THE ROLE OF HIGHLY COMPACTED BENTONITE IN LOCALIZED SUPPRESSION OF MICROBIAL ACTIVITY IN A NUCLEAR FUEL WASTE REPOSITORY

S. Stroes-Gascoyne and C. J. Hamon
Atomic Energy of Canada Limited, Whiteshell Laboratories
Pinawa, Manitoba, Canada

ABSTRACT

Highly compacted bentonite (HCB), through its inherent physical properties, is able to suppress microbial activity if dry density remains sufficiently high (i.e., preferably $\geq$ 1600 kg/m^3). At certain locations in a repository the HCB dry density may not be sufficiently high, which could result in increased microbial activity in these locations. An additional concern is the potential migration of microbes through desiccation-induced fractures in the HCB that have not healed rapidly or sufficiently enough upon re-saturation. If microbes are able to rapidly travel through such fractures, there is a risk that they could populate the area immediately around the containers and (temporarily at least) increase the potential for microbiologically influenced corrosion of the containers. Therefore, a series of experiments were carried out to address this possibility through the examination of the movement of fluorescent polystyrene microspheres of microbial sizes (0.2 to 0.6 μm) along interfaces and through fractures in HCB plugs. Results confirmed that these microspheres were able to travel along the interfaces between the HCB clay plugs and pressure cells, but not through the intact clay matrix. In those plugs containing an induced fracture at the start of the experiment, some microspheres were found inside the healed fractures in about 50% of the experiments, suggesting that microbial intrusion into healed or healing fractures is possible, but not massive. Further work would be required with actual microorganisms to confirm these results.

1. INTRODUCTION

Highly compacted bentonite (HCB)-based sealing materials are being developed for use in future deep geological repositories for high-level nuclear (fuel) waste in a number of countries, including Canada. Such materials would protect the waste containers physically and chemically, would ensure a diffusion-controlled solute transport, and would form a sorption barrier against radionuclide migration after container breach and waste form dissolution.

The possibility of microbial activity in bentonite-based sealing materials in a deep geological repository for high-level nuclear waste is of concern for a number of reasons (e.g., 1-4].

- Microbiological activity in bentonite may result in microbiologically influenced corrosion (MIC), which could affect the longevity of the containers. MIC would occur through the formation of corrosion-inducing aggressive environments under biofilms, through the formation of occluded areas, or through the production of corrosive metabolites. For the latter, sulphate-reducing bacteria (SRB) that produce sulphides are of specific concern for copper containers in a deep geologic repository.
- Microbial gas production (mainly CO_2 and CH_4) may cause a build-up of a gas phase in a repository, potentially reducing the effectiveness of the bentonite-based barriers.

- Mobile microbes may sorb radionuclides released from breached containers and act as colloids, potentially increasing the migration of radionuclides through flaws (e.g., unsealed or incompletely sealed fractures) in these engineered barriers.
- Microbial activity may lead to leaching of specific elements or dissolution of minerals from bentonite, leading to corrosion of the bentonite, which could potentially affect the effectiveness of this barrier.

Fortunately, because of the inherent and specific physical characteristics of highly compacted bentonite, such as small pores, low hydraulic conductivity, low water activity (a_w) and high swelling pressure, an additional role of HCB may be the suppression of significant microbial activity in such sealing materials. This idea has been explored for more than a decade [e.g., 4, 5] and recent work [6, 7, 8] unequivocally supports the microbial activity-suppressing attributes of HCB.

2. SUPPRESSION OF MICROBIAL ACTIVITY IN HIGHLY COMPACTED BENTONITE

Stroes-Gascoyne et al. [8, 9] carried out many laboratory experiments with Wyoming MX-80 bentonite plugs. These plugs were compacted in metal pressure cells, as shown in Figure 1, at 95% saturation, to dry densities of 800 to 2000 kg/m^3.

