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Microbiology In Nuclear waste Disposal

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Final synthesis report for WP2

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RE	Restricted to a group specified by the partners of the MIND project	
CO	Confidential, only for partners of the MIND project	

Publishable Summary

The MIND project targets a number of high urgency and high importance topics identified in the Strategic Research Agenda, by IGD-TP 2011. The scientific technical work programme of MIND was divided into two operative Work Packages (WP 1 and 2) where WP2 addresses the behaviour and technical feasibility and long-term performance of repository components. Key issues for the geological disposal of high-level waste concern the factors controlling sulphide production in the geosphere, including to what extent bacteria can accelerate canister corrosion in the near-field either by H_2 scavenging or by sulphide and/or acetate production. Further, it is important to identify conditions under which relevant bentonites inhibit bacterial activity, and to understand whether bacteria can accelerate degradation of bentonite-based buffers and influence the long-term behaviour of plug systems and seals.

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

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

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

It was tested if microbial activity had an influence on the stress from swelling in clay samples, but effects, if any, were too small for detection of the used system. In addition, it was found that

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corroding steel influenced the clay in the vicinity of the steel, but the results did not show any obvious effect from samples with SRB compared to sterile samples. The effect from sulphide on bentonite samples has been studied in MIND. It was found that sulphide reacts with ferric iron in the tested clays under the formation of FeS and elemental sulphur (S^0). Thus, the MIND project has found limited impact from microbial activity on bentonites with the exception of sulphide produced by active SPB that reacts with clay components, mainly ferric iron.

On partner found that the high pH conditions imposed by the cement inhibited microbial sulphate and nitrate reduction. However, the presence of intact cells in the suspension on top of cement and putative biofilm structures on the cement was indicated by scanning electron microscopy investigations. Interestingly, in sulphate-reducing conditions, a pH decrease from >12 to pH 10 was observed. It was also concluded by another partner that the alkaline solution leached from the concrete inhibited microbial activity due to an increased pH in the bentonite clay. Induced microbial activity in MX-80 with glucose led to a decreased in pH. A reason might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. In conclusion, the MIND project has demonstrated that microbial activity may decrease pH in high alkaline repository barriers.

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

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Table of contents

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1 Introduction

The MIND project targets a number of high urgency and high importance topics identified in the Strategic Research Agenda, (SRA) (IGD-TP, 2011), focusing mainly on Key topic 2: Waste forms and their behaviour and Key topic 3: Technical feasibility and long-term performance of repository components. The Scientific Technical Work Programme of MIND was divided into two operative Work Packages (WPs):

WP1 addresses SRA Key topic 2: Remaining key issues for the geological disposal of 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 intermediate level wastes (ILW) containing organics.

WP2 addresses SRA Key topic 3: Remaining key issues for the geological disposal of HLW (High Level Waste) 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 under which relevant bentonites inhibit bacterial activity, and to understand whether microorganisms can accelerate degradation of bentonite-based buffers and influence the long-term behaviour of plug systems and seals.

In this report the term *bacteri*a is used when only bacteria are meant. The term *microorganism/s* includes all prokaryotes (Bacteria and Archaea) and unicellular eukaryotes (fungi, yeast, algae and protozoa)

1.1 Participating partners

There was a total of 15 partners in MIND of which 10 partners from 7 countries participated in WP2 (Table 1-1).

Table 1-1: MIND partners participating in WP2

Acronym	Name	Country
MICANS	MICROBIAL ANALYTICS SWEDEN AB	Sweden
SCK•CEN	CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE FONDATION D'UTILITE PUBLIQUE	Belgium
NERC	NATURAL ENVIRONMENT RESEARCH COUNCIL	United Kingdom
VTT	TEKNOLOGIAN TUTKIMUSKESKUS VTT Oy	Finland
EPFL	ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE	Switzerland
TUL	TECHNICKA UNIVERZITA V LIBERCI	Czech Republic
CV REZ	CENTRUM VYZKUMU REZ S.R.O.	Czech Republic
GTK	GEOLOGIAN TUTKIMUSKESKUS	Finland
HZDR	HELMHOLTZ-ZENTRUM DRESDEN-ROSSENDORF EV	Germany
UNIMAN	THE UNIVERSITY OF MANCHESTER	United Kingdom

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1.2 Objectives and task list in work package 2

High-level radioactive waste, mostly spent fuel and waste from re-processed fuel, will be encapsulated in iron or copper canisters (Figure 1-1). In some concepts, so-called super containers will have a concrete barrier encapsulating iron shells that contain the waste. The canisters will either be surrounded by an engineered barrier consisting of swelling clay, or, they will be emplaced directly in the host rock. The metal and the clay barriers are commonly denoted engineered barrier systems (EBS) and are susceptible to deterioration processes. Possible microbial deterioration processes for the safety case are (a) metal corrosion, (b) illitization of smectite clay minerals such as montmorillonite and nontronite, and (c) degradation of concrete. Corrosion will eventually cause the canister to breach, leading to radionuclide release; illitization will compromise the clay buffer's swelling properties, reducing its barrier function; concrete degradation may prematurely destabilize plugs and seal system. Microbial activity could impact the rate of each of these barrier systems and thus impact the safety case by compromising a repository's isolation and containment functions. This work package aims at addressing these issues individually and providing actionable information to the implementers in order to improve their safety case.

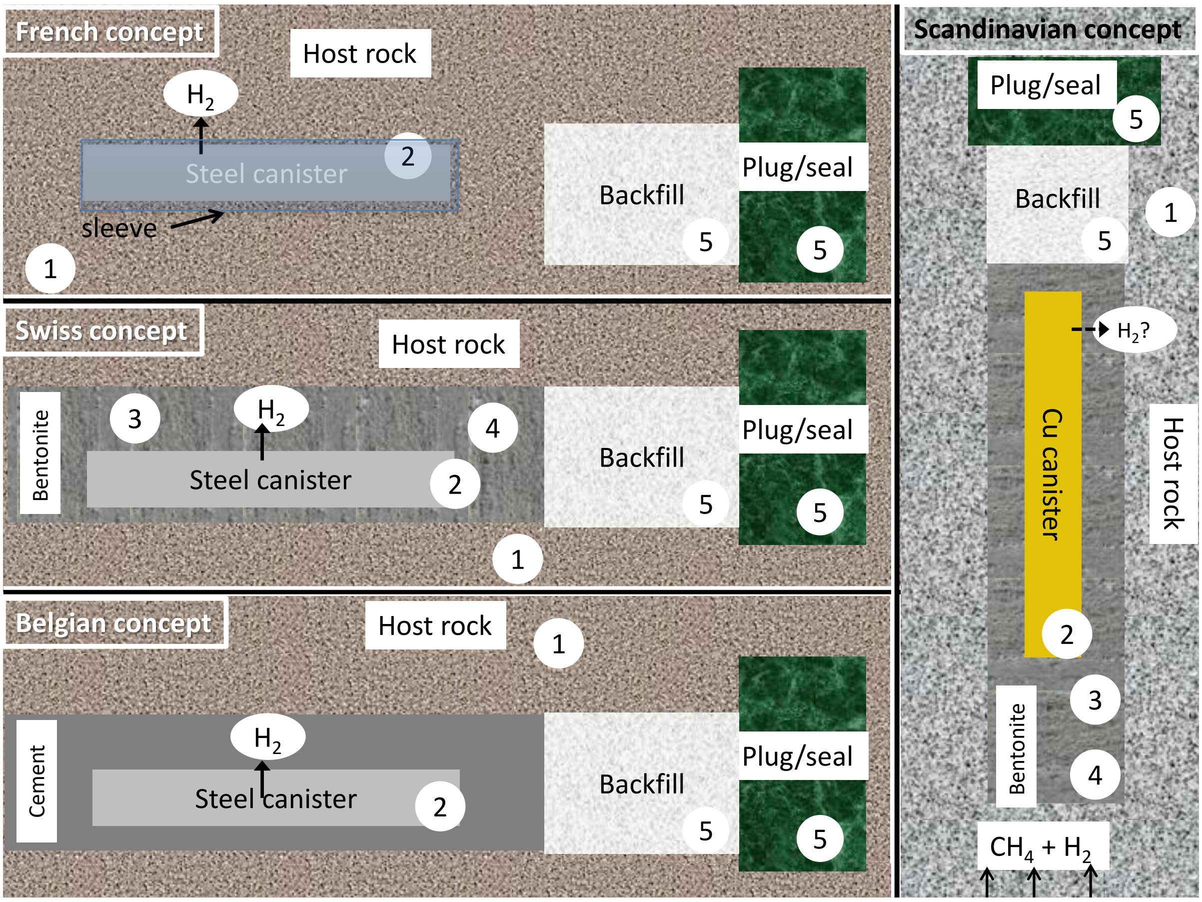

Figure 1-1. Representation of European high-level waste disposal concepts (not to scale). Microbial processes are possible at the numbered points, which correspond to task numbers. 1. Microbial generation of sulphide in the geosphere. 2. Microbially induced corrosion of canisters. 3. Microbial activity in bentonite buffer. 4. Microbial degradation of bentonite buffer. 5. microbial activity in backfill and plug/seals.

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The research objectives of WP2 were:

- 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).

Five tasks were formulated as described next.

1.2.1 Task 2.1 Microbial production of sulphide in the geosphere

The presence and activity of sulphide-producing bacteria in host rock groundwater and clays/shale is well documented. The sulphide they produce is not expected to reach the canisters because of slow diffusion through compacted bentonite buffers and the very low diffusion rate of sulphide in concrete. However, in the case of a failing clay buffer, e.g. due to erosion, or cracks in a concrete shell, sulphide will have a larger probability to contact with canisters and generate pitting corrosion. It is, therefore, important to understand what conditions foster sulphide production in the geosphere (often called the far-field) and its migration towards canisters. The action of competing iron reducing bacteria may mitigate sulphide production due to precipitation of iron sulphide phases. While the presence, numbers and diversity of sulphide-producing bacteria have been well documented, their activity is less well studied. There are cases when sulphide concentrations exceed the safety case limits more than 100 times and the underlying reasons for this accumulation are not fully understood. Hence, a remaining key issue for the safety case is to identify the factors controlling sulphide production in the geosphere, including man-made artefacts. Availability of electron donors, such as the H_2 and CH_4 from deep geological sources, is hypothesized to be one of several controlling factors. The longevity of waste canisters with respect to corrosion is a major objective of SRA Key topic 3.

1.2.2 Task 2.2 Microbially induced corrosion of canisters

Steel canisters will corrode under anaerobic, wet conditions, generating H_2. The build-up of H_2 is considered to depend on the corrosion rate, host rock permeability, and water consumption rate within the nearfield of the repository. Whether the same goes for H_2 generation from copper, has previously been debated. Many microorganisms use H_2 efficiently as an electron donor, among others for sulphide and acetate production. Hydrogen scavenging by microbes is believed to accelerate anaerobic corrosion of iron and sulphide, the product of microbial sulphate reduction, is corrosive for both iron and copper. Furthermore, copper is sensitive to acetate that may generate stress cracking. The remaining key issue is, therefore, to understand to what extent microorganisms can accelerate canister corrosion in the near-field either by hydrogen scavenging or by sulphide and/or acetate production. This issue is closely linked with the next task pertaining to the relationship between buffer and clay conditions and microbial activity. The longevity of waste canisters with respect to corrosion is a major objective of SRA Key topic 3.

