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Method development for assessing conditions for exclusion of bacterial sulphide production in bentonite clays

Karsten Pedersen, Andreas Bengtsson, Trevor Taborowski
Microbial Analytics Sweden AB

Daniel Svensson
Svensk Kärnbränslehantering AB

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Abstract

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. Besides rock movements, the biggest threat to the canister in the repository is corrosion. Bacterial activity in the buffer can produce chemical species that may accelerate the corrosion of copper. The most important type of bacteria are sulphate-reducing bacteria (SRB), that produce sulphide.

Micans has, in cooperation with SKB AB, developed a method for investigations of the relation between bacterial sulphide-producing activity and survival of sulphate-reducing bacteria (SRB) and other variables. Previous results indicate a correlation between increasing dry density versus decreasing bacterial sulphide producing activity. The method depended on the use of $^{35}SO_4$ to detect bacterial production of $H^{35}S^-$. However, the use of radiotracers strongly limited the variability of the method due to radiation issues, safety handling, waste processing and more. Therefore, the method has now been further developed using non-radioactive means to detect bacterial sulphide-producing activity. In total, 192 test cells have been loaded with a total of 10 different clays under varying conditions in a series of 9 consecutive tests which are presented in this report. The tested clays trade names were Asha, Bara Kade, Bulgarian, Calcigel, Georgian, Laponite, Moroccan, MX-80, Rokle and Turkish. The effects from varying dry densities ranging from 400 to 1600 kg/m^3, swelling pressures ranging from 80 to 9200 kPa, grain size and additions of lactate and sulphate on activity of SRB have been studied.

During the experiments, test cell pressures were measured continuously. Before and after the experiments, the following parameters were analysed, water content, dry and wet weight, content of soluble, leachable sulphate, Amount of leachable lactate and acetate and amount of sulphur on copper discs using X-ray fluorescence spectrometer (XRF) as well as Energy-dispersive X-ray spectroscopy (EDS). In addition, the swelling pressures of the clays were registered during the water saturation phase. Five different measurements or observations were applied to detect bacterial sulphide producing activity. They were the presence of S on copper discs in amounts exceeding the amount on copper discs in the background without additives, presence of black spots or layers in iron containing clays indicating iron sulphide, reduction of sulphate content compared with background measurements, reduction of lactate content compared with added amounts and production of acetate indicative of incomplete lactate oxidation to acetate by SRB.

Studies 1 to 6 tested different approaches in this method development including reproducibility tests. The summarized data of triple samples showed that data could be reproduced with small variations across identical test cells. The outcome was transformed to a comprehensive method applied in studies 7 to 9 with MX80, Bara Kade and Georgian clays where it was demonstrated that lactate, acetate and sulphate correlated as expected if SRB had been active in the clay cores.

Previous experiments using $^{35}SO_4$ analysed production of copper sulphide on copper discs which implies that most of the detected bacterial activity must have taken place close to the discs, plus possibly produced sulphide in the clay core that diffused towards the discs. The developed method analyses activity in the whole clay core, as consumption of lactate and sulphate plus production of acetate. It is also possible to analyse the presence of viable SRB as shown in study 8. In addition, XRF and EDS analyses of precipitates on the copper discs can be performed as well if required.

Because this method development addressed many issues, and solved challenges along the way, conclusions about cut-off ranges should be evaluated with caution. Still, the observed cut-off ranges for sulphide production were fairly consistent for the studied clays to between 1300 – 1400/1500 kg m^{-3} with exception for Bulgarian, Laponite and Rokle that deviated from this range. The 1300 – 1400/1500 kg m^{-3} range agrees with previously determined cut-off ranges for MX80, Asha and Calcigel.

Sammanfattning

I de finska och svenska slutförvarskoncepten för geodeponering av använt kärnbränsle har bentonitbarriären en viktig funktion för att upprätthålla integriteten hos kopparkapslarna genom att isolera bränslet. Förutom rörelser i berget utgör korrosion av kopparkapslarna det största hotet mot förvaret. Bakteriell aktivitet i bufferten kan producera kemiska ämnen som kan accelerera korrosion av koppar. Den viktigaste typen av bakterier är sulfatreducerande bakterier (SRB), som producerar sulfid.

Micans har, i samarbete med SKB AB, utvecklat en metod för att undersöka sambandet mellan bakteriell sulfidproducerande aktivitet och överlevnad av sulfatreducerande bakterier (SRB) samt inverkan av ett stort antal andra variabler. Tidigare resultat indikerade en korrelation mellan ökande torrdensitet kontra minskande bakteriell sulfidproducerande aktivitet. Metoden var beroende av användningen av $^{35}SO_4$ för att detektera bakteriell produktion av $H^{35}S^-$. Användningen av ett radioaktivt spårämne begränsade dock metodens möjligheter till metodvariation starkt på grund av strålningsproblem, säkerhetshantering, avfallshantering med mera. Därför har metoden nu vidareutvecklats med icke-radioaktiva medel för att detektera bakteriell sulfidproducerande aktivitet. Totalt har 192 testceller laddats med totalt 10 olika lertyper under varierande förhållanden i en serie av 9 på varandra följande tester vilka presenteras i denna rapport. De testade lerornas handelsnamn var Asha, Bara Kade, Bulgarian, Calcigel, Georgian, Laponite, Moroccan, MX-80, Rokle och Turkish. Effekten av varierande torrdensitet från 400 till 1600 kg m^{-3}, svälltryck från 80 till 9200 kPa, kornstorlek och tillsatser av laktat och sulfat på sulfidbildande aktivitet hos SRB har studerats.

Under experimenten mättes testcellerna tryck kontinuerligt. Före och efter experimenten analyserades följande parametrar: vattenhalt, torr- och våt-vikt, innehåll av lakbart sulfat, mängd lakbart laktat och acetat samt mängd svavel på kopparytor med hjälp av röntgenfluorescensspektrometer (XRF) samt energidispersiv röntgenspektroskopi (EDS). Dessutom registrerades svälltrycket hos lerorna under vattenmättnadsfasen. Fem olika mätningar eller observationer tillämpades för att detektera bakteriell sulfidproducerande aktivitet. Dessa var närvaron av S på kopparskivor i mängder som översteg mängden på koppar skivor i bakgrund utan tillsatser, närvaron av svarta fläckar eller lager i järnhaltiga leror vilket indikerar järnsulfid, minskning av sulfatinnehåll jämfört med bakgrundsmätningar, minskning av laktatinnehåll jämfört med tillsatta mängder och produktion av acetat som indikerar ofullständig laktatoxidation till acetat av SRB.

Studier 1 till 6 testade olika variabler i denna metodutveckling inklusive reproducerbarhetstester. De sammanfattade data från trippelprover visade att data kunde reproduceras med små variationer över identiska testceller. Resultaten lade grund till en slutlig metod vilken tillämpades i studierna 7 till 9 med MX80, Bara Kade och georgiansk lera där det visades att laktat och acetat samt sulfat korrelerade som förväntat om SRB hade varit aktiva i lerorna.

Tidigare experiment med $^{35}SO_4$ analyserade produktion av kopparsulfid på kopparskivor, vilket innebär att det mesta av den detekterade bakterieaktiviteten måste ha ägt rum nära kopparskivorna, plus möjligen producerat sulfid i lerkärnan som diffunderat mot skivorna. Den utvecklade metoden analyserar aktivitet i hela lerkärnan, som konsumtion av laktat och sulfat plus produktion av acetat. Det är också möjligt att analysera närvaron av livskraftiga SRB som visas i studie 8. Dessutom kan XRF- och EDS-analyser av utfällningar på kopparskivorna utföras vid behov.

Eftersom denna metodutveckling har hanterat flera tekniska problem och utmaningar längs vägen, bör slutsatser om gränsvärden när sulfidproduktion upphör utvärderas med försiktighet. De observerade gränsvärdena var dock ganska konsekventa för de studerade lerorna till mellan 1300–1400/1500 kg m^{-3} med undantag för Bulgarian, Laponite och Rokle som avvek från detta intervall. Intervallet 1300–1400/1500 kg m^{-3} överensstämmer med tidigare fastställda gränsvärden för MX80, Asha och Calcigel.

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 dry density between 1300 to 1600 kg m^{-3} is projected, corresponding to a saturated density between 1950 and 2050 kg m^{-3}. The bentonite is intended to hinder outward transport of radionuclides and inward transport of corrosive groundwater components, and to act as a buffer against rock movements. Bacterial activity in the buffer can produce chemical species that may accelerate the corrosion of copper. The most important type of bacteria are sulphate-reducing bacteria (SRB), that produce sulphide.

The prerequisites for significant viability of bacteria are sufficient availability of free water, nutrients, and space for living cells to grow. Mechanical forces, low water activity and small pore size will therefore affect bacterial activity in the buffer.

The presence and activity of sulphide-producing bacteria (SPB) have been detected in groundwater at repository depth (Bell et al. 2020; Drake et al. 2017; Hallbeck and Pedersen 2012; Pedersen et al. 2014) as well as in various types of commercially available bentonites including Asha, Calcigel and Wyoming MX-80 (Svensson et al. 2011) and Georgian bentonite (this report). Sulphide-producing bacteria have been found in a full scale demonstration repository (Arlinger et al. 2013), in various pilot and full scale tests of bentonite performance (Karnland et al. 2009; Lydmark and Pedersen 2011) and in the Boom Clay formation (Bengtsson and Pedersen 2016). The presence of SPB in commercial bentonite and their potential to be active after exposure to elevated temperature and salinity has been shown in Masurat et al. (2010a) and Svensson et al. (2011).

There seems to be a correlation between increasing dry density versus decreasing bacterial sulphide producing activity. This correlation has been investigated in detail in this report with focus on which buffer characteristics may limit bacterial sulphide producing activity. In the SR-Can safety assessment, the limit for controlling bacterial sulphide production was set as a dry density of 1250 kg m^{-3} (1800 kg m^{-3} saturated density) This gives a pore space and swelling pressure that lie close to the low pore space and high density and swelling pressure reported previously to suppress bacterial activity in Masurat (2006).

Micans has, in cooperation with SKB AB, developed a method for investigations of the relation between bacterial sulphide-producing activity and survival of sulphate-reducing bacteria (SRB) and large number of variables. The method depended on the use of $^{35}SO_4$ to detect bacterial production of $H^{35}S^{-}$ (e.g. Bengtsson et al. 2017a Figure 1-1). The lower limit of bentonite density for which the bacterial sulphate reduction becomes insignificant, was studied. Conclusions concerning dry density and potential additional constraints limiting bacterial activity were, however, somewhat incomplete. There are results that indicate that there may be a sharp limit in dry density where bacterial sulphate reduction ceases in MX-80 as well as in other bentonites (Bengtsson et al. 2017a; Bengtsson et al. 2017b; Bengtsson et al. 2015). These findings have been verified by Haynes et. al. (2019).

Figure 1-1. Copper surfaces from test cell 3 on the right and test cell 8 on the left analysed in a report by Bengtsson et al. (2015). Test cell 3 at saturated density of 1750 kg m^{-3} and added SRB had 1598 kBq surface activity after 47 days. Test cell 8 at a saturated density of 2000 kg m^{-3} and added SRB after 0.2 days had 0.2 kBq surface activity.

During the experiments, test cell pressures were measured continuously. Before and after the experiments, the following parameters were analysed:

- Water content.
- Dry and wet weight.
- Content soluble, leachable sulphate.
- Amount of leachable lactate and acetate.
- Amount of sulphur on copper surfaces using X-ray fluorescence spectrometer (XRF) as well as Energy-dispersive X-ray spectroscopy (EDS).
- Sulphide can be analysed if the content in clays is relatively high. However, the extractable sulphide concentrations did not go above the detection limit of the applied method in the experiments presented in this report.

Five different measurements or observations can detect bacterial sulphide producing activity:

- Presence of S on discs in amounts exceeding the amount on discs in the background without additives.
- Presence of black spots or layers in iron containing clays indicative of iron sulphide.
- Reduction of sulphate content compared with background measurements.
- Reduction of lactate content compared with added amounts.
- Production of acetate indicates activity of incomplete lactate oxidation by SRB.

1.1 Objectives

A total of nine consecutive studies is presented in this report. The line of studies represents a continuous development of a method that can assess what condition or conditions may exclude bacterial sulphide production in bentonite clays. Here follows a brief description of the respective objectives for each study.

An overview of he studied parameters are shown in Table 1-1.

1.1.1 Study 1 (2019)

The primary focus of study 1 was the XRF analysis of presence or absence of copper sulphide on copper discs at 6 different densities with three bentonite types: Bulgarian, Turkish and Moroccan. This approach was based on the assumption that bacterial sulphide production is an on/off process as function of dry density. The water content and the presence of leachable sulphate in the saturated clay cores were analysed at the end of the experiment.

1.1.2 Study 2 (2020)

In this study Bulgarian, Turkish and Moroccan clays were tested at one low and one high density. They were heat sterilised and bacteria or lactate was not added. Furthermore, the Moroccan clay (not sterilised) was tested at a higher density range than in study 1 with addition of lactate, sulphate and bacteria. Calcigel (not sterilised) was tested as well with these additions with reference to previous experiments where $^{35}SO_4$ was used as a trace substance for sulphide (Svensson et al. 2011). The primary focus was the presence or absence of copper sulphide on copper discs. In addition, the water content and the distribution of sulphate in the saturated clay core was analysed at the end of the experiment.

1.1.3 Study 3 (2020)

This study describes different approaches compared to studies 1 and 2 with Turkish and Moroccan bentonites plus the synthetic clay Laponite. The experiments focused on background, time dependency and grinding of the bentonite clays. The Turkish bentonite clay was compacted at two different dry densities without any additions and incubated for 1, 2 or 4 months. The Moroccan bentonite was tested with a coarse grain density and fine grinded grain density. This bentonite was also compacted at two different dry densities and incubated either 2 or 4 months. Furthermore, the Moroccan bentonite was spiked with SRB prior compactions and lactate was added after the water saturation phase. The synthetic clay Laponite was also compacted at two different dry densities and incubated 4 months. One set of two was spiked with lactate whereas the other two parallel densities had no additions.

1.1.4 Study 4 (2021)

In this study the bentonite clays MX-80, Asha and Rokle were compacted at two dry densities. All bentonite clays and densities were performed in triplets and was spiked with SRB prior compactions and lactate was added after the water saturation phase. The triplet density series had an additional background control sample that was without additions. The main goal with this test was to investigate reproducibility between test cells with identical density and additions in triplicates compared to a non-added control. Further, leachable lactate was analysed at the end of the experiment.

1.1.5 Study 5 (2021)

In this study the bentonite clays MX-80 and Rokle were compacted at two dry densities 450/470 and 1400 kg m^{-3}, corresponding to saturated densities of 1300 and 1900 kg m^{-3}. Every bentonite clay and density were performed in duplicates that were spiked with SRB prior compactions and lactate was added after the water saturation phase. All duplicate series had a background control test cell without additions.

1.1.6 Study 6 (2022)

Laponite has a low density but a high swelling pressure relative to the density. This clay was, therefore, deemed well suited for testing the effect of swelling pressure and density, which of these parameters does first knock out sulphide production in a series of increasing density / swelling pressure? Asha was compacted at the dry densities 1200 and 1280 kg m^{-3}. All bentonite densities were performed in triplets. Two test cells were spiked with SRB prior compactions and lactate was added after the water saturation phase. The third test cell in each series was used as a background control without additions.

1.1.7 Study 7 (2022)

The scope of work was determination of the threshold cut-off density of bacterial activity in MX80 and Bara-Kade (BK) bentonites. During the experiments, pressures were measured continuously. The following parameters were analysed: Water content, dry and saturated densities, content leachable sulphate and the amounts of leachable lactate and acetate.

1.1.8 Study 8 (2023)

Georgian bentonite clay was compacted at six different saturated densities. The test cells were spiked with SRB prior to compactions and lactate was added after the water saturation phase. All densities were performed in triplets and was spiked with SRB prior compactions and lactate was added after the water saturation phase. Each triplet density series had an additional background control sample without additions. Water content, dry and saturated densities, content leachable sulphate and the amounts of lactate and acetate were analysed.

1.1.9 Study 9 (2024)

The scope of work was to analyse the observed point of cut-off bacterial sulphide producing activity in Study 8 by a narrow density range based on the study 8 results with Georgian bentonite.

Table 1-1 Overview of studied variables in studies 1 – 9

Study no	Bentonites	Dry density range (kg m^{-3})	Swelling pressure range (kPa)	Additions	Analyses	Objectives
1	Bulgarian, Moroccan Turkish,	890–1500	800–3000	SRB, Lactate	Swelling pressure, Water content, dry density, sulphate	Copper sulphide on copper discs, post experiment leachable sulphate
2	Bulgarian, Calcigel, Moroccan Turkish	1000–1600	275–5400	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, sulphide	Sterilized clay test, copper sulphide on copper discs, pre- and post-experiment leachable sulphate, expanded density range
3	Laponite, Moroccan, Turkish	1200–1550	500–9200	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, SRB, sulphide	Time dependency, and grinding of the bentonite clay, densities
4	Asha, MX-80, Rokle	1090–1400	50–2650	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, lactate, sulphide	Statistics, reproducibility, background
5	MX-80, Rokle	450–1400	160–2000	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, lactate, sulphide	Grinding and small copper discs in the clay cores
6	Asha, Laponite,	645–1300	240–5800	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, lactate, sulphide	Swelling pressure versus density, artificial iron-free bentonite compared to iron-rich Asha bentonite
7	Bara Kade, MX-80	1000–1500	180–3050	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, lactate, acetate sulphide	Cut-off dry density for two similar types of bentonites. Acetate added to the analysis list
8	Georgian	1000–1500	300–5000	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, lactate, acetate	Cut-off dry density for Georgian betonites. Acetate added to the analysis list
9	Georgian	1250–1425	1200–3500	SRB, Lactate Na_2SO_4	Swelling pressure, Water content, dry density, sulphate, lactate, acetate	Fine tuning of the assumed cut-off density with the Georgian bentonite in study 8

2 Methods

2.1 Test cells

Identical test cells were used to create saturated bentonite cores in series with different densities. A test cell consisted of a titanium cylinder 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 35 × 20 mm (H × W) confined cavity was produced inside the cylinder (Figure 2-2). This cavity was filled with the respective bentonite powder (see section 0). By using spacers (not shown) on the screws running through the top lid the volume inside the test cell was kept constant. The pressure created by the swelling bentonite pushed the piston upwards and by doing so a force transducer mounted between the piston and top lid was compressed. The amount of compression, which stood in direct correlation to the bentonite swelling pressure, was recorded by a data collection system connected to a computer (see section 2.1.1). During the water saturation phase a water saturation (WS) bottom lid and piston were used. The lid had a 2 mm inlet hole which allowed water to enter the test cell and reach the bentonite. In addition, the piston had a longitudinal inside hole to get water inflow from both top and bottom. To stop the bentonite from swelling into the inlet holes, and also to get an evenly distributed inlet flow, a circular 40 µm pore size titanium filter (35 × 2 mm) was mounted with two Phillips screws on the inside of the saturation lid and piston. After the saturation phase the bottom lid and piston were replaced with a lid and a piston without inlet. The new piston was equipped with a removable ventilation plug to not trap gas inside of the test cell upon insertion of the piston. The titanium filter on the saturation bottom lid was replaced with a copper disc.

2.1.1 Force transducer and data collection

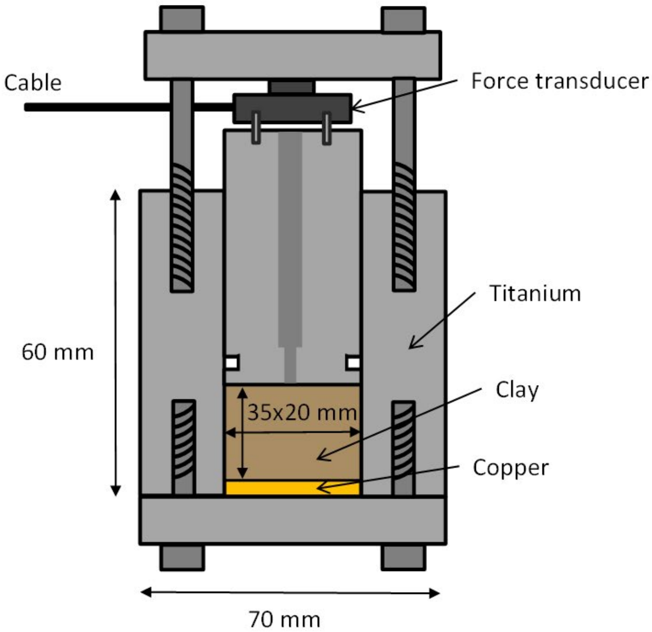
The force transducers used to register the swelling pressure from the bentonite were purchased from Stig Wahlström Automatik, Stockholm, Sweden and had a load range from 0 to 9200 kPa (AL131DL, Honeywell model 53). The force transducers were connected to a data collection system with a programmable logic controller and a computer with a custom-built software (CRS Reactor Engineering, Stenkullen, Sweden) for calibration and monitoring of the force transducer signals. The readings from the transducers were calibrated from 0 to 5 MPa against an externally calibrated manometer (Precision digital pressure model CPG1500, WIKA – AB Svenska Industri-Instrument, Göteborg, Sweden).

2.2 Bentonite slurries

2.2.1 Spiking

The bentonite clays were spiked with three different species of SRB. *Pseudodesulfovibrio aespoeensis* (DSM 10631), *Desulfotomaculum nigrificans* (DSM 574) and *Desulfosporosinus orientis* (DSM 765). *P. aespoeensis* was isolated from deep groundwater (Motamedi and Pedersen 1998), *D. nigrificans* is a thermophilic, spore-forming sulphide-producing bacterium and *D. orientis* is spore-forming sulphide-producing bacterium with the ability to grow with H_2 as source of energy. The bacteria were grown in appropriate medium and temperatures as specified by the German collection of microorganisms and cell cultures (DSMZ). At the start day of the experiments, bacterial numbers for each of the three bacterial cultures were determined in 1 mL samples using the acridine orange direct count method as devised by Hobbie et al. (1977) and modified by Pedersen and Ekendahl (1990). The three different bacterial cultures were mixed into one cocktail and mixed with the respective clay. This created batches of bacteria-doped bentonite with a bacterial content of approximately 1×10^7 SRB g^{-1}.

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.*



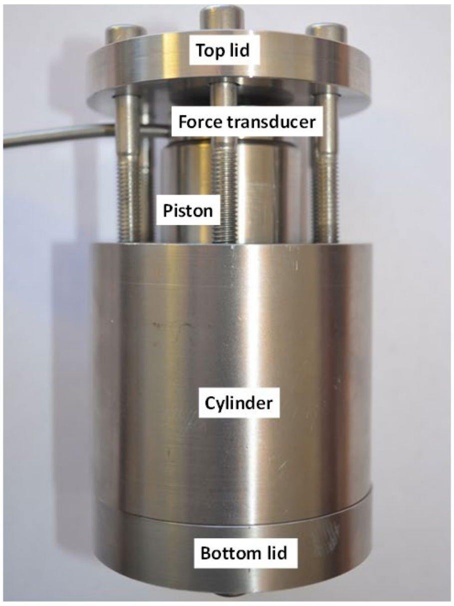


Figure 2-2. *Left: A schematic cross section of a test cell. Right: An assembled test cell, spacers are not mounted.*

2.3 Compaction of bentonite

The day before compaction of the bentonite the water content was determined by heating 3 × 1 g of each batch in aluminium bowls in 105 °C for 24 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 dry bentonite (m_{solids}) needed to obtain each saturated 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).

Each test cell was assembled with bottom lid and a titanium filter and placed on an analytical scale where the planned amounts of bentonite powder (m_{solids}), with or without added SRB, was weighed in. A water saturation piston was inserted in each test cell cylinder and in those cases where the bentonite powder volume was larger than the test cell volume (V_{total}) the bentonite powder was compacted with a workshop press (<25 kg cm^{-2}) (Biltema, Göteborg, Sweden, cat no.15-846).

2.4 Water saturation of bentonite

After compaction of the bentonite the test cells were assembled and mounted on a custom-built water saturation system (Figure 2-3). A sterile, anoxic salt solution was pushed into the evacuated (<10 Pa) system with 200 kPa total pressure. The solution consisted of NaCl, 120 mM; $CaCl_2 \times 2H_2O$, 7 mM; KCl 9 mM; NH_4Cl, 18 mM; KH_2PO_4, 1 mM; $MgCl_2 \times 6H_2O$, 2 mM; 3 mM Na_2SO_4; analytical grade water (AGW) to 1000 mL total volume. The solutions were produced as described by Widdel and Bak (1992) for preparing anoxic media. The pressures created by the swelling bentonites were monitored and the test cells were kept unaltered until stable swelling pressures presented themselves. Water could move freely in and out of the bentonite during the water saturation phase. In the second phase of the experiment where the two titanium filters were replaced with a copper disc and a 2 mm taller piston, respectively; external water was not in contact with the clay. By using an identical confined space before and after contact with water, the swelling pressures were approximately reproduced by spacers used to set the confined volume inside of the test cells. They were kept fitted during the whole experimental time and the reproduced swelling pressures (RSPs) were continuously registered.

Table 2-1. Saturation salt solution

Addition	Amount
Analytical grade water (mL)	1000
NaCl (g L^{-1})	7.0
$CaCl_2 \times 2H_2O$ (g L^{-1})	1.0
KCl (g L^{-1})	0.67
NH_4Cl (g L^{-1})	1.0
KH_2PO_4 (g L^{-1})	0.15
$MgCl_2 \times 6H_2O$ (g L^{-1})	0.5
Na_2SO_4 (g L^{-1}) optional yes or no	1.0

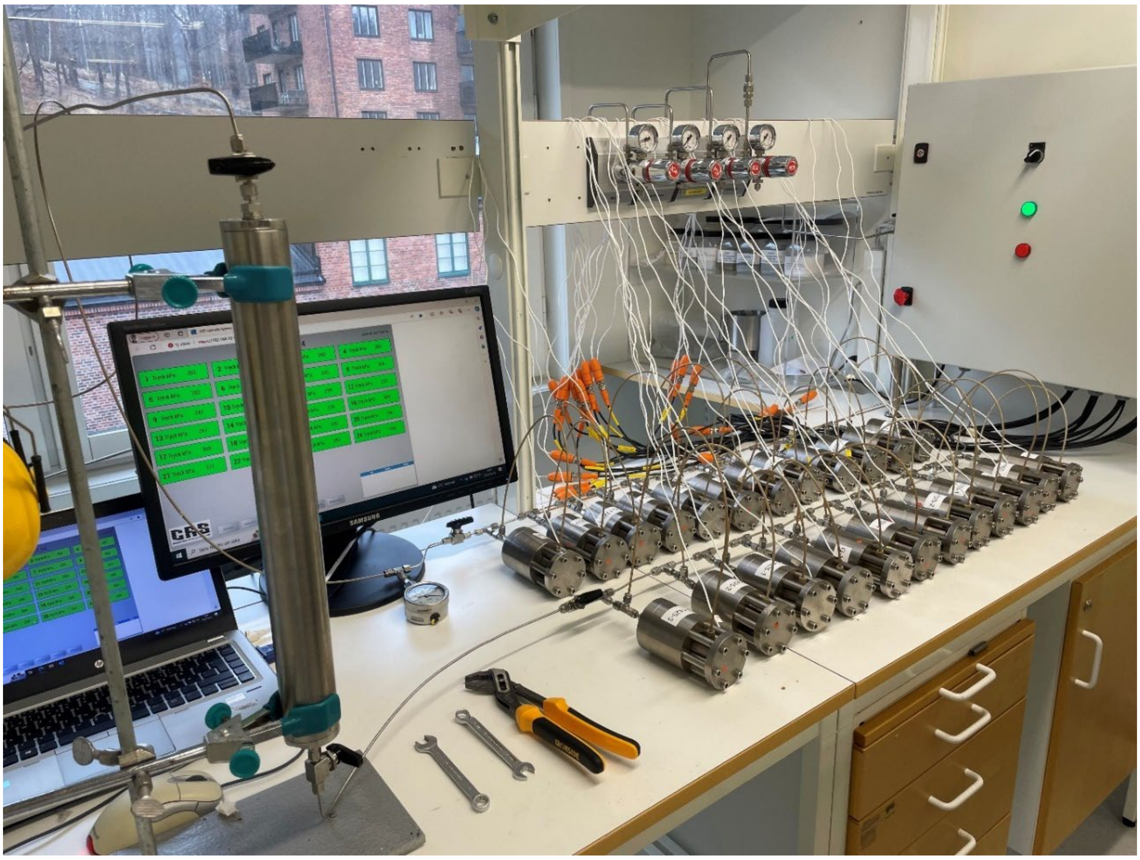


Figure 2-3. *Water saturation system with 24 test cells receiving the saturation salt solution from both top and bottom.*

2.5 Addition of lactate and copper discs

Circular 35 × 2 mm copper discs for XRF/EDS analyses were cleaned in an ultrasonic bath for 5 min in 99 % ethanol and then rinsed with sterile analytical grade water (AGW) (Millipore Elix Essential 3, Millipore, Solna, Sweden). Afterwards the discs were placed for 1 h in a beaker containing 250 mL 0.5 M sulfamic acid (Aminosulfonic acid, H_2NSO_3H) (cat.nr. 24 277-2, Sigma-Aldrich), according to procedures for chemical cleaning of copper in ISO 8407:2010 – Corrosion of metals and alloys – Removal of corrosion products from corrosion test specimens (ISO 847:2009, IDT). The procedure was finished by sequentially washing the discs four times in glass beakers containing 400 mL sterilized, anoxic AGW at pH 7.

All work performed with addition of lactate as well as the insertion of the copper discs were carried out in an anaerobic box (Figure 2-4).

The test cells were disconnected from the water saturation system and the bottom lids with titanium filters, together with top lids and force transducers were removed. In each cell a copper disc with the same size as the titanium filter was inserted in the cavity that the filter left in the bentonite.

A new bottom lid without inlet was attached to each test cell. A 5.7 M lactate solution was added on the core side opposite to the copper disc to a final calculated pore water lactate concentration of 23 mM in pore water of the spiked clays. The test cells were then reassembled with new pistons with ventilations plugs, force transducers and top lids. By once again attaching the spacers to the screws running through the top lids, the same inside confined as before was obtained.

Figure 2-4. *Addition of copper discs and lactate to the test cells in an anaerobic box.*

2.6 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 bottom discs were then carefully removed. The piston was pressed up by turning the screws so that the edge of the copper disc became visible.

2.6.1 Copper discs

The copper discs were removed with tweezers and were put in Petri dishes with AGW to remove the remaining clay particles by gently washing them. The copper discs were then transferred to the anaerobic box and left to dry. When dried the copper disc were photographed. Then the sulphur that had attached on the copper discs was located and quantified as mass % using X-ray fluorescence. Analyses were performed with the Delta XRF analyser (Scantec Nordic AB, Jonsered, Sweden) on the top of the copper disc that had been in contact with the bentonite clay (Figure 2-5). In addition, XRF and EDS analyses were performed by Daniel Svensson at the Äspö clay laboratory in study 2.

Figure 2-5. *Analysis of sulphur on the copper disc with the Delta XRF analyser.*

2.6.2 Bentonite samples

Bentonite samples, approximately 2 g each, were taken out from three core positions: Close to the copper disc, in the middle of the bentonite core and close to the piston. The positions were denoted 1, 2 and 3 where number 1 was close to the copper surface and so on. Samples at each position were taken for analysis of water content, distribution of sulphate, lactate and acetate in the bentonite.

2.6.3 Water content samples

Bentonite for water content analysis, approximately 2 g were carefully weighed in pre-weighed polypropylene tubes. The samples were then dried in a laboratory oven at 105 °C for 24 h and weighed again. The difference in weight before and after drying was taken as the bentonite water content.

2.6.4 Analysis of sulphate, lactate and acetate

The samples for sulphate, lactate and acetate analysis, approximately 2 g, were dispersed in 20 mL of a 10 % $MgCl_2$ solution on a shaker until totally dispersed.

Sulphate concentrations of the different positions of the bentonite core were determined using the turbidimetric SulfaVer4 $BaSO_4$ precipitation method (Method #8051, range 2 − 70 mg L^{-1}, HACH Lange, Sköndal, Sweden). Analyses were made on a HACH spectrophotometer model DR/2500 Odyssey (HACH Lange, Sköndal, Sweden). Sulphate analysis was also performed on raw bentonite before the start of the experiment to determine the amounts of leachable sulphate in each bentonite type. This was done by dispersing approximately 1 g of each bentonite in 20 mL of a 10 % $MgCl_2$ solution. The concentration of sulphate was then measured with the SulfaVer4 method on diluted or undiluted supernatant.

Lactate concentrations were determined with the enzymatic UV method (K-LATE, Megazyme, Wicklow, Ireland) using a Genesys 10 UV spectrophotometer (Thermo Electron Corporation) for detection. The concentration of lactate was measured on diluted or undiluted supernatant from the sulphate samples. Acetate concentrations were determined with the enzymatic UV method (K-ACET, Megazyme, Wicklow, Ireland) as done for lactate.

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 Study 1

3.1.1 Experiment plan

In this study, three different bentonite types and the three different species of added SRB exposed to varying levels of saturated density as shown in Table 3-1. The planned dry densities were calculated from planned saturated densities. The clays were added with SRB. Sulphate was not added but leachable sulphate was analysed for in each clay. Only Moroccan clay demonstrated leachable sulphate at a concentration of 1.22 mg SO_4 gdw^{-1}. Lactate was added when the copper discs were installed as shown in Figure 2-4 after a water saturation period of 16 days.

Table 3-1. List of test cells with bentonite and planned dry densities and additions. Y= Yes, N=no

Name of test cell	Bentonite	Planned dry densities (kg m^{-3})	Planned saturated densities (kg m^{-3})	Additions		
				SRB	Sulphate	Lactate
Bulgarian 1	Bulgarian	891	1600	Y	N	Y
Bulgarian 2	Bulgarian	965	1650	Y	N	Y
Bulgarian 3	Bulgarian	1039	1700	Y	N	Y
Bulgarian 4	Bulgarian	1113	1750	Y	N	Y
Bulgarian 5	Bulgarian	1188	1800	Y	N	Y
Bulgarian 6	Bulgarian	1262	1850	Y	N	Y
Turkish 1	Turkish	1114	1700	Y	N	Y
Turkish 2	Turkish	1186	1750	Y	N	Y
Turkish 3	Turkish	1261	1800	Y	N	Y
Turkish 4	Turkish	1343	1850	Y	N	Y
Turkish 5	Turkish	1418	1900	Y	N	Y
Turkish 6	Turkish	1495	1950	Y	N	Y
Moroccan 1	Moroccan	1066	1700	Y	N	Y
Moroccan 2	Moroccan	1142	1750	Y	N	Y
Moroccan 3	Moroccan	1219	1800	Y	N	Y
Moroccan 4	Moroccan	1295	1850	Y	N	Y
Moroccan 5	Moroccan	1371	1900	Y	N	Y
Moroccan 6	Moroccan	1447	1950	Y	N	Y

3.1.2 Water content, dry density, sulphate and swelling pressure

The test cells were opened and analysed after a total time of 66 days, i.e. 50 days after addition of copper discs and lactate. The results of the water content analysis and resulting dry densities are shown in table 3-2. The planned and analysed water contents differed at most 3 % and for the dry densities at most 6 %. Sulphate was absent in all clay samples (data not shown). Table 3-3 shows the registered swelling pressure at the end of the saturation phase, day 16.

Table 3-2. Weight, water content and dry density for the water saturated bentonites in each test cell. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Bentonite type	Amount of bentonite (gdw)	Planned water content (%ww)	Analysed water content (%ww)	Planned dry density (kg m^{-3})	Analysed dry density (kg m^{-3})
Bulgarian 1	Bulgarian	17.40	44.33	46.72	891	840
Bulgarian 2	Bulgarian	18.86	41.52	40.26	965	973
Bulgarian 3	Bulgarian	20.31	38.88	38.38	1039	1042
Bulgarian 4	Bulgarian	21.76	36.38	33.32	1113	1131
Bulgarian 5	Bulgarian	23.21	34.02	33.73	1188	1189
Bulgarian 6	Bulgarian	24.66	31.80	30.78	1262	1268
Turkish 1	Turkish	21.71	34.65	34.05	1111	1114
Turkish 2	Turkish	23.26	31.98	32.73	1190	1186
Turkish 3	Turkish	24.81	29.47	31.00	1270	1261
Turkish 4	Turkish	26.36	27.08	28.24	1349	1343
Turkish 5	Turkish	27.91	24.83	26.76	1428	1418
Turkish 6	Turkish	29.46	22.68	25.20	1508	1495
Moroccan 1	Moroccan	20.84	37.28	34.45	1066	1083
Moroccan 2	Moroccan	22.33	34.72	31.69	1142	1159
Moroccan 3	Moroccan	23.81	32.30	33.89	1219	1209
Moroccan 4	Moroccan	25.30	30.01	29.58	1295	1297
Moroccan 5	Moroccan	26.79	27.84	28.79	1371	1366
Moroccan 6	Moroccan	28.28	25.79	26.65	1447	1443

3.1.3 Copper discs

The copper discs showed decreasing black discolouration from S-Cu precipitates with increasing dry density (Figure 3-1) on the side that was in contact with the bentonite clays. However, the first copper disc of each tested bentonite clay showed less black discolouration than the second one which had a higher dry density. Possibly, the low density reduced contact between clays with SRB and the copper surface, but this is only speculation. Alternatively, SRB activity may have been so large at the lowest swelling pressure and density that lactate was consumed before it reached the vicinity of the copper disc which would reduce the amount of sulphide that could react with the copper disc. This is because sulphide readily reacts with bentonite and is, thereby, immobilized (Pedersen et al. 2017).

