Preliminary study on permeability reduction by enzyme-induced biopolymer formation

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ABSTRACT

Water leakage in earth structures, such as levee, embankments, and dams, is considered as geotechnical risks and hazards, thus it often requires the implementation of sealing. In an attempt to develop bio-sealing techniques based on microbial activities, this study explored the feasibility of using enzyme-induced biopolymer formation (EIBF) to induce and engineer bioclogging in soils. In particular, as accumulation of insoluble gel-like biopolymers in soil profoundly reduces soil permeability, we exploited formation of insoluble biopolymer, called dextran, by using dextrasucrase, the enzyme extracted from the model bacteria *Leuconostoc mesenteroides* in this study. First, a series of batch experiments was carried out to find the optimum EIBF conditions for enzyme extraction and biopolymer production, varying enzyme/sucrose ratio, sucrose concentration, number of treatment repetition. Second, the permeability reduction of a coarse sand pack was monitored while the optimized EIBF was implemented. In addition, the permeability reduction by EIBF was compared with that by microbially induced biopolymer formation (MIBF). The presented results provide fundamental and baseline data to further develop bio-sealing method using enzyme-induced biopolymer formation.

Keywords: Leakage; Bio-sealing; Bio-clogging; Permeability reduction; Enzyme; Insoluble biopolymer; Bacteria

1 INTRODUCTION

Water leakage in old earth structures, such as levee, embankments, and dams, becomes increasing problems, and it is considered as geotechnical risks and hazards, because it can lead to progressive failures and collapse of structures and floods. Thus, when such severe leakage is found in practices, sealing techniques are implemented as a countermeasure, which includes permeation grouting using cement-grout or partial re-construction. With recent intensive attempts to develop bio-based grouting techniques, bio-sealing methods based on microbial activities have garnered significant interest.

Indigenous soil bacteria in subsurface are capable of production of biofilms and/or insoluble polysaccharidic biopolymers either as their habitats or as byproducts of their metabolisms. In particular, use of such bacterial biopolymers and biofilms as a means to cause bioclogging, reduce overall permeability, and eventually seal cracks or leakage in geotechnical earth structures has been investigated in recent years (e.g., Cunningham et al. 1991; Kwon and Ajo-Franklin 2013; Noh et al. 2016; Jeon et al. 2017; Kwon et al., 2017; Kim et al. 2019).

Direct use of bacterial activities, either stimulation of indigenous bacteria or inoculation of model species into target grounds inevitably confront the problems with bacterial growth in field conditions, and the use is often limited to coarse-grained soils because of the micron-size of bacteria. Meanwhile, use of enzyme-induced reactions has also drawn much attention because of such challenges in exploiting bacterial activities (Park et al. 2014; Hamdan and Kavazanjian 2016). Instead bacterial culture or indigenous bacterial consortium habiting in subsurface, use of enzyme offers advantages in harsh environments. However, there has been no attempts using enzyme reactions to cause bioclogging in soils.

Therefore, in this manuscript, we for the first time report our attempt using enzyme-induced biopolymer formation (EIBF) to induce and engineer bioclogging in soils. First, we investigated formation and production of insoluble biopolymers, called dextran, using the enzyme (dextransucrase) extracted from bacterial culture of *Leuconostoc mesenteroides* (ATCC 14935). Herein, the enzyme-based dextran production method was optimized, varying the sucrose concentration and the mixing ratio of enzyme and sucrose. Second, a preliminary column experiment was performed to examine the efficiency and the extent of permeability reduction when the produced dextran accumulated in coarse sands.

2 MATERIALS AND METHODS

2.1 Bacterial culture preparation for enzyme extraction

In this study, *Leuconostoc mesenteroides* (ATCC 14935) was chosen as the model bacterium to extract enzyme. *L. mesenteroides* is a facultative anaerobe, which can be cultured in anoxic condition, and harmless to human (biosafety level 1) (Kwon and Ajo-Franklin, 2013; Ham et al. 2018). *L. mesenteroides* produces insoluble biopolymer, dextran, via lactic acid fermentation, when fed with sucrose-rich nutrients. The model bacteria produce the enzyme called dextransucrase and this enzyme promotes the synthesis of dextran from sucrose.