1.2.3 Task 2.3 Microbial activity in bentonite buffers

Previous laboratory and full-scale experiments considering microbial survival in compacted bentonite have found that microbial activity is correlated with bentonite density and its resulting water activity. It is expected that the inhibitory effect observed at higher densities is due to the space limitations resulting from higher swelling pressures. Individual commercial bentonites have been shown to display varying effectiveness in mitigating microbial activity at similar densities. This variability may be due to sulphate or organic matter content in the bentonites or it may be due to intrinsic differences in the swelling pressures obtained. It has been shown repeatedly that commercial bentonites contain large numbers of cultivable microorganisms including iron-reducing and sulphide-

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producing bacteria. An important remaining key issue is to identify conditions (including buffer density) under which relevant bentonites inhibit microbial activity. Because of the risk for loss of density and swelling capacity due to buffer erosion and illitization, a key issue is to document the lower limit for microbial activity in bentonite buffer and host clays. This issue is part of Key topic 2, subtopic 9 SRA defined as an urgent topic.

1.2.4 Task 2.4 Microbial degradation of bentonite buffers

Microbial iron-reducing bacteria have been shown to reduce ferric iron in nontronite to ferrous iron, thereby destroying the swelling properties of such clays. The presence of similar processes, i.e. microbial mineralogical alterations of bentonite is not well investigated. Attack of iron-reducing bacteria on the ferric iron component of bentonite buffers is expected to reduce the swelling capacity of the clay and thereby to open up for microbial activity inside the buffer, to increase diffusion of sulphide and possibly allow the discharge of radionuclides. A remaining key issue is to understand whether microorganisms can accelerate degradation of bentonite-based buffers. This issue is part of Key topic 2, subtopic 9 of the SRA and defined as an urgent topic.

1.2.5 Task 2.5 Microbial activity in backfill and influence on plugs and seals

The backfill of many repositories will consist of bentonite and crushed rock in various combinations. They will constitute a large source of electron acceptor and donors that can be utilized by microorganisms. Microbial activity in such compositions of backfill is not well studied but is expected to be of importance for the integrity of plugs and seals. Microorganisms are often active at interfaces and may therefore be active at the interfaces between cement plugs and seals and backfill. The long-term behaviour of seals and plug systems is an urgent priority issue under Key topic 3, subtopic 10 of the SRA. A remaining key issue is consequently how microbial activity in backfill will influence the long-term behaviour of plug systems and seals. Within different disposal concepts, cementitious materials can be an essential part of the plug and seal systems. The integrity of the cementitious materials should therefore be ensured over a long period of time. Microbial activity, although expected to be inhibited by high pH, might affect the performance of cement on the long-term, mostly by either the production of biogenic acids, thereby lowering pH and/or enhancing calcium leaching (a detrimental effect), (ii) the enhancement of carbonation, thereby clogging the cement pores (a desirable effect) or (iii) minor processes, like biologically induced sulphate release, triggering the production of the voluminous ettringite (also detrimental).

1.3 Peer-reviewed papers and reports

1.3.1 The following WP2-related papers have been published until May 2019:

Haynes, H.M., Pearce, C.I., Boothman, C., Lloyd, J.R., 2018. Response of bentonite microbial communities to stresses relevant to geodisposal of radioactive waste. Chem. Geol. 501, 58-67.

Miettinen, H., Bomberg, M., Vikman, M., 2018. Acetate Activates Deep Subsurface Fracture Fluid Microbial Communities in Olkiluoto, Finland. Geosciences 8, 399.

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

Smart, N.R., Reddy, B., Rance, A.P., Nixon, D.J., Frutschi, M., Bernier-Latmani, R., Diomidis, N., 2017. The anaerobic corrosion of carbon steel in compacted bentonite exposed to natural Opalinus Clay porewater containing native microbial populations. Corrosion Engineering, Science and Technology 52:101-112.

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1.3.2 Reports

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

Vikman, M., Matusewicz, M., Sohlberg, E., Miettinen, H., Tiljander, M., Järvinen, J., Itälä, A., Rajala, P., Raulio, M., Itävaara, M., Muurinen, A., Olin, M., 2018. Long-term experiment with compacted bentonite. (VTT Technology; No. 332). VTT Technical Research Centre of Finland. *https://cris.vtt.fi/en/publications/long-term-experiment-with-compacted-bentonite*

1.3.3 The following WP2-related work is in review or in preparation (May 2019)

Burzan, N., Frutschi, M., Diomidis, N, and R. Bernier-Latmani. The potential role of microbes in the corrosion of steel embedded in bentonite (in preparation).

Cernousek et al. Microbially influenced corrosion of carbon steel in the presence of anaerobic sulphate-reducing bacteria (in review).

Jakub Kokinda, Rojina Shrestha, Katerina Vizelková, Katerina Cerna, Jana Steinova, Alena Sevcu, Tomas Cernousek: Anaerobic microbial corrosion of Carbon steel in synthetic bentonite pore water of Czech Bam bentonite (in preparation).

Katerina Cerna, Rojina Shrestha, Jakub Kokinda, Hana Kovarova, Petr Polivka, Tomas Cernousek, Alena Sevcu: Survival of microorganisms in bentonite subjected to gamma radiation and the effect of anaerobic conditions on the microbial ecosystem (in preparation).

Nicole Matschiavelli, Sindy Kluge, Carolin Podlech, Daniel Standhaft, Georg Grathoff, Atsushi Ikeda-Ohno, Laurence N. Warr, Alexandra Chukharkina, Thuro Arnold, Andrea Cherkouk: Development of microorganisms in uncompacted bentonite and their relevance to the disposal of high-level radioactive waste (in review)

Rojina Shrestha, Jakub Kokinda, Katerina Cerna, Tomas Cernousek, Alena Sevcu: Effect of concrete on anaerobic microbial community in conditions relevant to deep radioactive waste repository (in preparation).

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1.4 Deliverables

The planned outcome of the research was summarised in 18 deliverables (Table 1-2).

Table 1-2: Deliverables in WP2

Deliverable Number	Deliverable Title	Lead beneficiary
D2.1	Inventory of reducing gases	MICANS
D2.2	Design, set up and operation of experimental equipment	NERC/VTT
D2.3	Interim report on deep gases and sulphur compounds as biogeochemical energy sources in crystalline rock	GTK
D2.4	Bacterial presence and activity in compacted bentonites	MICANS
D2.5	Microbial activation due to addition of electron donors/acceptors in deep groundwaters	VTT
D2.6	Microbial diversity in bentonite buffer of aged bentonite buffer experiment	VTT
D2.7	Microbial diversity in aged bentonite	TUL
D2.8	Long-term stability of bentonite in the presence of microorganisms	HZDR/VTT
D2.9	Evolution of stress in biotic and abiotic clay flow cells	NERC
D2.10	Microbial mobility in saturated bentonites of different density	CV REZ
D2.11	Cement deterioration boundaries	SCK•CEN
D2.12	Microbial activity in a concrete-bentonite clay interface	MICANS
D2.13	Anaerobic microbial corrosion of canister material	TUL
D2.14	Impact of bentonite dry density on the viability of organisms (in the context of steel corrosion)	EPFL
D2.15	Survival of microorganisms in bentonite subjected to different levels of irradiation and pressure	CV REZ
D2.16	Microbial activity and the physical- chemical and transport properties of bentonite buffer	NERC
D2.17	Sulphide production	GTK
D2.18	Rate of corrosion of carbon steel in bentonite under biotic and abiotic conditions	EPFL
D2.19	Synthesis of research into microbial processes affecting high level radioactive wastes and engineered barrier systems	MICANS

The main factors and processes addressed in each deliverable are shown schematically in Table 1-3. Three deliverables deal with conditions for microbial activity in the geosphere outside the

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repositories. Microbial presence, activity and processes in buffer and backfill are dealt with in 10 deliverables and 2 deliverables investigated concrete effects. Microbially influenced corrosion aspects are dealt with in 4 deliverables.

A synthesis of results and interpretations is presented next. First, this report summarizes data and results concerning the presence and diversity of microbes and conditions for life in buffer and backfill environments. Thereafter, the report reviews investigated processes such as stability of bentonite and concrete in the presence of microbial activity and microbially influenced corrosion.

The text refers to deliverables according to Table 1-2.

For the readers convenience, the respective summary of each deliverable can be found in the Appendix, chapter 6, of this synthesis report.

Table 1-3. Representation of investigated factors and processes in WP2 distributed over the 18 deliverables listed in Table 1-2.

Keywords	**D2.1**	**D2.2**	**D2.3**	**D2.4**	**D2.5**	**D2.6**	**D2.7**	**D2.8**	**D2.9**	**D2.10**	**D2.11**	**D2.12**	**D2.13**	**D2.14**	**D2.15**	**D2.16**	**D2.17**	**D2.18**
Geosphere (3)	o		o		o												o	
Electron donors and acceptors					x													
Sulphide			x		x												x	
Gases	x		x															
Geochemistry			x		x													
Buffer and backfill (10)		o		o		o	o	o	o	o				o	o	o		
Presence and diversity of microbes				x		x	x	x						x	x			
Limits of microbial activity				x										x	x	x		
Mobility of bacteria										x				x				
Bentonite stability/degradation		x		x		x		x	x									
Concrete-clay (2)											o	o						
Deterioration											x							
Microbial activity												x						
Canister (4)				o					o				o					o
Microbially influenced corrosion									x				x					x
Sulphide corrosion				x					x				x					x

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2 The geosphere

Geosphere issues have been addressed mainly by D2.1, D2.3, D2.5 and D2.17 (Table 1-3).

Metallic canisters will be used to ascertain the long-term isolation of spent nuclear fuel and other highly radioactive wastes in geological repositories. Iron (steel) and copper metals are in most cases considered as the canister materials. Complete containment and longevity with respect to corrosion are the performance targets of the metallic waste canisters. For the safe geological disposal of nuclear waste, sulphidic corrosion of copper is an important process to be evaluated in detail for copper canister. Neither can sulphide formation in corrosion of metallic iron be ruled out, but competitive reaction paths and reaction products are possible. Quantification of the canister corrosion requires that the constraints of microbially induced sulphide corrosion are well understood and are based on firm knowledge on the geochemical conditions at the disposal depth. D2.1 and D2.3 summarizes knowledge on occurrence of sulphur compounds in groundwaters of Finnish crystalline bedrock. The potential role of rock matrix fluids and gases for sulphide production was also identified as an important issue.

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

D2.5 describes results from simulations of deep groundwater mixing and infiltration, which introduces previously limited electron donors and acceptors for microbial communities and their activation. Activation of microbial populations with fluorescent redox dyes was an efficient screening method to study induced microbial metabolism. The two groundwaters studied with redox sensing fluorescent dye were very different from each other. OL-KR6 was a sulphate rich groundwater with high bacterial and archaeal diversity and clear activation with several electron donors and acceptors was detected. OL-KR15 on the other hand, had lower bacterial and archaeal diversity than OL-KR6 water and was activated with only acetate and acetate together with sulphate. Acetate was overall the most efficient activator of the studied microbial communities which indicates acetate's important role as an electron donor for different Olkiluoto deep subsurface groundwater communities (Miettinen et al. 2018).

D2.17 focussed on understanding of the limits of the deep life in the geosphere. Presence of electron acceptors in sufficient amounts (confer D2.5) is one of the most important controlling factors for sulphide formation. Nutrient limitation is not considered as an important limiting factor, because the bedrock environment provides an adequate source. In deep biosphere, recycling of essential elements and syntrophic interactions play key roles in sustaining microbial life. Transport limitations, based on advection and diffusion, seems to be an easily quantified way to estimate long-term sulphide production rates. Ideal conditions in terms of all other factors required by the microbial sulphide production would evidently lead to limited supply by the limited transport rate. Energetic calculations demonstrated that life, deep in the bedrock, has a plausible energy source supplied by the surrounding chemical energy. The life-sustaining energy flux, i.e. power, remains however low, and cannot be multiplied by many orders of magnitude in a sustainable way.

