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Impact of bentonite dry density on the viability of organisms (in the context of steel corrosion)

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Publishable Summary

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

Contents

1 Introduction

Nagra (National Cooperative for the Disposal of Radioactive Waste, Switzerland) is considering using carbon steel as a potential canister material for the disposal of high-level waste and spent fuel in a deep geological repository in Opalinus Clay rock. Bentonite clay with a dry density of 1,450 kg m^{-3} is proposed to backfill the emplacement tunnels and will be placed around and between the disposal canisters.

Here, we report on the corrosion experiment IC-A (iron corrosion) in the Underground Rock Laboratory (URL) Mont Terri, St.-Ursanne, Switzerland. The IC-A experiment is tasked with investigating the *in-situ* corrosion rates of steel with the overall long-term objective of providing measurements of the corrosion rate of carbon steel in compacted bentonite under simulated repository conditions. The results have been published by Smart *et al.* [1] and some are presented in this document, along with updated microbial results.

The potential role of the microbial community on the rate of corrosion is another goal of this experiment. The major goal of the work presented here is to study the effect of the bentonite buffer targeted dry-density on microbial viability and the extent of microbially influenced corrosion of carbon steel, especially by metabolically active sulfate-reducing bacteria (sulfide may result in pitting of carbon steel).

This report describes the set-up of the experiments and summarizes the results from a range of microbial analyses that were carried out after 12, 20, 33, and 66 months of exposure. *In-situ* corrosion experiments

1.1 Experimental layout

The experiment was installed in a vertical descending borehole in Opalinus Clay rock. Stainless steel modules, containing the carbon steel corrosion coupons (20 mm of diameter, thickness of 10 mm) embedded in compacted bentonite or in a pellet-powder mix of bentonite, with a range of controlled dry-densities, were prepared under anoxic conditions and inserted into the borehole, which contained natural Opalinus Clay porewater, and then sealed to maintain the long-term, low-temperature, anoxic conditions representative of those expected in a deep geological repository. The modules permitted the free exchange of water with the host rock. A set of 12 modules (Modules 1 to 12) was initially installed in the borehole; they will be removed and analyzed, and some will be replaced with newly prepared modules, according to a planned schedule over a 10-year period. In particular, Modules 13-15 were deployed 1.5 years into the experiment and Modules 16-18, 4 years into the experiment.

1.1.1 Bentonite preparation[1]

Volclay MX80 bentonite from Wyoming, U.S.A. was used throughout the experiment. Four different conditions of bentonite in two different formulations were used, as follows:

- 1250 kg m^{-3} , 1450 kg m^{-3} and 1550 kg m^{-3} compacted blocks: the compacted bentonite was provided by Clay Technology, Sweden. A 100 mm diameter mold that incorporated recesses for the corrosion coupons was manufactured. The bentonite was prepared to give a 95– 99% degree of saturation using deionized water. It was placed in the mold, flushed with nitrogen and then placed under vacuum during compaction, in order to minimize the amount of

[1] The preparation of the bentonite and of the corrosion coupons, as well as the assembly of the coupons and bentonite was carried out by AMEC, now Wood PLC, cf. Smart *et al.* [1]

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residual oxygen present in the bentonite at the start of the experiment. The blocks of bentonite were then handled and stored in a nitrogen atmosphere until the modules were assembled.

- 1450 kg m^{-3}, using a mixture of pellets and powder: this granular material was provided in the required density.

1.1.2 Assembly of corrosion test modules[1]

The test modules were fabricated from stainless steel and were 250 mm long with an external diameter of 126 mm. The sintered stainless steel filters had an external diameter of 106 mm, a wall thickness of 3 mm, a porosity of 30% and an average pore size of 18 µm. The housings and filters for each module were cleaned in acetone and deionized water and then the modules were assembled in an argon-filled glove box (<0.1 ppm oxygen) to ensure that the starting conditions for the experiments were anoxic. Four layers of coupons, with three specimens in each layer, positioned at 120° intervals were installed in each module (Figure 1). The outer edge of the coupons was located 5 mm from the outer circumference of the bentonite. The modules were then pre-saturated with anoxic artificial Opalinus Clay porewater by placing them in water-filled plastic containers for 2 days in the case of modules containing bentonite blocks, while the powder-pellet bentonite modules were soaked for 10 days. After soaking, the modules were heat-sealed into three layers of low permeability plastic (Mylar™) bags to retain an anoxic argon atmosphere during transport.

Figure 1: *Schematic cross-section through a test module showing the arrangement of corrosion coupons. Figure obtained from Smart et al.* [1]

1.1.3 Exposure

A 15 m deep vertical descending borehole was drilled in Opalinus Clay at the Mont Terri Underground Research Laboratory St. Ursanne, Switzerland on 20 March 2012. After drilling, the borehole was flushed with argon four times, pressurized at 3.5 bar and sealed with a 2.5 m long hydraulic packer to maintain anoxic conditions. After sealing, there was an inflow of natural Opalinus Clay porewater at a rate of 44 mL.day^{-1}. The modules were installed in the Mont Terri facility on 10 January 2013. At that time, samples from the porewater that had accumulated in the borehole were

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taken for analysis and an additional 9 L of anoxic synthetic Opalinus Clay porewater were added in the borehole. During exposure, the *in situ* borehole temperature was ~ 14°C.

1.2 Removal and analysis

1.2.1 Removal of test modules

The first set of three of the test modules (Modules 1-3) was removed for analysis on 30 September 2014 after 20 (~1.5 y) months of exposure. The second set of three modules (Modules 13-15) was removed for analysis on 4 July 2017 (after 33 months (~2.5 y)). The third set of six modules (Modules 16-18 and Modules 4-6) was removed in July 2018 after respectively 12 and 66 months (1 y and 5.5 y) of exposure (Figure 2). During removal, porewater samples were taken from the borehole for microbial analyses. The modules were removed from the borehole while purging with argon and put into purpose-built stainless steel transfer flasks that were filled with water from the borehole and purged with argon before transport to the UK for analysis, where they were placed into a pre-cleaned and sterilized (wiped with a 70% isopropyl alcohol solution) argon-purged glove box for dismantling.

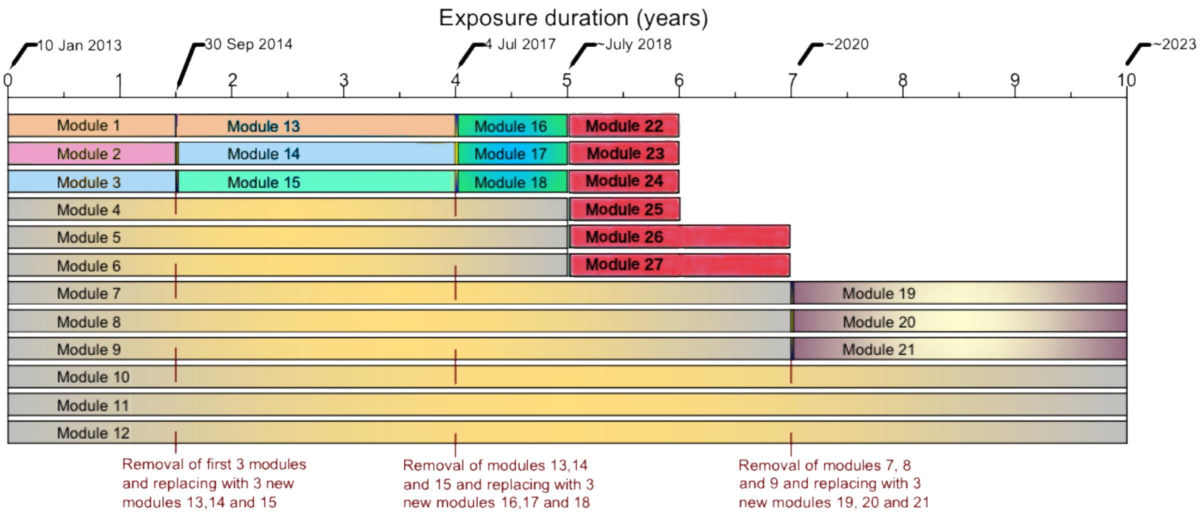

Figure 2: *Schematic of the deployment and retrieval schedule for the modules. In this report, modules 1-6, 13-15, and 16-18 are discussed.*

1.2.2 Analysis of corrosion coupons, porewater, and bentonite

The specimens were analyzed using a range of techniques that are summarized below.

