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Microbial activity in a concrete-bentonite clay interface

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Publishable Summary

In nuclear waste repositories cementitious materials can be an essential part of the plug and seal systems. In some concepts compacted bentonite clay will be used as backfill and will be in contact with the cementitious plug and seal. Microorganisms are often active at interfaces and may therefore be active at the interfaces between cement plugs and seals and backfill. A concern regarding the integrity is microbial degradation of concrete. However, microbial activity might be inhibited through an alkaline plume (pH > 13) generated by concrete diffusing into the bentonite. Therefore, concrete plugs were in contact with bentonite Volclay MX-80, which was compacted and saturated to a wet density of 1750 kg m^{-3}, in titanium test cells. The bentonite clay was inoculated with sulphide producing bacteria. Furthermore, glucose was added to induce microbial fermentation. The aim of this work was to analyse the effect of concrete on pH of the bentonite as well as microbial survival in the bentonite. The MX-80 was analysed in profile for water content, pH, ATP and CHAB.

The conclusion of the results was that the alkaline solution, leached from the concrete, lead to a deviation in wet density and increased the pH in the bentonite clay. However, the inhibition of microbial activity was dependent of the added carbon source. Induced microbial activity in MX-80 with glucose led to a decreased in pH. A reason might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. In the future bentonite clay samples should be analysed for acetate and cultivatable acetogenic bacteria to confirm this hypothesis.

Contents

1 Introduction

In nuclear waste repositories cementitious materials can be an essential part of the plug and seal systems [1]. Microbial activity in the repository will be of importance for the structural integrity of concrete and its hydraulic properties [2]. Microorganisms are often active at interfaces and may therefore be active at the interfaces between cement plugs and seals and backfill. A concern regarding the integrity is microbial degradation of concrete, which has been demonstrated on submerged and air exposed concrete [3] [4]. It has been oberserved that cements and concretes are degraded under aerobic conditions which prevail in the early phase of a repository [5]. Under anaerobic conditions microbial products like sulfuric acids, nitric acid or organic acids could be a significant factor in concrete disintegration [2] [6] [7]. In some concepts compacted bentonite clay will be used as backfill and will be in contact with the cementitious plug and seal. Sulphide-producing bacteria (SPB) are present and active in ground water in repositories [8] as well in proposed bentonites for backfill [9]. It has been shown that SPB in symbiotic relationship with sulphur oxidizing bacteria (SOB) cause microbial induced corrosion (MIC) of concrete in sewer systems [10]. This should be considered for the integrity of concrete structures in nuclear waste repositories. However, microbial activity of SPB might be inhibit through an alkaline plume (pH > 13) generated by hydroxyl ions from concrete diffusing into the bentonite [11].

The aim of this work was to analyse the effect of concrete on pH of the bentonite as well as microbial survival in the bentonite. Therefore, concrete plugs were set in contact with bentonite Volclay MX-80, which was compacted and saturated to a wet density of 1750 kg m^{-3}, in titanium test cells. The bentonite clay was inoculated with SPB. Furthermore, glucose was added to induce microbial fermentation.

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2 Material & Methods

2.1 Preparations of test cells

2.1.1 Concrete plugs

The plug and seal of a repository was simulated by a 5 mm concrete plug which was made with Bascement Skövde and sand (Table 2-1). The mixture consisted of 1-part water, 2-parts cement and 3-parts sand. The concrete plug was dried 24 hours.

Table 2-1: Material for the concrete plug.

Material	Composition
Cement	Total alkali, 1.3 %
Bascement Skövde	Sufite (SO_3), 3.3 %
	Chloride, 0.08 %
	Water soluble Cr^{6+}, < 2 ppm
Sand	SiO_2

2.1.2 Spiking bentonite clay

The SPB species *Desulfovibrio aespoeensis* (DSM 10631), *Desulfotomaculum nigrificans* (DSM 574) and *Desulfosporosinus orientis* (DSM 765) were used in this work. *D. aespoeensis* was isolated from deep groundwater [12], *D. nigrificans* is a mesophilic, spore-forming sulphide-producing bacterium and *D. orientis* is a spore-forming sulphide-producing bacterium with the ability to grow with H_2 as source of energy. The bacteria were grown in appropriate media and temperatures as specified by the German collection of microorganisms and cell cultures (DSMZ). 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. [13] and modified by Pedersen and Ekendahl [14].

