Impact of bacterial biopolymer formation on hydraulic conductivity, erosion resistance, and seismic response of sands

Impact de la formation de biopolymères bactériens sur la conductivité hydraulique, la résistance à l'érosion et les réponses sismiques des sables

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ABSTRACT: Use of microbial activities has garnered a huge interest in recent years, not only because of their ubiquitous processes occurring in natural geo-media, but also because of their versatility in applications to geo-engineering. In particular, *in situ* bacterial colonization and accumulation of biopolymers in subsurface have a profound effect on the physical and chemical properties of soils, influencing fluid flow and transport properties. However, the impact of bacterial biopolymer formation on soil properties as well as the physical properties of soft biopolymer itself remains poorly understood. This study presents detailed laboratory test results and interpretation of the insoluble polysaccharide biopolymer produced by model bacteria *Leuconostoc mesenteroides* and such biopolymer-grown and -accumulated sands. Firstly, the elastic characteristics of dextran with the shear modulus of ~0.1 Pa was confirmed by using particle-tracking microrheology. Secondly, the production and accumulation of insoluble biopolymer observed to lead to readily reduced hydraulic conductivity of sands by more than one order of magnitude and improved the erosion resistance of sands. Lastly, we examined the feasibility of using P- and S-wave responses for monitoring of biopolymer accumulation and permeability reduction in sands. This baseline experimental results presented here show how soft biopolymers formed by bacteria can modify and improve soil behaviors.

RÉSUMÉ : L'utilisation des activités microbiennes a suscité un vif intérêt ces dernières années, non seulement en raison de leurs processus ubiquitaires qui se produisent dans les géo-médias naturels, mais aussi en raison de leur polyvalence dans les applications à la géo-ingénierie. En particulier, la colonisation bactérienne in situ et l'accumulation de biopolymères en subsurface ont un effet profond sur les propriétés physiques et chimiques des sols, influençant l'écoulement de fluide et les propriétés de transport. Cependant, l'impact de la formation de biopolymères bactériens sur les propriétés du sol ainsi que sur les propriétés physiques du biopolymère doux lui-même reste mal compris. Cette étude présente des données d'essai de laboratoire sur le biopolymère (ou dextrane) de polysaccharide insoluble produit par les bactéries modèles *Leuconostoc mesenteroides,* et les sables accumulées et cultivées par tels biopolymères. Tout d'abord, on a confirmé les caractéristiques hautement élastiques du dextrane avec un module de cisaillement de ~0,1 Pa en utilisant une microrhéologie de suivi des particules. Deuxièmement, nous avons observé que la production et l'accumulation de biopolymère insoluble réduisait facilement la conductivité hydraulique des sables de plus d'un ordre de grandeur et améliorait la résistance à l'érosion des sables. Enfin, nous avons examiné la faisabilité de l'utilisation des réponses d'ondes P et S pour la surveillance de l'accumulation de biopolymères et la réduction de la perméabilité dans le sables. Ces résultats expérimentaux de base présentés ici montrent comment les biopolymères mous formés par les bactéries peuvent modifier et améliorer les comportements du sol.

KEYWORDS: Microbe, bacteria, biopolymer, bioclogging, hydraulic conductivity, erosion, shear modulus, P-wave, S-wave.

1 INTRODUCTION

Use of bacterial biopolymers and biofilms has garnered a huge interest in recent years, not only because of their ubiquitous processes occurring in natural geo-media, but also because of their versatility in the applications to geotechnical engineering Indigenous microbial communities in subsurface ubiquitously exhibit their ability to produce insoluble polysaccharidic biopolymers either as their habitats or as byproducts of their metabolisms. Owing to such ubiquity and versatility, it has been studied to utilize *in-situ* formation of bacterial biopolymers as a promising means to cause bioclogging, reducing permeability, increasing strength, and eventually to seal cracks or leakage in geotechnical earth structures (Blauw *et al.* 2009, Cunningham *et al.* 1991, Noh *et al.* 2016, Taylor and Jaffé 1990).

To date, the extent of the effect of bacterial biopolymers on soil properties still remains poorly known as well as the physical properties of soft biopolymer itself. Beginning in 2013, we undertook a series of laboratory measurements to determine the physical properties of insoluble polysaccharidic biopolymer produced by *Leuconostoc mesenteroides* and the geotechnical and geophysical properties of the biopolymer-associated sands. In this manuscript, we present experimental data on the shear moduli of bacterial biopolymer and on the changes in permeability, erosion resistance, seismic responses of sands caused by biopolymer formation (Table 1).

Table 1. The parameters measured and methods used.

