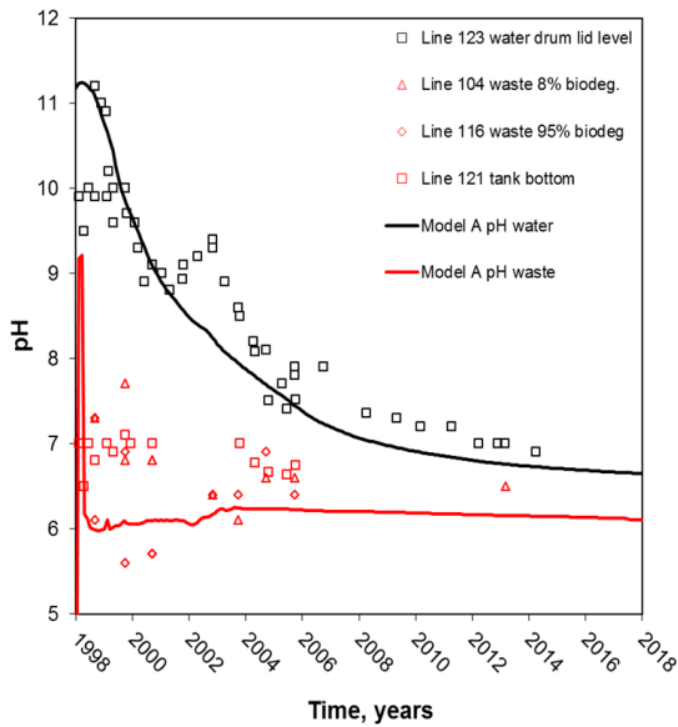


Figure 12: pH measurements from the waste drums and tank water regions of the TVO Gas Generation Experiment and modelled pH of the water and waste regions of the mode (Small, et al 2017).

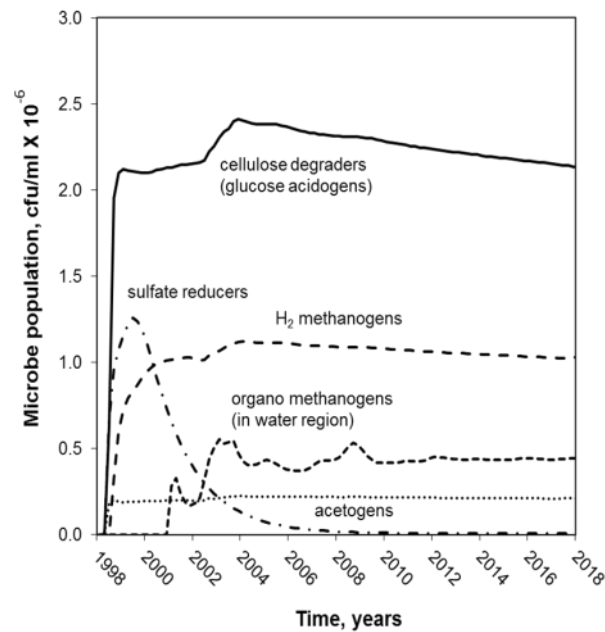


Figure 13: Modelled biomass concentration of the main anaerobic microbial processes simulated for the TVO Gas Generation Experiment (Small, et al 2017).

Within MIND Work package 1 Task 1.4, NNL is developing an Application Programming Interface (API) that implements the existing model approaches of the GRM code, so that it can be interfaced within

more modern, commercial modelling codes. The GRM microbial model considers a wide range of potential electron donors and acceptors, different microbial communities and is based on Michaelis-Menten kinetics (see Small *et al*, 2008). The API will enable the proven GRM microbial model to be coupled with geochemical and reactive-transport codes.

### 2.8.3 Conclusions

Good progress has been made with existing biogeochemical modelling methods to assist in the interpretation of data from *in situ* experiments. Code development work is in progress that will enable wider use of these modelling approaches by end users of the MIND project in performance assessment related studies.

## 2.9 WP1 - current status and intermediate conclusions

The work package WP1 comprises 4 tasks:

### **Task 1.1** *Review of ILW organic waste types and conditioning in Europe.*

This task was completed to plan at M18 through deliverable D1.1. "A Review of Anthropogenic Organic Wastes and Their Degradation Behaviour"

### **Task 1.2** *Rate and mechanism of biodegradation of ILW organic polymers affected by radiation, under repository relevant conditions.*

D1.2 "Effects of radiation and microbial degradation of ILW organic polymers" was delivered at M24 by UNIMAN and presents detailed studies of cellulose and PVC degradation as summarised in Sections 2.2 and 2.3 of this report. 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. 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 at starting hyperalkaline pH led to the production of H<sub>2</sub>, and acetate by fermentation processes and a decrease in pH. Concerning PVC materials, 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. A range of organic additives 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. 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 the lower energy yielding methanogenesis processes that result in microbial gas generation.

Deliverable D1.3 concerning biodegradation of bitumen radiolysis products has been produced. This work demonstrated that the 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 the reducing capacity of the Boom Clay and increase the mobility of certain radionuclides (Section 2.1).

Work concerning the biodegradation of irradiated ion exchange resin (Section 2.4) 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 H<sub>2</sub> and a number of other metabolisable organic compounds (EPFL). Studies examining the bacterial colonisation of irradiated resins (TUL/CV Rez) have demonstrated bacterial growth provided that the pH doesn't become too acidic by the resin irradiation. Related work examining the toxicity of Cs has shown no toxic effect of 0.5 mM of Cs<sup>+</sup> but toxic effects at higher concentration (1 and 5 mM) of Cs<sup>+</sup> were observed.

Studies of radionuclide interactions with cellulose degradation products is progressing and are reported in D1.4. Aqueous spectroscopy studies have furthered the knowledge of the UO<sub>2</sub><sup>2+</sup>-acetate system and the existence of two complexes. Studies of the U(VI)-ISA system in the acidic pH-range provide evidence of three dominant UO<sub>2</sub><sup>2+</sup>-ISA-complexes. Novel mechanisms of radionuclide (Se) interactions with bacteria isolated from bentonites, forming water insoluble complexes associated with the cells have been identified for experiments starting with either Se(IV) and Se(VI). 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.

### **Task 1.3 Microbiological metabolism under repository conditions**

This task includes two *in situ* experiments.

(1) An experiment at the Mont Terri URL examining hydrogen removal by microbial sulphate reduction processes, and potential transition to methanogenesis, in a borehole drilled into Opalinus Clay (Section 2.6). Results to date show the dominance of sulphate reduction processes in the Opalinus Clay system as a consequence of its high sulphate concentration. In addition thermodynamic considerations suggest that methanogenesis may not be feasible in this system. Microcosm experiments examining low sulphate concentrations again show the absence of methanogenic conditions. Sulphate reduction in this case results in formation of elemental sulphur by a reaction possibly involving Fe(III) containing minerals present in the Opalinus Clay (potentially, this has implications for MIND WP2 studies of copper corrosion by limiting sulphide concentration).

