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MIND

Microbiology In Nuclear waste Disposal

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DELIVERABLE 3.1-1

Year one evaluation report

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Publishable Summary

This year one evaluation report of MIND consists of two parts. The first part is a description of the state of the art of geological disposal of radioactive waste, with a special reference to microbiological issues. The second part is the first year evaluation of the microbial processes studied in MIND Work Packages 1 and 2.

The first introductory part describes the general principles and basic concepts of geological disposal. Three main geological options are currently chosen for detailed studies in different European countries, namely crystalline rock, clay rock and salt deposits. However, as none of the lead partners is specialized in salt deposits, this will not be considered within MIND. Crystalline rock and clay rock differ substantially from each other regarding the rock type and their respective disposal concepts, including with respect to their inherent microbiology. Solid crystalline rock originates from deep hot rock melt and provides a stable non-porous barrier to isolate radioactive wastes, but the effective fluid permeability and distribution of fracture systems must be considered carefully, including the role of fractures in enabling biogeochemical processes. There are plenty of studies showing the existence of a natural deep biosphere in the fractures of crystalline rock. On the other hand, clay rocks are originally deposited in marine biosphere environment. Fluid permeability of the dense (fine-grained) clay rock is generally very low, but the total porosity is high in comparison with the non-porous crystalline rock. Still, clay rocks have excellent retardation and sealing capability. The average pore size in dense clay rock is smaller than the size of typical microorganisms, consequently the existence of viable microbial populations in consolidated clay rock is still questionable.

The planned repository designs and engineered barrier systems differ for different bedrock concepts. So far the most advanced crystalline repository concepts for high-level waste are based on copper canisters embedded in bentonite buffer as diffusion and sorption barrier. The sealing capacity of the bentonite buffer and the long-term stability of copper canisters has been thoroughly studied and verified. It has been concluded that sulphidic corrosion of copper canister is a key process to be quantified. Contribution of microbially produced sulphide in the geosphere and in the repository materials has been identified as a key remaining question. The envisaged clay rock disposal designs are based on steel as canister material. Corrosion resistance of the iron-based canister materials is based on passivation of the metal surface by a protective oxide layer. In the Belgian supercontainer design for disposal of high-level waste and spent fuel, corrosion resistance is supported by the alkaline pH-value of the surrounding cementitious buffer.

In the MIND project, the key microbial processes of the geological disposal of radioactive wastes are addressed. Work Package 1 focuses on microbiological processes in organic containing long-lived low- and intermediate level waste that require geological disposal. The work package includes combined irradiation and biodegradation experiments of anthropogenic organics within the waste, including halogenated polymers, cellulose, bitumen and ion exchange resins and studies of gas generation and consumption under *in situ* conditions. A review of anthropogenic organic wastes and associated microbiological processes is provided in MIND Deliverable D1.1-1.

Work Package 2 focuses on the microbial processes in a high-level nuclear waste repository, especially the performance of bentonite buffers, metallic canisters and cementitious materials in the presence of microbes. Sources of sulphide and processes affecting sulphide formation are studied, as well as factors limiting the viability of microbes at the high swelling pressure of bentonite. Experiments are performed to study the corrosion of iron embedded in bentonite buffer and degradation of bentonite barriers.

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1 Introduction

1.1 General background

The Implementing Geological Disposal of Radioactive Waste Technology Platform (IGD-TP) has developed the vision, that by 2025, the first geological disposal facilities for spent fuel, high-level waste and other long-lived radioactive waste will be operating safely in Europe (Vision 2025). Long-term safety of a nuclear waste repository is demonstrated by means of a Safety Case containing system descriptions, models, calculations and complementary arguments. The general aim of the MIND project is to adequately include microbial processes in the Safety Case. The two main strategies to reach the target are to improve the understanding on the safety-relevant processes, and to integrate microbial processes in the Safety Case conceptualizations.

The Scientific Technical Work Programme is divided into operative Work Packages (WPs) focussing on the high importance topics identified in the Strategic Research Agenda, (SRA) (IGD-TP, 2011):

WP1 addresses SRA Key topic 2 (Waste forms and their behaviour): Remaining key issues for the geological disposal of intermediate level wastes (ILW) concerning the long-term behaviour, fate and consequences of organic materials in the waste along with H_2 generated by corrosion and radiolysis. The objectives of WP1 consequently are to reduce the uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from long-lived ILW containing organics.

WP2 addresses SRA Key topic 3 (Technical feasibility and long-term performance of repository components): Remaining key issues for the geological disposal of high level waste (HLW) concern the factors controlling sulphide production in the geosphere, including to what extent microorganisms can accelerate canister corrosion in the near-field either by hydrogen scavenging or by sulphide and/or acetate production. Further, it is important to identify conditions (including buffer density) under which relevant bentonites inhibit microbial activity, and to understand whether microorganisms can accelerate degradation of bentonite based buffers and influence the long-term behaviour of plug systems and seals.

WP3 will evaluate and integrate microbial processes towards the conceptualization and performance assessment of geological repositories and in the respective state of the art knowledge base. In addition, the impact of the inclusion of microbiology on expert conceptualisation and public perception of geological disposal will be estimated. The proper contextualization of results will be ensured and remaining key topics within and beyond the MIND project will be extracted by maintaining an active dialogue with stakeholders. Education and information exchange are an essential part of the project outcome.

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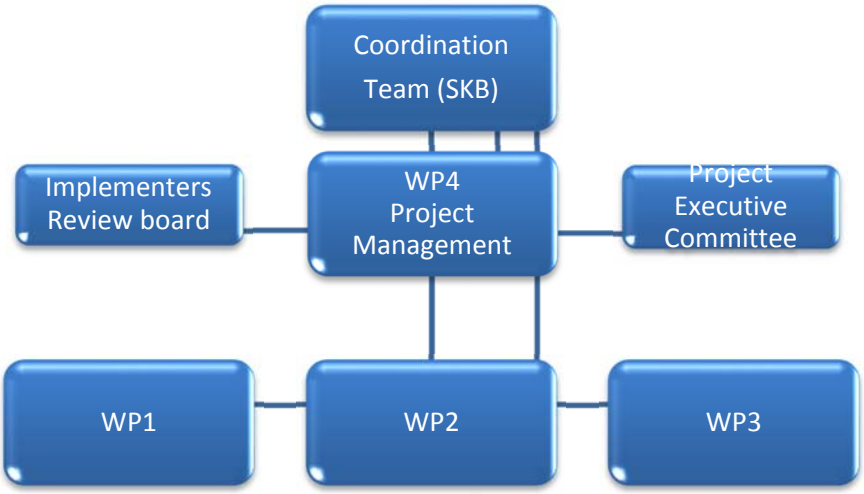

Figure 1: MIND project structure

1.2 Scope and structure of this report

This report is the year one evaluation report on the experimentally and computationally observed and predicted microbial processes in WP1 and WP2 (Deliverable D3.1-1). The first task of WP3, task 3.1, also includes a review of state of art knowledge base on geological disposal with a special reference to the microbiology issues (Milestone M3.1-2). The review aims at summarizing the background on how the research on the safety of nuclear waste disposal has progressed parallel to the evolution of the geological and technical concepts of the disposal. The need to understand geomicrobiological processes was recognized some decades ago, but the integration of different scientific disciplines and production of scientifically consistent models of the geological repository environment, both including microbiology, is still in progress.

The state of the art review is presented in chapter 2 of this report. The scope of the review was intended to be wide enough to demonstrate how different scientific disciplines and different backgrounds of people involved in the nuclear waste management have affected the development of the field. However, the special focus of the review is the interface between geosciences and biosciences. The review leads up to summarize the present state of the understanding of the key questions as a starting point of the MIND project.

Chapter 3, the year one evaluation, summarizes the progress of the work packages 1 and 2. The microbial processes studied and methods used are evaluated in the light of the initial premises.

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2 Geological disposal and microbiology

2.1 General background

Construction of commercial nuclear reactors for energy production started during the 1950's, and the next three decades were the time of rapid growth of the industry. Consequently, there was a growing need to develop concepts for the management of the radioactive wastes. Reprocessing of spent nuclear fuel applied in some European countries is an effective way to recycle useful components and to reduce the amount and activity of the waste, but many countries only have the option of direct disposal of the spent fuel. Interim storage is required by all spent fuel after the removal from the reactors, and the period of the interim storage may be extended if required by the progress of the final waste management program. Nevertheless, regardless of the waste management strategy, there will always be a need for a geological disposal repository. The recycling processes will inevitably generate waste containing the remains of long-lived radiotoxic products. In addition, other sources of long-lived radioactive wastes than those from nuclear energy production, are for example fuel from nuclear research reactors and decommissioning wastes.

General concepts of geological disposal have evolved parallel to the implementation of the nuclear power facilities. Primary requirements of safe geological disposal systems include general stability and good long-term predictability, good isolation and containment properties, as well as availability in terms of depth, volume and homogeneity of the geological formation. The continental crust of the Earth is a heterogeneous puzzle of rock units that have formed in different environmental conditions, evolved and migrated during the geological history, and finally aggregated and emplaced in their present positions. Consequently, different countries have to implement different concepts in developing their national programs for safe geological disposal.

Micro-organisms are known to be major players in geochemical conditions since the early geological history of the planet Earth. The subject of environmental microbiology has for a long time been examining the role of micro-organisms in subsurface environments, but the focus has been in the near-surface conditions of soil and aquatic systems. Marine microbiology has been studying biological processes even in very deep conditions, whereas deep drilling in aquifers and oil fields have confirmed the existence of life deep in the continental bedrock (e.g Onstott et al. 2009). Mining industry has studied the use microbes in extracting metals *in-situ* in the underground mines (e.g. Rossi 1990). A major boost to the study of the deep continental biosphere was, however, given by the introduction of the microbiology of nuclear waste disposal.

Here we aim at summarizing the state of the art of geological disposal with special reference to microbial processes:

- Disposal concepts: geological characteristics and microbiologically relevant properties
- Engineered barrier concepts and their main safety functions
- Microbial metabolism and its known boundaries

The background revised in this study is the earlier research on microbiological processes in nuclear waste disposal. Main source of information were the reports available from the nuclear waste management organisations. Other scientific literature was used, whenever the focus was in nuclear waste management. This review focuses on the geological disposal of HLW. Abrahamsen *et al*. reviewed concepts, processes and inventories of the ILW organic wastes (Deliverable D1.1).

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2.2 Geological concepts

Different potential host rocks have been identified to be suitable for the geological disposal of radioactive waste. In Canada, the proposed host rock for deep geological repository will be horizontally layered, undeformed sedimentary shale and limestone formations spread over large distances. The Yucca Mountain repository in Nevada is composed of altering layers of ignimbrite, non-welded tuff and semi-welded tuff. In European countries, three rock types are currently studied more in detail, namely salt deposits rock, crystalline and clay rock. However, However, as none of the lead partners is specialized in salt deposits, this will not be considered within MIND.

Salt deposits were the first geological formations considered for radioactive waste disposal already during the beginnings of the waste management of energy production. Currently the main site in operation is WIPP (Waste Isolation Pilot Plant) in New Mexico, where transuranic radioactive waste (TRU) from the research and production of nuclear weapons is to be disposed of. Other important sites based on the salt concept are Gorleben and Morsleben in Germany. Bedded salt deposits are free from fresh flowing water and practically impermeable, salt rock also seals all fractures and other openings relatively rapidly.

Even though free water activity is low in salt deposits, there are geomicrobiological aspects that have been discussed. Salt formations may be associated with entrapped brine formations, and the presence of halophile micro-organisms has been reported even in hypersaline environments. On the other hand, salt deposits are typically associated with organic-rich geological formations containing hydrocarbons and indigenous micro-organisms.

Crystalline rock has been considered as the geological disposal option in many countries. Decisions made in Finland and Sweden are firmly based on this concept. The Stripa mine in Sweden was one of the pioneering sites, where the geoscientific basis for nuclear waste disposal in crystalline rock was studied from different perspectives, including preliminary microbial observations. Since the 1980's research has focused on specific underground research laboratories (URL's) providing a more controlled environment for microbial sampling and research in crystalline rock: Grimsel in Switzerland, AECL in Pinawa, Canada, Äspö in Sweden, and Onkalo in Olkiluoto, Finland. Currently Olkiluoto is shifting from a research facility status to the construction site of the Finnish HLW repository. In Sweden the final repository site is planned to be in Forsmark. Czech Republic is also considering deep geological repository in crystalline rock. The concept will be studied and demonstrated in the Bukov Underground Research Facility (BURF) in metamorphic crystalline rock close to a uranium deposit.

Crystalline rocks, if available, are considered to provide stable host rock formations for nuclear waste repositories. Despite their hardness and very low porosity, crystalline rocks contain fractures possibly forming a more or less connected fracture network. Crystalline rock is often described by the concept of dual porosity: fracture porosity providing space for free-moving microbes (> about 1 µm) and matrix porosity, where only dissolved chemical substances and small colloids may exist. Due to the flow heterogeneity, fracture waters of crystalline rocks show compositional variation in different scales, possibly affecting the nutritional conditions and diversity in the immediate surroundings of the cells.

The ***Clay rock*** concept for geological disposal has also been one of the main options since the 1970's. Clay rocks originate from fine grained sedimentary material settling in undisturbed marine basins. Long-term sedimentation processes and subsequent consolidation to rock during millions of years have in many places resulted in the formation of deep and wide-spread homogenous formations, which are considered to be good candidates as host rocks for nuclear waste disposal.

In Switzerland, research focuses currently on Opalinus clay, while Callovo-Oxfordian clay deposit is likely the repository site in France and Boom Clay or Ypresian Clay in Belgium. Underground research

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laboratories have been active in all these countries (HADES in Mol, Belgium, Mont Terri in Switzerland and Meuse/Haute Marne in France).

Clay rocks have excellent properties for the isolation of radioactive wastes and other harmful substances from the biosphere. Dense, very fine-grained rock material is practically impermeable for water flow and advective transport. Clays also typically have the property of self-sealing, by which artificially generated open fractures or reactivated fault surfaces are closed again on saturation of the rock due to the swelling capability of certain clay minerals.

Porosity of a "fresh" clay is high (40-70 %), but decreases during compaction and diagenetic mineral growth (e.g. Opalinus clay has about 20 % porosity). Chemical and isotopic composition of clay pore waters indicate very long residence times. In fact, signs of the chemical composition of original seawater may be still observed in pore waters of clay rocks. Clay rocks might contain microbes from the depositional environments. However, the porosity of a clay can be a strong limiting factor for microbial activity if the pores are smaller than the size of an average bacterium, i.e. approximately 1 µm (Bengston and Pederson, 2016).

