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Preface

Long-term experiment with compacted bentonite was initiated already in 1997 by Muurinen and his co-workers to simulate storage in both oxic and anoxic repository conditions. The experiment was planned to be finished already after ten months, but two samples were stored and dismantled in 2012. This report describes the experimental set-up and the results from microstructural, chemical, mineralogical and microbiological analyses from the sampling at the end of the experiment.

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Writers

Contents

Abstract
Tiivistelmä

List of symbols

CEC	Cation exchange capacity
DNA	Deoxyribonucleic acid
EDS	Energy dispersive X-ray spectrometry
EPMA	Electron probe micro-analyzer
FESEM	Field emission scanning electron microscopy
HLW	High level radioactive waste
IC	Ion Chromatography
ICP-OES	Inductively Coupled Plasma- Optical emission spectrometry
IL	Interlamellar
ITS	Internal transcribed spacer
IRB	Iron reducing bacteria
ITS	Internal transcribed spacer
NMR	Nuclear magnetic resonance
Non-IL	Non-interlamellar
OTU	Operational Taxonomic Units
PCR	Polymer chain reaction
PTFE	Polytetrafluoroethylene
RNA	Ribonucleic acid
SAXS	Small-angle X-ray scattering
SEM	Scanning electron microscopy
SRB	Sulphate reducing bacteria

1. Introduction

Remarkable quantities of high-level radioactive waste (mainly spent fuel) is generated in the nuclear power units in Finland (Posiva, 2012a). Geological disposal is considered at present as the best method to dispose nuclear wastes. Several counttries prefer encapsulation of high-level radioactive wastes (HLW) in iron and steel canisters surrounded by highly compacted bentonite (Kaufholf et al. 2015). In Finland, at Olkiluoto, the cast iron canister's function is mechanical support and encapsulation is performed by a copper canister (Posiva, 2012b). Corrosion of iron and copper canisters surrounding the high radioactive wastes is considered as a major risk due to release of radionuclides, which would cause their migration to the surface or into the groundwater. Steel and copper canisters can corrode through chemical or microbiological processes in oxic and anoxic conditions (Enning et al., 2012); however, most of the studies consider only chemical corrosion (Posiva 2012a). Until now corrosion, mechanisms in the geological repositories are not fully understood.

Minerological composition and structure of different bentonites is complex and vary, the major properties of bentonite being due to montmorillonite. It swells and absorbs water several times its dry mass, in open volume, while in confined space it can form very high swelling pressure. Bentonite has extremely sorptive and thixotropic properties and is mainly used in industrial applications in two forms, calcium bentonite and sodium bentonite (Hughes, 2014). Bentonites have also adsorptive properties for several organic and inorganic compounds and can trap proteins. Minerals commonly found in bentonite include e.g. aluminosilicates, oxides, carbonates, sulphides and sulphates.

Because bentonites are natural clay minerals, the source of origin affects the mineralogical properties, buffering capacity and protective actions. Kaufhold et al. (2015) recently studied the effect of compacted Na- and Ca-bentonites on iron corrosion and found that bentonites with high charged smectites were less corrosive. Other studies have focused on the interaction of bentonite and corrosion of copper (Rosborg, et al., 2005; Pedersen, 2009; King, et al., 2002). Mineralogical composition of bentonite has also important impact on corrosion processes of Fe but also Cu (Rosborg et al. 2005). Fe-rich bentonites are considered more corrosive (Osacký, 2010). Moreover, corrosion products may also alter bentonite properties such as swelling and adsorption properties desired for bentonite in the repositories.

Organic carbon compounds in bentonite serve as nutrients for microorganisms and can result to acetate formation (Bengtsson et al., 2018; Marshall et al., 2015). Marshall et al. (2015) stated that the organic compounds in bentonite originated mainly from plant-derived waxes and lignin derived phenols and were highly aromatic structures, containing very low amount of smaller molecules, which would be preferable sources of nutrients for microorganisms.

Bentonite is expected to have also protective mode of action (safety function) and prevent microbial growth due to formation of swelling pressure[1], which will be formed after bentonite has been completely wetted after filling up the repository with water (Stroes-Gascoyne, 2010; Pedersen et al., 2000). Microbiological activity in the repositories has been considered as an important risk factor, which would at worst-case cause release of radionuclides.

Microbiological research on bentonite has been mainly focusing on studies of bacteria and especially of sulphate reducing bacteria (SRBs), which may form hydrogen sulphides and induce corrosion of copper canisters in the repositories (Haveman et al., 2002; Pedersen et al., 2009). Physicochemical properties of compacted bentonite and the small pore size of the mineral structures have been expected to prevent the growth of microorganisms (Rättö and Itävaara, 2012). On the other hand, bacteria have been shown to influence several properties of bentonite, e.g. cation exchange capacity (CEC), exchangeable cations, swelling and rheological properties of clay minerals (reviewed by Mueller, 2015).

In the KBS-3 concept bentonite is compacted into confined space at high dry density of about 1,600 kg/m^3, after which total wetting absorbs about 400 kg of water per cubic meter. However, even though the total porosity of the bentonite is comparable high (over 40%), the mass transport is limited to happen by molecular diffusion only and no water flow is taking place. In order to maintain the target density, the bentonite must be in confined volume and therefore it produces swelling pressure of typically few megapascals, and the experimental systems have to withstand the swelling forces. Therefore, many experiments carried out with highly compacted bentonite are batch type in that respect, that only destructive analysis are available and depending on the system size the experimental time is typically months or longer (diffusion type transport couples the equilibration time to square of the system size).

The experimental set up studied here was initiated in 1997. The aim of the experiment waste to get an insight of long-term effect of compacted bentonite and the changes in chemistry, mineralogy and microstructure in oxic and anoxic conditions. The interaction of compacted bentonite with low saline ground-water simulant and copper cylinder was studied during 15 years of storage. The experimental arrangement consist of the compacted Na-bentonite MX-80 enclosed in a copper cylinder allowed to react through a steel sinter with an external solution outside. One of the test parcels was kept under the nitrogen atmosphere and one under normal atmospheric conditions. The experimental setup was not initially designed for microbiological studies, which were performed the end of the experiment.

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[1] The high enough swelling pressure is a kind of indicator for conditions not good for microbial activity: very small pores compared to microbial size and slow mass transport by molecular diffusion.

2. Long-term experiment with bentonite

2.1 Experimental set-up

In order to study long-term processes (physical, chemical and mineralogical) of compacted bentonite, an experimental set up was designed (Muurinen et al., 1996; Melamed and Pitkänen, 1996) to simulate both oxic and anoxic repository conditions. The experiment was started in 1997, and was planned to be finished after ten months, but two samples (both one anoxic and one oxic) were stored for later analysis, which was finally carried out 2013 after dismantling September 19th, 2012.

The material used in the long-term experiment was Volclay MX-80 bentonite, which contained 85-95 w-% montmorillonite (Table 1). The elemental composition of MX-80 bentonite is shown at the table 2.

Table 1. The composition of bentonite (Melamed and Pitkänen, 1996). Studied by XRD and optical microscopy. + = detected

Mineral	Volume-%
Montmorillonite	85-95
Illite	+
Kaolinite	+
Quartz	3-6
Pyrite	1-3
Carbonates	1-3
Feldspars	1-3
Orthopyroxene	+
Amphibole	+
Micas	+
Gypsum	+
Iron oxides	+

Diameter of the bentonite samples enclosed in copper cylinders was 24 mm and the thickness 44 mm. The MX-80 bentonite was compacted to dry density of 1.5 g/cm^3 inside the copper cylinder, which was set inside the plastic bottle containing 100 mL non-saline groundwater simulant, Allard Water (Allard et al., 1983). The bentonite samples were pre-saturated before experiment with deionized water for two weeks before changing it to Allard water. Steel sinters were assembled at the ends of the copper cylinders to enable interaction between bentonite and external solution.

