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# DELIVERABLE 3.4 Year two synthesis report

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# **Publishable Summary**

Microbiology In Nuclear waste Disposal (MIND) is a multidisciplinary project with the goal of addressing key microbiology technical issues that must be tackled to support the implementation of planned waste disposal across the EU. The project addresses in specific 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 2 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. The kinetics of these processes are not featured prominently here, but will become an increasingly prioritised aspect of the MIND project throughout the remainder of its tenure, and a next update of this report.

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# **1** General introduction

# **1.1 The MIND project**

Microbiology In Nuclear waste Disposal (MIND) is a multidisciplinary project with the goal of addressing key microbiology technical issues that must be tackled to support the implementation of planned waste disposal across the EU. Whilst it is widely acknowledged that microorganisms are capable of transforming materials within nuclear waste and under the conditions of waste disposal, our understanding of what 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 on 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 two evaluation report on experimental and modelling research activities in WP1 and WP2 (Deliverable D3.4). 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. Some entries are shorter than others, reflecting their later starts and corresponding early stages of progress, whereas other projects began when the MIND project started, and have been completed with publications in preparation or in review.

The scope of the report is broad, covering all 'in progress' and completed scientific projects contained within MIND WP1 and WP2. However, owing to the number of projects still in their early stages, the emphasis of the work covered here is on processes and boundary conditions; kinetics will become an increasingly prioritised aspect of the MIND project throughout the remainder of its tenure, and as such will not be featured prominently here.

# 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  $H_2$  and  $CH_4$  under ILW repository conditions.
- To understand the effect of ILW **heterogeneity** on bioprocess pathways, pH and redox conditions, barrier degradation and radionuclide release.

# 2.1 Bituminized waste

Contributing partners: SCK•CEN

# 2.1.1 Problem statement & current knowhow

In Europe, at least five countries have to deal with significant bituminized long-lived low- and intermediate level (LILW-LL) radioactive waste. During the MIND project, the Belgian disposal concept with Boom Clay as reference host rock and Eurobitum as reference bituminized waste were selected to study more in detail. Eurobitum contains 60 % bitumen Mexphalt R85/40 and 40 % waste containing soluble and insoluble salts. Overall, bituminized 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 a safe geological disposal of the waste, a good understanding on 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 with Boom Clay as host rock was considered as reference. Within 50 years after closure of the gallery all pores and voids in the gallery would be filled with cement equilibrated Boom Clay pore water (Weetjens et al. 2010). Water will gradually infiltrate in the waste packages and uptake of pore water by the hygroscopic salts embedded in the waste results in the swelling of the bitumen what on its turn can lead to an increase of the pressure in and around the waste (Valcke et al. 2009). Water uptake of the bituminized waste results in the leaching of large amounts of NaNO $_3$  and other soluble salts in the Boom Clay. Scoping calculations indicate that, close to the waste drums the NaNO<sub>3</sub> concentration can reach up to a few molar and that during the first ~300 to ~1400 years the NaNO<sub>3</sub> concentration within the monolith will remain above 1 M and 0.5 M, respectively (Weetjens, Valcke and Mariën 2010), resulting in an increase of the ionic strength. Maximum  $NO_3^{-1}$ concentrations reach between 0.5 and 1 M at the gallery interface, and decrease also fast within the host formation (e.g. 0.1 M at a distance of 5 m in the Boom Clay) (Weetjens, Valcke and Mariën 2010). Moreover, bituminized 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 (in progress), a detailed overview of the degradation products is described. The extent of some of these processes will be affected by the continuous evolution of the rheological properties of the bitumen. This so called ageing results in harder bitumen, which tends to lose its binding capacity and becomes increasingly brittle.

The leaching products (NaNO<sub>3</sub> 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 putatively 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 oxidise pyrite and possibly dissolved organic matter from the Boom Clay host rock. Oxidation of these Boom Clay components, makes that its reducing capacity will be decreased and mobility of certain radionuclides can be increased. 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 bituminized waste (Bleyen et al. 2017). However, there can be a direct chemical reaction of nitrate with the steel and/or  $H_2$  present in the disposal facility.  $H_2$  will be present, produced by the anaerobic corrosion of steel drums and from radiolysis of water and bituminized waste. The produced steel and/or H<sub>2</sub> 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 disposal facility and the Boom Clay.

The fate and thus final impact of the leaching products (NaNO<sub>3</sub> and organics) released from the bituminised waste on the disposal facility will also be influenced by **biotic (microbial) processes**. Microorganisms will be ubiquitously present in the repository either as autochtonous species that are already present in 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 bituminized 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, putatively decreasing the reducing capacity of the clay formation (Bleyen, Mariën and Valcke). Or nitrite can be further biologically reduced to to gaseous nitrogen compounds (NO, N<sub>2</sub>O, N<sub>2</sub>) by denitrification. Denitrification could lead to a net pressure increase or decrease, 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, 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 (Harrington et al. 2012). Nitrogen fixating microbial species can reduce the N<sub>2</sub> to ammonia and then assimilate this into organic forms, such as amino acids and nucleotides. On the other hand, nitrite can also be reduced dissimilative 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 has been shown that in soils, the oxidation status of the soil and the  $C/NO_3^{-1}$  ratio are key parameters in regulation which microbial nitrogen conversion processes would occur or dominate. More reducing conditions and an excess of electron donor (such as biodegradable dissolved organic matter or hydrogen) as compared to nitrate (i.e. C/N ratio > 1), promote DNRA and thus ammonium production (Rutting et al. 2011). In contrast, in geological disposal conditions, the concentration of electron donors (biodegradable dissolved organic matter or hydrogen) in the water is estimated to be low (i.e. C/N ratio < 1), therefore, it is expected that dissimilative nitrate reduction to nitrite and denitrification to gaseous nitrogen compounds (NO, N<sub>2</sub>O, N<sub>2</sub>) will be the preferred processes in the disposal. If dissolved organic matter present in the clay formation or organic compounds leaching from the bituminized waste were used as electron donor during in situ experiments performed at the Mont Terri URL laboratory, indeed a net gas production has been observed (Bleyen et al. 2017). Even when low concentrations of acetate were added as additional easy metabolisable electron donor (C/N ratio 0.4 or 0.8) still dissimilative nitrate reduction and denitrification were dominant and no ammonium production was observed (Bleyen, Smets, Small, Moors, Leys, Albrecht, De Canniere, Schwyn, Elie and Valcke 2017). When hydrogen was used as electron donor, still the majority of the nitrate (34%) present at the start of the  $H_2$  pulse was reduced to nitrite after 24 days, while a small amount (8%) was converted to ammonium (Bleyen, Smets, Small, Moors, Leys, Albrecht, De Canniere, Schwyn, Elie and Valcke 2017). Denitrification to nitrogen gases (NO,  $N_2O$ ,  $N_2$ ) and/or ammonium assimilation can explain the remainder of the fast decrease in the nitrate concentration. On the other hand, the use of hydrogen as 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, Smets, Small, Moors, Leys, Albrecht, De Canniere, Schwyn, Elie and Valcke 2017).

