Report
P-19-07
September 2019

Verification of microbial sulfide-producing activity in calcigel bentonite at wet densities of 1750 and 1900 kg m^{-3}

Haydn M. Haynes
Sophie Nixon
Martin Birgersson
Jonathan R. Lloyd

SVENSK KÄRNBRÄNSLEHANTERING AB

SWEDISH NUCLEAR FUEL
AND WASTE MANAGEMENT CO

Box 3091, SE-169 03 Solna
Phone +46 8 459 84 00
skb.se

SVENSK KÄRNBRÄNSLEHANTERING

ISSN 1651-4416
SKB P-19-07
ID 1719753
September 2019

Verification of microbial sulfide-producing activity in calcigel bentonite at wet densities of 1 750 and 1 900 kg m^{-3}

Haydn M. Haynes, Sophie Nixon, Jonathan R. Lloyd
University of Manchester

Martin Birgersson
Clay Technology AB

Abstract

Research has shown that bentonite materials proposed for the disposal of nuclear waste contain sulfate-reducing bacterial communities. Such bacterial communities may accelerate the corrosion of the copper canisters intended for use in the disposal of high-level nuclear waste via the production of sulfide. Previous research by Bengtsson and Pedersen (2017) has identified a bentonite density threshold for Calcigel bentonite where sulfide-producing activity is halted, and this research intended to verify this threshold. Two Calcigel bentonite densities were investigated; a positive control (1 750 kg m^{-3}) was used to confirm that the sulfate-reducing bacteria were viable, and potentially active, as well as a target density (1 900 kg m^{-3}) at which sulfate-reducing activity was expected to be halted. Using an S-35 radioactive tracer we showed that sulfide-producing activity occurred at the 1 750 kg m^{-3} positive control bentonite density, and that sulfide-producing activity was prevented at the 1 900 kg m^{-3} target threshold. While the results were reproducible it was not clear if the swelling pressure could be measured accurately during the experimental stage of the research. The supplementation of the bentonite with additional SRB may have also increased the amount of corrosion observed at the positive control bentonite density. The S-35 radioactive tracer limited the follow up work that could be conducted on the bentonite samples, and a non-active alternative methodology could be considered for future research. A greater emphasis could also be placed on assessing the naturally-occurring bacterial communities for sulfate-reducing potential, as well as assessing the impact on a broader range of bentonite materials.

Sammanfattning

Tidigare forskning har visat att bentonitmaterial som föreslås användas i förvar för kärnavfall innehåller sulfatreducerande bakteriesamhällen. Sådana bakteriesamhällen kan påskynda korrosionen av de kopparkapslarna som ska användas för inneslutingen av kärnkraftsavfallet genom att de producerar sulfid. Tidigare forskning av Bengtsson och Pedersen (2017) har identifierat en gräns för densiteten hos Calcigel bentonit där aktiviteten hos de sulfidproducerande bakterierna stoppas. Den forskning som presenteras i den här rapporten syftar till att verifiera denna tröskel. Två Calcigel bentonitdensiteter undersöktes; en positiv kontroll (1 750 kg m^{-3}) användes för att bekräfta att de sulfatreducerande bakterierna var livskraftiga och potentiellt aktiva samt en måldensitet (1 900 kg m^{-3}) vid vilken den sulfatreducerande aktiviteten förväntades vara stoppas. Med användning av en S-35 radioaktivt spårämne visade vi att sulfidproducerande aktivitet pågick vid den positiva kontrollen med bentonitdensiteten på 1 750 kg m^{-3} och att sulfidproducerande aktivitet effektivt förhindrades vid måldensiteten för 1 900 kg m^{-3}. Medan resultaten var reproducerbara var dock det inte klart om svällstrycket kunde mätas korrekt under experimentfasen. Tillsatsen till bentoniten med ytterligare SRB kan också ha ökat den korrosion som observerats vid den positiva bentonitdensiteten. Den radioaktiva spåraren S-35 begränsade vilket uppföljningsarbete som kunde utföras på bentonitproverna, och en icke-aktiv alternativ metod kunde övervägas för framtida forskning. En större tonvikt kan också läggas på att utvärdera de naturligt förekommande bakteriesamhällena med avseende på sulfatreducerande potential, samt att bedöma påverkan på ett bredare urval av bentonitmaterial.