2.3 Disposal concepts and case examples

2.3.1 Crystalline rock

Crystalline rocks represent deep sections of the continental crust that is most typically seen on old continental shields, where the long-term erosion has exposed rocks originally crystallized at the depths of more than ten kilometers. Magmatic rocks, e.g. granites, and high-grade metamorphic rocks (gneisses) are crystallized at temperatures above 500 ^{o}C and at pressures of about 5 kbars. Migmatites are partially melted rocks that still may have preserved signs of original sedimentary or volcanic origin. With the uplift, denution and stress release during the more than billion years geological history, crystalline rock become brittle and fractures in different scales have been opened. Free water has occupied the fracture space since the very early geological history and fracture-controlled hydrothermal alteration is a common feature in crystalline rock.

The Olkiluoto site in Finland and the Forsmark site in Sweden are both situated within the about 1.8 Ga Svecofennian orogenic belt. In general terms, the rock type in Olkiluoto is migmatitic gneiss, varying from veined type with partially preserved supracrustal inclusions to a more homogeneous diatexic type. A SW-NE zone of pegmatitic granite inclusions and veins traverse the island, whereas the gneisses vary between tonalitic and micaceous composition (Figure 2). Both Olkiluoto and Forsmark sites have applied structural control principles in their site decision: a relatively uniform bedrock block is surrounded by major fracture zones. In Forsmark the host rock of the planned repository is a granite forming a tectonic lens between major shear zones, whereas in Olkiluoto the island itself constitutes the large scale block. Fractures and other structural feature within the Olkiluoto block have been studied in details (Figure 3), including the hydraulic properties of the fractures and hydrogeochemical characteristics of the fracture waters.

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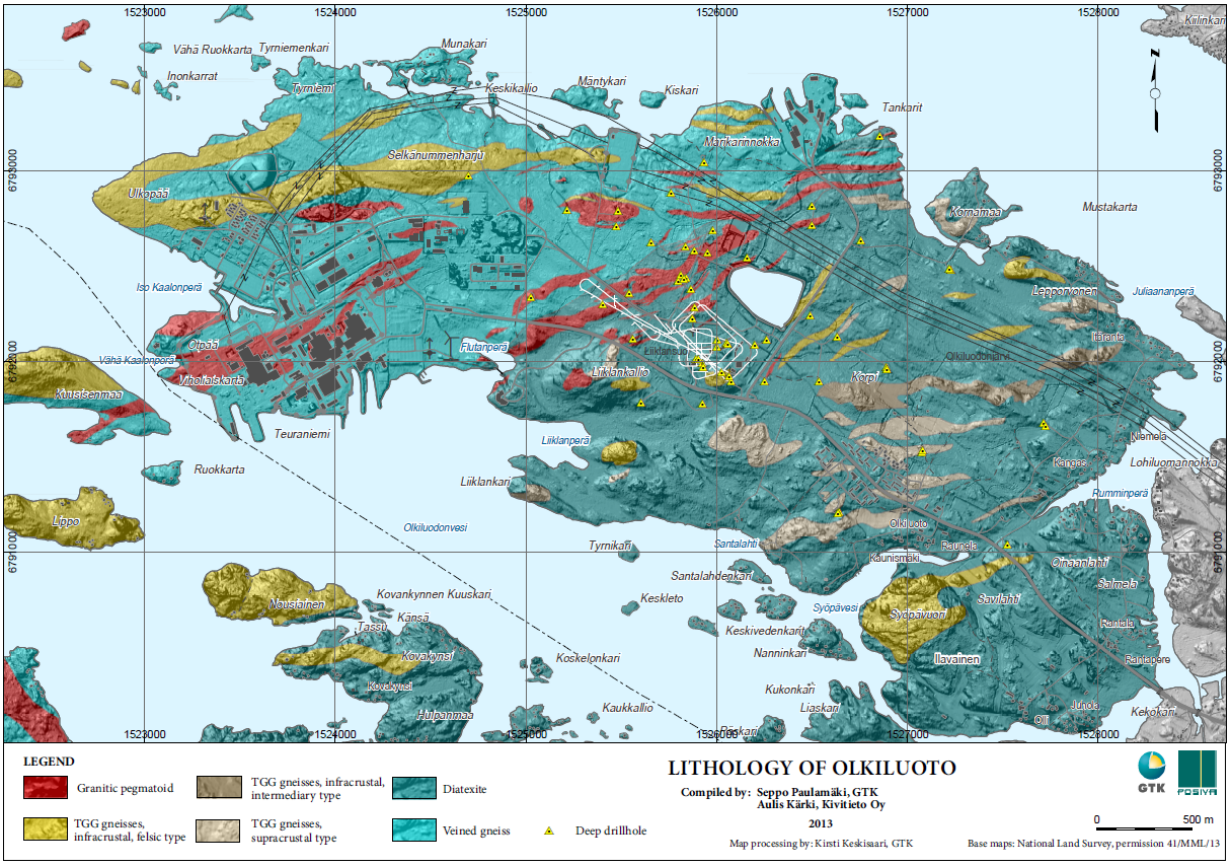

Figure 2: Geological map of Olkiluoto site, Finland (S. Paulamäki, GTK)

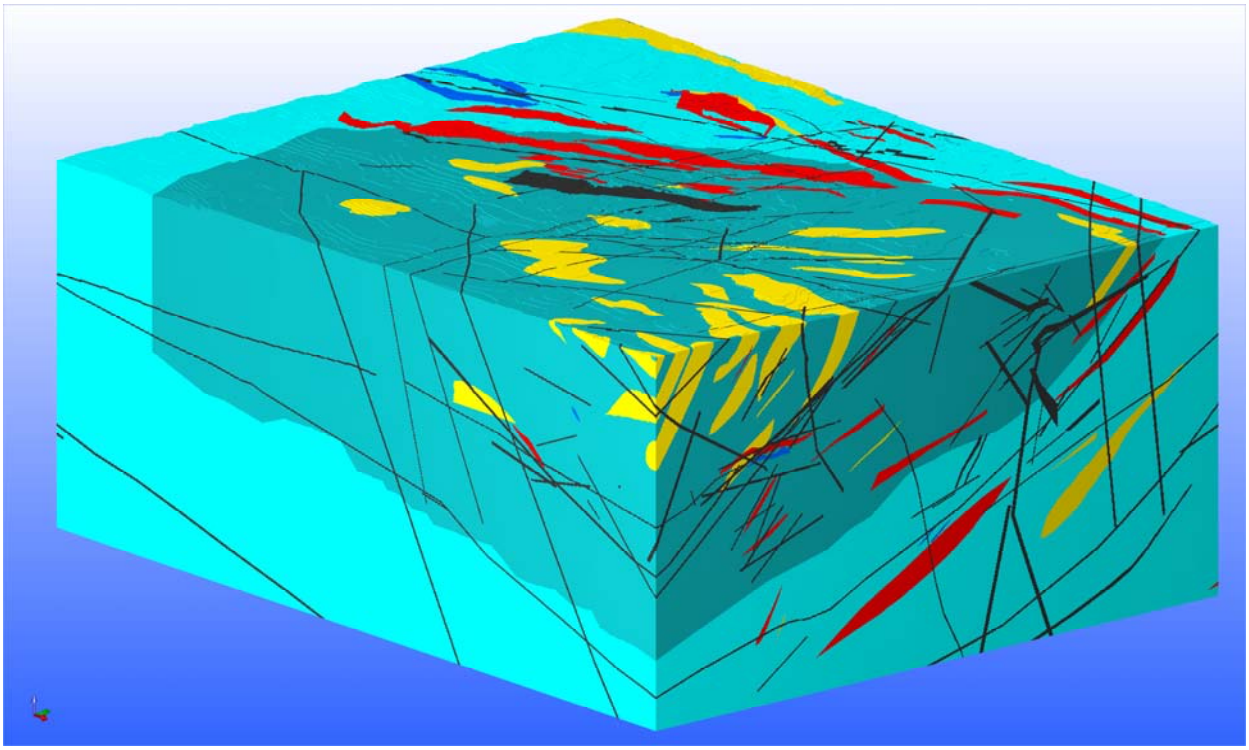

Figure 3: 3-D presentation of Olkiluoto bedrock and main brittle structures, seen from SE (M. Paananen, GTK)

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2.3.2 Clay concept

The Boom Clay in the north-east of Belgium has been intensively studied since 1974 because of its favorable properties to host a geological repository for high- and intermediate-level radioactive waste. Boom Clay provides both a physical (limited water flow) and a chemical (retention) barrier for radionuclide transport. The very low hydraulic conductivity and the low natural hydraulic gradient over the formation make molecular diffusion the dominant solute transport process.

Tertiary deposits form a regular sequence of alternating clay and sand layers where the Boom Clay is the uppermost main clay layer (Figure 4). The Boom Clay belongs to the Rupel group, which has been sedimented during the Lower Oligocene, Rupelian Stage, between 28.4 and 33.9 million years ago. It consists of an alternation of silty clay and clayey silt, with a high content of pyrite and glauconite in the silty layers. The Boom Clay has a typical banded nature, with variations in silt and clay content, carbonates (enriched in so-called septaria-bearing layers) and organic matter.

The Boom Clay is overlain by the Oligocene sands of Eigenbilzen and Voort, belonging to the Upper Rupelian. Above the Oligocene, the Neogene aquifer starts which consists of the Berchem Formation (glauconiferous fine sand, about 25 m thick) and Diestian Formation (about 100 m thick), containing the Diest sands (coarse sand) and Dessel sands (homogeneous fine sand), both glauconiferous.

The main aquifer beneath the Boom Clay is composed by the formation of Lede and Brussel (sand and calcareous sandstone). This aquifer is overlain with the Maldegem Formation, containing the Asse Clay layer, which hydraulically separates the Lede-Brussel aquifer from the sands of Ruisbroek (Zelzate Formation) and Berg (Bilzen Formation). Together, they are often referred to as the Lower-Rupelian aquifer.

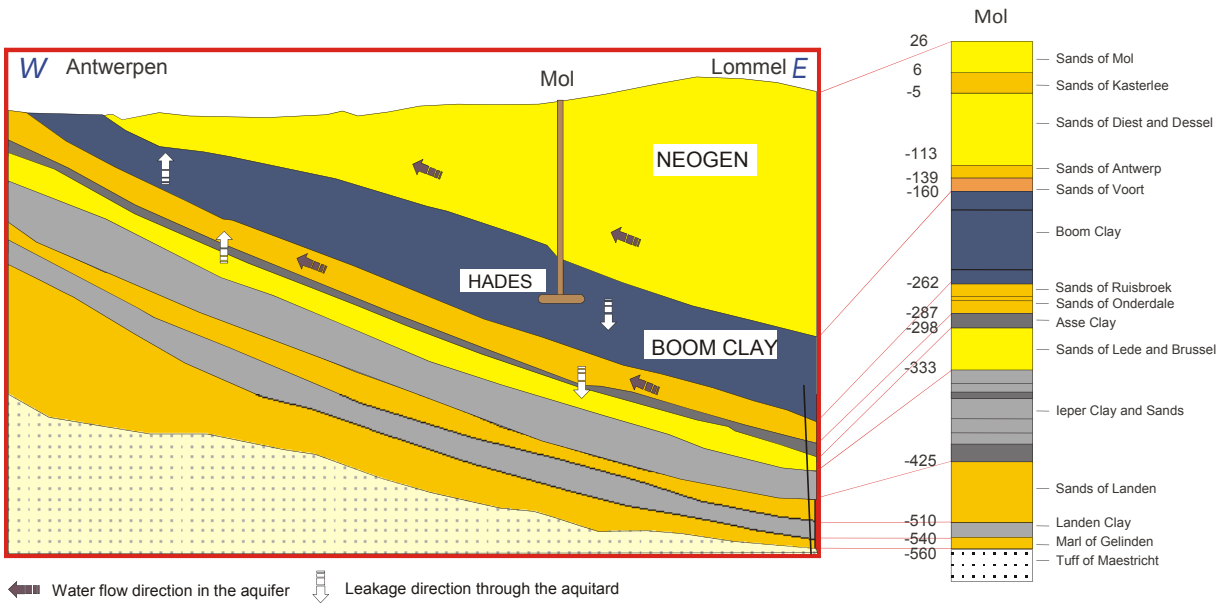

Figure 4: Geological cross-section through the Mol reference site, with indication of the HADES underground research facility.

As an alternative host rock, Ypresian clays are being considered in Belgium. The Ypresian clays sedimented during the first stage of the Eocene, between 55 and 49.6 million years ago, and form a clay sequence which groups the Kortrijk Formation and the Kortemark Member of the overlying Tielt Formation.

The Mont Terri rock laboratory, situated in the north-west of Switzerland, is located in the 180 million years old Opalinus Clay. Opalinus Clay is a mainly marly claystone with differing proportions of sand and carbonates. It is underlain by the calcareous layers of the Liassic and overlain by the Passwang Formation.

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The Opalinus Clay was formed in a shallow sea with an average water depth of 20 to 50 m. During storms or due to changes in the direction of the current, in some areas fine sand layers were washed in from the mainland. This resulted in the presence of three facies of Opalinus Clay, namely the shaly facies, the sandy facies and the carbonate-rich sandy facies. In the north, the shallow sea was bordered by the Rhenish Slate Mountains and, to the east and south, by the Bohemian Massif and the Alemannic Islands respectively. The deposition zone roughly covered a triangle between Bern, Munich and Strasbourg. Today, at the rock laboratory, the Opalinus Clay has a layer thickness of around 140 m. The period of deposition for this layer package was around 400,000 years.

The 130 m thick Callovo-Oxfordian clay layer, selected as potential host rock in France, is formed around 155 million years ago and is a deep clay layer in the Paris basin. This basin is shaped like a bowl filled with a succession of sedimentary layers the oldest of which are located on the edges and the bottom of the bowl, and the most recent at the centre. The Callovo-Oxfordian formation, located at a depth of between 400 and 600 m, is overlain and underlain by poorly permeable carbonate formations, respectively the Oxfordian and Dogger limestone formations. It comprises mostly argillite, a very fine-grained sedimentary rock formed at the bottom of seas and lakes.

2.4 Engineered barriers

2.4.1 Waste types and waste forms

Regarding the source, main categories of long-lived high-and intermediate-level nuclear waste are the spent fuel, reprocessing waste, operational waste from power plants and, finally, decommissioning waste. Primary spent fuel is composed predominantly of uranium dioxide (UOX) whereas MOX fuel is manufactured from plutonium recovered from used reactor fuel, mixed with depleted uranium.

