nagra.

Arbeitsbericht NAB 16-15

FEBEX-DP: Microbiological report

June 2017

Bengtsson Andreas, Blom Anders, Taborowski Trevor, Schippers Axel, Edlund Johanna, Kalinowski Birgitta and Pedersen Karsten

National Cooperative
for the Disposal of
Radioactive Waste

Hardstrasse 73
P.O. Box 280
5430 Wettingen
Switzerland
Tel. +41 56 437 11 11

www.nagra.ch

nagra.

Arbeitsbericht NAB 16-15

FEBEX-DP: Microbiological report

June 2017

Bengtsson Andreas[1], Blom Anders[1], Taborowski Trevor[1], Schippers Axel[2], Edlund Johanna[1], Kalinowski Birgitta [3] and Pedersen Karsten[1]

[1] Microbial Analytics Sweden AB, Mölnlycke, Sweden
[2] Bundesanstalt für Geowissenschaften und Rohstoffe, Hannover, Germany
[3] Swedish Nuclear Fuel and Waste Management Co., Stockholm, Sweden

KEYWORDS

Bacteria, bentonite, dry density, cultivation, sulphate-reducing bacteria, survival, viability

National Cooperative
for the Disposal of
Radioactive Waste

Hardstrasse 73
P.O. Box 280
5430 Wettingen
Switzerland
Tel. +41 56 437 11 11

www.nagra.ch

Nagra Arbeitsberichte ("Working Reports") present the results of work in progress that have not necessarily been subject to a comprehensive review. They are intended to provide rapid dissemination of current information.
This report was prepared on behalf of Nagra. The viewpoints presented and conclusions reached are those of the author(s) and do not necessarily represent those of Nagra.

Table of Contents

List of Tables

List of Figures

1 Abstract

High-level radioactive waste from energy production, mostly spent nuclear fuel and waste from re-processed fuel, will be encapsulated in metal canisters and disposed of in geological repositories. The canister in a repository must remain intact for 100'000 years or more before the radiation from the radioactive waste has decayed enough to be considered safe for the environment. Therefore, strict safety requirements are needed for the durability of the canisters. An issue that can threaten the canister integrity is a small but ongoing corrosion that eventually could create small holes in the canister metal that may lead to radionuclide emission. The anaerobic microbial corrosion process of concern is the dissimilatory reduction of sulphate to hydrogen sulphide by sulphate-reducing bacteria. FEBEX was a Full-scale Engineered Barrier EXperiment mimicking a typical high level radioactive waste repository concept for spent nuclear fuel disposal. The experiment has been excavated in two steps, the first, partial dismantling of the FEBEX "in-situ" test, FEBEX-I, was carried out during the summer of 2002, following 5 years of continuous heating. The second dismantling step, FEBEX-DP, took place in 2015. The microbiological results from FEBEX-DP are presented in this report. Bentonite samples for microbiological analysis were taken from six sections by two different laboratories. Cultivation of MPN for aerobic and anaerobic bacteria was performed. Microscopy, ATP-analysis, DNA extraction and sequencing were performed as well. Investigations during FEBEX-I have shown that the installed bentonite blocks carried a population of naturally occurring bacteria such as aerobic bacteria and anaerobic iron-reducing, nitrate-reducing and sulphate-reducing bacteria from the start. Cells could be cultivated in large numbers from moist, low temperature positions in FEBEX. The absence of cultivable bacteria in the samples from around the heater may be due to effects from desiccation, or, alternatively, viable cells were killed by the absence of water, possibly in combination with a high temperature. The data in this report show the presence of bacteria able to grow at 70 °C in FEBEX. Thermophiles may consequently appear in significant numbers in a warm repository if the conditions allow growth. Despite a large dry density (1.6 g cm^{-3}) and a high pressure (> 5 MPa) numerous bacteria could be cultivated after 18 years. Consequently, there does not seem to exist a cut-off density or pressure below these values where microorganisms in highly compacted bentonite are completely eradicated. Cultivability and viability of bacteria in compacted bentonite clays likely depend on several variables such as density, pressure, water availability and most importantly, the type of clay. The types and rates of metabolic activity by the bacteria found in the FEBEX clays, if any, is not revealed by the cultivation results and such investigations were out of scope of the investigation program. To study metabolic activity of bacteria in various ecosystems, including compacted bentonite clays, analysis of turnover of metabolites is commonly applied. Future repositories will very likely be installed with diverse bacterial populations and the possible safety issues from bacterial activity and the risk for microbial-influenced corrosion must be considered.

2 Introduction

2.1 The FEBEX project

FEBEX (Full-scale Engineered Barrier EXperiment in Crystalline Host Rock) is a research and demonstration project that was initiated by Enresa (Spain).

The aim of the project was to study the behaviour of near-field components in a repository for high-level radioactive waste in granite formations. The main objectives of the project can be grouped in two areas:

- Demonstration of the feasibility of constructing the engineered barrier system in a horizontal configuration according to the Spanish concept for deep geological storage (AGP-Almacenamiento Geológico Profundo), and analysis of the technical problems to be solved for this type of disposal method
- Better understanding of the thermo-hydro-mechanical (THM) and thermo-hydro-geochemical (THG) processes in the near field, and development and validation of the modelling tools required for interpretation and prediction of the evolution of such processes.

The project consisted of two large-scale tests (see Fig. 1) – "in situ" and "mock-up" (the latter is managed by CIEMAT in Spain) – a series of laboratory tests, and THM and THG modelling tasks.

The full-scale heating test ("in-situ" test), to which this document refers, was performed at the Grimsel underground laboratory in Switzerland, also known as Grimsel Test Site (GTS) or FelsLabor Grimsel (FLG in German). A complete description of the FEBEX project objectives and test program can be found in the "FEBEX Full-scale Engineered Barriers EXperiment in Crystalline Host Rock. Pre-operational stage summary report" (Fuentes-Cantillana et al. 1998).

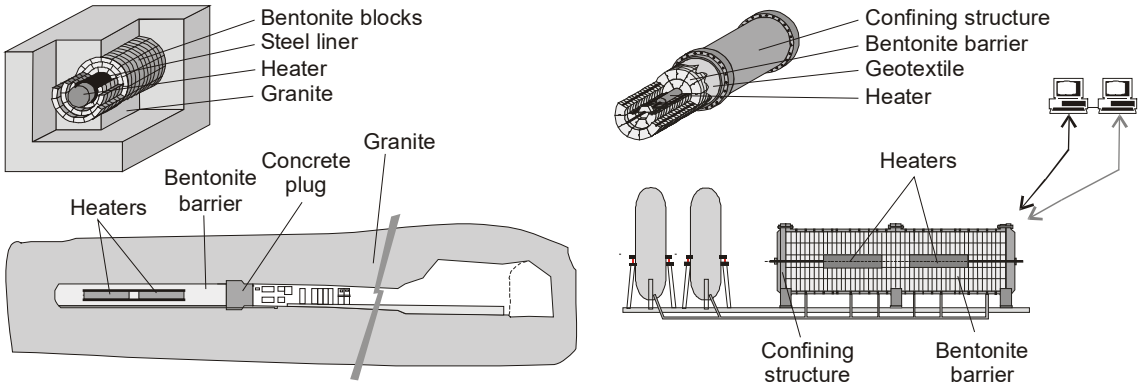

Fig. 1: Overall layout of FEBEX "in-situ" test (left) and "mock-up" test (right); after Fuentes-Cantillana et al. (1998).

The project started in 1994, and has been supported by the European Commission through consecutive contracts, identified as FEBEX I (Contract No FI4W-CT-95-0006) for the period January 1996 to June 1999, and FEBEX II (Contract No FIKW-CT-2000-00016), from September 2000 to December 2004. Afterwards, NF-PRO (near-field activities) took over from January 2005 to December 2007. Finally, in January 2008 the "in-situ" test was transferred from Enresa to a consortium composed of SKB (Sweden), POSIVA (Finland), CIEMAT (Spain),

Nagra (Switzerland) with the more recent addition of KAERI (South Korea), together forming the FEBEX Consortium, which currently supports the project.

The "in-situ" experiment excavation was carried out in 2015 and new partners, interested in taking part in the planned sampling and analysis operations, have been incorporated to the Consortium (now called FEBEX-DP) for that purpose, namely US DOE (USA), Obayashi (Japan), RWM (UK), Andra (France), BGR (Germany) and SURAO (Czech Republic).

2.2 The FEBEX bentonite

The bentonite used in FEBEX came from the Cortijo de Archidona deposit, developed by Minas de Gádor, S.A., in the zone of Serrata de Níjar (Province of Almería, Spain). Enresa had an extensive database on this deposit from the many studies prior to FEBEX. About 300 tons of raw material, which had been homogenized and conditioned, were acquired for the fabrication of the blocks for the barriers of the large-scale and mock-up tests and for all the laboratory tests. Some properties that characterise this bentonite are the following (Fuentes-Cantillana et al. 2000):

Montmorillonite content
88 % to 96 %

- Specific weight
  2.67 to 2.75 kg dm^{-3}

- Swelling pressure for a dry density of 1.60 g cm^{-3}
  4 MPa to 6 MPa

- Saturated hydraulic conductivity for a dry density of 1.60 g cm^{-3}
  4.2×10^{-14} to 7.6×10^{-14} m s^{-1}

The raw material prepared for the fabrication of the blocks was a granulate of bentonite with less than 5 % of the grains greater than 5 mm, with more than 85 % of the grains less than 74 μm.

The clay barrier was formed by blocks, weighing 20 to 25 kg each, of highly-compacted bentonite. The weighted average values of the dry density and water content of all the blocks fabricated were 1.70 g/cm^{-3} and 14.4 %, respectively. The construction of the barrier used 5'331 blocks, which corresponds to a mass of 115.7 ton. The in-place barrier had an average dry density, determined during construction, of 1.60 g cm^{-3} and a volume, when installed, of construction gaps (separations of variable magnitude) of 5.53 %. The gaps exist between blocks of the same slice; between slices; between blocks and the steel liner; and, systematically, between the clay barrier and the rock, where a gap of increasing width, up to as much as 3 cm, extends from the lower part to the crown.

2.3 Test configuration during FEBEX I

The installation of the "in-situ" test was carried out at the GTS. A horizontal drift with a diameter of 2.28 m was excavated in the Grimsel granodiorite especially for this experiment using a TBM (a tunnel boring machine). Two electrical heaters, of the same size and of a similar weight as the reference canisters, were placed in the axis of the drift. The gap between the

heaters and the rock was backfilled with compacted bentonite blocks, up to a length of 17.40 m, requiring a total 115'716 kg of bentonite. The backfilled area was sealed with a plain concrete plug placed into a recess excavated in the rock and having a length of 2.70 m and a volume of 17.8 m^3. Fig. 2 shows the dimensions and layout of the test components schematically. The material of the steel liner between the heaters was conventional alloyed steel for boilers and pressure vessels (15 Mo 3 after DIN 17155).

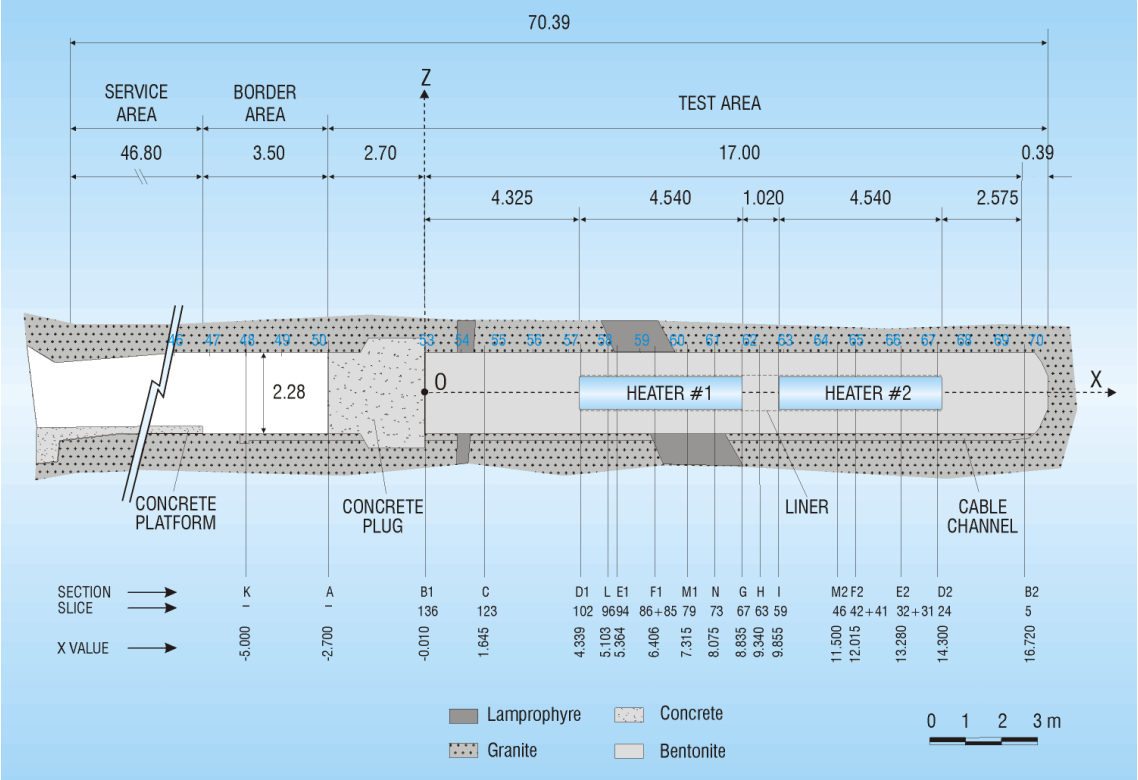

Fig. 2: General layout of the FEBEX "in-situ" test (FEBEX I configuration (Fuentes-Cantillana & García et al. (1998).

A total of 632 instruments were placed in the system along a number of instrumented sections, both in the bentonite buffer and in the host rock, to monitor relevant parameters such as temperature, humidity, total and pore pressure, displacements, etc. The instruments were of many different kinds and their characteristics and positions are fully described in the report titled "FEBEX Full-scale Engineered Barriers Experiment in Crystalline Host Rock. Final design and installation of the in-situ test at Grimsel" (Fuentes-Cantillana & García-Siñeriz 1998).

A Data Acquisition and Control System (DACS) located in the service area of the FEBEX drift collected the data provided by the instruments. This system recorded and stored information from the sensors and also controlled the power applied to the electrical heaters, in order to maintain a constant temperature at the heaters/bentonite interface. The DACS allowed the experiment to be run in an automated mode, with remote supervision from Madrid. Data stored at the local DACS were periodically downloaded in Madrid and used to build the experimental Master Data Base.

The construction of the concrete plug was completed in October 1996, and the heating operation started on 28. February 1997. A constant temperature of 100 °C was maintained at the heaters/bentonite interface, while the bentonite buffer was slowly hydrating with the water naturally coming from the rock. A complete report that includes both the installation of the test and the results gathered after two years of operation is given in "FEBEX full-scale engineered barriers experiment for a deep geological repository for high level radioactive waste in crystalline host rock Final Report" (Fuentes-Cantillana et al. 2000).

2.4 Dismantling of Heater 1 and test configuration afterwards (FEBEX II)

A partial dismantling of the FEBEX "in-situ" test was carried out during the summer of 2002, after 5 years of continuous heating. The operation included the demolition of the concrete plug, the removal of the section of the test corresponding to the first heater, and the sealing with a new shotcrete plug. A large number of samples from all types of materials were taken for analysis. A number of instruments were subsequently dismantled, and some new ones were installed. Accordingly, system design was adapted, and the physical layout was changed to ease the partial dismantling operation.

The buffer and all components were removed up to a distance of 2 metres from Heater #2 to minimise disturbance of the non-dismantled area. A dummy steel cylinder with a length of 1 m was inserted in the void left by Heater #1 in the centre of the buffer. Some new sensors were installed in that one additional metre of bentonite buffer.

Additional sensors were introduced in boreholes drilled in the buffer parallel to the drift. To simplify this operation, the new concrete plug was constructed in two phases: an initial temporary plug measuring just 1 metre in length, which was built immediately after dismantling, and a second section to complete the plug length to the 3 metres planned in the design of the experiment. Unlike FEBEX I, the new plug was a parallel plug, without a recess excavated in the rock, constructed by shotcreting.

The description of the partial dismantling operation is given in the report titled "Dismantling of the Heater 1 at the FEBEX "in-situ" test. Description of operations" (Bárcena et al. 2003). The configuration of the test, after completing the partial dismantling operation and construction of the full plug length, is shown in Fig. 3.

