Report
R-17-18
April 2018

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ISSN 1402-3091
SKB R-17-18
ID 1588702
April 2018

Bacterial sulphide- and acetate-producing activity in water saturated Calcigel bentonite cores in a wet density gradient from 1 750 to 2 000 kg m^{-3}

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Abstract

In a future repository for spent nuclear fuel, the dominant long-term corrosive agent may be sulphide from anaerobic bacterial processes such as the dissimilatory reduction of sulphate or sulphur to hydrogen sulphide, disproportionation of thiosulphate to sulphide and sulphate and desulfurylation of organic-S by various sulphide-producing bacteria (SPB). An issue that needs to be addressed is where bacterial sulphide-producing activity may take place in bentonite buffers surrounding copper canisters. One Calcigel bentonite core of low-density (1 750 kg m^{-3} wet density) and one core of high-density (2 000 kg m^{-3} wet density) were placed on top of each other which created a core with two different densities. The high and low-density cores were water saturated in separate test cells. Once full water saturation had been obtained, the low-density core was taken out and transferred to the test cell containing the high-density core. The two cores were identical in volume meaning that the merged core had twice the volume as the un-merged ones. This work seeks to investigate if bacterial sulphide-producing activity takes place only in the interface between the low-density bentonite and a simulated canister surface (copper disc) or in the whole, merged clay core.

The initial intention to compare bacterial sulphide-production in two communicating cores of bentonites with different densities could not be achieved due to a rapid re-distribution of water and wet density between the clay cores. Sulphate was reduced and lactate was oxidized to acetate in both test cells and in both density core parts. There were a large number of cultivable sulphate-reducing bacteria (SRB) in all samples positions which corroborates the observed sulphate-reduction and lactate oxidation. More copper sulphide was observed on the disc exposed the shortest time (33 days) despite the fact that more lactate was oxidised after 78 days compared to 33 days. This may be related to uneven distribution of growing SRB in the clay cores and to reactions between produced sulphide and iron in the clays that can immobilise sulphide as FeS and sulphur.

Contents

1 Introduction

In the Finnish and Swedish repository concepts for geodisposal of spent nuclear fuel (SNF) the bentonite barrier has an important function in maintaining the integrity of the copper canisters isolating the SNF (SKB 2010). In the repository, a highly compacted bentonite with a bulk wet density between 1 950 and 2 050 kg m^{-3} is projected. The bentonite is requiered to hinder outward transport of radionuclides and inward transport of corrosive groundwater components, and to act as a buffer against rock movements. The presence and activity of sulphide-producing bacteria (SPB) have been detected in groundwater at repository depth (Hallbeck and Pedersen 2012, Pedersen et al. 2014) as well as in various types of commercially available bentonites including Asha, Calcigel, Wyoming MX-80 and Rokle (Svensson et al. 2011). Sulphide-producing bacteria have been found in full scale demonstration repositories (Arlinger et al. 2013, Bengtsson et al. 2017b), in various pilot and full scale tests of bentonite performance (Karnland et al. 2009, Svensson et al. 2011) and in the Boom Clay formation (Bengtsson and Pedersen 2016). In a future SNF repository, the dominant long-term corrosive agent may, therefore, be sulphide. The anaerobic bacterial processes of concern are consequently 1) the dissimilatory reduction of sulphate or sulphur to hydrogen sulphide, 2) disproportionation of thiosulphate to sulphide and sulphate and 3) desulfurylation of organic-S by various SPB.

If given good growth conditions, i.e. carbon and energy sources and sulphate, the SPB can rapidly multiply several orders of magnitude and then the produced sulphide over time may become large. Therefore, it is required to test the present hypothesis that sulphide-producing activity of SPB will be very slow or nil in the planned bentonite density range and concomitant swelling pressures. Bacterial sulphide-producing activity has been demonstrated to decrease with increasing density of bentonite (Jalique et al. 2016, Motamedi et al. 1996, Motamedi 1999, Stroes-Gascoyne et al. 1997). Previous work with Wyoming MX-80 bentonite suggested that bacterial sulphide-producing activity and cultivability of SPB cease somewhere in the range of 1 900–2 100 kg m^{-3} wet density, but the exact cut-off density remained to determine (Masurat et al. 2010, Pedersen et al. 2000a, b). Variables of importance for such activity, in addition to bentonite density can be swelling pressure, pore space and pore water composition, transport conditions to and from the bentonite boundaries, usability of the naturally occurring organic matter present in the bentonite and H_2 from corroding metals, H_2 and CH_4 from geological sources and temperature. The type of bentonite may also have a large influence on survival and activity of SPB. Until now, our laboratory research on survival and activity of SPB in bentonite as functions of wet density has been performed with Wyoming MX-80, Calcigel, Asha, Rokle and GMZ bentonites at wet densities of 1 500 to 2 000 kg m^{-3} (Bengtsson et al. 2017a, Bengtsson and Pedersen 2017). In addition, we have investigated cultivability of bacteria in bentonite from the FEBEX project with a wet density of approximately 2 000 kg m^{-3} (Bengtsson et al. 2017b). The work performed has provided a good understanding of viability and activity of bacteria in the tested range of densities.