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3 Bentonite clays and conditions for microbial life

Bentonite clay will be used as a buffer material in engineered barrier systems (EBS) which will contain, protect and surround nuclear waste canisters in geological disposal concepts. The dissimilatory reduction of sulphate, thiosulphate and sulphur to sulphide by sulphide-producing bacteria (SPB) is a main concern for the safety case of a geological disposal (SRA key topic 3) since sulphide is a corrosive agent for metal waste canisters. The disposal concepts rely on the swelling capabilities of the bentonite clay when it becomes water saturated as one of the main protective features. To reach generally desired swelling pressures of >5 MPa a clay dry density of >1600 kg m^{-3} is commonly required. Depending on the minerology of the specific bentonite type and the groundwater composition, where salinity is an important factor, different swelling pressures are produced at the same wet density. A high density with a concomitant high swelling pressure is believed to have an inhibiting influence on bacterial activity of the natural bacterial populations in the bentonite clays, meaning that growth and metabolic activity will cease, but present bacteria will not necessarily die.

3.1 Presence and diversity of microbes in bentonite clays

Presence and diversity have been addressed mainly by D2.4, D2.6, D2.7 and D2.8 (Table 1-3).

There is an overwhelming evidence in the scientific literature that sulphide-producing bacteria (SPB) and many other types of microorganisms are present in commercial bentonite clays. For instance, cultivable SPB have been detected previously in various types of commercially available bentonites (Bengtsson and Pedersen 2017; Masurat et al. 2010a; Pedersen et al. 2000; Svensson et al. 2011). Cultivable sulphide-producing bacteria have also been found in full-scale demonstration repositories in Switzerland and Sweden (Arlinger et al. 2013; Bengtsson et al. 2017), in various pilot and full-scale tests of bentonite and copper canister performance (Johansson et al. 2017; Karnland et al. 2009; Smart et al. 2014; Svensson et al. 2011) and in the Boom Clay formation (Bengtsson and Pedersen 2016 and references therein). The microbes may originate from the mining sites or from industrial processing the clays because such processing open-up for introduction of bacteria from air, and all surfaces and materials that contact the clay on its way from the mines to the user. The bacteria likely survive in the dry clays as spores, or as completely desiccated cells. Bentonite or rather montmorillonite, has a verified high affinity for water and the cell membrane of bacterial cells is water permeable. If a bacterial cell is surrounded by bentonite, it is possible that the water affinity of montmorillonite will extract water from the cell, leaving it in a desiccated state. The phenomenon of drying cells for prolonged storage is well known and commonly used in microbiology (Gherna 1994). Slow desiccation can yield higher viability, after prolonged storage, than can fast desiccation (Laroche and Gervais 2003; Potts 1994) and also increased heat resistance and viability for both spores and vegetative cells (Fine and Gervais 2005). Bacteria consequently have several mechanisms to survive prolonged periods of exposure to heat and desication. When water saturation of the clay starts, spores and descicated cells can be activated and start to metabolize.

The principal methodologies to detect presence of bacteria are cultivation or visualisation using microscopy techniques. Diversity can either be analysed using methods for the determination of phenotypes (cultivation and classical taxonomy) or genotypes by extraction of nucleic acids and sequencing. Commonly, detection of presence and analysis of diversity to some taxonomic level are done simultaneously. Several deliverables deal with presence and diversity of microorganisms (Table 1-3). D2.4 and D2.14 show that cultivable sulphide-producing bacteria were present in all investigated clays, irrespective of density and swelling pressure. Presence of microorganisms in bentonites were reported in D2.6 and D2.7 as well. D2.6 and D2.15 report large bacterial and fungal diversities in bentonites using 16S rDNA sequencing. D2.7 specifically report representatives of genera *Nocardia*, *Pseudomonas*, *Pseudonocardia*, *Saccharopolyspora* and *Streptomyces*. D2.8 found the spore-forming SPB *Desulfosporosinus* to be common in the studied

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clay. Generally, analysis of DNA sequences in libraries of DNA extracted from various bentonites shows a large diversity of bacteria including SPB, and there is commonly a large proportion of spore-forming bacteria, a survival form well adapted to dry clays (Chi Fru and Athar 2008; Lopez-Fernandez et al. 2015).

Visualisation methods have been developed, tested and reported in D2.4 and D2.10. They both rely on a density gradient centrifugation step. In D2.4, clay samples from the FEBEX full-scale demonstration repository were separated and stained with DAPI. Bacterial cells could be extracted from the FEBEX clay and observed with microscopy (Figure 3-1). They had survived 18 years experimental time in the buffer. The deliverable D2.10 describes and alternative staining method with a so-called Live/Dead fluorescence staining method. Although the methods may need further optimization and testing of its general functionality on different bentonite types it will be very useful for future research of bacterial presence in various clay materials.

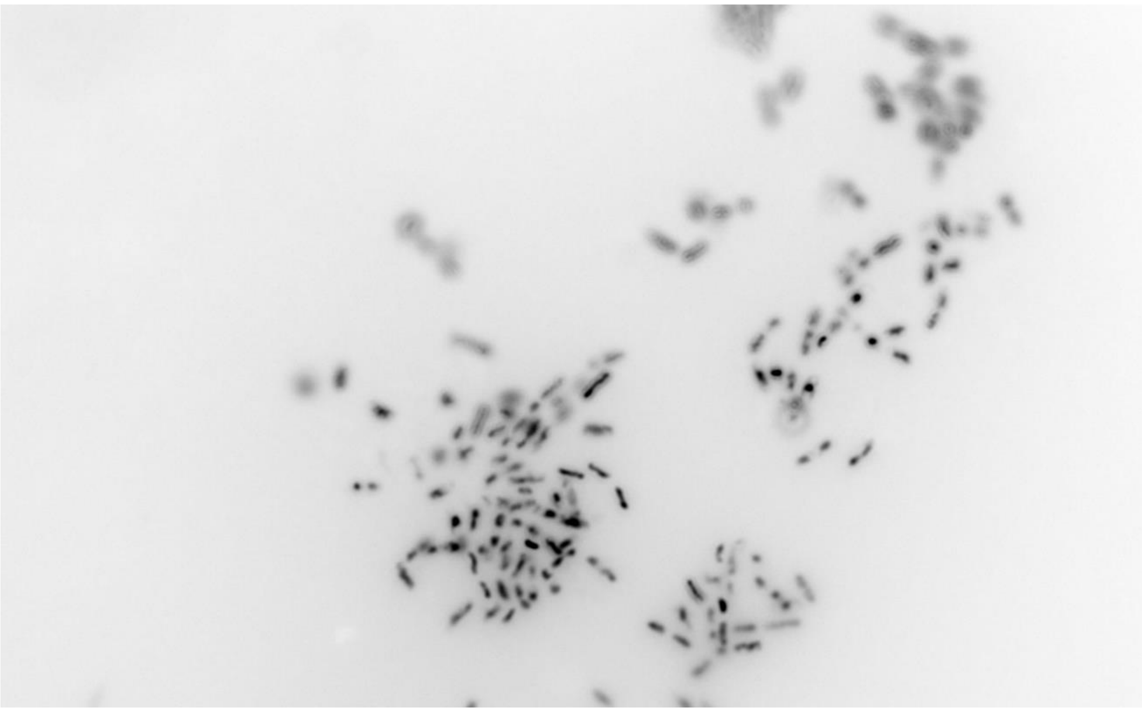

Figure 3-1. Images of extracted bacterial cells from non-spiked FEBEX clay sample B-C-60-18 after hydration. The clay was hydrated by incubation in 0.9% NaCl solution before cell extraction. The sample was stained with DAPI Vector shield mounting medium. Some of the cells have a blurred appearance due to that they are out of the focal plane of the microscope (Image from Bengtsson et al. 2017).

In conclusion, the MIND project thoroughly confirms previously published and reported data that has shown microbes to be present in commercial clays as well as in compacted clay in laboratory and field scale experiments simulating buffer and backfill conditions in future radioactive waste repositories.

3.2 Limits of bacterial activity in compacted clays and clay slurries

These limits have been addressed mainly by D2.4, D2.14, D2.15 and D2.16 (Table 1-3).

Bacterial life and survival, presence, viability and activity will depend on several different variables in a buffer or backfill that relate to the water saturation and swelling of bentonites compacted to high density. Consequently, it is not density *per se* that controls bacterial activity in compacted clays. Rather, other factors, that are related to the density and type of clay, control bacterial activity (Figure 3-2).

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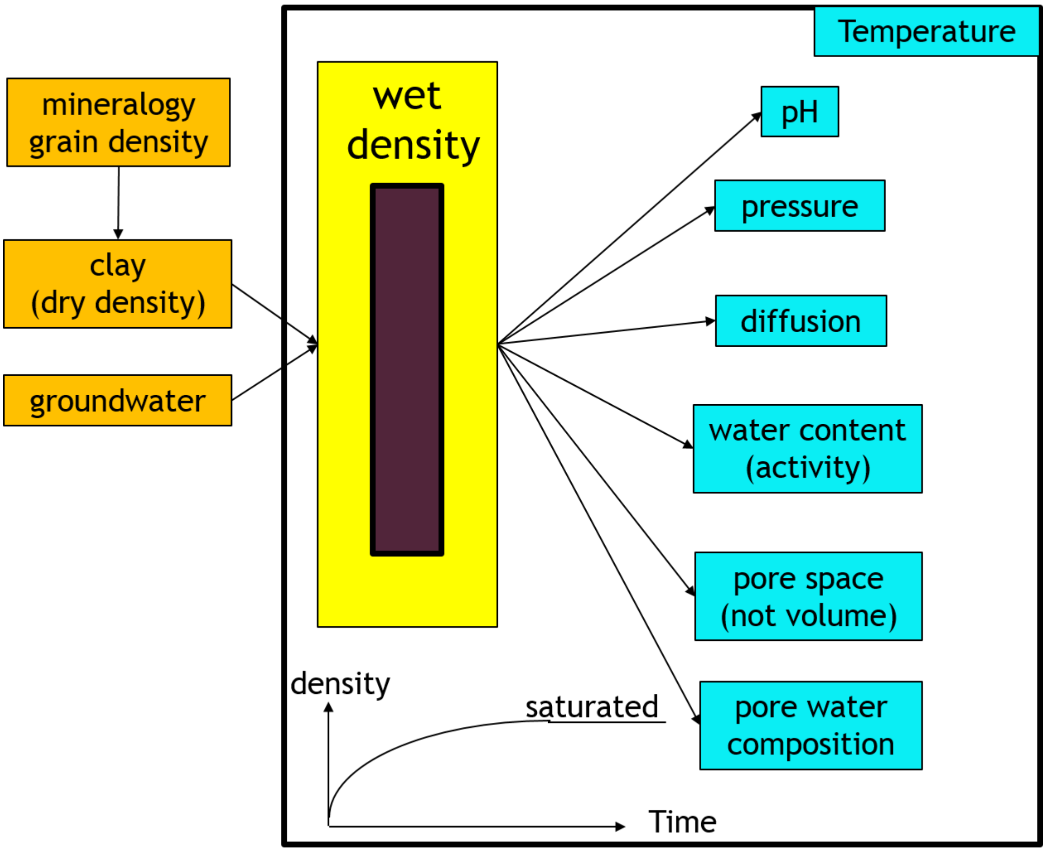

Figure 3-2. Variables setting the buffer characteristics (orange), that upon water saturation generate clay conditions (turquoise) except temperature not coupled to clay types, which may influence bacterial viability, cultivability and activity in the buffer and backfill.