(2) The Gas Generation Experiment (GGE) (Section 2.7) examining long term (~20 year) processes of microbial gas (methane) generation from cellulose and steel containing LLW. Modelling and interpretation of the experimental data concludes that the rate of gas generation has been affected by the homogenisation of chemical conditions over the 20 years of the experiment. In particular the initial alkaline pH of the tank water has been neutralised allowing methanogenesis to develop in all regions of the model of the experiment. The modelling also indicated that the increase in rate of gas generation observed coincides with the later establishment of organic consuming methanogens in the tank region of the experiment. Microbial characterisation of samples from the experiment also indicates the later occurrence of such organic consuming methanogens (Results presented in D1.6, delivered at M36). It is also possible that initially methanogenesis was partially inhibited by aqueous sulphide, formed by sulphate reduction processes, which has slowly declined as a result of equilibration with FeS (mackinawite).

**Task 1.4** *Modelling the impact of relevant biodegradations processes on the chemical and radionuclide source terms*

Modelling of *in situ* borehole experiments at the Mont Terri URL and the TVO Gas Generation Experiment has shown the capability to model these complicated experiments using reactive transport models that represent the microbial processes through Monod type kinetic processes coupled to equilibrium geochemical speciation and mineral reaction processes. The models have assisted in the interpretation of these experimental systems and have potential for wider application in performance assessment studies of repository systems.)

Work to date (M36) within WP1 has produced some significant results concerning organic degradation, radionuclide speciation and gas generation. Several peer-reviewed journal papers have been published from the WP concerning:

- The characteristics of alkaliphilic bacteria capable of ISA biodegradation at high pH (Bassil and Lloyd, 2017; 2018). Kuippers *et al* (2018) further discusses nickel complexation and immobilisation in the presence of cellulose degradation products. Nixon *et al* (2018) presents results of the PVC irradiation and biodegradation studies.
- Sanchez-Castro *et al* (2017a,b) has described the formation of Se(0) nanoparticles by bacteria from Spanish bentonite.
- Shrestha *et al* (2018) presents results of Cs toxicity studies.
- Small *et al* (2017) present modelling results and geochemical data from the TVO Gas Generation Experiment, while Vikman *et al* (submitted) presents detailed microbial characterisation information.

At the 2<sup>nd</sup> and 3<sup>rd</sup> Project Annual Meetings, the following summary points were collated from the WP1 research in progress presented:

- Anthropogenic polymers (e.g. bitumen, PVC, resins,) are biodegradable to an extent, and:
  - May affect pH buffering
  - Can produce many chemical degradation products (e.g. ISA) which are biodegradable at pH <11.5
  - Could fuel microbial gas generation processes
- Irradiation has various effects, including
  - Enhancing cellulose degradation
  - Making Irradiated PVC more recalcitrant
  - Generating acidity
- New data and findings regarding radionuclide interactions with organic degradation products
  - New understanding of uranyl ISA speciation.
  - Ni immobilisation by biogeochemical processes involving cellulose degradation products.
  - Radionuclide (Se)-microbe interactions
- H<sub>2</sub> is readily consumed by microbiological processes, which has significant implications for performance and safety assessment

- Fermentation processes are key to many scenarios
  - Cellulose degradation products, H<sub>2</sub> consumption, methanogenesis
  - Fermentation appears to occur in low pH niches of pH >12 microcosms
- Microbes can affect pH buffering
  - Heterogeneity important
  - Amount of concrete important
- Models being developed to help interpretation and for Performance Assessment

Over the remaining year, research on these topics will be finalised and assessed in the context of LILW disposal scenarios.

### 3 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.

#### 3.1 The availability of sulphide and its effects on canister corrosion

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

##### 3.1.1 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.

Microbial sulphate reduction  $4 \text{ H}_2 + \text{SO}_4^{2-} + 2 \text{ H}^+ \rightarrow \text{H}_2\text{S} + 4 \text{ H}_2\text{O}$  Equation 1

Copper corrosion  $2 \text{ Cu} + \text{H}_2\text{S} \rightarrow \text{Cu}_2\text{S} + \text{H}_2$  Equation 2

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 and 2. It should also be noted that Equation 2 occurs 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, Huttrunen-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  $\text{HS}^-$ , 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  $\text{H}_2$  produced by 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 ( $\text{Fe}^0$ ) and/or the  $\text{H}_2$  and reduce sulphates to sulphides ( $\text{S}^{2-}$ ) or  $\text{HS}^-$ , which can create corrosive agents such as  $\text{H}_2\text{S}$ ,  $\text{FeS}$  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  $\text{H}_2$  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  $\text{H}_2$  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 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)

### 3.1.2 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.

## **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<sub>2</sub> and CH<sub>4</sub> 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. 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<sub>2</sub> and 31 mL/L CH<sub>4</sub> in the porewater of the ONK-KR17 cores and the amount of H<sub>2</sub> and CH<sub>4</sub> increased with increasing depth in OL-KR58. While the concentration of CH<sub>4</sub> was within the range of what is found in deep groundwater, H<sub>2</sub> was much higher. H<sub>2</sub> is rapidly used by SRB, acetogens and methanogens in deep groundwater (Pedersen 2012). Therefore, any H<sub>2</sub> that leaves porewater to groundwater by diffusion has a great chance to be metabolised by bacteria down to concentrations below Km for H<sub>2</sub> (approximately 1 µM).

## **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 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<sub>2</sub> < 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.

qPCR analysis of both the corrosion experiments (laboratory temperature and 35°C) showed that the total 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, Czech synthetic bentonite pore water (SBPW) was used as the corrosion environment in order to simulate conditions in which the carbon steel container will be surrounded by compacted bentonite. VITA water from Josef URL was used as a microbial inoculum. The experiment is planned to last 24 months and it is still ongoing. The corrosion rate was determined by the weight loss method, and it was stable throughout the experiment in the case of the samples exposed to SBPW with VITA, whereas it decreased in time when the environment was sterile. After 12 months of the experiment, the calculated corrosion rate of the samples exposed to the non-sterile environment is 6.5 times higher compared to the samples not exposed to bacteria. The community composition in both biofilm and water changed after the period of 12 months. SRB nearly disappeared and were replaced by microorganisms capable of nitrate reduction such as *Acidovorax*, *Brevundimonas* and *Methyloversatilis*. 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. We observed a local corrosion attack on the samples after 6 months. Our results show that corrosion processes can be involved also in the environments dominating by NRB.

## 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. As a consequence 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 hours 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 ( $^{35}\text{SO}_4$ ) 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 water and instead 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. However, part of the sulphate reducers also produce acetate as do also acetogens detected in Olkiluoto.

