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/cm^3

NWMO TR-2010-06 **April 2010**

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ABSTRACT

Title: **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/cm^3**
Report No.: **NWMO TR-2010-06**
Author(s): S. Stroes-Gascoyne, C.J. Hamon, D.A. Dixon and D.G. Priyanto
Company: Atomic Energy of Canada Limited
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Abstract
This report presents and compares the results of a study on the culturability of indigenous microbes in compacted bentonite infused with calcium chloride ($CaCl_2$) porewater, with earlier data obtained with sodium chloride (NaCl) porewater. This study was carried out to assess whether, in saline Ca-dominated groundwaters, the salinity effects on microbes indigenous to Wyoming MX-80 bentonite would be similar to those determined with NaCl solutions in previous work. The results from this study show that aerobic culturability results obtained for porewaters with $CaCl_2$ are largely similar to the NaCl porewater results. Low aerobic culturability was observed for salt concentrations of ≥ 50 g $CaCl_2$/L. The lower culturability at a salt concentration of 50 g $CaCl_2$/L compared to 50 g NaCl/L would need to be confirmed.

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1. INTRODUCTION

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 of these materials such as low water activity (a_w), small pores and high swelling pressure, an important role of highly compacted bentonite is the reduction of significant microbial activity near the used fuel containers in a deep geologic repository (DGR), which would reduce or eliminate the possibility of significant microbially influenced corrosion (MIC). Previous work (Stroes-Gascoyne et al. 2006, 2010) determined that a dry density of ≥ 1.6 g/cm^3 or a porewater salinity of > 100 g NaCl/L (at dry densities of 0.8-2.0 g/cm^3) would keep microbial culturability at, or below, background levels in highly compacted bentonite buffer (i.e., ≤ 2 x 10^2 Colony-Forming Units/g). In a recent study (Stroes-Gascoyne and Hamon 2008), the effects of intermediate NaCl porewater concentrations (i.e., 60, 70, 80 and 90 g/L) on the culturability of microbes in compacted bentonite at target dry densities of 1.2, 1.4, 1.6 and 1.8 g/cm^3 were examined, as well as the effects of porewater salinities of 0 and 100 g NaCl/L on microbes in compacted bentonite, at intermediate dry densities (i.e., target dry densities of 1.1, 1.2, 1.4 and 1.5 g/cm^3). These additional data confirmed the previous requirements for bentonite dry density or porewater salinity to keep microbial culturability at, or below, background levels in the highly compacted bentonite buffer (i.e., ≥ 1.6 g/cm^3 or > 100 g NaCl/L). Although these additional data suggested that these requirements could possibly be lowered to ≥ 1.4 g/cm^3 for bentonite dry density or > 50 g NaCl/L for porewater salinity (Stroes-Gascoyne and Hamon 2008), a dry density of ≥ 1.6 g/cm^3 or a porewater salinity of > 50 g/L remain the preferred requirements.

Stroes-Gascoyne and Hamon (2008) recommended that porewater salinity studies with $CaCl_2$ be performed to ensure that in saline Ca-dominated groundwaters, the salinity effects on microbes indigenous in Wyoming MX-80 bentonite would be the same as those determined with NaCl solutions in the previous study (Stroes-Gascoyne et al. 2006, 2010). This report, therefore, presents the results of a study on the culturability of indigenous microbes in compacted bentonite infused with $CaCl_2$ porewater.

2. MATERIALS AND METHODS

Essentially the same materials and methods were used as reported previously (Stroes-Gascoyne et al. 2006, 2010; Stroes-Gascoyne and Hamon 2008), and a total of 8 experiments were carried out:

- The bentonite used was Wyoming MX-80 bentonite.
- The bentonite was compacted into ethanol-sterilized pressure cells to a number of target dry densities.
- The bentonite plugs were about 2 cm high with a diameter of 3.2 cm. (*Note that in previous reports (Stroes-Gascoyne et al. 2006, 2010; Stroes-Gascoyne and Hamon 2008) a diameter of 1.6 cm was specified; this should in fact have been a radius of 1 .6 cm, i.e., a diameter of 3.2 cm*).
- Before compaction, the bentonite was mixed with infiltration solutions such that, after compaction, the bentonite would be at about 95% saturation. The infiltration solutions consisted of sterilized, distilled deionized water or $CaCl_2$ solutions, with a range of concentrations.

- During the experiments, the plugs were infiltrated under pressure to saturation with the infiltration solutions.

- Four experiments were carried out at target dry densities of 0.8 g/cm^3 with porewater salinities of 50, 60, 80 and 100 g $CaCl_2$/L.

- Four experiments were carried out at target dry densities of 1.3 g/cm^3, with porewater salinities of 50, 60, 80 and 100 g $CaCl_2$/L.

The solutions used are referred to as infiltration water or porewater in this report, although the latter term is not strictly correct. The bentonite porewater may be somewhat different from the infiltration water, because of the possible presence of various salts in the as-bought "dry" bentonite.

The eight tests were carried out for 84-92 d, similar to the previous study (in which experimental durations ranged from 40 to 90 d, (Stroes-Gascoyne et al. 2006, 2010), at ambient laboratory temperature. 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 microbial and other analyses:

1. The plugs were weighed and measured to determine actual dry densities.

2. Water activity was measured on a subsample using a Decagon™ WP4 Dewpoint PotentiaMeter (Decagon Devices, Pullman, WA).

3. Water content was determined by subsequently drying this subsample in an oven at 110^o C to constant weight (ASTM D2216-05).

4. Aerobic and anaerobic heterotrophic bacteria were cultured on R2A medium (Reasoner and Geldreich 1985).

5. Sulphate-reducing bacteria (SRB) were cultured on modified Postgate B medium (Atlas 1993).

6. Swelling pressures were calculated from total measured pressures recorded at the end of each experiment.

3. RESULTS

Eight experiments with $CaCl_2$ porewater were completed successfully in this study.

Table 1 gives the results for measured dry density, measured effective montmorillonite dry density (EMDD), water content, a_w, swelling pressure and culturable aerobes, anaerobes, and SRB obtained in these experiments.

For comparison, Table 2 gives the same data for all corresponding experiments carried out with NaCl (from Stroes-Gascoyne and Hamon 2008, Tables A1 and A2).

Figures 1, 3, 5 and 7 show culturable aerobes as a function of measured dry density, and compare the previous NaCl data with the new $CaCl_2$ data, for salinities of 50, 60, 80 and 100 g/L respectively. Figures 2, 4, 6 and 8 show culturable aerobes, as a function of water activity, and compare the previous NaCl data with the new $CaCl_2$ data, for salinities of 50, 60, 80 and 100 g/L respectively.

Figure 9 shows culturable aerobes as a function of measured dry density and compares all previous NaCl data with the new $CaCl_2$ data, for all porewater salinities (0-200 g/L) used. Figure 10 shows the same data as Figure 9, but with only the $CaCl_2$ data highlighted, such that comparison is easier.

Figure 11 shows culturable aerobes, as a function of water activity, and compares all previous NaCl data with the new $CaCl_2$ data, for all porewater salinities (0-200 g/L) used. Figure 12 shows the same data as Figure 11, but with only the $CaCl_2$ data highlighted, such that comparison is easier.

Figure 13 shows culturable aerobes as a function of porewater salinity and compares all previous NaCl data with the new $CaCl_2$ data, for all dry densities (0.8-2.0 g/cm^3) used. Figure 14 shows the same data as Figure 13, but with only the $CaCl_2$ data highlighted, such that comparison is easier.

Figure 15 shows the swelling pressures (calculated from measured total pressures) as a function of measured dry density and compares corresponding previous NaCl results with the new $CaCl_2$ results. Figure 16 shows the same data as Figure 15, but with only the $CaCl_2$ data highlighted, such that comparison is easier.

4. DISCUSSION

The present results (Figures 1, 3, 5 and 7) appear to indicate that there is no significant increase in the aerobic culturability, above the culturability of the as-received bentonite material, for bentonite samples with dry densities of about 0.8 to 1.3 g/cm^3 in the presence of porewater salinities of 50 to 100 g $CaCl_2$/L. These results are largely comparable to those obtained previously with NaCl porewater. An exception is that in the previous study a noticeable increase in aerobic culturability was observed at a porewater salinity of 50 g NaCl/L (Stroes-Gascoyne et al. 2006, Stroes-Gascoyne and Hamon 2008). In the present study a porewater salinity of 50 g $CaCl_2$ /L appears to be sufficiently high to suppress aerobic culturability. It is recommended that some more experiments be carried out with porewater salinities of 50 g NaCl/L and 50 g $CaCl_2$/L to confirm this observation.

