Reducing Soil Permeability Using In-Situ Biofilm-Forming Bacteria: Overcoming Testing Apparatus Challenges

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ABSTRACT

Growing biofilm in saturated sand has been shown to reduce the permeability of soil by one or more orders of magnitude and may be a viable approach to reducing seepage in the field; however, obtaining laboratory permeability results in soil samples where biofilm is being developed is difficult. Adding nutrients results in the formation of biofilm in the soil but also the formation of biofilm in the piping and other areas of the permeability testing apparatus. In addition, some bacteria produce gas as a product of metabolism and this gas can collect in the apparatus and interfere with fluid flow. This paper presents an approach to permeability testing that effectively minimized the growth of biofilm and the collection of gas in the testing apparatus for multiple sand samples treated with a nutrient solution over a period of more than 60 days.

INTRODUCTION

Groundwater seepage is associated with failures of contaminant containment systems, dams, and levees. Traditional methods (e.g., grouting) to reduce seepage in the field can be expensive and may create significant environmental concerns (e.g., increase in greenhouse gasses associated with manufacturing cement). The use of biological processes to modify the properties of in-situ soils has shown significant potential; however, much of that work has focused on the improvement of strength and stiffness properties using microbially-induced calcite precipitation. Research on the use of biofilms to reduce soil permeability began in the late 1940's (Allison 1947). Many microbes form biofilms – a mixture of bacterial cells embedded in an extracellular matrix secreted by the microbe. If biofilm is present in sufficient amounts within a soil matrix, its presence occupies portions of the void spaces in the soil and reduces the ability of water to flow through the soil.

Biofilms have been an area of significant research in the past not because of the potential benefits of reducing permeability but because of complications caused by biofilm growth in equipment used for medical purposes, food processing, and water or sewage treatment among other fields. Biofilms stick to equipment surfaces and as a result may cause significant clogging of the equipment. In research to evaluate the potential benefits of the use of biofilms to reduce permeability of soils, biofilm growth in the apparatus can significantly impact the permeability results thereby making it difficult to separate the permeability reductions occurring in the soil from those occurring in the apparatus.

In our work to study the use of biofilms as a controlled means to reduce soil permeability, we have developed modifications to the apparatus that significantly reduce the negative impacts of biofilm growth in the apparatus on the permeability results. This paper presents an approach to permeability testing with a modified apparatus that significantly minimizes biofilm clogging at the interface where nutrients enter the soil sample. The modifications also reduce the buildup of gases within the apparatus and permit measurement of permeability along the length of the sample so that the distribution of biofilm formation and the resulting permeability changes can be evaluated.

BACKGROUND

Permeability reductions resulting from microbial activities of one or more orders of magnitude have been reported by a number of researchers (e.g., Cunningham et al. 1991, Vandevivere and Baveye 1992, Seki et al. 1996, Proto et al. 2016). There are two general approaches used to create the conditions that support the development biofilm in soils: adding known biofilm-forming bacteria to a soil and providing nutrients to support bacterial activity (a process known as augmentation) or simply providing nutrients to an untreated soil to support the activity of bacteria already present in the soil (a process known as stimulation). In the laboratory, and for either the augmentation or stimulation processes, the soil is confined in an apparatus that permits the inflow and outflow of fluids. To measure permeability, the apparatus requires not only a location where the soil is contained but also piping or other mechanisms that permit the measurement of pressure differences across the soil sample and of the volume of fluid passing through the sample.

A variety of nutrients have been used to support bacterial activity in soil samples and generally contain sugars, proteins, and other ingredients. These nutrients are combined with water and introduced to the soil sample as a fluid. While the addition of nutrients is required to support bacterial activities in the soil sample, the presence of nutrients in the testing apparatus supports bacterial activities in the apparatus itself, i.e., in the tubing, filters, and other parts of the apparatus that are in contact with the fluid. The growth of biofilm in the apparatus can result in clogging of the tubing and filters that may be significant enough to impact the ability of the apparatus to accurately measure the permeability of the soil. Problems associated with this type of apparatus clogging have not been reported by researchers although multiple researchers have noted that the biofilm growth is generally concentrated within a few centimeters of the location where nutrients are introduced to the soil sample (Gupta and Swartzendruber 1962, Vandevieve and Baveye 1992, Seki et al. 1996, Proto et al. 2016). Vandevieve and Baveye (1992) also noted that the significant reduction in permeability they reported was associated with the formation of a thick, bacterial mat at the point where nutrients entered a sand sample. A further challenge associated with the bacterial activities in the apparatus is the formation of gasses. Gas occlusion in the pore spaces of soil samples was noted by Seki et al. 1996, Seki et al 1998, and Ivanov and Chu 2008.