Parameter	*Material*	*Method*
Frequency-dependent complex shear moduli	Insoluble dextran	Particle tracking microrheology (PTM)
Hydraulic conductivity	Fine sand	Permeameter
Erosion rate	Fine sand	Erosion function apparatus (EFA)
P-wave velocity and attenuation	Fine sand	Ultrasonic transducer
S-wave velocity and attenuation	Fine sand	Bender element

2 MATERIALS AND METHODS

2.1 *Model bacteria and biopolymer dextran*

Leuconostoc mesenteroides (ATCC 14935) was chosen as the model bacterium. These model bacteria are non-motile and have a size of ~400–500 nm, so they can be readily transported by fluid flow through soils. These model bacteria are facultative anaerobe that can grow under anoxic, high pressure and low temperature conditions, which broadens the applicability. *L. mesenteroides* produces insoluble polysaccharide biopolymer, referred to as dextran, when metabolizing sucrose (Lappan and Fogler, 1996). It does not produce biopolymers when feeding nutrients based on glucose and fructose; this metabolism provides a good control on biopolymer formation.

Throughout this study, we used the defined growth medium, which was referenced from Lappan and Fogler (1996) and Noh et al. (2016). The growth medium contained sucrose, 10 g/L yeast extract, and 0.1M potassium phosphate (pH 7). The quantity of produced dextran differs with the sucrose concentration; thus, we varied the sucrose concentration in the growth medium in accordance with the experiment purpose, ranging from 15 g/L to 300 g/L.

2.2 *Methods*

In this section, the summary of each method is described. More details can be found in Jeon et al. (2017) for the particle-tracking microrheology test, Kim (2017) for the hydraulic conductivity test, Ham et al. (2016) for the erosion test, and Noh et al. (2016) for the seismic test.

2.3.1 *Particle-tracking microrheology*

The viscoelastic properties, in particular frequency-dependent complex shear moduli of insoluble bacterial biopolymer produced by *L. mesenteroides* were obtained using the particle-tracking microrheology (PTM) (Mason *et al.* 1997; Jeon et al., 2017). Herein, we used the growth medium containing 40 g/L sucrose. The inoculum of the model bacteria *L. mesenteroides* was prepared by mixing the culture grown for two days with the fresh growth medium, and the agar medium was prepared by mixing agar with the growth medium at 2% w/w concentration. A droplet of 200 μL inoculum in an exponential growth phase was placed on the agar plate, thereby, the insoluble biopolymer, dextran, was aerobically grown and incubated on an agar surface for 48 h at ~22–24°C. During this incubation, the fluorescent beads (500 nm diameter; F8812, Thermo Fisher) were added to evenly distribute and smear them in the dextran structure. Two-dimensional movements of the fluorescent beads were captured at 100 frames per second using the inverted fluorescent microscope instrumented with the high-speed CCD, and the rhodamine fluorescent light. The tests were performed under an ambient temperature of 25°C.

2.3.2 *Hydraulic conductivity measurement*

Reduction of hydraulic conductivity of sands caused by bacterial biopolymer was investigated using column tests (Kim 2017). A fine sand (Ottawa F110; D_{50} = 0.14 mm) was used as a host soil. The growth medium containing 40 g/L sucrose was used as nutrient to stimulate the growth of *L. mesenteroides* and production of insoluble dextran. An acrylic column (ID = 20 mm, height = 140 mm, internal volume = 44 cm^3) was designed for culturing bacterial biopolymers and monitoring hydraulic conductivity reduction. The column was equipped with one differential pressure transducer (DPT) installed at the two fluid ports in the column to monitor the differential pressure and to estimate hydraulic conductivity.

A sand pack was prepared by water-pluviation in the fresh growth medium and subsequent hand-tamping to achieve a fully saturated condition. Upon the completion of preparing the sand column, the baseline hydraulic conductivity was measured by injecting small volume of the fresh growth medium at constant flow rates. Thereafter, the fresh growth medium of ~five times of the pore volume was fed every 2–3 days for the fresh nutrient supply. This nutrient feed was carried out at a constant flow rate to monitor the pressure difference and the reduction in hydraulic conductivity. After each refilling, the flow was stopped and the column was kept static at the room temperature until the next refilling period. For the first 25 days (phase 1), the fresh growth medium without phosphate buffer was supplied. Thereafter, the fresh growth medium with phosphate buffer was fed to the column (i.e., phase 2; Kim 2017). All column tests were performed at the room temperature of ~25°C.