Table 3-3 shows the average results of sulphur on the copper discs from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured, corresponding to approximately 50 % och the disc area. The results show that for each bentonite the amount of sulphur decreases with increasing dry density. However, the first copper disc of each tested showed a lower amount of sulphur than the second copper disc, as mentioned above.

The copper discs were washed with AGW after removal from the test cells. Mechanical force to remove clay not washed away was avoided due to the risk of removing also corrosion products. The XRF analysis showed silicon from 0.6 – 11.7 % which stems from the bentonite clays. These clay residues might have obscured some sulphur, and the actual amount of sulphur might have been somewhat larger where silicon was measured. However, no obvious correlation between Si and S could be found, the distribution appeared random (Figure 3-2).

Figure 3-1. *Copper discs from every test cell after 50 days.*

Table 3-3. Swelling pressures at day 16 of the water saturation phase deduced from data obtained with force transducers for each test cell and average sulphur and silica values from XRF measurements (n = 5)

Name of test cell	Bentonite type	Analysed dry density (kg m^{-3})	Swelling pressure (kPa)	Average S (%)	Standard deviation S (± %)	Average Si (%)
Bulgarian 1	Bulgarian	840	740	4.04	1.64	2.7
Bulgarian 2	Bulgarian	973	890	9.64	1.55	7.9
Bulgarian 3	Bulgarian	1042	770	7.25	0.71	6.8
Bulgarian 4	Bulgarian	1131	1400	5.84	0.64	9.3
Bulgarian 5	Bulgarian	1189	1640	1.25	0.11	3.2
Bulgarian 6	Bulgarian	1268	2600	0.83	0.06	2.8
Turkish 1	Turkish	1114	980	1.75	0.70	2.9
Turkish 2	Turkish	1186	1030	8.90	2.96	4.4
Turkish 3	Turkish	1261	1200	1.29	0.14	3.3
Turkish 4	Turkish	1343	1830	0.80	0.20	9.7
Turkish 5	Turkish	1418	2170	0.79	0.09	9.6
Turkish 6	Turkish	1495	3000	0.34	0.19	1.6
Moroccan 1	Moroccan	1083	900	3.08	0.49	0.6
Moroccan 2	Moroccan	1159	1030	18.7	1.35	3.8
Moroccan 3	Moroccan	1209	1330	6.74	1.11	11.7
Moroccan 4	Moroccan	1297	1800	13.6	0.95	7.4
Moroccan 5	Moroccan	1366	2200	11.6	0.30	5.7
Moroccan 6	Moroccan	1443	2550	7.77	2.66	3.7

3.1.4 Summary of results and observations

There was a strong decrease in S over a dry density of 1200 kg m^{-3} (Bulgarian) and 1250 (Turkish) kg m^{-3} on the copper discs exposed to the Turkish and Bulgarian bentonites while relatively large amounts of S was found on all discs exposed to the Moroccan bentonite (Table 3-3, Figure 3-3). Other elements analysed were Si, Ca and Cu where Cu was the dominating substance detected (data not shown). Moroccan bentonite had significant amounts of leachable sulphate while sulphate could not be leached from the other two bentonites which may partly explain the high sulphide production in Moroccan bentonite. But still, the Turkish and Bulgarian results indicate sulphide production as well at low densities and the sulphate needed likely originated from gypsum. The copper disc exposed to lowest density of Turkish bentonite showed colony formed precipitates of black copper-sulphur precipitates typical for bacterial growth. Throughout the results of this report, appearance of black spots was frequently found when SRB were active in the bentonite.

The swelling pressures of bentonites should typically increase exponentially with increasing density which also was observed in this study (Figure 3-4). While Moroccan and Turkish dry densities followed a trend closely, the Bulgarian bentonite showed 300 – 400 kPa larger swelling pressures for similar Moroccan and Turkish dry densities.

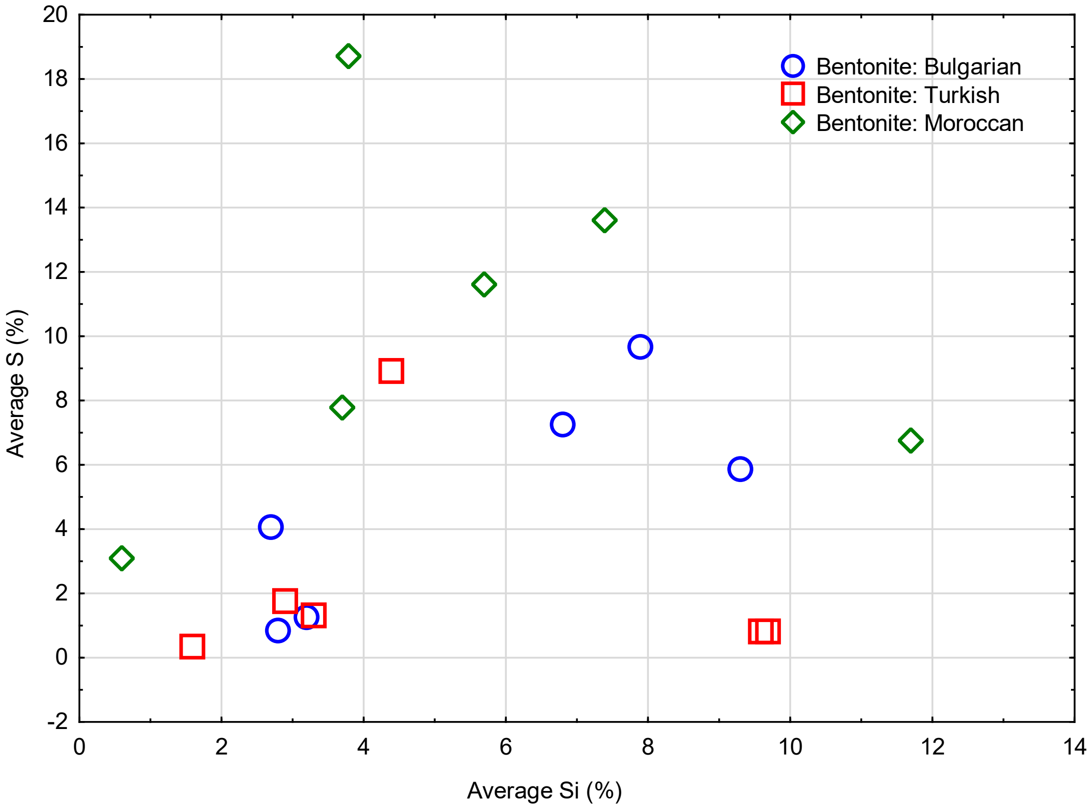


Figure 3-2. *Average Si versus average S on the copper discs on three bentonite types.*

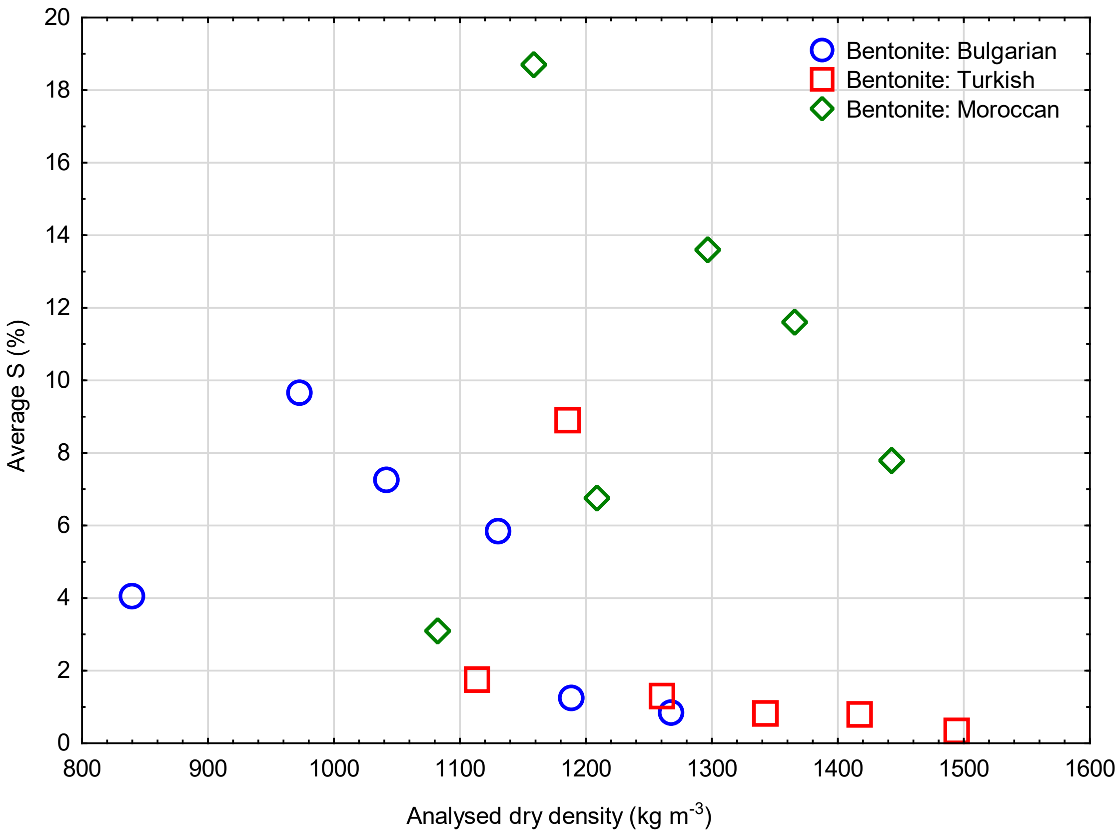


Figure 3-3. *The analysed dry densities versus average S on the copper discs of three studied bentonite types.*

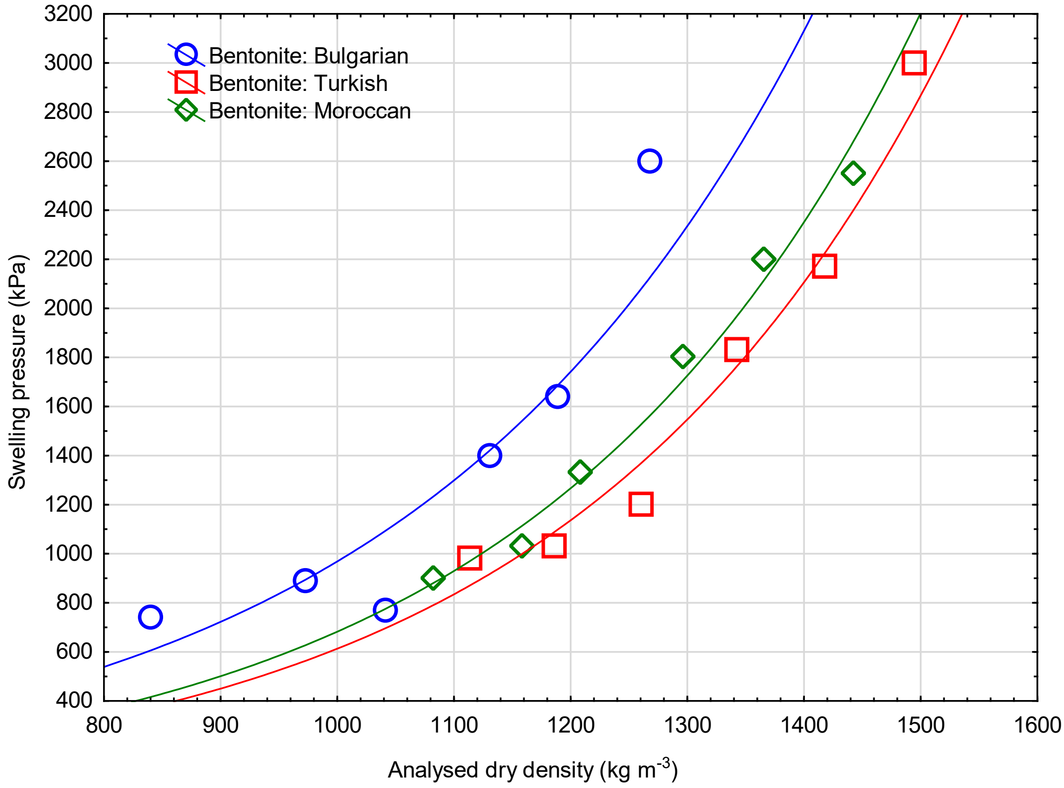


Figure 3-4. *Analysed swelling pressure versus analysed dry density for three bentonite types.*

3.2 Study 2

3.2.1 Experimental plan

This report describes two different approaches. In the first approach three different bentonite types were sterilised 160 °C for 10 hours and compacted at two densities without any additions except sulphate. In the second approach the Moroccan clay and Calcigel were spiked with the three different species of SRB plus lactate and sulphate and compacted at six different densities.

Table 3-4. List of test cells with bentonite and planned dry densities. Y= Yes, N=no

Name of test cell	Bentonite	Planned dry densities ($kg\ m^{-3}$)	Additions		
			SRB	Sulphate	Lactate
Turkish 1217	Turkish	1217	N	Y	N
Turkish 1541	Turkish	1541	N	Y	N
Moroccan 1181	Moroccan	1181	N	Y	N
Moroccan 1495	Moroccan	1495	N	Y	N
Bulgarian 1019	Bulgarian	1019	N	Y	N
Bulgarian 1332	Bulgarian	1332	N	Y	N
Moroccan 1308	Moroccan	1308	Y	Y	Y
Moroccan 1399	Moroccan	1399	Y	Y	Y
Moroccan 1452	Moroccan	1452	Y	Y	Y
Moroccan 1505	Moroccan	1505	Y	Y	Y
Moroccan 1551	Moroccan	1551	Y	Y	Y
Moroccan 1604	Moroccan	1604	Y	Y	Y
Calcigel 1250	Calcigel	1250	Y	Y	Y
Calcigel 1304	Calcigel	1304	Y	Y	Y
Calcigel 1351	Calcigel	1351	Y	Y	Y
Calcigel 1405	Calcigel	1405	Y	Y	Y
Calcigel 1452	Calcigel	1452	Y	Y	Y
Calcigel 1506	Calcigel	1506	Y	Y	Y

3.2.2 Water content, dry density, swelling pressure and sulphate

The results of the water content analysis and resulting dry densities are shown in Table 3-5. The planned and analysed water contents differed at most 3 % and for the dry densities at most 7 %. Table 3-6 shows the registered pressures for the sterile and the spiked clays at the end of the water saturation phase.

All bentonites were analysed for their natural leachable amount of sulphate. The results became:

- Turkish 3.2 $\mu mol\ gdw^{-1}$
- Moroccan 6.7 $\mu mol\ gdw^{-1}$
- Bulgarian 0 $\mu mol\ gdw^{-1}$
- Calcigel 0 $\mu mol\ gdw^{-1}$

Table 3-5. Weight, water content and dry density for the water saturated bentonites in each test cell. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Amount of bentonite (gdw)	Planned water content (%ww)	Analysed water content (%ww)	Planned dry density (kg m^{-3})	Analysed dry density (kg m^{-3})
Sterilized clays					
Turkish 1217	23.77	30.48	30.31	1217	1235
Turkish 1541	30.11	20.98	21.84	1541	1521
Moroccan 1181	23.07	32.53	32.06	1181	1171
Moroccan 1495	29.22	23.31	22. 60	1495	1496
Bulgarian 1019	19.91	38.25	39.27	1019	1026
Bulgarian 1332	26.03	27.98	29.49	1332	1327
Spiked clays					
Moroccan 1308	25.56	29.69	28.69	1308	1305
Moroccan 1399	27.34	27.13	28.54	1399	1353
Moroccan 1452	28.38	25.72	25.93	1452	1458
Moroccan 1505	29.42	24.35	24.91	1505	1501
Moroccan 1551	30.31	23.21	23.14	1551	1555
Moroccan 1604	31.35	21.93	20.52	1604	1660
Calcigel 1250	24.43	30.74	33.90	1250	1158
Calcigel 1304	25.49	29.10	28.54	1304	1287
Calcigel 1351	26.40	27.75	27.33	1351	1313
Calcigel 1405	27.46	26.22	28.13	1405	1320
Calcigel 1452	28.37	24.96	25.73	1452	1394
Calcigel 1506	29.44	23.53	23.98	1506	1484

Sulphate was present in the clay cores of the Turkish and Moroccan clay at the end of the experiment, but in lower amounts (Table 3-6) than what was observed at start. That may be due to bacterial sulphate reduction, or to inorganic immobilisation, or both.

3.2.3 Copper discs

Figure 3-5 shows the copper discs after 50 days of incubation. The discolouration of the copper discs was very subtle in comparison to study 1. Table 3-6 shows the average sulphur results from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured. The result for the sterilised clays (Turkish, Moroccan and Bulgarian) show that the higher dry densities had less sulphur on the copper discs in comparison to the lower densities. Results for the spiked bentonites (Moroccan and Calcigel) are not conclusive. There is no obvious increase or decrease of sulphur with increasing dry density, the amounts are comparable with what was observed for the sterilised clays.

It was observed that the bevel edges of the copper discs showed more discolouration than the top of the copper discs, for spiked bentonites. Table 3-6 also shows that indeed the amount of sulphur was larger on the bevel edges that on the top of the copper discs. The amount of sulphur decreased for the spiked Moroccan clay, from 6.87 % to 0.27 % with increase of dry density. Calcigel showed a decreasing trend with increasing density and also here, more sulphur was detected on the bevel edges than on the top of the copper discs.

Figure 3-6 shows the compacted, water saturated, sterilised bentonites after 50 days of incubation. A greenish patina was formed on top of the sterilised Moroccan 1495 and Bulgarian 1332 clays in contact with the copper discs the test cells. Such patina usually consists of varying mixtures of copper chlorides, sulphides, sulphates and carbonates, depending upon environmental conditions. The sterilised Moroccan was the only sterilised clay core that showed small black spots, indicating SRB sulphide producing activity. The spiked Moroccan bentonite cores showed black spots at all chosen densities and a brown discolouration of the clay at 1399, 1452, 1551 and 1604 kg m^{-3} dry density (Figure 3-7). Calcigel showed black spots as well, in various amounts and size at all chosen densities (Figure 3-8).

Table 3-6. Mean swelling pressures during the water saturation phase deduced from data obtained with force transducers for each test cell. Leachable amount of sulphate in the used clays and on average across the three sampling points for each test cell. (gdw = gram dry weight). Average sulphur values from XRF measurements on the top surface and bevel edge (n = 5)

Name of test cell	Analysed dry density (kg m^{-3})	Swelling pressure (kPa)	Micromole of sulphate per gram of bentonite (µmol gdw^{-1})	Average S on top (%)	Standard deviation S (± %)	Average S on bevel edge (%)
Sterilized clays						
Turkish 1217	1235	500	0.84	1.3	2.02	0.63
Turkish 1541	1521	4000	1.08	0.45	0.01	0.46
Bulgarian 1019	1026	500	0	0.58	0.16	0.81
Bulgarian 1332	1327	5400	0	0	0	0.46
Moroccan 1181	1171	400	5.47	0.32	0.03	0.39
Moroccan 1495	1496	2500	4.09	0.05	0.13	0.05
Spiked clays						
Moroccan 1308	1305	900	3.55	0.19	0.16	6.87
Moroccan 1399	1353	1700	5.29	0.48	0.12	3.05
Moroccan 1452	1458	1800	6.23	0.02	0.05	1.45
Moroccan 1505	1501	2900	4.17	0.51	0-23	1.7
Moroccan 1551	1555	4000	5.70	0.78	0.08	0.53
Moroccan 1604	1660	5400	3.61	0.27	0.05	0.44
Calcigel 1250	1158	275	0	0.24	0.48	7.34
Calcigel 1304	1287	300	0	0.47	0.09	9.09
Calcigel 1351	1313	500	0	0.37	0.07	0.64
Calcigel 1405	1320	700	0	0.46	0.04	4.72
Calcigel 1452	1394	1200	0	0.04	0.10	1.07
Calcigel 1506	1484	1300	0	0	0	2.33

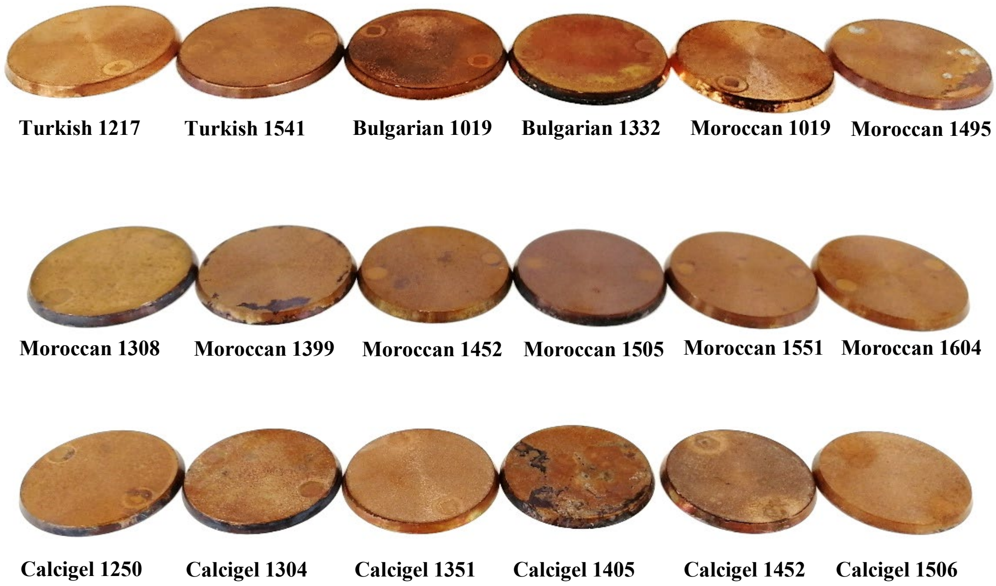


Figure 3-5. *Copper discs from every test cell spiked with SBR and lactate after 50 days.*

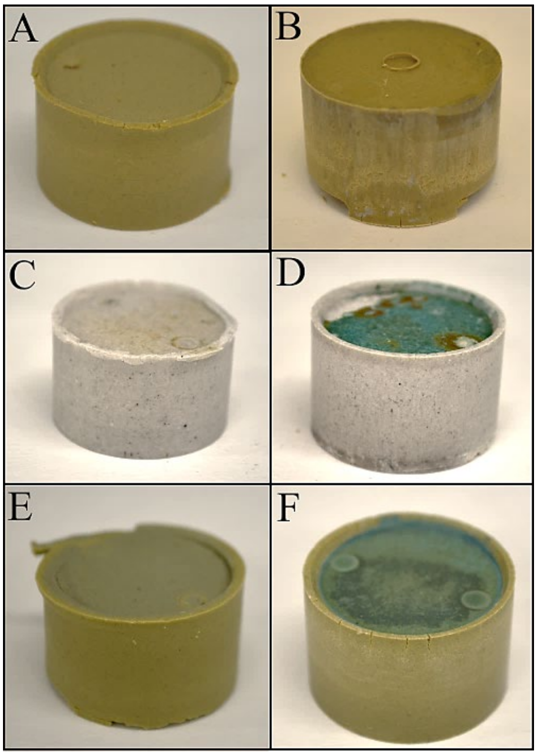


Figure 3-6. *Images of compacted water saturated bentonite cores after 50 days incubation. All clays were sterilised before compaction. A Turkish 1217; B Turkish 1541; C Moroccan 1181; D Moroccan 1495; E Bulgarian 1019; F Bulgarian 1332.*

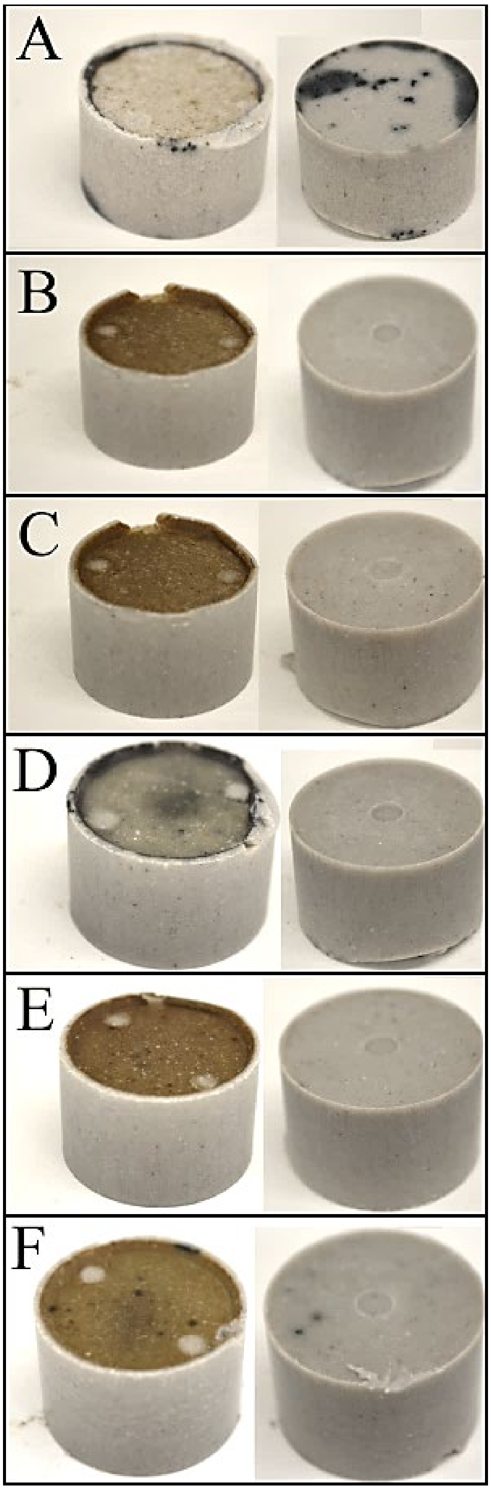


Figure 3-7. Images of compacted water saturated Moroccan bentonite cores after 50 days incubation. All clays were spiked with SRB and lactate was added as a carbon source. Left: bottom at the copper disc; Right: top where the lactate was added. A Moroccan 1308; B Moroccan 1399; C Moroccan 1452; D Moroccan 1505; E Moroccan 1551; F Moroccan 1604.

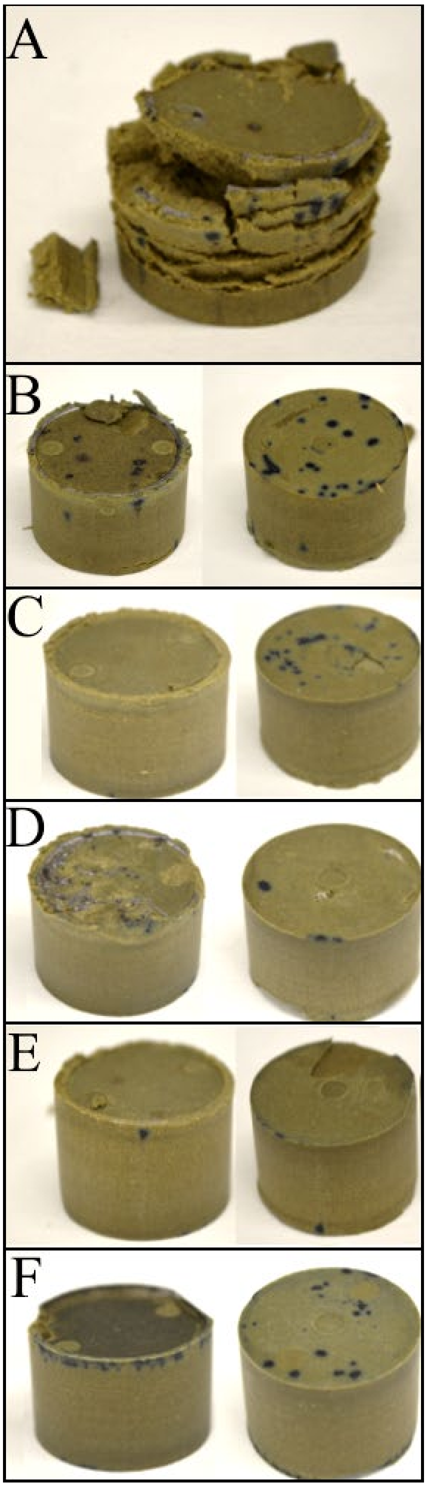


Figure 3-8. Images of compacted water saturated Calcigel bentonite cores after 50 days incubation. All clays were spiked with SRB and lactate was added as a carbon source. Left: bottom at the copper disc; Right: top where the lactate was added. A Calcigel 1250; B Calcigel 1304; C Calcigel 1351; D Calcigel 1405; E Calcigel 1452; F Calcigel 1506.

3.2.4 Summary of microbiology results and observations

Study 2 tested larger dry densities and swelling pressures for Moroccan bentonite compared to study 1 (Figure 3-9). The amount of sulphur on the copper discs was at background values, similar to what was observed for the sterilised clays. However, both clays, Moroccan and Calcigel had sulphur on the bevels at the lower densities (Figure 3-10). In addition, black spots were observed at the lowest Moroccan density (Figure 3-7) and in all of the Calcigel cores (Figure 3-8). These black spots most likely are iron sulphides that has formed due to microbial sulphide production. The explanation may be that density and swelling pressure was lower in the small slit between the copper discs and the test cell wall. It is rather clear that sulphate reduction may occur in the interface between the clay and the test cell wall under certain conditions, even if it is restricted inside the clay sample. Later studies in this report are directed towards tests that can detect SRB activity inside the cores.

In conclusion, SRB activity appeared to be restricted when the densities were larger than 1300-1400 kg m^{-3} (Figure 3-10). However, the positions with locally lower density at the bevel edge allowed bacterial sulphide production as judged from the presence of S on the copper discs.

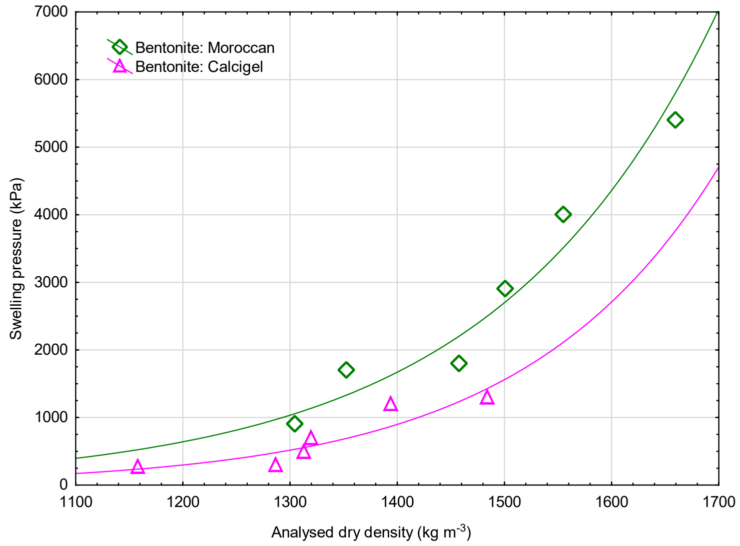


Figure 3-9. *Analysed swelling pressure versus analysed dry density for two bentonite types.*

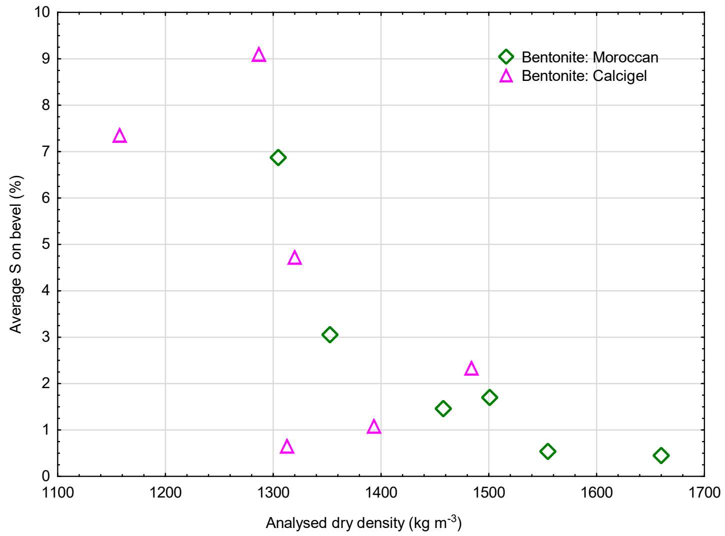


Figure 3-10. *The analysed dry density versus average S on the bevel of copper discs of two bentonite types.*

3.3 XRF and SEM studies with EDS on copper discs in study 2

The chemical and morphological changes on copper discs exposed to different bentonite samples under experimental conditions with bacteria in study 2 were investigated. Using XRF and SEM with EDS, variations in sulphur content and surface features were analysed to understand the effects of bentonite density on the amount and type of sulphur phases on the copper surfaces. XRD was used in an attempt to identify the formed corrosion product(s). The use of XRF to measure the content of the phases on the surface of the copper discs should be seen as semi quantitative as the discs are not homogenous because the amount of copper in relation to the amount of the elements on the surface will depend on the penetration depth of the measurement of the disc.

3.3.1 First approach of copper discs.

Chemical data collected through surface measurements using XRF were obtained from the copper discs (Table 3-7). In the Moroccan and Bulgarian bentonites, the sulphur content was noticeably lower in high-density bentonites and higher in low-density bentonites. A similar trend was observed in the Turkish bentonite, although the difference was less pronounced.

Table 3-7. First approach copper discs. XRF surface measurements (wt%)

	Dry density	MgO	Al_2O_3	SiO_2	S	Cl
Turkish Low	1235	0.034	0.308	0.58	0.146	0.049
Turkish Low	1235	0.045	0.381	0.707	0.175	0.043
Turkish High	1521	0.035	0.317	0.627	0.131	0.04
Turkish High	1521	0.03	0.305	0.574	0.131	
Moroccan Low	1171	0.025	0.332	0.484	0.111	
Moroccan Low	1171	0.036	0.418	0.628	0.082	
Moroccan High	1496	0.181	2.261	3.483	0.004	0.542
Moroccan High	1493	0.103	1.305	2.006	0.002	0.455
Bulgaria Low	1026	0.259	0.987	2.396	0.183	0.027
Bulgarian Low	1026	0.211	0.818	1.938	0.196	0.048
Bulgaria High	1327	0.079	0.333	0.798	0.049	1.581
Bulgarian high	1327	0.076	0.326	0.796	0.052	1.507

3.3.2 Second approach of copper discs

In the second approach, the results were less clear compared to the first approach. In all discs, the sulphur level exceeded the reference value measured from the backside of the copper disc, which had not been in contact with the bentonite experiment (Table 3-8). When the sulphur concentration was plotted as a function of the dry density, no correlation was observed between the amount of sulphur on the copper surface and the dry density of the bentonite (Figure 3-11 and Figure 3-12).

