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 **September 2008**

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ABSTRACT

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Report No.: **NWMO TR-2008-11**
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Company: Atomic Energy of Canada Limited
Date: September 2008

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 of these materials such as low water activity, 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 microbially influenced corrosion (MIC). Previous work determined that a dry density of $\geq$1.6 g/cm^3 or a porewater salinity of > 100 g NaCl/L would keep microbial culturability at or below background levels in highly compacted bentonite buffer (i.e., $\leq 2 \times 10^2$ Colony-Forming Units/g). In order to fill in some of the gaps left by the previous study, this report examines 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. In addition, the effects of a porewater salinity 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) were also examined. The additional data suggest that 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., $\geq$ 1.6 g/cm^3 or > 100 g NaCl/L) have been confirmed and that those requirements could likely be lowered to $\geq$ 1.4 g/cm^3 or > 50 g NaCl/L. However, in order to fully confirm that a dry density of $\geq$ 1.4 g/cm^3 and <1.6g/cm^3 is sufficient to suppress microbial activity and render MIC insignificant, it is recommended that actual pore sizes be measured in Wyoming MX-80 bentonite plugs of this range of dry densities. It is also 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 this study. It is further emphasized that the conclusions are valid for microbes indigenous in Wyoming MX-80 bentonite, but are not necessarily directly applicable to other brands of bentonite without further study.

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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, 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 microbially influenced corrosion (MIC).

In a previous study (Stroes-Gascoyne et al. 2006, 2008), laboratory experiments were carried out with Wyoming MX-80 bentonite, compacted (at 95% saturation) to a range of dry densities (0.8, 1.3, 1.6, 1.8 and 2.0 g/cm^3), and infiltrated (under pressure) with sterile NaCl solutions of 0, 50, 100, 150 and 200 g NaCl/L. During the tests (of 40 to 90 d duration), 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). Average pore size was measured on some samples using mercury intrusion porosimetry.

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 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 (Stroes-Gascoyne et al. 2006, 2008), which is smaller than the size range of known bacteria, including starved or ultramicrobacteria (0.2 – 0.4 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 back-ground levels (($2.1 \pm 0.2) \times 10^2$ Colony Forming Units per g dry bentonite). However, SRB and anaerobes did not increase significantly above background levels in any of the tests, due to the continued aerobic character of the test environments. 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 but in all combinations of dry density and porewater salinity tested, some culturability remained. It is hypothesized that these surviving organisms are either shrunken, almost inactive, dormant cells, or metabolically inactive spores. With respect to a_w and swelling pressure values, culturability increased exponentially around $a_w \geq 0.96$ (corroborating earlier studies) but decreased sharply at swelling pressures of ≥ 2 MPa. Both a_w and swelling pressure are influenced by the dry density of the bentonite. These results suggested that microbial activity in the bulk of compacted 100% bentonite can be controlled (i.e., suppressed) as long as the emplaced bentonite has a (uniform) dry density ≥1.6 g/cm^3, which ensures that the swelling pressure is well above 2 MPa, a_w is well below 0.96 and the average pore size is < 0.02 μm. Observations from several natural bentonite deposits corroborate these results. Therefore, depending on the specific requirements identified, it was concluded that dry density (and hence a_w and swelling pressure) might be tailored to provide a microbially unfavourable environment adjacent to the used fuel containers, ensuring that MIC is negligible (Stroes-Gascoyne et al. 2006, 2008).

Alternatively, a porewater salinity of > 100 g/L also appeared to control microbial culturability indigenous to the bentonite used for the study (Stroes-Gascoyne et al. 2006, 2008) and, therefore, host rock with a naturally high salinity porewater could be a favoured environment for a DGR from the perspective of reducing or avoiding MIC.

2. ADDITIONAL EXPERIMENTS

The salinity range used in the previous studies employed rather large concentration increments (i.e., 50 g/L) and results showed that microbes indigenous to the MX-80 bentonite could tolerate a salinity of 50 g/L but not a salinity of 100 g/L. Therefore, the current study focuses on the effects of intermediate NaCl concentrations (i.e., 60, 70, 80 and 90 g/L) on the culturability of microbes in compacted bentonite of target dry densities of 1.2, 1.4, 1.6 and 1.8 g/cm^3. In addition, the effects of a salinity of 0 and 100 g/L on microbes in compacted bentonite of intermediate dry densities (i.e., target dry densities of 1.1, 1.2, 1.4 and 1.5 g/cm^3) was also investigate in order to fill in some of the gaps left by the previous study.

3. MATERIALS AND METHODS

Essentially the same materials and methods were used as reported previously (Stroes-Gascoyne et al. 2006, 2008) and a total of 24 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 1.6 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 NaCl solutions with a range of concentrations
- During the experiments, the plugs were infiltrated under pressure to saturation with the infiltration solutions.
- Eight experiments were carried out at target dry densities of 1.1, 1.2, 1.4 and 1.5 g/cm^3 with porewater salinities of either 0 or 100 g NaCl/L
- Sixteen experiments were carried out at target dry densities of 1.2, 1.4, 1.6 and 1.8 g/cm^3, each with porewater salinities of 60, 70, 80 and 90 g NaCl/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 24 tests were carried out for times ranging from 45 to 80 d, similar to the previous study (in which experimental durations ranged from 40 to 90 d), 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^{TM} 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.

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).

In addition, a further dry “as-bought” bentonite sample was analyzed, as above.

4. RESULTS AND DISCUSSION

A total of 24 experiments were completed successfully in this study. Table 1 gives the additional data for measured dry density, water content, a_w and culturable aerobes, anaerobes and SRB obtained at the intermediate target dry densities of 1.1, 1.2, 1.4 and 1.5 g/cm^3 and porewater salinities of either 0 or 100 g/L.

Table 2 gives the additional data for measured dry density, water content, a_w and culturable aerobes, anaerobes and SRB obtained at the intermediate porewater salinities of 60, 70, 80 and 90 g/L at the target dry densities of 1.2, 1.4, 1.6 and 1.8 g/cm^3.

Table 3 gives the new and existing data for water content, a_w and culturable aerobes, anaerobes and SRB in dry, “as-bought” bentonite.

Table 4 gives the additional data for swelling pressure measurements.

In order to make comparisons with results obtained previously, Table A1 (Appendix A) gives all previously obtained values for target and measured water contents, target, measured and calculated (i.e., from measured water content) dry densities, target and measured effective montmorillonite dry density (EMDD) values and measured (i.e., calculated from total pressures recorded) swelling pressures in the previous experiments. Table A2 (Appendix A) summarizes the previously obtained measured values for a_w and the culture results for aerobes, anaerobes and SRB in the previous experiments. These data were reported previously in Stroes-

Gascoyne et al. 2006, and include additional data obtained in 2007.

Figures 1 and 2 show the aerobic culturability (with standard deviations) in compacted bentonite as a function of measured intermediate dry densities (target 1.1-1.5 g/cm^3) and the corresponding measured values for a_w respectively, at a porewater salinity of 0 g NaCl/L. In Figures 3 and 4 (with standard deviations) these new data are compared with the previous data (from Tables A1 and A2). Both these figures show that the new data are in excellent agreement with the previous data. Figures 1 to 4 show clear patterns relating aerobic culturability to measured dry density and a_w.

Figures 5 and 6 show the aerobic culturability (with standard deviations) in compacted bentonite as a function of measured intermediate dry densities (target 1.1-1.5 g/cm^3) and the corresponding measured values for a_w, at a porewater salinity of 100 g NaCl/L. In Figures 7 and 8 (with standard deviations) these new data are compared with the previous data (from Tables A1 and A2). Figures 5 and 6 show rather large standard deviations for aerobic culturability, but the data are either not significantly different from, or lower than, the previously determined background level of (2.1 $\pm$ 0.2) x 10^2 CFU/g for dry “as-bought” bentonite. Large standard deviations are often seen at low culturability samples and are presumably due to a non-homogeneous distribution of culturable cells in dry bentonite. Figures 7 and 8 show good agreement with previously obtained data for aerobes at a porewater salinity of 100 g/L. Figures 5 to 8 suggest a slight pattern relating aerobic culturability to measured dry density and a_w.

For completeness of this report, Figures 9 and 10 show the aerobic culturability (with standard deviations) in compacted bentonite as a function of measured dry densities and the corresponding measured values for a_w respectively, at a porewater salinity of 50 g NaCl/L (data obtained previously, Tables A1 and A2). The standard deviations appear much smaller in these graphs due to the large population and the logarithmic scale. As previously reported and similarly to Figures 1 and 2, clear patterns relating aerobic culturability to measured dry density and a_w are visible.

Figures 11 and 12, 13 and 14, 15 and 16, and 17 and 18 show the aerobic culturability (with standard deviations) in compacted bentonite as a function of measured dry densities and the corresponding measured values for a_w, at porewater salinities of 60, 70, 80 and 90 g NaCl/L, respectively. These figures show rather large standard deviations in the data due to the low aerobic culturability. However, in all but one of these experiments, the aerobic culturability data are either not significantly different from, or lower than, the previously determined background level of (2.1 $\pm$ 0.2) x 10^2 CFU/g in dry bentonite. The slightly higher aerobic culturability value at a dry density of 1.4 g/cm^3 and a porewater salinity of 70 g NaCl/L is unexplained. No clear patterns relating aerobic culturability to measured dry density and a_w are visible, suggesting that at salinities of 60, 70, 80 and 90 g NaCl/L, salinity (i.e., the osmotic effect) largely controls culturability, and not dry density.

Additionally, Figures 19 and 20 show the aerobic culturability (with standard deviations) in compacted bentonite as a function of measured dry densities and the corresponding measured water activity, at a porewater salinity of 150 g NaCl/L, respectively (data obtained previously, Tables A1 and A2). Again these figures show rather large error bars due to the low aerobic culturability, but the aerobic culturability data are either not significantly different or lower than the previously determined background level of (2.1 $\pm$ 0.2) x 10^2 CFU/g in dry bentonite. As expected, no clear patterns relating aerobic culturability to measured dry density and a_w are visible, because at such high salinities culturability is controlled entirely by salinity.

Finally, Figures 21 and 22 show the aerobic culturability (with standard deviations) in compacted bentonite as a function of measured dry densities and the corresponding measured values for a_w, at a porewater salinity of 200 g NaCl/L, respectively (data obtained previously, Tables A1 and A2). Again these figures show rather large error bars due to the low aerobic culturability, but the aerobic culturability data are either not significantly different or lower than the previously determined background level of (2.1 $\pm$ 0.2) x 10^2 CFU/g in dry bentonite. As expected, no clear patterns relating aerobic culturability to measured dry density and a_w are visible, because at such high salinities culturability is controlled entirely by salinity.

Figure 23 shows the aerobic culturability in dry "as-bought" bentonite as a function of time and suggests a slightly decreasing trend in culturability as a function of time. Figure 24 shows the aerobic culturability in dry "as-bought" bentonite as a function of a_w, with the earliest sample, analyzed (2004, Figure 23) having the highest culturability and the highest value for a_w. Table 3 shows a decrease in water content from 2004 (9.31%) to 8.93% in 2006 and 8.79% in 2008, with a corresponding decrease in culturability, probably due to storage in a non-sealed sack. From these data a new lower limit of aerobic culturability in dry bentonite (i.e., 75 CFU/g) can be derived. The upper and lower limits of aerobic culturability (230 and 75 CFU/g, respectively) in dry' "as-bought" bentonite are shown Figures 25, 28 and 30.

Figure 25 compiles all aerobic culturability data from this report (Tables 1, 2 and 3) and the previous data (from Tables A1 and A2 in this report) as a function of measured dry density. It is clear that aerobic culturability drops dramatically, from 10^5 – 10^7 CFU/g to < 10^3 CFU/g for all salinities in the range 0 to 200 g NaCl/L, once measured dry density increases to 1.4 g/cm^3. Most aerobic culturability data above a dry density of 1.4 g/cm^3 are at or below the upper dry bentonite levels (i.e., < 230 CFU/g).

Upon re-examination of the few data points in Figure 25 that appear to be above 230 CFU/g at dry densities $\geq$1.4 g/cm^3 (see Figures 1 to 22), it appears that virtually all those points have large error bars that indicate that they are not significantly different from the upper dry bentonite limit of 230 CFU/g. As already noted, one data point at a dry density of 1.4 g/cm^3 and a salinity of 70 g/L (Figures 13 and 14) and one data point at a dry density of 1.85 g/cm^3 and 0 g/L porewater salinity (experiment 1714-FC in Table A2) have slightly higher aerobic culturabilities than the upper dry bentonite level. The latter point was derived from an experiment in which a Cu coupon was embedded. For this experiment, culturabilities were determined in samples taken from several locations in this bentonite plug, but the overall dry density and a_w were measured for the whole plug. Therefore, local deviations of dry density in this plug could have caused this slightly deviating point. The compiled data in Figure 25, therefore, confirm the earlier conclusions (Stroes-Gascoyne et al. 2006) and in addition suggest that slightly lower dry densities ($\geq$ 1.4 g/cm^3) or a slightly lower porewater salinity (> 50 g/L, see also Figure 27) would be sufficient to keeping culturability levels at or below background levels in compacted bentonite.

Figure 26 compiles all aerobic culturability data from this report (Tables 1, 2 and 3) and the previous data (from Tables A1 and A2 in this report) as a function of a_w. Again, this graph confirms the earlier conclusion that aerobic culturability increases dramatically at a_w values above 0.96, as previously reported (Stroes-Gascoyne et al. 2006).

Figure 27 compiles all aerobic culturability data from this report (Tables 1, 2 and 3) and the previous data (from Tables A1 and A2 in this report) as a function of salinity and target dry density. It is clear that aerobic culturability decreases dramatically at a salinity of > 50 g/L, which is lower than the previous level of 100 g/L. Therefore, the additional data appear to allow the salinity requirements to be lowered to > 50 g/L, although at salinities between 50 and 100 g/L the aerobic culturability is occasionally slightly higher than the upper background level of 230 CFU/g, as shown in Figure 28, in which all new data are shown in relation to dry density and salinity.

Figure 29 gives the swelling pressure data as a function of the measured dry density (Table 4) for all additional experiments. A comparison of Figure 29 with Figure 8 in Stroes-Gascoyne et al. (2006) shows excellent agreement with previous data. At a measured dry density of ≥1.5 g/cm^3, swelling pressures are >2MPa for all salinities tested (0,60,70,80,90,and 100 g NaCl/L).

Figure 30 gives the aerobic culturability as a function of swelling pressure (Table 4) for all additional experiments. This figure shows that the swelling pressure is >2 MPa and the aerobic culturability is $\leq (2.1 \pm 0.2) \times 10^2$ CFU/g for dry densities of >1.4g/cm^3 at all intermediate salinities (60-90 NaCl/L) examined. A comparison with Figure 20 in Stroes-Gascoyne et al. (2006) shows good agreement with previous data.

5. CONCLUSIONS AND RECOMMENDATIONS

The additional data suggest that 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., $\geq$ 1.6 g/cm^3 or > 100 g NaCl/L) have been confirmed and that those requirements could likely be lowered to $\geq$ 1.4 g/cm^3 or > 50 g NaCl/L, Nutrient-rich environments such as typical soils, sludges and some sediments contain about 10^8 to 10^{10} viable bacteria per gram of which from 0.01 to 10% (10^4 to 10^7 cells/g) are typically culturable. Based on phospholipid fatty acid (PLFA) biomarker measurements in dry and saturated compacted bentonite (Stroes-Gascoyne et al. 2008), the viable population in the bentonite studied is about 10^6 cells/g, of which between about 0.001% and 10% could be cultured, depending on dry density and porewater salinity. At dry densities ≥ 1.4 g/cm^3 or salinities > 50 g NaCl/L, only about 10^1 to 10^2 cells could be cultured (or 0.001 to 0.01% of the PLFA-based viable cell count). Therefore, compacted bentonite constitutes an environment with very low microbial viability and culturability, which implies that microbial activity in such an environment will be extremely suppressed and likely would not cause significant microbially influenced corrosion (MIC).

In order to fully confirm that dry densities of ≥ 1.4 g/cm^3 and < 1.6 g/cm^3 are sufficient to suppress microbial activity and render MIC insignificant, it is recommended that actual pore sizes are measured in Wyoming MX-80 bentonite plugs of this range of dry densities, using mercury intrusion porosimetry. Pore sizes determined for compacted MX-80 bentonite with a measured dry density of ~1.63 g/cm^3 are shown in Figure 12 of Stroes-Gascoyne et al. (2006).

It is also recommended that 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 concentrations in this study. It is further emphasized that the conclusions are valid for Wyoming MX-80 bentonite, but are not necessarily directly applicable to other brands of bentonite without further study.

ACKNOWLEDGEMENTS

Many thanks are due to D. Drew, S. Jones and C. Kohle for expert assistance with the experiments, and to D. Priyanto for calculating the swelling pressures.