An issue that needs to be further addressed is where in the bentonite core the bacterial sulphide-producing activity takes place and if the mobility of sulphide is affected by bentonite density. In this experiment, one core of low (1 750 kg m^{-3} wet density) and one core of high (2 000 kg m^{-3} wet density) density Calcigel bentonite were placed on top of each other which created a core with two different densities. The high- and low-density cores were first water saturated in separate test cells. Once full water saturation had been obtained the low-density core was taken out and transferred to the test cell containing the high-density core. The two cores were identical in volume meaning that the merged core had twice the volume as the un-merged ones. Significant bacterial activity has previously been observed in Calcigel bentonite compacted to 1 850 kg m^{-3} wet density (Bengtsson and Pedersen 2017) which means that in 1 750 kg m^{-3} the assumption was that the activity would be equal or higher. In the same way has bacterial activity previously been observed to be close to nil in densities higher than 2 000 kg m^{-3} wet density.

This work seeks to investigate if bacterial sulphide-producing activity takes place in the whole, merged clay core or just in the interface between copper and the low-density bentonite core. If bacterial sulphide-producing activity only takes place in the interface, then with a high-density bentonite between the copper and low-density bentonite, which is how this experiment was set up, there would

be no sulphide produced close to the copper disc and no sulphide accumulated on the copper disc. A total of 4 test cells were used for the water saturation phase, subsequently these 4 were merged into 2 test cells that were used for the incubation phase. The test cells were harvested for analysis at two different incubation times, 33 and 78 days. Previous experiments have shown an ongoing bacterial sulphide-producing activity inside of the clay core at low and intermediate clay density levels (Bengtsson et al. 2017a, c). However, the accumulated amounts of sulphide on the copper discs were all through too low to be able to account for all the consumed sulphate. Therefore, in this experiment, analysis of lactate and acetate amounts after the experiment was carried out in profiles through the clay core. This provided a new insight on where in the clay core, and to what extent the added lactate had been consumed and acetate produced over the ranges of tested wet densities.

2 Material and method

2.1 Test cells

Four identical test cells were used to create saturated bentonite cores in series with two 1 750 and two 2 000 kg m^{-3} densities. A test cell consisted of a titanium cylinder of inner diameter 35 mm with top and bottom lid attached by six Allen screws for each lid. A piston operated inside the cylinder (Figure 2-1). When the piston was at its most extended position, a confined cavity of height 20 mm was produced inside the cylinder (Figure 2-2). This cavity was filled with Calcigel bentonite powder (see section 2.3). The test cells are described in detail in Bengtsson et al. (2017c).

Figure 2-1. *View of all parts included in a test cell. All parts in contact with the bentonites were made of titanium. See text for details. WS = water saturation.*

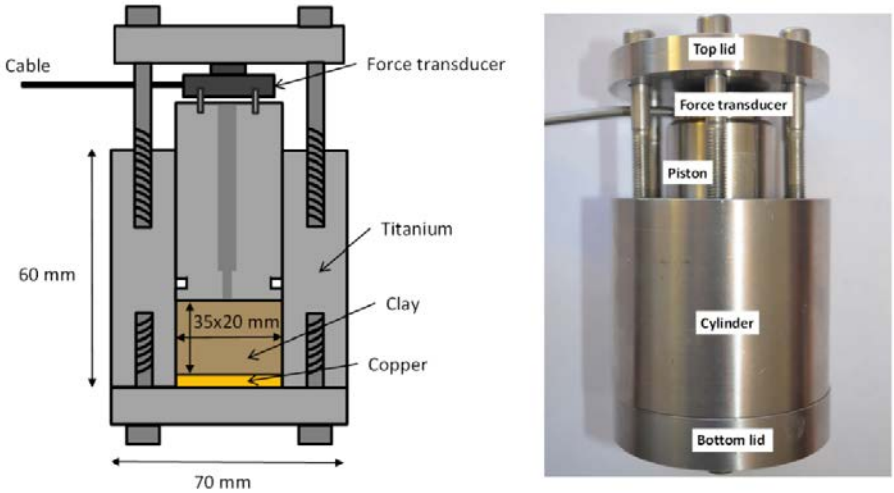

Figure 2-2. *Left: A schematic cross section of a test cell. Right: An assembled test cell, spacers are not mounted.*

2.2 Bentonite suspensions

Bentonite suspensions were prepared as described in Bengtsson et al. (2017c) with the following modifications.

The bacterial species *Desulfotomaculum nigrificans* (DSM 574) were exchanged by *Desulfotomaculum guttoideum* (DSM 4024) due to that it was difficult to produce viable cultures of *D. nigrificans*. *D. guttoideum* is a spore-forming sulphate-reducing bacterium that has many similarities with *D. nigrificans* that also form spores, but is easier to cultivate.

2.3 Compaction and water saturation of bentonite

The day before compaction of the bentonite the water content was determined on the SPB-doped bentonite batches by heating 3 × 1 g of each batch in aluminium containers in 105 °C for 20 h. The average of the weight difference before and after heating for the three replicates was thus equal to the initial water content of each bentonite batch. The amount of bentonite (m_{solids}) needed to obtain the planned wet density for each test cell was calculated using the following equation (from Karnland 2010).

$$m_{solids} = V_{total} \times \rho_m - m_{max\ water}$$

Where ρ_m is the saturated density, m_{solids} is the mass of the solids, $m_{max\ water}$ is the maximum possible mass of water, and V_{total} is the total volume of all components (solids and water). Grain densities were obtained from Karnland et al. (2006). The analysed dry and wet densities agreed with calculated values (± 1 %).

Each test cell was assembled and thereafter water saturated as described in Bengtsson et al. (2017c) with the following modification: instead of using spacers to create 20 mm thick clay cores the spacers used for this experiment were 10 mm shorter. This created clay cores with the dimensions 35 × 10 mm (width, height). After the water saturation phase, when the cores were merged the 20 mm spacers were used.