Figures 2, 4, 6 and 8 suggest that water activities in low dry density bentonite (target dry density 0.8 g/cm^3) appear to be higher for porewaters with $CaCl_2$ than for porewaters with the same concentrations of NaCl. The ionic strength of $CaCl_2$ solution is slightly higher than of a NaCl solution with the same mass of salt, e.g., for 100g $CaCl_2$/L the ionic strength is 3.6, whereas for 100 g NaCl/L it is 3.4. The fact that water activities appear to be slightly higher for bentonites infused with $CaCl_2$ porewater, than for bentonite infused with NaCl porewater with the same mass of salt, could perhaps be explained by diffuse double layer effects. A solution with a higher ionic strength will compress diffuse double layers around charged (clay) particles to a larger extent than a solution with a lower ionic strength. When diffuse double layers become more compressed, they will exclude previously bound water molecules, which will now be free in solution. This could perhaps counteract the effect of the slightly higher ionic strength of the $CaCl_2$ solution, and the end result would be more free water and a higher water activity for the $CaCl_2$ infused samples. This, however, does not explain the apparent suppression of aerobic culturability at a_w values > 0.96 in the experiments with 50 g $CaCl_2$/L (Figure 2). Again, some further experiments at porewater salinities of 50 g NaCl/L and 50 g $CaCl_2$/L are recommended.

Figures 9 and 10, 11 and 12, and 13 and 14 confirm the conclusions from Figures 1 to 8, i.e., they illustrate that the $CaCl_2$ porewater results for aerobic culturability are very similar to the NaCl porewater results for corresponding salt concentrations.

Figures 15 and 16, however, suggest that the swelling pressures, obtained at low dry density (0.8 g/cm^3) for $CaCl_2$ porewater, are significantly higher than for NaCl porewater. This is unexpected and, at present, there appears to be no explanation for this.

5. CONCLUSIONS

The results from this study show that aerobic culturability results obtained for porewaters with $CaCl_2$ are largely similar to the NaCl porewater results, as expected. Low culturability was observed for a salt concentration ≥ of 50 g $CaCl_2$/L This lower culturability at a salt concentration of 50 g $CaCl_2$/L compared to 50 g NaCl/L would need to be confirmed.

ACKNOWLEDGEMENTS

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Table 1: Results for $CaCl_2$ Experiments

Experiment #	1848	1849	1850	1851	1852	1853	1854	1855
Duration (d)	84	84	84	84	92	92	92	92
Target dry density (EMDD)(g/cm^3)	0.8 (0.65)	0.8 (0.65)	0.8 (0.65)	0.8 (0.65)	1.3 (1.10)	1.3 (1.10)	1.3 (1.10)	1.3 (1.10)
Measured dry density (EMDD) (g/cm^3)	0.86 (0.70)	0.96 (0.79)	0.89 (0.73)	1.07 (0.89)	1.32 (1.11)	1.34 (1.12)	1.29 (1.08)	1.37 (1.17)
Porewater salinity (g$CaCl_2$/L)	50	60	80	100	50	60	80	100
Water content (%)	68.2	69.7	60.5	66.3	41.9	41.8	44.4	34.9
Aerobes (CFU/g)	(1.71± 0.19) x10^2	(1.68 ± 0.83) x10^2	(1.37 ± 0.25) x10^2	(3.53 ± 0.57) x10^2	(8.43 ± 3.19) x10^1	(2.41 ± 0.40) x10^2	(2.66 ± 0.27) x10^2	(2.00 ± 0.46) x10^2
Anaerobes (CFU/g)	(6.63 ± 0.00) x10^1	(4.79 ± 2.07) x10^1	(4.93 ± 0.00) x10^1	(5.97 ± 3.76) x10^1	(6.02 ± 0.00) x10^1	(6.17 ± 5.34) x10^1	(5.31 ± 3.51) x10^1	(7.54 ± 1.54) x10^1
SRB (MPN/g)	<5.0	<5.4	<4.9	10.1	<3.6	3.3	30.6	<4.0
a_w	0.974	0.989	0.969	0.959	0.972	0.943	0.942	0.769
Swelling pressure (kPa)	580	630	480	525	1960	1120	1185	1022

Table 2: Corresponding Results for NaCl Experiments (from Stroes-Gascoyne and Hamon 2008)

Experiment	Measured Dry Density g/cm^3	EMDD g/cm^3	Salinity (NaCl) (g/L)	a_w	Aerobes (CFU/g)	Swelling Pressure (kPa)
1664	1.74	1.56	50	0.814	$(5.56\pm1.82)\times10^1$	21000
1655	1.79	1.62	50	0.893	$(6.99\pm3.07)\times10^1$	6200
1667	1.62	1.43	50	0.905	$(8.89\pm7.11)\times10^1$	8100
1640	1.57	1.38	50	0.934	$(1.76\pm0.13)\times10^2$	2600
1654	1.28	1.09	50	0.960	$(6.41\pm1.48)\times10^3$	1100
1637	0.8	0.65	50	0.959	$(1.26\pm0.11)\times10^6$	25
1815	1.15	0.95	60	0.962	$(2.19\pm0.54)\times10^2$	5
1816	1.27	1.08	60	0.953	$(8.89\pm3.85)\times10^1$	94
1817	1.52	1.31	60	0.929	$(7.90\pm2.89)\times10^1$	3848
1818	1.75	1.57	60	0.737	$(2.14\pm0.52)\times10^2$	9516
1819	1.22	1.02	80	0.948	$(1.46\pm0.07)\times10^2$	323
1820	1.44	1.23	80	0.931	$(1.45\pm0.06)\times10^2$	1722
1821	1.72	1.53	80	0.858	$(1.55\pm0.17)\times10^2$	2540
1822	1.76	1.57	80	0.824	$(2.91\pm1.10)\times10^2$	12974
1665	1.73	1.55	100	0.721	$(2.31\pm3.18)\times10^1$	28500
1666	1.64	1.46	100	0.764	$(7.47\pm2.28)\times10^1$	6600
1673	1.73	1.55	100	0.783	$(4.87\pm1.22)\times10^1$	11100
1672	1.62	1.43	100	0.880	$(5.69\pm2.81)\times10^1$	3350
1649	1.54	1.35	100	0.884	$(1.55\pm0.28)\times10^2$	1100
1650	1.30	1.11	100	0.936	$(3.84\pm0.61)\times10^2$	310
1670	1.31	1.12	100	0.940	$(4.94\pm0.78)\times10^1$	3250
1669	0.75	0.59	100	0.947	$(1.75\pm0.19)\times10^2$	20
1651	0.93	0.76	100	0.946	$(1.53\pm0.34)\times10^2$	20
1749T	1.60	1.40	100	0.824	$(2.12\pm0.12)\times10^2$	1706
1749M	1.65	1.47	100	0.820	$(1.61\pm0.43)\times10^2$	1706
1749B	1.61	1.42	100	0.830	$(1.22\pm0.19)\times10^2$	1706
1811	1.19	0.99	100	0.939	$(1.46\pm0.26)\times10^2$	126
1812	1.25	1.06	100	0.919	$(2.50\pm0.84)\times10^2$	239
1813	1.38	1.18	100	0.934	$(1.35\pm0.43)\times10^2$	failed
1814	1.46	1.25	100	0.934	$(1.67\pm0.53)\times10^2$	failed

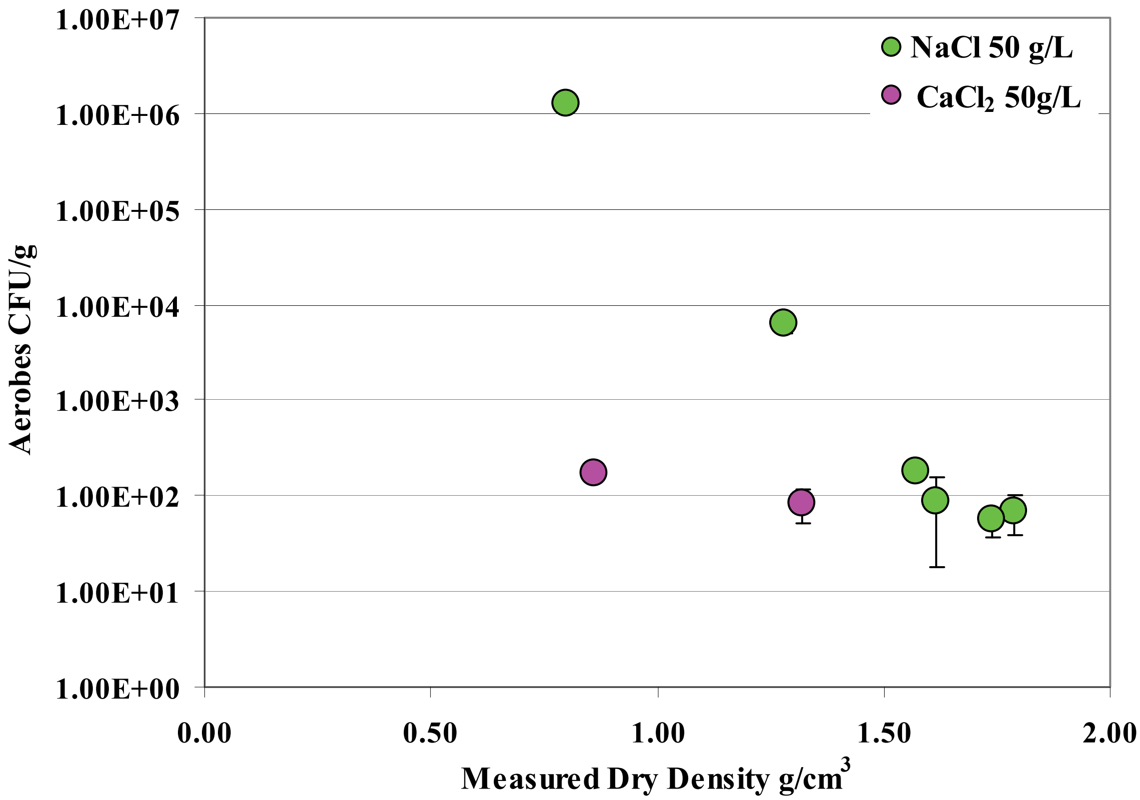

Figure 1: Heterotrophic Aerobes as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (50 g/L) or $CaCl_2$ (50 g/L)