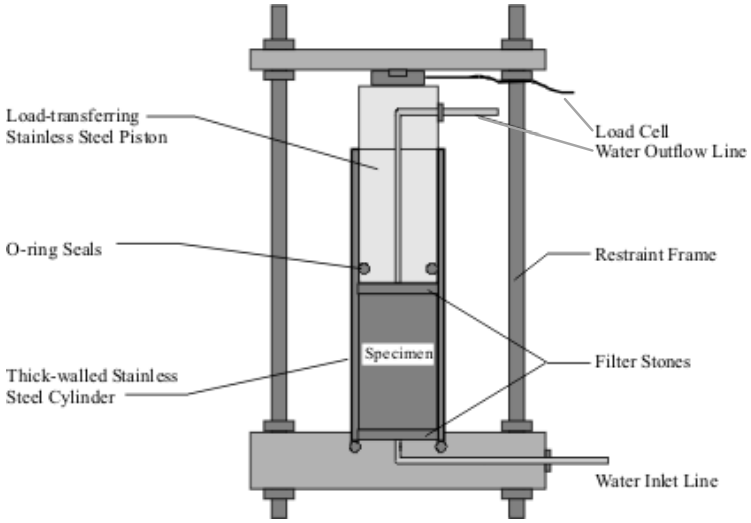


Figure 1. Schematic of a pressure cell.

The plugs were infiltrated (under pressure) with sterile distilled deionized water, and saline solutions of various compositions (NaCl and $CaCl_2$) and concentrations (0-200 g/L). After attaining a stable swelling pressure, the plugs were analyzed for the occurrence of culturable heterotrophic aerobic bacteria (by culturing) and for viable bacteria (by phospholipid fatty acid (PLFA) analysis. Microbial analyses were also carried out on HCB samples from the *in situ* large-scale long term test (LOT) at the Äspö Hard Rock Laboratory in Sweden that involved the placement of HCB (Wyoming MX-80) in a borehole [10].

Figure 2 compares equivalent data from [8] and [10] and shows that below a dry density of about 1300 kg/m^3, culturability of heterotrophic aerobic bacteria can be up to almost five orders of magnitude larger than background levels, which are about 10^2 colony forming units (CFU) per g

in as-received bentonite (powder). At high dry densities (1600 to 2000 kg/m^3), which ensure that the swelling pressure is > 2 MPa, a_w is < 0.96 and the average pore size is < 0.02 µm in HCB [8], all culturability remained at, or fell below, this background level. Figure 2 suggests that lower dry densities (1400 to 1500 kg/m^3) may also suppress microbial activity, but this needs further confirmation, because one data point from the LOT experiment [10] at a dry density of about 1500 kg/m3 shows high culturability. However, even at the highest dry density tested (2000 kg/m^3), some culturability remained, and viability (based on PLFA analysis, green circles in Figure 2) was only mildly affected by high dry density (factor of 3) [8]. Isolates from saturated HCB plugs (800 and 1600 kg/m^3) were identified by the BIOLOG^{TM} method (based on 96 substrate and enzyme reactions) and indicated a dominant presence of gram-positive, spore-forming bacteria [11]. This was also found in HCB material from the LOT experiment in Sweden (using DNA-based methods) [12]. The continued presence of viable bacteria and spores in HCB suggests the potential for increased microbial activity if a substantial reduction in the dry density of HCB were to occur in a repository.

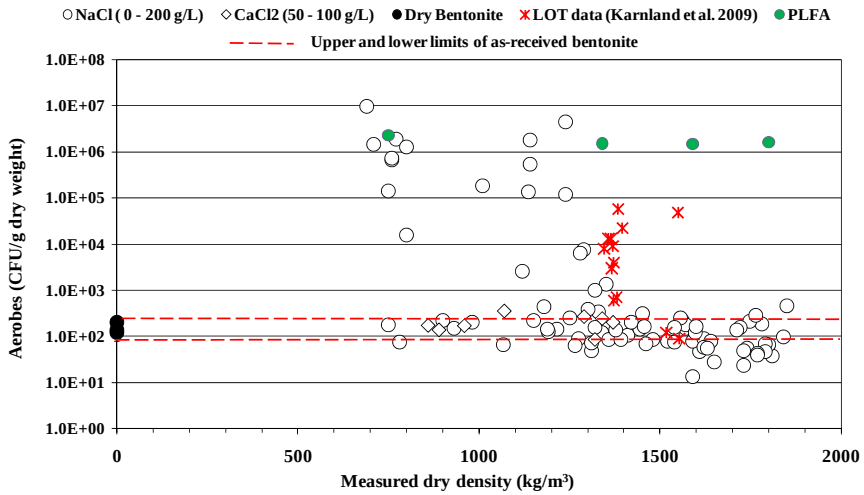


Figure 2. Comparison of the culturability of heterotrophic aerobic bacteria in highly compacted bentonite from small scale laboratory experiments (open circles and diamonds) and the *in situ* large scale LOT experiment (red stars) with phospholipid fatty acid (PLFA)-based viability (green circles) (black circles are for as received "dry" bentonite).