The growth medium containing 10 g/L yeast extract, 15 g/L sucrose, and 0.1 M potassium phosphate buffer was used to culture the model bacteria and stimulate the production of the enzyme dextransucrase (Noh et al. 2016). A frozen stock culture was resuscitated and cultivated in the defined medium at 26°C for ~24 hours. Then, the cultured inoculum was used for enzyme extraction.

2.2 Extraction of enzyme (dextransucrase) solution

As depicted in Fig 1, the culture centrifuged at 12,000 RPM for 15 min at 4°C to separate the supernatant from the biomass, such as bacterial cells and dextran. This procedure was repeated three times to ensure the exclusion of bacterial cells and dextran in the supernatants. Thereby, this biomass-free supernatant contained the enzymes, and it used as the enzyme solution for this study (Shukla et al. 2000; Ko et al. 2012).

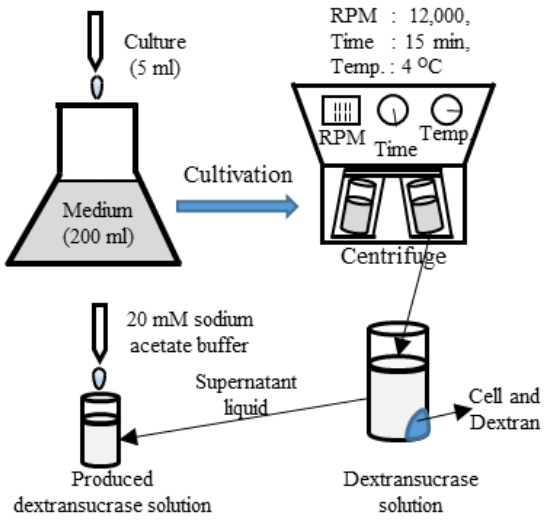

Fig. 1. Production of enzyme (dextransucrase) solution.

2.3 Batch test for optimization of EIBF

It was confirmed that addition of sucrose to the enzyme solution produced insoluble dextran, and the produced dextran had the similar fabric with that directly produced by *L. mesenteroides* (Fig. 2). Dextran production was investigated by controlling the sucrose concentrations and the enzyme solution/sucrose solution ratio to examine the amount of dextran production for optimization of enzyme-induced biopolymer formation (EIBF).

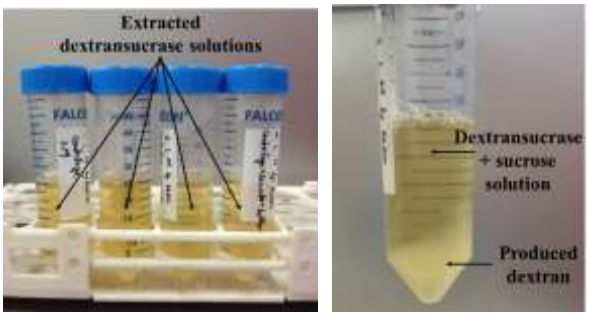

Fig. 2. (left) Extracted enzyme (dextransucrase) solution, and (right) dextran produced by mixing sucrose with dextransucrase solution.

The sucrose solutions were prepared by mixing de-ionized water with sucrose at four different concentrations (40, 80, 160, 320 g/L), as shown in Table 1. The sucrose solutions were autoclaved to prevent bacterial contamination before usage. Then, 50 mL of these sucrose solutions were mixed with 50 mL of the extracted dextransucrase solution (Fig. 2a). Thus, the final sucrose concentrations of the solution mixtures were 20, 40, 80 and 160 g/L, respectively. The solution mixtures were cured (or cultured) for 4 days at ambient room temperature. Thereafter, the quantity of dextran produced was measured.

