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Microbiology In Nuclear waste Disposal

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DELIVERABLE D4.7

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Publishable Summary

Microbiology In Nuclear waste Disposal (MIND) was an interdisciplinary project consisting of two experimental work packages and a third one that handles integration of society and policy-oriented studies. The fourth work package handled project management.

Work package 1 was focusing on the influence of microbial processes on organic containing long-lived Intermediate Level Waste (L/ILW) forms e.g. waste solidified with cement or bitumen. In work package 2 the influence of microbial processes on high level waste (HLW) and spent fuel geological disposal were studied. The emphasis was on quantifying specific measureable impacts of microbial activity on processes and the long-term performance of the system with its barriers under repository-relevant conditions to be considered in safety cases. The remaining uncertainties concerning the release of chemicals, gases and radionuclides that are important to the safety case for L/ILW disposal and near-field safety issues related to the engineered barriers in HLW disposal, are addressed respectively. Work package 3 was oriented towards interaction within (by e.g. sharing experience and results with other experts) and between different groups including both experts and the lay community through education and communication and thereby improving the general awareness of microbial issues. Work package 4 handled all administration related to reporting to the commission, project webpage, contact with the technical platform etc.

Microbial activity may have a significant impact on the physical and chemical evolution of repositories for radioactive waste. Therefore, what controls their activity under repository conditions must be well understood.

Contents

1 Introduction

The MIND project was a project addressing key technical issues that must be tackled to support the implementation of planned geological disposal projects for higher-level radioactive wastes across the EU. Geological disposal is a challenge as social and technical aspects blend and input from social science with regard to the design, implementation and post-construction management of the installation is required. In this respect, the impact of the inclusion of microbiology on expert conceptualisation and public perception of geological disposal, have been considered as well. Our current understanding of the impact of microbial metabolism on the safety of geological repositories has increased. This has resulted in an enhanced awareness that microorganisms may control influence on organic waste (e.g. solidified with cement or bitumen) evolution *in situ*, multi-barrier integrity and ultimately radionuclide migration from the repository. The project brought together, for the first time, 15 European groups from 8 countries working on the impact of microbial processes on the evolution/performance of the repository system and its barrier elements to be considered in safety cases for geological repositories across the EU, focusing on key questions posed by waste management organisations. The emphasis was on quantifying specific measureable impacts of microbial activity on barrier evolution under repository-relevant conditions. The knowledge obtained will be considered when choosing design and materials when constructing repositories for radioactive waste. The MIND project has thus contributed to that microbial processes in repositories are now being taken into account to greater extent than previously. This has resulted in significant refinements of safety case models currently being implemented to evaluate the long-term evolution of radioactive waste repositories. The project consisted of two experimental work packages, WP1 and WP2 and the third one WP3 handled distribution of knowledge obtained in WP1 and 2 including dissemination and education. WP4 handled all administration related to management and coordination of the project.

1.1 Work package 1

The overall objective of WP1 was to reduce uncertainty of safety relevant microbial processes controlling radionuclide, chemical and gas release from long-lived intermediate level wastes (ILW) containing organics. Development of gas in a repository for L/ILW has been studied previously by e.g. Wiborgh et al., [1] and more recently by Small et al., [2] who performed experimental and modelling investigations of the biogeochemistry of gas production from low and intermediate level radioactive waste in the GGE (gas generation experiment) in Olkiluoto. Organic waste materials present in ILW include a variety of natural (cellulose, bitumen) and anthropogenic polymers including: polyvinylchloride (PVC), polyethylene (PE) and ion exchange resins (D1.1). Effects resulting from the combined effects of hydrolysis, radiolysis and biodegradation of such organic wastes have been studied under the physical and chemical conditions relevant to organic ILW solidified with cement (waste forms) and disposal concepts. The range of microbial processes that can be fuelled by these organic wastes has been examined to assess the effect on processes of gas generation and radionuclide complexation. Laboratory and *in situ* large-scale experiments have been performed examining alkaline, cement buffered (pH 10-12.5) conditions and including actual organic waste materials to determine the types of microbially mediated chemical and gas generation processes that may proceed in heterogeneous cementitious waste forms with organic waste. Microbial processes, affecting gas generation (CH_4) and consumption (H_2) have been examined that have relevance to the pressurisation of the repository system and which are also important to the migration of gaseous radionuclides

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such as ^{14}C. Models of the microbiological, geochemical and transport processes have been developed to assist in the interpretation of the *in situ* experiments.
The main safety issues concerning radionuclide, chemical and gas release from ILW that are addressed by the work package are summarised as:

1. The degradation polymers including bitumen, PVC and ion exchange resins and hence their contribution gas generation and release of soluble organic species.
2. The biodegradation of cellulose hydrolysis products (e.g. ISA) and the overall effect on radionuclide complexation and transport.
3. Microbial gas generation (CH_4) and consumption (H_2) studied under *in situ* conditions.
4. The effect of high pH on limiting the above microbial processes in cementitious ILW.

