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EVALUATING THE PERFORMANCE OF BIO-CLOGGING ADDITIVES FOR SUSTAINABLE SOIL PERMEABILITY REDUCTION

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Bio-clogging, facilitated by bacterial activity, offers a sustainable and environmentally friendly alternative to traditional grouting methods for controlling soil seepage. However, the influence of bacterial presence and the use of culture medium during field implementation on setting times and their correlation with permeability reduction remains uncertain, posing a challenge to the advancement of this technology. This study examines the effects of bacterially induced bio-clogging on soil permeability, with a specific focus on conditions during and after the removal of the culture medium. Laboratory experiments reveal that microbial activity, driven by bacterial adhesion and extracellular polymeric substance (EPS) production, significantly reduces soil permeability by clogging soil pores. The observed reductions in permeability are comparable to those achieved with conventional grouting techniques but are attained without the associated environmental impacts. Importantly, even after the removal of the culture medium, dextran—a byproduct of microbial activity—was retained, and the permeability reductions remained significant. This finding underscores the potential for sustainable permeability reduction through bio-clogging, demonstrating the robustness of microbial biofilms, which persist and continue to influence soil properties despite the withdrawal of external nutrients. This research contributes to a better understanding of how biofilm development and EPS production influence soil permeability reduction, offering a step toward refining bio-clogging techniques for practical use.
Keywords: Bio-clogging; Extracellular polymeric substances (EPS); Microbial ground improvement; Permeability reduction.

1. Introduction

To achieve a substantial reduction in permeability, an essential aspect in the construction of geotechnical structures on permeable sand substrates, recent scholarly attention has turned towards eco-friendly and sustainable soil improvement methodologies. This focus has particularly been directed towards biological materials, such as plant systems and microbial agents, as evidenced by a body of research spanning the last decade (Leung et al., 2017; Kamchoom and Leung, 2018; Lim et al., 2020). This investigation explores a novel and potentially more sustainable method for reducing soil permeability. Current literature indicates that specific bacteria can form robust biofilms, comprising bacterial cells embedded in a matrix of extracellular polymeric substances (EPS). These substances facilitate bacterial adhesion to soil particles, and continuous cell division enhances biofilm thickness, which can significantly obstruct soil pores, thereby reducing water permeability (Taylor et al., 1990). Given its viscous and resilient nature, EPS might withstand physical deformation better than other materials, offering a promising alternative to traditional soil stabilization methods such as chemical grouting and slurry trenches. Nonetheless, the feasibility of this biological approach on a larger scale is limited by environmental conditions, such as temperature and nutrient concentrations, which affect the longevity and intensity of bacterial colonization (Mataragas et al., 2003). This research aims to quantify the effects of varying bacterial and nutrient concentrations on soil porosity and permeability, proposing a predictive model that could further elucidate the dynamics of bio-clogging in coarse-grained soils.

2. Material and method

2.1. *Test plan.*

A set of laboratory experiments was conducted to explore the impact of bacterial solutions and culture mediums on the setting time and the reduction of soil permeability. The primary objective of the initial series, referred to as Test BC, was to evaluate the decrease in soil permeability following the injection of 10 mL of bacterial solution, constituting approximately 0.7% of the total soil mass. Notably, this proportion is significantly lower than the 5% to 10% typically employed in conventional soil stabilization techniques such as cement and bentonite grouting or slurry trench construction (Kenney et al., 1992; ACI Committee 230, 2009). To account for microbial growth variation, six samples were prepared, comprising one control and five replicates. To investigate bacterial colonization and its associated effects, the experimental design included duplicate tests denoted as BC'. These duplicated tests maintained identical setups and conditions as their originals but were specifically employed to monitor dextran concentration and bacterial colonization patterns. Given that the culture medium incorporates a component of water-soluble sucrose, it is hypothesized that this could increase the fluid's viscosity and potentially contribute to a temporary reduction in soil permeability. To isolate and quantify the sole influence of the culture medium on soil permeability, an additional test series, Test C, was conducted using only the culture medium without the addition of bacteria. This series was designed to clearly differentiate the effects of the culture medium's properties from those induced by bacterial activity in reducing soil permeability

2.2. *Test setup*.