Thus, several microbial processes can potentially influence the fate of the nitrate in the host rock, and thus the final impact of nitrate on the performance and safety of the disposal. However, *in situ*, **several environmental parameters may stimulate or repress such microbial processes**. The supressing factors for microbial activity in a disposal facility with bituminized waste can be high salinity (caused by Na<sup>+</sup> from NaNO<sub>3</sub>), high pH (caused by the Ca(OH)<sub>2</sub> 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

Batch experiments were performed in hermetically closed and anoxic culture bottles to investigate more in detail the potential of microbes to reduce nitrate leaching from non-aged and thermally aged inactive bituminized waste in the presence or absence of relevant organic electron donors. In addition, to determine boundary conditions regarding nitrate and nitrite and salt concentrations, batch experiments were performed without inactive bituminized waste as source of nitrate, but in which known (higher) concentrations of sodium nitrate or sodium nitrite were added. Borehole water samples from different layers within the Boom Clay, which have been shown to comprise a highly diverse bacterial community of which a large fraction is active (Wouters *et al.* 2013), were collected and used as water environment and inoculum for these experiments. As additional electron donors, formate or acetate, known organic leaching products from bitumen, were tested. No steel, concrete or clay were added in the test at this stage. Experiments were performed at 30°C, which is higher than expected *in situ* disposal temperature, but needed to stimulate faster microbial metabolic activity, to allow gathering results within a reasonable time.

Without an additional electron donor, no nitrate reduction was observed after 50 days demonstrating that the organic material present in Boom Clay borehole water was a poor electron

donor. In the presence of formate, on average 11% of the nitrate leached from the inactive bituminized waste was reduced. In the presence of acetate, reduction increased up to 42% of the nitrate leached from the inactive bituminized waste, demonstrating that acetate is more favourable as electron donor compared to formate or the organic material present in Boom Clay borehole water. 16S DNA metagenomics showed that after 17 days, the microbial population was dominated by *Pseudomonas* when no additional electron donor or acetate was present, while *Acidovorax* was the most dominant genus with formate as electron donor. In addition, boundary conditions regarding the high salt (sodium nitrate) concentrations were investigated. Concentrations up to 4 M NaNO<sub>3</sub> were not lethal for the microbial community, as the viable cell count determined with flow cytometry, remained stable up over the full 80 days of testing. Nevertheless, concentrations of 1 M of NaNO<sub>3</sub> or NaNO<sub>2</sub>, or higher inhibited microbial activity.

# 2.1.3 Conclusions

In conclusion, the nitrate and soluble organics leached from bituminised waste can be removed by nitrate reducing microorganisms present in Boom Clay borehole water, with increasing rates in the presence of formate or acetate but very high concentrations of NaNO<sub>3</sub> or NaNO<sub>2</sub> are likely to inhibit microbial activity.

In summary, chemical and radiolytic degradation of bituminized waste will cause leaching of nitrate and soluble organics, which will be subject of abiotic and biotic processes and can have following impact:

- Some of the leached water-soluble organics (e.g. oxalate) could act as RN-complexing agents thereby modify the behaviour of radionuclides in disposal facility and the Boom Clay. This could be taken into account in the performance assessment.
- Nitrate can cause changes in the redox potential of the pore water in the disposal facility and host rock and therefore could increase the uncertainty on the speciation and thus mobility of some redox sensitive radionuclides (e.g. <sup>79</sup>Se). This can explicitly be taken into account in the performance assessment.
- Conversion of nitrate to NH4<sup>+</sup> abiotically via the interaction with steel or biotically by DNRA can have a significant impact on the solubility of some radionuclides (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. Similar assessments can be done for other concepts.
- 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, yet without any effect on the long-term radiological impact. Similar assessments can be done for other concepts. Besides, it is highly likely that hydrogen is consumed in the microbial denitrification process, leading to an overall gas pressure decrease with factor 5. These beneficial effects currently are neglected in conservative performance assessments, but could/should be evaluated.

# 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 Ca<sup>2+</sup> 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 radiologic 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.

Cellulose irradiation and biodegradation may enhance the rate of chemical cellulose hydrolysis by alkaline, 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 (RN). Acetate (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 agent for radionuclides (Glaus *et al.*, 1999). In the worst case, RN complexation with organic cellulose degradation products will lead to an increased mobility and a decreased retention of RN in the barriers of a nuclear waste disposal. In addition to complexation with soluble organic species, radionuclides can be sorbed and associated with microbial biomass. Therefore, the characterization of RN 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 <u>on cellulose degradation</u>

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).
- 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 not been identified yet.
- Microbial degradation of cellulosic material (Basil *et al.* 2015a) and ISA (Basil *et al.* 2015b) has been demonstrated at alkaline pH (pH 10)

### 2.2.3 Summary of experimental & computational results on RN complexation

# Contributing partners: HZDR & UGR

Aims:

- Aqueous RN speciation studies with cellulose degradation products as a result of both abiotic and microbial activities

Experimental setup:

- Systems: U(VI)-acetate, U(VI)-ISA
- Starting conditions: acidic pH range
- Synthesis of Ca(ISA)<sub>2</sub> based on a protocol from UNIMAN. Based on Ca(ISA)<sub>2</sub> the Na-form, NaISA, was prepared for the speciation studies. The purity of both salts was verified by <sup>13</sup>C- and <sup>1</sup>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. Future investigations will examine behaviour 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 (August 2016). Ongoing sampling and analysis of uranium and degradation products might 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 degradation products.

Key results so far:

- U(VI) acetate system (acidic pH range)
  - The speciation studies were performed by varying one parameter within a test series, whereas all other parameters were fixed. Direct spectroscopic (UV-VIS, TRLFS) and other methods (ITC) were applied. The data evaluation of the spectroscopic series was done for instance with factor analysis and peak deconvolution. As a result, we could characterize directly three U(VI)-acetate species (1:1, 1:2, and 1:3) based on their single component spectra and finally their individual stability constants (log  $\beta_{1:1}^0 = 2.94 \pm 0.27$ , log  $\beta_{1:2}^0 = 5.80 \pm 0.41$ , and log  $\beta_{1:3} = 7.29 \pm 0.39$ ).
- U(VI) ISA system (acidic pH range): first results
  - The spectroscopic and structural speciation experiments of U(VI) in the presence of ISA are combined with theoretical modelling (DFT calculations). Based on UV-VIS and ATR-FT-IR experiments, we found indications for a 1:1 and two structurally different 1:2 U(VI)-ISA complexes. These investigations are currently running.
- Long-term tissue paper biodegradation experiment under hyperalkaline conditions in cooperation with UNIMAN
  - Within the first three sampling points increasing U(VI) concentrations in solution, likely due to the influence of formed ISA and acetate as tissue degradation products,

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were measured. This experiment is currently running, and the samples will be analysed.