Table 3-8. Second approach Copper discs. XRF surface measurements (wt%)

Sample	Dry density	MgO	Al_2O_3	SiO_2	S	Cl
Copper Discs Calcigel 1250	1158	0.216	1.167	2.2	0.0556	
Copper Discs Calcigel 1250	1158	0.184	1.046	2.008	0.0576	
Copper Discs Calcigel 1300	1287	0.599	3.097	6.048	0.1856	0.019
Copper Discs Calcigel 1300	1287	0.64	3.318	6.507	0.2196	0.035
Copper Discs Calcigel 1350	1313	0.722	3.898	7.55	0.0664	0.059
Copper Discs Calcigel 1350	1313	0.794	4.254	8.377	0.0672	0.054
Copper Discs Calcigel 1400	1320	0.54	2.696	5.186	0.2504	0.045
Copper Discs Calcigel 1400	1320	0.626	3.159	6.058	0.364	0.029
Copper Discs Calcigel 1450	1394	0.546	2.925	5.65	0.194	0.037
Copper Discs Calcigel 1450	1394	0.611	3.262	6.268	0.2216	0.042
Copper Discs Calcigel 1500	1484	0.661	3.534	6.829	0.0884	0.026
Copper Discs Calcigel 1500	1484	0.638	3.443	6.762	0.0816	0.031
Copper Discs Moroccan 1300	1305	0.085	0.78	1.182	0.0636	
Copper Discs Moroccan 1300	1305	0.072	0.645	0.972	0.0584	
Copper Discs Moroccan 1400	1353	0.042	0.414	0.628	0.1644	0.026
Copper Discs Moroccan 1400	1353	0.059	0.539	0.818	0.1404	0.022
Copper Discs Moroccan 1450	1458	0.036	0.367	0.611	0.1044	0.031
Copper Discs Moroccan 1450	1458	0.034	0.383	0.617	0.1008	0.034
Copper Discs Moroccan 1500	1501	0.053	0.466	0.762	0.1688	
Copper Discs Moroccan 1500	1501	0.039	0.365	0.57	0.134	0.024
Copper Discs Moroccan 1550	1555	0.03	0.333	0.498	0.0588	0.066
Copper Discs Moroccan 1550	1555	0.027	0.336	0.515	0.0544	0.044
Copper Discs Moroccan 1600	1660	0.039	0.403	0.591	0.08	0.071
Copper Discs Moroccan 1600	1660	0.039	0.404	0.595	0.0744	0.051
Copper disc backside		0.01	0.237	0.307	0.0108	

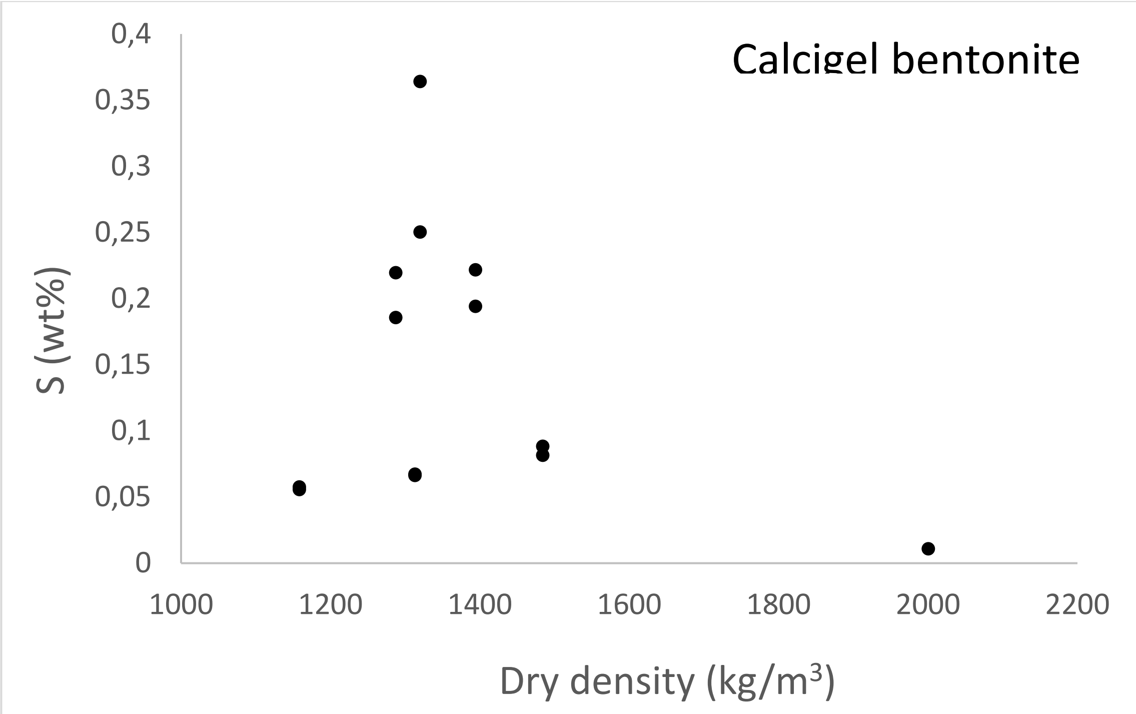


Figure 3-11. *Copper discs second approach showing XRF surface measurements of sulphur (wt%) as a function of the dry density for the Calcigel bentonite. The reference value obtained from the backside of the copper disc is positioned at 2000.*

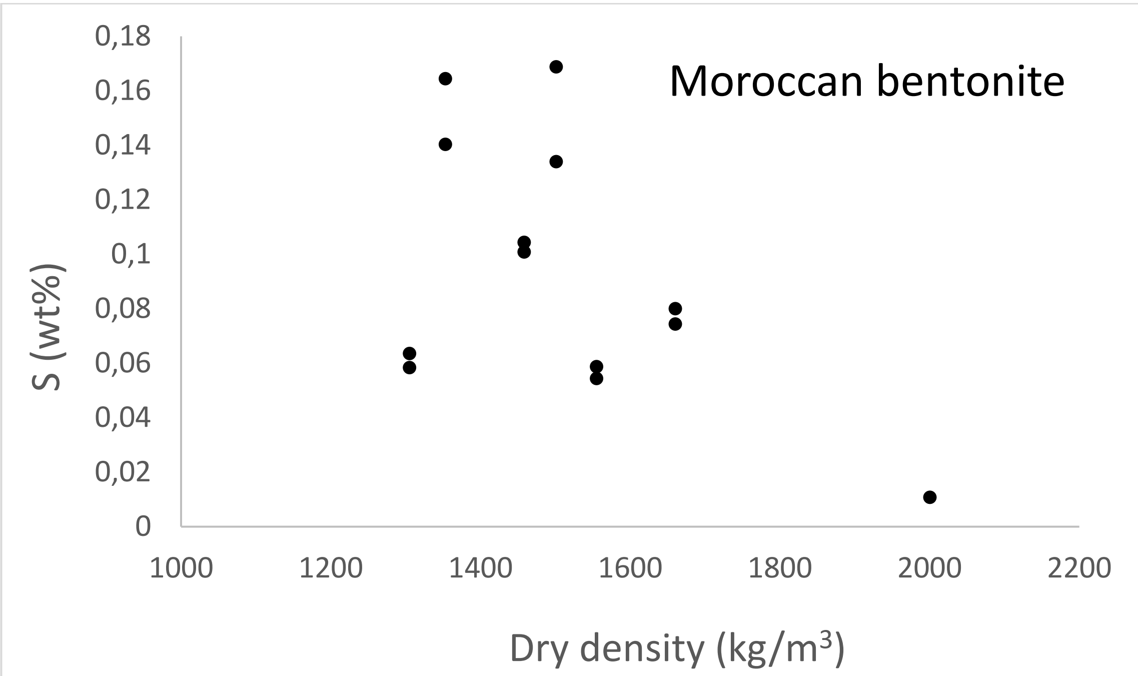


Figure 3-12. *Copper discs second approach showing XRF surface measurements of sulphur (wt%) as a function of the dry density for the Moroccan bentonite. The reference value obtained from the backside of the copper disc is positioned at 2000.*

The "Calcigel 1400 kg/m³" copper disc was selected for further investigation due to the presence of interesting surface corrosion features (Figure 3-13).

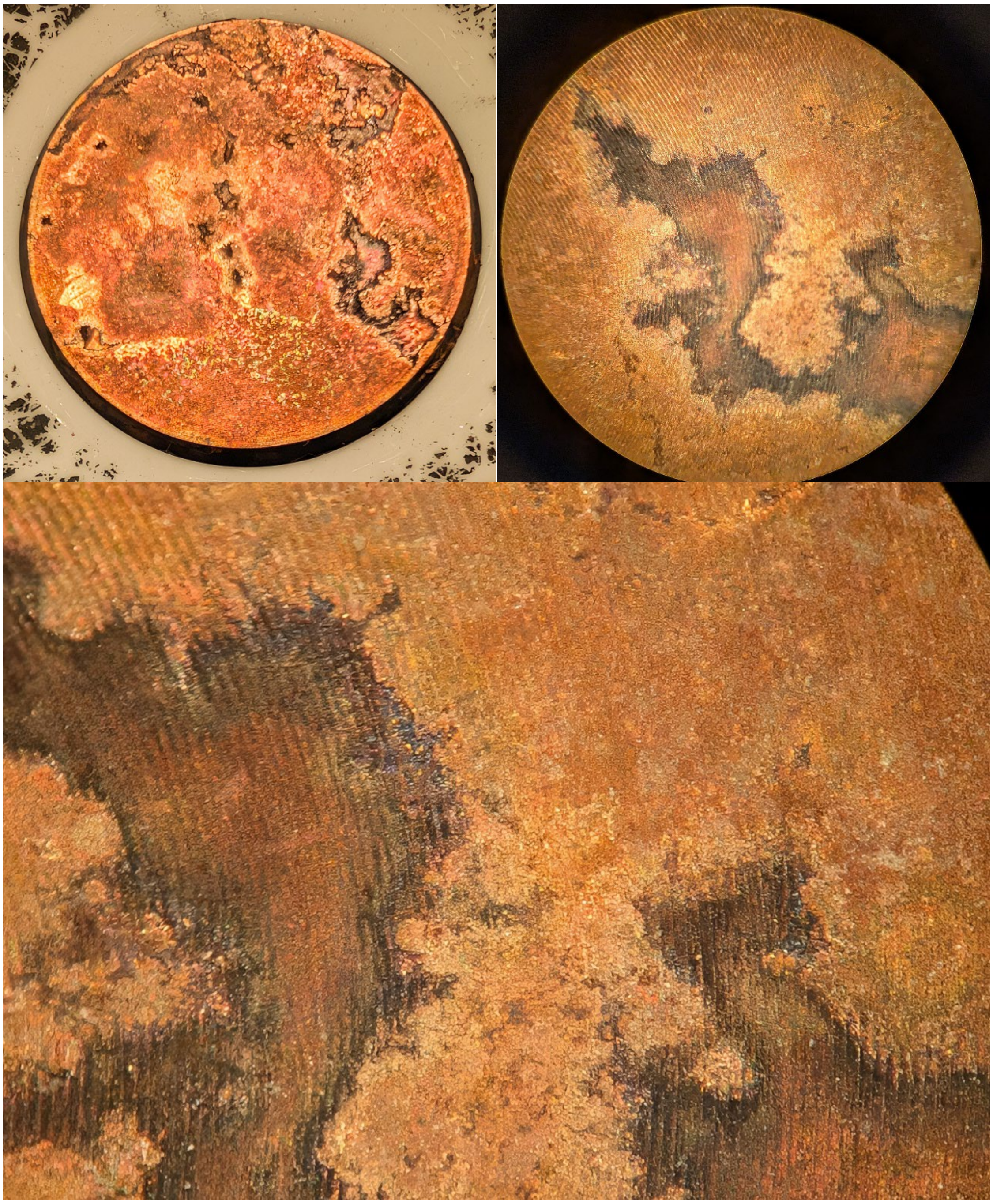
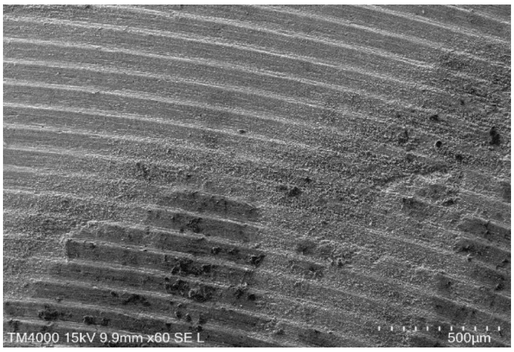
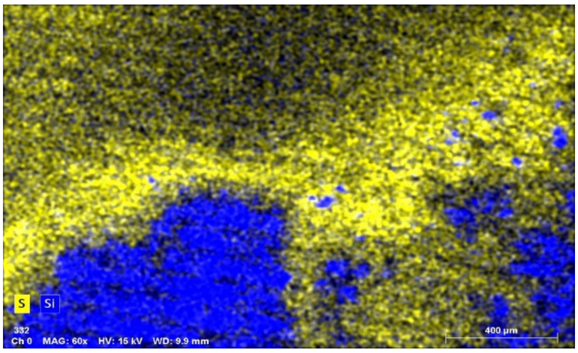


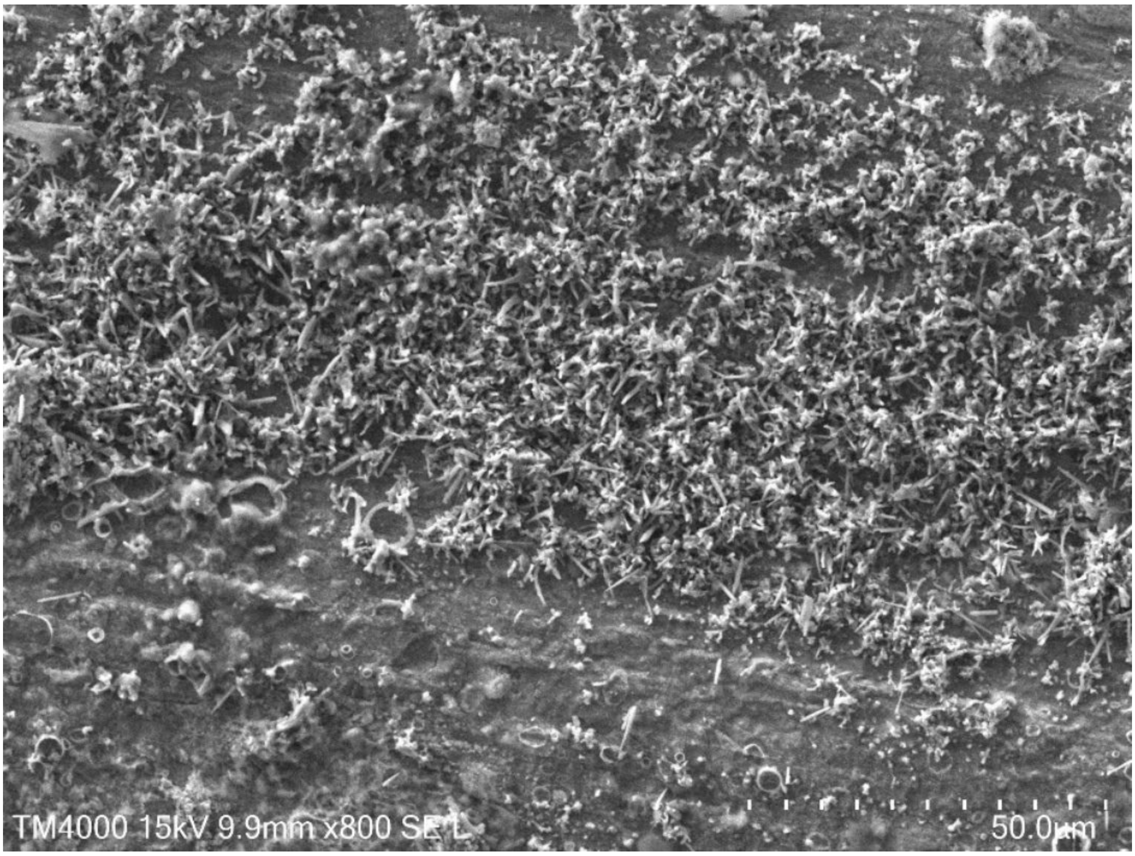
Figure 3-13. *Photos of the Calcigel 1400 kg/m³ disc. Top left: complete disc. Top right: microscope picture. Bottom: microscope picture with higher magnification.*

The Calcigel disc was analysed using a scanning electron microscope (SEM) with a connected EDS-analyser. The copper disc revealed several distinct phases on their surfaces. One clay-like phase (Figure 3-14a) was observed, coinciding with high silicon levels on the EDS map (Figure 3-14b) and most likely corresponding to bentonite. Another phase, appearing as elongated crystals (Figure 3-14c; Figure 3-15a), showed a strong correlation with sulphur in the EDS maps. Additionally, crust-like aggregates were identified (Figure 3-16), which were also associated with elevated sulphur levels.



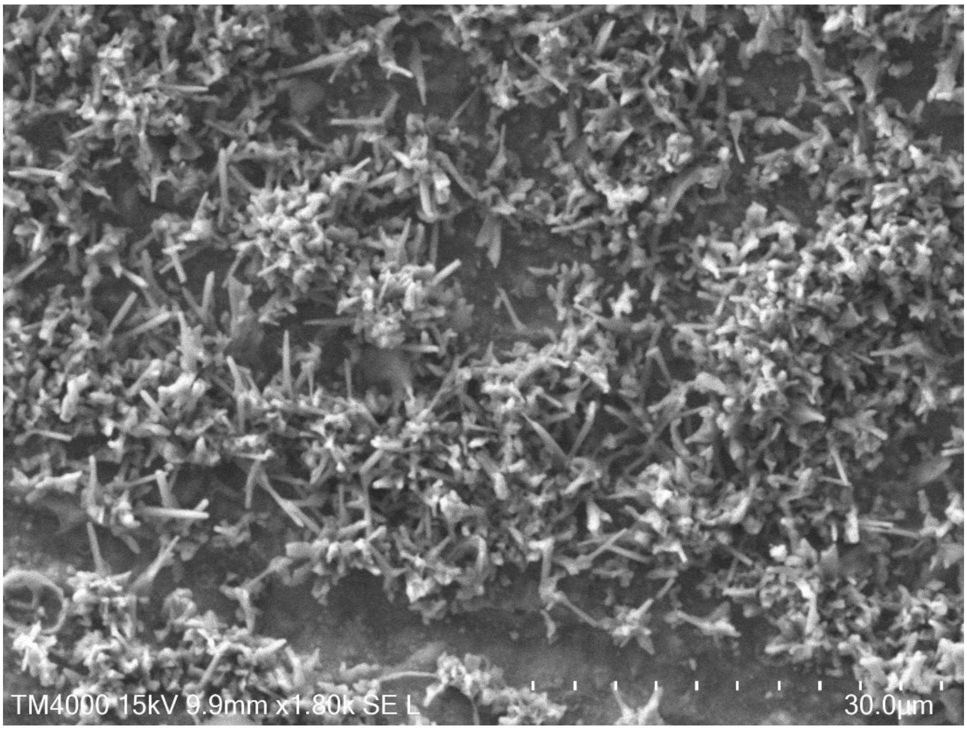


(a) (b)

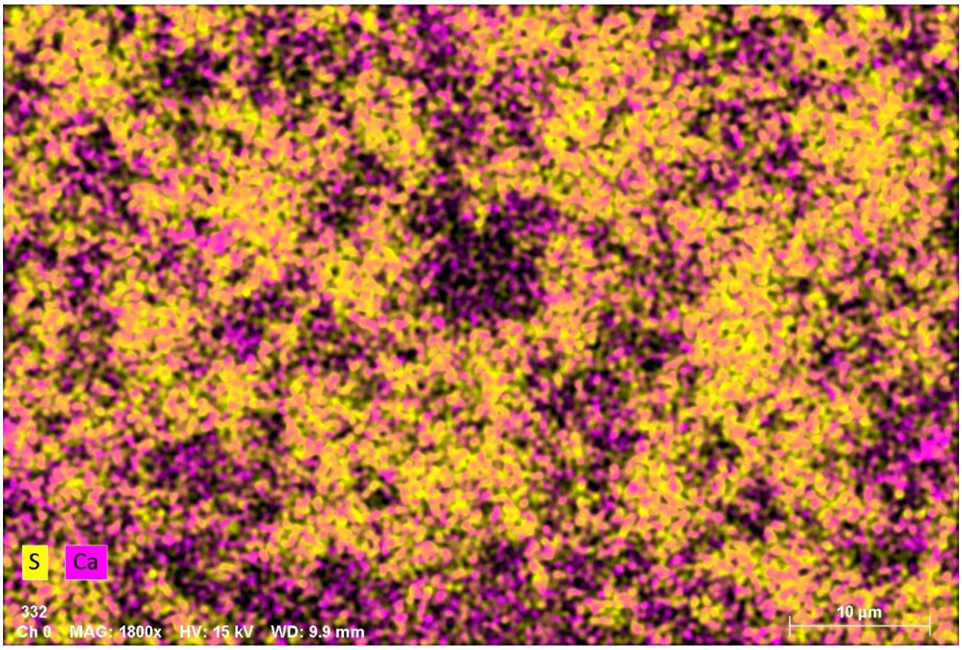


(c)

Figure 3-14. *(a) SEM micrograph of Calcigel 1400 kg/m³ copper disc at 60× magnification. (b) EDS elemental map showing S (yellow) and Si (blue) distribution. (c) 800× magnification.*

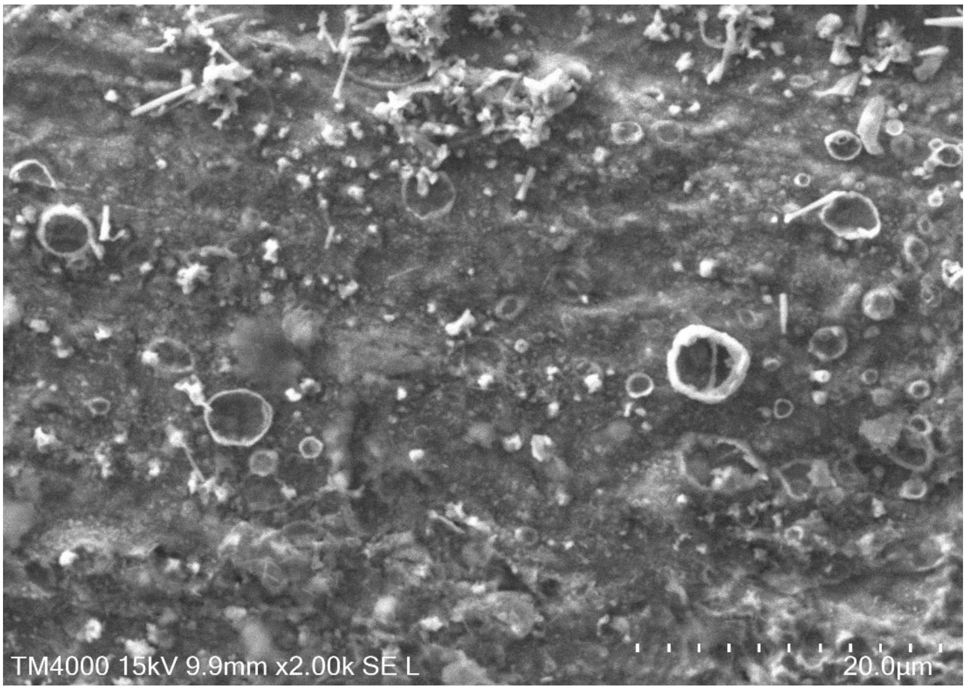


(a)

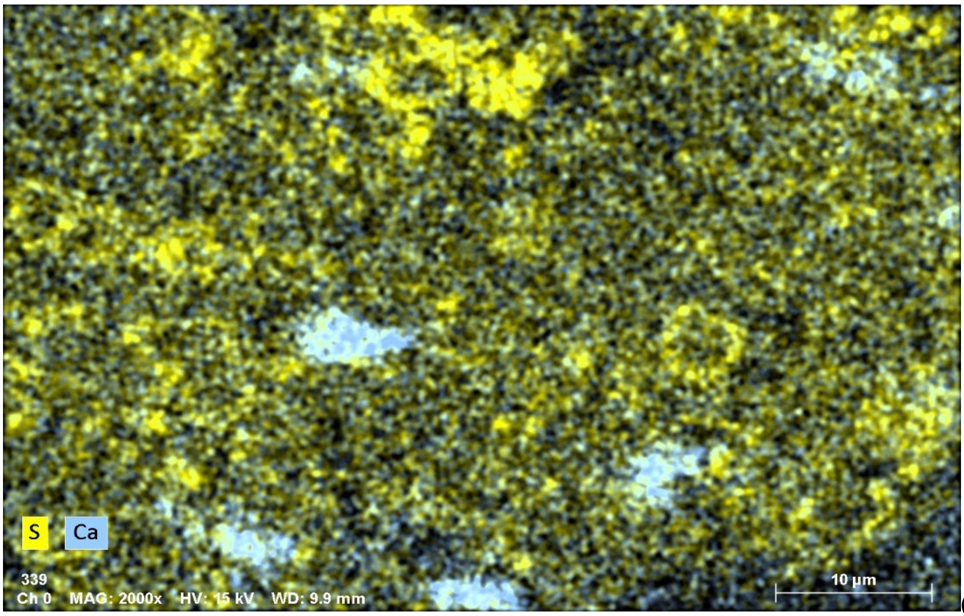


(b)

Figure 3-15. *(a) SEM micrograph of Calcigel 1400 kg/m^3 copper disc at 1800× magnification showing elongated crystals. (b) EDS elemental map showing S and Ca distribution.*



(a)



(b)

Figure 3-16. *(a) SEM micrograph of Calcigel 1400 kg/m^3 copper disc at 2000× magnification of crust like aggregates. (b) EDS elemental map showing S and Ca distribution.*

Spot and area analysis of the crystals show clearly that they are strongly associated with sulphur (Table 3-9).

As gypsum was a possibility, Ca was included in the EDS-maps, however the level was very low in all sites (Table 3-10).

The observed atomic ratios from Table 3-9 are Mg/Al = 0.83/0.92 = 0.90 and Al/Si = 0.92/1.97 = 0.47. For comparison, reference values for Wyoming montmorillonite, as reported by Karnland et al. (2006), are Mg/Al = 0.48/3.13 = 0.15 and Al/Si = 3.13/7.92 = 0.39. While the Al/Si ratio aligns with expectations, the Mg/Al ratio suggests a higher Mg content relative to Al than expected. However, if all sulphur were associated with magnesium in the form of magnesium sulphate, a significantly higher Mg content would be required (observed Mg/S atomic ratio is 0.83/12 = 0.07).

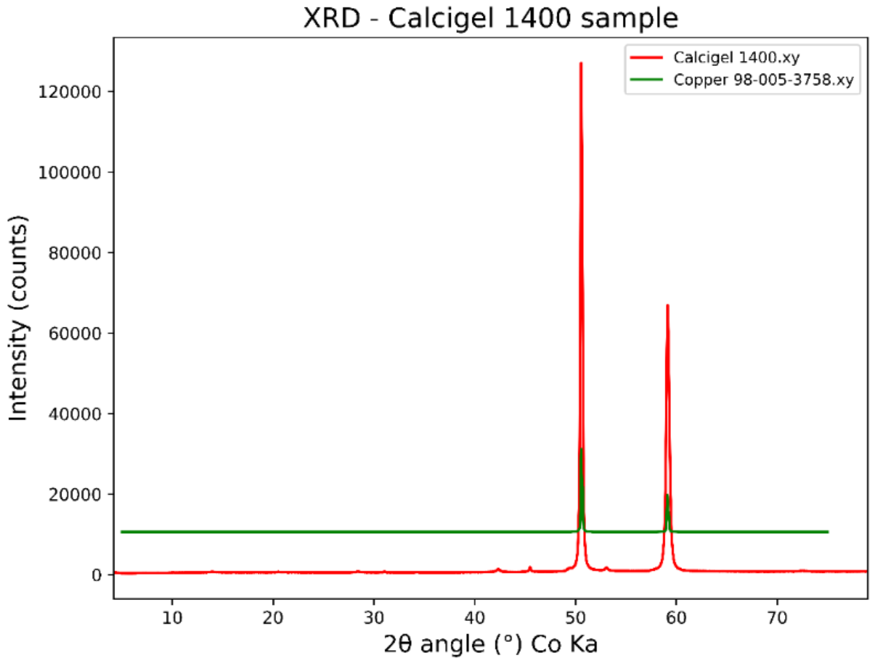
Example of possible phases for the crystals based on the chemical composition can be copper sulphide or copper sulphate.

Table 3-9. EDS analysis of area with crystals on the Calcigel 1400 kg/m^3 copper disc

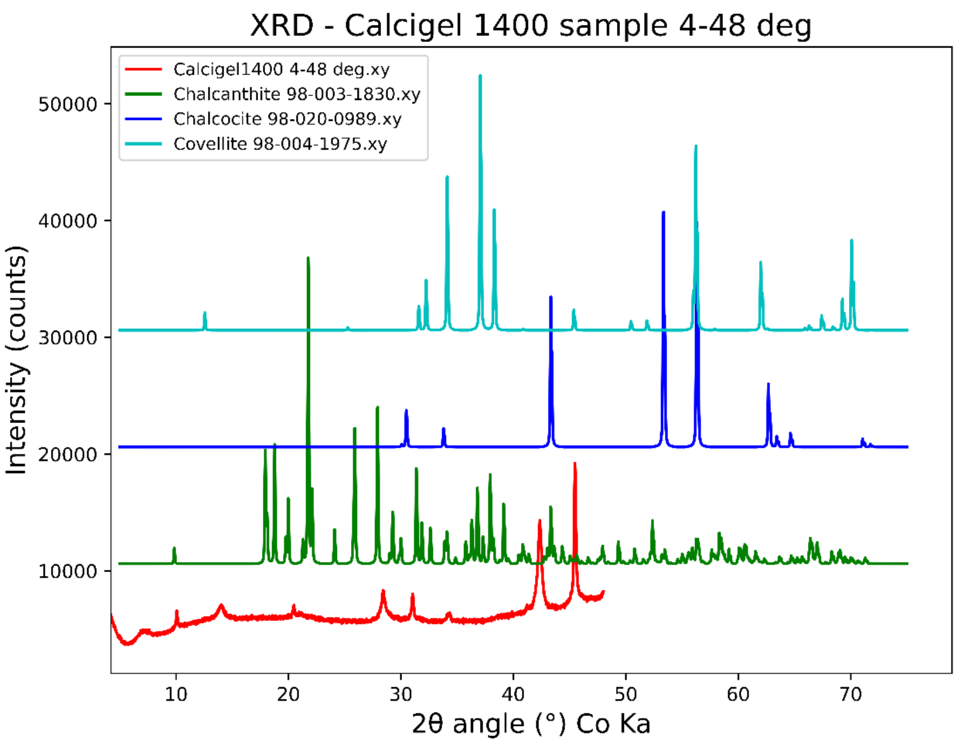
Element		Mass Norm. [%]	Atom [%]	Abs. error [%] (1 sigma)	Rel. error [%] (1 sigma)
Cu		79.34	54.63	2.52	3.36
O		5.69	15.56	1.04	19.40
C		3.85	14.05	1.12	30.84
S		8.82	12.03	0.34	4.14
Si		1.26	1.97	0.098	8.22
Al		0.56	0.92	0.072	13.44
Mg		0.46	0.83	0.073	16.87
Na		1.0E-12	2.0-12	0	10
Ca		1.0E-12	1.1-12	0	10
	Sum	100	100		

Table 3-10. EDS analysis of spot analysis of crystals on the Calcigel 1400 kg/m^3 copper disc

Spectrum	C	O	Na	Mg	Al	Si	S	Ca	Cu
Site 1	14.05	15.56	2.0E-12	0.83	0.92	1.97	12.03	1.2E-12	54.63
Site 2	14.37	13.75	0	0	1.14	1.46	13.69	0	55.57
Site 3	16.08	14.80	0	0	0.81	1.55	12.11	0	54.63
Mean	14.84	14.70	0	0.28	0.95	1.66	12.61	0	54.94
Sigma	1.09	0.91	1.2E-12	0.45	0.17	0.26	0.93	0	0.54
SigmaMean	0.63	0.52	0	0.28	0.098	0.16	0.54	0	0.31



(a)



(b)

Figure 3-17. *Powder XRD pattern of the surface of the Calcigel 1400 kg/m³ copper disc. (a) full pattern together with a copper diss reference, (b) Closeup of the 4-48 deg two theta range together with some selected Cu-S-reference phases.*

Powder XRD was measured of the surface of the Calcigel 1400 kg/m^3 copper disc. A full interval pattern was collected (Figure 3-17a) and to get more information of low intensity phases a separate data collection was done at lower angles than the strong copper reflections (a 3h measurement was done between around 4-48 deg two theta) to optimise the conditions to be able to identify the corrosion product (Figure 3-17b). The data was compared to some selected possible Cu-S-phases, but no matching patterns were observed.

3.3.3 Summary and conclusions of the XRF and EDX studies

Chemical and morphological analyses were performed on copper discs exposed to bacteria-bentonite experiments. The study aimed to investigate the effects of bentonite type, density, and bacterial activity on sulphur deposition and corrosion of copper surfaces. Key findings include:

Sulphur Content Analysis (XRF)

Differences in sulphur content were observed across bentonite types and densities. Moroccan and Bulgarian bentonites showed lower sulphur levels in high-density samples and higher levels in low-density samples. Turkish bentonites followed the same trend, but the differences were less pronounced.

In a second experimental approach, sulphur levels on all copper discs exceeded reference values from non-exposed disc surfaces. Hence, in the second approach no correlation was observed between sulphur deposition and bentonite density. The interpretation of this is that either sulphate reduction has occurred at all dry densities, or that the sulphate background on the discs is high. This raises questions whether XRF if a suitable method for the detection of sulphate reduction.

Morphological and Phase Analysis (SEM and EDS)

The Calcigel 1400 kg/m³ copper disc, selected for its distinct corrosion features, revealed several surface phases:

- A clay-like phase corresponding to high silicon content, likely bentonite.
- Elongated crystals correlating with sulphur, and crust-like aggregates associated with elevated sulphur levels.

Atomic Ratios from EDS

Atomic ratio analyses showed Mg/Al = 0.90 and Al/Si = 0.47. The Al/Si ratio aligns with reference values for Wyoming montmorillonite, but the Mg/Al ratio suggests an unexpectedly high Mg content. If sulphur were associated with magnesium as magnesium sulphate, a much higher Mg content would be required (Mg/S = 0.07 observed).

XRD Analysis

Powder XRD of the Calcigel 1400 kg/m³ copper disc surface was performed to identify corrosion products. Despite collecting data across a full interval and optimizing conditions for low-intensity phases, no matching patterns for potential Cu-S-phases were identified.

3.4 Study 3

3.4.1 Experiment

This study describes different approaches compared to studies 1 and 2 as shown in Table 3-11. The Turkish bentonite clay was compacted at two different dry densities without any additions and incubated for 1, 2 or 4 months. The Moroccan bentonite was tested with a coarse grain density and fine grinded grain density (Figure 3-18). The Moroccan bentonite was compacted at two different dry densities and incubated either 2 or 4 months. Furthermore, it was spiked with SRB prior compactions and lactate was added after the water saturation phase when the copper discs were added. The synthetic clay Laponite was also compacted at two different dry densities and incubated 4 months. One set of two was spiked and lactate was added, the other set of two had no additions.

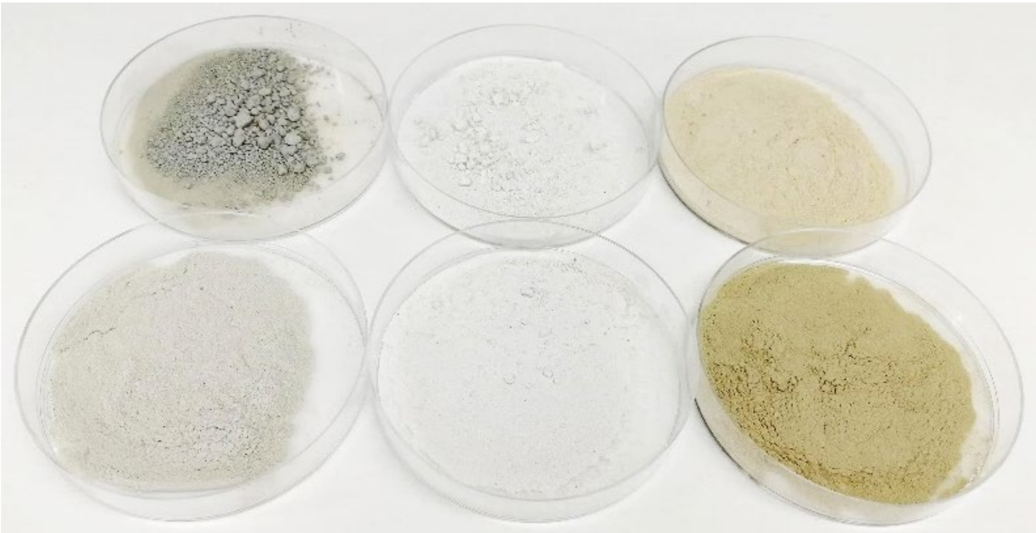


Figure 3-18. *From left to right, Bulgarian, Moroccan and Turkish bentonite. Top shows natural grain distribution at delivery to the laboratory. Bottom shows these bentonites after grinding.*

Table 3-11. List of test cells with bentonite and planned dry densities. Y= Yes, N=no

Name of test cell	Bentonite	Planned dry densities (kg m^{-3})	Additions		
			SRB	Sulphate	Lactate
Turkish 1200, 1 month	Turkish	1200	N	N	N
Turkish 1500, 1 month	Turkish	1500	N	N	N
Turkish 1200, 2 months	Turkish	1200	N	N	N
Turkish 1500, 2 months	Turkish	1500	N	N	N
Turkish 1200, 4 months	Turkish	1200	N	N	N
Turkish 1500, 4 months	Turkish	1500	N	N	N
Moroccan 1300, 2 months	Moroccan	1300	Y	Y	Y
Moroccan 1550, 2 months	Moroccan	1550	Y	Y	Y
Moroccan 1300, 4 months	Moroccan	1300	Y	Y	Y
Moroccan 1500, 4 months	Moroccan	1550	Y	Y	Y
Moroccan 1300 grinded, 2 months	Moroccan	1300	Y	Y	Y
Moroccan 1550 grinded, 2 months	Moroccan	1550	Y	Y	Y
Laponite 1100	Laponite	1100	N	N	N
Laponite 1300	Laponite	1300	N	N	N
Laponite 1100 spiked	Laponite	1100	Y	Y	Y
Laponite 1300 spiked	Laponite	1300	Y	Y	Y

3.4.2 Water content, dry density, swelling pressure, and sulphate

The results of the water content analysis and resulting dry density are shown in Table 3-12. The planned and analysed water contents differed at most 5.2 % (test cell Turkish 1200, 2 months) and for the dry densities at most 6.7 % (test cell Turkish 1550, 4 months).

