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 **July 2014**

S. Stroes-Gascoyne[1], C.J. Hamon[1], D. Priyanto[1], D. Jalique[2], C. Kohle[1], W. Evenden[1], A. Grigoryan[2], D.K. Korber[2]

[1] Atomic Energy of Canada Limited, Whiteshell Laboratories, Pinawa, Manitoba

[2] University of Saskatchewan, Department of Food and Bioproduct Sciences, Saskatoon, Saskatchewan

nwmo
NUCLEAR WASTE MANAGEMENT ORGANIZATION
SOCIÉTÉ DE GESTION DES DÉCHETS NUCLÉAIRES

Nuclear Waste Management Organization
22 St. Clair Avenue East, 6th Floor
Toronto, Ontario
M4T 2S3
Canada

Tel: 416-934-9814
Web: www.nwmo.ca

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

July 2014

S. Stroes-Gascoyne[1], C.J. Hamon[1], D. Priyanto[1], D. Jalique[2], C. Kohle[1], W. Evenden[1], A. Grigoryan[2], D.K. Korber[2]

[1]Atomic Energy of Canada Limited, Whiteshell Laboratories, Pinawa, Manitoba

[2]University of Saskatchewan, Department of Food and Bioproduct Sciences, Saskatoon, Saskatchewan

Document History

Title:	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		
Report Number:	NWMO TR-2014-20		
Revision:	R000	Date:	July 2014
[1] Atomic Energy of Canada Limited, Whiteshell Laboratories, Pinawa, Manitoba [2] University of Saskatchewan, Department of Food and Bioproduct Sciences, Saskatoon, Saskatchewan			
Authored by:	S. Stroes-Gascoyne[1] C.J. Hamon[1] D. Priyanto[1] D. Jalique[2] C. Kohle[1] W. Evenden[1] A. Grigoryan[2] D.K. Korber[2]		
Verified by:	S. Stroes-Gascoyne		
Approved by:	S. Stroes-Gascoyne		
Nuclear Waste Management Organization			
Reviewed by:	Jennifer McKelvie Alan Murchison		
Accepted by:	Chris Hatton		

ABSTRACT

Title: **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**
Report No.: **NWMO TR-2014-20**
Author(s): S. Stroes-Gascoyne[1], C.J. Hamon[1], D. Priyanto[1], D. Jalique[2], C. Kohle[1], W. Evenden[1], A. Grigoryan[2], D.K. Korber[2]
Company: [1]Atomic Energy of Canada Limited
[2]University of Saskatchewan
Date: July 2014

Abstract
Highly compacted, bentonite-based sealing systems are being developed for potential use in many nuclear fuel waste repository concepts. Due to the inherent physical characteristics such as small pores and high swelling pressure, one important role of compacted bentonite is the reduction of significant microbial activity near the used fuel containers, which would reduce or eliminate the possibility of microbially influenced corrosion (MIC). Many NWMO-sponsored laboratory experiments were carried out over the period 2005-2010 with highly compacted Wyoming MX-80 bentonite to determine the microbial occurrence, survival and viability in highly compacted bentonite. Results suggested that microbial activity in the bulk of compacted 100% Wyoming MX-80 bentonite can be controlled as long as the emplaced bentonite has a (uniform) dry density $\geq$1.6 g/cm^3, which ensures that the swelling pressure is ≥2 MPa, water activity is ≤ 0.96 and the average pore size is < 0.02 µm. Most of the previous experiments were of short duration (40-90 days). An experiment started in 2006, at a theoretical dry density of 2.0 g/cm^3 and infused with sterilized distilled deionised water, was continued for a period of 7 years 264 days (2811 days). On September 23 2013, this experiment was terminated and the bentonite plug analyzed as before. Results were compared with those obtained from the much shorter-duration experiments. The new results show that the number of viable cells in the compacted bentonite plug hovered around what is present in the "dry" clay. This was also observed for the previous experiments and these new results confirm that this remains so for almost 8 years. The cells that survive likely do so in a reduced viability form or in the form of spores: The few species that could be identified from 20 colonies on aerobic plates with the BIOLOG^{TM} method indicated the presence of soil bacteria with a high tolerance for environmental stress, spore formers and possibly some human-origin bacteria. Forty-six purified isolates from colonies on aerobic plates, identified by DNA extraction, amplification and sequencing, gave 30 positive identifications comprising 15 different species of *Bacillus*, *Paenibacillus* and *Brevibacillus*, all spore-forming organisms.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. RECAP FROM EARLIER WORK

Highly compacted, bentonite-based sealing systems are being developed for potential use in many nuclear fuel waste repository concepts. Due to the inherent physical characteristics, such as small pores and high swelling pressure, compacted bentonite is able to inhibit microbial activity near the used fuel containers, which would reduce or eliminate the possibility of microbially influenced corrosion (MIC). Many NWMO-sponsored laboratory experiments were carried out over the period 2005-2010 with Wyoming MX-80 bentonite, compacted (at 95% saturation) to a range of dry densities (0.8 to 2.0 g/cm^3), and infiltrated (under pressure) with sterile distilled deionised water or sterile NaCl solutions of 50 to 200 g NaCl/L. During the tests (durations from 40 to 90 d), total pressure values were recorded. Upon termination, the bentonite plugs were analyzed for water content, water activity (a_w) and dry density. Swelling pressure values were calculated from total pressure data. Concurrent microbial analyses included culturing for heterotrophic aerobes, anaerobes and sulphate-reducing bacteria (SRB). The average pore size was measured on some samples using mercury intrusion porosimetry. The effects of temperature on the microbial population in highly compacted Wyoming MX-80 bentonite were also studied (Stroes-Gascoyne et al., 2005, 2006, 2007, 2010a, b; Stroes-Gascoyne and Hamon, 2008, 2010).

The physical measurements confirmed that a_w is a function of both dry density and porewater salinity, with the latter becoming the dominant control on a_w at high salinities. Swelling pressure values at a given porewater salinity were low at dry density values of 0.8 and 1.3 g/cm^3, but increased significantly at dry density > 1.3 g/cm^3. An increase in porewater salinity caused a decrease in swelling pressure for a given dry density. The average pore diameter in bentonite samples with a dry density of 1.6 g/cm^3 appeared to increase slightly with increasing porewater salinity but remained unimodal and in the range of 0.01 to 0.02 µm. At dry densities of 0.8 and 1.3 g/cm^3 and porewater salinities of 0 and 50 g/L, culturability of heterotrophic aerobic bacteria increased by up to four orders of magnitude above background levels ($(2.1 \pm 0.2) \times 10^2$ Colony Forming Units (CFU) per g "dry" bentonite). However, SRB did not increase above background levels in any of the tests. At higher dry densities (1.6 to 2.0 g/cm^3) and higher porewater salinities (100 to 200 g/L) aerobic and anaerobic culturability remained at, or fell below, the background levels in "dry" bentonite. However, in all combinations of dry density and porewater salinity tested, some culturability remained. It is hypothesized that these surviving organisms are either almost inactive, dormant cells, or metabolically inactive spores.

With respect to a_w and swelling pressure values, culturability increased exponentially around $a_w \geq 0.96$ but decreased sharply at swelling pressures of $\geq$ 2 MPa. Both a_w and swelling pressure are influenced by the dry density of the bentonite. These results suggest that microbial activity in the bulk of compacted 100% bentonite can be controlled as long as the emplaced bentonite has a (uniform) dry density $\geq$1.6 g/cm^3, which ensures that the swelling pressure is ≥ 2 MPa, a_w is ≤ 0.96 and the average pore size is < 0.02 µm. Therefore, depending on the specific requirements identified, dry density (and hence swelling pressure and a_w) may be tailored to provide a microbially unfavourable environment adjacent to the used fuel containers, ensuring that MIC is negligible.

The purpose of the experiment described in this report was to determine if the conclusions drawn from the above reviewed experiments (40 to 90 days duration) are valid for much longer time periods. For this purpose, one experiment, started in 2006 at a theoretical dry density of 2.0 g/cm^3 (with infusion of sterilized distilled deionised water), was continued for a period of 7

years 264 days (2811 days). On September 23 2013, this experiment was terminated and the bentonite plug extruded and analyzed as before. This report gives the results obtained from this compacted bentonite plug and compares these with results obtained previously from the much shorter-duration experiments.

2. MATERIALS AND METHODS

2.1 EXPERIMENT 2063

This experiment was started on 2006 January 10. The experimental characteristics were:

- the bentonite used was Wyoming MX-80 bentonite,
- the bentonite was compacted into an ethanol-sterilized pressure cell at a target dry density of 2.0 g/cm^3,
- the bentonite plug was about 2 cm high with a diameter of 3.2 cm,
- before compaction, the bentonite was mixed with infiltration solution such that after compaction the bentonite would be at 95% saturation,
- during the experiment, the plug was infiltrated under pressure to saturation with the infiltration solution, in this case sterilized, distilled deionised water, and
- the experiment was continued for 2811 days (7.7 years).

The solution used is referred to as infiltration water or pore water in this report, although the latter term is not strictly correct. The bentonite pore water may be somewhat different from the infiltration water because of the possible presence of various salts in the as-purchased “dry” bentonite. Total pressure was recorded continuously during the experiment, and the swelling pressure was calculated from the total pressure minus the applied pressure.

Figure 1 shows a photograph of the setup as it remained for 7.7 years.

2.2 EXTRUSION OF PLUG 2063

After termination of the test on September 23 2013, the bentonite plug was extruded with a manual press into a sterile cup (Figure 2). Figure 3 shows the filter stones at the top and bottom of the plug before it was extruded. Previously, a hydraulic press was used for extrusion of plugs but due to the decommissioning of the Underground Research Laboratory (URL) over the past few years, the hydraulic press was no longer available.

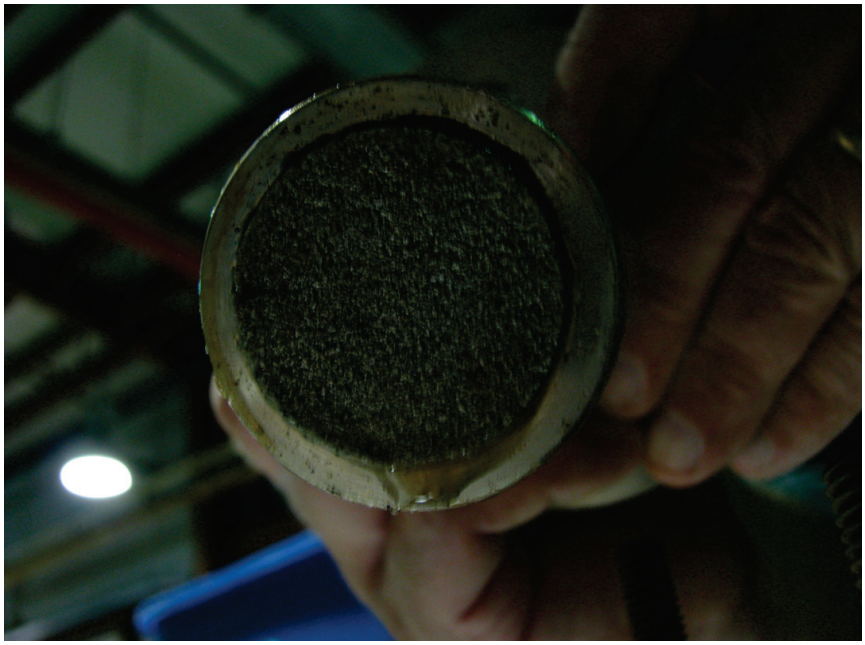
During extrusion of plug 2063, it apparently cracked. This had not happened before in the earlier tests, and it is possible that uneven pressure from the manual press caused the cracking. Figure 4 shows the extruded plug in the sterile cup. Figure 5 (A, B) shows the plug turned out on sterile foil. The crack is clearly visible. Also visible is the undisturbed bottom half of the plug, which is how previous plugs looked when extruded. This part of the plug is, therefore, most likely to give reliable results.

Figure 1: Sample Setup Showing the Pressure Cell containing Plug 2063

Putting plug apparatus on extractor

Figure 2: Extrusion of Plug 2063 with a Manual Press

Top Filter Stone

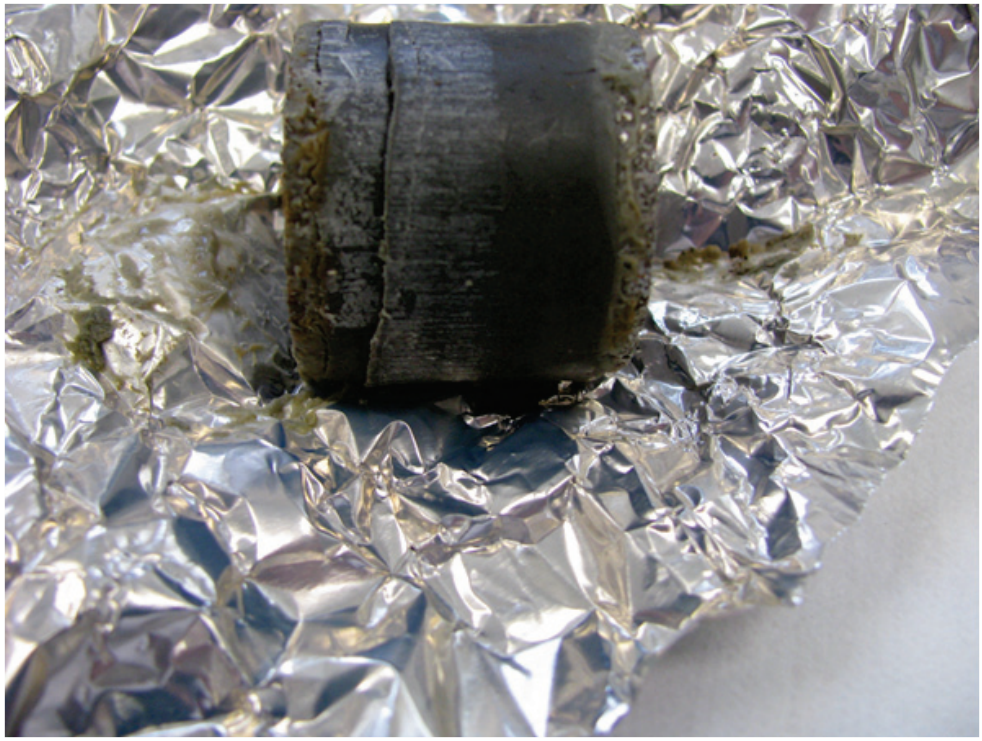


Bottom Filter Stone

Figure 3: Filter Stones at Top and Bottom of Plug 2063

Figure 4: Plug 2063 after Extrusion in Sterile Teflon Cup; top filter stone is visible

(A) plug top up on foil



(B) plug on side on foil

Figure 5: Top of and Side of Plug 2063

Figure 6 (A, B) shows the plug with the bottom and top filter stones removed. Since this was one of the earliest experiments in the series, the top part of the plug also contained a filter paper (filter papers were abandoned in later tests) which was also removed (Figure 6B). Figure 7 shows the plug without filter stones or filter paper on the sterile foil. The extent of cracking is clearly visible.

The plug was then wrapped in sterile foil and plastic, and taken immediately from the URL to the microbial laboratory at Whiteshell Laboratories (WL) for sampling in order to carry out a number of physical and microbial analyses.

2.3 SAMPLING OF PLUG 2063

Upon arrival of plug 2063 in the microbial laboratory at WL, the plug was weighed and measured, the latter being difficult and probably inaccurate because of the distortion of the plug due to the cracking (height of the plug varied from 20 to 24 mm).

Three samples were taken:

1. the outside of the plug (top, sides, bottom) was scraped with a sterile scalpel to determine microbial content at the interfaces (sample 2063a),

2. the plug was split open at the crack with a sterile knife and a sample was taken with sterile tools a little distance below the crack (top half of plug) (sample 2063b), and

3. a third sample was taken with sterile tools from the bottom half of the plug. This area looked undisturbed and probably will give the most accurate results (sample 2063c).