## 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  $\text{H}_2$  (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  $\text{H}_2$  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, 2 new *in situ* experiments were set-up in Opalinus clay, (1) to investigate the rate of microbial sulphate reduction (catalysed by  $\text{H}_2$  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 oxygen influx. The system monitors sulphate consumption and sulphide production as well as  $\text{H}_2$  consumption. The flow through cells were designed to allow a gradient of

sulphate and  $H_2$  to establish itself 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 1.5 and 2.5 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. Counts of aerobic heterotrophs tend to lower numbers for longer deployment times, as expected. Additionally, we found that the borehole had developed a sulphate-reducing microbial community since the initial drilling. This microbial community may have been responsible for the colonization of the bentonite or, alternatively, the indigenous bentonite microbial community may have grown due to the presence of a saturated environment. 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 2.5 years, the increase in complex organic matter degraders was reversed and the relative abundance of autotrophic sulphate-reducing primary producers increased again. Further durations of deployment, 1 year (corresponding to a shorter deployment time than is currently available) and 5 years (corresponding to double the deployment time of the currently available data) will help provide support for this data interpretation.

### **3.1.3 Conclusions**

#### **GTK**

Sulphide concentration in groundwater may be higher than that predicted by equilibrium solubility of iron sulphides, because microbial sulphate reduction may take place in iron-deficient conditions. However, the process is generally limited to a relatively narrow sulphate reduction zone. Present data indicates that sulphate and methane may coexist in deep fluids, indicating that methane is not a very effective electron donor for microbial sulphate reduction. It is known, that anaerobic oxidation of methane is a fairly complex microbial process depending on a complex syntrophic consortium of methanotrophic archaea and sulphate-reducing bacteria.

#### **TUL/CV Rez**

We demonstrated that the microbial activity highly influences carbon steel corrosion under anaerobic conditions. The biofilm formation differed depending on the temperature (~20 and 35 °C). Synthetic bentonite pore water prepared based on Czech BaM bentonite material, is rich in nitrates, and thus represents a suitable environment for the development of NRB consortia. NRB seems to be very capable to increase the corrosion rate. Further study is needed to confirm this.

## 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 the *in situ* experiments in the Opalinus clay in Switzerland, offer a partial view on the 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.

## 3.2 Microbial activity in and degradation of bentonite buffers

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

### 3.2.1 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 radionuclide migration 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 are still a number of unresolved questions related to the microbial survival and activity in such bentonites.

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 14.

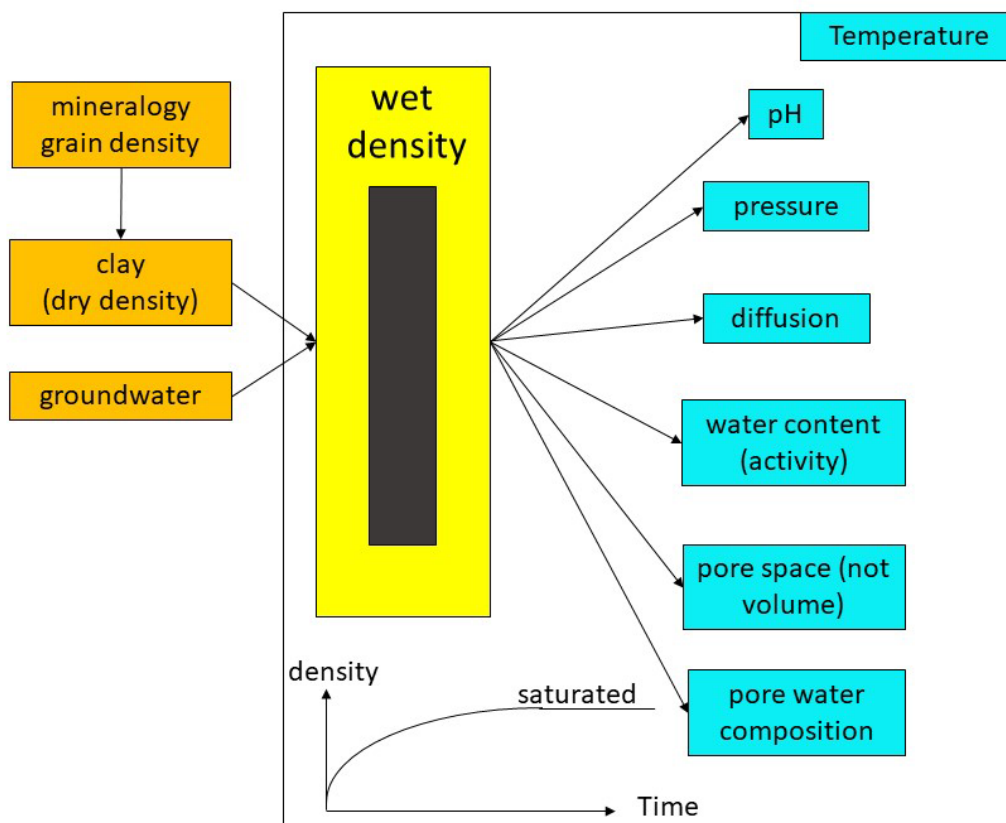


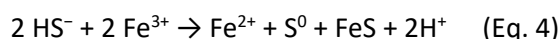
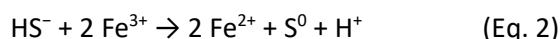
Figure 14: Variables influencing wet density that upon water saturation give rise to suggested factors influencing bacterial viability, cultivability and activity (turquoise) except temperature, not coupled to wet density. Bentonite clays of varying types will be saturated with groundwater over time and reach a final saturated wet density. The environmental conditions for microbial activity will be set by type of clay, groundwater composition and final wet density.

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 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<sub>2</sub>S 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<sub>2</sub>S gas. This fact is utilised to produce industrial filters for removal of H<sub>2</sub>S 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.



The dissociation constant for HS<sup>-</sup>/H<sub>2</sub>S is 10<sup>-6.98</sup> (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<sup>-</sup> 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 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<sup>3</sup> (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<sup>3</sup> 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  $\geq 67^{\circ}\text{C}$  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 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<sup>3</sup> 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.

### 3.2.2 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 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. 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). In addition, Boom Clay (Belgium) has been studied as a reference to a non-swelling clay. These clays had a varying element and mineral composition. In particular, the amount of montmorillonite varied from 66 % to 88 % and the iron content, analysed as  $\text{Fe}_2\text{O}_3$  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  $\text{kg/m}^3$ . 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 14). These variables need further attention for a full understanding of what conditions influence bacterial activity in compacted bentonite clays.