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Table 1 - Additional Data obtained at Intermediate Dry Densities

Exper-ment	Target Dry Density (g/cm^3)	Measured Dry Density (g/cm^3)	Porewater Salinity (g NaCl/L)	Water Content (%)	Aerobes (CFU/g)	Anaerobes (CFU/g)	SRB (MPN/g)	a_w
1807	1.1	1.01	0	58.95	$(1.81 \pm 0.22) \times 10^5$	$(4.02 \pm 2.30) \times 10^1$	4.67×10^0	0.995
1808	1.2	1.14	0	51.90	$(1.38 \pm 0.17) \times 10^5$	$(5.37 \pm 1.76) \times 10^1$	4.14×10^0	0.990
1809	1.4	1.41	0	27.42	$(1.05 \pm 0.42) \times 10^2$	$(4.28 \pm 1.78) \times 10^1$	1.09×10^1	0.932
1810	1.5	1.36	0	38.05	$(8.56 \pm 6.43) \times 10^1$	$(1.17 \pm 1.17) \times 10^1$	3.86×10^0	0.977
1811	1.1	1.19	100	57.56	$(1.46 \pm 0.26) \times 10^2$	$(5.41 \pm 2.60) \times 10^1$	9.24×10^0	0.939
1812	1.2	1.25	100	46.26	$(2.50 \pm 0.84) \times 10^2$	$(2.04 \pm 0.89) \times 10^1$	2.30×10^1	0.919
1813	1.4	1.38	100	40.53	$(1.35 \pm 0.43) \times 10^2$	$(4.50 \pm 2.55) \times 10^1$	4.42×10^0	0.934
1814	1.5	1.46	100	39.48	$(1.67 \pm 0.53) \times 10^2$	$(2.72 \pm 2.43) \times 10^1$	1.75×10^1	0.934

CFU/g = Colony-Forming Units/g dry weight
MPN/g = Most Probable Number of Cells/g dry weight
a_w = water activity
SRB = Sulphate Reducing Bacteria

Table 2: Additional Data Obtained at Intermediate Salinities

Experi-ment	Target Dry Density (g/cm^3)	Measured Dry Density (g/cm^3)	Porewater Salinity (g NaCl/L)	Water Content (%)	Aerobes (CFU/g)	Anaerobes (CFU/g)	SRB (MPN/g)	a_w
1815	1.2	1.15	60	51.06	$(2.19 \pm 0.54) \times 10^2$	$(5.42 \pm 4.29) \times 10^1$	$<4.29 \times 10^0$	0.962
1816	1.4	1.27	60	40.53	$(8.89 \pm 3.85) \times 10^1$	$(3.85 \pm 3.33) \times 10^1$	$<2.86 \times 10^0$	0.953
1817	1.6	1.52	60	30.58	$(7.90 \pm 2.89) \times 10^1$	$(4.33 \pm 1.17) \times 10^1$	$<2.29 \times 10^0$	0.929
1818	**1.8**	**1.75**	**60**	**18.77**	$(2.14 \pm 0.52) \times 10^2$	$(4.48 \pm 3.95) \times 10^1$	1.10×10^1	**0.737**
1819	**1.2**	**1.22**	**80**	**47.21**	$(1.46 \pm 0.07) \times 10^2$	$(8.33 \pm 14.4) \times 10^0$	4.50×10^0	**0.948**
1820	**1.4**	**1.44**	**80**	**36.47**	$(1.45 \pm 0.06) \times 10^2$	$(3.00 \pm 1.00) \times 10^1$	9.32×10^0	**0.931**
1821	**1.6**	**1.72**	**80**	**26.52**	$(1.55 \pm 0.17) \times 10^2$	$(2.78 \pm 3.47) \times 10^1$	2.59×10^0	**0.858**
1822	**1.8**	**1.76**	**80**	**22.54**	$(2.91 \pm 1.10) \times 10^2$	$(2.20 \pm 0.95) \times 10^1$	1.52×10^1	**0.824**
1837	**1.2**	**1.26**	**70**	**44.51**	$(6.47 \pm 2.24) \times 10^1$	$(4.58 \pm 0.72) \times 10^1$	3.75×10^0	**0.954**
1838	**1.4**	**1.45**	**70**	**35.35**	$(3.07 \pm 0.25) \times 10^2$	$(3.61 \pm 1.65) \times 10^1$	$<3.25 \times 10^0$	**0.941**
1839	**1.6**	**1.60**	**70**	**30.26**	$(1.21 \pm 0.29) \times 10^2$	$(2.13 \pm 0.38) \times 10^2$	2.86×10^0	**0.907**
1840	**1.8**	**1.79**	**70**	**21.64**	$(4.77 \pm 0.63) \times 10^1$	$(6.97 \pm 3.87) \times 10^1$	3.30×10^0	**0.823**
1841	**1.2**	**1.07**	**90**	**48.53**	$(6.54 \pm 1.62) \times 10^1$	$(6.54 \pm 1.62) \times 10^1$	5.04×10^0	**0.943**
1842	**1.4**	**1.39**	**90**	**35.36**	$(8.43 \pm 7.49) \times 10^1$	$(8.43 \pm 7.49) \times 10^1$	1.38×10^1	**0.926**
1843	**1.6**	**1.56**	**90**	**27.43**	$(2.55 \pm 0.74) \times 10^2$	$(2.55 \pm .743) \times 10^2$	1.15×10^1	**0.890**
1844	**1.8**	**1.71**	**90**	**21.54**	$(1.36 \pm 0.59) \times 10^2$	$(1.36 \pm .59) \times 10^2$	5.65×10^0	**0.809**

CFU/g = Colony-Forming Units/g dry weight
MPN/g = Most Probable Number of Cells/g dry weight
a_w = water activity

Table 3: Data for Dry, "as bought" Bentonite

Exper-iment	Target Dry Density (g/cm^3)	Measured Dry Density (g/cm^3)	Porewater Salinity (g NaCl/L)	Water Content (%)	Aerobes (CFU/g)	Anaerobes (CFU/g)	SRB (MPN/g)	a_w
1612	N/A	N/A	**0**	**9.31**	**$(2.07 \pm 0.23) \times 10^2$**	**$(5.51 \pm 1.91) \times 10^1$**	**1.03×10^1**	**0.471**
1733	**N/A**	**N/A**	**0**	**8.93**	**$(1.37 \pm 0.30) \times 10^2$**	**$(1.67 \pm 0.72) \times 10^1$**	**4.11×10^0**	**0.358**
1836	**N/A**	**N/A**	**0**	**8.79**	**$(1.20 \pm 0.39) \times 10^2$**	ND	**ND**	**0.383**

CFU/g = Colony-Forming Units/g dry weight
MPN/g = Most Probable Number of Cells/g dry weight
a_w = water activity
SRB = Sulphate Reducing Bacteria
N/A = Not Applicable

Table 4: Swelling Pressures in the Intermediate Dry Densities and Intermediate Salinities Experiments

Experiment	Target Dry Density g/cm^3	Target EMDD	Measured Dry Density	Salinity g/L	Swelling Pressure KPa
1807	1.1	0.92	1.01	0	515
1808	1.2	1.02	1.14	0	517
1809	1.4	1.11	1.41	0	426
1810	1.5	1.31	1.36	0	2748
1811	1.1	0.92	1.19	100	126
1812	1.2	1.02	1.26	100	239
1813	1.4	1.11	1.38	100	failed
1814	1.5	1.31	1.46	100	failed
1815	1.2	1.02	1.15	60	5
1816	1.4	1.21	1.27	60	94
1817	1.6	1.41	1.52	60	3848
1818	1.8	1.63	1.75	60	9516
1819	1.2	1.02	1.22	80	323
1820	1.4	1.21	1.44	80	1722
1821	1.6	1.41	1.72	80	2540
1822	1.8	1.63	1.76	80	12974
1837	1.2	1.02	1.26	70	364
1838	1.4	1.21	1.45	70	1518
1839	1.6	1.41	1.60	70	2809
1840	1.8	1.63	1.79	70	17553
1841	1.2	1.02	1.07	90	429
1842	1.4	1.21	1.39	90	1465
1843	1.6	1.41	1.56	90	4276
1844	1.8	1.63	1.71	90	13863

EMDD = Effective Montmorillonite Dry Density
Failed = Measurement failed due to sticking piston

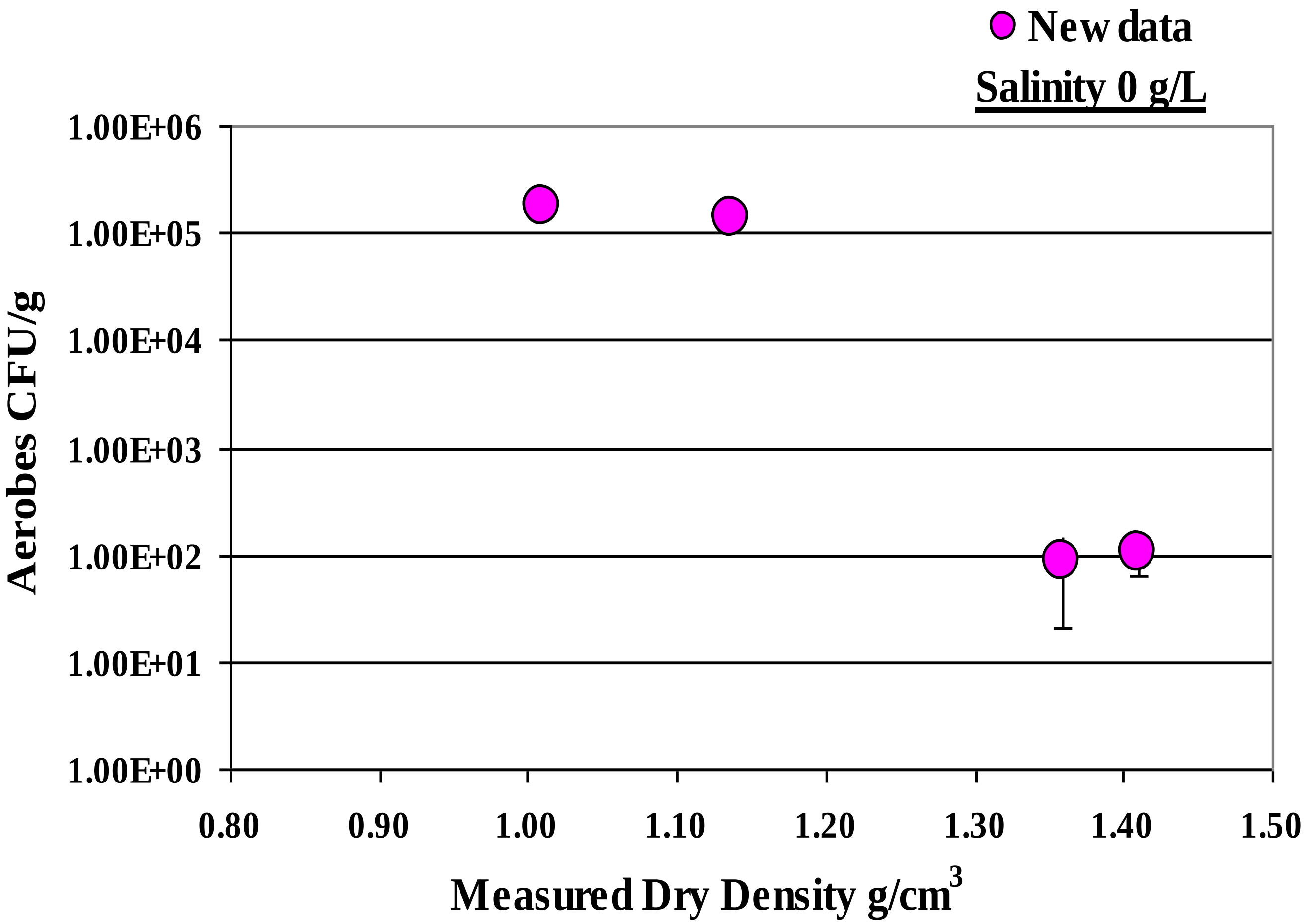

Figure 1: Aerobic culturability in compacted bentonite as a function of measured intermediate dry densities (target 1.1-1.5 g/cm^3) at a porewater salinity of 0 g NaCl/L

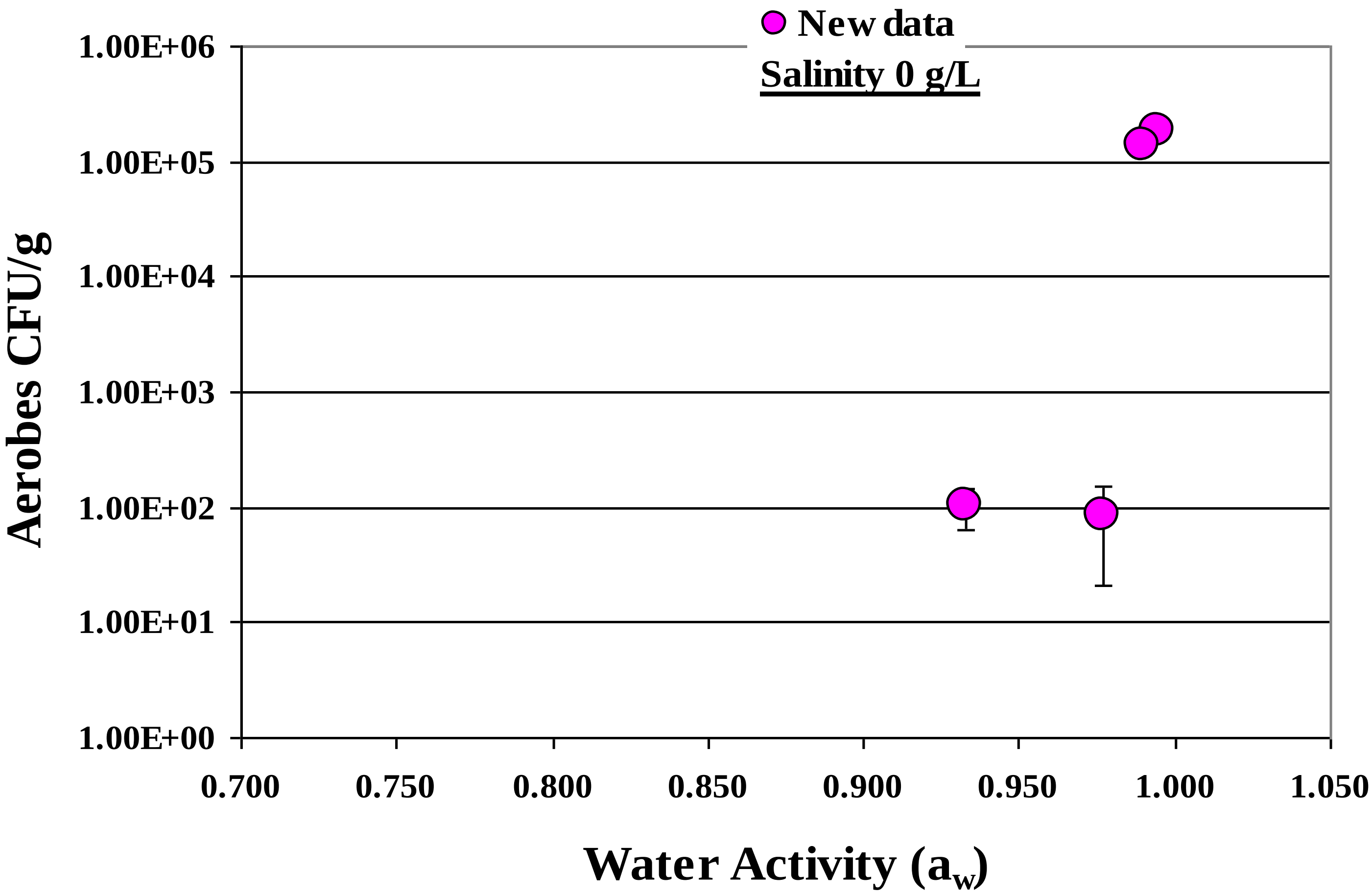

Figure 2: Aerobic culturability in compacted bentonite as a function of water activity at intermediate dry densities (target 1.1-1.5 g/cm^3) and at a porewater salinity of 0 g NaCl/L

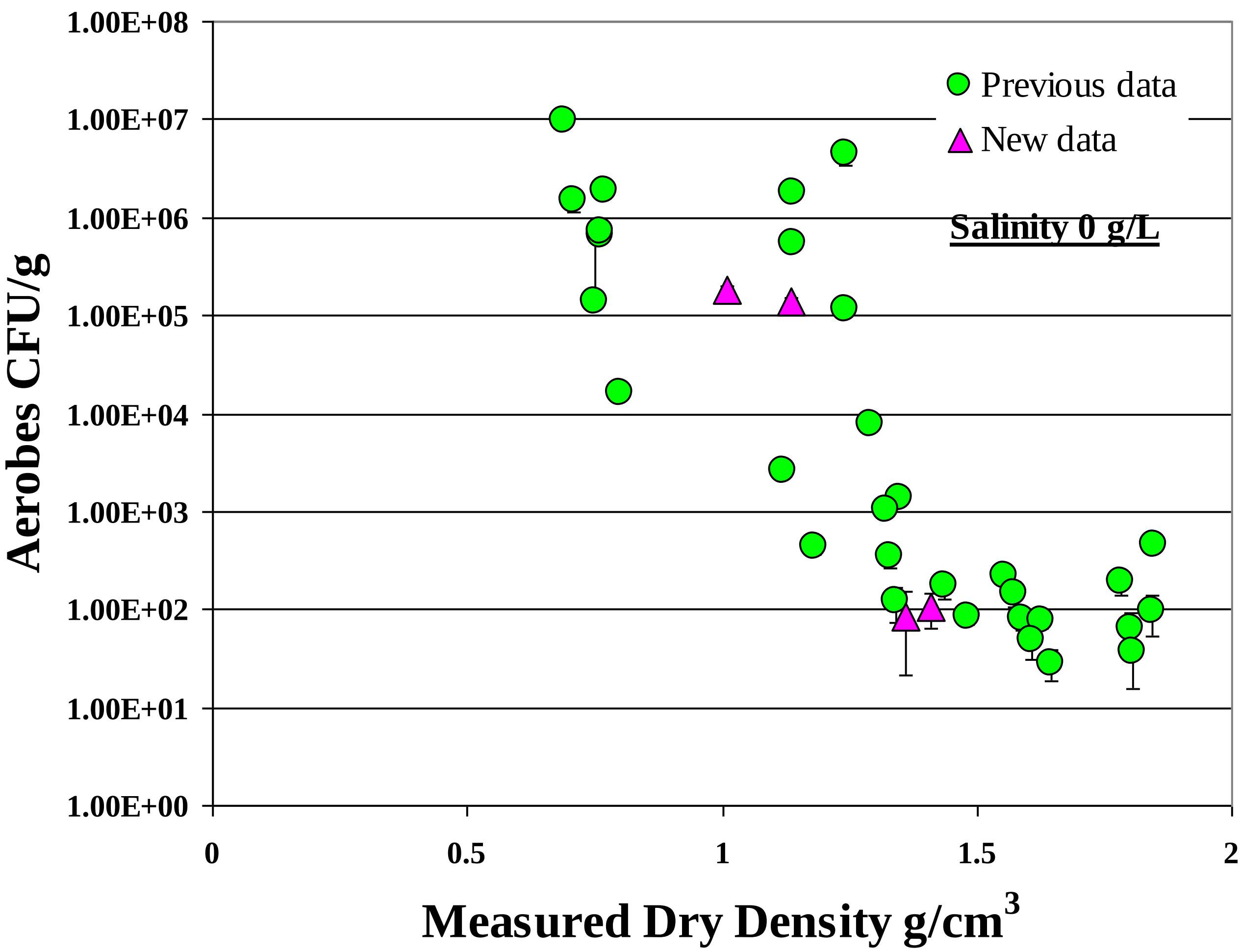

Figure 3: Comparison of aerobic culturability data in compacted bentonite obtained at intermediate dry densities (target 1.1-1.5 g/cm^3) with previous results, as a function of dry density and at a porewater salinity of 0 g NaCl/L

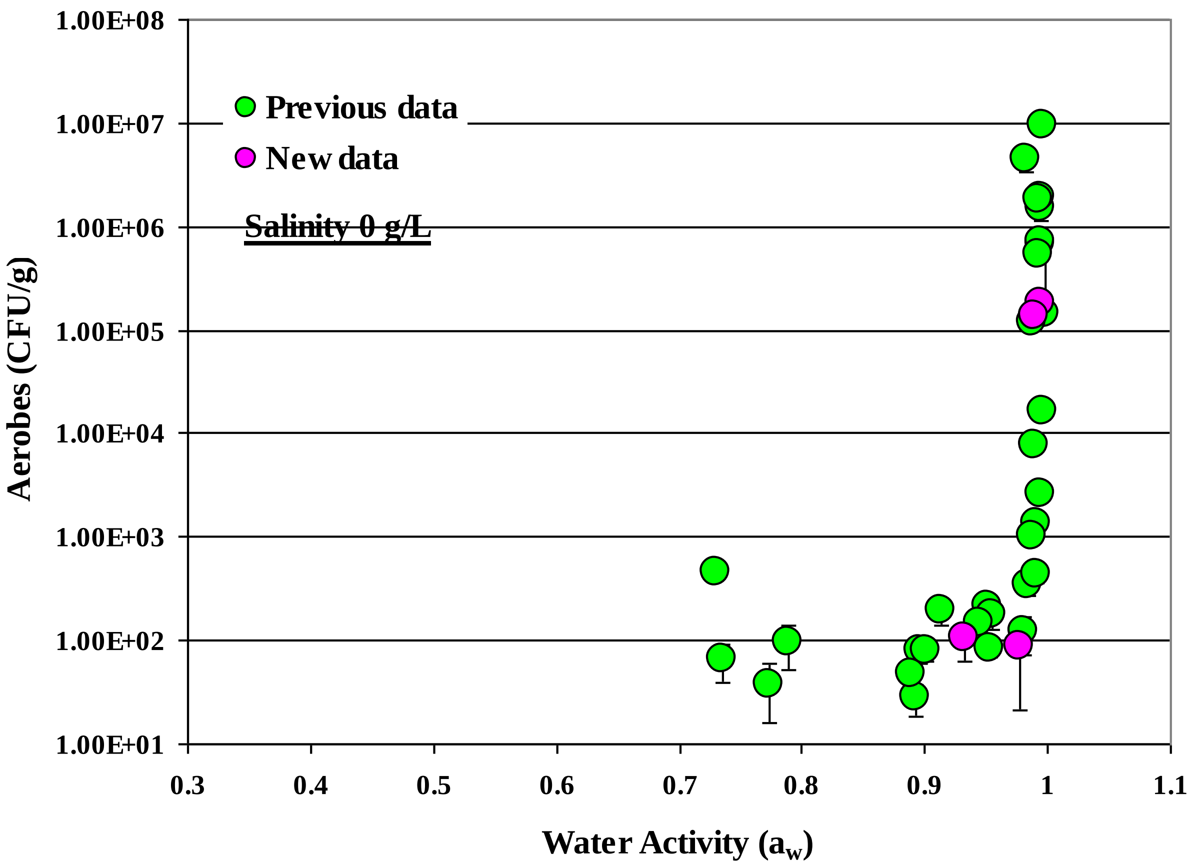

Figure 4: Comparision of aerobic culturability data in compacted bentonite obtained at intermediate dry densities (target 1.1-1.5 g/cm^3) with previous results, as a function of water activity and at a porewater salinity of 0 g NaCl/L