Table 3-12. Weight, water content and dry density for the water saturated bentonites in each test cell. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Amount of bentonite (gdw)	Planned water content (%ww)	Analysed water content (%ww)	Planned dry density (kg m^{-3})	Analysed dry density (kg m^{-3})
Turkish 1200, 1 month	23.46	31.34	29.20	1201	1251
Turkish 1550, 1 month	30.29	21.18	20.40	1550	1622
Turkish 1200, 2 months	23.46	31.34	36.54	1201	1133
Turkish 1550, 2 months	30.29	21.18	25.33	1550	1507
Turkish 1200, 4 months	23.46	31.34	35.36	1201	1153
Turkish 1550, 4 months	30.29	21.18	26.28	1550	1446
Moroccan 1300, 2 months, lactate, spiked	25.40	29.73	32.34	1300	1245
Moroccan 1550, 2 months, lactate, spiked	30.30	23.00	21.18	1551	1619
Moroccan 1300, 4 months, lactate, spiked	25.40	29.73	27.47	1300	1328
Moroccan 1550, 4 months, lactate, spiked	30.30	23.00	20.40	1551	1607
Moroccan 1300 grinded, 2 months, lactate, spiked	25.41	29.63	31.40	1300	1270
Moroccan 1550 grinded, 2 months, lactate, spiked	30.30	22.90	23.30	1550	1568
Moroccan 1300 grinded, 4 months, lactate, spiked	25.41	29.63	27.97	1300	1313
Moroccan 1550 grinded, 4 months, lactate, spiked	30.30	22.90	24.22	1550	1564
Laponite 1100	21.51	34.76	36.22	1101	1121
Laponite 1300	25.42	28.20	27.82	1301	1342
Laponite 1100 lactate, spiked	21.50	34.26	37.24	1100	1066
Laponite 1300 lactate, spiked	25.40	27.63	26.14	1300	1377

Table 3-13 shows the registered swelling pressures at the end of the saturation phase for all test cells. All bentonites were analysed for their natural leachable amount of sulphate before start of the experiments. The results became:

- Turkish 2.9 µmol gdw^{-1}
- Moroccan 3.7 µmol gdw^{-1}
- Laponite 5.8 µmol gdw^{-1}

Sulphate was present in almost all clay cores, but at lower amounts than observed for the bentonites at start (Table 3-13). The only exceptions were the Moroccan test cells with a dry density of 1245. Figure 3-19 to Figure 3-22 show the compacted, water saturated bentonites after incubation. The Turkish bentonite and Laponite showed no indication of SRB activity (Figure 3-19 and Figure 3-22). Figure 3-20 shows that the black discolouration increased over time in the Moroccan bentonite at a dry density of 1300 kg m^{-3}. However, the higher dry density did not show the same effect. In comparison the grinded Moroccan bentonite only showed black discolouration at the lower density (Figure 3-8).

Table 3-13. Swelling pressures at the end of the saturation phase deduced from data obtained with force transducers for each test cell. Leachable amount of sulphate in the clays, average across the three sampling points for each test cell (n=3). (gdw = gram dry weight). Average sulphur values from XRF measurements on the top surface (n = 5)

Name of test cell	Analysed dry density (kg m^{-3})	Swelling pressure (kPa)	Micromole of sulphate per gram of bentonite (µmol gdw^{-1})	Average S (%)	Standard deviation (± %)
Turkish 1200, 1 month	1251	470	0.70	0	0
Turkish 1550, 1 month	1622	4700	1.80	0	0
Turkish 1200, 2 months	1133	375	0.70	0	0
Turkish 1550, 2 months	1507	5760	1.60	0	0
Turkish 1200, 4 months	1153	460	0.30	0	0
Turkish 1550, 4 months	1446	4360	1.40	0.36	0.5
Moroccan 1300, 2 months	1245	930	0	7.22	2.78
Moroccan 1550, 2 months	1619	4470	2.70	0.16	0.10
Moroccan 1300, 4 months	1328	1160	0	15.9	0.58
Moroccan 1550, 4 months	1607	4780	4.68	0	0
Moroccan 1300 grinded, 2 months	1402	910	1.86	0	0
Moroccan 1550 grinded, 2 months	1421	5100	4.54	0.17	0.01
Moroccan 1300 grinded, 4 months	1313	880	3.45	0.02	0.06
Moroccan 1550 grinded, 4 months	1564	4120	2.87	0.20	0.04
Laponite 1100	1121	6730	2.90	0.38	0.05
Laponite 1300	1342	9240	4.00	0.47	0.02
Laponite 1100 spiked	1066	6730	3.20	0.22	0.07
Laponite 1300 spiked	1377	9240	5.0	0.55	0.20

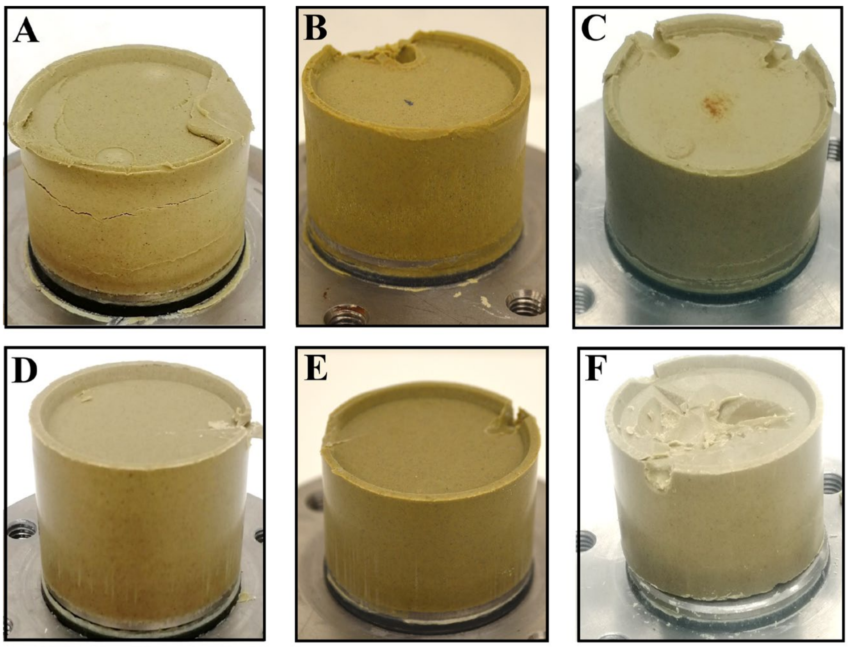


Figure 3-19. *Images of compacted water saturated Turkish bentonite cores after incubation. A Turkish 1200, 1 month; B Turkish 1200, 2months; C Turkish 1200, 4 months; D Turkish 1550, 1 month; E Turkish 1550, 2 months; F Turkish 1550, 4 months.*

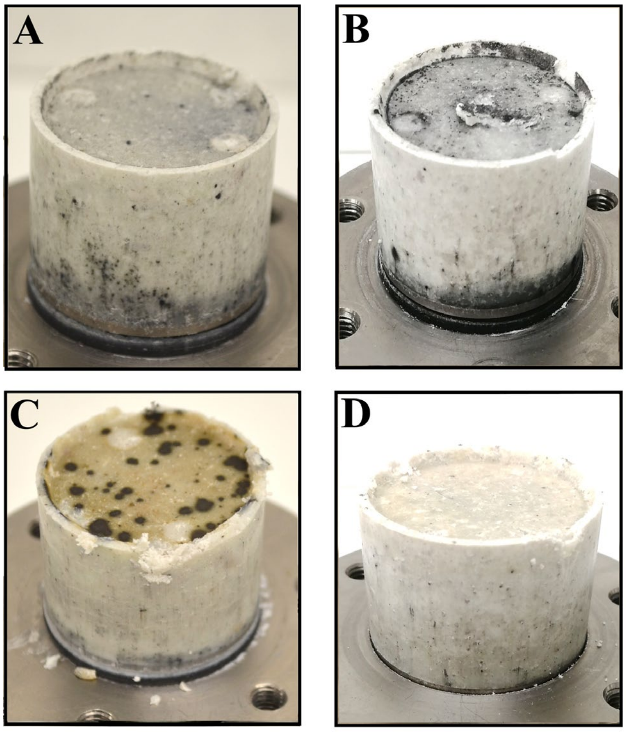


Figure 3-20. *Images of compacted water saturated Moroccan bentonite cores after incubation. A Moroccan 1300, 2 months; B Moroccan 1550, 2months; C Moroccan 1300, 4 months; D Moroccan 1550, 4 months.*

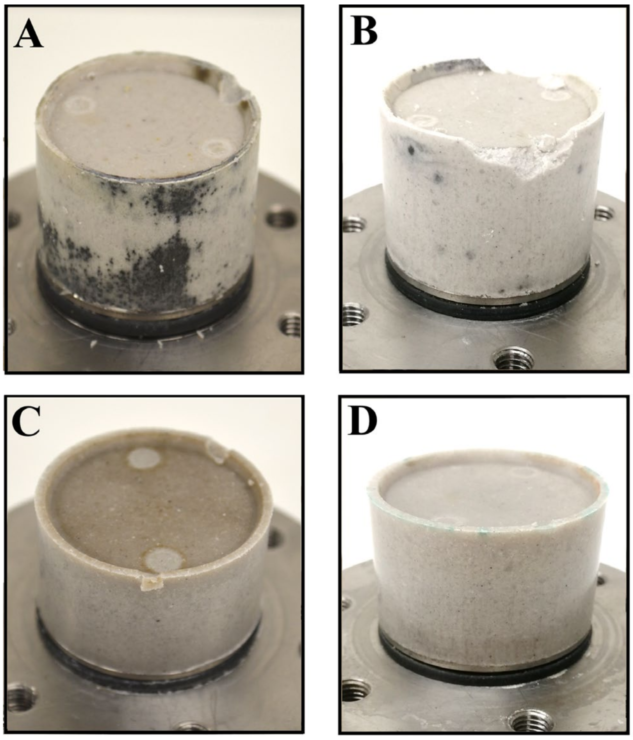


Figure 3-21. *Images of compacted water saturated grinded Moroccan bentonite cores after incubation. A Moroccan 1300 grinded, 2 months; B Moroccan 1550 grinded, 2 months; C Moroccan 1300 grinded, 4 months; D Moroccan 1550 grinded, 4 months.*

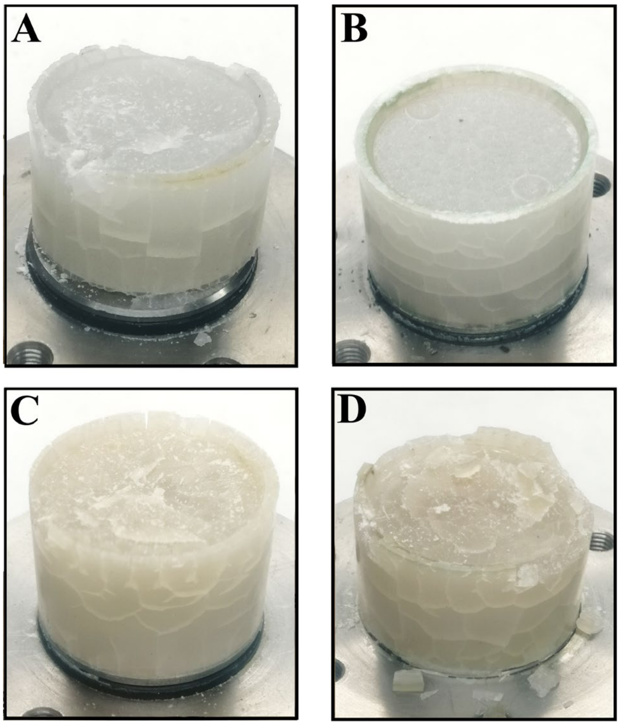


__Figure 3-22.__ Images of compacted water saturated Laponite cores after incubation. A Laponite 1300, 4 months; B Laponite 1300, 4 months; C Laponite 1100 spiked, 4 months; D Laponite 1300 spiked, 4 months.

3.4.3 Copper discs

Figure 3-23 to Figure 3-26 show the copper discs after incubation. Only the copper disc of the none-grinded Moroccan bentonite showed discolouration (Figure 3-24). Table 3-13 shows the average sulphur results from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured. The amount of sulphur increased by 45 % in 2 months in the test cell with Moroccan bentonite at a dry density of 1300 kg m^{-3}. All other test cells either showed no sulphur or only trace amounts (<1 %).

__Figure 3-23.__ Copper discs from every test cell with Turkish bentonite after 1, 2 and 4 months.

Figure 3-24. Copper discs from every test cell with Moroccan non-grinded bentonite after 2 and 4 months.

Figure 3-25. Copper discs from every test cell grinded Moroccan bentonite after 2 and 4 months.

Figure 3-26. Copper discs from every test cell with Laponite, after 4months.

3.4.4 Summary of results and observations

There were no signs of sulphide production in the Turkish, non-spiked clay cores or on the copper discs over the 1–4 months incubation period studied. Same results are valid for spiked and non-spiked Laponite. The Moroccan bentonite1300 had significant sulphide production deduced from black coloration and disappearance of sulphate. Non-grinded Moroccan bentonite had more of black clay cores and copper surfaces than grinded. Studies 1 and 2 has indicated a cut-off of sulphide production at around 1200 – 1300 kg m^{-3} dry density. Most of the dry densities tested here was around or above this number which may partially explain the absence of sulphide production, except for the Moroccan bentonite that has been somewhat of a worst-case bentonite, in particular, in a non-grinded form. Laponite was chosen due to its relatively high swelling pressure over density. Ther was no sign of sulphide production in Laponite samples with a dry density of around 1100 kg m^{-3} which is below the 1200 kg m^{-3} assumed cut-off dry density for other clays. The sulphur detected on the discs (Table 3-13) most likely is due to the presence of sulphate, not sulphide because there was no discolour on the discs (Figure 3-26). This report will return to Laponite in Study 6.

3.5 Study 4

3.5.1 Experiment

In this study the bentonite clays MX-80, Asha and Rokle were compacted at two dry densities (Table 3-14) corresponding to dry densities of 1090 and 1350 kg m^{-3}. Every bentonite clay and density were performed in triplets and were spiked with SRB prior compactions and lactate was added after the water saturation phase. Every triplet series had also a background sample that was without additions. The main goal with this test was to investigate reproducibility between test cells with identical density and additions in triplicates compared to a non-spiked control. In addition to study 1-3, here, leachable lactate was analysed at the end of the experiment.

Table 3-14. List of test cells with bentonite and planned dry densities

Test cell number	Bentonite clay	Planned dry/ saturated densities (kg m^{-3})	Replicate	Additions		
				Sulphate	Lactate	SRB
TC 1	MX-80	1087/1700	1	Y	Y	Y
TC 2	MX-80	1087/1700	2	Y	Y	Y
TC 3	MX-80	1087/1700	3	Y	Y	Y
TC 4	MX-80	1087/1700	Background	Y	N	N
TC 5	MX-80	1397/1900	1	Y	Y	Y
TC 6	MX-80	1397/1900	2	Y	Y	Y
TC 7	MX-80	1397/1900	3	Y	Y	Y
TC 8	MX-80	1397/1900	Background	Y	N	N
TC 9	Asha	1054/1700	1	Y	Y	Y
TC 10	Asha	1054/1700	2	Y	Y	Y
TC 11	Asha	1054/1700	3	Y	Y	Y
TC 12	Asha	1054/1700	Background	Y	N	N
TC 13	Asha	1356/1900	1	Y	Y	Y
TC 14	Asha	1356/1900	2	Y	Y	Y
TC 15	Asha	1356/1900	3	Y	Y	Y
TC 16	Asha	1356/1900	Background	Y	N	N
TC 17	Rokle	1047/1700	1	Y	Y	Y
TC 18	Rokle	1047/1700	2	Y	Y	Y
TC 19	Rokle	1047/1700	3	Y	Y	Y
TC 20	Rokle	1047/1700	Background	Y	N	N
TC 21	Rokle	1347/1900	1	Y	Y	Y
TC 22	Rokle	1347/1900	2	Y	Y	Y
TC 23	Rokle	1347/1900	3	Y	Y	Y
TC 24	Rokle	1347/1900	Background	Y	N	N

3.5.2 Water content, dry density and swelling pressure

The results of the water content analysis and analysed dry density are shown in Table 3-15. At day 19 the test cells were opened to add the copper discs and lactate. From day 72 the test cells were moved into Styrofoam boxes to reduce the effect of varying room temperature on the test cells. The pressure data from there on showed only minimal fluctuation (data not shown).

Table 3-15 shows the swelling pressure at the end of the water saturation period and mean reproduced swelling pressures deduced from data obtained with force transducers for each test cell from day 22 to 117.

Table 3-16 shows calculated statistics for Table 3-15 per studied clay and density. The standard deviations for each analysed parameter were generally low except for the measurement of reproduced swelling pressure at low densities, with exception for Rokle 1347 that was larger than for the other two clays at high density.

Table 3-15. Weight, water content and dry density for the water saturated bentonites in test cell 1-12. Swelling pressures at the end of the saturation period deduced from data (day 1 - 21) and the mean observed reproduced swelling pressure during the remaining experimental time, obtained with force transducers for each test cell. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Amount of bentonite (gdw)	Planned water content (%ww)	Analysed water content (%ww)	Planned dry density (kg m^{-3})	Analysed dry density (kg m^{-3})	Swelling pressure / Mean reproduced swelling pressure (kPa)
TC 1 MX-80 1087 1	21.24	36.07	39.03	1087	1022	180/197
TC 2 MX-80 1087 2	21.24	36.07	38.06	1087	1035	200/165
TC 3 MX-80 1087 3	21.24	36.07	40.25	1087	1006	200/367
TC 4 MX-80 1087 background	21.29	35.91	39.12	1089	1024	180/224
TC 5 MX-80 1397 1	27.30	26.46	29.35	1397	1332	1920/1983
TC 6 MX-80 1397 2	27.30	26.46	28.99	1397	1347	1680/1921
TC 7 MX-80 1397 3	27.30	26.46	28.57	1397	1356	1480/1952
TC 8 MX-80 1397 background	27.37	26.28	28.17	1401	1370	1760/1982
TC 9 Asha 1054 1	20.61	37.97	39.09	1054	1004	105/113
TC 10 Asha 1054 2	20.61	37.97	39.72	1054	1017	52/98
TC 11 Asha 1054 3	20.61	37.97	37.21	1054	1045	64/78
TC 12 Asha 1054 background	20.72	37.64	38.66	1060	1034	84/218
TC 13 Asha 1356 1	26.49	28.64	27.68	1356	1374	1090/968
TC 14 Asha 1356 2	26.49	28.64	27.65	1356	1381	870/1049
TC 15 Asha 1356 3	26.49	28.64	28.64	1356	1360	920/1494
TC 16 Asha 1356 background	26.63	28.26	27.02	1363	1390	1110/2021
TC 17 Rokle 1047 1	20.47	38.39	37.08	1047	1053	15/253
TC 18 Rokle 1047 2	20.47	38.39	37.18	1047	1062	250/435
TC 19 Rokle 1047 3	20.47	38.39	36.80	1047	1045	190/303
TC 20 Rokle 1047 background	20.57	38.08	35.56	1053	1077	290/101
TC 21 Rokle 1347 1	26.31	29.13	27.94	1347	1356	2400/2255
TC 22 Rokle 1347 2	26.31	29.13	28.90	1347	1331	2650/1457
TC 23 Rokle 1347 3	26.31	29.13	30.03	1347	1327	2190/2411
TC 24 Rokle 1347 background	26.45	28.77	29.78	1353	1325	2150/2622

Table 3-16. Statistics of Table 3-15

Analysed parameters	Analysed / planned/	Mean	Min	Max	SD	Standard deviation (± %)
MX80 1087						
Amount of bentonite (gdw)		21.3	21.2	21.3	0.0	0.12
Planned water content (%ww)		36.0	35.9	36.1	0.1	0.22
Analysed water content (%ww)	1.09	39.1	38.1	40.3	0.9	2.29
Planned dry density (kg m^{-3})		1088	1087	1089	1	0.09
Analysed dry density (kg m^{-3})	0.94	1022	1006	1035	12	1.17
Mean rep. swelling pressure (kPa)		238	165	367	89	37.42
MX80 1397						
Amount of bentonite (gdw)		27.3	27.3	27.4	0.0	0.13
Planned water content (%ww)		26.4	26.3	26.5	0.1	0.34
Analysed water content (%ww)	1.09	28.8	28.2	29.4	0.5	1.78
Planned dry density (kg m^{-3})		1398	1397	1401	2	0.14
Analysed dry density (kg m^{-3})	0.97	1351	1332	1370	16	1.18
Mean rep. swelling pressure (kPa)		1960	1921	1983	29	1.50
Asha 1054						
Amount of bentonite (gdw)		20.6	20.6	20.7	0.1	0.27
Planned water content (%ww)		37.9	37.6	38.0	0.2	0.44
Analysed water content (%ww)	1.02	38.7	37.2	39.7	1.1	2.76
Planned dry density (kg m^{-3})		1056	1054	1060	3	0.28
Analysed dry density (kg m^{-3})	0.97	1025	1004	1045	18	1.77
Mean rep. swelling pressure (kPa)		127	78	218	63	49.31
Asha 1356						
Amount of bentonite (gdw)		26.5	26.5	26.6	0.1	0.26
Planned water content (%ww)		28.5	28.3	28.6	0.2	0.67
Analysed water content (%ww)	0.97	27.7	27.0	28.6	0.7	2.41
Planned dry density (kg m^{-3})		1358	1356	1363	4	0.26
Analysed dry density (kg m^{-3})	1.01	1376	1360	1390	13	0.92
Mean rep. swelling pressure (kPa)		1383	968	2021	484	35.01
Rokle 1047						
Amount of bentonite (gdw)		20.5	20.5	20.6	0.1	0.24
Planned water content (%ww)		38.3	38.1	38.4	0.2	0.40
Analysed water content (%ww)	0.96	36.7	35.6	37.2	0.7	2.04
Planned dry density (kg m^{-3})		1049	1047	1053	3	0.29
Analysed dry density (kg m^{-3})	1.01	1059	1045	1077	14	1.30
Mean rep. swelling pressure (kPa)		273	101	435	138	50.55
Rokle 1347						
Amount of bentonite (gdw)		26.35	26.31	26.45	0.07	0.27
Planned water content (%ww)		29.04	28.77	29.13	0.18	0.62
Analysed water content (%ww)	1.00	29.16	27.94	30.03	0.95	3.25
Planned dry density (kg m^{-3})		1349	1347	1353	3	0.22
Analysed dry density (kg m^{-3})	0.99	1335	1325	1356	14	1.08
Mean rep. swelling pressure (kPa)		2186	1457	2622	509	23.28

3.5.3 Sulphate and lactate

Table 3-17 shows that sulphate was detected in all test cells except for test cells with Rokle. Further, the table shows that lactate was analysed in much larger concentrations in TC5 – TC7 which contained MX-80 at a planned dry density of 1397 kg m^{-3} and in all test cells with Rokle compared to low density test cells and for Asha test cells. The disappearance of lactate correlated with a decrease in sulphate concentrations which indicate bacterial sulphate reduction. A 5.7 M sodium lactate solution was added to a final calculated pore water lactate concentration of 23 mM in test cells with spiked clay which corresponds to approximately 10 µmol lactate gdw^{-1}.

3.5.4 Bentonite cores, copper discs and sulphide

Table 3-18 shows the average sulphur results from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured. The XRF measurements on copper discs from test cells with MX-80 showed only trace amounts of sulphur (<1 %). The XRF measurements on copper discs from test cells with Asha at a saturated density of 1700 kg m^{-3} showed that approximately 3–5 % of the surface is covered in sulphur. In comparison, the copper discs from the higher density test cells only showed trace amounts of sulphur. All copper discs from test cells with Rokle either showed no sulphur or only trace amounts for test cell 19 and 20. The sulphide analysis showed no measurable sulphide (<0.5 µmol gdw^{-3}) in any bentonite sample (data not shown).

Figure 3-27 shows the compacted, water saturated MX80 bentonites after 117 days of incubation. Black discolouration in the bottom bentonite core on the side of lactate addition (opposite to copper discs) indicate SRB activity. TC1 – TC3 and TC7 showed some discolouration of the clay. Figure 3-28 shows the copper discs after incubation. In the test cells with MX-80 only the copper discs with the lower density (TC1, 2 and 3) showed a patchy, slight discolouration. The background cores without any additions were clean without discolouration.

Figure 3-29 shows Asha TC9 – 11 at a planned dry density of 1054 kg m^{-3} with large areas with black discolouration at the bottom. This might be due to a lower density in this area where the clay is in contact with the piston. TC 13 – 15 with Asha at a planned dry density of 1356 kg m^{-3} also showed areas with black discolouration but less than at the lower density. In the test cells with Asha all copper discs showed black discolouration, except the background controls (Figure 3-30). The background cores did not show black discolouration.

Figure 3-31vshows TC17 – 19 with Rokle at a planned dry density of 1047 kg m^{-3} showed black discolouration at the bottom Figure 3-31. TC21 and 22 with Rokle at a planned dry density of 1347 kg m^{-3} were free of discolouration. However, TC 23 showed small areas of black discolouration. The background cores did not show black discolouration. In the test cells with Rokle none of the copper discs showed a black discolouration (Figure 3-32).

Table 3-17. Leachable amount of sulphate and lactate in the bentonite clays on average across the three sampling points for test cells 1-3. (gdw = gram dry weigh, - = not analysed). The amount of leachable sulphate from the natural clays are shown as well

Name of test cell	Micromole of sulphate per gram of bentonite (µmol gdw^{-1})	Mean of sulphate per clay and density (µmol gdw^{-1})	Standard deviation (± %)	Micromole of lactate per gram of bentonite (µmol gdw^{-1})	Mean of lactate per clay and density (µmol gdw^{-1})	Standard deviation (± %)
TC 1 MX-80 1087 1	24.7			<0.07		
TC 2 MX-80 1087 2	28.2	25.6	8.8	0.17	0.18	4.04
TC 3 MX-80 1087 3	24			0.18		
TC 4 MX-80 1087 background	30.3			-		
TC 5 MX-80 1397 1	22.5			9.29		
TC 6 MX-80 1397 2	21.2	19.9	17.6	6.82	7.4	22.5
TC 7 MX-80 1397 3	15.9			6.11		
TC 8 MX-80 1397 background	22.6			-		
Natural MX-80	51.1					
TC 9 Asha 1054 1	17.7			0.12		
TC 10 Asha 1054 2	19	18.6	4.0	0.10	0.10	14.8
TC 11 Asha 1054 3	19			0.09		
TC 12 Asha 1054 background	28.8			-		
TC 13 Asha 1356 1	22.3			0.19		
TC 14 Asha 1356 2	22.6	22.4	0.8	0.07	0.12	55.1
TC 15 Asha 1356 3	22.3			0.09		
TC 16 Asha 1356 background	34.6			-		
Natural Asha	37.7			-		
TC 17 Rokle 1047 1	<0.4			4.15		
TC 18 Rokle 1047 2	<0.4	<0.4	-	2.59	3.23	25.1
TC 19 Rokle 1047 3	<0.4			2.96		
TC 20 Rokle 1047 background	<0.4			-		
TC 21 Rokle 1347 1	<0.4			4.69		
TC 22 Rokle 1347 2	<0.4	<0.4	-	4.45	4.69	5.22
TC 23 Rokle 1347 3	<0.4			4.94		
TC 24 Rokle 1347 background	<0.4			-		
Natural Rokle	<0.4			-		

Table 3-18. Average sulphur values from XRF measurements on the top surface (n = 5)

Name of test cell	Average S (%) per test cell	Standard deviation (± %)	Average S (%) per clay and density	Standard deviation (± %)
TC 1 MX-80 1087 1	0.37	0.04		
TC 2 MX-80 1087 2	0.29	0.17	0.3	23.5
TC 3 MX-80 1087 3	0.3	0.17		
TC 4 MX-80 1087 background	0.37	0.06		
TC 5 MX-80 1397 1	0.67	0.04		
TC 6 MX-80 1397 2	0.75	0.07	0.7	2.9
TC 7 MX-80 1397 3	0.7	0.03		
TC 8 MX-80 1397 background	0.24	0.13		
TC 9 Asha 1054 1	3.44	0.97		
TC 10 Asha 1054 2	5.3	1.51	4.7	12.5
TC 11 Asha 1054 3	5.46	2.15		
TC 12 Asha 1054 background	<0.2	-		
TC 13 Asha 1356 1	0.29	0.48		
TC 14 Asha 1356 2	0.33	0.25	0.4	43.4
TC 15 Asha 1356 3	0.64	0.12		
TC 16 Asha 1356 background	<0.2	-		
TC 17 Rokle 1047 1	<0.2	-		
TC 18 Rokle 1047 2	<0.2	-	-	-
TC 19 Rokle 1047 3	0.34	0.33		
TC 20 Rokle 1047 background	0.35	0.09		
TC 21 Rokle 1347 1	<0.2	-		
TC 22 Rokle 1347 2	<0.2	-	-	-
TC 23 Rokle 1347 3	<0.2	-		
TC 24 Rokle 1347 background	<0.2	-		

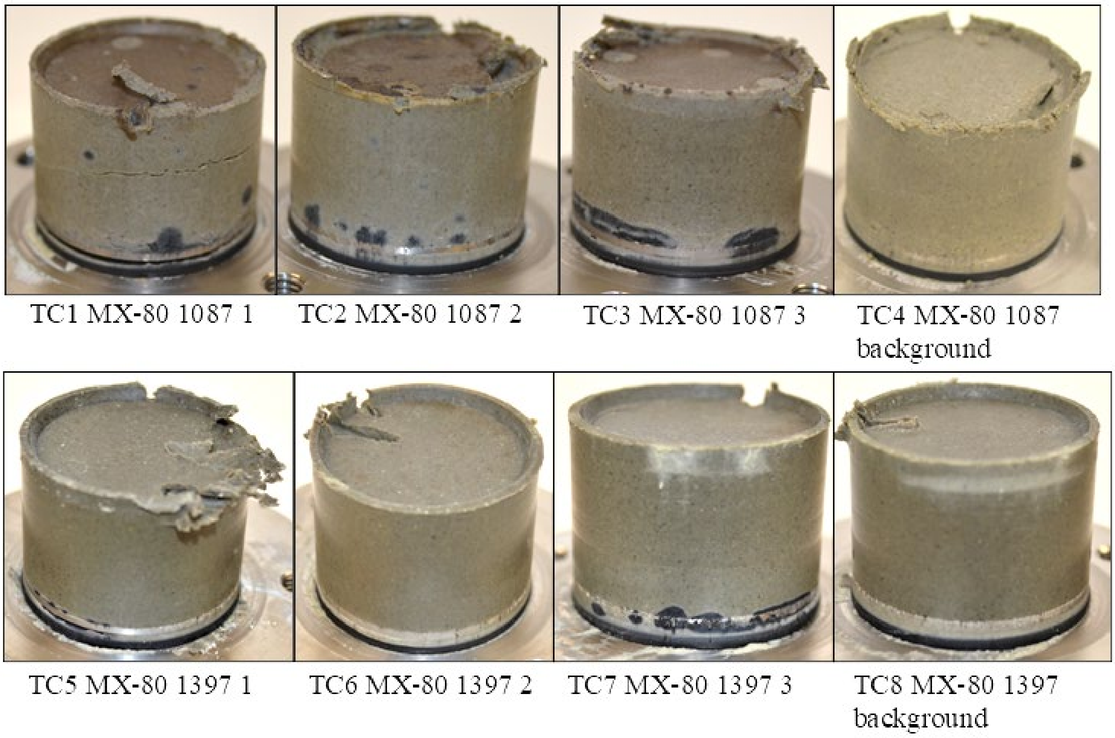


Figure 3-27. *Images of compacted water saturated MX-80 cores from TC1 – 8 after 117 days. Numbers show dry density.*

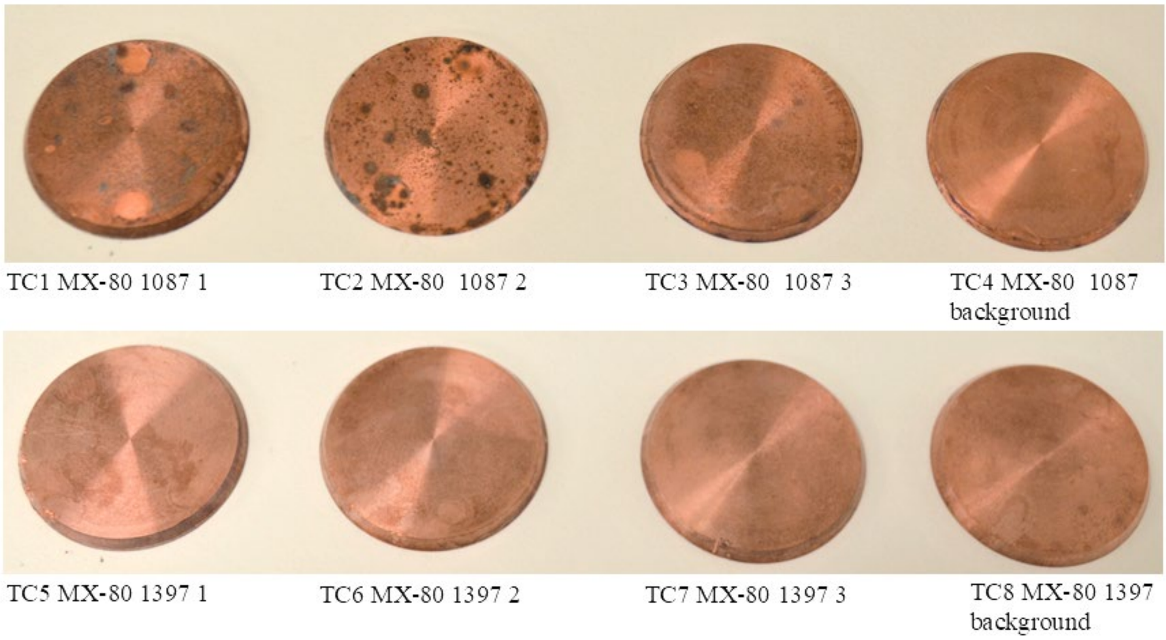
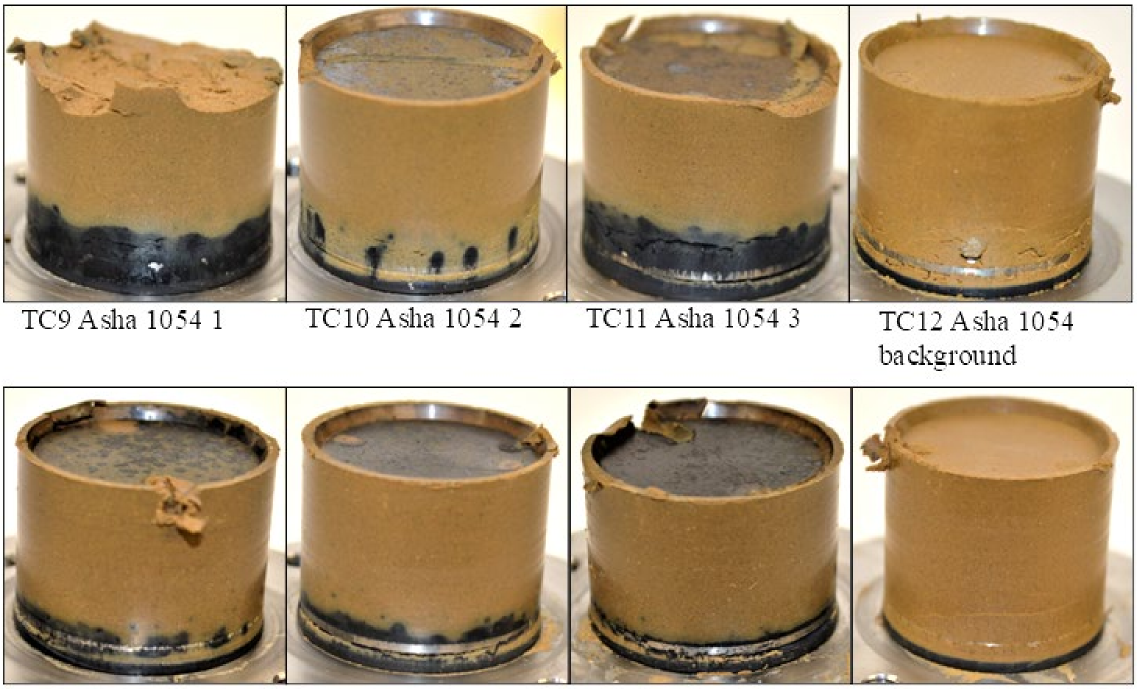


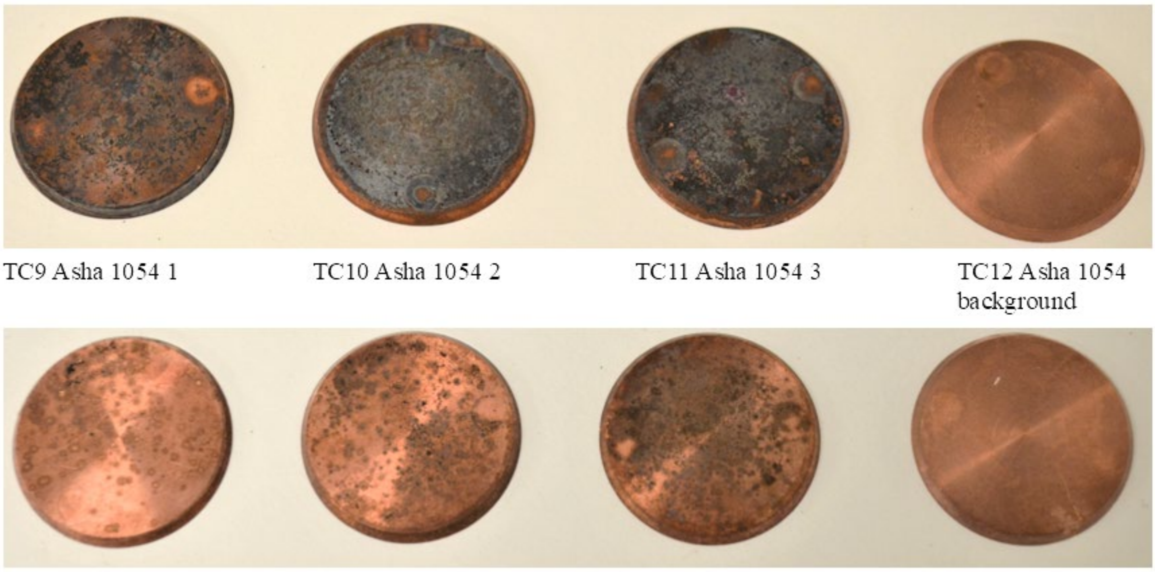
Figure 3-28. *Copper discs from TC1 – 8. Numbers show dry density.*