2.4 Addition of $^{35}SO_4^{2-}$, SO_4^{2-} and lactate

All work performed with additions of $^{35}SO_4^{2-}$ and lactate as well as the cleaning and insertion of the copper discs were carried out in the anaerobic box with an atmosphere consisting of 97 % N_2 and 3 % H_2, O_2 < 1 ppm (COY Laboratory Products, Grass Lake, MI, USA). The bottom lids for the 1 750 kg m^{-3} test cells were removed and the clay cores were extracted by pushing the piston out of the bottom of the cylindrical test cell completely. The clay cores were then transferred to the 2 000 kg m^{-3} test cells and inserted on top of the 2 000 kg m^{-3} clay core, on the piston side, which first was removed. To be able to push the 1 750 kg m^{-3} clay core down through the test cell so that it made contact with the 2 000 kg m^{-3} clay core at the bottom, a needle (BD Microlance, 0.6×25 mm, cat no. 240625, Next2Vet, Ystad, Sweden) was pushed through the 1 750 kg m^{-3} clay core which created a vent for the gas which otherwise would have been compacted upon insertion. The end results were two test cells with bentonite clay cores that measured 35 × 20 mm where half of the core consisted of bentonite with a wet density of 1 750 kg m^{-3} and the other half, closest to the copper disc, 2 000 kg m^{-3}.

Batches of $Na_2{}^{35}SO_4$ (PerkinElmer, cat. no. NEX041H010MC, 10 mCi (370MBq), specific activity: 1 050–1 600Ci (38.8–59.2 TBq $mmol^{-1}$ sodium sulphate in 1 mL water) was distributed over the samples by pipette. In addition, a 5.7 M lactate solution was added to a final calculated pore water lactate concentration of 28 mM in all test cells and the water saturation medium was added with 3 mM $Na_2SO_4^{2-}$. This concentration corresponds to from 1 to 1.5 µmole gdw^{-1} with the highest value in the highest density with the smallest pore water volume. All additions were done on the 1 750 kg m^{-3} side of the test cells. For more details see Bengtsson et al. (2017c). Briefly, at the bottom a Cu disc, then a high density bentonite core and finally a low density one, both with added SPB more or less homogeneously distributed within them. And at the top surface of the low density core lactate, radioactive and non-radioactive sulphate has been added.

2.5 Sampling and analysis

At the sampling date the pressure logging in the force transducer software was stopped, the force transducer was removed together with the top lid and screws. The top lid was then attached again, however with shorter screws to be able to push the piston all the way to the bottom. The test cells were moved to a fume hood and the bottom plates were carefully removed. The piston was then pressed up by turning the screws so that the edge of the copper disc became visible (Figure 2-3). An overview of analyses carried out on the copper discs and the bentonite cores is shown in Table 2-1. All analyses were performed according to the methods previously described in Bengtsson et al. (2017c) with the addition of analysis of lactate and acetate of clay samples taken in profiles through the clay core also described in Bengtsson et al. (2017c), described below.

Weight and volume parameters that was applied at end of the experiment for each test cell can be found in Table 2-2. In addition, the wet densities were calculated as total weight of the clay/volume of the test cell.

Table 2-1. Overview of performed analyses for all test cells.

Analysis	Method
$Cu_2{}^{35}S$ on copper discs	2D surface autoradiography
Most probable number	Cultivation
Distribution of ^{35}S in bentonite cores	Liquid scintillation counting
Distribution of sulphate in bentonite cores	Turbidimetric method by precipitation of $BaSO_4$
Distribution of acetate and lactate in bentonite cores	Enzymatic spectrophotometric methods

Table 2-2. Weight and volume parameters analysed at the end of the experiment for the water saturated Calcigel bentonite in each test cell. (gdw = gram dry weight, %ww = percent wet weight).

Bentonite type	Test cell code	Test cell volume (cm^3)	Amount of bentonite (gdw)	Pore water volume (mL)	Water content (%ww)	Water content SD (n=3)
Calcigel	TC49 1750/2000 (+) 33d.	19.54	26.41	10.63	28.69	0.2
Calcigel	TC50 1750/2000 (+) 78d.	19.54	26.41	11.60	31.75	1.6

Figure 2-3. *Sampling of a test cell in the previous experiment 2 (T8 2000 (+) 47d) as described in Bengtsson et al. (2017c). Left: pushing the piston up by tightening screws. Right: copper disc completely pushed out of the test cell.*

2.5.1 Acetate and lactate analysis

Acetate and lactate concentrations were determined with the enzymatic UV method (kit no. 10148261035 for acetate and kit no. 10139084035, for lactate; Boehringer Mannheim/R-Biopharm AG, Darmstadt, Germany) using a Genesys 10UV spectrophotometer (Thermo Fisher Scientific) for detection. The analyses were tested on two clays with increasing amounts of added lactate or acetate. There was a very good agreement between added and analysed amounts of these compounds. The background values for lactate and acetate in clays without addition of lactate or acetate were previously determined to be in the range of ~0.1 to ~0.7 µmol gdw^{-1} (Bengtsson et al. 2017a).