The samples were incubated under the room temperature in the anoxic chamber (anoxic experiment) or under ambient atmosphere leaving the plastic bottle open (oxic experiment). The anoxic samples were bubbled with nitrogen and the oxygen

was sucked away from bentonite under vacuum before starting the experiment. The work was performed inside the anaerobic glove box and the plastic bottle was stored inside hermetic box during the experiments.

Table 2. Elemental composition of MX-80 bentonite. Analyses used atomic adsorption spectrometry, neutron activation and gravimetric analyses (Melamed and Pitkänen, 1996).

Element	Content (ppm)	Element	Content (ppm)
Si	290 500 - 291 400	Sn	85 - 98
Al	108 000 -110 000	La	47 - 50
Fe	27 500 - 31 100	Cr	34 - 35
Na	17 500 - 19 800	Ni	33 - 35
Ca	10 000 - 11 000	Ba	360 - 370
Mg	1 500 - 1 500	Rb	10 - 13
As	5.9 - 6.5	Ta	2.7 - 3.1
Sb	1.2 -1.3	U	10 - 12
Zn	100 - 115	Th	34 - 36
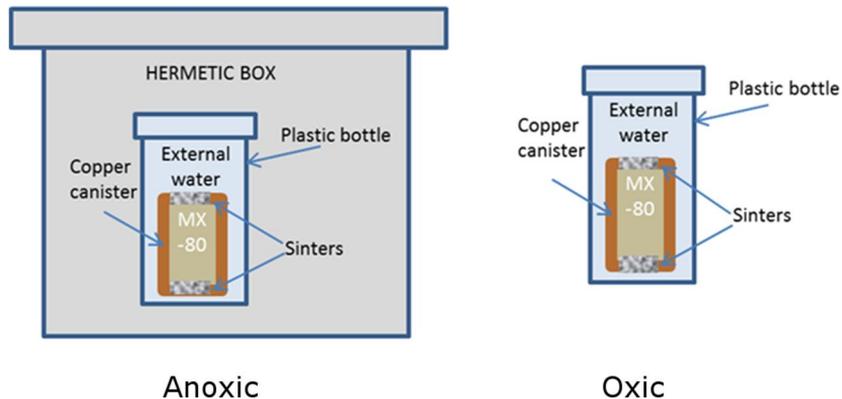
Figure 1. Experimental set-up for the long-term experiments in anoxic and oxic conditions.

2.2   Sampling

After 15 years of storage, the anoxic experiment was dismantled inside the glove box under argon atmosphere and the oxic experiment was dismantled under ambient atmosphere (Figure 2). The pH was measured immediately after finishing the experiment. The external water solution was decanted from the sample bottles, the samples from the surface of cylinder were taken and bentonite was pushed out from the cylinder. The cylindrical bentonite samples were cut into four 10 mm thick disks. The disks were cut into three pieces as a function of the distance from the copper cylinder wall (Figure 3). In addition, sample was also scrapped from copper cylinder surface for the microscopical analysis.

The samples for microbiological analyses were not originally planned when the experiment was started in 1997, and therefore estimation of the change in the microbial community during experimental time cannot be made. Sampling was performed aseptically from the copper bentonite interface and from inner parts of the bentonite as shown in Figure 3. The samples from external water solution were taken aseptically.

Figure 2. Sampling for chemistry, mineralogy, microscopy and microbiological analyses.
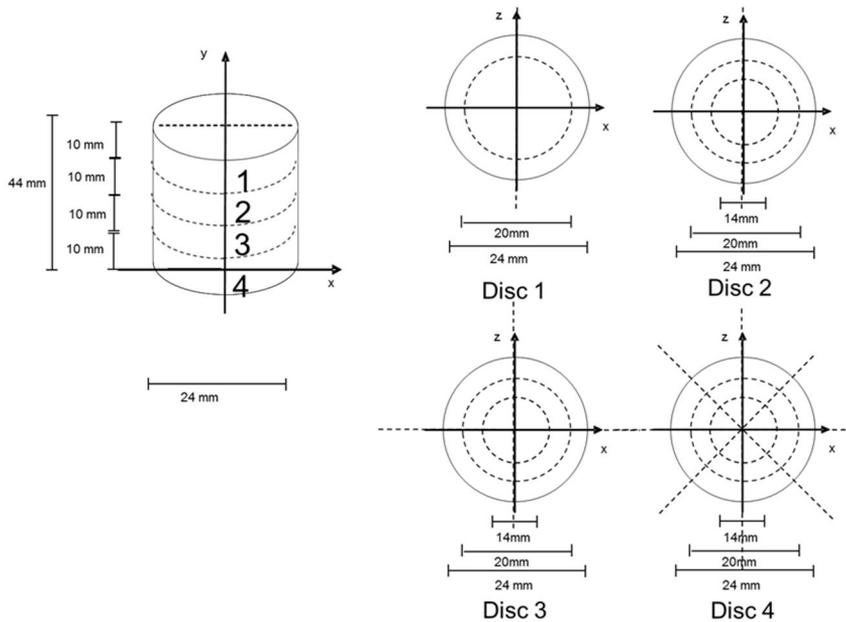
Figure 3. Schematic cutting of the bentonite samples for microstructure, chemical and microbiological analyses. The cylindrical samples were cut into four 10 mm thick disks. The discs were cut into three pieces as the function of the distance from copper wall.

2.3   Analyses

2.3.1   Microstructure

There is no direct method to determine the microstructure of compacted, water saturated swelling bentonite. Therefore, a set of three complementary methods were applied at the end of the experiment: nuclear magnetic resonance (NMR), small-angle X-ray scattering (XRD) and anion exclusion analysis.

Microstructure measurements were performed from wet bentonite samples. For NMR, cylindrical bentonite samples (0.4 cm in diameter, 1 cm in thickness) were probed from disc 2 (Figure 3) and sealed tightly in the glass NMR tubes and closed by PTFE caps. The measurement was carried out with a high-field Chemagnetics CMX Infinity 270 MHz NMR spectrometer using 1H NMR Carr-Purcell-Meiboom-Gill (CPMG) method with refocusing delay $\tau = 22$ µs. This is a spin-locking experiment measuring relaxation in the rotating frame T1ρ. Relaxation curves were analysed using XPfit software (SoftScientific). It was assumed that relaxation time of the water particles depends on the distance from the pore walls. However, during the measurement time there was enough time for some exchange between water particles

from pores of different size. This resulted in signal averaging and made interpretation of the results more difficult.

The samples for SAXS were probed perpendicularly in three different places and cut into 0.3 mm slices put in metal rings closed carefully with a polypropylene film. To reduce drying samples were prepared in a chamber with relative humidity around 80%. The X-rays were generated using a conventional sealed X-ray tube with Cu-anode. The beam was collimated and monochromated to Cu-kα radiation of wavelength 1.54 Å using Montel-multilayer mirror. The intensity of the scattered X-rays was collected using a Bruker Hi-Star area detector. Basing on the diffraction patterns average interlamellar distance was calculated. Combined with specific surface area of the bentonite it was used to calculate maximum volume of interlamellar pores (Matusewicz et al., 2013).

