Co-funded by the

European Commission

MIND

Microbiology In Nuclear waste Disposal

(GRANT AGREEMENT: 661880)

DELIVERABLE 4.6

Implementers' Review Board Evaluation Report

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Date of issue of this report: 21.05.2019

Report number of pages: 52

Start date of project: 01/06/2015
Duration: 48 Months

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

Publishable Summary

Microbiology In Nuclear waste Disposal (MIND) is an interdisciplinary project consisting of two experimental work packages and a third one that handles integration of society and policy-oriented studies. As a part of the project an Implementers Review Board (IRB) was organized consisting of representatives of the Nuclear Waste Management organisations in the countries contributing to MIND. In this evaluation report the IRB discusses microbial issues of potential importance to post closure safety, assess the contribution by MIND in resolving these issues and indicates potential needs for further research. These issues are:

- The swelling of bentonite and its role in preventing sulphate reduction, sulphide migration and "microbial induced" corrosion
- Microbially mediated gas generation including gas consumption and gaseous C-14 containing species
- Microbially induced organic waste matrix degradation - main factors of influence and major principles
- Microbially enhanced processes potentially affecting radionuclide transport / migration
- Environmental parameters that limit microbial impacts in different repository environments.

The MIND project has without doubt significantly enhanced our knowledge basis on microbial controlled processes. These processes where electron transfers occurs are by far the most relevant as microbial metabolism is based on the energy derived from these bio-(geo-)chemical redox reactions. Key reactions such as organic matter or hydrogen gas oxidation or oxyanion (nitrate, sulphate and carbonate) reduction do not occur without microbial catalysis. Furthermore MIND has helped to reduce uncertainties in regard to the limits of life within the engineered barrier system of waste disposal facilities and some host rock types (i.e. clay rocks). The limit in regard to pH (concrete barrier) and density of bentonite barriers or keys are now well established. The limit of life for compacted clay host rocks is also better constrained. The limit of life approach allows in the specific cases to distinguish a geochemical evolution without microbial catalysis from situations where the role of microbes, i.e. associated reaction catalysis, should be explicitly considered (i.e. biogeochemical evolution).

The project also contributed to our understanding and quantification of chemistry related transition periods, such as desaturation, of which the length is a function of hydraulics and of gas generation, the latter in part controlled by microbial activity either by gas oxidation (H_2) or formation (N_2, CO_2, CH_4) depending of course on the waste type and the inventory and the local conditions (host rock).

In some cases discrepancies persist between studies explicitly considering microbial activity and those looking mostly at global processes. The role of reduction of Fe(III) in smectite or of sulphate reduction on corrosion are two examples studied within the MIND project. It is clear that in microcosms in the presence of organic matter bioreduction of Fe(III) and sulphate can be observed but the related impact on bentonite swelling or corrosion has not been observed in underground rock laboratories. This indicates the need to couple the surface and URL experiments and to get as close as possible to repository conditions even in surface laboratory tests.

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1 Introduction

Project MIND (**M**icrobiology **I**n **N**uclear waste **D**isposal) is an interdisciplinary EU project that addresses the influence of microbial processes on nuclear waste forms and their behaviour, the technical feasibility and long-term performance of repository components. The project also organises an Implementers Review Board (IRB). The IRB consists of representatives of the Nuclear Waste Management Organisations (WMO) in the countries contributing to MIND and also from non-member countries such as Canada, USA and Japan

During the course of the MIND project the IRB has advised the Technical Coordinator and the Executive Committee of MIND with critical evaluation concerning research quality and significance of outputs in relation to the implementers needs, highlighted opportunities for networking with other international research activities and raised awareness of our research programme where appropriate. Members of IRB has participated in the MIND Annual Meetings, participated in discussions and also produced written feedback at the end of each annual meeting.

In operation the IRB developed a working table listing potential research gaps regarding Microbiology in Nuclear Waste Disposal. This table was a collation of FEPs (Features, Events and Processes) on different microbial processes of potential relevance to waste disposal and also addresses to what extent these “FEPs” were addressed in MIND. This GAP-table has then been communicated with the MIND WP leaders and was also discussed at a special session at the MIND annual meeting in Lausanne, Switzerland 2018. As a final product the IRB has produced this Evaluation Report where the contributions of MIND are assessed with respect to microbial issues, processes and conditions of relevance to Post Closure Safety of Nuclear Waste repositories.

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

2 Accomplishments of MIND in relation to Microbial issues, processes and conditions of relevance to Post Closure Safety of Nuclear Waste repositories

2.1 Introduction

The IRB has identified some overall areas where microbial processes may be relevant to Post Closure Safety of Nuclear Waste repositories. For each such area it is described why and when they could be of relevance, what achievements that have been made within and what remains to be resolved of common interest to WMOs.

2.2 The swelling of bentonite and its role in preventing sulphate reduction, sulphide migration and "microbial induced" corrosion

2.2.1 Introduction of issue

The swelling of bentonite and its role in preventing sulphate reduction, sulphide migration and "microbial induced" corrosion

Microbial activity in bentonite buffer and backfill can produce chemical species that may accelerate the corrosion of copper. The most important types of microbes are sulphate-reducing bacteria, which produce sulphide. The prerequisites for significant viability of microbes are sufficient availability of free water, nutrients, and space for living cells to grow. Mechanical forces, low water activity and pore size will therefore affect the microbial activity in the buffer (Motamedi et al. 1996, Stroes-Gascoyne et al. 1997, Pedersen et al. 2000, Pedersen, 2000, Masurat et al. 2010b, Stroes-Gascoyne et al. 2011). The presence of sulphate reducing bacteria (SRB) in commercial bentonite and their potential to be active after exposure to elevated temperature and salinity has been shown in Masurat et al. (2010a) and Svensson et al. (2011).

Because of the high temperatures, and desiccation effects after deposition of the canister, initial microbial activity close to the canister is not likely. The water content in the buffer will decrease near the canister. The heat from the spent nuclear fuel will introduce a stress on microbial populations. However, many microorganisms can survive and be active at high temperatures, provided that they have access to water, space, electron acceptors, carbon sources and energy that can sustain metabolic processes for repair of unavoidable heat damage in the cells.

There is a correlation between swelling pressure and microbial activity. However, some uncertainty remains regarding the importance of different buffer characteristics in limiting microbial activity during swelling. In the SR-Can safety assessment, the limit for controlling microbial sulphide production was set as a saturated clay density of MX-80 bentonite of 1,800 kg/m^3 (SKB 2006). This gives a pore space and swelling pressure that lie close to the low pore space and high swelling pressure reported to suppress microbes in Masurat (2006).

Conclusions concerning the swelling pressure/dry density and potential additional constraints limiting microbial activity are, however, somewhat incomplete. There are however results indicating that there is a sharp limit where microbial sulphate reduction ceases in MX-80 (Bengtsson et al. 2015).

In Bengtsson and Pedersen (2017) three different bentonite clays, Wyoming MX-80, Asha and Calcigel at saturated densities ranging from 1 500 to 2 000 kg/m^3 were investigated for microbial sulphide-producing activity as a function of bentonite density at full water saturation. The results for the three clays indicated intervals from 1 740–1 880 kg/m^3 in saturated densities within which sulphide-producing activity as analysed by the copper discs dropped from high to very low or below detection. It was observed that sulphide production, analysed as formation of copper sulphide on copper discs, ceased at a lower saturated density in the iron-rich Asha bentonite compared to Calcigel and MX-80 bentonites with lower iron contents. Bengtsson et al (2017) investigated the effect from one iron-rich Rokle bentonite and one iron-poor Gaomiaozi (GMZ) bentonite on sulphide producing activity over a range of three saturated densities, 1 750, 1 850 and 1 950 kg/m^3. The radiotracer methodology indicated a very low sulphide-producing activity in Rokle at all tested densities except for the sample with the lowest wet density that had a locally high sulphide production. For the GMZ, there was significant sulphide-producing activity at all three densities tested. The accumulations of $Cu_x{}^{35}S$ over wet density of the bentonite for copper discs fitted in test cells with bacterial additions from experiments in Bengtsson and

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Pedersen (2017) and Bengtsson et al (2017) are displayed in Figure 2-1. For each bentonite type there was a relatively small interval in wet density in which sulphide-producing activity registered as $Cu_x{}^{35}S$ dropped from high to very low or to absence of $Cu_x{}^{35}S$. The present range of each interval may just be a matter of the number of sampling points and the selected densities; analysis of more densities may possibly narrow the range.

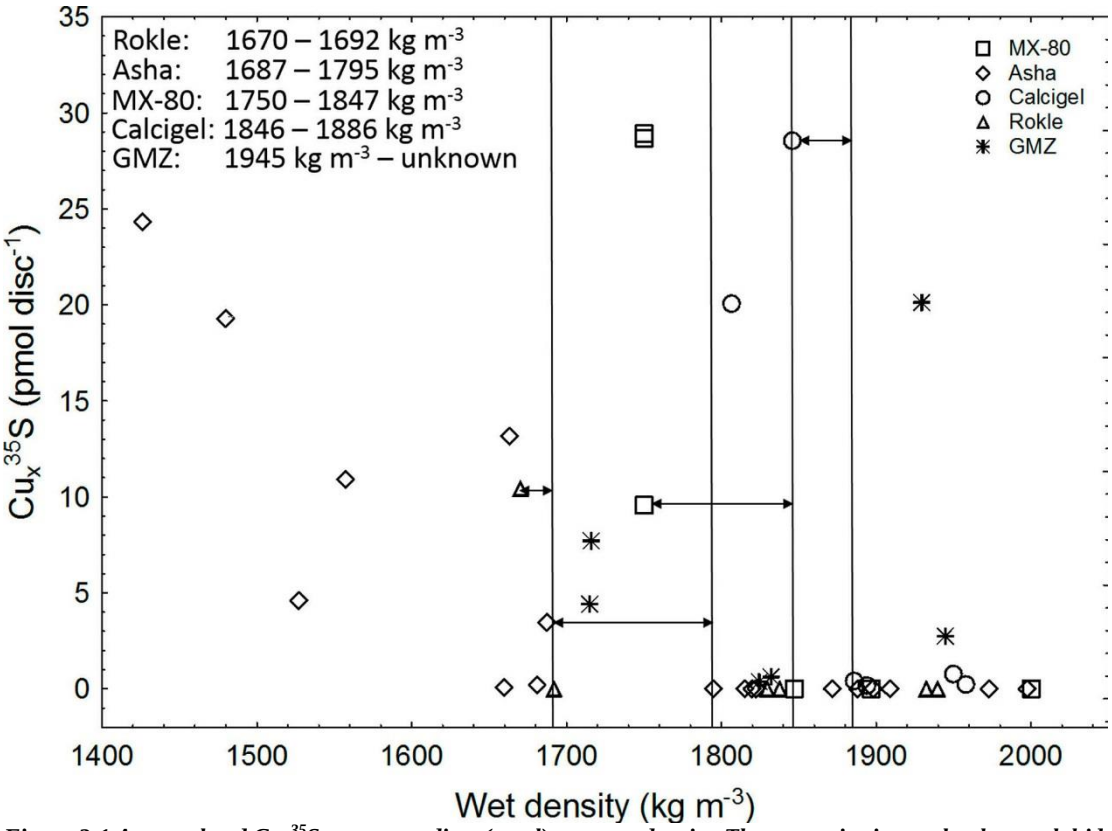

Figure 2-1 Accumulated $Cu_x{}^{35}S$ on copper discs (pmol) over wet density. The respective intervals where sulphide-production shifts from high to low are indicated with arrows. The corresponding analysed saturated density intervals are inserted, for GMZ all tested densities shows high sulphide production.

It is clear that there is a sharp boundary in saturated density for when microbial sulphide production can persist for a given bentonite material. There are however currently no clear understanding why this boundary is different between different bentonite materials. Montmorillonite content and swelling pressure are not sufficient explanations. There is an empirical correlation to the iron content, but this could be a coincidence. There is no mechanistic process that can explain why an iron rich bentonite should be less favourable for microbial sulphide production.

Redox reactions and clay stability

There are microbes in nature (iron-reducing bacteria, IRB) that can reduce trivalent iron as a part of their metabolism. IRB were also studied when the outer section of the Prototype Repository was excavated, where they were observed sporadically, especially near the contact with the rock (Svemar et al. 2016). There are indications that these microbes can in certain cases reduce the structural iron in montmorillonite and thereby also increase the probability of illitization (Kim et al. 2004).

Smectite is stable at low temperature and pressure, which are the typical conditions in soils and surficial sediments. As soils and sediments are buried, smectite becomes unstable and transforms into illite according to the following reaction:

smectite + Al^{3+} + K^{+} → illite + silica

Three important variables drive the smectite-to-illite (S-I) reaction: time, temperature, and potassium concentration. The S-I reaction is of special significance because the extent of the reaction, termed “smectite illitization,” is associated with a specific combination of temperature and time (Dong, 2012). In terrigenous sediments, the S-I reaction typically takes place over the temperature range of about 90–120 °C (Moore and

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Reynolds, 1997). However, in the presence of microbial activity, this reaction occurs via various mechanisms, at much lower temperatures and pressures over shorter time durations (Kim et al. 2004).

In general, methanogens are less efficient in reducing structural Fe(III) than iron-reducing and sulfate-reducing bacteria. The most important factors are the microbe/clay ratio, the clay mineral surface area, the chemistry of the aqueous medium, the presence or absence of electron-shuttling compounds, and temperature. Not all structural Fe(III) in clay is biologically reducible. When the extent of reduction is small (<30%), the smectite structure remains stable, but above this threshold, it becomes unstable and the clay mineral may dissolve. All previous experiments used nontronite to study the role of microbes in the S-I reaction (Dong 2012).

Much of our understanding of microbially mediated clay mineral transformation is qualitative. It is still poorly known to what extent these mineral reactions are mediated by microbial activity in nature. How can laboratory based results be extrapolated to complex field applications? What possible issues are involved in spatial and temporal scaling? These questions are especially relevant to the microbially catalyzed S-I reaction model, as it is not yet clear how widely this model is applicable to the overall picture of clay diagenesis. The laboratory-optimized conditions may be satisfied only in certain geological environments. The lack of these conditions may be the reason to explain why smectite in soils and sediments persists despite the presence of active microbial processes.

2.2.2 Why of relevance and when

In several repository concepts, like the KBS-3 method to be implemented in Sweden and Finland, or the concept considered by NWMO in Canada, spent fuel or HLW is emplaced in a copper coated container surrounded by a bentonite clay buffer, deposited at depth in the host rock. One of the very few threats to the integrity of the container would be copper corrosion by sulphide. It is therefore of importance to understand both to what extent microbial activity may affect the supply of sulphide and under what conditions this could occur.

2.2.3 Achievements within MIND

The swelling of bentonite and its role in preventing sulphate reduction, sulphide migration and "microbial induced" corrosion

Deliverable D2.4: Bacterial activity in compacted bentonites

The deliverable contain a summary of studies on the prevention of sulphate reduction performed prior to, or outside the MIND project. The deliverable does however not contain any new information from the MIND project on the issue.

Deliverable D2.6 Microbial diversity in bentonite buffer of aged bentonite buffer experiment

This deliverable describes the results obtained from the long-term experiment with bentonite buffer. The MX-80 Na-bentonite was compacted inside a copper cylinder, which was set inside a plastic bottle containing non-saline groundwater simulant. The aim of this experiment was to evaluate changes of chemical, mineralogical and microstructural parameters of bentonite in both oxic and anoxic conditions. Microbiological analyses performed at the end of the experiment included evaluation of bacterial and fungal communities by sequencing and visual evaluation microscopically. Living microbial cells were detected in the water samples by epifluorescent microscopy but the presence of living microbes on bentonite matrix and on copper surface could not be demonstrated. Microbial DNA was extracted from water, bentonite and copper surface of the capsule and used for analysis of bacterial and fungal community. Sequencing revealed the presence of sulphate and iron reducing bacteria in bentonite, water and copper surface.

This study did not consider sulphide production.

Deliverable D2.10: Microbial mobility in saturated bentonites of different density

The deliverable starts with a comprehensive review of issues and available literature covering:

- Microorganisms in groundwater
- Microorganisms in bentonite deposits
- Microorganisms under repository conditions
- Effect of pressure
- Effect of temperature
- Effect of desiccation
- Studies under repository conditions

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- Microbial migration in compacted bentonite

The aim of the study in the deliverable was to enhance the knowledge on the ability of bacteria to migrate through the compacted bentonite. The specific aims were to develop a reliable method for detection of bacteria in the bentonite (both viable and dead cells) and to monitor bacterial mobility in the compacted BaM bentonite.

A reliable method for direct detection of bacterial presence (both viable and dead cells) in compacted and saturated bentonite was developed. The method is based on the extraction of bacteria from bentonite using density gradient centrifugation and their subsequent Live/Dead staining.

This newly developed method was used to study microbial mobility within compacted BaM bentonite of two different dry densities – 1400 kg/m^3 and 1600 kg/m^3. Fourteen samples differing in their distance from the source of bacteria (VITA water) was searched for the presence of bacteria in each of both compacted bentonite samples and live cells were detected in all tested samples including the very innermost part and the bottom part of the bentonite samples of both densities. This finding indicates that bacteria are able to move through compacted bentonite of relatively high dry density (1600 kg/m^3).

The study was focussed on mobility and did not consider sulphide production.

Redox reactions and clay stability

Mineralogical alteration of bentonite in the presence of microbes has been studied by HZDR (MIND D2.8). The aim of this study was to investigate if microbes influence the beneficial properties of bentonites by either changing the solubility and/or composition of minerals and the reduction of important ions (e.g. ferric iron) of the studied bentonites.

The experiment with Bavarian bentonite consisted of an industrial/processed bentonite (B25) and a natural one (N01) and were performed at HZDR. The set-ups contained the respective bentonite and an anaerobic synthetic Opalinus-clay-pore water solution under an N_2/CO_2-gas-atmosphere. Acetate, lactate or hydrogen gas was used to stimulate potential microbial activity and geochemical effects.

The set-ups were incubated in the dark at 30 °C and 60 °C for one year. Bio-geochemical parameters were monitored and the microbial diversity as well as mineralogical composition were analysed.

The addition of substrates has a big influence on the evolution of microbial diversity and activity as well as on – connected – changes of geochemical parameters and even the mineral structure. The most striking effects showed the industrial and processed bentonite B25 at 30 °C. In microcosm-experiments including the natural bentonite N01 no striking effects were observed during the respective time frame of one year. Reasons for this could be that the microorganisms in bentonite B25 are already adapted to harsh conditions due to the several processing steps of bentonite B25 and/or that a different microbial population exists in the bentonites. Same is true for set ups of both bentonites at 60 °C, showing no significant changes with respect to the analysed parameters, regardless of the bentonite or the added substrate.