### 2.2.4 Conclusion

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 disolved 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 at hyperalkaline pH (pH 12.7) led to the production of H<sub>2</sub>, and acetate, while  $CH_4$  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 subject of abiotic and biotic processes and can have following impact:

- 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-RN mobility problem. This is currently further explored, in the running experiments.
- Other soluble organics are also produced, which can also from complexes with RN but it is so far unclear what impact they have on the final mobility of RN. 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 knowhow

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

Owing to strong intermolecular forces between polymer chains, PVC in its pure form is a rigid, mainly amorphous material with little flexibility. As such, PVC must be rendered flexible in order to be of use in the nuclear industry, which necessitates the addition of a variety of additives, including plasticizers, heat stabilizers, fillers, pigments, flame retardants, UV absorbers, colorants and anti-oxidants (Coaker, 2003). Of these, plasticizers are typically present in the largest quantities, accounting for between 30% and 50% by volume. Plasticizers are not covalently bonded to the PVC polymer, rather they sit between and serve to lubricate otherwise rigid polymer layers. There is concern that these additives may diffuse out of the PVC material under the conditions of a geological disposal facility (GDF), which may have implications for microbial activity and for radionuclide

mobility (Dawson, 2013). Indeed, phthalic acid, the degradation product of various phthalate ester plasticizers under high pH and irradiating conditions, is known to complex with uranium and other radionuclides (Vazquez *et al.*, 2008, 2009; Zhou *et al.*, 2004; Panak *et al.*, 1995).

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

# 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; 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 Ca(OH)2 and (from left to right) non-irradiated PVC powder; irradiated PVC powder; non-irradiated PVC sheet; irradiated PVC sheet. Note the change in colour of the PVC powder from white to black, and of the PVC sheet from clear translucent to light brown opaque.

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 Conclusion

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

# 2.4.1 Problem statement & current knowhow

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.

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 to anoxic conditions rapidly. Additionally, the range of analytical tools applied to characterize the products of resin irradiation was limited (Rebufa *et al*, 2015) and 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 Mt Terri underground rock laboratory), could produce chelating agents for radionuclides, could fuel sulfate reduction or methanogenesis. This potential is another aspect of the work that will be evaluated.

#### 2.4.2 Summary of experimental & computational results

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 ( $\text{Li}^+$ ,  $\text{BO}_3^{3^-}$ ,  $\text{SiO}_3^{2^-}$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{Mg}^{2^+}$ ,  $\text{K}^+$ ,  $\text{Ca}^{2^+}$ ,  $\text{F}^-$ ,  $\text{SO}_4^{2^-}$ ,  $\text{Br}^-$ ) 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 <sup>137</sup>Cs 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 H<sub>2</sub>, CH<sub>4</sub>, CO<sub>2</sub>, and CO were performed with a µ-GC. Furthermore, unirradiated resin that was otherwise treated identically was also analysed.

Comparison of the irradiated and unirradiated bead resins revealed the production of  $H_2$  and a number of chlorinated aliphatics, such as bromomethane, chloromethane, dichloromethane, and chloroethane, as well as aromatic compounds, such as benzene and toluene, in the gasphase. 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 has focused on the aqueous phase probably overlooked these compounds.

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 sulfate-reducing bacteria has been documented by several authors (Vogt *et al.*, 2011; Beller *et al.*, 1997). Sulfate reduction is likely to be the main metabolic process in the repository in Opalinus clay due to the presence of sulphate as well as H<sub>2</sub> from anoxic steel corrosion and resin irradiation (Bagnoud *et al.*, 2016).

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 total decline in bacterial biomass in two highest concentrations studied (20 and 60 g/l), probably because of 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.

# 2.4.3 Conclusion

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 a propitious environment for microbial metabolic activity.

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.

# 2.5 Radionuclide complexation

Contributing partners: HZDR & UGR

#### 2.5.1 Problem statement & current knowhow

As described in the various 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). RN complexation with such organic degradation products will lead to an increased mobility and a decreased retention of RN in the barriers of a nuclear waste disposal. Therefore the characterization of RN 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 and associated with microbial biomass. When sorbed to microbes in attached biofilms, the RN are removed from the water and the retention of RN in the barriers of a nuclear waste disposal is enhanced. The characterization of RN complexes 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.

#### 2.5.2 Summary of experimental & computational results

The experimental data obtained for uranium complexes with acetate and ISA, the degradation products of cellulose waste, are integrated with the respective cellulose degradations studies and reported above.

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. 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.

# 2.5.3 Conclusion

Novel mechanisms of radionuclide interactions with indigenous bacteria, forming water insoluble complexes associated with the cells may have the effect of limiting radionuclide mobility in groundwater. 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 knowhow

The accumulation of gases in the repository is a potential source of concern for waste management organizations. If the concentration of the produced gases would exceed the solubility limit of the gases in the water, a separate gas phase could be formed, putatively causing fissuring of the host rock, hence resulting in the formation of preferential pathways for radionuclide migration and release. In particular,  $H_2$  accumulation is expected in both HLW as well 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  $H_2$  production.

Microbial processes that consume H<sub>2</sub> could be highly beneficial to the safety case because it would result in decrease gas pressures. Microbial hydrogen consumption in Opalinus clay repository conditions has been evidenced in previous work (Bagnoud *et al.*, 2016a). So far, it has been clearly shown that in Opalinus clay porewater H<sub>2</sub> 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 H<sub>2</sub> + CO<sub>2</sub>  $\rightarrow$  CH<sub>4</sub> + H<sub>2</sub>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 sulfate. 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 sulfate-reducing bacteria. Geochemical data such as  $\delta^{34}$ S-sulfate and  $\delta^{13}$ C-methane confirmed the biological reduction of sulfate and the lack of methanogenesis. The sulfate 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 sulfate and Opalinus Clay were set-up and monitored over time after amendment with  $H_2$ . Interestingly, no methanogenesis was observed (likely due to the low bicarbonate concentration) but an increase in Fe(II) was observed in the medium in the presence of the clay. Further investigations of the samples are underway to uncover whether microbial iron reduction processes are responsible for this observation.

# 2.6.3 Conclusion

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 knowhow

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 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 <sup>14</sup>C in the form of <sup>14</sup>CH<sub>4</sub>.

The TVO gas generation experiment (GGE, see Figure 2) 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.



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

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Figure 2: 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 submitted for publication (Small et al., in revision) and discusses how the alkaline cellulose degradation processes occurring in waste drums has neutralised the initial alkalic 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 sulfate reduction occurring over the first 2 years, which resulted in peak concentrations of sulfide of ~ 0.2mM which have slowly declined to micromole concentrations that are saturated with respect to the Fe sulfide phase mackinawite. Bicarbonate concentration has increased progressively up to a concentration of 14 mM, while dissolved organic carbon (due to alkali cellulose degradation products) 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  $1m^3$  per year from the whole experiment. This is consistent with methanogenesis occurring throughout the GGE and in tank water once the pH had declined to below pH 9. It is also possible that methanogenesis was slowed, but not completely inhibited by sulfide present initially and the increased rate of methanogenesis is a consequence of the decline in aqueous sulfide concentration to very low values (< 3 X  $10^{-6}$  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 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.