TC9 Asha 1054 1 | TC10 Asha 1054 2 | TC11 Asha 1054 3 | TC12 Asha 1054 background

TC13 Asha 1356 1 | TC14 Asha 1356 2 | TC15 Asha 1356 3 | TC16 Asha 1356 background

Figure 3-29. *Images of compacted water saturated Asha cores from TC9 – 16 after 117 days. Numbers show dry density.*



TC9 Asha 1054 1 | TC10 Asha 1054 2 | TC11 Asha 1054 3 | TC12 Asha 1054 background

TC13 Asha 1356 1 | TC14 Asha 1356 2 | TC15 Asha 1356 3 | TC16 Asha 1356 background

Figure 3-30. *Copper discs from TC9 – 16. Numbers show dry density.*

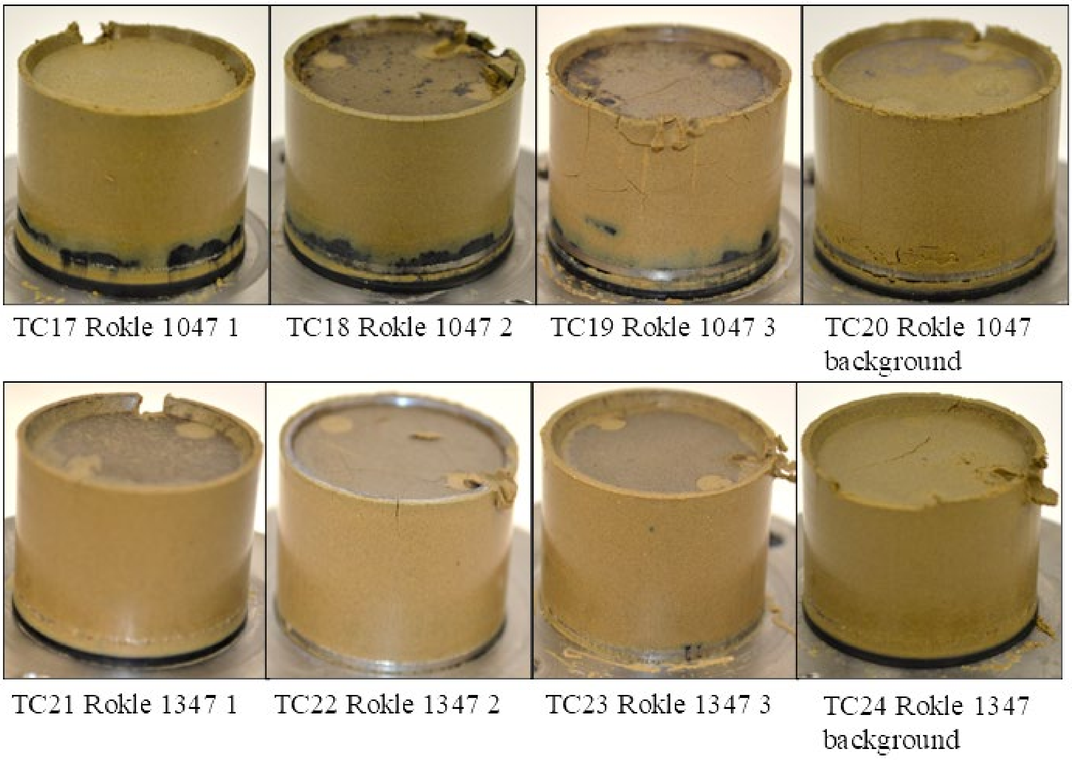


Figure 3-31. *Images of compacted water saturated Rokle cores from TC17 – 24 after 117 days. Numbers show dry density.*

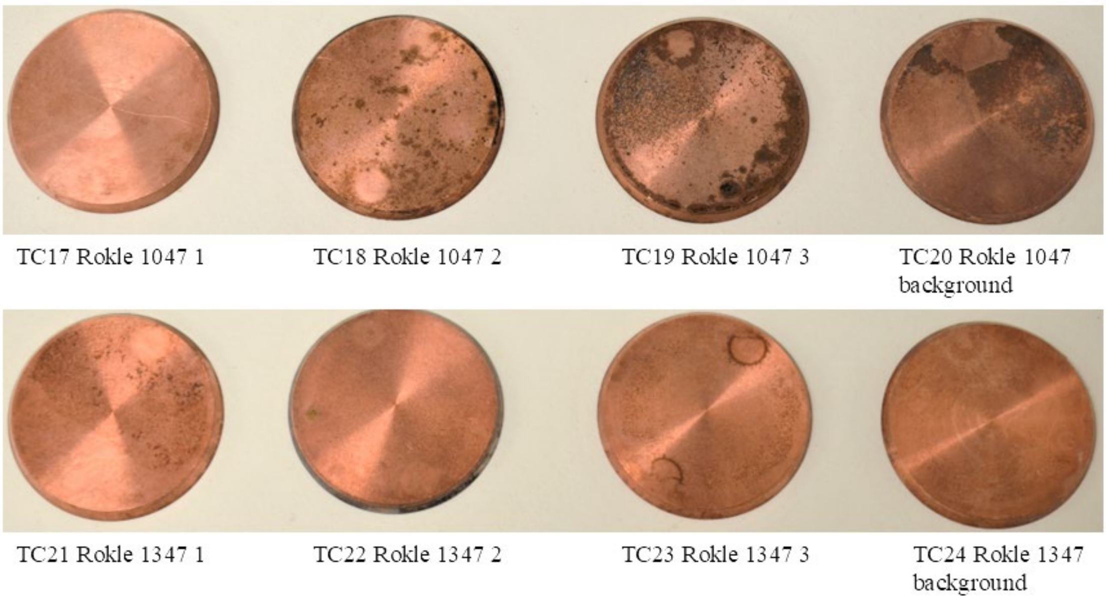


Figure 3-32. *Copper discs from TC17 – 24. Numbers show dry density.*

3.5.5 Summary of results and observations

In this study the precision of the method were determined with triple samples. The summarized data of the triple samples show that data can be reproduced with small variations across identical test cells (Table 3-16 to Table 3-18). Therefore, the system is ideal to reproduce data.

The bentonite cores and copper discs showed, as previous experiments have shown, that a higher density reduces SRB activity and sulphide production. Overall Rokle showed the lowest SRB activity and Asha highest. One explanation for the low SRB activity in Rokle and also the absence of lactate consumption, might be due to the absence of sulphate in Rokle itself. In comparison natural MX-80 contained 51.1 µmol gdw^{-1} and Asha 37.7 µmol gdw^{-1}. However, there are more factors that influence SRB activity than sulphate since Asha had more sulphur in average on the copper discs then MX-80 although containing less sulphate. Most of the blackening of Asha due to iron sulphide occurred in the interface and may, therefore be partly an artefact (see 3.2.4).

The pressure reading system seemed sensitive to exterior variations in room temperature that influenced the titanium the test cells are made of. In was observed that the pressure signal fluctuated less after moving the test cells to Styrofoam boxes to keep temperature stable. The fluctuation in signal may create movement in the test cells and loosens screws. This movement may create areas in the bentonite clay with lower density and swelling pressure. This can explain why there was black discolouration in the bentonite core of test cell 7 with MX-80 at a dry density of 1387 kg m^{-3} while there was no SRB activity in test cell 5 and 6 with identical parameters. Therefore, the test cells in the studies 5-9 were placed in an environment with a more stable temperature after water saturation compared to studies 1-4.

3.6 Study 5

3.6.1 Experiment

In this study the bentonite clays MX-80 and Rokle were compacted at two dry densities 450/470 and 1400 kg m^{-3}, corresponding to saturated densities of 1300 and 1900 kg m^{-3}. All bentonite clays and densities were performed in duplicates that were spiked with SRB prior compactions and lactate was added after the water saturation phase. All bentonite clays and densities had a background control test cell without additions. The study includes tests of the effect of granule size on MX-80 and Rokle as grinded and not grinded clays (confer Figure 3-18). Further, three small copper surfaces (1 × 5 × 13 mm) were added inside a set of MX-80 clay cores to test formation of copper sulphide inside the cores (Table 3-19). This approach was adopted from Pedersen (2010).

The study includes tests of the effect of granule size on MX-80 and Rokle as grinded and not grinded clays (confer Figure 3-18). Further, three small copper surfaces (1 × 5 × 13 mm) of the same material as the circular discs were added inside a set of MX-80 clay cores to test formation of copper sulphide inside the cores.

Table 3-19. List of test cells with bentonite and planned dry densities. Y=Yes, N=No

Test cell number	Bentonite clay	Planned dry densities (kg m^{-3})	Replicate	Additions/treatment				
				Sulphate	Lactate	SRB	Grinded	Small copper surfaces
TC 1	MX-80	470	1	Y	Y	Y	Y	N
TC 2	MX-80	470	2	Y	Y	Y	Y	N
TC 3	MX-80	470	Background	Y	N	N	Y	N
TC 4	MX-80	470	1	Y	Y	Y	N	N
TC 5	MX-80	470	2	Y	Y	Y	N	N
TC 6	MX-80	470	Background	Y	N	N	N	N
TC 7	Rokle	450	1	Y	Y	Y	Y	N
TC 8	Rokle	450	2	Y	Y	Y	Y	N
TC 9	Rokle	450	Background	Y	N	N	Y	N
TC 10	Rokle	450	1	Y	Y	Y	N	N
TC 11	Rokle	450	2	Y	Y	Y	N	N
TC 12	Rokle	450	Background	Y	N	N	N	N
TC 13	MX-80	470	1	Y	Y	Y	Y	Y
TC 14	MX-80	470	2	Y	Y	Y	Y	Y
TC 15	MX-80	470	Background	Y	N	N	Y	Y
TC 16	MX-80	1400	1	Y	Y	Y	Y	Y
TC 17	MX-80	1400	2	Y	Y	Y	Y	Y
TC 18	MX-80	1400	Background	Y	N	N	Y	Y

The test cells 13–18 had three small (1×5×13 mm) copper discs added (Figure 3-33). The volume of the three copper discs was calculated and then subtracted from the volume of the test cell to calculate the correct amount of bentonite powder. Circa 1/4 of the total amount from the bentonite clay was filled into the respective test cell cylinder and compacted with the workshop press. Then the first copper disc was added on top of the clay. This procedure was repeated for the other two copper discs and until the whole amount of bentonite powder was added.

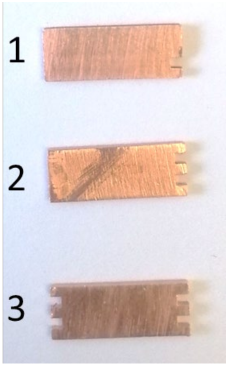


Figure 3-33. *Small copper discs. The numbers indicate in which order they were added with 1 being closest to the bottom.*

3.6.2 Water content, dry density and swelling pressure

The results of the water content analysis and resulting dry density are shown in Table 3-20. The swelling pressures at the end of the water saturation period day 21 is also shown. The test cells were opened and analysed day 126-129.

3.6.3 Sulphate and lactate

Table 3-21 shows that sulphate could be analysed in most of the test cells with MX-80 except for TC 1, 2, 4 and 14. In test cells with Rokle sulphate was under the detection limit as in study 4. Further, the table shows that lactate concentration was higher in test cells with grinded bentonite compared to test cells with non-grinded clay. The lactate concentration in TC16 and 17 with a dry density of 1500 kg m^{-3} was higher in comparison to TC13 and 14 with a dry density of 500 kg m^{-3}. The disappearance of lactate correlated with a decrease in sulphate concentrations which indicate bacterial sulphate reduction.

3.6.4 Bentonite cores, copper discs and sulphide

Table 3-21 shows the average sulphur results from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured. On the small copper discs the front and back side was measurement once. The XRF measurements on copper discs from TC1 approximately 7 % of the surface was covered by sulphur but the XRF measurement for the duplicate TC2 was below 0.2 %. A similar result was observed for the not grinded MX-80 series. All copper discs from test cells with Rokle showed no sulphur. The XRF measurements on copper discs from test cells with MX-80 and added small copper discs showed only trace amounts of sulphur (<1 %) for both densities.

There was no detectable difference in sulphur on the small copper plates between the two MX-80 densities (Table 3-22). The sulphide analysis showed no measurable sulphide (<0.5 µmol gdw^{-3}) in any bentonite sample (data not shown).

Figure 3-34 to Figure 3-40 show the compacted, water saturated bentonites with corresponding copper discs after 129 days of incubation. Black discolouration in bentonite clays and on the copper discs indicates SRB activity. The clay cores became rather brittle at low densities and did not keep shape when pushed out of the titanium cylinders.

TC1 and TC4 show black discolouration across almost the whole copper discs. However, this effect was not reproduced in the respective duplicate (TC2 and TC5). In the test cells with Rokle none of the copper discs showed a black discolouration. In the series with grinded Rokle only TC7 showed a spot of black discolouration. In contrast the whole clay core appeared slightly black in TC10 – 11 with non-grinded Rokle. TC13 and 14 with MX-80 at a planned dry density of 470 kg m^{-3} showed large areas with black discolouration at the bottom of the clay cores where lactate was added.

In the test series with MX-80 and added small copper plates only the copper plates with the lower density showed slight discolouration. However, the small copper plates of the higher density showed more black discolouration than the small copper plates of the lower density.

Table 3-20. Weight, water content and dry density for the water saturated bentonites in test cell 1-12. Swelling pressures at the end of the saturation phase deduced from data (day 1 - 20) obtained with force transducers for each test cell. (gdw = gram dry weight, %ww = percent wet weight, n.g. = not grinded, Cu = with copper discs inside the clay).

Name of test cell	Amount of bentonite (gdw)	Planned water content (%ww)	Analysed water content (%ww)	Planned dry density (kg m^{-3})	Analysed dry density (kg m^{-3})	Swelling pressure (kPa)
TC 1 MX-80 470 1	9.09	64.2	58.0	465	514	160
TC 2 MX-80 470 2	9.09	64.2	57.7	465	512	160
TC 3 MX-80 470 background	9.12	64.1	60.3	467	472	160
TC 4 MX-80 470 n.g. 1	9.11	64.1	63.9	466	425	180
TC 5 MX-80 470 n.g. 2	9.11	64.1	59.3	466	494	180
TC 6 MX-80 470 n.g. background	9.05	64.4	61.5	463	462	180
TC 7 Rokle 450 1	8.75	65.6	61.4	448	456	-
TC 8 Rokle450 2	8.75	65.6	59.7	448	471	180
TC 9 Rokle 450 background	8.82	65.3	59.6	452	448	80
TC 10 Rokle 450 n.g. 1	8.56	66.3	56.6	438	446	200
TC 11 Rokle 450 n.g. 2	8.56	66.3	61.4	438	400	200
TC 12 Rokle 450 n.g. background	8.62	66.1	56.6	441	403	250
TC 13 MX-80 470 + Cu 1	9.01	64.5	60.7	461	528	250
TC 14 MX-80 470 + Cu 2	9.01	64.5	64.5	461	472	250
TC 15 MX-80 470 + Cu background	9.04	64.4	65.1	463	465	114
TC 16 MX-80 1400 + Cu 1	27.02	27.2	25.4	1383	1480	2000
TC 17 MX-80 1400 + Cu 2	27.02	27.2	25.3	1383	1495	1700
TC 18 MX-80 1400 + Cu background	27.12	27.0	26.3	1388	1579	1500

Table 3-21. Leachable amount of sulphate and lactate in the bentonite clays on average across the three sampling points for each test cell. (gdw = gram dry weight, - = not analysed, n.g. = not grinded).

Name of test cell	Micromole of sulphate per gram of bentonite (µmol gdw^{-1})	Micromole of lactate per gram of bentonite (µmol gdw^{-1})	Average S (%)	Standard deviation (± %)
TC 1 MX-80 470 1	<0.4	0.12	6.62	1.96
TC 2 MX-80 470 2	<0.4	0.28	<0.20	-
TC 3 MX-80 470 background	17.3	-	0.26	0.01
TC 4 MX-80 470 n.g. 1	<0.4	<0.07	5.60	1.65
TC 5 MX-80 470 n.g. 2	12.9	<0.07	0.33	0.38
TC 6 MX-80 470 n.g. background	24.5	-	0.24	0.16
TC 7 Rokle 450 1	<0.4	0.39	<0.20	-
TC 8 Rokle 450 2	<0.4	0.30	<0.20	-
TC 9 Rokle 450 background	<0.4	-	<0.20	0.21
TC 10 Rokle 450 n.g. 1	<0.4	0.22	<0.20	-
TC 11 Rokle 450 n.g. 2	<0.4	0.30	<0.20	-
TC 12 Rokle 450 n.g. background	<0.4	-	0.28	0.01
TC 13 MX-80 470 + Cu 1	7	<0.07	1.09	1.36
TC 14 MX-80 470 + Cu 2	<0.4	<0.07	0.18	0.21
TC 15 MX-80 470 + Cu background	35.43	-	0.52	0.06
TC 16 MX-80 1400 + Cu 1	22.4	2.82	0.34	0.04
TC 17 MX-80 1400 + Cu 2	15.4	1.03	0.11	0.22
TC 18 MX-80 1400 + Cu background	18.81	-	<0.20	-

Table 3-22. Average sulphur values for the small copper plates from XRF measurements on the top and back side (n = 2).

Test cell	Average S (%)	Standard deviation (± %)
TC13 small copper plate 1	0.73	0.18
TC13 small copper plate 2	0.87	0.79
TC13 small copper plate 3	0.84	0.01
TC14 small copper plate 1	0.63	0.88
TC14 small copper plate 2	0.94	0.85
TC14 small copper plate 3	0.49	0.06
TC15 small copper plate 1	0.46	0.27
TC15 small copper plate 2	0.44	0.02
TC15 small copper plate 3	0.20	0.28
TC16 small copper plate 1	0.73	0.00
TC16 small copper plate 2	0.53	0.76
TC16 small copper plate 3	1.03	0.17
TC17 small copper plate 1	1.62	1.40
TC17 small copper plate 2	1.01.	0.01
TC17 small copper plate 3	1.12	0.00
TC18 small copper plate 1	0.15	0.21
TC18 small copper plate 2	0.32	0.02
TC18 small copper plate 3	0.21	0.30

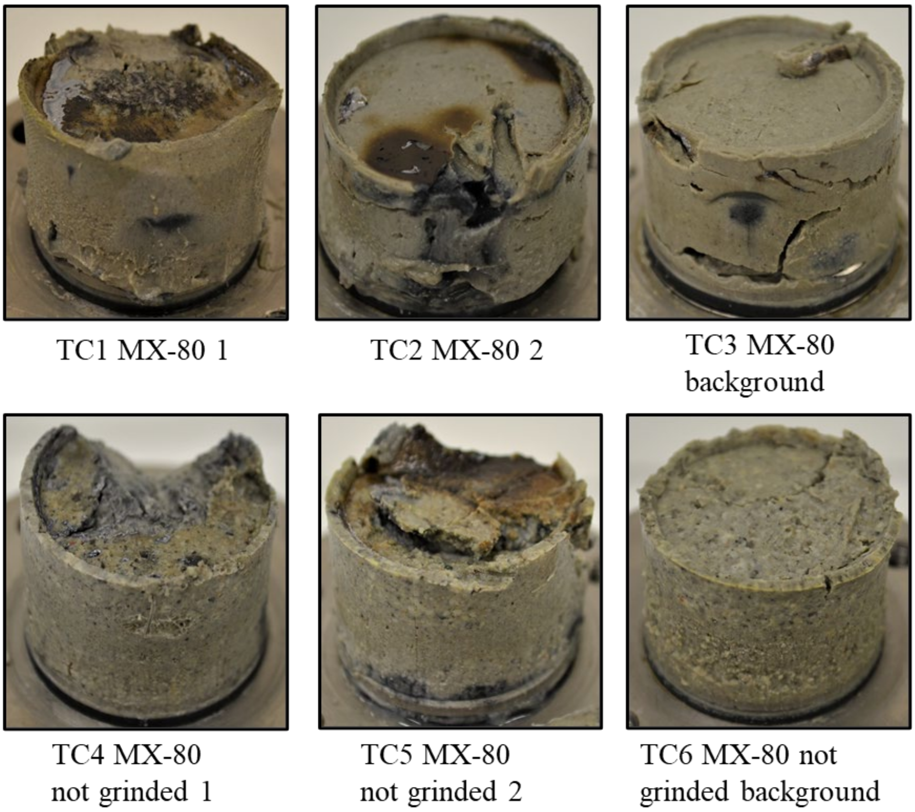


Figure 3-34. *Images of compacted water saturated MX-80 cores from TC1 – 6 after 129 days.*

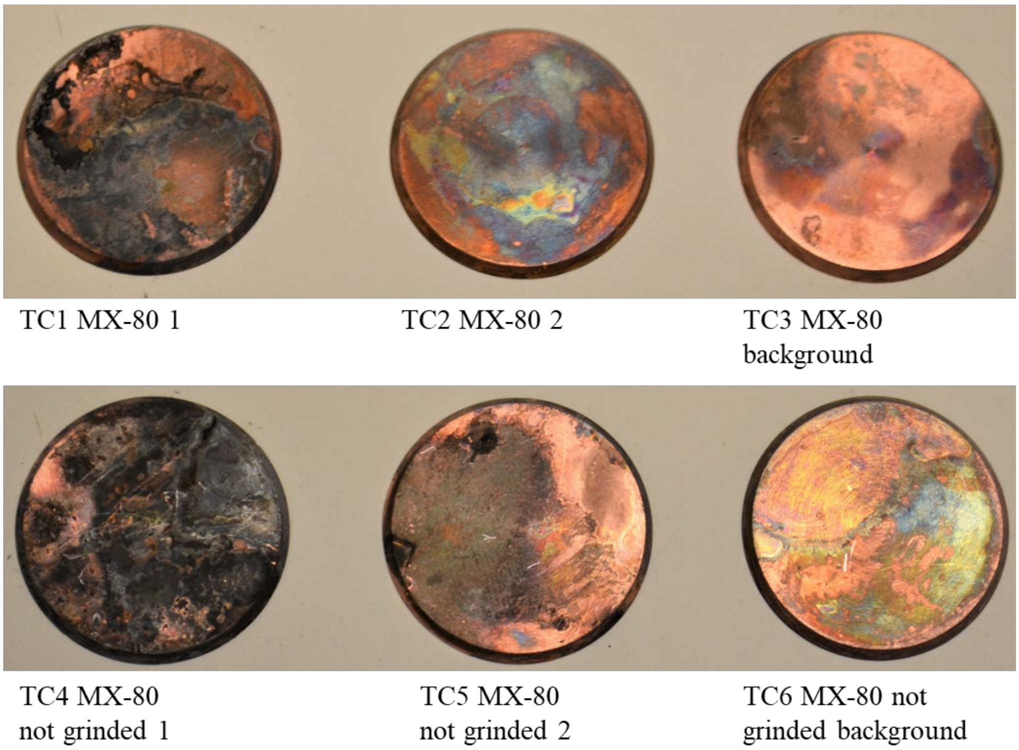


Figure 3-35. *Copper discs from MX-80 cores in TC1 – 6.*

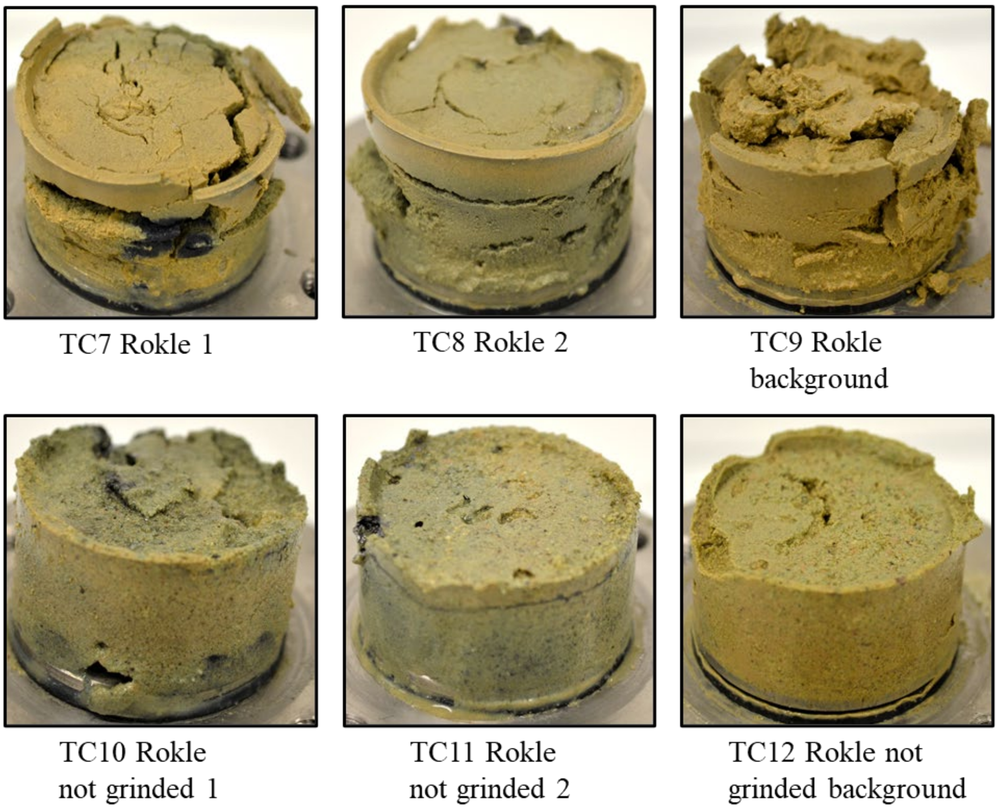


Figure 3-36. *Images of compacted water saturated Rokle cores from TC7 – 12 after 129 days.*

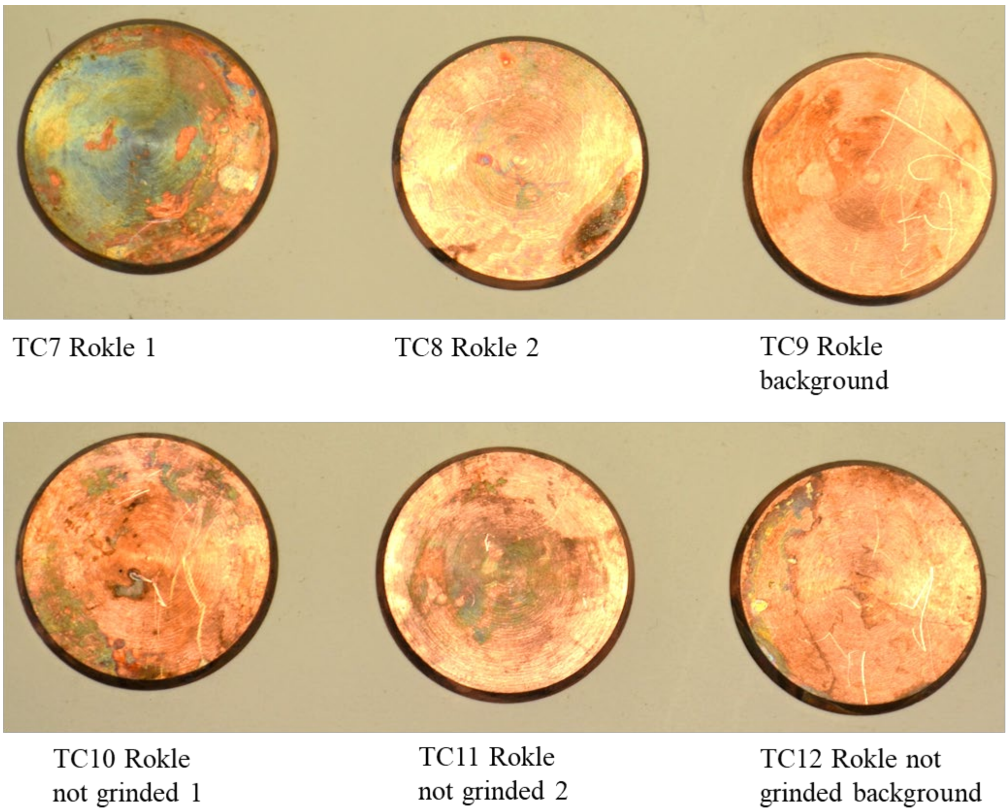


Figure 3-37. *Copper discs from Rokle cores in TC7 – 12.*

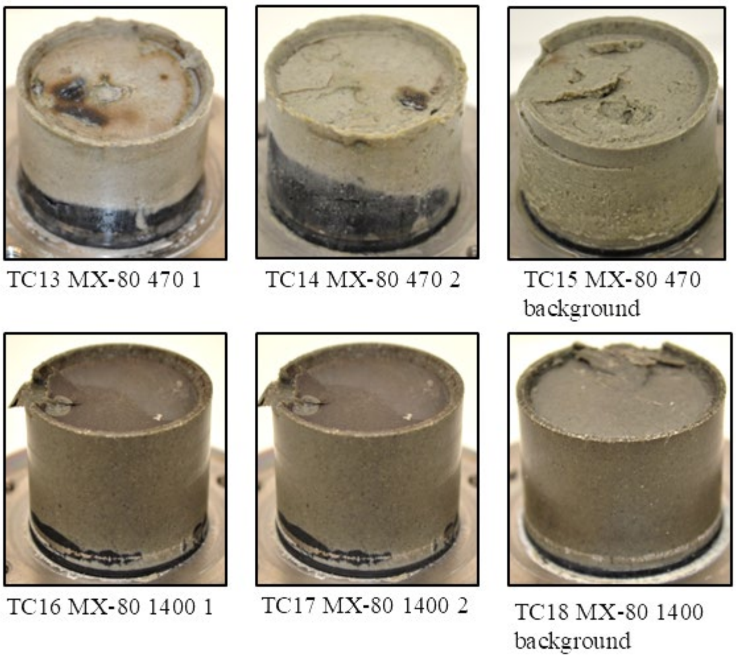


Figure 3-38. *Images of compacted water saturated MX-80 cores from TC13 – 18 after 129 days. Numbers show dry density.*

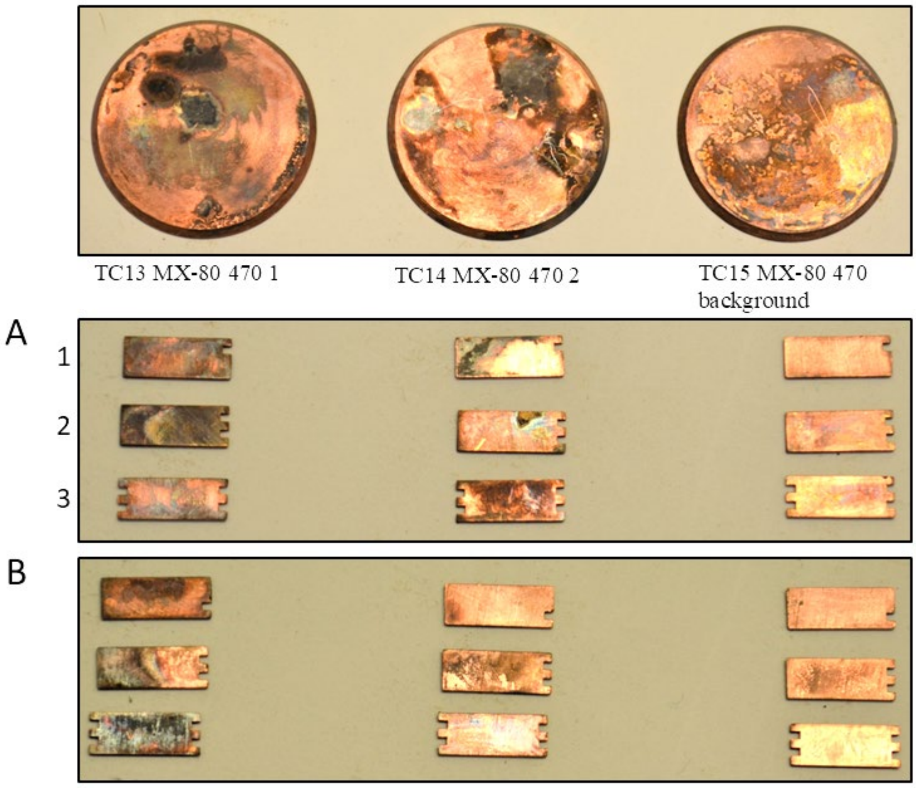


Figure 3-39. *Copper discs and small copper plates from TC13 – 15. A: topside of the three small copper plates. B: backside of the three small copper plates. 1-3 indicates in which order they were inside the bentonite with 1 closest to the bottom and 3 closest to the copper disc. Numbers show dry density.*

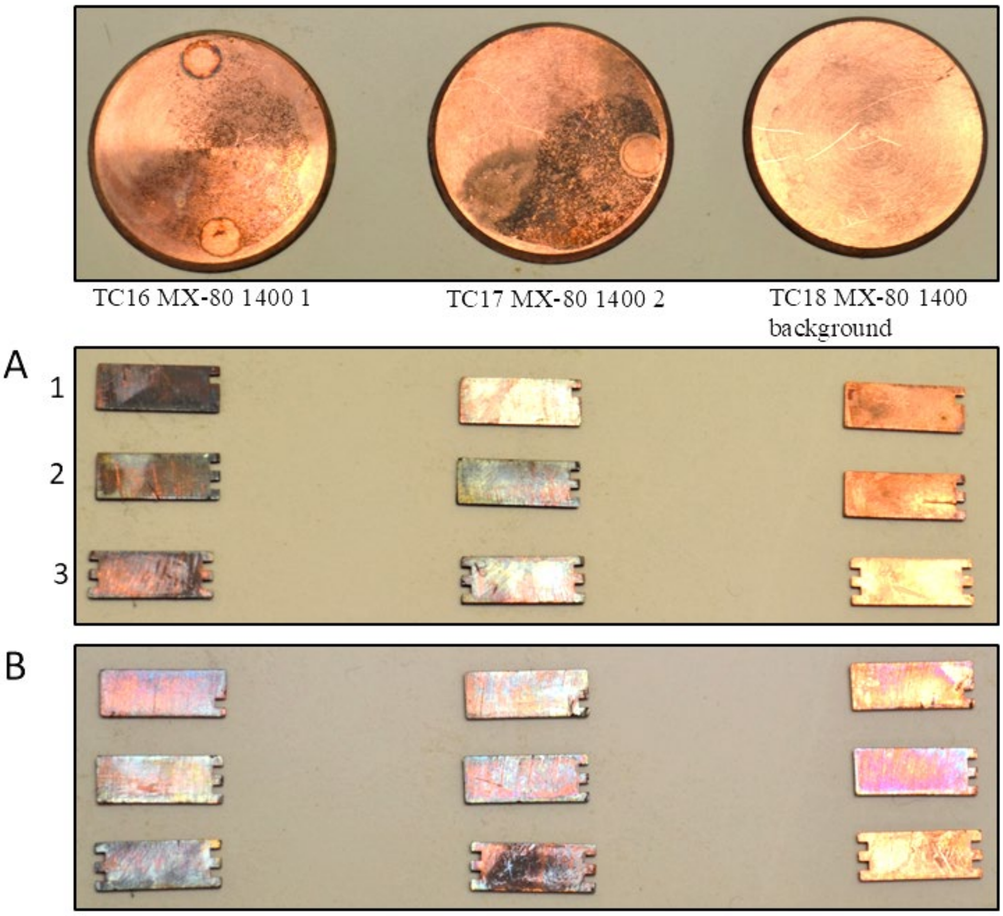


Figure 3-40. *Copper discs and small copper plates from TC13 – 15. A: topside of the three small copper plates. B: backside of the three small copper plates. 1-3 indicates in which order they were inside the bentonite with 1 closest to the bottom and 3 closest to the copper disc. Numbers show dry density.*

3.6.5 Summary of results and observations

There seems to have been an effect of granule size on the results of MX-80 and Rokle as judged from the disappearance of lactate and discolouration of the clay cores, but not when judged from amount of S on the copper discs. It is possible that measuring S on the copper disc may be an unreliable method for the detection of SRB activity if most of the activity occurs in the clay core and sulphide precipitates before reaching the discs. Rokle showed lower SRB activity than MX-80. One explanation for the low SRB activity in Rokle might be due to the absence of sulphate in Rokle itself.

In the series with small added copper plates, it could be shown again that a higher density reduces SRB activity analysed as S on the copper discs/ plates. There were only minimal differences in sulphur amounts on the three small copper plates in each test cell, indicating that SRB activity is not predominant at a specific location in the clay.