The obtained results clearly show, that the presence of hydrogen leads to presence of metabolically active sulphate-reducing organisms, being at least responsible for the formation of sulphides. Likewise, to the observations made in the hydrogen gas containing batches, the redox potential decreases with proceeding time, regardless of the supplied concentration of lactate. Again, an increase of ferrous iron and a simultaneous decrease of ferric iron as well as a decrease of sulphate concentration was observed with a concomitant formation of iron-sulphides that precipitated. SEM analysis revealed in the respective batches a significant accumulation of iron-sulphur compounds compared and in contrast to the control batches (data not shown), showing that the microbial formation of sulphides indeed effects the mineral composition of bentonites. Additionally, the formation of cavities and gas bubbles within the respective batches were observed, strongly arguing for the formation of gases, very likely also hydrogen sulphide gas, which was formed due to sulphate-reduction. Furthermore, the respective batches showed the consumption of lactate and a simultaneous formation of acetate in approximately equimolar amounts.

Structural effects on the bentonites themselves could not be demonstrated or verified so far, as well as the microbial influence on the swelling pressure and the cation exchange capacity. Setups without addition of substrates did not show any significant changes regarding geochemical or mineralogical changes. Although lactate and hydrogen gas containing batches showed clear effects on the geochemical and microbial point of view, significant structural effects on the bentonites were not observed, especially with respect to the process of illitization.

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2.2.4 What remains to be resolved of common interest to WMO:s

It was stated at the end of section 2.2.2 (below the fig 2-1.) "It is clear that there is a sharp boundary in saturated density for when microbial sulphide production can persist for a given bentonite material. There are however currently no clear understanding why this boundary is different between different bentonite materials. Montmorillonite content and swelling pressure are not sufficient explanations. The reason for this boundary should be investigated. For example, it has been suggested that the lower density limit for iron rich bentonites indicated by the experiments is not due to inhibition of the sulphide production, but that the sulphide reacts fast enough with iron, in order to never reach the copper disc.

It was also shown that microbial activity had an effect on geochemical parameters and even on mineral structure (section 2.2.3 *Redox reactions and clay stability*), and the main observations were formation of sulphide, reduction of iron and formation of iron sulphide precipitation as well as gas formation (likely also sulphide hydrogen). However, the structural effects on the bentonites themselves could not be demonstrated or verified so far, as well as the microbial influence on the swelling pressure and the cation exchange capacity. It is still unsolved if high sulphide concentrations or iron reduction rates could affect performance of bentonite if e.g. swelling properties will change.

2.3 Microbially mediated gas generation including gas consumption and gaseous C-14 containing species

2.3.1 Introduction

Anaerobic corrosion and degradation of the waste materials in the emplacement caverns of a repository for radioactive waste are associated with the formation and accumulation of a free gas phase and the build-up of gas pressure in the backfilled repository structures.

The accumulation and release of these repository-generated gases may affect a number of processes in the repository near-field that are relevant to post-closure safety. The long-term stability of the barrier system should be ensured over the so-called time frame for safety assessment, which is the main period of concern from the perspective of post-closure safety and which is usually defined based on the decrease in radiological toxicity that occurs over time. In the case of Nagra, it extends to 100'000 years for the L/ILW repository and to 1'000'000 years for the HLW repository. The specific issues to be considered are:

- The state conditions (water saturation, pore pressure, stress, porewater composition) in the backfilled emplacement rooms and in the surrounding host rock, which will be influenced for a period of thousands of years by gas accumulation.
- The integrity of the engineered barrier systems and the host rock, expressed in terms of safety-relevant properties, which may be impaired by the build-up of excessive gas pressures in the emplacement galleries and vaults.
- Gas generation and transport phases, and in some programmes transport of radioactive gases such as ^{14}C.

Repository-generated gases may interact chemically with the environment at their place of origin (e.g., carbonation of cement backfill by CO_2 or precipitation of H_2S as mackinawite or pyrite). Chemical sinks are assumed to completely consume the respective gas species since mass balance calculations have shown (Nagra, 2016) the availability of the required quantities of reactants, reactions to be thermodynamically favourable and not limited by kinetics considering the timeframe of assessment.

Repository-generated gases may also be consumed by microorganisms in the backfilled operational tunnels, where conditions are suitable for microbial activity (i.e., larger pores, higher water activity and circumneutral pH). A reduction of the H_2 gas pressure will likely be mediated by sulphate-reducing bacteria, which reduce sulphate in the porewater to HS^-. These conclusions are supported by observations from natural analogues. For instance, evidence from nature (e.g. ocean sediments, subglacial environments, deep mines) shows that microbial activity in the subsurface can take place over long periods of time (hundreds to thousands of years (e.g. Hoehler and Jørgensen 2013) and evidence from engineered underground structures (e.g. town gas underground storage systems) shows that microbial activity can occur at a large scale (millions of cubic metres of gas converted).
Moreover, studies from the underground rock laboratory at Mont Terri show that bacteria found in borehole water are efficient at oxidising hydrogen as long as sulphate is present in the borehole water.

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2.3.2 ^{14}C Origin, relevance and uncertainties

In many radioactive waste disposal programmes ^{14}C is an important radionuclide with a potential for significant dose contributions in safety assessments for a geological repository. In nuclear reactors, ^{14}C is mainly formed by neutron capture from ^{14}N, ^{17}O or ^{13}C. Little is known, however, on the chemical form of ^{14}C after release from the waste and the migration of the potential carrier compounds. This gives rise to a considerable uncertainty regarding the fate of ^{14}C and a conservative treatment in safety assessment. The major source of ^{14}C in a repository for low- and intermediate-level waste is activated steel. Others sources include graphite and organics. Under CASTWP2 (Mibus et al., 2018) a series of leaching experiments using samples of activated stainless steels, mild steel and some other, non-activated materials have been undertaken. Most experiments applied alkaline, anaerobic conditions but some tests involved neutral and acidic pH values as well as aerobic conditions. Different experiments provided different but complementary information on the speciation of ^{14}C or ^{12}C releases from steels on leaching, depending on the analytical approaches applied. In general, a fast-initial release of both ^{12}C and ^{14}C to the gas and solution phase is observed after immersion of steel samples into the leaching solution with the majority of the release being to solution. Later, within a few weeks, the release rate decreased significantly. As a first approximation, this release rate seems to correspond to the corrosion rate of steel, which is, however, subject to rapid changes in the early stage of the experiment. The measured speciation of ^{14}C comprises both organic, principally carboxylate species, and inorganic (carbonate) compounds in solution. Hydrocarbons, principally methane, and minor contributions of CO were found in the gas phase. No systematic differences in the behaviour of ^{12}C and ^{14}C were observed, although no rigorous comparison of the behaviour of bulk C and ^{14}C could be made. The ^{14}C inventories in selected samples have been estimated based on specified N contents and irradiation histories using different neutron flux and activation models. The measured activities were found to be higher than those calculated within an uncertainty factor of 2.4–4.6.

2.3.3 Gas generation, pressure build up and consumption

Gas generation

The gases generated by chemical or microbial processes (e.g. anoxic corrosion, degradation of organics) may be chemically or biologically consumed in the repository near-field, depending upon repository design and geological setting, see Figure 2-2. Taking into account the chemical environment in which these gases are released (high pH from the cementitious backfill in the L/ILW repository) and the solubility of gases in water, some gases will be present predominantly in the gas phase, while others will be largely dissolved. The solubility of gases is defined by Henry's law constant (K_H), which is the ratio of the equilibrium concentration of the dissolved gas to a given partial pressure of the gas. Gases such as CO_2, NH_3 and H_2S are readily soluble in water.

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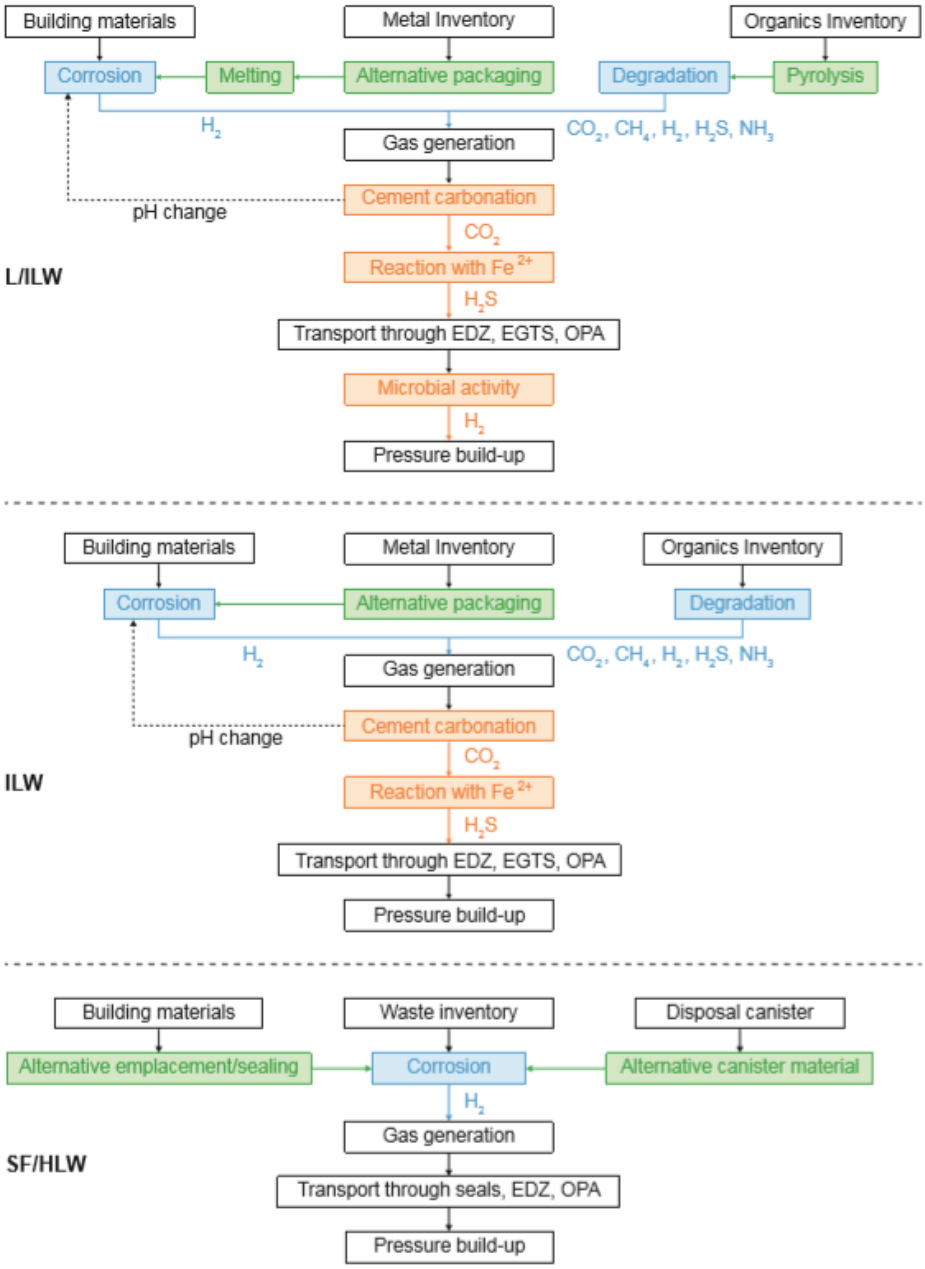

Figure 2-2: Schematic illustration of the expected sequence of processes and options related to gas produced in an L/ILW repository (top), and in the ILW (middle) and SF/HLW (bottom) parts of an HLW repository. Blue: processes leading to gas generation and the gas species generated. Orange: processes leading to gas consumption and the gas species consumed. Green: options leading to decrease of gas volumes produced. The generation of gas due to radiolysis of water is not addressed since the amount of gas produced is expected to be significantly lower than that produced by other processes. (Nagra, 2016).

Because of the high-pH porewater of the cementitious environment in a L/ILW repository, CO_2 and H_2S will be quickly deprotonated (HCO_3^- / CO_3^{2-} or HS^-/S^{2-}). The dissolved gases may react either with other minerals or may be consumed by microorganisms. An example of the interaction with other compounds is the carbonation of cement, in which dissolved CO_2 reacts to form calcite. NH_3 also dissolves easily in water, but given the alkaline environment, NH_3 will not be protonated to NH_4^+. Other gases such as CH_4 and H_2 dissolve less well in water and do not react with water upon dissolution and, therefore, are considered to contribute to the gas pressure build-up. (See Figure 2-3).

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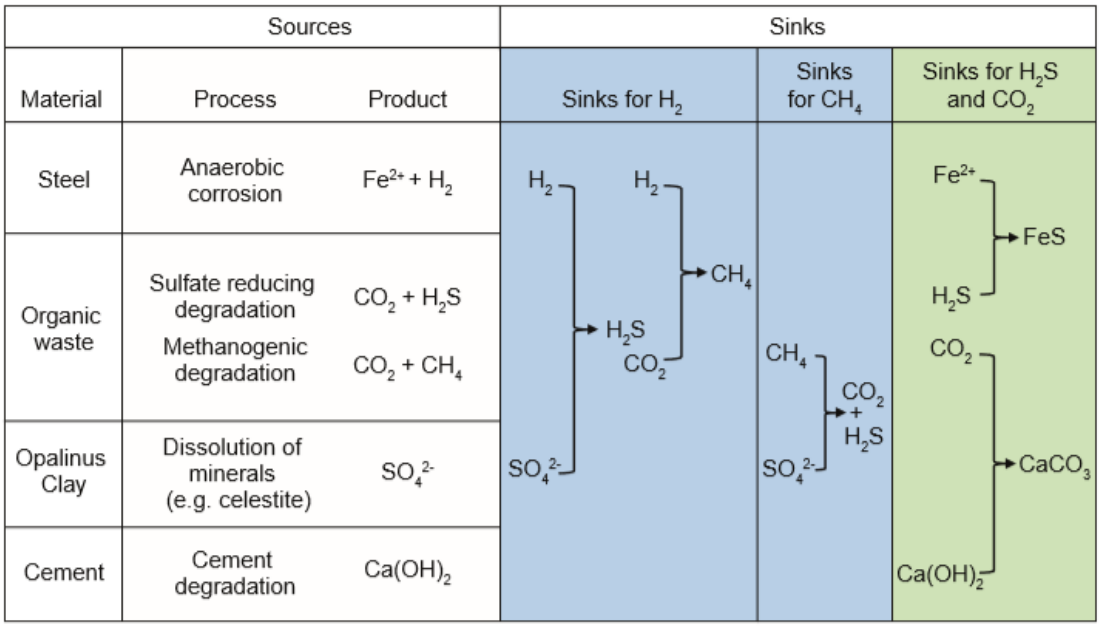

Figure 2-3: The main gas sources and sinks in a repository. The gas sources originate both from chemical and microbial reactions. Sinks in blue are microbial sinks, while sinks in green are chemical sinks. Acetogenesis as sink from H_2 and CO_2 is not mentioned because it is thermodynamically not favourable.

Production of H_2

Two chemical processes that produce H_2 gas can be distinguished: the anoxic corrosion of metals and the radiolysis of water. Depending on the specific inventory of the metallic compounds the gas production rate and gas amount might look different: e.g. Zn and Al corrode faster than Fe under repository conditions but usually Fe might be present in higher amounts in the inventory.

$$Me + xH_2O \rightarrow Me(OH) + xH_2$$

The radiolytic production of H_2 from water has been demonstrated in laboratory and field experiments (Sauvage et al. 2014). The gas production is related linearly to the applied radiation dose over four orders of magnitude. Due to the very low radioactivity level in L/ILW and in accordance with international practice (e.g. Rodwell & Norris 2003), the gas generation from the radiolysis of water is not typically considered to contribute significantly to H_2 gas production in a L/ILW repository, depending upon the inventory and water availability.

In addition to the chemical reactions there are a number of biochemical pathways which may lead to the production of H_2 through the fermentation of monomers. In particular, different types of fermentations, which proceed under O_2-limiting conditions, may occur in a L/ILW repository. To maintain the redox balance, fermenting microorganisms usually excrete reduced species such as acetate, ethanol, lactate or butanol. In addition, fermenting microorganisms may also produce H_2, a process that is catalysed by the enzyme hydrogenase. It should be noted that H_2 will usually only accumulate temporarily and will react eventually with oxidised species such as SO_4^{2-} (a process catalysed by sulphate reducers) or CO_2 (a process catalysed by methanogens, the latter occurs when sulphate reducers are not very active, i.e. at low sulphate levels, or acetogens).

Production of CO_2

As shown in the report by Poller et al. (2016) for the Nagra inventory and repository design, both carbon dioxide and methane resulting from the degradation of various organic compounds are produced in roughly similar quantities. From a microbiological point of view, CO_2 and CH_4 will be the final products of the oxidation, fermentation (and subsequent methanogenesis) of organic compounds present in the L/ILW inventory. Theoretically polymers and other high molecular weight organic molecules such as polystyrene, PVC, bitumen and cellulose (Figure 2-3) might be first degraded to monomers (or stripped off e.g. in the case of cellulose). The consecutive degradation of monomers by microbial activity might lead to the production of CO_2, H_2 and/or CH_4. Theoretically this oxidation is coupled to the reduction of oxidants in the order of decreasing reduction potential of the oxidant, starting from molecular oxygen, followed consecutively by nitrate, iron, manganese and sulphate.

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The L/ILW near-field in Nagra's repository concept mainly consists of cementitious materials and microbial activity is expected to be suppressed as a result of the high pH. Nevertheless, the degradation of organic material (e.g. after hydrolysis of cellulose) is conservatively (in the perspective of safety case) assumed to be microbially mediated. This assumption can be justified by considering the heterogeneity of the waste drums and the resulting expected variability of the pH inside the waste containers. Some waste containers will contain mainly metal waste in a cementitious matrix, while others will contain resin (polystyrene) and still other waste packages will be filled with bitumen. Since the influx of surrounding porewater into the waste container will depend on the degree of water saturation, the environment inside some waste containers may temporarily sustain microbial life, which will degrade the organic waste to CO_2 and CH_4. Small et al. (2008) reported the results of a large-scale gas generation experiment at the L/ILW repository in Olkiluoto (Finland) where gas generation from the waste drums was investigated. The rate and composition of the gas generated, as well as the aqueous geochemistry and microbial populations present at various locations within the experiment, were monitored over a period of nine years. Small et al. (2008) found considerable heterogeneity within the experiment: the pH varied from 5.5 to 10 between organic-rich waste and water equilibrated with concrete. The authors concluded that the observed heterogeneity resulted from competing anaerobic processes occurring together in the experiment but within different geochemical niches: microbial activity which was initially dominant in organic waste reduced the alkalinity of the concrete over time[1].

