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Manuscript title: Stimulated Microbial Growth for Permeability Reductions in Granular Soils

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Abstract

The controlled production of microbial growth has the potential to reduce groundwater flow in seepage and dewatering systems. Stimulating the growth of indigenous bacteria could clog the pore space and result in a substantial permeability reduction. This study investigated the spatial distribution of permeability reduction under different nutrient stimulation treatments of indigenous bacteria across 16 cm columns of Ottawa 50-70 sand. Spatially uniform permeability reductions of up to an order of magnitude were achieved using both a high glucose (50 mg L^{-1}) and a low glucose (10 mg L^{-1}) nutrient formulation. The overall permeability began to drop by day 2 and generally reached a minimum permeability by day 14. There was no noticeable difference in the final permeability nor the rate of permeability reduction between high and low glucose formulations. Upscaling of experiments is highly recommended for future studies on the spatial uniformity of microbial growth and biologically induced permeability reductions.

Keywords: Bio geotechnics; Geotechnical Engineering; Seepage

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Introduction

Groundwater seepage is associated with failures of contaminant containment systems, construction excavations, dams, and levees. Traditional methods to reduce seepage include slurry cut-off walls, sheetpile walls, grouting, and soil freezing (Xanthakos et al. 1994). However, these methods can be cost-prohibitive and may raise environmental concerns (Karol 2003, DeJong et al. 2010). The ability of bacteria to reduce the permeability of soils has long been recognized though it is typically viewed as a concern. For example, bioclogging in aquifers can lead to reductions in the performance of injection and recovery wells (Taylor et al. 1997) and biofilm growth in landfill drainage systems can be problematic (Cooke et al. 2001, Rowe 2005, McIsaac and Rowe 2007).

Many types of microbes can form a biofilm, which is a mixture of bacterial cells embedded in a self-produced, secreted, extracellular matrix (Watnick and Kolter, 2000). Establishment of a biofilm includes several steps beginning with reversible attachment to a living or non-living surface, followed by irreversible attachment, microcolony formation, maturation, and finally dispersal (Watnick and Kolter, 2000). Channels through the biofilm allow nutrients to circulate. Biofilms have been established in soil by using either a bio-augmentation process (adding known biofilm-forming bacteria to the soil) or a bio-stimulation process (adding nutrients to the soil to encourage the growth of indigenous biofilm-forming bacteria). Previous studies have shown significant reductions in permeability through the establishment of a biofilm by both bio-augmentation and bio-stimulation (e.g., Taylor et al. 1990, Dennis and Turner 1998, and Proto et al. 2016); however, when porewater pressure measurements were obtained during testing, the permeability reductions were typically observed within the first few centimeters of the soil sample near the nutrient injection point (e.g., Gupta and Schwartzendruber 1962, Mitchell and Nevo 1964, Vandevivere and Baveye 1992, Seki et al. 1996, and Proto et al. 2016).

Studies using bio-augmentation to initiate biofilm formation in soil are more common in the literature and have been shown to be effective. Bio-augmentation using starved bacteria has been used to achieve relatively constant biofilm distribution in a 50-foot (15.2 m) column of F-70 Ottawa sand (Sharp et al. 1999) and to create a 30-foot-wide (9.14 m) biofilm barrier along the centerline of a field-scale test, 130 feet wide (39.6 m), 180 feet long (54.9 m), 21 feet deep (6.40 m), that resulted in a 99% reduction of average permeability after three months of weekly or bi-weekly feeding (Cunningham et al. 2003). However, given the need to inject living, possibly non-native, bacteria into the subsurface, obtaining regulatory approval to use bio-augmentation in a field

application may prove challenging. In addition, there are substantial economic costs associated with producing the volume of bacterial inoculum required.

Bio-stimulation approaches may prove to be more favorable but little work has been done to systematically study how to achieve uniform bacteria growth. This study investigated altering the nutrient formulation that was added to a soil column under bio-stimulation conditions and measured the magnitude and spatial distribution of permeability reduction. The long-term goal of the research is to develop an approach that results in uniform permeability reductions over distances of at least one meter.