3.7 Study 6

3.7.1 Experiment

In this study the bentonite clay Laponite was compacted at six different dry densities (645 – 1080 kg m^{-3}) with corresponding relatively high swelling pressures (Table 3-23). Asha was compacted at the dry densities 1200 and 1280 kg m^{-3}. Every bentonite clay and density were performed in triplets. Two test cells were spiked with SRB prior compactions and lactate was added after the water saturation phase. The third test cell in each series was used as a background control without additions.

Table 3-23. List of test cells with bentonite and planned dry densities

Test cell number	Bentonite clay	Planned dry densities (kg m^{-3})	Replicate	Conditions		
				Sulphate	Lactate	SRB
TC 1	Laponite	645	1	Y	Y	Y
TC 2	Laponite	645	2	Y	Y	Y
TC 3	Laponite	645	Background	Y	N	N
TC 4	Laponite	665	1	Y	Y	Y
TC 5	Laponite	665	2	Y	Y	Y
TC 6	Laponite	665	Background	Y	N	N
TC 7	Laponite	685	1	Y	Y	Y
TC 8	Laponite	685	2	Y	Y	Y
TC 9	Laponite	685	Background	Y	N	N
TC 10	Laponite	700	1	Y	Y	Y
TC 11	Laponite	700	2	Y	Y	Y
TC 12	Laponite	700	Background	Y	N	N
TC 13	Laponite	760	1	Y	Y	Y
TC 14	Laponite	760	2	Y	Y	Y
TC 15	Laponite	760	Background	Y	N	N
TC 16	Laponite	1080	1	Y	Y	Y
TC 17	Laponite	1080	2	Y	Y	Y
TC 18	Laponite	1080	Background	Y	N	N
TC 19	Asha	1200	1	Y	Y	Y
TC 20	Asha	1300	2	Y	Y	Y
TC 21	Asha	1200	Background	Y	N	N
TC 22	Asha	1280	1	Y	Y	Y
TC 23	Asha	1280	2	Y	Y	Y
TC 24	Asha	1280	Background	Y	N	N

3.7.2 Water content, dry density, and swelling pressure

The results of the water content analysis and resulting dry density and the registered swelling pressures for all test cells at the end of the water saturation period are shown in Table 3-24.

3.7.3 Sulphate and lactate

Table 3-25 shows that sulphate could be analysed in all the test cells. The test cells with Laponite contained far less leachable sulphate then Asha did. However, Laponite (71.9 µmol gdw^{-1}) and Asha (72.9 µmol gdw^{-1}) contain naturally similar amounts of sulphate. But after compaction and water saturation, Laponite appeared to have lost the majority of leachable sulphate, likely in some kind of precipitations process. The table also shows that lactate was present in all Laponite test cells in amounts similar to what was added – lactate was not consumed. Asha had less lactate at the highest density and in the low-density test cells 19 and 20 (1205 kg m^{-3}), lactate was totally consumed.

Table 3-24. Weight, water content and dry density for the water saturated bentonites in test cell 1-24. Swelling pressures at the end of the water saturation period obtained with force transducers for each test cell. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Amount of bentonite (gdw)	Calculated water content (%ww)	Analysed water content (%ww)	Calculated dry density (kg m^{-3})	Analysed dry density (kg m^{-3})	Swelling pressure (kPa)
TC 1 Laponite 645	12.40	54.55	56.00	634	614	330
TC 2 Laponite 645	12.40	54.55	56.76	634	608	420
TC 3Laponite 645 background	12.63	53.68	53.20	647	655	390
TC 4 Laponite 665	12.77	53.57	53.53	654	637	450
TC 5 Laponite 665	12.77	53.57	54.24	654	635	450
TC 6 Laponite 665 background	13.02	52.69	52.28	666	668	430
TC 7 Laponite 685	13.15	52.61	52.53	673	664	460
TC 8 Laponite 685	13.15	52.61	52.75	673	650	280
TC 9 Laponite 685 background	13.40	51.71	51.16	686	686	380
TC 10 Laponite 700	13.52	51.67	51.95	692	674	480
TC 11 Laponite 700	13.52	51.67	51.85	692	685	500
TC 12 Laponite 700 background	13.78	50.74	51.52	705	690	700
TC 13 Laponite 760	14.68	48.85	47.88	751	756	840
TC 14 Laponite 760	14.68	48.85	49.52	751	741	850
TC 15 Laponite 760 background	14.96	47.87	47.40	766	763	970
TC 16 Laponite 1080	20.79	36.07	37.84	1064	1021	5200
TC 17 Laponite 1080	20.79	36.07	36.17	1064	1059	3900
TC 18 Laponite 1080 background	21.19	34.85	37.49	1084	1057	5800
TC 19 Asha 1200	23.55	33.05	31.50	1205	1232	200
TC 20 Asha 1200	23.55	33.05	31.44	1205	1237	240
TC 21 Asha 1200 background	23.68	32.69	33.73	1212	1188	240
TC 22 Asha 1280	25.02	30.79	28.22	1280	1339	270
TC 23 Asha 1280	25.02	30.79	30.90	1280	1305	250
TC 24 Asha 1280 background	25.15	30.42	30.32	1287	1256	360

Table 3-25. Leachable amount of sulphate and lactate in the bentonite clays on average across the three sampling points for each test cell. (gdw = gram dry weight). Average sulphur values for copper discs from XRF measurements on the top surface (n = 5)

Name of test cell	Micromole of sulphate per gram of bentonite (µmol gdw^{-1})	Micromole of lactate per gram of bentonite (µmol gdw^{-1})	Average S (%)	Standard deviation (± %)
TC 1 Laponite 645	3.4	15.38	0.13	0.28
TC 2 Laponite 645	1.8	15.80	<0.20	-
TC 3Laponite 645 background	4.0	-	0.90	0.14
TC 4 Laponite 665	3.1	15.90	0.21	0.2
TC 5 Laponite 665	4.2	14.40	<0.20	-
TC 6 Laponite 665 background	4.7	-	0.25	0.14
TC 7 Laponite 685	3.4	13.40	<0.20	0
TC 8 Laponite 685	4.4	13.50	0.08	0.19
TC 9 Laponite 685 background	2.1	-	<0.20	-
TC 10 Laponite 700	5.0	22.70	0.13	0.29
TC 11 Laponite 700	5.4	20.40	0.43	0.03
TC 12 Laponite 700 background	6.0	-	0.52	0.01
TC 13 Laponite 760	6.1	17.10	<0.20	-
TC 14 Laponite 760	4.54	19.00	<0.20	-
TC 15 Laponite 760 background	4.27	-	<0.20	-
TC 16 Laponite 1080	4.74	14.10	0.22	0.12
TC 17 Laponite 1080	6.64	12.50	0.22	0.12
TC 18 Laponite 1080 background	4.53	-	<0.20	-
Natural Laponite	71.9	-	0.44	0.05
TC 19 Asha 1200	60.41	< 0.07	1.29	0.75
TC 20 Asha 1200	35.99	< 0.07	0.35	0.78
TC 21 Asha 1200 background	47.93	-	<0.20	-
TC 22 Asha 1280	53.31	6.11	<0.20	-
TC 23 Asha 1280	51.35	3.78	<0.20	-
TC 24 Asha 1280 background	53.62	-		
Natural Asha	72.9	-		

3.7.4 Bentonite cores, copper discs and sulphide

Figure 3-41 to Figure 3-43 show the compacted, water saturated bentonites after 76 days of incubation. Black discolouration in bentonite clays indicate SRB activity. None of the test cells with Laponite showed black discolouration which should be expected because Laponite does not contain iron in any form. In contrast the test cells with spiked Asha showed black discolouration. However, with increase of density less spots were detected in the clay cores.

Figure 3-44 to Figure 3-46 show the copper discs after incubation. The copper disc from TC 3, 6, 8 and showed black discolouration. In the test cells with Asha TC 19 and 20 showed a black discolouration. Table 3-18 shows the average sulphur results from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured. The XRF measurements on copper discs from TC 1 – 18 with Laponite showed in general sulphur values below 1 %. The Laponite test cells at a dry density of 700 kg m^{-3} were the only series with sulphur below detection limit. There was no visible trend of increasing or decreasing sulphur with change of density of the Laponite. In contrast sulphur could be measured in the test cells with Asha at a dry density of 1200 kg m^{-3} but not in the test cells with a dry density of 1280 kg m^{-3}.

The sulphide analysis showed no measurable sulphide (<0.5 µmol gdw^{-3}) in any bentonite sample (data not shown).

3.7.5 Summary of results and observations

There was no bacterial activity in the Laponite test cells even at the lowest density because lactate was found in added concentrations. However, sulphate seemed to have decreased in all test cells with Laponite which was due to precipitation processes that immobilised sulphate. It was not possible to observe if swelling pressure or density stopped sulphide formation because of absence of measurable bacterial activity. Further, literature data suggest that Laponite may be unfavourable for microbial growth and survival (Niu and Zhang 2023; Malekkhaiat Häffner et al. 2019) which may explain the absence of microbial sulphide producing activity in Laponite.

In Asha an increase in density lead to the cessation of sulphide formation as shown previously. However, black iron sulphur formation was still visible in the higher density. One explanation could be that 1280 is just on the density limit and that the formation of black spots occurs in areas of the sample where the density is slightly lower than average such as at the interfaces between the test cells and the clay cores. Iron might be a factor that can prevent sulphide from reaching the copper discs (and canisters). This would be because of the formation of iron sulphide which stops the migration of sulphide.

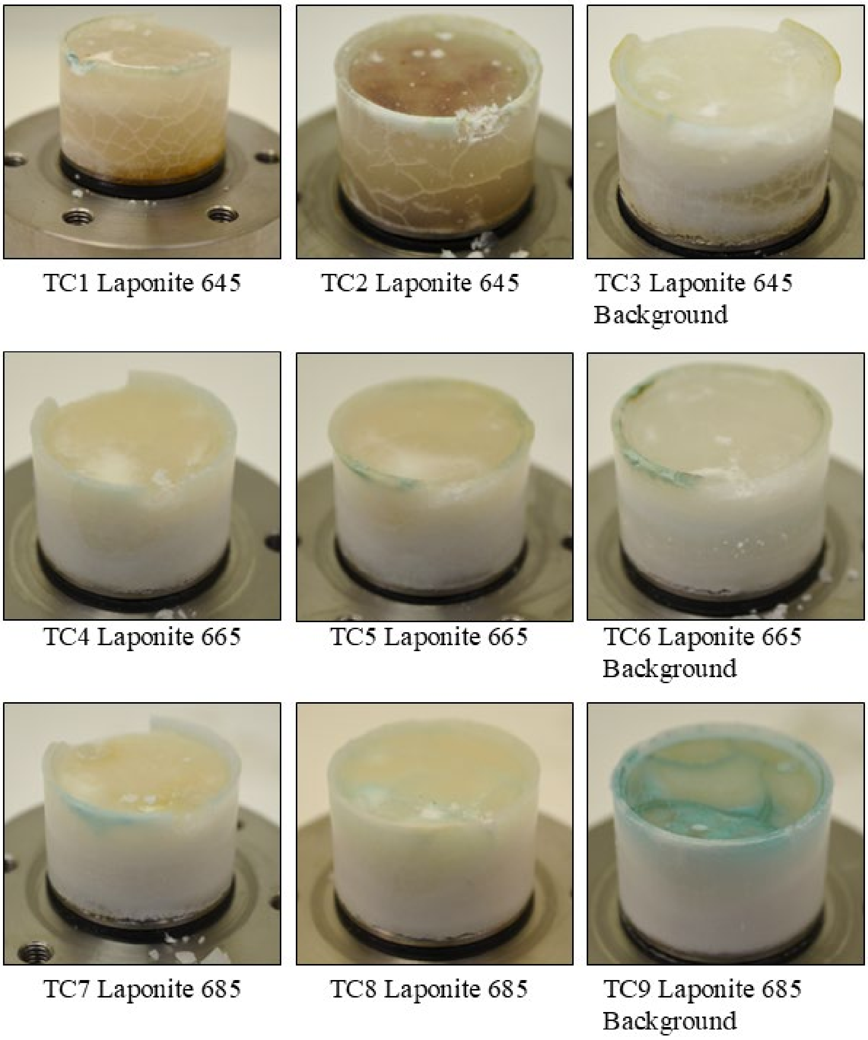


Figure 3-41. *Images of compacted water saturated Laponite cores from TC1 – 9 after 76 days.*

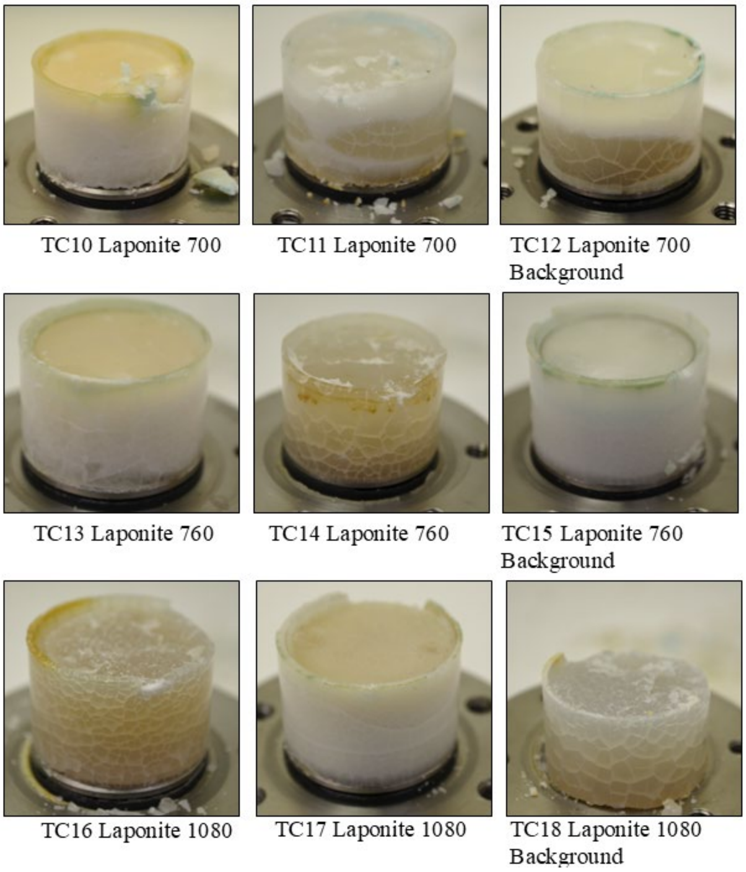


Figure 3-42. *Images of compacted water saturated Laponite cores from TC6 – 18 after 76 days.*

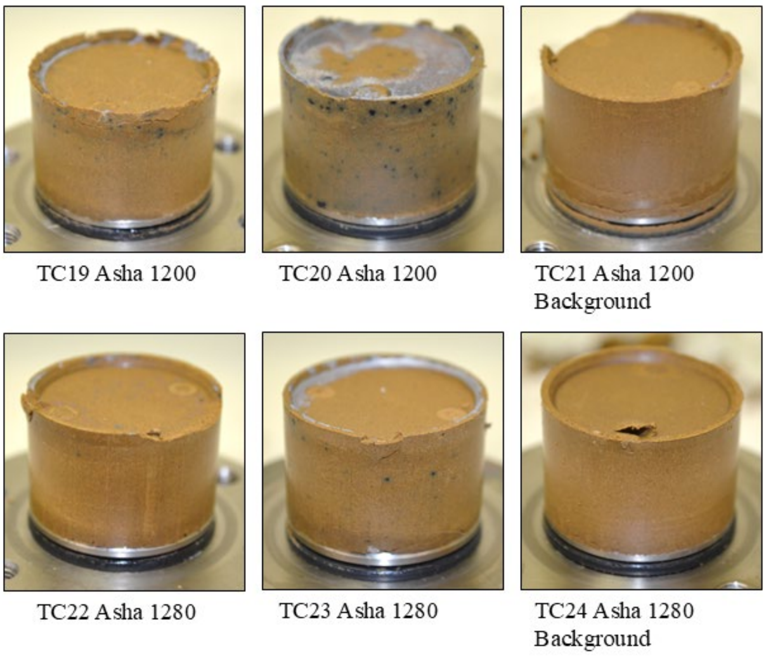
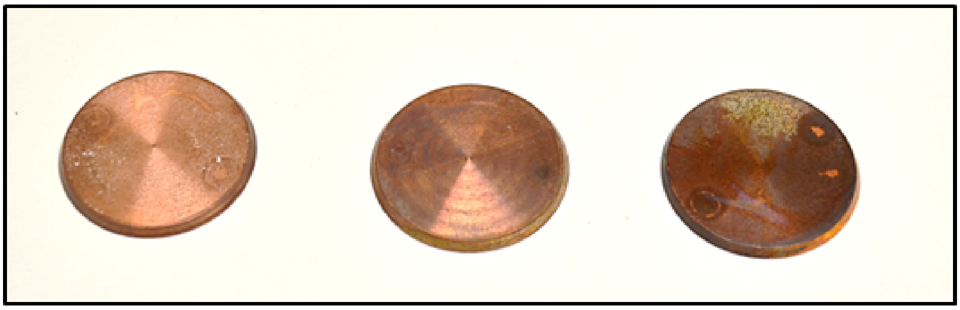


Figure 3-43. *Images of compacted water saturated Asha cores from TC19 – 24 after 76 days.*



TC1 Laponite 645 | TC2 Laponite 645 | TC3 Laponite 645 Background

TC4 Laponite 665 | TC5 Laponite 665 | TC6 Laponite 665 Background

TC7 Laponite 685 | TC8 Laponite 685 | TC9 Laponite 685 Background

Figure 3-44. *Copper discs from TC1 – 9.*

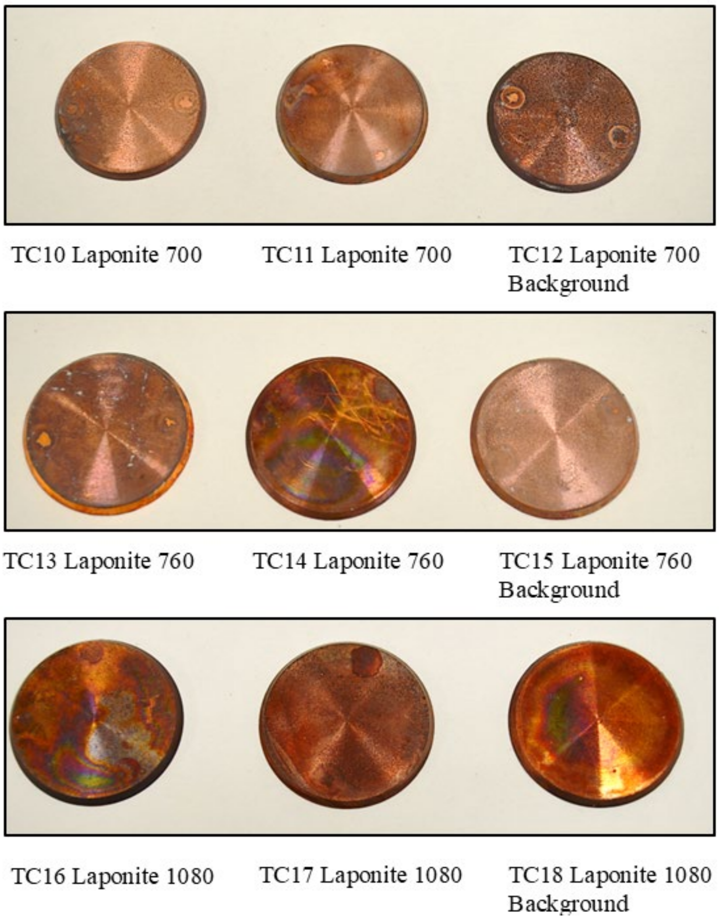


Figure 3-45. *Copper discs from TC10 – 18.*

Figure 3-46. *Copper discs from TC19 – 24.*

3.8 Study 7

3.8.1 Experiment

The bentonite clays MX80 and Bara Kade (BK) were compacted at six different dry densities. The test cells were spiked with SRB prior compactions and lactate was added after the water saturation phase according to Table 3-26.

Table 3-26. List of test cells with bentonite and planned dry densities. Y= Yes, N= No

Test cell number	Clay	Dry density (kg m^{-3})	Replicate	Test cell name	Additions		
					Sulphate	Lactate	SRB
1	MX80	1000	1	MX-1000-1	Y	Y	Y
2	MX80	1000	2	MX-1000-2	Y	Y	Y
3	MX80	1100	1	MX-1100-1	Y	Y	Y
4	MX80	1100	2	MX-1100-2	Y	Y	Y
5	MX80	1200	1	MX-1200-1	Y	Y	Y
6	MX80	1200	2	MX-1200-2	Y	Y	Y
7	MX80	1300	1	MX-1300-1	Y	Y	Y
8	MX80	1300	2	MX-1300-2	Y	Y	Y
9	MX80	1400	1	MX-1400-1	Y	Y	Y
10	MX80	1400	2	MX-1400-2	Y	Y	Y
11	MX80	1500	1	MX-1500-1	Y	Y	Y
12	MX80	1500	2	MX-1500-2	Y	Y	Y
13	BK	1000	1	BK-1000-1	Y	Y	Y
14	BK	1000	2	BK-1000-2	Y	Y	Y
15	BK	1100	1	BK-1100-1	Y	Y	Y
16	BK	1100	2	BK-1100-2	Y	Y	Y
17	BK	1200	1	BK-1200-1	Y	Y	Y
18	BK	1200	2	BK-1200-2	Y	Y	Y
19	BK	1300	1	BK-1300-1	Y	Y	Y
20	BK	1300	2	BK-1300-2	Y	Y	Y
21	BK	1400	1	BK-1400-1	Y	Y	Y
22	BK	1400	2	BK-1400-2	Y	Y	Y
23	BK	1500	1	BK-1500-1	Y	Y	Y
24	BK	1500	2	BK-1500-2	Y	Y	Y

3.8.2 Water content, dry density, and swelling pressure

The results of the water content analysis and resulting dry density are shown in Table 3-27 which also shows the mean swelling pressures during water saturation from day 6 during the water saturation phase and the reproduced swelling pressures after addition of lactate and copper discs deduced from data obtained with force transducers for each test cell. Figure 3-53 and Figure 3-54 show the registered pressures for all test cells during the water saturation phase and the incubation phase, respectively.

3.8.3 Sulphate, lactate and acetate

In studies 7-9 the amounts of analytes are given as total amount per test cell. Table 3-28 shows that sulphate could be analysed in all clays. Sulphate increased with increasing density due to increased total mass of the sample. There was three times more leachable sulphate in MX80 compared to BK clay. The three lowest BK density sulphate data is likely influenced by the addition of sulphate via the saturation water (confer Figure 3-50). Table 3-28 also shows that the added lactate was consumed to a large extent with concomitant production of acetate in the two lowest dry density test cells for both clay types. These data are shown graphically in Figure 3-47 to Figure 3-52.

3.8.4 Summary of results and observations

There was a clear drop in bacterial sulphide producing activity between dry densities of 1300 and 1400 kg m^{-3}. This was concluded from the lack of lactate consumption and acetate production at dry densities of 1400 and 1500 kg m^{-3}. Absence of sulphate consumption at these densities agrees with this conclusion. The production of acetate correlated with the consumption of lactate (Figure 3-51 and Figure 3-52) further corroborates the conclusion. The results from these tests with Wyoming bentonite seem to be consistent with the results from Bengtsson et al (2017a).

The results from these tests consequently show that measurements of lactate concentration give a good indication of if sulphate reduction has occurred.

Table 3-27. Weight, water content, dry density and swelling pressures of the water saturated bentonites in the test cells. Mean swelling pressures deduced from data obtained with force transducers for each test cell when the external water source was removed. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Amount of bentonite (gdw)	Calculated water content (%ww)	Analysed water content (%ww)	Calculated dry density (kg m^{-3})	Analysed dry density (kg m^{-3})	Mean 1 swelling pressure (kPa)	Mean 2 reproduced swelling pressure (kPa)	Mean 1 / Mean 2
MX-1000-1	19.36	39.4	39.97	991	987	275	118	2.3
MX-1000-2	19.36	39.4	40.82	991	973	267	158	1.7
MX-1100-1	21.27	35.9	36.39	1089	1077	302	190	1.6
MX-1100-2	21.27	35.9	35.93	1089	1093	311	202	1.5
MX-1200-1	23.24	32.5	33.16	1190	1202	466	857	0.5
MX-1200-2	23.24	32.5	32.28	1190	1216	509	416	1.2
MX-1300-1	25.16	29.5	30.39	1287	1303	759	813	0.9
MX-1300-2	25.16	29.5	30.20	1287	1315	804	833	1.0
MX-1400-1	27.10	26.6	25.69	1387	1430	1993	1632	1.2
MX-1400-2	27.10	26.6	25.64	1387	1429	2173	2548	0.9
MX-1500-1	29.07	23.8	23.52	1488	1526	4217	4114	1.0
MX-1500-2	29.07	23.8	23.56	1488	1523	4020	4021	1.0
BK-1000-1	19.45	39.2	40.38	995	981	216	148	1.5
BK-1000-2	19.45	39.2	40.24	995	974	180	89	2.0
BK-1100-1	21.37	35.6	37.09	1094	1070	265	177	1.5
BK-1100-2	21.37	35.6	36.88	1094	1071	239	125	1.9
BK-1200-1	23.35	32.2	33.74	1195	1183	318	330	1.0
BK-1200-2	23.35	32.2	33.71	1195	1193	414	454	0.9
BK-1300-1	25.27	29.2	30.54	1293	1290	801	686	1.2
BK-1300-2	25.27	29.2	30.13	1293	1292	753	834	0.9
BK-1400-1	27.22	26.3	27.71	1393	1411	1798	1539	1.2
BK-1400-2	27.22	26.3	27.47	1393	1416	1781	1875	0.9
BK-1500-1	29.20	23.6	24.68	1495	1517	3324	2404	1.4
BK-1500-2	29.20	23.6	25.06	1495	1512	3423	3045	1.1

Table 3-28. Leachable amount of sulphate, acetate and lactate in the bentonite clays for each test cell

Name of test cell	Total sulphate in test cell (µmol)	Total lactate in test cell (µmol)	Total acetate in test cell (µmol)
MX-1000-1	607	0	316
MX-1000-2	578	-4	297
MX-1100-1	719	-7	260
MX-1100-2	750	11	255
MX-1200-1	932	26	251
MX-1200-2	963	33	226
MX-1300-1	846	85	171
MX-1300-2	1117	82	225
MX-1400-1	1249	297	9
MX-1400-2	1256	340	5
MX-1500-1	1387	257	30
MX-1500-2	1347	291	5
BK-1000-1	188	-7	300
BK-1000-2	202	-5	296
BK-1100-1	204	-7	278
BK-1100-2	225	11	254
BK-1200-1	230	15	246
BK-1200-2	236	18	270
BK-1300-1	388	215	77
BK-1300-2	390	186	88
BK-1400-1	466	248	31
BK-1400-2	504	257	8
BK-1500-1	617	299	10
BK-1500-2	534	287	0

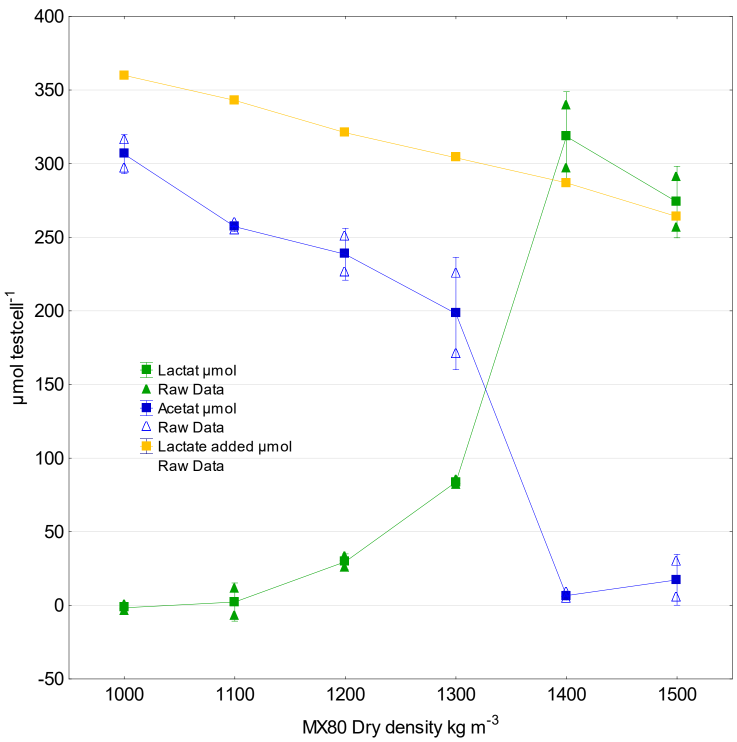


Figure 3-47. *Total average amount of added lactate and analysed lactate and acetate for two MX80 test cells as function of analysed dry density with raw data and error whiskers. The lines indicate trends over density. Bars show standard deviation.*

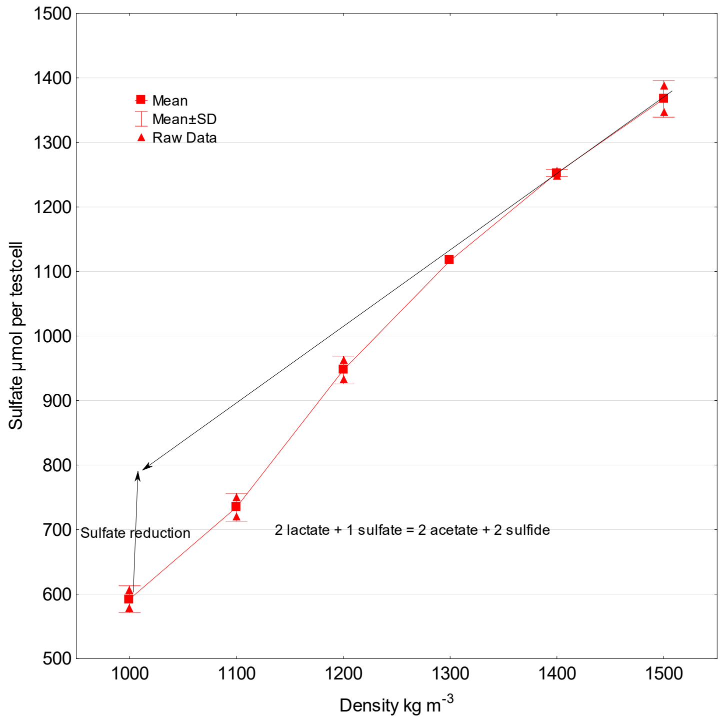


Figure 3-48. *Total average amount of sulphate for MX80 two test cells as function of analysed dry density with raw data and error whiskers. The lines indicate trends over density. Bars show standard deviation. The black lines show the calculated sulphate amount if no sulphate reduction occurred.*

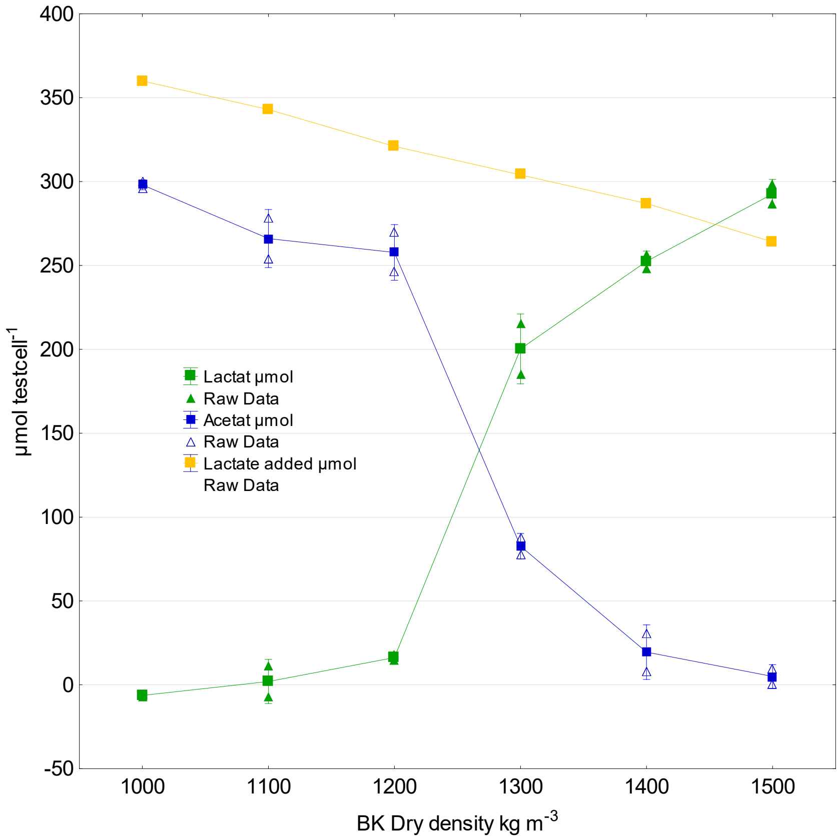


Figure 3-49. *Total average amount of added lactate and analysed lactate and acetate for two BK test cells as function of analysed dry density with raw data and error whiskers. The lines indicate trends over density. Bars show standard deviation.*

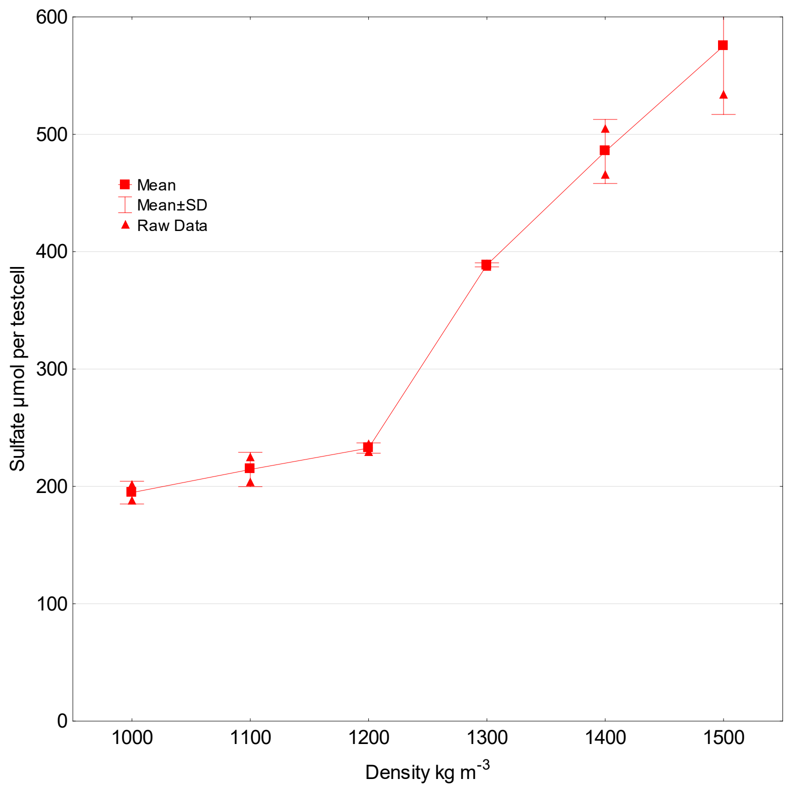


Figure 3-50. *Total average amount of sulphate for two BK test cells as function of analysed dry density with raw data and error whiskers. The lines indicate trends over density. Bars show standard deviation.*

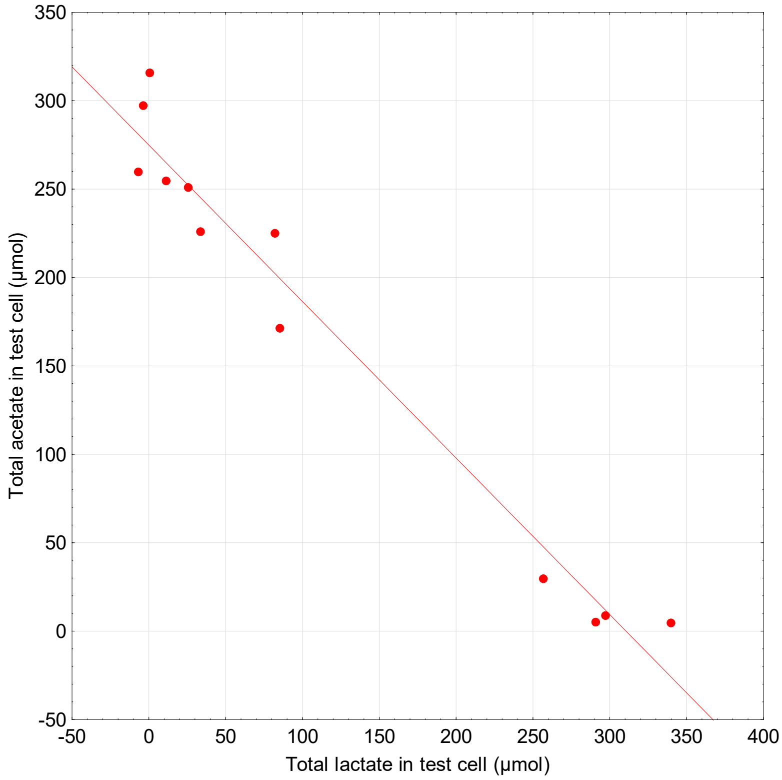


Figure 3-51. *Correlation between amounts of lactate and sulphate in the MX80 test cells, data from Table 3-28.*