1.2 Work package 2

In WP2 the objective was to better quantify the contribution of microbially produced sulfide in the geosphere and in buffers and backfill to the overall rate of canister corrosion. There is also a need to gain systematic information on the efficiency of specific bentonite buffer in inhibiting microbial activity. Already a few years before MIND started, it was shown that there would exist a threshold density over which sulfate reducers could not be active (e.g. Motamedi et al, [3], Stroes-Gascoyne et al. [4], Masurat et al.,[5]).

Remaining key issues for the geological disposal of HLW concern the factors controlling sulfide production in the geosphere, including man-made artefacts to what extent microorganisms can accelerate canister corrosion in the near-field either by hydrogen scavenging or by sulfide and/or acetate production. Further, it is important to identify conditions more in detail (including buffer density) under which relevant bentonites inhibit microbial activity and to understand whether microorganisms can accelerate degradation of bentonite based buffers and influence the long-term behaviour of plug systems and seals. To better understand the differences in behaviour between different bentonites.

Microbiology in work package 2 generally addresses near-field safety issues related to the engineered barriers canisters, bentonite clays and concrete. Three main safety issues can be postulated due to bacterial activity in the nearfield.

1. Bacterial sulfide production in buffer and backfill and the concomitant risk for sulfide corrosion of canisters.
2. Bacterial acetate production in buffer and backfill and the concomitant risk for acetate induced stress corrosion cracking of canisters.
3. Bacterial degradation of buffers and backfill, and of concrete constructions such as tunnel plugs.

1.3 Work package 3

The purpose with the third Work Package was to ensure that the output from WP1 and WP2 is well integrated. WP3 has evaluated and integrated microbial processes towards the conceptualization and performance assessment of geological repositories and in the

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respective state of the art knowledge base. The impact of the inclusion of microbiology on expert conceptualisation and public perception of geological disposal has been estimated (Deliverable D3.6). The proper contextualization of results will be ensured and remaining key topics within and beyond the MIND project will be extracted by maintaining an active dialogue with stakeholders (Deliverable D4.6).

In WP3 the outcome of the experimental work packages has been and will also be distributed to a broad audience, including students, professionals, the scientific community, stakeholders and the lay community via e.g. Euratom Fission and Training Scheme programme PETRUSIII. Synergies of the MIND program and consortium has, partly thanks to the IRB, been identified and cultivated with ongoing research lines of European laboratories and institutes outside the MIND consortium, with special attention for those in the less advanced member states.

1.4 Work package 4

The project has also initiated to form an Endusers' group called the Implementers Review Board (IRB). The IRB consists of representatives from the Nuclear Waste Management organisations and regulators with interest in MIND (SKB, Posiva, TVO, Andra, NWMO, Nagra, RWM, ONDRAF/NIRAS, NUMO, LANL, SURAO, CNSC, IRSN, FANC). During the course of the MIND project the IRB has advised the Technical Coordinator and the Executive Committee of MIND with critical evaluation concerning research quality and significance of outputs in relation to the implementers needs, highlighted opportunities for networking with other international research activities and raised awareness of our research programme where appropriate. Members of IRB have participated in the MIND Annual Meetings, participated in discussions and also produced written feedback at the end of each annual meeting.

2 Work package summaries

2.1 Work package 1

Outputs from laboratory and *in situ* experimental systems are reported in WP1 deliverables (D1.1- D1.8) and peer-reviewed journals, as discussed below.

The state of the art knowledge base concerning the biodegradation of organic polymers common in LLW/ILW was reviewed in D1.1, which includes a summary of information concerning the types of organic polymer present in ILW and some disposals of Low Level Waste (LLW) together with information concerning the European repository designs and concepts for their deep geological disposal. Deliverable D1.1 confirmed that the materials selected for study in the MIND project; bitumen, organic ion exchange resins and halogenated polymers (PVC), are present in significant amounts in the national inventories, but that biodegradation of these materials and their hydrolysis and radiolysis products was poorly understood. Cellulose is present in generally lower amounts in most inventories, but it is already established that it is of significance with regard to the strong complexation effect of its alkaline hydrolysis products. Recent findings concerning the biodegradation of the cellulose hydrolysis product isosaccharinic acid (ISA) using an alkaline inoculum from a high pH lime works [6,7] form the basis of further work within MIND WP1 using the inoculum to

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examine biodegradation of PVC (D1.2) and bitumen hydrolysis products (D1.3) at pH 10-11 conditions.