In the reprocessing option, uranium and plutonium are extracted for further use and the reprocessing waste is divided into different fractions. High-level reprocessing waste is about one order of magnitude less active than spent fuel, but still needs very long time of isolation from the biosphere. Consequently, vitrification into a glass or ceramic matrix is often considered as the principal immobilization technique for HLW. Due to its capacity to immobilize radionuclides, bitumen is often selected as an encapsulation matrix, mainly for liquid wastes and sludges. Similarly, cement based materials supports the containment of the radioactive materials, mainly attributable to the high pH of the cement.

In the following part, waste types produced by Belgian and Fennoscandian nuclear power industry are described. A detailed characterization of the organic waste types in European countries was given by Abrahamsen et al. (D1.1.).

In Belgium, radioactive waste of category B (long-lived waste) and C (heat-emitting waste) is destined for geological disposal in a suitable host formation. Category C waste consists of vitrified waste from former reprocessing contracts with AREVA (before 1993). Spent U in the OX and MOX fuel would also be classified as category C waste, however its status (waste or resource) is currently not established. Compacted cladding and technological waste from reprocessing is categorised as category B waste.

Apart from the waste from nuclear energy production at the nuclear power plants of Doel and Tihange, Belgium also has historic waste from the former Eurochemic reprocessing pilot plant at Dessel. This resulted in vitrified and cemented (PAMELA) waste, and bituminised (EUROBITUM) waste, classified as category B waste. The volumes of these waste streams after 40 years reactor lifetime are given below.

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Table 1: Waste type and volumes in Belgium

Type	Amount
Spent fuel UOX	4643 tHM / 10226 assemblies
Spent fuel MOX	66tHM / 144 assemblies
Vitrified waste (CSD-V)	390 canisters
Compacted waste (CSD-C)	432 canisters
EUROBITUM	13550 canisters
PAMELA vitrified	2201 canisters
PAMELA cemented	134 canisters

These amounts can increase to some extent in view of the recent prolongation of the service life of the Doel 1 and 2, and the Tihange 1 reactors. Furthermore, waste that does not comply with waste acceptance criteria for surface disposal will also be allocated to the geological disposal facility.

In Finland and Sweden, nuclear wastes from power plants is divided in two main categories: unprocessed spent fuel (UOX) and low-level waste (LLW)/ILW waste produced by the power plants during the operation. Spent fuel is in the form of uranium dioxide pellets packed in corrosion resistant metal tubes. These spent fuel rods are bundled together in fuel assemblies (Figure 5) that will be encapsulated in copper-iron canisters after the use and an initial cooling phase.

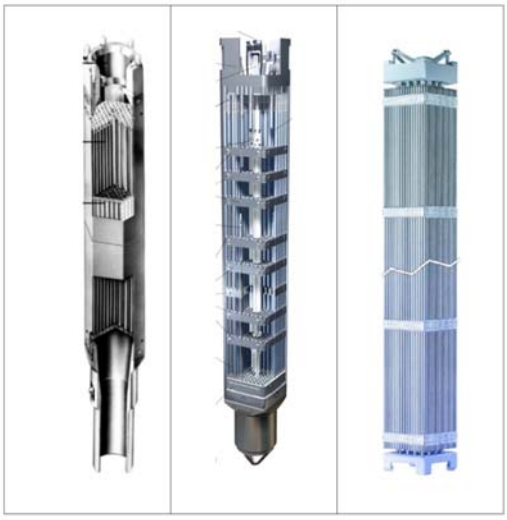

Figure 5: Spent fuel assemblies (Posiva Oy)

Both Sweden and Finland have final repositories for power plant operational waste. The Swedish SFR repository for low and medium level waste at Forsmark and VLJ-repositories in Finland (Olkiluoto and Loviisa) are tunnel – silo constructions about 50 – 100 m below the ground surface. The waste deposited in the LLW/ILW repositories comes from the operation of the nuclear power plants. It can include bituminized ion-exchange resins, filters that have collected radioactive substances from reactor water and maintenance waste, like tools and protective clothing. The waste is typically packed in steel drums and/or concrete boxes.

2.4.2 Canisters

All disposal concepts rely on different multi-barrier systems, in which the engineered and geological barriers aim at confining radionuclides. For example, in direct disposal, spent fuel pellets inside corrosion resistant claddings are encapsulated in metallic canisters supported by a cast iron insert which are surrounded by bentonite buffer and the host rock.

The main options of metals are iron (alloys) and pure copper. The primary reason for choosing copper was the general resistance against corrosion in oxygen free conditions. However, the strong affinity of copper to form sulphides remained an issue to be considered carefully. The present

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canister concept in Sweden and Finland has copper as the corrosion resistant outer wall and a cast iron insert (Figure 6). Steel canisters may be subjected to different types of corrosion phenomena. Contrary to copper, metallic iron can be oxidized in water, thereby producing hydrogen. The oxidation resistance of iron based alloys can be increased by alloying with components that are capable of forming a protective oxide layer on the surface that impedes further oxidation. Consequently, it is more difficult to form a protective oxide layer in reducing conditions, making the alloy less resistant to corrosion. However, the uniform anaerobic corrosion rate (i.e. the main corrosion mechanism under repository conditions) will be decreased at high pH as the steel becomes passivated.

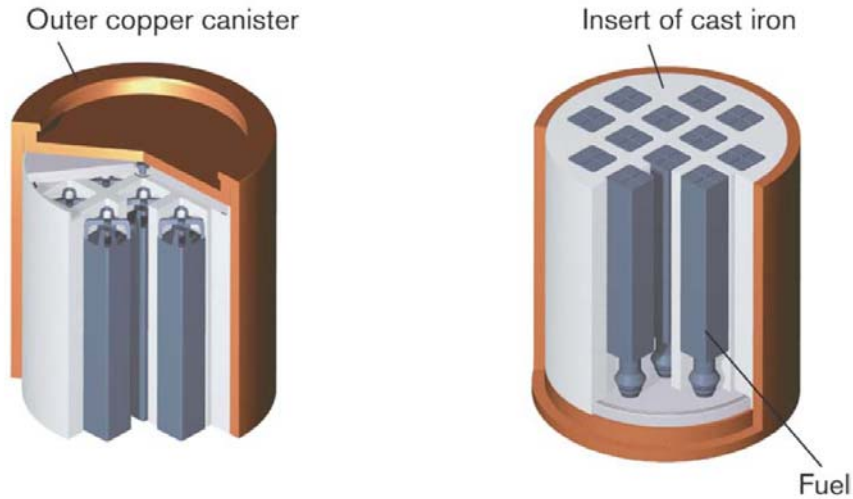

Figure 6: Spent fuel assemblies in copper-iron canister (King et al. 2010)

The ***Safety function*** of the canister is the containment of harmful substances. In terms of the service life, design bases of the canister materials are different: stability of copper canisters is based on the electrochemical nobility of the metal with respect to water. Copper canisters are aimed to maintain containment until the radionuclides of uranium and thorium series become predominant and mobile nuclides disappear, in 10^5 – 10^6 years. Iron canisters provide containment of the very highly radioactive components (e.g. isotopes of Pu, Cs, Sr) and creates a very reducing environment that keeps long-lived redox-active radionuclides immobile. In thermodynamic terms, corrosion of iron can be hampered by passivation due to an oxide coating as schematically shown in Figure 7.

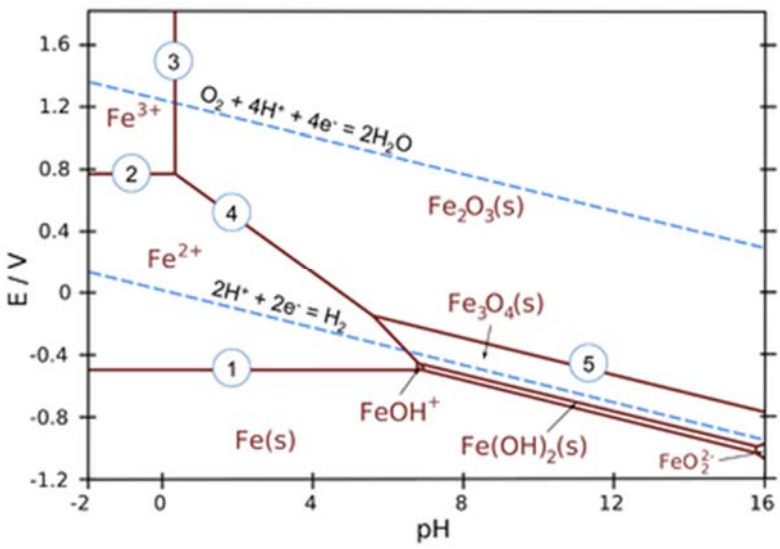

Figure 7: Pourbaix diagram of iron a ionic concentrations of 1 mM

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2.4.3 Buffer

Buffer material fills the open space between the deposition hole and the canister/waste form barrier. Bentonite is the typical buffer material in crystalline concepts, whereas cement buffer is used in clay rock repository concepts. By definition, bentonite is a clay material consisting predominantly of swelling smectite minerals (in practice montmorillonite). The ability to swell to a certain pressure is considered as a design criteria of the buffer. Compacted bentonite is practically impermeable to water and diffusion is considered as the only relevant transport mechanism in compacted bentonite.

The ***Safety function*** of the buffer is to protect the canister from a contact with free water and corrosive agents. The buffer also shields the canister mechanically against small bedrock movements. In case of some iron (steel) canister concepts, cement-buffer shields the canister effectively, because the high pH preserves passivating oxide coating on the surface of the canister (figure 7). However, it needs to be investigated to what extent microorganisms can accelerate the degradation of bentonite based buffers. Microbial activity is correlated with bentonite density and its resulting water activity. In addition, different commercial bentonites have been shown to display varying effectiveness in mitigating microbial activity at similar densities. This variability may be due to sulophate or organic matter content in the bentonites or to intrinsic differences in the swelling pressures obtained. Consequently, the conditions under which relevant bentonites inhibit microbial activity need to be identified. Both remaining issues are scrutinized within WP2 of MIND.

Bentonite clay has favourable properties as a diffusion barrier. The material consist of very thin montmorillonite flakes with a maximum diameter of about 200 – 400 nm. The flakes consist of stacked lamellas separated by interlamellar spaces (< 0.5 nm). Wetted bentonite swells developing high pressure in closed space. At high swelling pressures porosity is predominantly interlamellar. Additionally, at high swelling pressure (high dry density) much of the porosity is not available for anions because of the positive layer charge of the smectite structure (Figure 8).

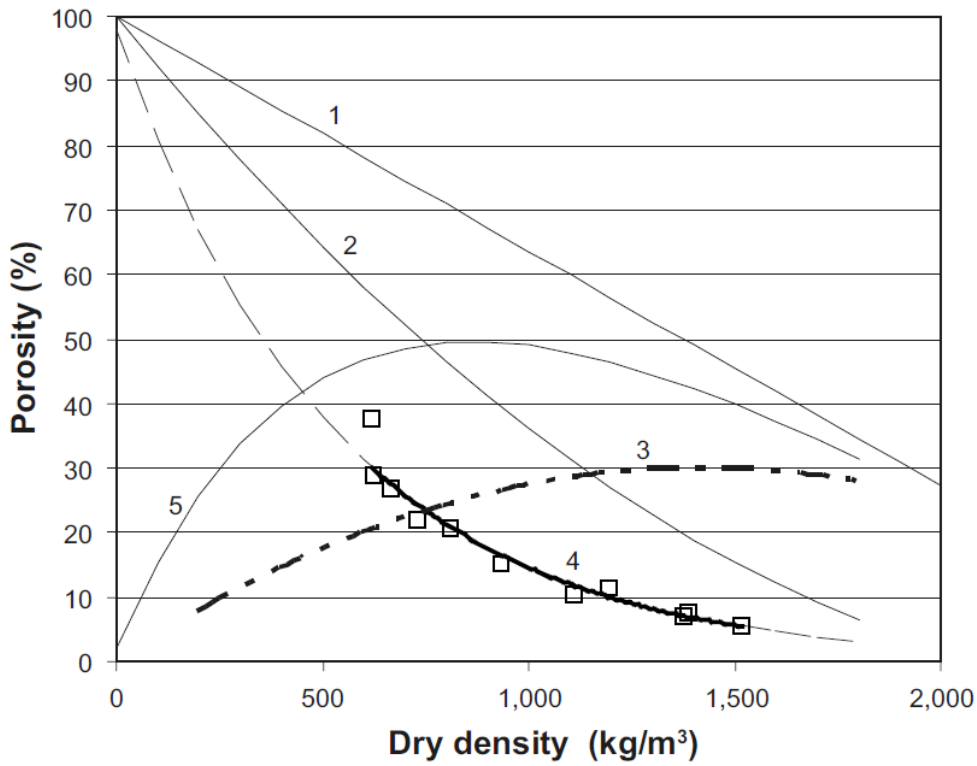

Figure 8: Different pore types in bentonite (Muurinen 2006). 1) total porosity; 2) external porosity (non-lamellar); 3) interlamellar porosity; 4) chloride porosity ; 5) porosity not accessible for chloride

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2.4.4 Excavated rock

Rock excavations are an essential part of the engineered repository system. Tunnels will stay open for long periods during the operation of the disposal facility. Excavation of the tunnels may produce some new fractures around the tunnels. Oxygenated conditions during the operation affect the excavated zone (EDZ) geochemically and microbiologically. Finally tunnels are filled, cementitious materials, crushed host rock and swelling clays are the main options for filling material. Concrete plugs are considered as additional barriers of transport. Oxygen-consuming microbes invaded during the construction phase can then only use up oxygen entrapped in the EDZ.

2.5 Repository designs

Geological boundary conditions, waste types and amounts, as well as an appropriate strategy in designing the technical barrier system has led to different repository designs. Different rock types have dissimilar properties, thus leading to different disposal concepts. Availability of rock volume in terms of well-defined homogeneity must match the space requirement. Repository depth have to meet the safety requirements in order to isolate the waste for man and environment as long as necessary. In the following, details are given for two different repository designs representing different geological conditions.

The envisaged repository in Boom Clay in Belgium consists of a series of rectilinear galleries, in which the waste will be disposed, situated around the midplane of the Boom Clay formation (as a working hypothesis). This corresponds to a depth of about 230 to 240 m at the reference site. Access to the disposal galleries is provided via the centrally located access gallery, to which all disposal galleries are linked (see Figure 9).