Masurat et al. [7] measured microbial sulphide production by SRB on small Cu plates in saturated compacted Wyoming MX-80 bentonite under repository-relevant conditions, but with the addition of radio-labelled sulphate and lactate. In all experiments increased bentonite density correlated with decreased sulphide production rates. They calculated that sulphide production rates measured in bentonite compacted to a wet density of 2000 kg/m^3 (equivalent to a dry density of 1600 kg/m^3) were a hundred to thousands of times below the rate needed to corrode through copper waste containers over 100 000 years [7]. Recent work by Pedersen [6] arrived at similar conclusions.

Confined, highly compacted bentonite with a dry density ranging from about 800 to 1600 kg/m^3 would have a hydraulic conductivity ranging from about 9 x 10^{-11} to 5 x 10^{-14} m/s while the swelling pressure would range from about 0.04 to 7 MPa [10]. Corresponding water activity

values would range from about 0.996 to 0.952 [9]. The bentonite microstructure changes continuously during compaction, hydration and swelling processes, and characterization of the pore structure in compacted hydrated bentonite is difficult [13]. Three different types of pores are generally distinguished. Mesopores and macropores mainly contain free pore water, which is charge balanced. The water in nano-scale pores is influenced by electrostatic surface forces. This bound water can be located in interlayers within the clay particles and/or in the external diffuse double layers on the surface of the clay particles (and other minerals) [13].

Using mercury intrusion porosimetry (MIP), it was determined that most of the pore diameters in highly compacted saturated bentonite with a dry density of about 1600 kg/m^3 were around 0.02 μm, with a very small population of macropores in the range of 5 to 200 μm [8, 14]. Unfortunately, conventional pore characterization methods such as MIP, nitrogen sorption (BET) and electron microscopy all require (oven- or freeze-) dried samples, which can introduce significant shrinking artefacts [13]. Recent 2-D and 3-D measurements on cryo-stabilized porewater with various electron microscope techniques and focused ion beam nanotomography have produced continuous pore size distributions, which give similar information as MIP but without the artefacts. For compacted bentonite with a dry density of 1580 kg/m^3, it was shown that the mesopores (1.5 volume %) do not form a continuously interconnected pore network. Therefore, transport of microbial nutrients and metabolic products cannot take place entirely by diffusion through the free water in the mesopores. For diffusion over distances above the micrometer scale, the pathways between the mesopores have to be bridged by nanopores in the interlayers or via the external diffuse double layers [13]. This could be one reason why microbial activity is highly suppressed in HCB with a dry density ≥ 1600 kg/m^3.

Van Loon et al. [15] studied the diffusion of $^{36}Cl^-$ in compacted bentonite, while varying both the bulk dry density of the bentonite and the external solution. Increasing the bulk dry density of the bentonite resulted in a decrease of both the effective diffusion coefficient and the Cl-accessible porosity, the latter due to anion exclusion effects. Up to a bulk dry density of 1300 kg/m^3, the interlayer contains three water layers. Between dry densities of 1300 and 1800 kg/m^3, the interlayer water is reduced from three to two layers. Above a dry density of 1800 kg/m^3, evidence of a further reduction to one water layer was found. These findings are corroborated with X-ray data, which show a decrease in the basal spacing of montmorillonite (the major component of bentonite) with increasing compaction [15]. Concurrently, around a dry density of 1300 to 1400 kg/m^3 culturability of heterotrophic aerobes was reduced to back ground levels, as shown in Figure 2 [8, 9]. It remains to be determined if this concurrent decrease in culturability is related to the reduction in the number of interlayer water layers.

