Microbial Analysis of Buffer Materials from the Alternative Buffer Material (ABM) Experiments at the Äspö Hard Rock Laboratory, Sweden

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S. Stroes-Gascoyne, C.J. Hamon and K. Stephenson

Atomic Energy of Canada Limited

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ABSTRACT

Title: **Microbial Analysis of Buffer Materials from the Alternative Buffer Material (ABM) Experiments at the Äspö Hard Rock Laboratory, Sweden**
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Abstract

The purpose of the Alternative Buffer Material (ABM) experiment, ongoing in boreholes at the Äspö Underground Hard Rock Laboratory in Sweden since December 2006, is to extend the knowledge base of alternative buffer materials to optimize issues such as availability, safety and cost. The ABM is focussed on differences in long-term buffer behaviour and stability between 11 different clay materials, under controlled, identical and repository-relevant conditions. The materials in the experiment include six Na-bentonites (Kunigel, Ibecoseal, MX-80 (LOT), Asha 505, Friedland and Ikosorb), three Ca-bentonites (Dep-CAN, Rokle and Calcigel), one Mg-bentonite (Febex) and one clay stone (argillite) (COX). Also included in the test packages are MX-80 granular material and MX-80-sand (30% quartz) (MX-80S) granular material. Three test packages were installed in 2006. Each package consists of at least 30 ten-centimetre thick buffer "rings", a central carbon steel heater pipe, heaters, sensors, and pipes for the artificial saturation system. The buffer rings were threaded onto the heater pipe in a predetermined pattern to give maximum mixture of materials. After installation, the packages were heated to a target temperature of 130^{o}C and saturated artificially or naturally. This report presents the results of the initial microbial analyses of the 11 clay materials included in this experiment as well as the results of the microbial analysis of a sample from ABM Test Package 1, which was retrieved in May, 2009.

The 11 Buffer Materials included in the ABM experiment were analyzed for the occurrence of culturable and viable microbes, to establish the initial microbial characteristics of these materials. Corresponding data for a MX-80 Na-bentonite batch used in Canada and for two carefully drilled Opalinus Clay (OPA) samples (from the Mont Terri Underground Rock Laboratory, Switzerland) were included in the comparisons between these ABM materials. The culture results showed that the highest to lowest culturability order (based on a summation of all culture results per sample, ignoring possible overlap between physiological groups) in all samples was: Asha 505 > Febex > MX-80 (LOT) > Ikosorb > Ibecoseal > Dep-CAN > Rokle > Friedland > MX-80 (Canada) > Calcigel > Kunigel > COX > OPA. COX and OPA are consolidated claystones which may explain their low results for culturable cells. Viable cell counts, based on phospholipid fatty acid (PLFA) analysis, showed a total cell range of 4 x 10^4 to 1 x 10^7 cell-equivalents/g. There was only a factor of about 15 difference in the PLFA-based biomass in all bentonite samples. The PLFA-based biomass in the argillite samples was about a factor of 10 lower than in the bentonite samples. The PLFA-derived community structure data suggested that the least diverse (and possibly least contaminated) samples were the natural argillites (COX and OPA), Calcigel and Kunigel. This appears to be in very good agreement with the low culturability in these samples. No clear correlations between heterotrophic culturability and total organic C content or SRB culturability and total S content were found. Considerable culturability was found at very low water activities (< 0.60) in some of these samples, suggesting that survival occurred as spores (or perhaps as dormant cells). The dominant presence of spore-formers is, therefore, suggested in these materials. A comparison with results from analyses on equivalent samples obtained by SKB showed good agreement

between the AECL and SKB data: The highest number of culturable cells (heterotrophic aerobes and SRB) was found in the Asha 505 and Febex samples; the lowest in the Kunigel and COX samples.

The sample from ABM test package 1 contained two layers of adjacent MX-80 and MX-80S granular materials and their interfaces with the rock, the carbon steel pipe (containing the heaters), a regular MX-80 bentonite layer and a Febex bentonite layer. The results from the microbial analysis confirmed yet again that interface locations are the most likely areas where microbial activity could occur in a repository. Despite temperatures near or at 100^{o}C a considerable population of viable heterotrophic aerobes and anaerobes were found at the interface between the rock, the granular MX-80 and the MX-80S materials. Survival occurred most likely in spore-form. Surviving spores are inactive and do not form a direct danger to the longevity of containers in a future repository. However, the presence of a large population of spores presents a potential for future increased microbial activity in a repository if conditions were to become more favourable.

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1. INITIAL MICROBIAL CHARACTERISTICS OF ABM MATERIALS

1.1 INTRODUCTION

An experiment is ongoing at the Äspö Hard Rock Laboratory (HRL) in which a number of alternative (bentonite-based) buffer materials are tested. The objectives of the ABM test include (Eng 2007):

- verification of laboratory results with in situ studies, with an emphasis on temperature, scale and geochemical conditions;
- discovering possible problems with block manufacturing and handling; and
- obtaining further data for verification of thermo-hydro-mechanical (THM) and geochemical models.

The experiment site is shown in Figure 1 (Eng 2007) and the experimental design, the package design, and the sensor types and locations are shown in Figures 2-5 (Eng 2007). Figure 4 (Eng 2007) shows the 11 buffer materials included in the ABM tests while Karnland et al. (2006) describe the mineralogy and sealing properties of some of those materials.

Many organisations and laboratories were involved in analyzing the ABM materials for a variety of parameters, including water content, mineralogy, cation exchange capacity and elemental (i.e., C and S) composition. The laboratories (and companies) involved in this testing were: Acuo Engineering (Sweden), Andra (France), BGR (Germany), Bo Rosborg Consulting (Sweden), Chech Technical University (Chech Republic), Clay Technology (Sweden), Enviros (Spain), Iko Minerals (Germany), KTH (Sweden), LTH (Sweden), Nagra (Switzerland), NWMO (Canada), Posiva (Finland), SKB (Sweden), University of Bern (Switzerland), University of Copenhagen (Denmark), University of Illinois (USA) and VTT (Finland).

The objective of the microbial work described in Part I of this report was to analyze these ABM materials for the occurrence of microbes, in order to establish the initial microbial characteristics of these materials for future use, when these tests are terminated over time and the materials are analyzed with respect to the development of microbial communities under the test conditions imposed. The materials were also analyzed for microbiological occurrence by SKB (Svensson et al. 2011) and a comparison between AECL and SKB results is included in this report.

1.2 ABM TEST MATERIALS INFORMATION

Tables 1 to 5 (Svensson, 2007) provide more information about the 11 ABM test materials. Table 1 gives the water content and appearance of the ABM materials included in the test. Table 2 gives mineralogical data and Table 3 gives the cation exchange capacity of these materials. Table 4 gives total, inorganic and organic C and total S content of the materials and Table 5 shows information on free swelling tests and colloid formation.

1.3 MICROBIAL ANALYSIS OF ABM MATERIALS AT AECL

Eleven samples were received from SKB at AECL Whiteshell Laboratories in April 2008. These materials were analyzed as described below. For comparison, data obtained for the MX-80 Wyoming bentonite batch used in Canadian laboratory studies are included in the results (Stroes-Gascoyne and Hamon 2008), as well as data obtained for two samples of carefully drilled Opalinus Clay (OPA 1 and OPA 2) (argillite) (Stroes-Gascoyne et al. 2008).

1.3.1 Water Activity

For each ABM clay sample, the water activity was measured on a subsample using a Decagon^{TM} WP4 Dewpoint PotentiaMeter (Decagon Devices, Pullman, WA).

1.3.2 Water Content

For each ABM clay sample, moisture content was determined by drying the water activity subsample at 110^{o}C to constant weight.

1.3.3 Heterotrophic Aerobes and Anaerobes (HAB and HAnB)

About 10 g (carefully weighed) of clay were added to 100 mL of Phosphate-Buffered Saline solution (PBS, i.e., 0.01M NaCl buffered to pH 7.6 with 9 mM Na_2HPO_4 and 1 mM $NaH_2PO_4.H_2O$). This suspension was stirred or shaken for 30 min. (under anaerobic conditions) after which serial dilutions were made to 10^{-3}. The dilutions were plated onto R2A agar (Reasoner and Geldreich 1985) and the plates were incubated at 30°C for 5-7 days (HAB) and up to 4 weeks (HAnB) before they were counted.

1.3.4 Sulphate Reducing Bacteria (SRB)

Degassed tubes containing sterile modified Postgate's B medium (Atlas 1993) were inoculated in an anaerobic glovebox (in triplicate) with the ABM PBS clay suspensions to 10^{-3} dilutions. The tubes were incubated at room temperature for about 4 weeks before they were scored.

1.3.5 Nitrate-Utilizing and Nitrate-Reducing Bacteria (NUB and NRB)

Nitrate-utilizing bacteria (NUB, that convert nitrate to nitrite) and nitrate-reducing bacteria (NRB, that convert nitrate to N_2) were enumerated. Sterile MPN tubes with degassed R2A medium (Reasoner and Geldreich 1985), amended with 0.1% nitrate, were inoculated with the ABM PBS clay suspensions in an anaerobic glovebox (in triplicate), serially diluted to 10^{-3} and scored for gas production (in inverted Durham tubes) or the presence of nitrite after about 4 weeks of incubation at 30°C.

1.3.6 Phospholipid Fatty Acid Analysis

A subsample of each ABM sample was submitted to Microbial Insights (Rockport, Tennessee, U.S.A.) for Phospholipid Fatty Acid (PLFA) analysis. Lipids were recovered using a modified Bligh and Dyer method (White et al. 1979). Extractions were performed using one-phase

chloroform-methanol- buffer extractant. Lipids were recovered, dissolved in chloroform, and fractionated on disposable silicic acid columns into neutral-, glyco-, and polar-lipid fractions. The polar lipid fraction was transesterified with mild alkali to recover the PLFA as methyl esters in hexane. PLFA were analyzed by gas chromatography with peak confirmation performed by electron impact mass spectrometry.

1.4 MICROBIAL ANALYSIS OF EQUIVALENT SAMPLES IN SWEDEN

The analyses conducted by AECL described in this report were carried out in 2008. SKB analyzed the same samples as AECL, with the exclusion of the MX-80 Canada and the Opalinus Clay materials which were added by AECL to the materials list to be analyzed at AECL. In 2011 SKB published the Swedish results of the microbial analyses of samples of the ABM materials (Svensson et al. 2011) and the AECL and SKB results for HAB and SRB are compared in this report.

Culture methods and growth media used by SKB are described in Svensson et al. (2011). Culture analyses for SKB included heterotrophic aerobic bacteria (HAB) by plating and Most Probable Number (MPN) analysis for iron reducing bacteria (IRB), autotrophic acetogens (AA), and sulphate-reducing bacteria (SRB). This allowed a comparison between the HAB and SRB data obtained at AECL and SKB.

Svensson et al. (2011) also report identifications of indigenous microbes from enrichment cultures with sulphide and acetate, by molecular methods.

1.5 RESULTS AND DISCUSSION

For the presentation of the results for the ABM test materials in this report, the materials are grouped into Na-bentonites, Ca-bentonites, Mg-bentonite and argillites. For comparison, data obtained for the MX-80 Wyoming bentonite batch used in recent Canadian laboratory studies are included in these results (Stroes-Gascoyne and Hamon 2008), as well as data obtained for two samples of carefully drilled Opalinus Clay (OPA 1 and OPA 2) (argillite) (Stroes-Gascoyne et al. 2008).

Water content, water activity and PLFA-based biomass results are given in Table 6. Culture results for HAB, HAnB, NUB, NRB and SRB are given in Table 7. PLFA-derived community structure results are given in Table 8, with an explanation of the community types given in Table 9.

Figures 6 to 19 show the PLFA-based biomass and culture-based biomass results for each material individually.

In Figures 20, 21, 22, 23, 24 and 25 the materials are compared for their content of PLFA-based biomass, HAB, HAnB, NUB, NRB and SRB, respectively.

In Figure 20 the materials are compared for their PLFA-based biomass content. The highest PLFA-based biomass (>5 x 10^6 cells/g) was found in the Rokle and Friedland samples. PLFA-based biomass ranging from 1 x 10^6 to 5 x 10^6 cells/g was found in Kunigel, Ibecoseal, MX-80 (LOT), MX-80 (Canada), Asah 505, Ikosorb, Dep-CAN, Febex and COX samples. Lower PLFA-based biomass (< 10^6 cells/g) was found in the Calcigel and OPA samples. It should be noted

that there was only a factor of about 15 difference in the PLFA-based biomass in all bentonite samples. The PLFA-based biomass in the argillite samples was about a factor of 10 lower than in the bentonite samples.

In Figure 21, the culturable heterotrophic aerobes in each sample are compared. The highest culturable heterotrophic aerobic populations (10^4 – 10^6 CFU/g) were found in Asha 505, Febex, MX-80 (LOT) and Ikosorb. Lower populations of culturable heterotrophic aerobes (10^2 – 10^4 CFU/g) were found in Ibecoseal, MX-80 (Canada), Friedland, Dep-CAN, Rokle and Calcigel. Very low culturable heterotrophic aerobic populations (detection limit to 10^2 CFU/g) could be detected in Kunigel, COX, OPA 1 and OPA 2 samples (Stroes-Gascoyne et al. 2008).

In Figure 22, the culturable heterotrophic anaerobes in each sample are compared. The highest culturable heterotrophic anaerobic populations (10^2 – 10^3 CFU/g) were found in Ibecoseal, MX-80 (LOT), Asha 505, Friedland, Ikosorb, Rokle and Febex. Lower populations of culturable heterotrophic anaerobes (10^1 – 10^2 CFU/g) were found in Kunigel, MX-80 (Canada), Dep-CAN, Calcigel, and COX. Very low culturable heterotrophic anaerobic populations (<10^1 CFU/g) were found in OPA 1 and OPA 2 samples (Stroes-Gascoyne et al. 2008).

In Figure 23, the culturable NUB populations in the samples are compared. The highest culturable NUB populations (10^4 – 10^5 MPN/g) were found in Ibecoseal, Asha 505, Ikosorb, Dep-CAN and Febex. Lower populations of culturable NUB's (10^3 – 10^4 MPN/g) were found in MX-80 (LOT), Friedland and Rokle while low NUB populations (detection limit – 10^3 MPN/g) were found in Kunigel, MX-80 (Canada), Calcigel, COX, OPA 1, and OPA 2 samples. Note that the NUB populations in Figure 23 will likely (partially) overlap with the heterotrophic aerobic populations in Figure 21. Nitrate-utilizing bacteria are facultative aerobes, that prefer to use O_2 as electron acceptor, but that will switch to nitrate in the absence of O_2. In these analyses, the HAB and NUB were both grown on R2A medium, but for the NUB cultures, 0.1% nitrate was added to the R2A medium. The HAnB were also grown on R2A under anaerobic conditions, but no nitrate was added.

In Figure 24, the culturable NRB populations in the samples are compared. Culturable populations of NRB > 10^2 MNP/g were found in MX-80 (LOT) and Febex. Lower NRB populations (10^1 –10^2 MPN/g) were found in Ibecoseal, Friedland and Ikosorb. Low (< 10^1 MNP/g) NUB populations were found in Kunigel, MX-80 (Canada), Asha 505, Dep-CAN, Rokle, Calcigel, COX, OPA 1, and OPA 2 samples.

In Figure 25, the culturable SRB populations in the samples are compared. Culturable populations of SRB > 10^2 MNP/g were found in Asha 505 and Friedland. Lower SRB populations (10^1 –10^2 MPN/g) were found in Ibecoseal, MX-80 (LOT), Dep-CAN and Febex. Low (< 10^1 MNP/g) SRB populations were found in Kunigel, MX-80 (Canada), Ikosorb, Rokle, Calcigel, COX and OPA 1 and OPA 2.

In general, the highest to lowest culturability order (based on a summation of all culture results per sample, ignoring possible overlap between heterotrophic aerobes and NUB) in all samples is: Asha 505 > Febex > MX-80 (LOT) > Ikosorb > Ibecoseal > Dep-CAN > Rokle > Friedland > MX-80 (Canada) > Calcigel > Kunigel > COX > OPA (1 and 2).

In Figure 26, the sum of all culturable species is compared against the PLFA-based viable cell count for each sample. This graph shows that in these samples, culturability increases as viability increases. The best-fit line through these data has a correlation coefficient of $R^2 = 0.5722$.

Figure 27 compares the PLFA-derived community structures in each material (Table 8). The highest percentages of general PLFA (found in all organisms) (i.e., > 58%) were found in Kunigel, Calcigel, COX and OPA samples. This suggests that these samples contained the least diverse populations. The highest percentages of PLFA indicative of Proteobacteria (Gram-negative, fast growing bacteria) (i.e., > 40%) were found in the Friedland, Ikosorb, Dep-CAN, Rokle and Febex samples. This could suggest that these samples contained a considerable population of ubiquitous bacteria such as *Pseudomonads*, which are often considered indicative of recent contamination. The lowest percentages of Proteobacteria ($\leq$20%) were found in the Kunigel, COX and OPA samples. PLFA analysis showed that no SRB were present in the Calcigel, COX and OPA samples, in agreement with low culturable SRB populations in these samples (Table 7 and Figure 25). No Firmicutes (mostly Gram-positive bacteria) occurred in the COX and OPA samples, and very low percentages of Firmicutes ($\leq$ 5%) occurred in the Calcigel and Dep-CAN samples. No anaerobic metal reducers were indicated by PLFA analysis in OPA samples and very low percentages (0.7%) were found in Calcigel and Dep-CAN. The highest Eukaryote populations (> 7%) were indicated in the Ibecoseal, MX-80 (Canada), MX-80 (LOT), Rokle and COX samples. This could indicate that these materials contained a considerable population of fungi. In general, the PLFA-based community structure data appear to indicate that the least diverse (and possibly least contaminated) samples are the natural argillites (COX and OPA), and Calcigel and Kunigel. This appears to be in very good agreement with the low culturability in these samples.

Figure 28 plots the relationship between water content (in weight %) and measured water activity in the ABM test materials. With the exception of the data points for Kunigel and Friedland (Table 6), there appears to be a linear relationship between water content and water activity for the bentonite samples and a different (linear) relationship between water content and water activity for the argillites. This is likely attributable to the different compositions of the materials, especially a low or absent montmorillonite content (Table 2) in the argillites.

In Figures 29 and 30, heterotrophic aerobic culturability is plotted against water content and water activity, respectively. These graphs show that there is a general increase in heterotrophic aerobic culturability with an increase in water content and corresponding water activity, but the correlation is low. Other relationships, such as between heterotrophic aerobic culturability and total organic C content and SRB culturability and total S content were explored but did not yield any clear correlations.

Generally, it is expected that water activity correlates well with aerobic culturability for vegetative cells, but not necessarily for culturable spores. Most Gram-negative bacteria become inactive and non-culturable around water activity values of about 0.96 and most Gram-positive bacteria become non-culturable around water activities of 0.90. The water activities measured for the ABM test materials are all below 0.80 and most are below 0.60, the value below which DNA material would naturally denature. The fact that considerable culturability was found at the very low water activities in these samples suggests that the organisms that could be cultured survived as spores or perhaps as dormant cells, that are much more tolerant of and resistant to low water activities. It is not likely that many of the cells that could be cultured survived as vegetative cells. The dominant presence of spore-formers and/or dormant cells is, therefore, suggested in these samples. Recently it was found that the viable species that could be cultured from Wyoming MX-80 bentonite retrieved from a 7 year old Canadian test were all spore-formers of the *Bacillus*, *Paenibacillus* and *Brevibacillus* type (Stroes-Gascoyne et al. 2014). However, Svensson et al. (2011) reported finding a number of spore-forming genera in the ABM materials, but also non-spore-forming species.

Figure 31 compares the HAB content in the ABM materials, as determined by AECL and SKB (Table 10). Considering that different growth media and methods were used, the agreement between the two sets of results is very good. Statistically only the results for Kunigel, MX-80 (LOT), Ikosorb and Rokle are different; the HAB contents for the other seven materials are statistically the same with respect to the AECL and SKB results.

Figure 32 compares the SRB enumerations obtained at AECL and SKB (Table 10). Considering that different growth media and methods were used, there was excellent agreement between the two data sets; statistically the SRB numbers obtained were the same for all 11 samples analyzed. The highest number of SRB was found in the Asha 505 and Friedland samples. The lowest numbers of SRB were found in Kunigel, MX-80, Deponit, Rokle and COX.

1.6 SUMMARY AND CONCLUSIONS

Eleven ABM test materials (archived aliquots of the materials used in the ABM in situ tests, provided to AECL by SKB) were analyzed for the occurrence of microbes, in order to establish the initial microbial characteristics of these materials for future use, when the in situ tests are terminated over time, and the materials in these tests are analyzed with respect to the development of microbial communities. Additionally, data for a MX-80 batch used in Canada and data for two carefully drilled Opalinus Clay samples were included in the comparisons between these ABM materials.