Background

The effect of biofilm formation on permeability in soils using one-dimensional permeameters was first quantified by Allison (1947). Proto et al. (2016) provides a summary of 14 studies conducted between 1947 and 2003 on a variety of particulate natural and synthetic materials. The results from those studies, as well as the work completed by Proto et al. (2016), generally indicate that it is possible to obtain permeability reductions of one or more orders of magnitude using bio-stimulation and that these reductions are reasonably robust with respect to varying hydraulic gradients and persistence over time given continuing injection of nutrients. However, the problem of bacterial growth primarily occurring within the first few centimeters of the location of the nutrient injection point remains.

Piezometers along the soil column length have shown biomass clogging to occur primarily within a few centimeters of the nutrient injection location (e.g., Raiders et al. 1986, Vandevivere and Baveye 1992, James et al. 1995, and Proto et al. 2016). Permeability reductions at greater distances from the inlet were occasionally observed but were of lesser magnitude. Clogging of the soil near the injection point during bio-stimulation may occur because the initial bacterial growth near the inlet depletes the oxygen and organic substrates in the supplied nutrients. The consumption of these resources within a short distance of the injection point limits bacterial growth at greater distances from the column inlet (Vandevivere and Baveye 1992).

The influence of soil types on biomass development using bio-stimulation has received limited attention in the literature. Allison (1947) studied samples of three soil types: Hanford Loam, Exeter Sandy Loam, and Hesperian Sandy Loam. The samples and fluids used in this work represented two conditions, sterilized and "reinoculated." The results of that work showed no significant impact of soil type on permeability reduction over time. The reduced permeability was attributed to microbial sealing with cells or microbially-produced polysaccharides. Cunningham et al. (1991) in a bio-augmented approach examined the results from sterilized

samples of 1 mm glass spheres, 0.7 mm sand, 0.54 mm sand, and a 0.12 mm glass and sand mixture. Their results indicated that biofilm thickness was a function of the initial permeability and particle size; however, final permeabilities for each sample stabilized at a minimum value between 3 and 7 x 10^{-8} cm^2, regardless of particle diameter. James et al. (1995) compared two sands, F70 (D_{50} = 0.212 mm) and F110 (D_{50} = 0.12 mm), inoculated with starved bacteria and then supplied with a sodium citrate medium. While similar final overall permeabilities were achieved in both materials, the permeability reductions were relatively uniform in the coarse sand but a gradation in permeability reductions was observed in the fine sand, with the greatest reduction occurring near the column inlet for the F70 sand (James et al. 1995).

Previous work related to flow conditions in the soil has looked at differences between continuous and batched (or stopped-flow) flow. Raiders et al. (1986) investigated the difference between continuously applying nutrients to cores of sterilized sandstone in a bio-augmentation experiment versus adding nutrients in batches. They observed that the resulting reductions in permeability (across an entire core) were less for the batched nutrients but were more uniform. Taylor and Jaffé (1990) investigated the use of two different continuous flow rates on columns of sterilized sand inoculated with a bacterial culture derived from sewage and observed that a higher flow rate resulted in more uniform decreases in permeability throughout the column. Seki et al. (1996) investigated two different hydraulic gradients (i = 10 cm/cm and i = 1 cm/cm) in 3 cm columns of paddy soil (35% clay, 15% silt, 50% sand) in a bio-stimulation test. In the high hydraulic gradient column (i = 10 cm/cm), no change in permeability was observed over 155 days following continuous application of a nutrient solution containing 50 mg L^{-1} glucose. They attributed the lack of permeability reduction to the higher initial bulk density of the soil and the reduced microbial growth due to the limited substrate supply. In the two low-flow columns (i = 1 cm/cm) significant permeability decreases occurred in the first centimeter over the first 10 days of feeding and a maximum reduction of two orders of magnitude occurred after 188 days. The permeability of the rest of the column (at a distance of 1 to 3 cm from the location where nutrients were introduced to the column) was much less than that in the first centimeter (Seki et al., 1996).