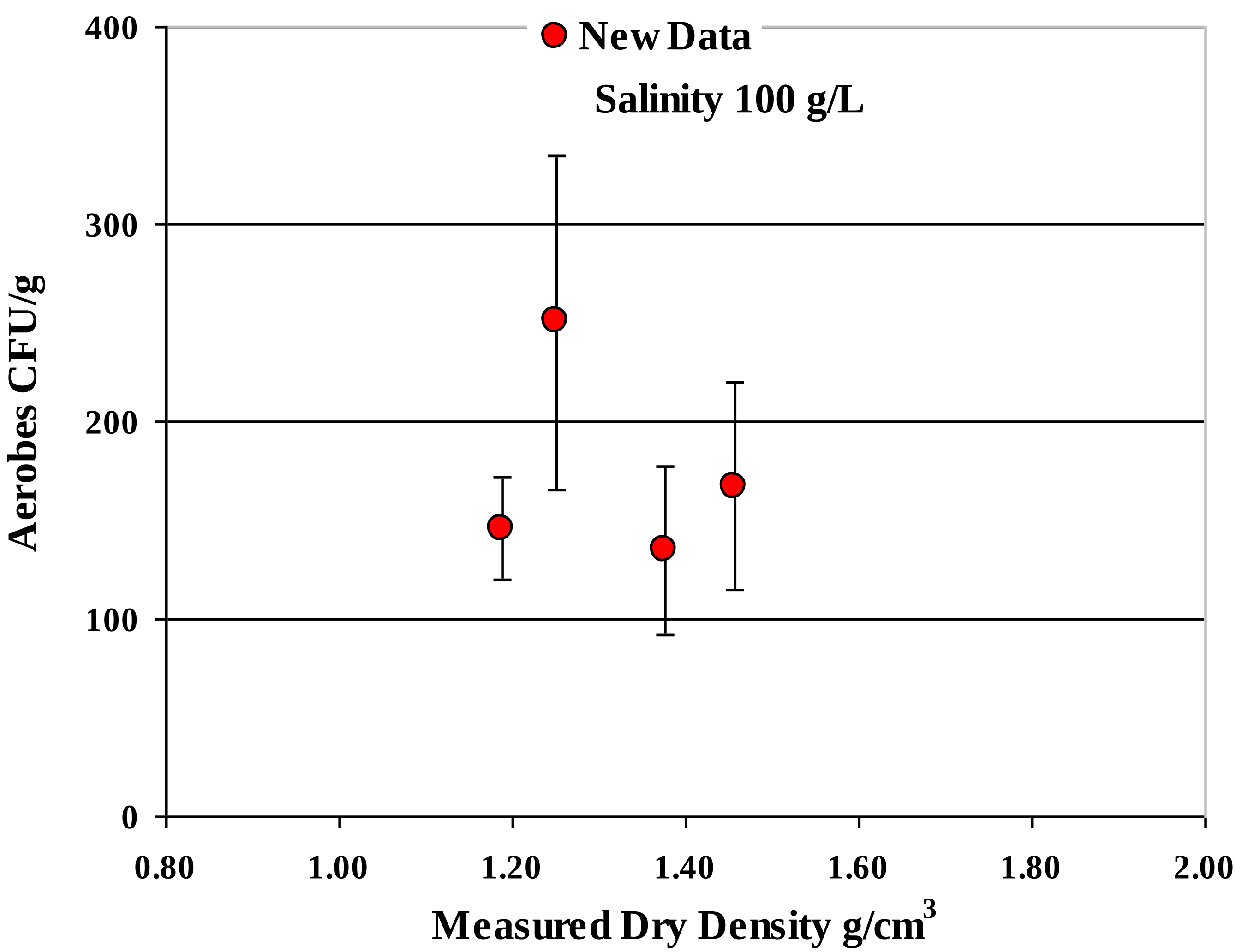

Figure 5: Aerobic culturability in compacted bentonite as a function of measured immediate dry densities (target 1.1-1.5 g/cm^3) at a porewater salinity of 100 g NaCl/L

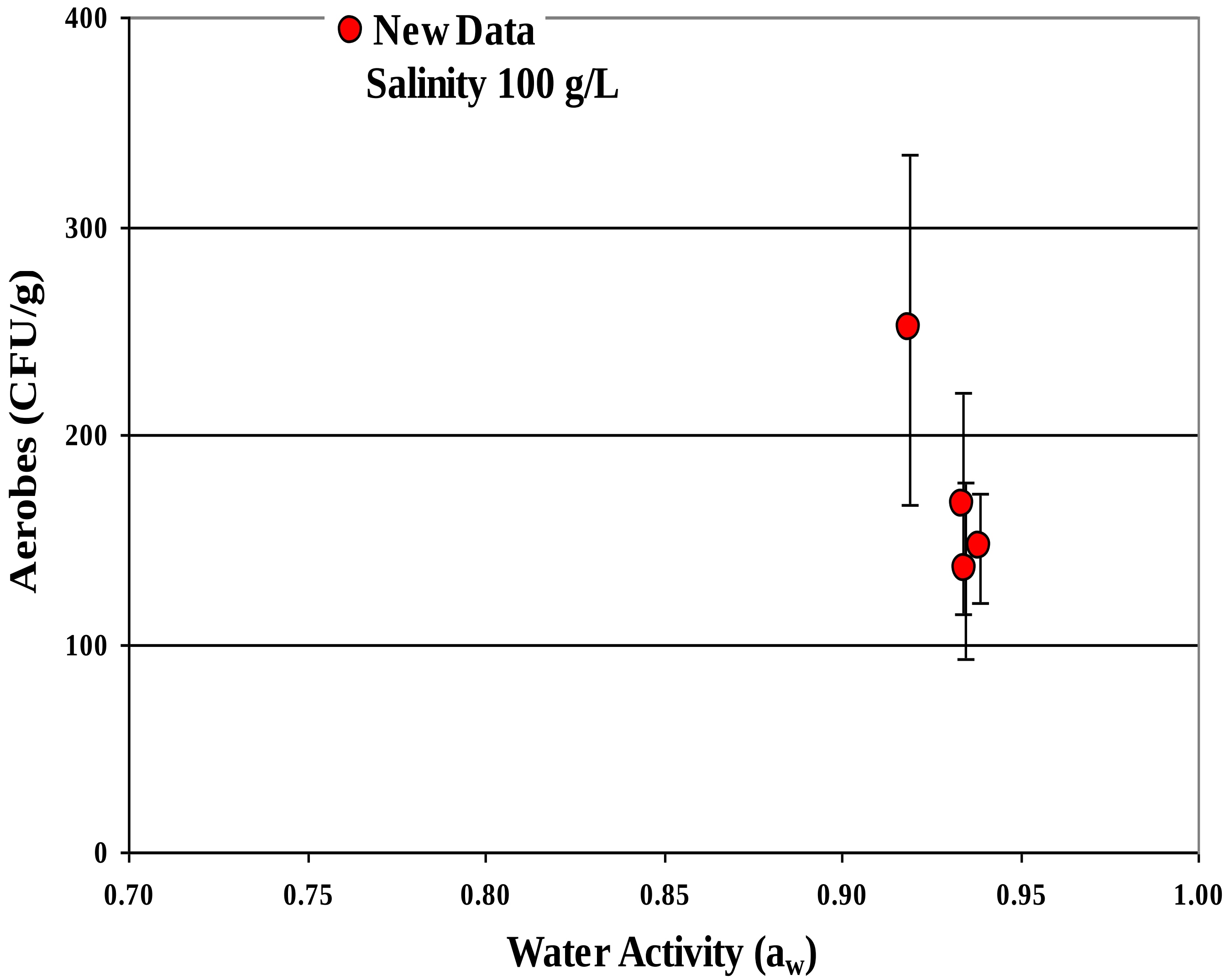

Figure 6: Aerobic culturability in compacted bentonite as a function of measured water activities at intermediate dry densities (target 1.1-1.5 g/cm^3) and at a porewater salinity of 100 g NaCl/L

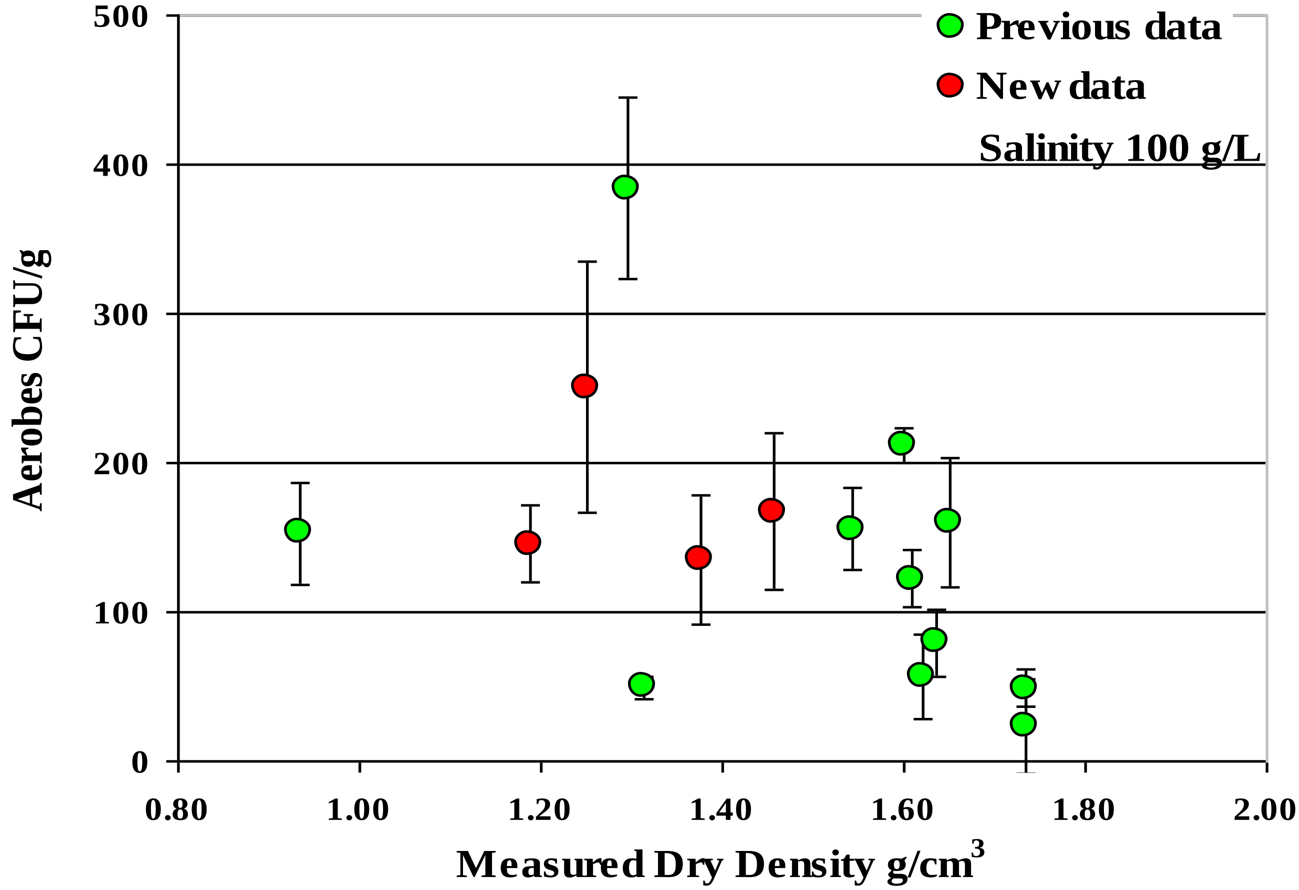

Figure 7: Comparison of aerobic culturability data in compacted bentonite obtained at intermediate dry densities (target 1.1-1.5 g/cm^3) with previous results, as a function of dry density and at a porewater salinity of 100 g NaCl/L

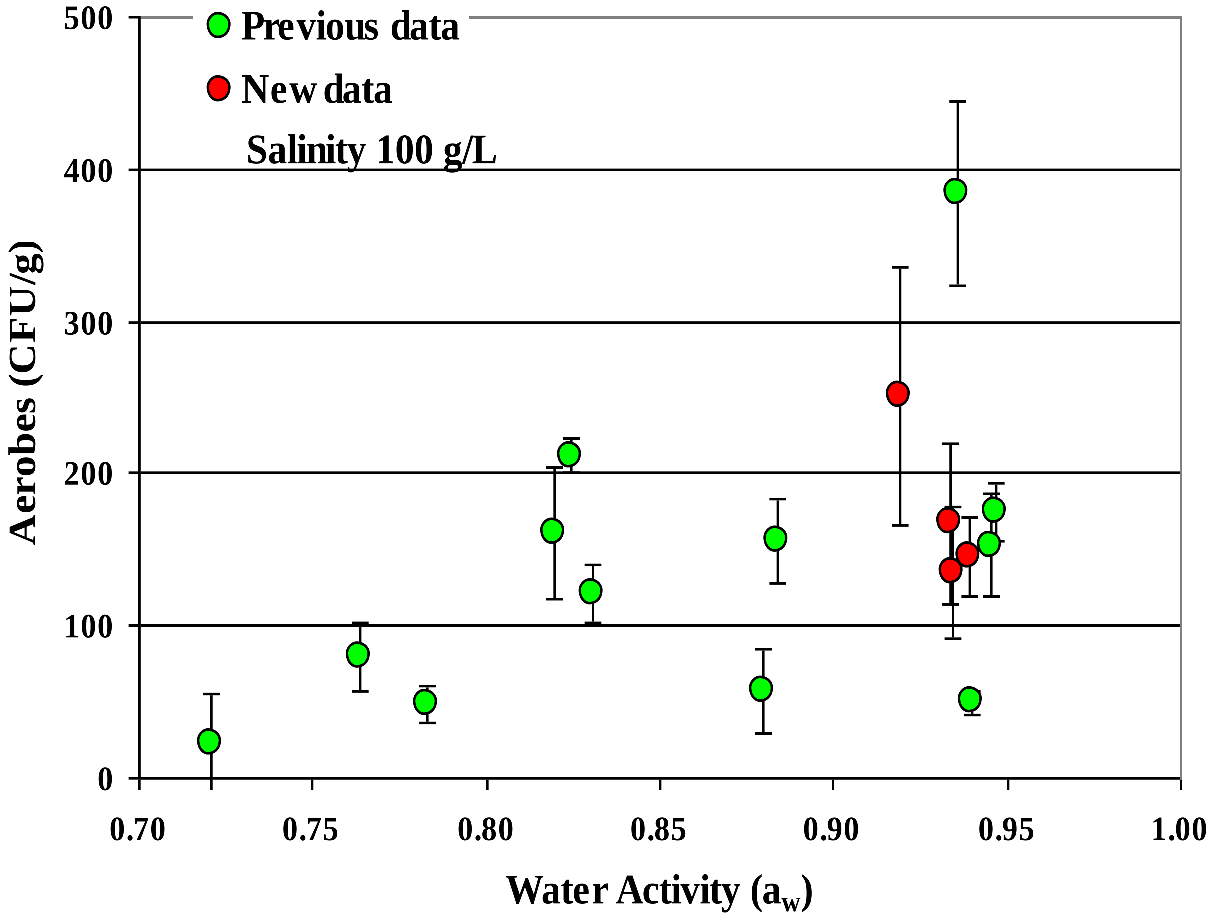

Figure 8: Comparison of aerobic culturability data in compacted bentonite obtained at intermediate dry densities (target 1.1-1.5 g/cm^3) with previous results, as a function of water activity and at a porewater salinity of 100 g NaCl/L

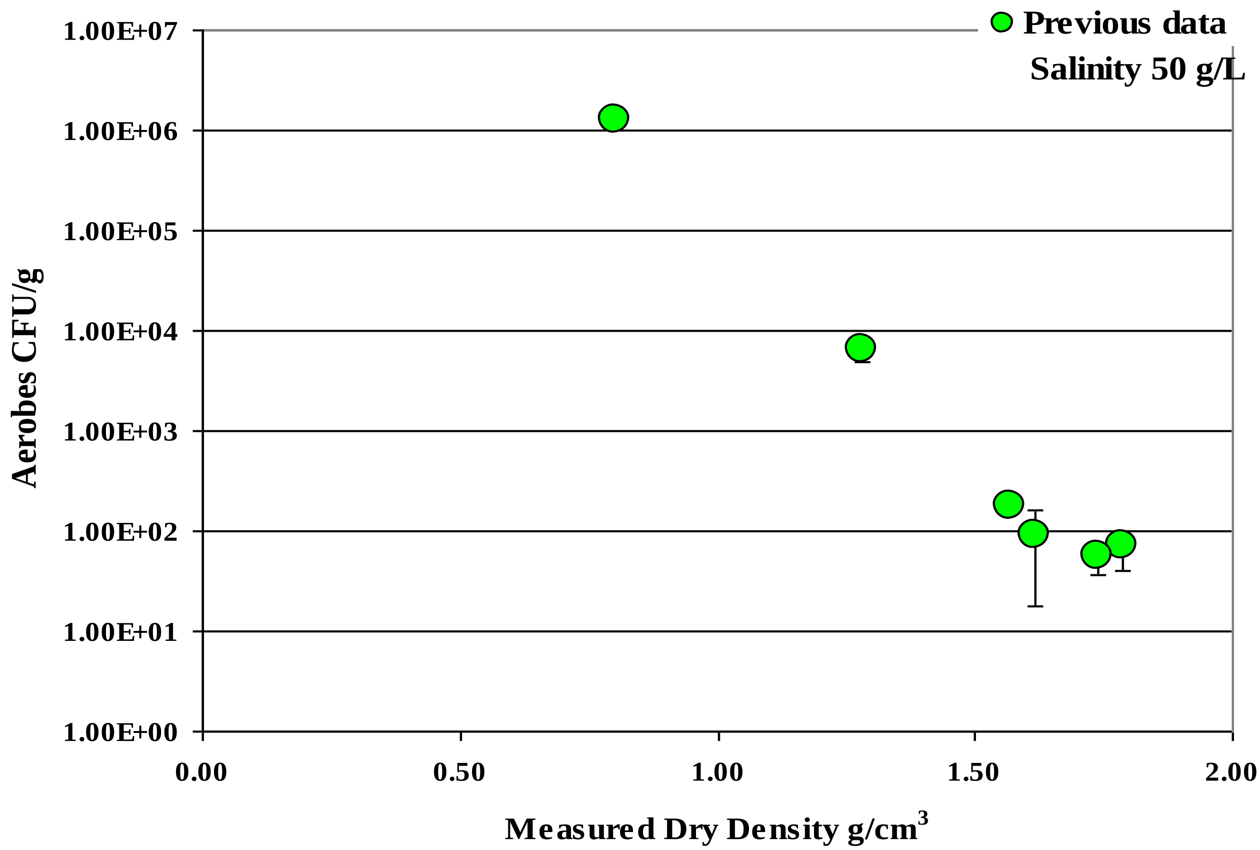

Figure 9: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 50 g NaCl/L (previous data)