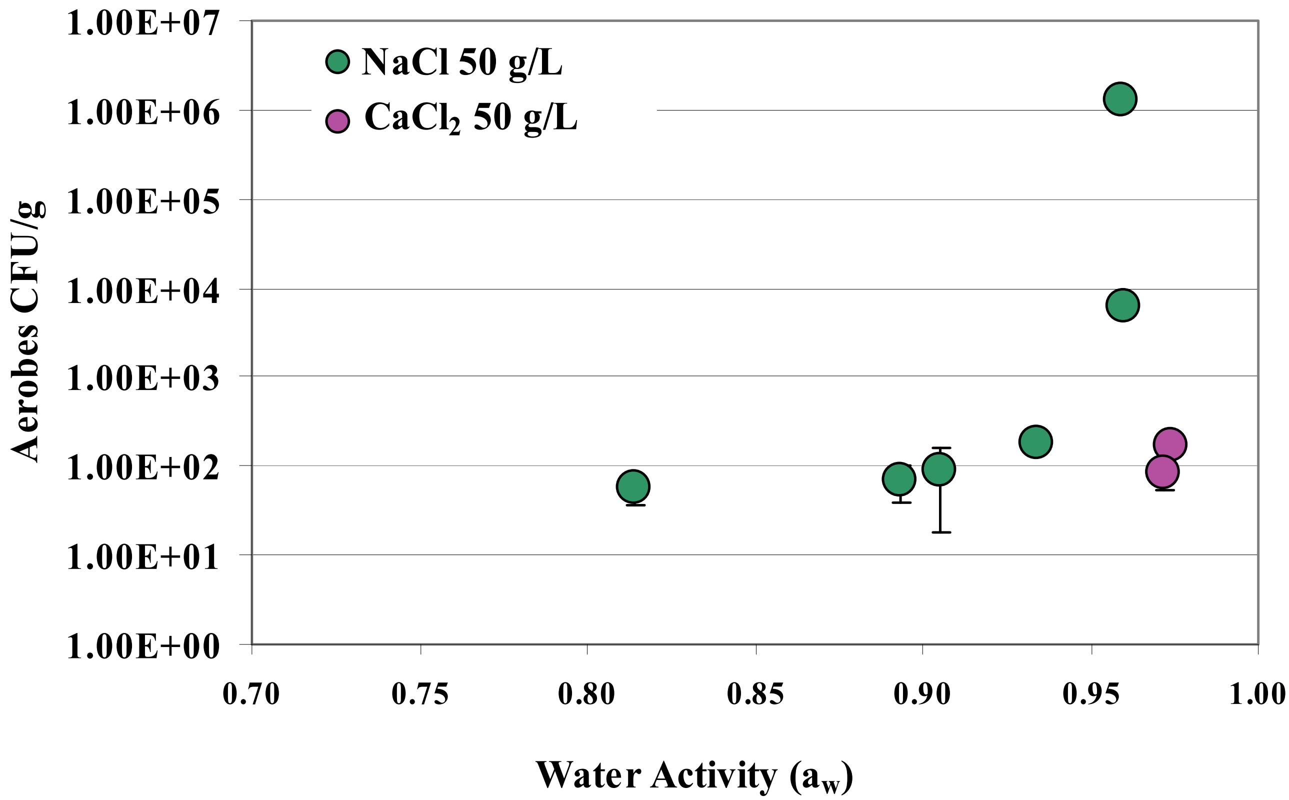

Figure 2: Heterotrophic Aerobes as a Function of Water Activity in Compacted Bentonite Infused with Porewater Containing NaCl (50 g/L) or $CaCl_2$ (50 g/L)

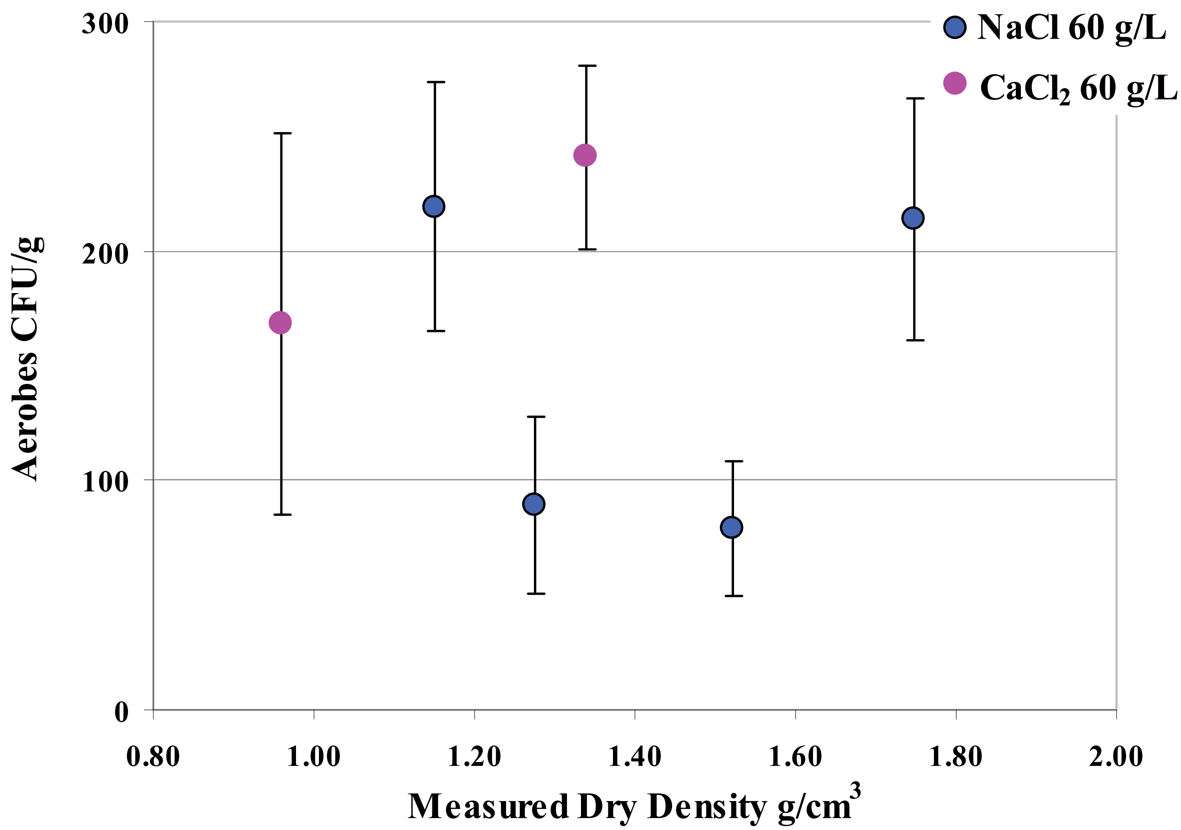

Figure 3: Heterotrophic Aerobes as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (60 g/L) or $CaCl_2$ (60 g/L)