Vandevivere and Baveye (1992) tested a nutrient solution containing four glucose concentrations (5 mg L^{-1}, 10 mg L^{-1}, 20 mg L^{-1}, and 100 mg L^{-1}) in short (4 cm) columns. When the bacteria were introduced through the sampling port located 8 mm above the inlet (in an upward flow environment), a three order of magnitude reduction in permeability occurred in 10 days using a nutrient solution containing 100 mg L^{-1} glucose while only a one order of magnitude reduction occurred in 22 days using a nutrient solution containing 20 mg L^{-1} glucose.

Clogging at the injection front is significantly delayed when bacteria are injected directly into the sand column. Scanning electron microscopy (SEM) images indicate that sand sampled from areas of oxygen- or glucose-limited growth conditions had no exopolymer and clogging appeared to be due to the presence of large aggregates of cells that formed local plugs within pores. Nutrient solutions with glucose concentrations as low as 10 mg L^{-1} were sufficient to lead to severe clogging at the sample inlet and the likelihood of clogging increased with increasing glucose concentration.

Seki et al. (1996) used a solution containing only glucose (50 mg L^{-1}) to encourage biofilm development in paddy field soil (35% clay, 15% silt, 50% sand). In one of the low flow columns (see above) where significant permeability reductions occurred, the glucose concentrations decreased from 45 mg L^{-1} to 12 mg L^{-1} in the first centimeter, and no further decreases in glucose occurred throughout the rest of the column. They concluded that most of the glucose was consumed by microorganisms in the top 1 cm of soil and also noted that glucose consumption would cause gas production (CO_2 and CH_4), which would further contribute to the permeability reduction. While CO_2 is highly soluble, they hypothesized that methane gas formation would be possible since the measured redox potential of the column was reduced to -150 mV after 39 days.

The goal of these experiments was to measure the spatial distribution of permeability reduction in Ottawa 50-70 sands under bio-stimulation conditions using a low (10 mg L^{-1}) and high (50 mg L^{-1}) glucose-containing growth media. Results indicate that the stimulation methodology used and both nutrient formulations produced a spatially uniform permeability reduction over a 16 cm column.

Material, Specimen Preparation, and Testing Equipment

Sand

Ottawa 50-70 (U.S. Silica Company) is a manufactured, uniformly graded, sub-rounded clean sand of white quartz mineralogy (D_{50} = 0.23 mm, D_{10} = 0.17 mm, Cu = 1.37). Ottawa 50-70 was selected based on its relatively high initial permeability (approximately 3 x 10^{-2} cm/s at e_{min}) and its prior use in bio-mediated soil improvement tests (e.g., Proto et al. 2016, DeJong et al. 2010).

Column Design and Set-up

A schematic drawing of the column configuration is shown in Figure 1. Each column was constructed from 7.62 cm (inside diameter) clear acrylic tubing. At the inlet of the sand column, copper screening was cut to fit inside the column and cover the sand surface. At the outlet of the sand column, a filter layer comprised of Porex was used to contain the sand; the Porex was stamped into circles and friction fit into the columns. The Porex filter

was held in place by a steel perforated sheet supported by three 2.5 cm pegs creating a space under the sand column for observation. The ratio of column diameter to grain diameter is about 330, which is sufficient to ensure that radial velocity variations due to wall effects are insignificant (Schwartz and Smith 1953). The sand was air pluviated into the column with a uniform 2.5 cm drop height and additional compaction occurred by gently tapping the column with a mallet during the pluviation process. The final sample height was approximately 16 cm and the final dry densities of the samples ranged from 1.56 to 1.58 g/cm^3 with void ratios ranging from 0.673 to 0.701 (average void ratio of 0.685). The permeability of three layers were monitored, two 5-cm layers and one 6-cm layer closest to where nutrients were introduced, using three piezometers located along the length of each column.