# 2.7.3 Conclusion

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 knowhow

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 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 studies include the T2GGM devloped to examine microbial gas generation in the Canadian deep geological repository (Suckling *et al*, 2015). Recently, NAGRA have developed models to assess the sources and sinks of gas generation (Leupin *et al*, 2016).

# 2.8.2 Summary of experimental & computational results

Within Task 1.3 (studied under *in situ* conditions) of MIND Workpackage 1, a PHREEQC model of sulfate 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 sulfate reduction by hydrogen developed by Maia *et al*, (2016), with coupling to a radial diffusion model to represent the supply of sulfate 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<sub>2</sub> 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 sulfide arising from sulfate 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 sulfate observed in the MA experiment. However, it is possible that the effect is mitigated by the precipitation of low-solubility iron sulfide phases, such as mackinawite (FeS).

Also within Task 1.3, NNL's GRM model application to simulate the suite of cellulose degradation processes leading to methanogenesis in the GGE (Small *et al*, 2008) has been further developed to consider diffusive transport processes. The model continues to be able to simulate the sequence of anaerobic microbial processes and the changes in methane gas generation rate that are coupled to neutralisation of the initially alkaline (pH 10-11) water present in the experiment (Small *et al*, in revision).

Within MIND Workpackage 1 Task 1.4, NNL is devloping 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. This work is ongoing and will be delivered in the final year of the MIND project.

# 2.8.3 Conclusion

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 workpackage 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. A further deliverable (D1.3) concerning biodegradation of bitumen radiolysis products will be produced shortly (Section 2.1). Work concerning the biodegradation of irradiated ion exchange resin is progressing as planned and has shown a variety of radiolysis products with potential to support microbial activity. Studies of radionuclide interactions with cellulose degradation products is progressing as planned, with some work published, and will be reported at M36 (D1.4, D1.5).

#### 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 sulfate reduction processe, and potential transition to methanogenesis, in a borehole drilled into Opalinus Clay (Section 2.6). This work will be supported with additional experiments examining the biodegradation of ion exchange resin radiolysis products under methanogenic conditions, *in situ* or in laboratory experiments. The work on methanogenesis and resin degradation will be reported at M36 (D1.7).

(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. This work has already resulted in the submission of a journal publication interpreting and modelling data collected to date and new microbiological data will be reported at M36 (D1.6).

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

This task has provided modelling support to both experiments within Task 1.3. Development of a new microbial modelling API is progressing as planned and will be delivered at M42 (D1.7, Section 2.8)

Work to date within WP1 has produced some significant results, particularly in the area of cellulose and PVC degradation, which together with the modelling study of the GGE are being prepared for publication. Two other papers are already accepted for publication from the MIND project concerning novel bacteria of relevance to geological disposal (Bassil and Lloyd, 2017; Sanchez-Castro *et al*, in press).

At the 2nd Project Annual Meeting, Prague, May 2017, 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
    - $\circ~$  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
  - o Generating acidity

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

- New data and findings regarding radionuclide interactions with organic degradation products
  - New themodynamic data
  - Radionuclide-solid 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
  - Microbes can affect pH buffering
    - Heterogeneity important
    - Amount of concrete important
- Models being developed to help interpretation and for Performance Assessment

Over the remaining two years, research on these topics will be finalised and assessed in the contextofLILWdiposalscenarios.

# 3 WP2 – Engineered barrier

WP2 lead: MICANS

The objectives of WP2 are to:

- Quantify the contribution of microbially produced **sulfide** 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. <u>microbial degradation</u> <u>of bentonites and cementitious materials</u>. (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 knowhow

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.

Both iron and copper have strong affinities to form sulfide compounds in geological environment. **Sulfidic 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 sulfide concentration strongly depends on the **geochemical conditions in the ground water** at the disposal depth. Dissolved sulfide (as HS<sup>-</sup>,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 sulfides in these conditions. These sulfide 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 sulfide oxidizing microbes**, and metals dissolve in the acidic sulfate solution thus formed. In changing hydrogeological conditions, sulfate-bearing waters may again enter anoxic conditions facilitating sulfate reduction to sulfide with the aid of **sulfate reducing microbes**. Relatively high sulfide concentration may develop in this transient sulfate reduction front, at depts 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 sulfide 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 sulfide minerals** directly on the metal surface and the **consumption of H**<sub>2</sub> produced by to the anoxic chemical corrosion of steel or copper. Sulfate-reducing bacteria play a key role in steel corrosion processes (Venzlaff *et al.* 2013). This group of microorganisms is able to take away electrons from the steel (Fe<sup>0</sup>) and/or the H<sub>2</sub> and reduce sulfates

to sulfides (S<sup>2-</sup>) or HS<sup>-</sup>, which can create corrosive agents such as H<sub>2</sub>S, FeS *etc.*. However, microbial enhanced corrosion of steel canisters is still a hotly debated point within the community. Microbial activity on the metal surface can accelerate the corrosion rate but can also act protectively. Careful design may harness the ability of microorganisms to consume H<sub>2</sub> rapidly, reducing an overpressure that is otherwise expected in the repository, while relegating sulfate reduction to an iron-rich porous medium that would sequester sulfide. 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 sulfide would precipitate, while relying on H<sub>2</sub> 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 sulfide on canister corrosion (Bagnoud *et al.*, 2016a). Moreover, it remains unclear to what extent does microbial activity, particularly sulfate-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 sulfide mineral as a source of aqueous sulfide
- sulfate sources and fluxes in the bedrock, as a source for sulfate-reducing bacteria
- electron donors (e.g. hydrogen) facilitating microbial sulfate 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

In the first phase, geochemical data of the ground water from available deep drilling sites in Finland was compiled in a database. In general the data is representative of Precambrian crystalline shield rock types: gneisses (high-metamorphosed argillaceous and volcanic rocks), granitoids and maficultramafic intrusives. Less typically, data is also available from Mesoproterozoic (Jotnian) sandstone and shale. Main part of the data originates from a nation-wide survey of deep drillholes (300 – 1200 m) in Finland using mainly drillholes for ore exploration purposes. Outokumpu deep drillhole (2500 m) and Pyhäsalmi underground mine were later the main targets of research. Geochemical data obtained during the Finnish site selection program for nuclear waste disposal was also included in the database, as well as that available from Olkiluoto site of Posiva Oy. All hydrogeochemical data are compiled to an Excel database, which is continuously updated and available for MIND project partners on request.

In general, dissolved sulfide data indicate a solubility control by solid iron sulfide phases (e.g. mackinawite). However, a clear "supersaturation" was observed in some deep samples. In Outokumpu, high sulfide concentration was associated with sulfate reduction front. Sulfate reduction fronts were frequently observed in groundwaters of deep crystalline rock, but the depth of the front varied substantially from site to site. A typical depth is about 300 – 400 m. However, in some geological environments sulfate reduction seems not to take place. The most distinctive rock type observed was the Jotnian shale having sulfate concentrations of several thousands of ppm's at the depth of 600 m. In fractured crystalline rocks, sulfate waters evidently have a near-surface origin. Special hydrodynamic conditions may assist deep intrusion of sulfate waters. For example, in some cases isotopic signature indicates mixing of sulfate waters with glacial melt waters.