## **EPFL**

Bioreactors to investigate growth in bentonite have been set up using a 80:20 % (wt.) sand:bentonite mixture. This porous media will provide insights into the hydrogen oxidation and sulphate reduction rates of SRB-biofilms under repository relevant conditions. Porewater from Opalinus Clay rock is flowing with a rate of approx. 20 mL/day through one bioreactor and microbial activity is facilitated by periodically addition of hydrogen gas. Throughout the course of a pre-test, porewater flow through one bioreactor has been blocked. To circumvent blocking, minor changes in the porewater inlet design are envisioned for the experimental phase (four modules, 3 month duration, daily application of 1 mL  $\text{H}_2$  at about 2.5 bar, 5 mL/day porewater flow per bioreactor). Results of these experiments are expected during the further course of the project.



## UNIMAN

UNIMAN has 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.
- 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. The first manuscript from this work has now been accepted by Chemical Geology, and focuses 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 will document the changes in clay mineralogical structure linked to microbial Fe(III) reduction in bentonites, and are in draft format; 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 has now been sequenced using an Illumina platform and the data is analysed. We hope to publish these data in due course, and are exploring joint publications with other FEBEX partners. Pressure cell systems have been constructed and high-pressure studies on Fe(III)-reduction in bentonites are reaching their conclusion. Haydn Haynes, the student completing this MIND-affiliated, RWM-funded PhD project will submit his thesis by the end of 2018, and we hope that this thesis will deliver at least three published manuscripts describing the work undertaken.

## BGS/NERC

BGS/NERC experimental work began early in 2017 as planned. Prior effort was concentrated on the design, manufacture, commissioning and calibrating of our experimental system to allow us to run experiments flowing artificial groundwater through compressed bentonite samples containing steel chips and to monitor the evolution of the stress regime in real time in this system (Figure 15). Two bespoke vessels were constructed from grade two titanium, each was fitted with 3 load cells (2 axial and 3 radial) to continuously monitor changes in stress. Filters at either end of the sample allow water to be injected and water to be collected. Injection and backflow HPLC pumps controls the pressure and flow. This allows hydration of the sample and creates a hydraulic gradient across the sample. Two experimental systems have been constructed and housed in temperature-controlled units.

All experiments used gamma-irradiated FE bentonite and compared the response of a non-inoculated “sterile” core (Test 1) to a core inoculated with a sulphate reducing bacteria enrichment from the bentonite prior to irradiation (Test 2). For the first pair of tests, the core was prepared to a dry density of 1400 kg/m<sup>3</sup> and contained 5 g unalloyed steel chips.

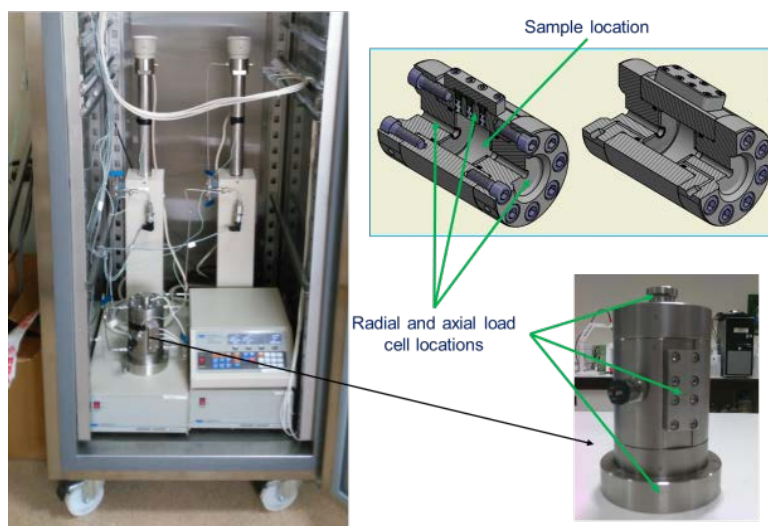


Figure 15: Images showing the experimental apparatus used for monitoring the impact of microbes on the physical properties of compacted bentonite samples.

In both tests, both pumps were set to 1000 kPa to allow the groundwater to flow into the clay, saturate and swell – as seen by the sharp rise in the load cell pressures seen from day 1 onwards (Figure 15). Once the swelling pressures were stable, the injection pressure was increased to 2507 kPa. In Test 1 this hydration period lasted 26 days and a swelling pressure of 1660 kPa was recorded. The application of the hydraulic gradient on day 26 resulted in an increase in total stress and the divergence of the data from the load cells reflected the gradient in hydraulic pressure across the sample. Following application of the hydraulic gradient, inflow asymptoted at around day 45 and flux remained constant thereafter. Permeability of the sample was constant at around  $1.8 \times 10^{-20} \text{ m}^2$ . A similar response was observed in the inoculated Test 2. In Test 2 Hydration occurred over a 25 day period. Swelling pressure is to be confirmed post-test upon recalibration of load cells. After application of the hydraulic gradient applied across sample on day 25 total stress increased in line with the hydraulic gradient applied across the sample. Following application of hydraulic gradient inflow asymptote was achieved around day 30. The permeability of the sample was  $1.8 \times 10^{-20} \text{ m}^2$ . For all subsequent tests, a similar strategy of initial hydration period with both pups set to 1000 kPa, followed by application of hydraulic gradient by increasing injection pressure to approximately 2500 kPa was used. The second pair of experiment was run, as described above, with the exception that the groundwater was amended with sodium lactate to encourage microbial activity. The third pair of experiments (currently being prepared) will also include lactate, but the core samples will be prepared to a dry density of  $1200 \text{ kg/m}^3$ .

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. At large scales this could reduce clay swelling capacity. Basal spacing appeared to be smaller in the presence of microorganisms, however more data will be required to confirm this.

SEM examination of the bentonite around the corroded steel in the uninoculated experiment without lactate, showed strong Fe enrichment, with Fe coating (or replacing) the bentonite. Calcium precipitation was observed, but no identifiable crystal morphologies observed. Initial stub analysis of the lactate-amended uninoculated experiment did not reveal any obvious iron enrichment or calcium precipitation. In comparison, in both of the inoculated experiments, in material around the corroded

steel calcium enrichment and acicular calcium carbonate crystals were observed within fractures in the bentonite next to the steel. A fibrous iron-rich phase was observed in the bentonite, and in the case of the lactate-amended, inoculated experiment, precipitating over the acicular calcium carbonate. Further experiments are required to confirm that microorganisms from the sulphate reducing enrichment community involved in the aragonite precipitation in the inoculated experiments.

There was a notable difference in the evolution of swelling pressure between experiments with and without lactate. In experiments without lactate, the values for swelling pressure at the end of testing were higher than those obtained prior to hydraulic testing, suggesting some form of reaction has occurred causing expansion of the fabric. It may be that this is related to the reduced amount of divalent substitution seen in the lactate amended samples by XRD analysis, possibly as a consequence of additional sodium ions coming from the sodium lactate. Unfortunately, any effect that microbial activity had on the evolution of stress in the clay samples was too small for detection using our system. Given the localised nature of corrosion within the clay, longer term testing of samples with iron dispersed within the sample are required to confirm and contextualise these results and its understand its potential impact on performance assessment.