The culture results showed that the highest to lowest culturability order (based on a summation of all culture results per sample, ignoring possible overlap between heterotrophic aerobes and NUB) in all samples was: Asha 505 > Febex > MX-80 (LOT) > Ikosorb > Ibecoseal > Dep-CAN > Rokle > Friedland > MX-80 (Canada) > Calcigel > Kunigel > Cox > OPA (1 and 2). COX and OPA are consolidated claystones which may explain their low results for culturable cells. Culture results obtained by AECL and SKB showed good to excellent agreement for most of the ABM samples, despite differences in culture conditions.

Viable cell counts, based on PLFA analysis showed a total cell range of 4×10^4 to 1×10^7 cell equivalents/g. There was only a factor of about 15 difference in the PLFA-based biomass in all bentonite samples. The PLFA-based biomass in the argillite samples was about a factor of 10 lower than in the bentonite samples. The PLFA-derived community structure data suggested that the least diverse (and possibly least contaminated) samples were the natural argillites (COX and OPA), Calcigel and Kunigel. This appears to be in very good agreement with the low culturability in these samples.

No clear correlations between heterotrophic culturability and total organic C content or SRB culturability and total S content were found and only weak correlations between heterotrophic culturability, water content and water activity. The fact that considerable culturability was found at the very low water activities in these samples suggests that the organisms that could be cultured survived as spores or perhaps as dormant cells that are much more tolerant of, and resistant to, low water activities than vegetative cells. The dominant presence of spore-formers is, therefore, suggested in these materials. Swedish molecular analysis of enrichment cultures of these ABM materials found many spore formers but also non-spore-forming species, while molecular analysis of culturable heterotrophic aerobes from a > 7 year old Wyoming MX-80 bentonite plug indicated the exclusive presence of spore-formers.

2. MICROBIAL ANALYSIS OF SELECTED SAMPLES FROM TEST PACKAGE 1

2.1 DESCRIPTION OF ABM TEST PACKAGES

The three ABM test packages were installed in 2006. Each package contained all test materials, had a diameter of 280 mm, and was emplaced in a 300 mm diameter borehole with a length of 3 meters. After installation, the packages were heated and saturated in a different manner. Figure 2 (Eng 2007; Sandén 2009) gives a schematic of the three packages, their test conditions and layout. Heating occurred to a target temperature of 130^{o}C and throughout the experiment the temperature was monitored using a computer data logging system.

Each package consisted of at least 30 ten-cm thick buffer "rings", a central steel heater pipe (carbon steel, P235TR1), heaters, sensors, and pipes for the artificial saturation system. The buffer rings were threaded onto the heater pipe in a predetermined pattern to give maximum mixture of materials. Table 11 (Eng et al. 2007) gives the sequence of the test materials in each package. Figure 5 gives the number and type of sensors in the ABM. A carbon steel pipe instead of a copper pipe (as usual) was used in order to also study the effects of rusting steel in close contact with the buffer materials.

On the outer edge of each package, four saturation pipes (titanium) were attached through which natural Äspö water could seep into packages 1 and 2. Package 3 was not saturated artificially, but in case there should be a change of plan, the saturation system was installed to speed up the saturation process. To further increase the water distribution along the packages, sand (with a uniform grain size to reduce clumping) was used to fill the small slot between the rock and the packages. The saturation system consisted of four pipes installed along the outer edge of each package. The pipes were connected to a water tank containing Äspö groundwater. At the location of imagined fractures, small holes were drilled in these pipes, to simulate water-bearing fractures in the rock. To avoid sand ingress and clogging, the pipes were covered in a perforated plastic sleeve. The pipes connected underneath the packages to make flushing of the pipe systems possible (Eng et al. 2007).

On May 11 2009, ABM test package 1 was retrieved. One of the samples taken was requested by AECL (for NWMO) for microbial analysis. This sample included two layers of adjacent MX-80 and MX-80S granular materials and their interfaces with the rock, the carbon steel pipe, a regular MX-80 bentonite and a Febex bentonite layer. The location of the AECL sample in Test Package 1 is shown in Table 11 and Figures 33 to 35 (Sandén 2009).

2.2 MATERIALS AND METHODS

2.2.1 Granular MX-80 and MX-80S MATERIALS

Granular materials MX-80 and MX-80S were also included in the ABM test packages. The two granular bentonite samples (about 50 kg each) were prepared by NAGRA for SKB on June 2 2006. Both the granular 100% MX-80 and the granular MX-80S (70% MX-80 + 30% quartz (0.1 – 0.5 mm)) contained grain sizes of 70% in the 7 to15 mm, and 30% in the 0.5 to 1.0 mm range. Specific details on how the materials were produced are given in Appendix A. In order to be able to install these materials, black steel wire cages (not corrosion protected by any means) were constructed that were wrapped with a fibre cloth, fastened to the steel frame with a thin steel wire. The steel frames held the weight of the blocks placed above the granular materials during installation and the cloth prevented the granular materials from falling out during package assembly.

2.2.2 Conditions in Test Package

Figure 33 (Sandén 2009) is a schematic of ABM test package 1 and shows detail as well as the type and number of blocks installed. The red box shows the intended block sequence to be sampled for the AECL sample. Figure 34 (Sandén 2009) shows the temperature development from Dec 4 2006, in three regions of the package. This was translated into a temperature profile for the whole package in Figure 35 (Sandén 2009), again with the red box showing the intended sample for AECL. Figure 36 (Sandén 2009) shows the artificial water saturation that took place in ABM test package 1. After the saturation system was initially activated it was observed that a large amount of water was inserted in the packages. It was concluded that this water must be disappearing through fractures in the rock wall. To further minimize the possibility of buffer erosion, the saturation was stopped and reactivated at a later point as shown in Figure 36.

2.2.3 RETRIEVAL OF TEST PACKAGE 1

ABM test package 1 was retrieved on May 11 2009. Retrieval was achieved by overcoring. The retrieval technique used was based on the methods developed during the LOT test at Äspö (Sandén 2009). First, the heater temperature was decreased in steps during the three to four weeks prior to package retrieval. To retrieve the package, percussion drilling was used subsequently, to drill 32 overlapping drill holes around the package, as shown in Figure 37 (Sandén 2009). Wire sawing was used to release the package from the bottom of the borehole, as shown in Figure 38 (Sandén 2009). The package was lifted out by using three steel wires placed around the package, while the steel tube and bentonite rings were secured to the rock parcel in order to keep the whole package intact during lifting, as shown in Figure 39 (Sandén 2009). In order to stabilize the rock column during lifting, wood beams were installed around the rock-bentonite package and straps were used to keep every part of the package in place during lifting, tilting and transport, as shown in Figures 40 and 41 (Sandén 2009). Figure 42 (Sandén 2009) shows how the rock was removed from the bentonite package, using a variety of hand tools. Note that the photos in Figures 38-42 were in fact taken during retrieval of a LOT package and do not actually show the retrieval of ABM test package 1, but that the same techniques were used during the retrieval of ABM test package 1.

2.2.4 THE AECL SAMPLE FROM ABM TEST PACKAGE 1

The red boxes in Figures 33, 34 and 35 show the intended ABM package 1 sample, as requested by AECL for NWMO. The objective was to determine microbial culturability at various locations in and around the granular MX-80-based materials. Emplaced, saturated and heated granular bentonite materials had not been analyzed before for microbial culturablity at AECL.

A detailed description of the actual sampling for AECL is given in the photos and record in Appendix B. The quadruple stack of blocks (18, 19, 20 and 21, Figure 34) was sealed in a plasticized aluminium bag at Äspö (photo B1 in Appendix B) on May 11 2009 and shipped to the Clay Technology Laboratory in Lund (Sweden) on May 13. On May 15 2009, sub-sampling for AECL of this stack of blocks was accomplished. Because of the size of the sample, it was not refrigerated. Appendix B (Figures B1 and B2) show that the plasticized aluminium bag was removed and a photo taken. The cloth surrounding the granular materials is clearly visible in Figure B2 of Appendix B. The stack of blocks was wrapped in plastic film immediately after removal of the aluminium bag. The horizontal black straps were applied at Äspö for support. Figure B3 shows the stack of blocks after removal of the MX-80 layer, which was removed by mistake, while the Febex layer (intended to be removed) was left in place. A clamp was used to keep the layers together during cutting of the wedge-shaped sample for AECL (Figure B4, Appendix B). To accomplish this, the outer steel bars of the two granulate cages were cut, using a tiger saw. The wedge-shaped sample was then cut out using a band saw. The wedge-shaped AECL sample is shown and described in Figures B5 and B6 of Appendix B. Figure B7 shows the sample prior to shipping, wrapped in plasticized aluminium, and vacuum-sealed. Figure B8 (Appendix B) shows the leftover block with the wedge-shaped AECL sample clearly missing.

The size of the package made it impossible to sample it under sterile or anaerobic conditions. However, at all times, the blocks were covered with plastic film to limit exposure to air and to bacterial contamination. Gloves and facemasks were worn by those persons doing the handling, cutting and wrapping of the sample. The sample handlers concluded that it was reasonable to assume that the inside of the bentonite sample was not contaminated during the lifting, parting and dissecting of the wedge-shaped sample for AECL.

The wedge-shaped sample (1896), together with samples from ABM block 10-11 (1893), ABM block 12-13 (1894) and block 18 (1895) were shipped from Clay Technology in Lund, Sweden on May 25 2009, in a cooler and surrounded by icepacks. The package arrived at AECL on June 5 2009. All samples were immediately stored unopened (i.e., sealed) at 4^{o}C until analysis could be initiated. Only sample 1896 was subsequently used for microbial analysis; the other samples (1893, 1894 and 1895) were stored at 4^{o}C for future analysis (if required).

2.2.5 SUB-SAMPLING OF AECL ABM SAMPLE 1896

Figure 43 shows a schematic of the wedge-shaped sample 1896 and how it was sub-sampled at AECL. Note that rubber gloves, sterilized with 70% isopropyl alcohol, were worn at all times during the sampling procedure. The plasticized aluminium package was opened carefully and the aluminium and plastic layers of the wrapping were spread out on the 70% isopropyl alcohol-sterilized surface of the laminar flow hood. The sample inside the package was wrapped in thin plastic and appeared to be in two pieces. The plastic was peeled back carefully and by the colour, texture and shape (Figure B6, Appendix B) it could be determined that the Febex layer had broken away from the rest of the sample. It was easy to distinguish between the materials.

The Febex material had a grey granite-like appearance. The granular MX-80 material had the appearance of dark clay. The granular MX-80S material appeared gritty, with sand.

Sample 1896-1 (Figure 43)
The interface of the Febex layer and the granular MX-80 layer was scraped with a sterile metal blade on both sides, in the centre of the layers, away from both the rock interface and metal tube (heated) interface. The material was scraped into a sterile glass dish and then placed in a sterile and labelled Teflon container.

Sample 1896-2 (Figure 43)
Layers MX-80 and MX-80S were stuck together. A sterilized long thin screwdriver was used to pry these layers apart. The exposed interfaces were then scraped (1896-2) with a sterile metal blade into a sterile and labelled Teflon container.

Samples 1896-3 and 1896-4 (Figure 43)
These samples came from the centre (matrix) of the MX-80 (1896-3) and MX-80S (1896-4) layers, respectively. A sterile metal blade was used to cut away layers to about 3.5 to 4 cm into the material, from the fibre cloth side, changing to new sterile blades between each cut. A sample was then dug out with a sterile spatula and put into a sterile and labelled Teflon container.

Sample 1896-5 and 1896-6 (Figure 43)
The interfaces between the steel tube and granular MX-80 (1896-5) and granular MX-80S (1896-6) were sampled by scraping those interfaces with a sterile steel blade. The samples were scraped directly into sterile and labelled Teflon containers.

Samples 1896-7 and 1895-8 (Figure 43)
The interfaces near the rock under the fibre cloth were sampled as follows. The cloth could be lifted easily from the clay surface, except for between the granular MX-80 and MX-80S layers where it was embedded about 2.5 cm into the clay. The granular MX-80 surface under the cloth (1896-7) was scraped in the middle and away from edges or the point where the cloth was embedded between the two layers. A sterile steel blade was used to scrape the sample into a sterile and labelled Teflon container. Subsequently the granular MX-80S surface under the cloth (1896-8) also was scraped in the middle and away from edges or the point where the cloth was embedded between the two layers. A sterile steel blade was used to scrape the sample into a sterile and labelled Teflon container.

Sample 1896-9 (Figure 43)
This sample was taken from the centre (matrix) of the Febex layer (1896-9). A sterile metal blade was used to cut away layers from the (band saw-cut) side of the material, changing to new sterile blades between each cut. A sample was then dug out with a sterile spatula and put into a sterile and labelled Teflon container.

2.2.6 WATER ACTIVITY MEASUREMENTS

For each ABM clay sample (1896-1 to 1896-9), water activity was measured on a sub-sample using a Decagon^{TM} WP4 Dewpoint PotentiaMeter (Decagon Devices, Pullman, WA).

2.2.7 WATER CONTENT MEASUREMENTS

For each ABM clay sample (1896-1 to 1896-9), the water content was determined by drying the water activity sub-sample at 110°C to constant weight.

2.2.8 HETEROTROPHIC AEROBES AND ANAEROBES

About 10 g (carefully weighed) of clay were added to 100 mL of Phosphate-Buffered Saline solution (PBS, i.e., 0.01M NaCl buffered to pH 7.6 with 9 mM Na_2HPO_4 and 1 mM $NaH_2PO_4.H_2O$). This suspension was stirred or shaken for 30 min. (under anaerobic conditions) after which serial dilutions were made to 10^{-3}. The dilutions were plated onto R2A agar (Reasoner and Geldreich 1985) and the plates were incubated at 30°C for 5-7 days (heterotrophic aerobes) and up to 4 weeks (heterotrophic anaerobes) before they were counted.

2.2.9 SULPHATE-REDUCING BACTERIA

Degassed tubes containing sterile modified Postgate's B medium (Atlas 1993) were inoculated in an anaerobic glovebox (in triplicate) with the ABM PBS clay suspensions to 10^{-3} dilutions. The tubes were incubated at room temperature for about 4 weeks before they were scored.

2.3 TEST PACKAGE 1 SAMPLES ANALYZED AT SKB

Three ABM materials retrieved from Test package 1 (sampled on May 11 and 12 2009) were analyzed for bacterial presence at SKB. These were Asha 505 (Block 14), Dep-Can (Block 27) and MX-80 (Block 2) (Svensson et al. 2011). Since these materials were different from the granular materials that were the focus of the AECL analyses, no comparison of results obtained at SKB and AECL could be made for this part of the ABM test. The results for the three blocks analyzed at SKB are given by Svensson et al. (2011).

2.4 RESULTS

Table 12 gives the results for water content, water activity, heterotrophic aerobes and anaerobes, and SRB in each of the nine 1896 samples. As well, the maximum temperature that each sample would have experienced in situ in ABM test package 1 could be estimated quite accurately from Figure 34 (assuming each ABM buffer "ring" was 10 cm high).

The water activity in all samples ranged from 0.980 to 0.983, except for the sample from the interface between the rock-fibre cloth and MX-80S (1896-8) that had a water activity of 0.988. These water activities are well above the value of about 0.95 to 0.96, below which microbial culturability in compacted clay-based materials is substantially reduced (Stroes-Gascoyne et al. 2006, 2008). The water content in the MX-80S layer or at interfaces containing MX-80S is generally lower than in the MX-80 and Febex materials, as a result of the sand content.

The (estimated) maximum temperatures for each sample indicated that all samples except the rock-cloth interface samples (1896-7 and 1896-8) experienced temperatures of > 100°C (i.e., 100-105°C, according to the colour regimes in Figure 4). The highest temperature (135°C) was recorded for the samples at the interface with the steel heater tube (1896-5 and 1896-6). The

lowest temperature (95-100^{o}C) was experienced by the samples at the rock-cloth interface. Such high temperatures should eradicate most or all vegetative cells and any surviving viable microbes would most likely be spores (e.g., Stroes-Gascoyne and Hamon 2010).

The smallest amount of clay analyzed was 0.1 g (i.e., the amount contained in 1 mL of a 10 g clay in 100 mL PBS suspension). Therefore, the detection limit was 10 colony-forming units per g clay or (at a water content of about 30%) about 7 CFU/g dry weight. Table 12 shows that the results for five of the nine samples analyzed for heterotrophic aerobes were below this detection limit, while the two samples that experienced the lowest maximum temperatures and came from the rock-cloth-MX-80 or MX-80S interfaces (1896-7 and 1896-8) contained a substantial amount of heterotrophic aerobes.

Sample 1896-8 contained (5.00 ± 0.58) x 10^4 CFU/g dry weight. This sample also had the highest water activity (0.988). Unfortunately, no background CFU/g level for the granular MX-80S was obtained, because this material was not provided for the background level tests (see part I of this report. The other rock-cloth-MX-80 interface sample (1896-7) contained (1.95 ± 0.24) x 10^2 CFU/g dry weight. No background level for this granular MX-80 material is available either. Regular non-granular MX-80 (LOT) material contained about 5 x 10^4 CFU/g (Stroes-Gascoyne and Hamon 2008).

Sample 1896-5, taken from the interface between the steel tube and granular MX-80 yielded only sporadic heterotrophic aerobic colonies, amounting to an insignificant number of 9.1 ± 7.9 heterotrophic aerobic CFU/g dry weight, close to the detection limit of 7 CFU/g dry weight (the starting level in MX-80 was likely > 10^4 CFU/g dry weight). This sample experienced a temperature of 135^{o}C. The heterotrophic aerobic cell count in the other sample that experienced a maximum temperature of 135^{o}C (1896-6) was below the detection limit of 7 CFU/g dry weight.

Sample 1896-3, taken from the centre (bulk) of the MX-80 layer, and having experienced maximum temperatures of 100-105^{o}C contained 9.2 ± 16 CFU heterotrophic aerobes per g dry weight. This number is also extremely low and is close to the detection limit (7 CFU/g dry weight). The heterotrophic aerobic cell counts in the other two bulk samples (i.e., in MX-80S and in Febex, samples1896-4 and 1896-9) were below the detection limit of 7 CFU/g dry weight.

Table 12 also shows that the results for five of the nine samples analyzed for heterotrophic anaerobes were below the detection limit (7 CFU/g dry weight), while only two samples contained substantial (well above the detection limit) levels of anaerobes. The sample with the highest water activity (0.988) that came from the rock-cloth-MX-80S interface (1896-8), that experienced the lowest maximum temperature (95-100^{o}C) and contained the highest number of heterotrophic aerobes, also contained the highest (and a substantial) number of heterotrophic anaerobes, i.e., (3.23 ± 0.30) x 10^3 CFU/g dry weight. Unfortunately, no background level for heterotrophic anaerobes in granular MX-80S is available, because this material was not provided for the background level tests.

Sample 1896-1 from the interface between granular MX-80 and Febex contained (2.60 ± 1.33) x10^2 CFU anaerobes/g dry weight, while having experienced a maximum temperature of 100 to 105^{o}C. Sample 1896-3, taken from the centre (bulk) of the MX-80 layer, and having experienced in situ maximum temperatures of 100-105^{o}C, contained a low number of culturable heterotrophic anaerobes ((1.38 ± 2.40) x10^1 CFU/g dry weight), just above the detection limit (7 CFU/g). Sample 1896-6 from the steel tube-MX-80S interface contained a very low level of

heterotrophic anaerobes, (4.27 ±7.3) CFU/g dry weight, indistinguishable from the detection limit.

All measurements for SRB were below the detection of 4 MPN/g dry weight. From Figure 25 and Table 7 it can be concluded that most ABM materials contained very few ($\leq$ 10 MPN/g dry weight) SRB, including the MX-80 material. The Febex material contained about 265 MPN/g dry weight SRB, but none were found in the Febex sample from ABM Test Package 1, possibly suggesting that the indigenous SRB cells may be very sensitive to temperatures of $\geq$ 100°C.

2.5 DISCUSSION

The only substantial amounts of culturable heterotrophic bacteria found occurred in samples 1896-1 (the interface between Febex and granular MX-80), in sample 1896-7 (the interface between the rock-cloth and MX-80), and especially in sample 1896-8, the interface between the rock-cloth and MX-80S. This latter sample had the highest water activity and experienced the lowest maximum in situ temperature, while the levels of heterotrophic aerobes and anaerobes in this sample are similar to background levels found in most ABM samples.

Stroes-Gascoyne and Hamon (2010) concluded that at low dry densities in compacted bentonite (0.8 g/cm^3), bacteria remained culturable at 80°C but not at 121°C and above. On the contrary, some bacteria in highly compacted bentonite (1.6 g/cm^3) remained culturable even after exposure to 130°C. In lower dry density saturated bentonites, bacteria are likely partially or even largely in a vegetative (and culturable) state, which makes them vulnerable to high temperatures. Under highly compacted conditions, most or all culturable bacteria are likely in dormant or spore-form, which makes them much more resistant to higher temperatures. The results from ABM test package 1 appear to at least partially confirm this pattern. In the bulk granular MX-80 (1896-3), some viability remained while in MX-80S (1896-4), which because of the sand content contained larger pore spaces, had a higher water activity and, therefore, presumably contained more vegetative cells (as suggested by sample 1896-8, compared to sample 1896-7), no viability remained. At the interface between Febex and MX-80 some viability remained, while at the interface between MX-80 and MX-80S, no viability remained.