Anion concentration per gram of dry bentonite was used to estimate the amount of water in large pore structures. It is assumed that during the time of the experiment sample reached equilibrium with the external solution, and that anions are not present in volume close to negatively charged clay surface (like in the interlamellar pore) due to electrostatic interaction (Kozaki et al., 2001). The volume of the pore space accessible for the anions was calculated to have ion concentration equal to this of the external solution. Remaining pore volume was assumed inaccessible for anions. In the calculations, the Cl^- ions were used.

2.3.2   Chemical analyses

The external water samples were analysed for the amounts of sodium, potassium, calcium, magnesium, copper and iron by ICP-OES (Inductively Coupled Plasma-Optical emission spectrometry, at Labtium Oy), sulphate and chloride by IC (Ion Chromatography, at Labtium Oy), pH and sulphide by electrodes, and bicarbonate by titration. The water samples were kept in airtight syringes before bicarbonate, pH and sulphide determination, and measurements were carried out inside an anaerobic argon atmosphere (CO_2 content below 0.04 ppm). The cations on the surface of plastic bottle were analysed by stripping. 20 mL of 1 M HNO_3 was poured to the plastic bottles and left into shaker for 1 week. The estimated uncertainties for IC and ICP-OES analyses were ±20% and ±10%, respectively.

Water content of the bentonite was determined by gravimetric measurement of weight loss (105°C, 24h). The bentonite samples were dried overnight at 105°C for chemical analyses. The cation exchange capacity CEC of the bentonite was determined by Cu(II)-triethylenetetramine method (Meier and Kahr 1999; Amman et al., 2005) from the disc 2 (Figure 3). The exchangeable cations were exchanged with a solution containing NH_4Cl in 80% ethanol. Then the solution was evaporated and the solids dissolved into water and Fe, Ca, K, Mg and Na ions were analysed by ICP-OES. Aqueous leachates were prepared by dispersing the bentonite sample in deionized water (4.0 g/400 mL) and allowed to dissolve for a week. Then the phases were separated by centrifuging, the solution phase was ultra-filtered, concentrated by evaporation and analysed with IC. The total amount of carbon and sulphur were

determined by combustion (up to 1400°C) using ELTRA CS 2000 device, with the detection limit of 0.05 %. The uncertainty of the carbonate analysis was ±25 % according to Labtium. The pH and Cl electrodes were manufactured, samples prepared and pH and Cl measured in samples according to Muurinen and Carlsson (2008) and Muurinen and Järvinen (2013). The pH in the bentonite pore water was measured with an IrOx electrode and Cl with AgCl electrode in a sample of 2 cm in diameter and 1 cm in height. Electrodes were calibrated before and after the measurement. The more detailed description of analyses and measurements can be found in Karnland (2009), Kumpulainen and Kiviranta (2010) and Muurinen (2010).

2.3.3   Mineralogy

The bentonite samples were dried overnight at 105°C for mineralogical analysis. Sample mounts for mineralogical analysis were made from the bentonite disc (4) of oxic and anoxic samples (Figure 3) by impregnating dried sample pieces into epoxy (15:2, EpoFix Resin: EpoFix hardener). Mounts were polished with diamond grinding plate (K1200) using alcohol based lubricants (DP Lubricant brown and DP Suspension A) and coated with carbon before electron optical analyses made at Geological Survey of Finland (GTK).

Scanning electron microscope (SEM) was used to identify the accessory mineral phases and to estimate the amounts of different mineral phases. Analyses were made in high vacuum with JEOL JSM 5900 LV, which was attached to an energy dispersive spectrometer (EDS) by Oxford Instruments controlled by INCA Feature software.

Electron probe micro-analyser (EPMA) was used for quantitative analyses from mineral particles. Analyses were made with Cameca SX100 instrument with five wavelength dispersive spectrometers (WDS) and one energy dispersive spectrometer (EDS).

2.3.4   Live/dead staining of microbiological samples

To visualize the microbial community, 4.5 mL of external water sample obtained from both oxic and anoxic copper cylinder was stained using Live/dead -staining kit (Molecular Probes) for 10 minutes at dark for microscopy. The Live/dead –staining kit contains two dyes: membrane permeable SYTO 9 labels all bacteria with green fluorescence and membrane impermeable propidium iodide labels membrane-compromised bacteria with red fluorescence. The stained water samples were filtered on membrane and examined with an Axio Imager.M2 epifluorescence microscope and using a 63✗/1.4 or 100✗/1.40 Oil Plan-Apochromat objective lens. The images were obtained by recording signal from two channels (λ_{ex}=470±40, λ_{em}=525±50 for Syto9 and λ_{ex}=550±25, λ_{em}=605±70 for PI). Images were captured using AxioCam MRm camera and AxioVision 4.8.2 software (Carl Zeiss Oy).

2.3.5   FESEM analysis of microbiological samples

Water samples of 4.5 mL were filtered on membrane prior to dehydration and preparation for field emission scanning electron microscopy (FESEM). The samples were fixed in phosphate (0.1 M, pH 7.2) buffered with 2.5% glutaraldehyde at +4°C for 2 to 3 h, and rinsed with phosphate buffer three times. Dehydration was carried out with an ethanol series from 30% to 50% to 70% to 80% to 96% and absolute, followed by hexamethyldisilazane. The samples were coated with Au/Pd and examined with Hitachi S-4800 FESEM operated at 1 kV.

2.3.6   Microbial community analysis

The composition of bacterial and fungal communities was studied from the surrounding external water samples, from bentonite (Figure. 3, Disc 2, outermost part) and from bentonite-copper interface of the copper cylinders. The composition of bacterial community was assessed by sequencing the universal small subunit ribosomal RNA gene, which is also known as the 16S rRNA gene in archaea and bacteria. The fungal internal transcribed spacer (ITS) gene markers were used to examine the total fungal diversity in samples.

   7.5 mL of external water surrounding the cylinders was filtrated on 0.2 µm polyethersulfone filters to concentrate microbial biomass. DNA extractions from bentonite were performed by using 0.9 g sample, which was diluted into water. The bentonite was frozen immediately on dry ice and stored at - 80°C until nucleic acid isolation. Biofilm on the surface of copper was scraped with sterile scalpels, and diluted in sterile phosphate-buffered saline solution and subsequently filtered on polyethersulfone-filters of 0.2 µm pore size and filters were maintained at - 80°C until DNA extraction.

   The DNA was extracted from water, bentonite and biofilm samples using ZR Soil Microbe DNA MidiPrep (Zymo Research) in accordance with the manufacturer's protocol. After extraction, the DNA was eluted in 50 µL elution buffer supplied by the manufacturer. Negative DNA extraction controls were included in the DNA extractions.

   The amplification libraries for high throughput sequencing on the Ion Torrent PGM platform were prepared by PCR from the DNA samples. Bacterial 16S genes were amplified with primers S-D-Bact-0341-b-S-17/S-D-Bact-0785-a-A-21 (Herlemann et al., 2011), targeting the variable region V3-V4 of the 16S rDNA gene, and fungal internal transcribed spacer (ITS) gene markers with primer pair ITS1 and 58A2R targeting the fungal ITS1 region (Buée et al., 2009).

   PCR amplification was performed in parallel reactions for every sample containing 1× MyTaqTM Red Mix (Bioline, London, U.K.), 20 pmol of each primer, 2 µL of template and nuclease-free water to a final reaction volume of 25 µL. The PCR program consisted of an initial denaturation step at 95 °C for 3 min and 35 cycles for bacteria and fungi. A final elongation step of 30 s was performed at 72 °C. Correct size of the PCR products was verified with agarose gel electrophoresis. Parallel

amplicon libraries were combined and sent to Bioser, University of Oulu (Finland) for sequencing on the Ion Torrent PGM sequencer (Thermo Fisher Scientific) using the 314 and 316 Chip Kit v2 with the Ion PGM Template IA 500 and Ion PGM Hi-Q sequencing kits. The amplicons were filtered for correct size (200 – 600 bp) and purified prior to sequencing.