## TUL

At CV Rez, 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 BaM bentonite compacted to 1400 kg/m<sup>3</sup> and 1600 kg/m<sup>3</sup> were saturated by underground water VITA using high pressure pump (up to 2 MPa). Saturation took place in the glove box under anaerobic conditions ( $C_{(O_2)} < 1$  ppm) and full saturation of both compacted bentonite samples was reached after 11 days in the case of 1400 kg/m<sup>3</sup> sample and after 55 days in case of 1600 kg/m<sup>3</sup> 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 modified protocol for Live/Dead staining of extracted bacteria.

The presence of both alive and dead bacteria was detected in all samples of both bentonite densities. This finding indicates that bacteria are able to survive and relatively quickly move through compacted bentonite of relatively high dry density (1600 kg/m<sup>3</sup>). Dry density of 1600 kg/m<sup>3</sup> equals to the wet density of over 2000 g/cm<sup>3</sup> 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.

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<sup>3</sup> 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.

## 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<sub>2</sub>/CO<sub>2</sub>-gas-atmosphere. For stimulating microbial activity and potential geochemical effects, suitable substrates were added (acetate, lactate or H<sub>2</sub>). The set-ups were incubated in the dark at 30°C and 60°C for one year. During this year, bio-geochemical parameters were monitored and the microbial diversity was analysed. Regarding these analyses, the B25 setups showed the most striking effects. The raw material of B25 revealed a very diverse microbial community whereas the set ups containing hydrogen or lactate were mostly dominated by spore-forming, sulphate-reducing *Desulfosporosinus* species. In the respective batches of B25, the lactate concentration dropped, as well as the sulphate concentration. Furthermore, the formation of acetate, as well as an increase of ferrous iron and a simultaneous decrease of ferric iron was observed. In the respective B25-batches, the colour of the bentonite material changed to different shades of grey with black spots in between. Samples from selected batches of B25 were analysed regarding its mineralogy with x-ray diffraction (XRD) and SEM, at the University of Greifswald. The SEM mapping analysis indicated iron-sulphur accumulations in lactate-containing B25-samples compared to the raw material and samples including hydrogen or no substrate. Thus, microbial activity under the applied conditions led to an alteration of mineral phases when the conditions were favourable.

## VTT

The 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 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 has 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 microbes and the metabolites they produce were able to change the bentonite structure 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. However, clear microbial activity in terms of ongoing sulphate reduction and sulphide formation as well as high number of sulphite reductase genes (*dsrB*) were detected in anoxic samples. Microbial activity also affected bentonite water phase chemistry and bentonite cation exchange capacity. These effects were not detected in sterile 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. Overall, microbial activity was lower in oxic than in anoxic microcosms and no sulphate reduction nor were sulphite reductase genes detected in oxic microcosms after one year. 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.

### **3.2.3 Conclusions**

Several experiments concerning microbial corrosion are still running, so, there are not yet final conclusions available.

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 se*, 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<sup>3</sup> 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.

## **3.3 Microbial activity in backfill and influence on plugs and seals**

Contributing partners: SCK•CEN, MICANS

### **3.3.1 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 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  $\text{Ca}^{2+}$  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.

## **MICANS**

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 chemical 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).

### 3.3.2 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 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 supernatants and putative biofilm structures on the cement. This suggests that the high pH environment does not completely eliminate the microbial population. Similarly, in sulphate reducing conditions, the high pH environment seems to inhibit microbial activity but does not eliminate the microbial community. Interestingly, flow cytometry indicated the presence of a microbial community in the supernatants of one of the replicates in sulphate reducing conditions. Sulphate concentrations decreased in all conditions, including the negative control. Lactate was supplemented as calcium lactate and in the presence of high Ca<sup>2+</sup> concentrations sulphate can build 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 supernatants. The extracellular polymeric substances (EPS) produced by the microbial community has been shown to interact with Ca<sup>2+</sup> and result in the precipitation of CaCO<sub>3</sub> (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.

### **MICANS**

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 closest 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 through CHAB cultivation and measuring of ATP. The control test cell showed the same order of magnitude ATP ( $1 \times 10^5$  amol mL<sup>-1</sup>) 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<sup>-1</sup>) 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.

### **3.3.3 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.

### **MICANS**

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.

## **3.4 WP2 current status and intermediate conclusions**

The workpackage WP2 consists of 5 tasks.

### **Task 2.1. Microbial production of sulphide in the geosphere**

The deliverables D2.1, “Inventory of reducing gases”, D2.3, “Interim report on deep gases and sulphur compounds” and D2.5 “Microbial activation due to addition of electron donors/acceptors in deep groundwater” have been submitted on time.



In the context of microbiological risks related to radioactive waste disposal, the geochemistry of gases in deep groundwater is an integral part of the determination of geochemical constraints of biological activity at disposal depths. Data on gas compositions and concentrations do exist from several separate locations in Finland and two locations in Sweden (D2.1, D2.3). In laboratory experiments with deep groundwater populations, acetate was overall 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. However, there were also activation effects from  $H_2$ ,  $CH_4$  and  $NO_3^-$  (D2.5).

The deliverable D2.17 "Sulphide production" will be delivered M45. This deliverable aims at defining the boundary conditions constraining the availability of sulphide in deep geological disposal conditions. Critical process to be analysed is the sulphate reduction to sulphide, which is constrained by the sulphate source and the availability of possible electron donors (e.g.,  $H_2$ ,  $CH_4$  and  $Fe^{2+}$ ).

### **Task 2.2. Microbial induced corrosion of canisters**

This task will produce two deliverables, D2.13 "Anaerobic microbial corrosion of canister material" due M42 and D2.18 "Rate of corrosion of carbon steel in bentonite under biotic and abiotic conditions" due M45.

The preliminary experiments for D2.13 started in December 2015 and was terminated after 240 days. Sulphate-reducing bacteria proliferation was detected in non-sterilized samples by using qPCR targeting functional genes *dsrA* and *apsA*. Uniform corrosion process was indicated on the stainless steel. More experiments with carbon steel under different conditions are ongoing. A long-term experiment with carbon steel exposed to the synthetic bentonite pore water inoculated with natural underground water, started in November 2016 and a corrosion experiment with carbon steel exposed to groundwater at 35°C is also ongoing. Most of the experiments are finished and the deliverable is being prepared.