The samples were saturated from the bottom up by submersion in tap water for a minimum of 48 hours. Physiological saline solution (0.9% NaCl) was then introduced to the samples during baseline testing for permeability.

Nutrients

A general nutrient solution was used to support the growth of many types of bacteria, including those capable of both aerobic and facultative anaerobic metabolism. Both high-glucose and low-glucose nutrient solutions were mixed daily and introduced to the columns from the top using gravity flow. The high-glucose solution consisted of 50 g glucose, 10 g casein peptone, and 5 g yeast extract, in 1 L of physiological saline solution (0.9% NaCl). The low-glucose nutrient solution consisted of 10 g glucose, 10 g casein peptone, and 5 g yeast extract, in 1 L of physiological saline solution. For each treatment, 800 ml of nutrient solution, approximately one and a half pore volumes, were added to the top of each column (Re = 0.18; the value represents the Reynolds number at the beginning of the experiment since the bulk porosity and permeability changes during the course of the experiment due to the growth of bacteria). The nutrient treatments were alternated daily with 800 ml of physiological saline, resulting in one nutrient treatment every two days. Physiological saline is isotonic to the bacterial cell allowing cells to maintain their osmoregulating potential in this solution. This approach had been developed in earlier testing (Roth and Caslake, 2019) to help reduce clogging of the testing apparatus above the soil surface.

Permeability Testing

Four column tests were constructed and run simultaneously to test the bulk and layer permeability reductions in the sand samples under high-glucose and low-glucose treatments. The effect of the treatment along the length of the columns was measured by monitoring changes in permeability using the three installed piezometers. A constant head testing approach (ASTM D2434-68) was used to measure permeability (Figure 2); the constant head approach allowed time for the fluid heights in the piezometers to stabilize under constant flow conditions before readings. For each permeability measurement, fluid height and volume discharged were measured three times and average values from the three measurements were used in the calculations. Permeabilities were measured on alternate days when the samples were treated with saline water. Bulk permeability was calculated using Darcy's law and the head loss between the inlet and outlet of the column. Bulk permeabilities, k (cm/s), were calculated using the equation:

$$k = \frac{QL}{Ath} \qquad 1.$$

where Q (cm^3) is the quantity of water discharged, L (cm) is the length of the soil sample between the measurement points for the head difference, A (cm^2) is the cross-sectional area of the sample, t (s) is the total time of discharge, and h (cm) is the difference in hydraulic head across the soil layer. Layer permeabilities were found by first calculating the hydraulic gradient, i (cm/cm), for each layer using the following equation:

$$i_{layer} = \frac{\Delta h_{layer}}{l_{layer}} \qquad 2.$$

where Δh (cm) is the head loss in each layer and l_{layer} (cm) is the height of each layer. Then, using the bulk permeability of the column, the layer permeability k_{layer} (cm/s) was computed using:

$$k_{layer} = \frac{k_{bulk} i_{bulk}}{i_{layer}} \qquad 3.$$

The permeabilities over the course of the 28-day experiment were normalized to the starting column value.

Results & Discussion

Biofilms have been shown to reduce flow in column tests (Taylor et al. 1990, Baveye et al 1992, Vandevivere and Baveye 1992, Seki et al. 1996, Dennis and Turner 1998, Cunningham et al 2003, Proto et al. 2016, Ostvar et al 2018) and using a batched flow method to deliver aerobically prepared nutrients was shown to create more uniform permeability reductions across the soil columns compared to continuously applying nutrients (Raiders et al., 1986). The research results presented here focused on whether altering the nutrient formulation during batch feeding would change the distribution of the observed permeability reductions in the soil. Permeabilities