Figure 1 delineates the schematic representation of the experimental setup utilized for bacterial injection studies. Each test sample was contained within a cylindrical container fabricated from polyvinyl chloride (PVC), measuring 15 cm in height and 10 cm in diameter. The base of each container was sealed with a PVC cap, and a drainage aperture of 1 cm in diameter was incorporated for fluid exit. A 2 cm-thick filter layer of graded pea gravel was placed at the base prior to sample preparation to prevent the loss of finer particles during testing. For BC', three additional sampling outlets were located at varying heights on the container walls to facilitate the periodic sampling of soil and to assess bacterial colonization throughout the duration of the experiments. The soil utilized for these tests was classified as Ottawa sand, recognized as a uniform and clean sand type (SP classification per ASTM D2487-17, 2017), with its primary properties summarized in Table 2. This sand was pre-heated to 80°C for a minimum of 24 hours to eliminate residual microbiological activity. Subsequently, the sand was conditioned to a water content of 14% and compacted to a target dry density of (1560 ± 10) kg/m3, achieved through the under-compaction method, corresponding to approximately 88% ± 1% degree of compaction (DOC). Following compaction, each sample underwent saturation with deionized water and was subjected to a constant head permeability test.Soil samples weighing 5 g were extracted from the top, middle, and bottom outlets, then oven-dried to quantify the initial water volume. Subsequent to this, the dextran, a byproduct of bacterial colonization, was isolated using aqueous extraction and centrifugation methods. Dextran quantification was performed using a refractometer (PAL-12S; ATAGO CO., LTD., Japan) capable of measuring concentrations from 0 to 15 g with an accuracy of ±0.2 g.

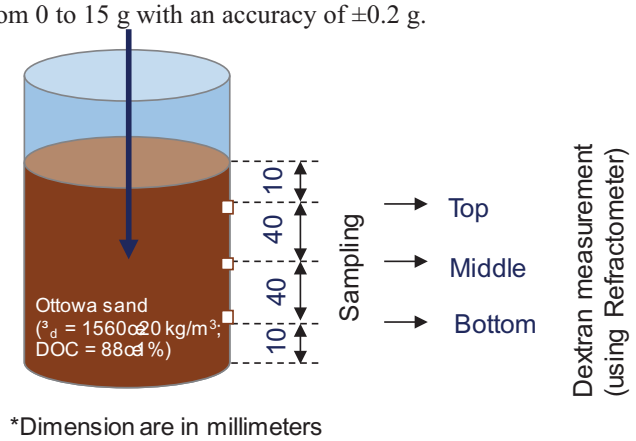

Fig. 1 Overview of test setup and sampling procedure

2.3. *Test procedure*.

The bacterial cultivation was similar to the previous studies (Treebupachatsakul and Kamchoom, 2021; Kamchoom et al., 2024). At the initiation of the experiment, a volume of 10 mL of this solution was applied to each saturated soil sample from the surface, utilizing a syringe over a duration of approximately 5 minutes to prevent the generation of excessive pore pressure within the sample. For Test BC, a two-stage procedure was

employed. During the initial phase, the medium was applied on the 3rd and 7th days of each week, spanning a total of 17 days. Subsequently, the samples underwent a 14-day period devoid of any medium to facilitate the assessment of bio-clogging dynamics during early microbial activity and the subsequent temporary withdrawal of the medium. The medium was applied by pouring a total of 320 mL directly onto the surface, allowing it to percolate through the sample by gravitational force. In contrast, for Test C, only the initial cultivation period of 17 days was performed. To evaluate the long-term persistence of bio-clogging, Test BC samples were subjected to a drying process at 80°C for 24 hours and subsequently re-compacted into their containers after the two-stage test had concluded. Permeability assessments of the bio-augmented soil were conducted using the constant head permeability test prior to the reintroduction of the culture medium. Dextran concentration measurements were routinely carried out every two days on Tests BC'. Additionally, selected soil samples from these tests were analyzed using scanning electron microscopy (SEM) at the conclusion of the medium application period to qualitatively assess the extent and pattern of bacterial colonization.

3. Temporal dynamics of dextran production

Figure 2 presents data comparing average dextran percentages at the top, middle, and bottom outlets from Test BC'. Over the initial five days, dextran production was minimal, indicating a bacterial setting time. Subsequent measurements showed dextran concentrations increasing to 15%–20%, which remained steady for nearly ten days. Without the addition of culture medium, dextran levels fluctuated yet stayed above 15% for several additional weeks. After recompaction, there was a notable reduction in dextran levels by approximately 10%. The observed lag in dextran production during the first five days reflects the adaptation period required for the bacteria to acclimatize to the soil environment, suggesting that immediate bacterial activity was not effective. The subsequent increase and stabilization of dextran levels indicate active bacterial colonization and bio-clogging of soil pores.

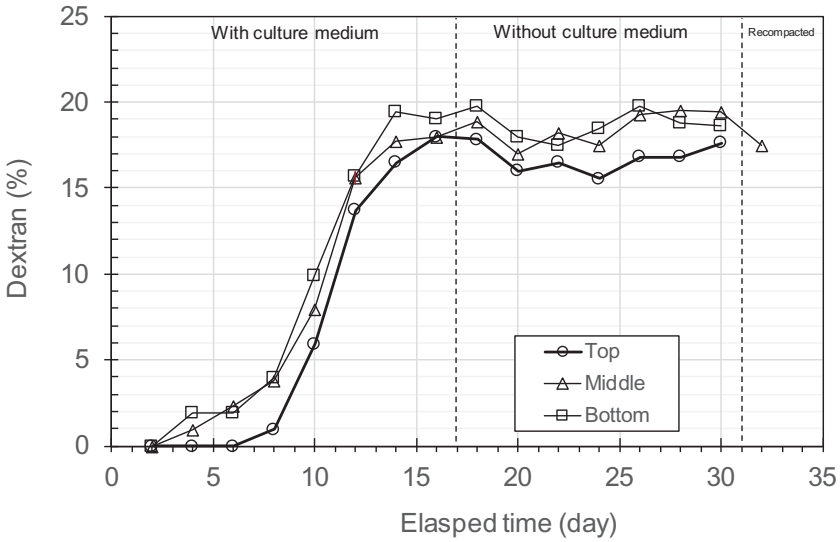

Fig. 2 Variation of dextran percentage with time at different outlets of container