Three complementary experiments were planned to address this task (D2.18). Two were laboratory based but designed to reproduce repository conditions, while another was based at a URL. The three experiments use complementary techniques to address the task. The rate of steel corrosion is evaluated in bentonite under *in situ* conditions. The investigation relies on the direct comparison of modules containing bentonite-embedded steel coupons that are either exposed to filter-sterilized porewater or to untreated porewater. Test modules have been retrieved for analysis and cultivation of heterotrophic aerobes and anaerobes, DNA extraction from forensic swabs, DNA extraction from black spots in bentonite, DNA extraction from 4 layers of bulk bentonite, DNA extraction from bentonite next to metal coupons. Limited microbial activity was found in the bentonite. The diversity of the microbial community was rather limited and was dominated by a *Pseudomonas*-related group. Sulphate-reducing bacteria were mainly found in the surrounding porewater, on canister surfaces, and at the interface between the filter and the bentonite. Analyses is still ongoing on additional samples and will continue throughout 2018.

### **Task 2.3. Microbial activity in bentonite buffers**

This task is scheduled for 4 deliverables, D2.4 "Threshold densities" (M36), D2.6 "Microbial diversity in bentonite buffer of aged bentonite buffer experiment" (M36), D2.14 "Role of bentonite density on the rate of corrosion of carbon steel" (M42) and D2.15 "Survival of microorganisms in bentonite subjected to different levels of irradiation and pressure" (M42). D2.4 and D2.6 have been delivered as planned.

The relation between water saturated clays at varying wet density and bacterial sulphide-producing activity is well studied (D2.4). However, wet density is just a value of the total amount of clay and water. That value does not reflect the conditions 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. These variables need further attention for a full understanding of what conditions control bacterial activity in compacted bentonite clays. Significant acetate formation from natural organic matter present in the clays was detected in the studied bentonites. This production occurred at all wet densities and suggests that bacterial activity, *per se*, 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.

The presence of living microbes on bentonite and on copper surfaces could not be demonstrated in the D2.6 study but microscopy studies revealed living microbial cells in the external water surrounding copper cylinders. According to the IonTorrent sequencing, SRB and IRB were detected in the bentonite matrix, water and copper surface. SRBs can produce corrosive sulphide and IRBs can have a role in processes that could be linked to the loss of swelling properties in bentonite. Fungal conidia and hyphae were detected by SEM in water and several groups of Ascomycetes and Basidiomycetes were identified by sequencing 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.

The current results obtained from the *in situ* experiments in the Opalinus clay in Switzerland, offer a partial view on the 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 and the results will be presented in D2.14.

BaM bentonite is currently being tested at high temperature and pressure for D2.14. Two DNA extraction methods were compared (based on protocols obtained from HZDR and SCK•CEN), another method involving ultrasonic disintegration of the material was also tested. Experiments with inoculated bentonite suspension under pressure 1, 2, 3 and 5 MPa together with atmospheric pressure control were performed. Experimental setup of temperature/pressure experiments is being finalised and the experiments started. Influence of radiation on microorganism's survivability in the bentonite suspension and water is also studied. Some experiments are still ongoing; they will be finished in November. The deliverable is being prepared.

#### **Task 2.4. Microbial degradation of bentonite buffers**

This task comprises 6 deliverables, D2.2 "Design, set up and operation of experimental equipment" (M18), D2.7 "Microbial diversity in aged bentonite" (M36), D2.8 "Long-term stability of bentonite in the presence of microorganisms" (M36), D2.9 "Evolution of stress in biotic and abiotic clay flow cells" (M36), D2.10 "Microbial mobility in saturated bentonites of different density" and D2.16 "Microbial activity and the physical-chemical and transport properties of bentonite buffer" (M44).

Sulphide has been found to reduce ferric iron in bentonites denoted Asha, MX-80 and Calcigel under the formation of elemental sulphur, ferrous iron and iron sulphide. This immobilisation effect can reduce the mass of sulphide that corrode metal canisters over repository life times, but the concomitant reduction of ferric iron may be problematic due to the destabilizing effect of ferrous iron on dioctahedral smectites such as montmorillonites (Pedersen *et al.*, 2017).

The results of the D2.7 analyses showed that there were bacteria present in all of the bentonite samples studied. Surprisingly, the microbial communities detected in the bentonite samples showed a high level of similarity. The bacterial profiles were characterised by the dominance of heterotrophs, aerobes or facultative anaerobes capable of respiring oxygen or nitrates. The majority of detected bacteria belong to common soil microorganisms or ubiquitous microorganisms with wide ecological amplitude enabling them to survive under various conditions. The results suggest that microorganisms found in the bentonite samples in this study were most probably present in the bentonite already before the start of the experiment.

The aim of the laboratory scale D2.2/D2.8 MX-80 bentonite storage experiment was 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. After one year, microcosms in MX-80 bentonite initiated both at aerobic and anaerobic conditions, no essential changes in bentonite mineralogy were found compared to the initiation of experiment. However, clear microbial activity in terms of ongoing sulphate reduction and sulphide formation as well as high number of sulphite reductase genes (*dsrB*) were detected in anaerobic samples. Microbial activity also affected bentonite water-phase chemistry and bentonite cation exchange capacity. These effects were not detected in sterile controls, demonstrating the microbial origin of these changes. The experiment is planned to continue for some years further as potential changes in bentonite mineralogy caused by microbial activity happens slowly in the studied conditions.

Given the limited duration of the D2.2/D2.9 tests, it is not surprising that the alteration of the bentonite was limited to a small zone adjacent to the iron. From a 'flow perspective' this will have little impact, as the bulk of the clay, and therefore the flowing porosity, remains unchanged. To understand the full impact of microbial action on the hydraulic properties of the clay, test durations would need to be increased substantially, or the iron should be dispersed within the entire clay matrix to increase reaction rates. Given these findings, the question of whether microbial activity alters the hydraulic behaviour of bentonite remains unanswered. Further work is required beyond the lifetime of this project to fully assess the impact of microbial induced changes on the hydraulic integrity of the clay.

The D2.10 study fulfilled important goals. First, a reliable method for direct detection of bacterial presence (both viable and dead cells) in the bentonite was developed, which has been missing. This method is based on the extraction of bacteria from bentonite using density gradient centrifugation and their subsequent Live/Dead fluorescence staining. Although the method needs further optimization and testing of its general functionality on different bentonite types, it is believed the method will be very useful for future research of bacterial presence in various clay materials.

The D2.16 work is progressing according to the plans. The experimental equipment that is being assembled and tested begins to generate the data needed. Collating data on the evolution of stress in biotic and abiotic flow cells is ongoing. The first two experiments have been dismantled, samples have been taken for DNA analysis, and culture-based enumeration have been set up. Culture-based analysis and DNA analysis is ongoing and assessment of physical, chemical and transport is ongoing as well.

#### **Task 2.5. Microbial activity in backfill and influence on plugs and seals**

This task comprises 2 deliverables, D2.11 "Cement deterioration boundaries" (M36) and D2.12 "Concrete stability" (M36).