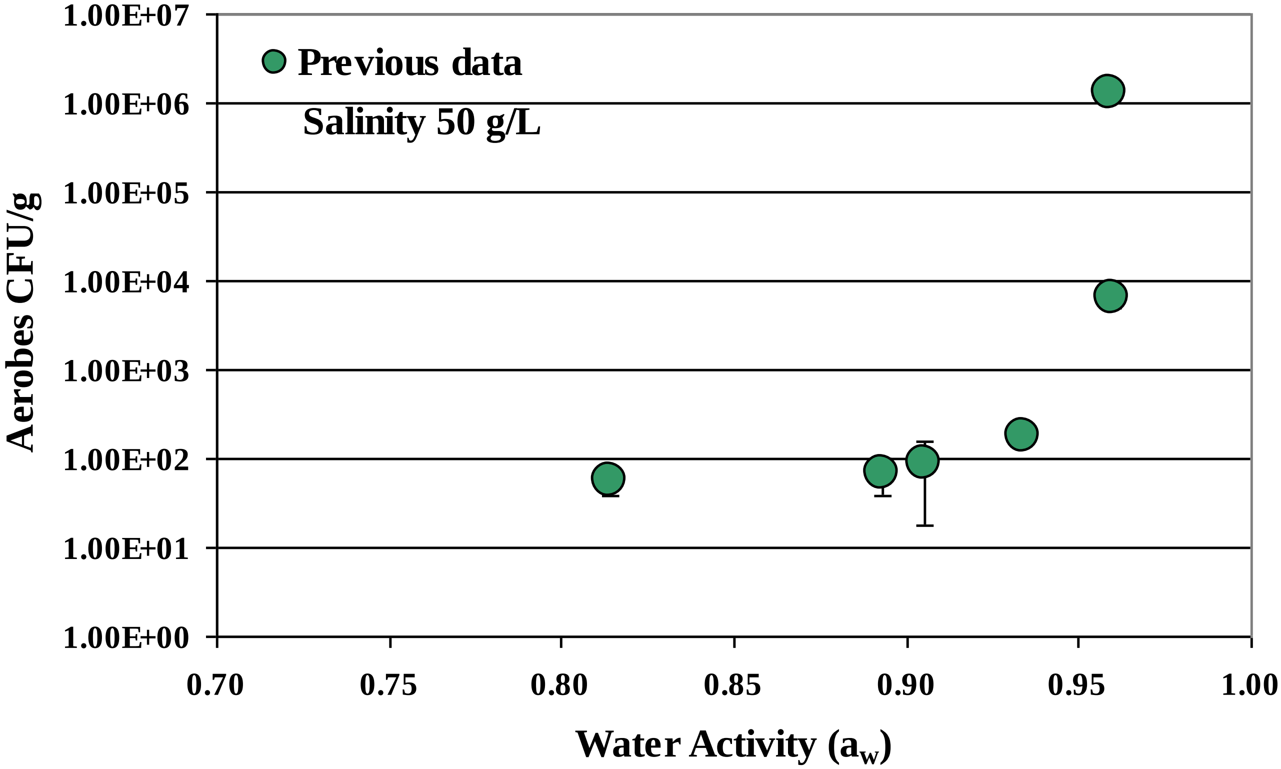

Figure 10: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 50 g NaCl/L (previous data)

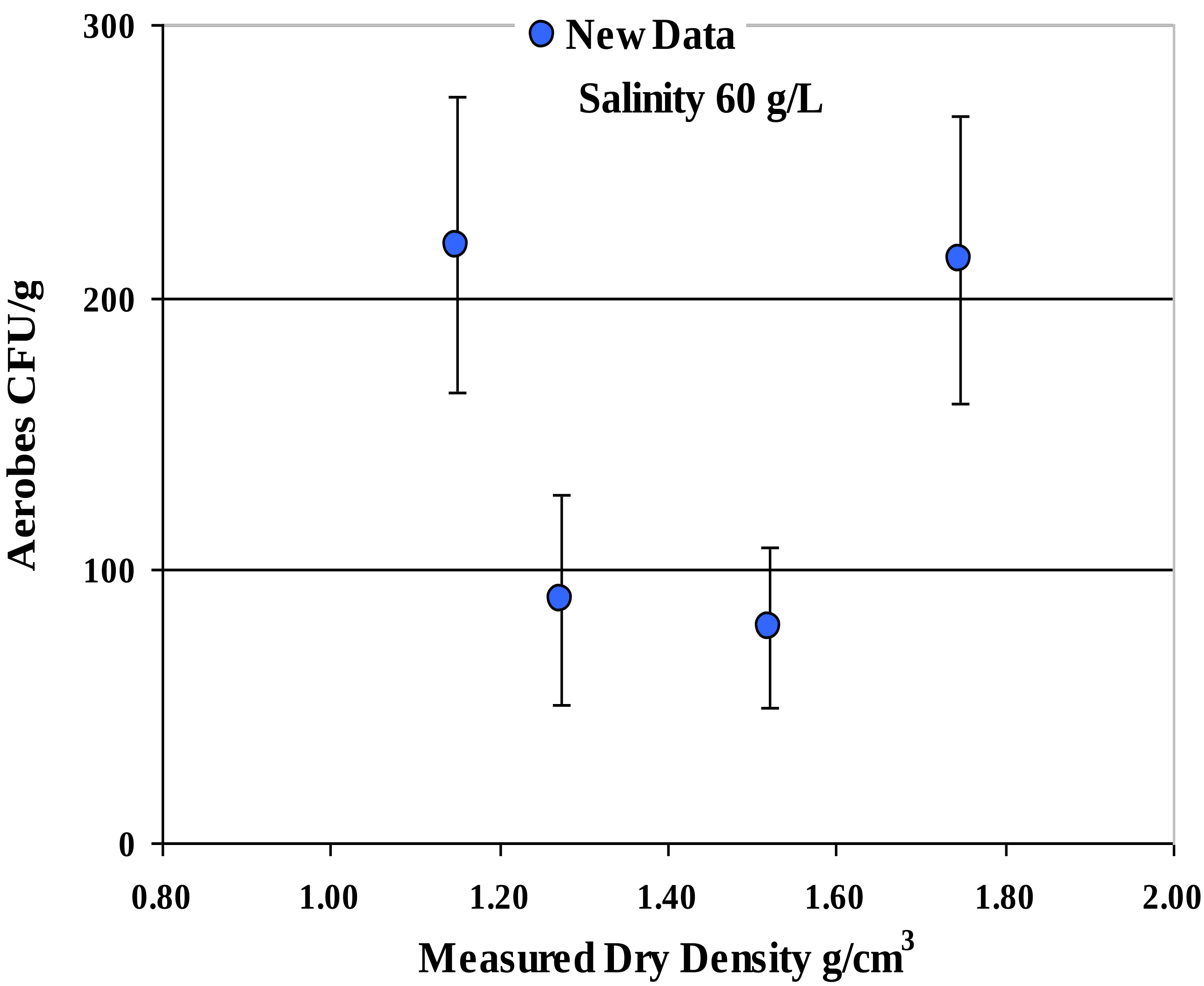

Figure 11: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 60 g NaCl/L (new data)

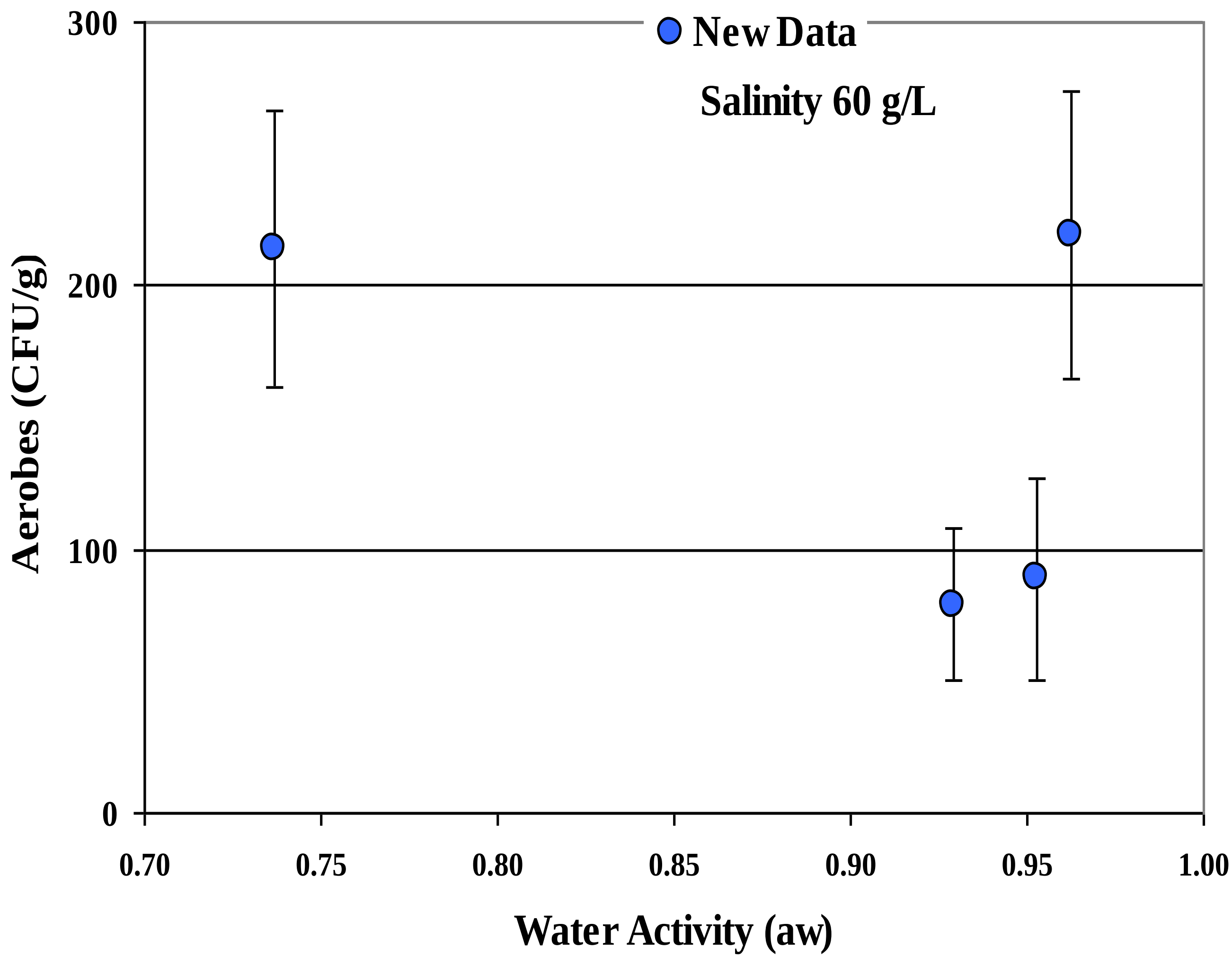

Figure 12: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 60 g NaCl/L (new data)

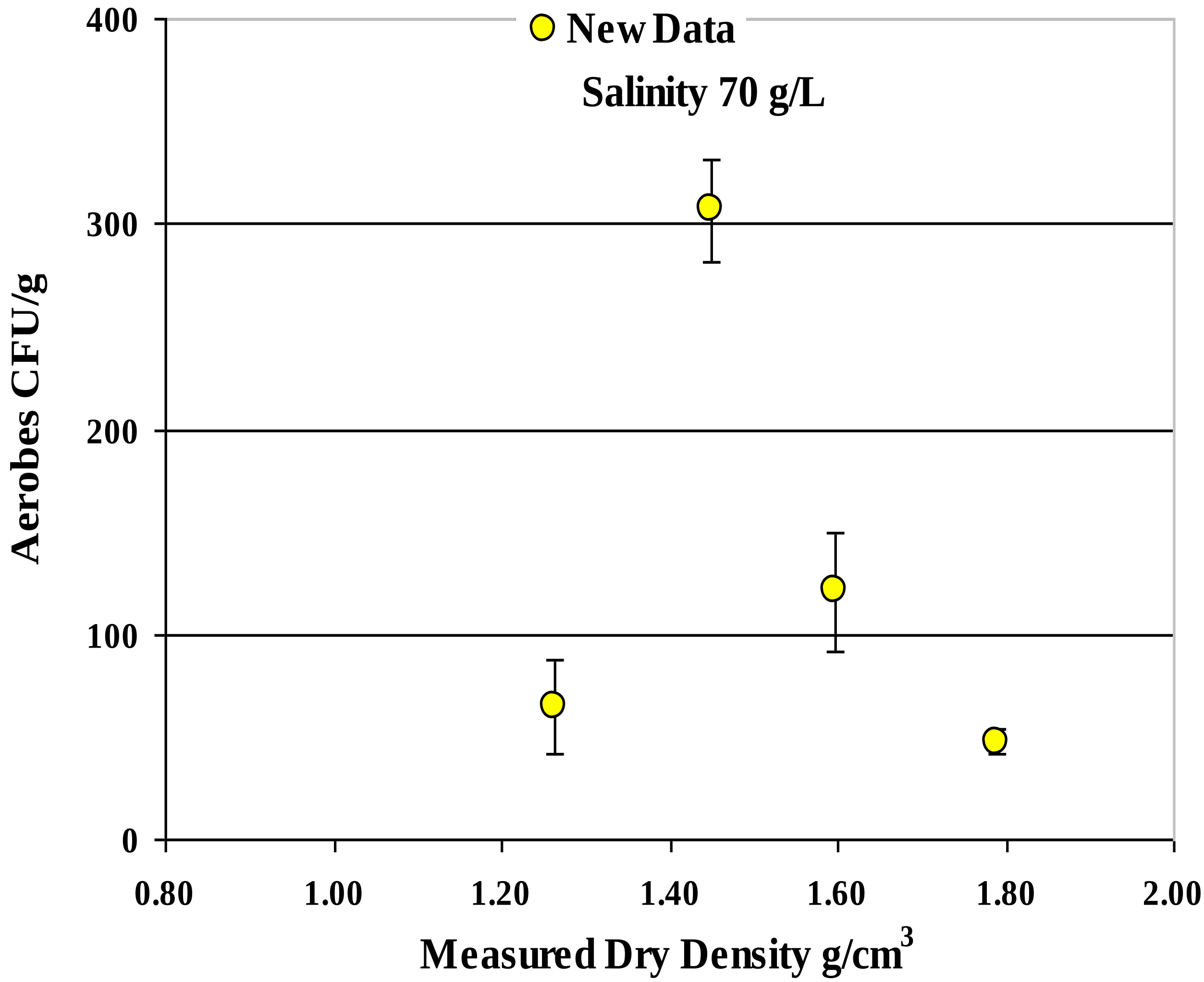

Figure 13: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 70 g NaCl/L (new data)

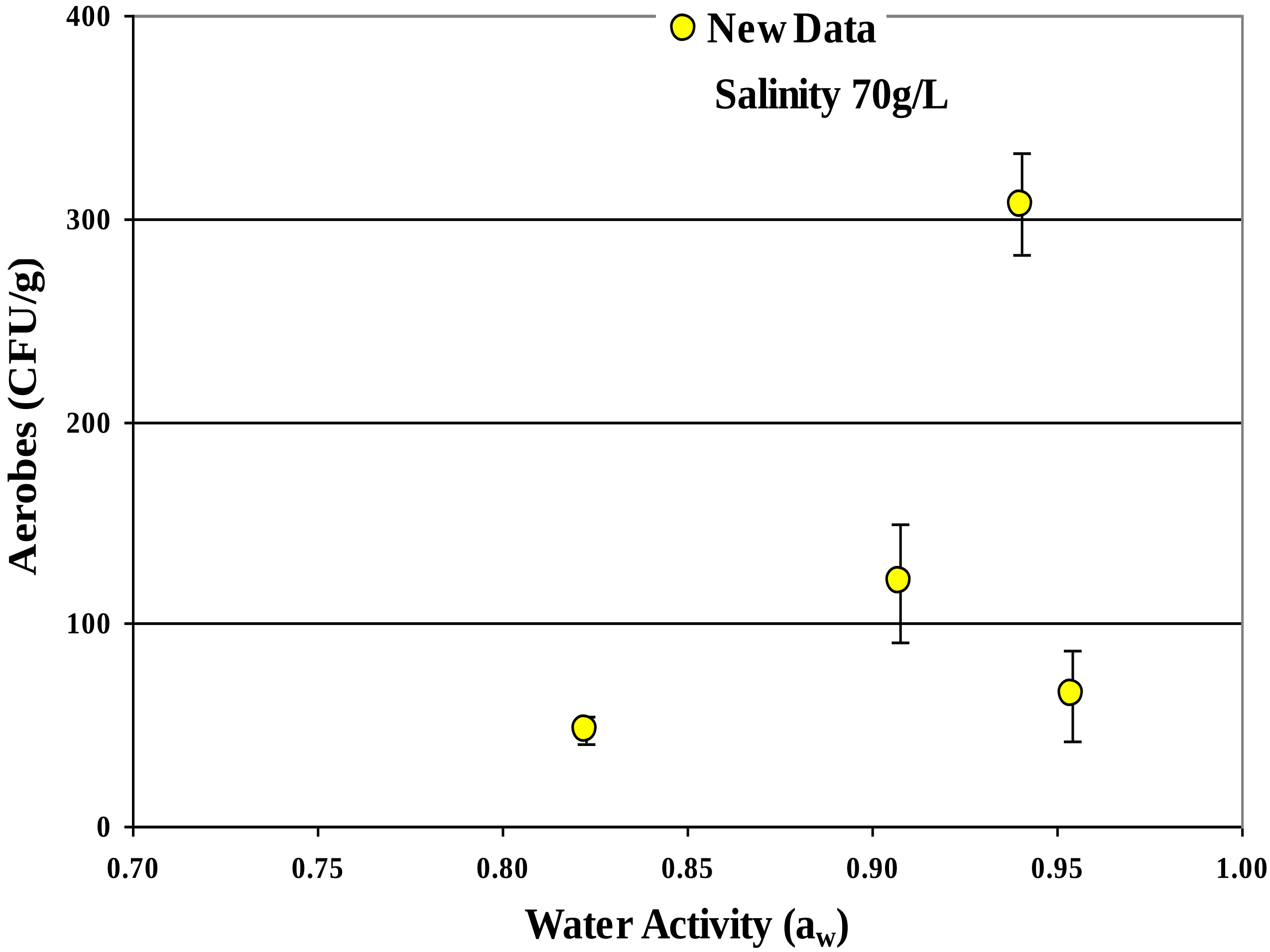

Figure 14: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 70 g NaCl/L (new data)