Main dissolved gas components of groundwaters in crystalline rocks are nitrogen and methane. Nitrogen forms a chemically stable covalent molecule  $N_2$ , having insignificant tendency to act in chemical reactions as electron acceptor or donor without microbial catalysis. Dissolved nitrogen can be a major component both in near-surface oxic waters as in deep anoxic waters together with

methane. Methane is often a major component of dissolved gases in crystalline rocks, concentration of methane increases typically with depth and with increasing salinity of waters. Evidently, hydrogen is the most reactive reducing gas component of groundwaters, but the analysed concentrations remain at < 2 mM in groundwaters of crystalline rock. Helium, formed in uranium and thorium series decay, may be a major component of dissolved gas phase in Precambrian crystalline rocks. As a slowly accumulating inert component it is indicative of the long residence times of deep groundwaters.

#### TUL/VTT

The deep geological disposal in the Czech Republic will be constructed in a suitable crystalline rock approximately 500 metres below surface. Czech concept of engineered barrier system assumes steel container (double-layer of stainless and carbon steel) and bentonite based sealing. The canister material was selected based on the requirement of "Reference project 2011 – SÚRAO" (SÚRAO - Radioactive Waste Repository Authority of Czech Republic). Therefore, corrosion behaviour of carbon steel 12020 has been investigated at CVR and TUL.

Corrosion behaviour of carbon steel was investigated under anaerobic conditions ( $O_2 < 1$  ppm) at 25°C in synthetic bentonite pore water (SBPW) (pH 9.0) inoculated with underground pore water from the VITA borehole dominated by sulfate reducing bacteria (SRB) (obtained from Josef URF) or in underground pore water of the VITA borehole only (pH 7.9) (without SBPW). Corrosion was measured using electrochemical impedance spectroscopy (EIS) and weight losses method. Surface analysis included metallography, SEM/EDS and Raman microspectroscopy. Composition and dynamics of microbial consortia were studied by molecular-biological tools (16S rRNA amplicon NGS sequencing, real time qPCR).

Biofilm and water samples were collected at the end of the experiments for the identification of bacterial communities. In case of SBPW inoculated with VITA water, sulfate reducing bacteria (SRB) survived in the experimental batches (their presence was confirmed also by NGS analysis), but no increase in time was detected by real-time qPCR. In contrast, real time qPCR and NGS results revealed a rapid increase of nitrate reducing bacteria (NRB). This observation can be explained by the chemical composition of synthetic bentonite pore water SBPW being rich in nitrates, an attractive terminal electron acceptor for nitrate reducing bacteria.

A second experiment using VITA water only, proved a rapid proliferation of SRB in both biofilm and water samples. This corresponds to observations from EIS measurements, which showed three corrosion stages of the steel sample studied, as well as SEM/EDS elemental mapping and Raman microspectroscopy which both proved the presence of S<sup>2-</sup>sulphur compounds - probably mackinawite - on the surface of the steel sample. Formation of black layer on the whole surface of the carbon steel samples could be possibly explained by the reaction of carbon steel with sulfide, an aggressive metabolic end product of SRB activity.

#### EPFL

Previous work has shown that the water in the boreholes in Opalinus Clay in Mt Terri harbour a microbial community that is able to rapidly and continuously reduce sulphate, while consuming  $H_2$  (Bagnoud *et al.*, 2016a; Bagnoud *et al.*, 2016b; Bagnoud *et al.*, 2016c). Primary producers in this system were identified as an autotrophic sulfate-reducing bacterium and a sulfide-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 sulfate-reducing organisms then utilized these organic acids as electron donors for further sulfate reduction. While we clearly showed **sulfate reduction in Opalinus borehole water**, the experimental design did not allow assessment of the rate of sulfate reduction (and hence  $H_2$  consumption) in porous media

where sulfate-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 sulfate reduction (catalized by  $H_2$  oxidation) and sulfide 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 flowthrough reactors with a sand and bentonite mixture. The system is entirely located within an anoxic chamber to minimize oxygen influx. The system monitors sulfate consumption and sulfide production as well as H<sub>2</sub> consumption. The flow through cells were designed to allow a gradient of sulfate and H<sub>2</sub> 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 sulfate and H<sub>2</sub> at which the growth is maximized. Finally, because the effluent is monitored, it will be possible to provide **a rate of sulfate reduction**, in combination with biological H<sub>2</sub> oxidation.

The second experiment (IC-A) entails the deployment of module 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 year of incubation in the borehole and the microbial community characterized by cultivation techniques. It was observed that the microbial biomass in the deployed bentonite was larger than that used to build the experiment. Additionally, we found that the borehole had developed a sulfate-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 sulfate reducing organisms. This suggests that electron donors from the rock formation may transiently fuel sulfate reduction but that these sources of energy are rapidly depleted. Further time points will help provide support for this interpretation of the data.

# 3.1.3 Conclusion

# GTK

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

# TUL/VTT

The experiments revealed accelerated corrosion rate of the carbon steel in synthetic bentonite pore water inoculated with ground water dominated by SRB and in ground water dominated by SRB after the period of 3 months and approximately 8 months, respectively. Biofilm formation was observed and the presence of corrosion product mackinawite on sample exposed only to groundwater was proved. This can be probably attributed to the metabolic activity of bacteria. Although the batch system of synthetic pore water inoculated with ground water was dominated by nitrate-reducing

bacteria (NRB) at the end of the experiment (due to the composition of synthetic bentonite pore water), we still assume that SRB, which were also proved to be present, played the main role in observed microbial induced corrosion process. Sulfide, produced by SRB, is a very strong corrosive agent resulting in different forms of corrosion such as general or local corrosion, and pitting corrosion. Our results show that even in environments favouring other microbial groups (in our case NRB), SRB are capable to influence corrosion processes of canister material. **EPFL** 

The current results obtained from the *in situ* experiments in the Opalinus clay in Switserland, 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 sulfide 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 knowhow:

Bentonites rich in swelling montmorillonite clay are used to construct engineered barriers, as buffer, backfill, plugs and seals, to absorb RN and retard RN 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 all 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 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 sulfate or organic matter content in the bentonites or it may be due to intrinsic differences in the swelling pressures 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 limit for microbial activity in bentonite buffer and host clays. Parameters important to investigate are shown in Figure 3.



Figure 3: 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 sulfide corrosion, and safety cases for radioactive waste disposal must, therefore, evaluate the risks involved with sulfide corrosion (King *et al.* 2011). The inorganic reduction of sulfate to sulfide is kinetically hindered at normal pressure and temperature (Cross *et al.* 2004). The main source of sulfide in geological repository environments is, therefore, past and present microbial reduction of sulfate, sulphur and thiosulfate to sulfide. **Sulfide-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. Attack of iron-reducing bacteria on the ferric iron component of bentonite buffers is expected to reduce the swelling capacity of the clay and thereby to open up for microbial activity inside the buffer, to increase diffusion of sulfide and possibly allow the discharge of radionuclides. A remaining key issue is **to understand whether microorganisms can accelerate degradation of bentonite based buffers**.