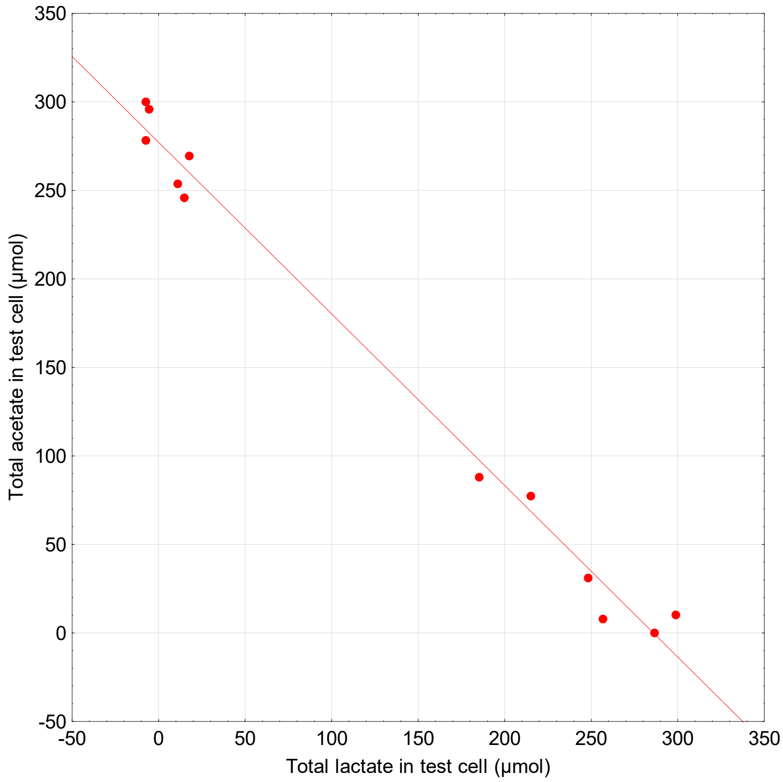


Figure 3-52. *Correlation between amounts of lactate and sulphate in the Bara Kade test cells, data from Table 3-28.*

3.8.5 Pressure curves

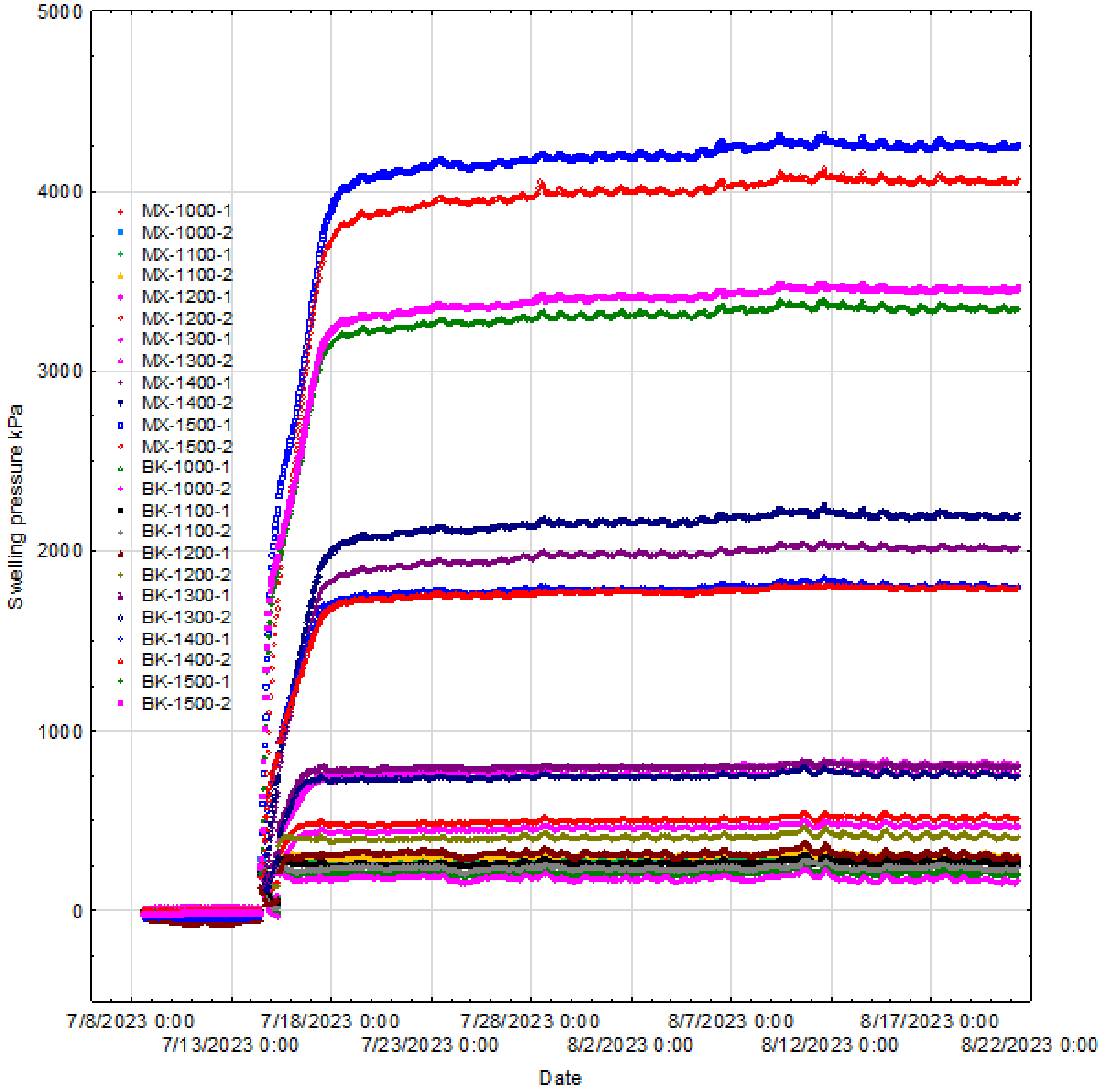


Figure 3-53. *Pressures registered by force transducers for test cells during the water saturation phase, lasting from start at 2023-07-08 to end 2023-08-22 when copper discs and lactate were added. See Table 3-27 for pressures.*

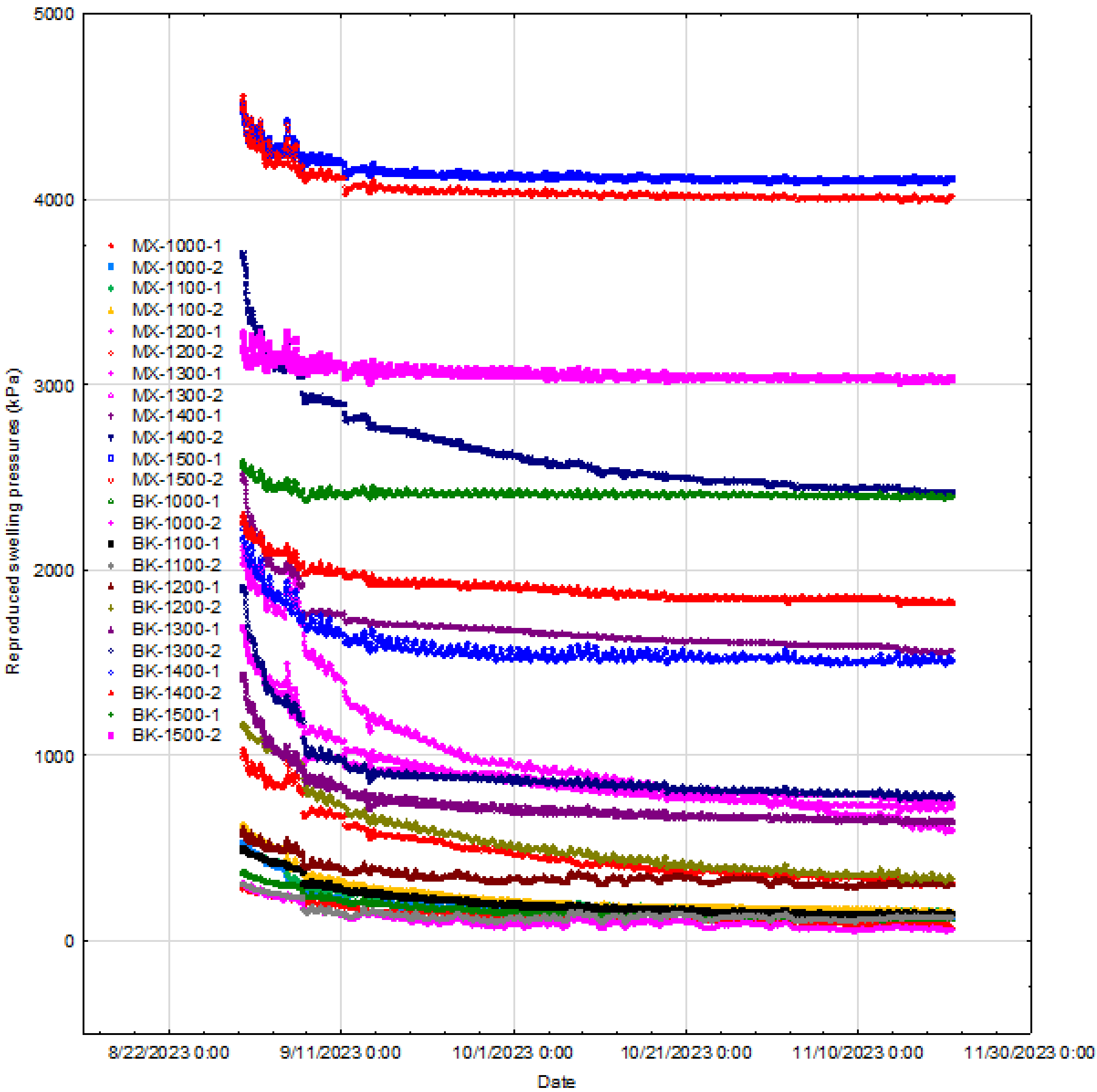


Figure 3-54. *Pressures registered by force transducers for test cells during 2023-08-30 to start of analyses of the experiment 2023-11-20. See Table 3-27 for average pressures.*

3.9 Study 8

3.9.1 Experiment

Georgian bentonite clay was compacted at six different saturated densities. The test cells were spiked with SRB prior compactions and lactate was added after the water saturation phase according to Table 3-29. The total amounts of lactate, acetate, sulphate were analysed.

Table 3-29. List of test cells with bentonite and planned dry densities. Y=yes, N=no

Test cell number	Clay	Dry density (kg m^{-3})	Replicate	Test cell name	Additions		
					Sulphate	Lactate	SRB
1	Georgian	1000	1	G-1000-1	Y	Y	Y
2	Georgian	1000	2	G-1000-2	Y	Y	Y
3	Georgian	1000	3	G-1000-3	Y	Y	Y
4	Georgian	1000	Background	G-1000-B	Y	N	N
5	Georgian	1100	1	G-1100-1	Y	Y	Y
6	Georgian	1100	2	G-1100-2	Y	Y	Y
7	Georgian	1100	3	G-1100-3	Y	Y	Y
8	Georgian	1100	Background	G-1100-B	Y	N	N
9	Georgian	1200	1	G-1200-1	Y	Y	Y
10	Georgian	1200	2	G-1200-2	Y	Y	Y
11	Georgian	1200	3	G-1200-3	Y	Y	Y
12	Georgian	1200	Background	G-1200-B	Y	N	N
13	Georgian	1300	1	G-1300-1	Y	Y	Y
14	Georgian	1300	2	G-1300-2	Y	Y	Y
15	Georgian	1300	3	G-1300-3	Y	Y	Y
16	Georgian	1300	Background	G-1300-B	Y	N	N
17	Georgian	1400	1	G-1400-1	Y	Y	Y
18	Georgian	1400	2	G-1400-2	Y	Y	Y
19	Georgian	1400	3	G-1400-3	Y	Y	Y
20	Georgian	1400	Background	G-1400-B	Y	N	N
21	Georgian	1500	1	G-1500-1	Y	Y	Y
22	Georgian	1500	2	G-1500-2	Y	Y	Y
23	Georgian	1500	3	G-1500-3	Y	Y	Y
24	Georgian	1500	Background	G-1500-B	Y	N	N

3.9.2 Water content, dry density, and swelling pressure

The results of the water content analysis and resulting dry density are shown in Table 3-30. Table 3-30 also shows the respective mean swelling pressures and reproduced swelling pressure deduced from data obtained with force transducers for each test cell. Figure 3-56 and Figure 3-57 show the registered pressures for all test cells during the water saturation phase and the incubation phase, respectively.

A pilot test with Georgian bentonites indicated that 8 weeks was not enough to obtain an even distribution of water in the test cells because there was up to 6-7 % variation in water content between the three sampled layers. Therefore, the water saturation time was increased to 12 weeks. The water content varied around 1 % or less between the three sampled layers for each test cell which is deemed very acceptable.

3.9.3 Sulphate, lactate and acetate

Table 3-31 shows that sulphate could be analysed in all clays except the G-1000 samples. Sulphate increased with increasing density. Table 3-31 also shows that lactate was consumed to a large extent with concomitant production of acetate in the two lowest dry density test cells.

3.9.4 Bentonite cores and copper discs

Table 3-31 shows the average sulphur results from the XRF measurements. On each copper disc five positions of approximately 1 cm^2 each were measured. The XRF measurements on copper discs showed significant sulphur values for the two lowest densities and occasional observations for the two next densities in weight.

Table 3-30. Weight, water content, dry density and swelling pressures of the water saturated bentonites in the test cells. Mean reproduced swelling pressures deduced from data obtained with force transducers for each test cell when the external water sours was removed. (gdw = gram dry weight, %ww = percent wet weight).

Name of test cell	Amount of bentonite (gdw)	Calculated water content (%ww)	Analysed water content (%ww)	Calculated dry density (kg m^{-3})	Analysed dry density (kg m^{-3})	Mean 1 swelling pressure (kPa)	Mean 2 reproduced swelling pressure (kPa)	Mean 1 / Mean 2
G-1000-1	19.47	39.54	38.9	996	1018	278	334	0.8
G-1000-2	19.47	39.54	39.5	996	1008	357	266	1.3
G-1000-3	19.47	39.54	38.8	996	1017	383	461	0.8
G-1000-B	19.54	39.24	39.6	1000	1003	297	379	0.8
G-1100-1	21.39	36.05	36.6	1095	1110	567	522	1.1
G-1100-2	21.39	36.05	35.5	1095	1117	519	618	0.8
G-1100-3	21.39	36.05	35.9	1095	1101	458	622	0.7
G-1100-B	21.48	35.72	35.9	1099	1107	550	659	0.8
G-1200-1	23.38	32.71	32.8	1196	1200	961	675	1.4
G-1200-2	23.38	32.71	33.2	1196	1171	849	806	1.1
G-1200-3	23.38	32.71	32.4	1196	1213	850	1020	0.8
G-1200-B	23.45	32.33	33.2	1204	1195	858	644	1.3
G-1300-1	25.30	29.71	29.8	1295	1308	1429	1903	0.8
G-1300-2	25.30	29.71	30.0	1295	1304	1512	1856	0.8
G-1300-3	25.30	29.71	30.7	1295	1293	1402	1212	1.2
G-1300-B	25.47	29.16	30.6	1304	1294	1267	1648	0.8
G-1400-1	27.25	26.86	26.5	1395	1411	2838	3495	0.8
G-1400-2	27.25	26.86	27.3	1395	1404	2786	3133	0.9
G-1400-3	27.25	26.86	27.0	1395	1407	2711	3508	0.8
G-1400-B	27.44	26.48	27.1	1404	1406	2353	4058	0.6
G-1500-1	29.24	24.17	25.7	1496	1486	4954	5116	1.0
G-1500-2	29.24	24.17	25.0	1496	1499	5450	4585	1.2
G-1500-3	29.24	24.17	25.4	1496	1490	5096	5506	0.9
G-1500-B	29.44	23.65	26.0	1506	1482	4898	5379	0.9

Table 3-31. Leachable amount of sulphate, acetate and lactate in the bentonite clays for each test cell. Average sulphur values for copper discs from XRF measurements on the top surface (n = 5)

Name of test cell	Total sulphate in test cell (µmol)	Total lactate in test cell (µmol)	Total acetate in test cell (µmol)	Average (%)	Standard deviation (± %)
G-1000-1	<1	<5	185	1.12	0.84
G-1000-2	<1	11.8	176	1.81	1.22
G-1000-3	<1	<5	168	1.45	036
G-1000-B	136	<5	<5	Not detected	-
G-1100-1	<1	6.9	163	1.38	0.26
G-1100-2	<1	8.1	160	3.61	0.25
G-1100-3	45.2	77.9	125	Not detected	-
G-1100-B	135	7.9	<5	Not detected	-
G-1200-1	<1	2.7	160	0.54	0.32
G-1200-2	<1	23.1	154	Not detected	-
G-1200-3	70	84.7	113	Not detected	-
G-1200-B	153	<5	29	Not detected	-
G-1300-1	120	182	17	Not detected	-
G-1300-2	74.8	103	73	0.14	0.32
G-1300-3	135	199	13	Not detected	-
G-1300-B	155	15.5	<5	Not detected	-
G-1400-1	133	197	11	Not detected	-
G-1400-2	144	167	9	Not detected	-
G-1400-3	142	186	10	Not detected	-
G-1400-B	157	<5	<5	Not detected	-
G-1500-1	146	156	8	Not detected	-
G-1500-2	144	172	7	Not detected	-
G-1500-3	141	234	<5	Not detected	-
G-1500-B	148	24.3	<5	Not detected	-

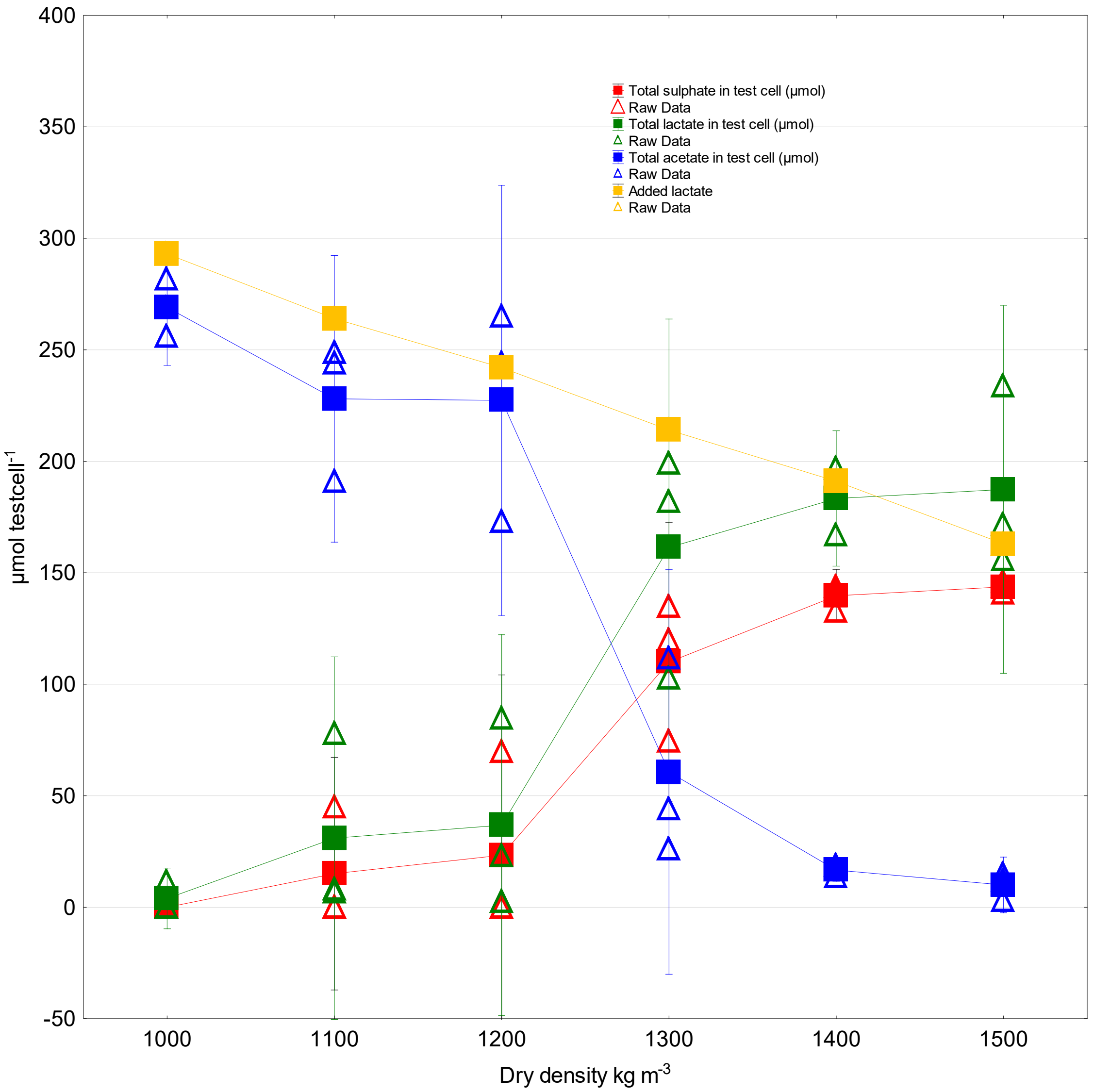


Figure 3-55. *Total average amount of sulphate, lactate and acetate for three test cells as function of analysed dry density with raw data and error whiskers. The lines indicate trends over density.*

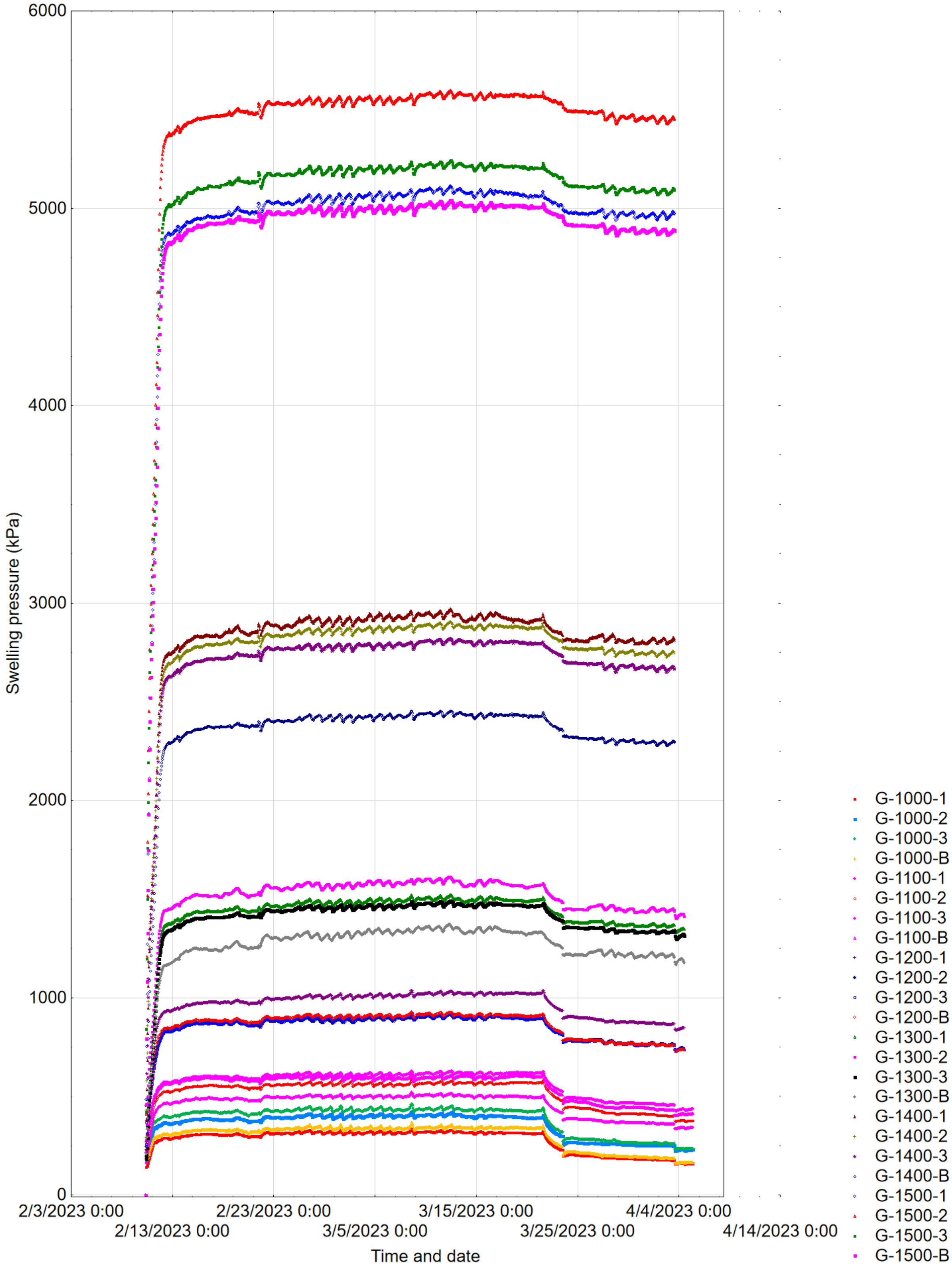


Figure 3-56. *Pressures registered by force transducers for test cells during the water saturation phase, lasting from start at 2023-02-10 to end 2023-04-05 when copper discs and lactate were added. The drop at the end of the curves is when the external pressure on the saturation water was removed.*

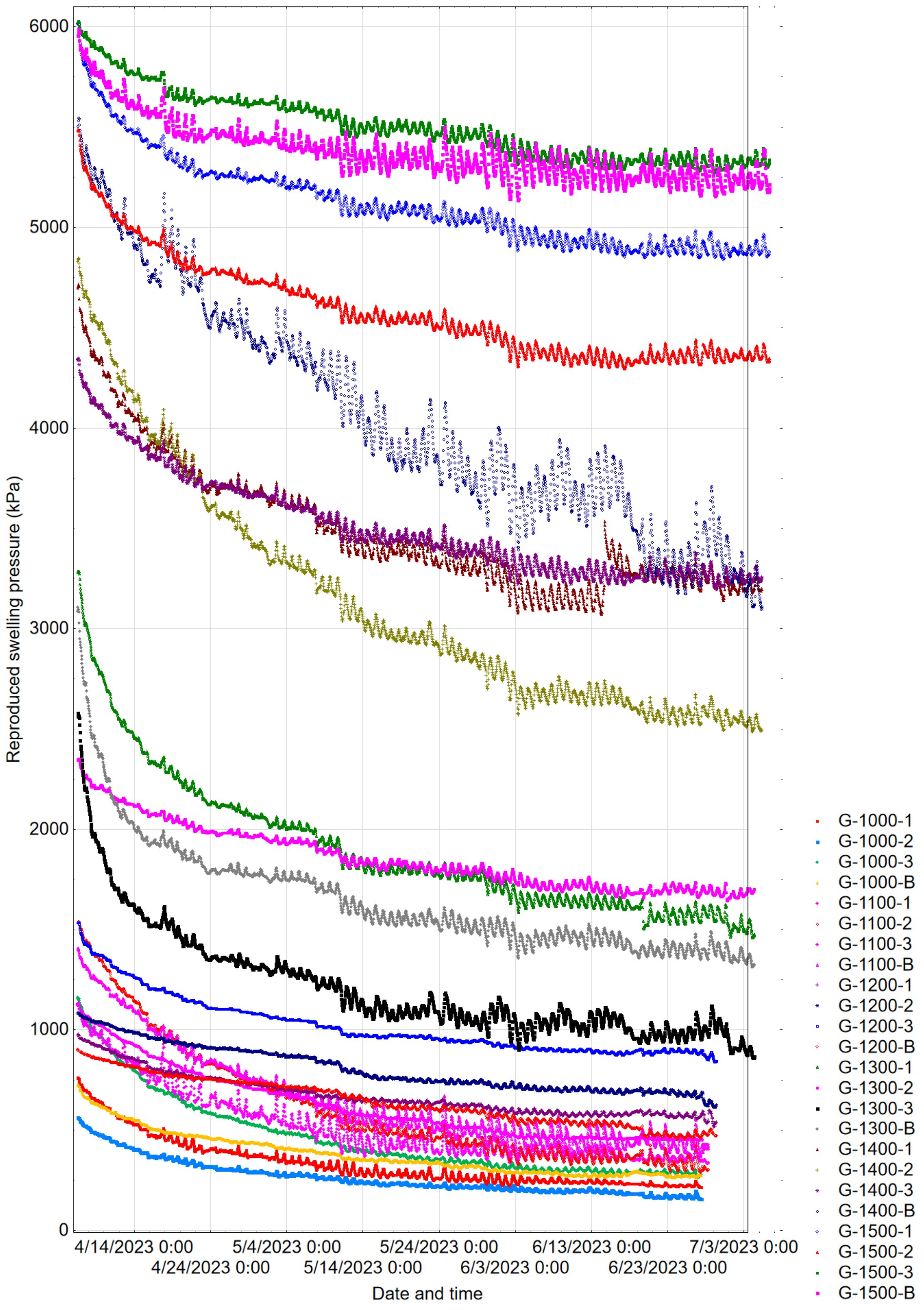


Figure 3-57. *Pressures registered by force transducers for test cells during 2023-04-06 to end of experiment 2023-07-06.*

3.9.5 Summary of results and observations

3.9.6 Data agreements

There were generally very good correlations between the analysed parameters with exception of two observed deviations.

1. Test cell G1100-3 deviated from the other two G-1100 test cells with bacteria and lactate. There was no detectable sulphur on the disc and there was sulphate left in the pore water and more lactate and less acetate compared to G1100-1 and 2 test cells. The reasons can be several, but as all clay parameters, densities etc. and pressures did not differ significantly, the reasons cannot be deduced from the available data. It can be noted that working with biological material may introduce unexpected variability.
2. G-1300-2 This test cell showed data similar to what was observed for G-1200-1.

In summary, data for sulphur on the copper disc, sulphate and lactate consumption and acetate production correlated as should be expected (Figure 3-55). If sulphur was present on a copper disc, there should be consumption of sulphate and lactate concomitant with the production of acetate. This was the case for all test cells with sulphur on the copper disc as illustrated here by G-1200-1 and G1300-2. The opposite, with no sulphur on the disc showed data where sulphate and lactate were present, and there was no production of acetate.

3.9.7 Swelling pressure and reproduced pressure

True swelling pressure is observed when water can move in and out of the clay in the test cells as was the case during the water saturation phase Figure 3-56. The water saturation was performed with a water pressure of approximately 200 kPa and when the pressure was released 2023-03-25, there was a drop in all registered pressures because the 200 kPa water pressure ads to the swelling pressure. When the copper discs and lactate were added water could not continue to be in contact with the clay. This is because there will be a vivid growth of SRB in the titanium filters needed to supply the clay evenly with water during water saturation. When the lids and the piston of the test cells were mounted pressure curves slowly decreased back to approximately the swelling pressure values (Figure 3-57). However, the registered pressures are only reproduced, not actual swelling pressures since no external water source is available at this stage. The force transducers are very sensitive to pressure and just the fluctuations of temperature in the laboratory produce small waves on the curves due to the effect of temperature on the titanium that expand and retract in response to the temperature. In any case, Table 3-30 shows that the average reproduced swelling pressures mimicked the true swelling pressures observed during water saturation reasonably well.

3.9.8 Density and water content

The target densities and water contents of the test cells were calculated before start of the experiment to ensure that correct amount of clay for each density was placed in the test cells. At the end of the experiment all densities and water contents were analysed. Table 3-30 shows that the agreement between calculated and observed densities and water contents were very good, in most cases within the precision of the measurements, i.e. < 1 %.

3.9.9 Interpretation of bacterial activity

The three SRB that were added are incomplete lactate oxidisers that oxidize lactate to acetate. Therefore, for porewater in the clays, it can be expected that decrease in the amount of lactate will correlate with increase of acetate concomitant with a decrease in the amount of sulphate. Figure 3-58 shows an inverse correlation between lactate and acetate which attests the activity of incomplete lactate oxidizing SRB when the correlation between lactate and sulphate in Figure 3-59. In theory, one mole of sulphate can oxidise 2 moles of lactate to two moles of acetate. However, this relation does not show up perfectly in Figure 3-59, where somewhat more sulphate than expected has been consumed, or, has precipitated out of solution. It is also possible that the intrinsic SRB present in the Georgian clay may have been complete oxidizers that oxidized some lactate to CO_2 with consumption of two moles sulphate per mole lactate. In any case, the combination of acetate production from lactate concomitant with the reduction in the amount of sulphate confirms that SRB have been active.

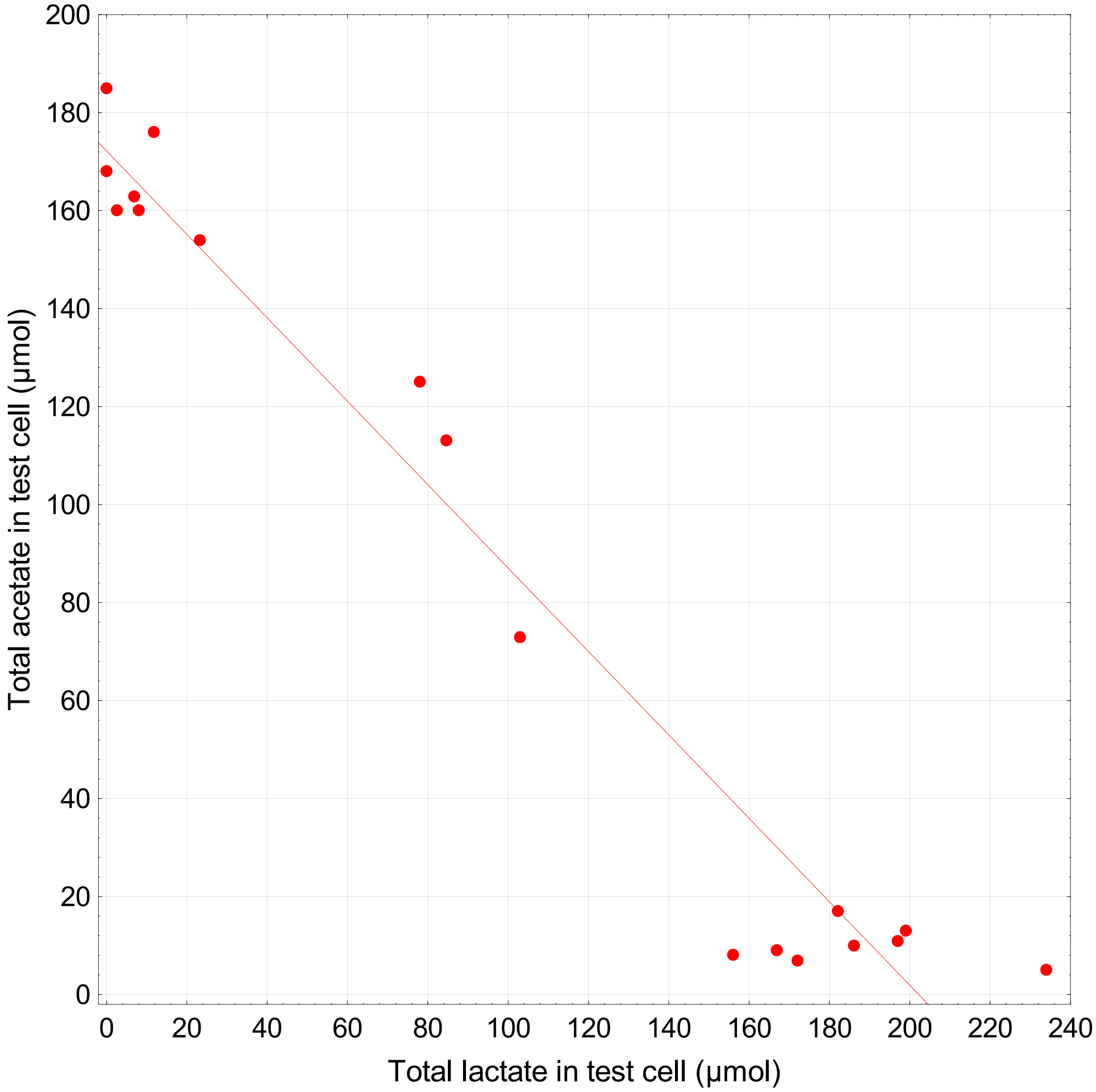


Figure 3-58. *Correlation between amounts of lactate and acetate in the test cells, data from Table 3-31.*

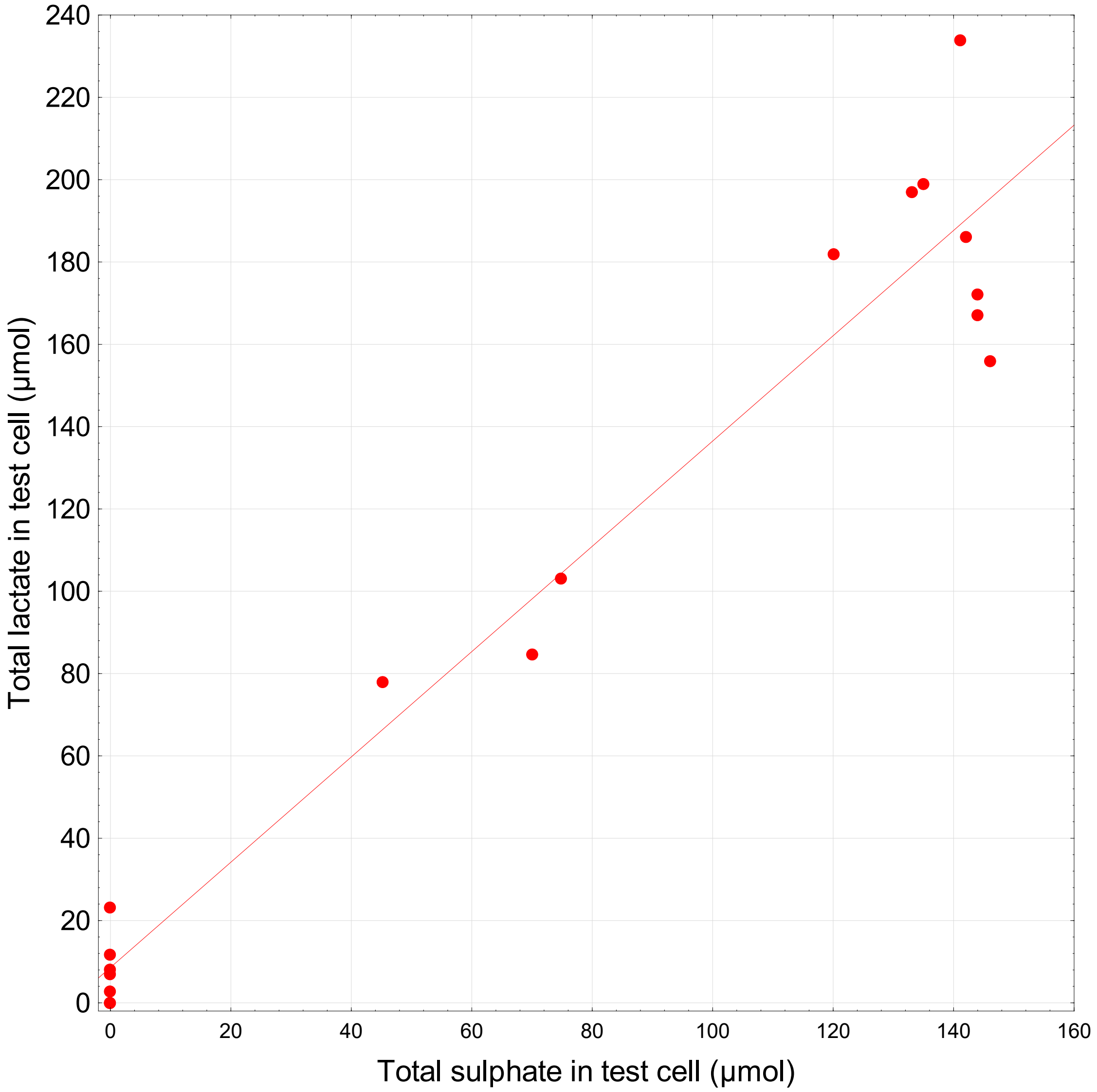


__Figure 3-59.__ Correlation between amounts of lactate and sulphate in the test cells, data from Table 3-31.

3.10 Study 9

3.10.1 Experiment

The Georgian bentonite clay was compacted at eight different dry densities in a narrower range compared to study 8. This study focussed on the observed region for cut-off densities observed in study 8. The clay was spiked with SRB prior compactions and lactate was added after the water saturation phase according to Table 3-32. The added copper discs were not analysed with XRF in this study.