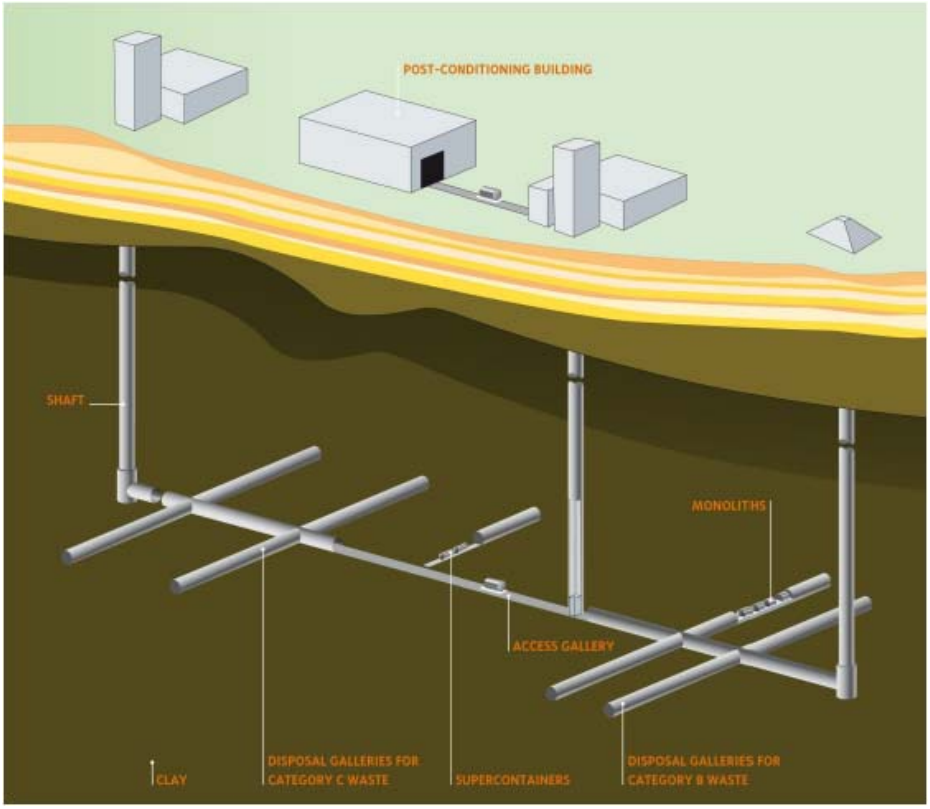

Figure 9: Indicative diagram of the geological repository envisaged for B&C waste and of the surface facilities for the production of supercontainers and monoliths B (figure taken from the ONDRAF/NIRAS RD&D plan, 2012)

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The engineered barrier design is based on the Contained Environment Concept, in which the waste is contained in a prefabricated concrete buffer. These shielded waste packages are called 'supercontainer' and 'monolith-B' for category C and B waste, respectively. In a supercontainer, two vitrified waste canisters or 4 UOX fuel assemblies (only 1 for MOX) are contained within a 30 mm-thick carbon steel overpack, which is inserted into a prefabricated concrete buffer based on ordinary Portland cement (Figure 10).

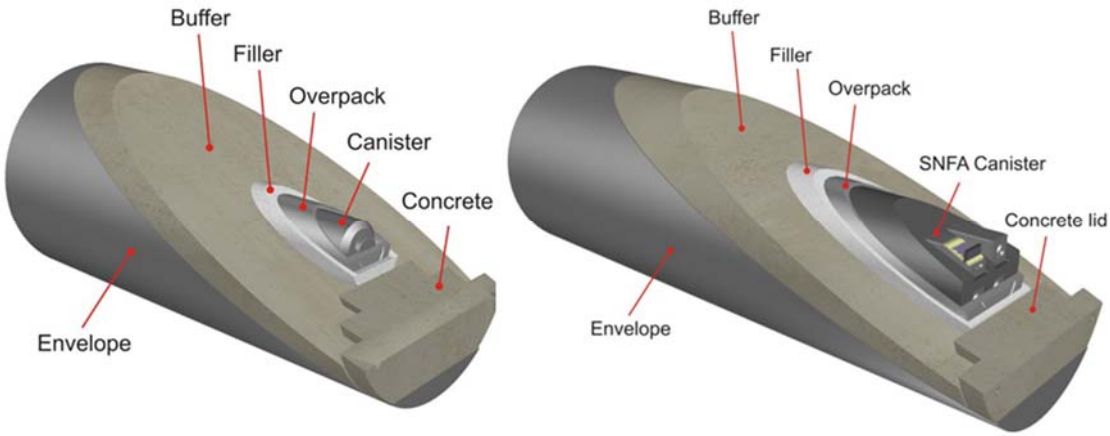

Figure 10: Supercontainer for vitrified high-level waste (left) and UOX irradiated fuel (right) (figure taken from the ONDRAF/NIRAS RD&D plan, 2012)

A monolith-B can accommodate 10 200 L drums (2x5 per cross-section) in case of EUROBITUM waste, or 8 CSD-C canisters in case of compacted waste (Figure 11).

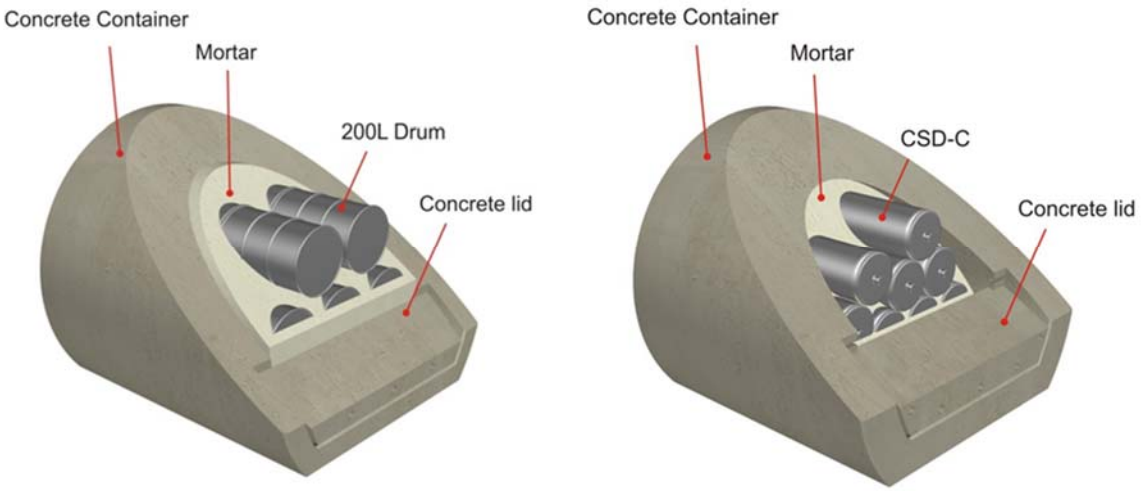

Figure 11: Monolith B for category B waste (200 L drums, left) and CSD-C (right). (figure taken from the ONDRAF/NIRAS RD&D plan, 2012)

The French concept for the disposal of B wastes is modular, permitting the separation of different types of package. Type B cells are sub-horizontal tunnels constructed , with a maximum length and diameter of 250 m and 12 m respectively at a depth of 500 m, in which packages are stacked on several levels (Figure 12). A concrete lining provides the structure with mechanical stability. Concrete also provides a chemical barrier function for the packages and thus contributes to the retention of radionuclides. There is limited clearance between the package and the disposal cell and this small volume will not be filled, in order to facilitate the closure operations and, if decided at a later date, possible retrieval of waste packages.

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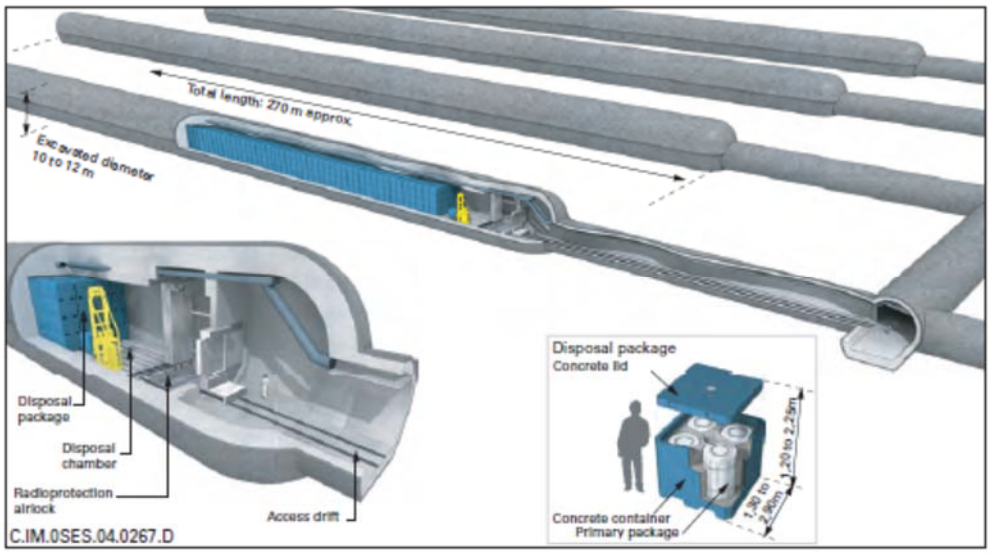

Figure 12: Illustration of disposal cells proposed for disposal of L-ILW in France (Andra, 2005).

In Switzerland, the plans are to dispose HLW and ILW in different areas of the same repository at a in the Jurassic-age Opalinus Clay. The repository features a ramp access system, with separate panels for emplacement of HLW and a zone for ILW waste disposal. The HLW repository comprises disposal tunnels with a diameter of 2.5 m at a maximum depth of 700 m. The repository for ILW consists of a test area with a facility for underground geological investigations, a pilot facility and a main facility with large disposal caverns**.** These installations are located at a depth of around 400 metres (Figure 13).

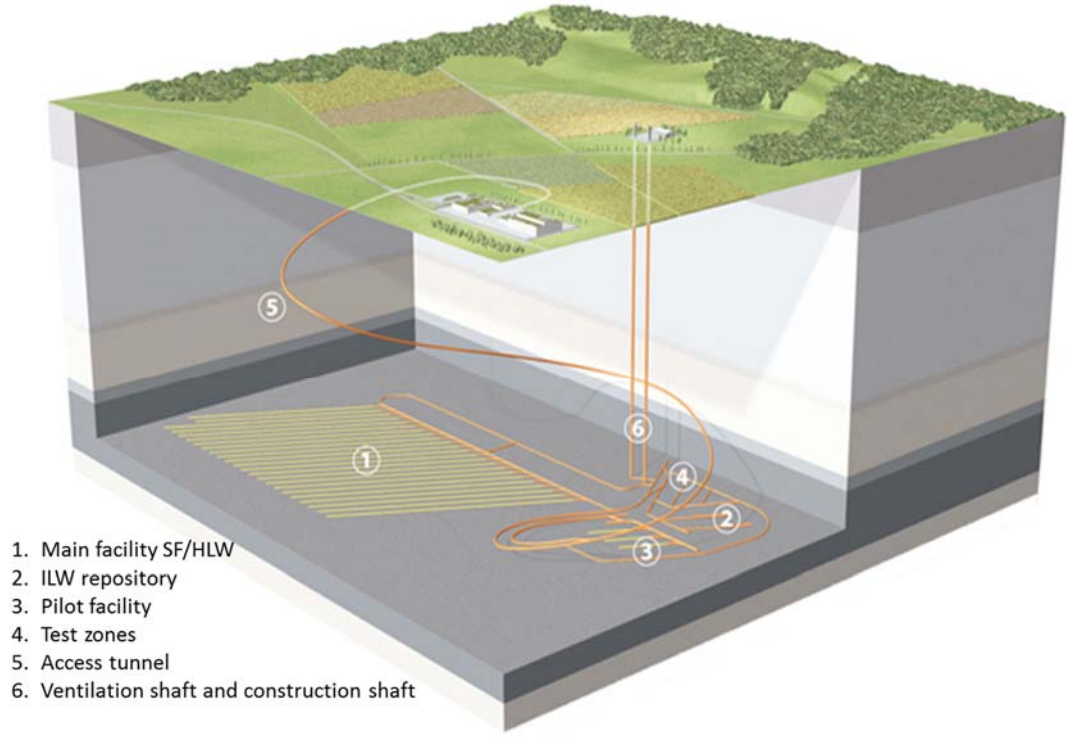

Figure 13: Illustration of Swiss concept for geological disposal of radioactive waste (www.nagra.ch)

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The crystalline repository concept planned in Sweden and Finland differs considerably from the clay repository concept. Site selection in the crystalline concept is based on defining the network of major fracture zones that surround a solid, non-porous bedrock block. However, small possibly water-conducting brittle fractures are always present to some extent.

Crystalline concepts in Sweden and Finland are based on a tunnel network excavated in the depth of several hundred meters below the surface. In Olkiluoto the access tunnel (Onkalo) is finished.

Each copper canister will be placed in an own deposition hole, and the space between the canister and hole wall is filled with precompacted bentonite blocks (Figure 14).