Stroes-Gascoyne et al. [16, 17] studied the migration of microbial cells into compacted buffer, a 50-50 mixture of bentonite and sand, with a dry density of 1600 kg/m^3, using radiation-sterilized buffer plugs in metal tubes in contact with viable microbes (*Pseudomonas stutzeri*) in a suitable growth medium. Results after 20 weeks showed that these bacteria did not migrate into the buffer any further than the smallest distance sampled (5 mm) but that migration along the metallic holder-buffer interface was very rapid. This suggested that interfaces (or areas in the buffer) with reduced density, such as the water-clay interface (where a gel layer forms) and the buffer-restraining surface interface, are pathways for microbial migration. The tests also showed that a naturally present *Bacillus* type, which survived the radiation sterilization as spores, did not migrate out of the buffer plugs into a sterile growth medium [16]. Similar results were found in a Japanese migration study [18]. These results suggest that migration of microorganisms through

the bulk of the buffer is either not likely or a very slow process but that cracks or interfaces may form preferred pathways for migration. The studies [16, 17, 18] were for compacted bentonite-sand mixtures and would have to be verified for 100% HCB.

Based on this discussion and the data shown in Figure 2, it seems justified to conclude that the dry density (and hence the swelling pressure and a_w) of HCB barriers may be tailored such that a microbially unfavourable (i.e., inactive) environment adjacent to used fuel containers can be created, which would ensure that MIC is negligible. However this conclusion is valid only as long as the HCB has a (uniform) dry density ≥ 1600 kg/m^3, because a high dry density does not kill but only deactivates the microorganisms present in the bentonite, as shown by the PLFA-viability in Figure 2. Attention must, therefore, be paid to those locations in a repository where a high dry density may not be maintained at all times. Of some concern is the possibility that the dry density of HCB will decrease at interface locations in a repository: (1) through expansion into (empty or bentonite pellet-filled) placement gaps at the container-buffer interface; (2) through compression of, and simultaneous expansion into, less-dense buffer or backfill materials; or (3) through loss of material into water-carrying fractures at rock-bentonite interfaces. A further concern is the possibility that microbial cells in a repository could migrate to container surfaces (where they could contribute to corrosion effects) through desiccation-induced cracks in the HCB, that have not healed rapidly or sufficiently enough upon re-saturation.

To address the first three concerns, additional experiments were carried out to examine the effects of a reduction in dry density from 1600 to about 1000 k g/m^3 on the microbial culturability (and hence activity) in HCB. The tests were performed in pressure cells as before (Figure 1) and after developing a stable swelling pressure at 1600 kg/m^3, a step-wise decrease in dry density was accomplished by allowing the compacted plugs to swell into an empty space, through repeatedly adjusting the top restraints of the pressure cells. Results showed that upon a step-wise decrease in dry density from 1600 to about 1000 kg/m^3, the culturable cell count in the bentonite increased by several orders of magnitude, on a time scale of a few hundred days, confirming that the suppressing effects of high dry density on microbial activity are reversible [11]. These results imply that a reduction in the dry density of HCB in a repository at interfaces could lead to local increases in microbial activity, if that reduction were sufficient to lower dry density to ≤ 1300 kg/m^3.

To address the possibility that microbial cells in a repository could migrate to container surfaces through desiccation-induced cracks in the HCB, that have not healed rapidly or sufficiently enough upon re-saturation, a number of new experiments were conducted that included the study of the movement of fluorescent polystyrene beads or microspheres of microbial sizes (0.2 to 0.6 μm) through interfaces and cracks in HCB. The advantage of using fluorescent beads is that these beads would be distinguishable from clay particles under the microscope. The clay naturally contains a microbial population and specifically labeled microbes would be required to make these distinguishable from both the clay particles and the microbes present. While this is possible, the initial set of experiments was performed with the fluorescent polystyrene beads.

3. MATERIALS AND METHODS

Two sets of experiments were conducted. The first set of six experiments was carried out to determine if fluorescent microspheres with a diameter of 0.2 μm could migrate along the interfaces and into compacted bentonite of dry densities ranging from 1200 to 1600 kg/m^3. The

size of the microspheres used was near the bottom range of known bacterial sizes (i.e., the size range of starved or ultra-microbacteria is 0.2 to 0.4 μm [19]).

