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FACTORS AFFECTING MICROBIAL ACTIVITY IN COMPACTED CLAY-BASED SEALING MATERIALS PROPOSED FOR USE IN A DEEP GEOLOGIC REPOSITORY FOR USED NUCLEAR FUEL

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ABSTRACT

Microbial activity in clay-based barriers immediately adjacent to metal used-fuel containers in a repository could affect the longevity of such containers. The current emphasis is, therefore, on reducing or minimizing microbial activity in such clay-based barriers through material composition design. Factors affecting microbial activity in clay-based materials were studied in large-scale and smaller-scale experiments. Results suggested that keeping water activity (a_w) values below ~0.95 may minimize microbial activity in clay-based barrier materials. A considerably higher effective montmorillonite dry density (EMDD), which partially controls a_w, is achievable for 100% bentonite than for previously proposed reference buffer materials, which contain only 50% bentonite.

I. INTRODUCTION

The Canadian Deep Geologic Repository (DGR) concept design for the long-term management of used nuclear fuel involves emplacement of corrosion-resistant, copper-shelled, used-fuel containers at a depth of 500-1000 m in a stable rock mass, surrounded by compacted clay-based barriers.

The potential for microbial activity in the clay-based barriers immediately adjacent to the used-fuel containers in a repository is of concern for a number of reasons[1]. Microbial activity may affect the longevity of used-fuel containers either through the formation of corrosion-inducing aggressive environments under biofilms or through the production of corrosive metabolites (such as sulphides, acetate and nitrite). Mobile microbes may sorb radionuclides released from breached containers and act as colloids, increasing the migration of radionuclides through these engineered barriers. Microbial gas production may cause a build-up of a gas phase in a repository, which could reduce the effectiveness of the clay-based barriers.[1]

However, clays and clay-rich deposits are considered in many international nuclear waste management programs as suitable engineered barrier materials or as a host rock environment for a repository. Laboratory tests, large *in situ* tests in underground research laboratories and the study of natural analogs have demonstrated the physical and geochemical containment capacity of clay, even in the presence of microbes. Physical factors that contribute to restricting the migration and metabolism of microbes in clays are the very small average pore throat diameters, the mostly small pore sizes and the low percentage of interconnected porosity found in clays. Another factor that affects microbial activity in clay-rich environments is the amount of available water, which is reduced by interactions with solute molecules (osmotic effect) and by adsorption to the surface of the clay particles (matric effect).

Microbiologists generally use the term water activity (a_w) to quantify the available water in materials such as soils and foods. The water activity of a solution or material sample is defined as 1/100 the relative humidity of air in equilibrium with that sample (when expressed as a percent) or in other words, a_w is the relative humidity of the sample, expressed as a fraction. Low a_w values are well-known deterrents for bacterial growth in the food industry (e.g., drying, high sugar or salt concentrations) and the relationship between a_w and microbial growth limits is well established.[2] Most bacteria flourish only at a_w values around 0.98 (the a_w for seawater) or higher and a value of about 0.95 is the lower limit for growth for most gram-negative bacteria.[2,3] Some specialized osmotolerant and halophilic organisms grow at much lower a_w values but no microbes are thought to be able to survive at a_w <0.6, because DNA becomes permanently disordered at this value.[2]

Because of the potential impact of microbial activity, especially on container longevity, an extensive study is underway to determine the extent of microbial occurrence and activity in various clay-based sealing materials considered in the DGR conceptual design, and to determine factors that may reduce or minimize this activity.

II. MICROBIAL POPULATIONS IN REFERENCE BUFFER MATERIALS

The occurrence of microbial populations in compacted reference buffer material (RBM, composed of 50% sand and 50% Na-bentonite) has been determined in a number of large-scale engineering tests at Atomic Energy of Canada Limited's (AECL)'s Underground Research Laboratory (URL). In these tests, the performance of compacted RBM was assessed under a variety of *in situ* conditions:

i) Unsaturated conditions at elevated temperature (Buffer-Container Experiment, BCE[4, 5]);

ii) Unsaturated and unheated conditions (Isothermal Test, ITT[6,7]);

iii) Almost fully saturated and unheated conditions (Buffer-Coupon Long-Term Test, BCLT.)[8]

Figure 1 shows the microbial population size of aerobic heterotrophs cultured on a dilute growth medium (R2A[9]) in <u>comparable</u> samples from these three large-scale tests. Also included in Figure 1 are the aerobic heterotrophic populations in archived RBM from the BCE[10] and ITT[6,10] (no microbial measurements were done prior to the start of these experiments) and freshly prepared RBM from the BCLT.[8] Figure 1 shows that RBM contains a sizable culturable (i.e., able to grow on R2A growth medium) population of aerobic heterotrophs prior to emplacement (between 10^5 and 10^7 Colony-Forming Units (CFU) per gram dry weight). However, after emplacement for various lengths of time, changes in culturability were observed. The aerobic heterotrophic populations in retrieved samples ranged from 10^3 to 10^5 CFU/g dry weight, with most values between 10^3 and 10^4 CFU/g dry weight. This constitutes a significant reduction in culturable population size (about two to three orders of magnitude). A reduction in microbial culturability as a result of external factors (compaction in this case) suggests that microbial metabolism and, therefore, viability and *in situ* activity, have been affected negatively.