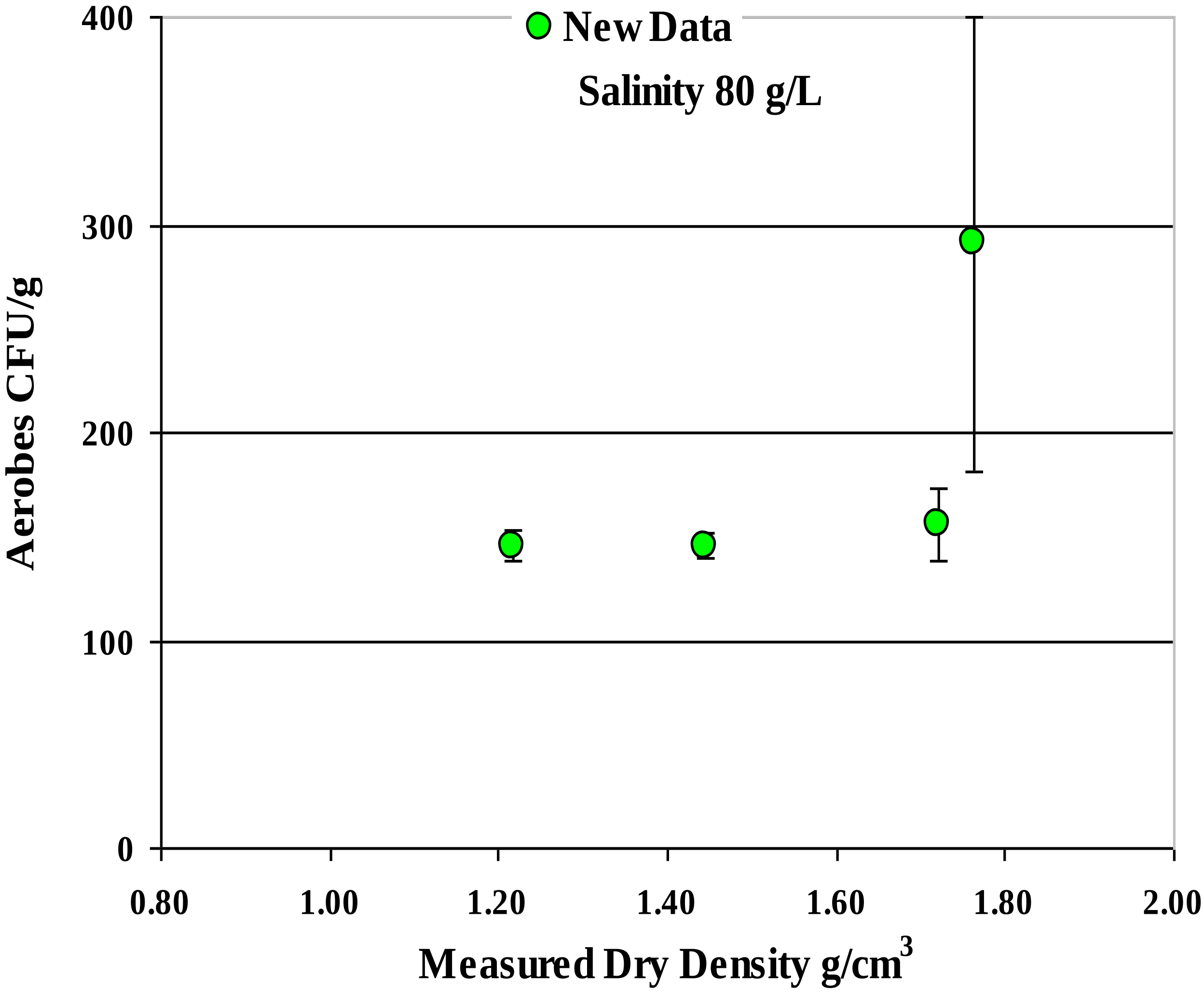

Figure 15: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 80 g NaCl/L (new data)

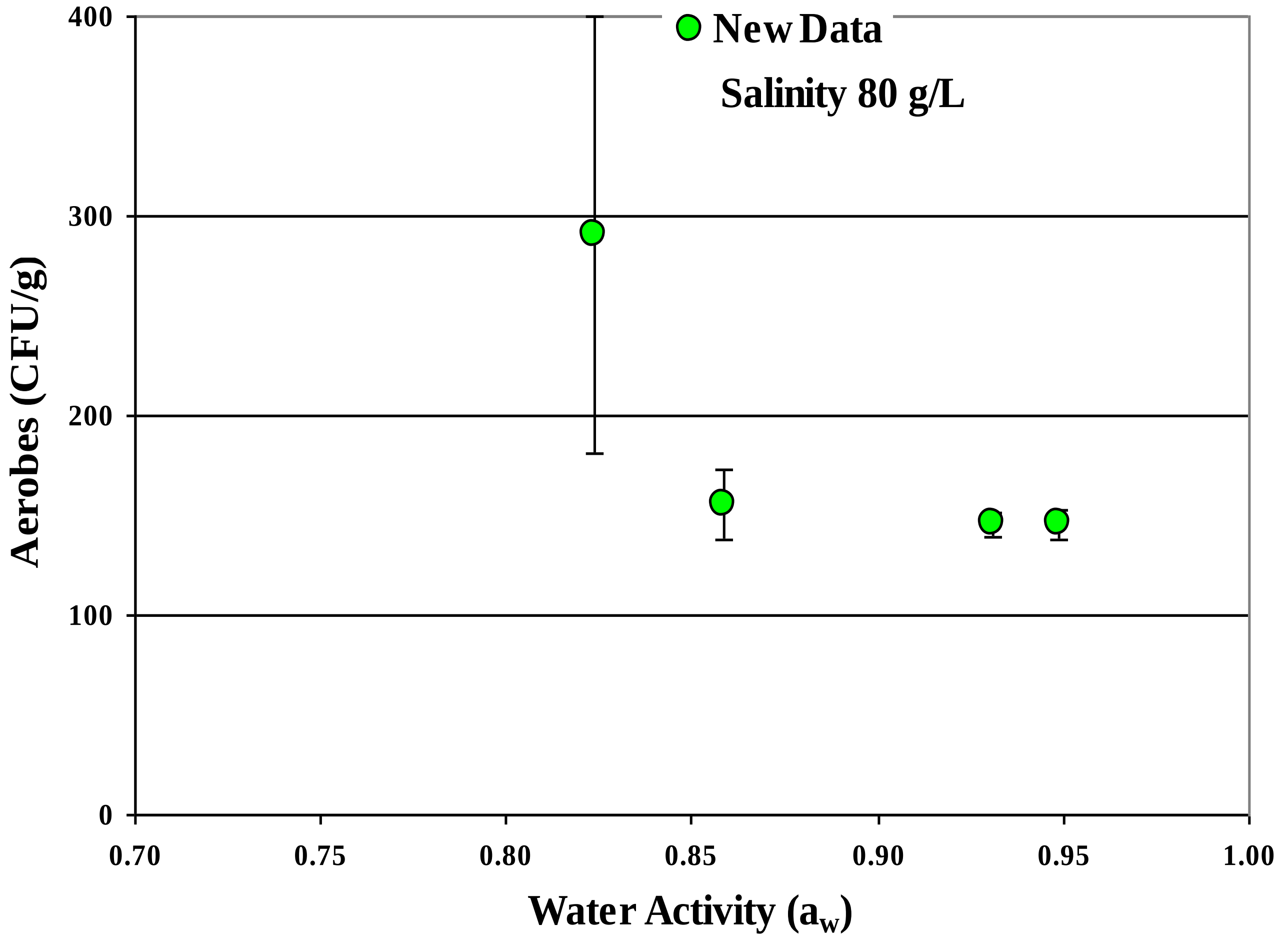

Figure 16: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 80 g NaCl/L (new data)

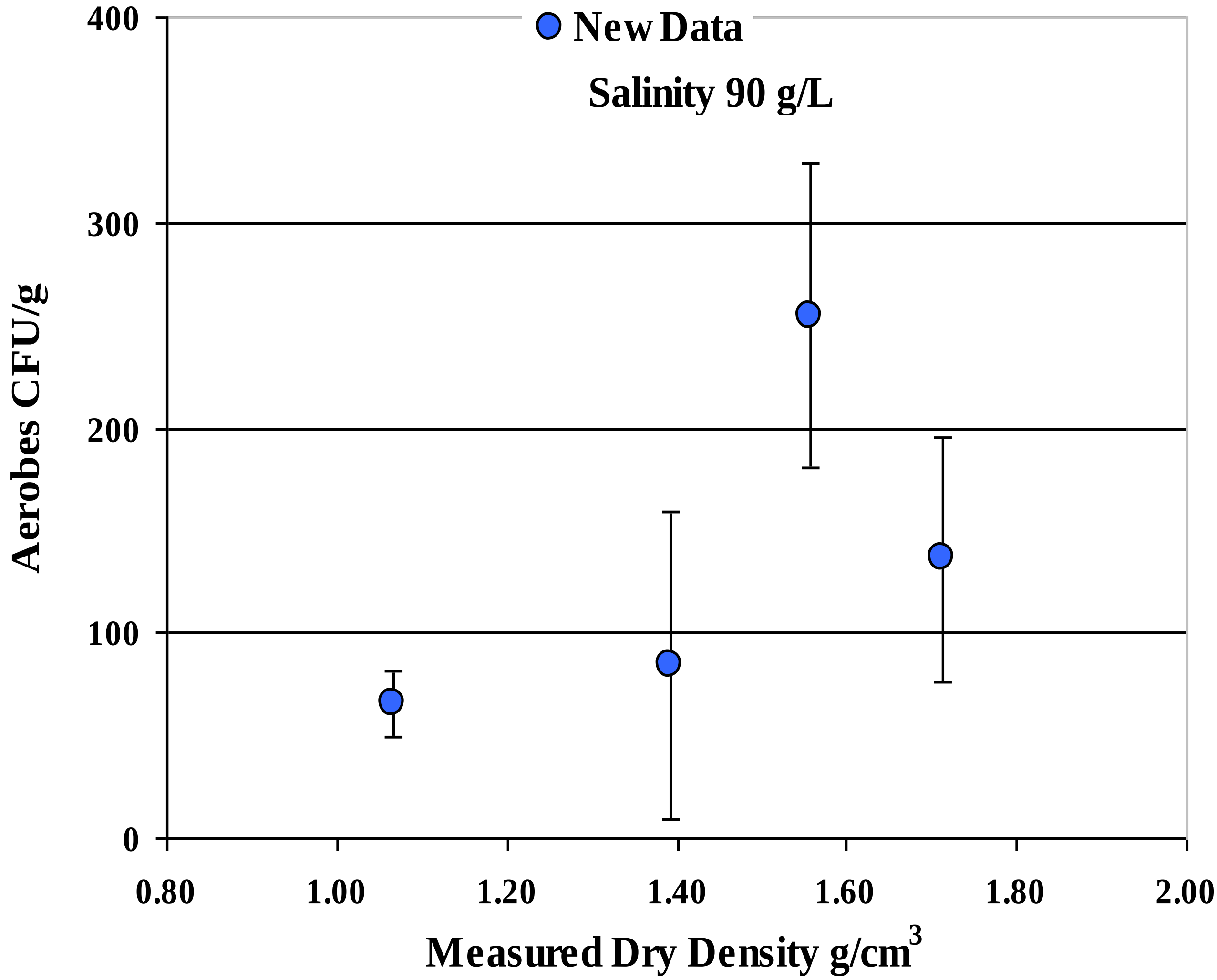

Figure 17: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 90 g NaCl/L (new data)

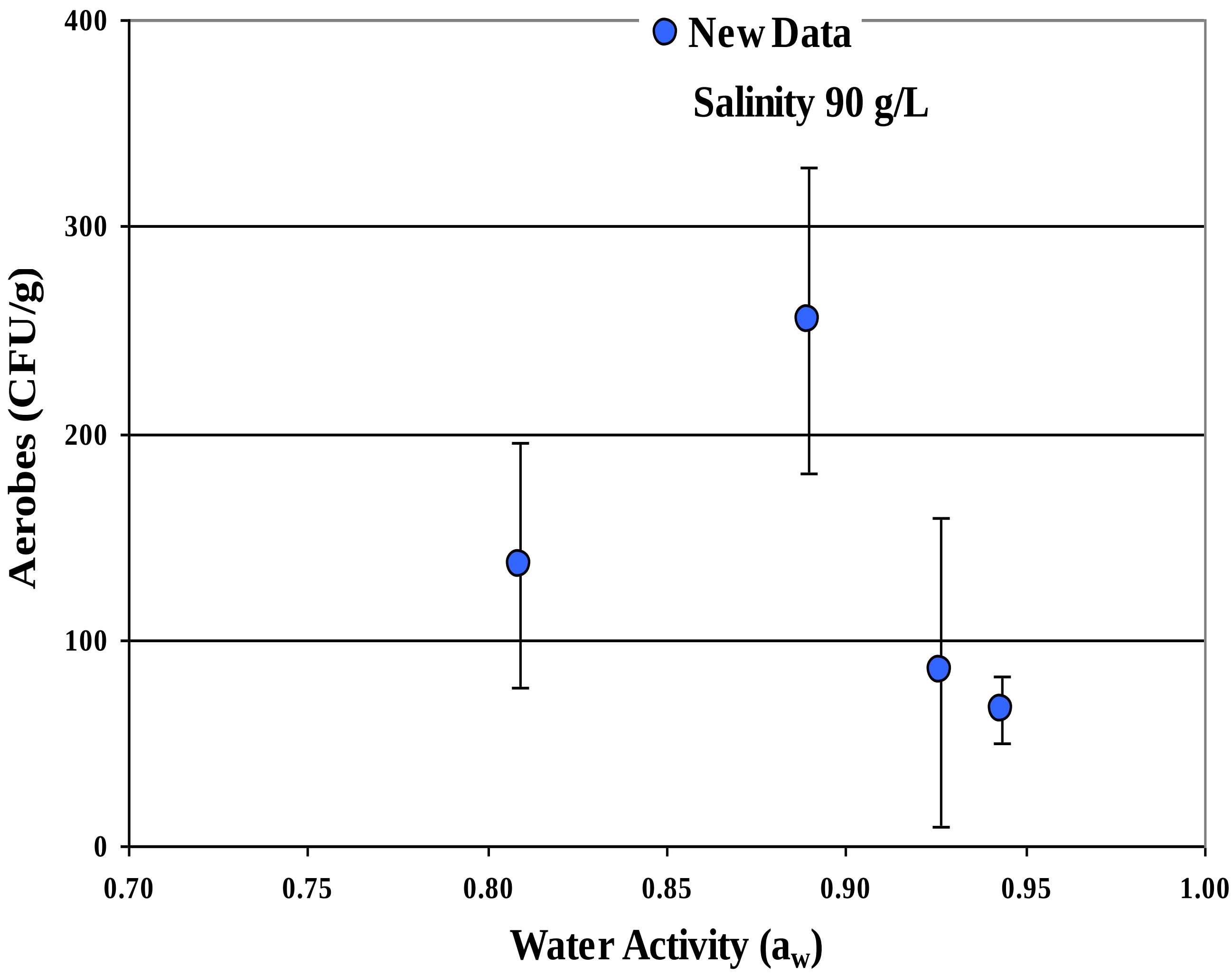

Figure 18: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 90 g NaCl/L (new data)

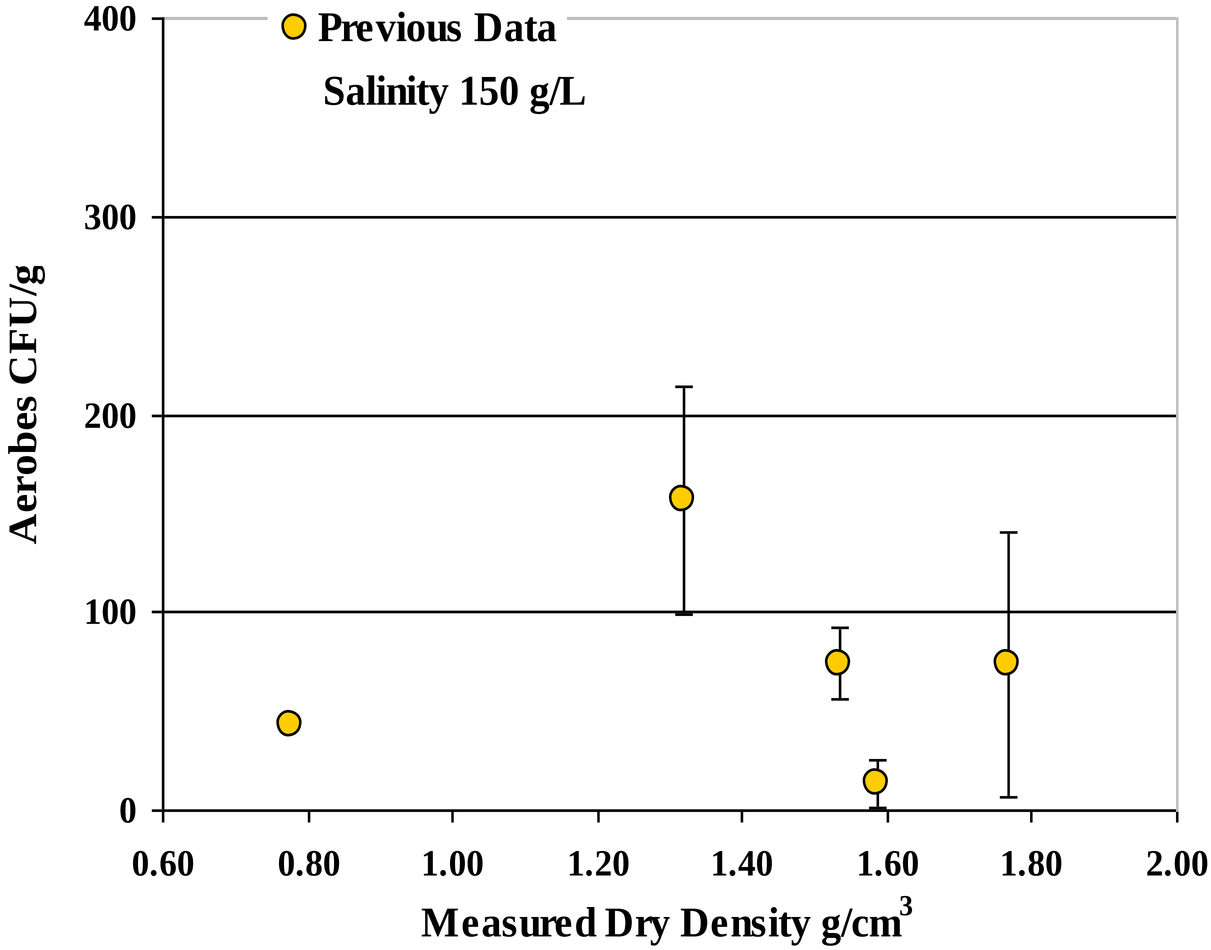

Figure 19: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 150 g NaCl/L (previous data)