Contents

1 Introduction

Microbes are found in bentonite deposits (López-Fernández et al. 2014) and may persist under geological disposal conditions (Stroes-Gascoyne et al. 2011). Many microorganisms are capable of sulfate reduction (Postgate 1979) which is a concern as sulfide production may lead to the corrosion of copper canisters intended for the disposal of nuclear wastes (Pedersen 2010). However, research has shown that microbial activity in bentonite buffers can be suppressed if a sufficient bentonite density is maintained (Stroes-Gascoyne et al. 2010). The threshold density of microbial sulfate-reducing activity has been shown to vary between bentonites as a result of swelling pressure (Bengtsson and Pedersen 2017), with montmorillonite content being a key criterion (Karnland 2010).

The aim of this work was to verify the wet density threshold of sulfate-reducing activity in Calcigel bentonite as described previously by Bengtsson and Pedersen (2017) in "Microbial sulfide-producing activity in water-saturated Wyoming MX-80, Asha and Calcigel bentonites at wet densities from 1 500 to 2 000 kg m^{-3}". The original experiment showed that sulfide-producing activity was minimal at a wet density of 1 900 kg m^{-3}, with a significant amount occurring at a wet density of 1 850 kg m^{-3}. Taking this into consideration we decided to use a Calcigel bentonite wet density of 1 750 kg m^{-3} as a positive control, with the aim of confirming that the 1 900 kg m^{-3} wet density would suppress microbial sulfide-producing activity.

2 Materials and Methods

The methodologies used in this report are largely comparable to those used in Bengtsson and Pedersen (2017). However, some adjustments were required to account for differences in equipment. The pressure cells used in this report were of an identical design to those used in the original experiment, but alterations were made to the saturation pistons so that they could accommodate flexible steel hosing. The flexible steel hosing was tolerant of higher pressures which was a requirement for calibrating the force transducers used. Saturation of the bentonites was achieved using a Teledyne ISCO (Lincoln, NE, USA) 260D syringe pump, as opposed to a gas-pressurised piston used in the original work. The syringe pump automatically adjusted the flow rate in the saturation system, and provided a stable, and accurate saturation system pressure. The piping for saturating the pressure cells, and the electronics used for monitoring the force transducers were designed, and built in house, but were not expected to influence the results. After reviving appropriate SRB pure cultures (identical to those used in the earlier study), their ability to produce iron sulfide in Postgate B medium was poor, they were therefore mixed with some SRB cultures grown from naturally-occurring bentonites (Haynes et al. 2018). Finally, the volume of the pressure cells during the experimental stage was reset to the volume used during the saturation stage instead of adjusting the volume so that the force transducer readings matched the readings obtained during the saturation stage. The pressure cells were reset in this manner as it was unclear if the force transducer readings during the experimental stage were relevant to the swelling pressure, meanwhile by resetting to the original volume we knew the wet density was consistent with the saturation stage.

2.1 Clay preparation and microbial inoculum

Bacterial communities were prepared by first growing pure cultures of *Desulfovibrio aespoeensis*, *Desulfotomaculum nigrificans*, and *Desulfosporosinus orientis* sourced from the German Collection of Microorgansisms and Cell Cultures (DSMZ, Brunswick, Germany) using the recommended media and conditions. The pure cultures were then further supplemented with SRB enrichments from FEBEX and Commercial bentonites (Haynes et al. 2018) in equal parts (1:1:1:1:1) to produce an "SRB cocktail". The Calcigel bentonite, and the SRB cocktail were transferred to an anaerobic chamber (Coylab, Grass Lake, MI, USA), and 1 g aliquots of Calicgel bentonite were spread evenly across five petri dishes. Each of the petri dishes was then spiked with 2 ml of the SRB cocktail using a sterile syringe, and then left to dry for approximately half an hour. The resulting SRB-doped Calcigel bentonite was then strained through a fine mesh, and mixed with more Calcigel bentonite to produce a final batch of approximately 100 g. The water content of the doped Calcigel bentonite was determined by inserting 1 g aliquots into preignited crucibles and heating them at 105 °C for an hour, before cooling the crucibles in a desiccator, and reweighing. The appropriate amount of Calcigel bentonite was then weighed the titanium pressure cells (previously described in Bengtsson and Pedersen (2017)) using the relationships described in Karnland (2010), and the bentonite compacted to the appropriate volume using a G-clamp (Figure 2-1). The final bentonite dimensions were 35 x 20 mm.