Analysis of corrosion coupons[2]

In order to determine the corrosion rate of the specimens, weight loss measurements were carried out in the glove box, according to a standard practice [2] and Clarke's solution (inhibited hydrochloric acid) was used as the descaling agent. The weight loss was converted to a corrosion rate given in units of µm/year. This was only done for the samples from Modules 1-3.

Porewater analyses

The sampled porewater was filtered on site for DNA analysis, using a sterile filtration device equipped with 0.2 µm polycarbonate membranes (Millipore Corporation, Billerica, U.S.A.). Four

[2] Analysis of the corrosion coupons was carried out by AMEC, now Wood PLC, cf. Smart *et al.* [1]

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filtrations of 250 mL each were performed. DNA was extracted from filtered water samples using a slightly modified protocol from the DNA Spin kit for Soil (MP Biomedicals, Illkirch, France) and then purified using the Genomic DNA Clean & Concentrator purification kit (Zymo Research, Freiburg, Germany). The total amount of DNA extracted was 19.5 ng and the final suspension had a concentration of 0.195 ng/µL. The relatively low amount of DNA recovered can be attributed to a low microbial activity in the borehole and/or the presence of clay particles that could have decreased the DNA extraction yield. The DNA collected from the borehole sample was amplified with universal bacterial 16S rRNA primers (28f 5′-GAG TTT GAT CNT GGC TCA G-3′ and 519r 5′-GTN TTA CNG CGG CKG CTG-3′), using 35 cycles with an annealing temperature of 50°C. The PCR products were purified with MSB® Spin PCRapace (TRATEC Biomedical AG, Birkenfeld, Germany) before sequencing at the Research and Testing Laboratory in Lubbock (U.S.A.) or at the sequencing facility at the University of Geneva. After sequencing was performed, data analysis was carried out using QIIME. The filtrate was used for chemical analysis. Sulfide, iron(II) and iron(III) were analyzed within 24 h after the sampling, using a UV-2501PC spectrophotometer (Shimadzu, Duisburg, Germany). Hydrogen sulfide (H_2S, HS^- and S^{2-}) was measured using the Cline method, and Fe^{2+} and Fe^{3+} with the ferrozine assay. The filtered borehole porewater was analyzed for the following:

- pH using an Orion 3-Stars pH meter (Thermo Scientific, Waltham, MA, U.S.A.).
- Major anions and cations by ion chromatography, using an ICS-3000 instrument (Dionex, Sunnyvale, CA, U.S.A.). For anions, 30 mM KOH was used as the eluent, with an IonPac AS11-HC column. The cations were measured with 40 mM of methanesulphonic acid as the eluent, with an IonPac CS16 column.
- Trace metals: Fe, Mn, Cr, Cu, Co, Ni, Zn, Sr, Si and Al were analyzed using inductively coupled plasma mass spectrometry on an Elan DRC II instrument (Perkin Elmer, Waltham, MA, U.S.A.).

1.2.3 Bentonite microbial analyses

The bentonite was cut and sampled with sterile knives and spatulas and packed in sterile sampling bags, then packed in two layers of Mylar™ bags under argon and kept at 4°C. In order to see any variation in the distribution of microbial activity in the bentonite and around the corrosion coupons, samples were taken at various locations (interior and exterior parts of the bentonite core). A single sample from each module was used for the measurement of water activity. Microbial enumeration was performed by preparing a suspension from the small pieces of sample section destined for cell culturing by adding a weighed amount of sample to a known volume of sterile, anoxic phosphate-buffered saline solution (1xPBS[3]) by shaking at 300 rpm for 30-60 minutes. Under sterile and anoxic conditions, the suspension was allowed to settle for 1 minute before a liquid sample was obtained from the middle of the bottle for cultivation. All following descriptions of cultivation were carried out under sterile, anoxic conditions (unless otherwise indicated). Serial dilutions (100-fold to 10^7 in PBS) of the suspensions were used in the enumerations.

Viable microbes were cultivated on R2A solid medium [3] under oxic and anoxic conditions (see

Table ***1*** for composition). The enumeration was carried out by plating in triplicates and counting colony forming units, as described elsewhere. A 1 ml solution or a 10-fold dilution thereof was added to an empty petri dish (culture plate) and ≈15 ml of semi-liquid R2A agar medium, kept at 45°C, was carefully added into the culture plate, followed by proper mixing according to the protocol. Aerobic plates were incubated at 30°C for 3–3.5 days in oxic conditions. Anaerobic heterotrophs were also

[3] 1L of 1x PBS contains 10 mmol disodium hydrogen phosphate, 137 mmol sodium chloride, 2.7 mmol potassium chloride and 1.8 mmol potassium dihydrogen phosphate.

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cultured on R2A medium in an anoxic glove box and incubated at 30°C under anoxic conditions for 17–28 days. Sulfate-reducing bacteria (SRB) were enumerated by the most-probable number (MPN) method in modified liquid Postgate' s B medium [4] and incubated at 30°C under anoxic conditions for 35–71 days.

Table 1: R2A medium for anaerobic and aerobic heterotrophs.

Total volume of 3.6 liters, aliquoted in 500 mL bottles, pH=7.2±0.1, anoxic medium flushed with sterile filtered nitrogen for 20 min.

Mass [g]	**Substance**
1.80	Yeast-Extract
1.80	Bacto-peptone
1.80	Vitamin Assay Casamino Acids
1.80	Glucose (Dextrose)
1.80	Soluble starch
1.08	K_2HPO_4
1.08	$MgSO_4 \cdot 7H_2O$
0.18	Na-pyruvate
6.00 per 500 mL aliquot	Agar

Table 2: Postgate's Medium B for sulfate reducing bacteria.

Total volume of 2 liters, pH=7-7.5.

Mass [g]	**Substance**
7.0	Na-lactate
4.0	$MgSO_4 \cdot 7H_2O$
2.0	NH_4Cl
2.0	$CaSO_4$
2.0	Yeast Extract
1.0	KH_2PO4
1.0	$FeSO_4 \cdot 7H_2O$
0.2	Ascorbic acid
0.2 (add as 20 mL solution)	Thioglycolic acid

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0.0008 (or 7mL of 0.025% solution)	Resazurin

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1.3 Results

1.3.1 Weight loss measurements

The results of the weight loss measurements are summarized in Figure 3. On the basis of the weight loss measurements, it can be seen that the corrosion rate increased in the following order:

Module 3, compacted block, 1550 kg m^{-3} < Module 2, compacted block, 1250 kg m^{-3} < Module 1, pellets, 1450 kgm^{-3}.