The sequence reads obtained from Ion Torrent sequencing were subjected to sequence analysis using the Mothur software version 1.39.5 (Schloss et al., 2009) using the standard operating protocol by Kozich et al. (2013) with some modifications. In quality control, a minimum sequence length of 250 bp was used. A maximum primer mismatch of two nucleotides (nt), maximum homopolymer stretches of 8 nt and quality score 25 was used. Adapters, barcodes, and primers were removed from the sequence reads, and chimeric sequence reads were removed from the data set with the VSEARCH algorithm (Rognes et al., 2016) by de novo detection and through similarity searches against the SILVA reference dataset (version 128) (Quast et al., 2013) with bacterial sequences, and UNITE reference dataset (version 7.2 2017-06-28) (Kõljalg et al., 2013) with fungal sequences.

Differing from the standard operating procedure developed by Kozich et al. (2013), no alignment for the fungal sequences was done but instead, a column-formatted distance matrix was calculated. The sequences were grouped into Operational Taxonomic Units (OTUs), following Mothur OTU-picking protocol using average neighbour algorithm to cluster sequence reads at 97% sequence similarity. Taxonomy from the domain- to species-level was assigned to OTUs via representative OTU sequences with the Wang algorithm at minimum confidence threshold of 80% (Wang et al., 2007) using SILVA database for the bacterial sequences and UNITE for the fungal sequences. Sequence reads obtaining no taxonomical assignments in the analyses and OTUs that were also found from the negative control sample were excluded from the datasets.

3. Results and discussion

3.1 Structural changes in bentonite

Structural changes in bentonite were studied using NMR, SAXS and anion exclusion analysis. The diffraction peaks of all samples in SAXS analysis were located close to 18.5 Å. This distance corresponds to three water layers in the interlamellar space. All the samples have a small peak corresponding to four water layers. No clear difference was seen between the bentonite samples stored in anoxic and oxic conditions. Samples were homogenous and no preferred orientation of the montmorillonite layers was observed.

The NMR relaxation curves do not show significant variation between samples. The results of the triplicate samples are scattered within the error margin, the average values for oxic and anoxic conditions are very close.

Chloride anions were applied in exclusion calculations. The values of interlamellar porosity calculated basing on the chloride concentration are comparable for the oxic and anoxic samples. The values of the proportion of the interlamellar and non-interlamellar pores are presented in Table 3.

Table 3. Results of the microstructural investigation: proportion of interlamellar and non-interlamellar porosity (partly from Matusewicz et al, 2017). IL =interlamellar and non-IL = non-interlamellar

	SAXS		NMR		Anion exclusion	
	Oxic	Anoxic	Oxic	Anoxic	Oxic	Anoxic
IL	82.9%	79.8%	92.3%	93.4%	64%	61%
non-IL	17.1%	20.2%	7.7%	6.6%	36%	39%

There were no big differences between porosity values of oxic and anoxic sample. Each method bases on a different measurement principle to estimate interlamellar and non-interlamellar porosity. Result from SAXS was literally the closest to the interlamellar porosity, as it is the volume of the periodic slit-like pores. The value of the interlamellar porosity obtained by NMR was the highest among the methods used as it measures the average distance of the water particles to the clay surface, hence including also volume close to not-stacked clay layers. Finally, anion exclusion thresholds total porosity as chloride accessible/not accessible, which was affected by salinity. In general, despite of the high porosity of the material it had very fine pore structure, with most of its pore volume being one-nanometer high slit-like pores.

Microstructural studies did not show significant differences between anaerobic and aerobic samples. Obtained results were comparable to those found in the literature (Matusewicz et al., 2016)

3.2 Mineralogical changes in bentonite

Some differences were noticed in the mineralogical composition between the two test series at the end of the experiment. Samples kept in oxic environment contained newborn secondary mineral phases. Especially bentonite samples taken from the middle part of the copper cylinder showed that after 15 years experiment two different secondary copper minerals, cuprite (Cu_2O) and malachite ($Cu_2(CO_3)(OH)_2$) had formed (Figure 4). This kind of copper mineral formation was not observed in anoxic environment.

The copper mobilization was also detected in the bentonite matrix. In the samples taken from oxic environment, there were reasonably high amounts of copper in the bentonite matrix. The copper content in clay matrix in the middle part of the copper cylinder varied from 1000 to 10000 ppm depending on the measurement distance of the copper minerals. Average value measured from the bentonite matrix away the range of copper minerals was 2200 ppm. In two reference points in bentonite matrix, taken from anoxic environment, the copper content was low (163 ppm) and below the detection limit (97 ppm).

Smectite (montmorillonite) content was over 95 weight-% in both bentonite sample. Most common accessories were quartz and feldspars together with carbonates and sulphides, iron oxides and sulphate minerals were common.

In sample taken from the oxic environment, especially in the bentonite sample collected from the middle part of the copper cylinder, where most of mineralogical changes happened compared to anoxic samples, identified accessory minerals were cuprite, malachite, quartz (SiO_2), plagioclase ($Na(AlSi_3O_8)$–$Ca(AlSi_3O_8)$), K-feldspar $(K,Na)(AlSi_3O_8)$, biotite $(K(Mg,Fe)_3(Al,Fe)Si_3O_{10}(OH,F)_2)$, apatite $(Ca_5(PO_4)_3(OH,F,Cl)$, ilmenite ($FeTiO_3$), barite ($BaSO_4$), calcite ($CaCO_3$), siderite ($FeCO_3$), pyrite (FeS_2) and sphalerite ($ZnFeS$). The mineralogy was similar when moving towards the copper cylinder wall, but less copper minerals were identified. Only cuprite was identified near the copper wall. Siderite is rather common accessory mineral, but calcite is rare and goethite ($Fe^{3+}O(OH)$) was missing in samples containing secondary copper minerals. Calcite together with goethite grains were clearly altered leached out-looking. Siderite and apatite grains had no marks of similar alteration.

The availability of copper was also seen in pyrite composition. Copper has partly replaced iron in pyrite lattice. All the samples had pure pyrites (FeS_2), but copper-rich pyrites were also detected. In some cases new Cu-Fe-S-phases were formed. In addition, pyrite with bornite (Cu_5FeS_4) rims was observed. Pyrites appear rarely as euhedral grains. They usually appear as small (5 µm) roundish grains, "pyrite balls", but also as large round grains (diameter over 100 µm).

Anoxic bentonite samples did not contain cuprite or malachite. Copper-rich pyrites were not identified in the middle part of the cylinder, but their amount increased towards the copper wall. In addition, calcite, goethite and apatite were more common near the copper cylinder wall. It is likely that part of these minerals have been leached out from the middle part of the cylinder.