of four test columns were monitored over a period of 28 days (14 treatments). Two columns were treated using the high-glucose medium (HG1 and HG2) and two columns were treated using the low-glucose medium (LG1 and LG2). The resulting normalized bulk permeabilities for these columns are shown in Figure 3 and the resulting normalized layer permeabilities are shown in Figures 4 and 5 for the high- and low-glucose columns, respectfully. Under batched flow conditions, with nutrients added every 48 hours, the 16 cm sand columns achieved a uniform permeability reduction. Bulk permeability reductions began in the first few days of treatment and generally approached the minimum by day 14 (7 treatments, ~10 pore volumes, Figure 3). Overall, bulk permeability was reduced by nearly an order of magnitude compared to the initial permeability and there was no noticeable difference in final permeabilities, nor the rate of permeability reduction, between the high and low glucose columns (Figure 3). These results are consistent with results in sandstone core samples (Raiders et al., 1986); however, unlike their results, permeability reductions did not initially occur at the inlet. It is possible that the long interbatch time might have allowed media to migrate deeper into the column, or the alternating saline treatment may have helped reduce clogging at the inlet.

Direct measurements of the pressure heads within the columns were provided by the piezometers and these measurements were used to calculate the permeabilities as described above. In the columns treated with the high-glucose medium, there was a slight increase in permeability in the top layer of HG1 and the lowest layer of HG2 over the first few treatments, but the permeability of all layers was decreasing after day 5 (~ 3 pore volumes) (Figure 4). The permeability of HG2 reached a minimum by day 7 (~ 5 pore volumes), much faster than the replicate column. Unlike the replicate column, HG2 had a slight increase toward the end of the treatment period.

In the columns treated with the low-glucose medium, the layers tracked the overall permeability reasonably well. In LG2, the layers tracked the overall permeability reduction and no obvious increase in permeability was observed toward the end of the 28-day experiment (Figure 5). In LG1, there was slightly more variability in the middle of the column and, similar to HG2 but more pronounced, a consistent increase in permeability in all layers after day 24. The increase in permeability at the end of the experiment could be due to the detachment of the accumulated biomass from the sand grains, which would open up the pore space. Over time, as microbial growth and the associated biomass and biofilm accumulates, the pore space would decrease resulting in a decrease in permeability and a concomitant increase in the shear stresses at the interface of the fluid and the biomass / biofilm. A steady state of biomass growth and detachment may have been established between days

14 and 26, resulting in a steady state permeability in support of the open pore model (Taylor et al. 1990). Near the end of treatment, detachment might have exceeded biomass growth resulting in an increase in the column permeability. It is also possible that the observed increase in permeability resulted from loss of entrapped air in the sample.

Two columns received a nutrient solution containing five times more glucose than the other two columns. Whether this led to an increase in microbial growth in the center of the column will be explored in future research. The fact that the layer permeability in both of our treatments was reduced over time suggests that microbial growth occurred throughout the column. Batched application of nutrients with a significant amount of time between the batches may allow the nutrients to reach further into the column encouraging microbial growth throughout. Ostvar et al. (2018) noted that the oxygen level in effluent from a low flow column (*Re* 0.1) was negligible indicating that the dissolved oxygen was consumed by the facultative anaerobe used in their studies. Under conditions of low oxygen availability, gas can be generated through the processes of fermentation and denitrification. Fermentation results in the production of hydrogen gas or carbon dioxide in addition to other organic products depending on the specific bacteria present in the column. Denitrification results in the production of nitrogen gas from the reduction of nitrate or nitrite. Seki et al. (1996) attributed a portion of the permeability reduction to carbon dioxide and methane gas, which was assumed to be formed from carbon dioxide, formic acid, acetic acid, and hydrogen gas. The formation of gas in our columns may have been partially responsible for permeability reductions within the soil samples. However, gas bubbles were only intermittently observed in the columns and occasionally, sufficient gas was generated that it could be observed escaping through the piezometers. The amount of gas observed did not correlate with the concentration of glucose in the medium; gas bubbles were no longer visible following the saline flush (on alternate days from feeding) and no accompanying permeability increase was observed when the gas bubbles were dissipated.