The three different bacterial cultures were mixed into one cocktail and poured or sprayed carefully out on a bed of MX-80 bentonite powder in a large glass Petri dish. The whole content of the Petri dish was then passed through a mesh. Two batches of doped MX-80 bentonite with a bacterial content of approximately 1×10^7 SPB g^{-1} were created. All the work was performed inside of an anaerobic box with an atmosphere consisting of 97 % N_2 and 3 % H_2, O_2 < 1 ppm (COY Laboratory Products, Grass Lake, MI, USA). Afterwards 0.1 % w/w glucose was added to one batch which is referred to as “spiked bentonite clay”. Benonite clay without the addition of glucose is refferred to as “none-spiked clay”.

2.1.3 Test cells

Six test cells were prepared for this work. The experimental parameters for each test cell are shown in Table 2-2. A test cell consisted of a titanium cylinder with top and bottom lid attached by six Allen screws for each lid. A 5 mm concrete plug at the bottom and a water saturation piston inside the cylinder. When the piston was at its most extended position, a 35 × 20 mm confined cavity was produced inside the cylinder (Figure 2-1). This cavity was filled with bentonite clay. 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 (Figure 2-2). 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 2.1.4). The bentonite clay was saturated trough a longitudinal inside hole in the water saturation piston which allowed water to enter the test cell and reach the bentonite (described in detail in section 2.1.6). To stop the bentonite from swelling

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into the inlet holes, and also to get an evenly distributed inlet flow, a 40 µm pore size titanium filter was mounted with two Phillips screws on the inside of the piston.

Table 2-2 Experimental parameters for each test cell.

Name	Aimed density	Glucose	Concrete plug
TC 11	1750 kg m^{-3}	No	No
TC 12	1750 kg m^{-3}	No	Yes
TC 15	1750 kg m^{-3}	Yes	Yes
TC 16	1750 kg m^{-3}	Yes	Yes
TC 23	1750 kg m^{-3}	Yes	Yes
TC 24	1750 kg m^{-3}	Yes	Yes

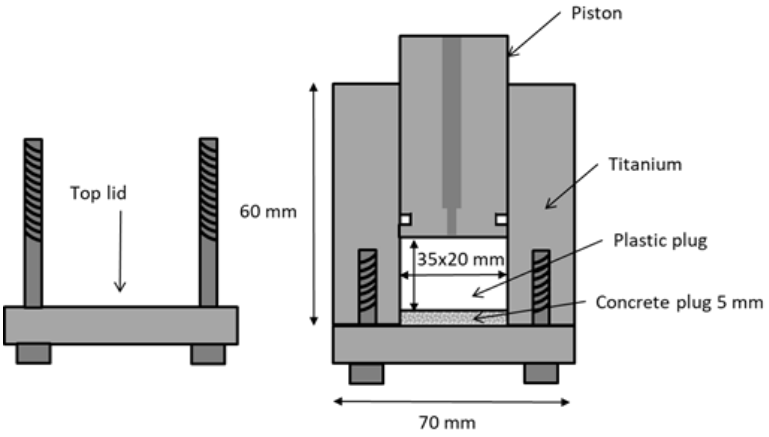

Figure 2-1 Scheme of a test cell with concrete- and plastic plug.

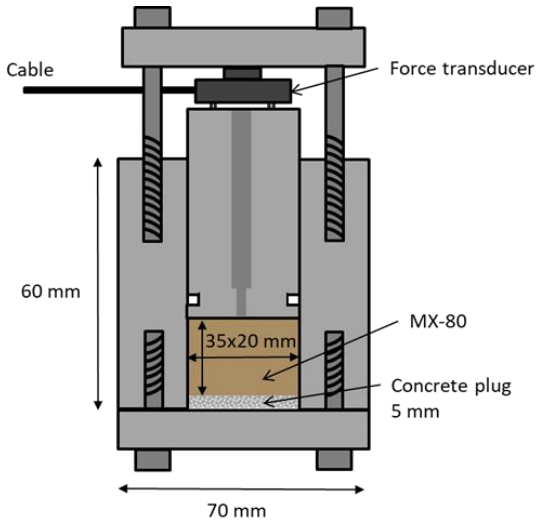

Figure 2-2 Scheme of an assembled test cell with MX-80 and concrete plug.