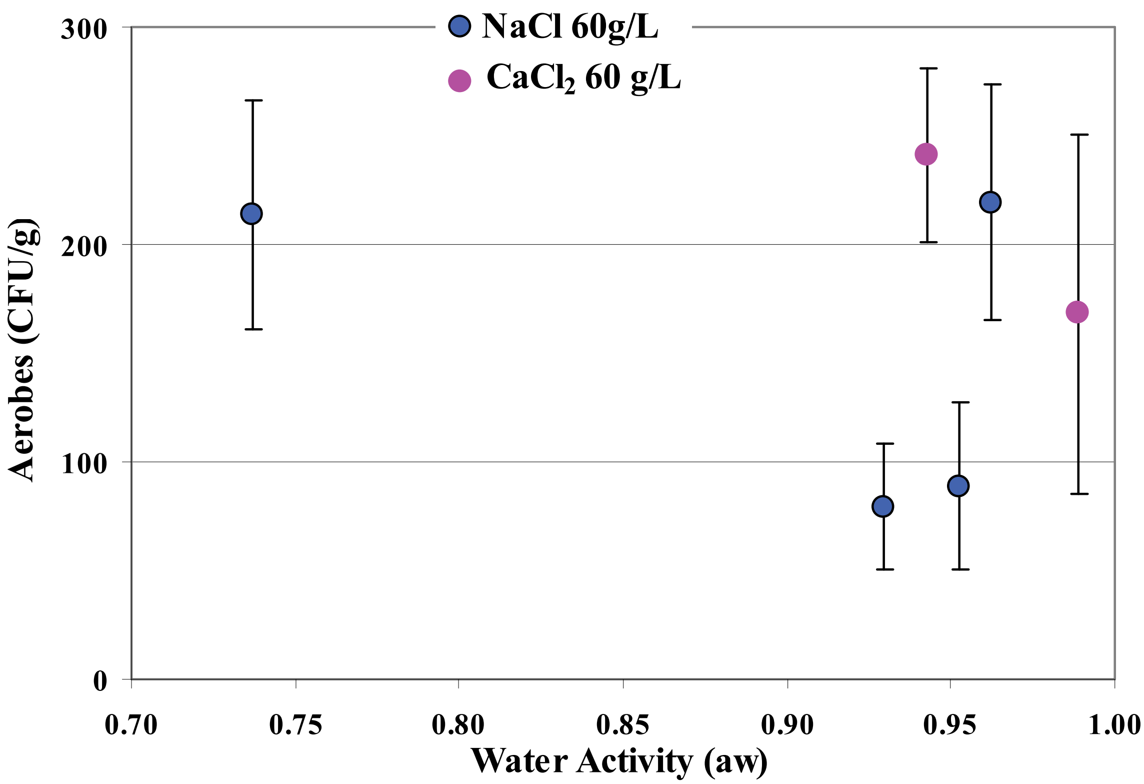

Figure 4: Heterotrophic Aerobes as a Function of Water Activity in Compacted Bentonite Infused with Porewater Containing NaCl (60 g/L) or $CaCl_2$ (60 g/L)

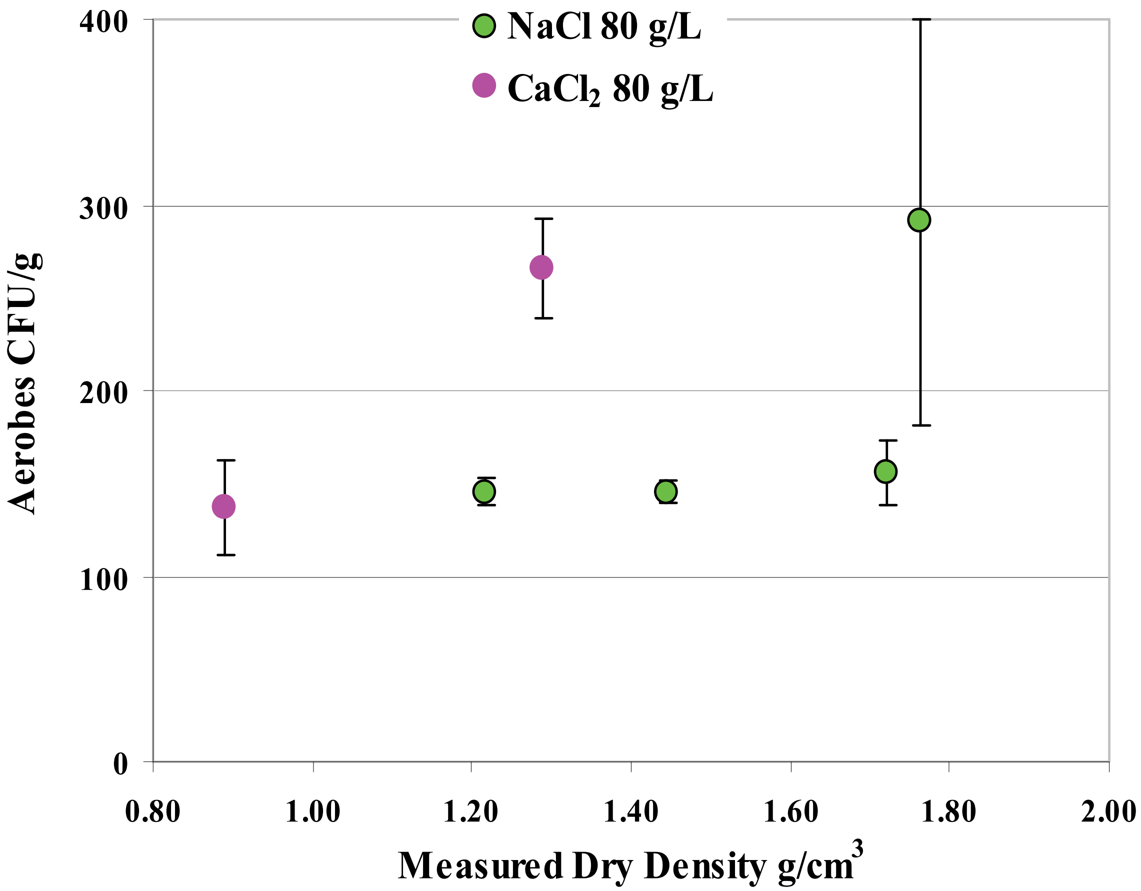

Figure 5: Heterotrophic Aerobes as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (80 g/L) or $CaCl_2$ (80 g/L)

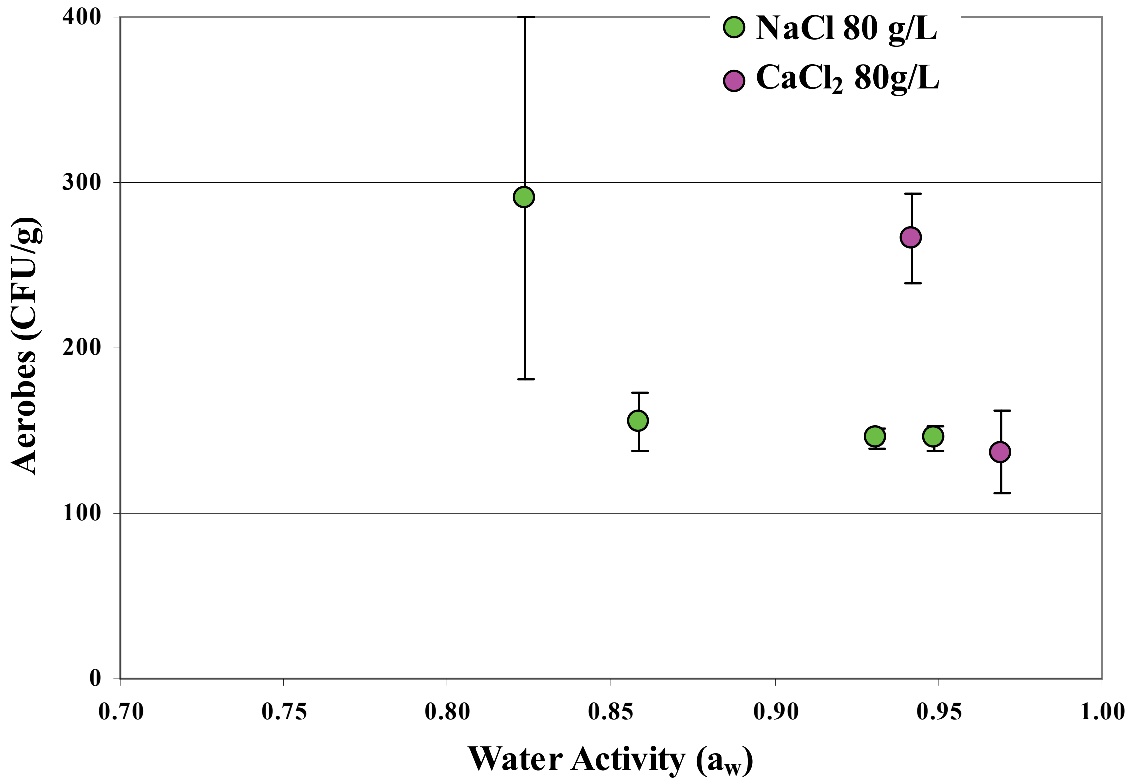

Figure 6: Heterotrophic Aerobes as a Function of Water Activity in Compacted Bentonite Infused with Porewater Containing NaCl (80 g/L) or $CaCl_2$ (80 g/L)