All data from this work is available from the corresponding author on request.

Conclusions

The controlled production of microbial growth and biomass has the potential to reduce groundwater flow in seepage and dewatering systems. We report:

- Batched flow of nutrients on an alternating day basis resulted in a nearly 10-fold reduction in permeability across 16 cm sand columns.

- The permeability reduction in the 16 cm sand columns was monitored through the installation of piezometers and was uniform across the column.
- Uniform permeability reduction resulted from the stimulation of growth of indigenous bacteria over 28 days. The growth of indigenous bacteria and the concomitant production of associated biomass, biofilm, and entrained air can clog the pore space and result in a substantial permeability reduction.
- Batched flow with higher glucose concentrations results in uniform permeability reductions along the soil column but the increased glucose and the resulting reduction in oxygen in the middle of the column may result in fermentation or denitrification.

This study provides new insights into the use of indigenous bacteria for permeability reductions that may be applicable to field applications where it is desirable to reduce the flow through granular soils. The results presented here increase understanding of the impact of the glucose level in the nutrient solution when using bio-stimulation processes to reduce soil permeability and confirm that uniform permeability reductions can be achieved in granular soils. Additional work is needed to study the influence of flow conditions and nutrient residence time required for sufficient biomass development and to understand the role of anaerobic microbial processes within the treated soil column. Upscaling of experiments is highly recommended for future studies on the spatial uniformity of biomass formation and biologically induced permeability reductions.

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Notation

A is the cross-sectional area of sample (cm^2)
Cu is the dimensionless coefficient of uniformity
D_{10} is the particle size (cm) of a granular material where 10% of the particles are smaller by weight
D_{50} is the particle size (cm) of a granular material where 50% of the particles are smaller by weight
e is the dimensionless void ratio
L is the length of flow path (cm)
Q is the volume of flow (cm^3)
$h(t)_e$ is the elevation head at time t (cm)
$h(t)_p$ is the pressure head at time t (cm)

i is the hydraulic gradient (cm/cm)
k is the permeability (cm/s)
k_o is the initial permeability (cm/s)
Re is the dimensionless Reynolds number
t is time (s)
Δh is the head loss (cm)
γ_w is the unit weight of water (g/cm^3)

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Figure captions

Figure 1. Schematic drawing of the configuration of the individual columns

Figure 2. Schematic drawing of the flow paths during treatments and permeability testing

Figure 3. Normalized bulk permeability results

Figure 4. Normalized layer permeability results for column HG1 (a) and column HG2 (b)

Figure 5. Normalized layer permeability results for column LG1 (a) and column LG2 (b)

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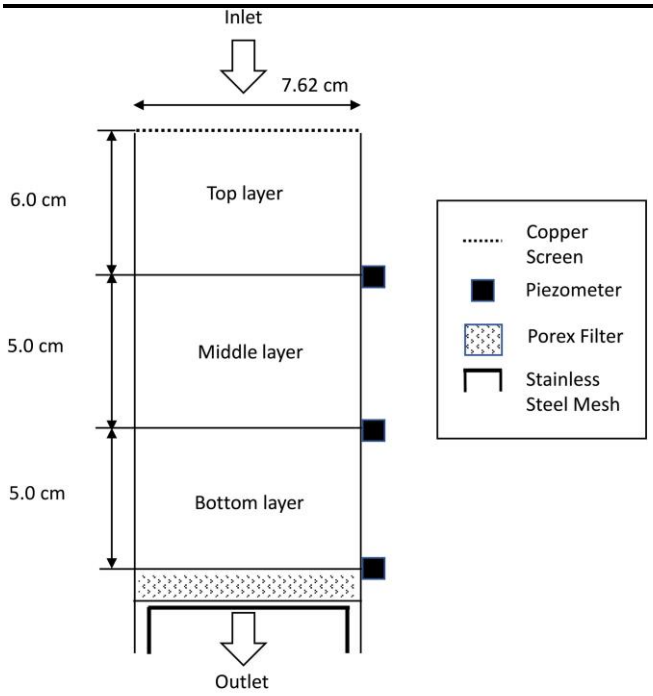