Then, the volumetric mixing ratio between the enzyme solution and the sucrose concentration (i.e., E/S ratio) was controlled. Different volumes of the enzyme solution (2, 4, 10, and 20 mL) were mixed with 20 mL of final 40 g/L sucrose solution. Thus, the E/S ratios were 0.1, 0.2, 0.5 and 1.0. The batch test conditions for the optimization of EIBF is listed in Table 1.

Table 1. Test conditions for EIBF optimization

Type	Enzyme solution vol. (mL)	Sucrose solution vol. (mL)	Sucrose conc. (g/L)	Final Sucrose conc. (g/L)	E/S ratio
A	50	50	40	20	1
A	50	50	80	40	1
A	50	50	160	80	1
A	50	50	320	160	1
B	2	20	44	40	0.1
B	4	20	50	40	0.2
B	10	20	60	40	0.5
B	20	20	80	40	1.0

Quantity of the produced dextran was estimated as

follows. Upon the complete reaction for 4 days, the produced dextran was extracted by centrifugation (RPM 12,000, Time: 15 min, Temp.: 4°C) for several times to obtain pure dextran (Dols et al. 1997). Then, the dextran was dried at 60°C in an oven to measure the mass of dry dextran.

2.4 Permeability test with EIBF

A column experiment was conducted to examine the permeability reduction upon the application of EIBF. Coarse silica sand (Ottawa 20/30) was used in the column test. The sand had the specific gravity of 2.65, the grain sizes of 0.6–0.85 mm with the mean grain size of 0.73 mm. For the EIBF treatment, the E:S ration of 1:2 with the sucrose solution of 40 g/L was chosen to prepare the treatment solution. Per each treatment, 60 mL of the treatment solution was re-injected.

Fig. 3 shows the column setup to monitor the permeability reduction. The sand column for this permeability test was prepared as follows. (i) The treatment solution was poured into the column. (ii) Then, the sterilized oven dried sand was water-pluviated into the column to achieve fully water-saturated condition, minimizing air trapped. Each layer of the sand pack was hand-tamped with a thickness of ~1–2 cm, and the final height of the sand column was ~6 cm. The initial void ratio and the pore volume of the sand pack were ~0.61 and 12.5 cm^3, respectively. (iii) The baseline permeability (or initial hydraulic conductivity; K_o) was measured by injecting 60 cm^3 of the treatment solution. (iv) Thereafter, the sand column was left for 3 to 4 days for production of dextran. (v) Fresh treatment solution of 60 mL was re-injected to the column every 3–4 days. This refilling was repeated for 21 days. Table 2 summarizes the permeability test condition.

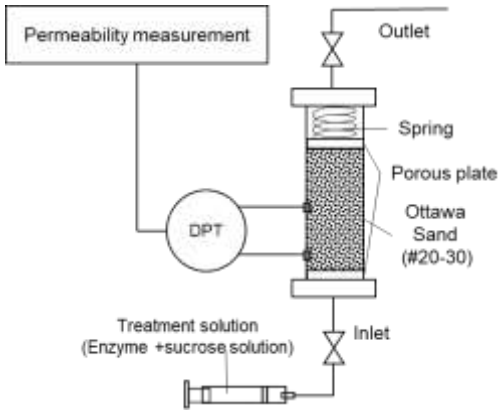

Fig. 3. A schematic drawing of the permeability test setup.

Table 2. Permeability test condition

Host sand	Coarse silica sand
Mean grain size	0.73 mm
Initial void ratio	0.61
Enzyme solution volume	20 mL
Final sucrose concentration of mixed treatment solution	40 g/L
Volume of sucrose solution	40 mL
Total injected volume of mixed treatment solution	60 mL
Treatment interval	3–4 days
Number of treatment	6

3 RESULTS

3.1 Optimization of treatment solution

Effect of sucrose concentration (20, 40, 80 and 160 g/L) on the dextran production quantity was investigated to determine the optimal sucrose concentration. The result showed that the dextran was formed most efficiently at the sucrose concentration of 40 g/L. Although the more dextran was produced with increasing sucrose concentration, a mass of dextran per 1 g of sucrose supplied decreased with an increase in sucrose concentration.