Each sample contained enough material for the following analyses:

- water activity,
- water content,
- aerobic and anaerobic heterotrophic bacteria,
- nitrate utilizing and nitrate reducing bacteria, and
- sulphate-reducing bacteria (SRB)

2.4 ANALYSIS OF SAMPLES FROM PLUG 2063

2.4.1 Water Activity

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

2.4.2 Water Content

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

(A) plug bottom end, no filter stone

(B) plug top end, showing paper filter

Figure 6: Bottom End of Plug, after Removal of Filter Stone (A); top end of plug after removal of filter stone, showing intact filter paper (B)

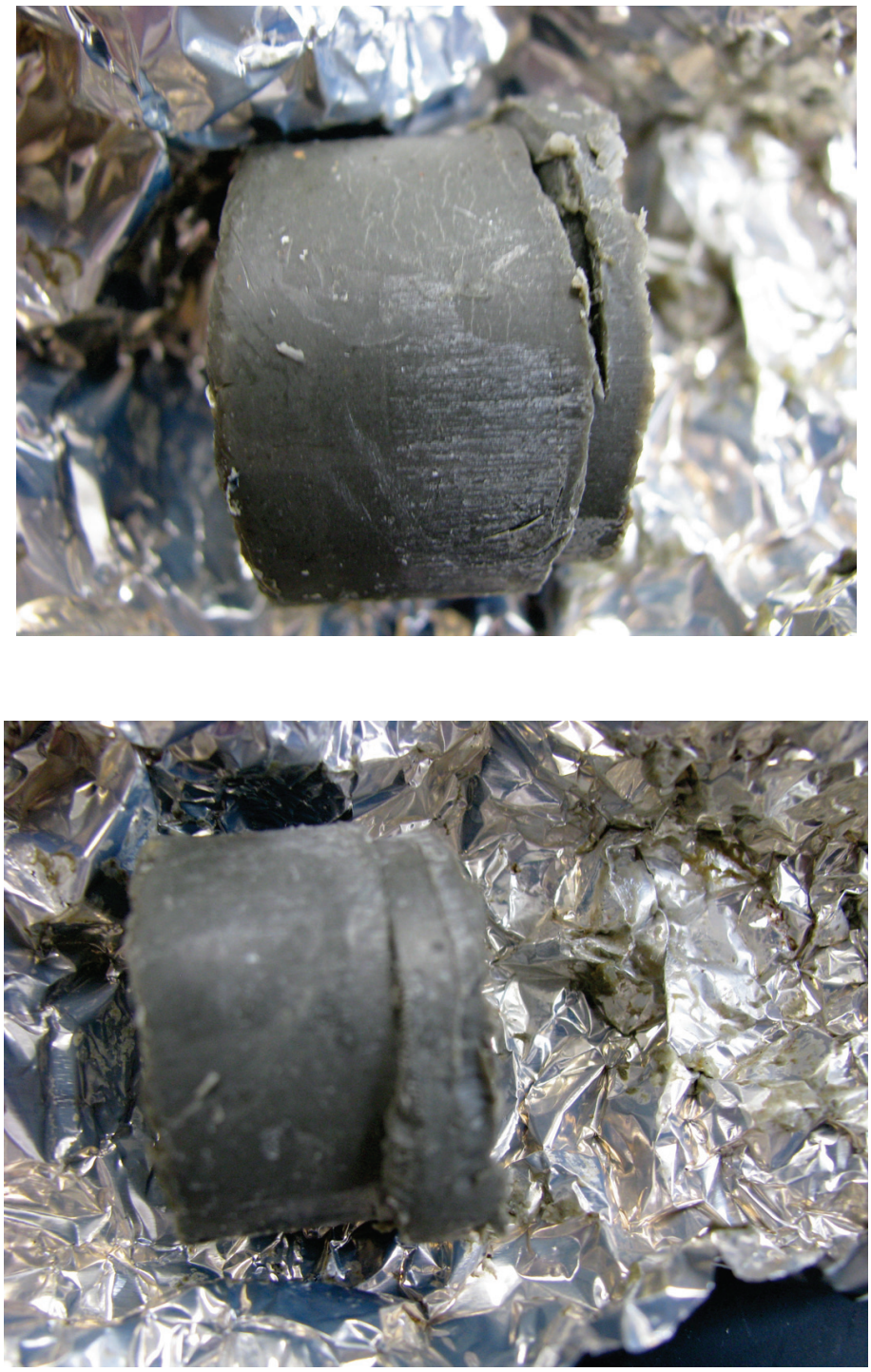


Figure 7: Plug 2063 with Filter Stones and Filter Paper Removed

2.4.3 Heterotrophic Aerobes and Anaerobes

About 10 g (carefully weighed) of bentonite 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. after which serial dilutions were made to 10^{-3}. The dilutions were plated onto R2A agar (Reasoner and Geldreich 1985) in triplicate 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.

2.4.4 Nitrate-utilizing and Nitrate-reducing Bacteria

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, containing inverted Durham tubes and degassed R2A medium (Reasoner and Geldreich 1985) that was amended with 0.1% nitrate, were inoculated with the above PBS bentonite suspension in an anaerobic glovebox (in triplicate), serially diluted to 10^{-3} and scored for gas production (visible in the Durham tubes, NRB) and/or the presence of nitrite (NUB) after about 4 weeks of incubation at 30°C.

2.4.5 SRB

Degassed tubes containing sterile modified Postgate's B medium (Atlas 1993) were inoculated in an anaerobic glovebox (in triplicate) with the PBS clay suspension to 10^{-3} dilutions. The tubes were incubated at 30°C for about 4 weeks before they were scored.

2.4.6 Identifications with BIOLOG™

All colonies (20) from 2063c aerobic triplicate plates A and B of dilution 10^{o} were put through the GEN III BIOLOG™ (2112 Cabot Blvd Hayward, Ca 94545) identification system.

The GEN III BIOLOG™ system comprises a standardized method that uses 96 physiological reactions to identify a broad range of bacteria. The method uses 71 carbon source utilization assays, 23 chemical sensitivity assays, and two control reactions. Nutrients and biochemicals are pre-filled and dried into the 96 wells of BIOLOG micro-plates. Each well can hold 100 µL of cell suspension. The cells must be freshly grown (4-24 hrs) since most organisms lose their viability in the stationary phase and the cell suspension must have a certain density depending on the Protocol used. Tetrazolium redox dyes are used in order to visualize the utilization of carbon sources in the micro-plate wells. Negative wells stay colourless after incubation, while positive wells turn purple. A computerized plate reader is used subsequently to read the plates after incubation. The positive purple wells give a "fingerprint" that is compared to BIOLOG's extensive library of species.

All isolated colonies recovered from sample 2063C aerobic triplicate plates A and B of dilution 10^{0} were characterized by sole carbon source utilization profiling using the BIOLOG GEN III system (according to manufacturer's instructions). Colonies on the plates were picked off with a sterile wood streak tool and streaked onto fresh R2A plates to further isolate and purify

colonies. Colonies that grew were then streaked onto BUG (Biolog Universal Growth) plates in order to obtain freshly growing cells for use in the identification reactions. After 24 hours cells were recovered from the BUG plates using sterile cotton-swabs and suspended in IF (Inoculating Fluid) until a turbidity of 90 to 98 % was obtained. Turbidity was measured with a BIOLOG turbidometer. Inoculating fluids IFA and IFB together with BIOLOG protocols A and B were used. One hundred uL of the cell suspensions were added to each of the 96 wells of the BIOLOG Gen III micro-plates. A computerized plate reader (BIOLOG microstation) was used subsequently to read the plates after 4 to 48 hours of incubation. Purple wells were indicative of a positive reaction, and together gave a fingerprint of the isolate that was compared with the BIOLOG library of species (GEN III database version 6.01)

2.4.7 Identifications with 16S-RNA

Three aerobic plates (one each from samples 2063a, 2063b and 2063c; dilution 10^{o}) were sent in a cooler on ice packs to University of Saskatchewan in Saskatoon for DNA extraction from the colonies on these plate, followed by amplification and sequencing.

The streak plate method was applied to isolate bacterial colonies from the diverse bacterial populations present on these plates. Distinct colonies, based on colour, size and morphological type, were selected for isolation and molecular identification. Bacterial isolates were subcultured at least four times to ensure pure cultures were obtained. A total of 46 bacterial isolates were isolated from the original plates. However, only 30 isolates were PCR amplified (see below).

Identification of bacterial isolates

Isolated bacterial colonies were subjected to DNA extraction following manufacturer's protocol (Feldan, Quebec, Canada). Thereafter, partial 16S rRNA gene was amplified using polymerase chain reaction (PCR) with One *Taq*® DNA polymerase (New England Bio Labs, MA, USA) and universal bacterial primers (8F and 531R (Hirkala and Germida, 2004)). The final concentrations of the reagents used for each 50-µL PCR reaction were as follows: 200 µM deoxyribonucleotide triphosphates (dNTP), 0.2 µM of each primer and 1.25 units of DNA polymerase. 34 µL of sterile Hyclone water (Thermo Fisher Scientific, MA, USA) was added to reach a total volume of 50 µL. Amplifications were performed in a Techne TC-412 thermal cycler (Bibby Scientific Limited, Staffordshire, United Kingdon) using the following conditions: initial denaturation for 10 min at 94ºC denaturation at 94°C; 35 cycles of (94°C for 45 s, annealing at 55^{o}C for 45 s, and elongation at 72^{o}C for 45s); and a final elongation step at 72^{o}C for 10 min. For sequencing, the *c*. 500 bp PCR amplicons were purified using Feldan gel extraction kit (Feldan, Quebec, Canada) according to the manufacturer's instructions. For identification and phylogenetic analysis, 16S rRNA gene sequences from the 34 bacterial isolated were assigned based on similarities to available strains within the NCBI 16S rRNA sequence database using the BLASTn search tool and Ribosomal Database Project (RDP II) (Bondici et al., 2013). Sequences of 16S rRNA were verified using the Chromas Explorer Lite software, version 3.3.0 (Heracle Biosoft S.R.L.). The sequences were also edited manually using Chromas Explorer Lite. Sequences alignments were edited manually using the Clustal Omega (EMBL-EBI; http://www.ebi.ac.uk/Tools/webservices/) (McWilliam et al., 2013). Identification of distinct bacterial isolates was based on the percent identity where 99-100% identical sequences were associated with the same taxon.

Alternative PCR amplification protocol

16 isolates did not produce any PCR products when the protocol above was used. The initial attempt was to use 1492R (Lane, 1991) and 338F (Muyzer et al., 1996) primers. However, no PCR products were produced again. A new PCR master mix was used containing 175 µl of 5x GC buffer (NEB), 56.5 µl of GC enhancer (NEB), 23 µl 10mM dNTP (GE), 17.5 µl HyClone water (ThermoFisher), 5.76 µl of OneTaq DNA polymerase (NEB), and 10 mM of 8F and 531R (Hirkala and Germida, 2004). Unfortunately, no PCR products were generated. Both attempts followed the same PCR conditions.

3. RESULTS AND DISCUSSION

The results from the physical and microbial analyses are given in Table 1. The results are compared with previous results (Stroes-Gascoyne et al., 2010a) in Figures 8 to 15. The previous data that were used to compose these graphs can be found in Appendix A (Tables A-1 and A-2).

Sample 2063a was taken (scraped) from the outside (interfaces) of the plug. Table 1 shows that this sample contained high numbers of heterotrophic aerobes (HAB) ((1.08±0.12) $x10^4$ CFU/g) and nitrate-utilizing bacteria (NUB) (1.83 x 10^5 CFU/g). This is not unexpected as higher numbers of culturable bacteria at bentonite-confining interfaces have been observed before (e.g. Stroes-Gascoyne et al., 2002) and are likely a result of the interfaces being wetter and more spacious (clay gel layer) than locations in the compacted bentonite matrix. The water activity in sample 2063a is quite low with respect to the high number of culturable HAB and NUB found, but may not reflect the true water activity at the interface, because it is influenced by the amount of matrix material scraped off the outside of the plug.

Sample 2063b (taken from the distorted top half of the plug) shows low numbers of HAB ((1.02±0.26) $x10^2$ CFU/g) but high numbers of NUB (1.16 x 10^4 CFU/g). The latter is unexpected and could point to possible contamination of this sample. Due to the cracking and distortion of the top half of plug 2063 it is possible that this sample was contaminated with material from the outside of the plug (the interface location that contained high numbers of HAB and NUB). Unfortunately, NUB and NRB were not enumerated in the previous experiments except for sample 1847 (Table 1), the dry bentonite (analyzed in 2008). This sample shows comparable numbers of HAB and NUB.

Because of the possibility that sample 2063b may have been contaminated as a result of the cracking and distortion of the plug, it was decided that the only trustworthy results are those from sample 2063c, the undisturbed bottom half of the plug. Therefore, only the results from sample 2063c are plotted Figures 8 to 15. Since the water content of the bentonite samples is likely the most accurately measurable parameter, the calculations of the dry density of sample 2063c based on that samples' moisture content is considered the most accurate value of the dry density of sample 2063c.

The calculations of dry density and effective montmorillonite dry density (EMDD) using the water content percentage from sample 2063c can be found in Appendix B.

Figure 8 (A and B) shows the water activity of sample 2063c as a function of water content and compares the measurement from this 7.7 year old experiment with previous experiments with

distilled deionised water (Figure 8A) and against all measurements, including the ones with high salt concentrations (Figure 8B) (data from Stroes-Gascoyne et al., 2010; see Tables A1 and A2 in Appendix A). The graph shows good agreement between the measurement from the 7.7 year old bentonite plug and the previous experiments (Stroes-Gascoyne et al., 2010a) which were carried out for mostly much shorter durations (40-90 days).

Table 1: Physical and Culture Results for Plug 2063

Sample	1847*	2063a	2063b	2063c
Description	bentonite powder	outside of plug	top half of plug (distorted)	bottom of plug
Water content (% o dry wt)	7.55	24.45	24.94	22.37
Water activity	0.393	0.913	0.928	0.874
Heterotrophic Aerobes (CFU/g)	$(6.24\pm2.34) \times 10^2$	$(1.08\pm0.12) \times 10^4$	$(1.02\pm0.26) \times 10^2$	$(1.28\pm0.65) \times 10^2$
Heterotrophic Anaerobes (CFU/g)	$(1.74\pm0.60) \times 10^1$	$(3.29\pm1.43) \times 10^1$	$(2.55\pm0.0) \times 10^1$	$(1.74\pm0.60) \times 10^1$
Nitrate utilizers (MPN/g)	9.62×10^2	1.83×10^5	1.16×10^4	6.43×10^2
Nitrate reducers (MPN/g)	<3.14	<7.5	<3.75	<4.29
Sulphate reducers (MPN/g)	<3.14	<7.41	<3.83	<4.26
Dry density** (g/cm^3)	N/A	1.85	1.85	1.85
Dry density*** (g/cm^3)	N/A	N/C	N/C	1.70
EMDD*** (g/cm^3)	N/A	N/C	N/C	1.52
Swelling Pressure (MPa)	N/A	23.3	23.3	23.3

*Previous Analysis (C.J. Hamon, unpublished results 2008)
**Averaged for whole plug (using plug thickness of 21 mm)
***Calculated from water content of sample 2063c, see Appendix A.

CFU = Colony Forming Units
MPN = Most Probable Number
N/A = Not applicable
N/C = Not calculated
MPa = Mega Pascals
EMDD = effective Montmorillonite dry density

Figure 9 (A and B) shows water activity as a function of dry density. For sample 2063c "measured" dry density was derived from the measured dimensions (i.e., total volume) of the plug, the weight and the water content. However, the volume obtained from the dimensions of

the plug was likely not accurate due to the cracking of the plug and the resulting lopsidedness of the top part of the plug. Therefore, the “measured” dry density value is likely less accurate than the “calculated” dry density value, which was calculated from the water content in the plug, as shown in Appendix B. Both values are shown on Figure 9A and B. The value calculated from the water content appears to fit better with the previous results.