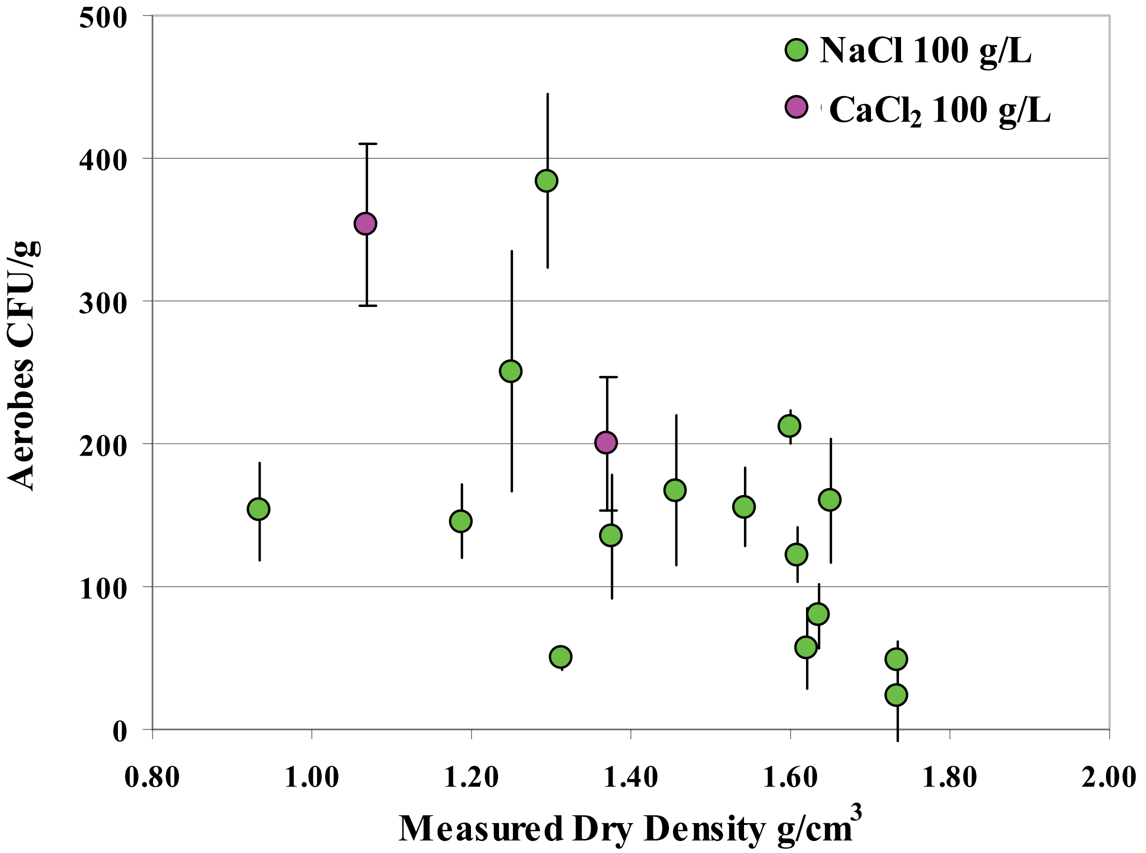

Figure 7: Heterotrophic Aerobes as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (100 g/L) or $CaCl_2$ (100 g/L)

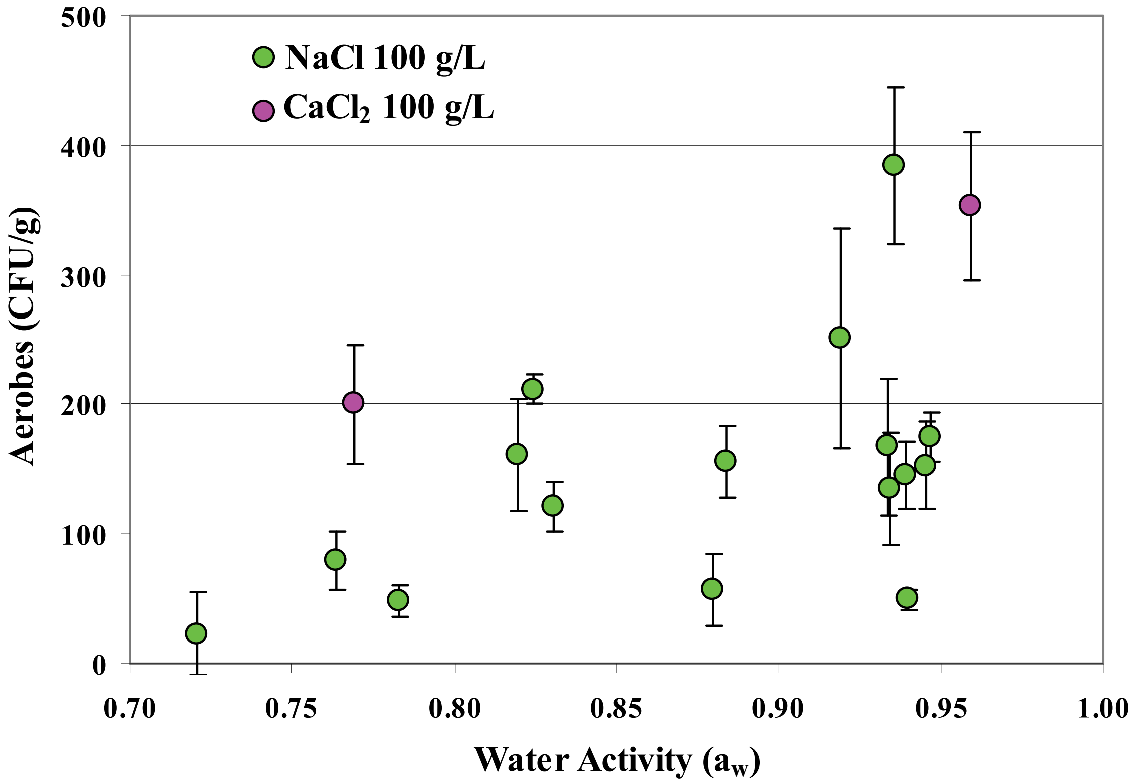

Figure 8: Heterotrophic Aerobes as a Function of Water Activity in Compacted Bentonite Infused with Porewater Containing NaCl (100 g/L) or $CaCl_2$ (100 g/L)

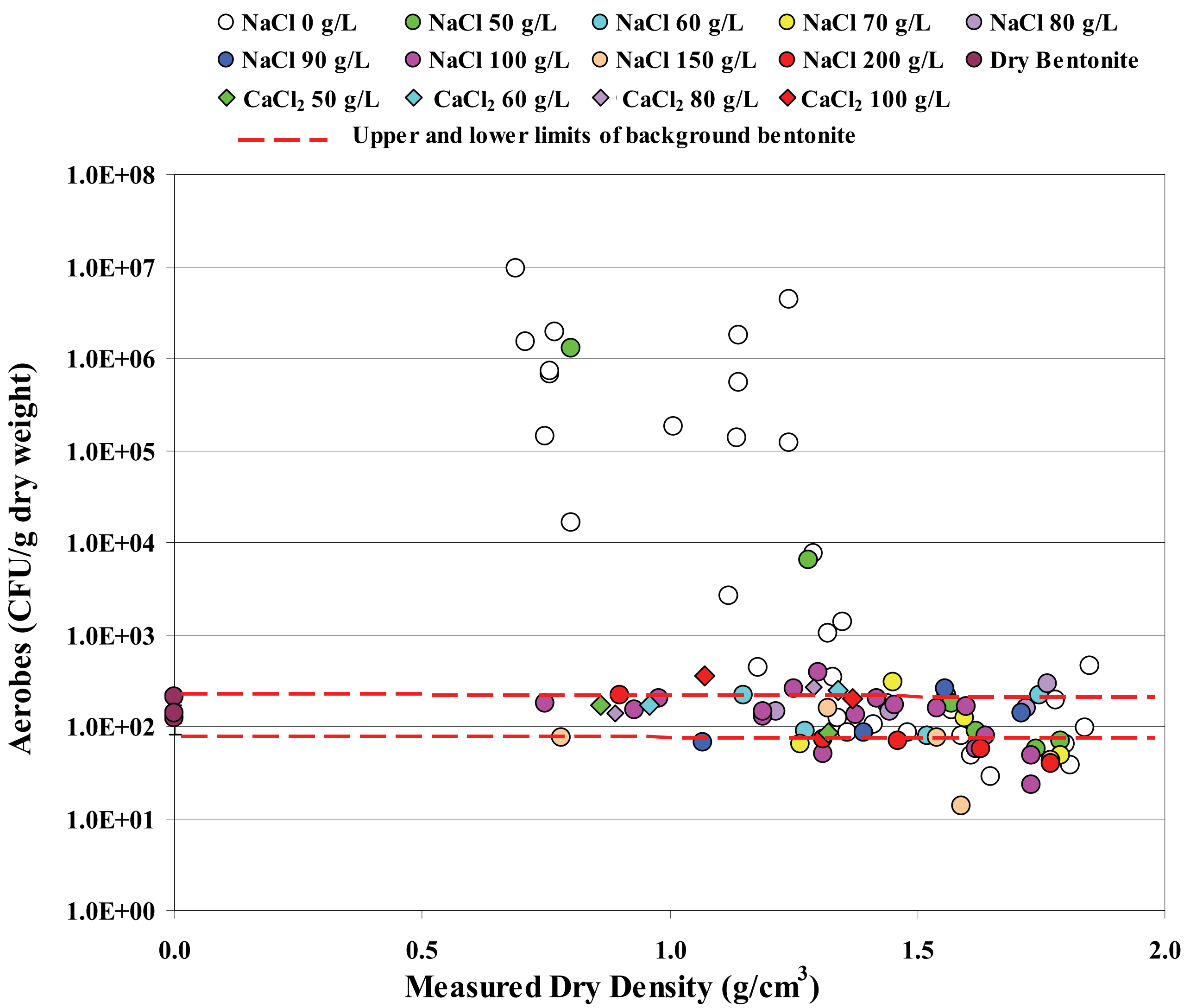

Figure 9: Comparison of Heterotrophic Aerobes as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (0, 50, 60, 70, 80, 90, 100, 150 and 200 g/L) or $CaCl_2$ (50, 60, 80 and 100 g/L)