While previous researchers have not noted issues associated with clogging or gas accumulation in the testing apparatus, the areas in the apparatus where this may occur are generally not visible. The work presented here provides evidence that the impact of bacterial activities in the apparatus on the permeabilities measured can be significant and that modifications to the equipment can reduce this impact.

METHODOLOGY

The research described here is part of a larger project that has a goal of establishing uniform biofilm formation in a one-meter column of soil. Initial tests for this project used an equipment setup similar to that used by Proto et al (2016), Figure 1. The soil samples tested were Ottawa 50-70 sand and were approximately 7.5 cm diameter by 15 cm tall. The nutrient solution used was a combination of glucose, casein peptone, yeast extract, and de-ionized water. Permeability measurements obtained with this equipment were made using the falling head method (Bowles 1992). For each treatment of the samples, the volume of nutrients used was approximately 1.5 times the pore volume of the sample.

Results from preliminary tests showed decreases in permeability in the samples receiving nutrients; however, within the first week of testing bacterial activity in the transparent tubing created a visible buildup of material. When the tubing was replaced with clean tubing, the observed decreases in permeability were significantly reduced. Modifications to the apparatus were made to reduce the amount of tubing and to permit observation of areas of the apparatus directly above or below the soil sample in order to observe any clogging that might occur in these areas, Figure 2. Initial observations and the permeability results of samples tested using this modified apparatus indicated that clogging in the remaining tubing (the tubing connecting the reservoir below the sample to the outlet point) was minimal and no longer impacted the permeability results. Some biofilm materials did build up in the area above the sample but that buildup was easily addressed by removing, cleaning, and replacing the screen that sat on the top of the sample. However, with this modified test set up we observed a buildup of gasses in the area directly below the sample. These gasses were created by bacterial activity occurring in the fluid after it passed through the sample. By drilling a small diameter hole into the apparatus and using a needle and syringe, we were able to remove the majority of gasses from this area for short periods of time and determined that the presence of these gasses below the sample were significantly impacting the permeability results.

Further modifications to the apparatus were then made to permit the continuous release of gasses from the area directly below the sample by positioning a piezometer at the base of the sample, Figure 3. Piezometers were also added at two points along the length of the sample so that fluid pressure measurements could be obtained at these locations and the apparatus was further modified so that permeability tests using the constant head method (Bowles 1992) could be conducted, Figure 4. While constant head tests were conducted infrequently, the results from these tests when combined with readings from the installed piezometers along the length of the sample permitted the calculation of the permeabilities of the top, middle, and bottom layers of the soil sample (Budhu 2000).

Six apparatus were constructed and used to measure permeabilities in six soil samples (Figure 5). Two of the samples (samples #1 and #2) were controls that received only de-ionized water throughout the testing period and four of the samples (samples #3, #4, #5, and #6) were treated with nutrient solution during the testing period. Dye was also added to the nutrient solution for two of the samples (#5 and #6) approximately weekly during the testing period so that the patterns of fluid flow in the samples could be observed.

Shortly after testing began, significant clogging caused by the buildup of biofilm above the soil samples developed in all the apparatus in which nutrients were being added. The impact of this clogging on the permeability results can be seen in Figure 6. The screens above each sample were removed, cleaned, and replaced and the fluid used in the daily permeability testing was then alternated between nutrients and de-ionized water to reduce the buildup of biofilm that occurred in the volume of fluid that remained above the samples between permeability tests.

RESULTS

The results of 60 days of testing are shown in Figure 6. After the clogging was addressed at the end of the first week, sustained permeability reductions from approximately 0.03 cm/s to 0.01 cm/s were observed in samples #3 and #4. The results for samples that were dye tested (# 5 and #6) exhibited greater decreases in permeability from approximately 0.03 cm/s to 0.033 cm/s. This result is not unexpected since in a biostimulated situation, the bacteria that grow in the sample are a function of the nutrients applied. Samples receiving the dye treatment received a slightly different nutrient mixture (the dye contained citrate and benzoate) and the bacterial activity occurring in the sample and the impact of that activity on permeability most likely reflect this modification to the nutrients used.