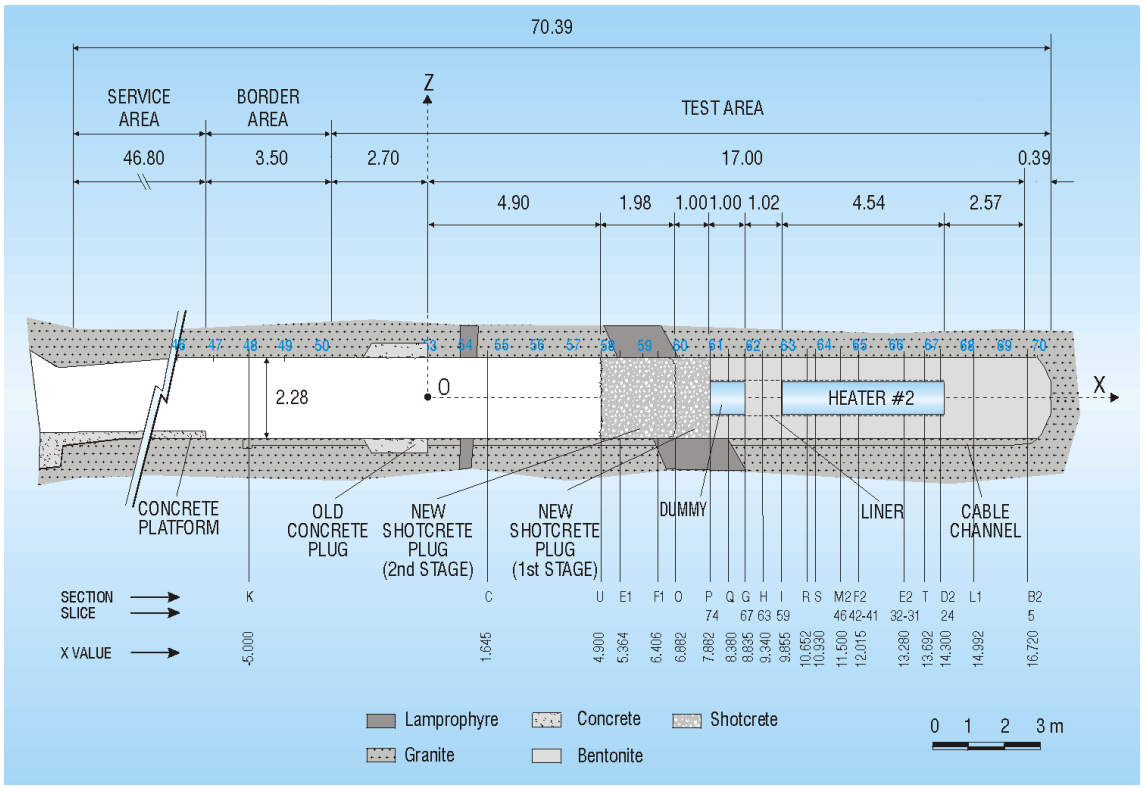

Fig. 3: Status of the FEBEX "in-situ" test after the partial dismantling (FEBEX II configuration) (Huertas et al. 2006).

A more complete report that describes the test from the conception up to two years of operation after the partial dismantling is given in the document titled "FEBEX Full-scale Engineered Barriers Experiment. UPDATED FINAL REPORT 1994 – 2004" (Huertas et al. 2006).

2.5 Concept of the dismantling of Heater #2

The objective of the second dismantling operation, carried out throughout 2015, was to dismantle all the remaining parts of the "in-situ" test, including Heater #2. This operation included carrying out a complete sampling of the bentonite, host rock, relevant interfaces, sensors, metallic components and tracers to allow the analysis of the barriers' condition after 18 years of heating and natural hydration.

Analytical results will be compared with data obtained from the partial dismantling (Huertas et al. 2006); the monitoring data (AITEMIN 2014) as well as with the results derived from modelling efforts (Lanyon & Gaus 2013). The results are expected to increase the current knowledge and confidence for the FEBEX-DP partners in bentonite performance with a focus on thermo-hydro-mechanical (THM) and thermo-hydro-chemical (THC) processes as well as on corrosion and bacterial activity. The reporting of the laboratory analysis and dismantling results is expected to be complete by the end of 2016 with a final integrated report issued in late 2017.

All details about the planned dismantling operation and sampling program are given in the reference documents: "FEBEX-DP (GTS) Full Dismantling Test Plan" (Bárcena & García-Siñeriz 2015a), "FEBEX-DP (GTS) Full Dismantling Sampling Plan" (Bárcena & García-Siñeriz 2015b) and its update (Rey et al. 2015).

All sample logs of the dismantling operation are documented in AN 15-578 Sample Log Book 34 to 62 FEBEX-DP (Abós & Martínez 2015).

2.6 Introduction to microbiological studies of the FEBEX project and objectives

The FEBEX-DP dismantling project has provided a second unique opportunity for characterisation of bacterial presence and processes in an engineered barrier and its components, that underwent continuous heating and natural re-saturation for 18 years.

High-level radioactive waste (HLRW) from energy production, mostly spent nuclear fuel (SNF) and waste from re-processed fuel, will be encapsulated in iron canisters in the present French and Swiss concepts and in copper canisters in the Swedish and Finnish concept (see http://www.igdtp.eu for details). A possible deterioration process is metal corrosion by sulphide that eventually may cause the canister to prematurely breach, leading to radionuclide release. Bacterial sulphide-producing activity could consequently impact the safety by compromising the canisters' isolation and containment functions.

The bentonite used in the FEBEX repository originated from El Cortijo de Archidona, Almeria, Spain and has been investigated in terms of bacterial diversity and bacterial numbers. Lopez-Fernandez et al. (2015) found high bacterial diversity in bentonite dominated by the phyla *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* in samples of FEBEX bentonite. The phylum *Proteobacteria* contains many species of the *Desulfovibrio* genus which are all sulphide-producers. During the partial dismantling of the FEBEX repository in 2002 (Enresa 2004) and from simulation in thermo-hydraulic cells (Enresa 2000), sulphate-reducing bacteria (SRB) that produce sulphide in their respiratory metabolism, could be cultivated from FEBEX bentonite samples in numbers ranging from 30 to 10'000 cells per gram depending on temperature and water content.

Many Gram-positive SRB species are able to form endospores (Castro et al. 2000) when the surrounding environment changes from favourable to unfavourable which temporarily stops metabolism and results in a very tough endospore wall. In this state, bacteria are able to withstand extreme drought and temperature while waiting for a favourable environment to return. Consequently, the previous results from bacterial diversity and number determination from FEBEX-I might not have been produced by bacteria that were active in the repository but came to life again when introduced to growth media in the test tubes for most probable number analysis (MPN). In addition, DNA is a very tough molecule that can remain intact long after the living cell has died (e.g. DNA in fossiles as described by Allentoft et al. (2012)). Hence, the bacterial diversity investigated previously using molecular analysis of extracted DNA samples could have originated from cells that were inactive or non-viable. However, it is clear from previous results that SRB inhabited FEBEX-I bentonite together with many other bacterial groups. These SRB may become active and produce sulphide which may damage metal waste canisters if the environment becomes suitable for bacterial activity as for instance a result of bentonite erosion that reduces density and hence swelling pressure.

Taking into account the initial objectives of the FEBEX experiment and the scientific developments in the field of microbiology in the context of radioactive waste since the 1990s the objectives of the FEBEX-DP microbiological studies were:

1. Contribute to the characterisation of the impact of bacterial activity on the long-term properties of the bentonite barrier, seals and plug systems in geological disposal concepts

2. Gain systematic information on the effectiveness of the bentonite buffer to inhibit bacterial activity by studying parameters such as density, temperature, water content and the related water activity
3. Investigate how the swelling pressure and water content affect the bacterial activity in bentonite
4. Gain information about how the bacterial activity and diversity vary in a cross-section of the bentonite from the wetter parts closest to the rock wall inward toward the heated canister

2.7 Microbiological sampling and analysis during the dismantling of FEBEX-DP

In 2015, the excavation of the second heater (Fig. 4) was initiated and a massive analysis program with the many different partners in the consortium started. This report will focus on microbiological studies performed on the excavated bentonite and relevant data from sensors such as water content and bentonite swelling pressure. The 9.5-meter-long tunnel containing the dummy, the heater and bentonite rings around, in front of and in the back of the heater were divided into sampling sections; with each section containing one or several bentonite block layers (Garcia et al. 2016).

Bentonite samples for microbiological analysis were taken from six of these sections: 42, 48, 52, 54, 60 and 62 (Fig. 5) by two different labs, Micans and BGR. Cultivation of MPN for aerobic and anaerobic bacteria was performed on Sections 42, 48, 52, 54, 60 and 62. Microscopy, ATP-analysis and DNA extraction and sequencing were performed on Section 60. Determination of pH and water content was performed on Sections 52, 54 and 62. An intact concrete-bentonite interface sample from Dismantling Section 36 (Mäder et al. 2016) was reserved too.

Additional microbiological analyses were also performed by Technalia (Madina 2016), SCK•CEN in Mol, Belgium and the university of Manchester (UNIMAN). The work done by SCK•CEN and UNIMAN is a part of the EU project MIND and will be reported as part of that project (www.mind15.eu).

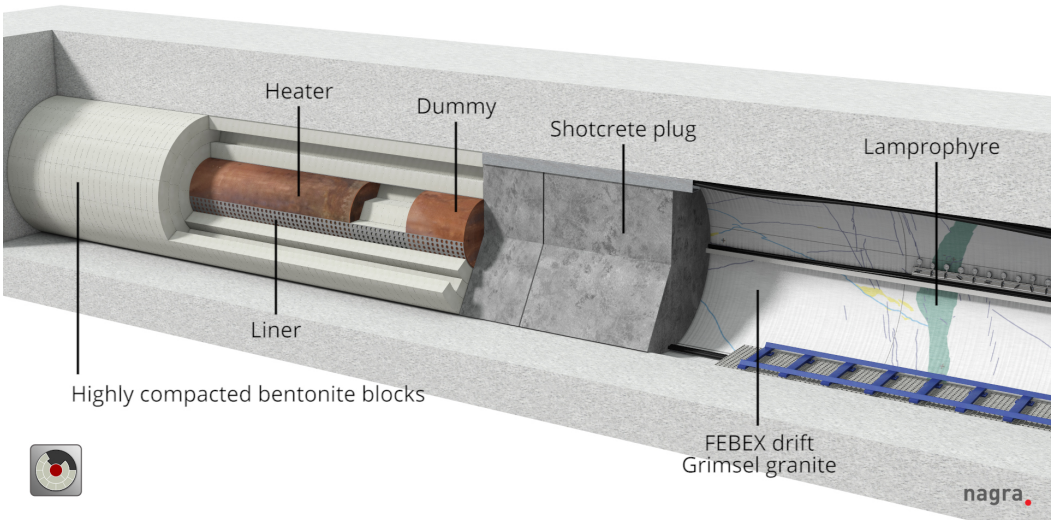

Fig. 4: 3D view of the FEBEX repository with Heater #2 situated horizontally in the centre of three rings of saturated bentonite clay.

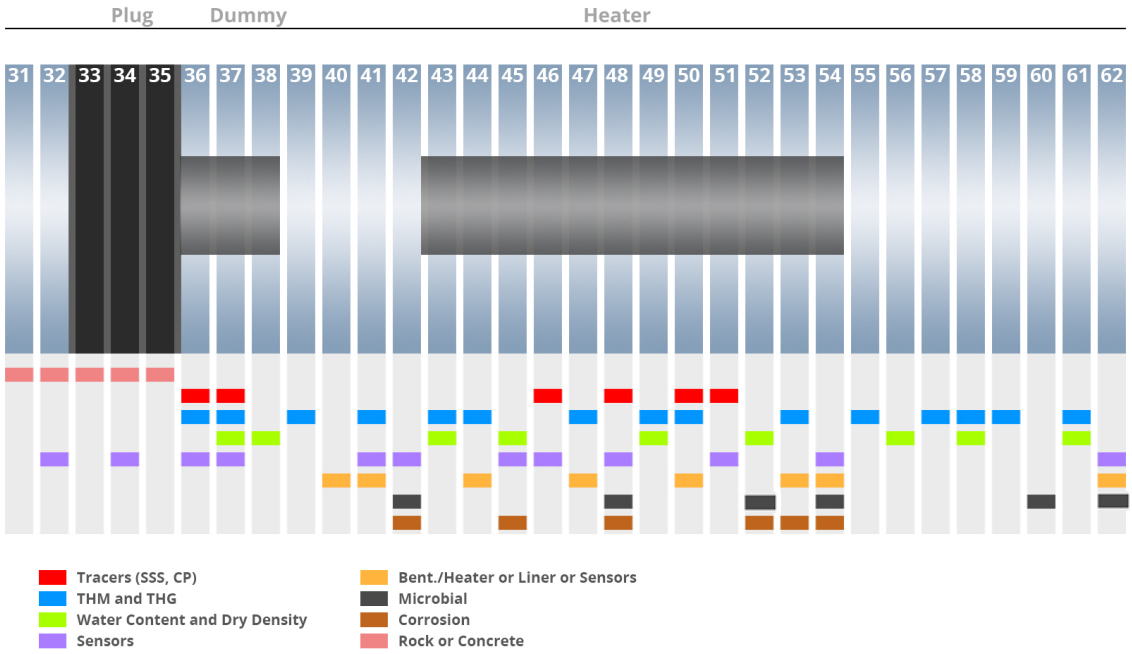

Fig. 5: Schematic figure of the FEBEX repository divided in dismantling sections.

Sections where samples for microbiological analyses were taken, including the interface between the bentonite and the shotcrete plug, are indicated with black horizontal bars.

3 Materials and methods

The materials and methods section is divided into two subsections depending on the consortium partner carrying out the analyses. Sections 2.1 and 2.2 explain Micans' procedures and Section 2.3 explains Bundesanstalt für Geowissenschaften und Rohstoffe's (BGR) procedures. A total of 126 core samples were taken of which 89 was handled by Micans and 37 by BGR.

3.1 Bentonite core samples – Micans Laboratory

A total number of 89 samples were taken from the four different dismantling sections (42, 48, 54 and 60) by a hole saw technique (Fig. 6). This was done by first drilling a circular core followed by insertion of a sterile metal tube (length 12 cm, Ø 8 cm) that was pushed into the dismantling section wall over the drilled core, leaving a sample inside the tube upon retraction. The sections were chosen to get a large resolution of bentonite exposed to both high and low water content and warm and cold temperatures. In addition, for each section bentonite samples were taken in straight lines from close to the rock wall, over the middle of the cross-section to the heater to investigate differences in bacterial viability as a function of distance to the heater or to the centre of the buffer. In many cases, samples were also taken from where the original bentonite block joints were situated, since swelling pressure in those positions might have been lower than in the centre of the block before full water saturation was reached. These positions could have favoured bacterial activity. Immediately after sampling, the metal tube with bentonite cores were wrapped in a sterile plastic bag to keep humidity and packed in double aluminium foil bags which were sequentially flushed with argon gas, evacuated at least three times, and then sealed in an evacuated state to keep samples in an inert, anoxic atmosphere. During the time awaiting shipment the samples were stored in a refrigerator at the GTS (Grimsel Test Site) at 4 °C. When all the dismantling sections had been excavated, the samples were transported to Micans' laboratory in Mölnlycke, Sweden, in a temperature controlled vehicle (+4 to +8 °C) to keep the samples as pristine as possible.

Each sample was given a code with information on the sample material, dismantling section and a serial number as references to each sample. For example, the first bentonite core sample taken from the Dismantling Section 42 was denoted B-C-42-1 (B= Bentonite C=Core Dismantling Section Number 42, Sample 1).

Fifteen of the 89 samples were analysed at Micans and the results are reported here. Twenty-four core samples were forwarded for analysis at SCK•CEN in Mol, Belgium, 12 samples were forwarded for analysis to the University of Manchester (UNIMAN), England and 8 samples were forwarded for analysis to Helmholtz-Zentrum Dresden-Rossendorf (HZDR), Germany; (Fig. 7 – 10) their results will be reported elsewhere. In addition to the samples displayed in Fig. 7 – 10, samples B-C-47-13 and 15 from Section 47 were also sent to HZDR (8 samples in total). Consequently, 30 samples are remaining as back-up samples if more analyses are deemed necessary. In addition, 9 samples (not included in the 89 samples handled by Micans) were sent directly from the sampling site to UNIMAN.

Fig. 6: Sampling of bentonite around the back end of heater in Dismantling Section 54. Top left: drilling with hole saw to create bentonite core sample. Bottom left: insertion of metal tube over drilled core. Right: packaging of sample in aluminium foil bag sequentially flushed with argon and evacuated.

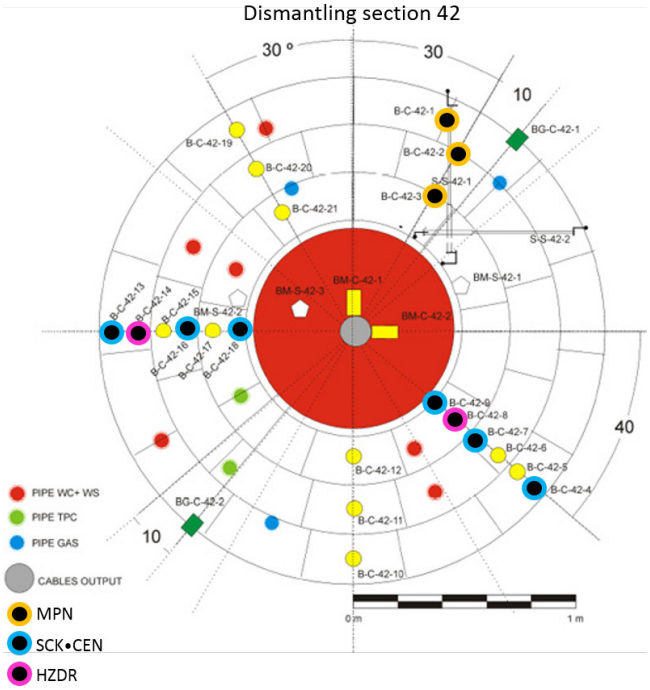

Fig. 7: Bentonite samples taken from Dismantling Section 42. Analysis performed by Micans as indicated by method designation or forwarded to other partner according to legend. Pipe and cable symbols can be ignored, they were not sampled for microbiology.