Third, bentonite clays have a significant capacity for adsorption of  $H_2S$  gas. This fact is utilised to produce industrial filters for removal of  $H_2S$  in low concentration gas streams. The absorbance capacity can be increased by the addition of iron to bentonite (Nguyen-Thanh *et al.* 2005; Stepova *et al.* 2009). The sulfide scrubbing mechanism is explained by **reactions between sulfide and ferric iron**. The reduction of ferric iron by sulfide from SPB was recently demonstrated for freshwater sediments (Hansel *et al.* 2015). The authors showed that sulfide was oxidised 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 sulfide reduces ferric iron to ferrous iron concomitant with the oxidation of sulfide to elemental sulphur (Eq 2). The ferrous iron could react with free sulfide and form FeS (Eq 3). The generalised sum of these processes is given in Eq 4.

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

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

These processes will cause an S immobilisation effect that can reduce the mass of sulfide 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).

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

#### MICANS

Micans has received samples from the FEBEX repository. Some of the samples have been forwarded to UNIMAN to increase the sample resolution in their experiment. MIND laboratory activities using

the FEBEX samples has started. Search for a threshold density has shown that the situation is more complicated than originally expected. By means of new methods, microbial activity is found at all tested densities, although the activity rate decreases with increasing density. Good survival of bacteria over the tested ranges are found as well. Survival and activity of bacteria in compacted bentonites likely depend on several different factors such as water activity, density, swelling pressure, pore space distribution, organic carbon content and characteristics of the different bentonites (Figure 3). Work continues to investigate the influence of these factors on bacterial life in clays. Micans has developed a method for DNA-extraction from microbes in bentonite clay using a phase separation technique.

In a study on the effect of sulfide on bentonites, sulfide was found to reduce ferric iron in montmorillonite type bentonites denoted Asha, MX-80 and Calcigel under the formation of elemental sulphur, ferrous iron and iron sulfide. These reactions demonstrated an immobilisation capacity of the clays that was 40  $\mu$ mole sulfide (g clay<sup>-1</sup>) or more, depending on the load of sulfide, and type of clay. The found immobilisation effect can reduce the mass of sulfide 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.

Presently, new experiments are on-going where the effect of sulfide-producing bacteria (i.e. sulphate reducing bacteria) and sulfide on bentonite performance and stability is investigated. It has recently been suggested that sulfide is incapable of diffusing through bentonite clay and that no sulfide can be analysed from bentonite doped with sulfide. Micans therefore started an experiment where sulfide from bentonite is analysed by a spectrophotometric precipitation method. Results indicate that sulfide in some cases are bound to the clay particles and is unanalysable below a threshold value that differs from clay to clay. A more detailed **sulfide diffusion experiment** is being setup where sulfide diffusion coefficients will be determined for bentonite clays of interest as buffer or backfill in a radioactive waste repository. The effective diffusion coefficients for sulfide in Asha bentonite, compacted to saturated wet densities of 1750 kg m<sup>-3</sup> and 2000 kg m<sup>-3</sup>, was determined to 2.74 × 10<sup>-11</sup> m<sup>2</sup> s<sup>-1</sup> and  $6.60 \times 10^{-12}$  m<sup>2</sup> s<sup>-1</sup>, respectively.Experiments are at present being run to study the process(es) behind the loss of analysable sulfide mixed with bentonite clays. A manuscript will be submitted on this work and the results. Some of these results were presented at the MontTerri meeting in February.

#### EPFL

**Bioreactors to investigate growth in bentonite** have been developed and built and experiments will be performed 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 has currently focused on the quantification, and identification of Fe(III) reducing and sulfate reducing microorganisms in bentonites, as well as investigating structural change in the clay minerals, after treatment e.g. irradiation. Work on this task has been progressing

at a steady rate, and a first draft for publication is nearing completion. Recent attempts to extract DNA from the FEBEX core samples have proved successful, and the extracted DNA has now been sequenced using an Illumina platform and the data analysed. Complementary FEBEX culturing work is ongoing. Pressure cell systems have been constructed and initial high-pressure studies are being planned.

#### **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 4). 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 injection 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.

The first pair of experiments is currently running. Both tests used gamma-irradiated FE bentonite and compared the response of a non-inoculated "sterile" core (Test 1) to a core inoculated with a sulfate reducing bacteria enrichment from the bentonite prior to irradiation (Test 2). Each core was prepared to a dry density of 1400 kg m<sup>-3</sup> and contained 5 g unalloyed steel chips.



Figure 4: 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 (Fig 5). 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  $1.8 \times 10^{-20}$  m<sup>2</sup>. A similar response was observed in the inoculated Test 2. Unfortunately, data recording was lost from several of the load cells, but it is anticipated that this data can be recovered by recalibration at the

end of the test. 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  $1.8 \times 10^{-20}$  m<sup>2</sup>. Both tests are ongoing and are planned to end between July and September 2017.

Data collection is ongoing, and initial results show that experimental systems are behaving as expected and reproducible results can be obtained. Monitoring will continue until the end of the experiments, when the post experimental materials (bentonite, steel and fluids) will be subjected to microbial, geochemical and mineralogical analysis.

#### **TUL & RCR**

TUL & RCR are finalising the preparation of the experiments focused on **survival of microorganisms in bentonite exposed to higher temperature, pressure and irradiation**. Newly obtained pressure cells were successfully tested (Figure 5). Optimal ratio of bentonite powder (Czech BaM bentonite) and water for bentonite suspension preparation is currently studied. Aged bentonite from Josef Underground facility will be analysed. Bentonite samples are planned to be removed in July 2017, in the meantime, DNA extraction protocol from bentonite is being improved. Experiments studying microbial mobility in bentonite are being prepared.



*Figure 5: A. Stresses and pressures recorded by the pumps (red and green lines) and load cells during uninoculated Test 1. B. Stresses and pressure recorded by the pumps and load cells in inoculated Test 2* 

#### HZDR

HZDR has set up several microcosm experiments to **test for microbial iron reduction in bentonite**, containing 20 gram bentonite (two Bavarian Bentonites, a processed one (B25) and a natural one (N01)) and 40 ml of synthetic, anaerobic Opalinus clay pore water solution under an  $N_2/CO_2$ -gas-atmosphere. Microbial activity and potential geochemical and biological effects were stimulated by adding various kinds of substrates (lactate, acetate or H<sub>2</sub>). The microcosms were incubated at 30°C and 60°C, respectively and the experiments are carried out in the dark without shaking for approximately one year. At specified time-points, samples are taken to analyse geochemical and biological parameters.