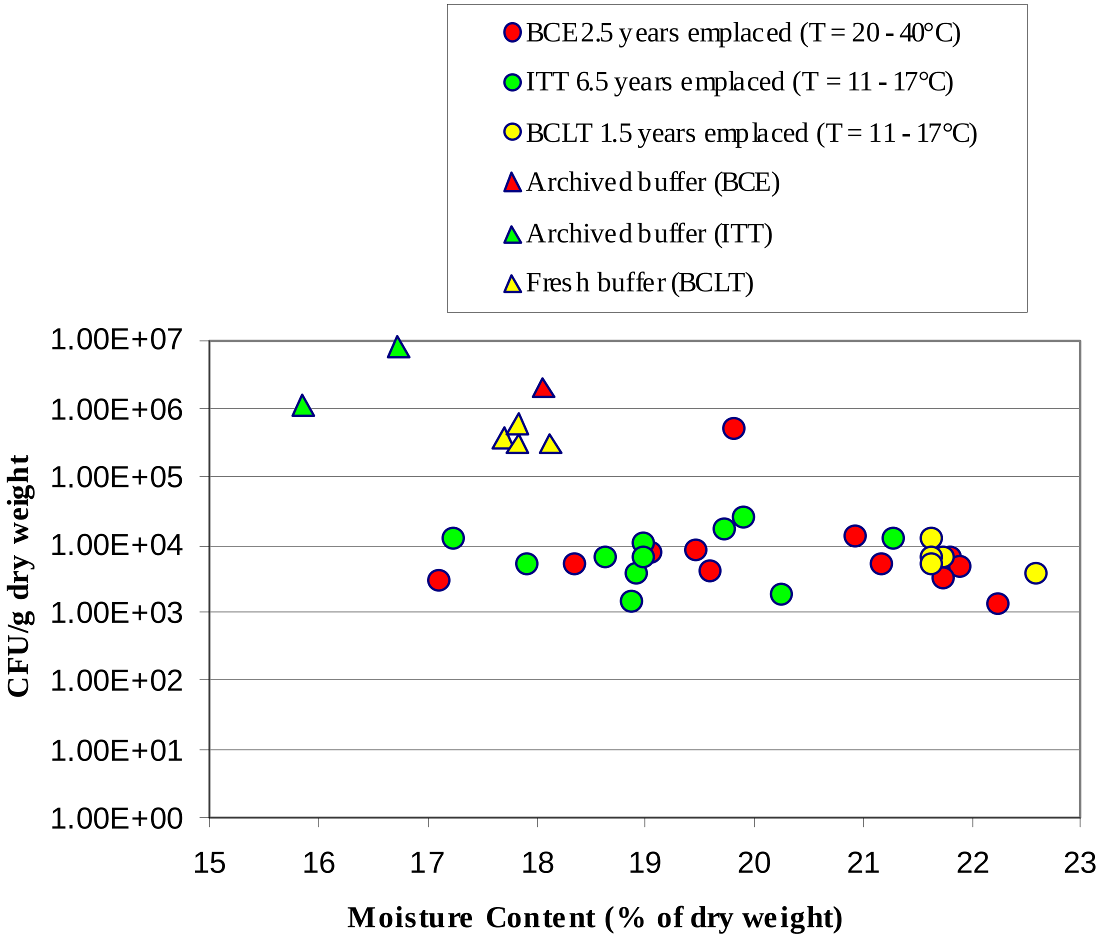

Figure 1: Comparison of culturable aerobic heterotrophic microbial populations (in CFU/g dry weight) in Reference Buffer Material (RBM) samples from various large-scale experiments (BCE, ITT and BCLT).

A small number of retrieved RBM samples from each test were also analyzed for phospholipid fatty acids (PLFA) content and ratios[4,6,8] (data not shown). PLFA's are components of intact cell walls and are thought to be indicative of potentially viable microbial cells, while the actual PLFA content can also be related to a potentially viable population size. Almost invariably the PLFA analyses indicated that the microbes in the retrieved RBM samples showed signs of decreased membrane permeability, indicating general stress, and shifted PLFA ratios, indicating starvation stress. The culture data combined with the PLFA data suggest, therefore, that microbial culturability and vigour are reduced for compacted RBM in an *in situ* environment. This probably indicates reduced metabolic activity, but not to the extent that the cells have died.