Mica in the bentonite samples was biotite and no transformation of smectite into illite was observed. Minor amount of illite was identified in SEM-Feature analyses and confirmed with EPMA. Illite grains occurred as single grains and are not formed due the alteration of smectite.
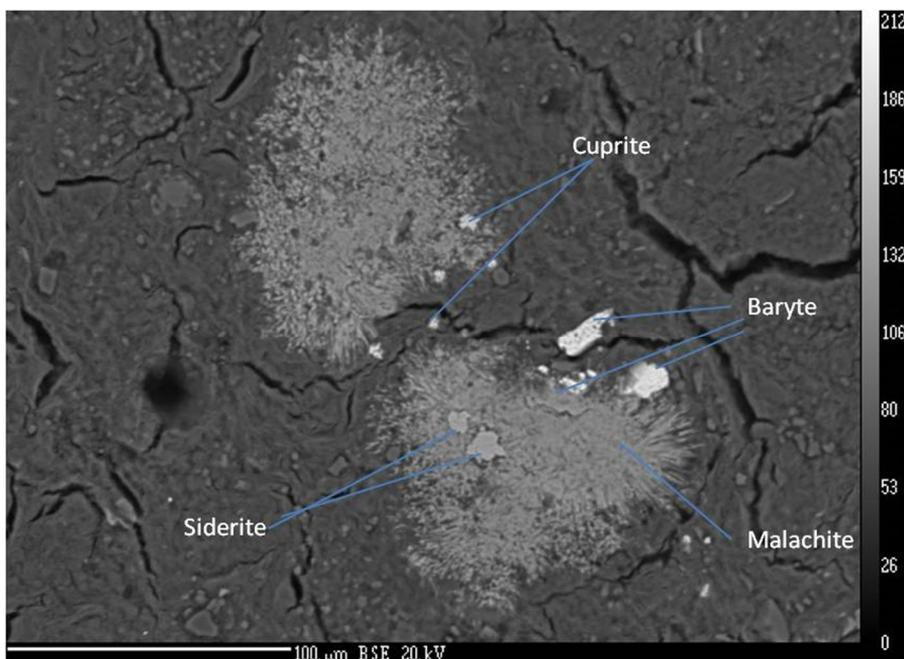

Figure 4. Secondary copper minerals in the bentonite sample taken from the middle of the copper cylinder kept in oxic conditions.

3.3   Elemental concentrations and contents

The pH, and cation and anion concentrations are given in the Table 4 in initial conditions and after 10 months and 15 years. The external water pH values were 9.1 and 9.5 in anoxic and oxic experiments, respectively. pH returned to its initial value in anoxic conditions, while in oxic conditions the pH has increased one unit. The most prominent composition change in external water is due to increase of Na_2SO_4, which is typically observed with MX-80 bentonite: gypsum is dissolved and calcium exchanges sodium as charge compensating cation, and sodium and sulphate start to diffuse out of the bentonite. These processes have mainly taken place during the first 10 months; the concentration of the sulphate was slightly greater in the oxic experiments. Similar processes are caused by dissolution of calcite, which cause

the increase of bicarbonate concentration. Some trace amount of copper was observed in external solution.

Table 4. Chemical conditions at the beginning of the experiment, after 10 months and at the end of the experiment. Values after in the beginning are from the report by Muurinen et al. (1996).

mg/L	Anoxic			Oxic		
	0	10 months	15 years	0	10 months	15 years
pH	9.2	8.5	9.1	8.4	9.5	9.5
Ca^{2+}	20.0	1.0	5.4	21.0	1.0	3.8
Na^+	57	890	970	57	968	1 200
Mg^{2+}	4.0	1.0	2.9	4.0	2.0	4.7
K^+	4.0	11.0	11.0	4.0	13.0	13.0
Cu^{2+}	0.01	0.13	0.19	0.01	0.27	0.82
HCO_3^-	116	659	970	128	428	950
Cl^-	57	88	83	57	106	74
SO_4^{2-}	10	620	1 200	10	725	1 400

3.4   Composition of bentonite

The water content (fraction of mass of water per mass of dry bentonite) of the bentonite samples were analysed from disk 4 (three different samples with increasing distance from the copper cylinder wall, Figure 3). The bentonite samples taken from both anoxic and oxic experiments had the same average water content of 0.31, but in anoxic sample the variation was larger (lower water content closer to the copper cylinder wall). The dry densities of the samples were calculated from water content to be 1.49 g/cm^3 for both conditions.

For anoxic and oxic conditions the CEC of the bentonite was measured to be 0.924 and 0.925 eq/kg (of oven-dry bentonite), respectively. The relative equivalent fractions in anoxic sample for Na, Ca, Mg, K and Cu were 0.63, 0.22, 0.13, 0.02 and 0.02, respectively, while in oxic conditions 0.65, 0.17, 0.14, 0.02 and 0.02. Copper showed clear increase in equivalent fraction closer to the copper wall in both cases: from 10 mm distance, to next to the copper surface the fractions increased from 0 to 0.004 in anoxic sample and from 0.003 to 0.026 in the oxic one. The exchangeable cation was mostly sodium, with calcium, magnesium and potassium present. The fraction of sodium, copper and magnesium where greater in oxic experiments, whereas fraction of calcium where greater in anoxic experiments in the cation exchange places. Iron was not found as an exchangeable cation from these experiments.

3.5   Microbial activity and diversity

3.5.1   Imaging of microbes

Microorganisms of the water samples were detected by Epifluorescence micros-copy (Figure 5) and by FESEM (Figures 6, 7, 8). LIVE/Dead staining and epifluo-rescence microscopy revealed living cells in the water samples. Fungal conidia were detected in the oxic water samples. In addition, some fungal hyphae were present. In the anoxic water bacterial spore forming microorganisms were observed (Figure 5D) as well as filamentous bacteria (Figure 5C). However, the number of microor-ganisms in this sample was low. The putative bacteria were showing signal only on green channel suggesting that these microorganisms had intact plasma membrane. The bacteria in water sample from oxic experiment had poor fluorescence, which might suggest that these organisms were not in active stage. In both water samples inorganic substance fluorescing on both channels was observed.
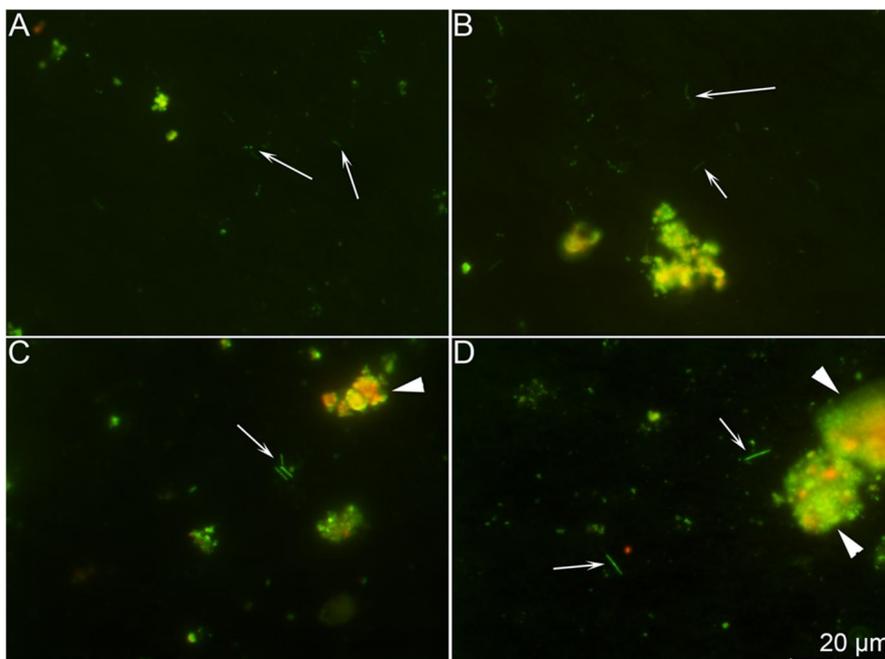

Figure 5. Epifluorescence micrographs of water samples from oxic (Panels A and B) and anoxic (Panels C and D) experiments filtered on membrane and stained using Live/dead -staining kit. In the water from the experiment incubated in oxic conditions, number of putative bacteria fluorescing poorly green (stained with Syto9) were observed (white arrows). In the water from the experiment incubated in anoxic conditions long, rod-like bacteria fluorescing green were observed. In both water samples lot of inorganic looking substance fluorescing green and red were observed (white arrowheads).