Deliverable D1.2 presents further studies of cellulose degradation following ^{60}Co ϒ irradiation (1 MGy) and hydrolysis at pH 12.7. Irradiation enhanced the rate of the abiotic alkaline hydrolysis of cellulose, which resulted in the production of higher concentrations of the radionuclide complexant ISA, compared to the unirradiated controls. Irradiation also led to an increase in the concentration of dissolved organic carbon, including ISA, and made the cellulose more bioavailable for microbial degradation at a starting pH value of 12.5. Fermentation of the cellulose degradation products at hyperalkaline pH (pH 12.5) led to the production of H_2, and acetate, while CH_4 was not detected. Fermentation at pH 12.5 is possibly due to the microbial activity in low pH niches associated with the cellulose tissue and which are further developed by acidity generated by fermentation. Characterisation, including 16S rRNA gene sequencing, of the bacteria (*Anaerobacillus isosaccharinicus*) isolated from the lime works inoculum establishes that it grew at pH 8.5–11, could utilise a number of organic substrates, and was able to reduce nitrate and arsenate (Bassil and Lloyd [8]). Deliverable D1.2 also presents studies of PVC biodegradation of pure PVC polymer (powder) and plasticised PVC sheet, following 1 MGy ^{60}Co ϒ irradiation in saturated $Ca(OH)_2$. Batch microcosm experiments were set up to test the ability of a high pH-adapted lime works inoculum to use these irradiated materials as the sole source of carbon and electron donors for nitrate reduction at pH 10 (Nixon et al [9]). The results from this work demonstrate that PVC plasticiser and fire retardant additives present in the PVC sheet are able to fuel microbial metabolism at pH 10 conditions using nitrate as an electron acceptor. Irradiation of plasticised PVC renders the material less bioavailable at pH 10, but microbial metabolism appears to be supported. No nitrate reduction was observed with non-irradiated PVC powder, although irradiated powder supported minor nitrate reduction. Separate tests on phthalate, the breakdown product of phthalate esters under irradiating and high pH conditions, and a potential radionuclide complexant was not found to support the metabolism tested, although it is clear that other additives present in plasticised PVC were degraded. These results highlight the bioavailability of volumetrically significant plasticised PVC under conditions relevant to its geological disposal in cementitious intermediate level nuclear waste.

Results of studies of bitumen degradation are presented in D1.3. This study concerned the degradation of Eurobitum, which also includes nitrate salts, which have the potential to oxidise the Boom Clay that is being considered as a possible host rock for nuclear waste disposal in Belgium. The study compared the potential of a microbial inoculum from the Boom Clay to metabolise nitrate leached from thermally aged inactive Eurobitum in the presence or absence of known bitumen degradation products (acetate, formate, oxalate). The study examined denitrification at pH 9-12.5 conditions and also included experiments inoculated with the high pH adapted lime works inoculum used in other MIND studies (D1.2, [3,4]). The study demonstrated that the nitrate leaching from Eurobitum rapidly stimulated microbial nitrate reduction. Different rates were observed depending on the organic compounds that were used as the electron donor to fuel the nitrate reduction process. Microbes were also able to use the Eurobitum as an electron donor to reduce nitrate. Microbial nitrate reduction resulted in a significant production of nitrite, which has the potential to oxidise the Boom Clay and thus affect the mobility of some radionuclides. Enhanced rates and extent of nitrate reduction were observed for the high pH adapted inoculum at pH 10.5, but the Boom Clay inoculum was also capable of nitrate reduction up to pH 10.5, reflecting conditions at the interface of the Boom Clay and the concrete liner of the disposal gallery.

Studies of ion exchange resin degradation are in progress in the Czech Republic and Switzerland and interim results are provided in the year 3 synthesis report D3.5. Both studies involve irradiation of cationic and anionic polystyrene based bead resins. Swiss studies have studied irradiation in Opalinus Clay groundwater conditions (pH 8) and under alkaline

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conditions using an electron linear accelerator (50 kGy) and at higher dose (200 kGy) using a ^{60}Co ϒ source. Analysis of the gas phase reveals the formation of H_2 and a number of chlorinated aliphatics, such as bromomethane, chloromethane, dichloromethane, and chloroethane, as well as aromatic compounds, such as benzene and toluene; many of these compounds have potential to fuel microbial processes. Studies in the Czech Republic have undertaken microcosm experiments of irradiated resins in groundwater inoculated with sulfate reducing bacteria. In this case a higher total dose was examined (^{60}Co ϒ, 1 MGy) and the proportion of resin to water varied. Degradation products of polystyrene resins detected in the liquid fraction mainly consisted of trimethylamine and dimethylaminacetatenitrile. Subsequent microbial microcosm experiments showed that the lowest concentration of irradiated anion exchangers (0.2 g/l) caused the highest relative increase in bacterial abundance (14 fold increase). At higher resin content microbial activity appears to have been inhibited by the low pH (3.1 - 3.4) following irradiation. The effect of Cs present in resins on microbial activity has also been examined, with no toxic effect being observed at 0.5 mM of Cs (stable) but with effects evident at higher concentrations of 1 mM and 5 mM Cs.