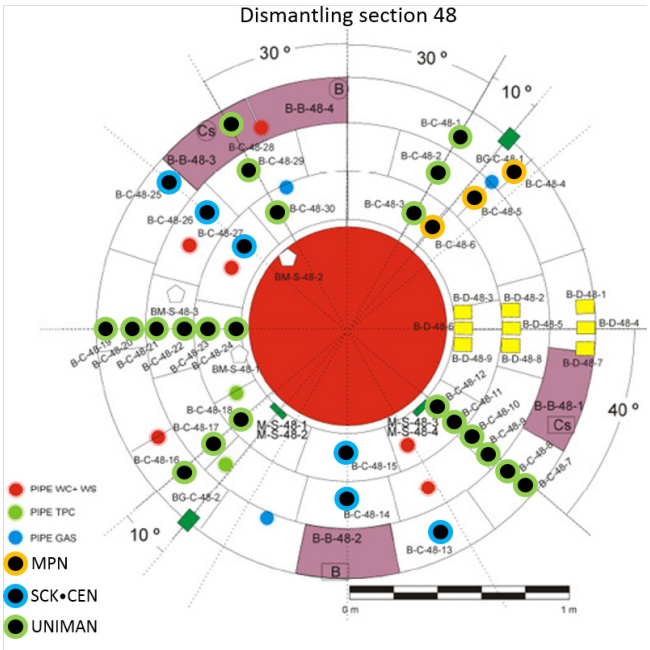

Fig. 8: Bentonite samples taken from Dismantling Section 48. Analysis performed by Micans as indicated by method designation or forwarded to other partner according to legend.

Pipe symbols can be ignored, they were not sampled for microbiology.

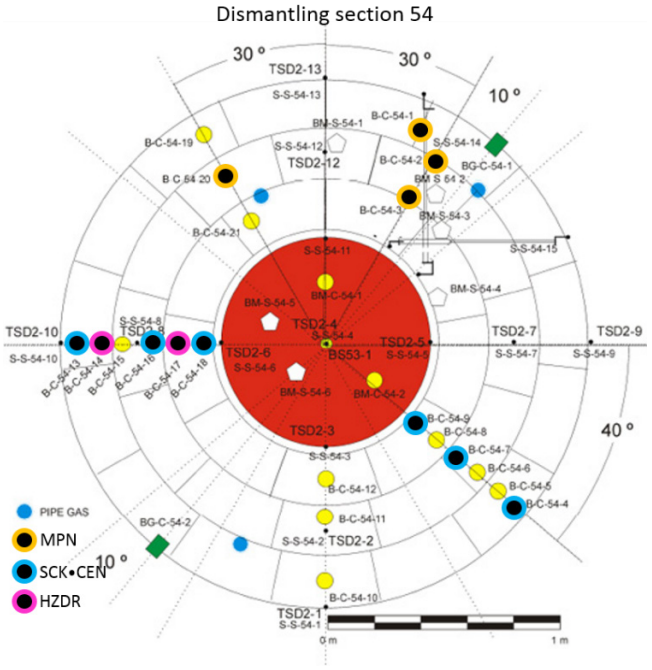

Fig. 9: Bentonite samples taken from Dismantling Section 54. Analysis performed by Micans as indicated by method designation or forwarded to other partner according to legend.

Pipe symbol can be ignored, they were not sampled for microbiology.

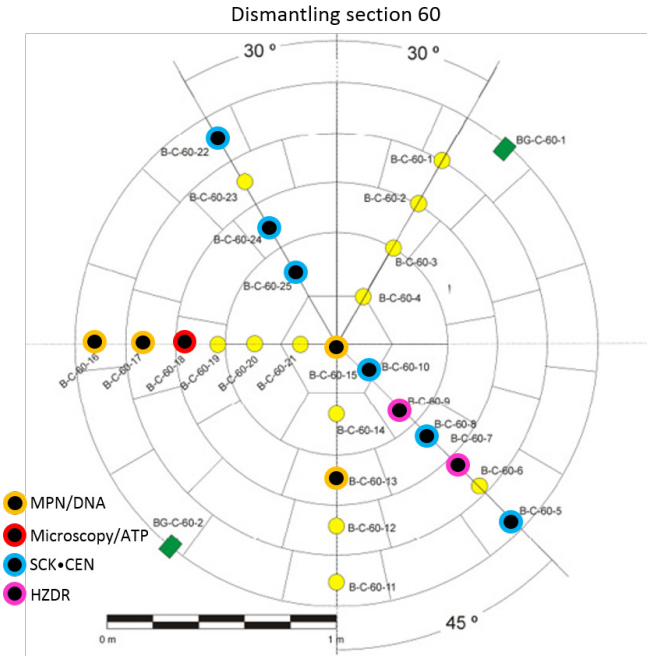

Fig. 10: Bentonite samples taken from Dismantling Section 60. Analysis performed by Micans as indicated by method designation or forwarded to other partner according to legend.

3.2 Materials and methods – Micans laboratory

3.2.1 Microbiological sampling

Upon arrival at Micans' laboratory the samples were stored in a temperature-controlled room at 18 °C. The aluminium foil bags were cut open with scissors and the inner plastic bags containing the metal tube with the bentonite core inside were taken out. All labels were double-checked to ensure that samples and bags had not been mixed up due to the large number of samples collected over time. The bentonite core inside of the metal tube was pushed out by using a workshop press (Biltema, Göteborg, Sweden, cat no.15-846) (Fig. 11). The exposed core was then split by using a sterile chisel and hammer (Fig. 11) producing two approximately similar size pieces with two new pristine bentonite surfaces. One of the pieces was randomly selected for analysis. One gram wet weight (gww) samples were taken from the middle of the core with sterile scalpels and put in 50 mL polypropylene tubes (Fig. 11). Immediately after sampling, the tubes were transferred to an anaerobic box with an atmosphere consisting of 97 % N_2 and 3 % H_2, O_2 < 1 ppm (COY Laboratory Products, Grass Lake, MI, USA) for further preparation.

Fig. 11: Microbiological sampling work flow.

Top left: visual inspection of the sample and labels. Top right: Extraction of bentonite core with workshop press. Bottom left: splitting of sample with sterile chisel and hammer. Bottom middle: sampling with sterile scalpels to a test tube on a balance. Bottom right: Inoculation of cultures for determination of most probable numbers of bacteria.

3.2.2 Most Probable Number (MPN) of bacteria determination

Samples for MPN-analysis were transferred to the anaerobic box where tubes containing 1 gww samples were filled with 20 mL sterile anaerobic 0.9 % NaCl solution and then put on a shaker to disperse the bentonite clay. After the bentonite clay had dispersed (> 2 h), the samples were inoculated in five tubes for each of total two 10-time dilutions, resulting in an approximate 95 % confidence interval lower limit of 1/3 of the obtained value and an upper limit of three times the value (Greenberg et al. 1992). The cultivation tubes were filled with 9 mL anaerobic SRB, IRB or NRB growth medium, mixed as described by Widdel and Bak (1992) for preparing anoxic media and modified as described elsewhere (Pedersen et al. 2008).

The MPN samples were cultured at 20 °C for eight weeks before analysis to ensure that slow-growing bacteria would be included in the results. SRB were detected by measuring S^{2-} production using the $CuSO_4$ method according to Widdel and Bak (1992) on a UV visible spectrophotometer (Genesys UV 10, Thermo Electron Corporation). Nitrate consumption by NRB was determined using a DR/2500 spectrophotometer (HACH, Loveland, CO, USA) with the chromotropic acid method (HACH Method No. 10020) for water and wastewater (0.2 – 30 mg L^{-1} NO^{3-} –N). IRB were detected by presence of the reduction product of Fe^{2+} by using the 1,10 phenanthroline method (HACH Method No. 8146).

3.2.3 Visualisation of bacteria

Bacteria from 20 gww of bentonite core sample B-C-60-18 were extracted by first detaching bacterial cells from clay particles followed by incrementing the electrostatic repulsion between the clay particles and the cells (method adopted from Kallmeyer et al. 2008). After several steps of centrifugation and phase separation a pellet was formed that was diluted in a series ranging from 10^0 to 10^{-2}. Two millilitres of each dilution step were filtered onto a 0.2 µm pore size polycarbonate filter. A small drop of 4',6-diamidino-2-phenylindole (DAPI) with Vectashield mounting medium was added on a glass slide and the polycarbonate filter was put on top. Another drop was added on top of the filter and covered by a cover slide. The mounted slide was incubated in the dark for 20-30 minutes. Bacteria on the filter were observed with a Zeiss Axio Scope.A1 microscope under UV-light at 1000 times magnification. Photographs were taken with a Axiocam 506 mono microscope camera with ZEN software and then processed for publication using Adobe Photoshop CS version 13.0 ×64.

3.2.4 ATP analysis

Adenosine-5'-triphosphate (ATP) transports chemical energy within cells for metabolism. ATP is a multifunctional nucleotide used in cells as a coenzyme. It is often called the "molecular unit of currency" of intracellular energy transfer. It is produced by cellular respiration, photosynthesis, or fermentation and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. One molecule of ATP contains three phosphate groups, and it is produced by ATP synthase from inorganic phosphate and adenosine diphosphate (ADP) or adenosine monophosphate (AMP). Metabolic processes that use ATP as an energy source convert it back into its precursors, so ATP is continuously recycled in organisms. The average active bacterium contains on average 1×10^{-18} moles (amol) of ATP, but this concentration can vary with cell size. Active cells have more ATP than do inactive, non-metabolizing cells. The analysis of ATP thus captures both biomass and activity (Eydal & Pedersen 2007).

The ATP Biomass Kit HS no. 266-311; BioThema, Handen, Stockholm) was used to determine total ATP in in the cells extracted from the bentonite of sample B-C-60-18 (see Section 2.2.3).

The ATP biomass method used here has been described and tested in detail, and evaluated for use with Fennoscandian Shield groundwater (Eydal & Pedersen 2007). The method has also been tested on bentonite samples previously with satisfactory results (unpublished work). The main objective with ATP analysis in this work was to investigate if viable bacteria were present.

3.2.5 DNA extraction and quantification

The MPN tubes with cultures of SRB, IRB and NRB with the highest and lowest (but still above detection limit) numbers of viable cells were selected for nucleic acid analysis together with cells extracted from B-C-60-18 (see Section 2.2.3). All samples analysed originated from cultures of bentonite from core Section 60 and from cores 13 and 15 – 18. Total genomic DNA was extracted from samples using the PowerWater® DNA Isolation Kit, (order no. 14900-100-NF, MO BIO Laboratories, Immuno diagnostics, Hämeenlinna, Finland).

Two other DNA extraction methods were also tested on raw clay from sample B-C-60-18. DNA from 10 gww samples were extracted using the methods described by Gabor et al. (2003) and by Selenska and Klingmuller (1991), from here on denoted as Method 1 and Method 2, respectively.

Extracted nucleotide eluates were first verified using a ND-1000 UV-vis spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), for quality control of the extraction efficiency and purity. Double-stranded (ds) DNA concentrations were then quantified fluorometrically using the Stratagene MX3005p fluorometer with MXPro software (Agilent Technologies Inc., Santa Clara, CA, USA) and the Quant-it™ Picogreen reagent kit from Molecular Probes (cat. no. P7589; Invitrogen, San Diego, CA, USA) according to the manufacturer's specifications. The extracted DNA was stored at −20 °C and subsequently used for sequencing.

3.2.6 Illumina sequencing

Amplicon library preparation of the 16S Bacteria rRNA gene hypervariable v6 region was performed by amplification with custom fusion primers containing Illumina Truseq adapter sequences, the i5 5′- AATGATACGGCGACCACCGAGATCTACAC-3′ on forward primer and the i7 5′ CAAGCAGAAGACGGCATACGAGAT- 3′ on reverse primer. Eight different inline barcodes were used on the read 1 forward primer containing a 9-nt (NNNNxxxxx) inline barcode followed by the 16S specific sequence. The read 2 primer contains a 6-nt index, 12 unique indexes, upstream of the sequencing primer binding site which is read in a separate indexing read, followed by the 16S specific region. The 16S specific target sequence; the forward primers used were (967F) 5′-CTAACCGANGAACCTYACC- 3′, 5′-CNACGCGAAGAACCTTANC- 3, 5′- CAACGCGMARAACCTTACC - 3, and 5′-ATACGCGARGAACCTTACC - 3 and the reverse primer used was (1064R) 5′-CGACRRCCATGCANCACCT-3 (Meyer et al. 2013).

The polymerase chain reaction (PCR) of the chosen region was carried out in triplicates with 33 µL reaction volume of 1.0 U Platinum Taq Hi-Fidelity Polymerase (Life technologies, Carlsbad CA), 1× Hi-Fidelity Buffer (Life technologies, Carlsbad CA), 200 µM dNTP PurePeak DNA Polymerase mix (Pierce Nucleic acid Technologies, Milwaukee, WI), 1.5 mM $MgSO_4$, 0.2 µM of each primer and ~ 10 ng template DNA. Each primer pair had control with no template for each Multiplex identifier. Cycling conditions were; an initial 94 °C 3 min. denaturation step followed by 30 cycles of: 94 °C for 30 s, 60 °C for 60 s and 72 °C for 90 s followed by a final 10 min. extension at 72 °C. The triplicate PCR reactions were pooled and checked on 1 %

agarose gel. The amplicons were the size of 236 base pairs. Amplicons were sent on dry ice to the sequencing facility. Upon arrival amplicons were checked for quality by capillary electrophoresis on a Fragment Analyser (Advanced Analytical) and quantified using Picogreen dsDNA reagent (Life Technologies, Carlsbad, CA) and pooled in equimolar amounts for optimal cluster concentration.

The library pool was sequenced on the NextSeq500 Instrument from Illumina using 150 base pairs paired-end run sequencing on high output mode. PhiX DNA at 25 % served as the control DNA for the run.

Raw data files were provided in FASTAQ format and were delivered through the Illumina cloud-based sequence Hub Basespace. Envonautics bioinformatics consultants Ltd. performed the bioinformatics analysis by using a phyton pipeline for 16S rRNA bacterial amplicon sequencing data, version 2.0.0. The source code and further information on the workflow for the pipeline may be found at: http://xapple.github.io/sifes/ . To resolve the dataset, reads were de-multiplexed by identifying reads by their index and barcode. Paired-end sequencing recovers DNA sequences from both ends of the DNA template giving a complete overlap between the reads. The algorithm implemented in the Mothur software version 1.35.1 was used for acquiring complete overlap of forward and reverse paired-end reads. Reads that did not assemble were rejected. A length cut-off algorithm rejected reads under 55 bases and over 140 bases. Further quality filtering to remove sequencing errors was done by searching for forward and reverse primer in reads. Sequences that did not have a perfect match in either of the forward or reverse read were discarded together with reads with undetermined bases (N´s). A chimera check was applied by using the UCHIME algorithm in deNovo and reference based mode (Edgar et al. 2011). All sources are available at http://drive5.com/uchime. The OTU clustering was performed by using the UPARSE pipeline at 3 % similarity threshold (Edgar 2013). Using the CREST classifier, the representative sequences for each OTU were searched against the SILVA 123 16S Database set to 97 % threshold. Bar charts detailing the composition at different levels were constructed by using the OTU table and taxonomic assignment.

Alpha diversity indices, observed richness and sequence similarities at different rarefaction depth were calculated by using the Chao1, ACE, Shannon and Simpson's estimates. Nucleotide sequences were submitted to the NCBI Sequence Read Archive (SRA) Repository and thereby given individual accession numbers.

3.2.7 Statistical analyses and data visualisation

Data graphics design and statistical analyses were performed in Statistica 13 (Statsoft Inc., Tulsa, OK, USA).

3.2.8 Droplet digital PCR

The extracted DNA from Methods 1 and 2 from B-C-60-18 was quantified with droplet digital PCR (ddPCR) by using the QX200™ Droplet Digital™ PCR system (Bio-Rad, Temse, Belgium). This is a new technology that enables the precise absolute quantification of target nucleic acids in a sample without the use of a standard curve.

Tab. 1: Primers used in ddPCR for assay of SRB.

PCR assay	Primer name	Primer sequence [5′-3]	Final concentration [nM]	Amplicon size [bp]
SRB	apsAF304	GAG CGT GTC TTC ATC GTC AA	200	112
	apsAR416	GCG TTG CAG GTG TAG ACG TA	200	112

SRB were detected with the primers apsAF304 and apsAR416 which are specific for the functional gene for the adenosine-5'-phosphosulphate (APS) reductase (Tab. 1). The SRB primers were designed by Ben-Dov et al. (2007). Protocols for ddPCR were optimized by running primers on an annealing temperature gradient at 55 – 68 °C.

A pre-sample solution was prepared containing 12.5 µL of 2 × QX200 ddPCR EvaGreen Supermix (Bio-Rad, cat no. 186-3010), 0.5 µL forward and reverse primers and 7.5 µL water. A volume of 4 µL was pipetted from each sample vial and mixed with 21 µL pre-sample solution. Twenty microliters of this solution were pipetted in eight compartments of the Droplet Generator Cartridge (Bio-Rad, cat no. 186-3008) and droplets were generated. The entire droplet emulsion volume of 40 µL was further loaded in a semi-skirted and PCR-clean 96-well PCR plate (Eppendorf, Leuven, Belgium). The loaded 96-well PCR plate was then heat-sealed with pierceable foil in the PX1™ PCR Plate Sealer and placed in a C1000 Touch™ Thermo Cycler (both from Bio-Rad). Thermocycling conditions were 95 °C for 10 min, followed by 40 cycles of 95 °C for 10 s and 58 °C for 30 s. A melting curve analysis was run as last segment at 95 °C for 1 min, 55 °C for 30 s and 95 °C for 30 s. After PCR amplification, the droplets were analysed in a QX200™ droplet reader (Bio-Rad), and the absolute quantification of PCR targets was analysed using QuantaSoft™ software version 1.7.4 with a threshold placed at an amplitude as low as possible for each sample. These data are then analysed using Poisson statistics to determine the target DNA template concentration in the original sample.