Small et al. (2008) modelled the results of this experiment with a biogeochemical reaction transport code (GRM). Microbial processes included in GRM are discussed in Humphreys et al. (1997). The model represented the main anaerobic microbial processes leading to methanogenesis and the observed spatial and temporal variations in aqueous and gaseous species. In order to model the experiment, its heterogeneity was considered such that individual waste containers were represented, and assumptions were made concerning transport rates of chemical species. Cellulose waste was oxidised by microbial activity, using for example sulphate as an oxidant, leading to CO_2 production. When no other oxidant (e.g. oxygen, nitrate, iron or sulphate) is present, CO_2 can be converted into CH_4 by methanogenesis.

Production of CH_4

Methane results from the degradation of various organic compounds. After depletion of oxidants with a higher energy yield such as O_2, NO_3^- , Fe^{3+} and SO_4^{2-}, the only oxidant remaining for the degradation of organics will be CO_2 and, therefore, ultimately CH_4 might be produced. Methanogenesis is a complex microbiological process. The initial steps are catalysed by fermenting microorganisms and this usually leads to an intermediate accumulation of CO_2, H_2 and acetate. These intermediates are subsequently converted to CH_4 by hydrogenotrophic methanoarchaea $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ and by acetotrophic methanoarchaea $CH_3COOH \rightarrow CH_4 + CO_2$. In the case of hydrogenotrophic methanogenesis, 5 moles of gas are converted into 1 mol of CH_4, whereas in the case of acetotrophic methanogenesis 2 moles of gas are produced from 1 mol of acetate.

Production of NH_3

The production rate for ammonia can be substantial and mainly results from the degradation of urea which is present in different waste types. Ammonia is however highly soluble in porewater and, therefore, will not contribute significantly to the overall gas pressure build-up.

Gas sinks in a L/ILW repository

The gases generated by chemical or microbial processes (e.g. anoxic corrosion, degradation of organics) may be chemically or biochemically consumed in the repository near-field. Taking into account the chemical environment in which these gases are released (e.g. high pH from the cementitious backfill in the L/ILW repository) and the solubility of gases in water, some gases will be present predominantly in the gas phase, while others will be largely dissolved. The solubility of gases is defined by Henry's law constant (K_H), which is the ratio of the equilibrium concentration of the dissolved gas to a given partial pressure of the gas. CO_2, NH_3 and H_2S dissolve readily in water. In a cementitious environment (such as in the Nagra reference concept) and the resulting high-pH porewater, CO_2 and H_2S will be quickly deprotonated (HCO_3^-/CO_3^{2-} or HS^-/S^{2-}). The dissolved gases may react either with other minerals or may be consumed by microorganisms. An example of the

[1] Compared to Swiss concept for L/ILW waste disposal the nearfield simulated in these experiments were buffered with only small amounts of concrete.

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interaction with other compounds is the carbonation of cement, with which dissolved CO_2 reacts to form calcite. NH_3 also dissolves easily in water, but given the alkaline environment, NH_3 will not be protonated to NH_4^+. Other gases such as CH_4 and H_2 dissolve less well in water and do not react with water upon dissolution and, therefore, are considered to contribute to the gas pressure build-up. In this section, the chemical and biochemical consumption (or sinks) of the three gases present in the largest volumes in a L/ILW repository (CO_2, CH_4 and H_2) is described.

The importance of waste heterogeneity on the fate of gases

Depending on the heterogeneity of the materials in a waste container, microbially favourable conditions may be present locally in some locations. These conditions will allow organic materials to be degraded by microorganisms to produce CO_2 and/or CH_4. Because of the large quantities of cementitious backfill surrounding the waste containers, it is, however, assumed that microbial activity will not be significant in the cementitious backfill of a L/ILW repository. Possible microbial sinks are, therefore, not considered to occur in the cementitious backfill, but instead in the backfilled operations tunnels.

Thermodynamic boundary conditions

Under standard conditions, the energy yields ($\Delta G°'$) of most anaerobic microbial reactions (e.g. litho or organotrophic reactions or fermentations) are negative, i.e. the reactions are exergonic, they release energy and are spontaneous. However, within the network of microbiologically catalysed processes, occasionally there are also endergonic steps or conversions with a very low energy yield.

Kinetics of microbial conversion

From a microbiological point of view, the basic modelling concept of the enzymatic conversion of a substrate is the Michaelis-Menten equation. The basic modelling concept of the growth of a microbial culture is the Monod equation. There is a huge body of literature on the applications and extensions of these equations, particularly with regard to complex environmental conditions, potential inhibitors and multi-substrate turnovers (Maragoni 2013).

In a L/ILW repository, the rates of gas production and consumption will probably not be dominated by microbiological kinetic constants (such as those in the above-mentioned modelling equations) but rather by the spatial characteristics, i.e. the diffusive and advective transport of the reactants. Major sources and sinks of reactants are spatially separated and transport becomes a major issue and the transport rates will dominate the turnover rates. In this context, the transfer of H_2 from the producer to the consumer may serve as an illustration. This transfer is important from a thermodynamic point of view and a fast transfer will speed up the overall reaction. Short spatial distances between producers and consumers are favourable and it has been observed that many H_2 consumers (e.g. methanogens) are tightly associated with H_2 producers (e.g. fermenters).

The organic waste in a L/ILW repository consists of polymers, which have to be converted to monomers before they can serve as an energy and carbon source for microorganisms. However, there is little information on the rate of this conversion and, therefore, the steady-state concentration of the readily bioavailable intermediates such as monomeric sugars and short chain fatty acids remains largely unknown (Warthmann et al. 2013). It cannot be excluded that these steady-state concentrations will be extremely low. This begs the question whether there is a lower concentration limit to sustain microbial life. It is unlikely that such a limit will be reached in a L/ILW repository. Studies in natural (open) systems (particularly marine sediments in oligotrophic ocean basins) revealed that microbial life can be sustainable over millions of years in systems where generation times of thousands of years can be expected (Hoehler and Jorgensen 2013).

Consumption of H_2

Hydrogen produced during the anoxic corrosion of metals may be used by the microbial populations present in the backfilled operations tunnels. In these tunnels, a more favourable combination of pore space, water activity and nutrients may be available (compared to the cement-filled near-field) to enable the microbial oxidation of hydrogen gas. In the section below are listed some of the microbiological sinks for H_2 that could be present in a L/ILW repository. Which of these processes will take place *in situ* will depend on the availability of electron acceptors and rates may be transport-dependent. The thermodynamic sequence will dictate the sequence of oxidation-reduction reactions taking place.

Bacterial chemolithotrophic oxidation of H_2 using O_2 will most probably not take place in a L/ILW repository. According to the expected repository evolution, the oxic phase will last only for a short time after repository

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closure, during which H_2 production will still be minimal. This type of bacterially catalysed process will thus not play a role in a L/ILW repository.

Methanogenesis and acetogenesis

During most of the repository evolution, anoxic and reducing conditions will dominate and, therefore, anaerobic microorganisms will constitute the bulk of the microbial population. There are two types of strictly anaerobic microorganisms that both use CO_2 as an electron acceptor and H_2 as an electron donor in their energy metabolism. On the one hand there is methanogens (CO_2 converted to CH_4) and on the other hand there is acetogens (CO_2 converted to acetate). Methanogens rely on the reductive acetyl-coA pathway for acetate-dependent energy generation. But from H_2 and CO_2, acetyl-coA pathway is used only for carbon fixation. Although both methanogenesis and acetogenesis consume H_2, the mass balance of the reactions should be carefully considered, since methanogenesis also produces gas (CH_4).

Methanogenesis is a complex microbial process that is carried out by a group of archaea termed methanogens. Two possible mechanisms for methanogenesis are possible. Either methane is produced from CO_2 and H_2, or methane is produced from acetate. The former mechanism is carried out by hydrogenotrophic Archaea and the latter by acetotrophic Archaea. The latter mechanism is not identical to acetogenesis, in which acetate is produced. Hydrogenotrophic methanogenesis also occurs in environments where other compounds such as formate, CO or even alcohols can supply the electrons for the reduction of CO_2. Hydrogenotrophic and acetotrophic methanogenesis processes were both observed to be the main mechanisms taking place in the underground town gas storage facility in Lobodice, leading to the loss of H_2 and an increase in CH_4 concentration.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

There is evidence for growth of methanogens at the Mont Terri URL in the MA-A experiment.

Acetogenesis is a process in which acetate is produced from 2 mols CO_2 and 4 mols H_2. Compared to methanogenesis, this reaction releases slightly less free energy ($\Delta G^{0'}$= -105 kJ/mol for acetogenesis; -131 kJ/mol for methanogenesis). The overall mass balance indicates a gas pressure reduction owing to acetate production.

$$4\ H_2 + H^+ + 2\ HCO_3^- \rightarrow CH_3COO^- + 4\ H_2O$$

Sulphate-reducing bacteria

Sulphate is an important electron acceptor in anaerobic respiration and sulphate-reducing micro-organisms are widespread in nature. They can use many different electron donors for the reduction of sulphate: hydrogen gas, lactate, acetate, propionate, pyruvate, etc. Sulphate-reducing bacteria can use hydrogen as an electron donor, whereas other electron donors are reported to be specific to limited number of species. The energy released by the following reaction is -152 kJ.

$$4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$$

The presence of such sulphate-reducing bacteria has been examined and proven in the MA and HT experiments at Mont Terri URL.

Consumption of CO_2

The major chemical sink for CO_2 is the abiotic carbonation of the cement used in the repository.

The major microbial sinks for CO_2 are methanogenesis and autotrophic carbon fixation. Methanogenesis was described previously in the section on hydrogen sinks; it forms methane from CO_2 and H_2. Autotrophic carbon fixation is a process in which CO_2 is reduced to make biomass instead of being used in energy metabolism. This process is essential for phototrophic microorganisms (that use light for their energy metabolism, which is not applicable in a L/ILW repository) and chemolithotrophic microorganisms.

Consumption of CH_4

In the older literature it was claimed that CH_4 can only be metabolised in the presence of molecular oxygen. The microorganisms and enzymes which catalysed the initial step (turnover of CH_4 to methanol) were recently

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characterised in detail. Two types of enzymes were found: a soluble methane monooxygenase (sMMO) and a particulate methane monooxygenase (pMMO) (Murrel and Smith 2010; Nazaries et al. 2013).

In the late 1980s there was increasing evidence that O_2 is not an essential co-substrate for the oxidation of CH_4. Particularly in marine sediments it was found that microbial CH_4 oxidation is also possible using SO_4^{2-} as a co-substrate (Alperin and Reeburgh 1985). The biochemistry of the reaction remained enigmatic and was only elucidated about 10 years ago (Thauer and Shima 2006, Thauer and Shima 2008 and Thauer et al. 2008). It was demonstrated that the last step in methanogenesis (CoM-S-CH_3 -> CH_4), which is catalysed by the methyl-coenzyme M reductase, is reversible. Later on it was demonstrated that not only O_2 and SO_4^{2-}but also NO_3^- can serve as an oxidant for CH_4 oxidation. From a thermodynamic point of view, CH_4 should also be oxidisable with Fe(III) and Mn(VI) as oxidants. Indeed, there is unequivocal biogeochemical evidence that these processes take place (Riedinger et al. 2014, Sivan et al. 2011, 2014, Beal et al. 2009, Egger et al. 2015).

2.3.4 Achievements within MIND

Corrosion and radiolysis and related formation of hydrogen gas

MIND delivered further evidence for H_2 gas oxidation, thus for a drop in associated gas pressures. The main question remains of course related to the sustainability of the catalysing microbial community.

In the GGE Experiment Vikman et al. (2019) reports that the gas produced consists of 80 - 90 % of methane. H_2 seems to be readily consumed by microbes. Methane seems to be partly produced by the utilization of acetate by methanogens. The main production of methane seems to be dominated by hydrogenotrophic methanogens converting H_2 + H_2O directly to methane. Vikman concludes based on the evolution of the relative ratio of SRB and methanogens is moving towards more methanogens. Biogeochemical processes in the GGE have been modelled using the Generalised Repository Model (GRM) by Small et al. (2017) and are consistent with the microbiological results (DNA pyrosequencing and qPCR). Vikman points out that heterogenic conditions in the waste and drums are ideal for creating microbial niches and activity.

Reduction of nitrate, carbonate and to some extent sulphate to form gases

The presence of H_2 gas, regardless of the type of electron acceptor, accelerates microbial growth (if viable) and catalysis; all the related redox reactions will lower the gas pressure.

In the Microbial Activity (MA) experiment in the Mont Terri underground rock laboratory, estimates of the hydrogen consumption rate were made (for more details on the MA experiment, see section 4.6). Bagnoud et al. (2016b) calculated *in situ* rates of microbial hydrogen oxidation and sulphate reduction based on field and laboratory experiments. More specifically, rates were calculated from two distinct in situ experiments (MA and Hydrogen Transfer (HT) experiments), using several measurement and calculation methods. Volumetric consumption rates were about 1.48 $\mu mol \cdot cm^{-3} \cdot day^{-1}$ for H_2, and 0.17 $\mu mol \cdot cm^{-3} \cdot day^{-1}$ for sulphate. From the calculations in this study it appeared that the sulphate:hydrogen stoichiometry was closer to 1:8.5 than to the theoretical value 1:4. This result suggested that, in Opalinus Clay, electrons derived from hydrogen oxidation not only reduce sulphate, but also carbon dioxide, via carbon fixation pathways, and probably also iron(III), through alternate respiratory processes (Bagnoud et al., 2016). This ratio between H_2 and sulphate consumed is comparable to the one reported by Vinsot et al. (2014) in the HT experiment at the Mont Terri URL in which H_2 consumption in Opalinus Clay also was monitored. In that study, it was estimated that 6 H_2 molecules were oxidised per molecule of sulphate reduced. Unfortunately, Vinsot et al. (2014) gave no information on the volumetric rates of H_2 and sulphate consumption in the porewater. It should be kept in mind that the rates calculated from the MA and HT experiments are optimum rates obtained in an aqueous environment. In a L/ILW repository, rates will likely be lower in the sand/bentonite backfill because of transport being limited by molecular diffusion.

Recent results from the underground rock laboratory of Mont Terri evidenced the proliferation of methanogens in porous medium composed of bentonite and sand and exposed to H_2 but in which sulphate was consumed. This result suggests that, should sulphate diffusion be limiting, methanogenesis, the next most thermodynamically favourable metabolism, will take place.

A compilation of natural systems with a turn-over of CH_4 and H_2 can be found in Leupin et al. (2016). These systems have persisted over thousands and even millions of years. Hoehler and Jorgensen (2013) compared the life phases in batch cultures with the microbial life in ocean sediments and found striking differences. A batch culture has doubling times in the range of hours and days and the microbial activities may extend up to weeks or

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a few years. In contrast, natural populations in ocean sediments have doubling times of several hundreds or thousands of years and the activities extend up to millions of years. Comparing these systems, Hoehler and Jorgensen (2013) also calculated the specific carbon turnover rates (expressed as moles of carbon assimilated per mole of cell carbon). It was found that even turnover rates in the range of 10^{-5}/day provided enough energy to sustain microbial life.

Subsurface ecosystems of interest are: (i) ocean sediments in oligotrophic and highly productive zones; (ii) ocean sediments in the vicinity of methane hydrates; (iii) ocean floors above zones where serpentinization is ongoing; iv) subglacial zones and (v) microbial communities in deep mining zones. In all these natural settings microbial activity has shown to last for millions of years at very low turnover rates.

Concluding remark

A summary of MIND contributions related to gases in provided in Table 2-1. Gases that are chemically or biologically generated in a geological repository for radioactive waste may interact chemically with the environment at their place of origin (e.g. carbonation of cement backfill by CO_2 or precipitation of H_2S as mackinawite or pyrite) depending on the repository concept. Chemical sinks (e.g. carbonation) are assumed to completely consume the respective gas species since mass balance calculations have shown the availability of the required quantities of reactants, reactions to be thermodynamically favourable and not limited by kinetics considering the timeframe of assessment.

Experimental results show that repository-generated gases may also be consumed by microorganisms, where conditions are suitable for microbial activity (i.e. larger pores, higher water activity and circumneutral pH). In a sand bentonite environment that is saturated with Opalinus porewater the reduction of the corrosion generated H_2 gas pressure will likely be mediated by sulphate-reducing bacteria, which reduce sulphate in the porewater to HS^-. *In situ* experiments show that the formed HS^- precipitates with Fe^{2+} present in the porewater.

These conclusions are also supported by observations from natural analogues. For instance, evidence from nature (e.g. ocean sediments, subglacial environments, deep mines) shows that microbial activity in the subsurface can take place over long periods of time (hundreds to thousands of years) and evidence from engineered underground structures (e.g. town gas underground storage systems) shows that microbial activity can occur at a large scale (millions of cubic metres of gas converted).

Degradation of polymers present in the (mainly ILW) waste may also contribute to the overall gas pressure build. However, this contribution is much less than the corrosion generated gases. Dixon. (2018) conclude that overall, PVC polymer is not biodegradable under alkaline (pH 10) conditions, but some additives present in PVC sheet are biodegradable. The rather limited overall biodegradation of such PVC materials suggests that these organic materials should not provide a significant source of microbial (methane) gas generation. An important next step is to assess which additives, or combinations of additives, are ultimately fuelling nitrate reduction, what effect these additives have on radionuclide mobility, whether they can fuel gas generation processes, and therefore constrain their overall impact on the safety case for nuclear waste disposal.

Table 2-1 Summary of MIND contributions related to gases

Substances under investigation	Process	Synthesis of results obtained in the MIND project and affiliated scientific work	General discussion
steel / organic matter	corrosion and radiolysis and related formation of hydrogen gas	MIND delivered further evidence for H_2 gas oxidation, thus for a drop in associated gas pressures. The main question remains of course related to the sustainability of the catalysing microbial community (cf. subject 2.6	Evidence suggests that H_2 pressure will be limited, although net gas pressures will ultimately be influenced by generation rates. In all repository systems niches should be present (low pH, sufficient space and water, etc.; where microbial life will prevail. However, asserting their sustainability over very long periods is difficult to do; which is why situations of increased gas pressure will have to be analysed.
other gas generating substances	reduction of nitrate, carbonate and to some extend sulphate to form gases	The presence of H_2 gas, regardless of the type of electron acceptor, accelerates microbial growth (if viable) and catalysis; all the related redox reactions will lower the gas pressure.	