Deliverables D1.4, D1.5 and published journal papers [10],[11],[12] report specific studies of <u>radionuclide interactions with cellulose degradation products and bio-reduction processes.</u> Deliverable (D1.4) presents results of spectroscopic studies of uranyl (UO_2^{2+}) speciation with ISA and with acetate, the latter representing a typical fermentation product of cellulose and ISA as revealed by studies reported in (D1.2). Uranyl (UO_2^{2+}) - acetate (AcO^-) speciation is described by a 1:1 complex; $[UO_2AcO]^+$ and a 1:2 complex ; $[UO_2(AcO)_2]$, with no evidence for a previously proposed 1:3 complex (D1.4). Fundamental information about the interaction of UO_2^{2+} with ISA were gathered by a variety of spectroscopic techniques: (i) three dominant UO_2^{2+}-ISA-complexes were discovered; (ii) two of the three complexes are probably polynuclear; (iii) 5- and 6-membered rings are the dominant binding motifs; and (iv) the limiting metal to ligand ratio is 1:2 (D1.4).

Studies of Ni interaction and immobilisation with biogeochemical processes associated with the metabolism of cellulose degradation products (e.g. ISA) related to studies reported in [D1.2] have also been published by Kuippers et al [10]. Here it has been shown that in an applied experimental system examining the fate of Ni complexed with ISA that the biodegradation of ISA at neutral pH resulted in the removal of complexed Ni from solution and incorporation in iron sulfide phases that were formed as a consequence of the microbial activity (sulfate reduction) that was stimulated.

High resolution electron microscopy studies have been undertaken, along with detailed microbial studies to characterise new species of bacteria from bentonites that are able to reduce selenate (SeO_4^{2-}) to less soluble forms (D1.5). Ruiz-Fresneda et al. [11] and Sanchez-Castro et al. [12] report about the bacteria *Stenotrophomonas bentonitica,* that is capable of reducing Se(VI) and Se(IV) to Se(0) forming nanoparticles, which with time crystallise to more insoluble forms morphologies (D1.5).

<u>Methanogenesis has been studied under *in situ* conditions</u> at the Mont Terri rock laboratory and through the long term LLW gas generation experiment (GGE) at Olkiluoto (D1.6, D1.7, D1.8, Small et al [13]). Studies at the Mont Terri Underground Rock Laboratory (URL) have examined the potential for methanogenesis to develop in Opalinus Clay as a consequence of reaction of H_2 with inorganic carbon (CO_2, HCO_3^-). This experiment relates to a scenario where H_2 is generated by anaerobic corrosion and radiolysis processes associated with the disposal of ILW and is relevant to <u>consumption of H_2</u> as well as furthering the understanding of methanogenesis. Results of the *in situ* borehole experiments show that methanogens and acetogens are found at very low abundance (<0.1%) as part of the microbial community, however no change to the relative proportion of these microorganisms was detected throughout the duration of the experiment. Although the sulfate concentration was successfully reduced from 12mM to less than 4 mM during the *in situ* experiment no evidence for methanogenesis and acetogenesis was recorded. Microcosm experiments were also setup to examine conditions with lower sulfate concentration. However these

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microcosms also show no evidence for the onset of methanogenesis. Methane levels were measured for the duration of the experiment however no increase in concentration was recorded. There was also no significant increase in the acetate concentration over time suggesting that acetogens were not active in the community. The microbial composition after the incubation period supports these conclusions as it is dominated by one family (*Desulfobulbaceae*), which accounts for over 98% of the total reads. No methanogenic or acetogenic microorganisms were identified. Further investigation suggests that the onset of methanogenesis is also controlled by the bicarbonate (HCO_3^-) concentration, which was too low in the *in situ* and microcosm experiments so methanogenesis remained thermodynamically unfavourable.

In situ study of methanogenesis from cellulose containing LLW under initial alkaline conditions has been studied through further microbiological characterisation and modelling studies of the 18 year gas generation experiment (GGE) at the VLJ Repository, Olkiluoto, Finland (D1.6, D1.8,[13]). The GGE was originally set up in 1997 as part of a previous EU project (PROGRESS) and is operated by Teollisuuden Voima Oyj (TVO) and examines microbial gas generation from 16 200 dm^3 drums of cellulose containing LLW from the Olkiluoto power plants, together with a 4 tonne concrete box in a 20 m^3 gas tight vessel initially filled with river water. Interpretation of the geochemical data and modelling (D1.7, D1.8) has shown that an observed decrease in pH of water present in the tank water in contact with the concrete from pH 10 to neutral occurred over a period of 8 years by cellulose degradation processes occurring within the waste drums. A biogeochemical model of the GGE (D1.8, [13]) represents the diffusion of alkali from the concrete and acidity from the waste drums generated by cellulose hydrolysis and fermentation processes. The model accurately represents the observed rate of CH_4 generation during the 18 years of operation (~ 0.5 to 1 m^3 CH_4 per year), including an observed doubling of gas generation that occurred after 8 years. Microbiological characterisation including genomic sequencing studies of samples from the GGE reveal that the increase in gas generation rate coincides with the presence of acetate utilizing Archaea from the family Methanosaeta; methanogens that consume organic carbon. Initially, the microbial community was characterised by sulfate reducers belonging to the order Desulfovibrionales and methanogens that utilise H_2 (Methanosarcinaceae). The increase in CH_4 gas generation rate also coincides with a decrease in concentrations of dissolved organic carbon (DOC) and volatile fatty acids in the tank water. Biogeochemical modelling ([13], D1.8) also simulated the effect of increased methanogenesis as being related to neutralisation of the initial alkaline tank water and delayed consumption of DOC. Consideration of concentrations of sulfide in the tank water [13] also raises the possibility that methanogenesis could be inhibited by sulfide, whose concentration was observed to decrease from 0.2 mM initially to < 3×10^{-6} M after 8 years as a result of slow equilibration with of iron sulfide (mackinawite).