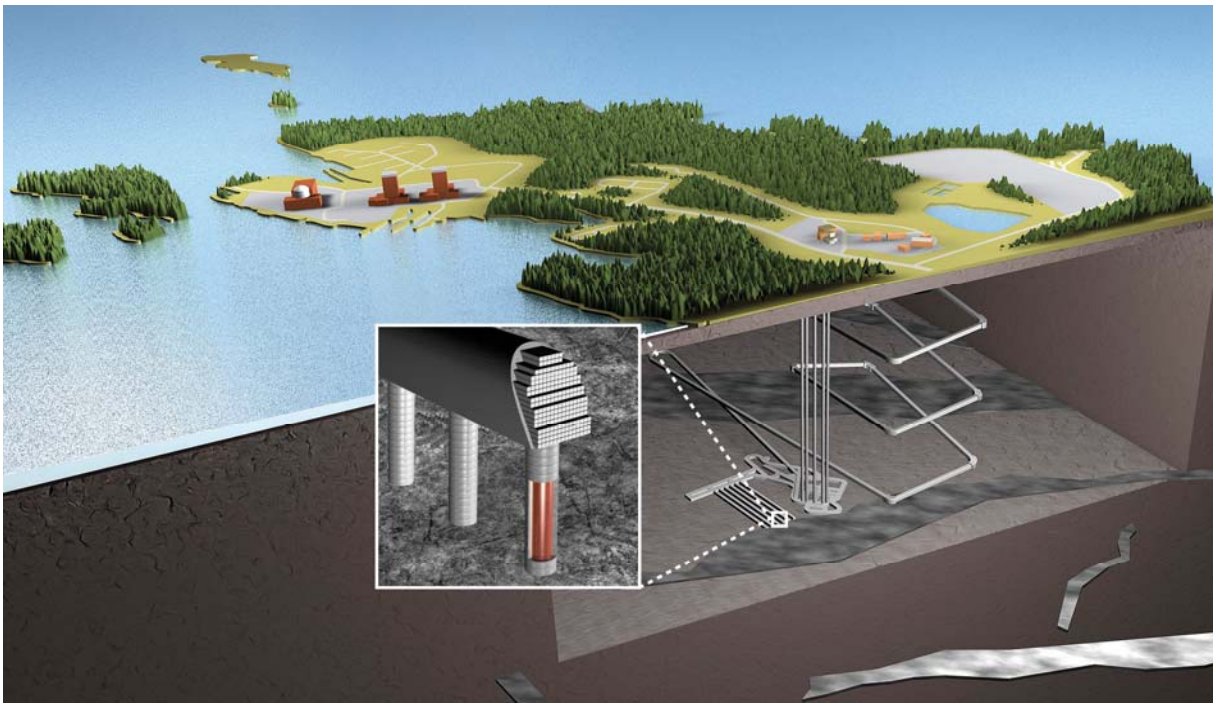

Figure 14: Final disposal at Olkiluoto (Posiva Oy)

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2.6 Microbiological studies in nuclear waste management

2.6.1 Microbial studies in Clay rock

The requirement of safe management and final disposal of radioactive waste launched studies on radionuclide migration during the early 80's. Possible effects of microbial activity on the safety of geological disposal were pointed out in a quite early phase of the research (e.g., West et al 1982; West & McKinley 1983). On the other hand, the multidisciplinary challenge to describe and model radionuclide migration in geological systems had to start from simplified concepts. For example, within the International project MIRAGE (Migration of radionuclides in the geosphere, coordinated by the Commission of European Communities (CEC)), microbes constituted a general geochemical agent that may be effective in both retardation and mobilization of radionuclides (Come et al. 1986). Thus transport modelling to describe migration phenomena considered "Colloids and microbes" as a passive parameter. Microbial metabolic processes were not accounted for.

McKinley *et al*. (1985) made an overview of the potential consequences of microbial activity in a generic (Swiss) nuclear waste repository for immobilized LLW/ILW in steel drums and for reprocessed HLW in thick-walled iron canister. Contribution of microbiological processes in degradation of structural materials was discussed and oxidative biocorrosion processes were emphasized (e.g., *Thiobacilli*). Enhanced mobility of key nuclides due to the complexation by microbial by-products was considered to be an important factor and resident micro-organisms in the far-field were considered to potentially act as “living colloids”. Iron corrosion due to sulphate reduction was considered as a reaction requiring either metallic iron or organic material as the electron donor. All in all, the importance of microbial processes for LLW/ILW wastes was emphasized.

McKinley *et al.* (1985) also summarized microbial sampling efforts performed by that time, much of the sampling being associated with the MIRAGE program. Sampling in the depth of 220 m in Boom Clay (Belgium) didn't show any detectable microbial activity in contrast to unconsolidated near-surface clay. In general the number and types of microorganisms was observed to decrease when salinity of water increased. Later on, biogeochemistry was included in the CEC-ANDRA project ARCHIMEDE-ARGILE, aiming at a better understanding of the water chemistry in clay environments, e.g., by testing and development of methodologies for water sampling and physico-chemical determinations. The HADES underground research laboratory in Boom Clay became a major target of the studies. Microbial sampling was done in the Boom Clay research tunnel at the depth of 224 m by means of horizontal core drilling (20 m) without water (Boivin-Jahns et al. 1996). The core was divided to sub-sections for microbial studies. The results indicated that bacterial densities and activities decreased with increasing depth from the tunnel wall.

Indigenous microbes in Opalinus Clay, Switzerland were also studied by coring ”undisturbed” clay samples in the Mont Terri Underground Laboratory (Mauclaire et al. 2007, Stroes-Cascoyne et al. 2007). Importantly, an indigenous community is not necessarily as ancient as the host rock deposit itself. Natural geological processes can introduce microorganisms within the host rock formations. Microbial studies of the aseptically cored clay rock sample did not indicate clearly viable microbial community in Opalinus clay, but the presence of a metabolically inactive (dormant) population was suggested. More recent attempts to provide an unambiguous answer on the question whether there is an indigenous microbial population present in the clay were also unsuccessful. In addition, physico-chemical evidence rather suggests that ancient undisturbed Opalinus Clay is far too restrictive (in pore size and water activity) to host microbiological life, except perhaps in areas where those restricting factors are less severe (e.g. fractures) (Leupin O. X *et al,* to be published in 2017). Nevertheless, it is clear that microorganisms are present and active in all borehole experiments performed at the Mont Terri Underground Laboratory, indicating that there are other sources of introduction of microorganisms in the repository (Bagnoud, 2016 a,b).

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Also in Boom Clay (HADES Underground facility) borehole water samples from different layers indicated the presence of a diversified and active microbial population in all water samples studied (Wouters et al. 2013). Although at least part of the community is probably introduced during the installation of the piezometers, the community can survive and be metabolically active and therefore has to be considered when radioactive waste will be disposed. Recently it was shown that a suphate reducing bacteria (SRB) community remained viable and active in Boom Clay with increasing densities. The amount of sulphide produced was reduced from a density of 1900 kg/m³ but the activity was not virtually inhibited, not even at a density of 2000 kg/m³. Although, most of the observed sulphate-reducing activity occurred with lactate, which is not present in Boom Clay, the study suggests that SRB can be active and produce sulphide in a Boom Clay repository for as long as sulphate is available. Nevertheless, there are some remaining open questions for future investigation (Bengtsson and Pedersen, 2016).

2.6.2 Microbial studies in Crystalline rock

2.6.2.1 Crystalline rock of the Fennoscandian Shield

The Stripa mine may be considered as one the first underground research sites for studying processes relevant to nuclear waste disposal in a natural environment. In the mine, there was access to a deep overflowing drill hole from the level 410 m to the depth of 1240 m. Deep sections of the drill hole were isolated by packers to collect inflowing waters to laminar-flow reactors. The results indicated that microbes have a strong tendency to attach on the surfaces. In addition, their metabolic activity was demonstrated and their genomic structure was studied by molecular biological methods (Ekendahl et al. 1994).

During the late 80's, microbiology established a firm foothold in Swedish and Finnish nuclear waste disposal programs. Progress during the first decades was summarized by Pedersen (2000). A strong network between research groups was established, including the Pinawa URL in Canada. Deep sampling sites for microbiology included four targets of Posiva's (formerly TVO's) site selection program and the Palmottu natural analogue site in Finland, several drill holes around Äspö, SW Sweden and the Stripa mine. Hydrogeochemical and microbiological sampling programs in both countries called for new methods to obtain representative fluid samples from deep bedrock fractures, and special equipments were constructed both by SKB and Posiva. A delicate (triple tube) drilling technique was taken in use. Careful monitoring of the drilling process (e.g. tracers in drilling water) became a common practice in drilling of deep drill holes for research purposes. As a result, an extensive and representative collection of microbial samples from the Fennoscandian Shield deep fracture waters became available.

Äspö, Sweden

Since its construction phase in the 1990's, the Äspö hard rock laboratory (HRL) in Sweden has been a major site for underground research of nuclear waste disposal, including a redox experiment, in which microbial oxygen consumption was studied (Puigdomenech et al. 1999). The microbe site at the depth of 450 m hosted the Microbe project (1999-2010) (Perdersen, 2013). The Microbe laboratory and the main outcome of the 10-year research programme were presented and summarized by Pedersen (2013). Fracture waters were collected from three packed-off drill hole sections (formation pressures around 26-32 bars and flow rates 0.4-1.5 L/min), and conducted into the microbe laboratory in the tunnel. Chloride and sulphate concentrations of the flowing water increased slightly during the 9 years period reaching the levels of 300 mM and 6 mM, respectively. The gas volume in the water was around 60 – 80 mL, with N_2 and He as the main components and CO_2, CH_4 and H_2 as minors. The total number of microbial cells (TNC) varied with time and between different fractures, having an arithmetic average of about 10^5 cells/mL. Microbial processes were also examined by a circulation system, in which the effects of different experimental parameters

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(additions of H_2, acetate and lactate) could be monitored. Sulphate reduction was demonstrated to be an active process in presence of the added electron donors. Viruses were found to be abundant in deep granitic groundwater at the Aspö Hard Rock Laboratory, and they were observed to be able to break down cell membrane of the indigenous bacterium Desulfovibrio aespoeensis in Aspö groundwater (Eydal *et al.* 2009).

Äspö HRL also hosted the "alternative Buffer Material (ABM) experiment", focussing on differences in long-term buffer behaviour and stability of different clay materials. In addition to bentonites, consolidated claystones were also tested (COX, OPA). Stroes-Gascoyne et al. (2014) reported the microbiological observations in these experiments. The culture results showed that the highest to lowest culturability order in all samples was: Asha 505 > Febex > MX-80 (LOT) > Ikosorb > Ibecoseal > Dep-CAN > Rokle >Friedland > MX-80 (Canada) > Calcigel > Kunigel > COX > OPA.

Olkiluoto, Finland

Within the Finnish site selection program, Olkiluoto site was chosen as the site for studies, and construction of an underground tunnel started in 2000. The tunnel, called ONKALO, was since then subjected to an extensive characterization program, which also included detailed microbial studies. The results of microbial characterization of ONKALO up to 2010 are summarized by Pedersen et al. (2012). The sampling procedures were adapted to drilling and tunnelling. Sampling methods included pumping of deep drill holes, sampling with down-hole samplers, and draining of pressurized drill holes.

The diversity of microorganisms in Olkiluoto groundwater was initially determined from the MPN analyses. In recent years, DNA-based analyses which included qPCR, DGGE, gene cloning and sequencing and 454 high throughput pyrosequencing were added with increasing frequency to the diversity analysis programme. Feasibility of each method was evaluated, including strengths, weaknesses, reproducibility and detection range. From 1997 up to the summer of 2010, a total of 153 microbiological samples were analysed. The deepest samples were taken from a depth of about 800 m, so that the data set is extensive both in space and time. The TNC numbers ranged from approximately 3×10^3 up to 2.5×10^6 cells/mL, and the cell numbers correlated well with ATP determinations. TNC numbers show a weak decreasing trend with the sampling depth, but the general decrease of TNC with time was more obvious (from about 4×10^5 to about 2×10^4 cells/mL).

This extensive data set allowed Pedersen *et al.* (op.cit) also to make a conceptual model of the depth distribution of different microbiological processes and corresponding physiological groups (Figure 15). Aerobic heterotrophs and nitrate-reducing bacterial prevail in the near surface bedrock, as well as methane oxidizing bacteria. There seems to be a minimum of bacterial activity between the clearly oxygenated zone and strictly anaerobic zone. This suboxic zone is occupied predominantly by acetogens and iron reducers. Sulphate reduction zone lies under iron oxidations. Methanogens seems to be distributed evenly through the depth range.

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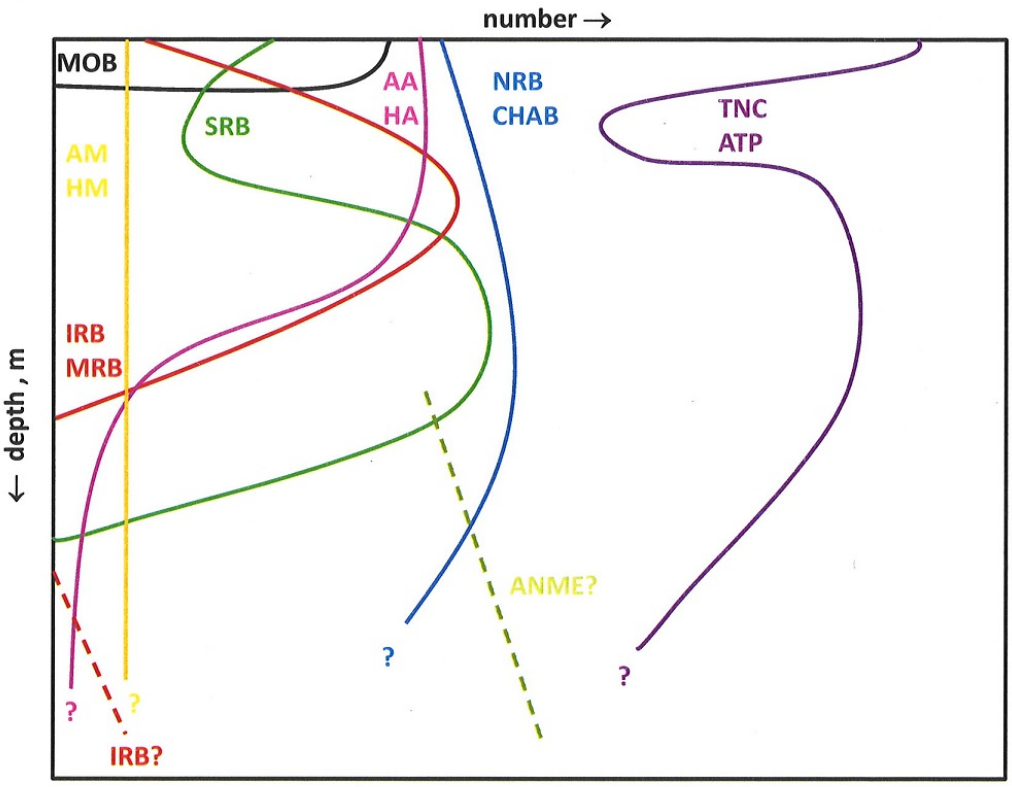

Figure 15: Relative depth distribution of microbes in Olkiluoto (Pedersen et al. 2012). TNC = total number of cells, ATP = Adenosine triphosphate, CHAB = cultivable aerobic heterotrophs, MOB = Aerobic methane oxidizers, NRB = nitrate reducing bacteria, IRB = iron

KYT-programmes in Finland, Outokumpu Deep Drill hole

Studies of deep life in crystalline bedrock have been a part of the Finnish research programmes on Nuclear Waste Management since 2007 (KYT2010, KYT2014, currently KYT2018). The main study site has been the 2500 m deep Outokumpu research drill hole in eastern Finland. Since the drilling in 2004 – 2005, the borehole has been a target of varied geoscientific studies (Kukkonen 2011), including hydrogeochemistry, gases and microbiology. Hydrogeologically Outokumpu Deep drill hole is slightly discharging in natural conditions. In the course of time, water in the borehole is becoming more saline due to brines discharging from several different fracture zones, and gases are continuously discharging from the borehole collar. Deep life and gases in Outokumpu have been studied in numerous samplings by taking tube profiles along the hole, pumping from packer-isolated sections, or using in-situ samplers. Water chemistry, gas composition and microbiology (Figure 16) show significant variation as a function of the depth. Currently, Purkamo (2015) summarized the microbiological research, and geochemistry of gases are presented by Kietäväinen (2016).