3.2.1 Pressure and density

The swelling pressure in the bentonite originates from separating flocs in the bentonite. This means that a mechanical pressure arises between the separating flocs, approximately equal to the swelling pressure. Even in low-density bentonites (1500 kg m^{-3}), a pore size in the nm range would theoretically not allow for bacterial existence unless the bacteria could withstand the mechanical pressure from the separating flocs (0.09 MPa at 1500 kg m^{-3}). Prokaryotic cells can compensate for the mechanical pressure in compacted bentonite by turgor pressure. Published data on turgor pressure in prokaryotic cells mention pressures between 0.08 MPa and 2 MPa (Potts 1994). An upper limit of 2 MPa turgor pressure would mean that cell integrity is possible, though limited, at bentonite swelling pressures below 2 MPa. Results from repeated experiments with different bentonite types suggested a limit for microbial activity at approximately 1 MPa (D2.4).). In experiments with BaM bentonite that is intended for the Czech repository, exposure to 2 and 5 MPa did not markedly reduce the total microbial biomass or change the community composition (D2.15). Similar results were obtained with Volclay MX80 during ongoing experiments in Mont Terri Underground Research Laboratory (URL) (D2.14). Irrespective of the targeted dry density, there was an initial increase in both aerobic and anaerobic heterotrophs followed by a steady decrease up to ~2.5 years. Increase in numbers was observed again at 5.5 years. There were little differences for SRB over time and clay density (Figure 3-3). Further, experiments by Haynes et al. (2018) also showed a limited effect of pressure (74 MPa 30 sec.) on survival of bacteria.

Consequently, swelling pressure, which relates to clay type and density, affected activity but not viability, cultivability or diversity. Clay density did not markedly influence viability and diversity.

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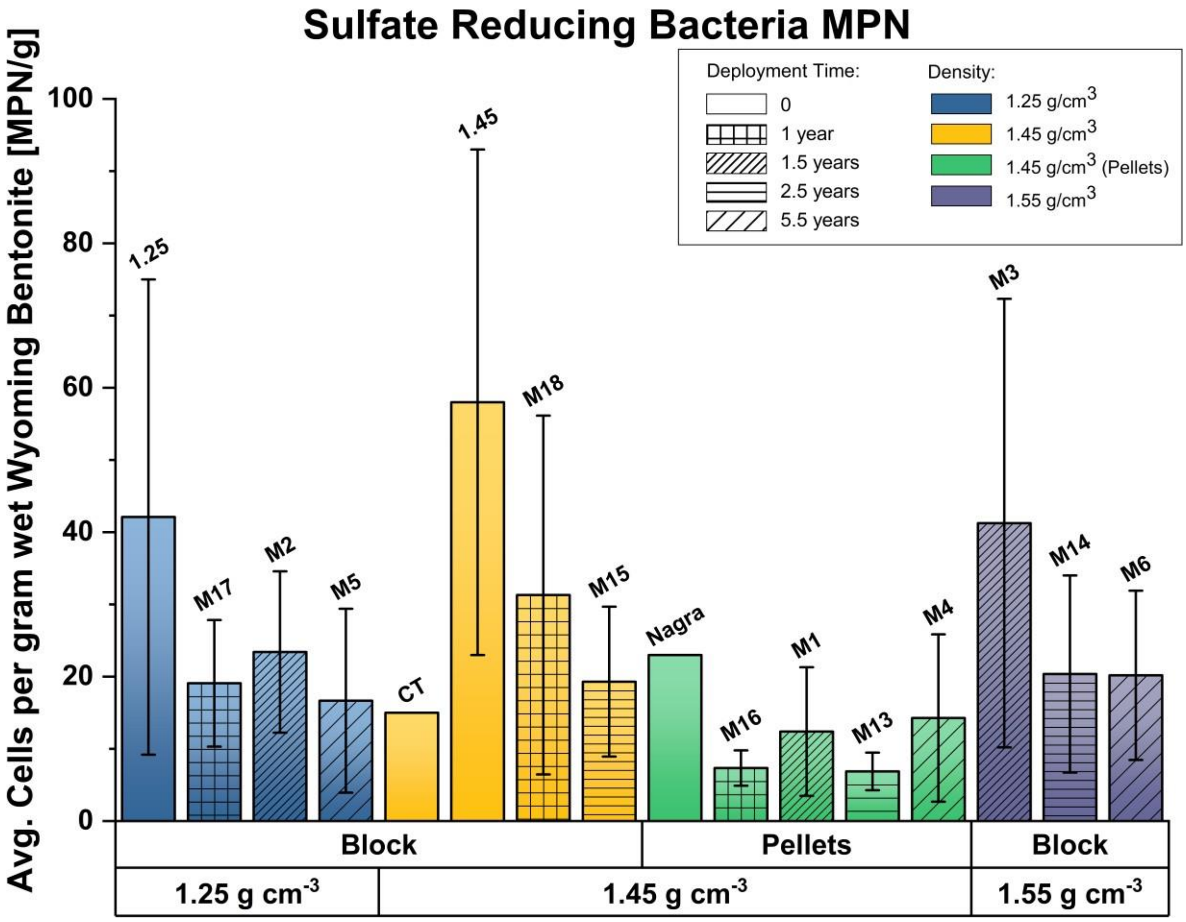

Figure 3-3. Average number of viable SRB versus the targeted dry density as a function of interior and exterior location within the samples. Number of viable SRB was averaged over all layers per module interior or exterior, respectively, and error bars represents standard deviation amongst layer. Modules from initial state are included (no pattern) and represented by the blocks from which the modules were made. The highest numbers of viable SRB are observed for the interior part of the initial state blocks 1.25 and 1.45, despite the three- to four-fold lower number observed for the CT pellets from which the blocks were made. Overall, the number of viable SRB remained stable irrespective of deployment time. Modules made out of bentonite pellets showed the least viability of SRB. No correlation with targeted dry density was apparent (Graph from D2.14).

3.2.2 pH and temperature

The pH of most bentonite clays is slightly alkaline but still well within the range of what most bacteria can tolerate. In radioactive waste repository concepts, the maximum surface temperature of canisters may not exceed 90 °C in order to avoid formation of steam when water come in contact with the canister. It was previously found that heat treatment at 120 °C for 15 h (Masurat et al. 2010b) or 110 C for 170 h (Bengtsson and Pedersen 2017) did fail to kill inherent bacteria in the bentonite. Heat was expected to be efficient, but that was still not enough to kill off sulphide-producing bacteria in the bentonite because intensive sulphide-producing activity and large numbers of cultivable sulphide-producing bacteria were observed in heat treated MX-80. Similar results were reported for exposing various bentonites to 90 °C, 24 h, by Haynes et al.

3.2.3 Diffusion

Transport of nutrients to, and metabolic products such as sulphide from bacteria will be diffusion limited due to the low porosity of buffers and backfill (See these references for data on sulphide diffusion Pedersen 2010; Pedersen et al. 2017). The diffusion rates were negatively correlated with increasing clay density. Bacterial activity will, consequently, be diffusion limited in backfill and buffers when the bentonite buffer and backfill are fully water saturated. The only position where bacteria

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are not affected by diffusion barriers will be the interface between rock/aquifers/engineered disturbed zone (ESZ) and buffer and backfill.

3.2.4 Water content

Water is needed for active bacterial life and this water must be present externally because bacteria (except spores) cannot keep water inside their cell membranes that are freely permeable for water. Low water content in clays, a_w, will inactivate or kill bacteria (Motamedi et al. 1996; Potts 1994). However, as was discussed for temperature above, many bacteria survive desiccation and can be activated again when there is enough water. It would appear from the D2.14 work that a single dry density threshold may not be applicable but rather than the ability of bentonite to inhibit microbial growth and activity may depend on the degree of saturation of the bentonite. Using the microbial results, it was hypothesized that full bentonite saturation may take several years and that oxygen may persist as adsorbed on the clay for long periods of time. Thus, microbes can thrive within the bentonite until it reaches full saturation and appear to persist at a stable number for extended periods. These results also raise the question of whether there is a resurgence of microbial activity after several years of exposure to a repository environment. The results from FEBEX where viable bacteria were found after 18 years (Bengtsson et al. 2017) (Figure 3-1) suggest resurgence of microbial activity is possible after long time.

3.2.5 Pore size and mobility

Bacteria are very small and if there are pores or other inhomogeneities in buffer and backfill with lower than planned pressures, local bacterial activity will be possible. In addition, there will be interfaces between rock engineered disturbed zone and bentonite and between bentonite and canisters at which pressures may differ from the bulk of buffer and backfill. Figure 3-4 shows that sulphide-producing bacteria can form local colonies in compacted clays, likely in positions were impurities of the clay offer enough large pore space for bacterial life. Similar black spots were observed in bentonite-SRB cultures as reported in D2.8. It is well known that bacteria generally grow in colonies, rather than evenly throughout their environment. The work with MX-80 bentonite in Mont Terri (D2.14) suggests that the bacteria found in the clay did not enter with saturating groundwater. Instead, it seems as if the bacteria present came with the clay. The opposite case is discussed in D2.10. There, it was concluded that bacteria were able to move in compacted bentonite. However, the authors also raise some doubts about their results as possibly related to experimental conditions. . The NGS sequencing results from subsequent experiment including similar bentonite and underground water imply that most of the bacteria from groundwater are indeed lost soon after their contact with the bentonite (D2.15).

3.2.6 Pore water composition

The pore water composition will vary with the type of bentonite and the composition of the saturating groundwater. Bentonites vary in composition with respect to elements and minerals and the type and content of natural organic matter (D2.4, D2.15). The conditions for survival and activity of bacteria may, consequently, vary significantly between different bentonites.

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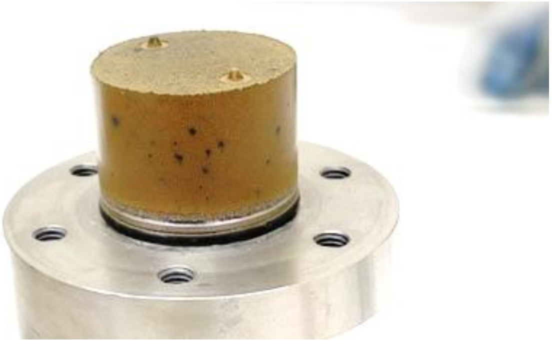

Figure 3-4. Black (FeS-stained) colonies of iron sulphide produced by sulphide-producing bacteria in Asha bentonite at 1750 kg m^{-3} wet density and with addition of lactate and sulphide-producing bacteria (From D2.4).

Figure 3-5. Anaerobic culture of sulphate-reducing bacteria with MX-80 bentonite. Black FeS-layers and areas with different intensities on top and inside of the bentonite.

Figure 3-6. In the bulk MX-80 bentonite and near the corrosion coupons, there is no evidence of abundant sulphate-reducing bacteria (SRB). In contrast, on the surface of the canister, and on the surface of the bentonite, in black spots, SRB are clearly present and growing (From D2.18).

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3.2.7 Irradiation

Radiation is expected to be very important factor presumably limiting microbial activity in the repository. However, the reaction of the microorganisms to the long-term exposure to low levels of ionizing radiation remains unclear. The average D_{10} dose for most vegetative microorganisms was calculated to be 0.420 kGy (van Gerwen et al., 1999), but several highly radiation-resistant microorganism are known. In accordance with this assumption,

Stress induced by irradiation (24.17 Gy min^{-1} for about 40 minutes) was found to have some effect on microbial activity in different bentonites. A reduction was seen in the microbial numbers and it was found that spore-formers had an advantage in survival (Haynes et al. 2018). (Haynes et al. 2018). On the other hand, application of nearly 20 kGy of total dose was not sufficient to completely eliminate the bentonite microflora, although the irradiation caused significant shift in the microbial community composition (D2.15). Further long-term irradiation experiments performed under repository relevant conditions are necessary to describe the development of microbial ecosystem and potential genetic changes caused by the radiation.

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4 Processes

The deliverables in the previous chapter discuss that microorganisms are present in all types of bentonites. Mainly, it appears as if the detected microorganisms follow with the clays, i.e. the clays are contaminated during processes, and possibly, some microorganisms may originate from the mined bentonites. It seems, consequently, important to evaluate the bentonite intended for repository use not only for its physical and mechanical properties but also for the potential for microbial activity that may risk to compromise the safety case.