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Substances under investigation	Process	Synthesis of results obtained in the MIND project and affiliated scientific work	General discussion
other gas generating substances	PVC degradation	The results by Nixon et al (2018) (D1.2) indicate that additives released from PVC sheet in Ca(OH)2 solution are biodegradable at pH 10. Additional microcosm experiments examining either TPP or phtalate ester as a carbon source indicate that these compounds are not responsible for the nitrate reduction (D1.2; Nixon et al, 2018) so the exact nature of the additives that fuel the observed nitrate reduction is not known. In the case of irradiated sheet (at maximum dose levels likely for ILW) it is possible that the additives become bonded to the PVC polymer and are less easily leached. In contrast, irradiation of the pure PVC powder liberates some DOC that can be utilised as an electron donor and carbon source for nitrate reduction.	

2.3.5 What remains to be resolved of common interest to WMO:s

Transport of H_2S to the canister under unsaturated conditions needs to be evaluated, particularly in disposal concepts using copper waste containers. Sulphide transport through the buffer towards the canister depends on several processes (e.g. dissolution and precipitation of minerals, microbial activity) and parameters (e.g. backfilling conditions, sulphate mineral inventory, and diffusion coefficient). Furthermore, under unsaturated conditions it must be assumed that non-isothermal conditions prevail. However, depending on the repository layout and the host-rock transport properties these conditions won't last for the whole period of assessment: if the transport properties are slow then building up a critical concentration gradient of HS^- will be slow as well. Assuming that HS^- will be produced in the EDZ then the resaturation of the nearfield and the closing of the EDZ will further limit the period of HS^- production. These aspects have to be carefully assessed for calculating the corrosion allowance considering microbially affected corrosion.

2.4 Microbially induced organic waste matrix degradation - main factors of influence and major principles

2.4.1 Introduction of issue

Intermediate Level and Low Level Wastes (ILW/LLW) are typically heterogeneous mixtures of radioactively contaminated materials, including polymeric substances. ILW and, in some cases, LLW requires geological disposal, where physical and chemical containment will ensure passive safety. The organics present in the radioactive waste inventory can originate either from the waste itself or from the immobilisation matrix.

- 'Waste organics': This group is very heterogeneous because a wide range of materials are used in the nuclear sector. The 'waste' organics, which are generally found in the highest quantities, are: cellulose (filters & flat cleaning sponges), polystyrene (ion-exchange resins), polyvinylchloride (gloves & protective clothing) and polyethylene (packing material). But also, fluoride polymers, polycarbonates, polyurethane, polyamide, acrylates and many more are known to be present.

- Immobilisation matrix: Organics are found in organic as well as inorganic immobilisation matrices. The most important organic immobilisation matrices are bitumen and polymers (for example, polystyrene). With respect to inorganic matrices, cement is most commonly used. Additives, often organic in nature, are added to this matrix in order adapt certain parameters of the cement, for example superplasticisers can reduce the water-cement ratio.

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The presence of organics in wastes needs particular consideration if they are soluble and able to bind radionuclides, for example via the formation of complexes, increasing radionuclide transport through the different barriers of a repository (e.g. OECD-NEA 1985). These soluble organics are either directly present in the waste ('as disposed') such as EDTA, or produced as a result of degradation processes such as isosaccharinic acid (ISA) (Humphreys *et al*. 2010).

The degradation of organics under repository conditions is a complex process of chemical, radiological and, if conditions allow sufficient activity, microbial degradation. There remains significant uncertainty surrounding organic degradation pathways and products of different materials, such as ion exchange resins, halogenated plastics and bitumen. The degradation of the organics can produce soluble products and gases such as H_2, CO_2, CO and CH_4 which are discussed in §2.3. For these two aspects (production of soluble, particularly complexing agents, and gas) it is extremely important to know the degradation rates to inform total system models and environmental safety cases.

Work Package 1 of the MIND Project provides a review of anthropogenic organic wastes and their degradation behaviour (Abrahamsen *et al*. 2015). Specifically, the review evaluates inventories for Belgium, Czech Republic, Finland, France, Spain, Sweden, Switzerland, The Netherlands and the UK. On the basis of these inventories, the following materials were put forward for evaluation in MIND:

- Halogenated plastics (e.g. PVC)
- Bitumen
- Organic ion exchange resins

Cellulose containing materials have been previously and extensively discussed in the literature so are not included in Abrahamsen *et al*. (2015) but were included for investigation by research teams in MIND. A summary of the key processes and principles for these materials is provided as follows:

Cellulose degradation

Cellulose is a relatively common material in the context of low and intermediate level radioactive repositories, due to its presence in everyday materials such as tissues, cloth and paper. It is a polysaccharide with a linear condensation polymeric chain with the formula $(C_6H_{10}O_5)n$. It therefore consists of glucose units (D-anhydroglucopyranose units) connected through β-1,4-glycosidic bonds and is also known as β-1,4-D-glucan (polyglucose) (Wertz et al. 2010).

Under alkaline, anaerobic conditions, isosaccharinate (ISA) is the main cellulose degradation product formed (Blears et al. 1957, Whistler and BeMiller 1958, Glaus and van Loon 2008). ISA is a general term for 3-deoxy-2-C-(hydroxymethyl)-D-aldonic acids, which has both α and β-diastereoisomers. α-ISA is a strong complexing agent and can bind to radionuclides thus altering their transport properties. If α-ISA is not broken down, either abiotically or biotically, sufficient α-ISA concentrations could arise in the waste with potentially negative consequences on radionuclide immobility.

α-ISA interacts strongly with certain radionuclides in a cementitious environment and reduces their sorption on the cement matrix. The hydrolysis degradation of cellulose is not completely understood at the time of writing, but is known to be governed by several reaction processes: 'peeling', 'stopping' and 'mid-chain scissioning' (Glaus et al., 2008; Humphreys et al., 2010). This is schematically shown in Figure 2-4. In addition to ISA, a large number of small chain aliphatic acids have been detected: formic, acetic, glycolic, pyruvic, for example. Each of these smaller organics contributes very little to the total mass of cellulose degradation products.

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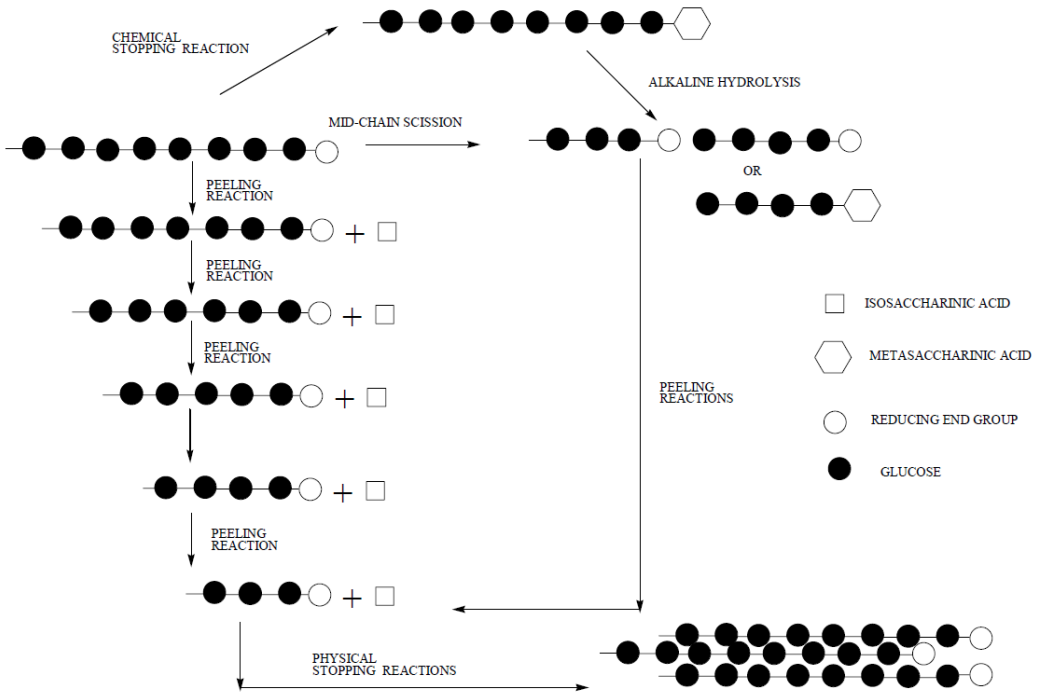

Figure 2-4 Model of the alkaline hydrolysis of cellulose (Humphreys, 2010)

α-ISA has been identified as a stronger complexant than degradation products derived from ion exchange resins, halogenated plastics and bitumen (Abrahamsen et al. 2015). Further understanding of mechanistic behaviour in a repository environment was, however, identified as a requirement of the project. Irradiation and biodegradation studies were subsequently undertaken to explore cellulose and ISA degradation (see Table 2.2).

Very limited studies had previously been performed on the biodegradation of ISA. Bassil *et al.* (2015a) was able to observe that at pH 10, aerobic and nitrate reducing bacteria are able to oxidise ISA. The possibility of ISA-degradation at high pH is also acknowledged by Humpreys *et al.* (2010) based on ISA degradation in high pH natural environments.

PVC degradation

PVC will be subjected to radiolytic and hydrolytic degradation processes. Hydrolytic degradation is increased in alkaline solutions with respect to neutral solutions. The radiolytic degradation is a very complex process in which total dose, dose rate and atmosphere play an important role as well as the potential of water to diffuse into the PVC matrix. There is a general paucity of pre-existing data exploring the effects of combined chemical and radiolytic stressors such as would be present in a repository (NDA 2013). In experiments it was observed that the degradation products were mostly coming from the additives added to the PVC-structure in the commercial products rather than from the 'PVC-backbone' (Colombani et al. 2007; Dawson, 2013). Because the additives aren't covalently bound with the PVC-matrix, they can also leach out more easily. Some of the identified organics are ketones, carboxylates, triphenyl phosphate and 2-ethylhexyl diphenyl phosphate and smaller hydrocarbons (less than five carbon atoms). Not all of the organic degradation products can be identified and a large fraction therefore stays unknown. In an experiment with extremely high PVC-cement ratio (2 g PVC/ 0.2 g cement), the degradation products of commercial PVC increased the solubility of certain radionuclides such as plutonium (Baston et al. 2017) and can form complexes with uranium and other radionuclides (Vazquez et al. 2008; 2009). This effect could not be attributed to the phthalate plasticisers or their degradation products, however.

To understand the potential for biodegradation of PVC additives in repository environments, microbial enrichment experiments were conducted by Nixon et al. (2018). This work explored PVC additives (e.g. plasticisers and flame retardants) as a source of energy and carbon for nitrate reducing bacteria at a high pH.

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Bitumen

Bitumen has been used in various countries as an immobilisation matrix such as Belgium, France and Sweden. The main radiolysis gas product is H_2, produced as a consequence of de-hydrogenation of cycloalkanes and related aromatisation. In presence of O_2, other gases produced include H_2O_2, N_2, CH_4, small hydrocarbons, CO and CO_2. In absence of O_2, these are largely the same with the exception of the absence of CO and CO_2 and the presence of higher quantities of the small hydrocarbons. The identified degradation products (alkaline hydrolysis and radiolytic) are (Walczak *et al*. 2001; Van Loon and Kopajtic, 1990; Kagawa et al., 2000):

- carboxylic acids, with usually higher concentrations of formic acid and acetic acid, and lower concentrations of longer (mono and poly) carboxylic acids (propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, …, until ~C18);
- linear alcohols;
- glycols;
- linear carbonyls;
- aromatic compounds such as (alkyl substituted) naphthalenes, substituted phenols or anilines;
- S-containing and N-containing compounds

Further work was found to be required to understand the behaviour and fate of components of bitumen leachate, released from wastes in a repository. Recent in situ studies at the Mont Terri underground laboratory in Switzerland, as part of the BN project, examined the reactions of nitrate with the Opalinus Clay together with acetate as a reference bitumen degradation product (Bleyen et al. 2017).

Bitumen is biodegradable both aerobically and anaerobically (neutral pH), provided that other conditions are favourable. The biodegradation of pure bitumen is a surface phenomenon due to the insolubility of the substrate. Many studied biofilms consisting of different strains have been reported. Preferentially, the saturated and aromatic hydrocarbons are consumed (Aït-Langomazino et al., 1991). There are no indications that bitumen would be completely biodegradable under disposal conditions. In unpublished research carried out by Andra in the 1980's and 1990's significant degradation and mobilisation of mobile organic substances from bitumen was never measured. Although, it should be mentioned that it wasn't possible to perform it to the extent necessary. The main gases that are produced during biodegradation of pure bitumen are CO_2 and CH_4 (i.e. fermentation), with minor quantities of H_2S and N_2. Under anaerobic and optimal growth conditions (i.e. in which carbon is the only growth limiting factor; pH 7, T= 30°C), about 0.2 – 0.6 g pure bitumen was degraded per year and per m^3 bitumen surface (Wolf and Bachofen, 1991). This is equal to a production of 0.15 -0.60 l CO_2 per year and per m^2 bitumen surface. Because of the highly alkaline environment of a repository, CO_2 will dissolve into the water to form H_2CO_3. This will in turn react with the portlandite in the cement and increase rates of dissolution. These values are for optimal growth conditions and should be considered as maximal values. Under less optimal conditions, such as highly alkaline environments, this will be significantly lower. Additionally, in the case where bitumen is used to solidify nitrate containing waste, such as for Belgian Eurobitum, an osmosis-driven swelling will take place due to contact of water with nitrate captured in a semi-permeable membrane (bitumen). The swelling will limit the space available for microbes once it has filled all free voids. Ageing of bitumen will also probably lower microbial degradation rates because of the relative increase of the recalcitrant asphaltene fraction.

Introduction to Ion-exchange resins

Polystyrene-divinylbenzene (PS-DVB) is used as backbone in many ion-exchangers commonly found in the nuclear sector. The surface is modified with for example sulfon or amine groups to produce a cation and anion ion-exchanger respectively. PS-DVB is an addition polymer and as such insensitive to OH^- (Van Loon & Hummel, 1995). Although PS-DVB has a high radiation resistance due to its aromatic nature, but less so than for example polyethylene, it is the more important long term degradation process than alkaline hydrolysis. Additionally, because PS-DVB is a co-polymer plasticisers are normally not present, this in contrast with PVC. The main identified radiolytic degradation products are oxalate and sulphate or amines depending on the ion-exchange resin. The concentration of the oxalate depends on the type of ion-exchanger (Van Loon & Hummel, 1995).

Whilst microbial processes may influence degradation associated with ion exchange resins, such processes are likely to be limited by the hostile conditions in a repository. Warthmann et al. (2013) concluded that there is no direct evidence that ion-exchangers would be readily biodegradable under anoxic conditions. Therefore the elements which could be used by microbes are the functional groups and radiolytic degradation products of the ion-exchange resins.

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2.4.2 Why of relevance and when

In the development of environmental safety cases, data obtained through scientific research provides a means of bounding uncertainty associated with repository processes. The greater our understanding of geo-microbial processes, the greater our confidence is in defining model parameters, thereby reducing uncertainty. This is highly relevant when considering organic waste (matrix) degradation. For example, one approach to modelling of cellulose is to assume that all cellulose degrades to ISA, which is then available to complex with radionuclides in the geosphere. This conservative approach could be more accurately defined, if we are able to take credit for among others, microbial ISA degradation in the near-field. For this example, reducing model uncertainty can be achieved by gathering robust evidence of cellulose degradation in repository conditions.

Internationally, there are a range of concepts, wastes, and host geologies, which, combined with programmes at different stages of development, makes it challenging to align research tasks. The identified themes in the gap lists developed in MIND have proven a useful mechanism of identifying shared priorities. In this section, the gap list identified a range of themes which are generically applicable to organics (wastes and/or matrices), regardless of programme specific factors. Such a collaborative platform allows us to share expertise and pool resources for common goals. As programmes develop, there is likely to be an increased need to focus upon programme specific factors, such as the behaviour of organic degradation products in specific host rocks.

2.4.3 Achievements within MIND

The following section discusses the key findings from research conducted in the MIND programme, with Table 2-2 providing a high-level summary, including discussion and parameters which may be relevant for safety case arguments.

Cellulose degradation

Cellulose materials present within a repository are expected to hydrolyse under high pH conditions, producing soluble ligands such as ISA. However, acetate producing microbial processes have been shown to cause acidification of simulated pore-waters, thereby reducing ISA production. Bassil et al (2015b) discovered that, while methanogenesis was minimal under repository conditions, other fermentation processes compete with ISA production, moderating the process. Small et al (2017) identified that, in a cellulose degradation product buffered system (from pH 10-11 to <9), methane rich gas production doubled. Under the near-steady state conditions which had established over the 18 year experiment, modelling suggested that methane production would continue until cellulose and steel materials become exhausted (Small et al. 2017).

ISA produced by chemical hydrolysis reactions in the repository may be moderated by microbial acetate production. This has positive implications for radionuclide mobility, since ISA is an important ligand which can increase radionuclide solubility. However, two notes have to be made:

1) This pH lowering affect would be present only in niches, since microbial activity is in general highly suppressed in a repository (high pH, low nutrient environment).
2) In these niches, sufficient nutrients will have to be present in order to produce enough acetate to overcome the high pH buffering capacity of the cement.

^{14}C release from activated steel is a concern, due to potential gas-phase mobility (Wieland & Hummel 2015). Non-radioactive methane production is important as ^{14}C carrier gas, although high pH environments have been shown to limit production (Bassil et al., 2015b). However, there is evidence from large scale tests suggesting that organic acids can buffer pH to <9, which can lead to increased methane production rate (Small et al., 2017). An alternative hypothesis from the study by Small et al (2017) is that the methanogenesis rate was influenced by aqueous sulphide, which also dropped during the same period.

Nixon et al. (2018) compared the effect of irradiation of cellulose prior to contact with a microbial community. H_2, acetate- and ISA-concentrations were more pronounced. Additionally a drop in pH was measured. No direct conclusions can be drawn on the effect of the microbial activity on the variation of the measured parameters.

ISA Degradation

Significant work has been undertaken to understand the production and degradation of ISA through the MIND programme and preceding work such as the BIGRAD consortia in the UK. In this programme, Bassil et al (2015a) found substantial microbial degradation of ISA occurred over short timescales (<10 days) in alkaline conditions up to pH 10-11. Nitrate reducing bacteria were identified as ISA degraders, with less or no

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degradation seen as conditions changed to iron and sulphate reducing conditions respectively (Bassil et al. 2015a). In contrast, a subsequent study at circum-neutral pH identified that ISA does degrade under iron and sulphate reducing conditions (Kuippers et al 2015). Conversion of ISA degradation products to methane was also observed, although the mechanics of these processes requires further investigation (Kuippers et al., 2015). Rout et al. (2015) first identified microbial ISA degradation up to pH 10, with methanogenic communities which had not previously encountered the substance.