Microbial culturability and derived potential microbial activity in clay-based sealing materials could be affected by a number of factors, including:

- Temperature (bacteria have minimum, optimum and maximum temperature ranges for metabolic activity; temperature affects moisture content).

- Moisture content (bacteria are affected by the availability of free water, as represented by the a_w value of the sample).

- Pore size distribution (compared to microbial cell size)

- Compaction density (which affects pore size distribution, water content and a_w values)

- Material composition of the clay-based buffer (e.g., bentonite content affects a_w and pore size distribution)

- Salinity of the pore water (which affects pore size distribution and a_w)

In the three large-scale tests (BCE, ITT and BCLT) summarized in Figure 1, factors of temperature and moisture content could have played a role, but not compaction density because all three experiments contained RBM of identical composition and the same dry density, i.e., 1.70 $\pm$ 0.05 Mg/m^3. Differences in material composition and dry densities were assessed in other large scale and smaller-scale experiments. The effects of the above factors on microbial culturability in clay-based sealing materials are discussed in Sections III and IV.

III. EFFECTS OF TEMPERATURE, MOISTURE CONTENT AND A_W

The BCE experiment included examination of the effects of temperature and related moisture content distributions on microbial culturability. The samples from the BCE included in Figure 1 were samples with temperatures $\leq 40^oC$. Figure 2 shows all aerobic heterotrophic culture results (on R2A medium[9]) from the BCE as a function of moisture content, with indication of ranges in sample temperature (i.e., 20-30^{o}C, 30-45^{o}C and 45-60^{o}C).[4] Figure 2 shows clearly that below a moisture content of 15% (of dry weight), no bacteria could be cultured in RBM from the BCE. The influence of temperature is less clear from this figure. Although all samples with moisture content below 15% had temperatures in the range of 45 to 60^{o}C, other samples with similarly high temperatures showed considerable microbial culturability, presumably because they had higher moisture contents. A statistical analysis of the BCE culture results was performed, taking all factors into account (i.e., temperature, moisture content, culture media and culture temperature[4,5]). This analysis showed that, although culture conditions had some influence, temperature (in the range 20 to 60^{o}C) did not significantly affect the amount of culturable organisms found, but that moisture content appeared to significantly affect microbial culturability in RBM from the BCE.

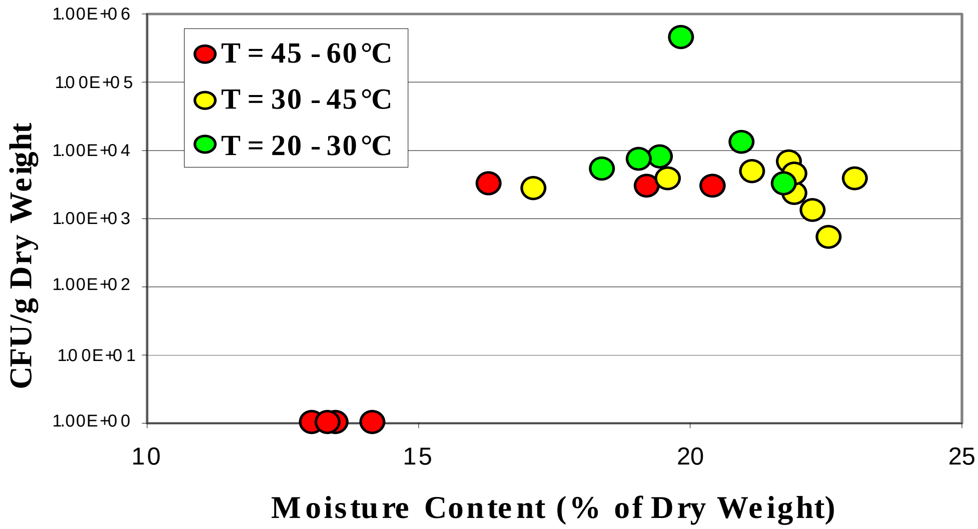

Figure 2: Culturable aerobic heterotrophs in the Buffer-Container Experiment (BCE) as a function of moisture content and temperature.