4.1 Microbial degradation of bentonite and concrete materials

4.1.1 Bentonite

D2.8 studied possible effects from microorganisms in bentonite slurries while D2.2, D2.4 and D2.9 investigated such effects in compacted bentonite (Table 1-3).

D2.8 found that the cation exchange capacity was stable with some small differences between anaerobic and aerobic treatments. Similar results were reported by D2.6. D2.9 tested if microbial activity had an influence of the stress from swelling in clay samples, but effects, if any, were too small for detection of the used system. In addition, D2.9 found that corroding steel influenced the clay in the vicinity of the steel, but the results did not show any obvious effect from samples with SRB compared to sterile samples. D2.4 studied the effect from sulphide on bentonite samples. It was found that sulphide reacts with ferric iron in the tested clays under the formation of FeS and elemental sulphur (S^0). These reactions will decrease the mass of sulphide that reach canisters because the sulphide is immobilised as FeS and S^0. However, the concomitant effects from these sulphide reactions on the clay properties remains to investigate.

4.1.2 Concrete

The possibility of microbial degradation of concrete has been addressed mainly by D2.11 and D2.12 (Table 1-3).

D2.11 reports on anoxic batch experiments that were performed to study the microbial community present in Boom Clay borehole water in a cementitious environment. It was found that the high pH conditions imposed by the cement inhibited microbial sulphate and nitrate reduction. However, the presence of intact cells in the suspension on top of cement and putative biofilm structures on the cement was indicated by scanning electron microscopy investigations. D2.12 concluded that the alkaline solution leached from the concrete inhibited microbial activity due to an increased the pH in the bentonite clay. However, the inhibition of microbial activity was dependent of added carbon sources. Induced microbial activity in MX-80 with glucose led to a decreased in pH. A reason might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. In the future bentonite clay samples should be analysed for acetate and cultivatable acetogenic bacteria to confirm this hypothesis. Similarly, D2.11 suggest that the high pH environment does not completely eliminate the microbial population. Interestingly, in sulphate reducing conditions, a pH decrease from > 12 to pH 10 was observed in one replicate harbouring clearly a larger microbial community in the suspension on top of the cementitious material compared to the other samples.

4.2 Microbially influenced corrosion of canisters

Canister corrosion has been addressed mainly by D2.4, D2.9, D2.13 and D2.18

4.2.1 Sulphide corrosion

The main corrodent for canisters will be sulphide. For a relatively short period, O_2 driven corrosion processes may occur, but the amount of enclosed O_2 will be limited. Once anaerobic conditions

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prevail, sulphide from SPB will be the main corroding species, provided it reaches the canisters through buffer and backfill.

The deliverable D2.13 reports investigations of anaerobic, microbially influenced corrosion (MIC) of canister materials (12020 carbon steel and 316 L stainless steel) in environments containing a natural microbial community dominated by SRB. The experiments were performed in parallel arrangements including sterile controls, and no nutrients were added. In other words, only naturally occurring nutrients and bacteria were included in the study. Biofilms at the higher temperature (35 °C) was observed to lead to an increase in the inhibitory action by a barrier effect of the biofilm, while biofilm at room temperature accelerated corrosion. Biofilms were consequently found to have a dual effect, both reducing and accelerating corrosion of the studied test specimens.

The deliverable D2.4 summarises work describing bacterial sulphide-production in bentonites compacter to various densities and swelling pressures. The sulphide produced in the clay was found to corrode copper specimens. The swelling pressure in the bentonite originates from separating flocs in the bentonite. The reviewed and compiled results suggest a swelling pressure limit of microbial activity at approximately 1 MPa. However, bacteria can survive a much higher pressure as inactive cells.

A larger scale experiment compared to the D2.4 and D2.13 experiments is presented in the D2.18. There, stainless steel modules with carbon steel corrosion coupons embedded in Volclay MX80 bentonite were installed in a vertical descending borehole in Opalinus clay the Mont Terri URL. . A pattern emerged from the results as a function of space. In the bulk bentonite and near the corrosion coupons, there was no evidence of abundant SRB. In contrast, on the surface of the canister, and on the surface of the bentonite, in black spots, SRB are clearly present and growing. The bulk bentonite harbours primarily aerobic microorganisms, suggesting the persistence of adsorbed oxygen in the bentonite for extended periods of time. Questions remain about the further evolution of the system as to whether SRB will progressively colonize an increasingly thicker layer of the bentonite. Further time points in this 10-year experiment will be able to address this question.

Effects from microbial activity on the swelling behaviour was studied in D2.9. A need to understand the limits to microbial growth and the potential for microbial activity to affect the swelling behaviour of the clay and metal corrosion was identified. Survival in bentonites, alteration of swelling capacity and microbial steel corrosion have all been investigated in isolated experiments. The D2.9 were designed to investigate all these processes in in a single set of laboratory scale flow experiments using compacted bentonite samples. The obtained results relate to the development of stress in the experiments, including discussion of petrological and mineralogical alterations that could influence the physical properties of the bentonite.

Given the limited duration of the tests, it was not surprising that alteration of the bentonite was limited to a small zone adjacent to the corroding iron. From a ‘flow perspective’ this will have little impact, as the bulk of the clay, and therefore the flowing porosity, remains unchanged. To understand the full impact of microbial action on the hydraulic properties of the clay, test durations would need to be increased substantially, or the iron should be dispersed within the entire clay matric to increase reaction rates. Given these findings, the question of whether microbial activity alters the hydraulic behaviour of bentonite remains unanswered. However, XRD and SEM analysis has identified alterations that appear to be related to the presence of microbial cells in the sample. This observation deserves further investigation. However, further work is required beyond the lifetime of this project to fully assess the impact of microbially induced changes on the hydraulic integrity of the clay.

In summary, all four deliverables report that SPB can be active and produce sulphide in buffer and backfill. They also identify constraining factors including time. The corrosion related processes observed are slow and several deliverables identified the need for longer-term experiments to be

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able to conclude on the extent and rates of MIC under repository conditions. These may vary from repository concept to concept and also between clays and canister materials.

Origins of sulphide

While the deliverables above focussed on SPB in buffer and back-fill, D2.17 addresses the SPB and their activity in the geosphere. The deliverable identifies several factors possibly controlling microbial sulphide production in the geosphere. The redox potential and solubility limitation of minerals, and limitations of availability of sulphur, electron donors and acceptors, nutrients and transportation.

Mobility of sulphide in compacted bentonite

Any sulphide produced in the geosphere or in buffer and backfill must diffuse through clay to reach the canister. New data suggest that sulphide reacts with bentonite clays which will limit the amount of a given sulphide concentration that can reach the clay. This sulphide buffering capacity seems to be significant as judged from the D2.4 results.

Figure 4-1. Transport of 2 different amounts of sulphide added to bentonite clays after 90 days of water saturation to 2000 kg m⁻³ wet density. There were significant amounts of S^0 in the black layers (see D2.4 for details).

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5 Acknowledgement

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6 Appendix – Deliverable summaries

6.1 D2.1 Inventory of reducing gases (GTK/Micans)

In order to provide data needed to address the question on geochemical constraints of biological activity at spent nuclear fuel waste repository sites, geochemical data of dissolved gases from deep drill holes and mines in Finland and Sweden were collected based on a literature and database survey. Gas data were found from 20 separate localities in Finland with the deepest drill holes extending down to 2500 m below surface. The gas phase is dominated by nitrogen and methane, although significant variation exists between different sites and with depth. At least partly this variation can be related to differences in lithology (rock types) and residence time of water within the bedrock. Corresponding data from Sweden were from 2 localities extending to at most 1000 m below surface.

In the context of microbiological risks related to SNF waste disposal, the geochemistry of gases in deep groundwater is an integral part of the determination of geochemical constraints of biological activity at disposal depths. Data on gas compositions and concentrations do exist from several separate locations in Finland and two locations in Sweden. Site to site as well as depth dependent variation should be taken into account in Finland and could possibly be used to predict changes related to for example different rock types. For Sweden, there is a need to increase the number of gas samples from the selected site in Forsmark and also the number of sites and geological types for comparisons and increased understanding of generation and transport of reducing gases in deep geological formations.

6.2 D2.2 Design, set up and operation of experimental equipment (NERC/VTT)

A laboratory scale experiment investigating the impacts of microbial activity on bentonite structure has been started at VTT. The experiment simulates a worst-case scenario where bentonite is not compacted, water, gases, nutrients and microorganisms are able to move along easily at temperature hospitable for microbial welfare. The aim is to find out if microorganisms and the produced metabolites are able to change the bentonite structure in favourable conditions and if these changes are significant for the bentonite ability to function in long-term scale. The experiment will be monitored with microbial sampling as well as with atomic force microscopy (AFM) once a year.

NERC (BGS) is responsible for carrying out experiments investigating microbial degradation of bentonite buffers (T2.4) and microbial induced corrosion of canisters (in T2.2). Bespoke vessels have been constructed from grade two titanium and fitted with 5 load cells (2 axial and 3 radial) for both of these tasks. This apparatus will continuously monitor total stress to see if the microbial activity and corrosion of steel has an effect on the hydromechanical and transport properties of the bentonite. All apparatus components have been manufactured and calibration is now underway. Experiments will begin as planned in Q1 of 2017.

6.3 D2.3 Interim report on deep gases and sulphur compounds as biogeochemical energy sources in crystalline rock (GTK/Micans)

Geochemical data on sulphur compounds and dissolved gases from deep drill holes and deep mines in Finland were compiled to a database. Data is used as a background of MIND work package 2, in which microbial production of sulphide in the geosphere will be assessed from the point of view of canister corrosion. This report summarizes the present knowledge on the mode of occurrence of sulphur compounds and dissolved gases in groundwaters of crystalline bedrock. Prerequisites of

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microbial sulphate reduction are discussed based on the data available. The most abundant sulphur compound in deep groundwaters is sulphate (SO_4). A distinct sulphate reduction zone is often observed, situating at different depths at different sites but most often above 400 m. Below the reduction zone sulphate concentration drop abruptly, and a transient sulphide-rich zone may exist. Sulphide concentrations observed are typically below 10^{-5} M, indicating that solubility of iron sulphide controls the concentration.

Methane is the most abundant dissolved gas, possibly acting as an electron donor for microbial sulphate reduction. Small amount of hydrogen is often observed in deep water samples and much larger amounts can be found in porewater of rocks. In some conditions sulphate shows distinct stability in deep anoxic systems, existing together with high amounts of methane. This was observed to be more common in groundwaters hosted by basic rocks (e.g. gabbro), which are generally considered to be less permeable than common granites and gneisses. Anomalous, high sulphate/high methane groundwater type was found in Jotnian metamorphosed clay rock of Muhos formation.

6.4 D2.4 Bacterial presence and activity in compacted bentonites (Micans)

The MIND project targets a number of high urgency and high importance topics identified in the Strategic Research Agenda, (SRA) (IGD-TP, 2011), focusing mainly on Key topic 2: Waste forms and their behaviour and Key topic 3: Technical feasibility and long-term performance of repository components. WP2 addresses SRA Key topic 3: Remaining key issues for the geological disposal of HLW (High Level Waste) 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 under which relevant bentonites inhibit bacterial activity, and to understand whether microorganisms can accelerate degradation of bentonite-based buffers and influence the long-term behaviour of plug systems and seals.

The work reported here aimed at 5 main objectives. 1) Analysis of the clay environment with respect to composition of minerals, elements and diversity of organic carbon. These characteristics may influence bacterial presence and activity differently in different clays. 2) Methods development regarding visualisation of bacteria in clays and analysis of diversity using nucleic acid sequencing methods. The methodological challenge was the extraction of cells and nucleic acids from the clay materials. The question if metagenome analysis reveal same diversity as does 16S rDNA gene sequencing was also addressed. 3) Parallel with the work reported here, work with compacted bentonite specifically addressed the effect of wet density on sulphide-producing activity. A new compilation of all data was done and interpreted. 4) The compacted clay work indicated that sulphide reacts with clays and is immobilised. Follow-up experiments with exposure of various bentonite clays to sulphide were performed to investigate if sulphide can degrade bentonite clays. 5) Previously, work with compacted bentonite was performed using radioactive sulphate ($^{35}SO_4$) which limited the possibility for downstream analysis. Next generation experimental configuration, without radioactivity, was developed and tested. This enabled investigation of bacterial presence, numbers and activity, swelling pressure effects and sulphide reactivity without the constrains imposed by the need to work with a radionuclide.