ISA is very likely to be a good candidate for microbial degradation, even under mildly alkaline repository conditions. Considering its degradation to less radionuclide complexing species will allow a more realistic approach to Performance Assessment. However, significant uncertainties concerning the sustainability of microbial life in deep geological repositories, in particular in cementitious environments, may not allow explicit consideration of such a process. To cover this Bassil et al (2015a) studied the effect of cellulose and ISA degradation with $Ca(OH)_2$ samples doped with microorganisms harvested from a limestone environment. Microcosms poised at pH 12.5, and inoculated using sediments from a legacy lime-kiln, showed biodegradation of the added cellulose and fermentation of the degradation products into acetate, while halting ISA production from the abiotic alkaline cellulose hydrolysis (Bassil et al. 2015a). Irradiation enhanced the rate of the abiotic cellulose hydrolysis by alkali, and further made the cellulose fibres more available for biodegradation and fermentation of the degradation products, led to the production of H_2, which was subsequently consumed. Methanogenic microorganisms and methane have not been detected in these systems yet (Bassil et al. 2015a). Enrichment cultures prepared at pH 10 and inoculated with sediments from the same legacy lime-kiln showed that alkaliphilic bacteria degrade ISA under aerobic and anaerobic conditions (Bassil et al. 2015b). An ISA-oxidising obligate alkaliphile belonging to the genus Anaerobacillus was isolated from these cultures, and was found to be the only species of this genus capable of ISA utilisation (Bassil and Lloyd 2018). Named Anaerobacillus Isosaccharinicis sp., the newly identified bacteria which can break down ISA under anaerobic, alkaline conditions was discovered through the course of this research programme (Bassil and Lloyd 2018). It exhibits growth between pH 8.5 and 11 and between 10 and 40°C. Importantly, it can tolerate salinity up to 6% w/v NaCl. Salinity tolerance of ISA degrading bacteria is significant, since it suggests that highly mineralised 'leachate' waters produced during waste re-saturation, as well as saline groundwaters, are unlikely to prohibit degradation of this important ligand.

Whilst data suggest that microbial ISA degradation in the near-field is likely to be inhibited only by very high pH conditions (>12), ISA degradation would appear to be possible both in the geosphere following dilution of alkaline pore-waters (i.e. down gradient of the near-field) and in the near-field over longer timescales, when first-flush of cementitious materials in the repository has passed. These findings provide us with greater confidence of the influence of ISA when conducting post-closure environmental safety assessments. While ISA degradation was not observed under high pH conditions, it was seen when conditions were circum-neutral. This implies that, as ISA containing waters migrate from the repository and are buffered/diluted by sulfidic groundwater, ISA degradation can occur. Furthermore, under sulphate reducing conditions, radionuclides will more readily form insoluble sulphides. Further research is, however, needed to underpin the behaviour of ISA under a greater range of environmental conditions.

PVC

Work by MIND researchers indicates that in spite of strong irradiation (albeit of limited relevance for intermediate-level waste) PVC degradation is most likely limited to the formation of some organic gases (benzene) and minor dissolved substances. Alkali hydrolysis of plasticised PVC led to dissolved organic carbon in all PVC enrichments to increase, supporting near-complete nitrate reduction, suggesting that this is an important step in PVC degradation (Nixon et al. 2018). Irradiation of PVC limited microbial nitrate reduction in plasticised PVC sheets, yet increased it in unplasticised PVC powder. Effects of radiation are unclear, although there are suggestions that plasticisers and other additives may become 'locked' into polymers by irradiation, thereby reducing bioavailability (Nixon et al. 2018).

Phthalate and phosphate plasticisers were targeted to assess whether these were the compounds responsible for sustaining nitrate reduction at high pH, although the study by Nixon et al (2018) found that they were not. Microbial nitrate reduction was therefore thought to be supported by additives other than triphenyl phosphate (TPP) or phthalate plasticisers in plasticised PVC. Where additives are not present, breakdown of polymer structure may release organic molecules which could be another mechanism of supporting nitrate reduction. Further work is required to further develop our knowledge of PVC and additive degradation processes.

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Nitrate is an important electron acceptor in a repository, before conditions become conducive to sulphate and iron reduction. This highlights the biodegradability of synthetic organic wastes and may have important knock-on implications on downstream (bio)geochemical processes.

Bitumen

In MIND, the focus was on nitrate-containing Eurobitum waste and is detailed in deliverable 1.3 (Mijnendonckx et al., 2018). As a first objective, the project focused on the ability of inoculated microorganisms (from the BC piezometers) to reduce the nitrate leaching from the Eurobitum. A small block of the non-radioactive Eurobitum (fresh or thermally aged) is put in to contact with Boom Clay pore water (either derived from piezometers or synthetic) and able to swell without restrictions. It is known that nitrate leaches through contact with (alkaline) water and limited release of organics is possible due to ageing (hydrolysis and radiolysis). It was shown that in presence of added organics (acetate, formate or oxalate) these additives are used to reduce the nitrate leaching from the Eurobitum. In absence of these organic additives, the difference has to be made between tests with non-aged Eurobitum and thermally aged Eurobitum. For the first no results indicated that nitrate reduction was taking placing. For the second, two tests were performed under slightly different conditions with contradicting results (no nitrate reduction versus limited nitrate reduction). It is debatable if the Eurobitum organics were used in the limited nitrate reduction or the H_2 in the glove box. The effect of pH on the nitrate reduction was a second objective. Results indicate that at pH 10.5 a considerable lower nitrate reduction was observed by microorganisms form the BC piezometer compared to pH 9 conditions. On the other hand, a microbial community which was already adapted to higher pH, was slightly more active at this pH 10.5 compared to pH 9. None of the communities actively performed nitrate reduction at the pH 12.5 conditions.

Table 2-2 Organic waste (matrix) degradation processes, key achievements in the EC MIND Project

Substances under investigation	Process	Synthesis of results obtained in the MIND project and affiliated scientific work	General discussion	Parameters relevant to safety case
Cellulosic materials	Degradation	Fermentation processes lower ISA-production (alkaline hydrolysis) and result in acetate lowering pH. If pH reaches 11-10, methanogenesis can become more important (Bassil et al 2015b; Small et al 2017)	ISA, an important complexant, is released from cellulose degradation at high pH. Fermentation and methanogenisis can lower the ISA-production. Although favourable environmental conditions (such as pH <11) are needed. Methane is an important carrier gas for gaseous (i.e. CO_2) ^{14}C.	Methanogenesis shown to double when pH decreases from 10-11 to <9 over decadal timescales (Small et al 2017 for details)
Isosaccharinic acid (ISA)	degradation (oxidation)	Rapid ISA degradation at high pH seen under oxic conditions (Bassil et al 2015a). Under Fe and S reducing conditions, pH limits ISA degradation (Kuippers et al 2015) although Rout et al (2015) observed methanogens degrading ISA up to pH 10.	ISA degradation has been observed under a range of conditions, excepting those where pH is very high. In terms of wider relevance to safety case, ISA passing through the engineered barrier might be expected to degrade (bio)chemically .	Microbial ISA degradation observed up to pH 11 and under salinity up to 6% w/v NaCl (Bassil and Lloyd 2018).
Polyvinyl chloride (PVC)	radiolysis / hydrolysis	Unknown organic additives in PVC are observed to fuel microbial nitrate reduction (Nixon et al 2018)	The uncertainty regarding the actual composition of commercial plastics seriously hampers the identification of organics responsible for their properties under GDF conditions	PVC additives can be used by microorganisms for nitrate reduction if pH <10

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Bitumen	radiolysis / hydrolysis	There is no evidence of nitrate reduction above pH 12.5 There is no evidence of degradation of the organic bitumen matrix which could fuel nitrate reduction Next to pH reduction rates depend on the present electron donor	Years of research on polymer and bitumen degradation as well as recent additions in the context of MIND do not show significant degradation of complex organic polymers, even if harsh conditions in terms of irradiation, oxidation and alkaline attack are applied. The role microbial degradation is therefore most likely insignificant. However nitrate leaching from specific bituminised waste types, such as Eurobitum, can cause geochemical perturbations depending on the environmental conditions.	Nitrate reduction is unlikely above pH 12.5 Microbial degradation of organic (Euro) bitumenised waste matrix is considered to be unlikely under repository conditions Nitrate reduction should be considered below pH 10.5 and presence of sufficient nutrients

2.4.4 What remains to be resolved of common interest to WMOs

MIND has contributed significantly to addressing knowledge gaps in the field of organic waste matrix degradation. In addition to the ongoing future research avenues identified through individual research programmes, there are future research needs which are of interest to a number of waste management organisations. These include investigating the impacts of pH in a cementitious repository and exploring the impacts of the microbial degradation of organics in more complex systems containing multiple potential degradation routes. Such work is likely to benefit from the use of underground research laboratories, where realistic environmental conditions can be simulated at large scales.

2.5 Microbially enhanced processes potentially affecting radionuclide transport / migration

2.5.1 Radionuclides in the waste and those making the dose

It is useful to get a feeling for the radionuclides present in the waste containers and those likely to eventually reach the biosphere. We will use here the example of the Andra 2005 Argile Dossier (Andra 2005b). Of the RN initially present in the waste, present after a defined period of time and making up the dose a significant difference can be seen (Figure 2-5).

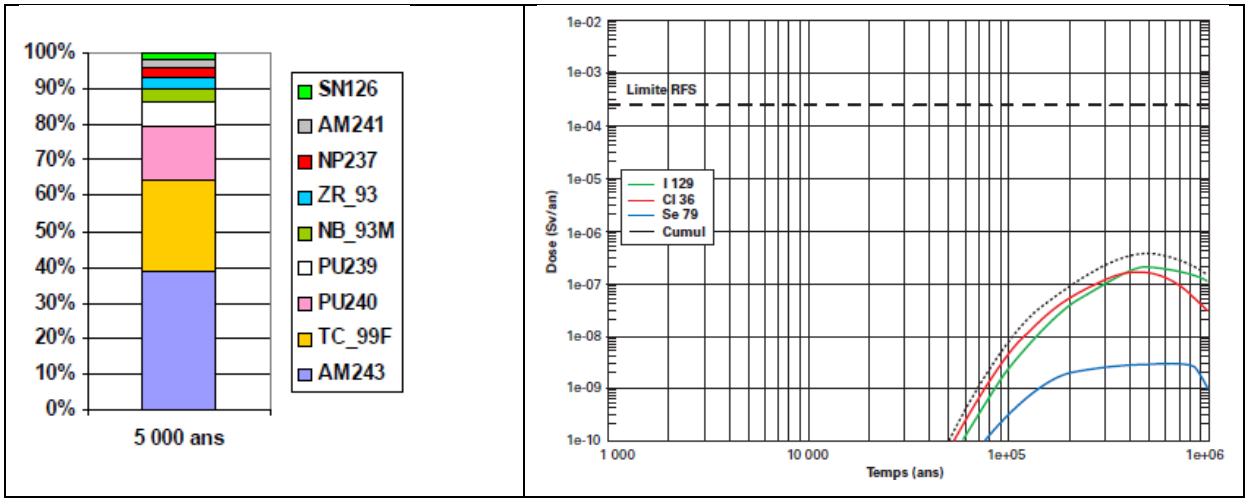

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Figure 2-5 Radionuclides present in vitrified waste after 5000 years of disposal (left) and the temporal evolution of the dose rate for RNs released from this vitrified waste simulated for the vicinity of the biosphere entrance point Saulx river (Andra 2005b).

The difference in the radionuclide present in the waste initially and after 5000 years is due to radioactive decay. After 5000 years the majority of the RNs remaining in the inventory (Figure 2-5, left) are expected to have very low mobility, either due to their low solubility or high sorption capacity. These RNs are therefore expected to remain within the engineered and geological barriers. The majority of the dose in the biosphere results from I-129, Cl-36 and Se-79 which are all expected to be present as anionic species and thereby exhibit high solubility and low sorption to the negatively charged surfaces of the barrier materials. While this example relates to specifically to Andra, and there may be variations from country to country due to differences in inventory and concept, the trends in behaviour observed here will be reflected internationally.

2.5.2 Microbiology and mobility of radionuclides

Introduction

The quick introduction to the RNs present in the waste and those likely to reach the biosphere alludes to concepts such as waste containment, engineered and host rock barriers and to possible pathways some RNs may take during their long migratory path to the surface. It also allows an appreciation of the broad range of chemical elements representing most major groups of the periodic table of the elements. Understanding the pathways of migration and the underlying functioning of the barriers requires a thorough understanding of material sciences, thus the need to know the organisation of glass, bitumen, steal, concrete, clay and finally of the host rock and the rocks of nearby groundwater systems. The behaviour of the RNs in these systems is governed by physical, biogeochemical and hydrological processes again embracing a very broad range of science and engineering. Within this complex system, microbiology plays a non-negligible role; some of the fields where microbial activity influences the phenomenological evolution and thus the migration of RNs are the following:

- Stability of barrier materials such as steel or concrete (see sections on corrosion and bentonite evolution); breaching of the container or the loss in integrity of concrete or bentonite would allow mobile RNs to migrate towards the biosphere faster.
- Some organic compounds are able to complex and mobilise usually immobile RNs. Their presence is a dependent on the waste, the waste treatment and on the barrier / host rock types, and their evolution with time depends strongly on their chemical reactions and the associated reaction catalysis performed by microorganisms (see section on organic matter).
- Build-up and removal of gas pressure is linked partly to physio-chemical processes, such as the production of dihydrogen via corrosion or radiolysis or its dual phase transport out of the production zone; but the production and fate of many key gases (oxidation of H_2, formation of methane, hydrogen sulphide, and dinitrogen) require microbial catalysis. Furthermore, the appearance and disappearance of gases is strongly coupled to resaturation and local hydraulics; knowing the role of water in all chemical reactions, it must be considered explicitly (see section on gas).
- The speciation of RNs, as we will see in more details below, is cardinal for some RNs. The related changes in chemical forms all depend on the availability ("activity") of electrons in the system. A system with substances able to accept electrons in their molecules, such as oxygen, nitrates, Fe(III), to a lesser extent sulphate or carbonates, the so called electron acceptors or oxidising agents tend to make a system more oxidizing. Substances such as dihydrogen, organic matter, sulphides, and reduced metals are able to donate electrons, giving them the ability to serve as reducing agents. Their presence is likely to make the system more reducing. The actual redox potential, a theoretical approach to quantify the electron potential, depends on the presence of electron acceptors and donors and on the kinetics of reactions between them. A positive redox potential is characteristic for an oxidising system, a negative redox potential for a reducing system. With few exceptions one can say that redox-sensitive RNs in their reduced state (*i.e.* U(IV), Tc(IV), Se(IV)) are more strongly retarded than their oxidised forms. This is one reason why it is essential to keep the near field of a repository for nuclear waste at a low redox potential. The persistence of a reduced system is thus a key pillar for the safety of a radioactive waste repository. Some important exceptions do exist (i.e. C in its most reduced form, CH_4). Many key reactions that define the redox potential are catalysed by microbial activity; many do not proceed without this catalysis.

The influence of microbiology on some of the above processes can be and has been studied without explicit consideration of RNs, as has been done within the MIND project. In this section, the behaviour of RNs will be explicitly considered. As discussed in the above bullet points there are some aspects where microbial reaction catalysis will have a positive impact on the evolution of the repository, the lowering of the dihydrogen gas

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pressure, the oxidation of complexing organic matter, or the catalysis of RN reduction and immobilization are some examples. For this reason, a concept of a "biobarrier" has been suggested and presented during the Prague MIND meeting. It draws a zone within the near field of a nuclear waste storage cell, where microbial activity is significant and plays a role in controlling safety-relevant processes. The extent of the "biobarrier" depends clearly on the waste type and the storage concept. From an implementer point of view, a barrier contributes to isolation and containment of the waste.

The explicit consideration of radionuclides

As was mentioned in the previous paragraph, microbially enhanced processes, in particular redox processes, have a clear role in reducing and maintaining gas pressures (oxidation of H_2; formation of CO_2 and/or methane), degrading (oxidising) organic matter and influencing the redox potential of the system, thus the redox state of RNs, their extent of sorption and their mobility. This section focuses on those processes directly affecting safety-relevant RNs. Gas-and organic matter-related issues have been discussed in previous sections (section 2.3 on gas and section 2.4 on organics).

It is not possible to treat the complexity of reactions likely to occur in waste cells as such; they need to be simplified, in both surface and *in situ* laboratory experiments. This approach is based on the concept that the reactivity in a complex system is the sum of the reactivity of all individual subsystems. The very recent MIND-related work on ISA degradation in a system with Fe(III) and sulphate reduction (with all reactions catalysed by microbial activity) and their role on Ni mobility (Kuippers et al. 2018) clearly shows that the concept can indeed be misleading. Microbial degradation of ISA occurs (see section 2.5.7 for more details); this should reduce the fraction of Ni (stable or radioactive) complexed by the ISA ligands, but in a more complex, more "realistic" system it also reduces the part of Ni which is sorbed to the Fe(III)-oxyhydroxides. This reduced Ni is released into solution in the process of Fe(III) reduction, an electron accepting process necessarily occurring parallel to the electron donating ISA oxidation. But parallel to Fe(III) reduction, sulphate reduction has also occurred in the experiments of Kuippers et al., forming sulphides that react with Fe(II) to form biogenic iron sulphides which control the fate of Ni. Ni is neither complexed by the remaining ISA ligands or its degradation products, nor immobilised by oxyhydroxides; it becomes immobilised within biominerals, which were initially not considered relevant in the simplified system.

It is therefore always necessary to build a comprehensive picture of what happens in a waste-cell. The systems studied in the laboratory have to be widened significantly. Considering the work that went into the research example referenced above (Kuippers et al. 2018) it is impossible to study explicitly all aspects that may play a role for any of the radionuclides. Some smart assessment is required to reduce the number of cases. As previously stated microbial activity essentially controls the kinetics of reactions. In some cases reactions proceed that would not happen under abiotic conditions. Kinetics do not generally play a significant role in nuclear waste safety assessments, particularly in the post-closure period and after the end of any potential period of retrieval due to the long timescales involved. In the search for safety-relevant situations where microbial reaction catalysis plays an essential role, it is valuable to point to situations

- where microbial catalysis plays an essential role in key biochemical reactions and,
- where safety-relevant radionuclides are implied.

This work is of course simpler for a situations, where

- the waste inventories are well established
- the components of the engineered barrier have been defined and quantified and
- the host rock is well characterised.

Furthermore, parameters and processes as given below should be kept in mind. They all affect microbial activity and the related reaction catalysis. They will also be discussed in the section on the limit of life and its sustainability (section 2.6), but they are important to understand the discussion of some case studies for specific RNs. Further aspects to keep in mind when considering the presence of microbiology and their role on RN speciation and mobility are:

- The presence of
    - electron acceptors (*e.g.* nitrate, sulphate, carbonate);

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- electron donors (*e.g.* organic matter, H_2, reduced metals, etc.);
- physical space and available water.