3.2.9 Overcoring sample of the concrete-bentonite interface

An overcoring sample was taken by drilling through the concrete plug (Dismantling Section 32) that sealed the FEBEX repository during the duration of the experiment, and into the first dismantling section around the dummy heater (Dismantling Section 36). The overcoring sample consisted of three different parts; one shotcrete part, one bentonite part and one part with the intact interface between the shotcrete and bentonite. The samples were cut into four equally large quarters and then preserved in epoxy resin to keep the samples unaltered. One of these quarters with all three parts was sent to Micans' laboratory for analysis of viable bacteria. For more information and details of the overcoring sample and sampling technique see Garcia et al. (2016). The 10 cm long quarter that contained the interface between shotcrete and bentonite (sample no. C-C-32-4-OC-cb-III) was sampled as in Section 3.2.2 were 1 gww samples of the bentonite were taken for MPN analysis of SRB, IRB and NRB. However, after analysis viable bacteria could not be found with any of the three growth methods, hence no further action was taken. Garcia et al. (2016) report temperatures around 30 °C in the interface between shotcrete and bentonite. Martínez et al. (2016) report a total pressure between 5 and 6 MPa in the same interface. Although the temperature was favourable for bacterial activity the pressure was high and may have restricted cultivability and activity. It is also well known that concrete (or shotcrete) can increase pH in nearby surroundings which may have had a negative impact on bacteria in the clay.

3.3 Materials and methods – BGR laboratory

3.3.1 Bentonite samples

A total of 37 samples were sent to BGR for analysis. Of these, 22 samples were processed and are reported here. The samples were shipped in air-tight bags in cooled containers by courier to the BGR geomicrobiology laboratory in September 2015 and kept in a cold room (8 °C) to minimise bacterial activity until subsampling for analyses.

Analyses performed included:

1. Numbers of aerobic living heterotrophic cells (MPN cultivation)
2. Numbers of anaerobic living heterotrophic cells (MPN cultivation)
3. Total cell counts (cell staining and fluorescence microscopy)
4. Real-time quantitative PCR (qPCR) quantification
5. pH- determination
6. Humidity determination
7. Residues

3.3.2 Bentonite core sample position in the dismantled sections

The cores were sampled and shipped as described in Section 2.1.

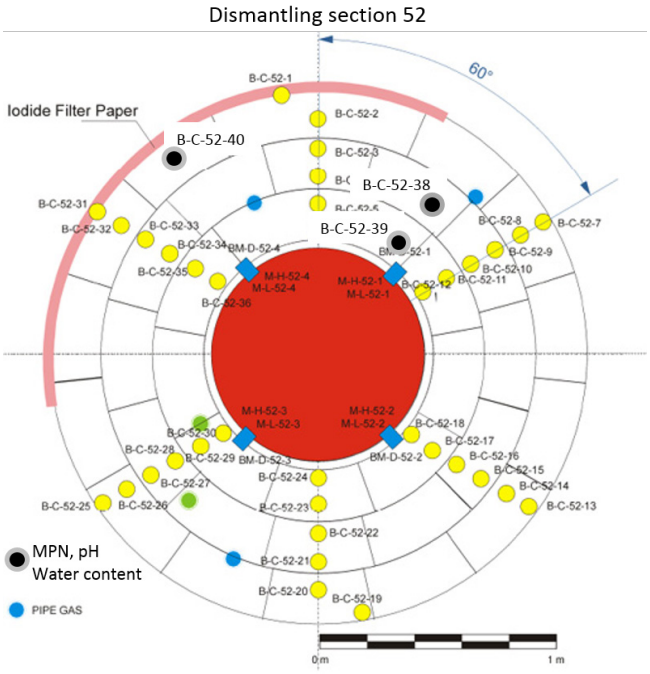

Fig. 12: Bentonite samples taken from Dismantling Section 52. Analysis performed by BGR according to legend.

Pipe symbol can be ignored, they were not sampled for microbiology.

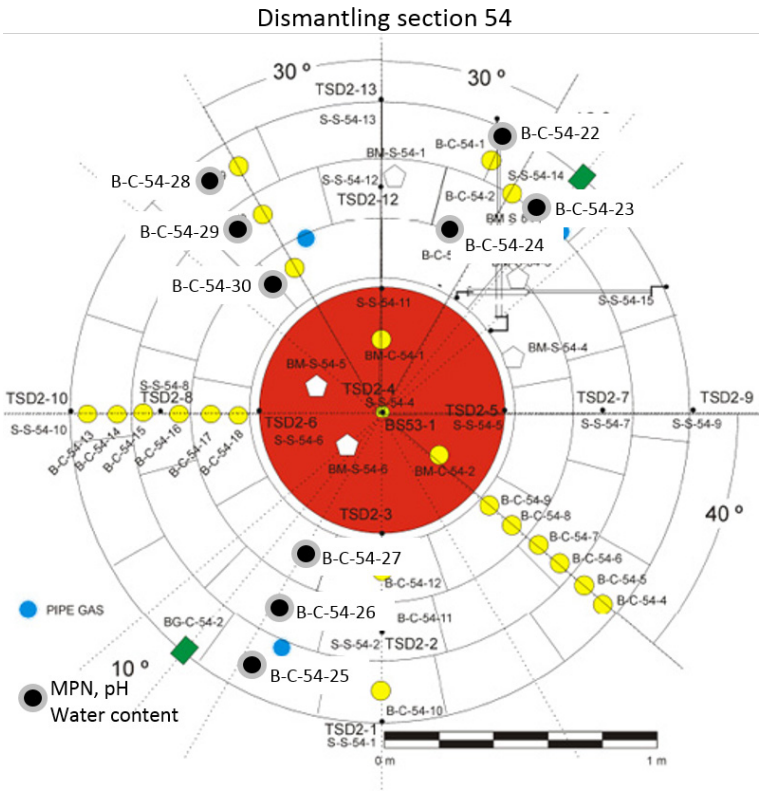

Fig. 13: Bentonite samples taken from Dismantling Section 54. Analysis performed by BGR according to legend.

Pipe symbol and yellow symbols can be ignored, they were not sampled for microbiology.

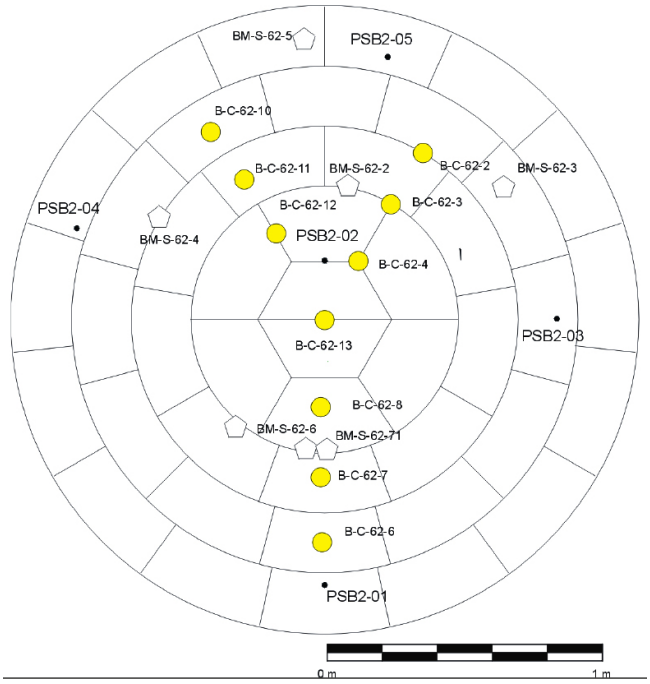

Fig. 14: Bentonite samples taken from Dismantling Section 62. Analysis performed by BGR according to legend.

3.3.3 Subsampling

The sampled core sections were opened under aseptic conditions and subsampled using the inner core part with sterile spoons or spatulas. A subsample per core sections was frozen at -20 °C for later qPCR analysis.

3.3.4 Aerobic analyses

3.3.4.1 Numbers of aerobic living heterotrophic cells (MPN cultivation)

Per core Section, 2 gww sample material (not dried) were shaken in 20 mL sterile physiological NaCl-solution (0.9 %) for 2 h at 100 rpm in 50 mL vials. Afterwards this liquid was used homogenously for inoculation via a dilution series (NaCl 0.9 %) of 3 parallel MPN tubes containing R2A-medium with glucose at pH 7.2 for enumeration of aerobic heterotrophic bacteria (Reasoner & Geldreicher 1985). Two parallel MPN-series were incubated at 30 °C and 70 °C for 21 to 35 days to detect mesophilic and thermophilic bacteria, respectively. MPN-tubes were counted as positive if the pH changed at least 0.5 unit and turbidity was observed (microscopic check for cells in some cases). The cell number was given per g sample dry weight.

3.3.4.2 Total cell counts (cell staining and fluorescence microscopy)

Samples of 0.5 gww were fixed in 2 % formaldehyde in phosphate buffered saline (PBS-buffer, i.e., 0.01 M NaCl buffered to pH 7.6 with 9 mM Na_2HPO_4 and 1 mM $NaH_2PO_4 \times H_2O$) as previously described (Pernthaler et al. 2001) for total cell counting. Total cell number determination was carried out by depositing cells on filters, staining with the bright fluorescent dye SYBR Green I and counting using a fluorescence microscope. Due to the fine-grained material the samples had to be diluted in order to distribute the material on filters. Therefore, the detection limit for the microscopic method was quite high at 10^6 cells mL^{-1} which resulted in total cell count values that were below the detection limit. Hence, no further action was taken regarding this analysis.

3.3.4.3 Quantitative, real-time PCR

High-molecular-weight DNA was extracted from the solid samples (which were first pounded in a mortar and pestle), following a modified FastDNA Spin Kit for Soil protocol (MP Biomedicals, Webster et al. 2003) for quantitative, real-time PCR (qPCR) analysis. Bacteria and Archaea were quantified via their 16S rDNA gene using TaqMan assays (Nadkarni et al. 2002, Takai & Horikosh 2000) as previously described (Schippers et al. 2005) using a StepOne™ instrument (Applied Biosystems). Reactions were performed in a total volume of 10 µL containing 1X master mix, 0.5 µM of each primer and different concentrations of DNA. Each DNA extract was measured in triplicate. PCR products obtained from DNA of pure culture cell extracts were used as DNA standards for qPCR assays. However, the DNA yield from the analysis did not reach sufficient amount for quantification. Hence, no further action was taken regarding this analysis.

3.3.4.4 pH- determination

12.5 ml KCl-solution was added to 5 gww sample material, shaken for 5 min. at 120 rpm at room temperature, and subsequently the pH was measured after 2 h.

3.3.4.5 Water content

The gravimetric water content (w) was determined as the ratio between the mass of water and the mass of dry solid expressed as a percentage. The mass of water was determined as the weight difference before and after sample drying at 105 °C to a constant weight (analyser Sartorius).

3.3.5 Anaerobic analyses

3.3.5.1 Numbers of anaerobic living heterotrophic cells (MPN cultivation)

The method as described under 2.3.4.1 was applied with the following modifications: Per core Section 2 gww sample material (not dried) were shaken in 20 ml sterile physiological NaCl solution (0.9 %) for 2 h at 100 rpm in serum vials flushed with dinitrogen gas. MPN Hungate tubes with R2A medium were flushed with dinitrogen gas before inoculation with the liquid in the serum vials.

3.3.5.2 Residues

Sample residues for potential future analyses were stored at 8 °C in air-tight glass flasks flushed with dinitrogen gas.

4 Results

4.1 MPN

Micans' MPN samples from Sections 42 to 54 showed very few cultivable cells. Most analyses reported values below the detection limit of 4 cells gww^{-1}, with some exceptions (Tab. 2). Water content, dry density and temperature data from sensors and laboratory analyses performed by other partners (Villar et al. 2016) showed that these dismantling sections around and in close adjacency to the heater were very dry and hot in a radial pattern from the heater out to the rock wall. This harsh environment for life would explain the low numbers of cultivable cells in these sections. Further in the gallery however, the environment was quite different with a relatively high water content and low temperature (approx. 20 °C). In bentonite samples from Dismantling Section 60 bacteria from all tested groups of SRB, NRB and IRB could be detected in various numbers ranging from a few to more than 2500 cells per gram wet weight (Tab. 2).

Tab. 2: Most probable numbers of SRB, NRB and IRB in FEBEX bentonite core samples with lower and upper confidence limits (CL).

Sample code	SRB gww^{-1}	Lower-Upper CL	NRB gww^{-1}	Lower-Upper CL	IRB gww^{-1}	Lower-Upper CL
B-C-42-01	< 4	-	8	2 – 34	< 4	-
B-C-42-02	< 4	-	< 4	-	< 4	-
B-C-42-03	< 4	-	< 4	-	< 4	-
B-C-48-04	< 4	-	< 4	-	< 4	-
B-C-48-05	< 4	-	< 4	-	< 4	-
B-C-48-06	< 4	-	< 4	-	< 4	-
B-C-48-13	< 4	-	< 4	-	< 4	-
B-C-54-01	< 4	-	< 4	-	< 4	-
B-C-54-02	< 4	-	16	6 – 48	< 4	-
B-C-54-03	< 4	-	< 4	-	< 4	-
B-C-54-20	< 4	-	< 4	-	< 4	-
B-C-60-13	4	2 – 20	< 4	-	< 4	-
B-C-60-15	12	4 – 36	2'600	1'000 – 7'800	< 4	-
B-C-60-16	60	20 – 220	120	60 – 360	4	2 – 20
B-C-60-17	46	18 – 172	2'200	800 – 6'000	22	8 – 58

gww = gram wet weight

The BGR cultivation technique used a broader medium (R2A) which suits a larger diversity of bacteria than a specific bacterial group-medium (i.e. SRB, NRB, IRB in Tab. 2) and resulted in higher cultivable bacterial numbers. To compare results in Tab. 2 and 3 the most correct way is to sum up the numbers for each bacterial group sample in Tab. 2 and compare that to the numbers in column 6, Anaerobic 30 °C, in Tab. 3. The greatest difference in negative and positive cultivability could be found in cores from Section 54 where the specific cultivation media technique showed positive growth only in one sample whereas the R2A technique picked up positive growth in five samples.

The results from BGR also reports a trend of increasingly higher bacterial numbers the further into the gallery the sample was taken. This agrees well with the results in Tab. 2. The most difficult bacterial group to cultivate were the anaerobic thermophilic bacteria. The highest number for this group could be found in sample B-C-52-38, located above the middle of the second half of the heater (Fig. 12) where the temperature close to the heater remained approximately 100 °C during the whole experimental time which might explain why thermophilic bacteria could be cultivated from that sampling position. The most abundant bacterial group were the aerobic mesophilic bacteria which probably also make up some of the cultivable bacteria in the anaerobic column since some species are facultative anaerobic bacteria, meaning that they can respire O_2 but are also able to survive and be active in O_2-free environments through fermentation or using alternative electron acceptors.

Taking all cultivation methods together, it is obvious that cultivability increased with increasing water content in the FEBEX bentonite. As is illustrated in Fig. 15, there was an increasing trend in cultivable aerobic and anaerobic cells with increasing water content while the numbers of cultivable cells were constant over water content. It should be noted that the water content values were analysed after shipping and preparations in the BRG laboratory and the values may, therefore, differ somewhat from the values determined on site (Villar et al. 2016). There is a tendency to lose water during handling of clay samples over time.

Tab. 3: Results of MPN cultivation performed by BGR for mesophilic and thermophilic, aerobic and anaerobic bacteria from three different dismantling sections.