In the visualization and DNA extraction study bacteria were detached from clay particles by replacing the polyvalent cations with monovalent cations and increasing the electrostatic repulsion between the clay particles and the negatively charged bacteria with a buffer that was chosen to estimate the recovery rate from spiked bentonite clays. This procedure made it also possible to detach, extract and visualize bacterial cells from hydrated FEBEX clay. These bacteria had survived in the FEBEX from 18 years. Even if it was possible to detach and extract bacterial cells the recovery rate for bentonite clay spiked with bacteria was very low. One possibility is that a lot of bacterial cells remained

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attached to the clay particles and were centrifuged down in the low centrifugation step together with the clay. Another possible reason for the low recovery rate could be that the majority of bacterial cells died through the spiking and the dehydration through the freeze-drying step. Vegetative cells are sensitive to drying. Furthermore, it could be that the bacterial cells needed longer time than applied to be hydrated before cell extraction, because it is a critical step for the revival of cells after drying. Dehydrated bacterial cell walls are less permeable to water.

Metagenomic sequence data can be analysed with many different aspects. Here, we show that there was a relatively good overlap and occurrence of genes in the metagenome and 16S rDNA libraries for Bacteria, but not for Archaea. The metagenome data suggests that only a fraction of the population in the biofilms belonged to Archaea. Both methods agreed on the occurrence of SRB.

The pore water composition will vary with the type of bentonite and the composition of the saturating groundwater. Various bentonite clays have small but significant amounts of natural and introduced (during mining) natural organic matter (NOM) than may be utilized by sulphide- and acetate-producing bacteria. Such NOM may also decrease or increase the mobility of radionuclides. There is a need in the long-term safety case for geological disposal radioactive wastes to explore if NOM in such buffers can promote activity of bacteria and influence radionuclide migration. A new method was been developed for the high-resolution determination and identification of low-molecular weight NOM in bentonite clays using solvent extraction and analysis by ion-trap mass spectrometry (MS) coupled with gas chromatography (GC). The studied bentonites varied in composition with respect to elements and minerals and the type and content of natural organic matter. The conditions for survival and activity of bacteria may, consequently, vary significantly between different bentonite types as inferred by the variation in the highest wet density at which sulphide production could be detected in compacted clays.

The swelling pressure in the bentonite originates from separating flocs in the bentonite. This means that a mechanical pressure arises between the separating flocs, approximately equal to the swelling pressure. Even in low-density bentonites (1500 kg m^{-3}), a pore size in the nm range would theoretically not allow for bacterial existence unless the bacteria could withstand the mechanical pressure from the separating flocs (0.09 MPa at 1500 kg m^{-3}). Prokaryotic cells can compensate for the mechanical pressure in compacted bentonite by turgor pressure. Published data on turgor pressure in prokaryotic cells mention pressures between 0.08 MPa and 2 MPa. An upper limit of 2 MPa turgor pressure would mean that cell integrity is possible, though limited, at bentonite swelling pressures below 2 MPa. Present results, reviewed and compiled here, suggest a limit at approximately 1 MPa. However, endospores can survive a much higher pressure.

Bacterial life and survival, presence, viability and activity were found to depend on several different variables in a buffer or backfill that relate to the water saturation and swelling of bentonites compacted to high density. Consequently, it was not density *per see* that controlled bacterial activity in the investigated compacted clays. Rather, other factors, that to some extent are related to the density and type of clay, controlled bacterial activity. Transport of nutrients to, and metabolic products such as sulphide from bacteria will be diffusion limited due to the low porosity of buffers and backfill. Here, diffusion rates were determined in compacted clays using a radiotracer methodology. The results agreed well with previously published data.

Bacteria are very small and if there are pores or other inhomogeneities in buffer and backfill with lower than planned pressures, local bacterial activity will be possible. In addition, there will be interfaces between rock engineered disturbed zone and bentonite and between bentonite and canisters at which pressures may differ from the bulk of buffer and backfill. Sulphide-producing bacteria were found to form local colonies in some of the investigated compacted clays, likely in positions were impurities of the clay offer enough large pore space for bacterial life.

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6.5 D2.5 Microbial activation due to addition of electron donors/acceptors in deep groundwaters (VTT)

Microbial communities utilize many energy sources in the deep biosphere. These energy sources and the biogeochemical cycles of elements, such as carbon, nitrogen and sulphur, are multiply connected to each other. It is highly important to understand these connections and the most important sources of energy used by microbes in order to evaluate the risks these microbial communities cause to the geological long-term storage of used nuclear fuel. Deep subsurface microbial communities in steady-state do not cause unidentified risks. In Finland, the Olkiluoto final disposal site contains different groundwater types including sulphate rich, brackish chloride and highly saline waters and their mixtures. In addition, gases such as methane can be found extensively in some water types. Mixing of these groundwater types causes metabolic activation of the resident microorganisms and in some cases for example corrosive sulphide accumulation takes place. The aim here was to study and simulate the deep groundwater mixing and infiltration, which introduces previously limited electron donors and acceptors for microbial communities and generate community activation.

The two deep groundwaters from Olkiluoto, studied with redox sensing fluorescent dye CTC were very different from each other. OL-KR6 was a sulphate rich groundwater with high bacterial and archaeal diversity and clear activation with several electron donors and acceptors was detected. OL-KR15 on the other hand, had lower bacterial and archaeal diversity than OL-KR6 water and was activated with only acetate and acetate together with sulphate. Acetate was overall the most efficient activator of the studied microbial communities which indicates acetate's important role as an electron donor for different Olkiluoto deep subsurface groundwater communities.

Activation of deep subsurface microbial populations detected with fluorescent redox dyes was an efficient screening method to study induced microbial metabolism. The used redox dyes (CTC and RSG) stained a large part of the studied groundwater populations without additions, indicating high activity level of the studied communities. However, for this reason, not all activations induced by electron acceptor and donor amendments were possible to detect. Another tested approach to study microbial community activation, the reverse transcriptase quantitative PCR (RT-qPCR) was tested. Bacterial and archaeal 16S rRNA gene transcripts were detected in the original and in all activated groundwaters but *dsr*B, *mcr*A and *nar*G gene transcripts to detect sulphate and nitrate reducers and methanogens, were below detection limits.

6.6 D2.6 Microbial diversity in bentonite buffer of aged bentonite buffer experiment (VTT)

This report describes the results obtained from the long-term experiment with bentonite buffer. The MX-80 Na-bentonite was compacted inside a copper cylinder, which was set inside a plastic bottle containing non-saline groundwater simulant. The aim of this experiment was to evaluate changes of chemical, mineralogical and microstructural parameters of bentonite in both oxic and anoxic conditions. Microbiological analyses performed at the end of the experiment included evaluation of bacterial and fungal communities by sequencing and visual evaluation microscopically.
Detected changes in the bentonite mineralogy included the observation of secondary copper minerals formation in the middle part of the bentonite matrix in oxic conditions. Copper content in pyrite increased when moving from the middle of the bentonite towards the copper cylinder surface in both oxic and anoxic experiments. Microstructural studies on bentonite did not show any significant differences in bentonite structure between samples taken from anoxic and oxic experiments. Chemical changes were typical dissolutions of gypsum and calcite, which released

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sodium, sulphate and carbonates into external water, while calcium exchanged sodium in the interlamellar spaces of bentonite.

The presence of living microbes on bentonite and on copper surface could not be demonstrated in this study but microscopical studies revealed living microbial cells in the external water surrounding copper cylinders. According to the IonTorrent sequencing, sulphate (SRB) and iron reducing bacteria (IRB) were detected in bentonite matrix, water and copper surface. SRBs can produce corrosive sulphide and IRBs can have role in processes that could be linked to the loss of swelling properties in bentonite. Fungal conidia and hyphae were detected by SEM in water and several groups of Ascomycetes and Basidiomycetes were identified by sequencing from bentonite samples. Many of the fungal genera detected are able to produce organic or inorganic acids that help fungi in solubilization of minerals from rock substrates.

6.7 D2.7 Microbial diversity in aged bentonite (TUL)

Bentonite is an integral part of the engineered barriers in geological repositories for low-, intermediate- and high-level radioactive wastes and hence it is crucial that its functionality is maintained for extremely long periods of time. However, the swelling ability, the most important feature of the bentonite barrier, can be affected by many factors including the presence of microorganisms.

Bacteria occurring in bentonites originate from two different sources. First, the microorganisms come from surrounding environment (underground water, host rock, human impact) and they can, under certain conditions, penetrate the bentonite barrier. Second source of microorganisms is the bentonite itself. All bentonite materials tested to be used as engineered barriers in nuclear waste repositories contained indigenous bacteria. Density of bacterial cells in the bentonite is generally decreasing with the level of the compactness of the bentonite, but it has been reported that microorganisms remain present even in densities above 2000kg m^{-3} (probably mainly in form of spores characterized by unbelievable durability).

In this work, we studied a diversity of the microbial communities present in the compacted bentonites originating from two independent long-term in situ experiments ("Mock-Up experiment" and "Bentonite95 experiment") performed under near-field conditions. The compacted bentonite buffer was exposed under conditions similar to a high-level radioactive waste repository in crystalline host rock. We employed 16S rDNA amplicon sequencing to determine the microbial profiles in different parts of bentonite samples.

The results of our analyses showed that there were bacteria present in all of the samples studied. Surprisingly, the microbial communities detected in the bentonite samples showed a high level of similarity. The bacterial profiles were characterised by the dominance of the heterotrophic, aerobic or facultatively anaerobic capable of respiring oxygen or nitrates. Almost no strict anaerobic or autotrophic bacteria were detected. The majority of detected bacteria belong to common soil or ubiquitous microorganisms with wide ecological amplitude enabling them to survive under various conditions. The commonest bacteria present in the bentonites were the representatives of genera *Nocardia*, *Pseudomonas*, *Pseudonocardia*, *Saccharopolyspora* and *Streptomyces*. Our results suggest that microorganisms found in the bentonite samples in this study were most probably present in the bentonite already before the start of the experiment. Further study is needed to determine whether the uncovered microbial diversity represents a metabolically active microbial community able to survive under harsh conditions present in the compacted bentonite rather than a DNA originating from dead cells, trapped and preserved between the layers of the clay minerals in the bentonite.

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6.8 D2.8 Long-term stability of bentonite in the presence of microorganisms (HZDR/VTT)

Due to high swelling capacity and a low hydraulic conductivity, bentonites are used as geotechnical barriers and in sealing and buffering functions in the nuclear waste repositories. The aim of this report was to study if microbes influence these beneficial properties of bentonites by either changing the solubility and/or composition of minerals and the reduction of important ions (e.g. ferric iron) of the studied bentonites. In order to elucidate the microbial potential within different bentonites, we performed two types of microcosm-experiments using two Bavarian bentonites and MX-80 bentonite.