Constant head permeability tests were conducted on one of the control samples (#2) and on three samples treated with the nutrients (#4, #5, and #6). The results of these tests are shown in Figure 7. These results provide evidence that the permeability varies slightly over the length of each of the samples but the results do not support the hypothesis that the permeability reduction is greatest where the nutrients enter the soil (the top layer of the samples). In particular, in sample #6 the permeability of the top layer was notably higher than the permeability of the middle and lower layers. Formation of gas was observed within the soil samples and it is hypothesized that the formation and release of gasses resulting from bacterial activity in this sample may have caused an increase in permeability of this top layer but further testing is needed to study this possibility.

CONCLUSIONS

A new approach to permeability testing that addresses the issue of clogging and gas buildup in the testing apparatus has been used to evaluate the permeability of multiple sand samples treated with a nutrient solution over a period of 60 days. Results from these tests indicate sustainable permeability reductions of up to one order of magnitude. While the observed permeability reductions are less than reductions found by some earlier researchers, reductions of permeability caused by biofilm buildup in the apparatus have been essentially eliminated in the results presented here through the use of modified permeability testing equipment. Additionally, measured hydraulic gradients along the samples indicate that the reductions observed in these tests are relatively uniform along the length of the samples. This uniformity of permeability reduction may indicate that the larger permeability reductions seen by previous researchers—who also noted that the permeability reductions occurred primarily at or near the location where nutrients entered the soil—may have been significantly impacted by clogging of the apparatus at the point where nutrients enter the sample. Additionally, the work presented here provides evidence that gasses may also build up in areas of the apparatus that are not visible and the presence of these gasses may also impact the permeability measurements but the presence of these gasses in the apparatus can be addressed by simple modifications.

If permeability reductions caused by biofilm formation in soils can be reliably studied in the laboratory using equipment modified to reduce clogging and the buildup of gasses and if issues associated with potential degradation of the biofilm over time can be addressed, future research may provide convincing evidence that biofilm growth in soils can be a low-cost alternative to traditional field methods of permeability reduction.

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REFERENCES

Allison, L.E. (1947). "Effect of microorganisms on permeability of soil under prolonged submergence." *Soil Science*, 63(6), 439-450.

Bowles, J.E. (1992). *Engineering Properties of Soils and Their Measurement*, 4 ed. McGraw-Hill, New York, 241 p.

Budhu, M. (2000). *Soil Mechanics and Foundations*. John Wiley & Sons, New York, 586 p.

Cunningham, A.B., Characklis, W.G., Abedeen, F., and Crawford, D. (1991). "Influence of biofilm accumulation on porous media hydrodynamics." *Environmental Science & Technology*, 25(7).

Gupta, R.P., and Swartzendruber, D. (1962). "Flow-associated reduction in the hydraulic conductivity of quartz sand." *Soil Science Society of America Journal*, 26(1).

Ivanov, V., and Chu, J. (2008). Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ." *Reviews in Environmental Science and bio/Technology*, 7(2), 139-153.

Proto, C.J., Dejong, J.T., and Nelson, D.C. (2016). "Biomediated permeability reduction of saturated sands." *Journal of Geotechnical and Geoenvironmental Engineering*, 142(12).

Seki, K., Miyazaki, T., and Nakano, M. (1996). "Reduction of hydraulic conductivity due to microbial effects." *Japanese Society of Irrigation Drainage and Reclamation Engineering*, 181, 137-144.

Seki, K., Miyazaki, T., and Nakano, M. (1998). "Effects of microorganisms on hydraulic conductivity decrease in infiltration." *European Journal of Soil Science*, 49(2)

Vandevivere, P., and Baveye, P. (1992). "Saturated hydraulic conductivity reduction caused by aerobic bacteria in sand columns." *Soil Science Society of America Journal*, 56(1).

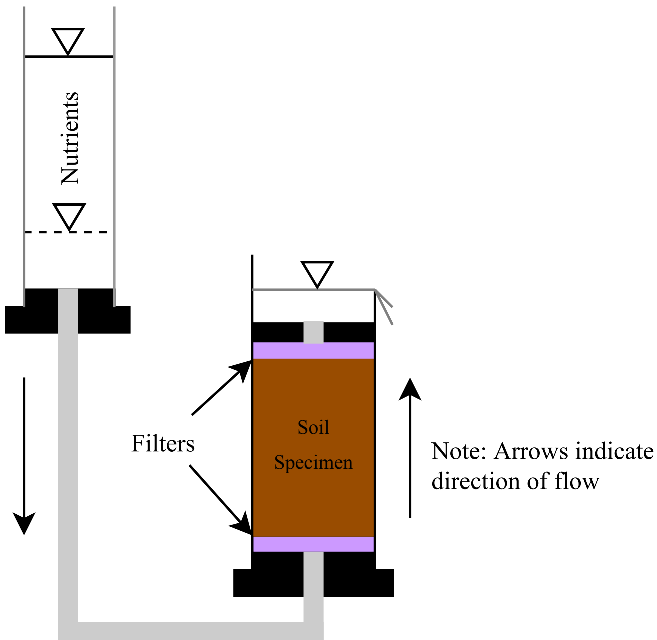

Figure 1. Initial testing setup (modified from Proto 2016).