2.6 Isotope calculations

Measured radioactivity counts were recalculated to mol ^{35}S in the form of $Cu_2{}^{35}S$ via equation (2-1), based on a maximum Instant Imager detector efficiency of 2 % (manufacturer's specification), as follows:

$$n = \left({}^{DPS}/_{\lambda} \Big/ N_A \right) \times 50 \qquad (2\text{-}1)$$

where n is the number of mols, λ is the decay constant for ^{35}S, N_A is Avogadro's constant and DPS is decays per second. The decay constant, λ, was calculated with equation (2-2), as follows:

$$\lambda = \frac{\ln(2)}{t} \qquad (2\text{-}2)$$

where $t_{1/2}$ is the half-life of ^{35}S (i.e. 7.55×10^6 s). Adjustment for decay of ^{35}S during the experimental time was made with equation (2-3), as follows:

$$A = A_0 \times e^{-\lambda t} \qquad (2\text{-}3)$$

where A is activity at time t, A_0 is activity at time zero and t is elapsed time. The amounts of $Cu_2{}^{35}S$ were then calculated using the total elapsed time between start of experiment and the measurement dates. The isotope dilution caused by the added, nonradioactive sulphate to the pore water was calculated by dividing the added concentration of sulphate (3 mM) by the concentration of radioactively labelled sulphate at the start of the experiments. The amount of $Cu_2{}^{35}S$ was multiplied by the isotope dilution factor to obtain the total amount of Cu_2S produced per copper disc. The factor of 50 is attributed to the Instant Imager's count efficiency of 2 %, as specified by the manufacturer.

2.7 Data processing, graphics and statistics

Data processing, statistical analyses and data visualizations were performed using Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, USA) and Statsoft Statistica v 13 (Statsoft, Tulsa, USA) software.

3 Results

3.1 Copper discs

There was a substantial difference in the amount of accumulated $Cu_2{}^{35}S$ on the copper discs of the two test cells (Table 3-1 and Figure 3-1) where TC49 had approximately ten times higher accumulation than TC50. This would be expected if the wet density and the pressure were lower in TC49 than TC50 but that was not the case, the analysed wet density for TC49 was actually slightly higher than for TC50. The accumulation of $Cu_2{}^{35}S$ was evenly distributed over the copper disc for both test cells (Figure 3-1).

Table 3-1. Analysed wet densities, pressures deduced from data obtained with force transducers for each test cell, radioactivity detected on the copper discs recalculated for half-life of the isotope and the total amount of copper sulphide on the discs calculated from the surface activity and the isotope dilutions ($[SO_4^{2-}]/[^{35}SO_4^{2-}]$).

Test cell code	Analysed wet density (kg m^{-3})	Pressure at end of experiment (kPa)	Surface activity (kBq)	Total amount of Cu_2S (nmole)
TC49 1750/2000 (+) 33d.	1897	800	296	310
TC50 1750/2000 (+) 78d.	1869	440	26.4	30.2

3.2 Pressure

After the water saturation phase of the experiment when the two clay cores with different densities were merged the pressure drops swiftly (Figure 3-2). This is probably due to rearrangement of water from the 1750 kg m^{-3} side to the 2000 kg m^{-3} side, and which concomitantly would have changed the volumes of the cores. After approximately 70 days the pressure stabilizes at around 440 kPa in TC50. The pressure was 800 kPa in TC49 when that cell was sampled after 33 days, a similar pressure was recorded for TC50 after 33 days.

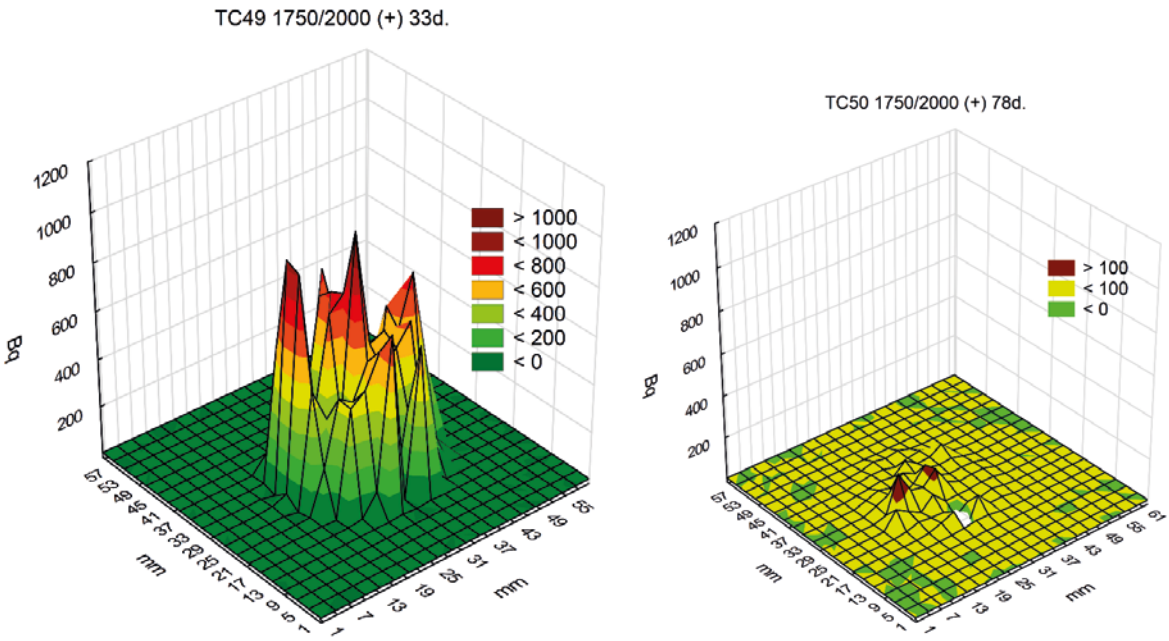

Figure 3-1. 3D raw data plots from autoradiography analysis of copper discs for test cells 49 (top) and 50 (bottom). Color scale according to legend for each image. Counts are adjusted for half-life of the isotope (87.4 days).

Previous experiments with Calcigel bentonite (Bengtsson et al. 2017c) showed that, with the current experimental setup, a wet density of 1 850 kg m^{-3} generated a pressure of approximately 400 kPa, This agrees well with the pressures and wet densities and the measured pressure in TC50 after 78 days of incubation (Table 3-1, Figure 3-2).