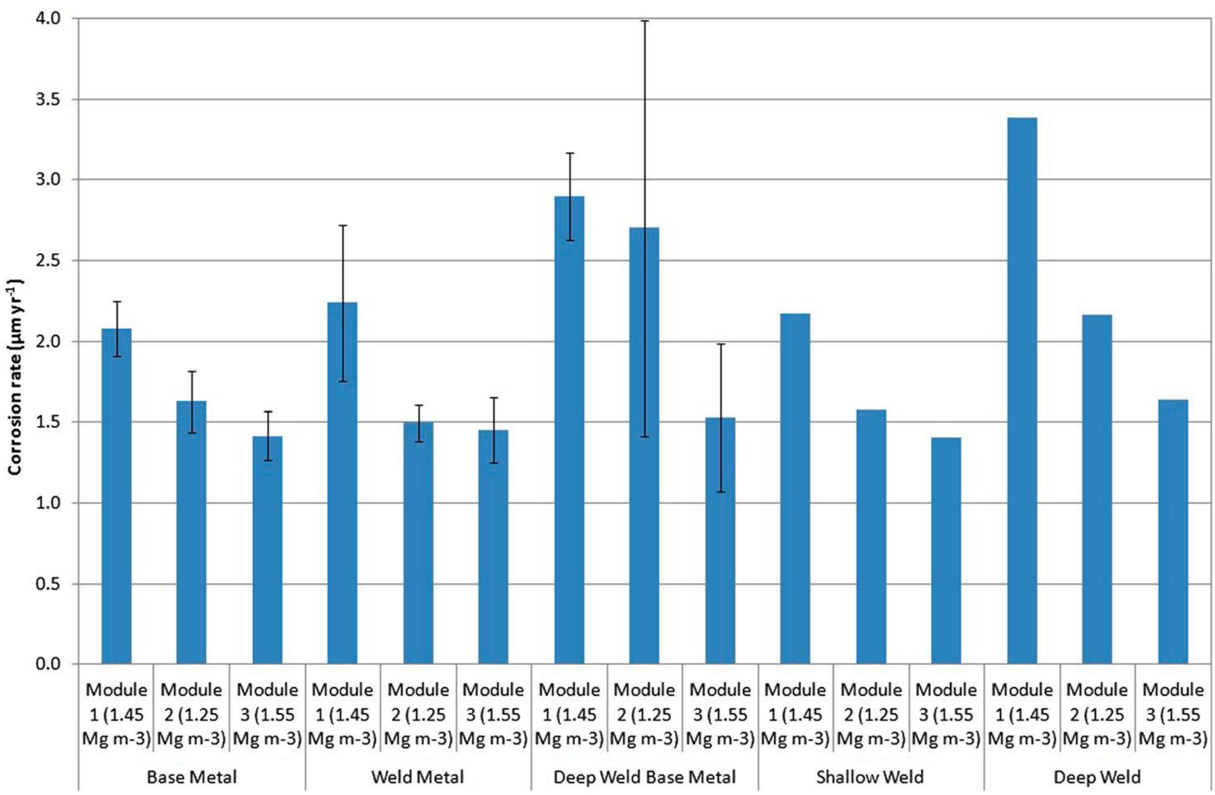

Figure 3: *Average corrosion rates based on weight loss measurements for the tested materials and bentonite densities. Figure obtained from Smart et al.* [1]

1.3.2 Porewater analyses

The results of the chemical analysis of the porewater sampled from the borehole are given in Table 3. These results show that this borehole was maintained under anoxic conditions, as substantial concentrations of reduced Fe and S were found for the first timepoint of retrieval (September 2014). For the second time point, a decrease in sulfide was observed which could be due to less sulfate reduction (the microbial community may suggest this explanation) or precipitation with metals (such as copper). There is an increase in the sulfate concentration in the two later time points, perhaps suggesting less active sulfate reduction. However, because the borehole has to be opened for sampling and exposure to oxygen is inevitable, conclusive interpretations of these geochemical data are not advisable .

The analyses of the microbial community in the porewater sample taken before exposure of the module to porewater showed that the great majority of the contributing genera fall within the Peptococcaceae family (~84%) and also within the functional category of sulfate-reducing bacteria (~66%) (Table 4). Two genera dominate the community: *Desulfotomaculum* sp. and

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Desulfosporosinus sp., which are both Gram-positive sulfate-reducing genera. In addition, there is evidence for *Pseudomonas* sp., representing 26% of the community. *Desulfotomaculum* sp., *Desulfosporosinus* sp., and *Pseudomonas* sp. have all been identified in Mont Terri borehole water previously [5]. The analysis of the porewater sample taken after 1.5 years of exposure of the modules to porewater (2014 sample) showed a significant change in the microbial community with a large increase in the number of *Pseudomonas* (87%) (Table 4). It is not known what caused this change but it had previously been suggested that bacterial necromass or Opalinus Clay organic matter could support growth of *Pseudomonas* sp. [5].

In 2017, the microbial community appears to balance back into a more diverse community, as indicated by a lower abundance of *Pseudomonas* (31%) and a higher abundance of sulfate-reducing bacteria (19%, combined). This indicates, that the community has changed: initially sulfate-reducing bacteria consumed easily accessible electron donors, originating from the surrounding host rock. Upon depletion of these, the fermenting *Pseudomonas* dominated the community, but several years later (2017), the community diversity increased and *Pseudomonas* sp. recede again.

Table 3*: Composition of the filtered porewater sampled from the borehole.* [a]*September 2014: Fe(II) and Fe(III) were measured before filtration and yielded 76.3 µM and no detectable Fe(III). After filtration and presumably oxidation, [Fe(II)] = 1.3 µM and [Fe(III)] = 22.2 µM.* [b]*July 2018: Total iron of 24 µM, Fe(II) concentration to be determined (tbd).*

Component	September 2014	July 2017	July 2018	Unit
Fe(II)	76.3[a]	0.3	24[b]	µM
H_2S	7.4	<0.1	tbd	µM
Co	<10	<10	<10	µg L^{-1}
Cu	13	<10	<10	µg L^{-1}
Sr	60657	18387	26467	µg L^{-1}
Ni	<10	69.3	<10	µg L^{-1}
Mn	53	125.9	<10	µg L^{-1}
Cr	31	<10	<10	µg L^{-1}
Zn	<10	<10	<10	µg L^{-1}
Al	<10	<10	<10	µg L^{-1}
Na^+	313.6	257.9	258.7	mM
NH^+_4	0.3	4.6	0.9	mM
Mg^{2+}	15.0	13.0	9.8	mM
K^+	1.9	3.8	3.4	mM
Ca^{2+}	18.8	12.3	13.4	mM
F^-	0.1	0.03	<0.005	mM
Cl^-	290.3	264.6	274.8	mM
NO^-_2	<0.005	<0.005	<0.005	mM
SO_4^{2-}	15.6	21.8	22.5	mM
Br^-	0.5	<0.005	<0.005	mM
NO^-_3	<0.005	0.16	0.09	mM
PO_4^{3-}	<0.005	0.04	<0.005	mM
pH	8.2	8.30	8.45	

Table 4*: Composition of porewater microbial community, operational taxonomical units with more than 1% contribution are listed below. A detailed analysis of the microbial community at the January 2013 timepoint has been reported in [5]. The initially (2013) abundant sulfate-reducing bacteria decreased in their contribution to the community for the 2014 timepoint, but represent in 2017 again a substantial contributor at about 19%. The community shows in 2017 a higher diversity, as indicated by the higher number of 1% contributors.*

January 2013	Rel. abundance	September 2014	Rel. abundance	July 2017	Rel. abundance
Desulfotomaculum	57.2%	*Pseudomonas*	87.3%	*Pseudomonas stutzeri*	30.9%
Pseudomonas	25.9%	*Desulfotomaculum*	2.4%	*Hydrogenophaga*	15.3%
Desulfosporosinus	4.2%	*Gracilibacter*	2.2%	*Desulfosporosinus meridiei*	6.9%
Desulfosporosinus	3.2%	*Desulfosporosinus*	1.7%	Bacteria	6.9%
Natronincola	2.3%	*Poseidonocella*	1.3%	*Sporotomaculum*	4.8%
Natranaerobiales	2.2%			Clostridia	3.8%
Desulfotomaculum	1.7%			*Thiobacillus*	2.5%
Peptococcaceae	1.0%			Peptococcaceae	2.5%
				Desulfosporosinus meridiei	2.4%
				Desulfobulbaceae	2.3%
				Natronincola anaerovirgula	1.7%
				Desulfitobacter	1.6%
				Beijerinckiaceae	1.5%

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				Desulfurispora	1.3%
				Desulfobulbaceae	1.1%
				Acidobacteria iii1-8 DS-18	1.0%
				Desulfococcus	1.0%

1.3.3 Bentonite analyses

The results of the measurements of bentonite moisture content and water activity are included in Table 5. Some data are not yet available.