These results led to the suggestion that controlling moisture content may be a tool in controlling microbial activity in clay-based sealing materials. However, the relationship between moisture content and culturability (Figures 1 and 2) only holds for the particular material (in this case RBM) for which the moisture content was measured. In order to make comparison of data sets obtained with different materials possible, several parameters have been defined to normalize data from materials with different compositions. One such parameter is the effective montmorillonite dry density (EMDD), defined as the dry mass of montmorillonite divided by the sum of the volume occupied by the montmorillonite and the volume of the voids.[11] The EMDD parameter, therefore, compares materials based on their montmorillonite content only (the latter is responsible for the swelling capacity of the clay-based materials). A related parameter is the effective montmorillonite water content (EMWC), defined as the water content divided by the dry mass of montmorillonite which, therefore, normalizes water content to montmorillonite content in a sample. Another more universally applicable parameter, used often by microbiologists, is a_w (already defined in Section I).

Water activity measurements are not available for the RBM samples from the BCE, ITT or BCLT. However, Figure 3 shows how a_w relates to EMWC in RBM of two EMDD values (1.08 and 1.21 Mg/m^3), derived from laboratory scale experiments.[12,13] The measurements were obtained with a Decagon™ CX-1 water activity meter (Decagon Devices Inc., Pullman, WA[12]) as part of laboratory experiments in which the ingress of bacteria in compacted sterilized RBM plugs was measured, to assess mobility in RBM. Since the materials used and the EMDD values achieved in these laboratory-scale experiments were comparable to the large-scale URL experiments (BCE, ITT and BCLT), i.e., 1.08 to 1.21 ± 0.05 Mg/m^3 versus ~1.15 ± 0.05 Mg/m^3, the a_w data in Figure 3 are considered valid for the BCE, ITT and BCLT materials. Figure 3 shows that the a_w value in RBM at about 37.5 % EMWC (or a moisture content of about 15% of dry weight) is around 0.95. Microbes could not be cultured in the samples from the BCE below a water content of about 15% (EMWC 37.5%) and, therefore, the a_w value boundary for significant culturability in RBM appears to be around 0.95. Figure 3 also indicates some scatter in the measured a_w data. This is possibly due to the nature of the analysis, i.e., the measurement is taken after an equilibration period in a sealed chamber. The length of the equilibration period depends on how fast the reading stabilizes and the judgement of this could lead to some subjectivity.

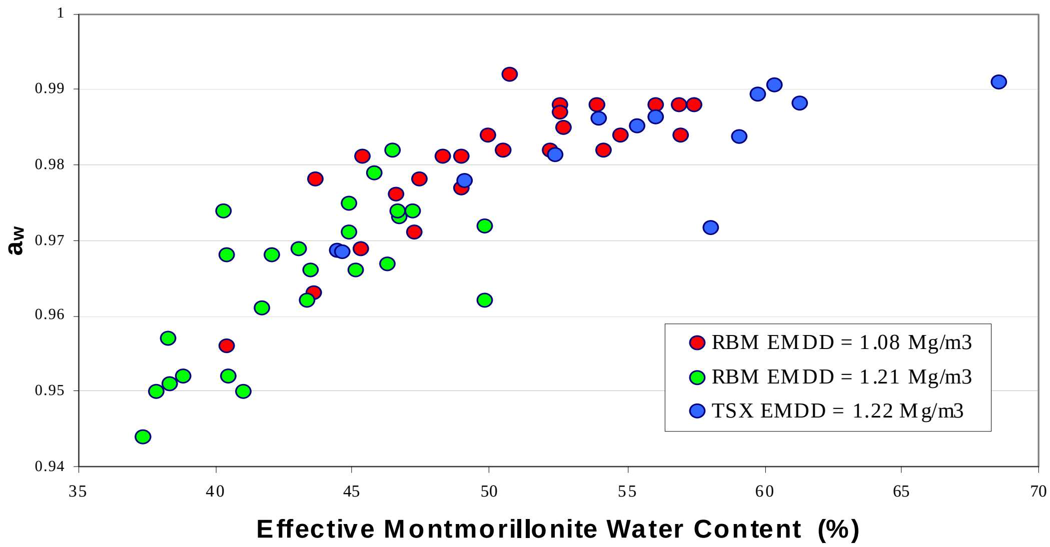

Figure 3: Measured water activity values as a function of Effective Montmorillonite Water Content (EMWC) in Reference Buffer Material (RBM) and Tunnel Sealing Experiment (TSX) clay samples.