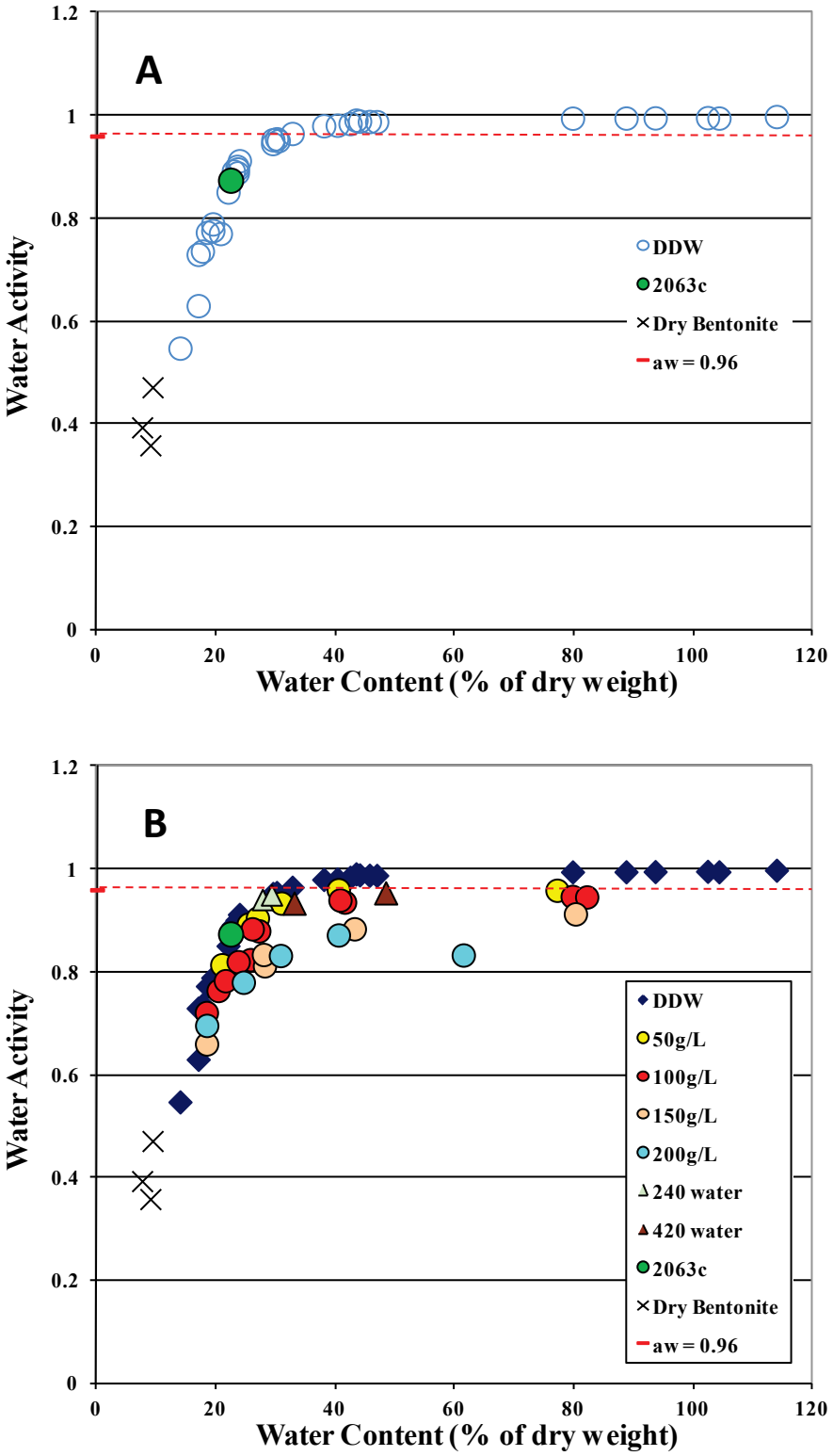


Figure 8: Water Activity Versus Water Content for DDW Experiments (A) and DDW + Saline Experiments (B). Data point from plug 2063 shown in green

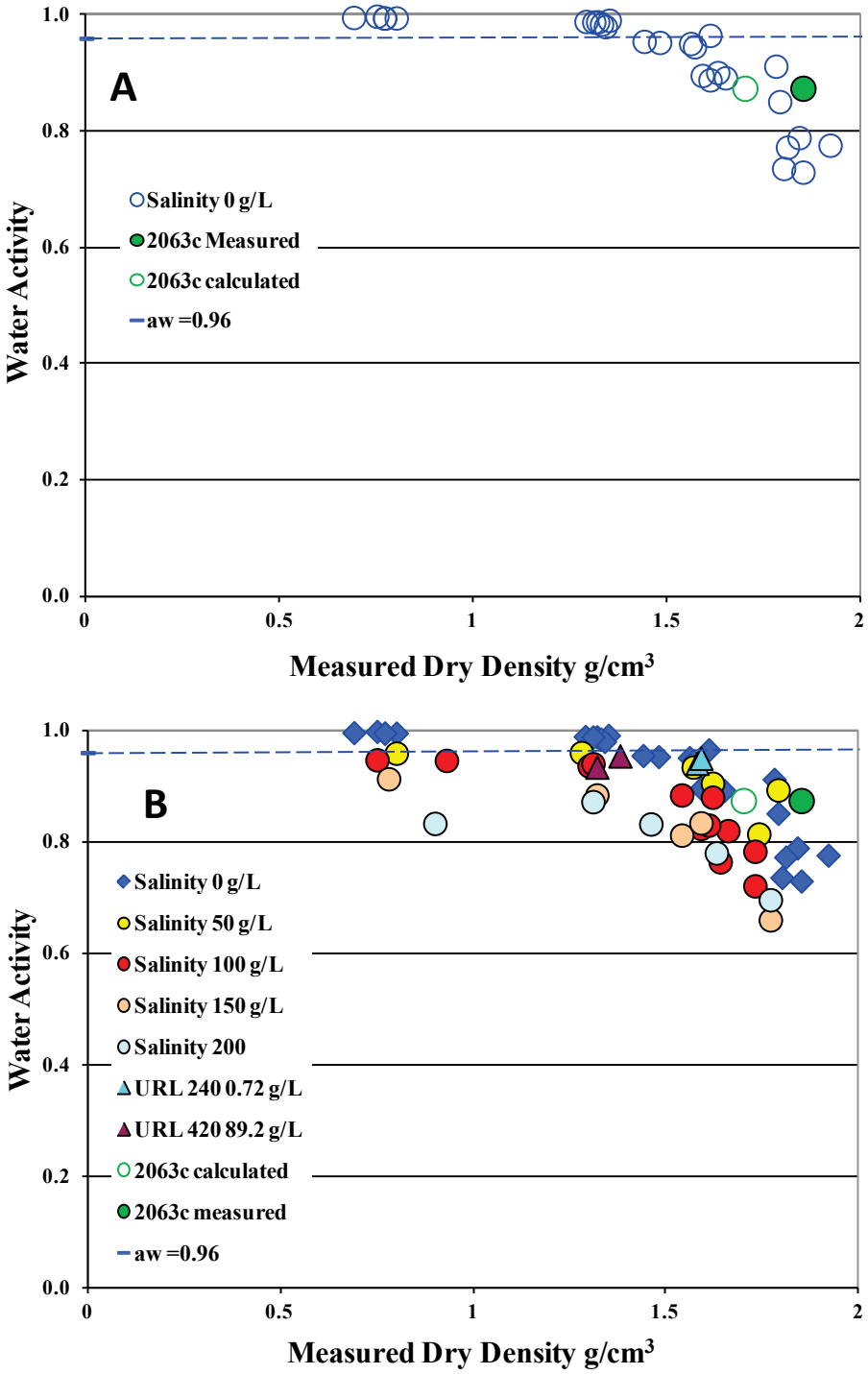


Figure 9: Water Activity Versus Measured Dry Density for DDW Experiments (A) and DDW + Saline Experiments (B). Data points from plug 2063 shown in green (open circle = dry density calculated from water content; closed circle = dry density calculated from measured volume of plug)

Figure 10 (A and B) shows the measured swelling pressure (Appendix B) against the dry density. Again, both the measured and the calculated values are plotted. Both values appear to agree reasonably well with previous measurements, both for distilled deionised water (Figure 10A) as well as for measurements in saline solutions (Figure 10B).

Figure 11 (A and B) plots the swelling pressure against the water activity measured in sample 2063c, both for distilled deionised water (Figure 11 A) and for all measurements (Figure 11 B). It appears that either the swelling pressure is somewhat on the high side or that the measured water activity was on the high side.

Figure 12 shows the number of aerobes (CFU/g) versus water activity for distilled-water infused samples (Figure 12A) and for saline water infused samples (Figure 12B). The measurements for sample 2063c agree well with the previous measurements (Stroes-Gascoyne et al., 2010a).

Figure 13 plots the number of aerobes (CFU/g) against measured dry density of previous experiments for distilled deionised-water infused samples (Figure 13A) and for saline water-infused samples (Figure 13B). Both the measured and calculated dry density for sample 2063c are shown on these graphs and good agreement is apparent.

Figure 14A plots number of aerobes (CFU/g) against swelling pressure for bentonite plugs with different (target) dry densities infused with distilled deionised water, while Figure 14B shows that comparison for saline-infused plugs. Again, good agreement is apparent.

Figure 15 (A and B) plots the number of aerobes (CFU/g) against the effective montmorillonite dry density (EMDD) of previous experiments. The EMDD is a unifying factor which makes comparisons between different bentonites possible on the basis of montmorillonite content. This graph (from Stroes-Gascoyne et al., 2010a) includes data from natural sites in Japan and shows there is good agreement for the measurements obtained from sample 2063c.

Figure 16A shows the colony identification results for aerobes (triplicate plate A of dilution10^0) from sample 2063c, while Figure 16B shows the results for aerobes (triplicate plate B of dilution10^0) from sample 2063c. Considerable quantities of molds were evident from both Figure 16A (many colony-sized molds) and Figure 16B (several large molds). The several large molds are likely from contamination with spores in the laboratory while the small molds could be present in the bentonite. Of the 12 colonies streaked from 2063C A, three gave an identification (ID), seven were molds and two gave simply no ID. The colonies identified were *Brevibacterium frigoritolerans*, and *Staphylococcus warneri*. The latter could have been present in the as-bought bentonite powder, added when the experiment was set up almost 8 years ago, added from non-detected contamination during the analysis of sample 2063c, or an actual presence in the sample, as Staphylococci are known to exist in soils. Of the eight colonies streaked from 2063c plate B dilution 10^0, five did not grow but the other three were identified as *Bacillus pumilus*, *Bacillus sp*. and *Paenibacillus polymyxa*, respectively. These species are soil bacteria with a high tolerance for environmental stress, spore formers and possibly some human-origin bacteria.

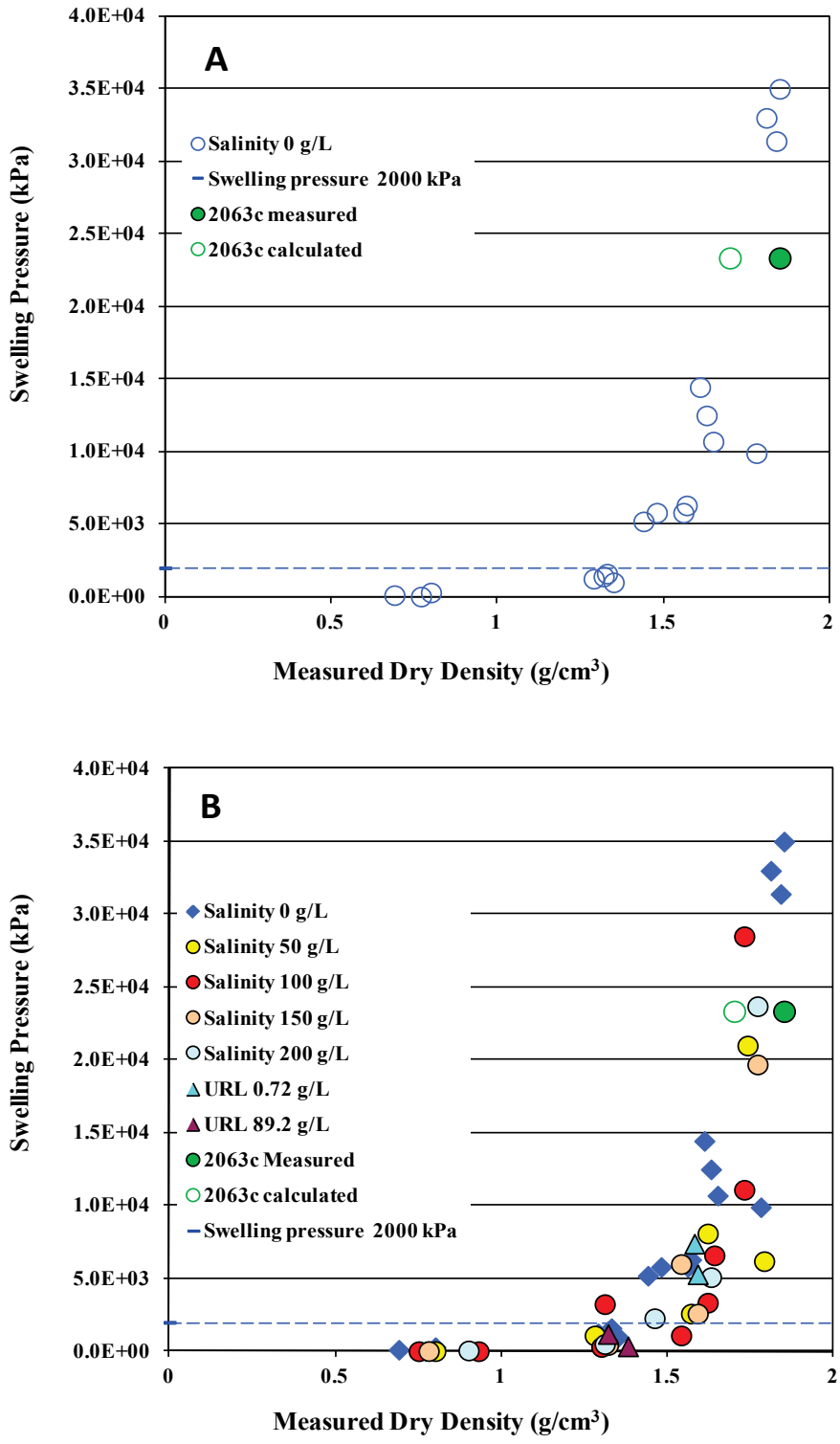


Figure 10: Swelling Pressure versus Measured Dry Density for DDW Experiments (A) and DDW + Saline Experments (B). Data point from plug 2063 shown in green (open circle = dry density calculated from water content; closed circle = dry density calculated from measured volume of plug)