Dismantling section	**Sample code**	**pH value**	**Water content** (w; %)	**Aerobic 30 °C** (cells gdw^{-1})	**Anaerobic 30 °C** (cells gdw^{-1})	**Aerobic 70 °C** (cells gdw^{-1})	**Anaerobic 70 °C** (cells gdw^{-1})
52	B-C-52-38	6.68	17.67	< 10	129	106	706
52	B-C-52-39	6.94	13.66	< 10	< 10	< 10	< 10
52	B-C-52-40	6.98	17.22	< 10	105	< 10	< 10
54	B-C-54-22	6.98	15.09	< 10	691	104	< 10
54	B-C-54-23	7.06	71.23	300	48	< 10	< 10
54	B-C-54-24	6.96	16.93	< 10	70	< 10	< 10
54	B-C-54-25	6.93	17.92	2'950	< 10	83	< 10
54	B-C-54-27	7.05	13.90	< 10	< 10	< 10	< 10
54	B-C-54-28	6.93	18.46	130'000	36	36	< 10
54	B-C-54-29	7.00	17.18	293	< 10	< 10	< 10
54	B-C-54-30	7.00	17.11	35	< 10	< 10	< 10
54	B-C-54-26	7.03	12.86	< 10	45	45	< 10
62	B-C-62-02	6.72	21.59	54'700	30'400	304	73
62	B-C-62-03	7.64	25.03	119'000	4'390	50	< 10
62	B-C-62-04	7.03	22.18	91'700	13'400	86	< 10
62	B-C-62-06	7.81	22.96	184'000	36'900	49	< 10
62	B-C-62-07	6.77	21.16	30'300	18'200	36	< 10
62	B-C-62-08	6.92	20.72	54'300	5'430	109	36
62	B-C-62-10	7.39	26.15	1'390'000	56'900	51	38
62	B-C-62-11	7.23	24.41	13'700	31'100	112	50
62	B-C-62-12	7.12	19.65	131'200	29'900	299	< 10
62	B-C-62-13	7.18	21.40	9'110	3'040	304	< 10

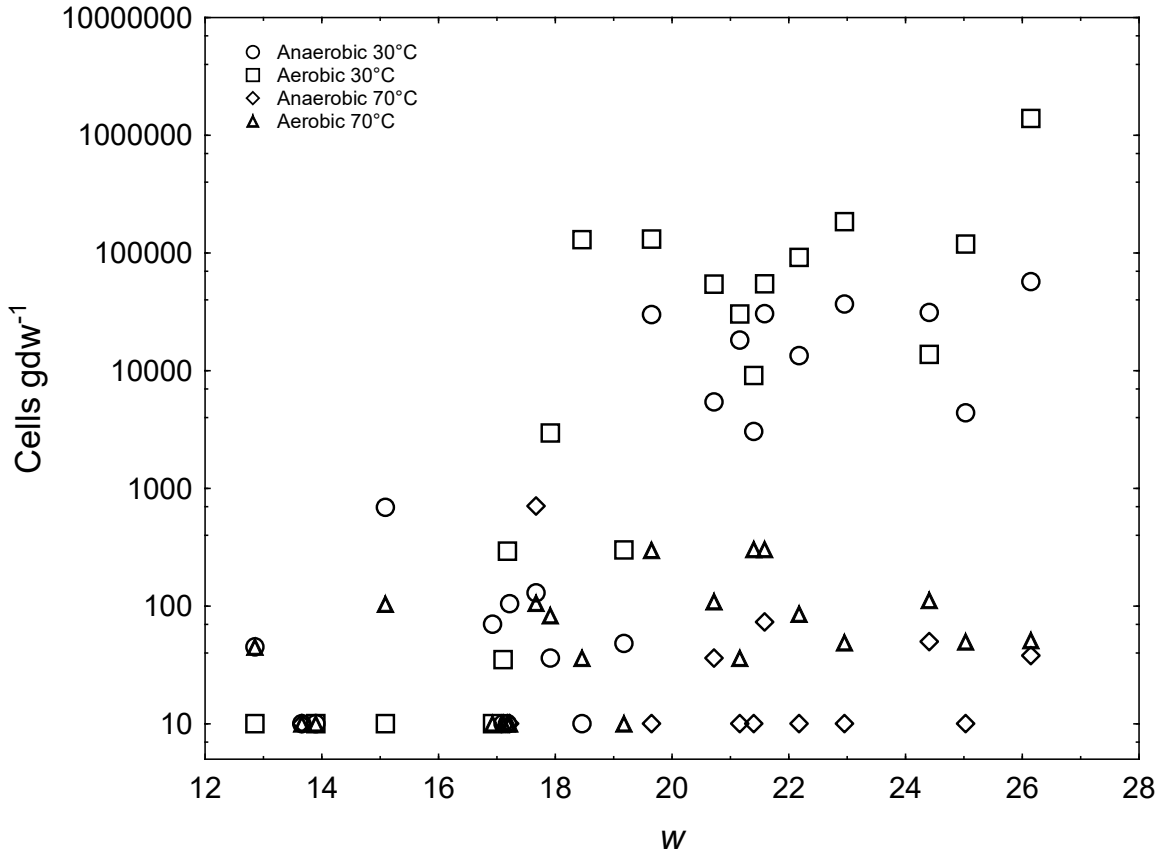

Fig. 15: Numbers of aerobic and anaerobic cells cultivated by BGR at 30 °C and 70 °C versus gravimetric water content (*w*), the ratio between the mass of water and the mass of dry solids expressed as a percentage (data from Tab. 3).

Data below detection < 10 cells gdw^{-1} are shown as 10 in the graph.

4.2 Visualisation of bacteria

Polyvalent cations are believed to play a major role in the formation of complexes between clay particles and organic matter such as bacteria (Jacobsen & Rasmussen 1992). Hence, it is often problematic to extract whole cells from soils and clays. Here, we used a method that increased the electrostatic repulsion between the clay particles and the bacterial cells (Kallmeyer et al. 2008). This method resulted in clear visual evidence of the indigenous bacteria in FEBEX bentonite core B-C-60-18 which could be observed in the microscope (Fig. 16).

4.3 ATP

A total of 1'465 amol gww^{-1} of ATP could be extracted from the 20 gww sample of core B-C-60-18 which approximately translates to 1'465 bacteria gww^{-1} assuming 1 amol per cel. The ATP result agrees well with the sum of MPN numbers (Tab. 2) which was approximately 2'000 MPN gww^{-1} in the dismantling section, since the ATP method includes all bacterial groups or species. In addition, since only living and active cells contain ATP whereas the MPN method takes into account also cells that can be dormant or inactive the somewhat lower number of active cells measured with ATP method compared to the MPN method was expected. However, it is possible that the sampled microbes were small and inactive, in that case, the ATP indicates a larger number than what was found with MPN analysis. Nevertheless, absolute

numbers are less important than the fact that ATP was found, which indicates the presence of living cells, something that is corroborated by the visualisation of cells performed on the same core (Fig. 16).

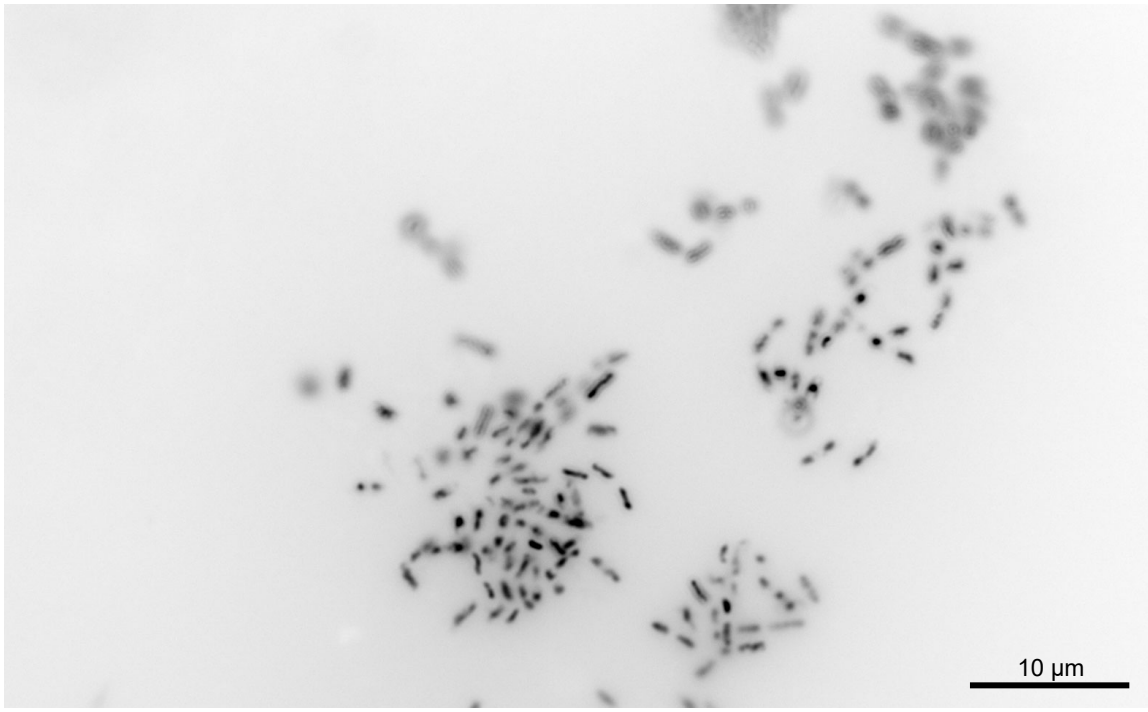

Fig. 16: Extracted cells from sample B-C-60-18 stained with DAPI in a 1'000 × magnification. Some of the cells have a blurred appearance because they are out of the focal plane of the microscope.

4.4 DNA extraction and quantification

4.4.1 From MPN tubes

DNA was extracted from MPN tubes in varying amounts ranging from half a ng per microliter to over 40 ng/µL^{-1}. The highest DNA concentrations were found within the NRB samples (Tab. 4) which correlates well with the MPN results, where a similar trend was observed (Tab. 2). Since only two MPN samples for IRB exhibited positive growth these were the only two subjected to DNA extraction from that enrichment type. All DNA extracted samples originated from Dismantling Section 60 since very few bacteria could be cultivated from the other analysed dismantling sections.

4.4.2 From raw clay (Method 1 and 2)

Method 1 and 2 resulted in 0.18 and 0.75 ng µL^{-1}, respectively, of extracted DNA from 10 gww samples. Both methods had problems with removing contaminates like proteins, phenol or carbohydrates resulting in low yields of extracted DNA. The samples were, however, analysed with ddPCR which resulted in 12 and 4 SRB gww^{-1} for Method 1 and 2, respectively. Since it is known that extracting cells from clays is a difficult task to perform (Kallmeyer et al. 2008), more samples should be analysed to increase the resolution and the statistical certainty.

However, the SRB MPN results reported cell numbers varying from 4 to 60 SRB gww^{-1} from dismantling section (#60), are in the same range as the ddPCR results, and taking into account some variability between samples the results correlate well.

Tab. 4: Extracted amounts of DNA from samples originating from MPN enrichment tubes.

Five tubes of specified dilution series with 10 mL culture each were pooled for the individual samples.

Sample	MPN enrichment	Dilution series	DNA concentration (ng/µL)
B-C-60-13	SRB	10^{-2}	3.84
B-C-60-15	SRB	10^{-0}	0.63
B-C-60-15	SRB	10^{-1}	18.22
B-C-60-16	SRB	10^{-0}	0.53
B-C-60-16	SRB	10^{-2}	1.72
B-C-60-17	SRB	10^{-0}	0.88
B-C-60-15	NRB	10^{-0}	5.82
B-C-60-15	NRB	10^{-1}	13.78
B-C-60-16	NRB	10^{-0}	12.75
B-C-60-16	NRB	10^{-2}	4.32
B-C-60-17	NRB	10^{-1}	42.52
B-C-60-17	NRB	10^{-2}	19.04
B-C-60-16	IRB	10^{-2}	1.21
B-C-60-17	IRB	10^{-0}	4.09

4.5 Illumina sequencing

The results from Illumina sequencing did not show any clear distinction in taxonomic abundance between the three different cultivation media, except the samples cultivated for SRB which had a somewhat divergent core population both at the order and genus level compared to NRB and IRB (Fig. 17 and 18). *Clostridia*, a class with the phylum *Firmicutes*, were protruding within the SRB samples. Bacteria within this group are Gram-positive obligate anaerobes and many are able to form spores, making them very tolerant to harsh environments. Many species of sulphate-reducing bacteria can also be found in the *Firmicutes* phylum which would agree well with the cultivation method, however, the taxonomic analysis of the data set have not been performed at species-level, therefore making such conclusions is only speculative. One of the NRB samples (B-C-60-15 NRB 10^{-1}) had a large group of *Corynebacteriales* which belong to the *Actinomycetes* phylum (Fig. 17). Many *Actinomycete* strains are able to convert nitrate or nitrite to nitrous oxide (N_2O) which correlates well with positive growth in NRB media tubes. The first IRB sample had a large group of *Sporolactobacillaceae* (Fig. 18). *Sporolactobacillaceae* are also part of the *Firmicutes* phylum of which the characteristics are described above. The genus *Sporolactobacillus* contains anaerobic, endospore-forming, Gram-positive, motile, rod-shaped bacteria which produce lactic acid from different kind of sugars by fermentation. Some anaerobic fermentative soil bacteria have been observed to reduce iron

(Bertrand et al. 2015) which would agree well with growth in an IRB medium. A large diversity of spore formers have been observed elsewhere as well as in MX-80 (Jalique et al. 2016).

For more information on the 20 most abundant predicted genera for each sample see Appendix 10.a.

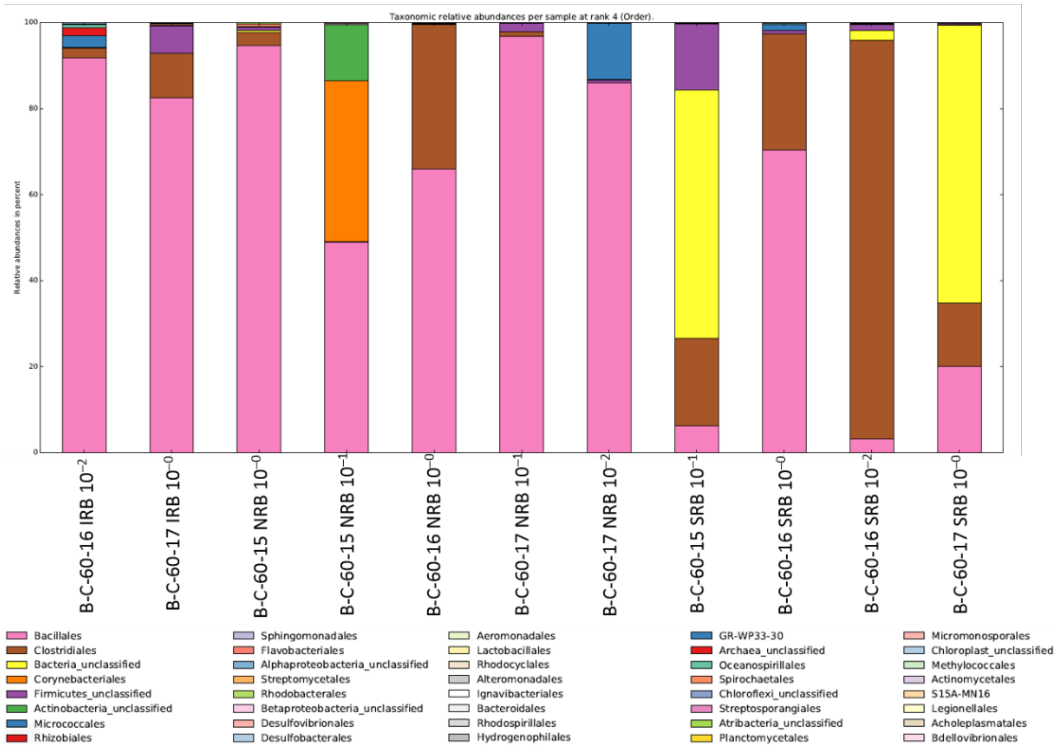

Fig. 17: Taxonomic relative abundance per sample at the rank of Order.
Colours specify orders according to legend.

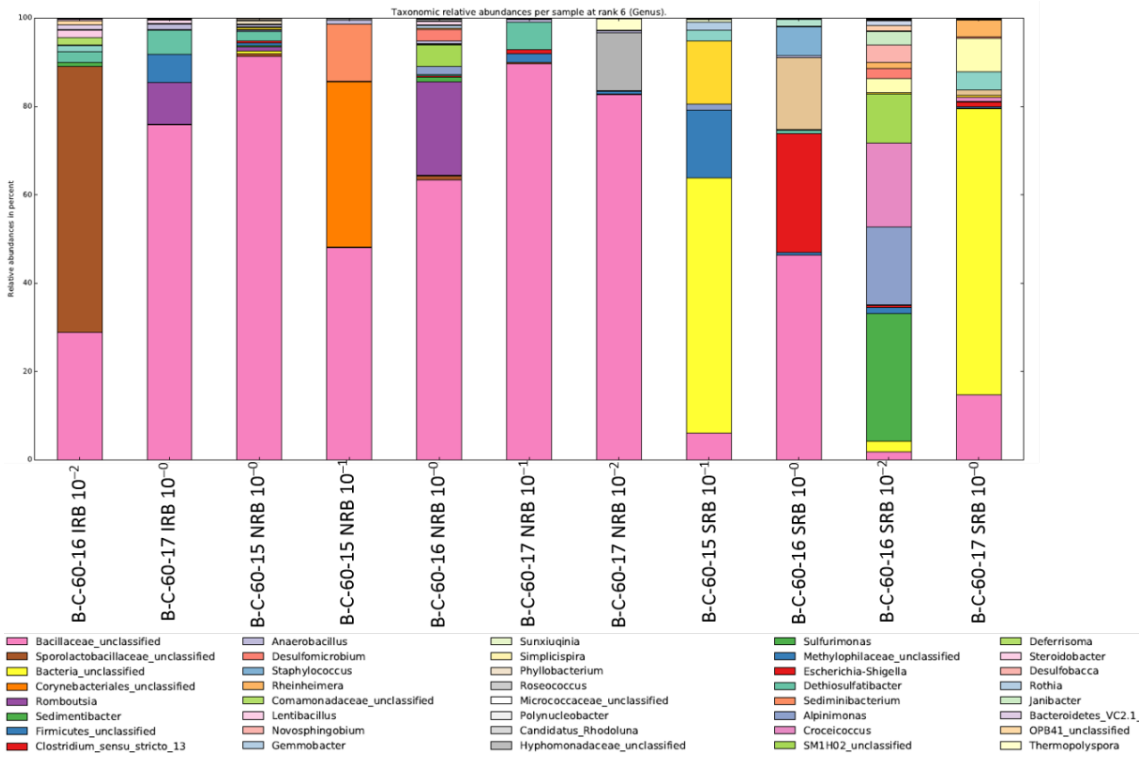

Fig. 18: Taxonomic relative abundance per sample at the rank of Genus.
Colours specify genera according to legend.

5 Discussion

5.1 Environmental conditions in FEBEX

The FEBEX clay was compacted to a dry density of 1.6 g cm^{-3} at the start of the experiment (Fuentes-Cantillana et al. 2000). During the FEBEX experiment there was a small redistribution of the dry density to an average value of 1.59 g cm^{-3}; extreme dry densities decreasing to 1.45 g cm^{-3} were found at the end of the gallery (Fig. 19 and 20). The reasons for this re-distribution are explained in further detail in Villar et al. (2016). There is an obvious inverse relationship between dry density and water content. The heat from the heater most likely caused these re-distributions by driving water towards the outer parts of the buffer, whereas water uptake occurred at the host-rock/bentonite interface. It should be noted that the data in Fig. 19 and 20 were obtained after the heater was turned off and some water may have migrated towards the heater during the cool-off period from 24th April to the beginning of August 2015. Positions closest to the heater may have been somewhat dryer than indicated on Fig. 19 and 20.

The temperature ranged from 22 °C in the gallery end up to 99 °C in the clay close to the middle of the heater (Tab. 5). Some of the pressure sensors still operative showed pressures > 5 MPa at several positions (Martínez et al. 2016).