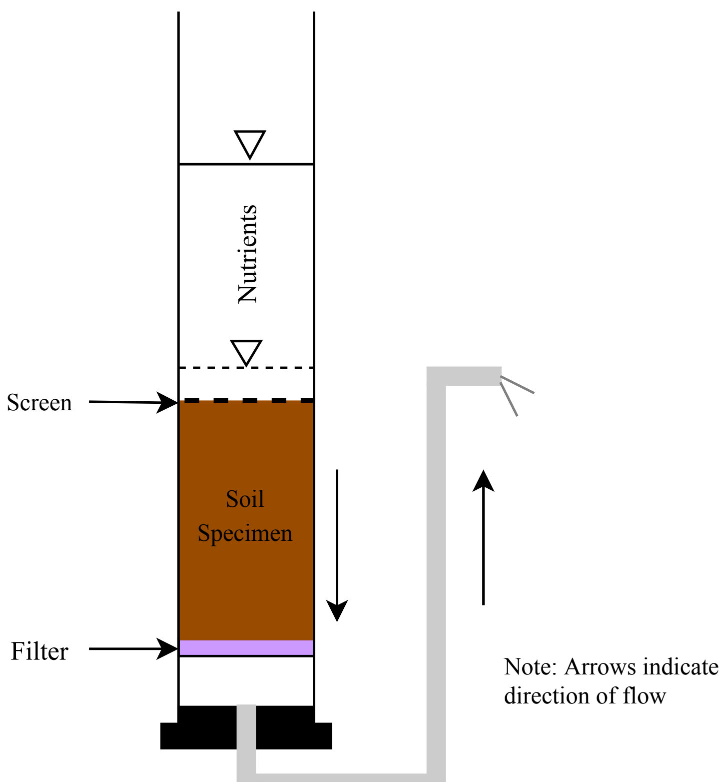

Figure 2. Revised testing setup to reduce clogging in piping.

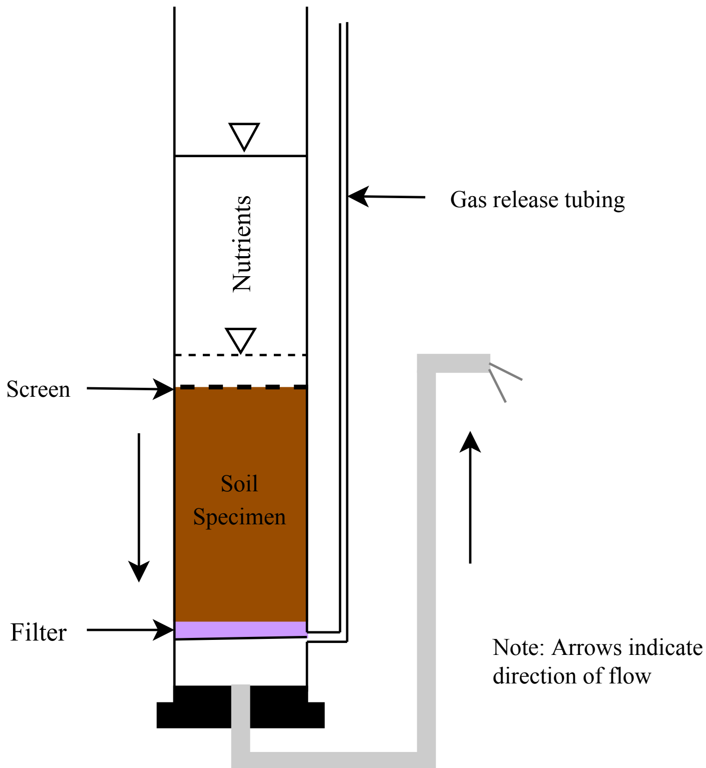

Figure 3. Testing setup to reduce buildup of gasses below sample.