Table 2-1. Target wet densities (kg m^{-3}) of the bentonites, as well as the target dry density (kg m^{-3}), Calcigel grain density (kg m^{-3}), water content of the bentonites inserted into the pressure cells, and the weight (including water content) of bentonite added to the pressure cells.

Target wet density (kg m^{-3})	Target dry density (kg m^{-3})	Grain density (kg m^{-3})	Water content (%)	Bentonite weight (g)
1750	1153	2760	14.06	25.81
1900	1383	2760	14.06	30.98

Figure 2-1. *Use of a G-clamp to compact the bentonite inside of the pressure cells. The tightening of the screw pushes the piston into the pressure cell reducing the volume to that required for the experiment.*

2.2 Load cell calibration

Calibration of the force transducers was achieved by attaching the empty pressure cells to the saturation system. The output of the force transducers was then recorded at a series of known fluid pressures set using a Teledyne ISCO (Lincoln, NE, USA) 260D syringe pump, and independently verified using a pressure gauge (Swagelok, Solon, OH, USA). Two force transducers were used to record the swelling pressures recorded in the pressure cells. A Load Cell – 200 kg, Disc (TAS606) (SEN-13332) (SparkFun, Boulder, CO, USA) was used on the 1 750 kg m^{-3} pressure cell, and a Miniature Button Load Cell, CDFM3 (Applied Measurements, Aldermaston, UK) on the 1 900 kg m^{-3} pressure cell. The force transducers were connected to a Raspberry Pi data logger (Raspberry Pi Foundation, Cambridge, UK) with custom logging software, and data collection was completed using PuTTY version 0.7.

2.3 Saturation phase

Following compaction of the bentonite plugs to their appropriate volumes the pressure cells were connected to the saturation system and gassed with nitrogen. The nitrogen was then evacuated using an Edwards (West Sussex, UK) E2M8 Rotary Vane High Vacuum Two Stage Pump. This process was carried out a further two times over the course of an hour, before the saturation system was left to evacuate for a further 23 hours. Artificial groundwater (AGW) for saturating the bentonites was produced by adding the following constituents to deionized water (18.2 MΩ); 120 mM sodium chloride, 7 mM calcium chloride dihydrate, 9 mM potassium chloride, 18 mM ammonium chloride, 1 mM potassium orthophosphate, 2 mM magnesium chloride hexahydrate, and 1 mM sodium sulfate. The AGW was subsequently corrected to a pH of 7 using 10 M sodium hydroxide, and made anoxic using a mixture of nitrogen, and carbon dioxide. After being autoclaved (126 °C, 20 mins) the AGW was inserted into a Teledyne ISCO (Lincoln, NE, USA) 260D syringe pump that had been cleaned with methanol, and deionized water (18.2 MΩ) prior to use. The AGW was delivered to the pressure cells at a constant pressure of 200 kPa, and the response from the force transducers was collected.