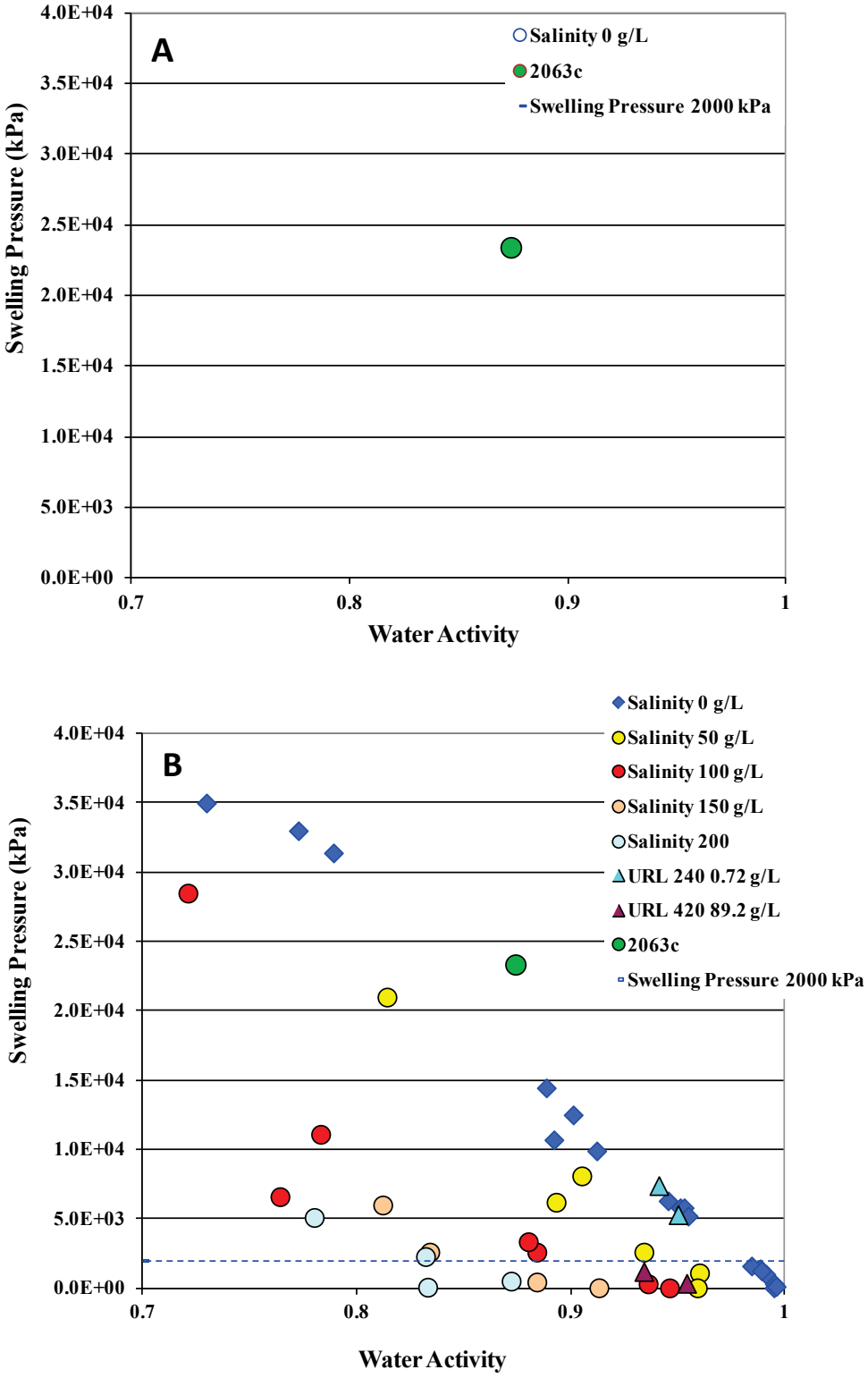


Figure 11: Swelling Pressure versus Water Activity for DDW Experiments (A) and DDW + Saline Experiments (B). Data point from plug 2063 shown in green

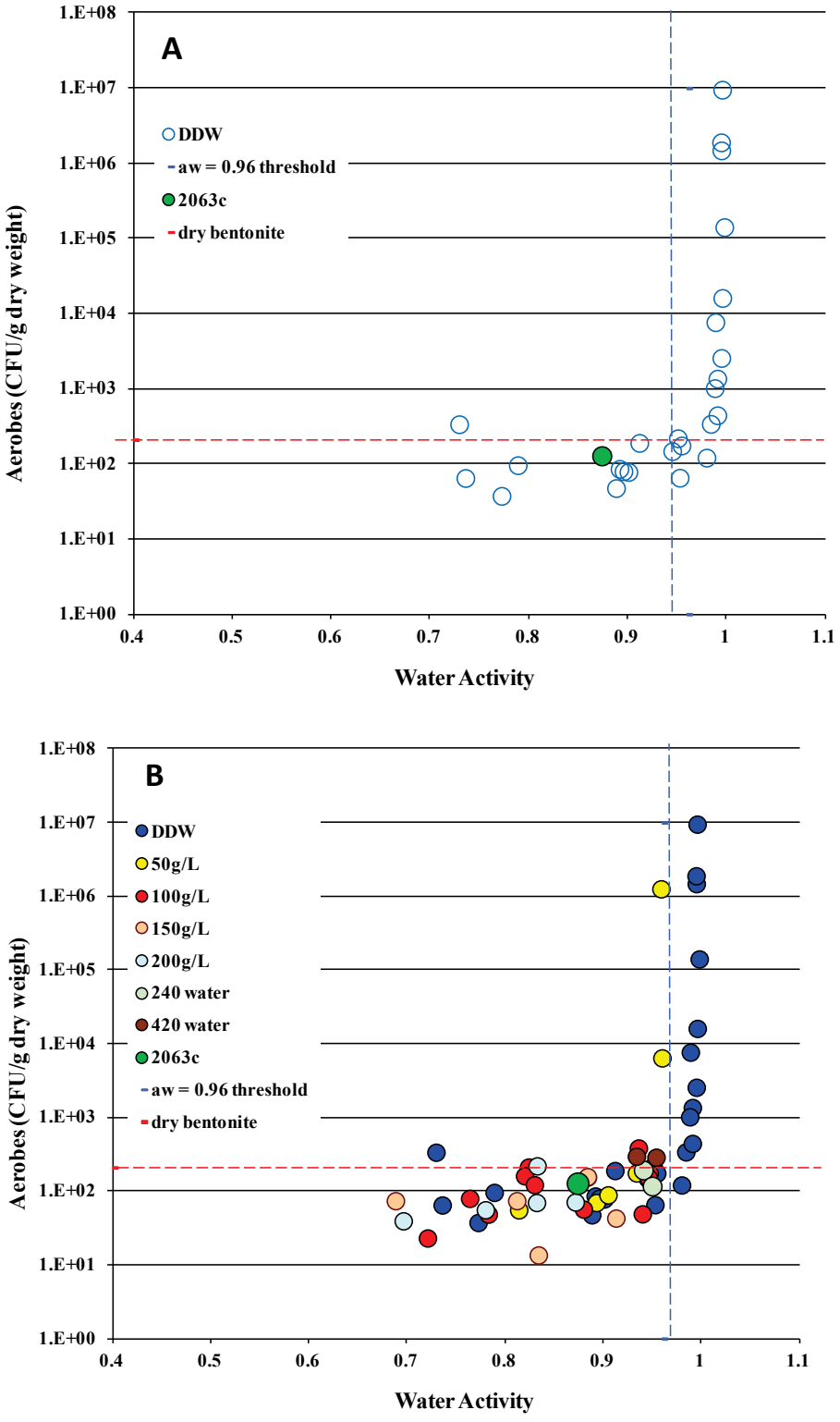


Figure 12: Culturable Heterotrophic Aerobes versus Water Activity for DDW Experiments (A) and DDW + Saline Experiments (B). Data point from plug 2063 shown in green

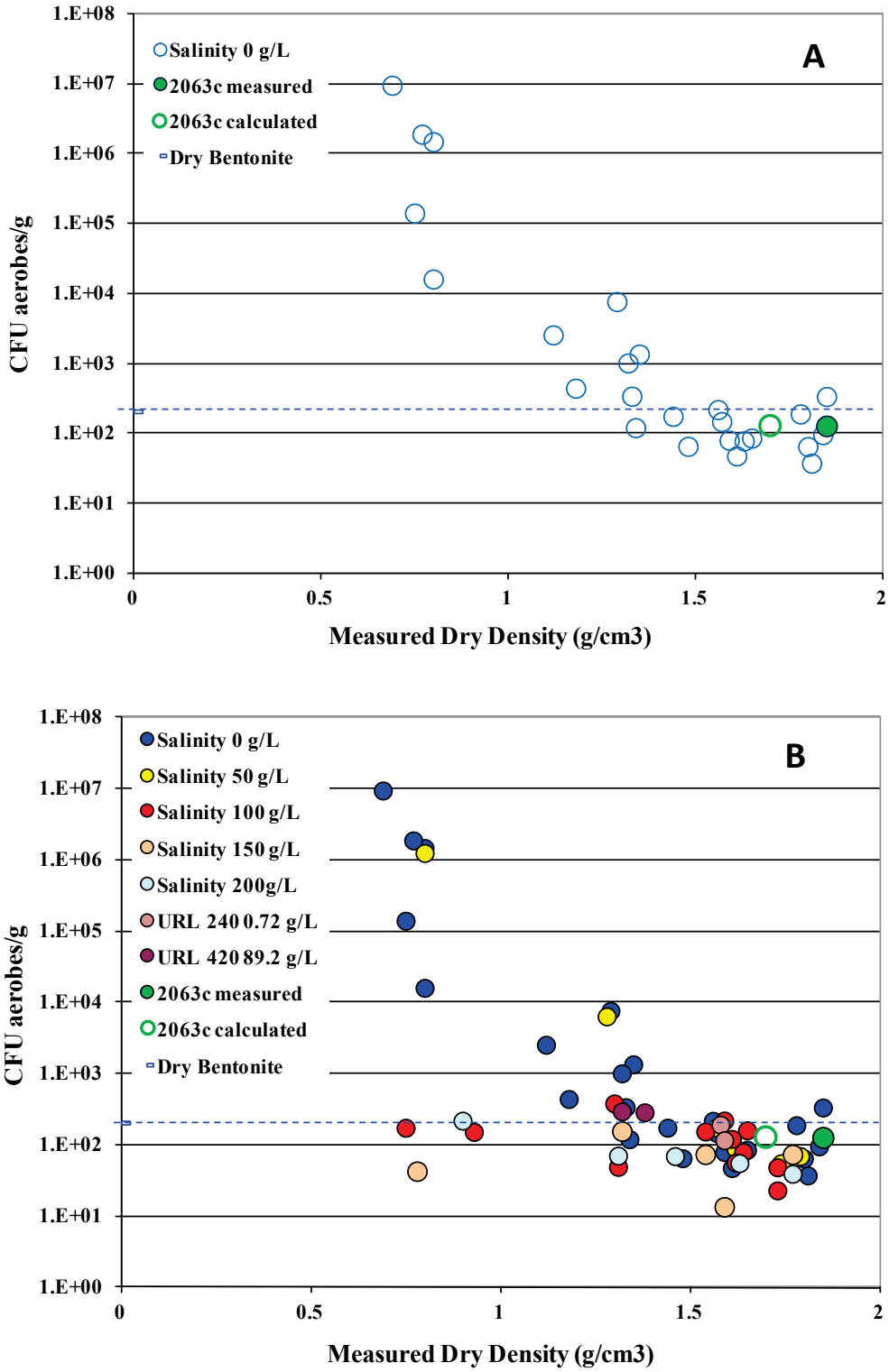


Figure 13: Culturable Heterotrophic Aerobes versus Measured Dry Density for DDW Experiments (A) and DDW + Saline Experiments (B). Data points from plug 2063 shown in green (open circle = dry density calculated from water content; closed circle = dry density calculated from measured volume of plug)

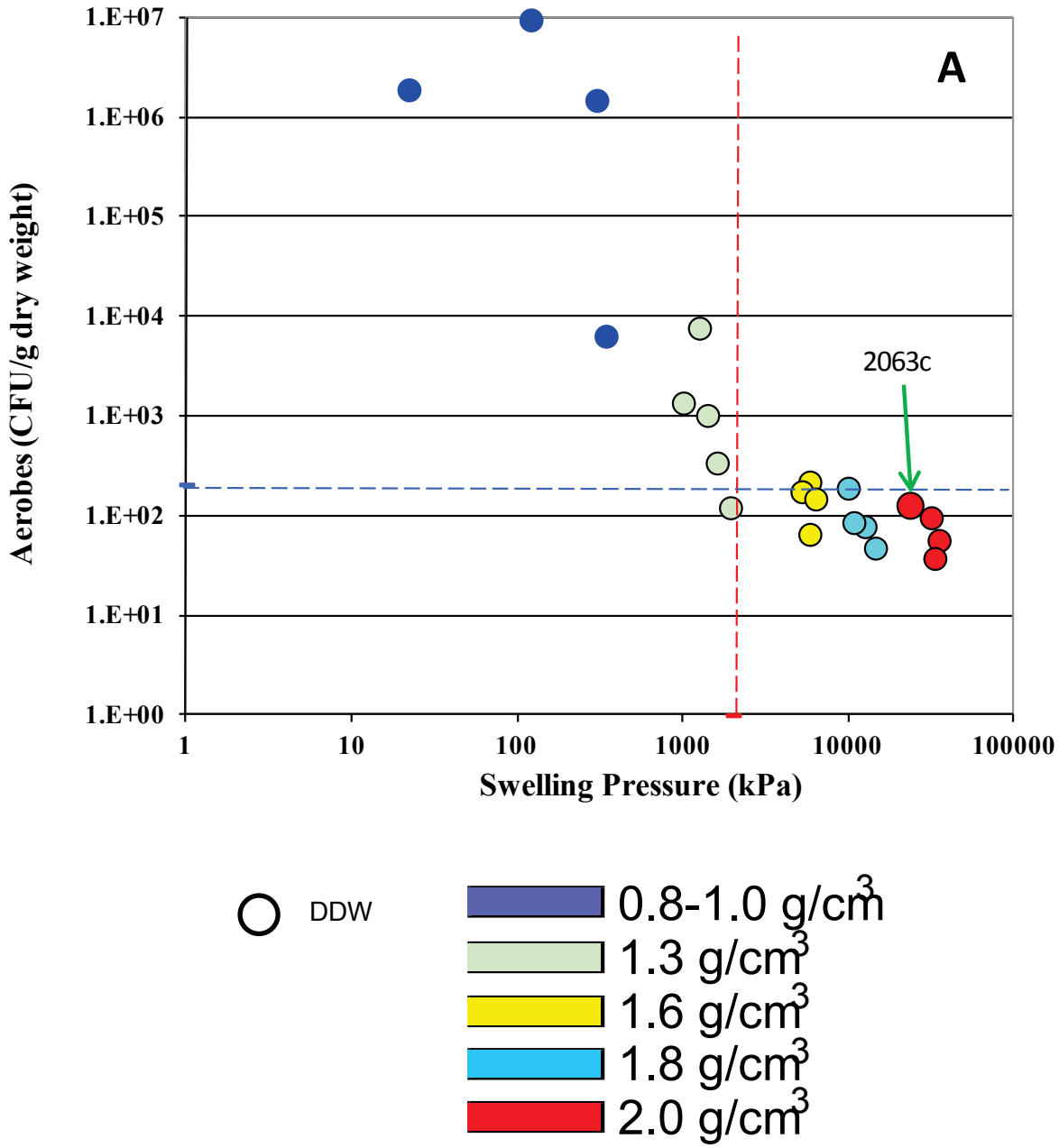


Figure 14A: Culturable Heterotrophic Aerobes as a Function of Swelling Pressure and Target Dry Density for DDW Experiments (A). Data point from plug 2063 shown in red with green arrow