4.Assessment of permeability reduction due to bio-clogging

Figure 3 delineates the variations in the permeability of bio-mediated soils from Tests BC. Initially, the permeability of the compacted bare soil was recorded at approximately 10^{-4} m/s. Following 10 days of bacterial treatment, permeability significantly decreased to about 5×10^{-6} m/s, indicating a substantial reduction by roughly an order of magnitude for each sample. This reduction aligns with existing literature that reports a decrease in permeability by two to four orders of magnitude following bacterial intervention in coarse-grained soils (Dashko and Shidlovskaya, 2016; Greer, 2018). Concurrently, the dextran measurements in Test BC' entered an exponential growth phase from the fifth to the fifteenth day post-cultivation, which correlated with a further drop in permeability to approximately 5×10^{-7} m/s, stabilizing after 14 days of bacterial growth. This permeability trend was consistent with the dextran variation illustrated in Figure 2, though a slight increase in permeability was observed between 14 and 17 days.

The measured permeability reductions and their correlation with dextran production phases highlight the impact of microbial bio-clogging on soil properties. The initial sharp decline in permeability reflects the effective pore space occlusion by biofilm formation, as indicated by the rapid increase in dextran concentration during the first ten days. The subsequent minor fluctuations in permeability, notably the slight increase observed from 14 to 17 days, may be attributed to changes in dextran distribution within the soil, as evidenced by its

decreased presence at the bottom of Test BC during the same period. Post-culture medium withdrawal, the measured permeability remained between 10^{-7} and 10^{-6} m/s, with diminished variability among samples, suggesting a stabilization of bio-clogging effects in the absence of additional medium. This phase showed less permeability fluctuation, corroborating the observed plateau in dextran levels. Upon drying and recompacting the soil, the permeability of the treated samples approached 10^{-6} m/s, comparable to the reductions achieved through traditional methods such as cement or bentonite grouting.

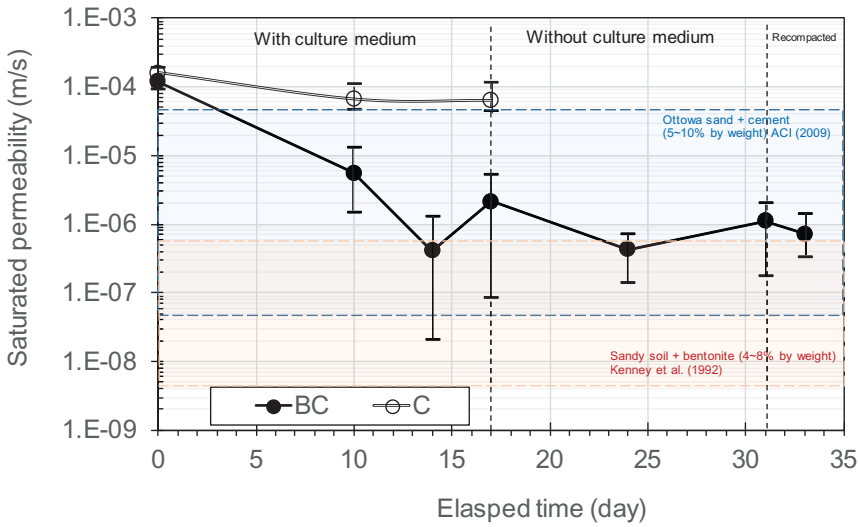

Fig. 3 Variations of measured permeability of bio-mediated soil with time

5. Summary

This study explored a sustainable method for reducing soil permeability through the utilization of bio-clogging. Laboratory experiments were conducted using bacterial solutions to evaluate their effects on the permeability of coarse-grained soils. The methods involved monitoring dextran production and bacterial colonization, and assessing the changes in soil permeability through constant head permeability tests. The key findings indicate that the introduction of bacterial solutions into the soil led to the formation of biofilms that effectively occluded soil pores, resulting in a substantial reduction in permeability. The initial permeability of the compacted bare soil significantly decreased from approximately 10^{-4} m/s to about 10^{-6} m/s after 10 days of treatment. These outcomes suggest that bio-clogging, facilitated by bacterial EPS, can reduce soil permeability comparably to traditional soil improvement methods like cement and bentonite grouting, but with the added advantage of being more environmentally sustainable. The efficacy of this method in maintaining reduced permeability levels, even after cessation of culture medium application, points to its potential long-term application in geotechnical engineering projects.

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