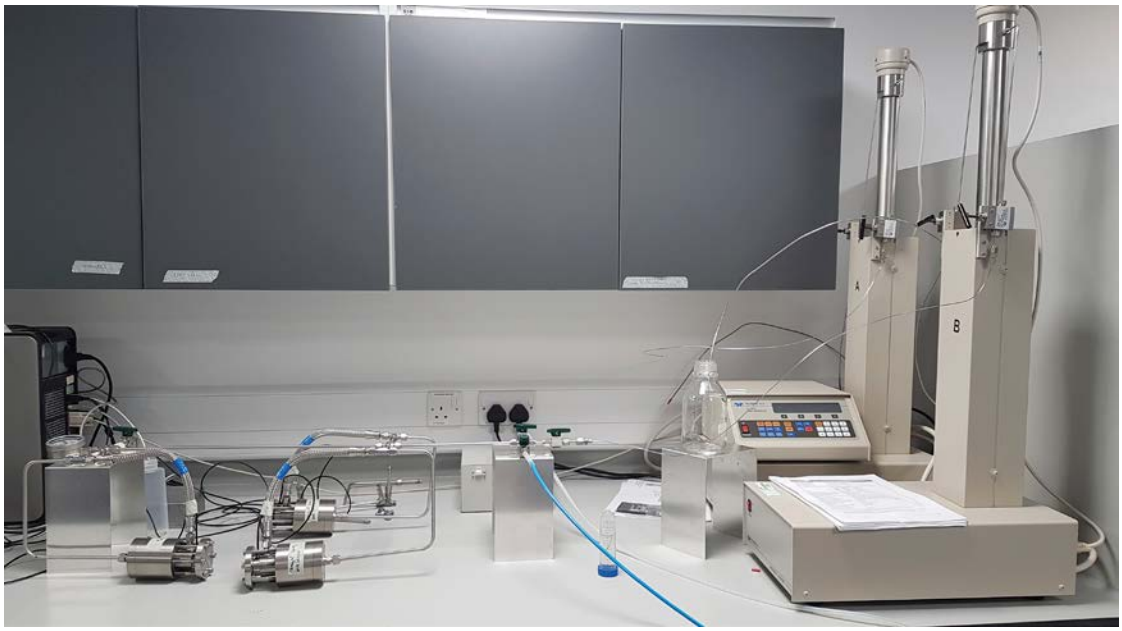

Figure 2-2. *Overview of the saturation system setup. A syringe pump located on the right of the image delivers fluid to the pressure cells towards the left of the image.*

Figure 2-3. *Close up view of the pressure cells, and the saturation system.*

2.4 Experimental phase

After 9 days of operation the pressure cells were removed from the saturation system and transferred to an anaerobic chamber. A set of copper discs were cut, and cleaned according to ISO 8407:2009. The bottom lids of the pressure cells were then opened, and the copper discs placed in the base. Following the addition of the copper discs the base plates were reattached, and the pistons removed. A syringe was used to insert 23 µl of filter-sterilised 60 % sodium lactate solution to the 1 750 kg m^{-3} pressure cell, and 20 µl to the 1 900 kg m^{-3} pressure vessel, before reinserting the pistons. The pressure cells were then transferred to a fume hood where the piston was removed, and 30 µl of 6 µM S-35 labelled sodium sulfate was deposited on the 1 750 kg m^{-3} bentonite surface, and 26 µl on the 1 900 kg m^{-3} bentonite surface. The pistons were then reinserted, and the volume reset to the value used in the saturation stage of the experiment.

2.5 Pressure cell dismantling

After 30 days the base plate of the pressure cells was removed, and the copper discs were collected. Bentonite residue was removed from the surface of the copper discs by bathing them in deionized water (18.2 MΩ) for 15 minutes in a petri dish. The copper discs were then transferred to empty petri dishes and air dried. After drying the copper discs were placed in grip seal bags and placed on a blank phosphor screen. The phosphor screen was shielded from natural light in a case and left to expose for 2 weeks. The phosphor screen was then immediately imaged (~ 10 minutes) using a FujiFilm (Tokyo, Japan) Fluorescent Image Analyzer FLA-3000.