Figure 1

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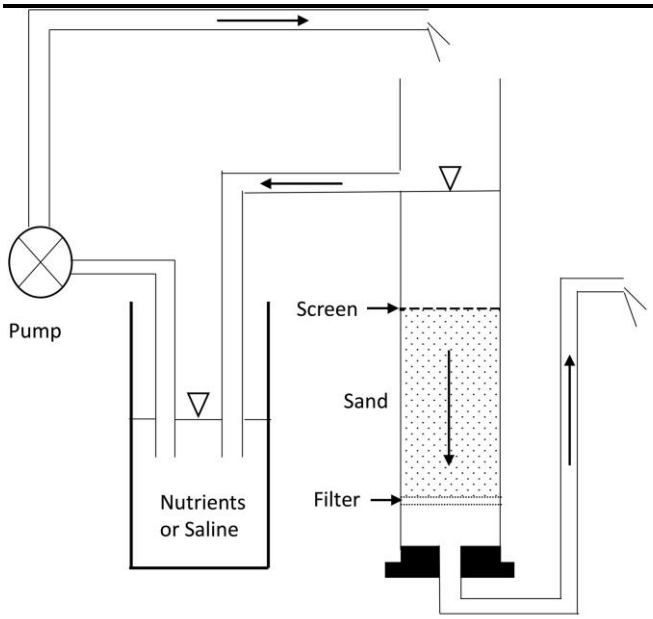

Figure 2

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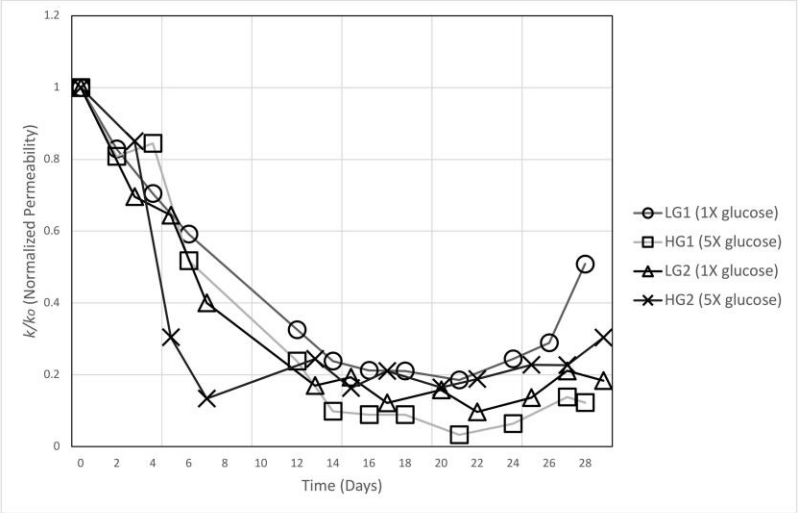

Figure 3

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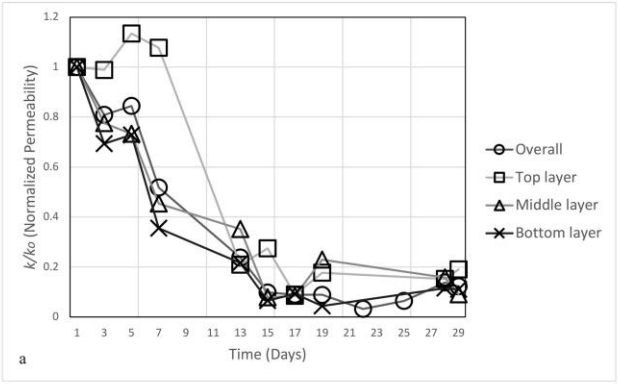
10.1680/jgrim.21.00011