3.3 Observation during sampling

When the clay cores were pushed up through the test cells as shown in Figure 2-3, a dark line was observed in the border between the two cores in both test cells with different densities (Figure 3-3). The dark colour likely is due to precipitation of FeS.

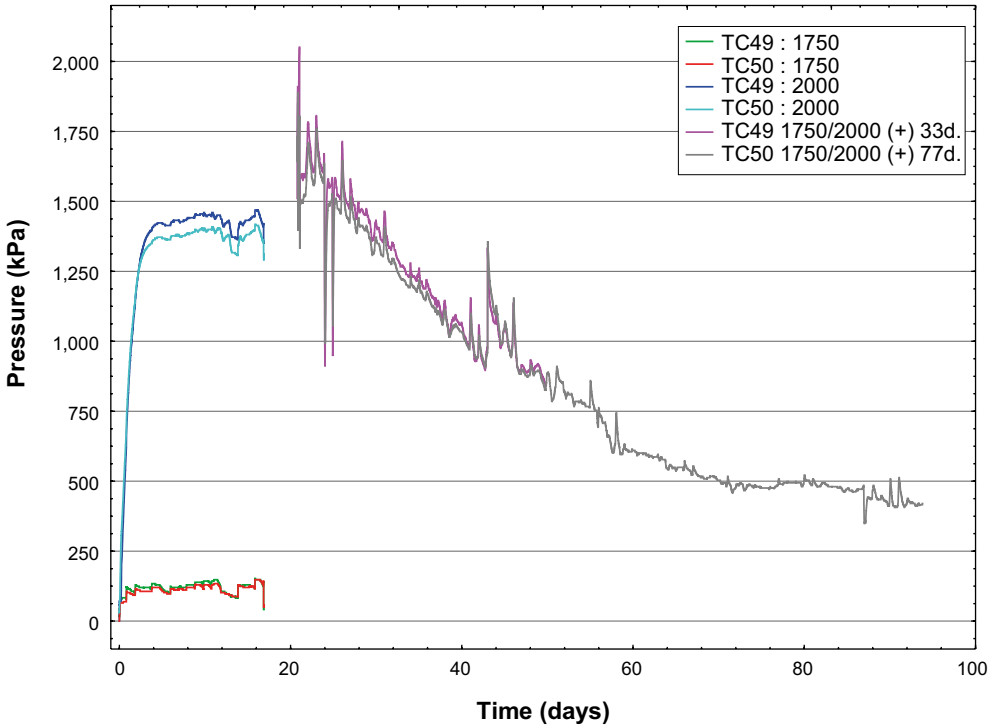

Figure 3-2. *Pressure for four test cells during water saturation phase (0– ~20 days) and then the two combined 1 750/2 000 kg m^{-3} test cells (pink and grey line).*

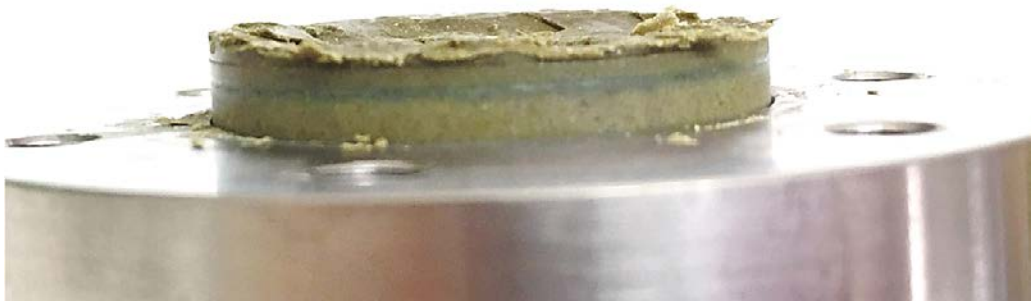

Figure 3-3. *Profile view of the clay core in TC49 during sampling after 33 days. The dark line indicates the border between the two clay cores.*

3.4 MPN-samples

The MPN results demonstrate how well the SPB community survived in the two different bentonite densities during incubation. TC50, which had a low amount of accumulated ^{35}S on its copper disc (Figure 3-4), had very high numbers of viable SPB in the 1 750 kg m^{-3} part of the clay core. However, viable SPB could be cultivated from all positions (Figure 3-4, Table 3-2). TC49 had a more even distribution of viable SPB with no significant increase or decrease in either of the two densities. Likely, the longer incubation time of TC50 had a favorable effect on the SPB which were able to proliferate significantly in the 1 750 kg m^{-3} part of the clay core but were inhibited in the 2 000 kg m^{-3} part.

Table 3-2. Most probable numbers of SPB in pore water of the bentonite cores.

	Position					
	Bottom (3 mm from copper discs)		Middle (8 mm from copper discs)		Top (13 mm from copper discs)	
Test cell code	10^{-6} × (MPN L^{-1})	Lower–upper 95 % confidence interval	10^{-6} × (MPN L^{-1})	Lower–upper 95 % confidence interval	10^{-6} × (MPN L^{-1})	Lower–upper 95 % confidence interval
TC49 1750/2000 (+) 33d.	54.7	20.5–171	3.52	1.41–14.1	11.7	4.83–33.1
TC50 1750/2000 (+) 78d.	18.6	6.19–74.2	48.5	18.2–152	>870	–

3.4.1 Distribution of ^{35}S in the bentonite cores

In Figure 3-5 the distributions of added ^{35}S in the bentonite cores are shown as nanomoles per litre pore water. Results were adjusted for the half-life of the ^{35}S isotope and the scintillation instrument efficiency (36 %). The analysis method does not separate $^{35}SO_4^{2-}$ from $H^{35}S^-$ in the bentonite pore water. Further, the analysis does not detect immobilised ^{35}S isotope, e.g. as $Fe^{35}S$. The results show that the isotope generally was evenly consumed or immobilised through the bentonite cores.