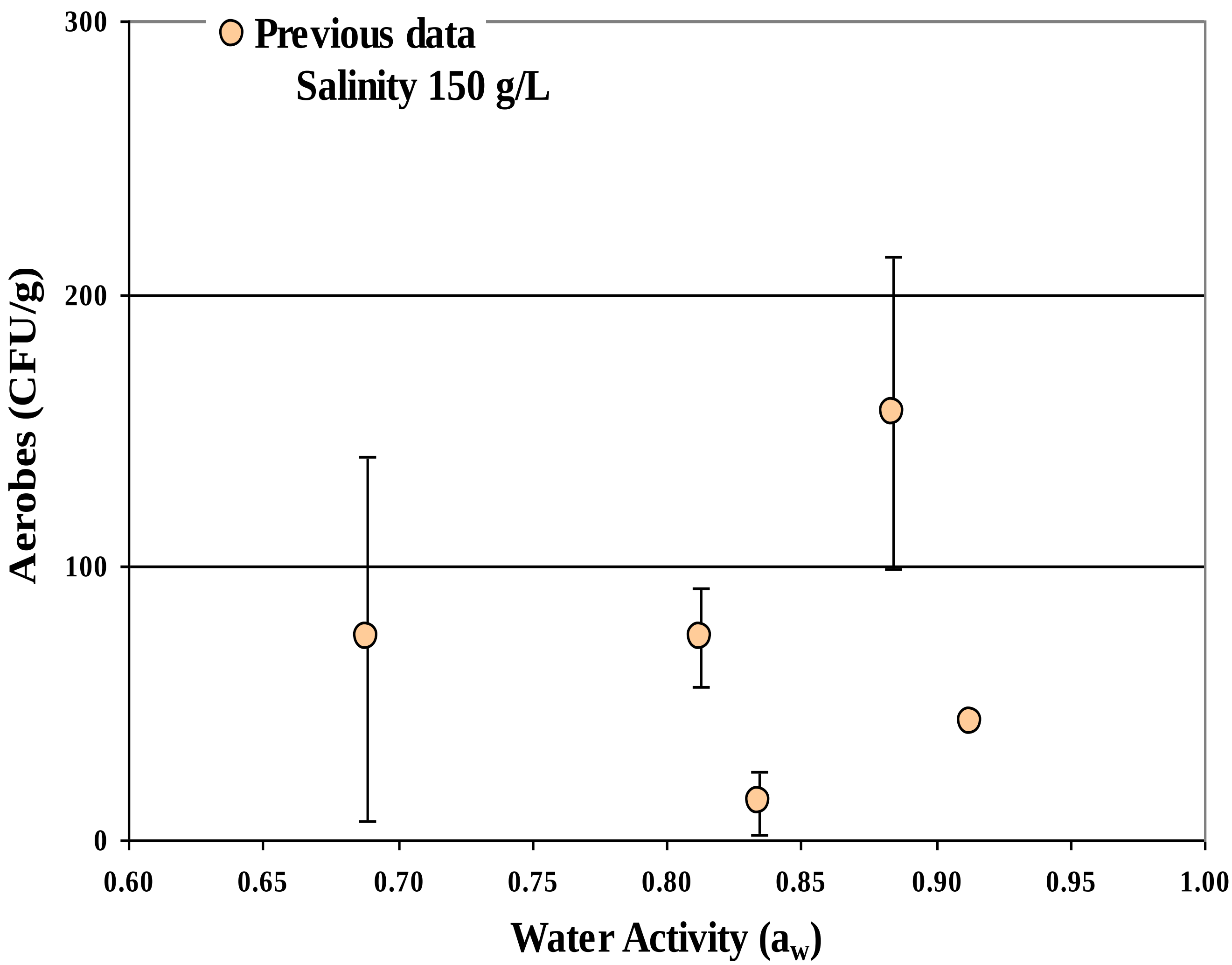

Figure 20: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 150 g NaCl/L (previous data)

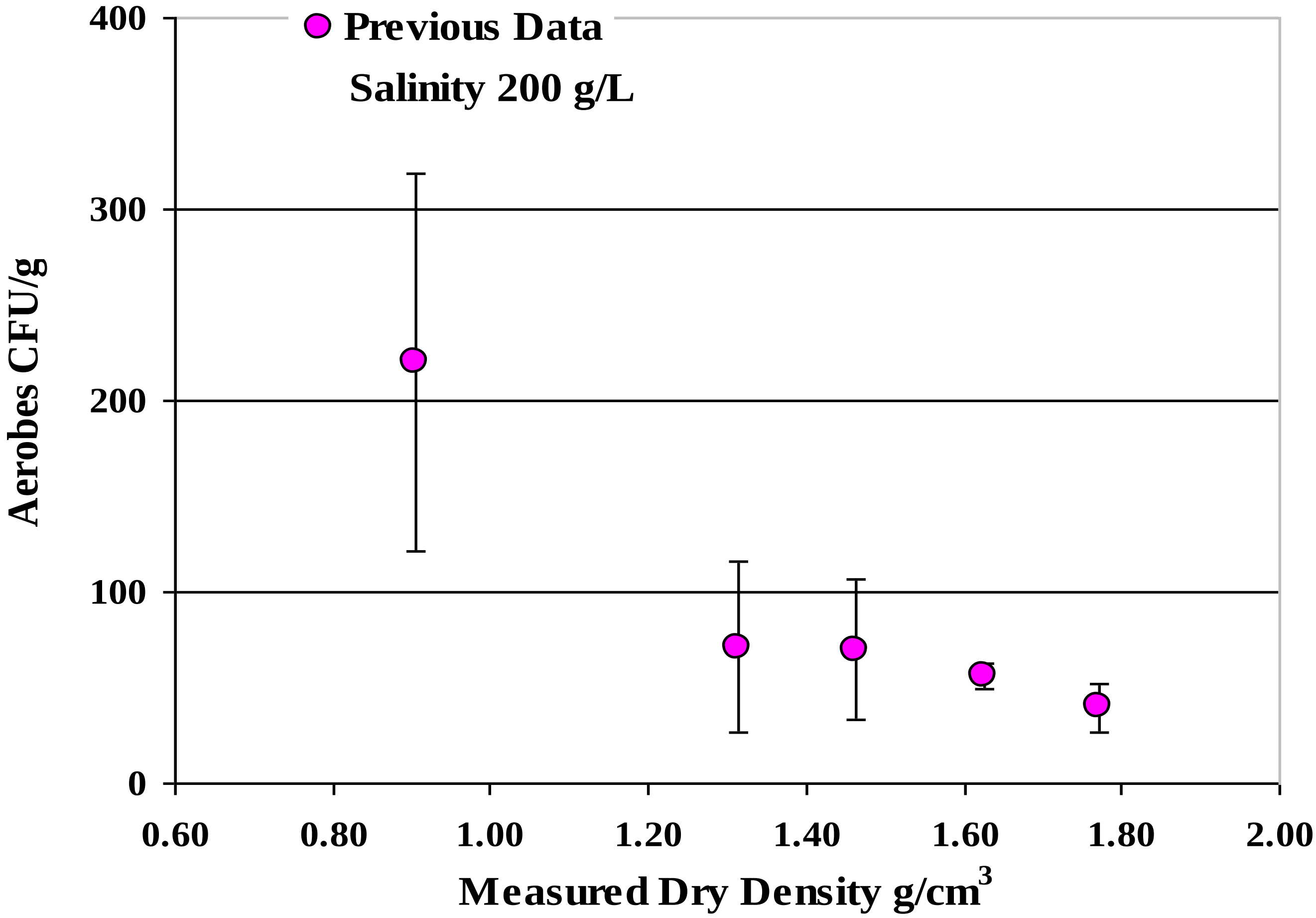

Figure 21: Aerobic culturability in compacted bentonite as a function of measured dry densities at an intermediate porewater salinity of 200 g NaCl/L (previous data)

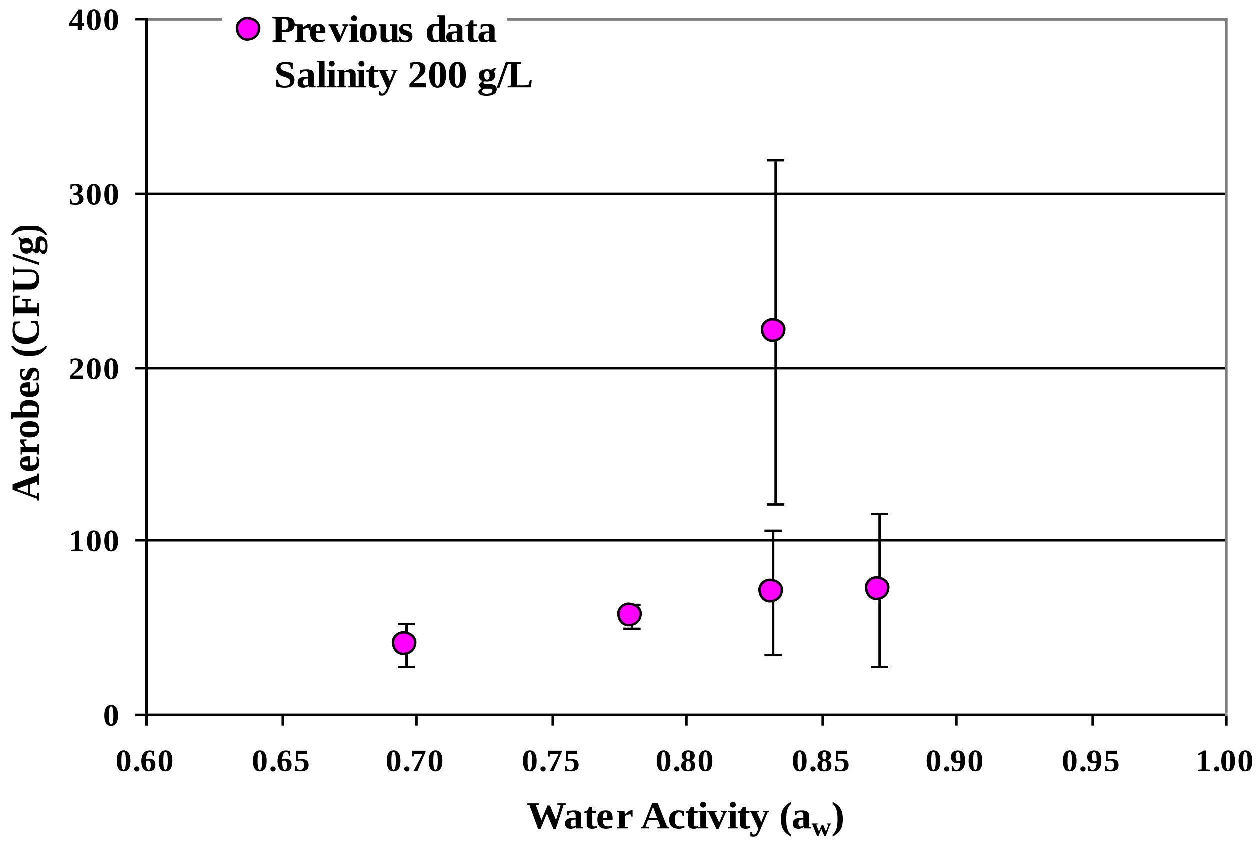

Figure 22: Aerobic culturability in compacted bentonite as a function of water activity at an intermediate porewater salinity of 200 g NaCl/L (previous data)

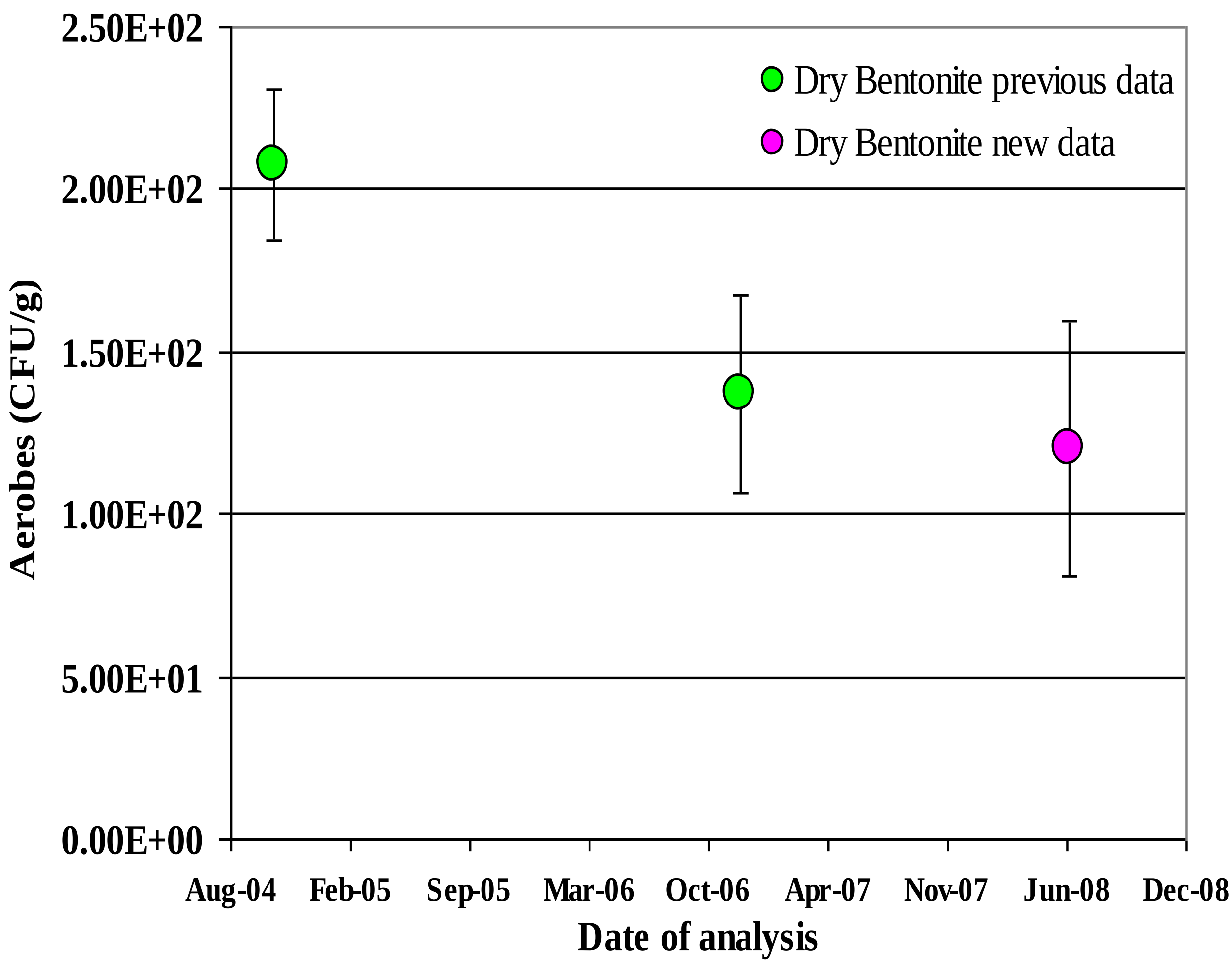

Figure 23: Aerobic culturability in dry, as bought Wyoming X80 bentonite as a function of time

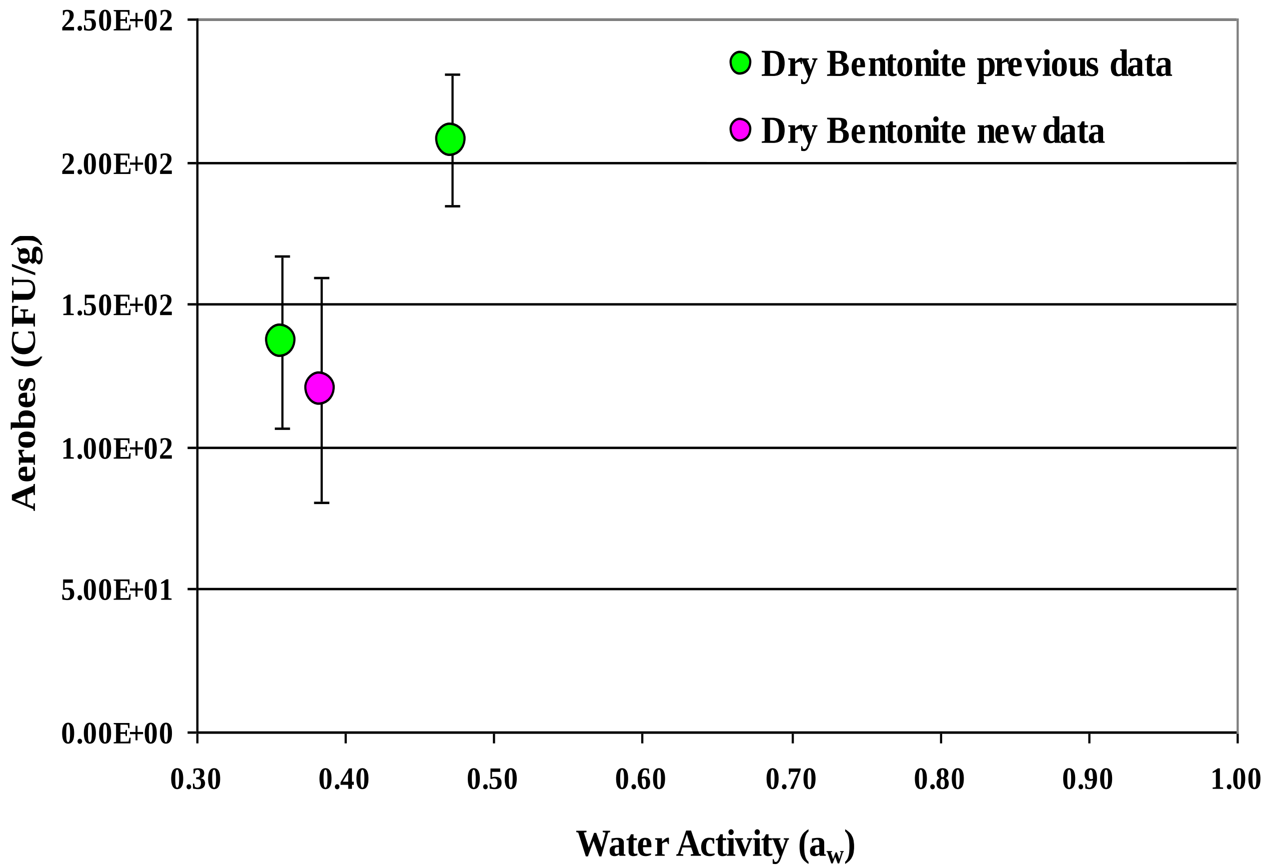

Figure 24: Aerobic culturability in dry, as bought Wyoming X80 bentonite as a function of water activity