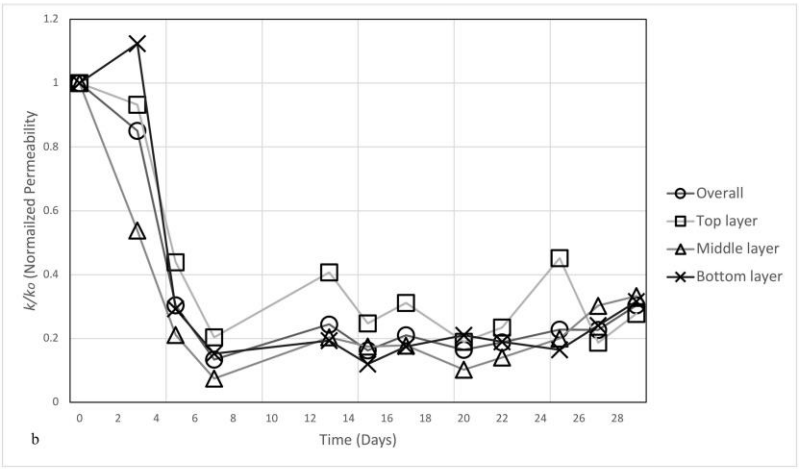


Figure 4

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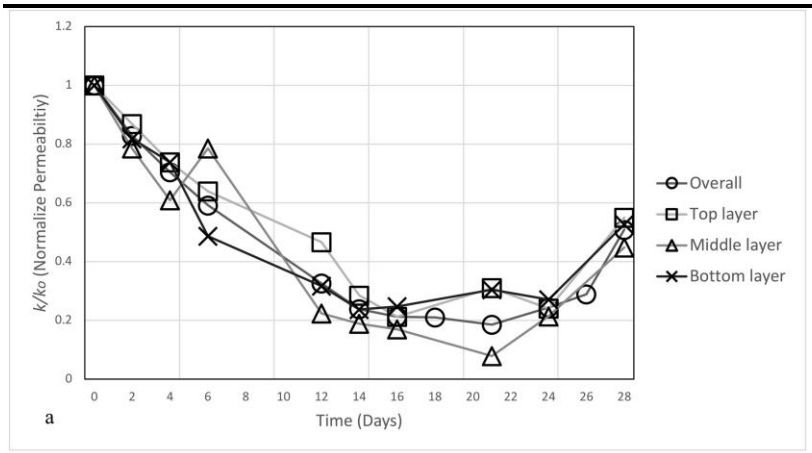

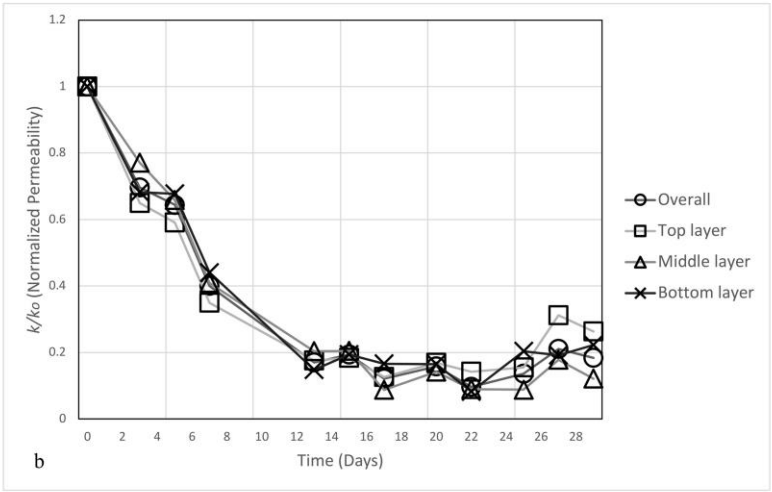

Figure 5