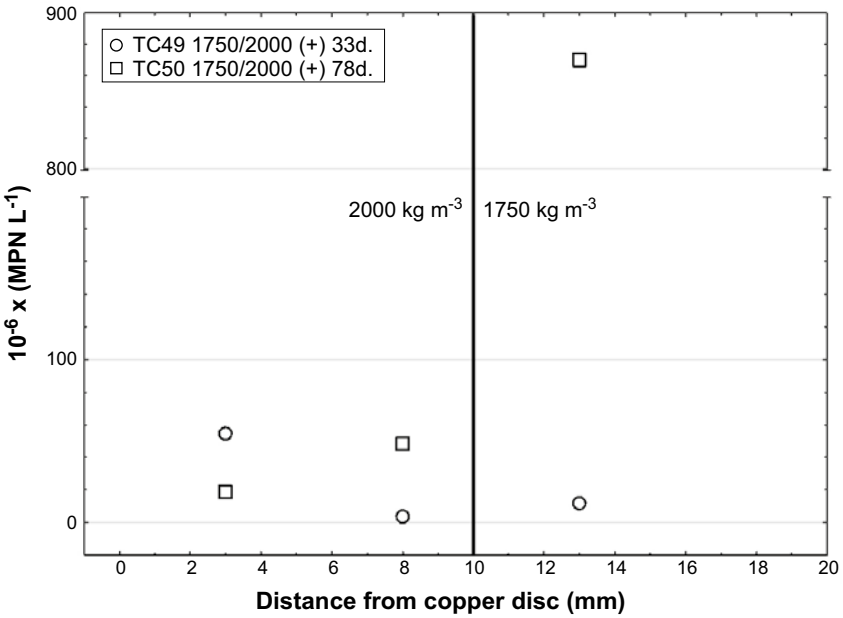

Figure 3-4. *Most probable numbers of sulphide producing bacteria in pore water of bentonite cores for both test cells over wet density. The vertical line shows where in the clay core the original border between the 1 750 and 2 000 kg m^{-3} wet densities was situated.*

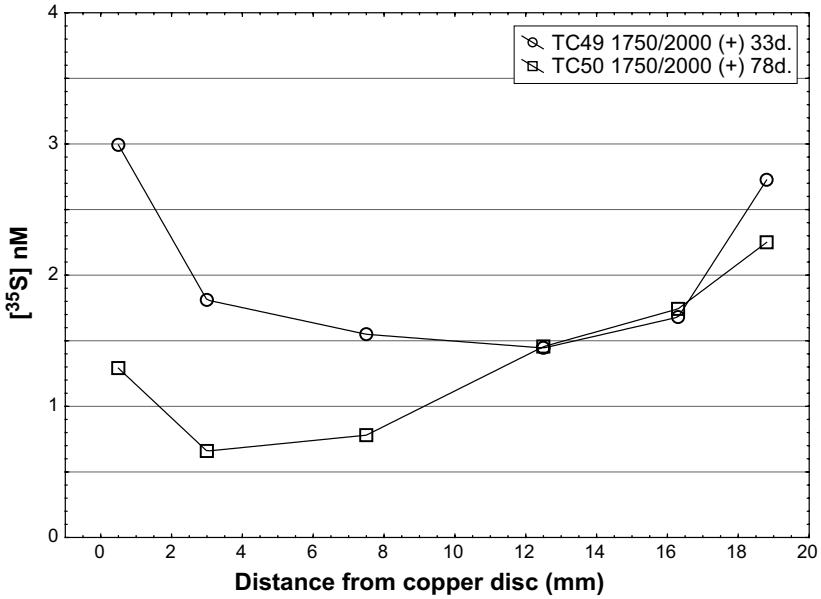

Figure 3-5. *Concentrations of ^{35}S in profiles in the bentonite cores for each test cell. Test cell number, bentonite density, addition of bacteria (+/−) and incubation time according to symbol description. The calculated added concentration of ^{35}S was 53 nM.*

3.5 Amounts and distribution of lactate, acetate and sulphate in the bentonite cores

The concentrations of leachable sulphate after the experiment are on the border to below detection (< 0.05 µmol gdw^{-1}) which shows that all of the added sulphate (1.21 to 1.32 µmol gdw^{-1}) likely were reduced to sulphide (Table 3-3). The profiles of lactate amounts were horizontal in both test cells over the core length after 33 days demonstrating that lactate diffused to all parts of the clay core within a month (Figure 3-6). In contrast, acetate amounts increased in the 1 750 kg m^{-3} part of the clay cores, indicating that bacterial activity was elevated in the low-density part of the clay core. TC50 which had a 45 day longer incubation time than TC49 also had higher values of produced acetate on every position through the clay core. In all positions the produced acetate levels were higher than the added lactate (green line in Figure 3-6) which shows that the active bacteria also were able to utilise naturally occurring organic matter in the clay as carbon source.

Comparing the lactate consumption and acetate production results with the accumulated $Cu_2{}^{35}S$ on the copper discs shows that the overall bacterial activity, analysed as sulphate consumption and acetate production, was higher in TC50 than TC49, even if TC49 had ten times higher values of accumulated $Cu_2{}^{35}S$ on its copper disc than TC50.