The experiment with Bavarian bentonite consisted of an industrial/processed bentonite (B25) and a natural one (N01) and were performed at HZDR. The set-ups contained the respective bentonite and an anaerobic synthetic Opalinus-clay-pore water solution under an N_2/CO_2-gas-atmosphere. Acetate, lactate or hydrogen gas were used to stimulate potential microbial activity and geochemical effects. The set-ups were incubated in the dark at 30° C and 60° C for one year. Bio-geochemical parameters were monitored and the microbial diversity as well as mineralogical analysis were performed. Only B25 set-ups at 30° C supplemented with lactate or hydrogen gas showed notable effects regarding microbial diversity, changes in bio-geochemical parameters as well as the formation of gases. The respective B25 microcosms where dominated by spore-forming, sulphate-reducing *Desulfosporosinus* spp. in contrast, the raw material of B25 (powder) revealed a very diverse microbial community, showing no significant presence of sulphate-reducing organisms. Thus, microbial diversity changed significantly within the analysed period of one year. In lactate-containing B25 microcosms, lactate and sulphate concentrations dropped. Furthermore, the simultaneous formation of acetate, as well as an increase of ferrous iron and a concomitant decrease of ferric iron was observed in the respective samples. Moreover, fissures were formed and the colour of the bentonite material changed to different shades of grey with sporadic black spots in the respective microcosms. Mineralogical analysis with Scanning Electron Microscopy indicated a significantly higher part of iron-sulphur accumulations in these samples. Similar observations were made when hydrogen gas served as an electron donor. Again, sulphate concentration decreased with a simultaneous increase of ferrous iron and a decrease of ferric iron with a concomitant formation of black precipitates.

The aim of the laboratory scale MX-80 bentonite storage experiment (VTT) was to simulate bentonite behaviour in circumstances that can take place in the interfaces of bentonite, host rock fractures and water flow in nuclear waste geological disposal. The bentonite was studied as a slurry in which water, gases, nutrients and microorganisms were able to move freely at the temperature hospitable for microorganisms. The objective was, to find out if microorganisms and the metabolites they produce are able to change the bentonite structure and if these changes could be significant for the bentonite stability in long-term. MX-80 bentonite microcosms after one year of storage initiated both, at aerobic and anaerobic conditions, showed no essential changes in bentonite mineralogy compared to the initiation of experiment. However, clear microbial activity in terms of ongoing sulphate reduction and sulphide formation as well as high number of sulphite reductase genes (*dsr*B) were detected in anaerobic samples. Microbial activity also affected bentonite water-phase chemistry and bentonite cation exchange capacity. These effects were not detected in sterile controls, demonstrating the microbial origin of these changes. In aerobic microcosm, oxygen was used steadily and after half a year, only trace of oxygen was left. Overall, microbial activity was lower in aerobic than in anaerobic microcosms and neither sulphate reduction, nor sulphite reductase genes were detected in aerobic microcosms after one year. The experiment is planned to continue for some years further as potential changes in bentonite mineralogy caused by microbial activity happen slowly in the studied conditions.

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6.9 D2.9 Evolution of stress in biotic and abiotic clay flow cells [(NERC)

Microbial activity has been implicated in both the corrosion of steel material and alteration of bentonite clays used in geological disposal facilities for radioactive waste. To understand the limits on microbial growth and the potential for microbial activity in this environment to affect the swelling behaviour of the clay and metal corrosion, a series of laboratory experiments were performed. The experiments described here were designed to investigate multiple effects of microorganisms (on steel corrosion, changes to bentonite swelling capacity, permeability and fluid flow) in a single set of laboratory scale flow experiments using compacted bentonite samples. Bentonite samples were prepared by mixing γ-irradiation-sterilized bentonite powder and artificial groundwater in a pre-calculated ratio, then compacting them using a hydraulic press to achieve a final dry density of 1400 kg m^{-3}. Five grams of steel chips were incorporated into the sample in a thin layer near the inlet end of fluid flow. Sterile samples were compared to samples inoculated with an enrichment of sulphate-reducing bacteria from the un-irradiated clay. Experiments were run in the presence and absence of sodium lactate to stimulate microbial growth. The results presented here relate to the development of stress in the experiments, including discussion of petrological and mineralogical alterations that could influence the physical properties of the bentonite.

There was evidence of steel corrosion in all experiments, detected by visual and microscopic observation and XRD analysis. XRD data showed an increase in the basal spacings of smectites in the zone immediately surrounding the steel compared to the starting material and material taken from further away from the steel, indicating replacement of monovalent cations with divalent ones. At large scales this could have the effect of reducing swelling capacity of the clay. There was also some indication that basal spacing was smaller in the presence of microorganisms, however more data from later experiments will be required to confirm this.

Close examination of the bentonite around the corroded steel by SEM in the uninoculated experiment without lactate (BUG021), showed strong Fe enrichment within the bentonite, with Fe coating (or replacing) the bentonite. Some calcium precipitation was observed by element mapping, but no identifiable crystal morphologies observed. Initial stub analysis of the lactate-amended uninoculated experiment (BUG023) did not reveal any obvious iron enrichment or calcium precipitation. In comparison, in both of the inoculated experiments, the material around the corroded steel was characterised by calcium enrichment and observation of acicular calcium carbonate crystals within fractures in the bentonite next to the steel. A fibrous iron-rich phase was observed in the bentonite, and in the case of the lactate-amended, inoculated experiment (BUG023), precipitating over the acicular calcium carbonate. In context of previous work in the literature, this was interpreted as being a result of cracks opening up around corroding steel as water within the bentonite is consumed by the corroding steel causing the clay to shrink back. This water movement may be responsible for the observed calcium enrichment and iron diffusion into these fractures. Further experiments are required to confirm that microorganisms from the sulphate reducing enrichment community involved in the aragonite precipitation in the inoculated experiments.
The continuous monitoring of the CVAF vessels allowed the evolution of swelling pressure and the permeability values for each sample to be monitored. There was a notable difference between the lactate amended experiments (BUG022 and BUG024) and unamended experiments (BUG021 and BUG022). In unamended experiments the values for swelling pressure at the end of testing were higher than those obtained prior to hydraulic testing, suggesting some form of reaction has occurred causing expansion of the fabric. It may be that this is related to the reduced amount of divalent substitution seen in the lactate amended samples by XRD analysis, possibly as a consequence of additional sodiums irons coming from the sodium lactate. Unfortunately, any effect that microbial activity had on the evolution of stress in the clay samples was too small for detection using our

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system. Given the localised nature of corrosion within the clay, longer-term testing of samples with iron dispersed within the sample are required to confirm and contextualise these results and its understand its potential impact on performance assessment.

6.10 D2.10 Microbial mobility in saturated bentonites of different density (TUL CZ Rez)

Indigenous bacteria are naturally present in bentonite materials to be used as an engineered barrier in deep geological repositories of nuclear waste. Their density is generally decreasing with the compactness of the bentonite, but they remain present even in densities above 2000 kg/m3 presumably in the form of spores characterized by unbelievable durability. Other factors, such as temperature or water availability do not limit the Gram-positive spore-forming bacteria predominating among indigenous bacteria as much as they do in the case of the non-spore-forming bacteria. Studies specifically focused on the spore-forming bacteria are thus highly needed.

The second source of bacteria that may affect bentonite in deep geological repository is contamination from the surrounding environment, mostly pore water with predominating Gram-negative non-spore-forming bacteria. Moreover, bacteria will be inevitably introduced from the surface during construction and operation of the repository, before its final closure. The speed of penetration of allochthonous bacteria into the bentonite barrier is strongly dependent on the swelling pressure and composition of the clay material, on hydrogeological conditions and on the stability of the bentonite/host rock interface.

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

Our second goal was to study microbial mobility within compacted BaM bentonite from Czechia of two different dry densities – 1400 kg m^{-3} and 1600 kg m^{-3}. Fourteen sections of saturated bentonite samples differing in their distance from the source of natural bacterial community (Josef URL, Czechia) were inspected for the presence of bacteria. Viable cells were observed in each section of both bentonite densities tested. This finding indicates that bacteria are able to move through saturated bentonite of even higher dry density (1600 kg m^{-3}) and thus higher swelling pressure of about 5 MPa. Czech BaM bentonite belongs to Ca-bentonites that might generally allow for better microbial mobility due to its physical properties, different from Na-bentonites. Based on previous studies, bacterial mobility in highly compacted Na-bentonite (≥ 1800 kg m^{-3}) is very limited. Therefore, further tests are needed to reveal whether the surprisingly high bacterial mobility detected in our experiment was caused solely by the lower bentonite density used, or by the unique properties of BaM bentonite (or Ca-bentonites in general), or if our novel method is more sensitive than previously used methods.

6.11 D2.11 Microbial cement deterioration boundaries (SCK CEN)

Cementitious materials have been used for centuries in many construction and engineering applications because of their long-term durability. Also for the geological disposal of radioactive waste, cementitious materials are used in many parts of the engineered barrier. Consequently, the interactions and the evolution of these materials with other repository materials, the host rock and its ground water, need to be assessed. Organic acids (e.g. acetate), carbon dioxide and sulphur compounds, originating from the waste and host rock or produced by microbes in the repository, can be corrosive towards cementitious materials resulting in Ca^{2+} leaching and a decrease in the original

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high alkaline pH. The latter will give rise to lower pH niches on the concrete were microbial activity will be enhanced and which in turn can have a possible impact on the mineralogy and chemistry of the cementitious materials. Interestingly, microbial processes can either have a detrimental effect on or be beneficial for the functional performance of the cementitious materials used within a geological disposal.

In this study, anoxic batch experiments were performed to study the microbial community present in Boom Clay borehole water in a cementitious environment. The objective of this study was to investigate whether this microbial community could affect in a positive or negative way the long-term evolution of the cementitious materials present in the engineered barrier of a geological repository for radioactive waste. This preliminary study demonstrated that the high pH conditions imposed by the OPC CEM I inhibit microbial sulphate and nitrate reduction. However, SEM analysis indicated the presence of intact cells in the suspension on top of cement and putative biofilm structures on the cement. This suggest that the high pH environment does not completely eliminate the microbial population. Interestingly, in sulphate reducing conditions, a pH decrease from > 12 to pH 10 was observed in one replicate harbouring clearly a larger microbial community in the suspension on top of the cementitious material compared to the other samples. However, the precise mechanism remains unclear and more detailed chemical and microbial analysis and of the structure of the OPC CEM I is planned.

6.12 D2.12 Microbial activity in a concrete-bentonite clay interface (Micans)

In nuclear waste repositories cementitious materials can be an essential part of the plug and seal systems. In some concepts compacted bentonite clay will be used as backfill and will be in contact with the cementitious plug and seal. Microorganisms are often active at interfaces and may therefore be active at the interfaces between cement plugs and seals and backfill. A concern regarding the integrity is microbial degradation of concrete. However, microbial activity might be inhibited through an alkaline plume (pH > 13) generated by concrete diffusing into the bentonite. Therefore, concrete plugs were in contact with bentonite Volclay MX-80, which was compacted and saturated to a wet density of 1750 kg m^{-3}, in titanium test cells. The bentonite clay was inoculated with sulphide-producing bacteria. Furthermore, glucose was added to induce microbial fermentation. The aim of this work was to analyse the effect of concrete on pH of the bentonite as well as microbial survival in the bentonite. The MX-80 was analysed in profile for water content, pH, ATP and CHAB.

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

6.13 D2.13 Anaerobic microbial corrosion of canister material (CV Rez, TUL)

The deep geological repository (DGR) was selected for disposal of spent fuel and high-level radioactive waste due to its high radioactivity and significant content of long-lived radionuclides. In the DGR, a series of engineered and natural barriers will work together to isolate radioactive waste from the environment. First barrier will be a metal canister that will prevent direct release of radionuclides into the repository environment for as long as possible (up to ten thousand years). Microbially influenced corrosion (MIC) is one type of corrosion that may limit the lifetime of nuclear waste canisters. Although the risk of MIC is rather low, convincing proof is still missing.

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Microorganisms are able to change the electrochemical conditions at the interface between a metal and an electrolyte solution by biofilm formation. These changes can range from acceleration of corrosion to complete corrosion inhibition. Sulphate-reducing bacteria (SRB) are the most important bacteria considered responsible for the MIC of carbon steel. On the other hand, archaeological analogues found under natural conditions with the presence of SRB confirm that these bacteria are not very aggressive for iron when the environment was strictly anaerobic.