Table 5*: Target bentonite densities, moisture content and measured water activity for different modules. [a]Water weight x 100/weight of original sample. [b]Water weight x 100/weight of dried sample. Table modified from Smart et al.* [1] *with added moisture values for the second, third and fourth set of test modules.*

Module	Deployment time (y)	Target dry bentonite density before emplacement (kg m^{-3})	Per cent moisture[a]	Moisture % dry weight[b]	Water activity a_w
1	1.5	1450	24.3	32.1	0.956 (±0.0104)
2	1.5	1250	28.9	40.6	0.984 (±0.027)
3	1.5	1550	22.6	29.3	0.950 (±0.014)
4	5.5	1450	22.3	29.7	-
5	5.5	1250	28.0	38.9	-
6	5.5	1550	23.0	29.8	-
13	2.5	1450	27.2	37.4	-
14	2.5	1550	24.0	31.6	-
15	2.5	1450	26.5	36.2	-
16	1.0	1450	23.8	31.5	-
17	1.0	1250	29.9	40.9	-
18	1.0	1450	24.6	32.6	-

Furthermore, microbial viability through cultivation assays is presented in Figures 4 through 9.

When comparing the number of anaerobic heterotrophs (i.e., microorganisms that use organic compounds as a source of carbon and that grow in the absence of oxygen) among the bentonite samples from modules sampled to date, there is a pattern that emerges. There is an initial increase in the number of viable microorganisms up until 1.5 years, which is followed by a decline (Figure 4). More specifically, it appears that the modules deployed for 1 year (M16-18) and 1.5 years (M1-M3) harbor more than 10-fold higher viable anaerobes than in the case of the initial samples (Figure 4). The modules deployed for 2.5 years (M13-M15) exhibit CFU/g numbers comparable to the initial values. Thus, we hypothesize that the bentonite was not fully saturated in the modules after 1.5 years while those deployed for 2.5 years were more saturated. After 5.5 years of deployment, there is the possibility that there is a slight increase in the number of viable microorganisms (particularly for the 1.55 g/cm^3 blocks and the 1.45 g/cm^3 pellets) but this remains to be confirmed with the samples to be retrieved after 7 years of incubation. Additionally, no trend correlating the dry density and number of viable heterotrophs could be discerned, except the 1.25 g/cm^3 block bentonite harbored generally higher viable counts than the other densities. Finally, there was no preferential location found for anaerobic heterotrophs (interior vs. exterior) (Figure 5).

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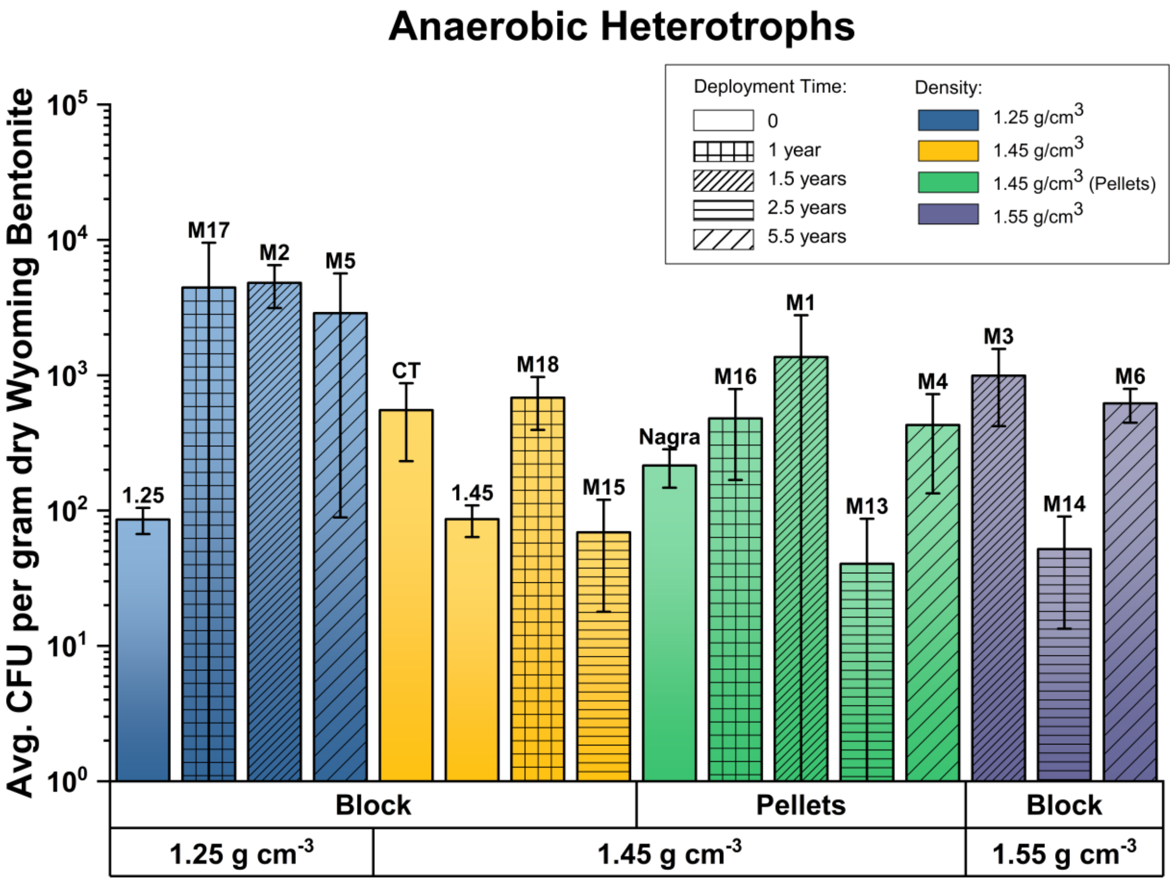

Figure 4: Average number of viable anaerobic heterotrophs as a function of time and the targeted dry density
The number of viable anaerobic heterotrophs was averaged over all layers per module, error bars represents standard deviation amongst layers. CT pellets were used to produce the different blocks, Nagra denotes the time zero sample of pellets provided by Nagra for the pellet modules (green).

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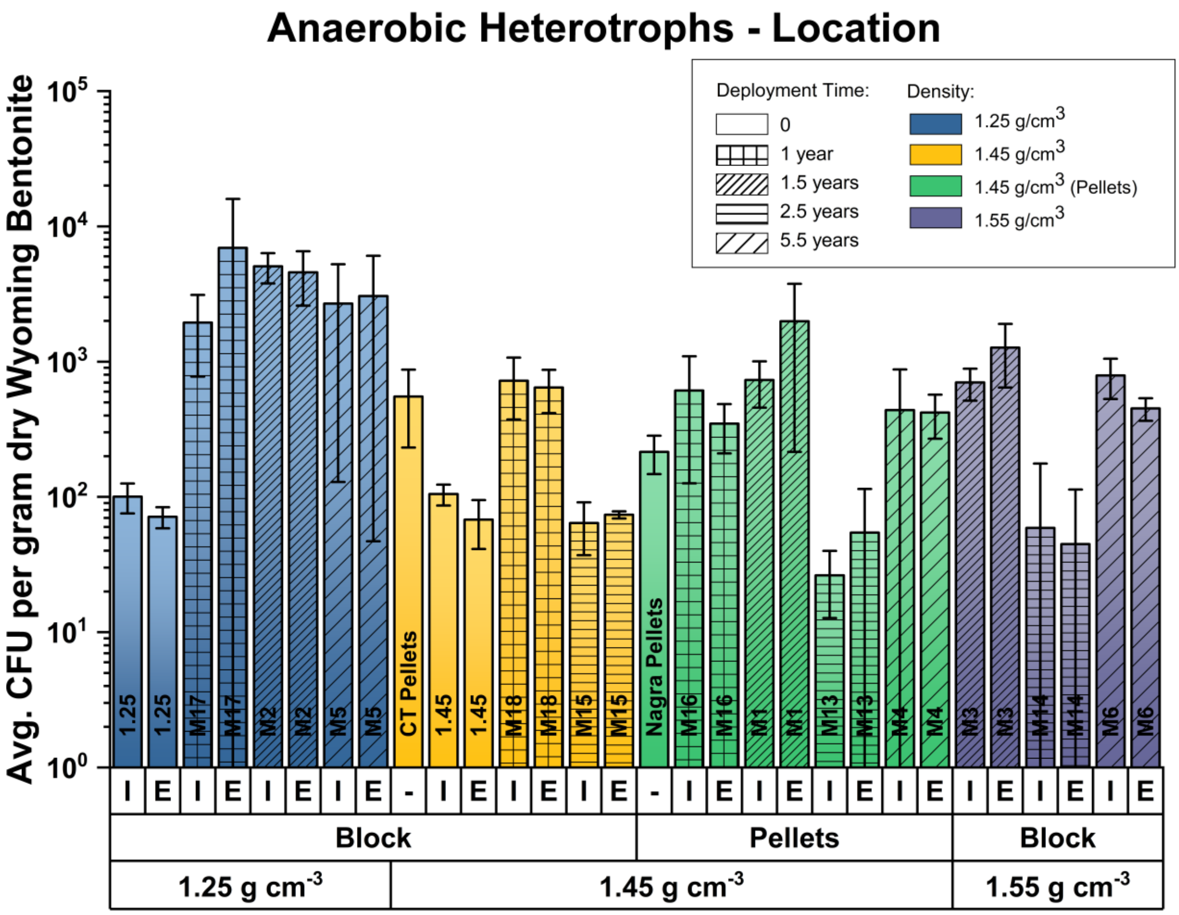