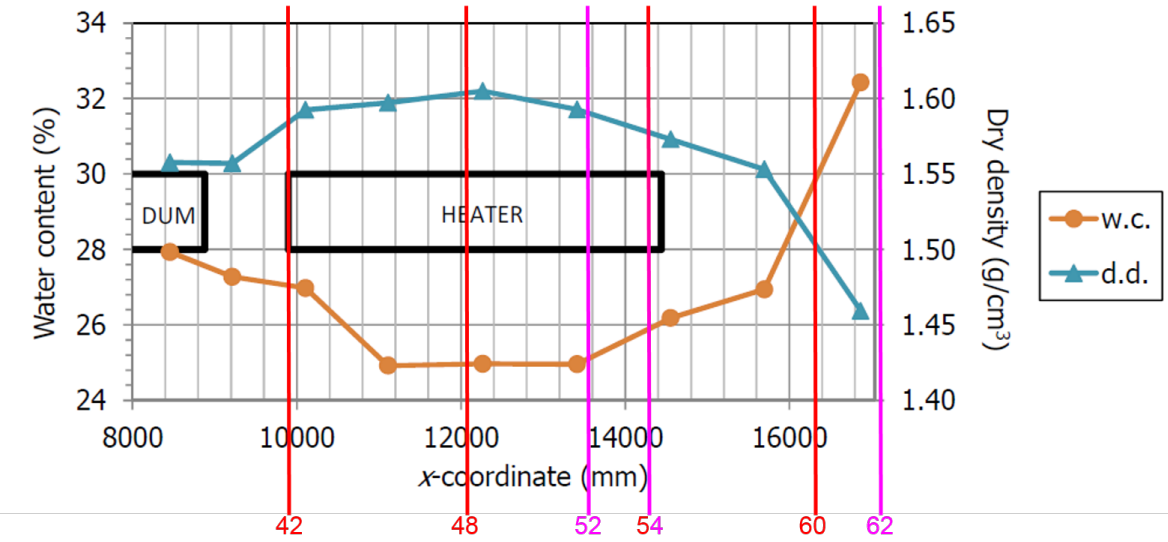

Fig. 19: Average water content (w.c.) and dry density (d.d.) for the sections sampled along the barrier as computed from the polynomial functions (see Villar et al. 2016 for details).

Sampled sections are marked with lines coloured pink for BGR and red for Micans. Sample Section 54 was analysed by Micans and BGR. Section 62 was the dismantling section at the end of the galley exhibiting the bentonite/host-rock interface.

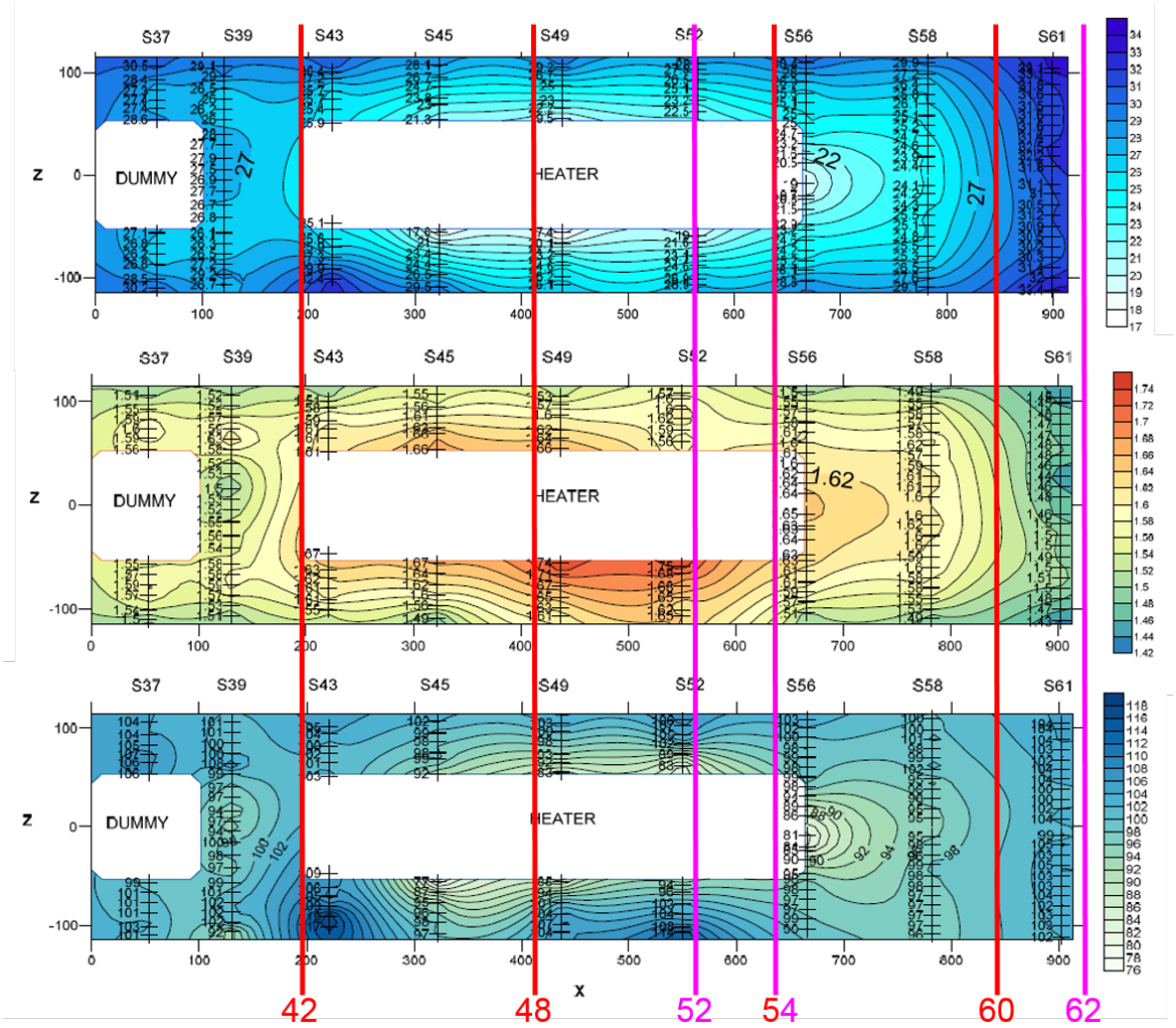

Fig. 20: Water content (top, in %), dry density (middle, in g cm−3) and calculated degree of saturation (bottom, in %) distribution in a vertical section along the gallery (see Villar et al. 2016 for details).

Sampled sections are marked with lines coloured pink for BGR and red for Micans. Section 62 was the clay next to the rock wall in the gallery.

Tab. 5: Temperature data from 24 April 2015 before the heater was turned off.

Numbers in brackets are the difference compared to the preceding recording. Table from Martínez et al. (2016).

	Section G (1 m from heater)	**Section I** (heater front)	**Section S** (1 m into heater zone)	**Section F2** (middle of heater)	**Section D2** (heater rear end)	**Section B2** (gallery end)
Outer ring	30 – 34 (0)	37 – 39 (0)	--	--	36 – 37	22 (0)
Intermediate ring	34 – 39 (0)	54 – 63 (+ 0.5)	72 (1)	75 (0)	54 – 56 (1)	20 – 22 (1)
Inner ring	36 – 41 (- 1)	84 – 87 (+ 0.5)	93 (1)	94 – 99 (+ 0.5)	83 – 88 (+ 1.5)	22 (0)

During the lifetime of a final repository for spent nuclear waste there will be several different stages. Initially it will be aerobic, unsaturated with temperatures of about 100 °C. Then the amount of oxygen will decrease gradually and some parts of the repository may start to become anaerobic. In a real repository, oxygen will eventually become depleted, the temperature of the canister will decrease, and then after a site-specific period the repository will be eventually fully saturated. However in FEBEX, fully saturated conditions were never reached and the oxygen level never dropped below 1 μmol L-1 implying that true anaerobic conditions did not prevail even after 18 years (Fernandez 2017). Thus, the results are only relevant for studying unsaturated conditions. Saturated conditions are relatively well studied, but regarding unsaturated, data are scarce which makes the findings from FEBEX interesting.

5.2 Presence, cultivability and activity of bacteria

Bacteria in bentonite clays can be present in different forms, as spores, dormant cells, metabolically active cells and as desiccated cells in dry clays. Their presence can be analysed using various cultivation methodologies and there are investigations that report a large diversity of bacteria in bentonites (Svensson et al. 2011, Masurat et al. 2010a). However, cultivation does not cover the full diversity of bacteria. There can be bacteria that for various reasons are difficult to cultivate. The use of DNA analysis can be helpful here as exemplified by Lopez-Fernandez et al. (2015) and Chi Fru & Athar (2008). The work presented in this report treats the cultivability of bacteria in different media which show the presence of cultivable bacteria. There may have been more life in the clays that did not grow in, or that was not activated by, the applied growth media. New methods are being developed for DNA extraction and analysis of life in bentonites. However, as mentioned in the introduction, DNA can remain intact long after the living cell has died (e.g. DNA in fossiles as described by Allentoft et al. 2012). Hence, a combination of cultivation, that has a very low limit of detection for cultivable cells, and DNA analysis should be performed and interpreted in parallel for the best possible result. Methods for DNA extraction and analysis of bentonites are presently being developed and tested within the MIND EU project (www.mind15.eu).

The types and rates of metabolic activity by the bacteria found in the FEBEX clays, if any, is not revealed by the cultivation results. Such investigations were out of scope of the investigation program. To study metabolic activity of bacteria in various ecosystems, including compacted bentonite clays, analysis of turnover of metabolites is commonly applied (Bengtsson & Pedersen 2016, Bengtsson et al. 2016). The presence and amount of ATP may also indicate the level of metabolic activity in microbial populations (e.g. Eydal & Pedersen 2007).

5.3 What influences cultivability of bacteria in compacted bentonites?

Bacteria can survive with very little water. Many can survive without water for long periods of time, a characteristic acquired over billions of years of evolution. Bacteria are what is often described as opportunistic, implying that they take advantage of a situation or environment when the proper circumstances present themselves. For example, a bacterial cell can lie dormant for hundreds of years as a spore, or a desiccated cell, and quickly come to life again when suitable conditions reappear (Potts 1994). This behaviour is especially important in the debate about the safety of a geological disposal, since environmental growth conditions can change during the long period of time (~ 100'000 years or more) for which the repository is designed and in which it should protect the biosphere from the encapsulated radioactive waste. A low bacterial activity and processes such as sulphide production can thus be detrimental to the long-term safety of a deep geological repository.

5.3.1 Water in FEBEX and bacterial cultivability

The results from both laboratories show very low amounts of cultivable cells from the drier part of the gallery, which was expected as bacteria need water in order to live and grow. However, in the sections further in, where temperatures are lower and the water content higher, (Sections 60 and 62) a significantly higher number of cultivable cells is found (Tab. 2 and 3). In comparison to the previous dismantling (Enresa 2000), the cell numbers reported here are similar. This can be explained by the fact that system conditions have remained relatively constant for a very long time resulting in a steady state environment. This would imply that the bacteria survive in a stationary (non-growth) phase. Their numbers do neither increase nor decrease, but the cells are cultivable and can be visualised (Fig. 16). Unhealthy or dead cells would be smaller (e.g. Watson et al. 1998) and they would not stain like the ones found in Section 60. DNA in dead cells are more densely packed than in living cells resulting in less efficient staining as the dye binds to the DNA (Hobbie et al. 1977). The detection of ATP in a sample from Section 60 suggests that the cells in Fig. 16 were active.

While there are many cultivable cells in Sections 60 and 62, fewer cells could be cultivated from Sections 42, 48, 52 and 54 (Tab. 2 and 3) which is related to the lower water content in these sections near the heater compared to Sections 60 and 62 (Fig. 20). Similar results have been found for other full-scale buffer tests (e.g., the Canadian full-scale buffer container experiment – BCE, Stroes-Gascoyne et al. 1997). Therefore, more bacteria may have been in dormant states (as spores or desiccated cells) than in the wet Sections 60 and 62. The phenomenon of drying cells for prolonged disposal is well known and commonly used in microbiology (Gherna 1994). Slow desiccation can yield higher viability after prolonged disposal, than fast desiccation (Laroche and Gervais 2003; Potts 1994) and also increases heat resistance and viability for both spores and vegetative cells (Fine and Gervais 2005). It is not known if there were desicated cells in the FEBEX clay although a large diversity of bacteria in that clay has been documented (Lopez-Fernandez et al. 2015). The absence of cultivable bacteria in the samples from around the heater may be due to the fact that they were viable but not cultivable due to effects from desiccation, or, that viable cells were killed by the absence of water, possibly in combination with a high temperature as discussed next. An example of the discrepancy between cultivable and total viable numbers of cells was previously described for a full-scale Canadian buffer experiment (Stroes-Gascoyne 2010).

5.3.2 Temperature in FEBEX and bacterial cultivability

Microorganisms belonging to the domains *Bacteria*, and *Archaea* can be active in temperatures from below 0 °C up to approximately 120 °C. This range overlaps with the temperature range expected in most high-level waste repositories, including the FEBEX experiment where the temperature ranged from 22 °C to 99 °C. Representatives of these domains have been reported in various underground environments. Geological disposal sites are generally cold to moderately warm with rock temperatures at repository depths between 15 to 20 °C, so thermophilic (i.e. heat-loving) organisms will not be common there before waste disposal. It is uncertain to what extent thermophilic *Bacteria*, and *Archaea* are present in clays, will invade from groundwater and/or multiply in a repository area in which the temperature will fall from > 80 °C to < 50 °C over the first 3'000 years. They certainly can be found active in all naturally occurring high-temperature groundwater. The consensus today is that thermophiles may appear in significant numbers in a warm repository if the conditions allow growth. The data from the BGR laboratory showed the presence of bacteria that were able to grow at 70 °C in Sections 52, 54 and 62 (Tab. 3).

5.3.3 Pressure and density in FEBEX and bacterial cultivability

It has been shown repeatedly that cultivability of SRB in compacted bentonite was negatively correlated with increasing density and swelling pressure (Masurat et al. 2010b, Pedersen 2010, Stroes-Gascoyne et al. 2010). Recent results corroborate this correlation for MX80 and Calcigel bentonites but not for Asha bentonite where the numbers of cultivable SRB were constant at densities above 1'500 kg m^{-3} (Bengtsson et al. 2016). Long-term experiments analysing cultivability of bacteria as a function of pressure and water availability in MX-80 bentonite confirmed that cultivability was supressed (Jalique et al. 2016). Based on previous and recent results, it appears safe to conclude that cultivability of SRB can be strongly reduced when the density is high and the related swelling pressure approaches 5 MPa (as in MX80 and Calcigel, but not in Asha). In FEBEX, where both the density and pressure were higher than in the work by Bengtsson et al. (2016) and Stroes-Gascoyne et al. (2010), larger numbers of bacteria could be cultivated after 18 years, including SRB. Consequently, there does not seem to exist a precise cut-off density or swelling pressure for when microorganisms in highly-compacted bentonite are completely eradicated. Therefore, cultivability and viability of bacteria in compacted bentonite clays likely depend on the combination of several variables such as density, pressure, water availability and most importantly, the type of clay.

5.4 Bacterial activity in FEBEX

After 18 years of simulation of a full-scale HLRW repository, anaerobic mesophilic bacteria were found in numbers of over fifty thousand per gram of bentonite in some core samples (Tab. 3). Specific bacterial groups important to the safety case of a HLRW repository such as SRB, NRB and IRB could be cultivated from several bentonite core samples (Tab. 2). However, this observation was mainly in dismantling sections where the water content was elevated and temperature and dry density was lower than in the hot and dry sections close to the heater (Fig. 20). As discussed above, cultivation techniques can only answer questions about bacterial viability, meaning, it provides information about how many bacteria can be cultivated, but not if they were actually active at the sample location. Hence, a method for extraction of living bacteria from the clay was developed and the extractant analysed for presence of ATP, a molecule that all living and active bacteria contain. When ATP was analysed on one of the samples in Section 60, where MPN analysis reported high bacterial numbers compared to numbers in the clay around the heater, ATP could be found in the same range as the cultivable numbers. Extracted cells from the same section were stained with a fluorescent marker and visualised under the microscope. The observed bacteria were mostly rod-shaped, and approximately 1 μm long (Fig. 16). Consequently, there were intact cells in Section 60 with significant amounts of ATP. This suggests that bacteria in the FEBEX bentonite were active. The DNA analysis may give some clarity about the taxonomic diversity of this sample.

5.4.1 Diversity of cultivable bacteria using DNA analyses

A new method for quantifying the bacterial number in a sample is ddPCR. The technique entails performing digital PCR that is based on water-oil emulsion droplet technology. A sample is fractionated into thousands of droplets, and PCR amplification of the template molecules occurs in each individual droplet. Each droplet is then analysed or read in a flow cytometer to determine the fraction of PCR-positive droplets in the original sample. The results from ddPCR can thus be compared with MPN since it provides similar information. However, different species of bacteria may have different copy numbers of the target genes for ddPCR. For best accuracy, the copy number of the target bacteria should be known, but that is often impossible when working with environmental samples with unknown bacteria. Here we used a target gene

specific for SRB which is called adenosine-5′-phosphosulphate reductase (*apsA)* which is involved in the reduction of sulphate to sulphide. Only bacteria that have this reduction capacity carry and express the *apsA* gene. However, since it is not known which species of SRB the samples contained the ratio of positive hits versus number of cells were set to 1:1. This approach is supported by literature (Friedrich 2002).