2.1.4 Force transducer and data collection

The force transducers used to register the swelling pressure from the bentonite clay were purchased from Stig Wahlström Automatik, Stockholm, Sweden. The force transducers had a load range from 0 – 2000 lbs (AL131DL, Honeywell model 53). They were connected to a data collection system

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consisting of two transducer amplifiers for strain gage (Stig Wahlström Automatik, model DR7DC), a programmable logic controller (model Direct Logic 06) and a PC with a custom build software (CRS Reactor Engineering, Stenkullen, Sweden) for calibration and monitoring of the force transducer signals.

2.1.5 Water content determination

The day before the beginning of the water saturation the water content of the two bentonite clay batches was determined. 3 × 1 g of spiked and non-spiked MX-80 was weighed into aluminium bowls and heated overnight at 105 °C. An average of the weight difference before and after heating was thus taken as the water content. This number was used in the mathematical density determination and determined how much bentonite clay each test cell should receive to reach a certain density at the specific volume.

2.1.6 Water saturation of bentonite clay

After the water content determination, the test cells were assembled with the top plate, force transducers, water saturation piston and the bottom lid and then mounted on a custom build water saturation system. The system was alternately evacuated to < 10 Pa total pressure and filled with nitrogen (N_2) to remove all oxygen (O_2). When the system was anaerobic it was left evacuated and an anaerobic saturation salt solution (Table 2-3) was added to the system with 200 kPa total pressure. The saturation salt solution was delivered to the test cells by a pressure transmitting device consisting of a steel cylinder with a piston made of poly-ether-ether-keton (PEEK). The piston was pressurized with nitrogen gas on the outer side of the piston. The inner side of the piston was filled with saturation salt solution under anaerobic conditions. All tubes and the pressure transmitter were sterilized in an autoclave at 121 °C for 20 minutes before use.

Table 2-3 Composition of 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

2.2 Sampling procedure

At the sampling date the pressure logging in the force transducer software was stopped, the force transducer was removed together with the top lid and screws. The top lid was then attached again, however with shorter screws to be able to push the piston all the way to the bottom. The test cells were moved to a fume hood and the bottom plates were carefully removed. The piston was then pressed up by turning the screws until the concrete plug was visible. The conncrete plug was removed and photo documented.

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2.2.1 Bentonite clay sampling

The piston was further pressed up until the bentonite core emerged fully. Bentonite samples were taken out at four positions. The positions were denoted:

- Position 1 (1 – 4 mm)
- Position 2 (5 – 8 mm)
- Position 3 (9 – 12 mm)
- Position 4 (13 – 16 mm)

Position 1 was closest to the concrete plug. Samples at each position were taken for analysis of pH, water content, ATP and culturable heterotrophic aerobic bacteria (CHAB).

2.2.2 Analysis of pH, ATP, CHAB and water content

pH was analysed by dissolving 1 g of bentonite clay from each position with 10 mL of AGW (analytical grade water) in a 50 mL Falcon tube. The bentonite clay was dissolved by shaking for 30 minutes at 200 rpm. Afterwards the clay slurry was analysed for pH with a pH meter (Scott, mod. CG 843P, VWR International AB, Stockholm, Sweden) equipped with a Hamilton electrode (Polilyte lab temp DIN, product no 242058/01, Genetec, Sweden).

ATP and CHAB was analysed by dissovling with 10 mL sterile 0.9 % NaCl solution. The bentonite clay was dissolved by shaking for 30 minutes at 200 rpm. CHAB was performed according to Hallbeck & Pedersen, 2012 [15] and Pedersen et al, 2008 [16]. Afterwards the samples were centrifuged at 4566 g for 15 minutes. The supernatant was decanted into a new 50 mL Flacon tube and analysed for ATP. ATP was analysed with a luminometer and the ATP Kit HS, Biothema, according to the manufactures instruction.

1 g of bentonite clay from each position was analysed for water content. Therefore, the samples were transferred and weight into pre-weighed 50 ml Falcon tubes. The samples were then dried at 105 °C for 24 h and weighed again. The difference in weight before and after drying was taken as the bentonite water content.

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3 Results

In the following the analysed test cells are referred to as TC 11, 12, 15, 16, 23 and 24. The experimental parameters for each test cell are shown in Table 2-2.

3.1 Density

The water content of the saturated bentonite clay was analysed to ensure that the aimed density of 1750 kg m^{-3} was reached. However, after 176 days of water saturation the densities deviated by 15 – 22 % from the aimed densities (Table 7-1).