Until now, an anaerobic atmosphere was created within all microcosms. Batches with Bentonite N01 at 60°C showed no significant changes in pH, Eh, sulfate- and iron-concentration after 200 days of incubation. However, low amounts of pyruvate have been formed after 60 days of incubation in N01 microcosms containing 50 mM lactate, indicating microbial activity within these batches. Similar observations have been made for Bentonite B25 at 60°C with one exception: Eh was decreasing from 300 mV to -170 mV within the first 140 days of incubation at 60°C when  $H_2$  was the electron donor. So far, for the 30°C incubations in the N01 microcosms no significant changes could have been

observed. However, differences are obvious in the B25 batches. Here, Eh decreased from 300 mV to - 300 mV, when H<sub>2</sub> was added and from 300 mV to -30 mV, when 10 mM lactate was supplemented. Additionally, B25 microcosms containing 10 mM lactate showed a decrease in sulfate- and lactate-concentration as well as the formation of acetate, suggesting metabolic activity of sulfate-reducing microorganisms in these batches. So far, no significant changes in the Fe(II)- and Fe(III)-concentration have been detected, in none of the experimental set ups. DNA was successfully extracted from the raw material of N01 and B25 and amplified 16S rDNA gene fragments were sequenced with MiSeq Illumina. The results show the presence of aerobic and anaerobic bacteria within the raw material, providing the potential to survive under these harsh conditions.

#### VTT

The VTT **long-term compacted bentonite experiment**, which began in 1997 and ended a couple of years ago, will be further studied for microbiology in the MIND project. The research has been ongoing as planned and sequencing for fungi has been done. The species diversity has been analysed and the manuscript is under work.

VTT has started a new **long-term microcosm** experiment in spring 2016 in order to study the **effect of early phase in the repositories** when the bentonite has not wetted enough and is not pressurized. The purpose of this research is also to simulate worst-case scenario, where organic compounds released from the surface may accelerate the microbial action in the repository. Commercial MX-80 bentonite (CETCO) was diluted with Olkiluoto surface water. A minor part of the dilution water was a mixture of three Olkiluoto drillhole waters. Low level of nutrients including acetate, formate and methanol and rock crushed originating from Olkiluoto were added into the sample bottles. A part of the samples was autoclaved and the water was sterile filtered to better observe the role of microorganisms in the experiment. The used bentonite has been earlier characterised and its possible changes during the experiment will be monitored with microbial and other methods including especially microscopy (atomic force microscopy). The gas composition of the bentonite samples has been followed with gas chromatography (GC-2xTCD). The first sampling of the microcosms is after one year. In collaboration of the Universidad de Granada, study of the bentonite samples with electron microscopy methods are planned.

# 3.2.3 Conclusion

Experiments and investigations have started as planned and results are starting to come. However, many of the experiments are long-term and few studies have, therefore been completed. Manuscripts are under production. It is expected that several papers will start to appear in open scientific press during 2017.

# 3.3 Cementitious materials

Contributing partners: SCK•CEN

# **3.3.1** Problem statement & current knowhow:

Cementitious materials have been used for centuries in many construction and engineering applications because of their long-term durability. Also during the geological disposal of radioactive waste, cementitious materials are used in many parts of the engineered barrier. In Belgium, the 'supercontainer' is selected as reference design for the disposal of high level radioactive waste (HLW). 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 . High alkaline conditions provided by the concrete buffer forms a protective passivating layer to ensure uniform corrosion of the carbon steel overpack. The supercontainer will

be placed in a disposal gallery lined with concrete wedge blocks and after waste emplacement, all voids will be filled with cementitious materials. 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. The integrity of the engineered barrier system has to be ensured at least for the duration of the thermal phase (several hundreds of years for vitrified HLW and a few thousands of years for spent nuclear fuel) of the waste of emplacement in the repository.

Several geochemical processes can affect the chemical evolution of concrete buffer such as chloride ingress, sulfate attack, alkali-silica reactions, carbonation and Ca<sup>2+</sup> leaching, which may result in an increased corrosion . Moreover, these processes can result in a local decrease in pH, leading to niches where growth of microorganisms will be possible. Microbial degradation of cementitious materials in aerobic conditions is commonly known , hence it can be possible in the early phase of geological disposal of radioactive waste. Direct anaerobic corrosion of concrete materials is not known . However, 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 (Zhu *et al.* 2016). In addition, organic acids (e.g. acetate) and carbon dioxide produced by microbes in the repository, can be corrosive towards cementitious materials and can lead to a further decrease in pH. On the other hand, microbial metabolic activity can lead to the formation of calcium carbonate which can result in partially clogging of the fractures as the molar volume of calcite is larger than that of portlandite .

# 3.3.2 Summary of experimental & computational results

The objective of this study is the investigation whether microorganisms could affect the long-term evolution of the cementitious materials present in the engineered barriers of a geological repository for radioactive waste, under relevant *in situ* conditions. Should this be possible, it will be assessed if this impact is positive or negative. To fulfil this task, several batch experiments will be performed in which microbial activity, biofilm development and overall shifts in microbial communities will be investigated. In addition, cement integrity will be monitored and chemical analysis of the liquid phase will be executed. During and/or after incubation of the experimental set-ups, microbial, molecular, chemical and microscopy analysis will provide an insight in the mechanisms affecting either deterioration or enhancement of cement integrity.

# 3.3.3 Conclusion

At this moment, no conclusions can be formulated as the experiments did not yet start.

# 3.4 WP2 current status and intermediate conclusions

The objectives of WP2 were to:

- Quantify the contribution of microbially produced **sulfide** 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 cementitous materials used in European geological disposal concepts, i.e. <u>microbial degradation</u> <u>of bentonites and cementitous materials</u>. (Tasks 4 and 5).

The overall conclusion is that all partners have started and performed experiments as planned for task 1, 2, 3 and 4. The work in task 1 has been reported in a deliverable, in two steps, according to plan foreseen in the grant agreement: deliverable D2.1 'Data report on the inventory of reducing

gases in host rock and their transport rates to groundwater and microbial ecosystems', and deliverable D2.1-2 'Interim report on deep gases and sulphur compounds as biogeochemical energy sources in crystalline rock'. For task 2, work is proceeding as planned and deliverable D2-2 'Design, set up and operation of experimental equipment has been submitted'. All the deliverables are available on the website of the MIND project (www.mind15.eu)

Task 3 will submit the deliverable D2.4 'Report on threshold densities for three commercial bentonites above which microbial activity including sulfide-producing activity is practically inhibited under specific clay properties' by the end of June 2017.

The concrete work (Task 5), will start in 2017 as planned.Deliverables for tasks 4 and 5 arescheduledtobesubmitterafteryear3.

# 4 General conclusions

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.

The aim of WP1 of the MIND project is (1) to gain more insights in the <u>degradation mechanisms</u> (i.e. radiolysis, hydrolysis and biodegradation) and the resulting <u>degradation products</u> from organics present in the waste, under relevant repository conditions, (2) to determine possible <u>boundary</u> <u>conditions</u> under which these reactions can occur, and (3) to assess their impact on the speciation and <u>mobility of radionuclides and gas generation</u> in de disposal facility.