Table 3-3. Average amounts of analysed sulphate in pore water of the clay cores, the calculated amount of added lactate at start of the experiment and the analysed amounts of lactate and acetate at end of the experiments. SD = standard deviation, n=6.

Test cell code	Average sulphate (µmol gdw^{-1})	SD	Added lactate (µmol gdw^{-1})	Average lactate (µmol gdw^{-1})	SD	Average acetate (µmol gdw^{-1})	SD
TC49 1750/2000 (+) 33d.	0.07	0.12	11.1	1.97	1.20	19.4	3.95
TC50 1750/2000 (+) 78d.	0.06	0.07	11.1	0.69	0.66	25.5	3.44

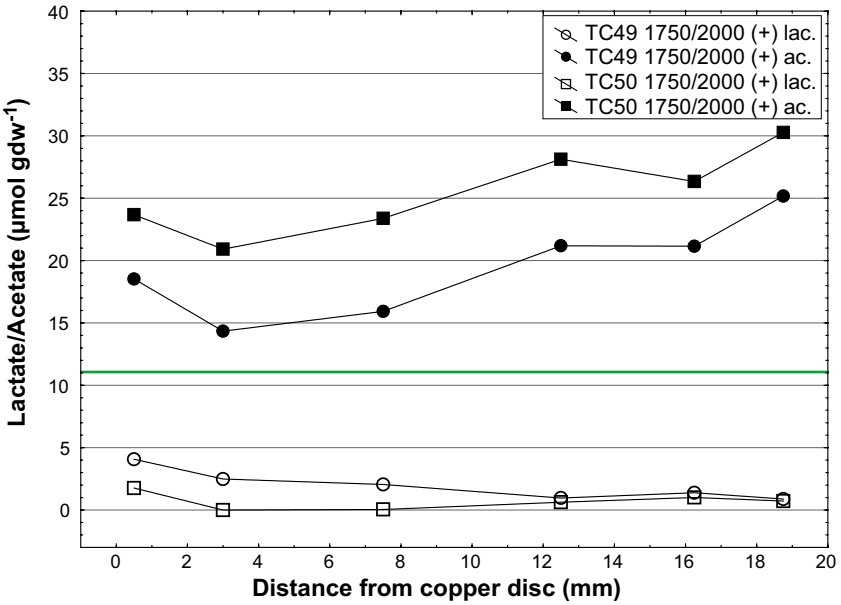

Figure 3-6. *Amounts of lactate and acetate in profiles of bentonite cores for each test cell. Test cell number, bentonite density, addition of bacteria (+/–) and incubation time according to symbol description. The green horizontal line indicates the level of added lactate for both test cells.*

4 Discussion

The original plan for this work, as designed by SKB, was that the densities of the two cores should not change over the experimental time period. Sulphide production, if any, was assumed to occur in an interface between low-density bentonite (1 750 kg m^{-3}) and the copper surface. This hypothesis was tested in this experiment by placing a high-density bentonite core closest to the copper surface which eliminated such an interface. The results in this report show that the experiment did not develop as planned with respect to stability of the densities. Still, important information can be obtained from the results.

4.1 Experimental set-up

4.1.1 Wet density and pressure

The average density of two equally sized, water saturated clay cores with 2 000 and 1 750 kg m^{-3} wet densities should theoretically be 1 875 kg m^{-3}. The analysed values were at most 1.2 % higher than planned (Table 3-1) which must be regarded as good precision of preparation of the two pooled clay cores. When the two cores were pooled, the pressure dropped rapidly due to re-distribution of water from the low-density to the high-density core part concomitant with a change in volume of the two clay cores. Although there was a high-density core next to the copper disc for some time, best represented by TC49 that was analysed after 33 days, it seems as if both cores had a similar density in TC50 after 78 days when the decrease in pressure had diminished.

4.1.2 Water distribution

There was a small difference in the analysed water content between the two cells (Table 2-2). Likely, this was due to that water was re-distributed from the low-density part of the test cell to the high-density part, thereby equalizing the water content in the clay core. Sampling 1 g clay for water content was done at 3 positions in the centre of the cores. Because there was a strong density gradient in TC49 due to re-distribution of water in the core, as judged from the rapidly decreasing pressure in that cell (Figure 3-2), the 3 samples may not have been representative for the average. In TC50, that was analysed for water content after the pressure had stabilised and re-distribution had diminished, the average may represent the average water content better than the value obtained for TC49.

4.2 Accumulation of $Cu_2{}^{35}S$

In the most recent experiments investigating sulphide-producing bacteria in compacted clays (Bengtsson et al. 2017a, Bengtsson and Pedersen 2017), it became obvious that sulphide did not migrate as a non-reactive monovalent anion in the clay cores inside the test cells. Non-reactive transport is generally assumed in modelling of sulphide transport through the buffer and backfill (King et al. 2010). A discrepancy was observed as the decrease in pore water sulphate concentration was much larger than the modelled decrease based on the amount of $Cu_2{}^{35}S$ on the copper discs. It was proposed that the investigated clays retarded transport of, or immobilised, HS^-. A similar effect was recently observed elsewhere for Wyoming MX-80 clay (Stone et al. 2016). Sulphide was found to reduce ferric iron in the montmorillonite type bentonites Asha, MX-80 and Calcigel under the formation of elemental sulphur, ferrous iron and iron sulphide. These reactions rendered an immobilisation capacity of the clays that was 40 µmole sulphide (g clay)$^{-1}$ or more, depending on the load of sulphide and type of clay.