FESEM analysis revealed that water samples contained substances resembling bentonite as well as other substances with inorganic appearance (Figures 6,7,8). In some areas, filter surface was heavily covered with these substances. However, microorganisms were observed in waters from both oxic and anoxic experiment. In the water collected from the cylinder incubated in oxic conditions, number of microorganisms with appearance of mould conidia were observed (Figure 6 A-D). In addition, some fungal hyphae were present. In the water collected from the cylinder incubated in anoxic conditions, some bacterial spore looking microorganisms (Figure 7C) as well as filamentous bacteria (Figure 7D) were observed. However, the number of microorganisms in this sample was low.
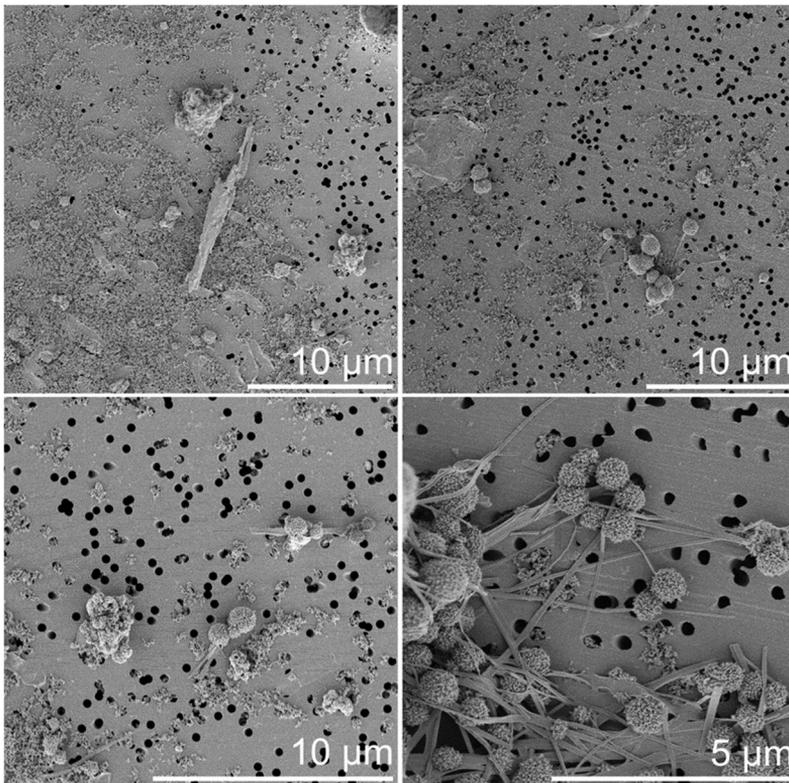

Figure 6. Scanning electron micrographs of water sample from oxic experiment filtered on membrane.
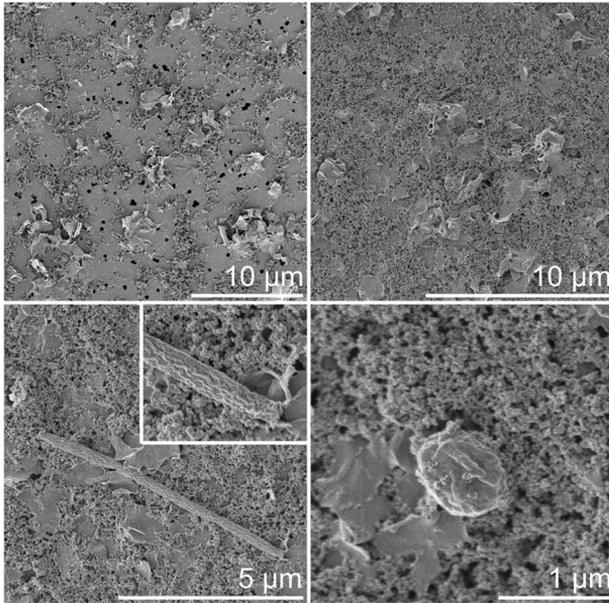
Figure 7. Scanning electron micrographs of water from anoxic experiment filtered on membrane.
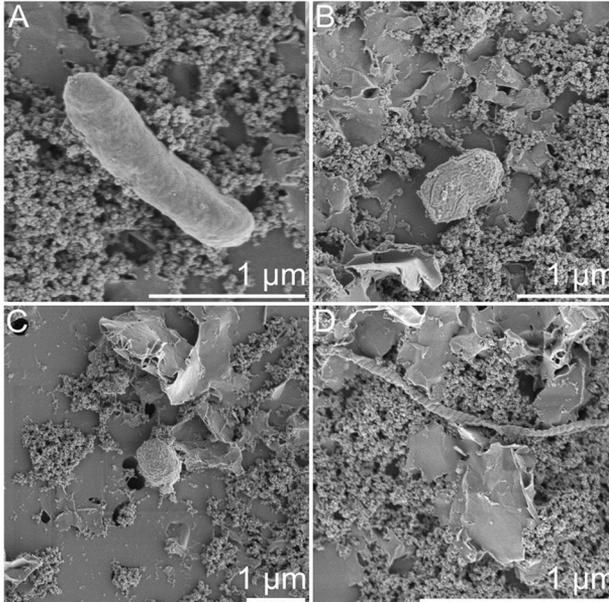

Figure 8. Scanning electron micrographs of water from anoxic experiment filtered on membrane.

A lot of bentonite was accumulated on the top of the sample that was scrapped from the copper cylinder surface (Cu-bentonite interface) and studied with FESEM (Figure 9). No microorganisms were observed in the sample but the accumulated bentonite might have prevented observing the microorganisms.
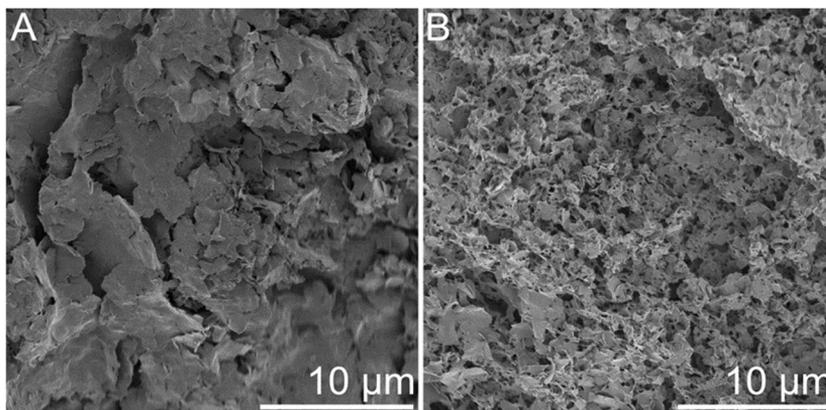

Figure 9. Scanning electron micrographs of the sample scrapped from Cu cylinder surface from the oxic experiment.

3.5.2   Microbial community structure

At the end of experiment, microbial DNA was extracted from water, bentonite and from biofilm on copper surface of the cylinder and used for analysis of bacterial and fungal community. DNA represents total microbial biomass including living, dormant and dead microbial cells but it does not give any information about the viability of the microbes. RNA would describe the active part of the microbial population but the analysed samples did not contain enough RNA for successful extraction. Nucleic acids tend to bind tightly to clay particles, which could be one reason for low RNA yield during extraction.