The pressure curves of the test cells fluctuated from 70 kPa to 180 kPa during the incubation time (Figure 3-1) and did not seem to stabilise. In addition, the curves of the TC 15, 16, 23 and 24 show no coherency in their course, albeit identical experimental parameters.

3.2 Changes in appearance, pH, ATP and CHAB

Figure 3-2 shows that spiked MX-80 close to the concrete plug had spots with a brown coloration. Furthermore, it was observed that the surface in contact with the spiked MX-80 of the concrete plug was brown as well. TC 12 which contained none-spiked MX-80 and a concrete plug did not show these colorations.

Table 3-1 shows that test cells with a concrete plug (TC 12, 15, 16, 23 and 24) had a change of pH from position 1 to 4 in comparison to TC 11 without a concrete plug. In test cells with spiked MX-80 (TC 15, 16, 23 and 24) the pH shifted more from position 1 to 4 in comparison to TC 12 with none spiked MX-80. The TC 11, without a concrete plug, showed no change in pH from position 1 to 4.

Figure 3-3 shows ATP in TC 15, 16, 23 and 24 which was higher at position 4 compared to the other positions. TC 11 showed the same order of magnitude ATP (10^5 amol mL^{-1}) at position 1 to 3 but a drop in measured ATP at position 4. In TC 12 ATP could not be detected.

Figure 3-4 shows that CHAB were only detected at position 4 for TC, except at position 3 for TC 24. The CHAB agar plates of TC 11 showed mould growth at position 1 to 3 (Figure 3-5).

Table 3-1 Analysed pH for each test cell.

Position	TC 11	TC 12	TC 15	TC 16	TC 23	TC 24
1	9.4	10.2	10.6	10.8	10.6	10.7
2	9.4	10.1	9.6	9.6	9.5	10.1
3	9.4	10	8.9	8.7	8.5	8.9
4	9.4	9.8	8.1	7.4	7.9	7.9

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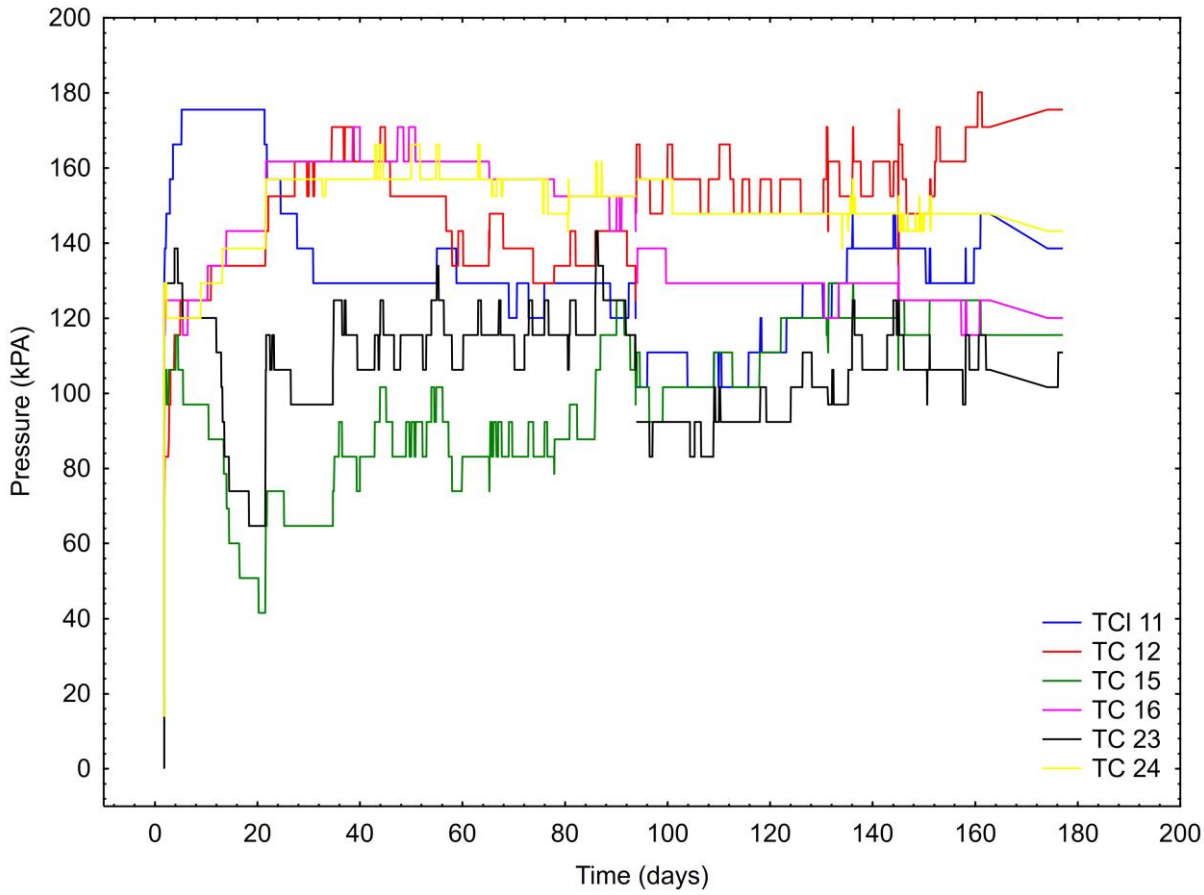