The D2.11 study demonstrated that the high pH conditions imposed by the OPC CEM I inhibited microbial sulphate and nitrate reduction. However, SEM analysis indicated the presence of intact cells in the suspension on top of cement and putative biofilm structures on the cement. This suggests that the high pH environment does not completely eliminate the microbial population. Interestingly, in sulphate reducing conditions, a pH decrease from > 12 to pH 10 was observed in one replicate harbouring clearly a larger microbial community in the suspension on top of the cementitious material compared to the other samples. Further studies to confirm this observation and to elucidate the precise mechanism are necessary.

The conclusion of the D2.12 results was that the alkaline solution, leached from the concrete, led to a deviation in wet density and increased the pH in the bentonite clay. However, the inhibition of microbial activity was dependent of the added carbon source. Induced microbial activity in MX-80 with glucose led to a decrease in pH. A reason might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. In the future bentonite clay samples should be analysed for acetate and cultivatable acetogenic bacteria to confirm this hypothesis.

## 4 General conclusions and 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 current status of the running 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 intermediate conclusions are provided above (Sections 2.9 and 3.4). 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

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

### 4.1 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 se* 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 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 ( $1500 \text{ kg/m}^3$ ), 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 ( $0.09 \text{ MPa}$  at  $1500 \text{ kg/m}^3$ ). 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  $0.08 \text{ MPa}$  and  $2 \text{ MPa}$  28. An upper limit of  $2 \text{ MPa}$  turgor pressure would mean that cell integrity is possible, though limited, at bentonite swelling pressures below  $2 \text{ MPa}$ . 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 where 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.

## 4.2 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, 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 submitted) 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 which 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 hydrogen. Methanogenesis is suppressed 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, and this is currently being investigated through microcosm experiments. 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 prep).

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).

### 4.3 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 of 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.
- 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 H<sub>2</sub> and a number of other metabolisable organic compounds. (Work ongoing).
- 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.

#### **4.4 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.

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 selenate and selenite by a bacterium isolated from Spanish bentonite, apparently as a mechanism to control Se toxicity.



- Bassil (in prep) similarly presents evidence for the association of U(VI) with biomass in cellulose degradation microcosm experiments potentially related to sporulation.
- 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 2.5.2) 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 4.2) 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  $^{14}\text{CH}_4$  by microbial processes is of primary importance. Additionally, the fate of  $^{14}\text{C}$  labelled soluble organics 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.

## 4.5 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 metabolism these definitions are important to follow:

- Activity: microorganisms are active and have an ongoing metabolism.
- Presence: microorganisms exist in the sample or at the sampled site and they are present as live or dead cells.
- Viability: microorganisms are alive and active or exist as dormant cells which have the power to become active when presented with a favourable environment.
- 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 ( $\text{pH} > 12.5$ ) 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 less clear cut as for cellulose containing waste 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). Irradiated cellulose microcosm experiments that had a starting pH of 12.5, developed fermentation processes, yielding  $\text{H}_2$  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.

## 5 References

Abrahamsen, L., T. Arnold, H. Brinkmann, N. Leys, M. Merroun, K. Mijndonckx, 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.

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

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 an anaerobic toluene activation by sulfate-reducing strain PRTOL-1. *Applied and Environmental Microbiology* 1997; 63(9):3729-3731.
- 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<sub>3</sub> 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., 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.

Bruggeman, C., N. Maes, M. Aertsens, J. Govaerts, E. Martens, E. Jacobs, 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.

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

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

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.

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.

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.

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>

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.

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

- 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.
- 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.
- 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.
- Posiva: Olkiluoto site description 2011, POSIVA 2011-02, 2013. [www.posiva.fi/tietopankki/olkiluoto\\_site\\_description\\_2011.1793.xhtml#.Wwz2Uqm-l0s](http://www.posiva.fi/tietopankki/olkiluoto_site_description_2011.1793.xhtml#.Wwz2Uqm-l0s)
- Pusch, R., *et al.* (2003). "Interaction of cement and smectitic clay—theory and practice." *Applied Clay Science* 23(1): 203-210.
- 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  $\gamma$ -radiolysis of ion exchange resins. *Radiation physics and chemistry* 2015, 106:223-234.
- 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.
- 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. Klemetsson (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>

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.

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. Fruttschi, 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., Dumas, 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>
- 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.
- 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.
- 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-Cabriaes JJ.2014.Effect of Iron-Reducing Bacteria on Carbon Steel Corrosion Induced by Thermophilic Sulfate –Reducing Consortia.
- Van Loon, L.R., Hummel, W. The radiolytic and chemical degradation of organic ion exchange resins 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.
- 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  $\alpha$ -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., Matusiewicz, 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 Itävaara, M. The biogeochemistry of gas generation from low-level nuclear waste: Microbiological characterization during 18 years study under *in situ* conditions. (Submitted to *Applied Geochemistry*).
- Vogt, C., Kleinstuber, 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  $\alpha$ -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)- $\alpha$ -isosaccharinic acid and gluconic acid complexes. *Radiochim Acta* 92, 897–902.

Warwick, P., Evans, N. & Vines, S. (2006). Studies on some divalent metal  $\alpha$ -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.

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  $\alpha$ -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.

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

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.

## 6 Acknowledgement

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon 2020) under grant agreement 661880 The MIND-project.

## 7 Appendix

### WP 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 <sup>+</sup>	H <sub>2</sub> gas removal; Gas volume decrease		Gas pressure decrease	Bleyen <i>et al.</i> 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 <i>et al.</i> 2017a, b	
	Nitrate/nitrite reduction to nitrogen gas	N <sub>2</sub>	Gas volume increase	pH limit < 12.5?	Gas pressure increase	Bleyen <i>et al.</i> 2017a, b	<b>1f</b>
	Nitrate/nitrite reduction to ammonium	NH <sub>4</sub> <sup>+</sup>					
Small soluble organics & Nitrate or nitrite	Organotrophic Nitrate/nitrite reduction	CO <sub>2</sub>	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	<b>1a</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	This study	<b>1g, 1c</b>

	Nitrate/nitrite reduction to nitrogen gas	N <sub>2</sub>	Gas volume increase	pH limit < 12.5; Electron donor, C/N ratio	(-) Gas pressure increase	This study	<b>1f</b>
	Nitrate/nitrite reduction to ammonium	NH <sub>4</sub> <sup>+</sup>				Bleyen <i>et al.</i> 2017a, b	<b>1g, 1c</b>
Hydrogen & Sulphate	Hydrogenotrophic Sulphate reduction	S <sup>2-</sup>	Generation of corrosion-aggressive species	If nitrate as electron acceptor is depleted E <sup>0'</sup> = -217 mV	Corrosion	Not this study	<b>1j</b>
Hydrogen & CO <sub>2</sub>	Hydrogenotrophic Methanogenesis	CH <sub>4</sub>	Gas volume decrease	If nitrate and sulphate as electron acceptor are depleted E <sup>0'</sup> = -238 mV	Gas pressure decrease	Not this study	<b>1f</b>