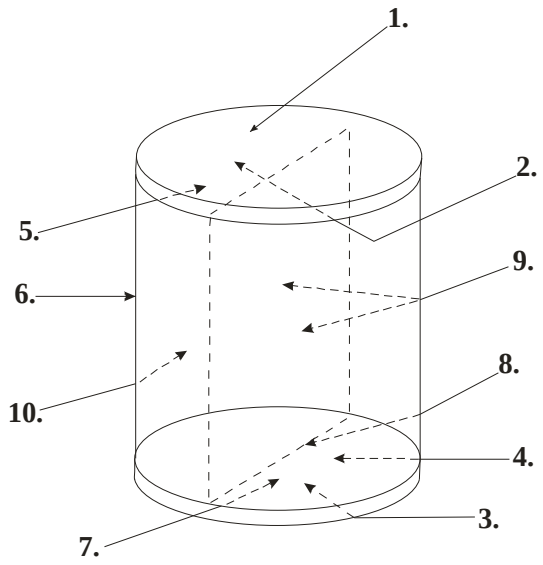
- The bentonite used was Wyoming MX-80 bentonite.
- The bentonite was compacted into ethanol-sterilized pressure cells (Figure 1) to target dry densities of 122, 1400 and 1600 kg/m^3.
- Two plugs of each target dry density were prepared.
- The bentonite plugs were about 2 cm high with a diameter of 3.2 cm.
- Before compaction, the bentonite was mixed with sterilized distilled deionized water such that, after compaction, the bentonite would be at about 95% saturation.
- During the experiments, the plugs were infiltrated under pressure to saturation with sterilized, distilled deionized water, containing fluorescent microspheres with diameters of 0.2 μm.

The fluorescent micospheres were obtained from Molecular Probes^{TM}. They were manufactured using high-quality, ultraclean polystyrene microspheres. The spheres were internally labelled with proprietary fluorescent dyes. The microspheres used in this experiment were blue fluorescing, carboxylate-modified microspheres (FluoSpheres) with a diameter of 0.2 μm. A quantity of 100 μL of the stock solution of these beads was added per litre of infusion water.

The plugs were infused for 173 days. After termination of the tests, the bentonite plugs were extruded onto clean sterilized foil, wrapped, and taken immediately to the laboratory for a number of analyses:

- The plugs were weighed and measured to determine actual dry densities.
- Water activity was measured on a subsample using a Decagon^{TM} WP4 Dewpoint PotentiaMeter (Decagon Devices, Pullman, WA).
- Water content was determined by subsequently drying this subsample in an oven at 110^o C to constant weight.
- The surface areas and the interior of the plugs were examined for the occurrence of microspheres, with an Olympus BX40 microscope with fluorescence attachment and using Image-Pro image analysis.

The tops, bottoms, sides and centers of each plug were sampled by lightly scraping some material from those locations onto a microscope slide, adding a drop of immersion oil and a coverslip and examining these slides under the microscope for blue fluorescent spheres. Prior to initiation of the microspheres experiment, suspensions of aliquots of bentonite and of the microsphere stock solution were prepared and examined under the microscope to determine if the bentonite particles would interfere with their detection but no significant interference was found at that stage. The locations of the interface and bulk samples examined (locations 1 to 4) are shown in Figure 3.



1. **Top filterstone exposed face**
2. **Top filterstone clay face**
3. **Bottom filterstone water face**
4. **Bottom filterstone clay face**
5. **Top of clay plug**
6. **Sides of clay plug**
7. **Bottom of clay plug**
8. **Fracture location at bottom**
9. **Inside of fracture**
10. **Center of clay plug matrix**

Figure 3. Schematic of the location of the samples taken from the highly compacted bentonite plugs for examination of the occurrence of microspheres.

The second set of six experiments was carried out to attempt to determine if and how far microspheres of a bacterial size range can migrate into a fracture while it is re-sealing. Two sizes of microspheres were used in this experiment, the 0.2 μm microspheres described above and larger microspheres with diameters of 0.6 μm. The latter were manufactured by Interfacial Dynamics Corporation, and were surfactant-free fluorescent blue CML latex beads. A quantity of 100 μL of the stock solutions of each these beads was added per litre of infusion water. The experiments were carried out as described for the first set, with two plugs each of target dry densities 1200, 1400 and 1600 kg/m^3. After compaction into the pressure cells, each plug was extruded and cut in half lengthwise as shown in Figure 4.