Then, the optimal mixing ratio of enzyme solution to the sucrose solution (i.e., E/S ratio) was determined by controlling the enzyme solution volume to be added to 20 mL of 40 g/L sucrose solution, as listed in Table 1. The E/S ratios were 0.1, 0.2, 0.5 and 1.0, which means the addition of 2, 4, 10, and 20 mL enzyme solution, respectively. As a result, the mass of dextran per 1 g of sucrose supplied increased linearly with the increasing E/S ratio, because of the more enzyme and the greater enzyme activity at the higher E/S ratio. The produced dextran with the E/S ratio of 1.0 was slightly more than that with the E/S ratio of 0.5.

Consequently, the treatment solution was determined to be prepared by mixing the enzyme solution with final 40 g/L sucrose solution at the volumetric ratio of 1:2, which is the E/S ratio of 0.5.

3.2 Permeability reduction test

With the optimized treatment solution, the column experiment was conducted to measure the variation of permeability reduction. The permeability (K) was estimated from differential pressure response using Darcy's law, assuming the laminar flow.

Fig. 4 shows the permeability variation of the sand caused by the formation of insoluble biopolymer by the enzyme reactions. Herein, the permeability was normalized with the initial value. The baseline permeability (initial hydraulic conductivity) was measured as 4.7×10^{-4} m/s. After ~21 days of the experiment, the hydraulic conductivity decreased to 2.0×10^{-5} m/s, which corresponds to ~96% reduction. This permeability reduction of coarse sand by more than one order of magnitude shows the feasibility of using EIBF for bio-sealing applications. During the nutrient refilling process and permeability measurement, the flow was kept at 1 mL/min for ~60 mins. There is possibility that the flow may have washed the produced biopolymers out, therefore, further investigation is warranted to quantify such wash-out effect on

sustainability of bioclogging.

This result can be compared to the result in Kim et al. (2019), in which the bioclogging was caused by bacterial biopolymer formation by the same model bacteria *L. mesenteroides*. It was found that the EIBF reduced the permeability the faster with the fewer treatment times for the similar permeability reduction.

To be applicable in the fields, the behavior and performance of the EIBF method needs to be identified, regarding to effect of enzyme activity, economic production of enzyme, and storage of extracted enzyme solution. Furthermore, the detachment and wash-out of produced biopolymers is required to be examined to identify the durability and sustainability of the induced clogging.

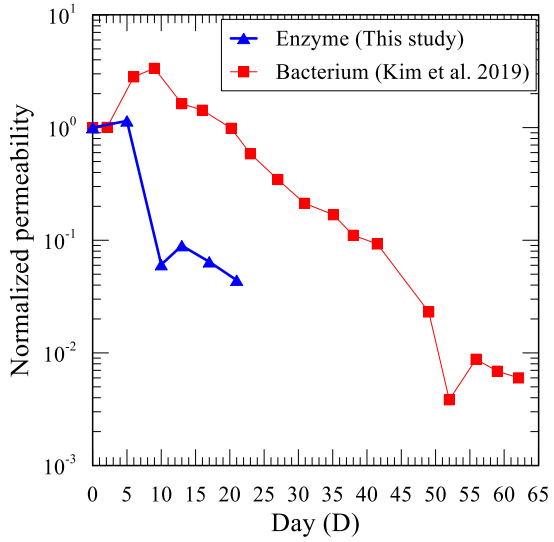

Fig. 4. Variation of permeability during the column experiment.

4 CONCLUSION

In this study, the method to reduce hydraulic conductivity of sands by using enzymes extracted from L. mesenteroides was developed, and named as the enzyme-induced biopolymer formation (EIBF) method. The effects of sucrose concentration and enzyme solution concentration were tested, and it was found that the optimal treatment was achieved at the E/S ratio of 0.5 with 40 g/L sucrose solution. The permeability reduction test was conducted by using the EIBF method, and the permeability was reduced by more than one order of magnitude with three times of the treatment within 12 days.

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