Included in Figure 3 are data from another full-scale *in situ* experiment, the Tunnel Sealing Experiment (TSX). This international experiment (also performed at AECL's URL) was funded by Canada, Japan, France and the USA. The objective of the TSX was to assess the applicability of technologies for construction of full-scale concrete and clay bulkhead seals; to evaluate the performance of each bulkhead; and to identify and document the parameters that affect that performance[14,15]. The clay bulkhead seal consisted of pre-compacted clay blocks, made from 70% bentonite (in this case Kunigel™ which has a somewhat different montmorillonite content than the bentonite used in the RBM of the BCE, ITT and BCLT) and 30% sand at an initial moisture content of 14.5% (of dry weight) and a dry density of 1.9 Mg/m^3 (EMDD 1.22 Mg/m^3, comparable to the RBM materials in Figure 3). Many samples, especially from the bulk clay blocks and the clay block-granite interfaces, were taken during the decommissioning of the TSX and analyzed for culturable aerobic heterotrophs and other organisms.[16] Moisture content and a_w (using a Decagon™ WP4 Dewpoint PotentiaMeter (Decagon Devices, Pullman, WA)) were also measured[16]. Figure 3 shows that, based on EMWC and comparable EMDD values, the a_w measurements for the TSX material are in good agreement with the RBM measurements.

Figure 4 shows the amount of aerobic heterotrophs (on R2A medium[9]) found in TSX samples as a function of moisture content (% of dry weight). Culture data from freshly compacted clay blocks (obtained in 1997) are also included. The data in Figure 4 show a drop of about an order of magnitude in culturability in the bulk clay block samples after emplacement (about 7 years) compared to freshly prepared clay block samples. Aerobic heterotrophic culturability was also determined for clay block-clay block interface samples and for clay-granite interface samples from near the tunnel wall. During emplacement of the clay-bulkhead seal, the granite tunnel wall was coated with a layer of shot clay (to facilitate emplacement of the clay blocks) of the same composition as the clay blocks but with a lower dry density. The clay-granite interface, therefore, had a somewhat lower dry density than the bulk clay block samples. Figure 4 shows that clay block-clay block interfaces sampled contained similar-sized culturable aerobic heterotrophic populations to those at the start of the experiment in the freshly prepared clay blocks. At the clay block-clay block interfaces, the average moisture content did not increase compared to the starting moisture content values. It may, therefore, be that the somewhat larger space allowance at these interfaces prevented a drop in culturability compared to the bulk clay block samples.

Clay block-granite interfaces showed a clear increase in culturability by up to an order of magnitude compared to the values in freshly prepared clay blocks, giving culturabilities of at least two orders of magnitude higher than in the bulk clay block samples after 7 years of emplacement. At the clay block-granite interface, water from the hydraulically pressurized chamber entered the clay blocks from the shotclay that was placed between the clay blocks and the rock, which has clearly increased moisture content in these samples, in addition to the larger space availability at these interfaces. Both factors appear to have led to a significant increase in aerobic culturability at the clay block-granite interfaces. Similar observations of significantly larger (order of magnitude range) aerobic heterotrophic populations were also made for RBM-granite interfaces in the BCE and ITT. It is, therefore, possible that clay-rock interfaces (and possibly interfaces between clay and other materials) in a repository may be conduits for microbial activity and perhaps mobility. This could be of consequence for the sealing ability of clay-based seals and needs to be investigated further.