At a temperature of 135°C, virtually all viability was lost both in the MX-80 and MX-80S materials (samples 1896-5 and 1896-6). The only result that does not appear to fit this pattern is sample 1896-9. Some remaining viability would have been expected in the bulk of the highly compacted Febex layer. An explanation is not obvious. A factor to consider is the possibility that densities in the highly compacted materials such as Febex could have changed as a result of swelling into the sand-filled space between the rock and the test package. Dry densities of the ABM Test package 1 materials analyzed in this report were not measured at AECL. Another factor that could have played a possible role is the fact that the Febex material is the only Mg-bentonite included in the ABM experiment and contained a high amount of both aerobic and anaerobic heterotrophs (Table 7 and Figures 21 and 22) compared to most other materials included in the ABM experiment. If most of these bacteria were in vegetative state, a temperature of 105°C could have eradicated them. Yet another factor to consider is that, depending on the swelling pressure in these materials, a temperature of 105°C could mean a localized boiling of the porewater, which could have affected cells on a local scale much more than a temperature just under or at 100°C at the rock interface where large amounts of culturable cells were found.

The results from these analyses confirm again that interface locations are the most likely areas where microbial activity could occur in a repository. Despite temperatures near or at 100^{o}C a considerable population of viable heterotrophic aerobes and anaerobes were found at the interface between the rock-cloth and the MX-80S and (to a lesser extent) the MX-80 materials. A number of factors could have supported this survival. A temperature of 95-100^{o}C is not likely to kill all spore-forming organisms (e.g., Stroes-Gascoyne and Hamon 2010); the fibre cloth could have created a supporting environment for bacteria, through providing nutrients, humidity and larger pore spaces; the MX-80S would contain larger pore spaces in which bacteria can survive. Also, the water activity was highest in this sample. Very few bacteria were found in the samples that had experienced 135^{o}C, a temperature at which little or no survival is expected. The very low culturable cell numbers found in these samples were at or below the detection of the method.

Microbial survival in this test package occurred most likely and largely as spore-forming organisms because temperatures were > 95^{o}C in all regions, reaching as high as 135^{o}C near the heater tube. At such temperatures little survival of vegetative cells is expected. Survival in spore-form does not pose a direct danger to the longevity of containers in a future repository because spores by their nature are not active. The presence of a significant population of such spore-formers does present, however, the potential for future increased microbial activity, if and when conditions become more favourable, at which time spores could become vegetative cells, with an active metabolism that could produce corrosion-inducing metabolic by-products, such as organics acids, or in the case of SRB, sulphides.

The results from the SKB analysis of Asha 505 (Block 14), Dep-Can (Block 27) and MX-80 (Block 2) (Svensson et al. 2011) showed that bacterial survival in test package 1 was very low as a result of the extreme temperatures in this package (90-130^{0}C). The only bacteria surviving were mesotrophic heterotrophic aerobic bacteria, which were found in the order of $10^2 – 10^3$ cells/g dry weight. However, Svensson et al. (2011) pointed out that the ABM test temperatures were up to 40^{o}C higher than those that would occur in a repository of current design, and that previous studies have indicated that bacteria can survive both high swelling pressure and high temperatures in bentonite (e.g., Masurat et al. 2010; Karnland et al, 2009)

2.6 CONCLUSIONS

The results from these analyses confirm yet again that interface locations are the most likely areas where microbial activity could occur in a future repository. Despite temperatures near or at 100^{o}C a considerable population of viable heterotrophic aerobes and anaerobes were found at the interface between the rock-cloth and the MX-80S or MX-80 materials, as well as at the interface between Febex bentonite and MX-80. Although not examined in this work, it is expected that the surviving organisms are largely spore-formers. Survival in the form of spores, which are not actively metabolizing, does not form a threat to the longevity of containers in a repository unless conditions improve and the spores become vegetative cells with an active metabolism that could produce corrosive metabolic by-products. The potential for such activity at interfaces needs to be taken into account when assessing the performance and safety of a repository.

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REFERENCES

Atlas, R.M. 1993. Handbook of Microbiological Media, (L.C. Parks, Editor). CRC Press Inc.

Eng, A. 2007. Presentation given at ABM Project Meeting # 3, Lund , Sweden, November 29, 2007.

Eng, A., U Nilsson and D. Svensson. 2007. Äspö Hard Rock Laboratory. Alternative buffer material installation report. SKB International Progress Report IPR-07-15.

Karnland, O., S. Olsson, and U. Nilsson. 2006. Mineralogy and sealing properties of various bentonites and smectite-rich clay materials. SKB Technical Report TR-06-30.

Karnland, O., S. Olsson, A. Dueck, M. Birgersson, U. Nilsson, T. Hernan-Hakansson, K. Pedersen, S. Nilsson, T. Eriksen and B. Rosborg. 2009. Long term test of buffer material at the Äspö Hard Rock Laboratory, LOT project. Final report on the A2 test parcel. SKB Technical Report TR-09-29.

Masurat, P., S. Eroksson, and K. Pedersen. 2010. Microbial sulphide production in compacted Wyoming bentonite MX-80 under in situ conditions relevant to a repository for high-level radioactive waste. Applied Clay Science 47, 58-64.

Reasoner, D.J. and E.E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49 1-7.

Sandén, T. 2009. Alternative Buffer Material, Planning the termination of test parcel 1. Presentation given on February 3, 2009, at Äspö.

Stroes-Gascoyne, S., C.J. Hamon, C. Kohle, and D.A. Dixon. 2006. The effects of dry density and porewater salinity on the physical and microbiological characteristics of highly compacted bentonite. Ontario Power Generation Report 06819-REP-01200-10016-R00. November 2006.

Stroes-Gascoyne, S. and C.J. Hamon. 2008. The effect of intermediate dry densities (1.1-1.5 g/cm^3) and intermediate porewater salinities (60-90 g NaCl/L) on the culturability of heterotrophic aerobic bacteria in compacted 100% bentonite. NWMO TR-2008-11, Nuclear Waste Management Organization, Toronto.

Stroes-Gascoyne, S. and C.J. Hamon. 2010. The effects of elevated temperatures on the viability and culturability of bacteria indigenous to Wyoming MX-80 bentonite. NWMO TR-2010-08, Nuclear waste Management Organization, Toronto.

Stroes-Gascoyne, S., C. Sergeant, A. Schippers, C.J. Hamon, S. Nèble, M.-H. Vesvres, S. Poulain, C. Le Marrec. 2008. Microbial analyses of PC water and overcore samples: Synthesis of results. Mont Terri Project Technical Note TN2006-69.

Stroes-Gascoyne, S. and C.J. Hamon, P. Maak and S. Russell. 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., C.J. Hamon, D. Priyanto, D. Jalique, C. Kohle, W. Evenden, A. Grigorya and D.K. Korber. 2014. Microbial analysis of a highly compacted Wyoming MX-80 bentonite plug infused under pressure with distilled deionised water over a period of almost eight years. NWMO TR-2014-20, Nuclear Waste Management Organization, Toronto.

Svensson, D. 2007. Presentation given at ABM Project Meeting # 3, Lund , Sweden, November 29, 2007.

Svensson, D., A. Dueck, U. Nilsson, S. Olsson, T. Sandén, S. Lydmark, S. Jägerwall, K. Pedersen and S. Hansen. 2011. Alternative buffer material. Status of the ongoing laboratory investigation of reference materials and test package 1. SKB Technical Report TR-11-06.

White, D., C.W.M. Davis, J.S. Nickels, J.D. King and R.J. Bobbie. 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51-62.

Table 1: Water Content and Appearance of the ABM Test Materials

ABM Material	Water Content Wt. %	Appearance
Na-bentonites		
Kunigel	7%	fine powder
Ibecoseal	13%	powder
MX-80 (LOT)	11%	powder
Asha 505	35%	coarse chunks
Friedland	5%	powder
Ikosorb	12%	powder
Ca-bentonites		
Dep-CAN	13%	powder
Rokle	5%	fine powder
Calcigel	8%	powder
Mg-bentonites		
Febex	13%	small granules
Argillite		
Callovo-Oxfordian (COX)	3%	crushed rock

Table 2: Mineralogical (XRD) Data for ABM Test Materials

Na bentonites

Mineral	Kunigel	Ibecoseal	MX-80 (LOT)	Asha 505	Friedland	Ikosorb
Montmorillonite	+	+	+	+	+ - (S)	+
Mixed layers					+ - (B)	
Trioct. Smectite				? (S)		
Muscovite/Illite		-	- (B)		+ -	
Quartz	+ -	-	+ -		+	-
Feldspar	? (S)		- (S)			-
Clinoptilolite	-					
Calcite	-	-				
Dolomite						
Kaolinite				+ -	+ -	
Chlorite						
Cristobalite		- (S)	- (B)			-
Gypsum						
Goethite				-		
Siderite					-	
Anatase						
Apatite						
Pyrite			- (S)			

Ca-bentonites Mg-bentonite Argillite

Mineral	Dep-CAN	Rokle	Calcigel	Febex	COX
Montmorillonite	+	+	+	+	?
Mixed layers					?
Trioct. Smectite					
Muscovite/Illite	? (S)	-	+ -		+ -
Quartz		-	+ -	- (S)	+
Feldspar			-		-
Clinoptilolite					
Calcite	-		-	- (S)	+
Dolomite	-		-		-
Kaolinite	? (S)		? (S)		-
Chlorite			- (B)		- (B)
Cristobalite				- (S)	
Gypsum					
Goethite		? (S)			
Siderite					
Anatase		- (S,1)			
Apatite		? (S)			
Pyrite	? (S)				- (S)

(S) = SKB
(B) = BGR

Table 3: Cation Exchange Capacity of ABM Test Materials

	Cation Exchange Capacity	
ABM Material	**SKB**	**BGR**
Na-bentonites		
Kunigel	65	62.5
Ibecoseal	88	87.3
MX-80 (LOT)	83	84.2
Asha 505	86	87.5
Friedland	25	23.2
Ikosorb	93	90.2
Ca-bentonites		
Dep-CAN	80	80.1
Rokle	62	72.8
Calcigel	63	63.9
Mg-bentonite		
Febex	96	98.3
Argillite		
Callovo-Oxfordian (COX)	7 (17 with acid)	11.1

Table 4: Total, Inorganic and Organic C and Total S Content of ABM, Test Materials

ABM Test Material	Total C (wt. %)	Organic C (wt.%)	Inorganic C (wt.%)	Total S (wt.%)
Na-bentonites				
Kunigel	0.43	0.07	0.36	0.34
Ibecoseal	0.79	0.17	0.62	0.23
MX-80 (LOT)	0.26	0.17	0.09	0.27
Asha 505	0.03	0.01	0.02	0.02
Friedland	0.79	0.35	0.44	0.61
Ikosorb	0.23	0.02	0.21	0.07
Ca-bentonites				
Dep-CAN	0.91	0.02	0.89	0.78
Rokle	0.27	0.17	0.10	0.02
Calcigel	0.42	0.03	0.39	0.02
Mg-bentonite				
Febex	0.12	0.02	0.10	0.03
Argillite	3.94	0.65	3.29	0.68
Callovo-Oxfordian (COX)				

Table 5: **Free Swelling Tests and Colloid Formation for ABM Test Materials**

ABM Test Material	Colloids	Swelling Volume (% Increase)
Na-bentonites		
Kunigel	++	16
Ibecoseal	++	18
MX-80 (LOT)	++	23
Asha 505	++	10
Friedland	0	20
Ikosorb	++	11
Ca-bentonites		
Dep-CAN	0	24
Rokle	0	9
Calcigel	0	11
Mg-bentonite		
Febex	+	10
Argillite		
Callovo-Oxfordian (COX)	n.a.	n.a.

Table 6: Water Content, Water Activity and PLFA-based Cell Counts in ABM Test Materials

AECL Sample Number	ABM	Water content (% of dry weight)	Water Activity	PLFA (cell equivalent/g)
Na-bentonites				
1831	Kunigel	8.30	0.526	2.04×10^6
1829	Ibecoseal	15.07	0.586	3.49×10^6
1832	MX-80 (LOT)	12.21	0.542	3.12×10^6
1847	MX-80 (Canada)(1)	8.93	0.358	1.36×10^6
1823	Asha 505	14.29	0.504	4.98×10^6
1828	Friedland	5.33	0.574	9.94×10^6
1830	Ikosorb	14.75	0.551	2.83×10^6
Ca-bentonites				
1826	Dep-CAN	19.11	0.750	1.83×10^6
1833	Rokle	4.16	0.069	1.16×10^7
1825	Calcigel	9.75	0.334	7.50×10^5
Mg-bentonite				
1827	Febex	15.13	0.452	3.26×10^6
Argillites				
1824	COX	1.72	0.304	1.07×10^6
1752	OPA (1)(2)	7.93	0.946	4.91×10^4
1758	OPA (2)(2)	7.90	0.931	1.85×10^5

(1) From Stroes-Gascoyne and Hamon (2008)
(2) From Stroes-Gascoyne et al. (2008)

Table 7: Culture Results for ABM Test Materials

ABM Test Material	Aerobes (CFU/g)	Anaerobes (CFU/g)	Nitrate-utilizing bacteria (MPN/g)	Nitrate-reducing bacteria (MPN/g)	Sulphate-reducing bacteria (MPN/g)
Na-bentontites					
Kunigel	$(4.69\pm3.48)\times10^{1}$	$(2.89\pm0.63)\times10^{1}$	2.27×10^{1}	$<3.2\times10^{0}$	3.89×10^{0}
Ibecoseal	$(5.81\pm0.32)\times10^{3}$	$(2.48\pm0.90)\times10^{2}$	1.67×10^{4}	2.55×10^{1}	1.67×10^{1}
MX-80 (LOT)	$(5.22\pm0.45)\times10^{4}$	$(2.16\pm0.06)\times10^{2}$	8.38×10^{3}	1.23×10^{2}	1.03×10^{1}
MX-80 (Canada)[1]	$(6.24\pm2.34)\times10^{2}$	$(1.74\pm0.60)\times10^{1}$	9.62×10^{2}	$<3.13\times10^{0}$	$<3.14\times10^{0}$
Asha 505	$(6.36\pm1.98)\times10^{5}$	$(4.35\pm0.30)\times10^{2}$	1.23×10^{5}	8.15×10^{0}	2.68×10^{2}
Friedland	$(1.84\pm0.69)\times10^{3}$	$(1.78\pm0.64)\times10^{2}$	1.57×10^{3}	1.57×10^{1}	2.51×10^{2}
Ikosorb	$(3.04\pm0.34)\times10^{4}$	$(1.50\pm0.40)\times10^{2}$	2.36×10^{4}	4.73×10^{1}	$<3.33\times10^{0}$
Ca-bentonites					
Dep-CAN	$(1.32\pm0.05)\times10^{3}$	$(7.95\pm0.69)\times10^{1}$	1.79×10^{4}	4.29×10^{0}	1.10×10^{1}
Rokle	$(2.28\pm1.02)\times10^{3}$	$(3.26\pm0.47)\times10^{2}$	6.38×10^{3}	9.97×10^{0}	7.18×10^{0}
Calcigel	$(2.73\pm0.62)\times10^{2}$	$(8.15\pm1.23)\times10^{1}$	4.67×10^{2}	7.76×10^{0}	9.78×10^{0}
Mg-bentonite					
Febex	$(9.54\pm1.24)\times10^{4}$	$(2.85\pm0.46)\times10^{2}$	1.66×10^{4}	1.02×10^{2}	2.55×10^{1}
Argillites					
COX	$(4.57\pm1.13)\times10^{1}$	$(1.63\pm0.57)\times10^{1}$	1.96×10^{1}	$<2.94\times10^{0}$	$<2.94\times10^{0}$
OPA (1)[2]	<10	<10	<2	<2	<2
OPA (2)[2]	<10	<10	10	<2.5	<3

(1) Stroes-Gascoyne et al. unpublished (2007)
(2) Stroes-Gascoyne et al. (2008)

Table 8: PLFA-derived Community Structure in ABM Materials

	% of Total PLFA					
ABM Test Material	**Firmi-cutes (TerBr Sats)**	**Proteo-bacteria (Monos)**	**Anaerobic metal reducers (BrMonos)**	**SRB/ Actino-mycetes (Mid BrSats)**	**General (Nsats)**	**Eukaryotes (Polyenoics)**
Na-bentonites						
Kunigel	8.1	20.1	1.1	4.3	64.3	2.0
Ibecoseal	10.9	38.4	1.0	5.0	36.6	8.1
MX-80 (LOT)	10.5	33.0	1.2	5.2	42.4	7.7
MX-80 (Canada)[(1)]	13.1	38.6	1.2	3.6	35.0	8.5
Asha 505	27.4	25.6	1.5	10.8	31.4	3.3
Friedland	14.0	43.0	1.1	5.0	33.8	3.1
Ikosorb	11.7	48.6	1.0	11.5	25.2	2.0
Ca-bentonites						
Dep-CAN	5.2	51.9	0.7	4.9	36.0	1.4
Rokle	13.7	41.9	2.7	6.7	26.2	8.9
Calcigel	3.3	34.8	0.7	0	58.8	2.4
Mg-bentonite						
Febex	12.6	44.2	3.2	8.4	26.4	5.2
Argillites						
COX	0	14.7	1.5	0	75.0	8.8
OPA (1)[(2)]	0	0	0	0	100.0	0
OPA (2)[(2)]	0	11.4	0	0	88.6	0

(1) Stroes-Gascoyne et al. unpublished (2007)
(2) Stroes-Gascoyne et al. (2008)

Table 9: Description of PLFA Structural Groups

PLFA Structural Group	General Classification	Specifics
Monoenoic (Monos)	Abundant in Proteobacteria (Gram negative bacteria), typically fast growing, utilize many carbon sources, and adapt quickly to a variety of environments.	Proteobacteria is one of the largest groups of bacteria and represents a wide variety of both aerobes and anaerobes.
Terminally Branched Saturated (TerBrSats)	Characteristic of Fimicutes (Low G+C Gram-positive bacteria) and some Gram-negative bacteria (especially anaerobes).	Fimicutes are indicative of presence of anaerobic fermenting bacteria (mainly *Clostridia/Bacteriodes*-like).
Branched Monoenoic (BrMonos)	Found in the cell membranes of micro-aerophiles and anaerobes, such as sulfate- or iron-reducing bacteria.	In contaminated environments high proportions are often associated with anaerobic sulfate and iron reducing bacteria.
Mid-Chain Branched Saturated (MidBrSats)	Common in sulfate reducing bacteria and also Actinobacteria (High G+C Gram-positive bacteria).	In contaminated environments high proportions are often associated with anaerobic sulfate and iron reducing bacteria.
Normal Saturated (Nsats)	Found in all organisms.	High proportions often indicate less diverse populations.
Polyenoic	Found in eukaryotes such as fungi, protozoa, algae, higher plants, and animals.	Eukaryotic scavengers will often rise up and prey on contaminant utilizing bacteria.