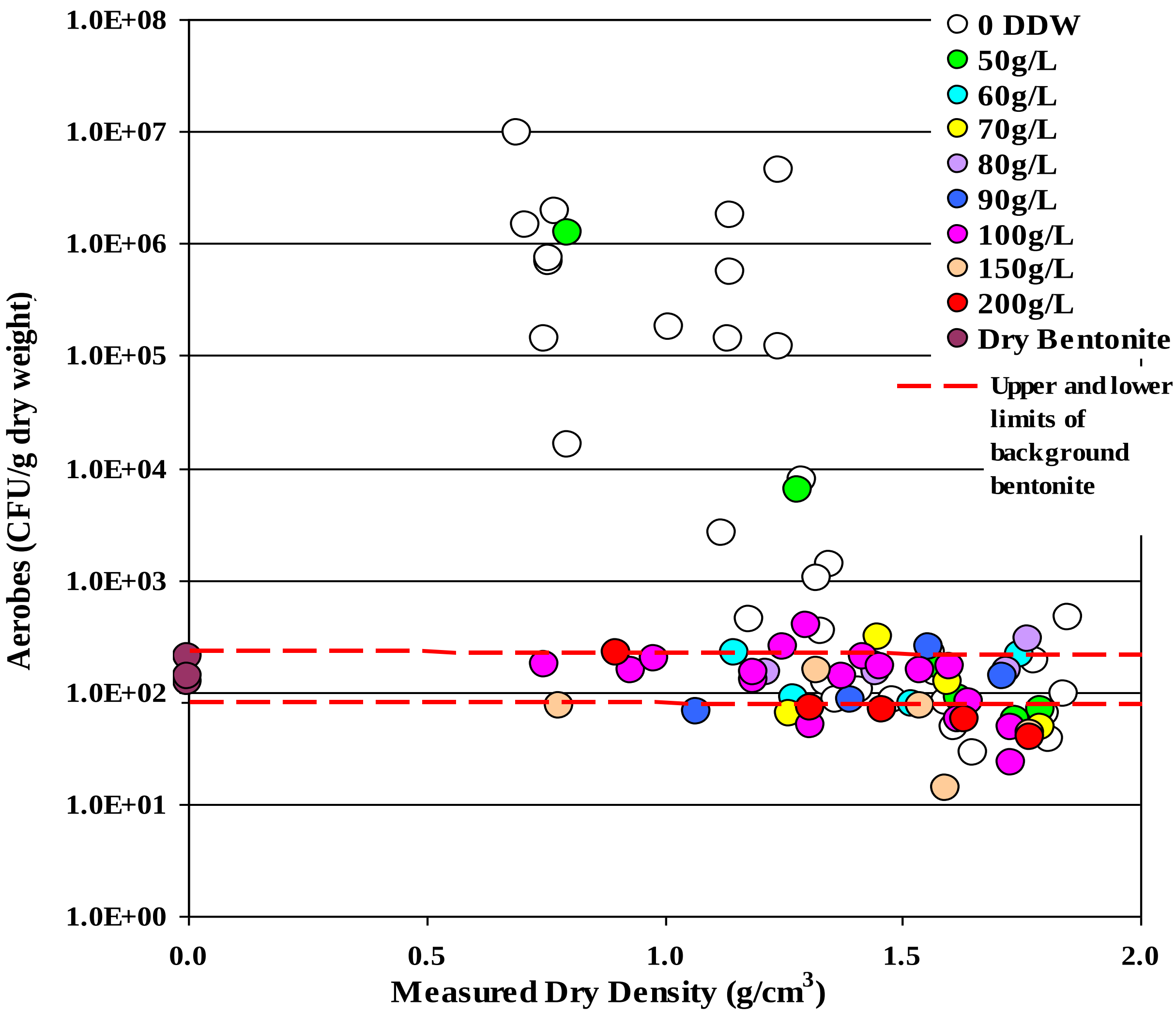

Figure 25: Aerobic culturability in compacted bentonite as a function of measured dry densities at porewater salinities ranging from 0-200 g NaCl/L; comparison of new and previous data

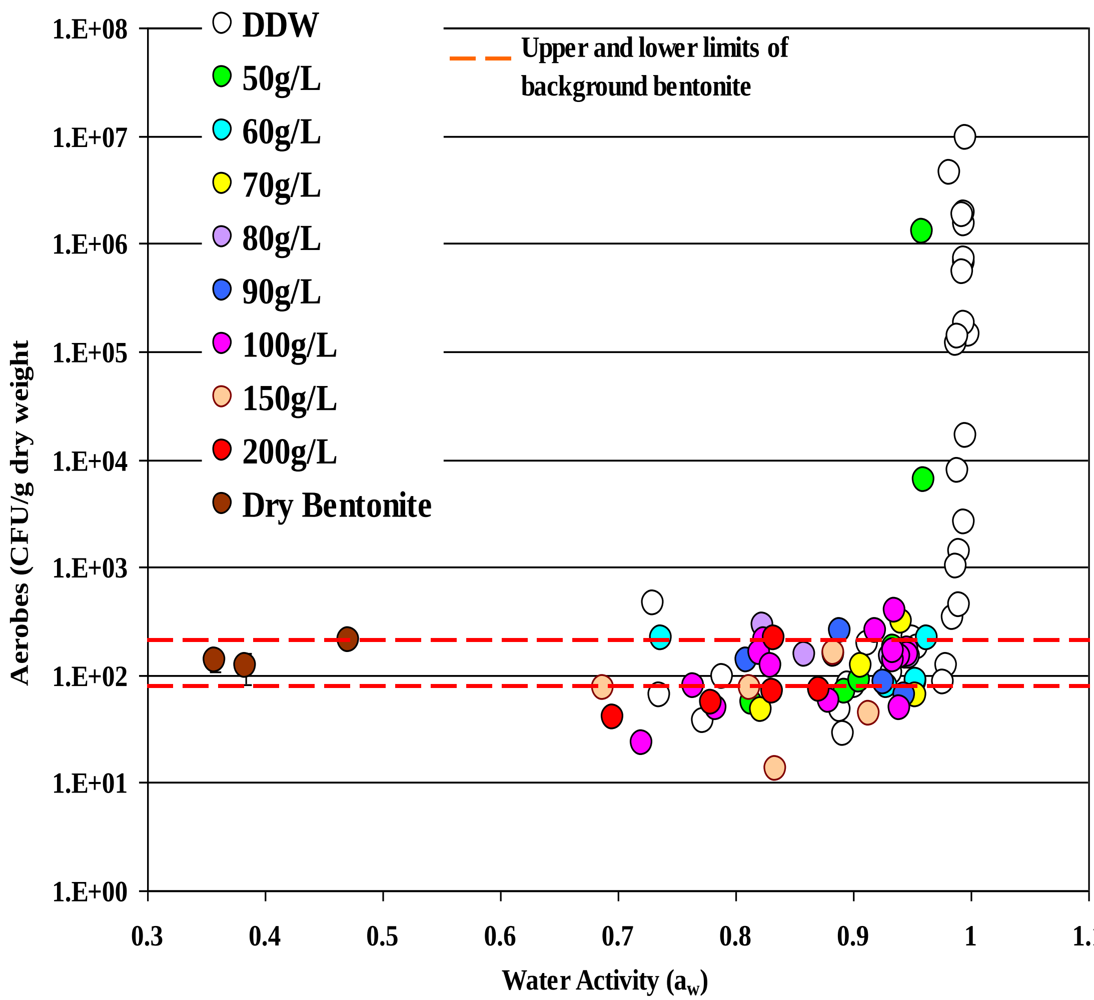

Figure 26: Aerobic culturability in compacted bentonite as a function of water activity at porewater salinities ranging from 0-200 g NaCl/L; comparison of new and previous data

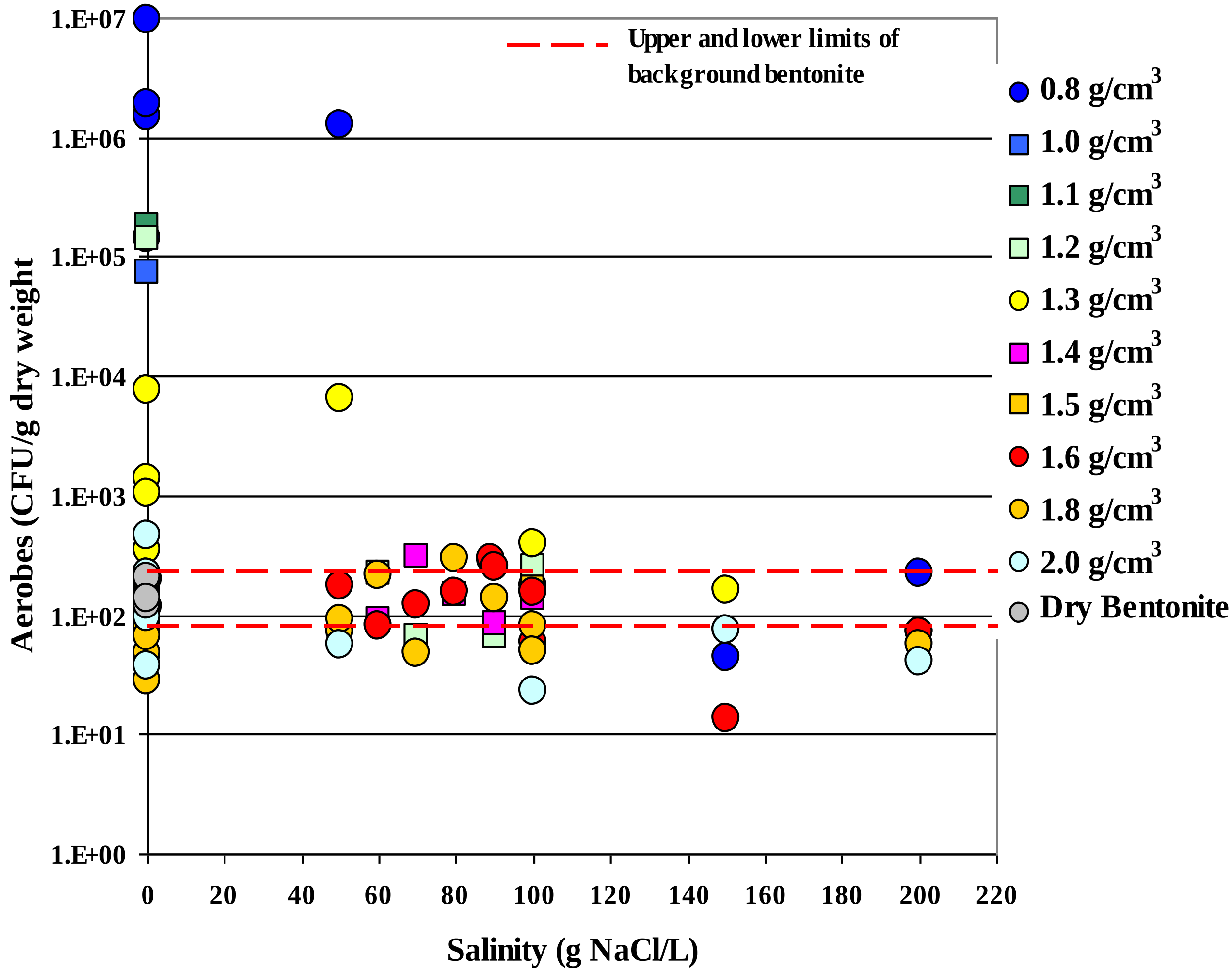

Figure 27: Aerobic culturability in compacted bentonite as a function of salinity at target dry densities ranging from 0.8 to 2.0 g/cm^3

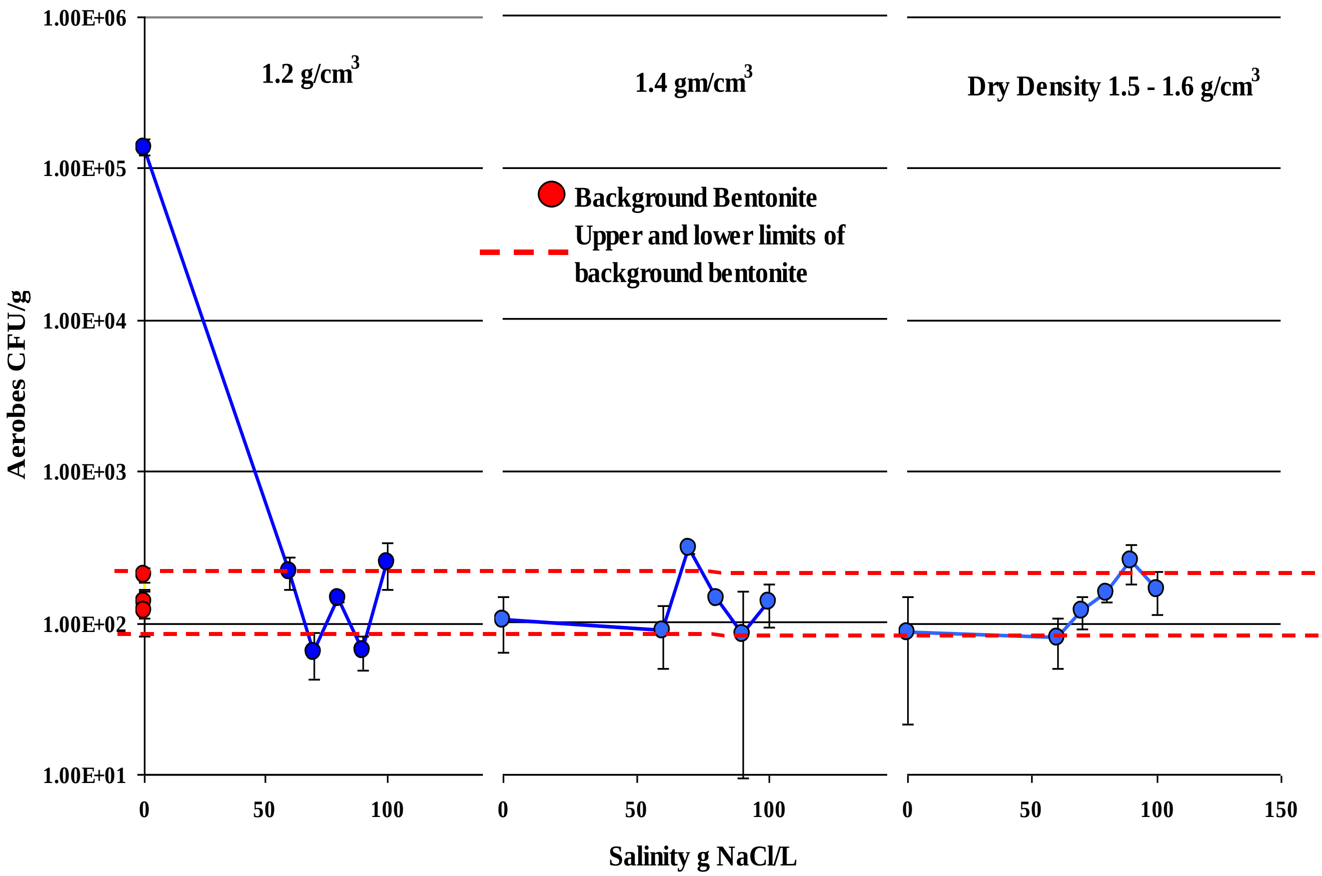

Figure 28: Aerobic culturability in compacted bentonite as a function of intermediate dry densities and intermediate salinities (comparison of new data)

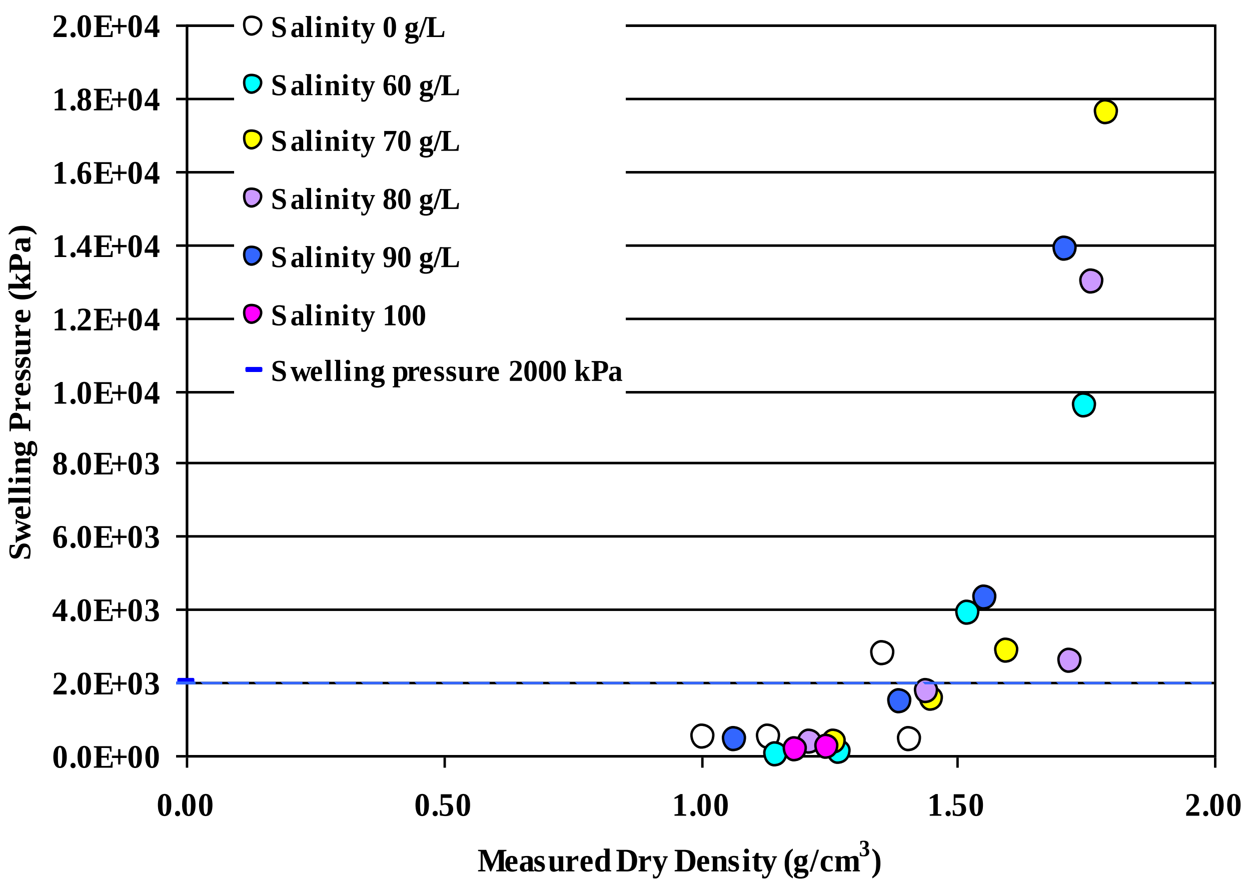

Figure 29: Swelling pressure in compacted bentonite as a function of measured dry density and salinity (new data).

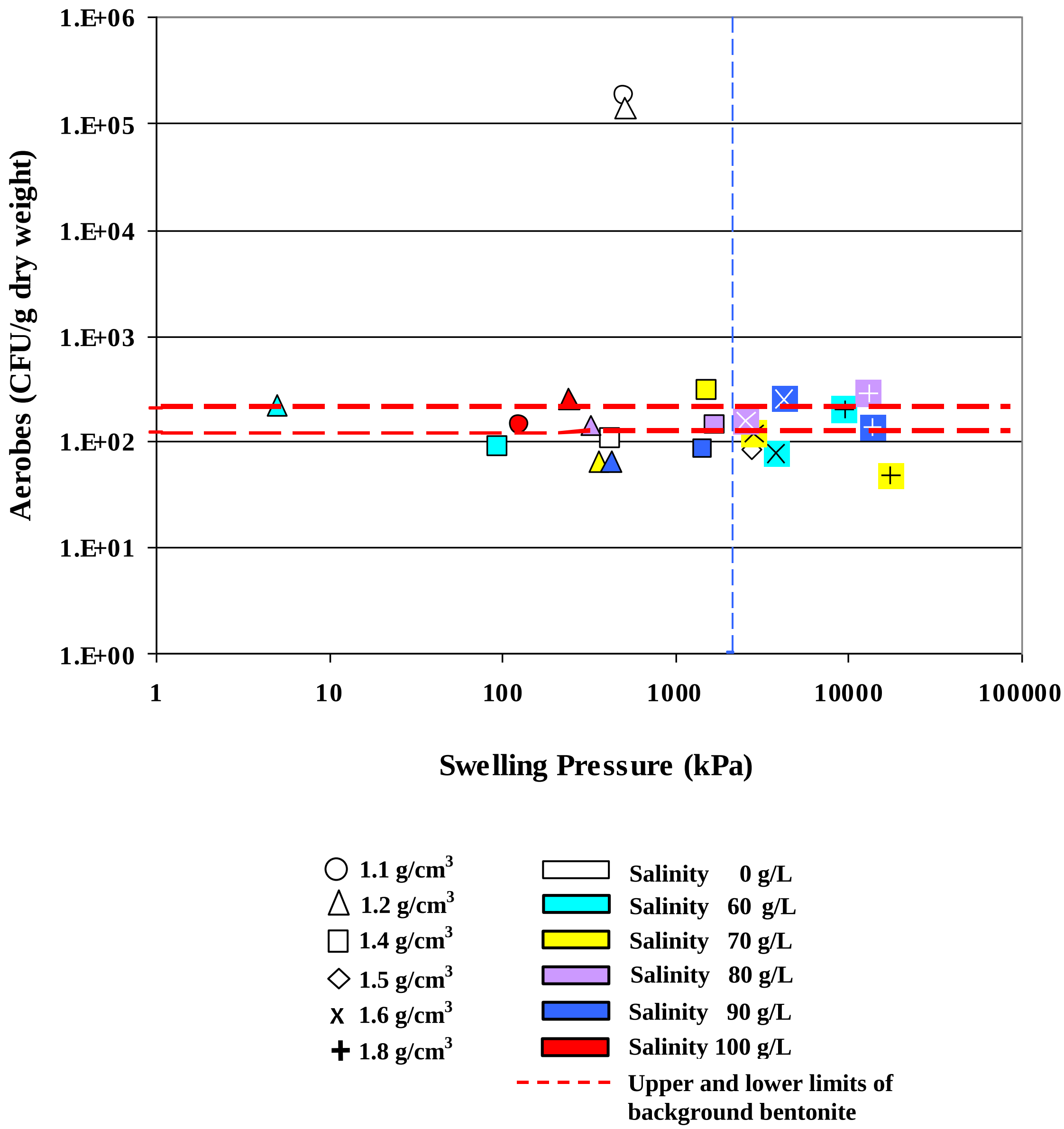

Figure 30: Aerobic culturability in compacted bentonite as a function of swelling pressure (new data).