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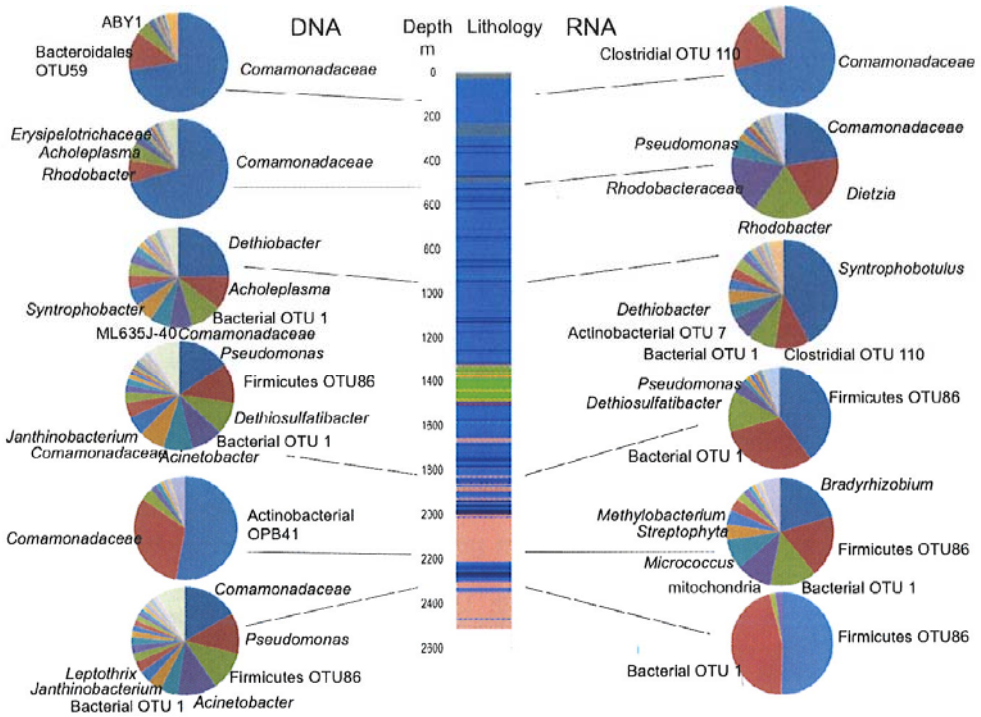

Figure 16: The bacterial community structure of the studied Outokumpu bedrock fractures (Purkamo 2015).

2.6.2.2 Crystalline rock of the Canadian Shield

The underground laboratory of the Atomic Energy of Canada Limited (AECL URL, Pinawa, Manitoba) was in operation from the mid 80's to 2010, providing facilities for the Canadian research program on microbiological studies of the nuclear waste management program (Stroes-Gascoyne and West 1997). As summarized by the authors *"This microbial program focuses on answering specific questions in areas such as the survival of bacteria in compacted clay-based buffer materials under relevant radiation, temperature, and desiccation conditions; mobility of microbes in compacted buffer materials; the potential for microbially influenced corrosion of containers; microbial gas production in backfill material; introduction of nutrients as a result of vault excavation and operation; the presence and activity of microbes in deep granitic groundwaters; and the effects of biofilms on radionuclide migration in the geosphere".*

The direct disposal concept of AECL for spent fuel was based on crystalline rock (granite) and copper as canister material. Dimensions and thermal load of the Canadian (CANDU) canister differ from the SKB-Posiva concept and the repository was planned to be based on a vault concept. The vault would allow either in-room emplacement of several canisters or placing each canister in its own deposition hole in vault floor relatively close to each other's. Peak thermal and radiation conditions would thus be extreme for microbial life, and buffer desiccation was a matter of consideration. Harsh conditions will most probably sterilize the canister surface, but at a short distance (25 – 50 cm) from the canister surface microbes will be able to survive the radiation field and microbially induced corrosion (MIC) due to diffusing metabolic processes must be considered. Assessment of the possible MIC requires mass balance corrosion model and knowledge on the locality of sulphide production by SRB.

Compactness of the bentonite buffer was discussed by Stroes-Gascoyne and West (op.cit): most observed pore diameters center around 10 nm with very few larger pores up to diameter of 20 µm. The material studied was dried compacted bentonite and the large pores were between material aggregates. Effects of excavation related and other man-made nutrient supply for microbes was also discussed: nitrogen compounds due to excavation, hydrocarbons residues and synthetic polymers from the construction materials and operation, drilling water etc. It was pointed out that radiation

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and heat will break out organic material in the near field. Decomposition of organic matter to methane and CO2 was further considered.

Significant numbers of microbes (about 10^5 /mL) were observed from the groundwaters of granitic rock of AECL URL. Scanning electron microscopic studies indicated different morphologies. Culturable anaerobic heterotrophs predominated over aerobes and observed metabolic processes included denitrification, nitrogen fixation, sulphate reduction and iron reduction, but not methanogenesis. Preliminary results of biofilm growth on a flow-through reactor were given and the role of biofilms on radionuclide retardation was discussed.

Currently, the Canadian nuclear waste management, organized by NWMO, is based on an Adaptive Phased Management approach. Adaptability implies that there may be different options how to proceed within or after each phase. Consequently, there was a need to review the general State of Science in all relevant fields of nuclear waste research. Near-field microbiological processes were reviewed by Wolfaardt and Korber (2012), far-field microbiology consideration were reviewed by Sherwood Lollar (2011). These reports provide an up-to-date summary of deep geomicrobial processes in general and microbiological methods applied today in those studies. A good general discussion on near-field microbial issues and potential impacts was given by Wolfaardt and Korber (op.cit) and, finally recommendations were given for a path forward. The review by Sherwood Lollar (op.cit) deals with the deep and 'ultra-deep', biosphere from a wider geoscientific perspective. However, certain 'deep life' environments, e.g. deep aquifers, differ substantially from those of the currently planned nuclear waste repository environments.

2.6.3 Microbial studies in salt deposits

A review by McGenity et al (2000) describes the origin and existence of microbes in rock salt deposits. These deposits are formed in evaporation of water basins and concurrent precipitation of dissolved salts. The hypersaline evaporation basins become enriched in halophilic microorganisms, but as the concentration of salt increases the overall microbial diversity decreases. Halophilic microbes have been isolated from salt deposit brines, but it remains unclear if they are they descendants of the original population of the evaporation basin or associated with the water source of the brine

2.6.4 Microbial studies related to the engineered barriers

2.6.4.1 Cementitious materials

Cementitious materials will be used in geological disposal facilities for the containment of the ILW/HLW wastes, buffer around the canisters and as bulk concrete for construction purposes. Cementitious buffer has a strong alkaline effect on its surrounding, initial phase pH-value of fresh cement containing excess alkali hydroxides may be higher than 13. In the United Kingdom, the proposed concept for intermediate level radioactive waste (ILW) includes package in steel containers emplaced in a cementitious backfill. Consequently, a time-dependent geochemical gradient developes around cement, if in contact with bedrock. Biogeochemical processes within the gradient zone were studied in details in the project BIGRAD (Small et al. 2016). Within BIGRAD Rizoulis et al (2012) and Williamson et al (2013) investigated the effect of pH on anaerobic microbial processes and Williamson et al (2104, 2105) examined radionuclide bioreduction processes under cement buffered conditions. Rout et al (2014) and Bassil et al (2015) have demonstrated the biodegradation of the cellulose degradation product and strong radionuclide complexant, isosaccarhinic acid (ISA) under anaerobic pH 10 conditions.

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2.6.4.2 Bituminised waste

Bitumen is used as encapsulation matrix for ILW waste. In some countries, it accounts for a significant amount of the organics present in the waste. For example, about 90 % of the organics present in the Belgian ILW waste inventory comprises bitumen. The possible degradation of bitumen by micro-organisms is already known and studied since at least 1935 (Hundeshagen, 1935). Biodegradation rates depend on the chemical composition of the bitumen substrate, the metabolic character of the microorganisms involved and various biological and physico-chemical parameters (Ait-Langomazino et al, 1991). Degradation experiments showed that in aerobic conditions biodegradation rates are much higher compared to anaerobic conditions (Wolf et al, 1991; Jacquot et al 1997). Nevertheless, also in anaerobic conditions a distinct production of N_2 and CO_2 was observed after one year and all short strain carboxylic acids (C_5-C_{10}) leached from the bitumen were degraded by microorganisms, showing a clear activity of microorganisms (Jacquot et al, 1997). In addition, in aerobic as well as anaerobic growth conditions biofilm formation on the bitumen was often observed (Ait-Langomazino et al, 1991; Wolf et al, 1991; Springael et al, 1997). It was shown that blown 'hard' bitumen is more sensitive to biodegradation compared to a distilled 'soft' bitumen, comprising a lower amount of saturates (Ait-Langomazino et al, 1991). This is in agreement with the fact that the resins and asphaltenes are much more resistant to biological degradation compared to the saturates, although organisms able to use asphaltenes as carbon source are reported several times (Pineda-Flores et al, 2004; Ali et al, 2012; Tavassoli et al, 2012).

More importantly, during disposal conditions the bitumenised waste will be prone to chemical and radiolytic degradation (discussed in deliverable D.1.1). High amounts of salts, carboxylic acids, glycol, aromatic compounds, oxidised compounds, sulphur and nitrogen compounds, ketones and phenols have been identified in leaching experiments of bitumen and bituminized waste (Walckzak, 200; Kagawa e al, 2000; Valcke et al, 2000). Most of these degradation products are theoretically degradable by microorganisms if proper growth condtions are attained.

After backfill and closure of the gallery, the repository will re-saturate with groundwater. Generally, repositories located in crystalline hard rock will saturate rapidly, where those located in indurated clay rocks (e.g. France) are anticipated to take 100 000 years to return to a state of hydrogeological equilibrium (Andra, 2005). In contrast, the Boom Clay formation is expected to re-saturate much faster allowing water to saturate the bituminised waste after 50 years (Weetjens et al, 2010). Consequently large amounts of $NaNO_3$ will be released which will induce an osmotic pressure on the present microbial and archaeal communities. Scoping calculations indicate that during the first ~300 to ~1400 years, the $NaNO_3$ concentration within the monolith will remain above 1 M and 0.5 M, respectively (Weetjens et al, 2010). Closer to the waste drums, the concentrations can reach up to a few molar (Weetjens et al, 2010). Consequently, the high salinity together with the absence of void space and porosity disfavour the development of microbial activity within the bitumen matrix. However, nitrate will provide a strong electron acceptor for microbial processes and consequently conditions of interest are those at the interface of the bituminised waste canister and cement container/backfill material (depending on the disposal concept design and its temporal evolution). There, microbial activity utilising the organic degradation products and soluble electron acceptors (nitrate and sulphate leaching from the waste) as energy sources could develop. Bitumen-Nitrate-clay interactions are studied in an experiment at the Mont Terri Rock Laboratory (BN-experiment) and also in MIND, biodegradation of bitumen degradation products in the presence or absence of different electron acceptors, bitumen, different pH will be scrutinized (WP1 task 1.2).

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2.7 Microbial processes, constraints of life

Life on planet Earth started to evolve during more than 3.5 billion years ago. Since then, through the evolution, adaption and competition, practically all possible ecological niches have been occupied by living matter. Deep life has been observed to challenge many of the typical limits of life, as high temperature, high water salinity etc. It is thus more practical to define what actually is required to live: nutritional *elements* in appropriate compounds for cellular synthesis, *liquid water*, *space* and *energy* to sustain the metabolic processes.

2.7.1 Nutritional elements

As a rule, living populations recycle all elements required to sustain the system in a steady state, but lack of some essential nutrients may become the limiting factor in a fast growing system. Microbes in bedrock-groundwater environment have all essential major and minor components of life in their environment, but the bioavailability of some components may be restricted. Identification of these possible “bottle necks” in deep geological disposal systems is important from the safety point of view.

2.7.2 Space

The space requirement of a unicellular microbe is not large, typically of the order 1 μm^3. In crystalline rock, fracture apertures clearly exceeding the space requirement of microbes are common. Visually unbroken rock specimens typically show microfracture apertures of tens of micrometers when studied by petrographic methods (X-ray tomography, impregnation etc.). Hydrogeochemically, this micro-scale matrix porosity may differ substantially from the larger scale flow porosity due to open fractures.

Clay rocks have high porosities up to tens of percentages, but the individual pores are small. Pore size distribution in clays typically has a log-normal characteristics, so that most of the pore volume is in the nanometer range. During the lithification and diagenesis of a sedimentary clay to a clay rock, pore size will decrease by compression and precipitation. Bentonite is a special type of clay with a high content of clay mineral group smectites, which expand when wetted. Expansion and compression lead to decreasing pore size. Space limitation in compacted clay materials for microbial activity and mobility is a key process to be demonstrated.

Construction of the repository systems for nuclear wastes implies, however, that open space and a mechanically and chemically disturbed zone will be created in the bedrock.

2.7.3 Liquid water

Deficiency of liquid water is probably not a major limitation for the microbial processes in the bedrock. However, water in clays is at least partly electrostatically bound to the mineral grains, implying the activity of water is distinctly decreased. In salt formations, availability of free liquid water is evidently the most important constraint of microbial activity.

2.7.4 Energy

Energy limitation is evidently a salient point for deep life. Energy flux in biogeochemical system can be considered as a sequence of electron transfers, “redox ladders” (Figure 17).