Figure 5: Average number of anaerobic heterotrophs versus the targeted dry density as a function of exterior and interior location in the samples.

The number of anaerobic heterotrophs was averaged over all layers per module but split into interior and exterior parts. The error bars represent standard deviation amongst layers. For some layers, only 1 CFU/g was found (M4, M13, M14, M17). Modules from initial state are included (no pattern) and represented by the blocks from which the modules were made and the initial pellets (Nagra). Also included are the pellets used to make the blocks (CT, block made by Clay Technology). The highest counts for anaerobic heterotrophs are observed for the 1- and 1.5-years samples, whereas longer exposure time to Opalinus clay porewater results in lower counts of viable anaerobic heterotrophs. No interior/exterior pattern is not observable for the 12 modules analyzed.

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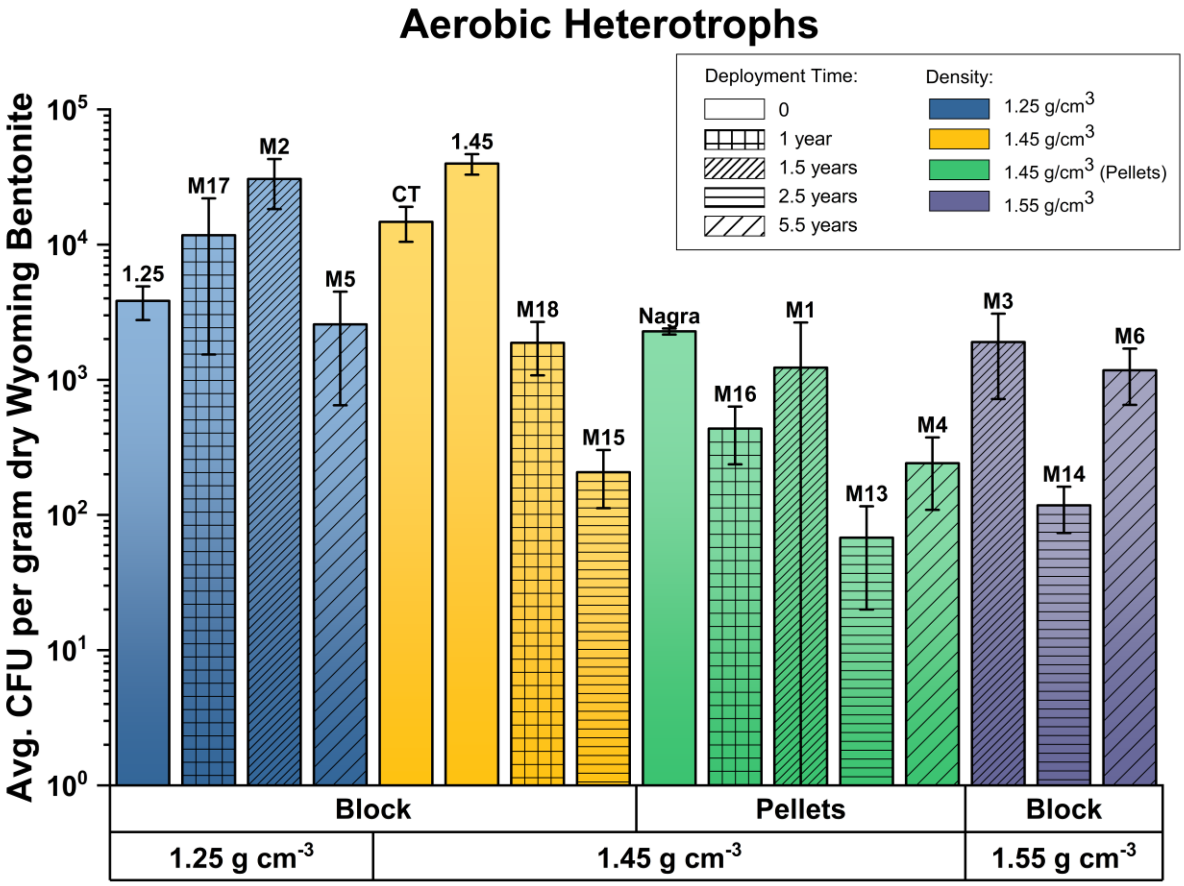

Figure 6: Average number of viable aerobic heterotrophs as a function of time and the targeted dry density.
The number of viable aerobic heterotrophs was averaged over all layers per module, error bars represents standard deviation amongst layers.

Aerobic heterotrophic bacteria, which use oxygen as an electron acceptor, exhibit a similar behavior as the anaerobes, with the difference that cell counts were typically greater for the former (Figure 6). There is an increase in the number of viable microorganisms up until 1.5 years and a decrease at 2.5 years of exposure. However, just like we observed for anaerobes, after 5.5 years, the number of viable aerobes increases slightly. Whether this is an artifact will be evaluated at the 7-year sampling time. As observed for the viable anaerobic heterotrophs, the 1.25 g/cm^3 dry density condition appears to harbor larger number of viable microorganisms relative to other dry densities. No particular trend in a preferential location was observed (Figure 7).

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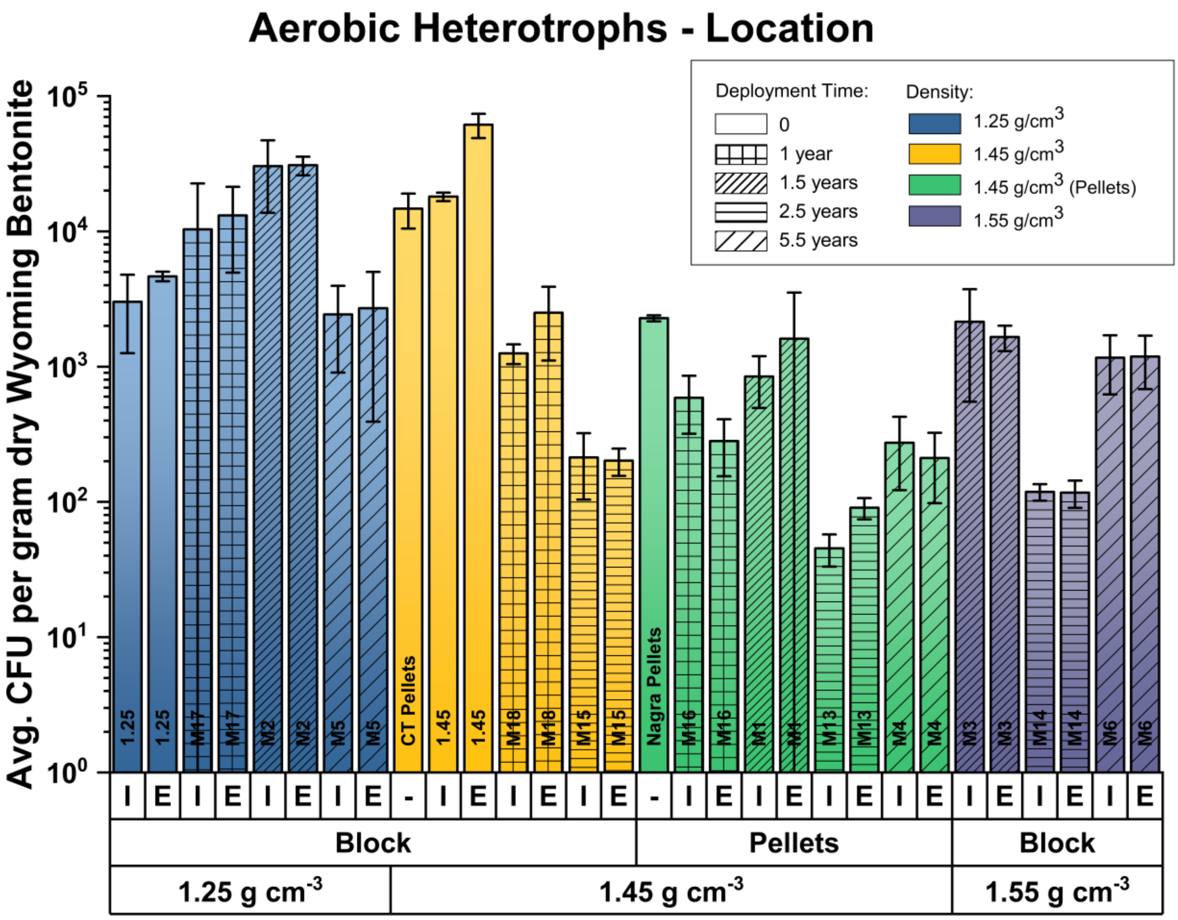