Although the presence of active microbes in bentonite and on copper surface could not be demonstrated, spore-forming bacteria have been shown to survive in compacted bentonite as dormant spores and are able to resume a metabolically active state after decompaction (Stroes-Gascoyne et al., 2011).

According to the sequencing results, the bacterial community in the water, bentonite and copper surface consisted mainly of Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Flavobacteria and Actinobacteria (Figure 10). No big differences between oxic and anoxic conditions were detected. In addition, bacterial community structure in water and copper surface was quite similar when assessed in class-level. The bacterial community in bentonite differed to some extent from

microbial community in water and copper surface. Because the experiment was not originally planned for microbiological studies, no samples were taken in the beginning of the experiment. Therefore, information of the changes in microbial communities as a function of time could not be achieved.

Sulphate reducing bacteria (SRB) are one of the main concerns for the safety case of a geological disposal because sulphide formed by SRBs is a corrosive agent for copper cylinders. SRBs consist of a diverse, distantly related assembly of Bacteria and Archaea characterized by the use of sulphate as a terminal electron acceptor during anoxic respiration (Muyzer and Stams, 2008). SRBs have been shown to grow in various extreme environmental conditions including high pressure (Fichtel et al., 2015) and they also contain several spore-forming gram-positive species (Castro et al., 2000). Sequencing analysis revealed several potential SRBs in bentonite samples including families of *Desulfobacteraceae* and *Desulfofurellaceae*. Copper and water samples contained SRB-bacteria belonging family of *Desulfobulbaceae*.
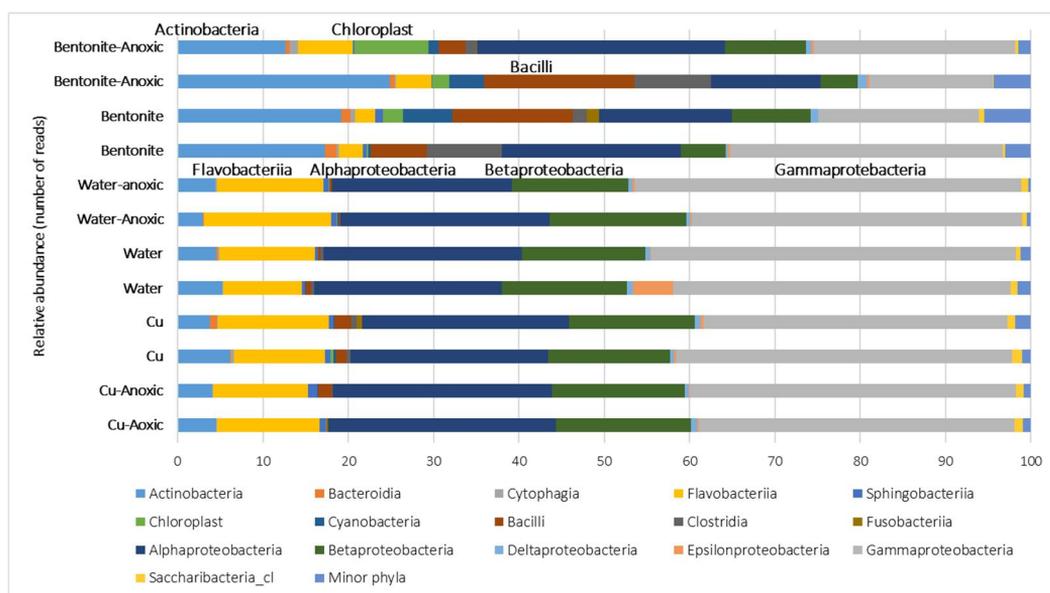
Figure 10. Bacterial community structure obtained by high throughput sequencing presented as class-level. Minor phylum refers to the taxa with a maximum abundance of <0.1% in any sample.

Another interesting microbial group in deep geological environment are iron-reducing bacteria (IRB). The reduction of Fe (III) to Fe (II) in swelling bentonite with an associated replacement of H_2O by K^+ in the interlayer space can result in the conversion of smectite to illite and loss of swelling properties in bentonite (Mueller, 2015; Pentráková et al., 2013). The ability to reduce Fe (III) is widely available within

the domains of both Bacteria and Archaea. For example, Shewanellaceae belonging Gammaproteobacteria has been shown to be potential iron reducing bacteria IRB (Lovley, 1993). Interestingly this microbial group form 0.2-5.7% of the sequences in studied samples.

According to the sequencing, fungi were identified to belonging to several groups of Ascomycetes and Basidiomycetes (Figure 11). Fungi can produce acids and cause mineralogical changes in substrates. Because pH was maintained alkaline during the experiment, it indicates low fungal activity in compacted bentonite.
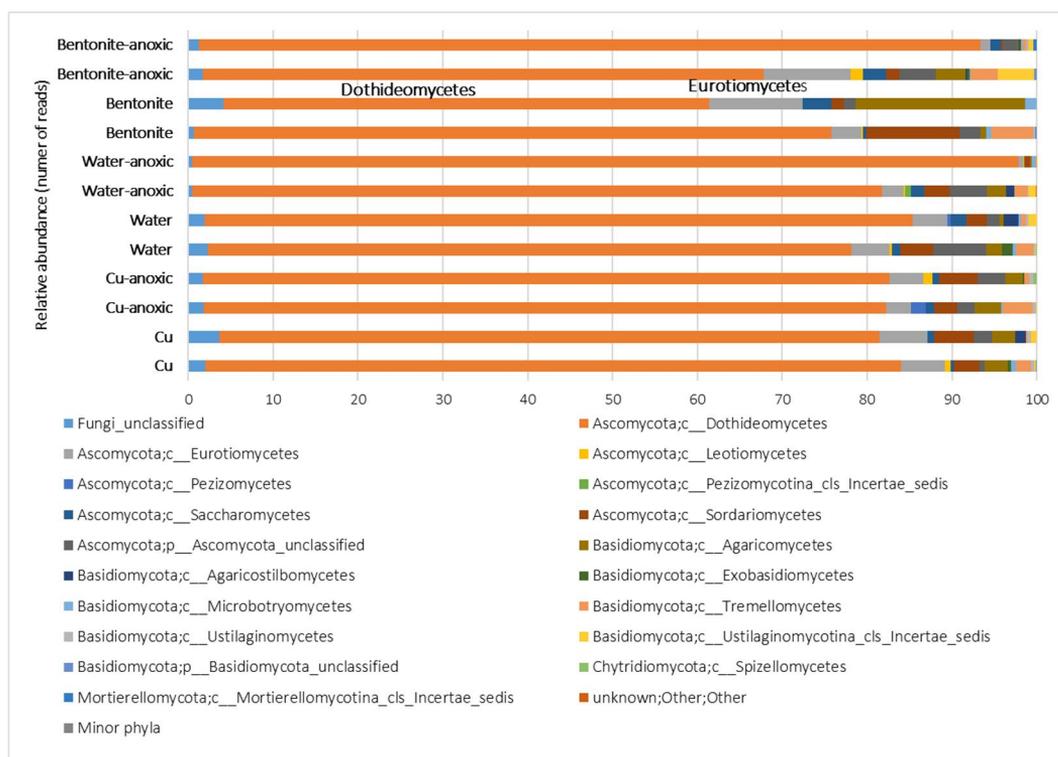

Figure 11. Fungal community structure obtained by high throughput sequencing presented as class-level. Minor phylum refers to the taxa with a maximum abundance of <0.1% in any sample.