Table 10: Comparison of results for Heterotrophic Aerobic Bacteria and Sulphate-Reducing Bacteria Obtained by AECL and SKB

ABM Test Material	HAB (CFU/g dry wt)	HAnB (CFU/g dry wt)	SRB (MPN/g dry wt)	SRB (S) (MPN/g dry wt)
Na Bentonites				
Kunigel	$(4.7\pm3.5) \times 10^{1}$	0	3.9 (0.6-27)	<10
Ibecoseal	$(5.8\pm0.3) \times 10^{3}$	$(5.8\pm4.9) \times 10$	6.7 (4.5-57.8)	61 (23-178)
MX-80 (LOT)	$(5.2\pm0.4) \times 10$	$(6.6\pm1.6) \times 10^{3}$	10.3 (2.6-41.4)	<10
Asha 505	$(6.4\pm2.0) \times 10^{5}$	$(8.4\pm10.9) \times 10^{4}$	268 (75-960)	91 (38-40
Friedland	$(1.8\pm0.7) \times 10^{3}$	$(1.7\pm0.9) \times 10^{3}$	251 (70-900)	68 (26-198)
Ikosorb	$(3.0\pm0.3) \times 10^{4}$	$(4.5\pm4.6) \times 10^{3}$	<3.3	<10
Ca-Bentonites				
Dep-CAN	$(1.3\pm0.1) \times 10^{3}$	$(1.0\pm0.6) \times 10^{3}$	11.0 (2.7-44.1)	9 (4-44)
Rokle	$(2.3\pm1.0) \times 10^{3}$	$(2.0\pm3.4) \times 10^{2}$	7.2 (1.6-31.9)	9 (5-46)
Calcigel	$(2.7\pm0.6) \times 10^{2}$	$(4.2\pm4.9) \times 10^{2}$	9.8 (2.4-39.3)	56 (21-152)
Mg-Bentonite Argillite	$(9.5\pm1.2) \times 10^{4}$	$(6.9\pm2.6) \times 10^{4}$	25.5 (7.3-89.8)	10(5-50)
Cox	$(4.6\pm1.1) \times 10^{1}$	$(2.3\pm4.0) \times 10^{2}$	2.9 (0.5-24.5)	<10

(S) = SKB Data (from Svensson et al. 2011)
HAB = Heterotrophic Aerobic Bacteria
SRB = Sulphate Reducing Bacteria

Table 11: The Block Order in Each of the Three ABM Packages

The Positions with "Callovo Oxfordian Discs" Represent Two Discs*

Block Number	Package 1	Package 2	Package 3
31	Not Installed	MX80	MX80
30	MX80	MX80	MX80
29	MX80	Febex	Ibeco Seal
28	Ikosorb	Ikosorb	Rokle
27	Deponit	MX80 Granulate	Febex
26	Ibeco Seal	Deponit	MX80
25	Friedland	MX80 Granulate + Quartz	Friedland
24	Asha 505	Rokle	Callovo-Oxfordian Discs
23	Calcigel	Friedland	Callovo-Oxfordian
22	Callovo-Oxfordian	Kunigel V1	Kunigel V1
21	Febex	Asha 505	Deponit
20	MX80 Granulate	Callovo-Oxfordian Discs	Calcigel
19	MX80 Granulate + Quartz	Callovo-Oxfordian	MX80 Granulate
18	(MX80)	Calcigel	Asha 505
17	Kunigel V1	MX80	Ikosorb
16	Rokle	Callovo-Oxfordian	MX80 Granulate + Quartz
15	Deponit	Ibeco Seal	Friedland
14	Asha 505	MX80 Granulate + Quartz	MX80 Granulate
13	Rokle	Kunigel V1	Ibeco Seal
12	Callovo-Oxfordian	Ikosorb	Kunigel V1
11	MX80	Ibeco Seal	Febex
10	Ikosorb	Asha 505	Deponit
9	Friedland	Febex	Callovo-Oxfordian
8	Febex	MX80 Granulate	Ikosorb
7	MX80 Granulate + Quartz	Rokle	Rokle
6	Ibeco Seal	MX80	Calcigel
5	Calcigel	Deponit	MX80 Granulate + Quartz
4	Kunigel V1	Friedland	Asha 505
3	MX80 Granulate	Calcigel	MX80
2	MX80	MX80	MX80
1	MX80	MX80	MX80

*From Eng et al. (2007)
Red = AECL Sample
ABM = Alternative Buffer Materials

Table 12: Enumeration, Water Content, Water Activity and Temperature Results

Sample	Description	Water Content %	Heterotrophic Aerobes CFU/g dry wt.	Heterotrophic Anaerobes CFU/g dry wt.	SRB MPN/g dry wt.	Water Activity (a_w)	Estimated Temperature* (^{0}C)
1896-1	Interface Between FEBEX and MX-80	35.37	BDL	$(2.60\pm1.33) \times 10^2$	< 4	0.983	~ 100 - 105
1896-2	Interface Between MX-80 and MX-80S	35.36	BDL	BDL	< 4	0.983	~ 100 - 105
1896-3	In Center of MX-80	39.48	$(9.2\pm16) \times 10^0$	$(1.38\pm2.40) \times 10^1$	< 4	0.981	~ 100 - 105
1896-4	In Center of MX-80S	30.64	BDL	BDL	< 4	0.981	~ 100 - 105
1896-5	Interface Steel MX-80	38.63	$(9.1\pm7.9) \times 10^0$	BDL	< 4	0.980	~ 135
1896-6	Interface Steel MX-80S	28.60	BDL	$(4.27\pm7.3) \times 10^0$	< 4	0.981	~ 135
1896-7	Interface Cloth MX-80	42.51	$(1.95\pm0.24) \times 10^2$	BDL	< 4	0.983	~ 95 - 100
1896-8	Interface Cloth MX-80S	31.39	$(5.00\pm0.58) \times 10^4$	$(3.23\pm0.30) \times 10^3$	< 4	0.988	~ 95 - 100
1896-9	In Center of FEBEX	34.71	BDL	BDL	< 4	0.982	~ 100 - 105

CFU = Colony-forming Units
SRB = Sulphate-Reducing Bacteria
MPN = Most Probable Number
*Estimated from Figure 35
BDL = $\leq$ 7 CFU/g dry wt.

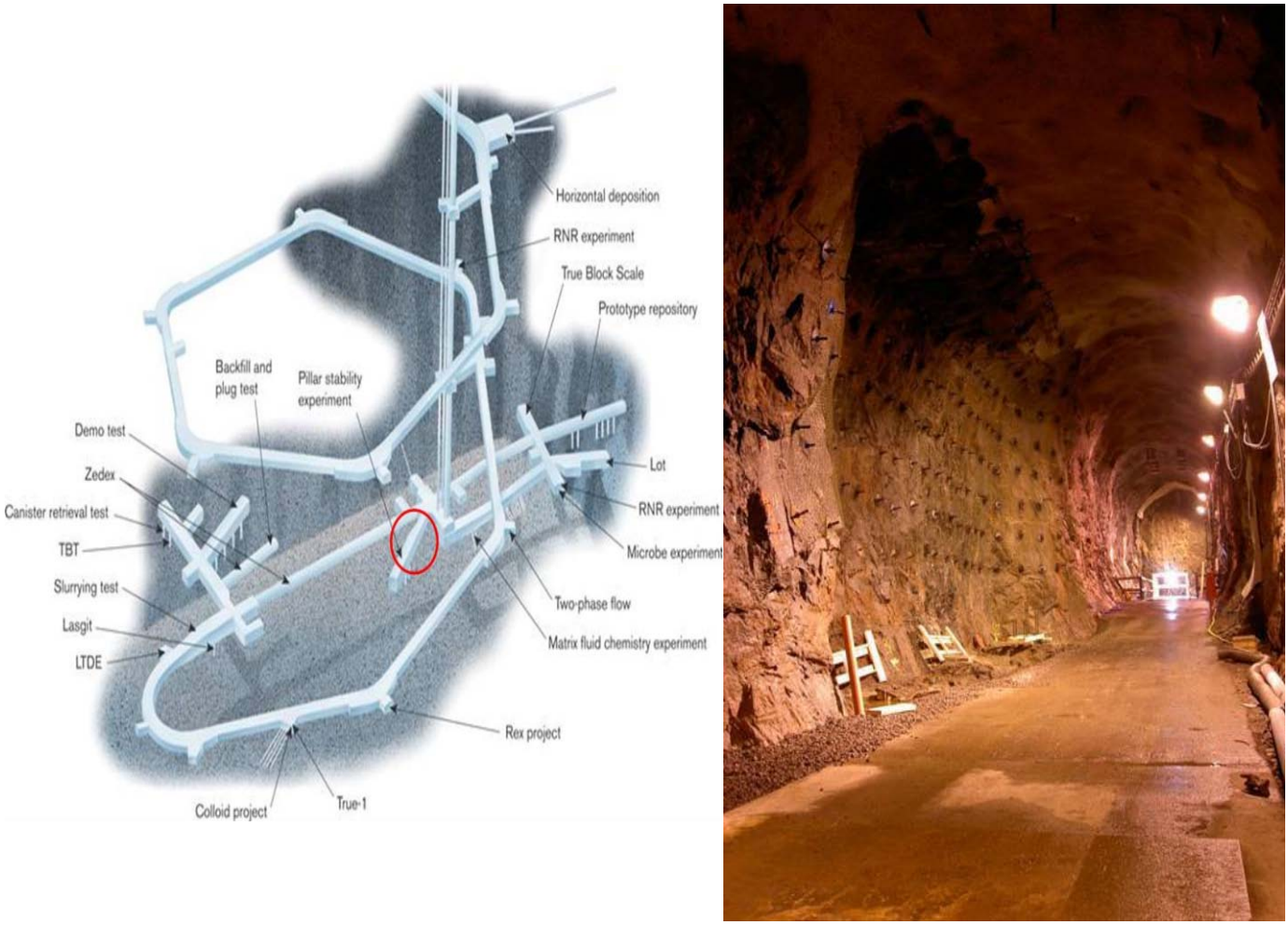


Figure 1: Location of the ABM Experiment in the Äspö Hard Rock Laboratory

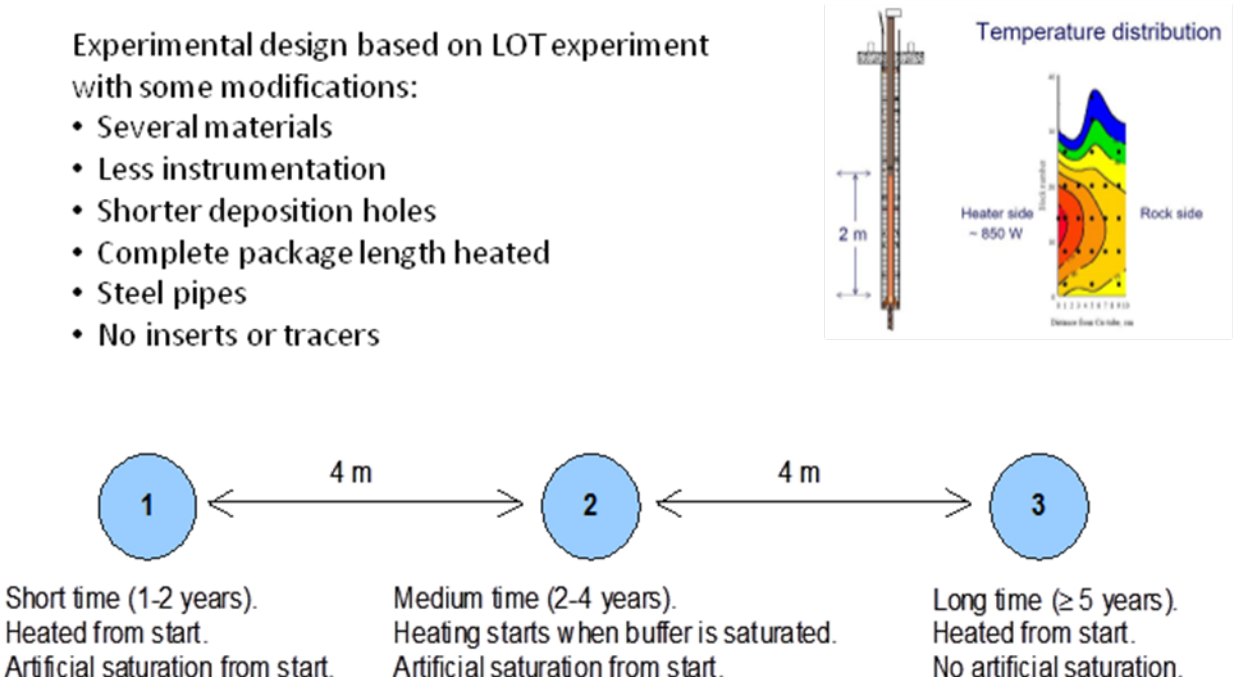


Figure 2: Experimental Design for the Three ABM Experiments at Äspö

Package parts:

- Steel pipe
- Buffer blocks
- Saturation system
- Instrumentation
- Heater system
- ”Backfill”

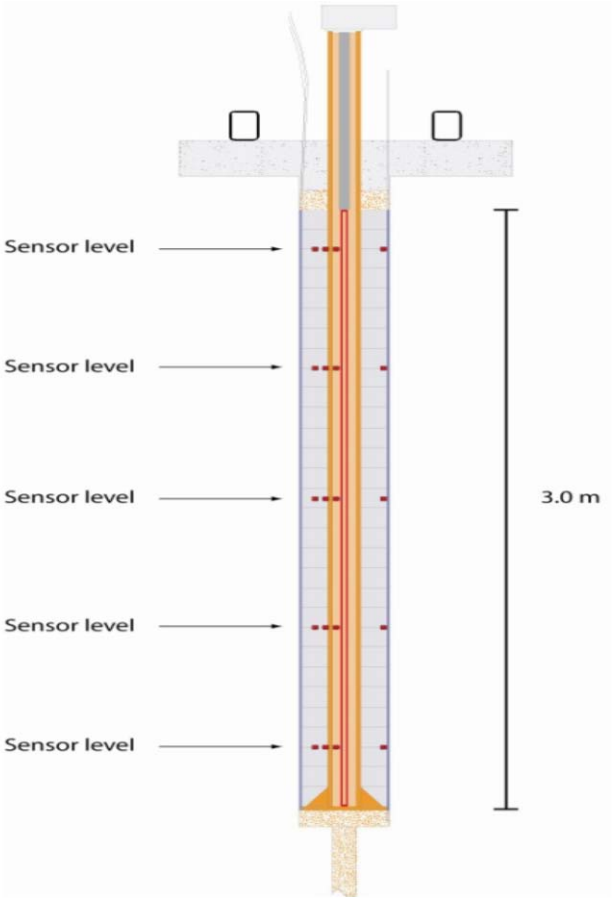


Figure 3: Package Design for the ABM Experiment at Äspö

Block material:

- Ikosorb
- Deponit CA-N
- Ibeco Seal M-90
- Friedland
- Asha 505
- Calcigel
- Febex
- Kunigel V1
- Callovo Oxfordian (both discs and remoulded)
- Rokle
- Mx-80

Granulates:

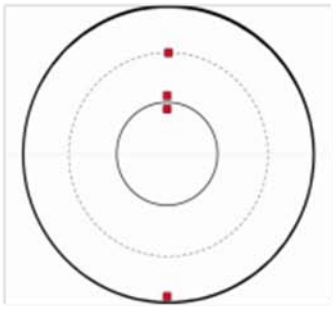
- MX-80
- MX-80 with quartz

Total of 14 variations of 11 materials !

Figure 4: Buffer Materials used in the ABM Experiments

Monitoring temperature and humidity:

- Thermocouples
  - 20 per package
- Temperature indicators
  - 2 between each block
- Relative humidity sensors
  - 4 in package number 2



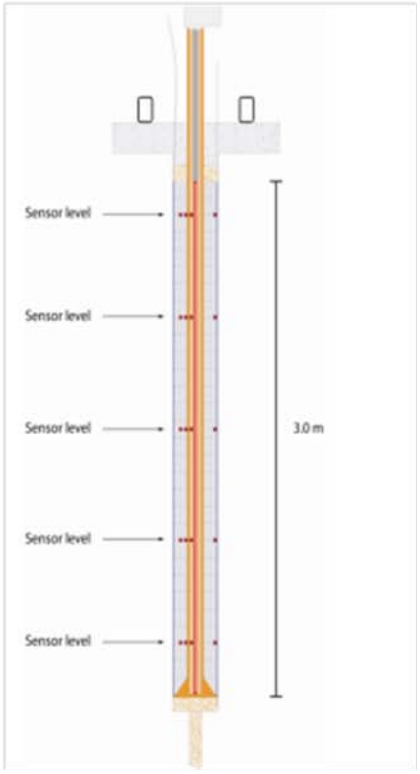


Figure 5: Number and Type of Sensors in ABM Package Design

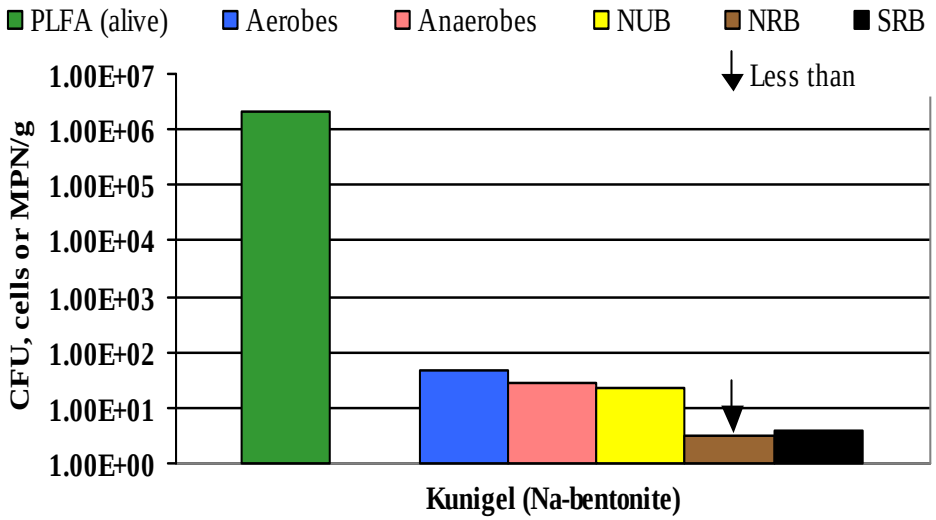


Figure 6: Viable and Culturable Cell Counts for Kunigel Na-bentonite Sample

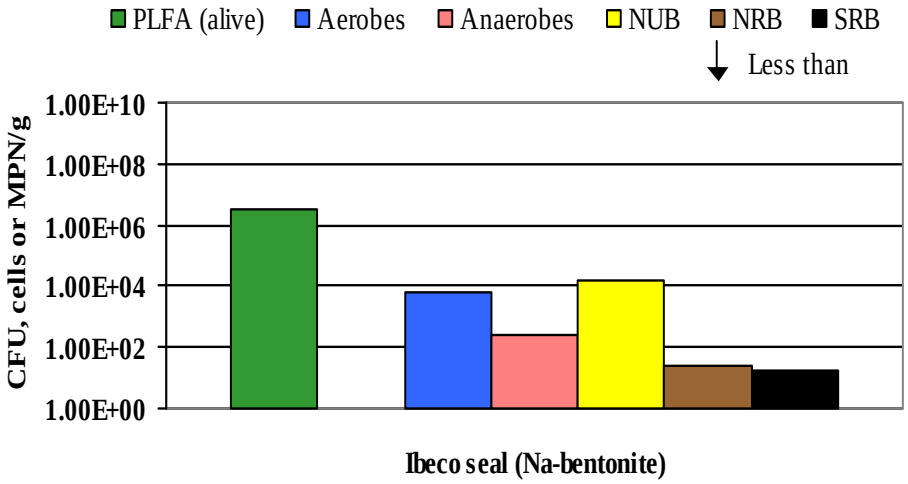


Figure 7: Viable and Culturable Cell Counts for Ibeco seal Na-bentonite Sample

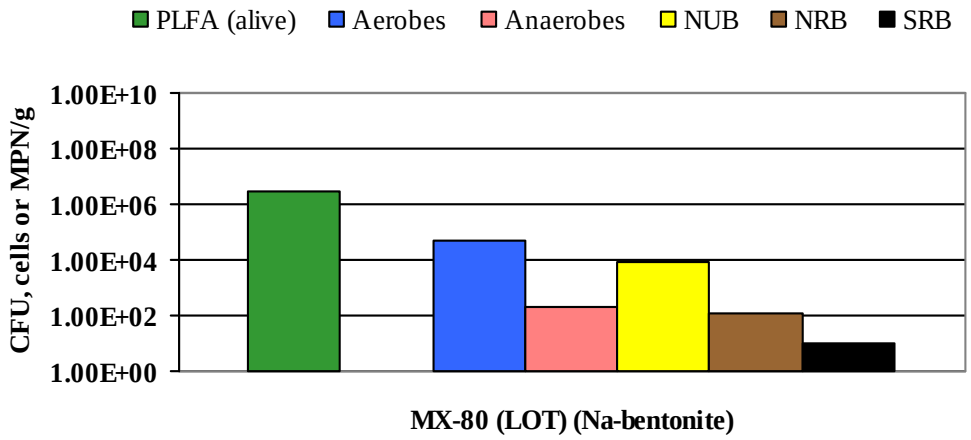


Figure 8: Viable and Culturable Cell Counts for Wyoming MX-80 (LOT) Na-bentonite Sample.