Table 2: Results of microbial experiments

Test set-up (batch, <i>in situ</i> , computationally predicted, ...)	Process investigated	Organisms studied	End products	Boundary conditions
Anoxic batch experiment	Biofilm formation on Eurobitum	Boom Clay borehole water	NO <sub>2</sub> <sup>-</sup> , N <sub>2</sub> , 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 <sub>2</sub> <sup>-</sup> , N <sub>2</sub> , 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 <sub>2</sub> <sup>-</sup> , N <sub>2</sub> , +?	pH 12.5 & Phosphate can become limiting

## 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 <sub>2</sub>	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	<b>2f</b>
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	<b>2f</b>
Cellulose ( & ISA)	Fermentation and respiration	CO <sub>2</sub>	Potentially drop the pH in the GDF. Potentially Encapsulate or sorb radionuclides	Chemical toxins Radioactivity		Bassil <i>et al.</i> , 2015a, b; and this study	<b>2f</b>

Table 4: Results of microbial experiments

Test set-up (batch, <i>in situ</i> , 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 <sub>2</sub> , CO <sub>2</sub> , Acetate	pH

## 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	<b>1a</b>
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	<b>1a</b>
Phosphate ester	Phosphate ester degradation	Unknown	Reduced phosphate ester concentration	pH ≤10	unknown	This study	<b>1a</b>

Table 6: Results of microbial experiments

Test set-up (batch, <i>in situ</i> , 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

## 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 <sub>2</sub>	Sulfate reduction	HS <sup>-</sup>	Consumption of H <sub>2</sub> and SO <sub>4</sub> <sup>2-</sup> ; Establishment of sulfate-reducing community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	This study	
H <sub>2</sub> and CO <sub>2</sub>	Hydrogenotrophic methanogenesis	CH <sub>4</sub>	Consumption of H <sub>2</sub> and CO <sub>2</sub> ; Shift in microbial community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	This study	<b>1f</b>

Table 8: Results of microbial experiments

Test set-up (batch, <i>in situ</i> , computationally predicted, ...)	Process investigated	Organisms studied	End products	Boundary conditions (pH, temp, IS, chemical, rad)
ongoing				



## 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 <sub>Start</sub> 12.2, [Ca(OH) <sub>2</sub> ] 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		<b>1a</b>
Se and Spanish bentonite bacterial isolate	Se(VI) and Se(IV) reduction to and Se(0) nanostructure formation	Monoclinic and trigonal Se(0) nanostructures	Increased insolubility of Se oxyanions		Positive impact by the Immobilization of Se oxyanions	Ruiz-Fresneda et al. 2018)	<b>1h</b>
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)		<b>1h</b>

Table 10: Results of microbial experiments

Test set-up (batch, <i>in situ</i> , computationally predicted, ...)	Radionuclide investigated	Organisms studied	End products	Boundary conditions

## 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 <sub>2</sub> and SO <sub>4</sub> <sup>2-</sup>	Sulfate reduction	HS <sup>-</sup>	Consumption of H <sub>2</sub> and SO <sub>4</sub> <sup>2-</sup> ; Establishment of sulfate-reducing community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	Bagnoud et al., 2016	<b>1f</b>
H <sub>2</sub> and CO <sub>2</sub>	Hydrogenotrophic methanogenesis	CH <sub>4</sub>	Consumption of H <sub>2</sub> and CO <sub>2</sub> ; Shift in microbial community	pH 8 and Opalinus Clay conditions	Positive impact by consuming gases	This study	<b>1f</b>
	Methanogenesis	CH <sub>4</sub>	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 <sup>-</sup>	Formation of corrosive sulphides, consumption of H <sub>2</sub>	Availability of sulphate	Reduction in gas generation, enhancement of corrosion		

Table 12: Results of microbial experiments

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

## WP2 – Engineered barriers

### The availability of sulphide and its effects on metal canister corrosion (VTT)

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 <sup>2-</sup>	Production of corrosive agents	Availability of energy source and electron donor. pH > 6	General/local corrosion		1j
Sulphate	Metallic Iron oxidation	S <sup>2-</sup>	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, <i>in situ</i> , 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 <i>Geobacteraceae</i>	N <sub>2</sub> S <sup>2-</sup> Fe(II)	O <sub>2</sub> < 1 ppm pH <8.6; 9.1>
Batch experiment with carbon steel in groundwater VITA	Biofilm formation, corrosion	SRB <i>Geobacteraceae</i>	S <sup>2-</sup> Fe(II)	O <sub>2</sub> < 1 ppm pH <7.9; 9.0>

## 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 <sub>2</sub> and SO <sub>4</sub> <sup>2-</sup>	Hydrogenotrophic Sulfate reduction	HS <sup>-</sup>	Removal of gas H <sub>2</sub>	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 <sup>-</sup>				
H <sub>2</sub> and CO <sub>2</sub>	Hydrogenotrophic methanogenesis	CH <sub>4</sub>	Removal of H <sub>2</sub> and CO <sub>2</sub>	pH 8 and Opalinus Clay conditions; presence of methanogenic archaea	Positive impact by consuming gases	This study	1f

Table 16: Results of microbial experiments

Test set-up (batch, <i>in situ</i> , computationally predicted, ...)	Process investigated	Organisms studied	End products	Boundary conditions
In situ	methanogenesis	Microbial community	HS <sup>-</sup>	pH 8, in situ conditions
batch	methanogenesis	Microbial community	Fe(II)	pH 8, in situ conditions

## 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 <sub>2</sub> 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, <i>in situ</i> , computationally predicted, ...)	Process investigated	Organisms studied	End products	Boundary conditions
'worst-case scenario experiment', batch, aerobic, slurry, nutrients	degradation of bentonite	indigenous	?	
'worst-case scenario experiment', batch, anaerobic, slurry, nutrients	degradation of bentonite	indigenous	?	

## 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 <sub>2</sub> 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, <i>in situ</i> , 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	indigenous including SRBs, iron reducers, fungi	FeS	
'Worst-case scenario experiment', batch, anaerobic, slurry, nutrients	degradation of bentonite: sulfate and iron reduction	indigenous including SRBs, iron reducers, fungi	FeS	
Long-term experiment with compacted bentonite, batch, aerobic/anaerobic	changes in bentonite structure and mineralogy	SRBs, iron reducing bacteria, fungi		

## 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
$\text{Ca}^{2+}$ , $\text{CO}_2$	Carbonation	$\text{CaCO}_3$	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	
$\text{SO}_4^{2-}$ , $\text{H}_2$ or organic matter	Sulphate reduction	Sulphide	Lowering external $\text{SO}_4^{2-}$ 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, <i>in situ</i> , 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