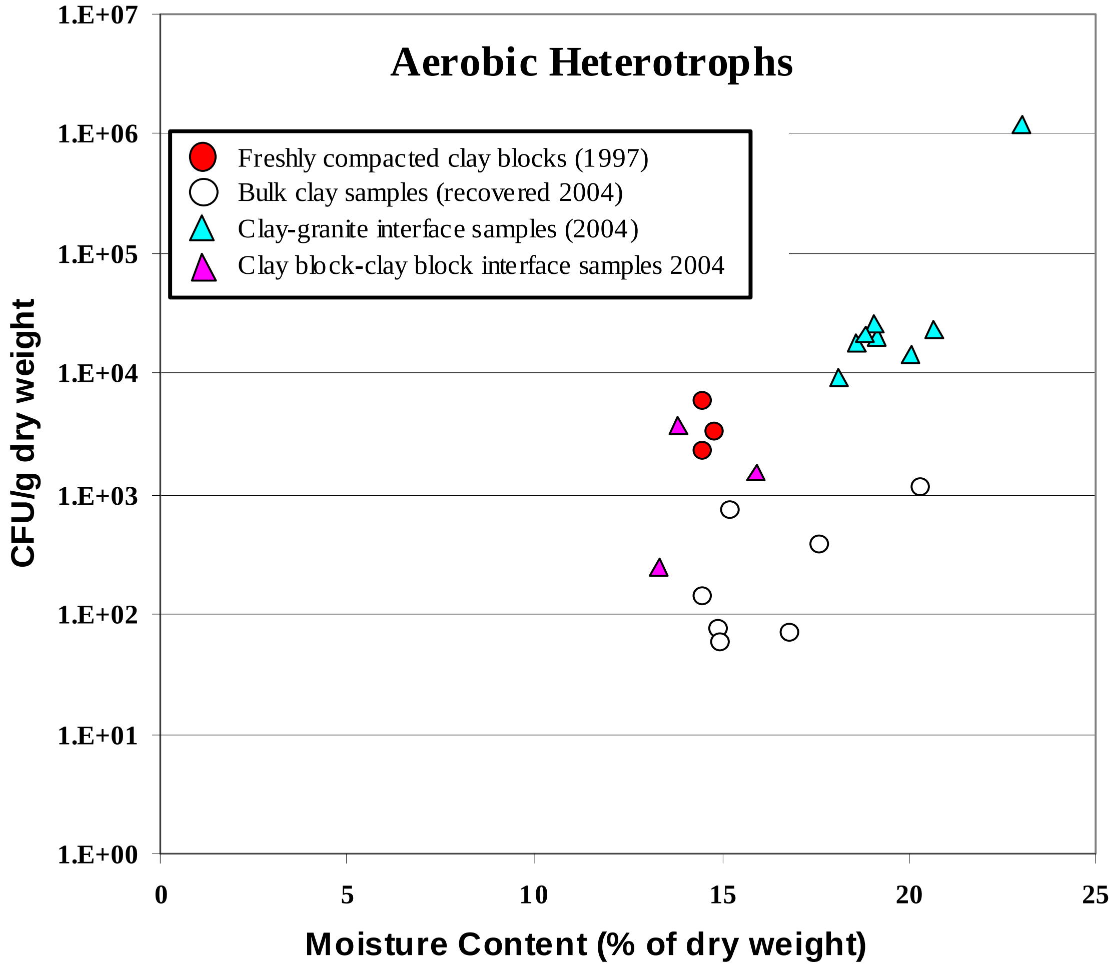

Figure 4: Culturable aerobic heterotrophs in samples from the Tunnel Sealing Experiment (TSX) as a function of moisture content and sample location.

IV. EFFECTS OF DRY DENSITY AND SALINITY IN 100% BENTONITE

The BCE results suggest that bacteria could no longer be cultured in RBM samples with a_w <0.95. Clearly a substantial change in culturability (and therefore metabolism) occurred in the relatively short time span (2.5 year) of the BCE. However, Figure 3 has shown that saturated or nearly saturated RBM of dry densities up to 1.8 Mg/m^3 (EMDD 1.21 Mg/m3) always has an EMWC value > 37.5% (or moisture content well above 15% of dry weight) and a_w values > 0.95 It would, therefore, be very difficult to obtain sufficiently low moisture contents (and a_w values) to curtail microbial activity within RBM in a saturated repository.

A Swedish study[3] has reported that microbial survival of laboratory-grown bacteria (isolated from granitic groundwater) was severely reduced and may be non-existent in water-saturated 100% bentonite compacted to a dry density of 2 Mg/m^3 as a result of lower a_w values (<0.96) and possibly the low frequency of pore spaces large enough to accommodate microbes. Therefore, it appears that highly-compacted 100% bentonite may be a more suitable material to control microbial activity near metal used-fuel containers. However, in the Swedish study[3], laboratory-grown microbes were used which may not have been as well adapted to the nutrient-poor and harsh conditions in a clay-based environment. Any further testing of compacted 100% bentonite for microbial survival, therefore, should be conducted with organisms naturally occurring in the (uncompacted) bentonite and in the groundwaters that would saturate the compacted bentonite in a repository.

Compacted 100% bentonite (dry density 1.8 Mg/m^3; EMDD 1.6 Mg/m^3), mixed with URL Fracture Zone 2 groundwater to ~95% saturation was manufactured with the intent of emplacing it as part of the BCLT. Microbial analysis of this freshly prepared and compacted 100% bentonite suggested that this is indeed a less favourable environment for microbial culturability than RBM, with culturable aerobic heterotrophic populations of around 4×10^2 CFU/g dry weight[8] (data included in Figure 6) which is lower than culturability in RBM (10^3-10^4 CFU/g dry weight, Figure 1). However, effects of long-term emplacement on microbial culturability in 100% bentonite (e.g., a possible further reduction) could not be assessed in the BCLT because the test was terminated before emplacement of this material could take place[8].