To obtain more information about which types of bacteria were cultivated in the MPN tubes, Illumina sequencing was performed. Since each of the growth medium types, SRB, NRB and IRB, were developed to enrich for the respective metabolic type of bacteria the assumption was that only genera matching those bacterial groups would come up in the bioinformatics analysis. However, the vast majority of sequences in each sample could not be classified to a certain genus. This commonly occurs if matching sequences are unavailable in the sequence libraries to which the data is compared. Consequently, there was a large fraction of sequences in the culture tubes that did not get a good hit against the sequence database (Fig. 17 and 18). Valuable conclusions could, however, still be drawn from the bioinformatics analysis (Section 3.5). The genus *Desulfosporosinus* was found in many of the cultures. This genus includes spore-forming, mesophilic sulphide-producing bacteria with the ability to grow with molecular hydrogen as source of energy, e.g. *Desulfosporosinus orientis* (Stackebrandt 2014, Stackebrandt et al. 2003). The detection of a large diversity of spore-forming genera was expected because spores will survive for a long time in dry clay until water saturation is reached. When water is available, spores can germinate to active bacteria.

5.4.2 Bacterial life in the FEBEX in-situ test over 18 years

Enresa (1998, 2000) performed initial investigations of bacterial life in the clay intended for the FEBEX test during the pre-operational phase. Approximately 10'000 SRB cells gdw^{-1} were found in hydrated test cells with intermediate temperatures (35 – 40 °C) and approximately 50 SRB gdw^{-1} were detected with intermediate heating (40 – 50 °C) with MPN analysis. In hot test cells (50 – 80 °C) numbers of SRB were below the detection limit. NRB could be found at all test cell temperatures however with strong bias towards clay without compaction and heating where the MPN of NRB was 28'000 cells gdw^{-1}. Similarly, the numbers of aerobic bacteria were very high, 100'000 cells gdw^{-1} in the clay before compaction. The numbers of different bacteria found during the pre-operational FEBEX phase corroborate the numbers found and reported here. It is obvious that bacteria in general have the ability to survive for a long time in compacted bentonite, likely as spores as discussed above.

The dismantling of the first canister, FEBEX-I took place during 2002 and the project included microbiological investigations. However, the number of samples and the attention to bacterial issues was minute. During the FEBEX-DP, the final dismantling, the microbiological program was given much more room and interest. In the corrosion report of Madina et al. (2004) extensive corrosion damage was observed on several sensors after the partial dismantling. The investigators found sulphur-rich corrosion products and 377 SRB gdw^{-1} in the bentonite covering the sensors. The SRB in combination with high humidity of the bentonite blocks housing the sensors were responsible for significant corrosion damage in those components. The rest of the dismantled sections only had slight generalized corrosion for the heater, liner and corrosion coupons. In FEBEX-DP the corrosion damage on sensors was limited to the carbon steel components anchoring the sensors to the rock and to the liner (Madina 2016). The corrosion products were suggested to be magnetite (Fe_2O_3) and siderite ($FeCO_3$) that are mainly generated under low oxygen pressure. Even if the corrosion morphology resembled that of MIC (microbial induced corrosion), bacteria could not be cultivated from the bentonite housing the sensors (Madina 2016).

6 Performance assessment

The results from both Micans and BGR show very few or no cultivable cells from the sections around and adjacent to the heater (Sections 42, 48, 52 and 54) where the dry density in general varied between 1'600 and 1'700 kg m^{-3} (measured in Section 43 and 49 and therefore assumed to be valid for the Sections 42 and 48). A significantly higher number of cultivable cells are found in the sections further in (Section 60 and 62), where temperatures were lower, the dry density slightly lower 1'400 – 1'500 kg m^{-3}) and the water content higher (Fig. 19, Tab. 2 and 3). Therefore, it can be concluded that the harsh conditions around the heater were not favourable for bacteria. H_2S release from the bentonite was not detected (Fernandez 2017) indicating that there was no bacterial sulphate-reducing activity going on in the clay. The porewater analysis further supports that there was no or only a small production of sulphide as both sulphate and methane were found in rather high quantities in the porewater and released from the bentonite respectively (Fernandez 2017). The isotope signatures further support that the methane was produced by methanogens. In addition, the low acetate levels may indicate occurrence of acetoclastic methane production. Methanogenesis is generally suppressed by sulphate reduction so extensive production of sulphide would have resulted in no biogenic methane.

For 18 years, a rather hostile environment was prevailing in the mid sections (e.g. Section 49) around the heater with high density and temperatures slightly above 100 °C. However, in the section (Section 43) closest to the first heater, temperatures were 28-40 °C during the 2nd operational phase, i.e. after excavating the first heater, meaning that temperatures in the clay were closer to the ambient conditions in the innermost sections (Sections 60 – 62). The operational temperature was measured as 23 °C in Section 61 (Villar et al. 2016). However, the densities of Section 43 were about 1'600 – 1'700 kg m^{-3} compared to 1'400 – 1'500 kg m^{-3} in Section 61. Since there was no data on density for the microbial section, we assume that the densities were similar in the adjacent sections that were sampled for microbiology. This indicates that cultivability could be affected by density.

Bengtsson et al. (2016) investigated the influence of buffer density on the sulphate reducing activity of SRB. The results showed that the sulphate reduction was below detection above a well-defined density. Similar results were reported by Stroes-Gascoyne et al. (2010). However, in the FEBEX-DP sulphide production was not studied, only the cultivability of certain microbial groups like NRB, IRB and SRB using MPN. From the MPN analyses it is not possible to conclude that the SRB were actually reducing sulphate *in situ*; we only know that they were present in the buffer and that they could be cultured. Some SRB are also able to use ferric iron as electron acceptor and may, therefore, not reduce sulphate. ATP was detected in the clay in this study indicating that there was ongoing microbial activity in the clay. However, ATP-analysis is a measure on microbial activity, showing that ATP is being produced, but not by which type of microbe.

The hypothesis, supported by the studies by both Bengtsson et al. 2016 and Stroes-Gascoyne et al. 2010, is that there is no sulphate-reducing activity as long as the buffer has a density that is above the threshold density. This value varies from clay to clay (Bengtsson et al. 2016) and lies somewhere between 1'660 and 1'950 kg m^{-3} wet density (i.e. above 1'470 kg m^{-3} dry density) for the investigated clays, MX80, Calcigel and Asha. The threshold density for sulphate reduction for the FEBEX or other clays is not possible to state without investigation of the specific clay. However, the results from the current study may indicate that there is a density limit for microbial cultivability in the FEBEX clay if it is assumed that the only thing that differs between Section 42 (43) and 60-62 is the density (temperatures were about the same). Whether

there is an actual threshold density or a gradual decrease in culturability with increased density, cannot be deduced from this study. The culturability in the FEBEX clay appears to be within the same range as the activity of SRB for the clays in the study by Bengtsson et al. (2016). If the same applies for sulphate reduction in FEBEX is still under investigation within the MIND project. From the perspective of safety assessment, not only production of sulphide is of interest, also the transport of sulphide to the canister is of interest. Recent studies by e.g. Stone et al. (2016) and Svensson et al. (2017) indicate that the clay itself has the ability to absorb sulphide to a certain degree and thereby preventing some of the sulphide to reach the canister.

In comparison to the previous dismantling (Enresa 2000) the cell numbers are similar. This can be explained by the fact that the system conditions have remained more or less constant for a very long time resulting in an environment that is very close to equilibrium. This implies that the microbes are in, what is called, a stationary phase.

The FEBEX project has confirmed the existence of viable cells after 18 years under harsh conditions found in the clay in the arid and heated parts of the gallery. However, around and adjacent to the heater, very few cultivable cells are reported and most analyses were below the detection limit, with some exceptions (Tab. 2). This indicates that a long period of heating (of at least 18 years) seems to exhaust the cells in such a way that they cannot be recovered enough to be cultivated.

From a performance-assessment perspective, the sulphide production is the process that determines corrosion of the waste container, not the presence of microbes *per se*. Thus, it is interesting to understand both what is limiting the production, and what parameters can be measured for an understanding of the system.
Measuring the number of cells (MPN) and the culturability of the microbes gives an indication of the prerequisite conditions for microbial activity. The microbial activity can be estimated by measuring the products, e.g. the change in amounts of sulphide, acetate, methane or sulphate, but then disturbing or competing factors need to be taken into account, like sulphide "uptake" in the bentonite, and other sources of methane and sulphate. Establishment of a threshold buffer density (or alternatively expressed in terms of swelling pressure) for a specific clay can be used as support for specifying requirements of a buffer or clay material used in a repository. Thus, the FEBEX microbiological investigations provide relevant and unique information, by covering the early phase (in the FEBEX case: 18 years) of a repository re-saturation cycle.

7 Conclusions

Cells could be cultivated in large numbers from moist, low temperature positions in FEBEX. The absence of cultivable bacteria in the samples from around the heater may be because they were viable but not cultivable due to effects from desiccation, or, that viable cells were killed by the absence of water, possibly in combination with a high temperature.

The types and rates of metabolic activity by the bacteria found in the FEBEX clays, if any, is not revealed by the cultivation results and such investigations were out of scope of the investigation program. To study metabolic activity of bacteria in various ecosystems, including compacted bentonite clays, analysis of turnover of metabolites is commonly applied.

The data in this report showed the presence of bacteria able to grow at 70 °C in FEBEX. Thermophiles may consequently appear in significant numbers in a warm repository if the conditions allow growth.

Despite a high dry density (1.6 g cm^{-3}) and pressure (> 5 MPa) numerous bacteria could be cultivated after 18 years. Consequently, there does not seem to exist a cut-off density or pressure below these values for which microorganisms in highly compacted bentonite are completely eradicated. Cultivability and viability of bacteria in compacted bentonite clays likely depend on several variables such as density, pressure, water availability and most importantly, the type of clay.

8 Acknowledgments

This work has been financially supported by the Full-Scale Engineered Barrier Experiment – Dismantling Project (FEBEX-DP) consortium (http://www.grimsel.com/gts-phase-vi/febex-dp). The work with visualisation received funding from the Euratom research and training program denoted microbiology in nuclear waste disposal (MIND), 2014-2018, under grant agreement No. 661880.

9 References

Abós, H. & Martínez, V. (AITEMIN) (2015): Sample Log Book 34 to 62 FEBEX-DP. AN 15-578. September 2015.

AITEMIN (2014): "Sensors Data Report, In Situ Experiment", Report Nº: 8 – Full Report, June 2014.

Allentoft, M.E., Collins, M., Harker, D., Haile, J., Oskam, C.L., Hale, M.L., Campos, P.F., Samaniego, J.A., Gilbert, M.T., Willerslev, E., Zhang, G., Scofield, R.P., Holdaway, R.N. & Bunce, M. (2012): The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. Proc Biol Sci 279, 4724 – 4733.

Bárcena, I. & García-Siñeriz, J.L. (2015a): FEBEX-DP (GTS) Full Dismantling Sampling Plan. Nagra Arbeitsbericht NAB 15-14.

Bárcena, I. & García-Siñeriz, J.L. (2015b): FEBEX-DP (GTS) Full Dismantling Test Plan. Nagra Arbeitsbericht NAB 15-15.

Bárcena, I., Fuentes-Cantillana, J.L. & García-Siñeriz, J.L. (2003): Dismantling of the Heater 1 at the FEBEX "in situ" test. Description of operations. Enresa Technical Report 9/2003.

Ben-Dov E., Brenner A. & Kushmaro A. (2007): Quantification of sulfate-reducing bacteria in industrial wastewater, by real-time polymerase chain reaction (PCR) using dsrA and apsA genes. Microbial Ecology 54:439-51.

Bengtsson A., Blom A., Hallbeck B., Heed C., Johansson L., Stalén J. & Pedersen K. (2016): Microbial sulphide-producing activity in water saturated MX80, Asha and Calcigel bentonite at wet densities from 1'400 to 2'000 kg m^{-3}, SKB Report TR-16-09, Swedish Nuclear Fuel and Waste Management Co, Stockholm, Sweden.

Bengtsson A. & Pedersen K. (2016): Microbial sulphate-reducing activity over load pressure and density in water saturated Boom Clay. Applied Clay Science, 132 – 133, 542 – 551.

Bertrand J.-C., Caumette P., Lebaron P., Matheron R., Normand P. & Sime-Ngando T. (2015): Environmental Microbiology: Fundamentals and Applications: Microbial Ecology Springer.

Castro H.F., Williams N.H. & Ogram A. (2000): Phylogeny of sulfate-reducing bacteria. FEMS Microbiology Ecology 31: 1 – 9.

Chi Fru, E. & Athar, R. (2008): In situ bacterial colonization of compacted bentonite under deep geological high-level radioactive waste repository conditions. Appl. Microbiol. Biotechnol. 79, 499 – 510.

Edgar R.C. (2013): UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10: 996 – 998.

Edgar R.C., Haas B.J., Clemente J.C., Quince C. & Knight R. (2011): UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194 – 200.

Enresa (1998): FEBEX - Full-scale engineered barriers experiment in crystaline host rock - Pre-operational stage summary report PT-01-1998.

Enresa (2000): FEBEX project - full-scale engineered bariers experiment for a deep geological repository for high level radioactive waste in crystalline host rock - final report, PT 01-2000.

Enresa (2004): FEBEX project Post-mortem Analysis: Corrosion Study. Enresa PT 08-2004.

Eydal H.S. & Pedersen K. (2007): Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield groundwater from depths of 3-1000 m. J Microbiol Methods 70: 363 – 373.

Fernandez A. (2017): FEBEXe/FEBEX-DP Gas-Water Analysis Report. Nagra Arbeitsbericht NAB 16-013.

Fine, F. & Gervais, P. 2005: Thermal destruction of dried vegetative yeast cells and dried bacterial spores in a convective hot air flow: strong influence of initial water activity. Environ. Microbiol. 7, 40 – 46.

Friedrich, M.W. (2002): Phylogenetic Analysis Reveals Multiple Lateral Transfers of Adenosine-5'-Phosphosulfate Reductase Genes among Sulfate-Reducing Micro-organisms. Journal of Bacteriology 184: 278 – 289.

Fuentes-Cantillana, J.L. & García-Siñeriz, J.L. (1998): "FEBEX Full-scale Engineered Barriers Experiment in Crystalline Host Rock. Final Design and installation of the "in situ" Test at Grimsel". Enresa Technical Report 12/98.

Fuentes-Cantillana, J.L., García-Siñeriz, J.L., Franco, J.J., Obis, J., Pérez, A.; Jullien, F.; Alberdi, J., Barcala, J.M., Campos, R., Cuevas, J., Fernández, A.M., Gamero, E., García, M., Gómez, P., Hernández, A., Illera, A., Martín, P.L., Melón, A.M., Missana, T., Ortuno, F., Pardillo, J., Rivas, P., Turrero, M.J., Villar, M.V., Mingarro, M., Pelayo, M.; Caballero, E., Cuadros, J., Huertas, F., Huertas, F.J., Jiménez de Cisneros, C., Linares, J.; Bazargan-Sabet, B., Ghoreychi, M.; Jockwer, N., Wieczorek, K.; Kickmaier, W., Marschall, P.; Martínez, M.A.; Carretero, P., Dai, Z., Delgado, J., Juncosa, R., Molinero, J., Ruiz, A., Samper, J., Vázquez, A.; Alonso, E., Carrera, J., Gens, A., García-Molina, A. J., Guimera, J., Guimaraes, L.do N., Lloret, A., Martínez, L., Olivella, S., Pintado, X., Sánchez, M.; Elorza, F.J., Borregón, J.L., Canamon, I., Rodriguez Pons-Esparver, R.; Fariña, P. & Farias, J. & Huertas, F. (2000): FEBEX full-scale engineered barriers experiment for a deep geological repository for high level radioactive waste in crystalline host rock Final Report. Enresa, Technical Report 1/2000.

Gabor E.M., Vries E.J. & Janssen D.B. (2003): Efficient recovery of environmental DNA for expression cloning by indirect extraction methods. FEMS Microbiology Ecology 44: 153 – 163.

Garcia J.L., Abós H., Martinez V., De la Rosa C., Mäder U. & Kober F. (2016): FEBEX-DP Dismantling of the heater 2 at the FEBEX "in situ" test., Nagra Arbeitsbericht NAB 16-11.

Gherna, R.L. (1994): Culture preservation, in: Gerhardt, P., Murray, R.G.E., Willis, A.W., Noel, R.K. (Eds.), Methods for General and Molecular Bacteriology. Society for Microbiology, Washington, DC, USA, pp. 278 – 292.

Greenberg A.E., Clesceri, L.S. & Eaton, A.D. (1992): Estimation of Bacterial Density, Standard methods for the examination of water and wastewater. American Public Health Association 18: 9 – 49.

Hobbie, J.E., Daley, R.J. & Jasper, S. (1977): Use of nuclepore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology 33, 1225 – 1228.

Huertas, F., Fariñas, P., Farias, J., García-Siñeriz, J.L., Villar, M.V., Fernández, A.M., Martín, P.L., Elorza, F.J, Gens, A., Sánchez, M., Lloret, A., Samper, J. & Martínez, M.A. (2006): "Full-scale Engineered Barriers Experiment. Updated Final Report 1994 – 2004" December 2006. Enresa, Technical Report 05-0/2006.

Jacobsen, C.S. & Rasmussen, O.F. (1992): Development and Application of a New Method To Extract Bacterial DNA from Soil Based on Separation of Bacteria from Soil with Cation-Exchange Resin. Applied and Environmental Microbiology 58: 2458 – 2462.

Jalique, D.R., Stroes-Gascoyne, S., Hamon, C.J., Priyanto, D.G., Kohle, C., Evenden, W.G., Wolfaardt, G.M., Grigoryan, A.A., McKelvie, J. & Korber, D.R. (2016): Culturability and diversity of microorganisms recovered from an eight-year old highly-compacted, saturated MX-80 Wyoming bentonite plug. Applied Clay Science 126, 245 – 250.

Kallmeyer, J., Smith, D.C., Spivack, A.J. & D'Hondt, S. (2008): New cell extraction procedure applied to deep subsurface sediments. Limnology and Oceanography - Methods 6, 236 – 245.