Deliverable [D1.8] concerns modelling of microbial processes in *in situ* experiments such as the GGE and borehole experiments at the Mont Terri URL. Deliverable D1.8 describes the basis to represent the growth of anaerobic microbes through Monod kinetics and coupling to geochemical speciation and solute transport. Examples are provided using the open source geochemical code PHREEQC, for wider application to assist the interpretation of such biogeochemical experiments and to underpin performance assessment models.

2.2 Work package 2

This work package consists of 5 tasks.

1. Microbial production of sulfide in the geosphere
2. Microbial induced corrosion of canisters
3. Microbial activity in bentonite buffers
4. Microbial degradation of bentonite buffers

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5. Microbial activity in backfill and influence on plugs and seals

Microbial production of sulfide in the geosphere: In the context of microbiological risks related to radioactive waste disposal, the geochemistry of gases in deep groundwater is an integral part of the determination of geochemical constraints of biological activity at disposal depths. Data on gas compositions and concentrations do exist from several separate locations in Finland and two locations in Sweden (D2.1, D2.3). In laboratory experiments with deep groundwater populations, acetate was overall the most efficient activator of the studied microbial communities which indicates acetate's important role as an electron donor for different Olkiluoto deep subsurface groundwater communities. However, there were also activation effects from H_2, CH_4 and NO_3^- (D2.5). The deliverable D2.17 "Sulfide production" aims at defining the boundary conditions constraining the availability of sulfide in deep geological disposal conditions. Critical process to be analysed is the sulfate reduction to sulfide, which is constrained by the sulfate source and the availability of possible electron donors (e.g., H_2, CH_4 and Fe^{2+}).

Microbial influenced corrosion (MIC) of canisters: This task has produced two deliverables, D2.13 "Anaerobic microbial corrosion of canister material" and D2.18 "Rate of corrosion of carbon steel in bentonite under biotic and abiotic conditions". The D2.13 report includes a description of MIC and methods that can be used to study corrosion and microbiology. Anaerobic MIC were studied in experiments with stainless steel and carbon steel in three types of water, granitic water from the VITA source and HV1 source in Frans Josephs underground research facility together with synthetic bentonite porewater which was inoculated with VITA water. Corrosion was studied in inoculated samples and in sterile controls with a range of electro-chemical methods; electrochemical impedance spectroscopy (EIS), open circuit potential and polarization curves. Weight loss studies were done and two DNA-techniques for identification of microorganisms were used. Also surface analyses were performed. Two temperatures, lab temperature and 35 °C were used in some studies. The duration of the experiments was from 111 days to 2 years. It was clearly shown that the corrosion rate of carbon steel in the presence of bacteria, SRB, was several times higher than in sterile controls. This was shown by weight loss experiments and illustrated with SEM.

Microbial activity in bentonite buffers: This task was scheduled for 4 deliverables, D2.4 "Threshold densities" (M36), D2.6 "Microbial diversity in bentonite buffer of aged bentonite buffer experiment" (M36), D2.14 "Role of bentonite density on the rate of corrosion of carbon steel" (M42) and D2.15 "Survival of microorganisms in bentonite subjected to different levels of irradiation and pressure" (M42).
The relation between water saturated clays at varying wet density and bacterial sulfide-producing activity is well studied (D2.4). However, wet density is just a value of the total amount of clay and water. That value does not reflect the conditions in compacted clay where several variables of importance for bacterial life can be of importance, such as clay type, pH, temperature, transport conditions, water content, pressure, pore space and pore water composition. These variables need further attention for a full understanding of what conditions control bacterial activity in compacted bentonite clays. Significant acetate formation from natural organic matter present in the clays was detected in the studied bentonites. This production occurred at all wet densities and suggests that bacterial activity, *per se*, was possible also at densities where sulfide-production could not be detected. Acetate is known to induce stress-corrosion cracking of copper and other metals and the possible formation of acetate should, therefore, be further investigated.
The presence of living microbes on bentonite and on copper surfaces could not be demonstrated in the D2.6 study but microscopy studies revealed living microbial cells in the external water surrounding copper cylinders. According to the IonTorrent sequencing, SRB and IRB were detected in the bentonite matrix, water and the copper surface. SRBs can produce corrosive sulfide and IRBs can have a role in processes that could be linked to the loss of swelling properties in bentonite. Fungal conidia and hyphae were detected by SEM in