- Temperature
- pH
- Salt concentrations
- Enhanced pressure
- Reduced water activity
- Irradiation.

In the upcoming sections on cases studies, a variety of cases are discussed mostly distinguishing the intermediate-level nuclear waste concept from a high-level waste concept. The former is characterised by concrete thus by an alkaline pH, the latter by neutral pH and the presence of higher temperature, irradiation and bentonite as part of the engineered barrier.

2.5.3 Case study 1: C-14

The issue has been discussed as part of the gas subject (§ 2.3), the focus here is on the fate of C-14. Microbiological reaction catalysis can be quite striking in the case of carbon, which appears in very different forms: a solid (^{0}C) in graphite, in gaseous forms of widely different valence ($C^{IV}O_2$, $C^{-IV}H_4$) or present as a variety of solid or dissolved organic substances. Many of these chemical forms have widely varying migration behaviour. Carbon-14 present as CO_2 or HCO_3- is known to exchange isotopically with carbonates or to be bound during carbonation of concrete, thus experiencing significant retardation (Dayal and Reardon, 1994). If the redox potential drops and no other electron acceptors are present, carbonate may become an electron acceptor and in the presence of methanogenic bacteria (Kotelnikova, 2002) ^{14}C can be converted to gaseous CH_4, known to be potentially mobile (Francis et al., 1980). The fate of C-14 is thus essentially related to the question of whether some fraction of C-14 can be converted to methane, its most mobile form.

The study of the speciation of C-14 in nuclear waste has not been part of the MIND project, but recent advancements in the field show how essential such information can be for further C-14 migration assessments and the role of microbiology therein. Considering stainless steel present in the reactor vessel of a nuclear power plant, where thermal neutrons cause (n,p) reactions converting stable ^{14}N, a steel impurity, to ^{14}C, knowledge of the speciation of the C-14 formed is of course essential. The most recent work carried out as part of the Euratom project CAST (https://www.projectcast.eu/) shows the delicateness of such an analysis (Cvetković et al. 2018a). A few grams from an irradiated steel nut (from the Gösgen NPP; highly radioactive, mainly because of the presence of Co-60) was introduced into an artificial cement pore water solution to simulate its contact with alkaline water in a concrete repository cell. As the concentrations of the C-14 phases were extremely low (in the pico- and femto-molar range), only the combination of ion chromatography with accelerator mass spectrometry allowed the speciation at such low levels. C-14 bearing formate and acetate where the main organic leaching product, a result confirming what has been found on leaching tests with non-irradiated zero-valent iron (Cvetković et al. 2018b). What happens to these C-14 containing organic acids is then a multidisciplinary question where microbiology (organic acids are electron and organic carbon sources), plays as much a role as hydrology, chemistry, material sciences and others.

C-14 in intermediate and high-level waste

The presence of hydrogen gas, which if reacting in biotic systems (not in abiotic systems at ambient temperature), can create a very low redox potential. Dihydrogen can therefore be considered a key element in allowing methanogenesis to occur.

In systems with sufficient "sulphate, there was no detectable methane production. The potential for methane production in (such systems) was only expressed when the hydrogen partial pressure was artificially increased, either by the addition of hydrogen or by the inhibition of sulphate reduction with molybdate" (Lovley 1985). "SRB (sulphate-reducing bacteria) outcompete MB (methanogenic bacteria) by maintaining the hydrogen partial pressure below a minimum threshold necessary for methane production" (Lovley 1985). More recent work by MIND partners (Bagnoud et al. 2016a) has shown in a very detailed analysis how the carbon cycle is driven by autotrophic hydrogen oxidisers with organic matter processed by fermenters, followed by complete oxidation to carbon dioxide by heterotrophic sulphate-reducing bacteria. But sulphate remains in the system because it diffuses in from the clay-rich host rocks (Nagra, and Andra cases) (Bagnoud et al. 2016b). However, what happens if hydrogen gas pressures increase above the minimum threshold or even beyond, to partial pressures rarely studied because of safety-related issues. As the production of H_2 is essentially given by the corrosion rates, which are rather small, higher pressures are unlikely. For what-if safety evaluation, some agencies have to

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consider accumulation of H_2 and increase in hydrogen partial pressure though unlikely from a biogeochemical point of view.

Methane formation and the associated enhanced mobility of C-14 may thus not be an issue for waste repositories, where the presence of sulphate does not allow the transition from sulphate to carbonate reduction.

C-14 in low-level waste

In addition to the question on whether C-14 can be part of mobile gaseous forms, there is also the general question on C-14 in solid or dissolved organic forms. There is little data available on the fraction of C-14 initially present in organic substances compared to the fraction present in the inorganic form. The issue has raised more concern for low-level waste repositories situated at or near the surface (Francis et al. 2008) than for high or intermediate-level waste. The subject is extremely broad and touches of course on the general question on organic waste (section 2.4). There has been no work within the MIND project on C-14, though it is often used as a radioactive tracer to understand a variety of biogeochemical and biochemical processes occurring in the environment.

The Finnish *in situ* experiment, a low- and intermediate level waste mock-up site, has been mentioned before (Small et al. 2017). Methane has been found in the gas phase, essentially because the system quickly evolved to lower pH and had low sulphate concentrations; it was thus prone to methanogenesis. The evolution of the dissolved organic phases and their load of C-14 has been studied much less. However, it is an important research field that needs to be elaborated further in the future. As many of the transitions of organic radioactive waste, regardless if they are purely chemical, oxidative or fermentative, are likely to occur in low and intermediate repositories, in particular in niches with more neutral pH, and as microbial catalysis plays an important role here as well, the issue should be given significant priority.

2.5.4 Case study 2: Se-79

Selenium is a perfect example of an element that has relevance in both the nuclear and non-nuclear fields and whose multiple redox transitions are strongly catalysed by microbial activity. The relevance to nuclear waste disposal may of course vary considerably depending on the type of waste and treatment (i.e. reprocessing). Choosing the Andra B2 bituminous waste, a waste type rich in nitrate (see discussion below) one can see that Se-79 is present at significant activities (*i.e.* 1.1 to 2.9e7 Bq/waste container). However, when compared to other RNs such as Tc-99 or Ni-63 that are present at activities up to 3 orders of magnitude higher (Andra 2005a), Se-79 becomes secondary in the case of French bituminous waste. For the vitrified waste, its role is indeed pertinent; in the Andra 2005 Dossier it was part of the main RNs making up the dose (Figure 2-5 right).

Memoirs have been written on Se and its behaviour in nuclear waste cell systems (Cannière et al. 2010); the relevant literature is particularly rich because stable Se is an essential as well as a toxic element (Stolz et al. 2006). For the nuclear waste cell, it is useful to know the behaviour both in abiotic and biotic systems. We focus here on near neutral rather than the concrete-dominated systems because the barrier function of the host rock is more relevant that the one of the engineered barrier. Se is redox sensitive with the most oxidised form, Se(VI) known to have higher solubility and reduced sorption. Within the waste Se is more likely present in the mobile oxidized forms. Redox changes are strongly catalysed by microbial activity, particularly for the transition from Se(VI) to Se(IV) there is hard evidence for the need of microbial reduction; reduction does not take place in sterile control experiments (Chung et al. 2006).

After reduction of oxygen or nitrate, the overall redox potential of the waste cell will reach a value where reduction of Se(VI) becomes thermodynamically favourable. If nitrate is still present in the system, *i.e.* in the presence of nitrate bearing waste, and reactive, the redox potential is increased and Se, present in the oxidised form, will not be reduced, even if catalysing bacteria exist (Oremland et al., 1999). In cases where Se(VI) is at such high concentrations that it becomes toxic for microorganisms, the more toxic Se(VI) can be reduced to the less toxic Se(IV) even in the presence of nitrate (Subedi et al. 2017). This information has recently been confirmed by laboratory experiments carried out by SCK●CEN (Hendrix et al. 2018), a research laboratory associated to the MIND project.

Two situations could be of concern:

- In the case of nitrate present in the waste and a host rock without current microbial activity, such as the Andra Callovo-Oxfordian clay rock (COx) or the NAGRA Opalinus Clay (Opa), the nitrate would stabilise the oxidised form of Se, Se(VI). If Se(VI) reaches the host rock where no microbial reduction will occur, the more mobile oxidised form of Se will persist. This mobile form of Se would thus be able to migrate through the host rock with a much-reduced retardation. The reduced forms, such as Se(IV), on the other hand, would be immobilized by different minerals and different mechanisms.

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- In the case of methanogenesis occurring within the near field, Se could actually be reduced to the –II state and form the gaseous dimethylselenide. This process has been observed in soils (Zhang et al. 1999) but has thus far not been observed nor considered in the near field.

The MIND-related research on the existence of Se(VI) reducing bacteria present in naturally occurring bentonite is useful, but not essential because Se(VI) can be reduced abiotically. Speeding up the reaction catalysis, as microorganisms can do, is not relevant for a repository system where time is not an issue. The reduction to more negative valence states and the likelihood of methylation does remain an important issue that should be investigated further (see section 2.5.8).

2.5.5 Case study 3: Tc-99, uranium, other actinides and analogues

Tc-99, as well as the actinides have multiple redox states. As a rule of thumb, the most oxidised form is the most mobile; in common with Se-79. Generally speaking the redox reactions of these RNs occur with faster kinetics if microbial catalysis is active (Lloyd 2003). In contrast, however, to Se-79, where the reduction of selenate (SeVI) does not occur without microbial catalysis, the RNs belonging to this group do react in abiotic systems.

Technetium can be used as an example. Several publications indicate the role of microorganisms in the reduction process of the mobile form pertechnetate [$Tc(VII)O_4^-$] (Chernyh et al. 2007; Fredrickson et al. 2000; Lloyd et al. 2000) but the abiotic catalysis has also been documented (Morris et al. 2008; Zachara et al. 2007; Peretyazhko et al. 2008; Peretyazhko et al. 2012). The most informative studies are those for Tc(VII) reduction at pH>12, thus clearly abiotic (Asmussen et al. 2018; Arai et al. 2018); they indicate without a doubt that purely chemical abiotic reduction processes do occur.

With the above information, it is possible to anticipate the phenomenological evolution of Tc in a waste cell. This of course depends on the type of waste and the storage concept. It is thus limited to a situation of a specific waste agency.

In case of Tc released as pertechnatate Tc(VII), if it is not reduced by a more rapid biocatalysis, for example at interfaces, it will be reduced within the reducing environment of the host rock (Callovo-Oxfordian clay rock in case of the Andra concept) by a chemical process. This reduction may be slower, but in view of the time scales involved, this is of limited relevance. The same argument applies to the other RNs that are known to be chemically reduced (U, Np, Pu, ...).

Within compacted clay host rocks we are thus within an abiotic system where equilibrium thermodynamics can be applied to estimate the redox potential of the system and the related stable redox state of each RN and its speciation and sorption behaviour.

In the context of intermediate-level long-lived waste containing large masses of nitrates, the latter will impose oxidising redox conditions if nitrates react. However, this is only the case in biotic systems. When nitrate is not reacting but merely diffusing into the abiotic system (Dagnelie et al. 2017) they cannot influence the redox conditions of the system which remains in the case of the COx host rock defined by the Fe(III)/Fe(II) couple (Silvester et al. 2005). In the engineered barrier system, if the pH remains below 12, nitrates will indeed control the redox conditions and thus the speciation of redox sensitive RNs. In the presence of nitrates, the behaviour is quite comparable to what has been discussed for Se in the previous section (section 2.5.4). At 10 mM concentration, the reduction of Tc(VII) is hardly impacted, but if the concentration of nitrate reaches 100 mM Tc(VII) reduction is inhibited (Li and Krumholz 2008). Comparable reduction inhibition has also been observed for U(VI). Independent studies have shown that the oxidised form maintains its stability at a critical concentration threshold located somewhere between 1 à 100 mM (Istok et al. 2004; Senko et al. 2002).

In the presence of nitrates, Tc and U are thus more likely to migrate through the engineered barrier and to reach the clay host in their oxidised form. However, based on the hypothesis that nitrates only exert their oxidising potential in the presence of microbial reaction catalysis, which is not present in the host rock, the return to reducing conditions in the host rock should allow for the reduction of Tc and U and thus bring about increased sorption and diminished mobility.

2.5.6 Why of relevance and when

As this section relates to the mobility of RNs the “when” is clearly defined by the release of RNs from the waste containers. This broad issue depends in a complex way on the type of waste, engineered barrier and host rock. However, even after release it is not possible to approach this question from a generic point of view. As mentioned above the extent and the role of the “biotic zone”, loosely defined as the volume within the near-field where microbial activity occurs, is important to know, but depends on the type of waste, the concept and the host rock. The role of microbially enhanced redox reactions (reduction of mobile Se-79(VI) or Tc-99(VII) to less mobile reduced form, C-14 mobilised in the CH_4 form or Se-79 as a gaseous methylated form) also depend on

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the concept and the type of situation. In the case of an abiotic clay host rock (French and Swiss cases), the sites of bioactivity have to be located within (in case of organic acids locally neutralising the concrete matrix) or between the waste containers and at the interfaces to the pristine host rock. It is within these zones that microbial reaction catalysis controls the redox conditions and thus the speciation of redox-sensitive RNs.

The waste, engineered barrier material and host rock

- supply the electron donors (H_2, organic matter) and acceptors (nitrate, FeIII, sulphate), major components that fuel redox reactions;
- define the environment in which microbial redox reaction catalysis will be significant or not (pH, irradiation, temperature, space and water availability,…; see section 2.6 for further details).

With the information given in the paragraphs of this RN-related chapter we can hypothesis that the microbial influence on RN mobilisation is minor in particular if the following conclusions can be confirmed:

- Continuous influx of sulphate from the host rock will inhibit methanogenesis and thus the formation of mobile gaseous forms of C-14 and Se-79 ;
- The host rock as the major barrier will remain a reducing environment in which all redox-sensitive RNs will be transformed into their less mobile reduced form, catalysed in this case by chemical processes.

A remaining issue is linked to the need of microbial reduction of Se(VI), the most mobile form of selenium. If the "biotic zone" is too restricted, (i.e. no microbial activity in the host rock) it is possible that Se-79(VI) will remain partly in the more mobile form and thus migrate through the engineered and geological barriers.

The very informative paper by Kuipers et al. mentioned above (Kuippers et al. 2018) adds a requirement of caution. The disappearance and appearance of mineral phases that are related to microbial reaction catalysis (biominerals) will likely influence RN speciation and migration in ways that are difficult to quantify. Fe-oxides and – hyroxides, initially present or formed during corrosion serve as RN scavengers (Ni in the work by Kuipers et al.). Their dissolution, because of microbial FeIII reduction, will liberate a fraction of RNs, but if sulphate reduction occurs at the same time with associated metal sulphide precipitation, a new RN scavenger appears in the system. These complexities, so far seldom considered, will have to be taken into account more explicitly with or without consideration of microbial reaction catalysis.

2.5.7 Achievements within MIND (incl. cooperation)

The achievements within MIND are summarised in table form (Table 2-3); this avoids repetition of information already given in earlier paragraphs. In some cases, additional information on research other than MIND has been added when MIND-associated research teams have been involved. Some IRB-relevant information remains hidden in the text. In this case, the related subject has not been given special focus during the evaluation; it yields nonetheless some useful information.

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Table 2-3: Achievements within the MIND project and associated research

Subject	***authors / institutions (References)***	***MIND (et al.) key findings***	***IRB comments***
RN and metal toxicity	Manchester (Kuippers et al. 2018) and TUL Czech Republic	Not only radionuclides (Cs-137 at 0.5 to 5 mM, Sevcu, TUL unpublished), but also stable metals (Ni > 0.1 mM) can be toxic to microorganisms and lead to death or a halt in metabolism.	In a real waste cell, a multitude of toxic metals and RNs will be present. It is not known yet if bacteria can survive in such an environment.
RNs and redox	Lloyd et al., UManch (Kuippers et al. 2018)	The impact of microbiology is more complex than initially thought. In a system ISA – Fe – Ni it is actually the presence of sulphate reduction products (precipitated sulphide; biominerals) that control the fate of a RN (i.e. Ni)	The overall "real" system must be studied; research should move away from simplified systems.
RNs, organic matter and complexation	Brinkmann, Moll HZDR; Merroun U Granada	Attempts have been made to apply spectroscopic methods to unravel the sorption of RNs (U) on organic surfaces (ISA or microorganisms)	Data on well-defined organic substance (ISA) are useful to complete thermodynamic databases; the characterisation of RN interactions with cell surfaces has not yet shown applicability.
BN Se(VI) reduction in the presence of nitrate	SCK●CEN Bleyen, Moll et al. unpublished (Hendrix et al. 2018)	The presence of nitrate does indeed inhibit Se(VI) reduction; after nitrate reduction Se(VI) will be reduced more readily. Earlier work on the ability of Se(VI) reducing bacteria to carry out the reduction process even in the presence of nitrate has been confirmed. This is only the case if Se(VI) is present at toxic levels. It is reduced to the less toxic Se(IV) or Se(0) forms.	The in situ work showed again the importance to evaluate systems that are as close as possible to a waste cell situation. In situ experiments within possible host rocks are thus most promising and should be encouraged.
Modelling redox reactions and transfer	Small et al., NNL (Small et al. 2017)	Software is by now readily available to treat fast reactions based on thermodynamic equilibria and slower microbial catalysed reactions with kinetic laws and related parameters.	It is difficult though to obtain the kinetic rate constants for systems where reactions are likely to be rather slow and which cannot be studied easily in the laboratory.
Se bentonite	Merroun et al., U. Granada (Ruiz Fresneda et al. 2018)	Se(IV) reducing bacteria are present within bentonites (Spanish samples); details on the reduction pathways and the different structures of Se(0) nanoparticles are now available.	Reduction of Se(IV) to Se(0) is both a chemical and microbial reaction; this transition and associated reaction kinetics are well known; a remaining issue is in regard to Se(VI) reduction, which indeed does require microbial catalysis. The occurrence of Se(VI) reduction in the presence of sulphate or nitrate is indeed poorly known and does require further research.

2.5.8 What remains to be resolved of common interest to WMO's

It is not simple to pinpoint a single issue where microbial activity directly and essentially controls or influences RN mobility. This is even less so when considering generic systems comparable or useful to waste management organisations. Of course, one can mention corrosion, bentonite degradation, or organic matter complexations, as has been done in the previous sections, but when focusing on the essential issues the site, waste and concept specificities become obvious. There may not be an issue solely related to microbes and radionuclides. Even the role of microbes in catalysing redox reactions involving RNs depends strongly on the components present in the system (waste matrix, barrier materials). What remains to be solved is multi component and multi subject and relates just as well to questions on corrosion, organic matter or concrete degradation, gas formation, etc.