The main goal of this study was to assess the impact of microbial activity on corrosion behaviour of carbon steel and stainless steel in an anaerobic environment containing natural microbial community dominated by SRB. Therefore, granite groundwater from Josef Underground Research Center (Josef URC) containing SRB and synthetic bentonite pore water (bentonite BaM) inoculated with granite groundwater were used in our experiments. Electrochemical measurement, surface analysis, weight loss measurement and molecular biological tools were employed to evaluate the impact of MIC. This comprehensive analysis has given us a detailed overview about the impact of microorganisms and the processes of corrosion. Moreover, we were able to distinguish when biofilm formation began by comparison of sterile and non-sterile impedance spectrum.

To summarize, biofilm dualism was observed at the higher temperature (35°C) leading to an increase in the inhibitory action by barrier effect of the biofilm, while biofilm at T_{LAB} accelerated corrosion. The seeding dispersal related to formation of biofilm at T_{LAB} revealed in the form of second layer of biofilm, which was observed independently using EIS and SEM measurements. The unstable passivity and MIC inhibition of stainless steel was observed in the granite groundwater under anaerobic conditions. Severe local attack was observed only in the case of weight loss experiments with carbon steel using synthetic bentonite pore water inoculated with VITA water for samples taken after 3, 6 and 12 months. The samples collected after 18 months of exposure were without local attack under non-sterile conditions.

6.14 D2.14 Impact of bentonite dry density on the viability of organisms (in the context of steel corrosion) (EPFL)

A long-term *in situ* corrosion experiment is ongoing in the Mont Terri Underground Research Laboratory in Switzerland to (i) measure the *in situ* corrosion behaviour of carbon steel in compacted bentonite under simulated repository conditions and (ii) study the effect of the bentonite buffer density on microbial activity and microbially-influenced corrosion. Here, we investigate the presence and distribution of a microbial community in the bentonite in the vicinity of steel coupons. The local anoxic groundwater was shown to contain a viable microbial community. After either 1, ~1.5, ~2.5, or 5.5 years of exposure, the bentonite was sampled and viable aerobic and anaerobic heterotrophs as well as sulfate-reducing bacteria were quantified in the bentonite. A pattern emerges from the results as a function of time: there is an initial increase in both aerobic and anaerobic heterotrophs followed by a steady decrease up to ~2.5 years. This was observed irrespective of the targeted dry density. Furthermore, an increase in numbers was observed again at 5.5 years, but it is premature to draw any firm conclusions on this point. As for sulphate-reducing bacteria (SRB), we observe little difference in viable numbers despite variable dry density and deployment time. In contrast, bentonite form impacts the number of viable SRB, with pellet formulations harbouring fewer viable counts as compared to bentonite blocks. Finally, the location within the bentonite block (whether closer to the borehole water or not) did not impact viable counts significantly. Within the range of conditions considered in this experiment, the dry density of the bentonite appears to play a role in determining the viability of heterotrophic microorganisms as there is a decrease in viable heterotrophs at 1.45 and 1.55 g cm^{-3} relative to 1.25 g cm^{-3} targeted dry density.

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6.15 D2.15 Survival of microorganisms in bentonite subjected to different levels of irradiation and pressure (TUL, CVRez)

In the geological repository of radioactive waste, combined effects of high pressure, elevated temperature and a certain low-level radiation on the bacteria can be expected. Nowadays, we have reasonably good understanding of the effect of each particular variable separately, however their synergic effect and especially the effect of radiation and exceptionally long-time frame remains rather unclear. Long-term studies on bentonite irradiation under realistic repository conditions are thus highly needed, although their conduction remains technologically very challenging. Our study aimed to improve the knowledge on the effect of Gama radiation and pressure on indigenous microbial community in bentonite inoculated by natural porewater. Therefore, we studied these effects using bentonite type BaM (Keramost, Czech Republic) enriched with granitic porewater VITA from Josef Undeground Research Center (Czech Republic), a natural source of anaerobic sulphate-reducing bacteria.

The composition of microbial communities within the bentonite suspension samples was changing continuously during the irradiation experiment. At the beginning, indigenous microorganisms present in the underground VITA water and BaM bentonite went through bottleneck during adaptation to specific environment of bentonite suspension. Most of the bacterial genera occurring in the VITA water disappeared and both total biomass and species richness markedly decreased during this phase. Subsequently, gradual changes in microbial community composition were observed reflecting the prevailing conditions in the samples. In the samples influenced by the presence of oxygen, the community changes were driven by the available electron donors evolving from heterotrophs (chemoorganotrophs) to autotrophs (chemolithotrophs) with the decrease of available organic material. In anaerobic control, limitation by both available electron acceptors and donors was identified. The communities evolved from heterotrophic facultative anaerobic nitrate reducers to autotrophic anaerobic iron or sulphur reducers and fermenting microorganisms. Community changes did not reflect species richness, which remained rather constant after the initial bottleneck. Microorganisms that adapted and survived under harsh conditions underwent further selection caused by the Gama radiation. Notably, application of 19,656 Gy absorbed dose at the constant dose rate 13 Gy h^{-1} did not completely eliminate bacteria in bentonite suspension. However, decline in total microbial biomass, but not species richness, was observed together with minor changes in the microbial community structure. Gram-negative non-spore-forming microorganisms dominated in the aerobic irradiated samples as well as aerobic controls although spore-formers are generally more radiation resistant. We ascribed this unexpected pattern to the presence of oxygen. In anaerobic control samples dominated Gram-positive spore-forming bacteria that are generally more resistant to radiation, thus we presume that even higher absorbed doses of ionizing radiation would be needed to eliminate microbial activity in anaerobic conditions. The results of irradiation experiment further showed a key role of iron reduction in the microbial processes occurring in the bentonite under anaerobic conditions. Ferric ions naturally occur in the bentonite molecular structure and their reduction might cause decrease in bentonite swelling capacity and thus negatively influence the safety of the repository. Exposure to 2 and 5 MPa pressure did not markedly reduce the total microbial biomass nor did substantially change the composition of the microbial community present in the bentonite suspension.

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6.16 D2.16 Microbial activity and the physical-chemical and transport properties of bentonite buffer (NERC)

A series of experiments have been carried out into microbial survival and activity in compacted FEBEX bentonite compressed to dry densities of 1200 kg m^{-3} and 1400 kg m^{-3}. Samples were prepared containing steel chips and an artificial groundwater. Samples were then inserted into a constant volume axial flow vessel. The use of this set up allowed the simultaneous investigation of interactions between the steel, bentonite and microorganisms, whilst also monitoring bentonite permeability and swelling pressures. By using this set up the influence of microorganisms on the performance of the bentonite as a buffer material could be investigated. The bentonite was sterilised by irradiation, and sulphate-reducing bacteria were reintroduced into half the tests so that the influence of microbial activity could be investigated. The number of sulphate-reducing bacteria and, anaerobic and aerobic heterotrophic bacteria were monitored in all tests. Bacteria were seen to persist and increase in inoculated tests while bacterial numbers remained low in uninoculated tests. Previous reports of higher microbial survival in lower density bentonite samples have been confirmed for FEBEX bentonite, with survival and growth detected in samples with an initial dry density of 1200 kg m^{-3} and 1400 kg m^{-3}. This indicates that in FEBEX bentonite microorganisms survive in the upper range of dry densities reported for microbial survival. Initial tests run using samples with a dry density of 1400 kg m^{-3} suggested that the presence of microorganisms is associated with particular iron phases and the development of aragonite crystals. The basal spacing of bentonite increased during three of these tests, indicating replacement of monovalent cations by divalent ones in experiments. Lower density experiments did not show this same pattern; iron and calcium alteration were observed in both tests at 1200 kg m^{-3} with greatest alteration being observed in the uninoculated test along with iron sulphide precipitation. In the lower density samples the shift from monovalent to divalent cations was much less pronounced.
In all of the tests, permeability was lower in the inoculated sample, and in the low-density tests (1200 kg m^{-3}) a 20% reduction in permeability was observed in inoculated samples. Other than this reduction of permeability there was no consistent pattern associated with the presence or absence of microorganisms. However, the possibility that microorganisms could influence the mineralogical and petrological character of the bentonite has been highlighted. These changes could affect its performance as a barrier material and further studies need to be undertaken to confirm the role of microorganisms in steel corrosion and its impact on bentonite behaviour.

6.17 D2.17 Sulphide production (GTK)

This report summarizes the outcome of the Task 2.1 dealing with the availability of sulphide in the bedrock around the repository. Sulphide is an important constituent affecting metal corrosion and, consequently, processes controlling the access of sulphide into the repository near field must be understood and quantified. Sulphide production is dealt here in a broad sense including the natural presence of sulphide in bedrock groundwater, release from sulphide minerals and transport to the repository. The final aim of this study is to quantify the contribution of microbial sulphate reduction to sulphide. Lack of sulphur sources can hardly be considered as a limiting factor, even though sulphur bearing minerals are not always common in all rock types, as for example in granitic rocks. Aqueous sulphate is not solubility-limited, it is a common constituent of near-surface waters and provides the source for microbial sulphide formation, if coming in contact with deep anoxic zone. Redox conditions of the groundwater-bedrock system are considered as a qualitative measure of the sulphide production capacity of the system. Very low redox values indicate the abundance of electron donors, whereas high redox potentials indicate the presence of oxygen or other strong electron acceptors suppressing the sulphate reduction to sulphide. Solubility limitation provides the general abiotic constraint on the concentration of sulphide in the groundwater-bedrock system. Relatively high sulphide concentrations occasionally observed in deep groundwaters are evidently due to active microbial sulphate reduction fronts, but transient in space and time. Presence of

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electron donors is one of the most important controlling factors for microbial sulphide formation. Dissolved molecular hydrogen is the most effective electron donor, whereas methane is the most common one. Currently, more attention has been paid to the role of organic C-2 compounds, especially acetate. Nutrient limitation is not considered as an important limiting factor, because the bedrock environment provides an adequate source. In deep biosphere, recycling of essential elements and syntrophic interactions play key roles in sustaining microbial life. Transport limitations, based on advection and diffusion, are easily quantified ways to estimate long-term sulphide production rates. Ideal conditions in terms of all other factors required by the microbial sulphide production would evidently lead to limited supply by the limited transport rate. Energetic calculations demonstrate that life deep in the bedrock has a plausible energy source supplied by the surrounding chemical energy. The life-sustaining energy flux, i.e. power, remains however low, and cannot be multiplied by many orders of magnitude in a sustainable way.

6.18 D2.18 Rate of corrosion of carbon steel in bentonite under biotic and abiotic conditions (EPFL)

A long-term *in situ* corrosion experiment is ongoing in the Mont Terri Underground Research Laboratory in Switzerland to (i) measure the *in situ* corrosion behaviour of carbon steel in compacted bentonite under simulated repository conditions and (ii) study the effect of the bentonite buffer density on microbial activity and microbially-influenced corrosion. Here, we investigate the composition of the microbial community in the bentonite in the vicinity of steel coupons and in other locations. After 1, ~2.5, or 5.5 years of exposure to the porewater, the bentonite was sampled and the microbial community characterized using molecular tools. A pattern emerges from the results as a function of space. In the bulk bentonite and near the corrosion coupons, there is no evidence of abundant sulphate-reducing bacteria (SRB). In contrast, on the surface of the canister, and on the surface of the bentonite, in black spots, SRB are clearly present and growing. The bulk bentonite harbours primarily aerobic microorganisms, suggesting the persistence of adsorbed oxygen in the bentonite for extended periods of time. Questions remain about the further evolution of the system as to whether SRB will progressively colonize an increasingly thicker layer of the bentonite. Further time points in this 10-year experiment will be able to address this question.

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