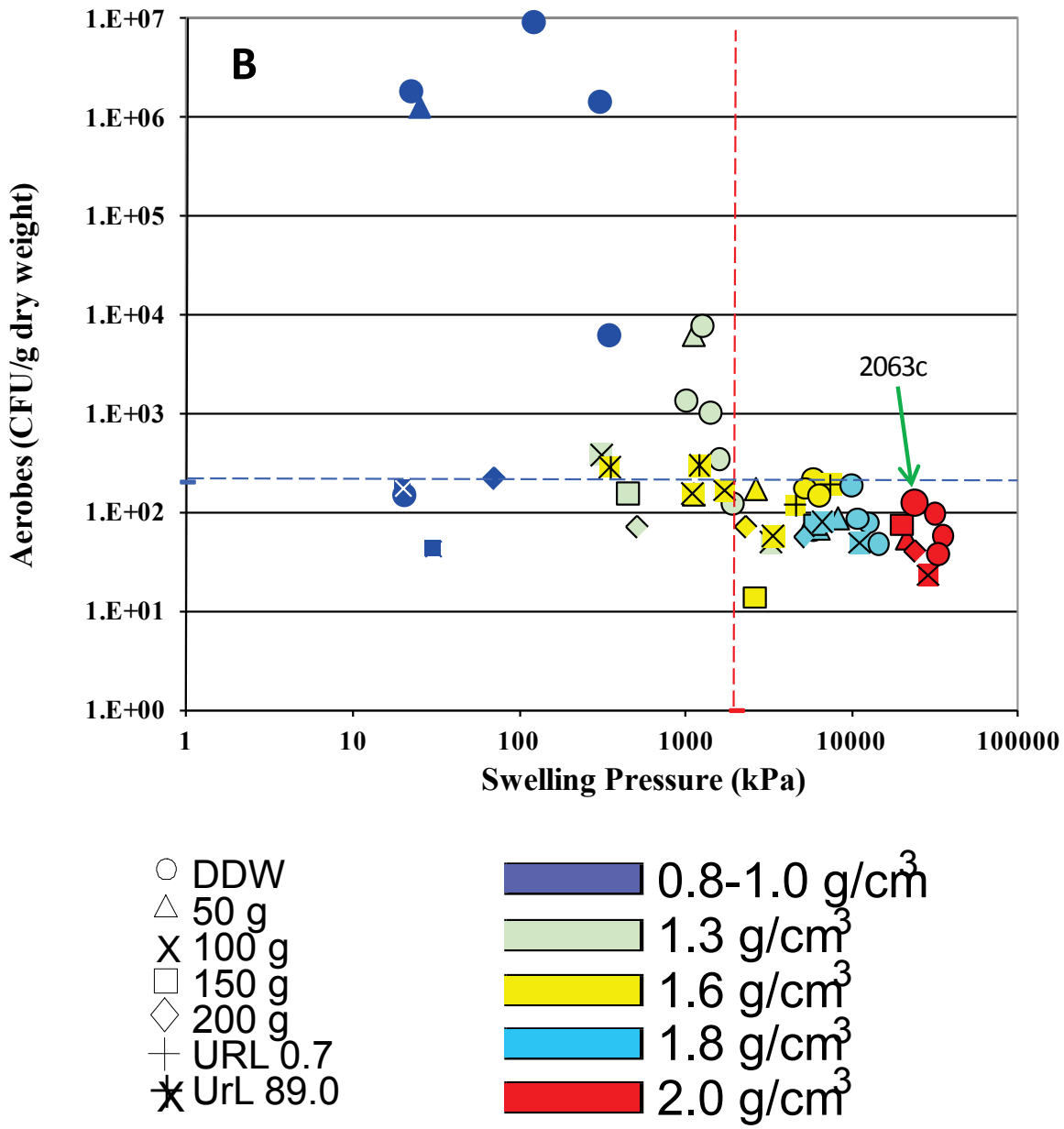


Figure 14B: Culturable Heterotrophic Aerobes as a Function of Swelling Pressure and Target Dry Density for DDW + Saline Experiments (B). Data point from plug 2063 shown in red with green arrow

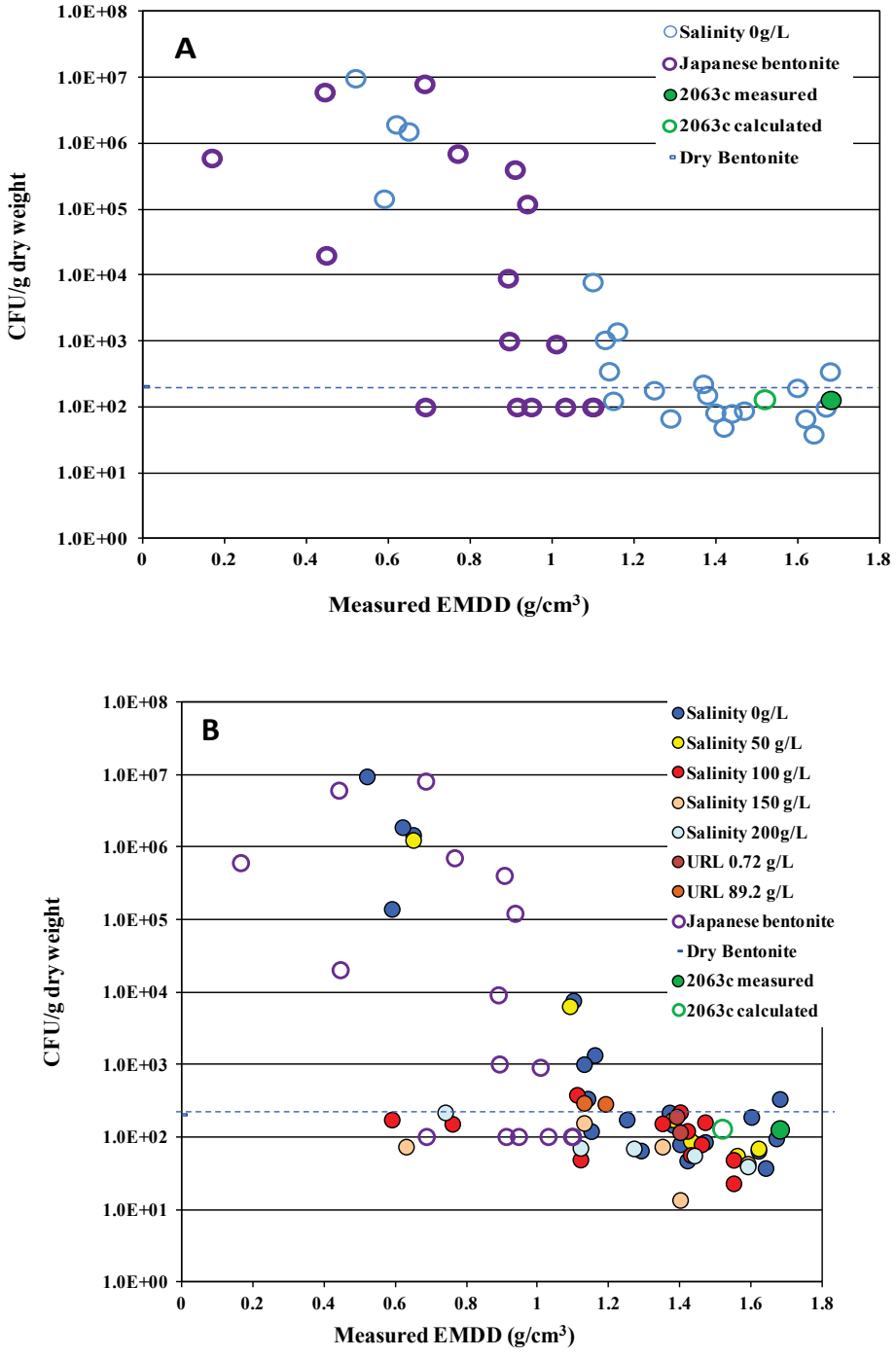


Figure 15: Culturable Heterotrophic Aerobes versus Measured Effective Montmorillonite Dry Density (EMDD) for DDW Experiments (A) and DDW + saline experiments (B). Data points from plug 2063 shown in green (open circle = dry density calculated from water content; closed circle = dry density calculated from measured volume of plug)

PLATE SAMPLES BIOLOG

Sample number 2063C (A)

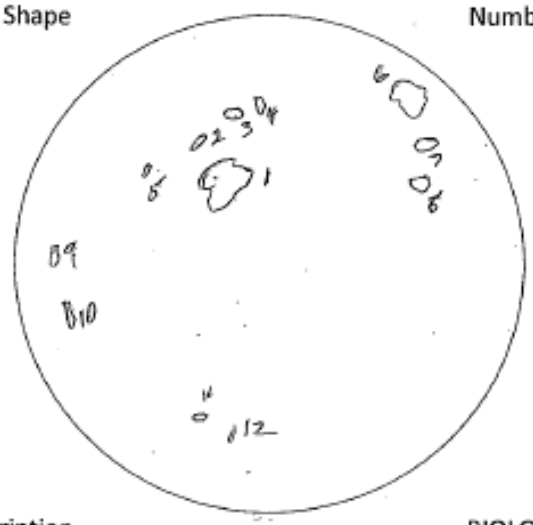
Sample Date Nov 28 2013

Plate dilution 10^0

Date picked Nov 28 2013

Plate Colony, Position, Shape

Number of Colonies: 12



Selected Colonies description	BIOLOG Identification
1 Soft, dirty white surface – soft cream pics	*Brevibacterium frigoritolerans*
2 Soft, embedded – soft cream pics	*Brevibacterium frigoritolerans*
3 Soft, embedded -mold	Mold
4 Soft, embedded- mold	Mold
5 Soft, embedded – soft, cream	No ID
6 Soft, white, embedded – soft cream	*Staphylococcus warnery*
7 Soft, embedded – mold	Mold
8 Soft, embedded – mold	Mold
9 Soft, embedded – mold	Mold
10 Soft, embedded – mold	Mold
11 Soft, embedded – mold but colonies	Mold
12 Soft, embedded near surface – mold but colonies	No ID

Note: A lot of these turned out to be molds. Original colony count was 4; 5 bacterial colonies were found.

Figure 16A: BIOLOG™ Identification Results for Heterotrophic Aerobic Colonies from Sample 2063C Plate A, dilution 10^0

PLATE SAMPLES BIOLOG

Sample number 2063C (B)

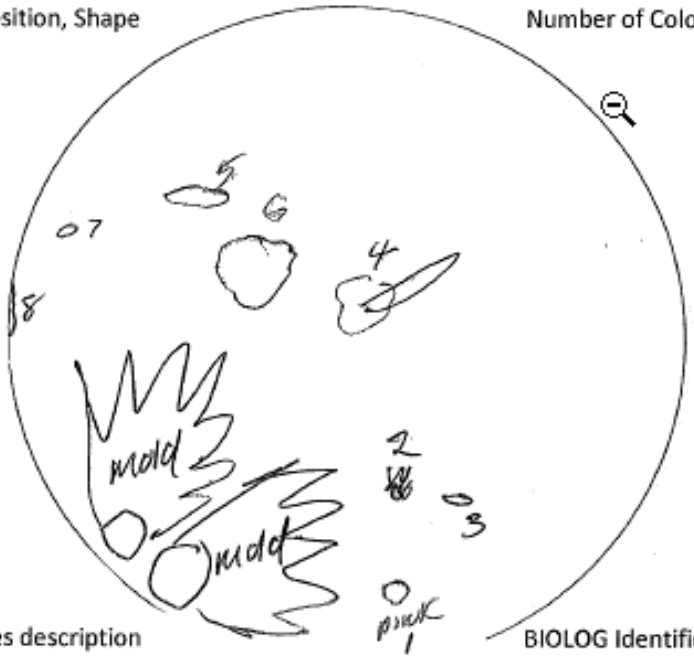
Sample Date Nov 28 2013

Plate dilution 10^0

Date picked Nov 28 2013

Plate Colony, Position, Shape

Number of Colonies: 8



Selected Colonies description	BIOLOG Identification
1. soft pink	No growth
2. cluster of colonies –cream soft	*Bacillus pumulus*
3. large colony splitting agar	No growth
4. embedded, large, soft – soft cream	Bacillus sp.
5. large, soft, embedded	No growth
6. large, round, in bottom – hard	*Paenibacillus polymyxis*
7. embedded, soft	No growt
8. on edges – contamination?	No growth

The original count was 10, some colonies are probably obscured by the mold (black)

(B)

Figure 16B: BIOLOG™ Identification Results for Heterotrophic Aerobic Colonies from Sample 2063C Plate B, Dilution 10^0

Table 2 shows the results from the molecular identifications. Of the 46 isolates, 30 could be identified and showed a total of 15 different species of *Bacillus*, *Paenibacillus* and *Brevibacillus*, all spore forming organisms. These results agree quite well with the much more limited BIOLOG identifications. Both results appear to confirm the hypothesis that the culturable bacteria in highly compacted bentonite survive as spores. Spores are inactive until they germinate and become vegetative cells.

4. CONCLUSIONS

The results from this experiment show that considerably more bacteria could be cultured from the outside of plug 2063 than from the inside matrix part of the bottom half of plug 2063. The top half of the plug was distorted during extrusion and results from that subsample may not be reliable due to possible contamination from the outside of the plug. Therefore, only results from the bottom half were compared to results obtained previously. The new results show that the number of viable cells in the compacted bentonite plug hovered around what is present in the "dry" clay. This was also concluded for the previous experiments lasting mostly between 40 and 90 days and these new results confirm that this remains so for almost 8 years. The cells that survive likely do so in a reduced viability form or in the form of spores. The molecular identifications showed a total of 15 different species of *Bacillus*, *Paenibacillus* and *Brevibacillus*, all spore forming organisms. The species identified with BIOLOG indicate the presence of soil bacteria with a high tolerance for environmental stress, spore formers and possibly some human-origin bacteria, corroborating the molecular identifications. Both results appear to confirm the hypothesis that the culturable bacteria in highly compacted bentonite survive mainly as spores. Spores are inactive and as such do not contribute to MIC or other detrimental effects until they germinate.

ACKNOWLEDGEMENTS

We are grateful to the NWMO for funding the work described in this report and to OPG for providing financial support in 2006 when this experiment was initiated.

Table 2: Sequence Identities (closest match) of the 30 Isolates Taken from Heterotrophic Aerobic Plates

(aligned using Clustal Omega and matched against NCBI database using BLASTn algorithm and Ribosomal Database Project (RDP II))

Sample ID	Closest Match		Identity		Phylum/Class
			BLASTn	RDP	
AA1	*Bacillus soli*		99	0.923	*Firmicutes/ Bacilli*
B7			99	0.952	
A11			99	0.917	
B3			99	0.960	
B2W			99	0.953	
AA2	*Bacillus drentensis*		100	1	*Firmicutes/ Bacilli*
C5			100	1	
C4			100	1	
C14			100	0.952	
AA3	*Bacillus simplex*		100	1	*Firmicutes/ Bacilli*
A1	*Bacillus licheniformis*		99	0.97	*Firmicutes/ Bacilli*
A2			100	0.987	
B4	Putative	*Bacillus soli*	98		*Firmicutes/ Bacilli*
A12			98		
C8			99		
A14			99		
B4		*Bacillus niacini*		0.904	*Firmicutes/ Bacilli*
A12				0.91	
C8				0.935	
A14				0.917	
C2	*Bacillus cereus*		100	1	*Firmicutes/ Bacilli*
A3	*Bacillus niacini*		99	0.892	*Firmicutes/ Bacilli*
B2			99	0.949	
AA4	*Paenibacillus prosopidis*		99	1	*Firmicutes/ Bacilli*
A6			99	0.954	
A4			99	0.805	
A7			100	0.986	*Firmicutes/ Bacilli*
A8			99	0.907	
C19	*Paenibacillus* sp. EGI 80148		99	0.971	*Firmicutes/ Bacilli*
C21	*Paenibacillus borealis*		99	0.92	*Firmicutes/ Bacilli*
B1	*Paenibacillus firmus*		100	1	*Firmicutes/ Bacilli*
C7			100	0.979	
A9	*Paenibacillus naphthalenovorans*		99	0.99	*Firmicutes/ Bacilli*
C16	*Brevibacillus limnophilus*		100	0.923	*Firmicutes/ Bacilli*

REFERENCES

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

Bondici, V.F., J.R. Lawrence, N.H. Khan, J.E. Hill, E. Yergeau, G.M. Wolfaardt, J. Warner and D.R. Korber. (2013). Microbial Communities in Low Permeability, High pH Uranium Mine Tailings: Characterization and Potential Effects. Journal of Applied Microbiology 114(6):1671-1686.

Hirkala, D.L.M. and J.J. Germida. (2004). Field and Soil Microcosm Studies on the Survival and Conjugation of a *Pseudomonas putida* Strain Bearing a Recombination Plasmid, pADPTel. Canadian Journal of Microbiology 50: 595–604.

Lane, D.J. (1991). 16S/23S rRNA Sequencing. In Nucleic Acid Techniques in Bacterial Systematics; Stackebrandt, E., Goodfellow, M., Eds.; John Wiley & Sons: New York, NY, USA. pp. 115-175.

McWilliam, H., W. Li, M. Uludag, S. Squizzato, Y.M. Park, N. Buso, A.P. Cowley and R. Lopez. (2013). Analysis Tool Web Services from the EMBL-EBI. Nucleic Acids Research 41(W1): 597-600.