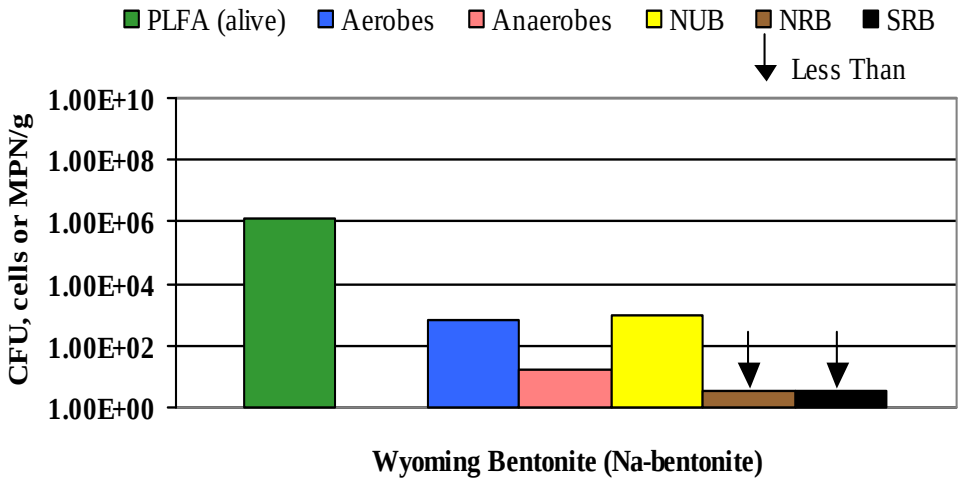


Figure 9: Viable and Culturable Cell Counts for Wyoming MX-80 (Canada) Na-bentonite Sample

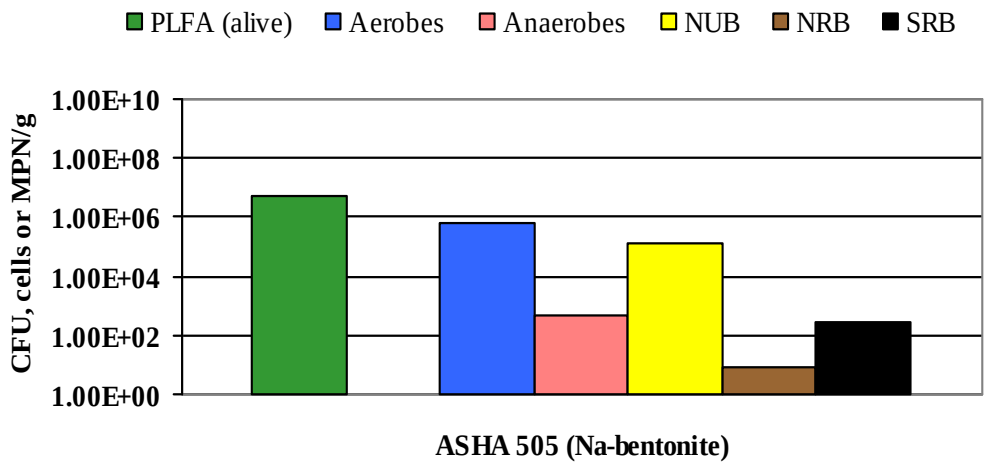


Figure 10: Viable and Culturable Cell Counts for Asha 505 Na-bentonite Sample

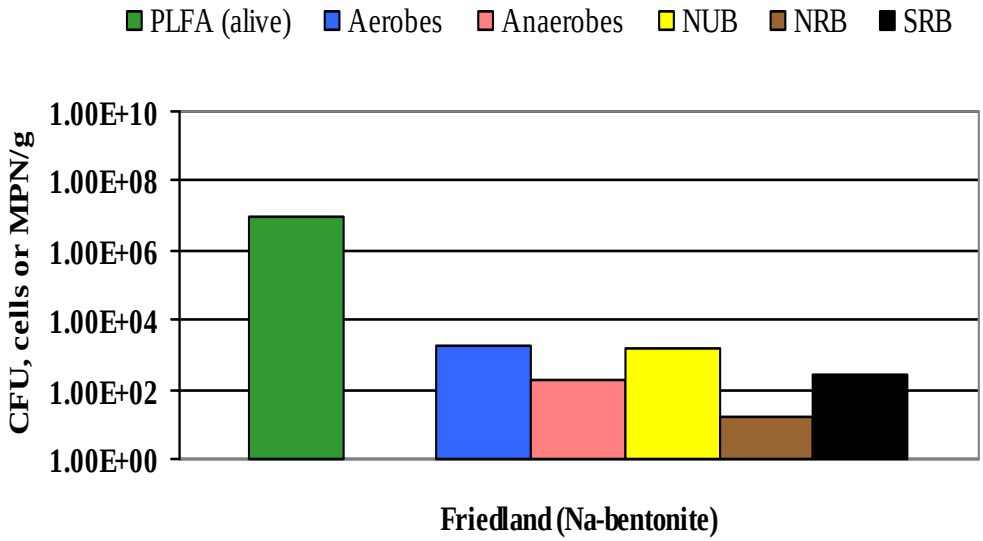


Figure 11: Viable and Culturable Cell Counts for Friedland Na-bentonite Sample

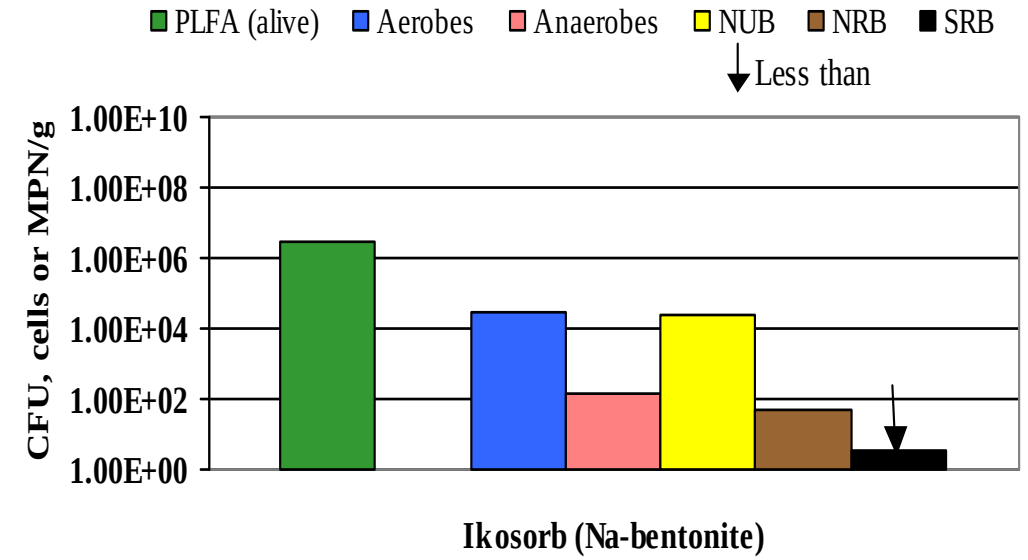


Figure 12: Viable and Culturable Cell Counts for Ikosorb Na-bentonite Sample

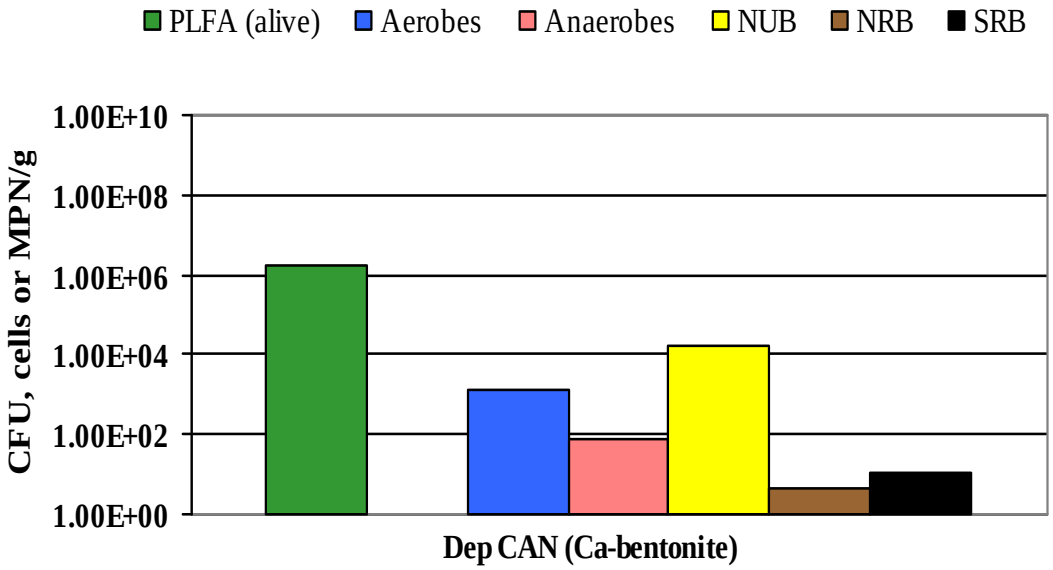


Figure 13: Viable and Culturable Cell Counts for Dep-CAN Ca-bentonite Sample

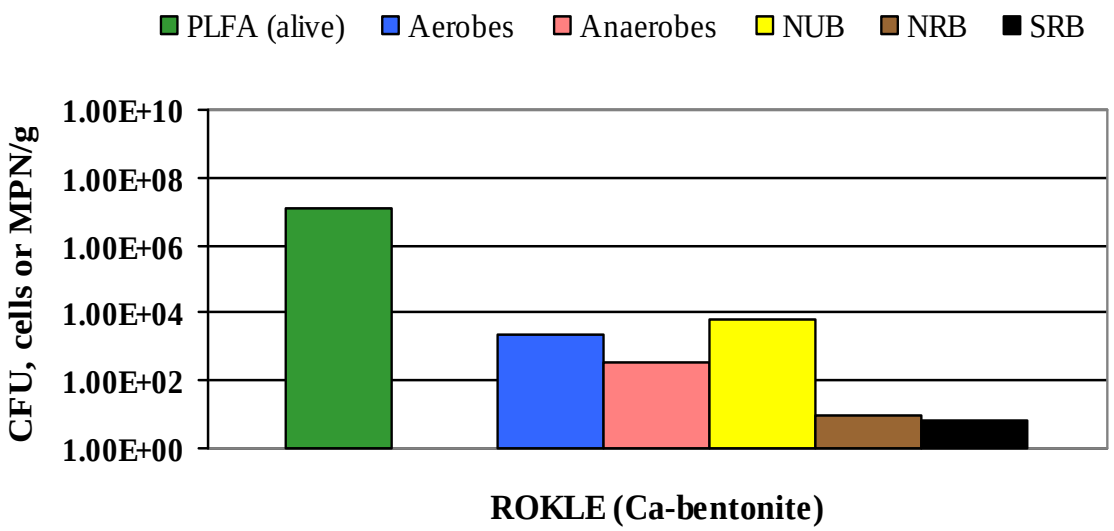


Figure 14: Viable and Culturable Cell Counts for Rokle Ca-bentonite Sample

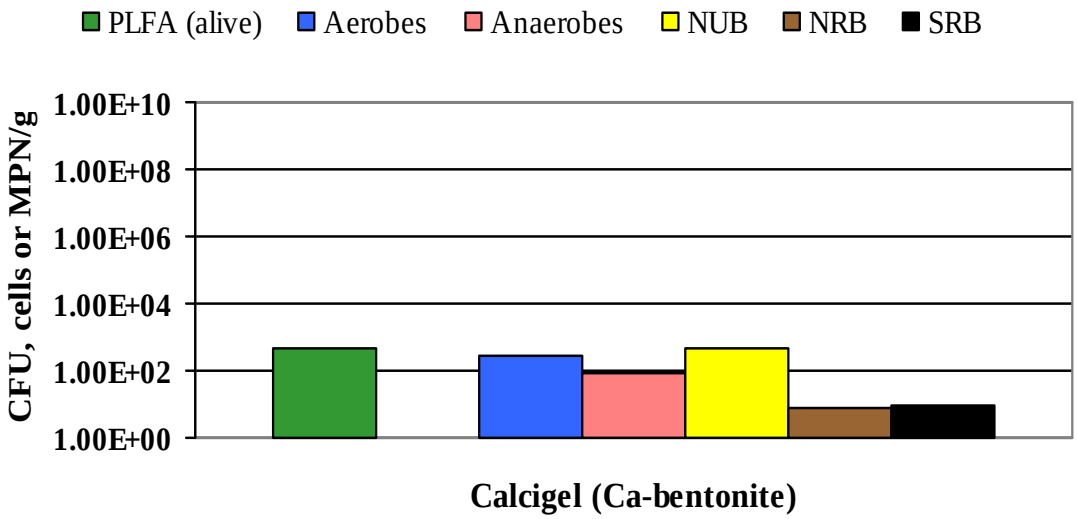


Figure 15: Viable and Culturable Cell Counts for Calcigel Ca-bentonite Sample

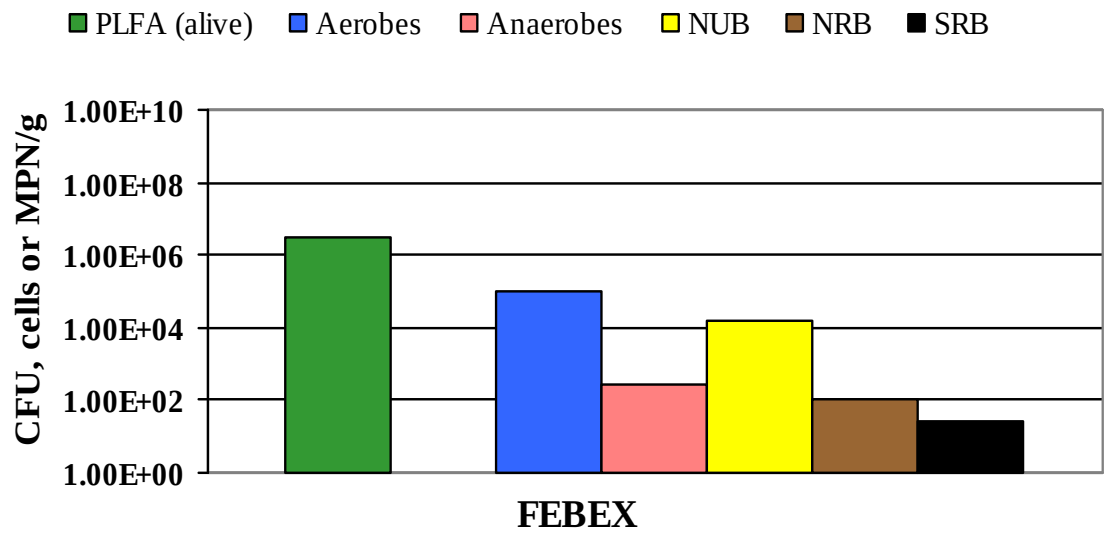


Figure 16: Viable and Culturable Cell Counts for Febex-Mg-bentonite Sample

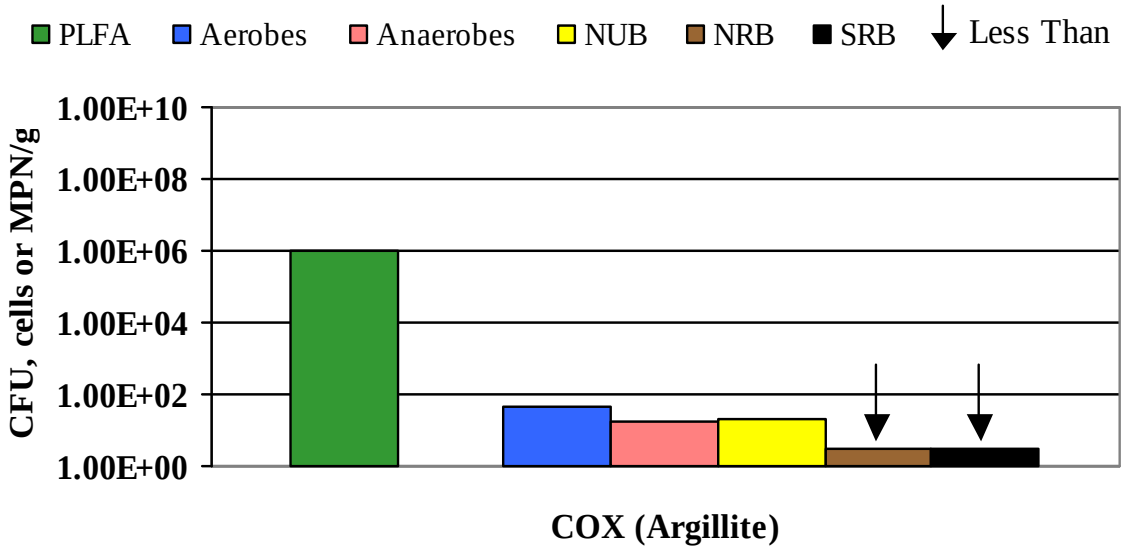


Figure 17: Viable and Culturable Cell Counts for COX (Callovo-Oxfordian) Argillite Sample

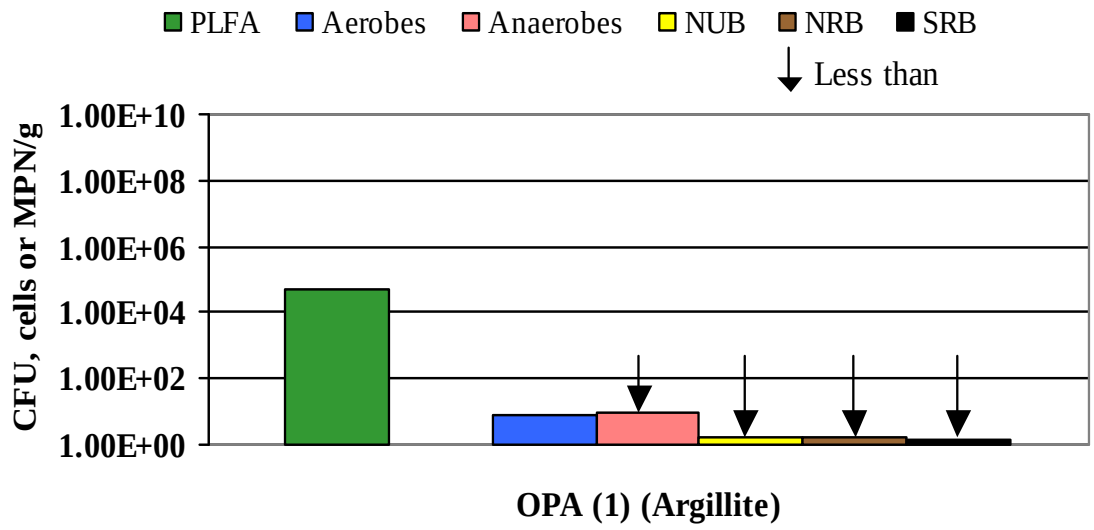


Figure 18: Viable and Culturable Cell Counts for Opalinus Clay (OPA 1) Argillite Sample

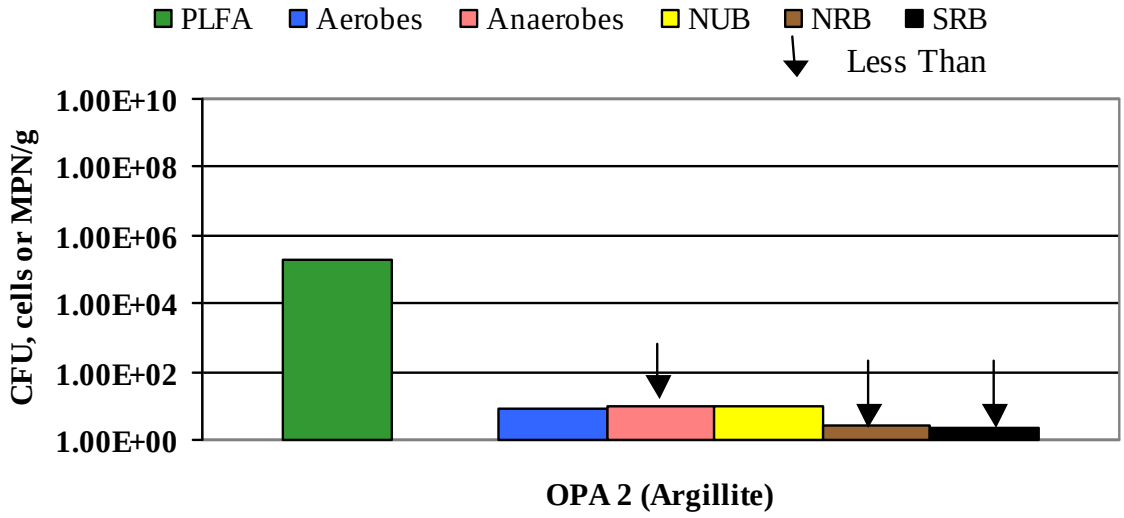


Figure 19: Viable and Culturable Cell Counts for Opalinus Clay (OPA 2) Argillite Sample