Figure 7 - Average number of aerobic heterotrophs versus the targeted dry density as a function of interior and exterior location within the samples.

The number of aerobic heterotrophs was averaged over all layers per module but split into interior and exterior parts. The error bars represent standard deviation amongst layers. For some layers, only 1 CFU/g was found (M1, M17). Modules from initial state are included (no pattern) and represented by the blocks from which the modules were made and the initial pellets (Nagra). Also included are the pellets used to make the blocks (CT, block made by Clay Technology). The highest counts for anaerobic heterotrophs are observed for the 1- and 1.5-years samples, whereas longer exposure time to Opalinus clay porewater results in lower counts of viable anaerobic heterotrophs. No interior/exterior pattern is not observable for the 12 modules analyzed.

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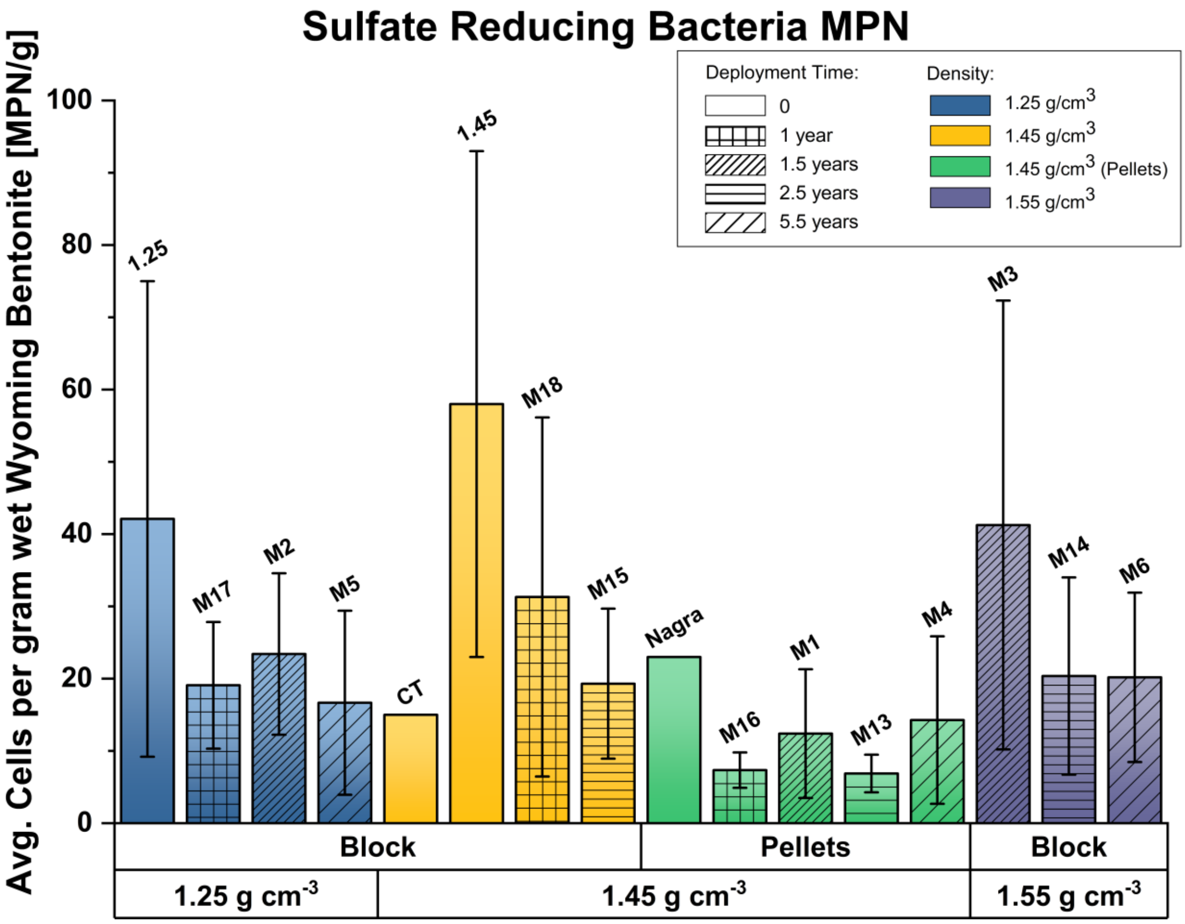

Figure 8: Average number of viable sulfate-reducing bacteria (SRB) as a function of time and the targeted dry density. Number of viable SRB is averaged over all layers for each module and error bars represent standard deviation amongst layers. Modules from initial state are included (no pattern) and represented by the blocks from which the modules were made. The highest SRB counts are observed for the initial samples (1.25 and 1.45). The longer the exposure time to Opalinus clay porewater, the lower the counts of viable SRB. There is no observed trend correlating dry density to SRB number.

SRB numbers were evaluated by the MPN method. Surprisingly, the number of SRB was slightly higher in Module 3 (highest dry density, 1.55 g/cm^3) after 1.5 years of exposure than in the other modules. However, the number of viable SRB remains higher in the highest tested dry density (1.55 g/cm^3) for the longer deployment times (2.5 and 5.5 years) compared to the lower dry densities (1.25 and 1.45 g/cm^3). The number of SRB found viable in pellet-containing modules are generally the lowest numbers observed. The most likely explanation for this behavior is the presence of spore-forming SRB and their uneven distribution in the bentonite, leading to a higher SRB load for blocks due to the compaction procedure. The SRB numbers were generally higher than those obtained for the Febex experiment in Grimsel Test Site [6].

The SRB counts were observed to be higher in the interior than the exterior of some sample (Figure 9), particularly for Module 1 and Module 4 (Pellets), Module 3 and Module 6 (1.55 g/cm^3 blocks) and Module 15 (1.45 g/cm^3 blocks). However, and perhaps most importantly, SRB counts appeared to be 2-3 times more abundant in the initial block samples than in the modules after 1, 1.5, 2.5 or 5.5 years (Figure 8).