Muyzer, G., S. Hottentrager, A. Teske, and C. Wawer. (1996). Denaturing Gradient Gel Electrophoresis of PCR-Amplified 16S rDNA: A New Molecular Approach to Analyse the Genetic Diversity of Mixed Microbial Communities. In Molecular Microbial Ecology Manual; Akkermans, A., Ed.; Kluwer Academic Publ.: Norwell, MA, USA. p. 23.

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

Stroes-Gascoyne, S., C.J. Hamon, P. Vilks and P. Gierszewski. 2002. Microbial, Redox and Organic Characteristics of Compacted Clay-based Buffer after 6.5 Years of Burial at AECL's Underground Research Laboratory. Applied Geochemistry 17, 1287-1303.

Stroes-Gascoyne, S. C.J. Hamon, D.A. Dixon and B. Kjartanson. 2005. Factors affecting microbial activity in compacted clay-based sealing materials proposed for use in a deep geologic repository for used nuclear fuel. Proc. Canadian Nuclear Society Waste Management, Decommissioning and Environmental Restoration Conference, May 8-12, Ottawa, Canada

Stroes-Gascoyne, S.,C.J. Hamon, C. Kohle, 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 No: 06819-REP-01200-10016-R00. November 2006.

Stroes-Gascoyne, S., C.J. Hamon, D.A. Dixon, C.L. Kohle and P. Maak. 2007. The effects of dry density and porewater salinity on the physical and microbiological characteristics of compacted 100% bentonite. Materials Research Society Symposium Proceedings 985, 505-510.

Stroes-Gascoyne, S., C.J. Hamon, P. Maak and S. Russell. 2010a. 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.A. Dixon and D.G. Priyanto. 2010b. The effect of $CaCl_2$ porewater salinity (50-100 g/L) on the culturability of heterotrophic aerobic bacteria in compacted 100% bentonite with dry densities of 0.8 and 1.3 g/cm3. NWMO TR-2010-06, Nuclear waste Management Organization, Toronto.

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 Wypming MX-80 bentonite. NWMO TR-2010-08, Nuclear waste Management Organization, Toronto.

APPENDIX A: COMPLETE DATA SET FROM EARLIER WORK

CONTENTS

Page

Table A.1: Target and Measured Values for Water Content, Dry Density and EMDD and Measured Values for Swelling Pressure

Experiment and Sample No.	Target Water Content (%)	Measured Water Content (%)	Target Dry Density (g/cm^3)	Measured Dry Density (g/cm^3)	Calculated Dry Density (g/cm^3)	Target EMDD (g/cm^3)	Measured EMDD (g/cm^3)	Swelling Pressure (kPa)
Uncompacted bentonite (1612)		9.31						
Uncompacted bentonite (1733)		8.93						
Uncompacted bentonite (1847)		7.55						
2.0 – DDWL (1663)	13.0	19.38	2.0	1.84	1.77	1.84	1.67	31400
2.0 – DDWLC (1714)	13.0	16.94	2.0	1.85	1.85	1.84	1.68	35400
2.0 – DDWL (1728)	13.0	18.48	2.0	1.81	1.80	1.84	1.64	33000
2.0 – DDWL (1728-60°C)	13.0	13.90	2.0	nm	1.96	1.84	nm	n/a
2.0 – DDWL(1737)(60°C)	13.0	19.40	2.0	1.92	1.77	1.84	1.76	28973
1.8 – DDWL (1644)	18.5	23.85	1.8	1.78	1.64	1.62	1.60	9900
1.8 – DDWPH (1668)	18.5	23.44	1.8	1.63	1.65	1.62	1.44	12500
1.8 – DDWL C (1712)	18.5	22.83	1.8	1.65	1.67	1.62	1.47	10700
1.8 – DDWL (1727)	18.5	23.51	1.8	1.61	1.65	1.62	1.42	14450
1.8 – DDWL – (1727-60°C)	18.5	16.95	1.8	nm	1.85	1.62	nm	n/a
1.8 – DDWL (1732) (PLFA)	18.5	17.65	1.8	1.80	1.83	1.62	1.62	Failed
1.8 – DDWL(1735)(60°C)	18.5	21.95	1.8	1.79	1.70	1.62	1.61	16972
1.6 – DDWL (1645)	25.5	30.36	1.6	1.56	1.48	1.41	1.37	5800
1.6 – DDWLC (1710)	25.5	29.45	1.6	1.48	1.50	1.41	1.29	5800
1.6 – DDWL (1717)	25.5	30.08	1.6	1.44	1.49	1.41	1.25	5200
1.6 – DDWL (1726)	25.5	29.41	1.6	1.57	1.51	1.41	1.38	6300
1.6 – DDWL (1726-60°C)	25.5	20.66	1.6	nm	1.73	1.41	nm	n/a
1.6 – DDWL (1731) (PLFA)	25.5	23.53	1.6	1.59	1.65	1.41	1.40	Failed
1.6 – DDWL (1738)(60°C)	25.5	32.70	1.6	1.61	1.43	1.41	1.42	4464
1.3 – DDWL (1643)	39.9	43.39	1.3	1.35	1.24	1.11	1.16	1000
1.3 – DDWLC (1708)	39.9	45.64	1.3	1.32	1.21	1.11	1.13	1400
1.3 – DDWL (1716)	39.9	42.40	1.3	1.33	1.26	1.11	1.14	1600
1.3 – DDWL (1719)	39.9	43.99	1.3	1.29	1.23	1.11	1.10	1250
1.3 – DDWL (1719-60°C)	88.0	40.25	1.3	nm	1.29	1.11	nm	n/a
1.3 – DDWL (1730) (PLFA)	39.9	37.98	1.3	1.34	1.33	1.11	1.15	1925
1.3 – DDWL (1734)(60°C)	39.9	45.86	1.3	1.31	1.21	1.11	1.12	1189

Experiment and Sample No.	Target Water Content (%)	Measured Water Content (%)	Target Dry Density (g/cm^3)	Measured Dry Density (g/cm^3)	Calculated Dry Density (g/cm^3)	Target EMDD (g/cm^3)	Measured EMDD (g/cm^3)	Swelling Pressure (kPa)
0.8 – DDWL (1638)	88.0	104.21	0.8	nm(0.71)*	0.71	0.65	0.65 (t)	300
0.8 – DDWL (1707)	88.0	79.67	0.8	0.77	0.86	0.65	0.62	22
0.8 – DDWL (1718)	88.0	102.30	0.8	0.69	0.72	0.65	0.52	120
0.8 – DDWL (1718-60^{0}C)	88.0	93.5	0.8	nm	0.77	0.65	nm	n/a
0.8 – DDWL (1729) (PLFA)	88.0	113.85	0.8	0.75	0.66	0.65	0.59	175
0.8 – DDWL (1736)(60^{o}C)	88.0	88.64	0.8	0.77	0.80	0.65	0.62	335
2.0 – 50L (1664)	13.0	20.90	2.0	1.74	1.72	1.84	1.56	21000
1.8 – 50L (1655)	18.5	25.33	1.8	1.79	1.60	1.62	1.62	6200
1.8 – 50L PH(1667)	18.5	26.87	1.8	1.62	1.57	1.62	1.43	8100
1.6 – 50L (1640)	25.5	30.87	1.6	1.57	1.47	1.41	1.38	2600
1.3 – 50L (1654)	39.9	40.43	1.3	1.28	1.29	1.11	1.09	1100
0.8 – 50L (1637)	88.0	77.07	0.8	0.8(t)	0.88	0.65	0.65 (t)	25
2.0 – 100L (1665)	13.0	18.32	2.0	1.73	1.81	1.84	1.55	28500
1.8 – 100L PH (1666)	18.5	20.29	1.8	1.64	1.75	1.62	1.46	6600
1.8 – 100L (1673)	18.5	21.50	1.8	1.73	1.71	1.62	1.55	11100
1.6 – 100L 1672)	25.5	27.10	1.6	1.62	1.56	1.41	1.43	3350
1.6 – 100L (1649)	25.5	26.00	1.6	1.54	1.59	1.41	1.35	1100
1.6 – 100L (1749T)	25.5	25.68	1.6	1.60	1.59	1.41	1.40	1706
1.6 – 100L (1749M)	25.5	23.64	1.6	1.65	1.65	1.41	1.47	1706
1.6 – 100L (1749B)	25.5	25.07	1.6	1.61	1.61	1.41	1.42	1706
1.3 – 100L (1650)	39.9	41.48	1.3	1.30	1.27	1.11	1.11	310
1.3 – 100L (1670)	39.9	40.70	1.3	1.31	1.29	1.11	1.12	3250
0.8 – 100L (1669)	88.0	79.62	0.8	0.75	0.86	0.65	0.59	20
0.8 – 100L (1651)	88.0	82.05	0.8	0.93	0.84	0.65	0.76	20
2.0 – 150L (1704)	13.0	18.35	2.0	1.77	1.81	1.84	1.59	19700
1.8 – 150L (1662)	18.5	28.06	1.8	1.54	1.74	1.62	1.35	6000
1.6 – 150L (1661)	25.5	27.88	1.6	1.59	1.54	1.41	1.40	2600
1.3 – 150L (1660)	39.9	43.11	1.3	1.32	1.25	1.11	1.13	445
0.8 – 150L (1703)	88.0	80.16	0.8	0.78	0.85	0.65	0.63	30
2.0 – 200 L (1698)	13.0	18.37	2.0	1.77	1.81	1.84	1.59	23700
1.8 – 200 L (1699)	18.5	24.51	1.8	1.63	1.63	1.62	1.44	5100

Experiment and Sample No.	Target Water Content (%)	Measured Water Content (%)	Target Dry Density (g/cm^3)	Measured Dry Density (g/cm^3)	Calculated Dry Density (g/cm^3)	Target EMDD (g/cm^3)	Measured EMDD (g/cm^3)	Swelling Pressure (kPa)
1.6 – 200 L (1700)	25.5	30.73	1.6	1.46	1.48	1.41	1.27	2280
1.3 – 200 L (1701)	39.9	40.44	1.3	1.31	1.29	1.11	1.12	510
0.8 – 200 L (1702)	88.0	61.37	0.8	0.90	1.02	0.65	0.74	900
1.6 – 240StL (1652)	25.5	27.70	1.6	1.58	1.55	1.41	1.39	7400
1.6 – 240NStL (1671)	25.5	29.23	1.6	1.59	1.51	1.41	1.40	4550
1.6 – 420StL (1658)	25.5	48.36	1.6	1.38	1.17	1.41	1.19	350
1.6 – 420NStL (1659)	25.5	33.02	1.6	1.32	1.43	1.41	1.13	1200

DDW = Distilled Deionised Water
50 = 50 g/L
100 = 100 g/L
150 = 150 g/L
S = Short-duration
L = Long-duration
PH = Samples for Porosimetry
St = Sterile
NSt = Non-sterile
240 = 240 level URL water

N/A = Not Applicable (short experiment)
(t) = Target value
EMDD = Effective Montmorillonite Dry Density
C = Copper Coupon Present
* Calculated from measured water content using the equation:
water content = (water density/dry density) – (1/specific gravity)
(specific gravity bentonite = 2.70)
T, M, B, E = Top, Middle, Bottom, Exterior of bentonite plug
Failed = Measurement failed due to load cell problems

Table A.2: Results for Water Content, Water Activity, Aerobes, Anaerobes and SRB

Experiment and Sample No.	Duration (d)	Solution	Water Content (%)	a_w	Aerobes CFU/g dry wt.	Anaerobes CFU/g dry wt.	SRB MPN/g dry wt.	PLFA Cell Eq/g dry wt.
Uncompacted Bentonite (1612)	-	-	9.31	0.471	$(2.07\pm0.23)\times10^{2}$	$(5.51\pm1.91)\times10^{1}$	1.03×10^{1}	
Uncompacted Bentonite (1733) (PLFA)	-	-	8.93	0.358	$(1.37\pm0.30)\times10^{2}$	$(5.56\pm2.94)\times10^{1}$	4.1	1.36×10^{6}
Uncompacted bentonite (1847)			7.55	0.393	$(6.24\pm2.34)\times10^{2}$	$(1.74\pm0.60)\times10^{1}$	<3.14	
2.0 – DDWL (1663)	54	DDW	19.38	0.789	$(9.60\pm4.33)\times10^{1}$	$(1.67\pm0.72)\times10^{1}$	4.32	
2.0 – DDWL (1714)C*	62	DDW	16.94	0.730	$(1.77\pm0.15)\times10^{0}$	0	$< 3.3\times10^{-3}$	
2.0 – DDWL (1714-AC)	62	DDW	16.94	0.730	$(2.17\pm0.34)\times10^{2}$	$(6.00\pm2.60)\times10^{1}$	10.3	
2.0 – DDWL (1714-FC)	62	DDW	16.94	0.730	$(4.56\pm0.34)\times10^{2}$	$(3.84\pm2.52)\times10^{1}$	6.8	
2.0 – DDWL (1728)	156	DDW	18.48	0.773	$(3.75\pm2.17)\times10^{1}$	$(3.33\pm1.44)\times10^{1}$	< 3.76	
2.0 – DDWL (1728-60°C)	8	DDW	13.90	0.547	$(4.44\pm2.22)\times10^{1}$	$(4.81\pm2.31)\times10^{1}$	< 4.6	
2.0 – DDWL (1737)(60°C)	52	DDW	19.40	0.776	$(5.70\pm2.74)\times10^{1}$	$(4.17\pm2.22)\times10^{0}$	< 3.75	
1.8 – DDWL (1644)	38	DDW	23.85	0.912	$(1.90\pm0.53)\times10^{2}$	$(4.76\pm1.65)\times10^{1}$	5.27	
1.8 – DDWL (1668)	53	DDW	23.44	0.901	$(7.81\pm1.42)\times10^{1}$	$(3.33\pm0.72)\times10^{1}$	9.13	
1.8 – DDWL (1712)C*	61	DDW	22.83	0.893	0	0	$< 3.3\times10^{-3}$	
1.8 – DDWL (1712-AC)	61	DDW	22.83	0.893	$(1.43\pm0.77)\times10^{2}$	$(3.58\pm1.77)\times10^{1}$	1.57×10^{1}	
1.8 – DDWL (1712-FC)	61	DDW	22.83	0.893	$(2.82\pm0.98)\times10^{1}$	$(1.69\pm0.85)\times10^{1}$	3.4	
1.8 – DDWL (1727)	79	DDW	23.51	0.888	$(4.76\pm1.65)\times10^{1}$	$(2.38\pm1.65)\times10^{1}$	10	
1.8 – DDWL (1727-60°C)	8	DDW	16.95	0.630	$(7.41\pm6.42)\times10^{0}$	$(3.70\pm6.42)\times10^{0}$	< 4.6	
1.8 – DDWL (1732) (PLFA)	44	DDW	17.65	0.736	$(6.48\pm2.64)\times10^{1}$	$(2.59\pm1.70)\times10^{1}$	< 3.4	1.61×10^{6}
1.8 – DDWL (1735) (60°C)	51	DDW	21.95	0.851	$(5.90\pm1.47)\times10^{1}$	$(5.90\pm2.55)\times10^{1}$	< 4.29	
1.6 – DDWL (1645)	38	DDW	30.36	0.951	$(2.19\pm0.00)\times10^{2}$	$(1.17\pm0.60)\times10^{2}$	1.50×10^{1}	
1.6 – DDWL (1710-C)*	61	DDW	29.45	0.963	$(7.80\pm1.35)\times10^{-1}$	0	$< 3.31\times10^{-3}$	
1.6 – DDWL (1710-AC)	61	DDW	29.45	0.953	$(4.61\pm1.54)\times10^{1}$	$(1.43\pm0.77)\times10^{2}$	8.5	
1.6 – DDWL (1710-FC)	61	DDW	29.45	0.953	$(8.47\pm0.47)\times10^{1}$	$(4.37\pm1.25)\times10^{1}$	9.2	
1.6 – DDWL (1717)	45	DDW	30.08	0.955	$(1.75\pm0.46)\times10^{2}$	$(5.84\pm2.68)\times10^{1}$	< 5.2	
1.6 – DDWL (1726)	79	DDW	29.41	0.945	$(1.48\pm0.41)\times10^{2}$	$(1.43\pm0)\times10^{1}$	< 4.4	
1.6 – DDWL (1726-60°C)	8	DDW	20.66	0.770	$(2.96\pm2.31)\times10^{1}$	$(1.85\pm1.28)\times10^{1}$	4.5	
1.6 – DDWL (1731) (PLFA)	44	DDW	23.53	0.896	$(7.98\pm1.93)\times10^{1}$	$(3.33\pm0.72)\times10^{1}$	< 3.9	1.48×10^{6}
1.6 – DDWL (1738) (60°C)	52	DDW	32.70	0.965	$(1.16\pm0.50)\times10^{1}$	$(3.03\pm5.25)\times10^{0}$	2.73	
1.3 – DDWL (1643)	37	DDW	43.39	0.991	$(1.36\pm0.14)\times10^{3}$	$(9.09\pm0.91)\times10^{1}$	< 2.78	
1.3 – DDWL (1708-C)*	57	DDW	45.64	0.988	$(7.96\pm0.80)\times10^{-2}$	$(5.31\pm0.46)\times10^{-2}$	$< 3.3\times10^{-3}$	
1.3 – DDWL (1708-AC)	57	DDW	45.64	0.988	$(9.52\pm8.25)\times10^{0}$	$(2.86\pm1.43)\times10^{1}$	9	
1.3 – DDWL (1708-FC)	57	DDW	45.64	0.988	$(1.02\pm0.39)\times10^{3}$	$(1.16\pm1.16)\times10^{1}$	< 4.6	
1.3 – DDWL (1716)	45	DDW	42.40	0.984	$(3.40\pm0.71)\times10^{2}$	$(2.96\pm1.28)\times10^{1}$	< 6.2	
1.3 – DDWL (1719)	77	DDW	43.99	0.989	$(7.67\pm0.54)\times10^{3}$	$(2.38\pm2.97)\times10^{1}$	< 4.7	

