Improvement of Surface Erosion Resistance of Sand by Microbial Biopolymer Formation

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Abstract: Direct use of naturally occurring microbes for soil improvement has recently gained attention due to their ubiquitous and versatile characteristics in subsurface soil. Microbes produce soft and sticky extracellular polymeric substances (or biopolymers) that are known to alter the hydrological characteristics of soils; however, the mechanisms and extent of such soft biopolymers in altering soil erosion resistance remain scarcely explored. This study explored the role of microbial biopolymers in soil erosion resistance. The surface erosion resistance of sandy soils was evaluated by using a hybrid erosion function apparatus, in which the model bacteria *Leuconostoc mesenteroides* were stimulated to produce an insoluble biopolymer. The results revealed that the microbial biopolymer formation increased the critical shear stress and surface erosion resistance, which the researchers attributed to the increased cohesion by grain-coating biopolymer slimes and the reduced seepage flows due to pore clogging. This study provides baseline but promising results on how microbially grown biopolymers can be used to improve soil erosion resistance. **DOI: 10.1061/(ASCE)GT.1943-5606.0001900.**

Author keywords: Erosion; Microbial activity; Biopolymer; Soil improvement.

Introduction

Soil erosion occurs once shear stress exerted by moving fluids exceeds a critical value, referred to as the critical erosion shear stress (Jacobs et al. 2011). The erosion of soils is a ubiquitous phenomenon that often leads to scouring and failures of underwater geostructures (Khwairakpam and Mazumdar 2009; Prendergast and Gavin 2014). Accordingly, several techniques have been used to increase the soil erosion resistance, including chemical feeding methods (Agassi and Ben-Hur 1992; Sherwood 1993; Basha et al. 2005), use of cover materials for sea or river floors (Vick 1984; Ahn et al. 2002), and piling (Chiew 1992; Heidarpour et al. 2010).

Direct use of naturally occurring bacteria to strengthen the soil, mitigate earthquake-induced liquefaction, and clean up polluted sites has received increased attention because of not only their ubiquitous occurrence in natural soils but also their versatility in the applications to geoengineering (Karol 2003; Mitchell and Santamarina 2005). Bacterial activity can alter the mechanical and hydrological properties of soils, such as the interparticle bonding, stiffness, strength, and permeability, by producing insoluble biomaterials (Taylor and Jaffé 1991; Mitchell and Santamarina 2005; DeJong et al. 2006; Martinez and DeJong 2009; Kwon and Ajo-Franklin 2013; Noh et al. 2016; Jiang et al. 2016; Jiang and Soga 2016). A biofilm is a mixture of bacterial cells and gel-like extracellular polymeric substances (EPSs). These soft gel-like EPSs (or biopolymers) fill the pores of soils and cause bioclogging by reducing hydraulic conductivity (Baveye et al. 1998; Bouwer et al. 2000; Dunsmore et al. 2004). The potential engineering uses of such bioclogging by biopolymers have been discussed in several previous studies (e.g., Baveye et al. 1998; Ivanov and Chu 2008; Nugent et al. 2010; Chang and Cho 2012; Chang et al. 2015, 2016). On the contrary, the use of biopolymers for improvement of erosion resistance has been limited to few studies (e.g., Orts et al. 2000; Tolhurst et al. 2002; Parsons et al. 2016). However, the mechanisms and the extent of such soft EPS formed by in situ microbial activities in altering soil erosion resistance remains scarcely explored.

This study reports that the soft but insoluble biopolymers formed by in situ microbial activities improve surface erosion resistance of soils. The model bacteria *Leuconostoc mesenteroides* were cultured and stimulated to produce insoluble polysaccharide biopolymer, known as dextran, in fine sand. The surface erosion rates of these biopolymer-grown sand specimens were measured at different flow velocities of water. The contribution of the microbially produced biopolymer to erosion resistance and critical shear stress was quantified in relation to pore saturation of the biopolymer formed in soils, and the possible mechanisms in enhancing erosion resistance are discussed.

Experimental Program

Model Bacterium

Leuconostoc mesenteroides (ATCC 14935) was chosen as the model bacterium because of its advantages. It is nonmotile and