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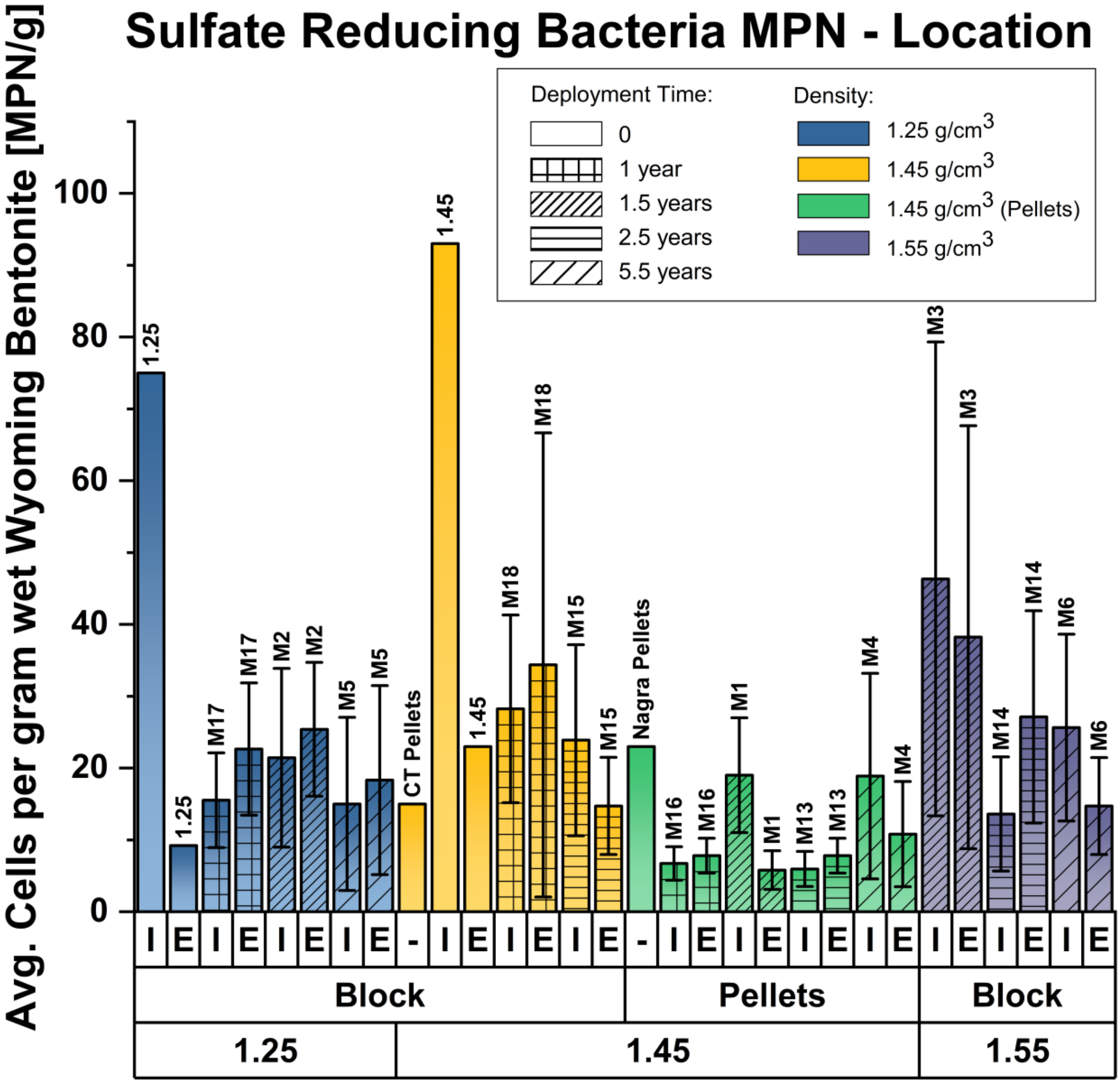

Figure 9: Average number of viable SRB versus the targeted dry density as a function of interior and exterior location within the samples.

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

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2 Discussion

2.1 Corrosion rates

The analyses of the corrosion coupons have demonstrated that surface damage of the carbon steel had developed due to corrosion in the bentonite over a period of ~1.5 years. The corrosion rates were derived from weight loss measurements and range from 1.21 to 3.38 µm/year. It is apparent that both bentonite density and the initial form of the bentonite have a significant influence on the rate of corrosion, across all materials. The corrosion rates for all specimens in the module with the greatest block density (Module 3, 1550 kg m^{-3}) were lower than those in the other modules with lower bentonite density. The influence of the initial bentonite form can also be seen, as the corrosion rate was lower for all specimens in the modules containing compacted bentonite blocks compared to the specimens in the module containing pelletized bentonite. This is despite the fact that the bentonite density of one of the modules containing block bentonite (Module 2, 1250 kg m^{-3}) was lower than that of the module containing pelletized bentonite (Module 1, 1450 kg m^{-3}). In contrast to the bentonite density, the effects of the steel composition and the presence or absence of welds on the corrosion rate are minor.

2.1.1 Comparison with laboratory corrosion experiments

The results from the *in situ* Mont Terri experiment can be compared to those from a concurrent laboratory program to measure the long-term corrosion rate of carbon steel [7], by measuring the rate of gas evolution. It should be noted that the environmental conditions for the Mont Terri specimens differ significantly from those used in the laboratory experiments, particularly with regard to the temperature of the Mont Terri experiment which is approximately 15°C compared to 60°C for the laboratory experiments, as well as the presence of a microbial population characteristic of a water-filled borehole in Opalinus Clay rock.

Corrosion rates derived from the monitoring of hydrogen evolution in the laboratory, assuming the formation of $Fe(OH)_2$ and Fe_3O_4 over durations similar to those of the Mont Terri experiment, were similar. The corrosion rate of laboratory-based experiments in bentonite saturated with synthetic Opalinus Clay porewater over a comparable exposure period ranges from 2.65 to 3.92 µm/year, which is slightly higher than the values measured *in situ* (1.21– 3.38 µm/year). Such corrosion rates are in general agreement with rates reported in the literature for similar exposure conditions and durations [6].

2.2 Microbial analyses

The analyses of the porewater indicate that the conditions in this borehole (anoxic, reducing) are favorable for the growth of sulfate-reducing bacteria. The increase in the contribution of *Pseudomonas* to the community during the module exposure period is largely accounted for by the decrease in a member of the Peptococcaceae family, to which pertains to the genus *Desulfotomaculum*. Based on the original metagenomic study, it was determined that this SRB was capable of heterotrophic sulfate reduction, probably using organic matter from the breakdown of microbial necromass as well as using H_2 as an electron donor [5]. The *Pseudomonas* species was thought to use hydrolyzed organic matter from the Opalinus Clay and degrading microbial necromass. Its emergence as the dominant organism suggests that the availability of H_2 may have decreased and organisms dependent on that electron donor (such as *Desulfotomaculum* spp.) may have given way to organisms presumed to utilize the refractory organic matter in Opalinus Clay (such as *Pseudomonas* sp.). The microbial community developed further into a more complex ecosystem as observed in 2017. Members of the Peptococcaceae family contributed about 19% to the observed community while the *Pseudomonas* species relative abundance decreased. This may indicate a more complex microbial metabolic network than observed and described in 2013 [5].

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In the bulk bentonite, the cultivation of (an)aerobic heterotrophs showed that higher viable microbial counts were found for shorter experimental durations (i.e., for 1 or 1.5 years after deployment) than in the initial state. This finding suggests microbial growth in the early experimental stages. Thus, early time points, in which full saturation has not yet been reached, may provide microbes with sufficient pore space and water to sustain activity, independently from dry density. The experiment was designed to attain saturation by immerging the modules in anoxic water for 2 to 10 days (depending on the bentonite formulation). These results suggest that this amount of time is insufficient for full saturation. After 2.5 years of deployment, there is a large drop in the number of viable microbes, suggesting that within the year between 1.5 and 2.5 years of deployment, saturation has been reached and microbes are inhibited and inactivated. However, at 5.5 years, there is a slight increase in the number of organisms relative to 2.5 years. Surprisingly, this is particularly clear in the 1.55 g/cm^3 targeted dry density blocks. The data available are too few to conclude that this is a representative trend. A conceptual model based on the findings thus far is presented in figure 10. The dashed line is intended to convey the preliminary nature of the conclusions for the 5.5-year time point, which will be re-evaluated at the 7-year time point.