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water and several groups of Ascomycetes and Basidiomycetes were identified by sequencing from bentonite samples. Many of the fungal genera detected are able to produce organic or inorganic acids that help fungi in solubilisation of minerals from rock substrates. The current results obtained from the *in situ* experiments in the Opalinus clay in Switzerland, offer a partial view on the microbial activity, and thus the potential impact of microbial processes on canister corrosion, under repository relevant conditions. Further evidence of the role of microbes in sulfide production and steel corrosion and the results arepresented in D2.14.
BaM bentonite is currently being tested at high temperature and pressure for D2.14. Two DNA extraction methods were compared (based on protocols obtained from HZDR and SCK•CEN), another method involving ultrasonic disintegration of the material was also tested. Experiments with inoculated bentonite suspension under pressure 1, 2, 3 and 5 MPa together with atmospheric pressure control were performed. Temperature/pressure experiments have been completed. Influence of radiation on microorganism's survivability in the bentonite suspension and water has also been studied (Deliverable D2.15)..

<u>Microbial degradation of bentonite buffers:</u> This task comprises 6 deliverables, D2.2 "Design, set up and operation of experimental equipment" (M18), D2.7 "Microbial diversity in aged bentonite" (M36), D2.8 "Long-term stability of bentonite in the presence of microorganisms" (M36), D2.9 "Evolution of stress in biotic and abiotic clay flow cells" (M36), D2.10 "Microbial mobility in saturated bentonites of different density" and D2.16 "Microbial activity and the physical-chemical and transport properties of bentonite buffer" (M44).
Sulfide has been found to reduce ferric iron in bentonites denoted Asha, MX-80 and Calcigel under the formation of elemental sulphur, ferrous iron and iron sulfide. This immobilisation effect can reduce the mass of sulfide that corrode metal canisters over repository life times, but the concomitant reduction of ferric iron may be problematic due to the destabilizing effect of ferrous iron on dioctahedral smectites such as montmorillonites.
The results of the D2.7 analyses showed that there were bacteria present in all of the bentonite samples studied. Surprisingly, the microbial communities detected in the bentonite samples showed a high level of similarity. The bacterial profiles were characterised by the dominance of heterotrophs, aerobes or facultative anaerobes capable of respiring oxygen or nitrates. The majority of detected bacteria belong to common soil microorganisms or ubiquitous microorganisms with wide ecological amplitude enabling them to survive under various conditions. The results suggest that microorganisms found in the bentonite samples in this study were most probably present in the bentonite already before the start of the experiment.
The aim of the laboratory scale D2.2/D2.8 MX-80 bentonite storage experiment was to simulate bentonite behaviour in circumstances that can take place in the interfaces of bentonite, host rock fractures and water flow in nuclear waste geological disposal. After one year, microcosms in MX-80 bentonite initiated both at aerobic and anaerobic conditions, no essential changes in bentonite mineralogy were found compared to the initiation of experiment. However, clear microbial activity in terms of ongoing sulfate reduction and sulfide formation as well as high number of sulphite reductase genes (*dsrB*) were detected in anaerobic samples. Microbial activity also affected bentonite water-phase chemistry and bentonite cation exchange capacity. These effects were not detected in sterile controls, demonstrating the microbial origin of these changes.
Given the limited duration of the D2.2/D2.8 tests, it is not surprising that the alteration of the bentonite was limited to a small zone adjacent to the iron. From a 'flow perspective' this will have little impact, as the bulk of the clay, and therefore the flowing porosity, remains unchanged. To understand the full impact of microbial action on the hydraulic properties of the clay, test durations would need to be increased substantially, or the iron should be dispersed within the entire clay matric to increase reaction rates. Given these findings, the question of whether microbial activity alters the hydraulic behaviour of bentonite remains unanswered. Further work is required beyond the lifetime of this project to fully assess the impact of microbial induced changes on the hydraulic integrity of the clay.

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The D2.10 study fulfilled important goals. First, a reliable method for direct detection of bacterial presence (both viable and dead cells) in the bentonite was developed, which has been missing. This method is based on the extraction of bacteria from bentonite using density gradient centrifugation and their subsequent Live/Dead fluorescence staining. Although the method needs further optimization and testing of its general functionality on different bentonite types, it is believed the method will be very useful for future research of bacterial presence in various clay materials.
In D2.16 a series of experiments have been carried out to investigate microbial survival and activity in compacted FEBEX bentonite compressed to dry densities of 1200 kg m^{-3} and 1400 kg m^{-3}. It was indicated that FEBEX bentonite microorganisms survive in the upper range of dry densities reported for microbial survival. In addition, the results indicate that presence of microorganisms is associated with particular iron phases and the development of aragonite crystals.