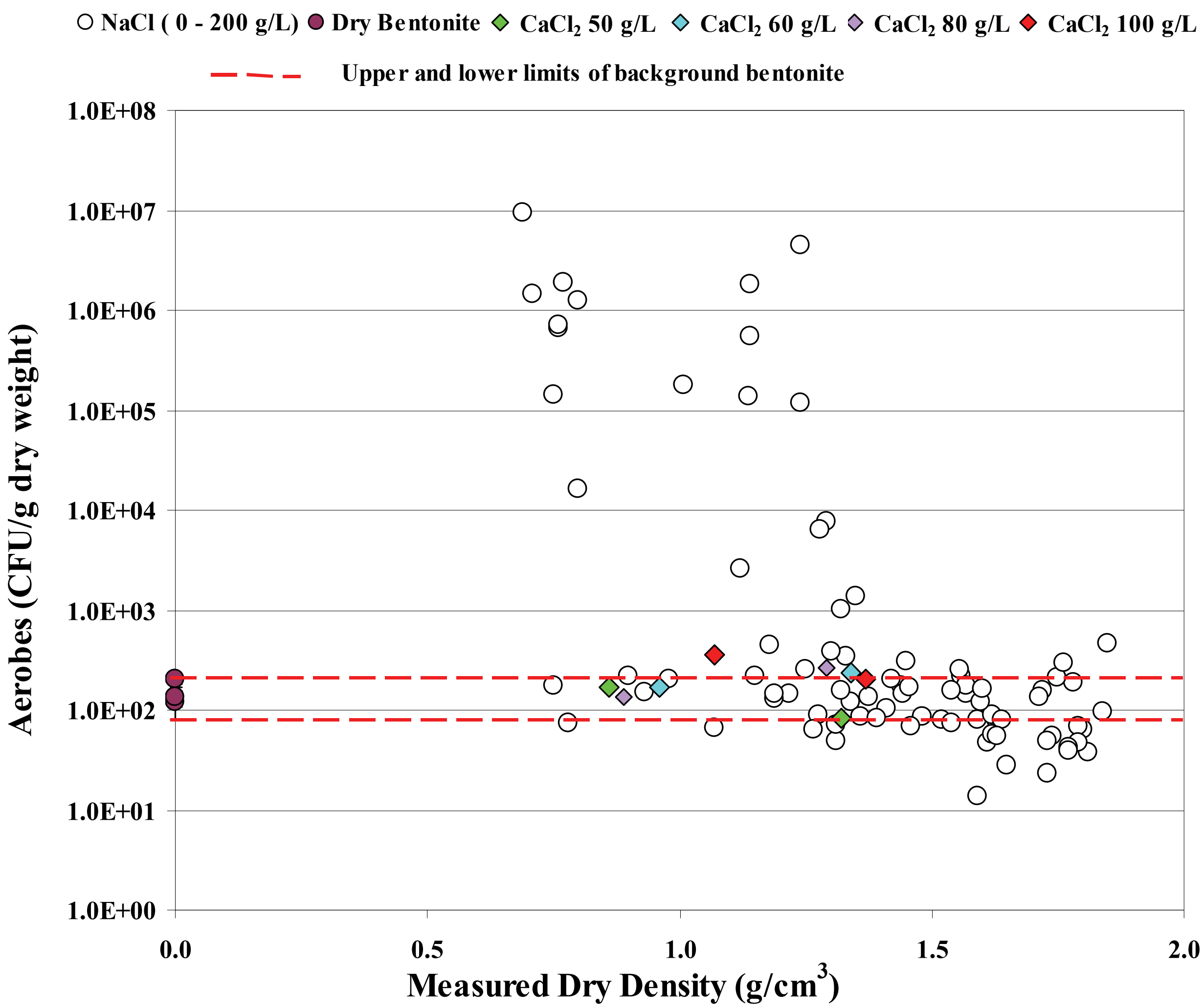

Figure 10: Comparison of Heterotrophic Aerobes as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (0 - 200 g/L) or $CaCl_2$ (50, 60, 80 and 100 g/L) ($CaCl_2$ data shown in colour)

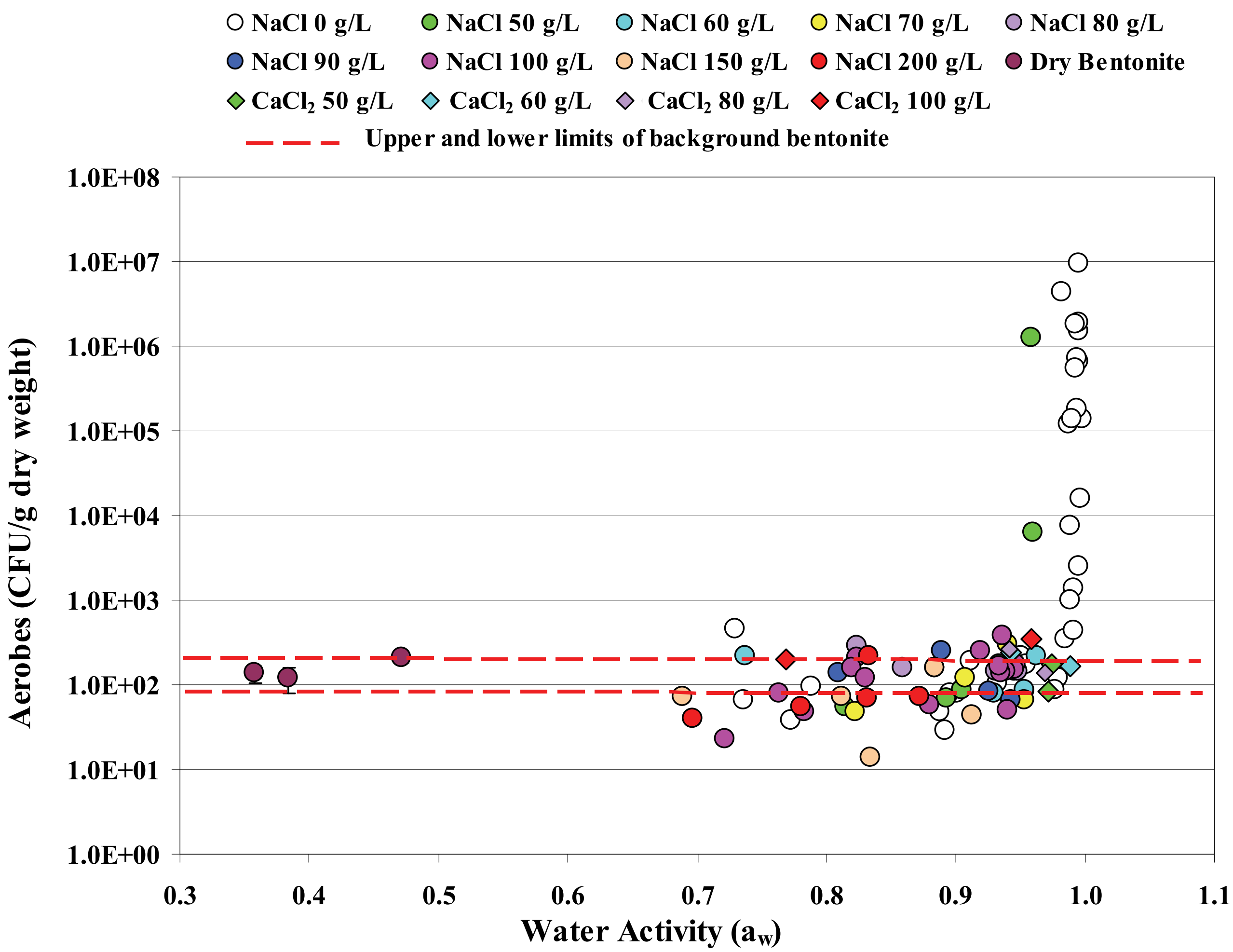

Figure 11: Comparison of Heterotrophic Aerobes as a Function of Water Activity in Compacted Bentonite Infused with Porewater Containing NaCl (0, 50, 60, 70, 80, 90, 100, 150 and 200 g/L) or $CaCl_2$ (50, 60, 80 and 100 g/L)