Way forward

The information given in the previous paragraphs and their link to the associated gap analysis indicate that detailed process understanding remains certainly an important pillar for constructing scenarios on what will likely happen in a repository. To reduce uncertainties on what actually happens inside a waste repository requires

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more dedicated work on mock-ups as close to reality as possible. This indicates the need for an enforced link between microbiological analysis and engineering work. The so-called demonstrators or 1 to 1 large-scale feasibility experiments, such as the FEBEX in situ bentonite-heating test, represent perfect examples (Zheng et al. 2011). The FEBEX experiment informs about the presence or absence of microorganisms in compacted bentonite and the extent to which they are able to catalyse chemical reactions (sulphate reduction, organic matter degradation; hydrogen gas oxidation) (Fernandes 2017). However, the experiments are long-term experiments and difficult to be carried out within the realm of short-term joint research activities.

It will be difficult to guarantee sustainable microbial activities over several thousands of years. In safety assessment, an increase of H_2 pressure therefore will have to be considered. In this regard, it will be important to know what the effect of increased H_2 pressures will have on microbial activity, most likely enhanced sulphate reduction associated with the increased availability of hydrogen gas in solution. If indeed enhanced hydrogen gas pressure with associated increased solubility leads to increased sulphate reduction, the system will be able to reduce hydrogen pressure again and thus serve as a safety valve helping to avoid further hydrogen pressure increase. Anoxic hydrogen oxidation studies at higher H_2 gas pressure are therefore needed.

The gap analysis and the editorial work on this Evaluation Report clearly show that it will be more difficult to justify small experimental activities in surface laboratories. The need to move closer to real waste cell situations with further activities in underground rock laboratories (URLs) in close cooperation with engineering work and based on a thorough understanding of material science and the geosciences is required. It must be considered that different countries are at different stages in their development (*i.e.* no decision on site or host rock). When comparing agencies that have reached a stage of maturity (SKB-Posiva vs. Andra-Nagra) one can easily see the diverging concepts (different host rocks) that of course can bring about considerable differences in their assessment of the role of microbiology.

On the other hand, the joint work within MIND as well as ongoing in different URLs clearly shows the advantage of a broad international approach, in spite of the different concepts, levels of understanding and progress. The role that microbial catalysis plays in these complex systems has been shown by the MIND project. In spite of the fact that detailed reaction analysis remains superficial because of inherent complexity, microbial catalysis in some key redox reactions is essential. A general discussion of such a complex system cannot be done generically; it is essential to consider the mass of substances involved (present in waste, engineered components and host rock) as well as the likelihood of microbial activity and its sustainability (i.e. granitic vs clay-rich sedimentary rock) (Hoehler and Jørgensen 2013). Ways have to be found to agree upon common issues that can then be fused into a common research agenda in which microbiology shall play a similar role than any other contributing science.

As microbiology is necessarily linked to redox reaction or electron transitions, a unified comprehension of all related issues may be a valuable but courageous goal. The years of misunderstanding and the resulting diverging views of electrochemists and geochemists on *in situ* corrosion makes a quick success not likely. It may be simpler in the short term to continue evaluating redox states, to couple their transitions with processes occurring in the near field and the geological barrier (with biotic or abiotic control), and to model these evolutions as a function of time. This may allow us to evaluate with less uncertainty the behaviour of redox-relevant RNs in this system, possibly with a somewhat more explicit consideration of microbiology.

2.6 Environmental parameters that limit microbial impacts in different repository environments

2.6.1 Introduction of issue

Considering the important role of microbiology, knowledge on the limit of life for microbes is of course cardinal. Work in this field has been carried out from different perspective. Looking at the extra-terrestrial point of view, as suggested by S. Nixon, University of Manchester (Cockell and Nixon 2012) helped considerably to focus the issue and to digest work already done. Both theoretical and experimental approaches have been proposed. The thermodynamic approach is valuable and allows quantification of the theoretical energy yield of a chemical reaction. This is relatively simple for well-defined inorganic substances; for organics, that play a significant role in some waste cells, simplifications must be considered (LaRowe and van Cappellen 2011). Of course, there is still a debate on how much energy a bacterium really requires for its metabolism and the kind of reaction pathways likely preferential (Lever 2011).

A combination of environmental factors will limit microbial growth and activity in a DGR. Table 2-4 summarizes the extreme tolerances that have been demonstrated for a variety of microorganisms (West and McKinley, 2002). The influence of these environmental parameters will be design and site-specific. For each

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DGR, the influence of these environmental parameters on microbial growth and activity in the far-field should be assessed during site characterization and on potential impacts on the near-field should be accounted for in during repository design.

Table 2-4 Summarized range of tolerances of microorganisms to a variety of subsurface environmental conditions (West and McKinley, 2002; Wolfaardt and Korber, 2012)

Condition	Range of tolerance
Temperature	-20 to 113°C
Radiation	Up to 17-30 kGy
Water activity	Minimum of 0.62
pH	0 to 12
Pore Size	Minimum of 0.2 µm
Pressure	Up to 180 MPa
Salinity	Up to 50% w/w

2.6.2 Characterization of life in host rock of near and far-field

To assess the potential impacts of the repository host rock in the **near** and **far-field**, traditional microbial analysis methods must be adapted for the low biomass expected at potential repository sites. The search for microbial metabolism, even at a much-reduced level in a possible host-rock candidate, the highly compacted Opalinus Clay (Opa), was initiated more than a decade ago. The question of indigenous microorganisms in the Opalinus Clay was first been raised in the thesis of Sébastien Poulain (Poulain, 2006) of which some results have been published (Poulain et al., 2008). They "suggested the presence of a few sparse autochtonous microbial cells in the Opalinus Clay". No information was given if they were viable. Parallel to his thesis a benchmark study has been carried out with the same goal in mind to investigate the occurrence of indigenous microbes in the same host rock. The results were published in 2 papers (Stroes-Gascoyne et al., 2007; Stroes-Gascoyne et al., 2011), but cannot be specified as conclusive and were formulated with much caution: "The microbial characterization of Opalinus Clay suggests, therefore, that unperturbed Opalinus Clay contains only a small viable microbial community, which is probably metabolically (almost) inactive (dormant), due to space and water restrictions".
This research was also synthesised in the PhD thesis of Alexandra Bagnoud (Bagnoud, 2015; § 2.3.3). In the same document one can also find information on operational taxonomic units (OTUs) from 16s rRNA analysis carried out throughout the Mont Terri underground rock laboratory. These OUTs seem to indicate the presence of viable microorganisms within the Opalinus Clay, but the attempts carried out as part of the thesis to extract DNA from 50 g of rock have not been too successful. Only "a small amount of DNA could be extracted, with great difficulty", (Bagnoud, 2015).

The question relevant to radioactive waste disposal is not the existence of fragments of microbial cells in old samples such as those found in a 250 Ma old Permian salt crystal (Vreeland et al., 2000), but their viability. The latter is controlled in the case of clay rocks by size, water and nutrient availability. The pore size of the Opalinus Clay ranging from one to 25 nM is much smaller compared to the size of microorganisms (0.3 to 5 µm). This of course could be used as an argument against colonisation; even ultramicrobacteria ranging from 200 to 300 nm in size or the recently found ultra-small bacteria that passed through 200 nm filter (Luef et al., 2015), do not seem capable to live in such a restricted environment. Indeed, as indicated by Bagnoud (2015), the pore size given for rocks indicate averages, allowing for areas with larger porosity, where microorganisms may survive in a somewhat dormant state. Further work within MIND (EPFL, in preparation) seems to indicate, that indeed highly compacted clay rocks are void of microbial activity. A study on clay rock diagenesis within the Callovo-Oxfordian (COx) clay rock (French host rock and an Opalinus Clay analogue) suggested a halt in microbial activity based on geochemical and mineralogical evidence. As soon as porosity and permeability dropped below a threshold porosity value during compaction, diagenesis (Lerouge et al. 2011) microbial metabolism and the associated sulphate reduction ceased. MIND therefore comforted the position of Nagra and Andra that microbial catalysis within the pristine host rocks (COx and Opa) is insignificant / none existing.

Very significant work has been carried out in the field of microbial metabolism in clays and clay-rich rocks (for work done within MIND see section 2.2. In spite of the difficulties to detect microbial cells or related DNA in a clay environment - because of the strong sorption of bacteria to the negatively charged surface - the presence and the limitations of microbial life in clay systems is now much better understood. For example, researchers' within MIND have developed additional new methods to detect and characterize microbial communities in the Opalinus Clay at Mont Terri. In addition, an international exercise is underway to compare nucleic acid extraction and

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sequencing techniques and collectively determine the best method. This activity is allowing participants to benchmark the methods that they will be able to use to assess microorganisms at their repository sites.

2.6.3 Characterization of life in the near-field

Clays and clay rocks

Repository designs use bentonite to surround the used fuel container in order to minimize the activity and mobility of microorganisms in the near-field. Microbial activity has the potential to affect container integrity (i.e., by microbiologically influenced corrosion of the container). Within the repository, there is likely no single environmental condition that can be relied upon entirely to create a sterile environment, however a nearly sterile environment may occur locally near the used fuel container, as a result of high temperature and radiation fields. However, heat and radiation cannot be relied upon to limit microbial activity within the entire sealing system, and their impact will also decrease over time. To control microbial growth and activity within the repository, the sealing system design should create a combination of environmental factors that can collectively prevent microbial growth and activity. These could include:

- limiting water availability (a_w) so as to inhibit microbial activity during the unsaturated and saturated periods of a repository;
- limiting the "pore size" and "connected porosity" of the sealing materials to minimize transport of microorganisms; and
- maximizing contact pressure at the interfaces of the container and the sealing system components, and the sealing system components and the host rock to inhibit microbial activity.

The compaction density of bentonite is an important design criteria for DGRs, as it directly relates to the a_w, pore size and swelling pressure that will occur upon saturation. Within the MIND project, much work has been performed on the limit of microbial activity in swelling clays. The latest article (Bengtsson and Pedersen 2017) in a long series of work (Stroes-Gascoyne et al. 2010) gives further experimental evidence for the threshold clay density beyond which microbial activity, e.g. sulphate reduction comes to a hold. Analysis of the FEBEX engineered analogue gives further evidence for the much-reduced microbial activity in such systems (Fernandes 2017). This research has a followed-up within MIND, where clay samples have been used for a comparative analysis of microbes initially present in different bentonites (FEBEX and others) and where the clay samples have been subjected to a variety of stresses likely to occur in a waste cell: pressure, temperature, ionic strength, and irradiation. This work clearly shows that there is already little microbial activity within the clay samples (a few thousand cells per g) and that each individual stress reduces the activity further, with some exceptions (Haynes et al. 2018). The limiting factors temperature, ionic strength and the role of irradiation have indeed not been studied much. Of particular interest seems to be the role of temperature; the presence of sulphate-reducing bacteria in systems containing steel, bentonite and clay host rock is much reduced at 80°C with mostly meso- and thermophilic species present (Necib et al., 2017).

Research within the MIND project has shown that the dry density at which microbial growth and activity ceases is dependent on bentonite type as discussed in section 2.2. In addition to selecting a bentonite density that is restrictive, energy and nutrient sources required for microbial metabolism can be controlled. Microorganisms require a carbon source, nutrient source and electron donors and acceptors to grow and be active. To further control growth and activity, these components should be minimized by:

- selecting materials with low carbon and nutrient content and bioavailablity;
- minimizing the addition of carbon and nutrients to the sealing materials beyond natural occurring values (i.e., during mining, processing, handling, blending and placement of the materials); and
- minimizing the mechanism of transport for nutrients and metabolites within the placement-room-sealing system by ensuring creation of a diffusion-limited environment.

Concrete-containing components of the waste containers and engineered barriers

Nuclear waste repositories for both low and intermediate level wastes are characterised by the presence of cementitious phases; they are also present in high-level waste repositories either as part of seals or additions to bentonite. The presence of both electron donors and electron acceptors, ingredients of redox reactions, should call for the initiation of redox reactions, but the need of microbial catalysis of most of these reactions, requires a specific analysis of the microbiology of these "unnaturally" high pH environments.

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The presence of both electron donors and electron acceptors, ingredients of redox reactions, should call for the initiation of redox reactions, but the need of microbial catalysis of most of these reactions, requires a specific analysis of the microbiology of these "unnaturally" high pH environments.

Quantification of reaction dynamics in alkaline systems involving Fe(0), H_2 or organic matter as electron donors and nitrates / sulphates (if present) as well as carbonates or water (and RN in their possibly oxidised form) as electron acceptors indeed requires microbial catalysis. There are many analogues for testing simulation approaches for microbial catalysis of related redox reactions, but few are in alkaline systems. With H_2 almost omnipresent as an energy source, essential and trace nutrients most likely present in the heterogeneous waste cell environment, with space and water available depending on depth, architecture and re-saturation, the high pH will become the most critical parameter controlling microbial activity in space and time. In this paragraph, we will summarise the knowledge in relation to alkaliphilic microbial activity, in some cases derived from existing natural analogues or laboratory microcosm experiments in others based on thermodynamics.

The high alkalinity is likely to be the main parameter controlling microbial activity in disposal systems where concrete is part of the engineered barrier material. The limiting influence of high pH is well known, but it depends on the potential energy present within the system (O_2 or nitrates being more energetic than sulphate or carbonate, bioavailable organic matter more reactive than recalcitrant organics). Some bibliographic research has been done in search for natural analogues, but the ideal case with widely comparable phenomenology has not been found. The hyper-alkaline spring waters of Maqarin in Jordan (Alexander et al. 1992) or Allas Springs on Cyprus (Rizoulis et al. 2016) or some extremely alkaline ground waters (polluted site near Chicago, (Roadcap et al. 2006) are relevant, because the pH of some of the springs surpasses 12. The Maqarin site has been studied both as a natural analogue of concrete-clay interaction (Gaucher and Blanc 2006, pp. 776–788) and for the migration of RNs (i.e. Re, Trotignon et al. 2006, pp. 755–761). Microbiological laboratory studies found the presence of microorganisms at all three sites even in waters with a pH > 12 (Pedersen et al. 2004; Rizoulis et al. 2016; Roadcap et al. 2006) , but it is not clear if they were only present (i.e. "naked survival") or metabolically active and catalysing redox reactions. It is also possible that they have been washed in by less alkaline waters. It is clear that the cellular protection and the required active transport mechanisms across the cell wall have a high energy demand and that these bacteria (alkalophiles) are characterised by strongly reduced metabolism compared to bacteria leaving in pH neutral conditions (neutrophiles) (Horikoshi 1999, pp. 735–750).

Directly applicable, more recent work has been carried out by members of the MIND consortium (Rizoulis et al. 2012) using microorganisms collected from a lime production site near Manchester, where waters of pH > 12 are present. They combined microbiological, analytical and thermodynamic approaches. Indeed the energy theoretically available from key electron acceptor redox half reactions are quite different. Nitrate reduction to dinitrogen or nitrite liberates much more energy than the reduction of sulphate. Furthermore, the related free energies drop as a function of pH (Figure 2-6).

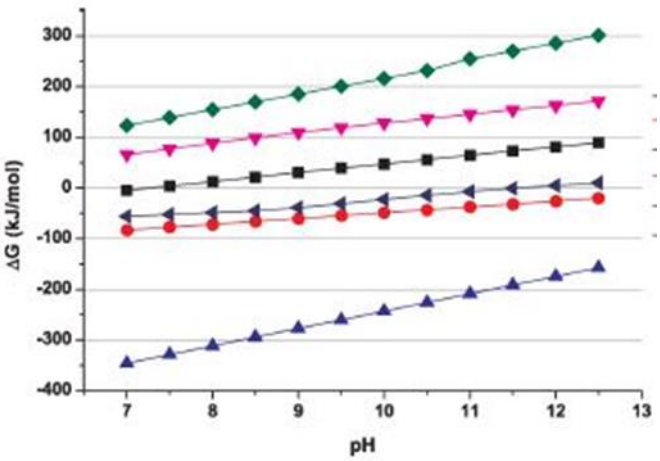

Figure 2-6:Thermodynamic calculation of the Gibbs free energy (ΔG, the lower the value, the more energy available for microbial metabolism) for different key electron acceptor reduction reactions (e.g. $NO_3^-//N_2$ blue triangle; NO_3^-/NO_2^- red circle and SO_4^{2-}/H_2S green diamond) as a function of pH (Rizoulis et al. 2012).

Considering these thermodynamic data and knowing that microbial metabolism becomes more and more energy demanding with increasing pH (Horikoshi 1999), it is not surprising that nitrate reduction can occur at high pH of at least 11, whereas sulphate reduction is not measurable if pH becomes larger or equal to 10 (Figure 2-7). In both cases, the reaction does require microbial reaction catalysis.

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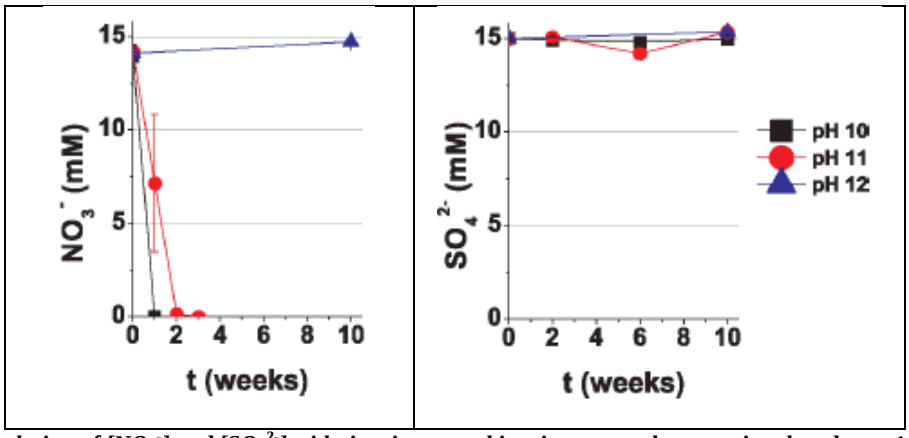

Figure 2-7 : Evolution of [NO_3^-] and [SO_4^{2-}] with time in anaerobic microcosms that were incubated over 10 weeks, set up at pH 10, 11 and 12, using lactate and acetate as carbon sources and nitrate or sulphate as sole electron acceptors (Rizoulis et al. 2012).

It is not possible to predict the evolution in space and time of the various microbial species capable of influencing key processes occurring in concrete-dominated repository systems. It is thus not possible to predict reaction kinetics controlled by microbial activity. However, a biogeochemical simulation exercise will help at least in tracing possible reactionary pathways.