Table 3-32. List of test cells with bentonite and planned dry densities. Y=yes, N=no

Test cell number	Clay	Dry density (kg m^{-3})	Replicate	Test cell name	Additions		
					Sulphate	Lactate	SRB
1	Georgian	1250	1	G-1250-1	Y	Y	Y
2	Georgian	1250	2	G-1250-2	Y	Y	Y
3	Georgian	1250	3	G-1250-3	Y	Y	Y
4	Georgian	1275	1	G-1275-1	Y	Y	Y
5	Georgian	1275	2	G-1275-2	Y	Y	Y
6	Georgian	1275	3	G-1275-3	Y	Y	Y
7	Georgian	1300	1	G-1300-1	Y	Y	Y
8	Georgian	1300	2	G-1300-2	Y	Y	Y
9	Georgian	1300	3	G-1300-3	Y	Y	Y
10	Georgian	1325	1	G-1325-1	Y	Y	Y
11	Georgian	1325	2	G-1325-2	Y	Y	Y
12	Georgian	1325	3	G-1325-3	Y	Y	Y
13	Georgian	1350	1	G-1350-1	Y	Y	Y
14	Georgian	1350	2	G-1350-2	Y	Y	Y
15	Georgian	1350	3	G-1350-3	Y	Y	Y
16	Georgian	1375	1	G-1375-1	Y	Y	Y
17	Georgian	1375	2	G-1375-2	Y	Y	Y
18	Georgian	1375	3	G-1375-3	Y	Y	Y
19	Georgian	1400	1	G-1400-1	Y	Y	Y
20	Georgian	1400	2	G-1400-2	Y	Y	Y
21	Georgian	1400	3	G-1400-3	Y	Y	Y
22	Georgian	1425	1	G-1425-1	Y	Y	Y
23	Georgian	1425	2	G-1425-2	Y	Y	Y
24	Georgian	1425	3	G-1425-3	Y	Y	Y

3.10.2 Water content, dry density, and swelling pressure

The results of the water content analysis and resulting dry density are shown in Table 3-33 which also shows the respective mean swelling pressures/pressures deduced from data obtained with force transducers for each test cell. Here, every third test cell for each density was compressed to a pressure close to the limit for the force transducers, approximately 9500 kPa.

3.10.3 Sulphate, lactate and acetate

Table 3-34 and Figure 3-60 shows that sulphate, lactate and acetate could be analysed in all test cells. However, the changes in concentrations were small, as could be expected from comparison with data from previous work with this clay shown in Figure 3-55.

Figure 3-60 suggests a complete cut off at around 1325 kg m^{-3} dry density but of course, this is open for interpretation. It is clear that the sulphate reduction has been rather limited at all densities in this test series. The data from highly pressurized test cells (every third cell) is somewhat ambiguous to interpret (Figure 3-61). During sampling it was observed that the high pressure had forced some water up on top of the copper discs. Because lactate was dissolved in the water, losing some water to the top of the copper disc may have decreased the concentration of lactate in the pore water of those test cells. Another possibility is that there actually was bacterial lactate oxidation ongoing because the acetate concentration increased in these test cells. These explanations are not possible to confirm but if there was an unexplained high-pressure artefact occurring, the high-pressure test cells do indicate a cut of at around 1300 kg m^{-3} almost similar to test cells with pressures approximating the swelling pressures observed during water saturation.

A final note: This Georgian clay was more difficult to work with compared to MX 80 and other clays. Pressures and data spread more, i.e. larger standard deviations, with the Georgian clay compared to other clays we have worked with.

Table 3-33. Weight, water content, dry density and pressures of the water saturated bentonites in the test cells. Mean swelling pressures deduced from data obtained with force transducers for each test cell before the external water source was removed and mean pressure after the external water source was removed. (gdw = gram dry weight, %ww = percent wet weight)

Name of test cell	Amount of bentonite (gdw)	Calculated water content (%ww)	Analysed water content (%ww)	Calculated dry density (kg m^{-3})	Analysed dry density (kg m^{-3})	Mean 1 swelling pressure (kPa)	Mean 2 pressure (kPa)
G-1250-1	24.16	31.70	32.55	1236	1236	1470	1400
G-1250-2	24.16	31.70	33.29	1236	1230	1070	1280
G-1250-3	24.16	31.70	33.39	1236	1217	1180	**8790**
G-1275-1	24.65	30.92	30.88	1261	1280	1650	1490
G-1275-2	24.65	30.92	32.04	1261	1259	1660	1680
G-1275-3	24.65	30.92	31.51	1261	1267	1440	**9540**
G-1300-1	25.13	30.19	30.91	1286	1288	1710	1650
G-1300-2	25.13	30.19	33.63	1286	1245	1720	1826
G-1300-3	25.13	30.19	30.66	1286	1285	1850	**9070**
G-1325-1	25.60	29.48	29.76	1310	1320	2320	2240
G-1325-2	25.60	29.48	30.10	1310	1314	2000	2080
G-1325-3	25.60	29.48	29.94	1310	1330	1790	**9330**
G-1350-1	26.08	28.78	30.85	1335	1335	2000	2230
G-1350-2	26.08	28.78	29.30	1335	1355	2510	2230
G-1350-3	26.08	28.78	28.98	1335	1354	2170	**9100**
G-1375-1	26.57	28.09	28.13	1360	1390	2720	2150
G-1375-2	26.57	28.09	28.32	1360	1383	3100	2620
G-1375-3	26.57	28.09	29.86	1360	1381	2090	**9360**
G-1400-1	27.07	27.37	27.56	1385	1406	3440	2440
G-1400-2	27.07	27.37	28.41	1385	1405	2830	2640
G-1400-3	27.07	27.37	28.00	1385	1404	1800	**9310**
G-1425-1	27.54	26.70	26.95	1410	1445	1780	2850
G-1425-2	27.54	26.70	26.71	1410	1440	3320	2850
G-1425-3	27.54	26.70	27.16	1410	1411	3420	**9460**

Table 3-34. Leachable amount of sulphate, acetate and lactate in the bentonite clays for each test cell

Name of test cell	Total sulphate in test cell (µmol)	Total lactate in test cell (µmol)	Total acetate in test cell (µmol)
G-1250-1	132	222	106
G-1250-2	139	245	94
G-1250-3	171	290	32
G-1275-1	138	256	61
G-1275-2	145	299	62
G-1275-3	165	258	37
G-1300-1	156	272	51
G-1300-2	144	254	98
G-1300-3	172	303	70
G-1325-1	172	294	47
G-1325-2	174	291	25
G-1325-3	176	257	63
G-1350-1	174	300	No data[a]
G-1350-2	163	296	No data
G-1350-3	148	237	No data
G-1375-1	142	258	No data
G-1375-2	170	294	31
G-1375-3	168	245	60
G-1400-1	144	273	65
G-1400-2	167	291	17
G-1400-3	159	293	26
G-1425-1	146	248	75
G-1425-2	143	261	51
G-1425-3	139	255	52

a: analysis failure.

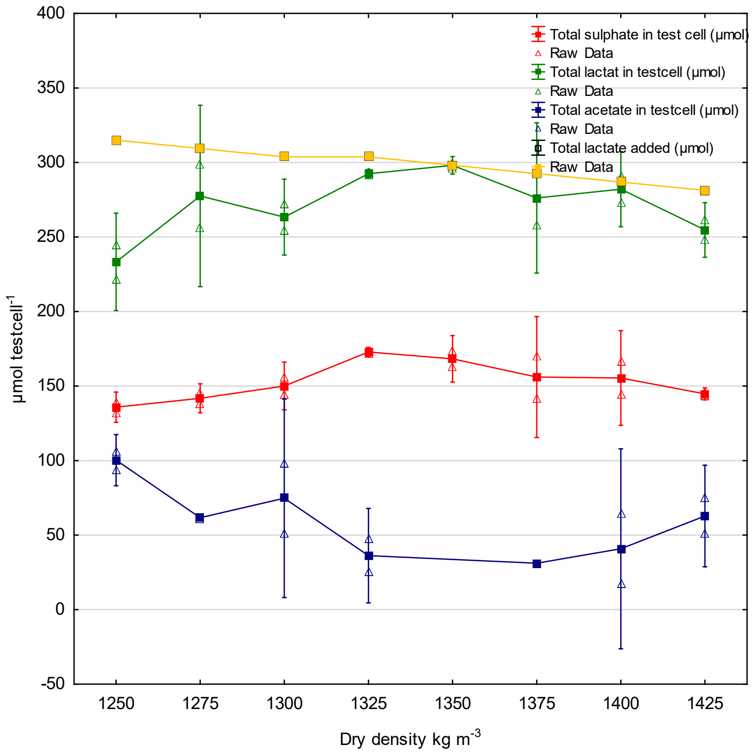


Figure 3-60. *Total average amount of added lactate and analysed lactate and acetate for replicates 1 and 2 as function of analysed dry density with raw data and error whiskers. The lines indicate trends over density. Bars show standard deviation.*

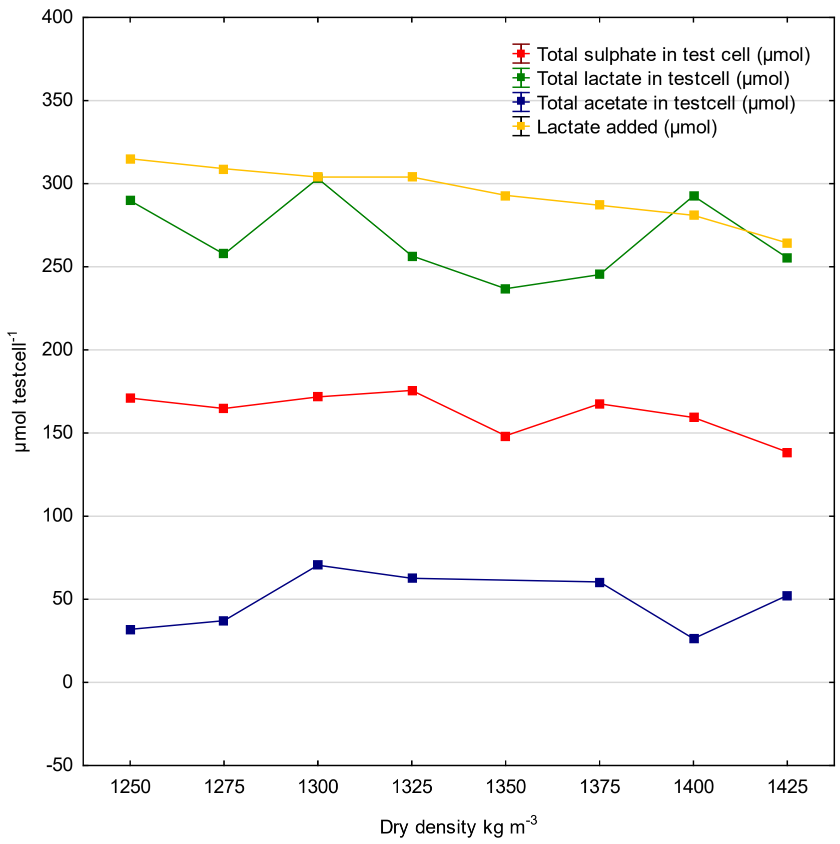


Figure 3-61. *Total average amount of added lactate and analysed lactate and acetate for replicate 3 as function of analysed dry density. The lines indicate trends over density.*

4 Discussion

4.1 Studied clays

Bacterial sulphide production and other related aspects of the bentonite clays studied in this report have been reported previously. For detailed information about the characteristics, origin etc of the studied clays (Table 1-1) we refer to other reports on the respective clay (Publications - SKB.com). For this report, the specific focus was on finalization of a method for assessing conditions for exclusion of bacterial sulphide production in bentonite clays.

4.1.1 4.1 Experimental set-up and procedures

The present experimental set-up has passed through several development steps, starting three decades ago with steel test cells and silver foils and later copper discs (Motamedi et al. 1996; Pedersen et al. 2000). A series of different technical approaches have been tested since then using both addition of pure cultures of SRB in the laboratory, *in situ* addition of SRB present in groundwater at Äspö hard rock laboratory (Masurat et al. 2010b; Pedersen 2010) and concomitant control experiments without additions of SRB. The control experiments have repeatedly demonstrated the presence and activity of indigenous SRB in the studied bentonites as have investigations of various bentonites (Masurat et al. 2010a; Svensson et al. 2011). The methodology now comprises a reproducible experimental configuration that enables detailed and controlled experiments on the activity of sulphide-producing bacteria in compacted bentonite as a function of a large array of independent variables such as electron donor and acceptor, temperature, carbon source, species of microorganisms, type of bentonite, groundwater composition and density. The work presented in this report was designed to compare bacterial sulphide-producing activity under varying dry densities and corresponding pressures which were the only variables applied for each clay. All other conditions, concentrations of the electron donor and carbon source (lactate), temperatures and diversity of added SRB were kept constant.

The present experimental set-up was developed based on experiences from previous experiments with Wyoming MX-80 bentonite using test cells made of stainless steel (Masurat et al. 2010b; Pedersen 2010; Pedersen et al. 2000). The test cells used in these experiments were made of titanium because this metal does not easily react with S^{2-} (Persson et al. 2011), which may be the case with stainless steel. Further, in earlier experiments, it was found that bacterial sulphate-reducing activity in free water adjacent to the bentonite became very intensive and the produced S^{2-}interfered with the analysis of the S^{2-}production rate inside the bentonite (Pedersen 2010). Therefore, the experimental equipment was constructed to exclude any free water in contact with the bentonite clays after the addition of the carbon source, sulphate and SRB. Any production of S^{2-} in the present experiments must have originated from bacterial sulphide-producing activity inside the clay samples. The pressures in the test cells with bentonite relied mainly on the swelling properties of montmorillonite, a main component of swelling bentonites, which may approach 90 % of the weight (Tajeddine et al. 2015). Because different clays have a different content of montmorillonite, the clays may not generate similar high swelling pressures.

4.2 Measurements

4.2.1 Weights

The clay weights were measured both before and after the experiments. It is a straightforward procedure. Of great importance is to avoid unwanted exchange of water. The absolute dry weights are measured immediately after desiccation in oven at 105 °C. Likewise, wet weight must be measured directly after sampling to avoid evaporation of water. There was a good agreement between planned and analysed densities and water contents as demonstrated in study 4 (Table 3-12). Standard deviations for 4 replicates were not more than at most a couple of percent.

4.2.2 Pressures

During the experiments, test cell pressures were measured continuously. The first phase with water saturation, the pressures measured are true swelling pressures caused by the suction of water by the clay (Figure 3-53). In the second phase after lactate was added the analysed pressures (e.g. Figure 3-54) reproduced the swelling pressures in many cases (Table 3-15, Table 3-27 and Table 3-30).

In study 9 one test cell the pressure was mechanically increased (Table 3-33) close to the upper limit of the force transducers (9.2 MPa). There was no obvious effect on the analysed parameters (confer Figure 3-60 and Figure 3-61). Increasing the pressure will just increase hydrostatic pressure, it has no effect on the swelling pressure, i.e. the suction effect caused by the clay. The suction will remain approximately the same as during the water saturation phase because water is not removed or added to the clays.

4.2.3 Sulphur on copper discs

The presence of sulphur on the copper discs have been registered by three different methods. The first method used ^{35}S on the discs as indication of sulphide production in the clays (e.g. Figure 4-1). That method was abandoned due to the strong restrictions working with radionuclides, such as radiation issues, safety handling, waste processing and more. The second method utilized XRF for analysis of elemental S on the discs, assumed to originate from sulphide produced by bacteria. A drawback with XRF is that the instrument only registers what is on top of the surface, it cannot see through clay remnants or other material that cover the copper sulphide. Further, if sulphate has attached to the copper surfaces the XRF cannot distinguish it from sulphides. The third method that notes black discolouration of the discs may support XRF results because it will only be copper sulphide that discolour the copper, sulphate will not.

All three methods rely on transport of sulphide from the clay core. In cases where sulphide production occurs opposite the copper disc, the produced sulphide will likely be precipitated in the clays as black spots or layers (e.g. Figure 3-27 and Figure 3-28) and the copper discs may look shiny without any detectable sulphur. However, Figures 3-2 an 3-3 in Bengtsson et al (2017a) show no local accumulation of ^{35}S inside the clay cores which indicates that sulphide is free to diffuse throughout the clay.

A way around was tested in study 5 by placing small copper discs in the clay which demonstrated sulphide production in the clay core. However, placing the discs and harvesting them became laborious.

4.2.4 Black spots in the clay cores

Presence of black spots or layers in the clay have been frequently observed with low and intermediate densities. These spots and layers are the results of sulphide reacting with iron in the clays (Pedersen et al. 2017). Layers were mostly observed at the clay core end that contacted the piston (Figure 2-1). It was found that variations in environmental temperature had a large effect on the registered pressures. Likely, expansion and retraction of the piston may have caused a small slit between the clay core and the piston surface which would have enabled local bacterial sulphide producing activity. Just a couple of μm would be enough for most bacteria that average around 1 μm in size. This was particularly obvious in Study 4 and 5. Study 6 - 9 were, therefore, performed in temperature-controlled environments such as isolated Styrofoam boxes and later, study 7-9 in an airconditioned room. The "layer effect" did not appear after this method update.

Bacteria often grow in colonies. The black spots in the clays may indicate local positions where growth conditions for bacterial sulphide production have been favourable, perhaps due to small variations in the clay core structure.

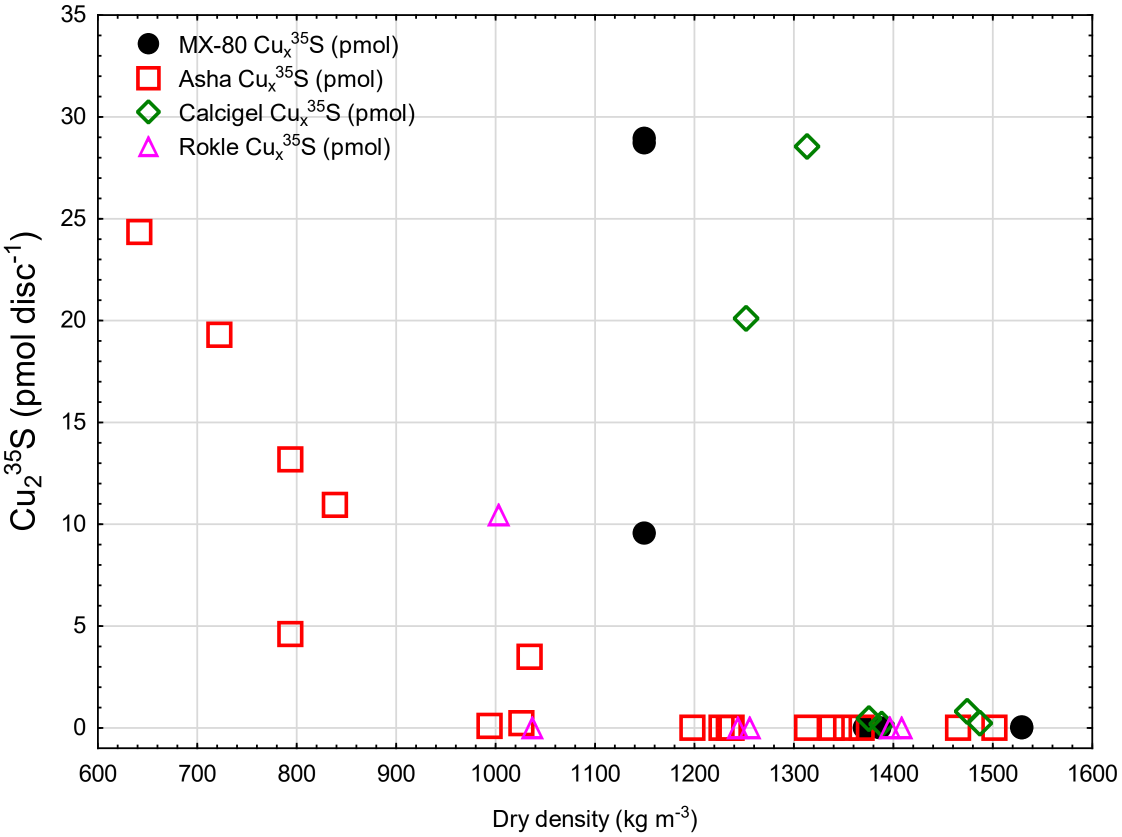


Figure 4-1. *Accumulated Cux35S on copper discs (pmol) over dry density. The corresponding analysed dry density intervals are inserted, and for GMZ all tested dry densities show high sulphide production. Data for Asha, MX-80 and Calcigel from Bengtsson et al. (2017a) and for GMZ and Rokle from Bengtsson et al. (2017b).*

4.2.5 Leachable sulphate

All clays except Rokle and Calcigel had varying amounts of leachable sulphate in the natural clay. A decrease in this amount can be taken as evidence of sulphate reduction, but there are other possibilities as well. Precipitation during the experimental time could reduce the amount of leachable sulphate. Another non-bacterial effect, difficult to predict, is the ion exchange between the saturation water and the pore water during the saturation phase. In many cases, there is much more leachable, i.e. soluble, sulphate in the porewater than what is present in the saturation water (3 mM). Consequently, it has been observed previously that sulphate is diffusing out from the top and bottom of the clay cores to the saturation water in the titanium filters during the water saturation phase, exemplified for Asha in Figure 4-2. This effect will lower the total amount of leachable sulphate in the clay cores, as compared with the amount in the natural clays. The use of background clay cores without addition of lactate and bacteria should have experienced this effect as well and, therefore, a decrease in amount of leachable sulphate in added clay cores compared to the background clay is very likely caused by sulphate-reducing bacteria.

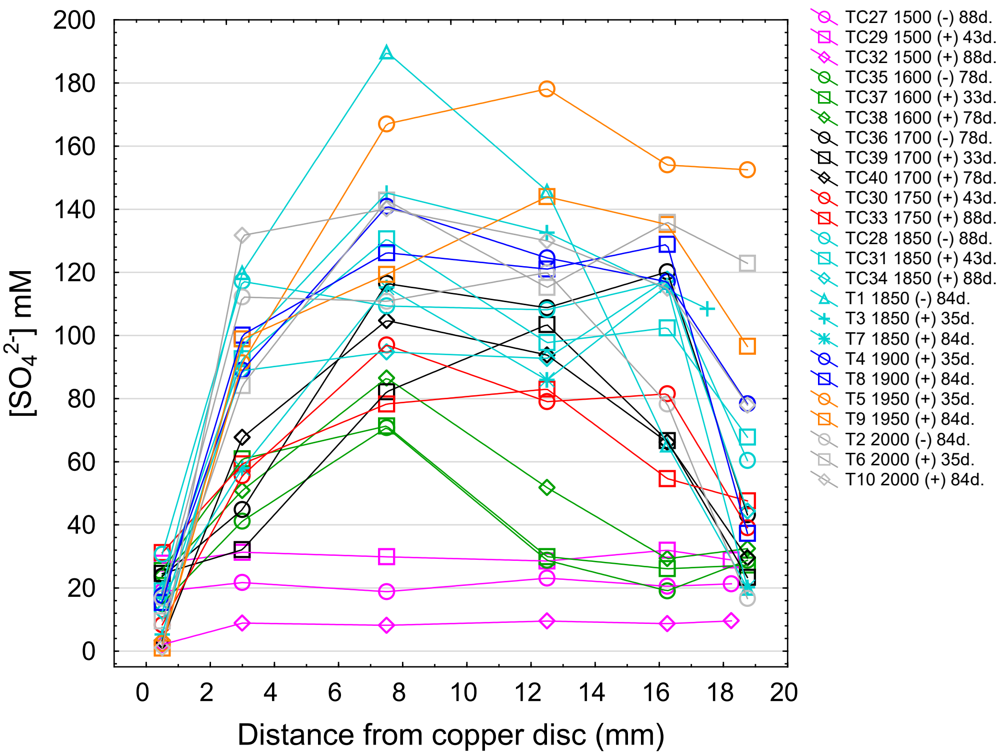


Figure 4-2. *Concentrations of sulphate in pore water shown as profiles of Asha bentonite cores for each test cell. Test cell number, bentonite saturated density, addition of Bacteria (+/−) and incubation time according to symbol description. Figure 3-6 from Bengtsson et al. (2017a).*

4.2.6 Leachable lactate and acetate

The concentration of added lactate was kept constant in the pore water. With increasing density, the total amount of added lactate was concurrently decreased as demonstrated in Figure 3-47. In cases where no consumption of lactate was observed, it can be concluded that there was no bacterial production of sulphide. In opposite, if the amount of lactate decreased significantly below the amount of added lactate, it can be taken as an indication that there was bacterial production of sulphide. The added SRB oxidise lactate to acetate which means that there should be an increase in the amount of acetate concomitant with a decrease in lactate, as was the case in studies 7, 8 and 9.

4.2.7 Sulphide

Sulphide can be analysed if the clay content is relatively high. However, the extractable sulphide concentrations did not go above the detection limit of the applied method in the experiments presented in this report.

4.2.8 Indicators for sulphide production

This report presents 9 studies where a combination of measurements has been applied on 10 different bentonite clays under varying densities. The combination of analyses and measurements attest each other meaning that conclusions can rely on more than one analysed factor. Although the consumption of lactate appeared to be the best indicator, analysis of sulphate, acetate, S on copper discs and black spots generally confirmed presence or absence of sulphide production. A disadvantage with the use of lactate as an indicator is that it cannot be used for studies of sulphate reduction in “natural” systems when no lactate is added.

The cut-off ranges found in studies 1-9 are discussed next and summarised in Table 5-1.

4.3 Method and cut-off indications

4.3.1 MX-80

Study 4 included the MX-80 dry densities 1087 and 1397 kg m^{-3}. Lactate was consumed at the low density, but not in two of three test cells at the higher density. The third high density test cell had black spots at the lower core end where lactate was added (Figure 3-27), likely due to SRB growth in the interface between the test cell and piston and the clay. Apart from that interface growth, study 4 suggests a cut-off for MX-80 between 1087 and 1397 kg m^{-3}.

Study 5 included the MX-80 dry densities 470 and 1400 kg m^{-3}. Lactate was totally consumed at the low density, and also in the two test cells at the higher density. Again, black spots appeared in interface between the test cell piston and the lower end of the clay core (Figure 3-38), while the background core was free from black spots. There was no significant S on the copper discs suggesting the lactate was only consumed at the lower core end resulting in bacterial sulphide production.

Study 7 tested a range of six densities from 1000 to 1500 kg m^{-3}. There was no lactate and sulphate consumption or acetate production at 1400 and 1500 kg m^{-3} (Figure 3-47). Study 7 consequently narrowed the range in Figure 4-1 to a cut-off for MX-80 between 1300 and 1400 kg m^{-3}.

In conclusion, the results from these tests with Wyoming bentonite seem to be consistent with the results from Bengtsson and Pedersen (2017).

4.3.2 Bara Kade

Study 7 tested a range of six dry densities from 1000 to 1500 kg m^{-3}. There was no lactate and sulphate consumption or acetate production at 1500 kg m^{-3} (Figure 3-49). Study 7 consequently suggested a cut-off range for Bara Kade between 1400 and 1500 kg m^{-3}.

4.3.3 Georgian

Study 8 tested a range of six dry densities from 1000 to 1500 kg m^{-3}. There was no lactate and sulphate consumption or acetate production at 1400 and 1500 kg m^{-3}. Study 8 consequently suggested a cut-off range for Georgian between 1300 and 1400 kg m^{-3}.

Study 9 attempted to fine-tune the cut-off range at 8 dry densities ranging from 1250 to 1425 kg m^{-3}. The outcome was somewhat difficult to interpret. Figure 3-60 indicate a cut-off range between 1325 and 1350 kg m^{-3} where lactate and sulphate consumption or acetate production ceased, but the data also indicated some lactate and sulphate consumption or acetate production at densities above 1300. The Georgian clay water saturation stabilized very slowly which may have resulted in some bacterial sulphide production activity before full saturation was obtained in the complete clay core. Follow up experiments should use much longer saturation times, or, even better, use a "faster" clay such as MX-80.

4.3.4 Rokle

Study 4 included the Rokle dry densities 1047 and 1347 kg m^{-3}. This clay does not have leachable sulphate above the detection limit of 0.4 µmol gdw^{-1} (Table 3-17). That may be the limiting factor for sulphide producing bacteria irrespective of the density. However, the low-density clay cores did show evidence of sulphide production as black spots at the bottom of the clay core (Figure 3-31), almost absent at the high density. Apart from that interface growth, study 4 suggests a cut-off for Rokle between 1087 and 1397 kg m^{-3}.

Study 5 included the Rokle dry density of 450 kg m^{-3}. Here lactate was consumed, but there was no sulphur on the copper discs (Figure 3-37). This low density likely allowed bacterial consumption of all lactate before it had diffused to the vicinity of the copper discs. Figure 3-36 shows that more than half of the clay cores were discoloured blackish from the lower core surface. Further, Rokle has a very high iron content which may be the reason for the sulphide scavenging.

4.3.5 Asha

Study 4 included the Asha dry densities 1054 and 1397 kg m^{-3}. There was lactate and sulphate consumption at both densities, mainly at the bottom of the clay cores. Copper discs all carried sulphur and the lower density had more than the high density (Figure 3-30). The cut-off density was obviously not reached.

Study 5 included the Asha dry densities 1200 and 1280 kg m^{-3}. Here, there was lactate and sulphate consumption at both densities at quite different levels, mainly visible as blackening at the bottom of the lower density clay cores, and as lactate consumption for both densities.

The radiotracer experiments suggested a a cut-off range between 1050 and 1200. However, that method only analysed the copper discs. Here, analysis of lactate involved the total clay core (three layers analysed) which is important especially for a iron-rich clay like Asha where diffusion of sulphide towards the copper discs may be significantly mitigated by FeS precipitation and, therefore, not registered on the discs.

4.3.6 Laponite

Study 3 included Laponite at 1100 and 1300 kg m^{-3}, spiked and not spiked with sulphate-reducing bacteria. There was no significant evidence for sulphide production in any of the bacteria and density combinations (Table 3-13). Black spots were absent in the cores (Figure 3-22) and on the discs (Figure 3-27).

Study 6 included Laponite at six dry densities from 665 to 1080 kg m^{-3}. Lactate was found in added concentrations at all densities and sulphur was absent on the discs (Table 3-25).

For reasons not confirmed, it became obvious that sulphide producing bacteria could not be active in difference to all other tested clays in this report. However, literature data suggest that Laponite may be unfavourable for microbial growth and survival (Niu and Zhang 2023; Malekkhaiat Häffner et al. 2019).

4.3.7 Calcigel

Study 2 included Calcigel at six dry densities from 1250 to1560 kg m^{-3}. Lactate was not analysed in this study. Sulphate was absent in all densities and there was only very small amounts of sulphur on the discs. However, the bevel edges showed clear evidence of sulphide production. But because the density in the slit between the copper disc and the titanium test cell was unknown, that observation was not conclusive. There were black spots at all densities suggesting a cut-off for Calcigel >1500 kg m^{-3}.

4.3.8 Turkish

Study 1 included Turkish clay at two dry densities from 1114 to1495 kg m^{-3}. Lactate was not analysed in this study. There was a decreasing trend of S on the copper discs which approached the detection limit above 1400 kg m^{-3} (Figure 3-3).

4.3.9 Moroccan

Study 1 included Moroccan clay at six dry densities from 1083 to 1443 kg m^{-3}. Major amounts of S were found on all copper discs (Table 3-3, Figure 3-1, (Figure 3-3).)

Study 2 included Moroccan clay at six dry densities from 1308 to1604 kg m^{-3}. In this experiment, there were less sulphur on the copper discs but a clear cut-off could not be identified.

Study 3 included Moroccan clay at two dry densities 1300 to1500 kg m^{-3}, grinded and not grinded. The not grinded clay at 1300 kg m^{-3} had large amounts of S on the discs as was also found in study 1. However, after grinding, there was no signs of sulphide production after 2 and 4 months incubation with 1500 kg m^{-3}.

4.3.10 Bulgarian

Study 1 included Bulgarian clay at six dry densities from 840 to1268 kg m^{-3}. There was a decreasing trend of S on the copper discs which approached the detection limit above 1200 kg m^{-3} (Figure 3-3).

5 Summary and conclusion

A total of nine consecutive bentonite clay studies is presented in this report. The line of studies represents a continuous development of a method that can assess what condition or conditions may exclude bacterial sulphide production in bentonite clays. The studies 1 to 6 tested various configurations with several different clays including statistical evaluations. The outcome was transformed to a comprehensive method applied in studies 7 to 9 with MX80, Bara Kade and Georgian clays.

Previous experiments using $^{35}SO_4$ analysed production of copper sulphide on copper discs which implies that most of the detected bacterial activity must have taken place close to the discs, plus possibly produced sulphide in the clay core that diffused towards the discs. The present method analyses activity in the whole clay core, as consumption of lactate and sulphate plus production of acetate. It was also possible to analyse the presence of viable SRB as tested in study 8. Of course, if deemed important, XRF and EDS analyses of precipitates on the copper discs can be performed as well.

Because the studies addressed many issues, and solved challenges along the way, conclusions about cut-off ranges should be evaluated with caution. Still, the cut-off ranges observed were fairly consistent for all tested clays to between 1300 – 1400/1500 kg m^{-3} with exception for Bulgarian, Laponite and Rokle (Table 5-1). These ranges agree with previously determined cut-off ranges for MX80, Asha and Calcigel (Bengtsson et al. 2017a).

Table 5-1. Table summarizing observed cut off ranges

Bentonite	Cut-off range dry density (kg m^{-3})	Studies
MX-80	1300 – 1400	4, 5, 7
Bara Kade	1400 – 1500	7
Georgian	1300 – 1400 maybe 1325 – 1350 (uncertain range)	7, 8
Rokle	1047 – 1347	4, 5
Asha	>1397	4, 6
Laponite	No sulphide producing activity at densities from 650 – 1300	3, 6
Calcigel	>1500	2
Turkish	1300 – 1400	1
Moroocan	1300 – 1500 (for grinded clay)	1,2,3
Bulgarian	1200	1

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