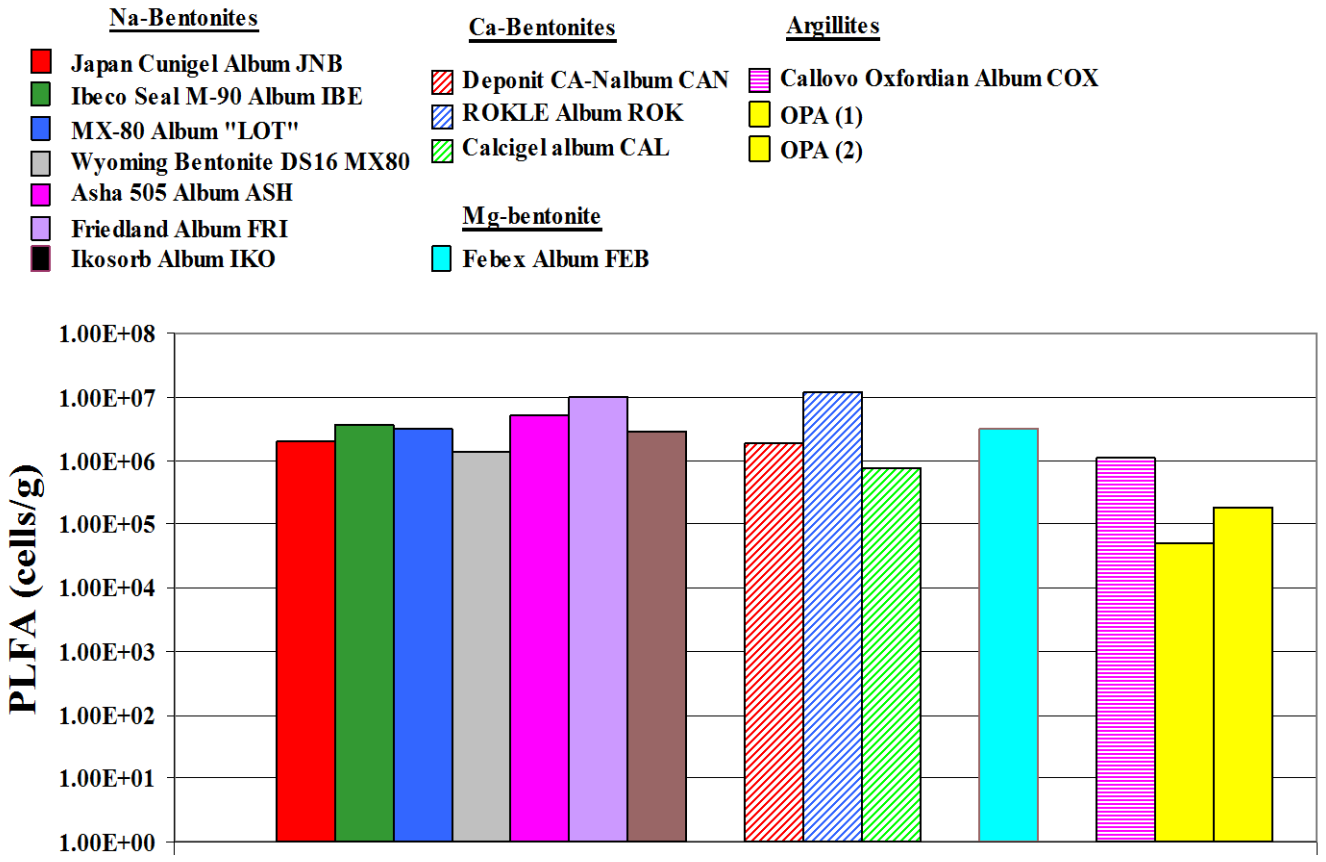


Figure 20: Comparison of PLFA-based Biomass (viable cells) in all ABM Samples

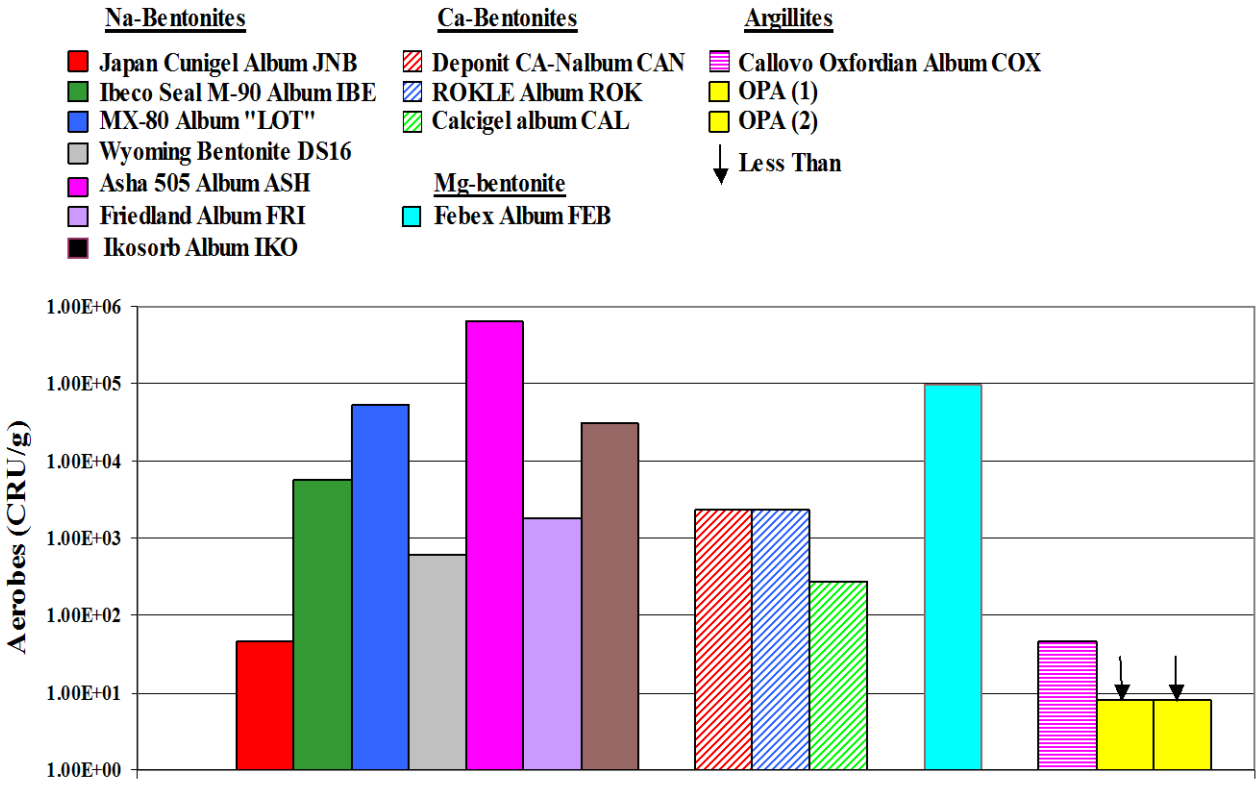


Figure 21: Comparison of Culturable Heterotrophic Aerobic Cell Content in all ABM Samples

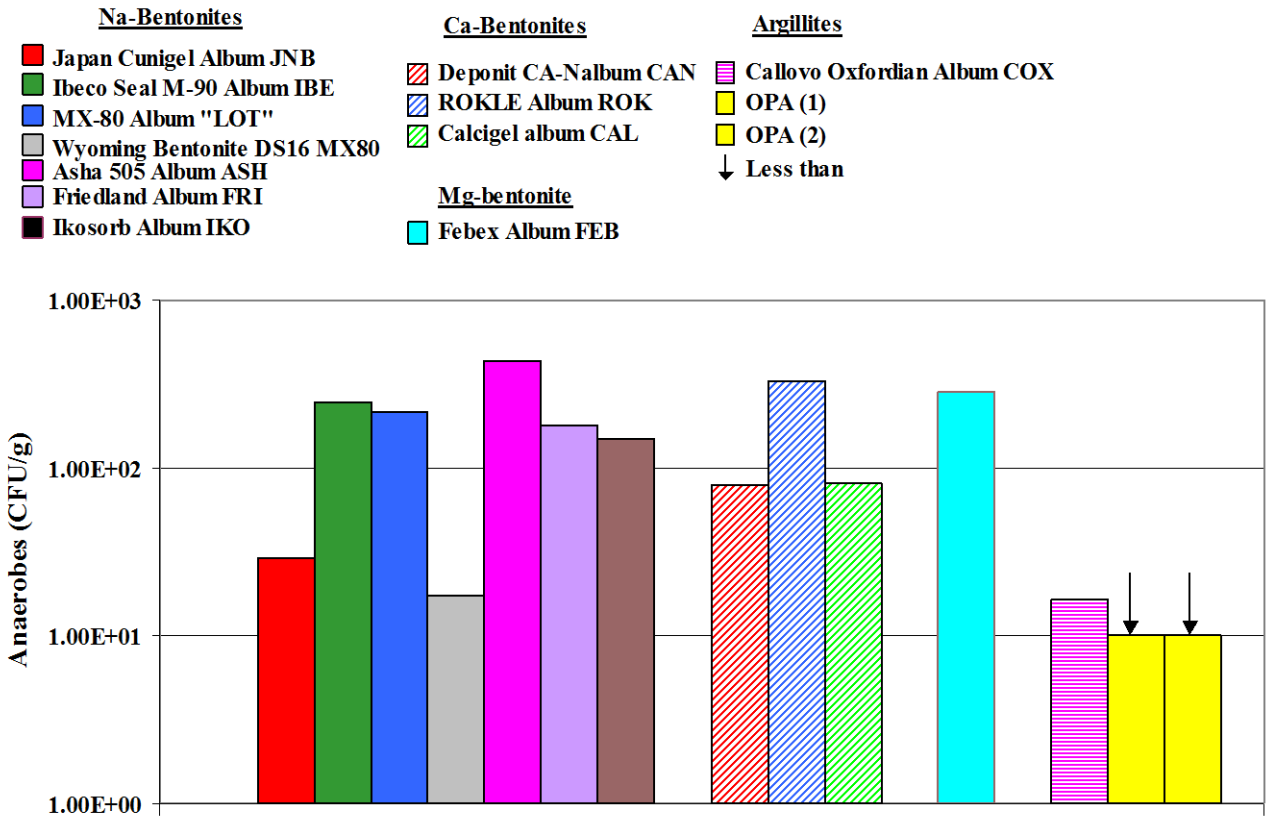


Figure 22: Comparison of Culturable Heterotrophic Anaerobic Cell Content in all ABM Samples

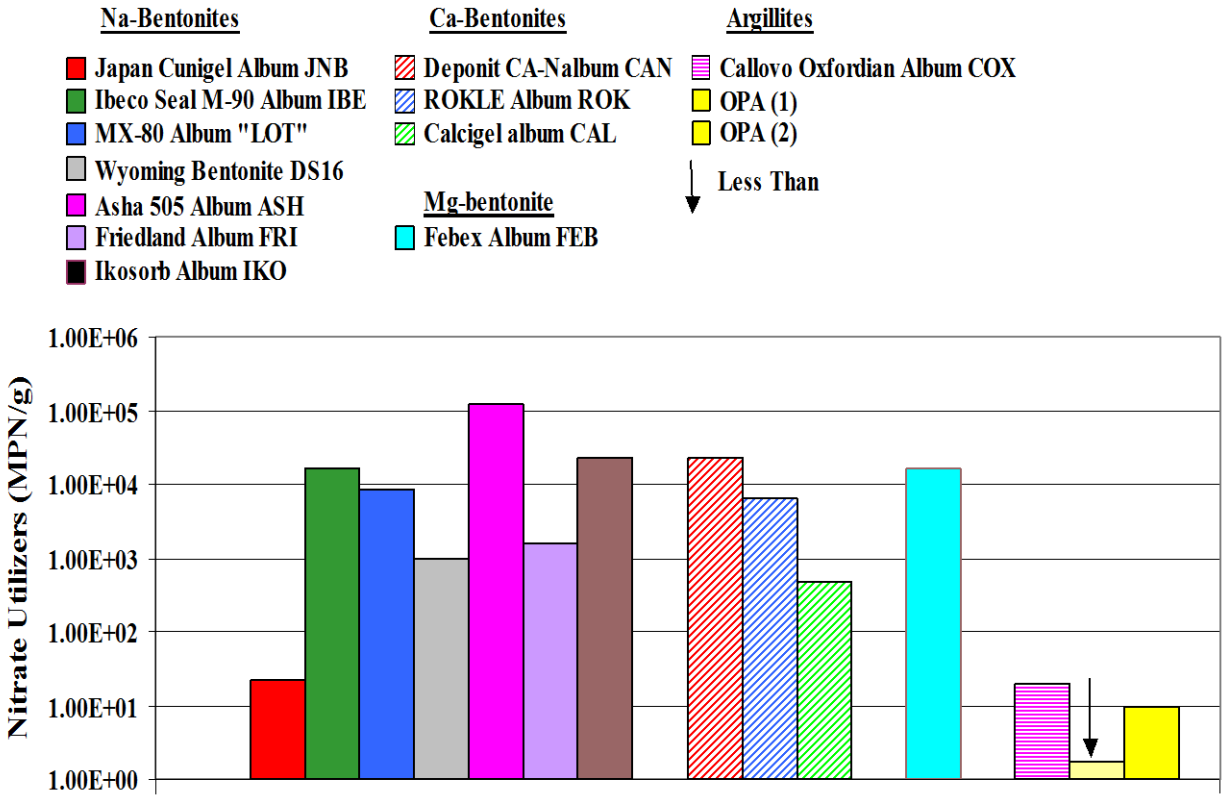


Figure 23: Comparison of Culturable Nitrate-utilizing Bacteria (NUB) Content in all ABM Samples

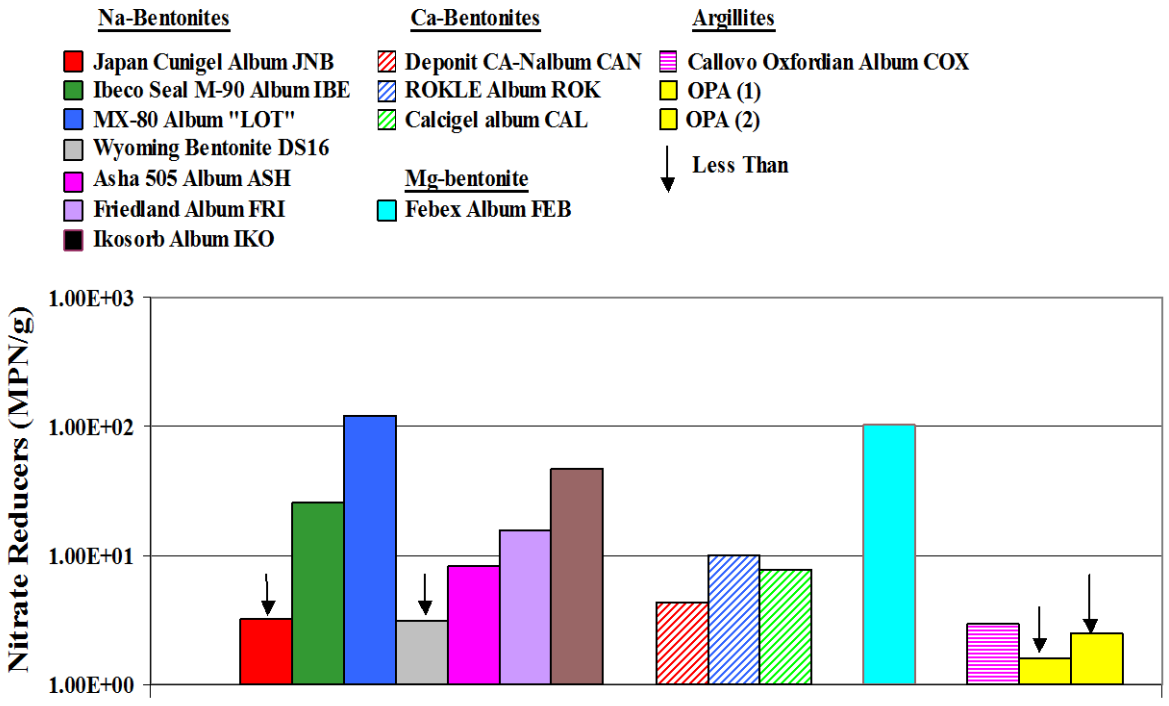


Figure 24: Comparison of Culturable Nitrate-reducing Bacteria (NRB) Content in all ABM Samples

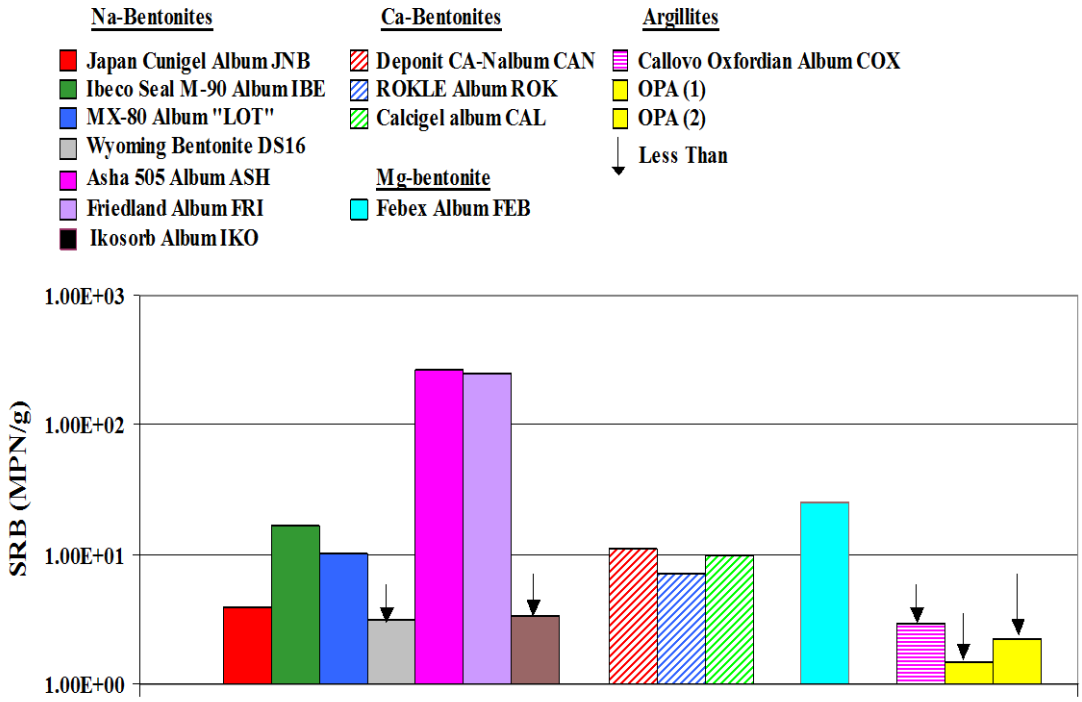


Figure 25: Comparison of Culturable Sulphate-reducing Bacteria (SRB) Content in all ABM Samples

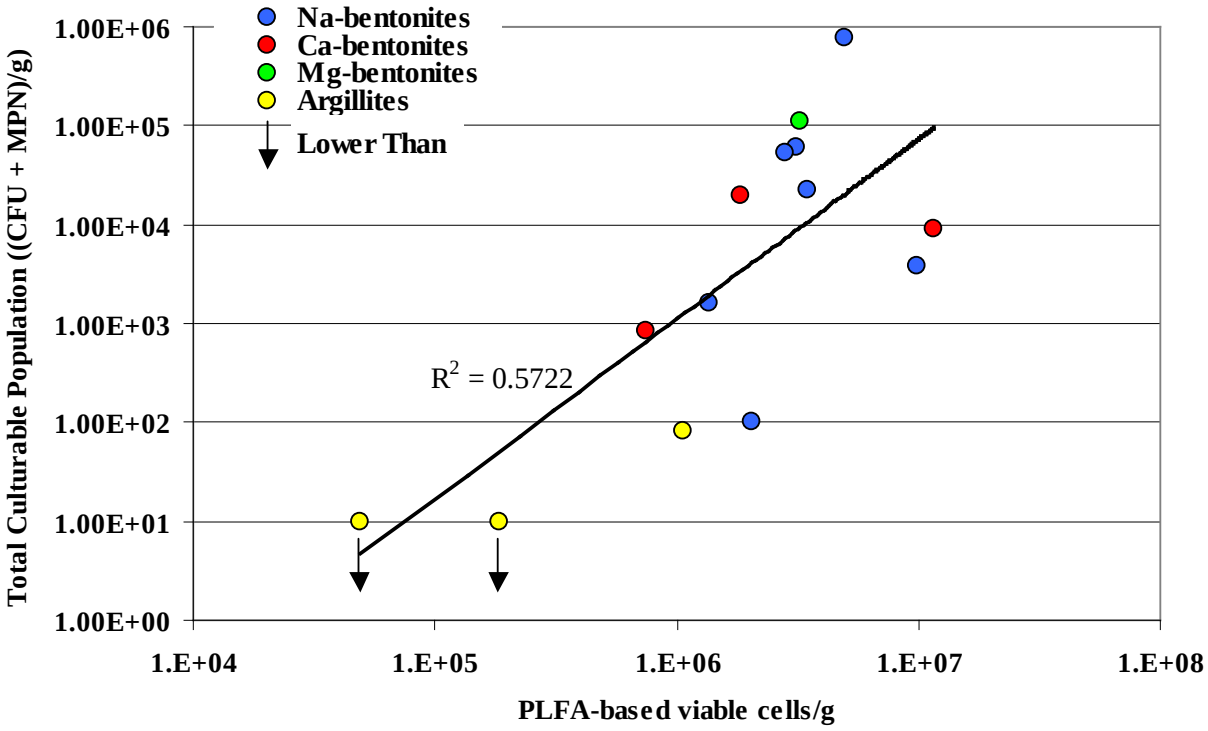


Figure 26: Comparison of the Sum of Culturable Cells ((CFU + MPN)/g) Versus PLFA-based Viable Biomass in all ABM Samples