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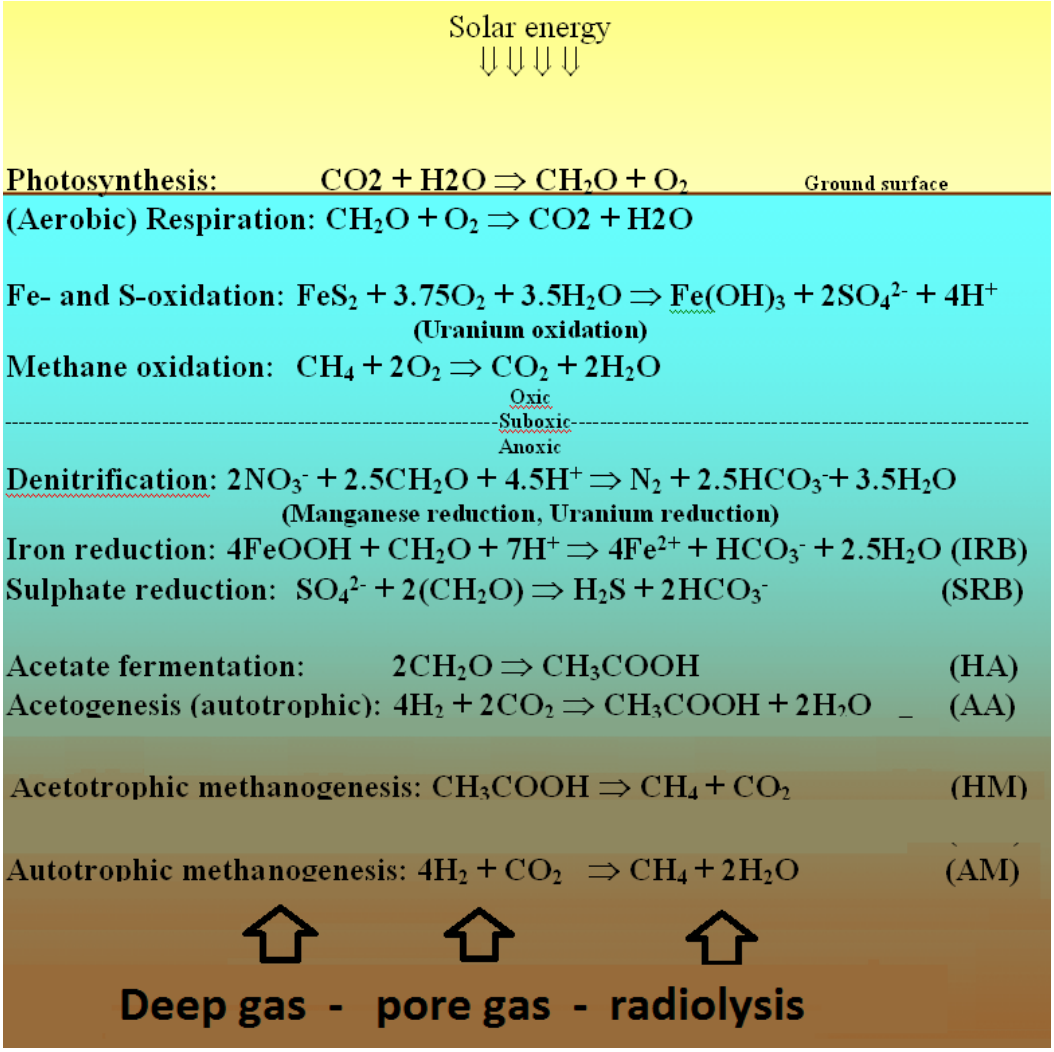

Figure 17: Redox ladders

Solar energy has been the main driving force of life since the emergence of first photosynthetic organisms more than 3500 million years ago. In the absence of light, the opposite process i.e., respiration consumes oxygen and provides energy for life in deeper layers of the biosphere in the continental subsurface and oceans. Aerobic respiration uses typically carbohydrates as electron donor, but methane can also be oxidized in metabolic processes (aerobic methanotrophy). Aerobic chemolithotrophy extends the operational environment of the microbes to the rock: microbes can use certain inorganic constituents as electron source (sulphide minerals, ferrous iron etc.) and fix inorganic carbon (CO_2) for cellular chemosynthesis. Iron-sulphur system (often acidophilic) microorganisms are known to be involved in metal corrosion processes and some species may also utilize ferric iron as electron acceptor instead of oxygen.

Anaerobic respiration processes determine a reduction sequence towards the deeper layers of the ground surface. Nitrate reducing microbes are primarily heterotrophs using mainly organic compounds as electron donors. Nitrate thus reduced to ammonium compounds is easily available for microbes, but certain microbes can also fix molecular N_2 directly for cellular synthesis. As a result, uncontaminated deep groundwaters are typical devoid of nitrogen compounds other than N_2.

Microbial reduction of Fe(III), Mn(IV) and U(VI) with concurrent oxidation of organic matter are important biogeochemical reactions in sub-oxic or anoxic aquatic sedimentary environments. Fermentative processes which need no external electron acceptor become predominant in deeper anoxic layers. Fermentative reaction chain is based on electron transfers between organic molecules

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and gradual consumption of organic matter, finally leading towards a mixture of methane and carbon dioxide. Hydrogen is an intermediate in the fermentative process chain, but – depending on the source material – may also remain as a constituent in the final product.

Sulphate and aqueous dinitrogen (N_2) are the two most resistant electron acceptors in deep groundwaters. The resistivity evidently depends both on their thermodynamic stability (low energy gain) and on the stable electron configuration of the molecules (covalence). Reduction of sulphate to sulphide requires microbial enzymatic catalysis, and takes place effectively within the uppermost tens of meters in strictly anaerobic sedimentary systems. However, in fractured crystalline rock the depth of the sulphate to sulphide transition zone varies substantially.

Microbial methane from fermentative reactions is frequently observed in anoxic near-surface organic sediments (e.g. peat, anoxic aquatic sediments). Deep methanic environment, however, typically shows dissimilar characteristics compared to methane from fermentative pathway. Deep methane typically correlates inversely with CO_2, indicating that CO_2 acts as an electron acceptor in the presence of a strong electron donor (e.g., H_2). Interestingly, N_2 is the last remaining electron acceptor, which seems not to be consumed effectively even if strong electron donors are present.

Finally, energetic prerequisites of life deep in the continental geosphere decrease, if no intrinsic and/or replenishing reducing power is available. The existence of a self-sustaining, hydrogen-driven deep biosphere has been discussed in the scientific literature (Onstott 2016), but the required primary gas source and renewal rate still need to be verified.

2.8 Discussion and conclusions

Currently, there are no more doubts about the existence of a deep biosphere in the continental upper crust. Artefacts due to contamination have been excluded or at least reduced by various methodologies. Microbial communities have been observed to vary consistently with geochemical environments, but there are indications that the population density decreases towards the depths of two kilometres and more. The origin and "reasons" of the unicellular life to occupy the harsh, low-energy conditions may depend on the geological conditions. In case of the clay rocks, a microbial community may even originate from the depositional environment of the rock, whereas in fractured crystalline rock the deep anoxic environment may just provide an appropriate refuge for anaerobic micro-organisms. Space requirement is a key question especially in dense clay rocks, where the average pore size is clearly smaller than the size of typical microbe. Consequently, it can be questioned, if there can actually be a viable indigenous community in clay rock. Water-free salt deposits are evidently hostile environments for life, but associated brines may provide an ecological niche for halophilic microorganisms.

The present report aims at discussing the geomicrobiological processes in the bedrock systems considered for nuclear waste disposal in Europe. Each country has its own geological disposal concept and there is quite some variety depending on the geological conditions available. Three different host rock types are considered in detail of which two are considered within MIND, namely crystalline rock and different types of clay rock. Both types of host rock have their specific characteristics, however, the general function is to isolate the radioactive waste for man and environment as long as necessary. Next to the host rock, different engineered barriers are developed in different countries. Again, they all have specific characteristics but the main safety function of the engineered barrier system is the delay and attenuation of the release to retain the contaminants for as long as required within the disposal system. Microbial activity can have an impact on the long term integrity of the engineered barrier systems and consequently, it is important to demonstrate the limits of viability of microbes both in buffer material and in the near field of the canister. Microbial activity can influence to the mobilisation of radionuclides either by immobilising them or by enhancing their mobilisation, it can contribute to the gas build-up of the gas phase by the production of gas or they can reduce the gas pressure build-up resulting from the anoxic corrosion of

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the waste containers or microbial activity by SRB may influence the corrosion of the metal canisters. Understanding all these processes is of paramount importance for the geological disposal of radioactive waste.

Deep sedimentary aquifer systems were not included. Porous sandstone formations are an essential part of sedimentary rock sequences. Regional scale, topography driven flow of water within the aquifers can reach the depth of several kilometres, providing an allochthonous source of deep populations in these permeable formations. The most important general constraints of deep life in continental rock systems are evidently energy and space. In general, dense clay formations seems to be able to inhibit microbial mobility, because of the small size of pores in the inter-granular space. Repositories constructed in non-porous crystalline rock also utilize the impermeability of compacted bentonite.

Despite the ambiguity related to the possible presence of an indigenous microbial population in the host rock, microorganisms will be ubiquitously present in the repository as it will not be possible to build a repository in a sterile manner. Moreover, it has been shown that just providing space – what will occur during construction of the repository – is a sufficient condition for bacterial activity what is shown during almost all borehole experiments. It has been shown that those communities can survive and be metabolically active, hence they has to be considered when radioactive waste will be disposed. Next to space, a microbial community needs to obtain energy to fuel their metabolic processes. As discussed in the MIND deliverable D1.1, these can be provided by the organics present in the radioactive waste.

Construction and operation of the repository produces a transient oxic environment and an aerobic microbial population, which can consume oxygen readily after the closure of the repository. However, sulphate and nitrogen compounds will be available for a longer period and the initial oxic and sub-oxic period must be accounted for, keeping in mind the early-phase high heat generation of the waste. A primary performance function of the buffer is to wet and swell to a target pressure protecting the canisters from early-phase oxic corrosion and later sulphidic corrosion. Anoxic corrosion of steel canister can theoretically take place as a reaction between iron metal and water, with reaction products being iron oxides and hydrogen. However, in repository where they use cementitous materials as backfill, anaerobic corrosion of the steel will be decreased as the steel will become passivated by the high pH originating from the concrete.

In case of radionuclide release from the repository, transport and retardation processes in the host rock become important. Many of the long-lived nuclides are effectively retarded and accumulated by biomass, both by reduction and precipitation and by binding in strong complexes with the some functional groups of biomass. However, there are some radioisotopes (e.g. C, Se) that are involved in metabolic processes. Understanding their transport behaviour requires understanding of the microbiology in terms of metabolism and overall activity.

The MIND project deals with two different waste categories. In Work Package 1, intermediate-level waste with a high content of organics is investigated, including variable waste forms, activity and package concepts. In Work Package 2, issues concerning high-level waste, predominantly spent fuel encapsulated in metal canisters and finally embedded in bentonite or cementitious buffer in the deposal galleries, are addressed.

Overall, the project is multidisciplinary covering essential fields of microbiology, geochemistry and numerical modelling. The final aim of the project is to combine the information from these different starting points to understand in detail all biogeochemical processes relevant for nuclear waste management.

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3 First year progress and results

3.1 Work package 1

WP1 works under the theme "Improving the geological safety case knowledge of the behaviour of organic containing long-lived intermediate level wastes", covering the six objectives:

- To reduce uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from long-lived intermediate level wastes (ILW) containing organics (SRA Key topic 2 sub topic 2).
- To quantify the combined rates of biodegradation, radiolysis and hydrolysis of anthropogenic organic polymers and cellulose present in ILW under disposal conditions.
- To identify key chemical species resulting from organic ILW biodegradation, radiolysis and hydrolysis and their effects on radionuclide speciation and mobility.
- To establish the in situ chemical and physical conditions that may limit microbial activity in EU repository concepts for ILW utilising cementitious materials within a neutral pH host rock.
- To examine the microbial generation and consumption of CH_4 and H_2 under ILW repository conditions.
- To understand the effect of ILW heterogeneity on bioprocess pathways, pH and redox conditions, barrier degradation and radionuclide release.

3.1.1 Task description

To reach the objectives WP1 is organized in four subtasks. Progress in the tasks is summarized in the following:

Task 1.1 (Review of ILW organic waste types and conditioning in Europe) has collated information concerning the inventory of different types of organic materials present mainly in ILW repositories (D1.1). The three most important groups of organic waste types are:

- Ion exchange resins used in collecting dissolved radioactive elements from the primary cooling water of the nuclear reactors. The resins are organic polymers, different types of polymers are required to remove cationic and anionic forms of radionuclides. The resins are regenerated, and the resulting effluent can be concentrated by evaporation.
- Halogenated polymers, especially polyvinyl chloride (PVC), are frequently used materials in the construction of the nuclear facilities.
- Bitumen is obtained as a by-product of petroleum industry, but is also found as natural occurrences. Bitumen is considered as a good material in immobilizing radionuclides.

The review (Abrahamsen et al, 2015) gives detailed information on the inventory of these organic wastes in European countries. The review covers the key aspects of WP1, including principles of biodegradation of different polymeric structures, attached functional groups and loose-bound additives in hydrocarbon polymers. Aliphatic and aromatic base structures of polymers are resistant against biodegradation, but a strong radiation field can damage the structure and possibly promote biodegradation. In addition to radiolysis, also other effects due to the storage conditions on the stability of organic waste were discussed in the review: high pH due to cementitious materials, strong electron acceptors (e.g. nitrate) and evolution of hydrogen. The Task 1.1 review provides a starting point to the Task 1.2, in which biodegadation of relevant irradiated organic wastes will be studied experimentally.

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Task 1.2 (Rate and mechanism of biodegradation of ILW organic polymers affected by radiation, under repository relevant conditions) examines the combined effects of radiation and biodegradation on the organic materials in ILW repositories. Materials selected for irradiation studies are:

- 4 different ion exchange resin (EPFL)
- PVC, as powder and sheet (UNIMAN)
- Cellulose (UNIMAN)
- Bitumen (SCK•CEN)

PVC and cellulose materials have been irradiated at the Dalton Cumbria Facility (DCF) UK at pH 12.5 cement buffered conditions and ~ 1MGy ^{60}Co for bidegradation studies by UNIMAN. Further irradiations of PVC and cellulose will be performed on pH 7 solutions and at lower doses (100 kGy). Ion exchange resins used in Switzerland have also been irradiated at DCF at ~ 1MGy under pH 8 Opaainus Clay groundwater conditions for study by EPFL. Further irradiation studies of ion exchange resins have been performed by RCR with a ^{60}Co source in the Czech Republic.

Microcosm experiments of the irradiated PVC materials have been undertaken with Harpur Hill, high pH, lime kiln waste, sediment inoculum (see Rizoulis et al, 2012) at pH 10. Bioreduction has been assessed by studying nitrate reduction Phthalate and triphenyl phosphate representing plasticisers present in PVC show negligible nitrate reduction at pH 10, but increased nitrate reduction under neutral pH. Microcosm experiments using the Harpur Hill inoculum at pH 10 using irradiated and unirradiated PVC powder (no additives present) also show nitrate reduction to nitrite. In particular, unirradiated PVC powder is not bioavailable for nitrate reduction, but irradiated PVC powder supports minor nitrate reduction. PVC film (with plasticers additives) is bioavailable for nitrate reduction whether irradiated or not, but rates are higher with unirradiated PVC film. Further analyses including DNA analysis, 16S rRNA gene sequencing and chemical analysis of the unirradiated and irradiated PVC materials are in progress. Dissolved organic carbon (DOC) analyses shows that DOC increases over the course of the experiment in all microcosms, regardless of whether a live inoculum was added or nitrate reduction detected. The DOC in microcosms containing PVC film is higher than in those containing PVC powder. These data suggest that hydrolysis of PVC materials took place over the course of the experiments. These first results indicate that the structure of the PVC, either original or after irradiation and the presence of additives is important to the biodegradtion of PVC materials and their potential to fuel anaerobic microbial processes.