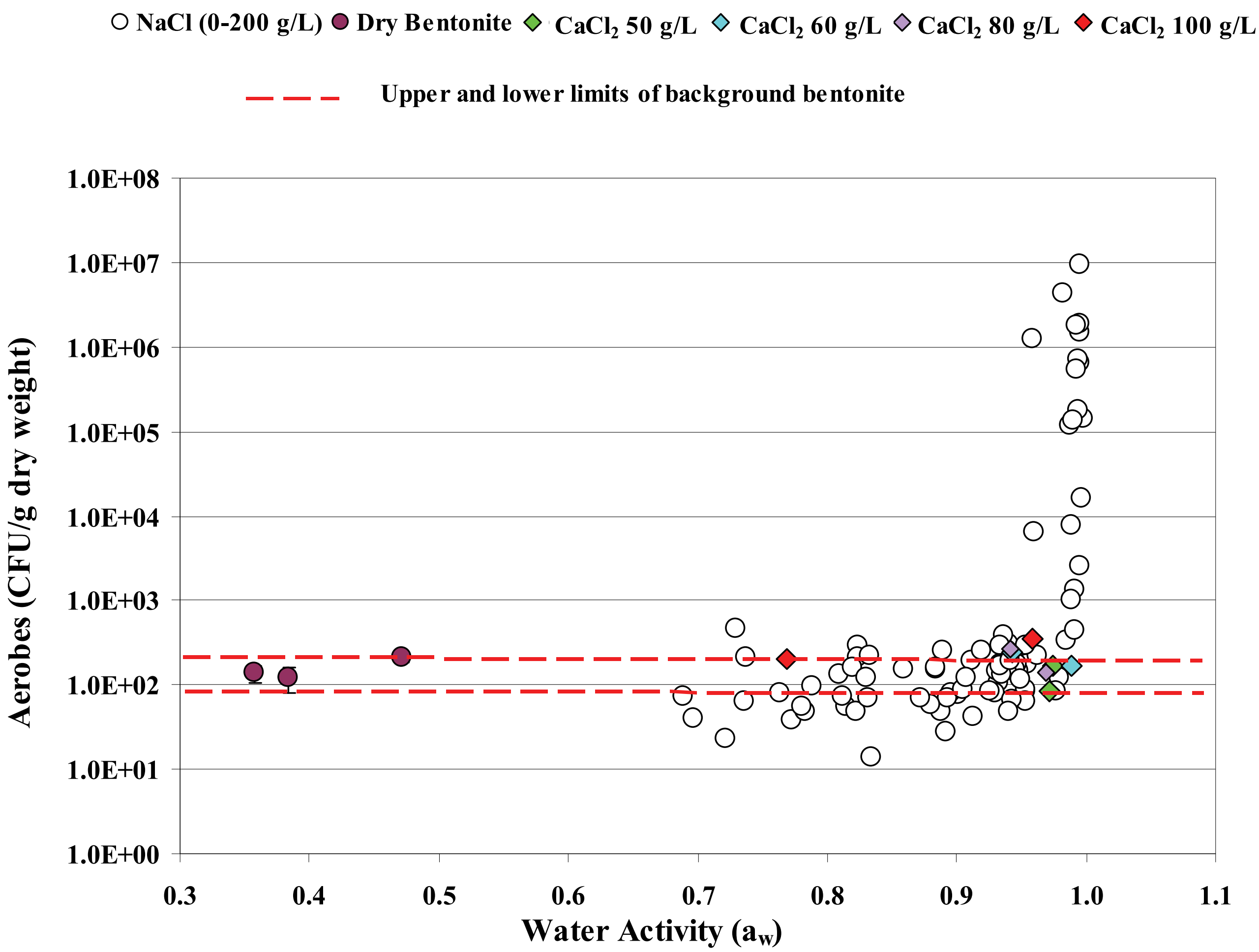

Figure 12: Comparison of Heterotrophic Aerobes as a Function of Water Activity in Compacted Bentonite Infused with Porewater Containing NaCl (0 – 200 g/L) or $CaCl_2$ (50, 60, 80 and 100 g/L) ($CaCl_2$ data shown in colour)

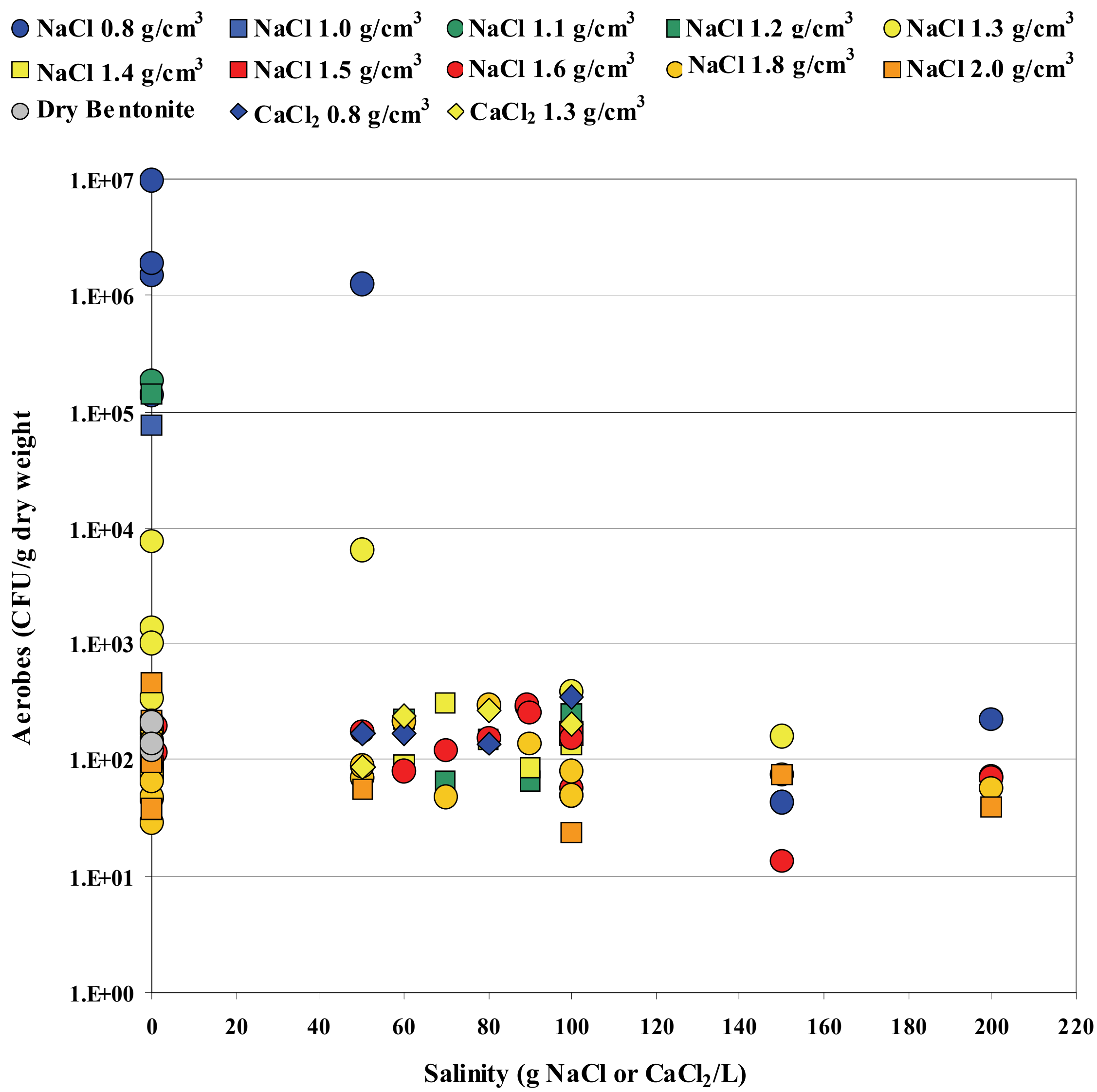

Figure 13: Comparison of Heterotrophic Aerobes as a Function of Porewater Salinity ($NaCl$ or $CaCl_2$) in Compacted Bentonite with Dry Densities of 0.8, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.8 and 2.0 g/cm^3 (as shown above graph after each symbol)