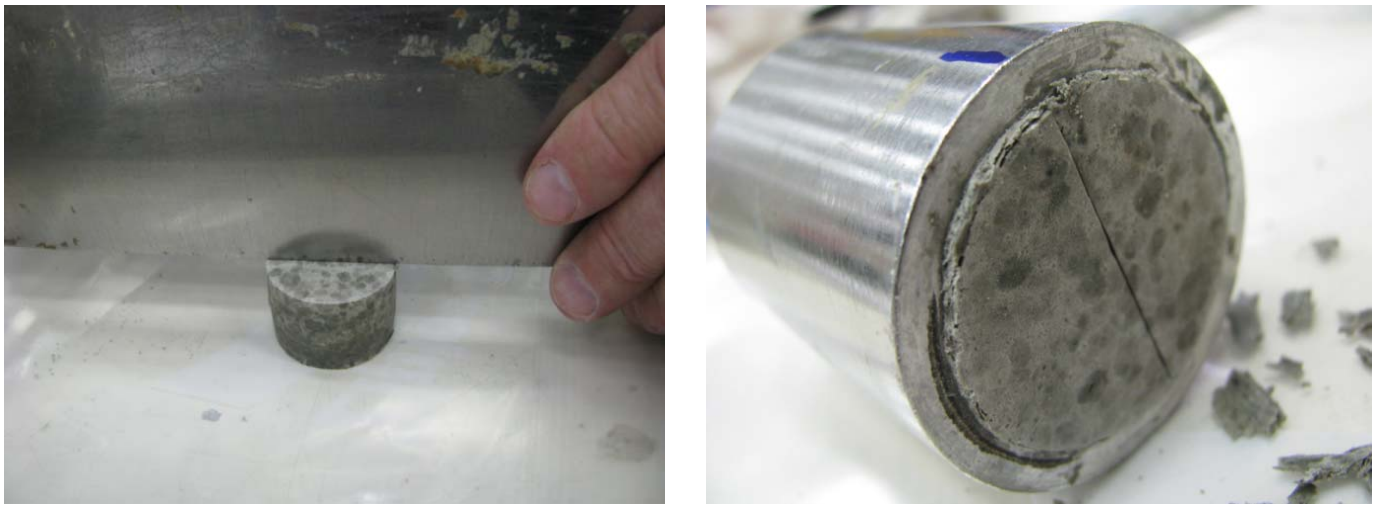


Figure 4. Preparation of "fractured" highly compacted bentonite plugs.

This removed sufficient material (the weight of which was recorded) to create a "fracture". The two halves of each plug were put together and the "fractured" plug was reintroduced into the pressure cell, and marked such that the location of the fracture in each plug was known (Figure 4). The plugs were infiltrated under pressure to saturation with sterilized, distilled deionized water, containing the fluorescent microspheres with sizes of 0.2 and 0.6 μm. Initially, a quantity of 100 μL of the stock solutions of each of these beads was added per litre of infusion water. However, because of some detection problems experienced with the first set of experiments at this concentration of microspheres, the intrusion water chambers were drained after about 3 weeks and new water was added with an order of magnitude higher concentration of microspheres of both sizes, in an attempt to make detection after termination of the experiments easier.

This second set of plugs was analyzed after 90 days of infiltration. Samples were taken as for the first set at locations 6 to 10 shown in Figure 3, with special focus on the created fracture in each plug. The plugs were split in half along the original cut. Subsequently, both sides of the (re-sealed) "fracture" were scraped off onto a microscope slide, which was examined to determine if any microspheres did enter the fracture.

4. RESULTS AND DISCUSSION

Table 1 gives the (partially inconclusive) results for the first set of experiments with microspheres (plugs 1941-1946).

It became apparent that, although a trial observation of microspheres in a suspension of bentonite was successful prior to the start of these experiments (as shown in Figure 5), it was extremely difficult to actually detect any microspheres after the experiments, even at the bottom of the plugs where they should have been easy to find.