Figure 3-1 Pressures registered by force transducers over time, test cells according to symbol description.

Figure 3-2 Image of compacted, water saturated and spiked MX-80 of test cell 15 after incubation (left) and the concrete plug surface which was in contact with the bentonite clay (right).

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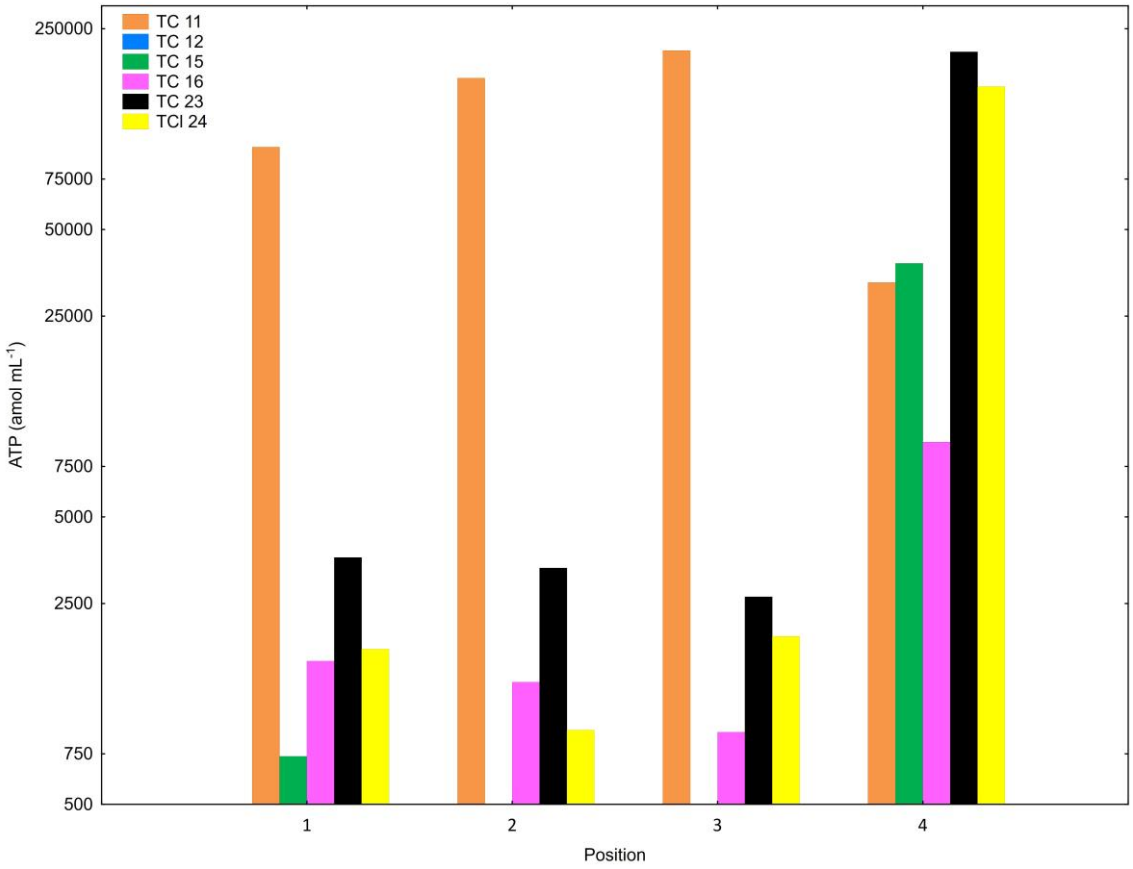

Figure 3-3 Analysed ATP at four positions, test cells according to symbol description.