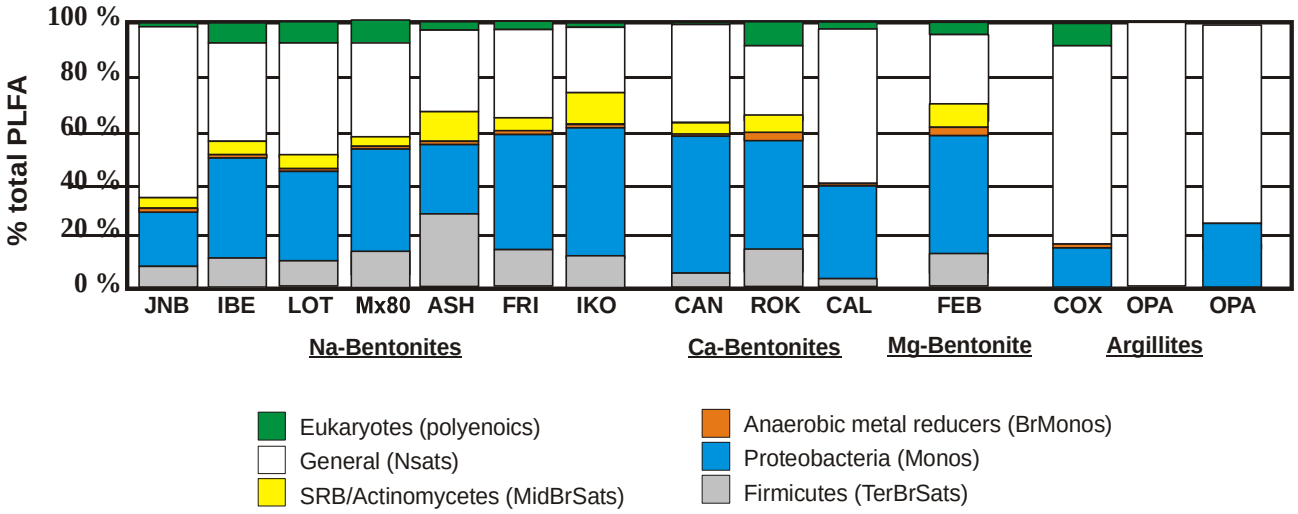


Figure 27: Comparison of the PLFA-based Community Structure in all ABM Samples

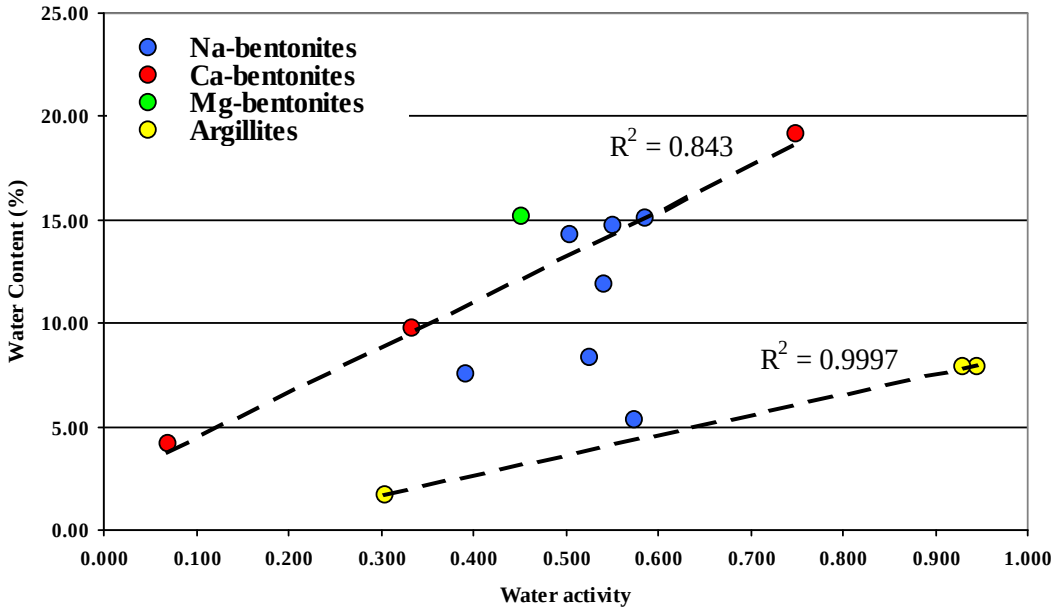


Figure 28: Water Activity as a Function of Water Content in ABM Samples

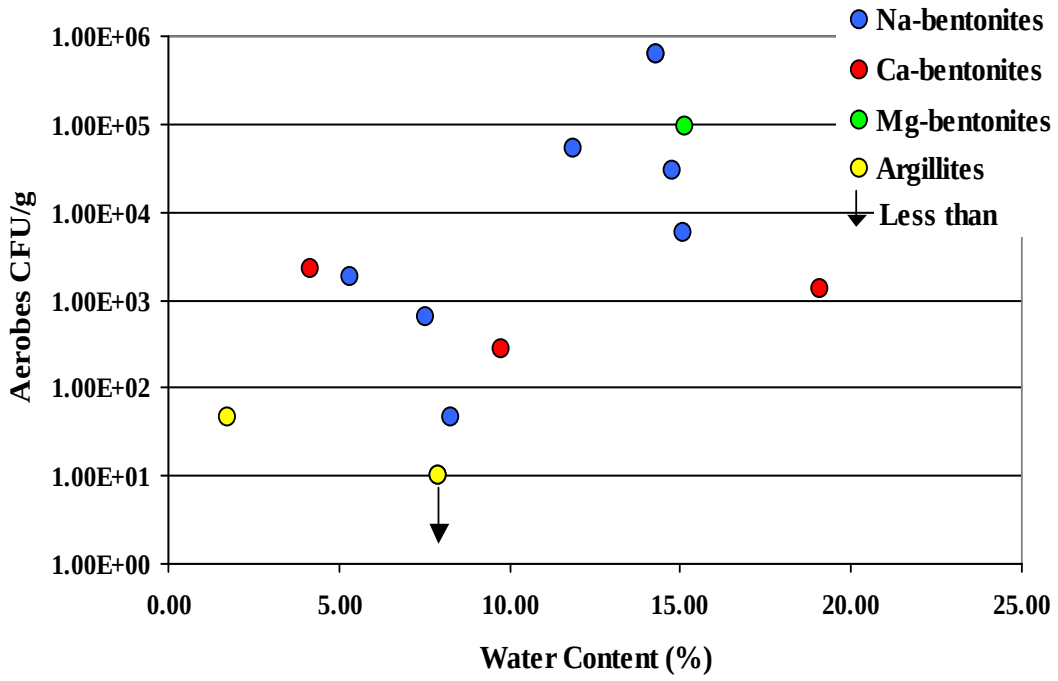


Figure 29: Culturable Heterotrophic Aerobes as a Function of Water Content in ABM Samples

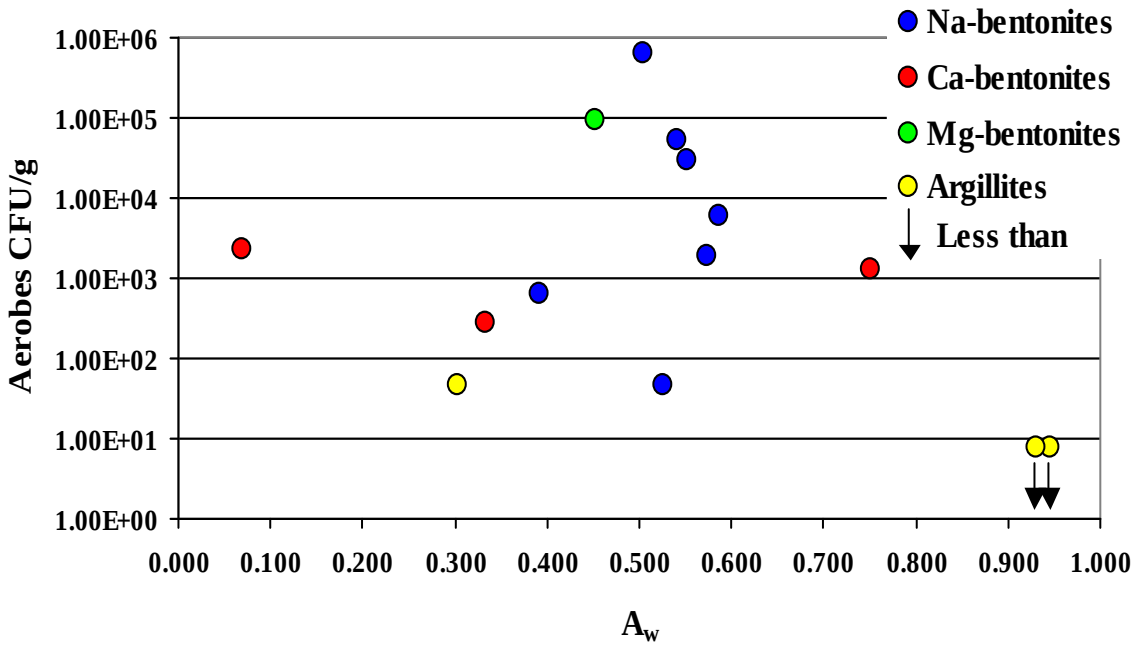


Figure 30: Culturable Heterotrophic Aerobes as a Function of Water Activity in ABM Samples

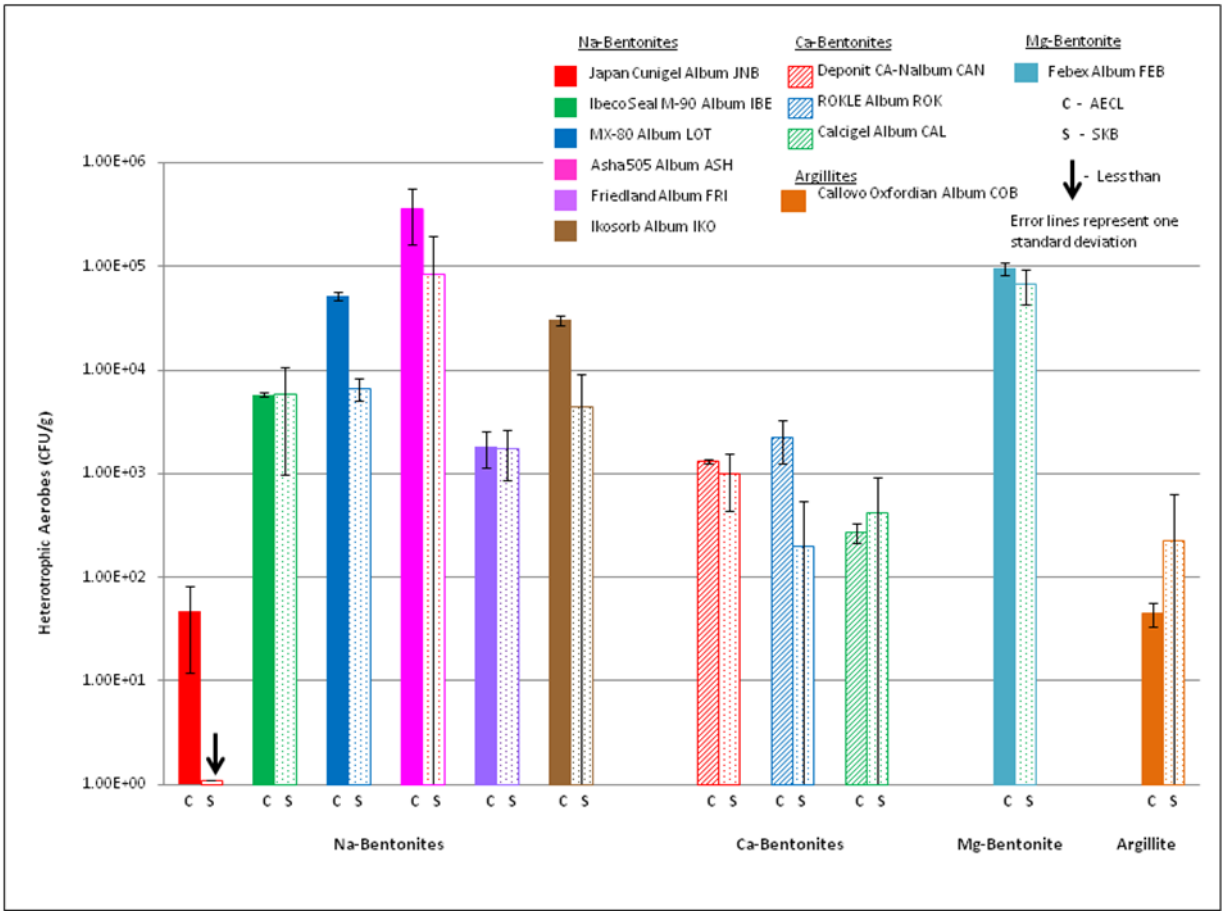


Figure 31: Comparison of the Numbers of Culturable Heterotrophic Aerobic Bacteria in ABM Samples Obtained by AECL and SKB

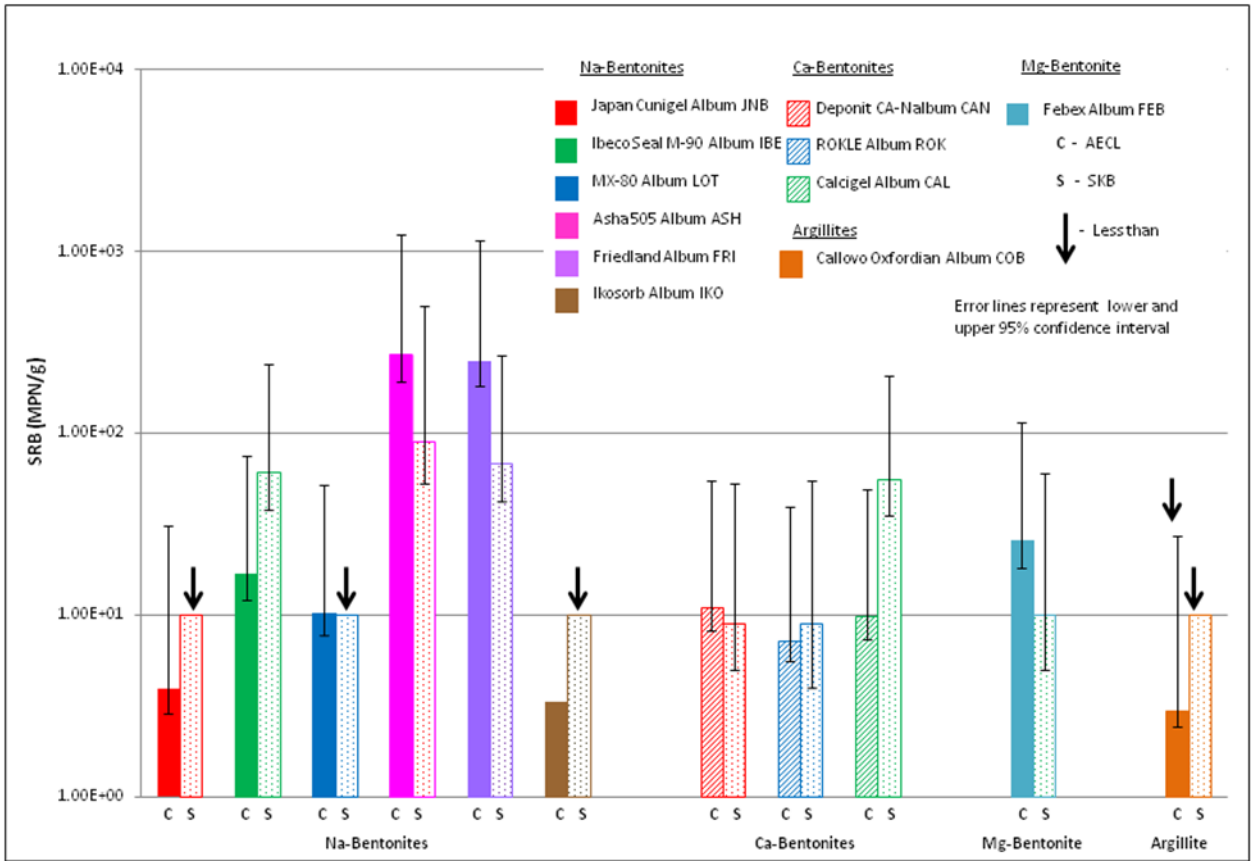


Figure 32: Comparison of the Numbers of Culturable Sulphate-reducing Bacteria in ABM Samples Obtained by AECL and SKB