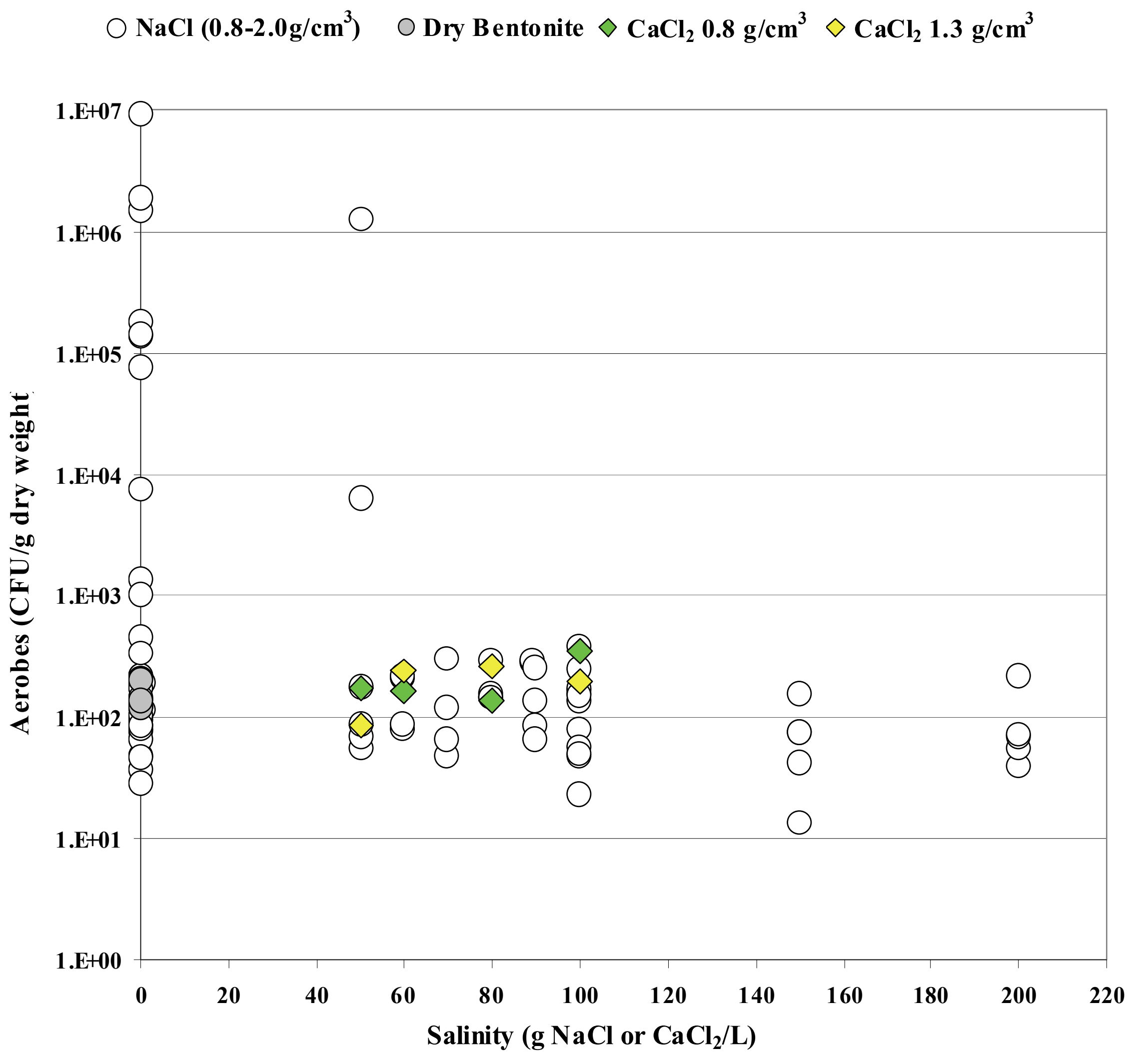

Figure 14: Comparison of Heterotrophic Aerobes as a Function of Porewater Salinity ($NaCl$ or $CaCl_2$) in Compacted Bentonite with Dry Densities Ranging from 0.8 to 2.0 g/cm^3 (as shown above graph after each symbol) ($CaCl_2$ data shown in colour)

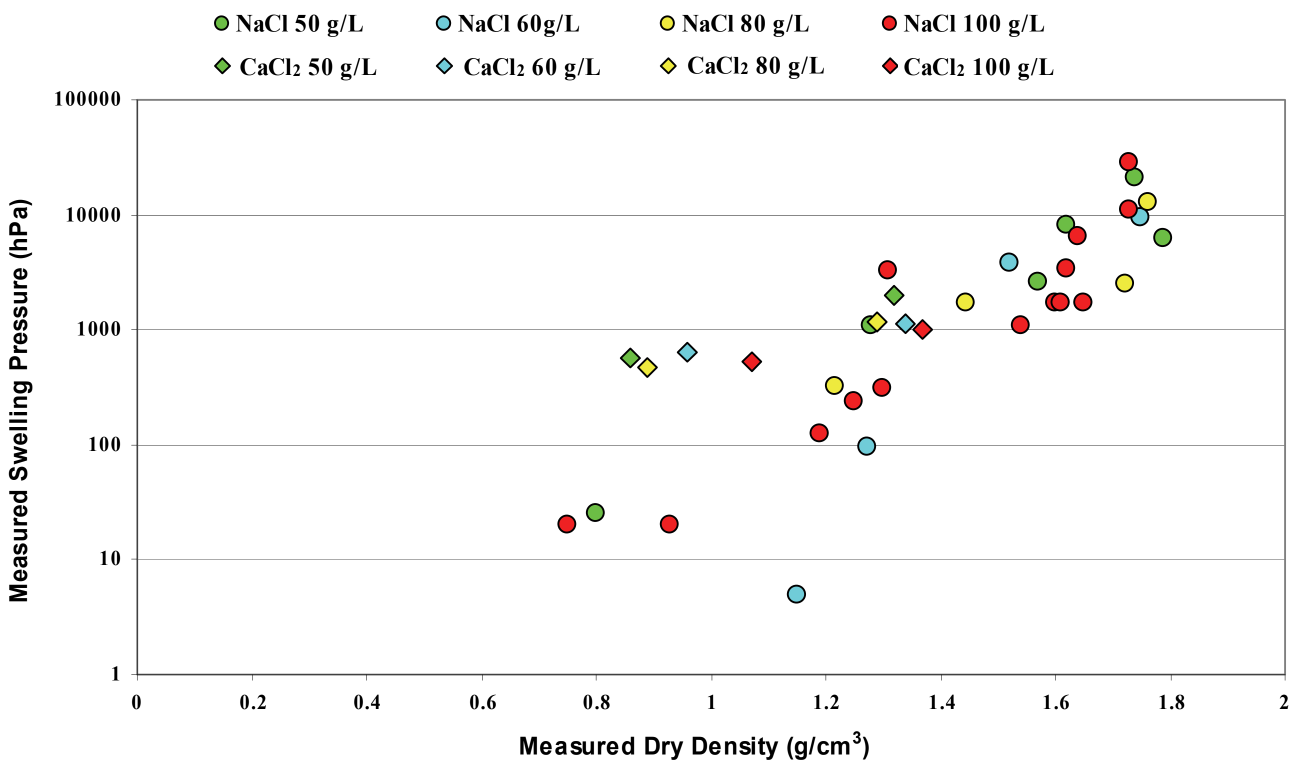

Figure 15: Measured Swelling Pressure as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (50, 60, 80 and 100 g/L) or $CaCl_2$ (50, 60, 80 and 100 g/L)

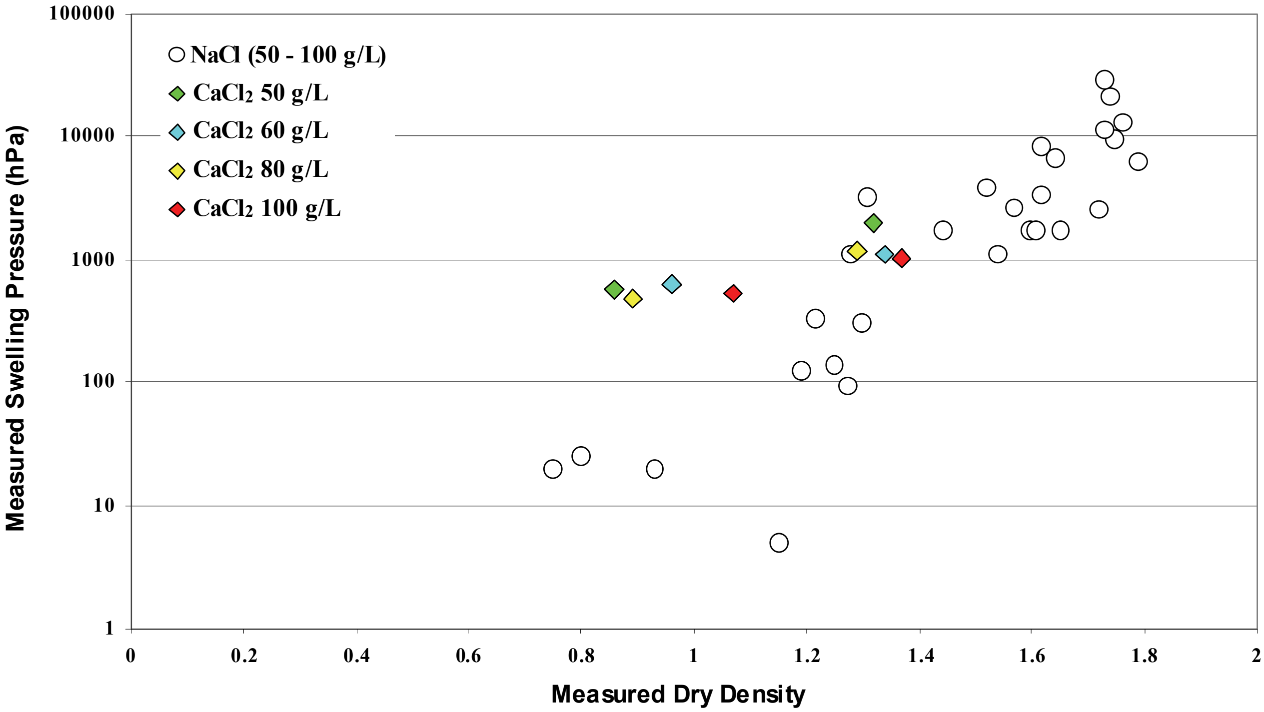

Figure 16: Measured Swelling Pressure as a Function of Measured Dry Density in Compacted Bentonite Infused with Porewater Containing NaCl (50-100 g/L) or $CaCl_2$ (50, 60, 80 and 100 g/L) ($CaCl_2$ data shown in colour)