Upon further examination using ultrasound treatment of the filterstones, it was apparent that many of the microspheres became stuck in the filterstones at the bottom of the plugs, where they were introduced. This likely "diluted" the quantity of microspheres actually getting through to the clay plugs to such an extent that they became difficult to detect. Additionally it is possible that too few microspheres were added at the beginning of the experiments, making them difficult to detect amongst the clay particles, which also possess some fluorescence. After much effort, microspheres were detected at the bottom and top of the plugs for four of the six plugs (1941, 1943, 1944 and 1945). Curiously, for the other two plugs (1942 and 1946) no microspheres could be detected at the bottom or top. Neither could microspheres be found at the sides of the plugs or in the centers of the plugs. However, finding microspheres at the top of four of the plugs implies either transport along the outside of the plugs or through the plug matrix, or both. Unfortunately, the pathways of these microspheres could not be confirmed with these results. Table 1 also shows that the measured dry densities deviated from the targeted values by from 3.3 to as much as 42.1% (plug 1944). It is not clear what caused such large discrepancies. Upon termination of experiments, the compacted bentonite plugs usually are measured and their water activity and content determined before other analyses take place. In this instance, however, samples were scraped from the tops, sides and bottoms of the plugs first and the other measurements were delayed, which could have caused some of the discrepancies. Overall, the first set of experiment with microspheres was limited in success.

Table 1. Results from microsphere migration experiments

Clay plug #	1941	1942	1943	1944	1945	1946
Target dry density (kg/m3)	1200	1200	1400	1400	1600	1600
Actual dry density (kg/m3)	1160	1300	1210	810	1360	1450
Water content (%)	50.7	46.4	50.7	83.1	42.4	32.0
Water activity	0.991	0.990	0.991	0.995	0.985	0.963
Location of samples (*)						
Top of clay plug (5)	+	-	+	+	+	-
Sides of clay plug (6)	-	-	-	-	-	-
Bottom of clay plug (7)	-	-	+	+	+	-
Centre of clay plug (10)	-	-	-	-	-	-

Clay plug #	1949	1950	1951	1952	1953	1954
Target dry density (kg/m3)	1200	1200	1400	1400	1600	1600
Actual dry density (kg/m3)	1170	1170	1340	1340	1530	1560
Water content (%)	55.0	53.2	43.1	46.1	39.4	36.4
Water activity	0.990	0.992	0.986	0.989	0.981	0.975
Location of samples (*)						
Top filterstone exposed face (1)	n/a	n/a	n/a	n/a	n/a	n/a
Top filterstone clay face (2)	-	-	n/a	n/a	++	+
Bottom filterstone water face (3)	+	n/a	n/a	n/a	n/a	n/a
Bottom filterstone clay face (4)	n/a	+	+	n/a	n/a	n/a
Top of clay plug (5)	+	-	++	+	+	+
Sides of clay plug (6)	+	+	+	++	++	++
Bottom of clay plug (7)	+	+	+	+	++	+
Fracture location at bottom (8)	+	n/a	n/a	+	n/a	n/a
Inside of fracture (9)	-	-	++	+	+	-
Centre of clay plug (10)	n/a	-	-	-	-	-

*For location of samples, see Figure 4; n/a = not analyzed
- = No spheres observed (0 per frame); + = sporadic spheres observed (1-2 per frame);
++ = frequent spheres observed (> 2 per frame);

Table 1 also gives the results of the second set of experiments for which ten times more microspheres were used. Actual dry densities were within 5% of the targets in these experiments. Most samples examined from these experiments showed the presence of microspheres, especially at the bottoms, tops and sides but no spheres were found in the matrix of these plugs. This was also observed in experiments with bentonite-sand plugs [16, 17, 18]. These results, therefore, suggest rapid transport from the bottom to the top along the interface between the HCB clay plugs and pressure cells, but not through the intact clay matrix. In those plugs containing an induced fracture at the start of the experiment, some microspheres were found inside the healed fractures (as shown in Figure 5) in about 50% of the experiments performed. This suggests that microbial intrusion into healed or healing fractures is possible, but that this intrusion is not massive. However, further work is required to confirm these results with actual microorganisms (labeled for easy detection). If it were confirmed that microbes are able to

travel through healing or healed desiccation fractures, they could populate the area immediately around the containers and (temporarily at least) increase the potential for corrosion (MIC) of the containers.