Experiment and Sample No.	Duration (d)	Solution	Water Content (%)	a_w	Aerobes CFU/g dry wt.	Anaerobes CFU/g dry wt.	SRB MPN/g dry wt.	PLFA Cell Eq/g dry wt.
1.3 – DDWL (1719-60°C)	8	DDW	40.25	0.981	$(5.62\pm1.03)\times10^{3}$	$(6.19\pm4.36)\times10^{1}$	< 4.6	
1.3 – DDWL (1730) (PLFA)	44	DDW	37.98	0.980	$(1.21\pm0.47)\times10^{2}$	$(1.94\pm0.84)\times10^{1}$	< 4.4	1.52×10^{6}
1.3 – DDWL (1734) (60°C)	51	DDW	45.86	0.988	$(3.60\pm6.23)\times10^{0}$	$(1.08\pm1.08)\times10^{1}$	< 3.4	
0.8 – DDWL (1638)	35	DDW	104.21	0.995	$(1.48\pm0.37)\times10^{6}$	$(4.25\pm0.16)\times10^{3}$	6.92	
0.8 – DDWL (1707)	41	DDW	79.67	0.995	$(1.89\pm0.05)\times10^{6}$	$(1.00\pm0.35)\times10^{2}$	< 5.7	
0.8 – DDWL (1718)	77	DDW	102.30	0.996	$(9.50\pm0.50)\times10^{6}$	$(3.42\pm3.99)\times10^{2}$	< 7.9	
0.8 – DDWL (1718-60°C)	8	DDW	93.52	0.995	$(5.53\pm4.62)\times10^{1}$	$(4.00\pm2.00)\times10^{1}$	< 4.6	
0.8 – DDWL (1729) (PLFA)	44	DDW	113.85	0.998	$(1.41\pm0.05)\times10^{5}$	$(1.82\pm0.7)\times10^{2}$	<6.6	2.29×10^{6}
0.8 – DDWL (1736) (60°C)	51	DDW	88.64	0.995	$(6.68\pm1.67)\times10^{1}$	$(5.00\pm1.67)\times10^{1}$	< 0.5	
2.0 – 50L (1664)	55	50g NaCl/L	20.90	0.814	$(5.56\pm1.82)\times10^{1}$	$(2.08\pm2.60)\times10^{1}$	4.29	
1.8 – 50L (1655)	56	50g NaCl/L	25.33	0.893	$(6.99\pm3.07)\times10^{1}$	$(9.54\pm9.54)\times10^{0}$	< 2.86	
1.8 – 50L PH (1667)	53	50g NaCl/L	26.87	0.905	$(8.89\pm7.11)\times10^{1}$	$(5.00\pm1.25)\times10^{1}$	4.37	
1.6 – 50L (1640)	42	50g NaCl/L	30.87	0.934	$(1.76\pm0.13)\times10^{2}$	$(1.56\pm0.62)\times10^{2}$	3.78	
1.3 – 50L (1654)	56	50g NaCl/L	40.43	0.960	$(6.41\pm1.48)\times10^{3}$	$(3.91\pm1.69)\times10^{1}$	< 4.40	
0.8 – 50L (1637)	41	50g NaCl/L	77.07	0.959	$(1.26\pm0.11)\times10^{6}$	$(2.79\pm1.32)\times10^{2}$	< 3.89	
2.0 – 100L (1665)	55	100g NaCl/L	18.32	0.721	$(2.31\pm3.18)\times10^{1}$	$(6.67\pm5.77)\times10^{0}$	4.29	
1.8 – 100L PH (1666)	53	100g NaCl/L	20.29	0.764	$(7.97\pm2.28)\times10^{1}$	$(3.33\pm1.11)\times10^{1}$	4.1	
1.8 – 100L (1673)	56	100g NaCl/L	21.50	0.783	$(4.87\pm1.22)\times10^{1}$	$(5.42\pm3.81)\times10^{1}$	1.83×10^{1}	
1.6 – 100L (1672)	56	100g NaCl/L	27.10	0.880	$(5.69\pm2.81)\times10^{1}$	$(2.44\pm0.00)\times10^{1}$	4.39	
1.6 – 100L (1649)	55	100g NaCl/L	26.00	0.884	$(1.55\pm0.28)\times10^{2}$	$(3.17\pm1.45)\times10^{1}$	< 4.20	
1.6 – 100L (1749T)	38	100g NaCl/L	25.68	0.824	$(2.12\pm0.12)\times10^{2}$	$(8.20\pm7.10)\times10^{1}$	< 6.36	
1.6 – 100L (1749M)	38	100g NaCl/L	23.64	0.820	$(1.61\pm0.43)\times10^{2}$	$(1.21\pm0.17)\times10^{2}$	< 5.34	
1.6 – 100L (1749B)	38	100g NaCl/L	25.07	0.830	$(1.22\pm0.19)\times10^{2}$	$(1.68\pm2.91)\times10^{1}$	< 3.90	
1.3 – 100L (1650)	56	100g NaCl/L	41.48	0.936	$(3.84\pm0.61)\times10^{2}$	$(2.29\pm0.34)\times10^{2}$	< 4.38	
1.3 – 100L (1670)	54	100g NaCl/L	40.70	0.940	$(4.94\pm0.78)\times10^{1}$	$(3.33\pm2.18)\times10^{1}$	1.24×10^{1}	
0.8 – 100L (1669)	54	100g NaCl/L	79.62	0.947	$(1.75\pm0.19)\times10^{2}$	$(2.22\pm1.92)\times10^{1}$	6.08	
0.8 – 100L (1651)	56	100g NaCl/L	82.05	0.946	$(1.53\pm0.34)\times10^{2}$	$(6.00\pm0.94)\times10^{1}$	5.89	
2.0 – 150L (1704)	91	150g NaCl/L	18.35	0.688	$(7.41\pm6.68)\times10^{1}$	n/a	6.67	
1.8 – 150L (1662)	54	150g NaCl/L	28.06	0.812	$(7.41\pm1.83)\times10^{1}$	$(4.67\pm1.15)\times10^{1}$	5.71	
1.6 – 150L (1661)	50	150g NaCl/L	27.88	0.834	$(1.36\pm1.17)\times10^{1}$	$(4.00\pm0.00)\times10^{1}$	1.50×10^{1}	
1.3 – 150L (1660)	50	150g NaCl/L	43.11	0.884	$(1.57\pm0.57\times10^{2}$	$(5.56\pm0.96)\times10^{1}$	< 5.63	
0.8 – 150L (1703)	91	150g NaCl/L	80.16	0.913	$(4.30\pm0.00)\times10^{1}$	$(7.62\pm2.18)\times10^{1}$	5.16	

Experiment and Sample No.	Duration (d)	Solution	Water Content (%)	a_w	Aerobes CFU/g dry wt.	Anaerobes CFU/g dry wt.	SRB MPN/g dry wt.	PLFA Cell Eq/g dry wt.
2.0 – 200L (1698)	90	200g NaCl/L	18.37	0.696	$(3.95\pm1.24)x10^{1}$	$(4.67\pm0.62)x10^{1}$	3.88	
1.8 – 200L (1699)	90	200g NaCl/L	24.51	0.780	$(5.58\pm0.69)x10^{1}$	$(2.50\pm1.25)x10^{1}$	< 3.71	
1.6 – 200L (1700)	90	200g NaCl/L	30.73	0.832	$(7.00\pm3.61)x10^{1}$	$(2.00\pm1.00)x10^{1}$	< 3.04	
1.3 – 200L (1701)	91	200g NaCl/L	40.44	0.872	$(7.11\pm4.43)x10^{1}$	$(1.13\pm0.29)x10^{2}$	< 3.15	
0.8 – 200L (1702)	91	200g NaCl/L	61.37	0.833	$(2.20\pm0.99)x10^{2}$	$(1.19\pm0.08)x10^{2}$	$1.43x10^{1}$	
1.6 – 240StL (1652)	55	TDS 0.72 g/L	27.70	0.941	$(1.93\pm0.38)x10^{2}$	$(3.94\pm3.48)x10^{1}$	< 3.94	
1.6 – 240NStL (1671)	55	TDS 0.72 g/L	29.23	0.950	$(1.17\pm0.19)x10^{2}$	$(3.33\pm1.91)x10^{1}$	$1.15x10^{1}$	
1.6 – 420StL (1658)	56	TDS 89.2 g/L	48.36	0.954	$(2.88\pm1.28)x10^{2}$	$(6.00\pm2.00)x10^{1}$	< 5.63	
1.6 – 420NStL (1659)	56	TDS 89.2 g/L	33.02	0.934	$(2.97\pm1.12)x10^{2}$	$(6.14\pm2.16)x10^{1}$	5.10	

DDW = Distilled Deionised Water
TDS = Total Dissolved Solids
50 = 50 g/L
100 = 100 g/L
150 = 150 g/L
200 = 200 g/L
St = Sterile
NSt = Non-sterile
240 = 240 level water
420 = 420 level water
S = Short-duration
L = Long-duration
PH = Samples for Porosimetry

a_w = Water activity
CFU = Colony-Forming Units
MPN = Most-probable Number
SRB = Sulphate-Reducing Bacteria
na = not available
C = Copper Coupon Present
AC = Around Coupon
FC = Further away from Coupon
* = CFU or MPN/mm^2 (Coupon surface area)
PLFA = Phospholipid Fatty Acid Analysis

T,M,B,E = Top, Middle, Bottom, Exterior of bentonite plug
Rehydr. = Desiccation at 60°C, rehydrated at RT
RT = Room Temperature

APPENDIX B: SWELLING PRESSURE OF LONG-TERM MICROBIAL STUDY (SPECIMEN 2.0 DDW)

CONTENTS

Prepared by:
Deni Priyanto, Ph.D, P.Eng

Test conducted by:
Connie Hamon, Bill Evenden, and Cliff Kohle

Report provided for:
Sim Stroes-Gascoyne & Connie Hamon

2013 November 27

B.1 INTRODUCTION

This Appendix describes the swelling pressure of the specimen 2.0 DDW (9). The specimen was installed on 2006 January 10 and dismantled 2013 September 16. Total duration of the test was 7.7 years. Solid component of the clay was MX-80 bentonite, fluid component was distilled water.

B.2 METHODOLOGY AND RESULTS

B.2.1 Swelling Pressure Measurements

The swelling pressure was measured using a swelling pressure cell.

Load cell (LC) was used to measure total pressure and a pressure transducer (PT) was used to measure the water pressure applied to the specimen. The general information regarding LC and PT, including serial number and calibration coefficient is provided in Table B-1. The raw data from the LC and PT recorded from the data logger have units of mV and were converted using the calibration coefficient to units [kg] for LC and [kPa] for PT (Figure B-1).

During the long duration of the test, several events occurred, but they did not affect the swelling pressure result of the test.

- Several times during the test, the argon tank to apply hydraulic pressure was low and was replaced. During these times, low pressure was recorded. Swelling pressure was back to its original value after the argon tank was replaced.
- In 2008, during the maintenance of the URL laboratory, the top of the cell was accidentally disturbed, but it came back to its original pressure afterward.
- In 2011, the pressure transducer was recalibrated and the pressure was not measured. Although the pressure was not recorded in the data logger, Hydraulic pressure was still applied to the cell.

Figure B-2 shows the total pressure (P_{total}), hydraulic pressure (u) applied to the cell, and swelling pressure (P_s). These values were determined as follows.

$$P_{total} = (LC\ [kg] \times g\ [m/s^2]\ ) / A\ [m^2] \quad (1)$$

$$u = PT\ [kPa] + P_{\Delta H}\ [kPa] \quad (2)$$

$$P_{\Delta H} = \rho_w \times g \times \Delta H \quad (3)$$

where
LC = load cell reading in [kg]
PT = pressure transducer reading to measure the argon pressure applied to the top of water reservoir [kPa]
A = contact area of the specimen, equal to the specimen area, diameter ~32 mm.
$P_{\Delta H}$ = hydrostatic pressure due to the location of water reservoir with respect to the cell.
g = gravitational acceleration [m/s^2]
ρ_w = water density [kg/m^3]
g = gravitational acceleration [m/s^2]
ΔH = vertical distance of the cell to the water reservoir (= 1.5 m).

At the beginning the top valve was open, until it was completely saturated. After 2007, the top valve of the cell was closed, so that hydraulic pressure was assumed to be uniform throughout 21 mm thickness of the specimen. Using Terzaghi's effective stress principle, the swelling pressure (Ps) is equal to the effective stress, and

$$P_s = P - u \quad (4)$$

Since 2007, the swelling pressure of the cell is relatively constant and equal to approximately ~23,350 kPa (see Figures B-2 and B-3). The changes of the interruption described previously did not affect the swelling pressure results. Possible lines showing an increase of the pressure prior to 2007 were added in the figures for purposes. Again, the most significant data was the last period of the swelling test.