<u>Microbial activity in backfill and influence on plugs and seals:</u> This task comprises 2 deliverables, D2.11 “Cement deterioration boundaries” (M36) and D2.12 “Concrete stability” (M36). The D2.11 study demonstrated that the high pH conditions imposed by the OPC CEM I inhibited microbial sulfate and nitrate reduction. However, SEM analysis indicated the presence of intact cells in the suspension on top of cement and putative biofilm structures on the cement. This suggests that the high pH environment does not completely eliminate the microbial population. Interestingly, in sulfate reducing conditions, a pH decrease from > 12 to pH 10 was observed in one replicate harbouring clearly a larger microbial community in the suspension on top of the cementitious material compared to the other samples. Further studies to confirm this observation and to elucidate the precise mechanism are necessary. The conclusion of the D2.12 results was that the alkaline solution, leached from the concrete, 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 decrease in pH. A reason might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. In the future bentonite clay samples should be analysed for acetate and cultivatable acetogenic bacteria to confirm this hypothesis.

2.3 Work package 3

This work package includes 3 tasks:

1. Synthesis, evaluation, abstraction and integration of experimental and computational output
2. The impact of the inclusion of microbiology on expert conceptualization and public perception of geological disposal
3. Knowledge and information exchange

<u>Synthesis, evaluation, abstraction and integration of experimental and computational output:</u> Within this task of work package 3, all experimental work obtained within WP1 and WP2 was synthesized on a yearly basis and reported in two deliverables: D3.4 and D3.5. The first year, an evaluation of the initial state of the art knowledge base was reported as deliverable D3.1. In addition, next generation sequencing based on the 16S rRNA amplicon, a technique that is frequently used by most partners to identify the present microbial community was compared and evaluated among different partners. This because it is known that the analysis of microbial communities via next generation sequencing based on the 16S rRNA amplicon can be biased due to the DNA extraction protocol, primer design, PCR amplification, sequencing artefacts and bioinformatics analysis procedures. Therefore, care should be taken in comparing results obtained using different methods. Also within MIND, DNA extraction procedures, sequencing processes and bioformatics pipelines are highly diverse

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among the different partners. In order to evaluate the impact of the different strategies on the identified microbial community, a commercial microbial community standard of ZymoBiomics, consisting of eight different bacterial species was used (note that all data were analyzed blind i.e. involved partners didn't know the composition of the mock community).
In first instance, the sequencing process and bioinformatics pipelines were compared and evaluated. To this end, the DNA of the mock community was sent around to eight different microbiology laboratories involved in the MIND project. The different laboratories processed these samples with their own sequencing and data analysis tools. In total, five laboratories had sent in their sequencing data. Two different sequencing technologies were used i.e. Illumina MiSeq and IonTorrent, and the V3-V4 or V4 region of the 16S rRNA gene was selected as amplicon. If different labs analyzed their own data and predicted the mock community, variation was observed in the amount of Operational Taxonomic Units (OTUs) between the different research groups, ranging between 10 and more than thousand OTUs. However, the majority of this variation could be explained by the bioinformatics pipeline used, rather than the amplicon region, the quality of the sequencing data or the type of sequencing technology (Illumina MiSeq versus IonTorrent). This could be confirmed by applying the same 16S rRNA gene amplicon sequencing analysis pipelines on the five different datasets as comparable results were obtained from the different datasets. It was also observed that longer amplicons (e.g. V3-V4) returned a higher sequencing error rate than the approaches focusing on one region (e.g. V4). Additionally, most artefacts in the sequencing data originated from the presence of chimeric sequences, even for the best performing bioinformatics pipeline. Overall, it was clear that the different state-off-the-art sequencing and data analysis approaches used by the different partners in the MIND community are adequate and comparable, providing high quality scientific data. Chimera removal should be a point of attention whenever analyzing sequencing data. Knowledge and experience on how to address and process such samples and data was shared among the partners.
In a next step, an experiment was carried out to compare different DNA extraction protocols to recover DNA from a clay-rich environment. This matrix – although hampers the efficiency of DNA extraction – was selected as it is essential to have robust DNA extraction techniques from relevant host rock formations to assess the microbial impact on the safety of deep geological radioactive waste repositories. Seven laboratories received a 10 g aliquot of Opalinus Clay rock collected from the Mont Terri Underground Rock Laboratory in St. Ursanne, Switzerland spiked with the Zymobomics cell mock. Every participating laboratory extracted DNA using their own method. Afterwards, DNA amplification (16S rRNA gene V4 region) was performed by a single laboratory and Illumina MiSeq sequencing was carried out by a single commercial laboratory. In addition, the same bioinformatics pipeline was used to process and compare the DNA sequences obtained. Highly different results were obtained with the different DNA extraction methods. The amount of extracted DNA ranged from 0.66 ng to 418 ng, while in theory ~ 4.5 µg DNA could be obtained. In addition, there was one method outstanding in reassembling the mock community, while for other methods, an over- and underrepresentation of different OTUs was observed. The results of this study will be further elaborated on in future work. Nevertheless, these results indicate that care should be taken with comparison of data obtained with different methods and that testing the efficiency of the used DNA extraction protocol is essential.