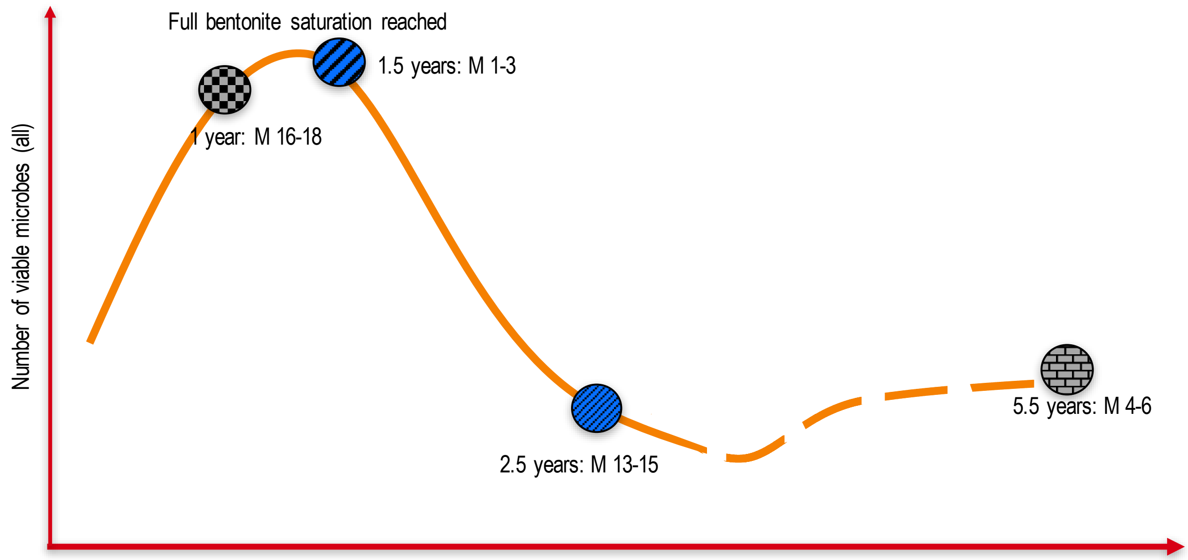

***Figure 10**: Conceptual model of evolution of the number of viable microbes in the bentonite as a function of time of exposure.* The dashed line represents a tentative finding. More data will be needed to support what is currently a conjecture.

One of the goals of the experiment was to identify the bentonite preparation that would be most inhospitable to microorganisms. Thus, the bentonite dry density was varied (1.25 g/cm^3, 1.45 g/cm^3 and 1.55 g/cm^3) and so was the bentonite preparation (blocks vs. pellets). Interestingly, there was little impact of the dry density on the viable counts for the two highest dry densities considered (1.45 and 1.55 g/cm^3). Comparing, for instance, 1.45 g/cm^3 blocks with 1.55 g/cm^3 blocks, there was no significant difference in the viable counts at 2.5 years for both anaerobic and aerobic heterotrophs (the only time point that is present for both datasets) (Figures 4 and 6). However, a comparison of the 1.25 g/cm^3 and the 1.55 g/cm^3 blocks at 5.5 years of deployment suggests greater viable counts for both aerobes and anaerobes at the lower targeted dry density (Figures 4 and 6). Considering the preparation of the bentonite, the blocks appear to harbor either the same or more anaerobic heterotroph viable counts as compared to the pellets at 1 y and 2.5 y at the common dry density of 1.45 g/cm^3.

The bentonite sampling was carried out in such a way as to allow evaluation of the impact of location of individual samples on cell counts. There was no clear systematic trend observed from the data.

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The lack of a clear trend may suggest that microorganisms present in the bentonite rather than microorganisms entering from the borehole were present and viable in the modules, despite the fact that the filter pore size was large enough (18 µm) to allow entry of microorganisms.

The heterotrophic aerobes were present in much higher numbers than anaerobes and grew up to 1.5 years (as previously discussed). Given the anaerobic conditions extant in the borehole, this finding is surprising. Of course, some of the anaerobes enumerated could also be facultative anaerobes, which would mean that they can also grow under aerobic conditions. However, that explanation is unsatisfactory because there is a greater number of aerobes than anaerobes. Thus, we propose that molecular oxygen remains adsorbed to the clay surface and allows the persistence and growth of aerobes in the bentonite.

By comparing the module deployment times, we conclude that a longer residence corresponds to fewer viable SRB, although the viable counts appear to stay constant after 1.5 years. There is no clear trend in the number of viable cells relative to the observed dry density for these modules, contrary to might be expected. This may be due to the fact that the effect of dry density is superimposed onto the impact of the degree of saturation of bentonite. The latter is likely to increase until saturation. In addition, fewer viable SRB cell numbers were observed in the pellet formulation as compared to the bentonite block formulations of similar dry density, suggesting that the block pressing procedure might be a source of SRB. More specifically, the bentonite pellet batch (CT, Clay Technology AB) used to produce the blocks (for all dry densities) showed up to 3-fold lower SRB numbers than the corresponding blocks (only initial block samples for the 1.25 g/cm^3 and 1.45 g/cm^3 formulation were provided) (Figure 8 and 9). No difference was observed when comparing the two bentonite pellet batches used in this experiment, namely the CT and the Nagra pellets (Figure 8).

When comparing the location of the sampled bentonite (i.e., the interior vs. the exterior (Figure 9), we observe that the initial block samples have up to 7-times greater SRB counts in the interior compared to the exterior part. Two out of three 1.5 years modules (M1-M3) and two out of the three 5.5 years modules (M4-M6) showed the same trend, higher interior vs. exterior counts. For two out of three 2.5 years modules (M13-15), this trend is reversed, higher exterior vs. interior counts. All three 1 year modules (M16-18) showed higher counts at the exterior location compared to the interior. The lack of consistency in the number of viable SRBs as function of their location and the high deviation within the module layers does not allow to answer the question whether SRB can migrate from the surrounding porewater into bentonite. In addition, the observation might be an experimental artefact because when the bentonite is sampled for culturing, a cheese knife and a wide spatula are used, cutting first through the outer parts of the bentonite core and hence potentially distributing SRB from the filter-bentonite interface into deeper layers of bentonite. A change in the sampling procedure, e.g., by removing the outer-most millimeter of bentonite before cutting the core, could help circumvent this potential artefact.

The analyses conducted here provide only information about the viability of microorganisms in bentonite. The finding, which is in line with previous work [8], shows that viable microorganisms are present in bentonite. The question of whether a specific bentonite density might preclude microbial survival has been investigated by several groups and the results do not provide a clear density threshold. For Febex [6], the threshold is around 1600 kg m^{-3}, whereas for MX-80 [8], the threshold appears to be closer to 1500 kg m^{-3}. It would appear from this work that a single dry density threshold may not be applicable but rather than the ability of bentonite to inhibit microbial growth and activity may depend on the degree of saturation of the bentonite. Using the microbial results, we can hypothesize that full bentonite saturation may take several years and that oxygen may persist as adsorbed on the clay for long periods of time. Thus, microbes can thrive within the bentonite until it reaches full saturation and appear to persist at a stable number for extended periods. These results

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also raise the question of whether there is a resurgence of microbial activity after several years of exposure to a repository environment.

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3 Conclusions

An *in situ* corrosion experiment was set up and operated in the Mont Terri Underground Research Laboratory and a first set of three modules was removed for detailed analysis after ~ 20 months of exposure, a second set of three modules after ~ 33 months of exposure and a third and fourth set after 12 and 66 months, respectively. Corrosion analyses have been carried out on the first set of modules. The main conclusion from this analysis is as follows:

The bentonite with the lowest density (1.25 g/cm^3) exhibited the highest numbers of anaerobic and aerobic heterotrophs. In contrast, there was little difference between 1.45 and 1.55 g/cm^3 in terms of viable heterotrophs. Viable SRB counts were similar across the bentonite targeted dry densities. Viability in bentonite varies over deployment time but not the expected manner. The maximum microbial viability is reached at 1.5 years and decreases only after 2.5 years. This behavior is hypothesized to be linked to the degree of saturation of the bentonite, which may require several years.

4 Acknowledgement

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