The copper surface radioactivity in the experiments reported here was 10 times higher in TC49 after 33 days compared to the surface radioactivity in TC50 after 78 days. Sulphide-production was clearly ongoing in both test cells, as judged by the surface radioactivity on the copper discs and the decrease in sulphate, but the amount of sulphide that reached the copper disc varied. If the growth and activity of SPB occurs away from the copper disc, sulphide can be trapped as FeS and sulphur and that sulphide will not reach the copper discs. Previously, it was found that growth of SPB occurred in colonies (Bengtsson et al. 2017a) and in the present experiments, FeS production by SPB was visible in the border between the clay cores (Figure 3-3). Random distribution of sulphide-producing colonies close or distant to the copper discs in TC49 and TC50, respectively, may explain why the TC49 copper disc were more radioactive than was the TC50 copper disc.

Sulphate concentrations in the porewater were at or below the detection limit of the method, there was no leachable sulphate left in the porewater. Likewise, the leachable amount of ^{35}S was very low. Bacterial sulphate-reduction to sulphide can explain the disappearance of sulphate concomitant with precipitation of the produced radioactive sulphide as FeS, or immobilisation as elemental sulphur.

4.3 Lactate comsumption

Lactate was consumed in both test cells in the 1 750 kg m^{-3} core parts. There were also lower concentrations of lactate in the 2 000 kg m^{-3} core parts of both test cells compared to the expected average concentration (Figure 3-6). However, less lactate was found in the TC50 close to the copper disc after 78 days compared to TC49 after 33 days. The experimental design and sampling do not allow a solid conclusion about where this lactate consumption occurred. It may have been ongoing in both clay core parts. The radioactivity on both copper discs suggests that some sulphide-production occurred close to the discs, i.e. in the 2 000 kg m^{-3}. Alternatively, did lactate consumption and sulphide-production take place in the 1 750 kg m^{-3} core parts under continuous re-distribution of consumables and products via diffusive transport to and from the 2 000 kg m^{-3}. The latter alternative requires that at least part of the produced sulphide could move to the copper discs without being trapped as FeS and sulphur.

4.4 Acetate production

Just as for lactate, acetate was detected in approximately similar values for all analysed positions in each test cell although the values tended to be slightly higher in the 1 750 kg m^{-3} core parts. The added SPB were all incomplete lactate oxidizers that expel acetate that is not further metabolised. When the consumed amounts of lactate are compared with the produced amounts of acetate, both test cells had approximately twice as much acetate compared to the added amount of lactate. Consequently, there must have been more organic carbon available in this clay than the added lactate.

Acetogenic bacteria are a diverse group of strictly anaerobic bacteria that play an important part in the global carbon cycle by their production of acetate. Most members also show an outstanding metabolic flexibility for utilizing a vast variety of different substrates, including lactate, carbohydrates and alcohol (Schuchmann and Müller 2016). Metabolic flexibility is a key ability of acetogens to compete in ecosystems and might explain the almost-ubiquitous distribution of acetogenic bacteria in anoxic environments such as the clay cores in the experiments described here. Previously, cultivable acetogens were found in large numbers in various bentonite clays (Svensson et al. 2011) and they are reported to occur in deep Forsmark groundwater at occasionally large numbers (Hallbeck and Pedersen 2012). In the experiments reported here, acetogens must have competed with SPB over the lactate in the clays but also over other organic carbon compounds in the clays. High-resolution analysis of the diversity of organic carbon in bentonite clays, and quantification of specific compounds is needed to fully explain the observed acetate production (Marshall and Simpson 2014). Different clays may have different composition and amounts of organic carbon.

4.5 Numbers of sulphate-reducing bacteria

The MPN analysis was directed towards sulphate-reducing bacteria (SRB) with sulphate as the only electron acceptor in the medium. Other SPB would not grow in the medium, e.g. bacteria that disproportionate thiosulphate to sulphide and sulphate, sulphur-reducing bacteria, and bacteria that desulphurylate of organic-S to sulphide. The MPN were similar in all but one sample from 1 750 kg m^{-3} core part after 78 days (Table 3-2). In that core SRB were present at a much larger number than what was observed for the other five samples. All clay cores were added with similar numbers of SRB (section 2.2) which suggests that the difference was due to growth between sampling TC49 after 33 days and TC50 after 78 days. However, as we deal with living entities, it is difficult to explain in detail how this difference appeared. One possible explanation of the large number of SRB in one sample position and time could be that the sample included a large colony of growing SRB. Such colonies were suggested to be present in clay cores of Rokle and GMZ bentonites (Bengtsson et al. 2017a). There was only one test cell for each sampling time and several parallels would be needed for a statistically significant conclusion. Irrespective of this difference, it can be concluded that SRB survived and were viable (cultivable) in both test cells and in both density core parts.

5 Conclusions

The initial intention to compare bacterial sulphide-production in two communicating cores of bentonites with different densities could not be achieved due to a rapid re-distribution of water and wet density between the clay cores.

Sulphate was reduced and lactate was oxidized to acetate in both test cells and in both density core parts. There were a large number of cultivable sulphate-reducing bacteria in all samples positions which corroborates the observed sulphate-reduction and lactate oxidation. More copper sulphide was observed on the disc exposed the shortest time (33 days) despite the fact that more lactate was oxidised after 78 days compared to 33 days. This may be related to uneven distribution of growing SPB in the clay cores and to reactions between produced sulphide and iron in the clays that can immobilise sulphide as FeS and sulphur.

Since lactate was oxidized and acetate was produced on all positions through the merged clay cores, microbial activity seems to be possible not only at an interface between copper and low-density bentonite but in the whole bulk. Yet, if this activity was sulphide-producing or not cannot be answered by the current experimental setup.

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Arkitektkopia AB, Bromma, 2018

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