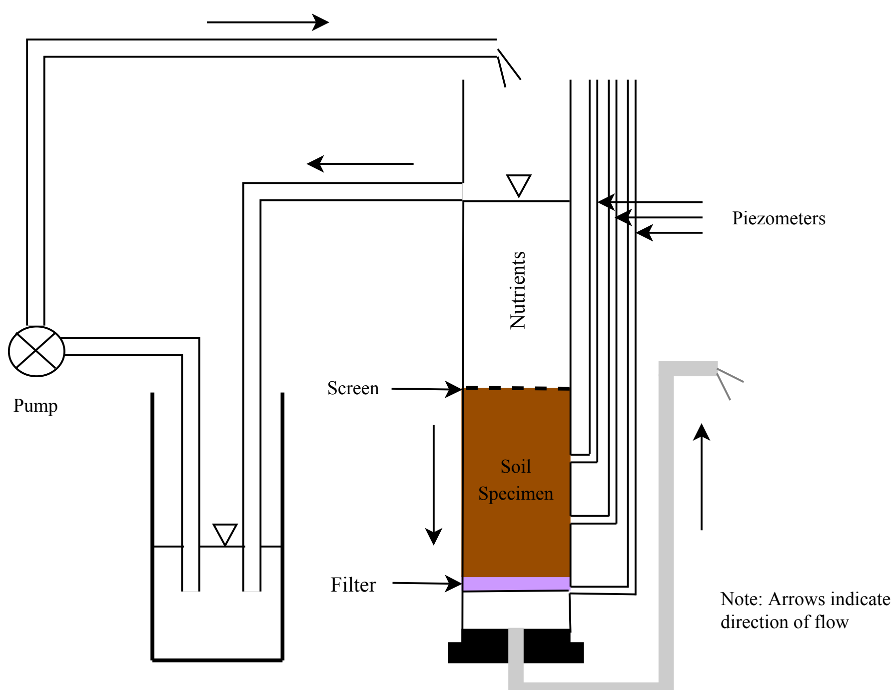

Figure 4. Testing setup for constant head permeability measurements.

Figure 5. Six sample columns.

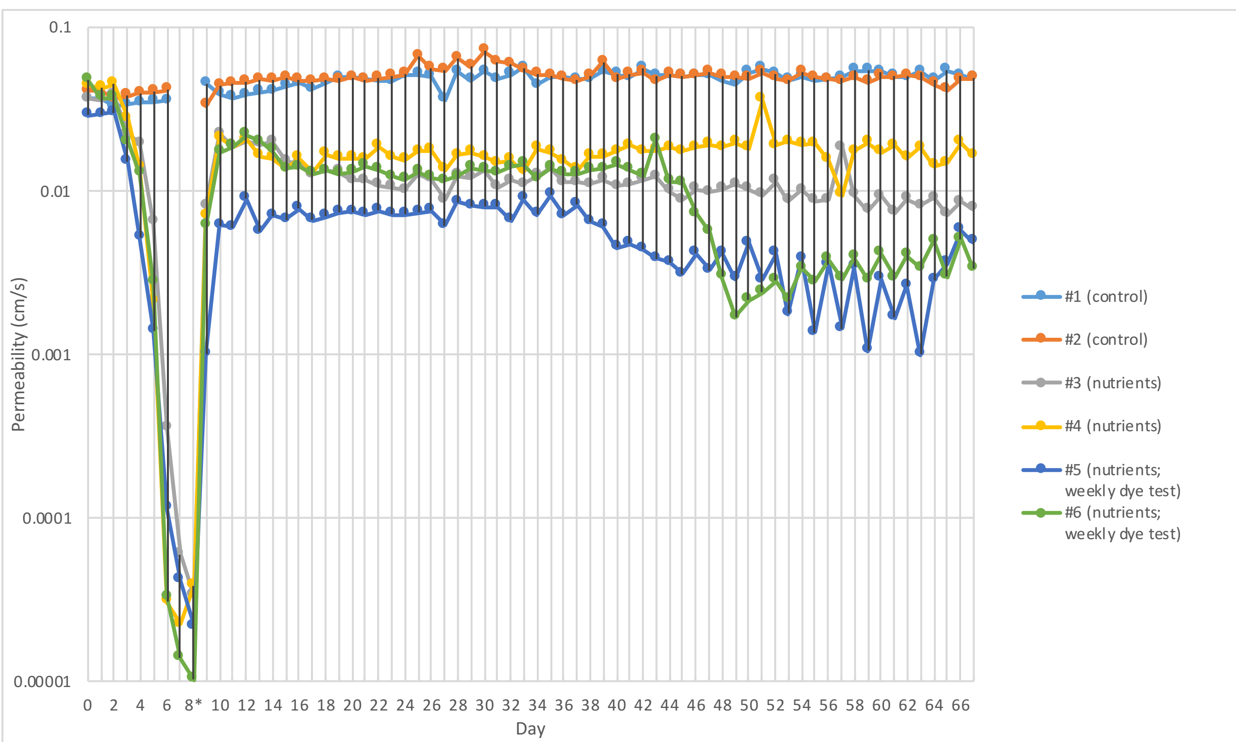

Figure 6. Permeability results.