The presence of highly saline pore water (resulting partially from saline ground waters in granitic host rock and partially from the precipitated minerals in bentonite that would dissolve during the saturation process with groundwater) could change swelling pressures and a_w values in 100% bentonite, unless the EMDD is sufficient to minimize these effects. Saline pore fluid will open up free pore space and thus potentially facilitate microbe migration and activity in highly compacted bentonites. The effects of opening up of pore space and decreasing the swelling pressure will be, at least partially, offset by a decrease in a_w of the pore fluid as a result of the high salinity. There is, therefore, a need to improve the understanding of the effects of saline pore water solutions on the physical and microbial characteristics of compacted 100% bentonite. Experiments are now in progress, in which the effects of salinity, EMDD and resulting swelling pressures on a_w values and microbial culturability are measured. Initial results are shown in Figures 5 and 6.

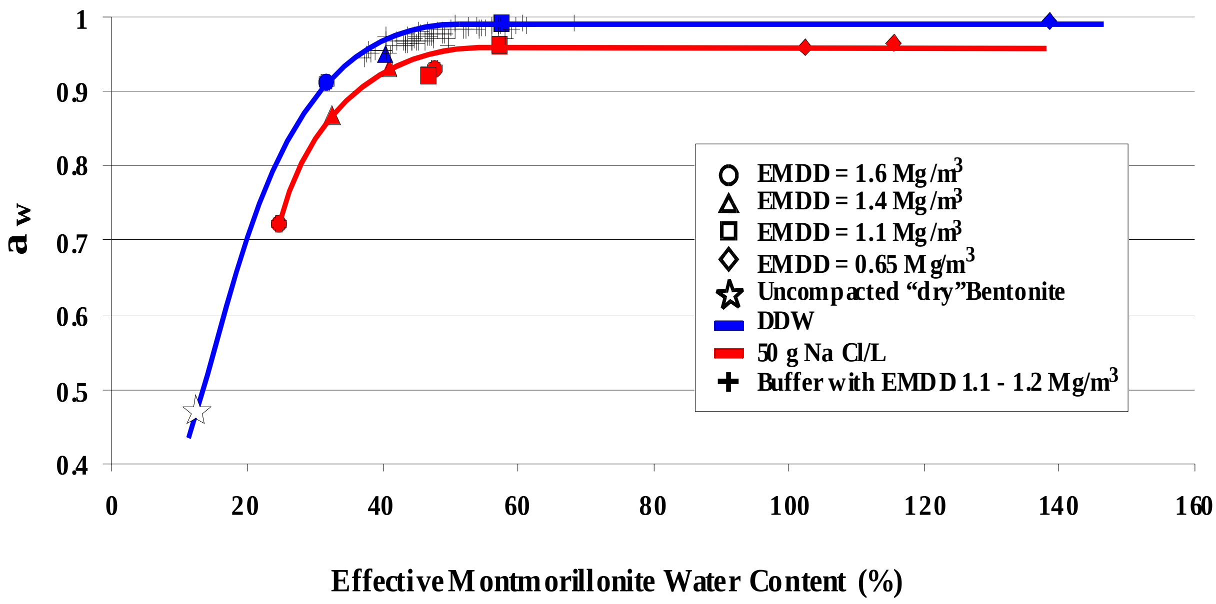

Figure 5: Effects of Effective Montmorillonite Dry Density (EMDD) and salinity on water activity values as a function of Effective Montmorillonite Water Content (EMWC).

Figure 5 shows the effect of dry density (in terms of EMDD) on measured a_w values as a function of EMWC in compacted 100% bentonite, in the presence of sterilized distilled deionized water (DDW) and in the presence of sterilized DDW to which 50 g NaCl/L was added. The a_w values were measured with a Decagon™ WP4 Dewpoint PotentiaMeter (Decagon Devices, Pullman, WA). The presence of salinity (50 g NaCl/L) appears to suppress a_w by ~0.03 to 0.04 units.

The effects of EMDD on a_w are also apparent. For the comparable EMDD values of 1.1- 1.2 Mg/m^3 and with DDW as pore water, 100% bentonite, RBM and TSX material (data from Figure 3) reach similar a_w values at saturation. Figure 5 shows clearly that compacted 100% bentonite at the higher EMDD value of 1.6 Mg/m^3 gives lower a_w values than the RBM tested

Figure 6 shows the corresponding aerobic heterotrophic culturability data as a function of EMWC (including the RBM culture data from the BCE, ITT and BCLT from Figure 1 for comparison). Note that any microorganisms in the 100% bentonite (with DDW and 50 g NaCl/L) originated from the bentonite (since sterilized solutions were used for saturation) and may, therefore, have been well-adapted to the harsh conditions in a clay environment. A slightly higher culturability in DDW compared to saline pore water is observed in most cases but the effect is not very large. The culturability in 100% bentonite is generally at least a factor of 10 lower than in RBM at the higher EMDD values. At the lower EMDD values (1.1 Mg/m^3 and especially 0.65 Mg/m^3) where the bentonite contains more water, culturability values in 100% bentonite become comparable to RBM values. Therefore, these initial results show that 100% bentonite compacted to high EMDD values indeed reduces the culturability of microorganisms contained in the bentonite, but a severe reduction in, or total lack of, culturability, as reported in the Swedish experiments[3] was not observed.