<u>The impact of the inclusion of microbiology on expert conceptualization and public perception of geological disposal:</u> In order to understand how MIND experts (MIND researchers, members of the MIND Implementer Review Board) and experts outside the project (e.g. geologists, waste management regulators, policy makers) conceptualize the role of microbes in radioactive waste disposal, social scientists conducted semi-structured interviews with MIND members, participated as observers and presenters at MIND meetings, and analyzed guiding RWM documents within the MIND project. Elicited findings served as inputs for communication with project participants and civil society. To this end, group discussions, interviews and an interactive workshop with implementers, project participants and civil society were organized.

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Recurrent issues concerned the possibility and scope of interdisciplinary collaboration within and outside the MIND research community, the importance of public communication (particularly the communication of uncertainty), and the institutional recognition for microbiology in radioactive waste governance at large. In a concluding interactive workshop, members of civil society reproduced and reiterated these issues, while also shedding new light on them, by stressing that communicating uncertainty can generate (rather than erode) public trust in science, and by probing the relationship between MIND research and public safety. These observations should sensitize MIND stakeholders to their own research concepts and practices, including the value all stakeholders, without exception, ascribe to public communication – as research on the role of microbes in radioactive waste is characterized by a considerable degree of exoticness, uncertainty, and ambiguity. Although communication has been recognised as a challenge due to multidisciplinarity, trandisciplinarity, diverse risk perceptions and complexity, the new communication tools and opportunities offer a lot of possibilities to reach out with scientific results and to enable stakeholders to make informed decisions.

<u>Knowledge and information exchange</u>
Education and training activities were initiated (D3.3) to cultivate awareness of the relevance of microbial issues in otherwise typically abiotic fields of expertise, and to dissipate the knowledge gained in the MIND project beyond the known geomicrobiology expert circles. To this end, two courses related to microbiology in nuclear waste disposal were organized during the project, an exchange program for Master and PhD students has been developed and focused communication on these initiatives has been undertaken.

2.4 Work package 4

The MIND webpage (D4.1) was created by WP4 and has been updated continuously with information about project meetings, exchange opportunities, links to publications (see also D4.5), courses organized by the project, conferences related to the topic of the project etc, The Implementers' Review Board has developed a table listing potential research gaps regarding Microbiology in Nuclear Waste Disposal. This table is a collation of FEPs (Features, Events and Processes) on different microbial processes of potential relevance to waste disposal and also addresses to what extent these “FEPs” were addressed in MIND. This list has been used as a support when putting together the IRB Evaluation Report (D4.6) where the contributions of MIND are assessed with respect to microbial issues, processes and conditions of relevance to Post Closure Safety of Nuclear Waste repositories.

3 Conclusions

The MIND project was one of the first projects, investigating the impact of microbial processes on organic material in cementitious waste forms and their behaviour, on the technical feasibility and on long-term performance of repository components including clay and canister materials. The research was focussed on key questions posed by waste management organisations. Overall, many experiments have been conducted providing further insights into different microbial processes that can be expected during the geological disposal of radioactive waste. Chemical conditions that may limit methanogenesis and hydrogen consumption in LLW/ILW repositories were elaborated on in more detail. The research related to H_2 consumption by sulfate reduction processes is also of relevance to microbial induced corrosion of metal canisters. In addition, the knowledge base concerning the range of organic polymers and additives that may contribute to gas generation from

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LLW/ILW including the combined effects of irradiation and biodegradation under alkaline conditions was increased. Further knowledge has also been obtained regarding the biodegradation of potential radionuclide complexants, including ISA under alkaline conditions. Furthermore, several experiments showed that enhanced microbial activity could result in solid partitioning of radionuclides, which could affect their mobility in ground water. Different experiments resulted in a more detailed understanding of microbial activity in backfill material and bentonite buffers. In addition to the experimental work, the MIND project focussed on education and communication. All the expertise gathered within the project is summarized in yearly synthesis reports deliverables [D3.1, D3.4, D3.5] which can be valuable for waste management organizations to define further knowledge gaps that could be elaborated on in future projects.

The evaluation report (D4.6) by the IRB concludes that MIND has been invaluable to assemble the competence on microbiological issues of relevance to repository safety and has helped to clarify what issues are of potential importance and what that mere has scientific interest. Thereby MIND forms an essential platform for how further efforts in the areas should be prioritized.

The findings in the MIND project has contributed to that microbial processes in repository environments are now being considered e.g. when setting the requirements on engineered barriers such as buffer and backfill. This has resulted in significant refinements of safety case models currently being implemented.

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D1.5, Merroun et al. (UGR) “Microscopy and element mapping characterization studies of radionuclide microbe organic interactions”, 2015.

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