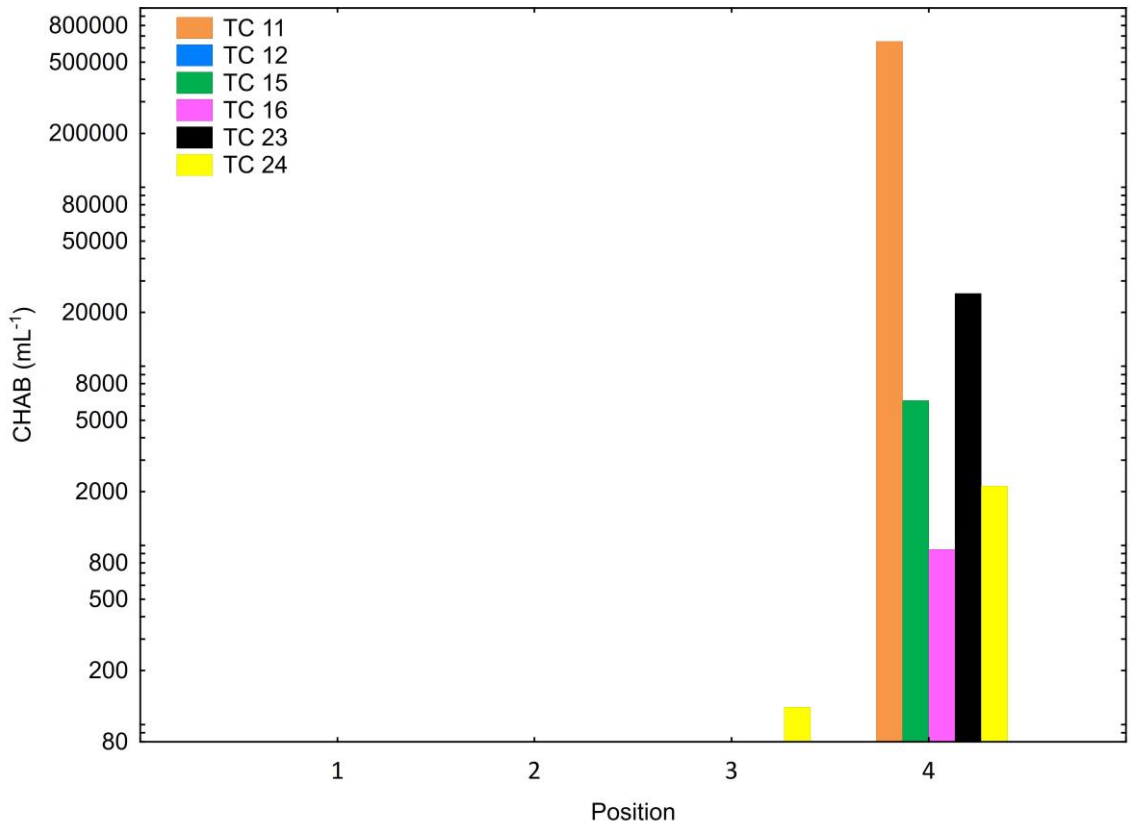

Figure 3-4 Analysed CHAB at four positions, test cells according to symbol description.