Fermentation microcosms with cellulose tissue paper (2 months $Ca(OH)_2$) with and without 1MGy irradiation have been set up. Preliminary results indicate that irradiation at high pH induces mid chain scissions, which enhances the rate of cellulose hydrolysis by alkali and ISA production. Samples inoculated with the Harpur Hill sediment show significant H_2 production. Studies of ISA complexed to uranium show promising results with microbial metabolism leading to uranium precipitation. Similar experiments with lanthanum, to study radionuclide-immobilisation mechanisms in the novel bacterium, are being set up. Bioinformatics studies have explored the metabolism of cellulose degradation products, comparing ISA and gluconic acid, and are still on-going. Enrichment cultures at pH 10.5 and containing ISA as the only electron donor and either Fe(III), or sulphate as electron acceptors have been set up to try to isolate Fe(III)- and sulphate-reducing, ISA-degrading bacteria. Characterisation studies are being conducted to help with precise phylogenetic placement of the novel bacterial isolate from the Harpur Hill site responsible for ISA degradation. Phylogenetically related bacteria have also been shown to degrade ISA under similar growth conditions. The genomes of these bacteria have been sequenced and submitted for publication.

SCK•CEN has studied biodegradation of bitumen in laboratory batch experiments. Irradiated bitumen samples from previous and on-going experiments have been collected for the experimental work. The work to characterize the soluble organic fraction in bitumen has started in cooperation with

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UNIMAN. Other tests including visual characterization of microbe colonization have been successful and have been undertaken in preparation for 3 month duration biodegradation tests.

Characterisation of irradiated ion exchange resin materials has begun, preliminary gas chromatography mass spectrometry (GC-MS) results indicating time dependent behaviour in solutions after irradiation. Once the organic irradiation products are characterized microcosm experiments will be undertaken studying their biodegradation and potential to drive sulphate reduction and methanogenesis under Opalinus Clay conditions (related to EPFL studies in Task 1.3). Studies of ion exchange resins in the Czech Repbulic will utilize an anthropogenic source of alkaline (cement impacted) water sampled 30 m below ground in a Prague underground metro tunnel as a water inoculum. This follows chemical and microbial (16S rRNA gene) characterization of a number of natural groundwaters from underground laboratories, which did not identify any suitable nautral alkaline waters.

HZDR and UGR focus on the characterization of the materials and products of the irradiation tests. This includes development and testing of methods to study U(VI) complexation with ligands produced in the irradiation experiments. UGR also studies microbial reduction and speciation of selenium, which is on the one hand an important radiotoxic component of nuclear wastes and on the other hand a possible component of metabolic processes. HZDR have collaborated with UNIMAN to set up experiments to test uranium speciation with cellulose and cellulose degradation products under hyperalkaline conditions (in $Ca(OH)_2$ solution) over a period of 1 year. The experiment will be examined using spectroscopic methods to investigate the fate of uranium and the influence of uranium on this complex system.

Task 1.3 (Microbiological metabolism under repository conditions) aims at verifying the environmental constraints that limit microbial processes in nuclear waste repositories. The task utilises two existing *in situ* experiments.

The large-scale long-term gas generation experiment (GGE) operated by TVO, Finland simulates the processes related to the storage of low and intermediate level waste in the VLJ repository (Olkiluoto, Finland). A sampling plan was devised by VTT (M1.3-1) and the first sampling campaign undertaken. The amount and activity of most relevant microbial groups (e.g. methanogens, sulphate reducers) have been analysed by qPCR. Volatile fatty acids and other metabolites were analyzed and total number of microbial cells in the water and the attachment on waste materials were studied by microscopy. A data set of gas generation rate, gas and water composition for 18 years operation of the experiment has been interpreted and modelled (linked to Task 1.4). The data records the neutralization of pH of the experiment from initial pH 11, buffered by concrete, to neutral pH by microbially mediated organic degradation processes.

The Mont Terri MA (Microbial Activity) experiment (funded by ANDRA, Nagra, BGR and NWMO) examines hydrogen utilisation processes under in situ conditions in the Opalinus Clay. EPFL started an experiment based on injection of hydrogen into a borehole (M1.3-2). The aim of the experiment is to test if the reducing conditions will favour microbial methanogenesis. Methane formation could further lead to sulphate reduction using methane as the electron donor. Results from sequencing and isotopic analysis of methane revealed that methanogenesis is not yet extant in the borehole. Clearly, microbial activity is being stimulated by the H_2 amendment but the sulphate concentration remains too high for methanogenesis.

Task 1.4 (Modelling the impact of relevant biodegradations processes on the chemical and radionuclide source terms) aims at developing models experimental work of WP1 to aid interpretation and for consideration in performance assessment. Modelling codes e.g PHREEQC are versatile tools in demonstration and prediction of geochemical processes in aqueous phase (speciation), in mineral phase boundaries (dissolution-precipitation) and in coupled transport

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processes (reactive flow). Microbial processes are kinetic processes that must be coupled to conventional and existing reactive transport models.

NNL is developing subroutines to model microbial growth and the reaction of electron donor and acceptor species in the existing Generalised Repository Model (GRM) FORTRAN code (Small et al, 2008). Specific subroutines will simulate different metabolic processes, which can be interfaced with the PHREEQC geochemical modelling code and other reactive transport models. A prototype interphase has been developed that can model the growth of an unlimited number of microbial groups in a batch reactor (e.g. microcosm) system.

3.1.2 Overview of the WP1 progress during year 1.

The work package started with an exhaustive review (D1.1), giving an overview of the organic waste materials, material properties and processes to be studied further. Sample materials for the laboratory test are chosen, and laboratory test of biodegradation have been started. Data from large scale URL experiments is being interpreted and modelling tools are being developed for further use.

Milestones defined for the year one have been reached:

- List of polymers for irradiation studies
- Microbial inocula for exchange
- TVO gas experiment sampling plan
- Hydrogen injection into MA borehole (Mt Terri)

3.2 Work package 2

WP2 works under the theme ”Improving the safety case knowledge base about the influence of microbial processes on high level waste and spent fuel geological disposal”, covering the three objectives:

- Quantify the contribution of microbially produced sulphide in the geosphere and in buffers and backfill to the overall rate of canister corrosion (SRA Key topic 3).
- Characterize the impact of microbial activity on the long-term performance of bentonites and seals and plug systems in European geological disposal concepts (SRA Key topic 3, sub-topics 9 and 10).
- Gain systematic information on the effectiveness of specific bentonite buffers and their properties (density, pH) in inhibiting microbial activity (SRA Key topic 3).

3.2.1 Task description

To reach the objectives WP2 is organized in five tasks. Progress in the tasks is summarized in the following:

Task 2.1 (Microbial production of sulphide in the geosphere) aims at identifying the factors controlling sulphide production in the engineered barrier system and in the surrounding geosphere. Availability of electron donors, such as the H_2 and CH_4 facilitating microbial reduction of sulphate to sulphide was identified as a key controlling factor.

Partners within the task have different, complementary approaches: Micans co-operate with Posiva Oy studying gas emission from fresh drill core samples obtained from Olkiluoto site. First results obtained by M12 indicate gas release from the drill cores placed in closed brass tubes. Geochemical approach of GTK deals with the general existence of gases, sulphur compounds and other essential components of deep life in the crystalline bedrock. Data covers about 35 deep drill holes (maximum depth range 400 –2500 m) from different localities and different geological environments in Finland. VTT is studying biochemical process of sulphide production in laboratory conditions in the presence of added electron donors. During the first year analytical methods were searched and evaluated.

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Sampling and planning for laboratory tests together with Posiva Oy was underway by the end of first year.

Task 2.2 (Microbially induced corrosion of canisters) deals with microbially assisted sulphidic corrosion of copper canister and iron corrosion enhanced by microbial consumption of the corrosion product hydrogen (hydrogen scavenging). These both processes are theoretically possible in anoxic conditions. Consequently, the remaining question is to understand to what extent microorganisms can survive and accelerate corrosion processes in the near-field conditions.

EPFL investigates the rate of steel corrosion in bentonite under in situ conditions. Experiments going on at the Mt Terri URL allow the comparison of corrosion rate of steel in bentonite matrix in "normal" conditions and in a parallel experiment in which microbial attack is inhibited by a 0.2 µm mesh. Another experiment planned to start within the MIND project studies steel in bentonite emplaced in a borehole, in which hydrogen evolution would be monitored. This drill hole at Mt Terri is finished and the system is ready for the start of the experiment.

NERC is running an experiment with steel wire embedded in bentonite blocks in a laboratory scale flow-through cell. The experiment is performed in controlled conditions (P, T etc), allowing comparison between sterile and inoculated systems.

TUL & CV REZ perform laboratory experiments on a new canister design having a stainless steel inner part and carbon steel outer layer. In the first phase stainless steel was tested for possible anaerobic corrosion using natural groundwater as an inoculum, and UV-sterilized groundwater as a control. The experiment was held under anaerobic conditions for 111 days. Current experiment focuses on the carbon steel corrosion under anaerobic conditions.

Task 2.3 (Microbial activity in bentonite buffers) focuses on the performance of the bentonite buffer, with a special reference to its sealing capability against microbial processes. Bentonite materials may contain indigenous microbes of the depositional environment, microbial population related to the changing post-depositional environment, as well as contamination during excavation and manufacturing of the product. The technical use of bentonite as a buffer is based on compaction of the dry material. By subsequent emplacement and swelling of the precompacted bentonite in the limited space of the deposition holes the sealing capability is finally established. The aim of this task is to verify that the safety function of the bentonite buffer is fulfilled in the presence of – and with respect to – the microbial activity.

The task is utilizing bentonite samples from the international FEBEX experiment performed at the Grimsel test site, Switzerland during 1994 – 2016, in which the behaviour of bentonite buffer was studied in a full-scale experiment. Precompacted bentonite blocks were installed in a horizontal deposition hole in crystalline rock, and electrical heaters embedded in the buffer simulate the heat-generating HLW canister. Water saturation and development of swelling pressure were monitored during the long-term experiment, which now is in the dismantling phase. Samples are delivered for microbial studies to MICANS and UNIMAN. Micans has started to develop a method for DNA-extraction from microbes in bentonite clay using a phase separation technique. UNIMAN has focused on the quantification, and identification of Fe(III) and sulfate reducers in bentonites, as well as investigating structural change in the clay minerals, after treatment e.g. irradiation. These techniques will be applied on FEBEX samples extracted from the Grimsel Test Site.

One of the safety functions of bentonite is the resistance against anion diffusion because of the positive layer charge of smectites. Micans is therefore performing experiments on the existence and diffusion of sulphide in bentonite. TUL & CV REZ focused on the preparation of the experimental cells for higher temperature and pressure tests. TUL optimised the method of DNA extraction from bentonite samples specifically from the Czech bentonite. Typical soil bacteria as well as chemolitotrophs were present in both bentonite samples.

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VTT performed a 20 years laboratory experiment, in which compacted bentonite was placed inside a copper vessel. Microbial studies of the material will be done by VTT within the MIND project. Sequencing of fungi has been done and analysis of the results is underway.

Task 2.4 (Microbial degradation of bentonite buffers) aims at scrutinizing the scenario that bentonite would fail to maintain its safety function. Natural bentonites have shown chemical stability in their natural geological setting. The age of the well-known Wyoming bentonite is of the order of 100 million years, but the primary mineralogical and textural characteristics are still preserved. Bentonite, wetted and swelled in the nuclear waste deposition holes must not only keep the physical properties but also remain chemically stable, because alteration the material may affect the long-term performance. Important minor components in bentonites are iron and sulphur, existing in variable amounts and in different redox states in different bentonite deposits. Microbiologically catalyzed redox reactions of these components are key processes to be studied in this task.

MICANS has analysed the effect from adding acetate to buffer materials thereby stimulate iron-reducing bacteria. A first set of samples with the clays indicated the release of ferrous iron from some of the clays as a result of microbial activity growing on added acetate.

The work plan for NERC is to investigate the microbiology at the buffer/canister interface, specifically understanding the direct impact of microbial processes on the mineralogy, microstructure and physical properties of bentonite. By the end of first year, the experimental rig for the experiment has been designed and built ahead of schedule and is being commissioned. The press for manufacturing samples was delivered, but it still required modifications delaying the start of the testing period.

Laboratory work at HZDR and VTT are based on batch incubation of bentonite slurries. Bentonite materials used by HZDR are from mineral deposits in Germany, and VTT is using commercial Wyoming bentonite. Besides the microbial community geochemical and mineralogical analysis will be performed.

Task 2.5 (Microbial activity in backfill and influence on plugs and seals) deals with geo-engineered materials of a mixed and variable composition. The target properties of the tunnel backfill are to support the bedrock and to restrain the radionuclide migration. Important materials in the backfill are expanding clay materials, crushed rock and cementitious materials (e.g. concrete plugs).

SCK•CEN addresses the effects of microbial processes in cementitious materials. Depending on the prevailing metabolic processes, microbial effects on cementitious materials may be either negative (acid production => leaching) or positive (clogging of the pores in cement). By the end of year one, DNA-extractions have been tested with success on the FEBEX samples.

3.2.2 Overview of the WP2 progress during year 1.

The overall target of this work package is defined as ”Improving the safety case knowledge base about the influence of microbial processes on high level waste and spent fuel geological disposal”. In compliance with the research plan, the work performed emphasizes behaviour of the buffer-canister system: Sealing properties of bentonite buffer with respect to diffusion, corrosion of canister embedded in bentonite, chemical reactions affecting long-term stability of bentonite. This work package aims at testing and demonstrating the role of microbes in these processes.

Several tests were started, in which microbially inoculated process will be compared with sterile controls. Inoculum used in most cases is the bentonite material itself or natural groundwater from appropriate conditions was added. A key question for the interpretation is to ascertain comparability of the experimental results. Therefore, a common molecular protocol was developed by the MIND partners to probe the microbial community in bentonite.

In general, WP2 is well focused on the key research area as defined in the work plan. Planning and preparation, including technical development and testing, have been an important part of the first year experimental work. Modelling activities have not started yet, but data compilation is underway.

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