3 Results

3.1 Saturation phase

The Calcigel bentonite plugs were left to saturate for 9 days (previous work with the setup have observed saturation after 3 days (unpublished). Initial swelling pressures during day one were 211 kPa for the 1 750 kg m^{-3} pressure cell, and 433 kPa for the 1 900 kg m^{-3} pressure cell (Figure 3-1). Markings on the pressure cell pistons indicated that the volume of the internal cylinder was too high, and this was corrected on day 1. A final measurement of the piston height with a ruler on day 8 indicated the internal volume of the cylinder was still too high and was readjusted. The final swelling pressures were 384 kPa and 1 452 kPa for the 1 750 kg m^{-3}, and the 1 900 kg m^{-3} pressure cells respectively (Figure 3-1).

3.2 Experimental phase

Following the introduction of the lactate as an electron donor, and S-35 labelled sodium sulfate as additional electron acceptor, the pressure cells were left to incubate for a further 30 days at room temperature. No adjustments were made to the pressure cell pistons once set, and the pressure cells had steady (considering temperature fluctuations) mechanical pressures of 320 kPa in the 1 750 kg m^{-3} pressure cell, and 1 000 kPa in the 1 900 kg m^{-3} pressure cell (Figure 3-2).

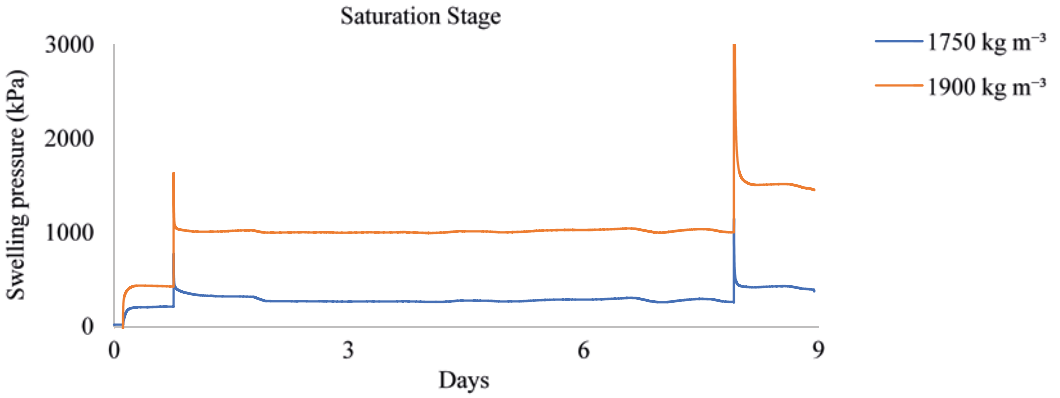

Figure 3-1. *Swelling pressure readings (kPa) from the force transducers attached to the pressure cells during the saturation period. Spikes in the swelling pressure occurred as a result of decreasing the pressure cell volume to the intended volume required for the chosen wet densities.*

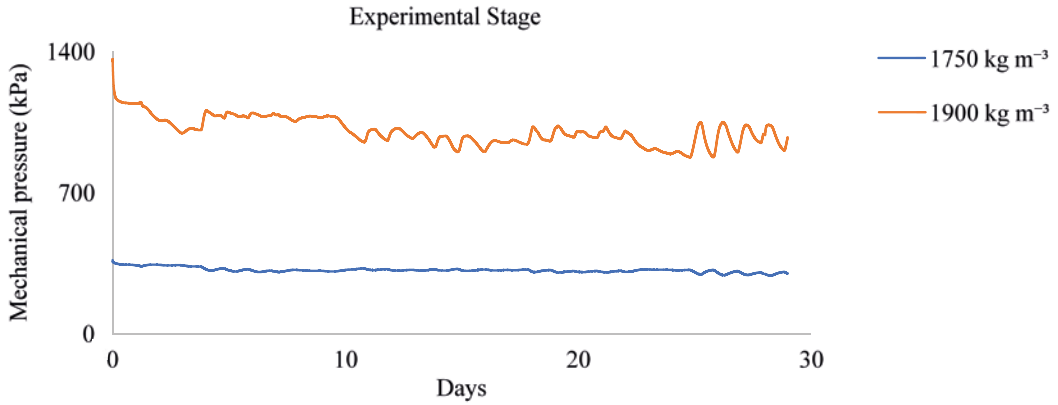

Figure 3-2. *Pressure readings (kPa) from the force transducers during the experimental stage of the work.*