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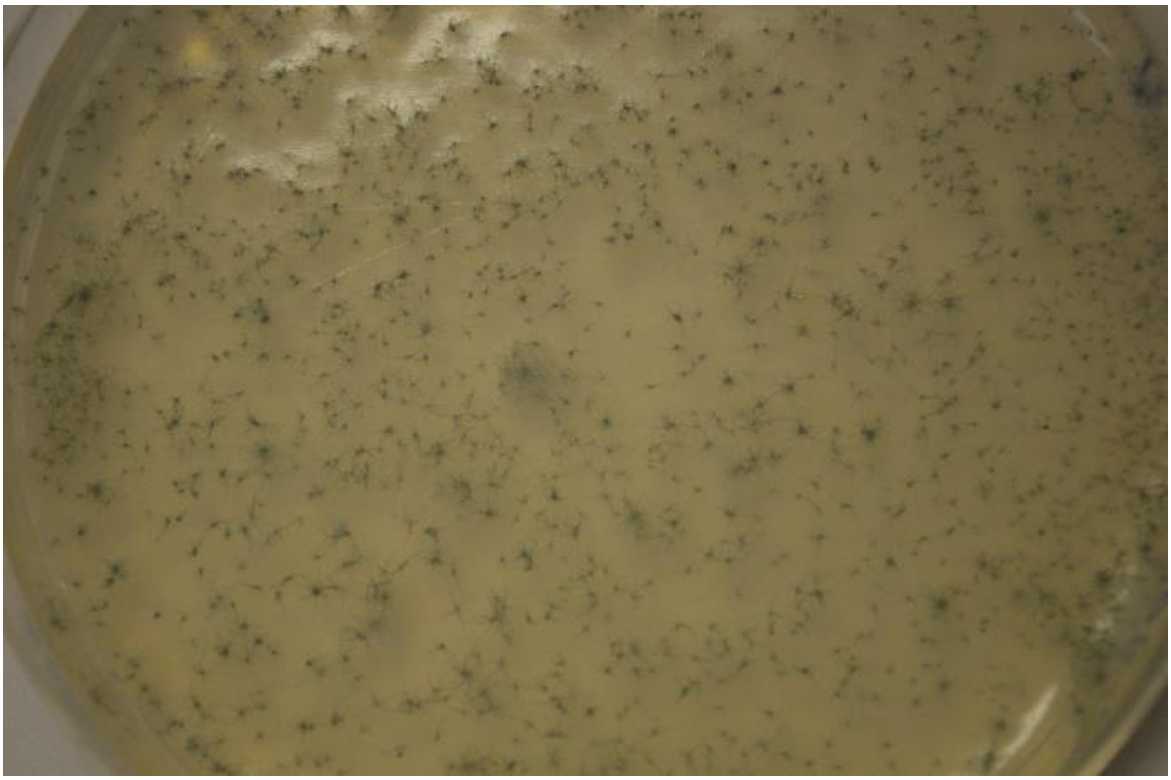

Figure 3-5 CHAB agar plate with mould growth from TC 11 position 2.

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4 Discussion

In this work the influence of a concrete plug on the pH and microbial survival in compacted and saturated MX-80 was investigated.

4.1 Deviation of density by alkaline solution

The analysed densities deviated from the aimed density of 1750 kg m^{-3} probably due to the concrete plug. The saturation lead to a leaking of an alkaline plume from the concrete as the pH change in TC with a concrete plug showed. It was shown before that concrete will initially generate a high-pH water (pH > 13) rich in K, Na, Ca ions [11]. This alkaline solution leached from the concrete induced dissolution and precipitation in the bentonite clay [17] which may have influenced the swelling pressure. Furthermore, the cementation by secondary minerals such as zeolites may lead to degradation of the desirable properties of the buffer and explains why the analysed density deviated from the aimed one [18]. The effect of leached alkaline solution from concrete should be taken into consideration in future investigations.

4.2 pH shift by microbial activity

The concrete plug increased the pH in bentonite clay and inhibited microbial. However, it was depending on if it was spiked or none-spiked bentonite clay. In spiked bentonite clay the pH decreased after position 2 possibly due to microbial activity induced by the added carbon source. In none-spiked bentonite clay microbial growth was inhibited due to the high pH and lack of additional carbon source. A reason why the pH decrease in spiked bentonite clay might be that natural occurring acetogenic bacteria produced organic acids from the added carbon source. Svensson et al. showed that high numbers of cultivable acetogens can be found in various clays [9]. In future investigations bentonite clay samples can be analysed for acetate as done in Bengtsson et al. and cultivatable acetogenic bacteria to confirm this hypothesis [19].

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5 Acknowledgement

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7 Appendix

Table 7-1 Calculated and measured, total weight, water content and density for every test cell.

Test cell	Calculated water content (%)	Measured water content (%)	Calculated Density (kg m-3)	Measured Density (kg m-3)
Test cell 15, MX-80, spiked, concrete	35.04	34.85	1750	1370
Test cell 12 MX-80, non-spiked, concrete	35.47	37.70	1750	1451
Test cell 11, MX-80, non-spiked	35.47	33.95	1750	1488
Test cell 16, MX-80, spiked, concrete	35.04	37.99	1750	1473
Test cell 23, MX-80, spiked, concrete	35.04	36.31	1750	1372
Test cell 24, MX-80, spiked, concrete	35.04	37.69	1750	1431

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