ABM. Test layout, Package 1

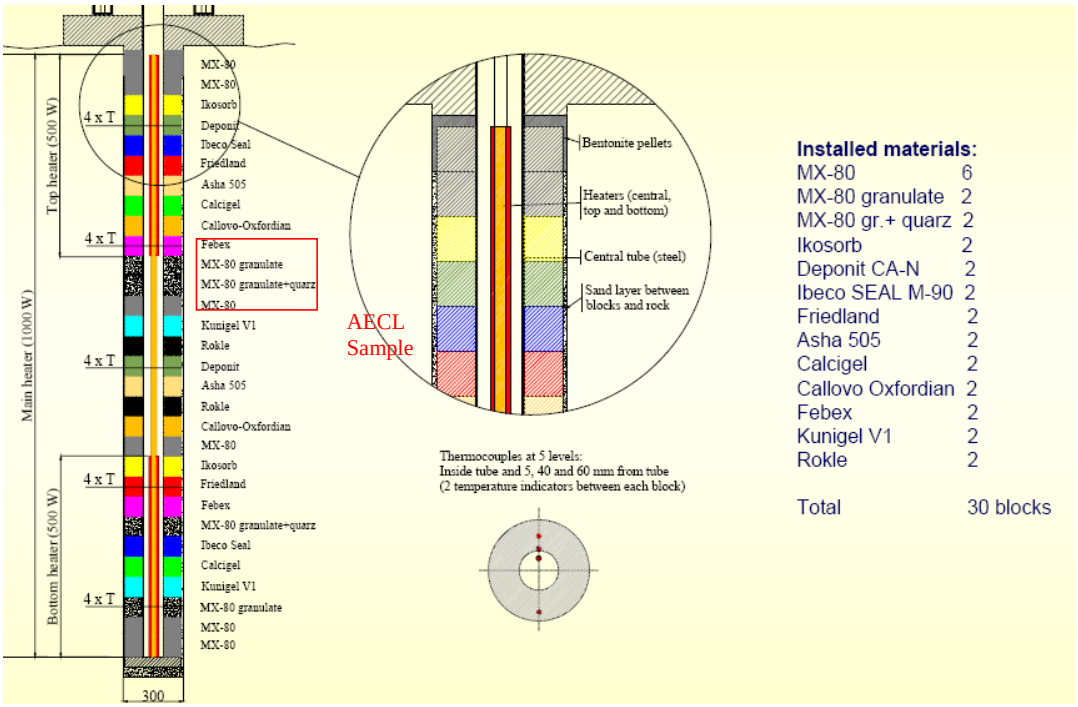


Figure 33: Detailed Schematic of ABM Test Package 1

Package 1, Temperature developement

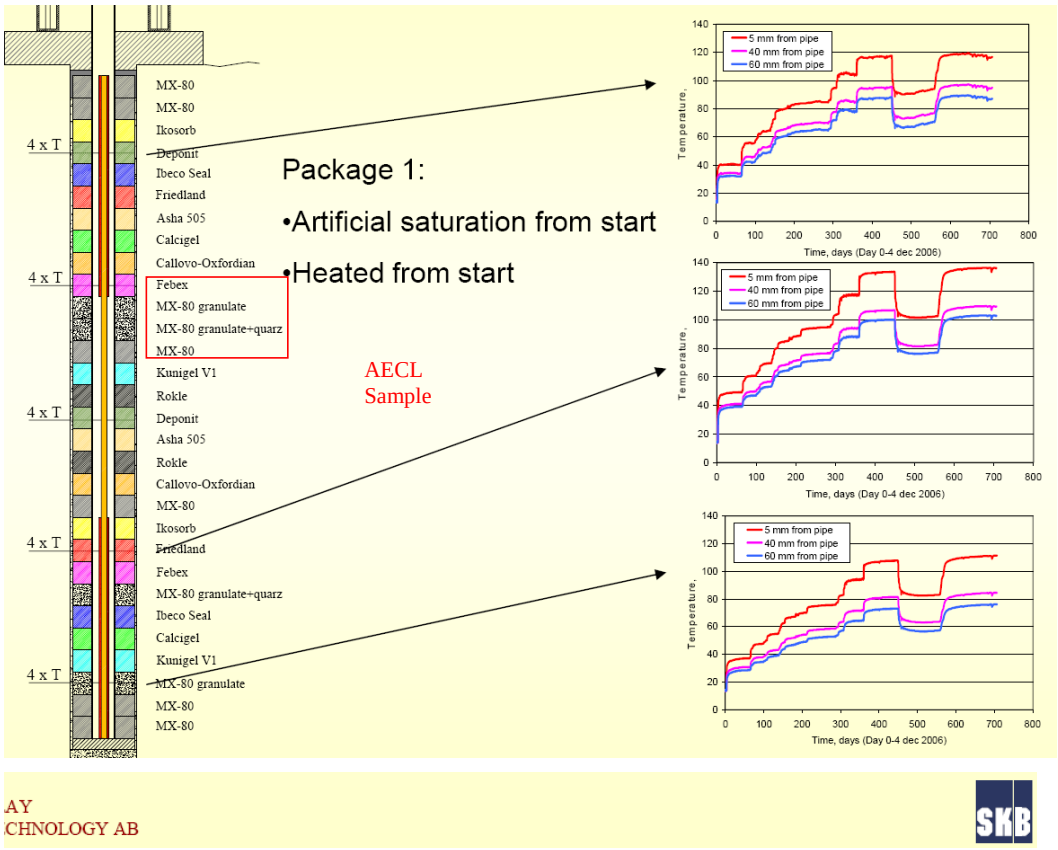


Figure 34: Temperature Development in Various Regions of ABM Test Package 1

Package 1, Temperature distribution

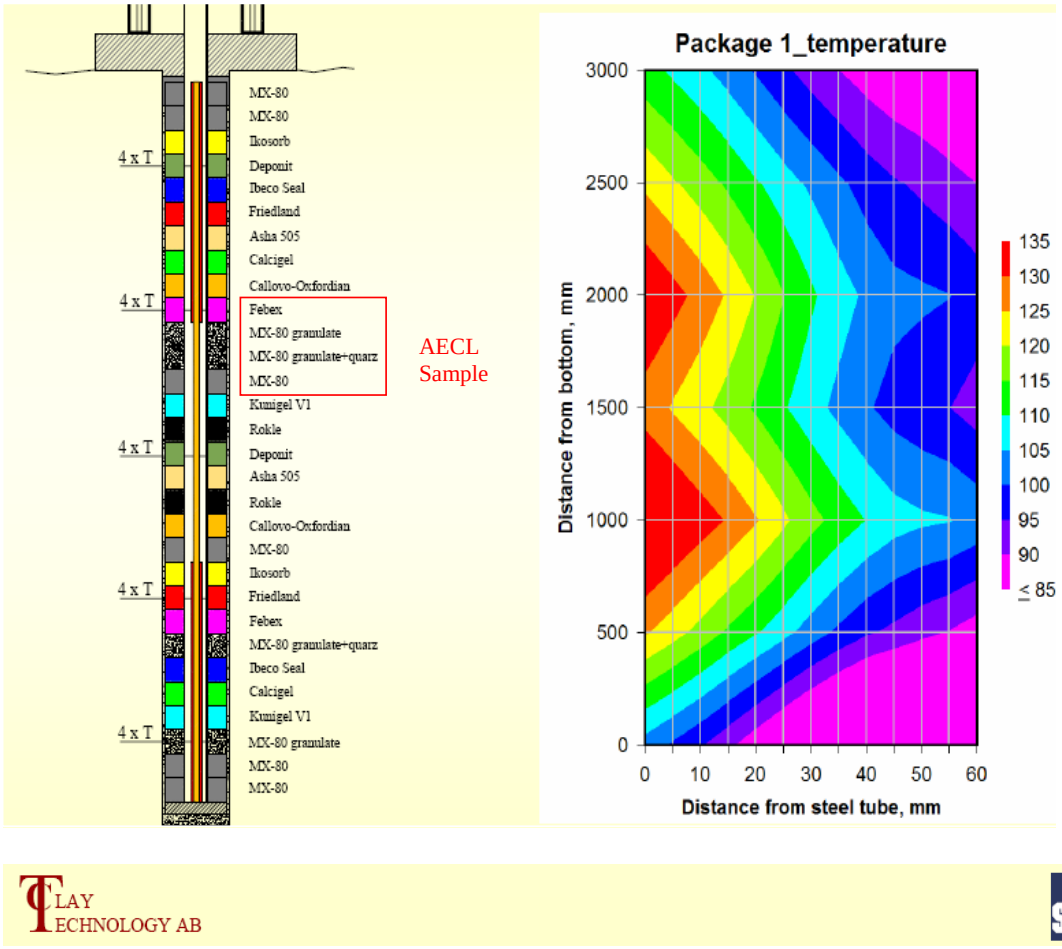


Figure 35: Detailed Temperature Distribution in all of ABM Test Package 1

Artificial water saturation

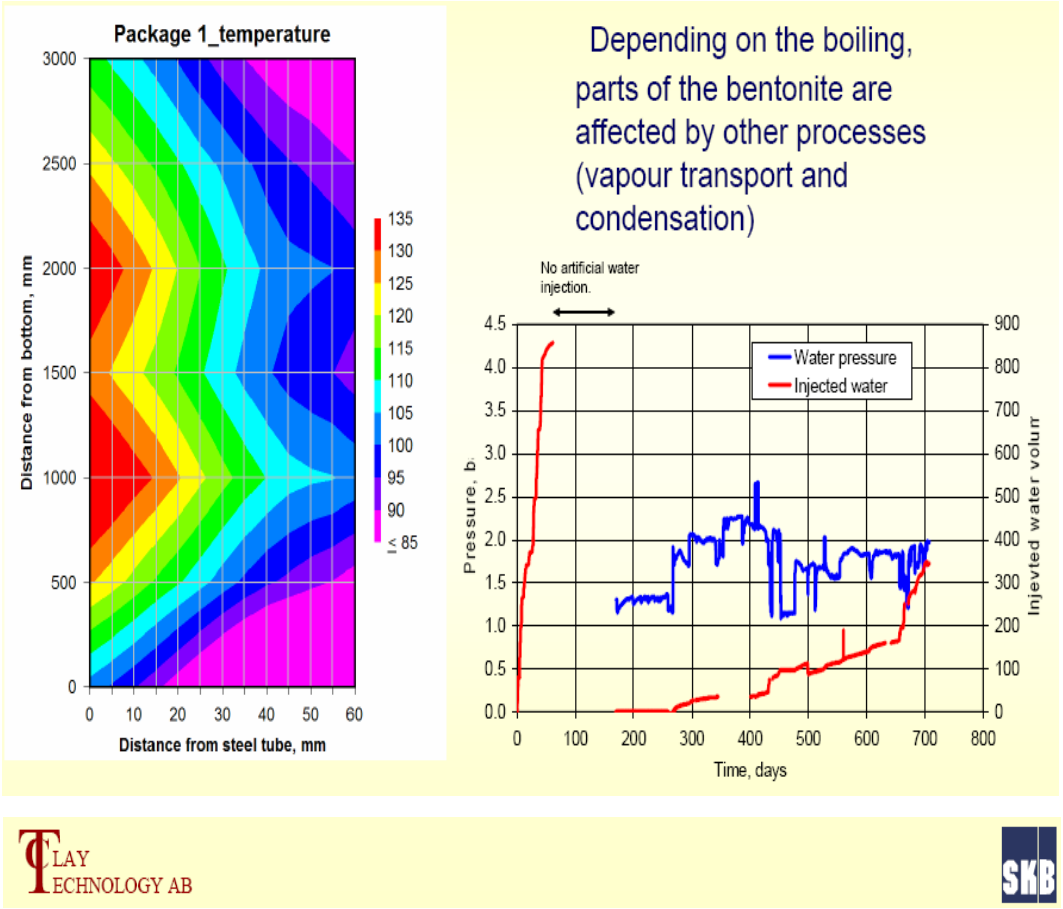
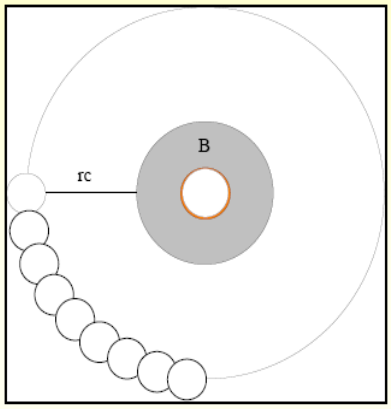


Figure 36: Artificial Water Saturation in ABM Test Package 1

Termination of Package 1

Technique used for the LOT parcels:

- Heater decrease in steps during 3-4 weeks.
- Overlapping drilling around the test hole (percussion drilling)



Drilling:

Depth	3.1 m
Diameter	89 mm
Number of cores	32
Total length of cores	ca 98 m
Rock cover (rc)	175 mm

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Figure 37: Planned Method for Overcoring of ABM Test Package 1

Termination of Package 1

- 2 x core drilled holes (d=300 mm)
- Wire sawing in order to release the package in the bottom

LOT Technique

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SKB

Figure 38: Overcoring Technique as Applied to the LOT Experiment at Äspö

Termination of Package 1

- The lifting is made by use of steel wires placed in three directions around the package
- Due to the sand filter in ABM, it is important to secure the steel tube and the bentonite relative the rock parcel. A scenario could otherwise be that the bottom of the rock column falls off and the bentonite and tube with it.

LOT Technique

CLAY TECHNOLOGY AB

SKB

Figure 39: Package Lifting Technique as Applied to the LOT Experiment at Äspö

Termination of Package 1

In order to stabilize the rock column during lifting, beams are positioned around it and straps are used to keep everything in place.

Test parcel:

Length	3.1 m
Diameter	~0.65 m
Weight	~2800 kg

Figure 40: Package and Rock Column Stabilizing Technique as Applied to the LOT Experiment at Äspö

Transport to a dry niche

Lot Technique

TLAY TECHNOLOGY AB SKB

Figure 41: Horizontal Package Transport Technique as Applied to the LOT Experiment at Äspö

Releasing the bentonite from the rock

The rock will be removed by use of existing fractures and by making new cuts with an angle grinder. Other tools are drilling machine and wedges.

Lot Technique

CLAY TECHNOLOGY AB SKB

Figure 42: Rock Removal Technique as Applied to the LOT Experiment at Äspö

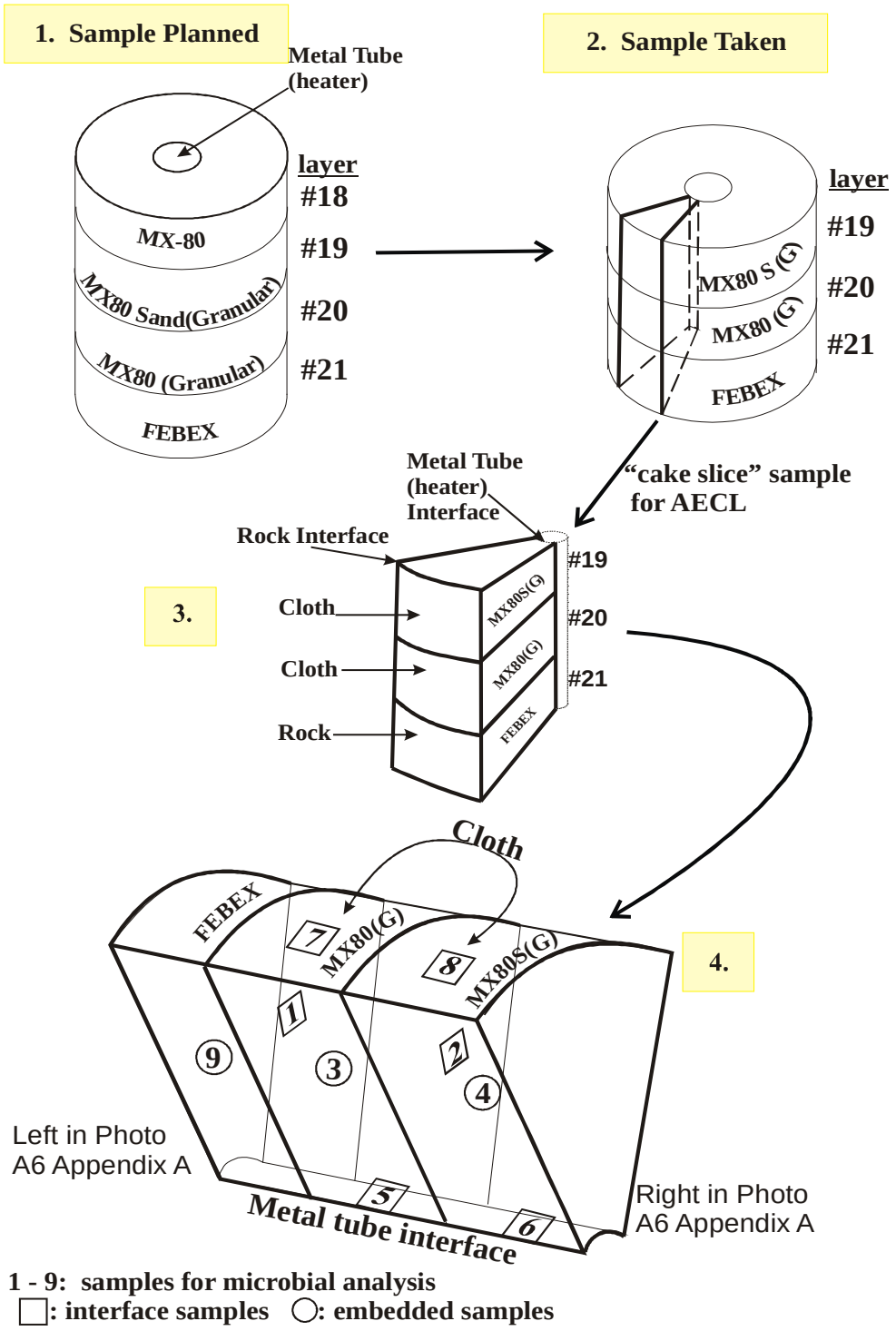

Figure 43: Schematic of the ABM Test Package 1 Sample Analyzed by AECL and Location of the Nine Sub-samples for Microbial Analysis

APPENDIX A: INFORMATION REPORT ON PREPARATION OF BENTONITE GRANULATE SAMPLES

From Hanspeter Weber, Nagra, Oct. 26, 2009

According to NAGRA order, no. 905.070.962.09, we produced two bentonite granulate samples of ca. 50 kg each as specified below. We sent the samples on 02.06.06 to the following address:

Anders Eng
Project Manager
Swedish Nuclear Fuel and Waste Management Co
Äspö Hard Rock Laboratory
PL 300
SE-572 95 Figeholm
Sweden

Product I

Material: 100% MX80

Particle grain size: binary system 70% 7-15 mm
30% 0,5 1,0 mm

Production conditions:

1. compaction with roll press, specific compression force 56 kN/cm, compact size ca. 80×20×10mm;
2. screening of press product, > 10mm to be crushed, < 10mm to be recycled;
3. crushing of press product with jaw crusher;
4. screening of crushed product into fractions 7-15mm, 0,5-1,0mm; and
5. spheronizing of coarse fraction in IfB- test drum (20 rev.)

Product quality:
- apparent density of coarse grains (7-15mm) 2,16 g/cm^3
- installation density of binary granulate system 1,243 g/cm^3, (measured under pouring conditions)

Product II

Material: 70% MX80 + 30% quartz (0,1-0,5mm)

Particle grain size: binary system 70% 7-15 mm
30% 0,5 1,0 mm

Production conditions:

1. compaction with roll press, specific compression force 64 kN/cm, compact size ca. 80×20×10mm;

2. screening of press product, > 10mm to be crushed, < 10mm to be recycled;
3. crushing of press product with jaw crusher;
4. screening of crushed product into fractions 7-15mm, 0,5-1,0mm; and
5. spheronizing of coarse fraction in IfB- test drum (10 rev.).

Product quality:

- apparent density of coarse grains (7-15mm) 2,27 g/cm3
- installation density of binary granulate system 1,282 g/cm3, (measured under pouring conditions)

APPENDIX B: INFORMATION ON SAMPLE TAKEN FOR AECL FOR MICROBIOLOGY STUDIES

Sampling team: Urs Mäder, Torbjörn Sanden, Ulf Nielsson, Margariata Koroleva

Notes by Urs Mäder

Lifting of ABM Parcel #1: May 11, 2009, 23:00

On-site sectioning of ABM Parcel #1 at Äspö: May 12, 2009

Transport to CT / Lund: May 13, 2009

Sampling date: May 15, 2009

Objective: Sample of 3 blocks with interfaces between blocks not disturbed or exposed to atmosphere.

Figure B1: ABM #1 Blocks 18/19/20/21 (15.05.2009, 08:08) Quadruple pack of blocks sealed in the original plasticized aluminum bag applied at Aspo. The height is about 40 cm and it was heavy.

Figure B2: ABM #1 Blocks 18/19/20/21 (15.05.2009, 08:11) Quadruple pack of blocks in upside down position, from top to base:
Block 18 (MX-80)
Block 19 (MX-80 s/b granulate)
Block 20 (MX-80 granulate
Block 21 (FEBEX)
Sand can be seen stuck to the outer surface of the top and bottom blocks. There is hardly any clay material surrounding the two middle blocks (granulated cages) – the fabric of the cages is exposed. The vertical grooves were hosting either water saturation lines or wire lines to sensors. The black straps were already applied at Aspo for support. The opened stack was wrapped in plastic film immediately after opening and taking the picture.

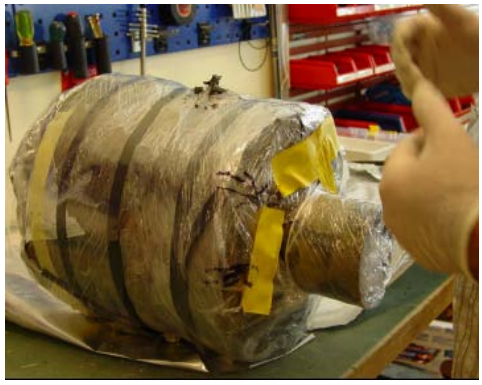

Figure B3: Packet of three blocks (19/20/21) after removal of MX-80 bentonite block (18). Block 18 was removed by mistake – it should have been block 21 (FEBEX). The packet was protected by plastic film immediately after it was unpacked from the plasticized aluminum foil (e.g. after image 19 was taken).

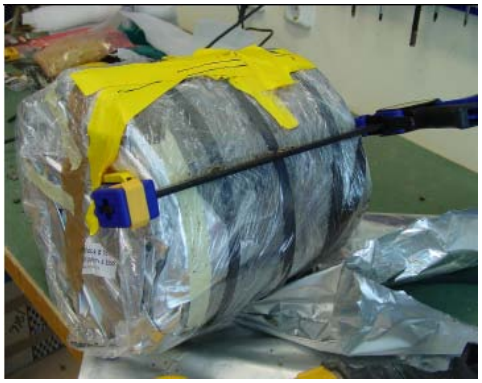

Figure B4: Packet of three blocks (19/20/21) after removal of MX-80 bentonite block (18). A clamp was used to ensure that the blocks would not part during sample handling. Sample handling included cutting through the outer steel bars of the two granulate cages. This was done with a tiger saw and involved some force. The "piece of cake" to be cut out was located between the radial "spokes" of the cage placed at 90º angles.

Figure B5: After cutting the outer steel bars of the two granulate cases (block 19 and 20), the stack of 3 blocks could be cut radially with a band saw. After these two radial cuts the "piece of cake" could be gently separated from the inner steel tube. The result is shown above with the clamp still attached. The sample was placed on plastic film.

Figure B6: Close up of the "piece of cake". There are no separation lines visible between the blocks. The slightly greenish colour of the FEBEX bentonite (block 21) is visible on the left. The MX-80 granulate is in the middle (block 20), and the MX-80 s/b granulate is on the right side (block 19). There is fabric still attached to the outer surface of the granulate material. This fabric was placed there initially to allow filling of the granulate into the stack.

Figure B7: AECL sample after vacuum packaging with plasticized aluminum. Two sealed bags were applied for better physical stability of the sample. There is some plastic film as an innermost wrapping.

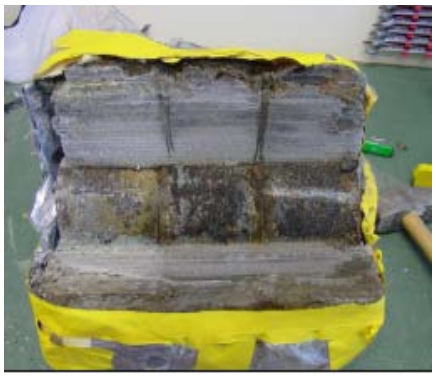

Figure B8: View of the triple block after cutting out the "piece of cake" for AECL studies. The FEBEX bentonite block (21) is on the left, MX-80 granulate (20) in the middle, and MX-80 s/b granulate on the left.

The above is a subset of images taken to document the sampling of the quadruple stack of blocks (18-21). More details to follow in the notes including all images.

Storage and handling conditions: the quadruple block was stored at ambient conditions inside its plasticized aluminum vacuum pack between Tuesday and Friday. The sampling as described above was conducted on Friday morning. It was impossible to conduct the work under sterile conditions or in a glove box due to restrictions imposed by the weight (>40 kg) and the heavy work required to cut through the steel cages. The exposure to atmosphere was minimized by applying plastic film on exposed surfaces. Handling was performed wearing gloves. Work on exposed bentonite was performed with a face mask on. It is reasonable to assume that the inside of the inside of the bentonite sample was not contaminated during the lifting, parting and dissecting.