3.3 Copper disc analysis

Accumulation of the copper sulfide corrosion product on the copper discs was identified by exposing a phosphor image plate to the corroded copper surface, and therefore the radioactive S-35 that was introduced to the bentonite after the saturation stage. The 1 750 kg m^{-3} tested positive for S-35 accumulation accounting for 99.96 % of the illumination (Table 3-1). The 1 900 kg m^{-3} produced a negative result (0 %), lower than the background signal of the phosphor image plate (0.04 %) (Table 3-1). The copper discs were also inspected using a beta radiation dose rate monitor which detected 120 cps (counts per second) on the 1 750 kg m^{-3} copper disc, and 9 cps on the 1 900 kg m^{-3} copper disc (Table 3-1). The background count for the room was 9 cps.

Table 3-1. Images of the copper discs after removal from the pressure cells, along with counts per second (cps) using a beta radiation dose monitor, as well as fluorescence intensity of the discs when placed on the phosphor plate.

	Background	1750 kg m^{-3}	1900 kg m^{-3}
			
Beta radiation count (cps)	9	120	9
Flourescence Intensity	0.04 %	99.96 %	0 %

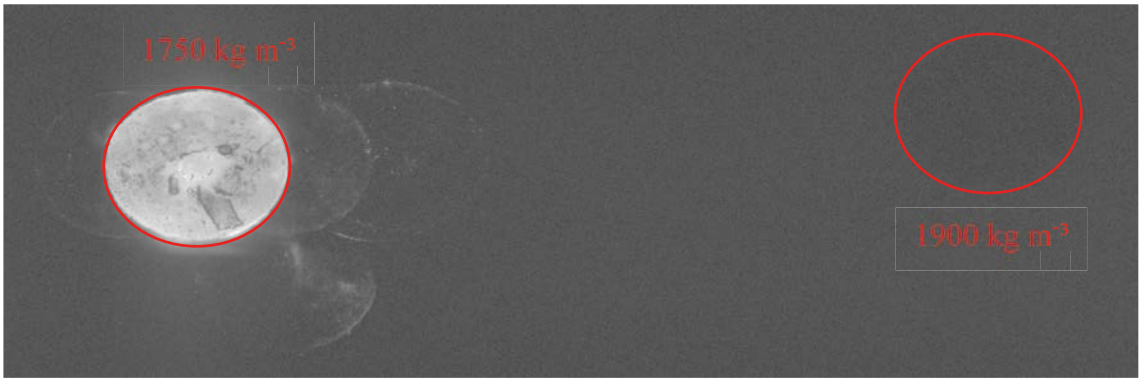

Figure 3-3. *Phosphor plate exposed to the copper discs used in the pressure cells. A lighter colour indicates a greater exposure to radiation from S-35.*

4 Discussion

Using the experimental setup described in Bengtsson and Pedersen (2017), the aim of this work was to confirm the bentonite density threshold of sulfide-producing activity in Calcigel bentonite. The setup consisted of a 35 x 20 mm bentonite plug encased in a titanium pressure cell. The bentonite was inoculated with an SRB cocktail and contained a copper disc (mimicking a copper HLW container) with sodium lactate, and S-35 labelled sodium sulfate added at the opposite end. Two bentonite densities were used to verify the results of the previous research; 1 750 kg m^{-3}, and 1 900 kg m^{-3}. These densities were calculated using equations described in Karnland (2010). The experimental densities may differ slightly from the calculated values, although sample weights, and volumes were maintained. Measurement of the true wet densities was not possible due to the radiological risk of the S-35 label.