The biggest fungal groups that were detected in all samples were Cladosporium and Alternaria belonging class Dothideomycetes. Most of the species detected have been encountered in rock substrates according to literature (Gadd, 2007; Guieidan, 2011). Many of the fungal genera detected for example Penicillium (class Eurotiomycetes), Trichoderma (Ascomycota), Cladosporium (class Dothideomycetes) and Exophiala (Ascomycota) produce organic or inorganic acids that help fungi in

solubilization of minerals from rock substrates (Sterflinger, 2000). In addition, Alternaria, Cladosporium and Penicillium strains that were detected in copper samples are able to oxidase iron and manganese (Sterflinger, 2000). Conclusions

Bentonite is an important buffer material between the copper canister and bedrock in KBS-3 method for the final disposal of spent nuclear fuel. The KBS-3 repository is planned to have the copper canisters safe for thousands of years. Therefore, long-term experiments aimed to study chemical and mineralogical changes of bentonite in contact with copper are valuable for safety studies of a KBS-3 repository.

Long-term experiments were successfully carried out for bentonite-copper systems both in oxic and anoxic conditions. Despite the experimental setup was designed to investigate chemical, physical and mineralogical changes as a function of time and between oxic and anoxic samples, microbiological studies were also conducted at the end of the experiment. Because the experiment was not originally planned for microbiological studies and no samples were taken in the beginning of the experiment, information of the changes in microbial communities as a function of time could not be achieved.

The main conclusions from the experiment were:
- Microstructural studies did not show significant differences in bentonite structure between samples taken from anoxic and oxic experiments.
- Mineralogical alterations were more extensive in the bentonite matrix in samples taken from the middle part of the copper capsule. Changes in the bentonite mineralogy were observed as a formation of secondary copper minerals in the oxic experiment.
- Copper content in pyrite increased when moving from the middle of the bentonite matrix towards the copper surface of the cylinder in both oxic and anoxic experiments.
- Chemical changes were typical dissolutions of gypsum and calcite, which release sodium, sulphate and carbonates into external water, while calcium exchanges sodium in the interlamellar spaces of bentonite.
- Epifluorescence microscopy revealed living microbial cells in the external water surrounding copper cylinders.
- The presence of living microbes on bentonite and on copper surface could not be demonstrated in this study. The type of bentonite can also influence the survival of microbes.
- Microbiological studies revealed the presence of SRBs and IRBs in bentonite, water and copper surface. SRBs can produce corrosive sulphide and IRBs can be related to processes that could be linked to the loss of swelling properties in bentonite.
- Fungal conidia and hyphae were detected by SEM in water and several groups of Ascomycetes and Basidiomycetes were identified by sequencing from bentonite samples.

- Extraction of nucleic acids (DNA and RNA) from bentonite is essential step for the evaluation of microbial communities. This was shown to be very challenging and more research is needed for develop more efficient extraction methods.

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Title	**Long-term experiment with compacted bentonite**
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Abstract	Bentonite is an important buffer material between the copper canister and bedrock in geological disposal of spent nuclear fuel in Finland. The long-term experiment with compacted Na-bentonite with low saline ground-water simulant and copper canister was initiated in 1997. The aim of the experiment was to evaluate changes in chemical, mineralogical and microstructural parameters in bentonite in oxic and anoxic conditions. The experiment was planned to be finished after ten months, but two samples were stored and dismantled in 2012. Although the experimental setup was not initially designed for microbiological studies, they were performed the end of the experiment. Changes in the bentonite mineralogy included the observation of secondary copper minerals formation in the middle part of the bentonite matrix in copper cylinder in oxic conditions. Copper content in pyrite increased when moving from the middle of the bentonite towards the copper cylinder surface in both oxic and anoxic experiments. Microstructural studies on bentonite did not show any significant differences in bentonite structure between samples taken from anoxic and oxic experiments. Chemical changes were typical dissolution of gypsum and calcite, which releases sodium, sulphate and carbonates into external water, while calcium exchanges sodium in the interlamellar spaces of bentonite. The presence of living microbes on bentonite and on copper surface could not be demonstrated in this study but microscopical studies revealed living microbial cells in the external water surrounding copper cylinders. According to the sequencing, sulphate (SRB) and iron reducing bacteria (IRB) were detected in bentonite, water and copper surface. SRBs can produce corrosive sulphide and IRBs can be related to processes that could be linked to the loss of swelling properties in bentonite. Fungal conidia and hyphae were detected by SEM in water and several groups of Ascomycetes and Basidiomycetes were identified from bentonite samples.
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Nimeke	**Pitkäkestoinen koe kompaktoidulla bentoniitilla**
Tekijä(t)	Minna Vikman (ed.), Michal Matusewicz, Elina Sohlberg, Hanna Miettinen, Mia Tiljander, Joonas Järvinen, Aku Itälä, Pauliina Rajala, Mari Raulio, Merja Itävaara, Arto Muurinen & Markus Olin
Tiivistelmä	Bentoniitti on tärkeä kuparikapselin ja kallioperän välissä käytettävä puskurimateriaali ydinjätteen loppusijoituksessa Suomessa. Pitkäaikainen koe kompaktoidun Na-bentoniitin, kuparikapselin ja vähäsuolaisen pohjaveden vuorovaikutuksista käynnistettiin vuonna 1997. Kokeen tavoitteena oli selvittää kemiallisia, mineralogisia ja mikrorakenteessa tapahtuvia muutoksia kompaktoidussa bentoniitissa hapellisessa ja hapettomassa ympäristössä. Koe oli suunniteltu lopettavaksi kymmenen kuukauden jälkeen, mutta kaksi näytettä säilytettiin ja purettiin vasta vuonna 2012. Koetta suunniteltaessa ei oltu varauduttu mikrobiologisiin analyyseihin, jotka tehtiin kokeen lopussa. Minerologisissa analyyseissä havaittiin, että sekundäärisiä kuparimineraaleja muodostui kuparisylinterin sisällä olleen bentoniitin keskelle hapellisissa olosuhteissa. Pyriitin kuparipitoisuus kasvoi kompaktoidun bentoniitin keskiosista reunoille siirryttäessä sekä hapellisessa että hapettomassa koeympäristössä. Bentoniitin mikrorakenteessa ei havaittu merkittäviä muutoksia. Kemialliset muutokset liittyivät kipsin ja kalsiitin liukenemiseen, jolloin vapautui natriumia, sulfaattia ja karbonaatteja ympäröivään liuokseen kalsiumin vaihtaessa paikkaa natriumin kanssa bentoniitin interlamellaaritiloissa. Eläviä mikrobeja ei tässä tutkimuksessa havaittu bentoniitissä tai kuparin pinnalla. Mikroskooppiset tutkimukset osoittivat, että eläviä mikrobeja oli havaittavissa kuparisylinteriä ympäröivässä vesiliuoksessa. Sekvensointitulosten mukaan sulfaattia (SRB) ja rautaa pelkistäviä bakteereita (IRB) todettiin bentoniitissä, vedessä ja kuparin pinnalla. Sulfaatinpelkistäjät voivat tuottaa korrodoivaa sulfidia ja raudanpelkistäjät voivat vaikuttaa bentoniitin paisuntaominaisuuksiin. Sienirihmastoja ja konidioita havaittiin elektronimikroskoopilla vedestä ja useita Ascomycetes ja Basidomycetes ryhmiin kuuluvia sieniä identifioitiin bentoniitista.
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