APPENDIX A:

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						
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
1.6 – DDWL (1748T)	25.5	47.35	1.6	nm(1.18)*	1.18	1.41	0.98	341
1.6 – DDWL (1748M)	25.5	52.60	1.6	nm(1.12)*	1.12	1.41	0.93	341
1.6 – DDWL (1748B)	25.5	87.69	1.6	nm(0.80)*	0.80	1.41	0.65	341

continued . . . .

Table A.1 (cont'd): 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)
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
1.2 – DDWL (1781T)((Rehyd.)	46.3	40.88	1.2	1.24	1.28	1.01	1.09	860
1.2 – DDWL (1781B) (Rehyd.)	46.3	44.12	1.2	1.24	1.23	1.01	1.04	860
1.0 – DDWL (1750E)(60°C)	63.0	52.94	1.0	1.10	1.11	0.77	0.93	450
1.0– DDWL (1750T)(60°C)	63.0	57.54	1.0	1.10	1.06	0.77	0.85	450
1.0 – DDWL (1750B)(60°C)	63.0	61.43	1.0	1.10	1.02	0.77	0.77	450
1.0 – DDWL (1778T)(60°C →RT)	63.0	57.99	1.0	1.03	1.05	0.77	0.83	~ 400→350
1.0 – DDWL (1778M)(60°C→RT)	63.0	57.38	1.0	1.03	1.06	0.77	0.85	~ 400 →350
1.0 – DDWL (1778B)(60°C→RT)	63.0	56.05	1.0	1.03	1.07	0.77	0.87	~ 400→350
1.0 – DDWL (1780T) (Rehyd.)	63.0	58.92	1.0	1.14	1.04	0.77	0.82	260
1.0 – DDWL (1780B) (Rehyd.)	63.0	63.72	1.0	1.14	0.99	0.77	0.76	260
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°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°C)	88.0	88.64	0.8	0.77	0.80	0.65	0.62	335
0.8 – DDWL (1779T) (Rehyd.)	88.0	90.39	0.8	0.76	0.78	0.65	0.63	220
0.8 – DDWL (1779B) (Rehyd.)	88.0	95.07	0.8	0.76	0.76	0.65	0.60	220

continued . . . .

Table A.1 (cont'd): 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)
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
1.8 – 50S (1625)	18.5	18.51	1.8	1.8(t)	1.80	1.62	1.62 (t)	N/A
1.6 – 50S (1627)	25.5	25.54	1.6	1.61	1.60	1.41	1.42	N/A
1.3 – 50S (1628)	39.9	35.15	1.3	1.32	1.39	1.11	1.13	N/A
0.8 – 50S (1626)	88.0	86.89	0.8	0.8(t)	0.81	0.65	0.65 (t)	N/A
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

continued . . . .

Table A.1 Concluded: 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)
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
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 – 240StS (1646)	25.5	26.95	1.6	1.56	1.56	1.41	1.37	N/A
1.6 – 240NStS (1647)	25.5	24.73	1.6	1.60	1.62	1.41	1.41	N/A
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
1.6 – 420StS (1656)	25.5	26.91	1.6	1.62	1.56	1.41	1.43	N/A
1.6 – 420NStS (1657)	25.5	28.86	1.6	1.61	1.52	1.41	1.42	N/A

DDW = Distilled Deionized 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
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	$(2.07 \pm 0.23)10^{2}$	$(5.51 \pm 1.91)x10^{1}$	$1.03x10^{1}$	
Uncompacted Bentonite (1733) (PLFA)	-	-	8.93	0.358	$(1.37 \pm 0.30)x10^{2}$	$(5.56 \pm 2.94)x10^{1}$	4.1	$1.36x10^{6}$
2.0 – DDWL (1663)	54	DDW	19.38	0.789	$(9.60 \pm 4.33)x10^{1}$	$(1.67 \pm 0.72)x10^{1}$	4.32	
2.0 – DDWL (1714)C*	62	DDW	16.94	0.730	$(1.77 \pm 0.15)x10^{0}$	0	$< 3.3x10^{-3}$	
2.0 – DDWL (1714-AC)	62	DDW	16.94	0.730	$(2.17 \pm 0.34)x10^{2}$	$(6.00 \pm 2.60)x10^{1}$	10.3	
2.0 – DDWL (1714-FC)	62	DDW	16.94	0.730	$(4.56 \pm 0.34)x10^{2}$	$(3.84 \pm 2.52)x10^{1}$	6.8	
2.0 – DDWL (1728)	156	DDW	18.48	0.773	$(3.75 \pm 2.17)x10^{1}$	$(3.33 \pm 1.44)x10^{1}$	< 3.76	
2.0 – DDWL (1728-60^{o}C)	8	DDW	13.90	0.547	$(4.44 \pm 2.22)x10^{1}$	$(4.81 \pm 2.31)x10^{1}$	< 4.6	
2.0 – DDWL (1737)(60^{o}C)	52	DDW	19.40	0.776	$(5.70 \pm 2.74)x10^{1}$	$(4.17 \pm 2.22)x10^{0}$	< 3.75	
1.8 – DDWL (1644)	38	DDW	23.85	0.912	$(1.90 \pm 0.53)x10^{2}$	$(4.76 \pm 1.65)x10^{1}$	5.27	
1.8 – DDWL (1668)	53	DDW	23.44	0.901	$(7.81 \pm 1.42)x10^{1}$	$(3.33 \pm 0.72)x10^{1}$	9.13	
1.8 – DDWL (1712)C*	61	DDW	22.83	0.893	0	0	$< 3.3x10^{-3}$	
1.8 – DDWL (1712-AC)	61	DDW	22.83	0.893	$(1.43 \pm 0.77)x10^{2}$	$(3.58 \pm 1.77)x10^{1}$	$1.57x10^{1}$	
1.8 – DDWL (1712-FC)	61	DDW	22.83	0.893	$(2.82 \pm 0.98)x10^{1}$	$(1.69 \pm 0.85)x10^{1}$	3.4	
1.8 – DDWL (1727)	79	DDW	23.51	0.888	$(4.76 \pm 1.65)x10^{1}$	$(2.38 \pm 1.65)x10^{1}$	10	
1.8 – DDWL (1727-60^{o}C)	8	DDW	16.95	0.630	$(7.41 \pm 6.42)x10^{0}$	$(3.70 \pm 6.42)x10^{0}$	< 4.6	
1.8 – DDWL (1732) (PLFA)	44	DDW	17.65	0.736	$(6.48 \pm 2.64)x10^{1}$	$(2.59 \pm 1.70)x10^{1}$	< 3.4	$1.61x10^{6}$
1.8 – DDWL (1735) (60^{o}C)	51	DDW	21.95	0.851	$(5.90 \pm 1.47)x10^{1}$	$(5.90 \pm 2.55)x10^{1}$	< 4.29	

continued…

Table A.2 Continued: 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.
1.6 – DDWL (1645)	38	DDW	30.36	0.951	$(2.19\pm0.00)x10^{2}$	$(1.17\pm0.60)x10^{2}$	$1.50x10^{1}$	
1.6 – DDWL (1710-C)*	61	DDW	29.45	0.963	$(7.80\pm1.35)x10^{-1}$	0	$< 3.31x10^{-3}$	
1.6 – DDWL (1710-AC)	61	DDW	29.45	0.953	$(4.61\pm1.54)x10^{1}$	$(1.43\pm0.77)x10^{2}$	8.5	
1.6 – DDWL (1710-FC)	61	DDW	29.45	0.953	$(8.47\pm0.47)x10^{1}$	$(4.37\pm1.25)x10^{1}$	9.2	
1.6 – DDWL (1717)	45	DDW	30.08	0.955	$(1.75\pm0.46)x10^{2}$	$(5.84\pm2.68)x10^{1}$	< 5.2	
1.6 – DDWL (1726)	79	DDW	29.41	0.945	$(1.48\pm0.41)x10^{2}$	$(1.43\pm0)x10^{1}$	< 4.4	
1.6 – DDWL (1726-60°C)	8	DDW	20.66	0.770	$(2.96\pm2.31)x10^{1}$	$(1.85\pm1.28)x10^{1}$	4.5	
1.6 – DDWL (1731) (PLFA)	44	DDW	23.53	0.896	$(7.98\pm1.93)x10^{1}$	$(3.33\pm0.72)x10^{1}$	< 3.9	$1.48x10^{6}$
1.6 – DDWL (1738) (60°C)	52	DDW	32.70	0.965	$(1.16\pm0.50)x10^{1}$	$(3.03\pm5.25)x10^{0}$	2.73	
1.6 – DDWL (1748T)	37	DDW	47.35	0.991	$(4.43\pm0.80)x10^{2}$	$(6.25\pm4.64)x10^{1}$	$1.34x10^{1}$	
1.6 – DDWL (1748M)	37	DDW	52.60	0.995	$(2.56\pm0.32)x10^{3}$	$(6.69\pm1.05)x10^{1}$	< 3.90	
1.6 – DDWL (1748B)	37	DDW	87.69	0.996	$(1.61\pm0.17)x10^{4}$	$(5.85\pm1.53)x10^{2}$	6.87	
1.3 – DDWL (1643)	37	DDW	43.39	0.991	$(1.36\pm0.14)x10^{3}$	$(9.09\pm0.91)x10^{1}$	< 2.78	
1.3 – DDWL (1708-C)*	57	DDW	45.64	0.988	$(7.96\pm0.80)x10^{-2}$	$(5.31\pm0.46)x10^{-2}$	$< 3.3x10^{-3}$	
1.3 – DDWL (1708-AC)	57	DDW	45.64	0.988	$(9.52\pm8.25)x10^{0}$	$(2.86\pm1.43)x10^{1}$	9	
1.3 – DDWL (1708-FC)	57	DDW	45.64	0.988	$(1.02\pm0.39)x10^{3}$	$(1.16\pm1.16)x10^{1}$	< 4.6	
1.3 – DDWL (1716)	45	DDW	42.40	0.984	$(3.40\pm0.71)x10^{2}$	$(2.96\pm1.28)x10^{1}$	< 6.2	
1.3 – DDWL (1719)	77	DDW	43.99	0.989	$(7.67\pm0.54)x10^{3}$	$(2.38\pm2.97)x10^{1}$	< 4.7	
1.3 – DDWL (1719-60°C)	8	DDW	40.25	0.981	$(5.62\pm1.03)x10^{3}$	$(6.19\pm4.36)x10^{1}$	< 4.6	
1.3 – DDWL (1730) (PLFA)	44	DDW	37.98	0.980	$(1.21\pm0.47)x10^{2}$	$(1.94\pm0.84)x10^{1}$	< 4.4	$1.52x10^{6}$
1.3 – DDWL (1734) (60°C)	51	DDW	45.86	0.988	$(3.60\pm6.23)x10^{0}$	$(1.08\pm1.08)x10^{1}$	< 3.4	

continued…

Table A.2 Continued: 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.
1.2 -DDWL (1781T) (Rehyd)	40	DDW	40.88	0.982	$(4.41\pm0.98)x10^{6}$	0	< 4.8	
1.2 -DDWL (1781B) (Rehyd)	40	DDW	44.12	0.987	$(1.18\pm0.16)x10^{5}$	$(7.76\pm6.72)x10^{0}$	< 4.7	
1.0 – DDWL (1750E)(60°C)	40	DDW	52.94	0.989	$(9.53\pm8.25)x10^{0}$	$(9.53\pm16.5)x10^{0}$	< 4.4	
1.0 – DDWL (1750T)(60°C)	40	DDW	57.54	0.992	$(1.14\pm1.31)x10^{1}$	$(8.58\pm14.9)x10^{0}$	< 3.6	
1.0 – DDWL (1750B)(60°C)	40	DDW	61.43	0.993	$(1.15\pm1.15)x10^{1}$	$(7.69\pm13.3)x10^{0}$	< 2.4	
1.0 – DDWL (1778T) (60°C→RT)	(40+76)	DDW	57.99	0.990	$(4.36\pm2.55)x10^{2}$	$(5.29\pm0.51)x10^{2}$	< 4.4	
1.0 – DDWL (1778M) (60°C→RT)	(40+76)	DDW	57.38	0.990	$(6.55\pm0.92)x10^{2}$	$(4.08\pm1.00)x10^{2}$	< 4.1	
1.0 – DDWL (1778B) (60°C→RT)	(40+76)	DDW	56.05	0.989	$(1.98\pm0.17)x10^{5}$	$(3.62\pm0.30)x10^{2}$	< 4.6	
1.0 –DDWL (1780T)(Rehyd)	40	DDW	58.92	0.991	$(1.80\pm0.24)x10^{6}$	$(1.18\pm2.05)x10^{1}$	< 5.6	
1.0 –DDWL (1780B)(Rehyd)	40	DDW	63.72	0.993	$(5.41\pm0.61)x10^{5}$	$(1.97\pm2.25)x10^{1}$	< 4.6	
0.8 – DDWL (1638)	35	DDW	104.21	0.995	$(1.48\pm0.37)x10^{6}$	$(4.25\pm0.16)x10^{3}$	6.92	
0.8 – DDWL (1707)	41	DDW	79.67	0.995	$(1.89\pm0.05)x10^{6}$	$(1.00\pm0.35)x10^{2}$	< 5.7	
0.8 – DDWL (1718)	77	DDW	102.30	0.996	$(9.50\pm0.50)x10^{6}$	$(3.42\pm3.99)x10^{2}$	< 7.9	
0.8 – DDWL (1718-60°C)	8	DDW	93.52	0.995	$(5.53\pm4.62)x10^{1}$	$(4.00\pm2.00)x10^{1}$	< 4.6	
0.8 – DDWL (1729) (PLFA)	44	DDW	113.85	0.998	$(1.41\pm0.05)x10^{5}$	$(1.82\pm0.7)x10^{2}$	<6.6	$2.29x10^{6}$
0.8 – DDWL (1736) (60°C)	51	DDW	88.64	0.995	$(6.68\pm1.67)x10^{1}$	$(5.00\pm1.67)x10^{1}$	< 0.5	
0.8 –DDWL (1779T)(Rehyd)	40	DDW	90.39	0.995	$(6.63\pm0.28)x10^{5}$	$(1.87\pm3.24)x10^{1}$	< 8.4	
0.8 –DDWL (1779B)(Rehyd)	40	DDW	95.07	0.994	$(7.18\pm0.29)x10^{5}$	$(9.97\pm9.14)x10^{1}$	10.8	

continued…

Table A.2 Continued: 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.
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	
1.8 – 50S (1625)	1	50g NaCl/L	18.51	0.723	$(1.13\pm0.33)\times10^{2}$	$(1.67\pm0.72)\times10^{1}$	4.5	
1.6 – 50S (1627)	1	50g NaCl/L	25.54	0.868	$(1.56\pm0.09)\times10^{2}$	$(1.67\pm0.19)\times10^{2}$	4.50	
1.3 – 50S (1628)	1	50g NaCl/L	35.15	0.921	$(7.08\pm2.60)\times10^{1}$	$(1.25\pm1.25)\times10^{2}$	3.88	
0.8 – 50S (1626)	1	50g NaCl/L	86.89	0.966	$(2.17\pm0.58)\times10^{2}$	$(7.78\pm0.96)\times10^{1}$	6.00	
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	

continued…

Table A.2 Concluded: 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.
2.0 – 200L (1698)	90	200g NaCl/L	18.37	0.696	$(3.95 \pm 1.24) \times 10^{1}$	$(4.67 \pm 0.62) \times 10^{1}$	3.88	
1.8 – 200L (1699)	90	200g NaCl/L	24.51	0.780	$(5.58 \pm 0.69) \times 10^{1}$	$(2.50 \pm 1.25) \times 10^{1}$	< 3.71	
1.6 – 200L (1700)	90	200g NaCl/L	30.73	0.832	$(7.00 \pm 3.61) \times 10^{1}$	$(2.00 \pm 1.00) \times 10^{1}$	< 3.04	
1.3 – 200L (1701)	91	200g NaCl/L	40.44	0.872	$(7.11 \pm 4.43) \times 10^{1}$	$(1.13 \pm 0.29) \times 10^{2}$	< 3.15	
0.8 – 200L (1702)	91	200g NaCl/L	61.37	0.833	$(2.20 \pm 0.99) \times 10^{2}$	$(1.19 \pm 0.08) \times 10^{2}$	1.43×10^{1}	
1.6 – 240StL (1652)	55	TDS 0.72 g/L	27.70	0.941	$(1.93 \pm 0.38) \times 10^{2}$	$(3.94 \pm 3.48) \times 10^{1}$	< 3.94	
1.6 – 240NStL (1671)	55	TDS 0.72 g/L	29.23	0.950	$(1.17 \pm 0.19) \times 10^{2}$	$(3.33 \pm 1.91) \times 10^{1}$	1.15×10^{1}	
1.6 – 240StS (1646)	2	TDS 0.72 g/L	26.95	0.940	$(1.33 \pm 0.32) \times 10^{2}$	$(2.00 \pm 2.00) \times 10^{2}$	< 3.05	
1.6 – 240NStS (1647)	2	TDS 0.72 g/L	24.73	0.921	$(1.92 \pm 0.44) \times 10^{2}$	$(2.00 \pm 0.15) \times 10^{3}$	4.42	
1.6 – 420StL (1658)	56	TDS 89.2 g/L	48.36	0.954	$(2.88 \pm 1.28) \times 10^{2}$	$(6.00 \pm 2.00) \times 10^{1}$	< 5.63	
1.6 – 420NStL (1659)	56	TDS 89.2 g/L	33.02	0.934	$(2.97 \pm 1.12) \times 10^{2}$	$(6.14 \pm 2.16) \times 10^{1}$	5.10	
1.6 – 420StS (1656)	1	TDS 89.2 g/L	26.91	0.885	$(2.25 \pm 0.50) \times 10^{2}$	$(1.25 \pm 0) \times 10^{1}$	4.49	
1.6 – 420NStS (1657)	1	TDS 89.2 g/L	28.86	0.896	$(2.83 \pm 0.52) \times 10^{2}$	$(3.78 \pm 0.65) \times 10^{1}$	< 3.40	

DDW = Distilled Deionized 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