Microbially induced corrosion as a result of sulfate-reducing activity (and subsequent sulfide production) was identified in the bentonite plugs by the accumulation of S-35 labelled sulfide on the copper disc surface. A black coating was observed across much of the copper disc in contact with the 1 750 kg m^{-3} bentonite plug consistent with corrosion of the copper disc, and accumulation of a copper sulfide product (Table 3-1). A check with a beta radiation dosimeter confirmed the accumulation of a radioactive product, with the only potential source being the S-35 labelled sodium sulfate (Table 3-1). Further confirmation of a positive result was established by exposing a phosphor screen to the corroded surface of the copper disc. The phosphor image showed a strong relation between intensity, and the location of the corrosion products on the copper disc confirming the products were the source of the radioactivity (Figure 3-3). Having established the positive control experiment (1 750 kg m^{-3}) was successful, the target 1 900 kg m^{-3} experiment was then investigated. The 1 900 kg m^{-3} came out of the pressure cell relatively pristine, but upon exposure to the atmosphere some tarnishing of the surface was observed (Table 3-1). Inspection of the 1 900 kg m^{-3} copper disc with a beta radiation dosimeter provided a result consistent with the background radiation of the room (9 cps) (Table 3-1). Exposure of the phosphor screen to the 1 900 kg m^{-3} copper showed that no S-35 accumulated on the copper disc (a blank image was recorded), and therefore no sulfide-related copper corrosion occurred at the target Calcigel bentonite density threshold of 1 900 kg m^{-3} (Figure 3-3). However, studies have shown that produced sulfide can be trapped in the bentonite (Stone et al. 2016), and therefore we can not discount that sulfate-reduction in the bentonite did not take place.

In terms of comparability to a geological disposal facility (GDF) scenario, the experimental setup is suitable for testing the effects of swelling pressure, and bentonite density on sulfide-producing activity. However, it is not clear if the mechanical pressure imposed on the bentonite plugs during the experimental stage is directly relatable to swelling pressure. The decrease in pressure after the initial reset (Figure 3-2) may be due to rearrangement of the macro-structure, or the removal of an external water source. More testing is required to examine this issue. The use of an SRB cocktail to increase the amount of viable SRB in the bentonite is also not representative of the amount of SRB that would be present in a bentonite intended for the disposal of nuclear waste. This might exaggerate the amount of sulfide-production that occurs when the bentonite is not at its target density threshold. Future research should investigate if the SRB naturally occurring in the bentonite would be enough to initiate sulfide-production in the material. The radioactive S-35 tracer was suitable for determining the accumulation of microbially-produced sulfide on the copper discs. However, the added radiological risk places restrictions on follow-up analyses that can be conducted, and increase administration, and clean-up times. These issues can be circumvented by looking for non-active ways to investigate sulfide-producing activity in the bentonite, and sulfide accumulation on the canister material (copper disc). Plausible options may include quantification of sulfate in the bentonite prior to, and after incubation, as well as imaging techniques including electron microscopy approaches to identify corrosion products on the copper disc (Figure 4-1).

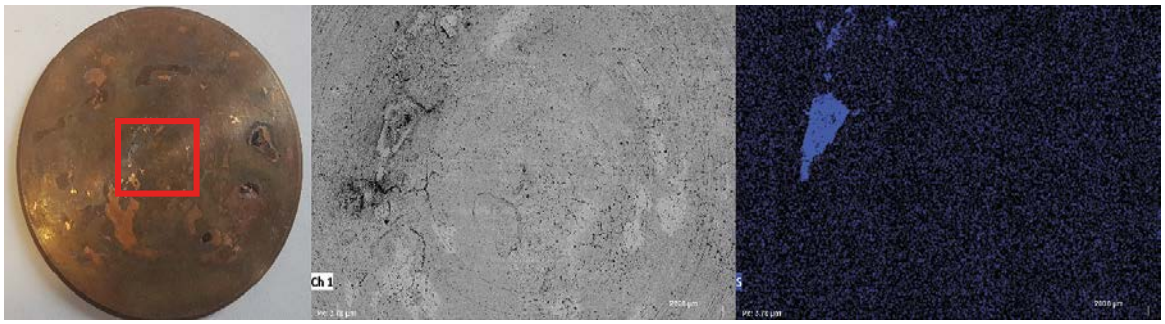

Figure 4-1. *Example of an electron microscopy approach to analyzing copper corrosion. The image on the left depicts a corroded copper disc, with the middle image depicting a false colour ESEM image, and the right image the same area with a map of sulfur accumulation on the surface (unpublished).*