It was shown with various experiments that both natural and anthropogenic organics (e.g. cellulose, and, e.g. bitumen, PVC, resins,) are prone to radiolytical, chemical, and microbial degradation. In addition, biodegradation of the organic waste degradation products can occur at neutral, alkaline (pH 10) and hyperalkaline (pH 12.5) pH. A commonly observed effect is that (bio)degradation of this organic waste may lower the pH of the water environment. This pH reduction enhances microbial activity resulting in turn in an increased biodegradation rate.

The resulting degradation products (e.g. nitrate, ISA, acetate, trimethylamine, PVC plasticisers) in turn have shown to be subject of various abiotic and biotic processes. This is especially important for the soluble organic ISA – known to have a significant impact on radionuclide mobility – but which is susceptible to biodegradation even at alkaline pH. A lot work has been done to unravel the precise mechanisms of the ISA biodegradation process, and in a next step it will be interesting to explore reaction kinetics and boundary conditions in further experiments. On the other hand, it has been shown it is highly unlikely that microbial activity would reduce this risk for another known radionuclide complexant, phtalic acid. In addition, it has been shown that depending on the environmental conditions, microbial nitrate reduction can lead the production of  $NH_4^+$ , which can sorb onto clay minerals and therefore compete with some radionuclides for sorption. However, 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. Similar assessments can be done for other concepts.

In addition, microbial degradation of organics may enhance  $CH_4$  or  $N_2$  gas generation (e.g. methanogenesis or via denitrification). But, several experiments have also shown efficient microbial  $H_2$  gas consumption, which can potentially occur in combination with methanogenesis or denitrification, and which then results in an overall gas pressure decrease.

Additionally, novel mechanisms of radionuclide interactions with indigenous bacteria from bentonite have been observed, forming water insoluble complexes, and thus potentially reducing the radionuclide mobility in groundwater. The reaction rates and possible boundary conditions should be investigated more in detail.

Most of the abiotic organic waste degradation processes have a more negative impact (e.g. increase radionuclide mobility), while microbial activity can have both negative and positive implications. Currently performance assessment studies do not take into account such microbial beneficial effects, but this could/should be evaluated.

One of the key questions posed in WP2 of the MIND project was to what extent microbial activity can influence the corrosion rate of steel within the constraints of the repository conditions, including the bentonite barrier. In this aspect, it has been shown that the sulfate reducing activity is of paramount

importance it could result in the production of sulfide species that are known to enhance corrosion. In addition, microorganisms could form a biofilm on the surface of the canister and enhance corrosion via the direct precipitation of iron sulfide minerals on the surface and the consumption of  $H_2$ . The overall aims of WP2 are (1) the quantification of the contribution of microbially produced sulfide in the geosphere and in buffers and backfill to the overall rate of <u>canister corrosion</u>, (2) gaining insights on the effectiveness of specific <u>bentonite buffers to inhibit microbial activity</u>, and (3) to characterize the impact of microbial activity on the long-term <u>performance of bentonites and cementitious</u> seals and plug systems in European geological disposal concepts.

A nationwide survey of deep drillholes in Finland showed that sulfide concentration in groundwater may be higher than that predicted by equilibrium solubility of iron sulfides, because microbial sulfate reduction may take place in iron-deficient conditions. However, the process is generally limited to a relatively narrow sulfate reduction zone.

In synthetic bentonite pore water inoculated with ground water dominated by sulphate reducing bacteria (SRB), a clear accelerated corrosion rate of the carbon steel was demonstrated. Even in water environments favouring other microbial groups, SRB are capable to influence corrosion processes of canister material. To complement such kind of batch experiments, *in situ* experiments have been started to monitor and determine rates sulfate reduction, together with H<sub>2</sub> production, and to investigate steel corrosion mechanisms *in situ*.

The question remains if bacteria, such as sulphate or iron reducing bacteria, can also be active in compacted bentonite buffer materials. Experiments have shown microbial survival and activity of bacteria in several compacted bentonites. However, survival and activity of bacteria in compacted bentonites depends on several different factors such as water activity, density, swelling pressure, pore space distribution, organic carbon content and characteristics of the different bentonites.

Other experiments have shown the microbial mediated immobilisation of sulfide on montmorillonite type of bentonites. This can reduce the mass of sulfide that corrode metal canisters over repository life times. However, the concomitant reduction of ferric iron to ferrous iron, may be problematic due to the destabilizing effect of ferrous iron on dioctahedral smectites such as montmorillonites.

To elucidate more in detail the different microbial processes that can be expected in nuclear waste and in a repository, within a reasonable time frame, for many of the experiments performed until now, more optimal growth conditions were selected in terms of for example temperature and water availability, etc.. Some test parameters therefore could be seen as less representative for in situ conditions (e.g. neutral pH, temperature, dose rates during irradiation experiments), however, we believe it is more useful to initially gain more insights in possible reaction mechanisms at optimal conditions before determining boundary conditions. In that way, the most important factors determining microbial activity can be defined. Moreover, conditions will not be homogenous throughout the repository and there will always be niches that are more favourable for microbial activity. The in situ TVO gas generation experiment that has been in operation for nearly 20 years studying methanogenic gas generation from operating LLW from the Olkiluoto power plants experiment is good example showing that. Here, heterogeneities in the initial chemical conditions have permitted the development of microbial processes that led to a rapid decrease of the pH, which led to an overall increased gas generation rate. The microbial community that was active resembled a typical community for anaerobic digestion. Also in the Mont Terri URL, an in situ experiment is carried out to investigate the potential for microbial methane gas generation and the associated kinetics under current in situ conditions (at neutral pH). The goal is to attempt a maximum rate of gas production and to understand the mechanism of methane generation and consumption within the microbial community present in such an engineered and geological setting. These long-term *in situ* experiments are highly relevant, if not essential, to confirm microbial modelling methods. Modelling of microbial processes relevant to geological disposal is not common; however, it can help the interpretation and prediction of biogeochemical processes. Moreover, it can be implemented in performance assessment studies to examine the significance of specific microbial processes.

A more detailed knowledge of the microbial processes, their boundary conditions and reaction kinetics in different conditions is however, needed to be able to integrate such processes more correctly in the modelling methods. As an example, performance assessment was done specifically for Eurobitum waste in the Belgian disposal concept with Boom Clay as reference host rock and with the abstraction of potentially relevant microbial processes and associated uncertainties in the assessment through appropriate model parameterisation and/or conservative hypotheses. The outcome suggests that no adverse long-term effects are to be expected, and that some (microbial) processes might even have a beneficial impact on repository safety. Similar performance assessments, taking into account the microbial processes acting on the bituminsed waste, would be relevant for other disposal concepts.

In conclusion, in both experimental work packages the research partners within MIND are working hard to investigate the variety of research questions defined at the beginning of the project. To fulfil these tasks, combination of laboratory batch experiments and *in situ* experiments, as well as computer based modelling, are carried out. Currently, many of these experiments are still on-going or even still to be started, making it difficult to make a throughout analysis and interpretation towards performance assessment at this moment. At this stage, it too early to make a proper list of features, events and processes (FEP's) regarding microbial processes relevant for geological disposal of radioactive waste as suggested by the implementers review board. However, when more results are generated, throughout the remaining next 2 years of the project, this could be attempted.

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