2.3.3 *Erosion resistance using erosion function apparatus*

The improvement in soil erosion resistance by bacterial biopolymers formed by *L. mesenteroides* was explored by measuring the erosion rates of the biopolymer-grown soil specimens at different flow velocities using the erosion function apparatus (or EFA) (Briaud et al. 2001; Ham et al. 2016). We cultured and stimulated the model bacteria *Leuconostoc meseteroides* to produce insoluble polysaccharide biopolymer, known as dextran, in fine silica sand (D_{50} = 0.32 mm, C_u = 1.77, C_c = 1.16). The sucrose concentration of the inoculum was controlled to be 80, 150, and 300 g/L. The dry and sterilized silica sand was mixed with liquid inoculum, and it was poured and hand-tamped in a thin-walled. The bacteria in the inoculum of each sand-pack specimen was cultured for more than 2 days to produce dextran under an ambient room temperature. For the reference specimen (REF) with no bacteria, a fresh growth medium of 40 g/L sucrose concentration was used as the pore fluid. The erosion rate was obtained at various flow velocities and plotted against the corresponding shear stress to produce the erosion curve.

2.3.4 *Seismic monitoring*

Seismic response, both P- and S-wave, of sands during *in situ* accumulation of bacterial biopolymers were obtained by conducting column experiments (Kwon and Ajo-Franklin 2013, Noh et al., 2016). P-wave response at an ultrasonic frequency range (~hundreds of kHz), and S-wave response at several kHz were acquired by using ultrasonic transducers and bender elements while *L. mesenteroides* were stimulated to produce insoluble dextran. Fine sand (Ottawa F110; D_{50} = 0.11 mm) was used as a host soil. The fresh growth media with 15 g/L sucrose was used for these column experiments, and the sterilized sand was wet-packed by hand-tamping in the column filled with the liquid inoculum. The column experiments continued for more than 20 days up to 40 days. The periodic feed of the fresh media was conducted every 48 h (refilling process), and the volume of the injected fresh growth medium was ~2–3 times of the pore volume in the sand pack. The differential pressure response (ΔP) across the two fluid ports, induced by the fluid flow, was measured to monitor the changes in permeability during the refilling processes based on Darcy′s law. Upon the completion of each refilling process, the column was kept under static conditions until the next refilling process, i.e., pulsed-flow mode. During the injection process, the fluid pressure was elevated and maintained at higher than 300 kPa by using a back-pressure regulator. The transmitted and received P- and S-wave signals were acquired daily during bacterial growth and biopolymer formation. The observed changes in permeability, P- and S-wave velocity, and P- and S-wave attenuation were correlated with the amount of biopolymer produced.

3 RESULTS AND ANALYSES

3.1 *Complex shear moduli of insoluble dextran*

The PTM uses the Brownian motions of small particles caused by thermal energy. The trajectories of these particles can be converted to complex shear modulus G*. The real component G' is the storage modulus and the imaginary component G" is the loss modulus. Figure 1 shows the complex moduli of insoluble dextran *in situ* conditions. The storage modulus and the loss modulus were ~0.1 Pa and ~0.001–0.01 Pa for the frequency range of 0.1–100 Hz. The storage modulus G' was fairly constant over the range of frequency, and its value was larger than the loss modulus G" by more than one order of magnitude. Over the tested frequency range, the results indicate that the insoluble dextran shows the highly elastic behavior. Our shear modulus values were comparable to those of *Staphylococcus aureus* biofilms also measured by PTM though some values from the conventional rheometers were much higher than ours by more than one order of magnitude (e.g., Rogers et al., 2008; Choppe et al., 2010).

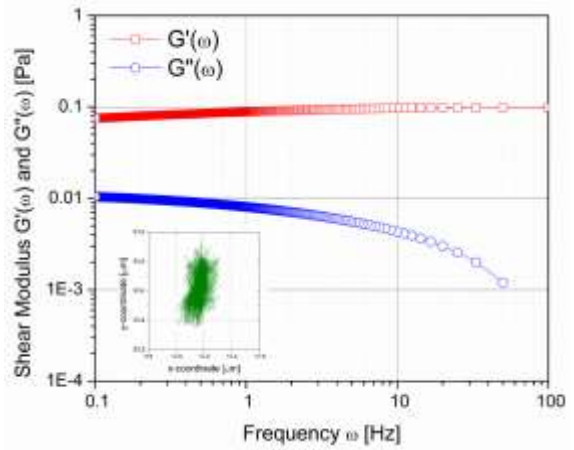

Figure 1. Frequency-dependent complex shear moduli of insoluble dextran matrix. The inset shows the captured trajectories of a fluorescence bead.