Kim J., Dong H., Seabaugh J., Newell S.W. & Eberl D.D. (2004): Role of microbes in the smectite-to-illite reaction. Science 303: 830 – 832.

Lanyon G.W. & Gaus Irina (2013): Main outcomes and review of the FEBEX In Situ Test (GTS) and Mock-Up after 15 years of operation. Nagra Arbeitsbericht NAB 13-96.

Laroche, C. & Gervais, P. (2003): Unexpected Thermal Destruction of Dried, Glass Bead-Immobilized Microorganisms as a Function of Water Activity. Appl. Environ. Microbiol. 69, 3015 – 3019.

Lopez-Fernandez M., Cherkouk A., Vilchez-Vargas R., Jauregui R., Pieper D., Boon N., Sanchez-Castro I. & Merroun M. (2015): Bacterial Diversity in Bentonites, Engineered Barrier for Deep Geological Disposal of Radioactive Wastes. Environmental Microbiology 70: 922 – 935.

Mäder, U., Detzner, K., Kober, F., Abplanalp, H., Baer, T. & Cloet, V. (2016): FEBEX-DP – Plug Overcoring and Concrete-Bentonite Interface Sampling prior to Dismantling. Nagra Arbeitsbericht NAB 16-10.

Madina, V., Azkarate, I. & Insausti, M. (2004): Corrosion of several components of the "in-situ" testperfomed in a deep geological granite disposal site. In: Proceedings of the 2nd International Workshop on Prediction Of Long Term Corrosion Behaviour in Nuclear Waste Systems, Nice, France, September 2004, European Federation of Corrosion.

Madina, V. (2016): Corrosion study of FEBEX-DP components. Nagra Arbeitsbericht NAB 16-54.

Martínez, V., Abós, H. & García-Siñeriz, J.-L. (2016): FEBEX/FEBEX-DP. Final sensors data report. Nagra Arbeitsbericht NAB 16-19.

Masurat, P., Eriksson, S. & Pedersen, K. (2010a): Evidence of indigenous sulphate-reducing bacteria in commercial Wyoming bentonite MX-80. Applied Clay Science 47, 51 – 57.

Masurat, P., Eriksson, S. & Pedersen, K. (2010b): Microbial sulphide production in compacted Wyoming bentonite MX-80 under in situ conditions relevant to a repository for high-level radioactive waste. Applied Clay Science 47, 58 – 64.

Meyer, J.L., Akerman, N.H., Proskurowski, G. & Huber, J.A. (2013): Microbiological characterization of post-eruption "snowblower" vents at Axial Seamount, Juan de Fuca Ridge. Front Microbiol 4: 153.

Nadkarni, M., Martin, F.E., Jacques, N.A. & Hunter, N. (2002): Determination of bacterial load by real-time PCR using a broad range (universal) probe and primer set. Microbiol. 148: 257 – 266.

Pedersen, K., Arlinger, J., Eriksson, S., Hallbeck, A., Hallbeck, L. & Johansson, J. (2008): Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4-450 m in Olkiluoto, Finland. The ISME journal 2: 760 – 75.

Pedersen, K. (2010): Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. J. Appl. Microbiol. 108, 1094 – 1104.

Pernthaler, J., Glockner, F.O., Schonhuber, W. & Amann, R. (2001): Fluorescence in situ hybridization (FISH) with rRNA-targeted oligonucleotide probes. Marine microbiology 30: 207 – 226.

Potts, M. (1994): Dessiccation tolerance of prokaryotes. Microbiology and Molecular biology Reviews 58:755 – 805.

Reasoner, D.J. & Geldreicher, E.E. (1985): A new medium for the enumeration and subculture of bacteria from potable water. Applied and Environmental Microbiology 49:1 – 7.

Rey, M., Bárcena, I. & García-Siñeriz, J.L. (2015): Full Dismantling Sampling Plan. Rev. 5. AITEMIN. May 2015.

Schippers, A., Neretin, L.N., Kallmeyer, J., Ferdelman, T., Cragg, B.A., Parkes R.J. & Jörgensen, B.B. (2005) Prokaryotic cells of the deep sub-seafloor biosphere identified as living bacteria. Nature 433: 861 – 864.

Selenska, S., Klingmuller, W. (1991): Direct detection of nif-gene sequences of Enterobacter agglomerans in soil. FEMS Microbiological Letters 80: 243 – 246.

Stackebrandt, E. (2014): The Emended Family Peptococcaceae and Description of the Families Desulfitobacteriaceae, Desulfotomaculaceae, and Thermincolaceae, in: E. Rosenberg, et al. (Eds.), Firmicutes and Tenericutes, Springer, Heidelberg New York Dordrecht London. pp. 285 – 289.

Stackebrandt, E., Schumann, P., Schuler, E. & Hippe, H. (2003): Reclassification of *Desulfotomaculum auripigmentum* as *Desulfosporosinus auripigmenti* corrig., comb. nov. Int J Syst Evol Microbiol 53: 1439 – 43.

Stroes-Gascoyne, S., Hamon, C.J., Maak, P. & Russell, S., 2010: The effects of the physical properties of highly compacted smectitic clay (bentonite) on the culturability of indigenous microorganisms. Applied Clay Science 47, 155 – 162.

Stroes-Gascoyne, S., Haveman, S.A., Hamon, C.J., Delaney, T., Pedersen, K., Arlinger, J., Ekendahl, S., Hallbeck, L., Jahromi, N., Dekeyser, K. & Daumas, S. (1997): Occurrence and identification of microorganisms in compacted clay-based buffer material designed for use in a nuclear fuel waste disposal vault. Can. J. Microbiol. 43, 1133 – 1146.

Svensson, D., Dueck, A., Nilsson, U., Olsson, S., Sandén, T., Lydmark, S., Jägerwall, S., Pedersen, K. & Hansen, S. (2011): Alternative buffer material. Status of the ongoing laboratory investigation of reference materials and test package 1, SKB Technical Report TR-11-06. pp. 146.

Svensson, D., Lundgren, C. & Wikberg, P. (2017): Experiments with bentonite and sulphide – results from experiments 2013 – 2016. SKB Report P-16-31, p. 26.

Takai K. & Horikoshi K. (2000): Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Applied and Environmental Microbiology 66: 5066 – 5072.

Villar, M.V., Iglesias, R.J., Abós, H., Martínez, V., de la Rosa, C. & Manchón, M.A. (2016): FEBEX-DP on-site analyses report. Nagra Arbeitsbericht NAB 16-12.

Watson, S.P., Antonio, M. & Foster, S.J. (1998): Isolation and characterisation of Staphylococcus aureus starvation-induced, stationary-phase mutants defective in survival or recovery. Microbiology 144, 3159 – 3169.

Webster, G., Newberry, C.J., Fry, J. & Weightman, A.J. (2003): Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale. Journal of Microbiological Methods 55:155 – 164.

Widdel, F. & Bak, F. (1992): Gram-negative, mesophilic sulphate-reducing bacteria, in: Balows. The prokaryotes 4: 3352 – 3378.

Appendix

a) Illumina sequencing

Tab. A-1: The 20 most abundant predicted genera in sample B-C-60-15 SRB 10^{-1}.

#	**Genera**	**Reads**
1	Bacteria_unclassified	1'024'894
2	Firmicutes_unclassified	271'028
3	Clostridiales_unclassified	255'301
4	Bacillaceae_unclassified	108'169
5	Desulfosporosinus	41'502
6	Peptococcaceae_unclassified	32'009
7	Proteiniclasticum	23'407
8	Clostridium_sensu_stricto_1	9'575
9	Hydrogenophaga	1'405
10	Gammaproteobacteria_unclassified	1'327
11	Halolactibacillus	1'144
12	unknown_unclassified	1'050
13	Sporolactobacillaceae_unclassified	878
14	Clostridiaceae_1_unclassified	806
15	Corynebacteriales_unclassified	525
16	Xenophilus	519
17	Desulfuromonadaceae_unclassified	441
18	Flavobacterium	251
19	Desulfitobacterium	204
20	Bacillales_unclassified	194

Tab. A-2: The 20 most abundant predicted genera in sample B-C-60-16 SRB 10^{-0}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	658'623
2	Clostridium_sensu_stricto_13	382'404
3	Paenibacillaceae_unclassified	231'291
4	Cohnella	93'292
5	Actinotalea	21'032
6	Bacillales_unclassified	10'227
7	Firmicutes_unclassified	9'204
8	Halolactibacillus	6'081
9	Clostridiales_unclassified	2'919
10	Hydrogenophaga	2'801
11	Gammaproteobacteria_unclassified	644
12	Bacilli_unclassified	407
13	Xenophilus	367
14	Bacteria_unclassified	316
15	Clostridiaceae_1_unclassified	312
16	Flavobacterium	277
17	Sphingorhabdus	198
18	Romboutsia	182
19	Pseudomonas	178
20	Bacillus	140

Tab. A-3: The 20 most abundant predicted genera in sample B-C-60-16 SRB 10^{-2}.

#	**Genera**	**Reads**
1	Sedimentibacter	485'333
2	Clostridium_sensu_stricto_7	317'750
3	Proteiniclasticum	296'701
4	Clostridium_sensu_stricto_3	186'760
5	Clostridium_sensu_stricto_18	66'016
6	Alkaliphilus	52'565
7	Clostridiaceae_1_unclassified	50'854
8	Bacteria_unclassified	40'101
9	Lachnoclostridium_5	39'289
10	Bacillaceae_unclassified	30'117
11	Paenibacillus	22'004
12	Gracilibacter	21'908
13	Firmicutes_unclassified	21'895
14	Lachnospiraceae_unclassified	16'228
15	Clostridium_sensu_stricto_13	8'763
16	Tissierella	4'179
17	Clostridiales_unclassified	3'283
18	Gammaproteobacteria_unclassified	1'169
19	Desulfosporosinus	1'068
20	Burkholderiales_unclassified	1'050

Tab. A-4: The 20 most abundant predicted genera in sample B-C-60-17 SRB 10^{-0}.

#	**Genera**	**Reads**
1	Bacteria_unclassified	864'730
2	Bacillaceae_unclassified	197'333
3	Alkaliphilus	101'535
4	Desulfosporosinus	53'055
5	Paenibacillus	50'320
6	Paenibacillaceae_unclassified	17'668
7	Clostridium_sensu_stricto_13	14'382
8	Clostridium_sensu_stricto_7	12'043
9	Clostridiales_unclassified	6'597
10	Firmicutes_unclassified	4'373
11	Lachnoclostridium_5	2'950
12	Peptococcaceae_unclassified	2'891
13	Mobilitalea	2'723
14	Corynebacteriales_unclassified	2'285
15	Halolactibacillus	1'824
16	Actinobacteria_unclassified	786
17	Clostridiaceae_1_unclassified	575
18	Bacillales_unclassified	440
19	Cohnella	99
20	Pseudomonas	65

Tab. A-5: The 20 most abundant predicted genera in sample B-C-60-15 NRB 10^{-0}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	2'781'354
2	Bacillales_unclassified	64'182
3	Romboutsia	27'545
4	Firmicutes_unclassified	22'616
5	Bacilli_unclassified	21'017
6	Sporolactobacillaceae_unclassified	19'148
7	Bacteria_unclassified	15'318
8	Clostridiales_unclassified	14'593
9	Halolactibacillus	13'440
10	Proteiniclasticum	12'338
11	Clostridium_sensu_stricto_13	11'477
12	Clostridia_unclassified	9'106
13	Sedimentibacter	8'670
14	Desulfosporosinus	4'678
15	Paenibacillus	4'432
16	Lachnospiraceae_unclassified	2'882
17	Peptoclostridium	2'709
18	Peptococcaceae_unclassified	1'131
19	Lachnoclostridium_5	1'080
20	Micrococcales_unclassified	1'065

Tab. A-6: The 20 most abundant predicted genera in sample B-C-60-15 NRB 10^{-1}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	1'441'091
2	Corynebacteriales_unclassified	1'122'213
3	Actinobacteria_unclassified	390'524
4	Halolactibacillus	23'397
5	Nocardioides	9'141
6	Bacteria_unclassified	2'928
7	Bacillales_unclassified	1'728
8	Hydrogenophaga	1'262
9	Gammaproteobacteria_unclassified	1'040
10	Firmicutes_unclassified	1'036
11	Sporolactobacillaceae_unclassified	983
12	Pseudomonas	793
13	Nocardiaceae_unclassified	641
14	Rhodococcus	529
15	Streptomycetaceae_unclassified	467
16	Desulfuromonadaceae_unclassified	435
17	Xenophilus	356
18	Clostridiales_unclassified	299
19	Flavobacterium	243
20	Bacilli_unclassified	227

Tab. A-7: The 20 most abundant predicted genera in sample B-C-60-16 NRB 10^{-0}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	1'504'227
2	Romboutsia	502'878
3	Clostridium_sensu_stricto_3	113'345
4	Lachnoclostridium_5	61'737
5	Proteiniclasticum	41'968
6	Sedimentibacter	24'941
7	Sporolactobacillaceae_unclassified	23'469
8	Peptostreptococcaceae_unclassified	18'173
9	Halolactibacillus	15'489
10	Peptococcaceae_unclassified	13'069
11	Bacillales_unclassified	9'015
12	Psychrobacillus	7'799
13	Paenibacillus	6'007
14	Desulfosporosinus	5'411
15	Clostridium_sensu_stricto_13	5'380
16	Clostridiales_unclassified	3'435
17	Hydrogenophaga	2'711
18	Clostridiaceae_1_unclassified	2'302
19	Firmicutes_unclassified	1'831
20	Desulfitobacterium	1'721

Tab. A-8: The 20 most abundant predicted genera in sample B-C-60-17 NRB 10^{-1}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	2'183'305
2	Bacillales_unclassified	152'704
3	Firmicutes_unclassified	46'783
4	Clostridium_sensu_stricto_13	23'341
5	Halolactibacillus	14'903
6	Sporolactobacillaceae_unclassified	6'006
7	Bacilli_unclassified	2'787
8	Ruminiclostridium_1	1'617
9	Pseudomonas	678
10	Romboutsia	653
11	Bacteria_unclassified	459
12	Lentibacillus	282
13	Bacillus	231
14	Intrasporangium	225
15	Aminobacter	195
16	Intrasporangiaceae_unclassified	155
17	Clostridiales_unclassified	154
18	Desulfosporosinus	135
19	Georgenia	103
20	Burkholderiales_unclassified	94

Tab. A-9: The 20 most abundant predicted genera in sample B-C-60-17 NRB 10^{-2}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	1'249'560
2	Intrasporangium	197'207
3	Paenisporosarcina	39'125
4	Firmicutes_unclassified	9'566
5	Halolactibacillus	8'225
6	Bacilli_unclassified	2'665
7	Bacillales_unclassified	2'189
8	Sporolactobacillaceae_unclassified	1'073
9	Bacteria_unclassified	905
10	Actinobacteria_unclassified	579
11	Intrasporangiaceae_unclassified	419
12	Romboutsia	353
13	Pseudomonas	195
14	Planococcaceae_unclassified	121
15	Clostridium_sensu_stricto_13	113
16	Clostridium_sensu_stricto_3	89
17	Burkholderiales_unclassified	61
18	Micrococcales_unclassified	51
19	Lachnoclostridium_5	45
20	Aminobacter	30

Tab. A-10. The 20 most abundant predicted genera in sample B-C-60-16 IRB 10^{-2}.

#	**Genera**	**Reads**
1	Sporolactobacillaceae_unclassified	2'789'195
2	Bacillaceae_unclassified	1'336'503
3	Bacillales_unclassified	114'133
4	Aminobacter	82'418
5	Intrasporangiaceae_unclassified	74'120
6	Desulfosporosinus	58'294
7	Georgenia	50'306
8	Sedimentibacter	39'306
9	Burkholderiales_unclassified	38'476
10	Gemmatimonadetes_unclassified	13'336
11	Peptococcaceae_unclassified	7'613
12	Halolactibacillus	6'896
13	Aeribacillus	6'063
14	Bacilli_unclassified	4'714
15	Cytophagales_unclassified	1'873
16	Actinotalea	1'413
17	Bacteria_unclassified	1'264
18	Firmicutes_unclassified	1'226
19	Tuberibacillus	1'094
20	Bacillus	1'001

Tab. A-11: The 20 most abundant predicted genera in sample B-C-60-17 IRB 10^{-0}.

#	**Genera**	**Reads**
1	Bacillaceae_unclassified	933'156
2	Romboutsia	116'314
3	Firmicutes_unclassified	78'687
4	Bacillales_unclassified	66'878
5	Halolactibacillus	15'232
6	Peptostreptococcaceae_unclassified	8'289
7	Bacilli_unclassified	2'185
8	Pseudomonas	1'880
9	Intrasporangium	883
10	Gammaproteobacteria_unclassified	794
11	Hydrogenophaga	750
12	Bacteria_unclassified	683
13	Desulfuromonadaceae_unclassified	535
14	Actinotalea	451
15	Sedimentibacter	304
16	Clostridiales_unclassified	273
17	Clostridium_sensu_stricto_13	255
18	Desulfomicrobium	237
19	Sporolactobacillaceae_unclassified	211
20	Clostridium_sensu_stricto_3	205

Jalique, D.R., Stroes-Gascoyne, S., Hamon, C.J., Priyanto, D.G., Kohle, C., Evenden, W.G., Wolfaardt, G.M., Grigoryan, A.A., McKelvie, J. & Korber, D.R. (2016): Culturability and diversity of microorganisms recovered from an eight-year old highly-compacted, saturated MX-80 Wyoming bentonite plug. Applied Clay Science 126, 245 – 250.

Kallmeyer, J., Smith, D.C., Spivack, A.J. & D'Hondt, S. (2008): New cell extraction procedure applied to deep subsurface sediments. Limnology and Oceanography - Methods 6, 236 – 245.