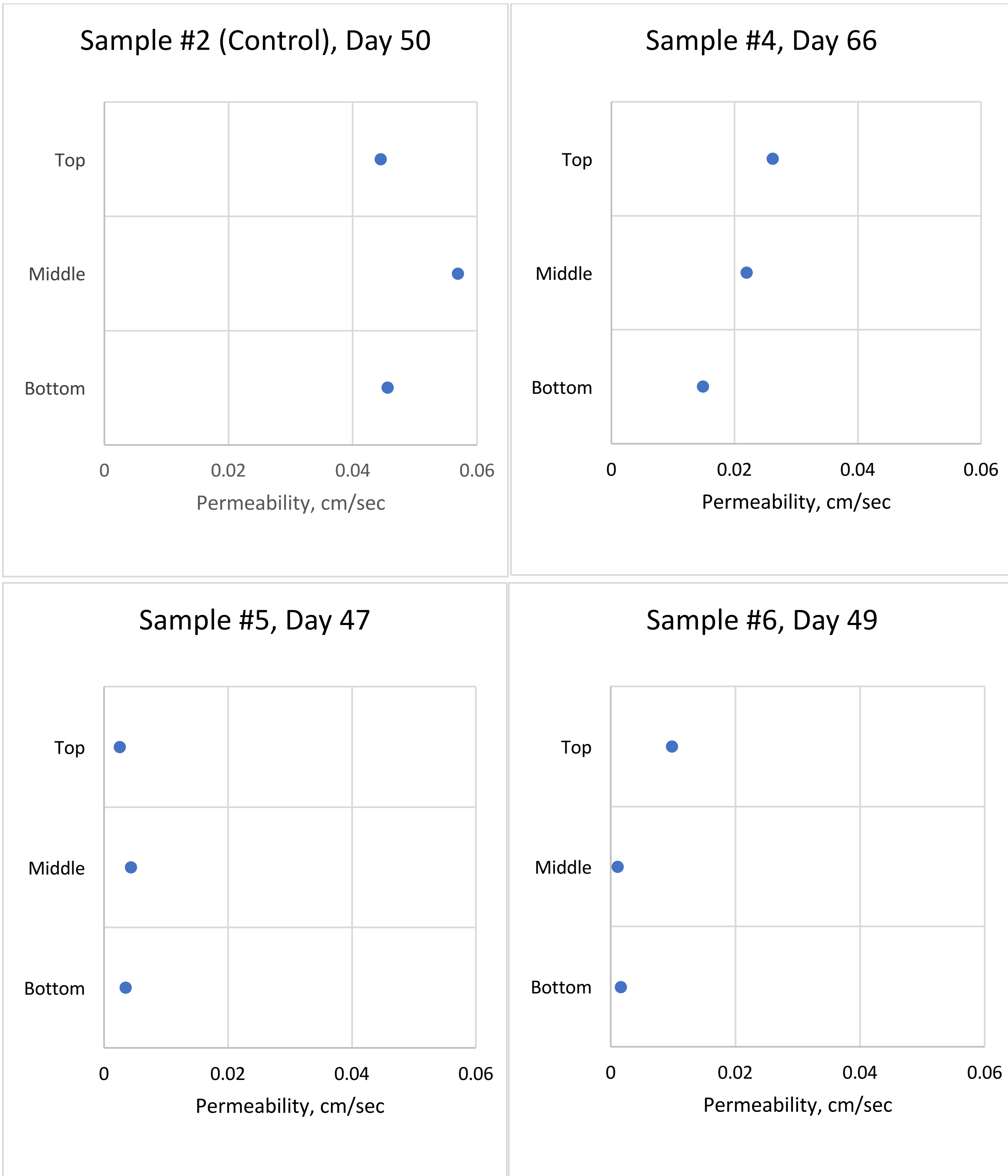

Figure 7. Layer permeability results from constant head testing.