3.2 *Reduction in hydraulic conductivity*

The reduction of hydraulic conductivity (or permeability) can be expressed with the orders of magnitude or the reduction ratio in percent. For example, 90% reduction is equivalent to the reduction by one order of magnitude (e.g., from 1 to 0.1 m/s) and 99.9% reduction corresponds to the reduction by three orders of magnitude (e.g., 1 to 0.001 m/s). For successful application of bio-sealing practices, the permeability reduction to be achieved at minimum is usually set as 99% or two orders of magnitude reduction. It appears that the bacterial dextran can readily reduce the hydraulic conductivity of sands by more than one order of magnitude, as shown in Figure 2. The baseline hydraulic conductivity of the fine sand was approximately 4–5×10^{-5} m/s at the beginning. The hydraulic conductivity was observed to decrease to 1–2×10^{-6} m/s (Figure 2b) though the rate of such reduction varied in accordance with the growth medium composition. Moreover, the tendency of hydraulic conductivity reduction seems not converged, which implies that the hydraulic conductivity could be reduced further if the test continued. Figure 2b shows the hydraulic conductivity normalized with the baseline value (k/k_o) with respect to the pore saturation of biopolymer (S_{bp}) in sands. Given the soft but insoluble biopolymer that does not change the void ratio or porosity of the sand-packs, the pore saturation of biopolymer can be correlated to the normalized hydraulic conductivity k/k_o, following the relation suggested by Masuda et al. (1997):

$$k/k_o = \left(1 \quad S_{bp}\right)^n, \tag{1}$$

where n is the empirical fitting exponent. As can be seen in Figure 2b, the exponent n appears to range ~30–90 for the particular type of biopolymer dextran used in our study. Although precisely estimating the quantity of the produced bacterial biopolymers *in situ* is challenging, such assessment in well-controlled laboratory conditions can provide useful information to establish a hydraulic conductivity reduction model and to predict efficacy and applicability of bio-sealing techniques using *in situ* bacterial biopolymer formation at the field scale.

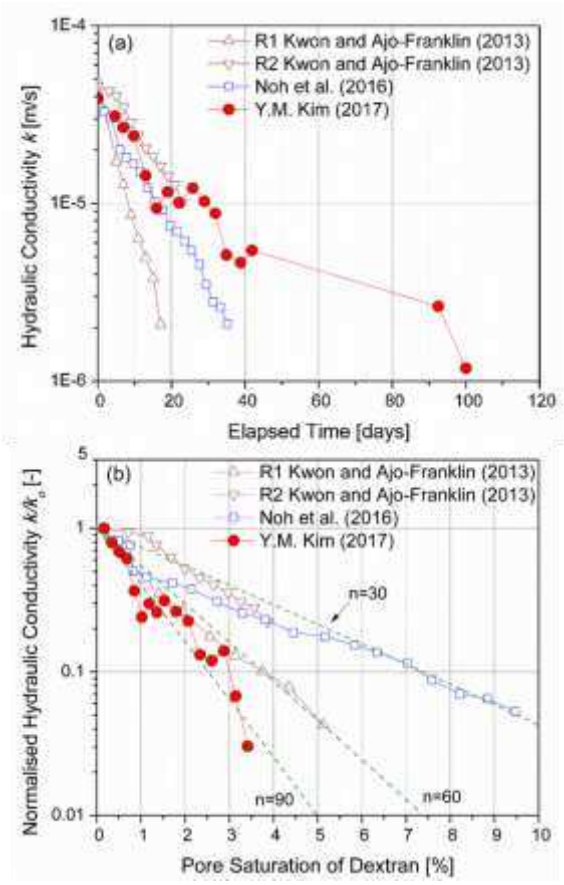

Figure 2. (a) Reduction in the hydraulic conductivity with the time elapsed, and (b) variations in the normalized hydraulic conductivity with the pore saturation of dextran accumulated over the courses of experiments.

3.3 *Improvement in erosion resistance*

Figure 3 shows the erosion rates measured from the EFA tests under various flow velocities. The critical shear stress (τ_c) was found to increase with the amount of dextran. In specimens REF and S80 (sucrose 80 g/L), the erosion began to occur at the shear stress greater than ~0.1 Pa. Only minimal difference between REF and S80 was found in terms of the erosion rates and critical shear stress, possibly owing to the small amount of biopolymer produced in S80. For specimens S150 (sucrose 150 g/L) and S300 (sucrose 300 g/L), the critical shear stress was ~0.18 Pa and 0.23 Pa, respectively, larger than the values for REF and S80, which are attributed to the biopolymers (dextran) formed in soils. The erosion resistance of soils (or erodibility), which is the sensitivity of the erosion rate $\dot{z}$ to the excess shear stress ($\tau-\tau_c$), was also observed to be affected by the bacterial biopolymer formation. In the biopolymer-grown soils, the erosion resistance was seen to be improved as the more dextran produced.