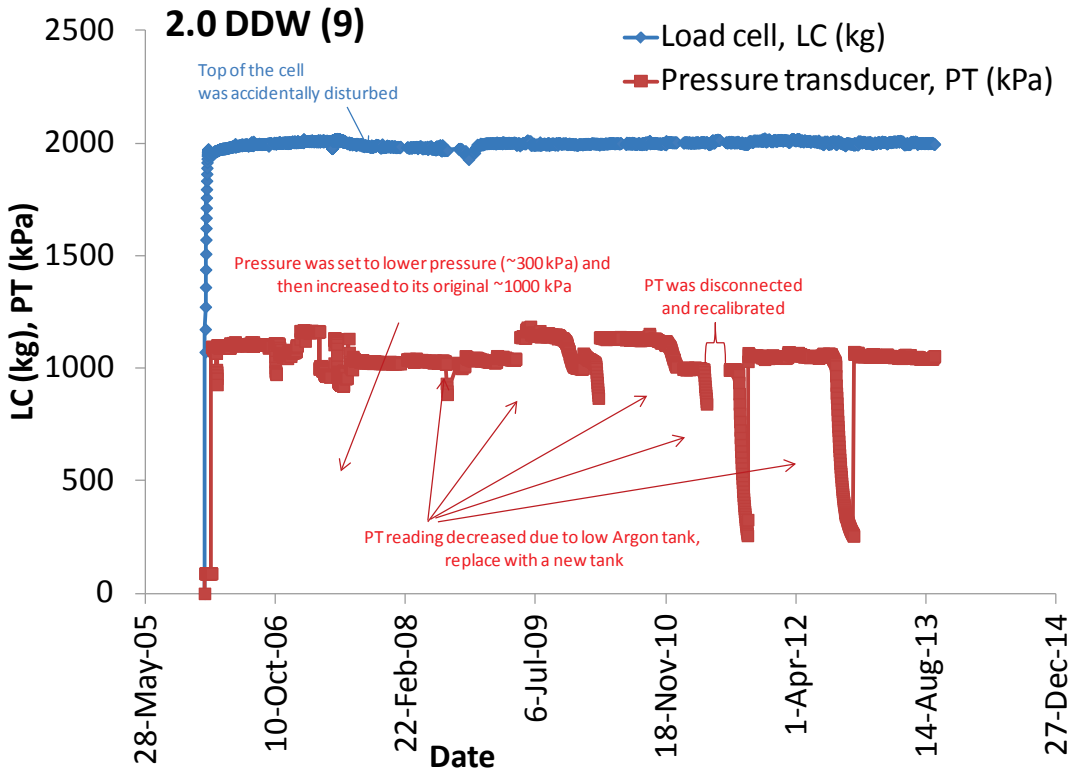
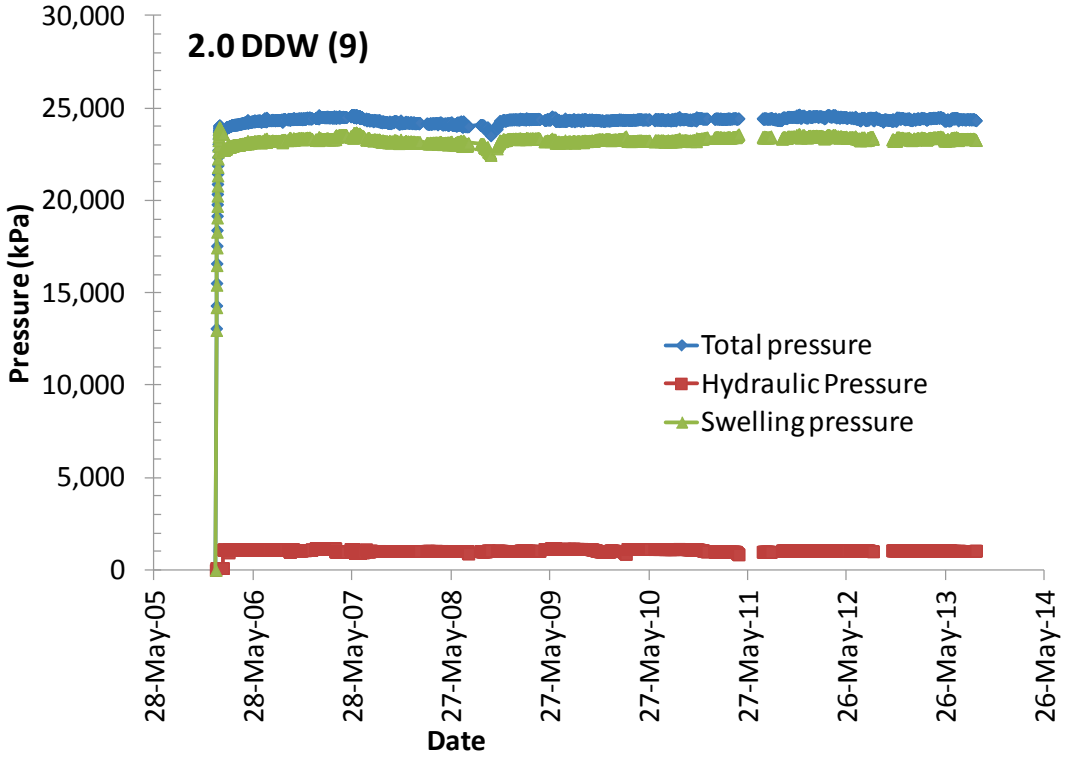


Figure B-1: Load Cell and Pressure Transducer Data for Swelling Pressure Test of Specimen 2.0 DDW (9)



-

Figure B-2: Evolution of Total Pressure, Hydraulic Pressure and Swelling Pressure of Specimen 2.0 DDW (9)

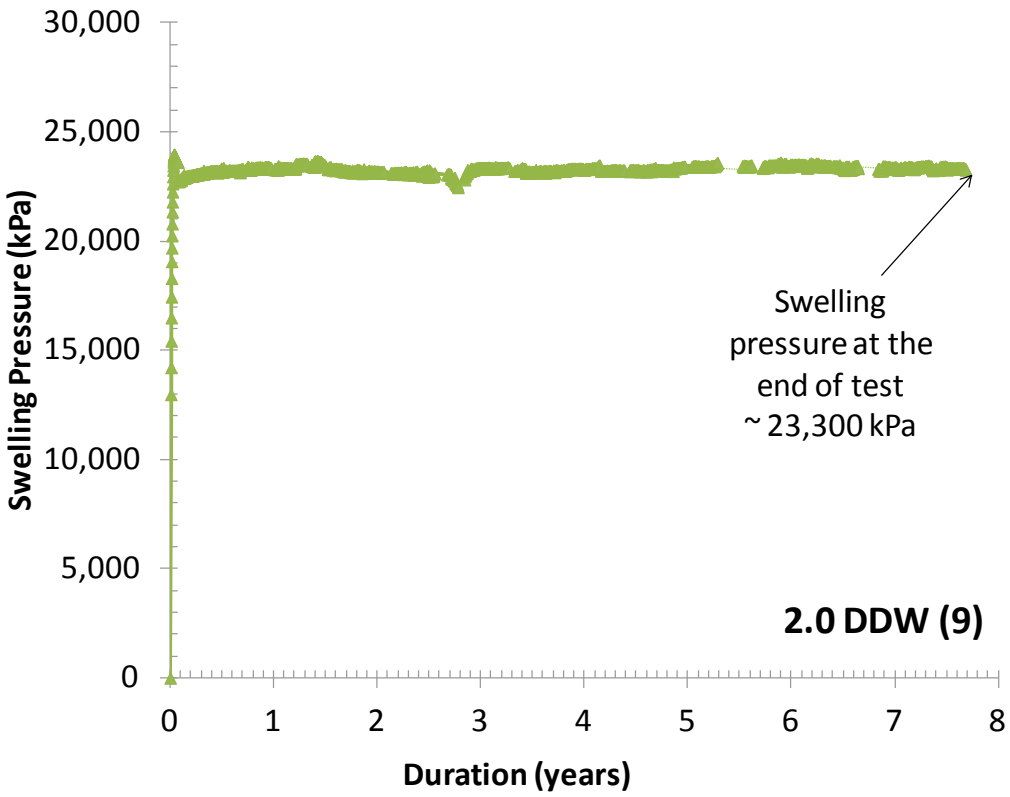


Figure B-3: Evolution of Total Pressure, Hydraulic Pressure and Swelling Pressure of Specimen 2.0 DDW (9)

B.2.2 Calculation of the Dry Density and Effective Montmorillonite Dry Density (EMDD)

The dry density and EMDD were calculated based on the measurements at the end of test after the plug was dismantled. The spread sheet used to calculate the dry density and EMDD of the specimen is provided in Table B-2.

Dry density calculated from the gravimetric water content measurement and the assumption that the specimen was fully saturated. Specimen is assumed to be fully saturated and dry density (ρ_{dry}) was calculated using the following equation:

$$\rho_{dry} = \frac{G_S \times \rho_w}{1 + \left(\frac{w \times G_S}{S} \right)} \quad (5)$$

G_s = specific gravity of soil solid (=2.745 for this specimen),
ρ_w = density of water = 1000 kg/m^3
S = degree of saturation = 100%
w = w_{sat} = saturated water content (=22.37 % for specimen 2.0 DDW (9))

Dry density calculated using method was 1.70 Mg/m^3.
This dry density was lower than what was targeted (~2.0 Mg/m^3), because specimen thickness was greater than the target during installation. There has not been a drastic change in the

swelling pressure measured during the test (Figure B-4). Since the most important measurement in this test was the end of test properties, since it corresponds to the final swelling pressure, the dry density at the end of test was used for further analysis.

B.2.2.1 Calculation of EMDD

The calculation of the EMDD in this document was done using the following method.

$$EMDD = \frac{M_m}{(V_m + V_v)} = \frac{f_m \cdot f_c \cdot \rho_d}{\left[1 - \left(\frac{(1-f_c)\cdot \rho_d}{G_a \cdot \rho_w}\right) - \left(\frac{(1-f_m)\cdot f_c \cdot \rho_d}{G_n \cdot \rho_w}\right)\right]} \quad (6)$$

where: ρ_d = dry density of soil (kg/m^3);
ρ_w = density of water (kg/m^3);
f_c = mass fraction of clay in dry solids;
f_m = mass fraction of montmorillonite in clay fraction f_c;
G_a = specific gravity of aggregate solid;
G_n = specific gravity of non-montmorillonite component in clay;
G_s = specific gravity of soil solid;
M_m = mass of montmorillonite component (kg);
V_m = volume occupied by montmorillonite component (m^3); and
V_v = volume of void (m^3).

The following coefficients were used to calculate EMDD in this document:
f_m = 0.75, f_c = 1.0, G_a = 2.745, G_n=2.645, G_s = 2.745, ρ_w = 1 g/cc
Substitution of the ρ_{dry} of 1.70 g/cm^3 to Equation (6), results in the EMDD for specimen 2.0 DDW (9) equal to 1.52 Mg/m^3.

Figure B-4 shows plot of EMDD versus swelling pressure of the specimen 2.0 DDW (9), compared to the trend line of the AECL's swelling pressure test data base for specimen using distilled water. Data points from AECL data base also shown in Figure B-4.
In addition, 3 data points from previous swelling pressure tests for microbial study using target dry density of 2.0 Mg/m^3 are also shown.

The result of the swelling pressure and EMDD measurement of the long-term test of specimen 2.0 DDW (9) was comparable to the trend line of the data base.

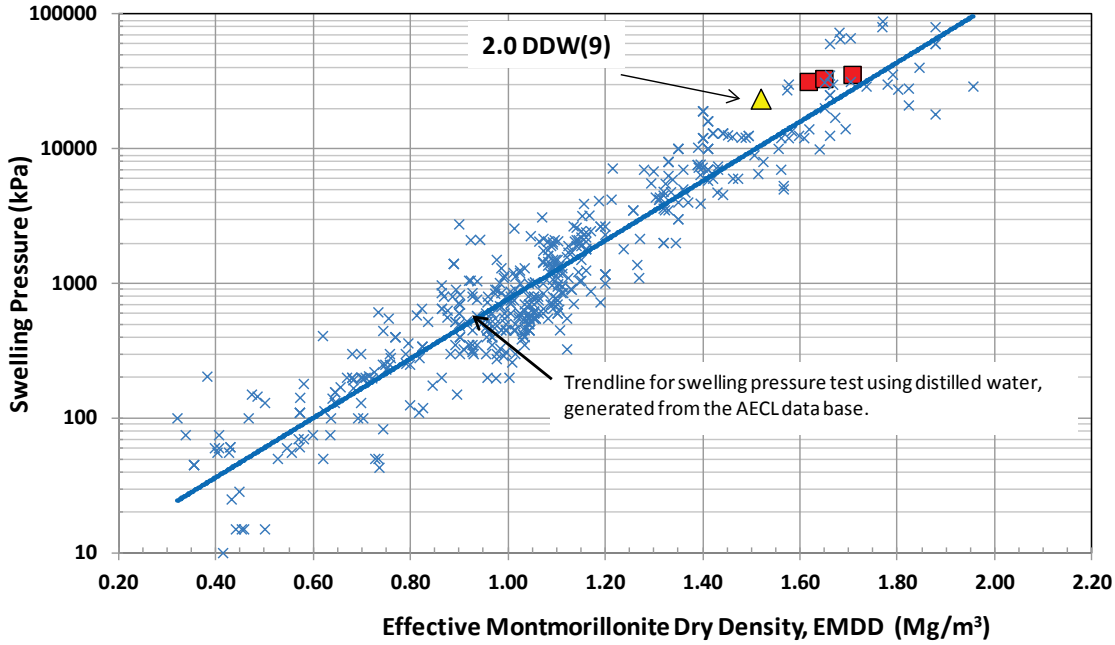


Figure B-4: Comparison of the Long-term Microbial Study Result with the Trend Line Generated from AECL's Swelling Pressure Test Database using Distilled Water (>500 data points)

B.3 SUMMARY

- Swelling pressure of the specimen 2.0 DDW (9) has been stabile at 23,350 kPa since 2007.
- Dry density and EMDD of the specimen were determined from the measurement at the end of the test and equal to 1.70 Mg/m^3 and 1.52 Mg/m^3, respectively.
- These measurements were comparable with the AECL's data base.

REFERENCE

ASTM D2216 -10, Standard Test Methods for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass

Table B-1: Load Cell and Pressure Transducer used to Test Specimen 2.0 DDW (9)

SWELLING PRESSURE OF THE LONG-TERM SWELLING PRESSURE - MICROBIAL TEST	
Test conducted by:	C. Hamon, B. Evenden, & C. Kohle
Date:	2013 October 31
Specimen Name:	2.0 DDW (9) - channel 87, Stn. 30
Target dry density (Mg/m^3):	2
Mixing Liquid:	DDW
Location, Stn.:	30
Installation date:	10-Jan-2006
End of test:	16-Sep-2013
Duration (years):	7.7
Note: Two different pressure transducer was used in this test to measure Argon Pressure	
INSTRUMENTS CALIBRATION INFORMATION	
Load cell	
Serial No.	185690
Range:	0-4000 lb
Calbration date:	21-Mar-2005
Channel No.	87014
Fitting coefficients (mV-kg)	
A =	83.629
B =	-29.07
Press. Transducer 1.	from Jan 2006 to 19 May 2011
Serial No.	286780
Range:	0-3500 kPa
Calbration date:	N/A
Channel No.	87023
Fitting coefficients (mV-kPa)	
A =	108.73
B =	107.42
Press. Transducer 2.	from 19 May 2011 to end of test September 2013
Serial No.	286780
Range:	0-3500 kPa
Calbration date:	19-May-2011
Channel No.	87023
Fitting coefficients (mV-kPa)	
A =	115.83
B =	21.937

Table B-2: Dry Density and EMDD of Specimen 2.0 DDW (9)
Specimen 2.0 DDW (9)

Dry density at the end of test	
Specimen:	2.0 DDW (9)
Analyst by:	D. Priyanto
Date:	2013 November 02
Data provided by:	Connie Hammon & Sim Stroes-Gascoyne
Date:	2013 November 01
Dry density calculation - version 2 **Assuming that the plug was completely saturated**	
Measured-Water content (%) =	22.37
Degree of saturation (%)=	100
Specific gravity, Gs =	2.745
Density of water (g/cc) =	1
Dry density (g/cc) =	**1.70**
EMDD Calculation	
Specimen:	**2.0 DDW (9)**
Note: calculated from target dry density	
	EOT
fm:	0.75
fc:	1
rw (g/cc):	1
Ga:	2.745
Gn:	2.645
Gs:	2.745
Dry density, target(g/cc)	1.70
A-EMDD	0.000
B-EMDD	0.16
EMDD, target (g/cc)	**1.52**

Note:					
3 measurement of water content was done.					
The hydraulic press that was usually used for sample dismantling has been removed from the URL.					
Manual press was used for dismantling of this specimen.					
As a result, specimen was disturbed when dismantled, because of uneven pressure applied.					
Top 1/2 of specimen was extremely wet and disturbed, while bottom of the specimen was normal & undisturbed.					
The water content measurement was done in 3 part of specimens.					
Top and side (%)=	24.45	Disturbed			
Top 1/2 (%) =	24.94	Disturbed			
Lower 1/2 (%)=	22.37	Undisturbed.			
Final dry density measurement was done from the undisturbed sample from the lower 1/2 of specimen.					