5 Conclusions

Using methods previously described in Bengtsson et al. (2015), and Bengtsson and Pedersen (2017) we were able to confirm that microbial sulfide-production in Calcigel bentonite would be prevented if the wet density of the bentonite was maintained at 1 900 kg m^{-3} or greater. Proving that sulfide-producing activity can be prevented at a target bentonite density is critical in proving that such activity can not occur in a bentonite buffer intended for nuclear disposal, and therefore the impact of microbially-induced corrosion of copper on canister integrity will be minimised. This threshold bentonite density will vary between different bentonite materials, as a result of montmorillonite content, and other factors, and therefore needs to be considered when selecting a candidate bentonite for use. While the original experiments of Bengtsson and Pedersen (2017) were repeatable, some aspects of them could potentially be refined. For instance, it is unclear how the mechanical pressure applied during the experimental stage of the work relates to the swelling pressure generated during saturation of the bentonite. The supplementation of the natural sulfide-producing bacteria community with additional SRB may also increase the amount of sulfide accumulation observed on the copper discs when the threshold bentonite density is not achieved, and the use of the S-35 radioactive tracer limits the follow up research that can be conducted on the samples.

Future research could therefore investigate the target bentonite density of the naturally occurring sulfide-producing bacteria communities. Refinement of the techniques used to investigate the accumulation of the corrosion products may also provide more information on the extent of corrosion observed.

References

SKB's (Svensk Kärnbränslehantering AB) publications can be found at www.skb.com/publications.

Bengtsson A, Pedersen K, 2017. Microbial sulphide-producing activity in water saturated Wyoming MX-80, Asha and Calcigel bentonites at wet densities from 1 500 to 2 000 kg m^{-3}. Applied Clay Science 137, 203–212.

Bengtsson A, Edlund J, Hallbeck B, Heed C, Pedersen K, 2015. Microbial sulphide-producing activity in MX-80 bentonite at 1 750 and 2000 kg m^{-3} wet density. SKB R-15-05, Svensk Kärnbränslehantering AB.

Haynes H M, Pearce C I, Boothman C, Lloyd J R, 2018. Response of bentonite microbial communities to stresses relevant to geodisposal of radioactive waste. Chemical Geology 501, 58–67.

ISO 8407:2009. Corrosion of metals and alloys – Removal of corrosion products from corrosion test specimens. Geneva: International Organization for Standardization.

Karnland O, 2010. Chemical and mineralogical characterization of the bentonite buffer for the acceptance control procedure in a KBS-3 repository. SKB TR-10-60, Svensk Kärnbränslehantering AB.

López-Fernández M, Fernández-Sanfrancisco O, Moreno-García A, Martin-Sánchez I, Sánchez-Castro I, Merroun M L, 2014. Microbial communities in bentonite formations and their interactions with uranium. Applied Geochemistry 49, 77–86.

Pedersen K, 2010. Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. Journal of Applied Microbiology 108, 1094–1104.

Postgate J R, 1979. The sulphate-reducing bacteria. Cambridge: Cambridge University Press.

Stone W, Kroukamp O, Moes A, McKelvie J, Korber D R, Wolfaardt G M, 2016. Measuring microbial metabolism in atypical environments: Bentonite in used nuclear fuel storage. Journal of Microbiological Methods 120, 79–90.

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

Stroes-Gascoyne S, Hamon C J, Maak P, 2011. Limits to the use of highly compacted bentonite as a deterrent for microbiologically influenced corrosion in a nuclear fuel waste repository. Physics and Chemistry of the Earth, Parts A/B/C 36, 1630–1638.

Arkitektkopia AB, Bromma, 2019

SKB is responsible for managing spent nuclear fuel and radioactive waste produced by the Swedish nuclear power plants such that man and the environment are protected in the near and distant future.

skb.se