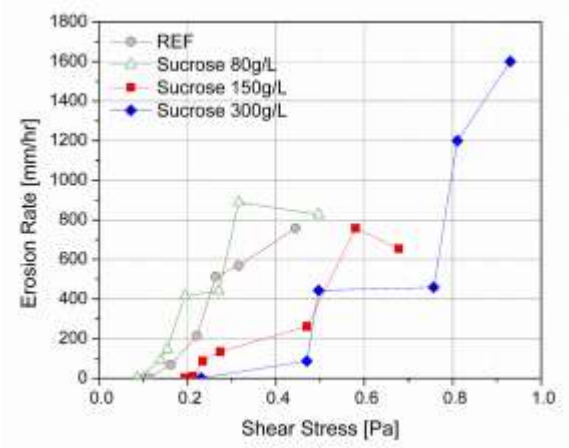

Figure 3. The erosion rates against shear stresses for biopolymer-grown sand specimens.

3.4 *Seismic responses due to biopolymer accumulation*

The identification of spatial and temporal distribution of bacterial biopolymers is critical for successful application of microbial soil treatment in the field. Herein, we present the complied lab test data on high-frequency seismic responses (P- and S-wave velocity and attenuation) during the accumulation of the insoluble bacterial biopolymers (Kwon and Ajo-Franklin, 2013; Noh et al., 2016), as shown in Figure 4.

During the biopolymer accumulation to approximately 10% pore saturation, the P-wave velocity remained constant. Whereas, the P-wave attenuation increased by 1.6 times with significantly reduced amplitudes in the signatures. This is because the insoluble dextran creates new solid-liquid interfaces that cause additional frictional energy loss during wave propagation Note that 10% pore saturation of dextran caused the permeability reduction by 95% (see Figure 2). Meanwhile, the S-wave velocity appeared to increase from 55 m/s to ~80–95 m/s when the effective confining stress was minimal. Therefore, for very near-surface soils, S-wave velocity can be an indicator for biopolymer accumulation. However, it should be interpreted with care because the response may differ for deeper soils with higher effective confining stress. Similar to P-wave attenuation, the S-wave attenuation is seen to increase by 1.6 times.

Theses results suggest that the P- and S-wave attenuation is more relevant to soft and insoluble bacterial biopolymer accumulation in sands and S-wave velocity may have benefits for near-surface soils. Our data is limited to a high frequency (ultrasonic) range; several hundreds kHz for P-wave and several kHz for S-wave. Further investigation should be conducted to identify low-frequency seismic responses during bioclogging.

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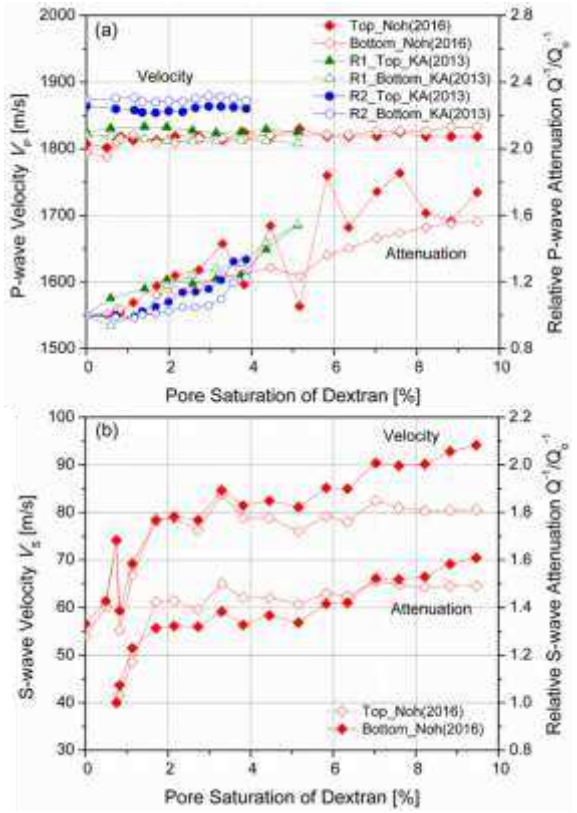

Figure 4. Seismic P- and S-wave responses during biopolymer accumulation: (a) P-wave velocity, and (b) S-wave velocity. KA(2013): Kwon and Ajo-Franklin (2013); Noh(2016): Noh et al. (2016).