An example of a simulation exercise is for a section of a waste cell, where pH has dropped below 10 where and sulphate is released from the waste container, FeIII is present initially in the system or produced via corrosion, and organic matter and H_2 are available as electron donors. The exercise was carried out by the National Nuclear Laboratory on behalf of Andra (Small et al. 2008a) with the biogeochemical reaction and transport code GRM (Small et al. 2008b, pp. 1383–1418). The variation of the redox potential with time is given to illustrate the simulation approach and some major results (Figure 2-8).

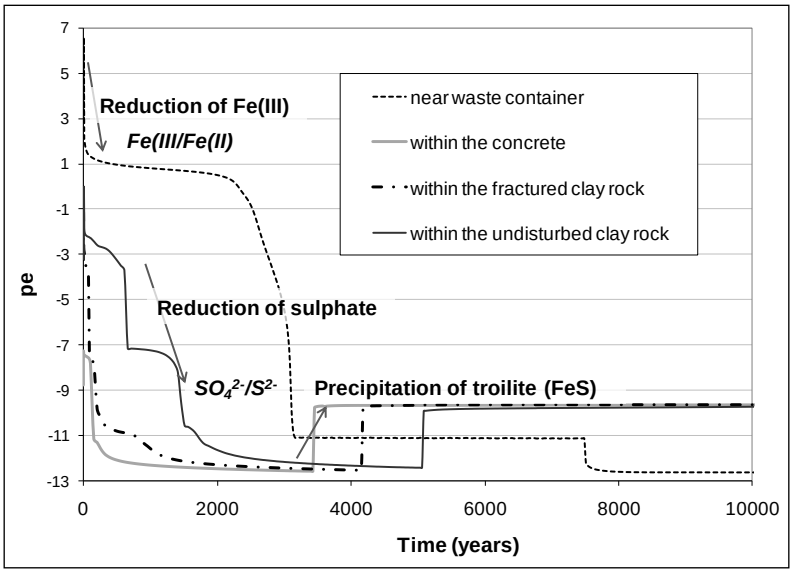

Figure 2-8. Simulation of the redox potential in a waste cell where pH has dropped < 10 using the biogeochemical reaction and transport (here diffusion) code GRM (Generalised Repository Model); main reactions controlling redox potential are reduction of FeIII and sulphate and the precipitation of troilite (FeS) (Small 2009).

This type of modelling has been carried out extensively, partly within the MIND project (Small et al. 2008b; Small et al. 2017). The impact of the pH on the kinetics of key redox reactions (reduction of nitrate, sulphate or carbonate; oxidation of H_2 and organic matter of varying complexity and bioavailability) can nowadays be explicitly considered using parameters for quantification of microbial growth and death rates (Small and Abrahamsen-Mills 2018).

Additional research, in some cases related to the MIND project, furthermore helps to gain confidence in the pH limit for microbial activity. In the presence of nitrate pH 12 seems to be the limit (Durban et al. 2018; Rafrafi et al. 2017). If only lower energy electron acceptors such as Fe(III) or sulphate are present, the pH limit is much

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lower < 10 (Lloyd 2003, Lloyd and Macaski, 2002; Durban et al., 2018; Rafrafi et al., 2017; Rizoulis et al., 2012).

2.6.4 Characterization of life in repository interfaces

Within the repository near and far fields, interfaces are areas where there is the greatest potential for microbial activity. In the case of a high-level waste repository, microbial activity may be limited to the interface host rock / engineered barrier, or within the fractures in the host-rock. Within these regions, the likelihood for microorganisms to exist as biofilms will also require further assessment. The role of biofilms particularly under harsh conditions is well known (Boltz et al. 2017; Azeredo et al. 2017), however, very little work has been done to verify this possibility under repository conditions. Classical *in situ* work has essentially looked at the solution circulating in a borehole. The classical work to investigate *in situ* the pore water chemistry may serve as an example. It was in these experiments that microbial sulphate reduction significantly altered the natural system (Vinsot et al. 2008; Stroes-Gascoyne et al. 2011b). Access to the solid, either to the host rock or to the metal filter (present in all experimental setups in the clay host rock environment) has only been able after over coring, but little microbiological work has been done on these samples. It is only recently that the experiments have been equipped with clay and filter loops, through which the solution circulates just as through the borehole but which can be opened and sampled for direct surface analysis. Both the BN (bitumen-nitrate-clay interaction) and the MA (microbial analysis) experiments currently running at the Mont Terri URL in Switzerland (with MIND members involved in both projects) have looked at the solids to understand the geochemical and mineralogical changes, but also to study the biofilms. Such work needs to be intensified as the biofilms are more resistant to unfavourable conditions and possibly even more sustainable than isolated suspended mobile microorganisms.

2.6.5 Achievements within MIND

Through the research conducted in the MIND project, the tolerances of microorganisms (Table 2-4) has been updated. Table 2-5 summarizes the updated understanding on the limits of life that has been gained through the MIND research program.

Table 2-5: Range of tolerances of microorganisms to a variety of subsurface environmental conditions that has been gained through MIND

Environmental Parameter	Process	Key conclusion and threshold demonstrated in the MIND project	MIND Reference
Temperature	After disposal of spent fuel, the temperature will increase in surrounding host-rock. Local sterilization may occur near the containers. Within the rest of the repository is microbial activity stimulated?	A heat treatment of the bentonite at 110 °C was performed for 170 h intended to sterilize the bentonite from bacteria; prolonged exposure to heat was expected to be efficient, but that was still not enough to eradicate sulphide-producing activity in the bentonite because intensive sulphide-producing bacteria (SPB) and large numbers of cultivable SPB were observed in the heat-treated MX-80. The heat tolerance has been reported up to 120 °C for SRB.	D2.4 D1.6 D2.7 Bengtsson and Pedersen (2017)
Radiation	After disposal of spent fuel, radiation fields will increase in surrounding host-rock. Local sterilization may occur near the containers. Within the rest of the repository does radiolysis of water produce H_2 that can stimulate microbial activity?	Results indicate that stress induced by radiation does not completely inhibit microbial activity, but a reduction in cell numbers was seen in most cases. The concern with regard to the positive effect of H_2 production through radiolysis on microbial activity still persist	D2.15 D2.1 D1.6 Haynes et al, (2018)

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Water activity	Reduction of pore space through bentonite compaction results in loss in free water. Is there a consistent threshold for a_w below which microbial activity is limited in bentonite clay?	Arguments based on the threshold density, the limit of water activity <0.96, remain valid. This limit seems to vary with the type of clay and the overall biogeochemistry.	D2.4 D1.6 D2.7
pH	Reactions liberating/consuming H^+ or OH^-, thus influencing the pH and microbial metabolism	There are both thermodynamic and additional (i.e. MIND) research indicating that only in the presence of NO_3^-) is microbial activity beyond pH 10 possible; the general upper limited is at ~ pH 12.	D2.11
Pore size	Reductions in pore space create a diffusion-dominated environment for transport that restricts movement of microorganisms, as well as geochemical components needed for metabolism.	Even in low-density bentonites (1.500 g cm^{-3}), a pore size in the nm range would theoretically not allow for bacterial existence unless the bacteria could withstand the mechanical pressure from the separating flocs (0.09 MPa at 1.5 g cm^{-3}).	D2.4
Pressure	Swelling of bentonite clay puts mechanical stress on microorganisms and restricts growth and activity.	An upper limit of 2 MPa turgor pressure would mean that cell integrity is possible, though limited, at bentonite swelling pressures below 2 MPa. However, endospores can survive a much higher pressure. In a different experiment, the bentonite dry density was varied (1.25, 1.45 and 1.55 $g.cm^{-3}$). The bentonite with the lowest density (1.25 $g.cm^{-3}$) exhibited the highest numbers of anaerobic and aerobic heterotrophs. In contrast, there was little difference between 1.45 and 1.55 $g.cm^{-3}$ in terms of viable heterotrophs. Viable SRB counts were similar across the bentonite targeted dry densities.	D2.4 D2.14
Salinity	Salinity can play an important role to suppress microbial activity through decreaing the water activity due to interaction of water molecules with solute ions.	Experiments using higher water salinities inhibited microbial growth at lower bentonite densities than those that used pure water. It is important to note that even though salinity has an inhibitory effect on microbial growth and activity, it can also decrease the swelling ability of bentonite clay.	D.2.4
Electron donors and acceptors	Availability of electron donors and acceptors (e.g. H_2, CH_4, SO_4, NO_3, Fe(III),	Limiting of essential compounds may lower microbial activity. On the one hand, low S and Fe containing clays may inhibit SRB and IRB activity, but	D1.6 D1.8

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		on the other hand, low iron content may not then play as a sink for sulphide (formed e.g. in rock matrix outside the clay) (cf. Subject 2 and 3).	
Carbon and nutrients	Organic carbon could be an energy source for microbial activity	Heterogeneity in the chemical conditions in the repository can create optimal niches (e.g. lower pH, more organic carbon) for microbial activity and can lead to gas generation.	D1.6

2.6.6 What remains to be resolved of common interest to WMO:s

Limit of life

Within the MIND program, the influence of irradiation, lack of water and space, pH, ionic strength and pressure on microbial metabolism have been studied, but mostly individually (Table 2-5). As we have seen in Section 2.5, it is necessary to take into account the toxicity of further elements also present in the system. Ni, Se, Cs have been mentioned, but considering elements present in the waste, stainless steel or in concrete further toxicity issues need to be added as well. It is thus quite likely that the zone of microbial activity in the near field (bio-barrier) will be reduced even further because of combined toxicities.

Sustainability of microbial life

Most of the research within MIND has assessed the presence of microorganisms. Measuring the activity is much harder in these low biomass systems, which exist over geological timeframes. The main question that remains open, yet difficult to resolve, is the sustainability and prediction of microbial activity over thousands of years, the period when corrosion and hence hydrogen gas production will in principle take place. In some publications, it has been postulated that isolated microbial life persisted for thousands of years (Lin et al. 2006), but it is difficult to assess the value of this statement and to apply it to a repository scenario. Some experts (i.e. Rizlan Bernier-Latmani oral communication) have indicated that long-term sustainability is possible, but solid evidence, for example from natural analogues is not available.

In addition to the microbiological evaluation, rock mechanical evidence will have to be considered jointly. Clay-rich rocks such as both French and Swiss host rocks are considered to partially self-sealing barriers, a process that helps closing fractures in the host rock, and thereby reducing the amount of open space available for microbial life (Bastiaens et al. 2007). However, it is likely that some open spaces persist between the engineered barriers and the host rock, at least for a time and as indicated above this certainly depends on the repository design, the materials used, and the type of host rock.

Role of biofilms

It is likely that the development of major biofilms, possibly at interface zones, allow microbial activity to remain sustainable in systems where planktonic species are likely to be inactive. The development of biofilm systems has this far not been studied extensively, but in most cases where microbial follow-up has been carried out biofilms have been detected. Biofilms have been found on concrete surfaces (Alquier et al. 2014) and on host rock surface (Mont Terri BN and MA, unpublished results). There is however the need to investigate biofilms further, in particular in view of their efficiency and sustainability under extreme conditions and over very long periods (thousands of years).

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3 Other contributions by MIND

The MIND project has investigated the impact of microbial processes on low and intermediate-level nuclear waste forms, the technical feasibility and the long-term performance of repository components. Research topics focussed on key questions raised by different waste management organisations. Under MIND project, extensive numbers of experiments have been conducted to provide further insights into different microbial processes relevant to a geological disposal of nuclear waste. In addition to the experimental and evaluation work, the MIND project dedicated significant amount of time on education, training, communication, and data management.

The direct funding of the research projects by the European Commission has been an important means of financial support, but also it allowed a valuable networking directly with a broad range of researchers and experts worldwide. Guidelines and recommendations provided by international waste management organisations will not only be important for developing new and innovative programs, but would also serve as valuable resources for future generations. International cooperation is extremely essential for sharing knowledge and expertise in certain areas that might be lacking within other individual programs. In short, participation in international workshops creates an engaging and inspiring environment for both internal staff and academics, and hence desirable for the future endeavour.

The IRB concludes that MIND has been invaluable to assemble the important microbiological issues relevant to repository safety. Moreover, MIND has been instrumental to clarify what issues are of potential importance and what topics remained to be further investigated in future. In particular, IRB believe that MIND formed an essential platform as to how the future efforts in this area should be prioritized and navigated to effectively demonstrate the safety of radioactive waste repositories.

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4 Issues that remain to be resolved

Generally, the IRB concludes that microbial processes cannot be assessed in isolation to other THMC processes affecting repository evolution. An integrated assessment is needed. Nevertheless, the IRB has identified some areas where further work could be warranted.

Corrosion may directly affect the containment of the EBS and the gas generation resulting from corrosion may also affect EBS and host rock containment functions. Generally microbial activity mainly affect corrosion by changing the chemical environment (e.g. formation of sulphide) but there may be other processes that could also be worth studying, including:

- Potentially there may be other microbial corrosion effects and their dependence on e.g. H_2S, temperature and radiation) .
- Validation experiment on corrosion in high pH environments

Bentonite is used as a barrier material in a wide range of repository concept. There are several issues related to microbiology concerning the stability and evolution of the clay material itself and how processes inside the bentonite affect the chemical environment. Examples of issues where more studies could be warranted include:

- Further developing the understanding on what is affecting the microbial activity in high density bentonite
- Microbe-clay interactions
- Effect of bentonite saturation on microstructure and microorganisms
- Sulphide reactions in bentonite and the role of iron – effect on performance.
- How do colonies re-establish in bentonite following a drop in swelling pressure
- Microbial activity on compacted bentonite – e.g. impact of heterogeneity – processes during emplacement
- Effect of salinity on swelling pressure and microbial activity
- What is the index of buffer to prevent the microbial activity (swelling pressure, water activity)
- Conditions in which methanogenesis will prevail.
- Chemical condition of porewater when microbe is active in buffer (pH, Eh, Fe^{3+})

Modelling the **sulphide/sulphate system** is essential for many safety assessments since, e.g. sulfide is a corroding agent of Cu. Examples of issues where more studies could be warranted include:

- Mass evaluation formation of Ni, Fe, sulphides, space, modelling
- Biogeochemical evolution of groundwater just after closure
- Sulphide reactivity related to mineralogy and geochemistry
- Oxidation of pyrite dying exploitation (O_2 present) – impact on corrosion
- Critical parameters for modelling sulphide issue (sulphide precipitation, organic carbon, H2 amounts, HS- transportation) what is the limiting factors
- Sulphates in groundwater – what is the effect on Cu corrosion
- Sulphide toxicity effects on SRB
- Kinetics of precipitation of iron-sulphate in nearfield

Gas formation and migration may have large impacts on repository performance since it affects EBS and host rock properties. Gas can also be a direct path for radionuclide migration (e.g. C-14 as CO_2). Gas production in the repository originate both from chemical and microbially induced processes. Examples of issues where more studies could be warranted include:

- Conditions in which methanogenesis can prevail
- Methanogenesis – its boundary conditions in situ, carbonate, and link to modelling
- Implications of methanogenesis for ^{14}C
- Availability of gases in the geosphere
- Bioavailability of gaseous organic phases
- H_2 – CH_4 processes and SO_4 complexation
- H_2 diffusion through seals (into gallery) O_2 diffusions through seal into waste
- Limitations of gas production
- Transformation of nitrate to N_2 gas or ammonium

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Organic waste matrix degradation has received significant attention throughout the MIND research programme, focussing on fundamental processes in cellulose degradation, bitumen and PVC, as well as degradation processes of the ligand ISA. Future research needs were identified through the course of the MIND programme include:

- investigating the impacts of pH in a cementitious repository
- exploring the impacts of the microbial degradation of organics, particularly ISA, in more complex systems containing multiple potential degradation routes.

Microbes could also affect **radionuclide migration**. Examples of issues where more studies could be warranted include:

- Modelling RN migration induced by microbial activity (role of colloid formation)
- Can biofilms have potential to act as colloid (source) and thus enhance RN migration

Finally, it is recognized that even if a process is observed in laboratory it may not necessary imply that it would be of importance for repository post closure safety. The actual RTHMC conditions at the repository is often controlling to what extent processes can be active. **Joint *in situ* activities** should be considered including

- Long term in situ experiments on i) degradation and complexation of waste organic matter on 2) fate of organics and 3) waste substrate evolution
- Scale of pH heterogeneity in ILW/LLW
- Consider heterogeneity also in modelling
- Characterisation of organic material – potential substrate for e.g. SRB

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5 Conclusions

The MIND project has without doubt significantly enhanced our knowledge basis on microbial controlled processes. These processes where electron transfers occurs are by far the most relevant as microbial metabolism is based on the energy derived from these bio-(geo-)chemical redox reactions. Key reactions such as organic matter or hydrogen gas oxidation or oxyanion (nitrate, sulphate and carbonate) reduction do not occur without microbial catalysis. Furthermore MIND has helped to reduce uncertainties in regard to the limits of life within the engineered barrier system of waste disposal facilities and some host rock types (i.e. clay rocks). The limits of life in regard to pH in cement and density of bentonite barriers are now relatively well established. The limit of life for compacted clay host rocks is also better constrained. The limit of life approach allows in the specific cases to distinguish a geochemical evolution without microbial catalysis from situations where the role of microbes, i.e. associated reaction catalysis, should be explicitly considered (i.e. biogeochemical evolution).

The project also contributed to our understanding and quantification of chemistry related transition periods, such as desaturation, of which the length is a function of hydraulics and of gas generation, the latter in part controlled by microbial activity either by gas oxidation (H_2) or formation (N_2, CO_2, CH_4) depending of course on the waste type and the inventory and the local conditions (host rock). The fate of the cellulose degradation product and ligand ISA is more clearly understood, with research conducted through MIND highlighting the impact of pH and redox conditions on microbial degradation rates.

In some cases discrepancies persist between studies explicitly considering microbial activity and those looking mostly at global processes. The role of reduction of Fe(III) in smectite or of sulphate reduction on corrosion are two examples studied within the MIND project. It is clear that in microcosms in the presence of organic matter bioreduction of Fe(III) and sulphate can be observed but the related impact on bentonite swelling or corrosion has not been observed in underground rock laboratories. This indicates the need to couple the surface and URL experiments and to get as close as possible to repository conditions even in surface laboratory tests.

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

6 Acknowledgement

The authors wish to thank (in alphabetical order): Irina Gaus (Nagra), Takahiro Goto (NUMO), Jennifer McKelvie (formely NWMO representative), Don Reed (LANL), Satoru Suzuki (NUMO), Kirsi Weckman (TVO), Charles Wittebroodt (IRSN) and all that have participated and contributed at the IRB-meetings. The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880 The MIND-project.

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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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