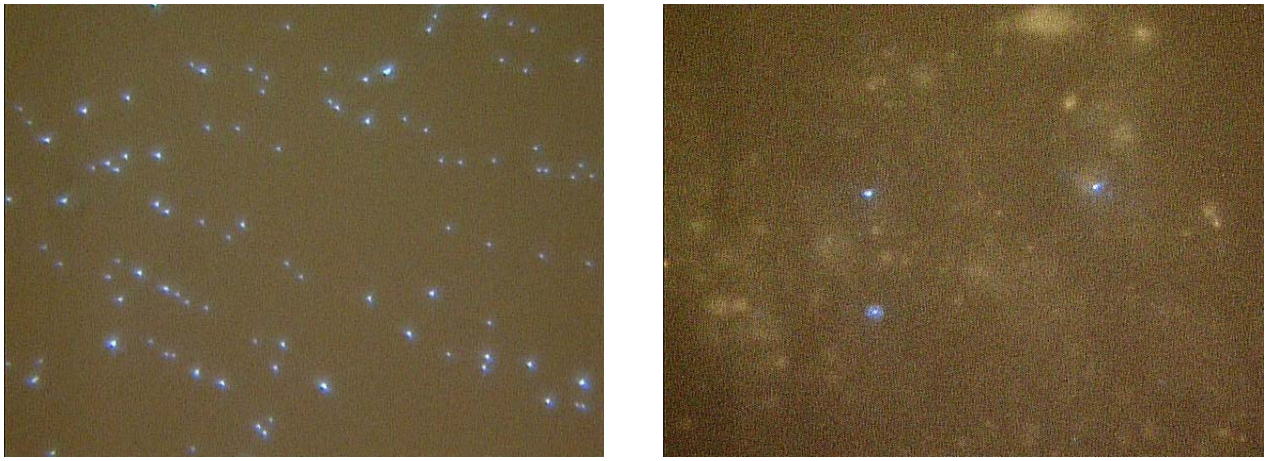


Figure 5. Suspension of bentonite and stock solution of microspheres (left) and microspheres detected inside the healed fracture of plug 1951 (right) (the microspheres are 0.2 µm in diameter).

The potential for increased microbial activity at interface locations can be minimized or eliminated by adequate design and placement methods of HCB. Materials compliance models [e.g., 20] can be used to determine the required as-placed dry density of HCB in order to achieve specific targets for long-term equilibrium dry density for various container placement designs. Migration through desiccation cracks, if confirmed, may be more difficult to prevent and potential consequences of such migration may need further assessment.

5. CONCLUSIONS

HCB, through its inherent physical properties, suppresses microbial activity if the bentonite dry density remains sufficiently high (i.e., preferably ≥ 1600 kg/m^3). There are locations in a repository where the bentonite dry density may not be sufficiently high and materials compliance models should be used to determine the required as-placed dry density of HCB in order to achieve specific targets for long-term equilibrium dry density for various container placement designs. A further concern is the possibility that microbial cells could migrate to container surfaces (where they could contribute to corrosion effects) through desiccation-induced cracks in the HCB, that have not healed rapidly or sufficiently enough upon re-saturation. Therefore, a series of experiments were carried out to address this possibility through the examination of the possible movement of fluorescent polystyrene microspheres of microbial sizes (0.2 to 0.6 µm) along interfaces and through fractures in HCB.

Results showed that most samples from these experiments that were examined contained microspheres, especially those samples from the bottoms, tops and sides of the plugs but no microspheres were found in the intact matrix of the plugs. This was also observed in previous experiments with bentonite-sand plugs. These results, therefore, confirmed that the microspheres

travelled along the interface between the HCB clay plugs and pressure cells, but not through the intact clay matrix.

In those plugs containing a fracture at the start of the experiment, some microspheres were found inside the healed fractures in about 50% of the experiments performed, which suggests that microbial intrusion into healed or healing fractures is possible but not massive. Further work is required to confirm these results with actual microorganisms. If it were found that microbes are able to travel through healing or healed desiccation fractures, they could populate the area immediately around the containers and (temporarily at least) increase the potential for MIC of the containers.

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