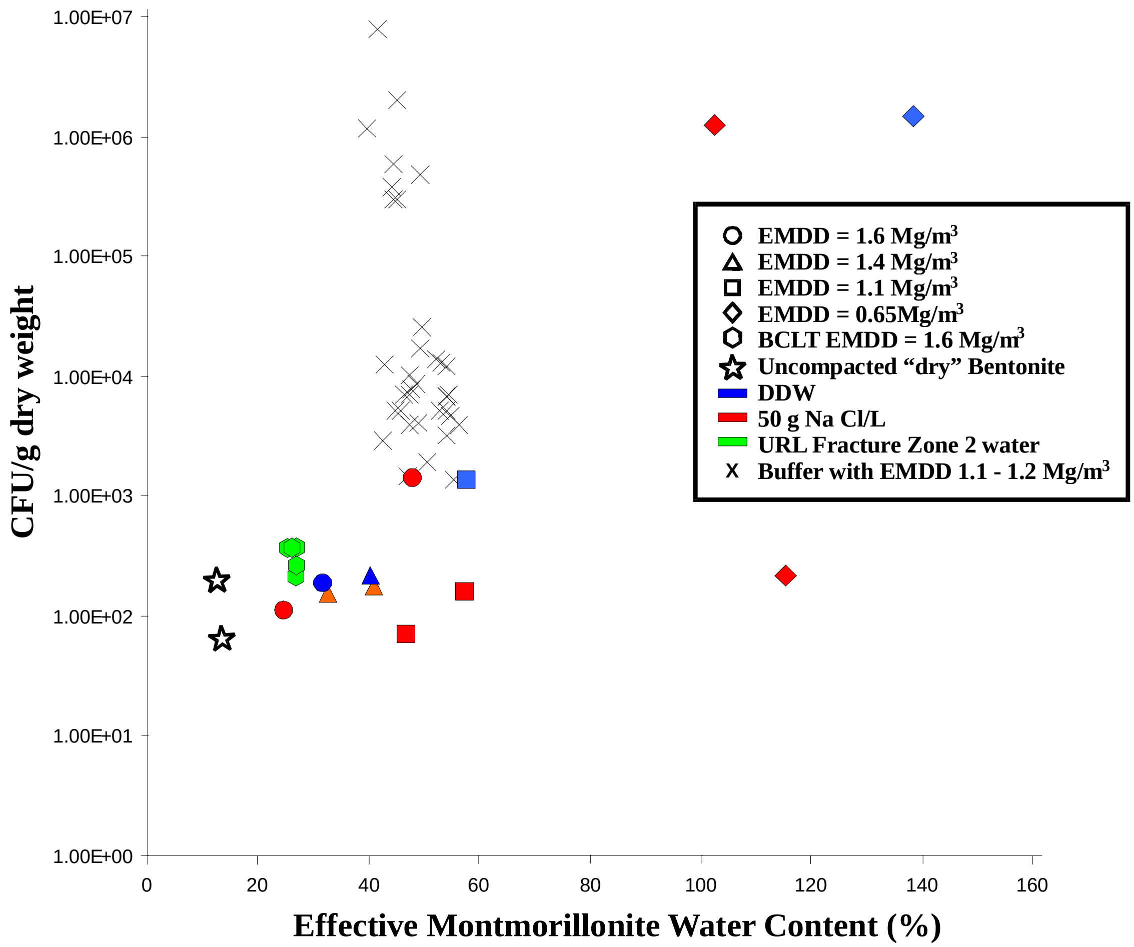

Figure 6: Culturable aerobic heterotrophs as a function of Effective Montmorillonite Water Content (EMWC), Effective Montmorillonite Dry Density (EMDD) and pore water salinity in compacted 100% bentonite.

V. CONCLUSIONS

Although much work remains to be carried out with compacted 100% bentonite and a variety of pore water salinities (up to 100g/L), it is apparent from the initial results that compacted 100% bentonite would be a better sealing material than RBM in order to minimize microbial culturability because the achievable EMDD values in 100% bentonite are much higher than for RBM. The DGR conceptual design now incorporates a dense layer of compacted 100% bentonite immediately around the waste containers. This is intended in part to impose low enough a_w values, high enough swelling pressures and small enough pore sizes to keep microbial activity and thus microbially induced corrosion effects to a minimum.

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