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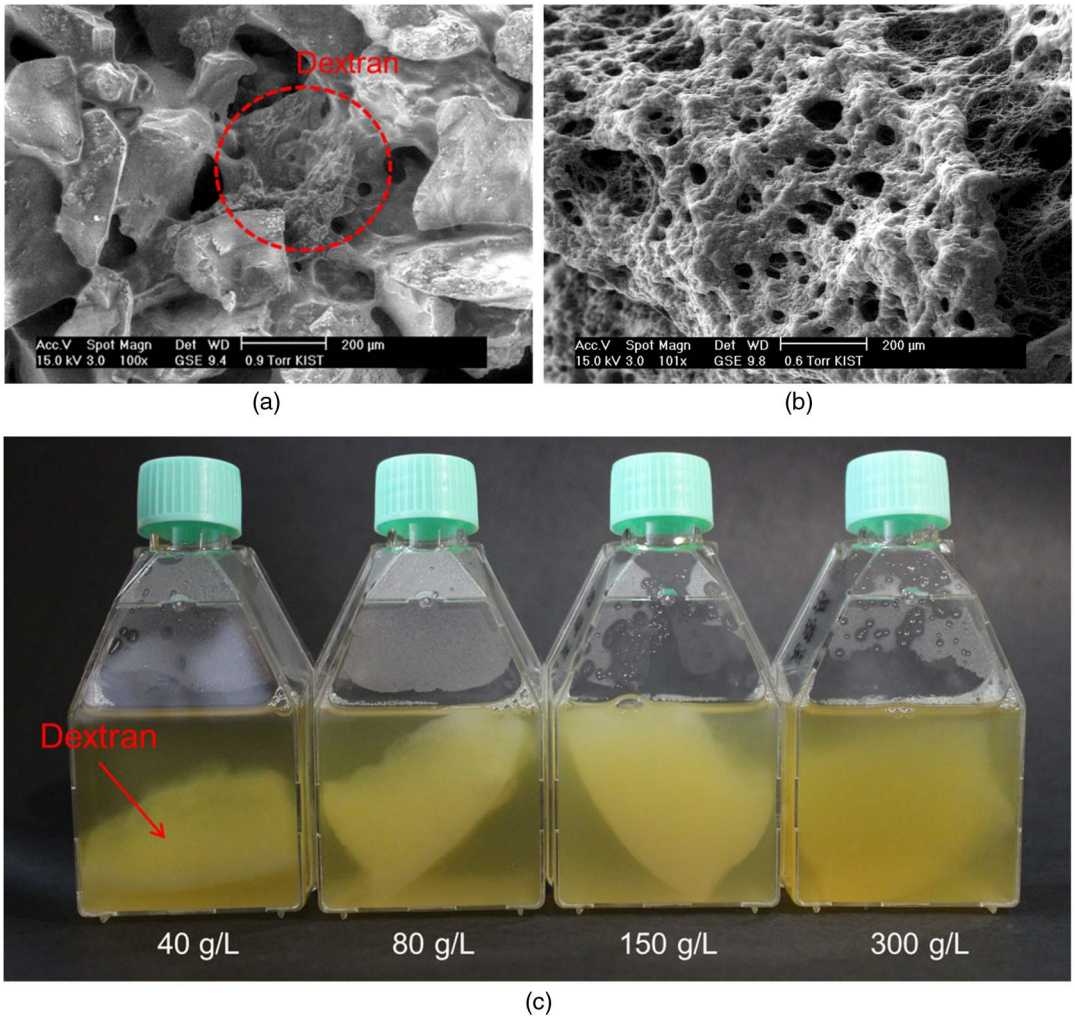

Fig. 1. Images of dextran produced by *L. mesenteroides*: (a) an environmental scanning electron microscopy (ESEM) image of dextran produced in silica sand; (b) an ESEM image of dextran itself; and (c) a digital image of dextran cultured in different sucrose concentrations.

has a size of approximately 400–500 nm, so it can be transported through pores by advective fluid flows. This bacterium is a facultative anaerobe that can grow under anoxic conditions and a high pressure and low temperature, which broadens the applicability. It is edible and harmless to humans (biosafety Level 1). *L. mesenteroides* produces insoluble polysaccharide biopolymer when metabolizing sucrose, as shown in Fig. 1.

The growth medium was defined for bacterial growth and dextran production; it contained 10 g/L yeast extract, 0.1 M potassium phosphate buffer, and sucrose with varied concentrations of 40, 80, 150, and 300 g/L (Table S1) (Lappan and Fogler 1996; Noh et al. 2016). Because increasing the sucrose concentration stimulates the bacteria to produce more dextran [Fig. 1(c)], we chose four sucrose concentrations of 40, 80, 150, and 300 g/L, hereafter, referred to as S40, S80, S150, and S300, respectively. The majority of the produced EPS was identified as dextran via Fourier transform infrared spectroscopy.

L. mesenteroides (ATCC 14935) was acquired from the American Type Culture Collection in a freeze-dried condition. After resuscitation in 100 mL nutrient broth, it was stored as glycerol stocks [2:3 volume/volume (v/v)] at −70°C. Before the experiment, a frozen stock was slowly melted at a low temperature and transferred into the defined growth medium of 10 mL, and this was aerobically incubated at an ambient room temperature. This aerobic culture was used as the start culture for preparing a liquid inoculum that was used for specimen packing.

Specimen Preparation

Fine silica sand, of which grain size ranged from 0.13 to 0.4 mm, was used as the host soil (Fig. 2). Five test specimens with inner diameter of 71 mm and height of 300 mm were prepared by wet-packing the dry and sterilized silica sand in liquid inoculums, as listed in Table 1. The liquid inoculums were mixtures of the start culture at an exponential growth stage and the fresh growth media (1 : 10 v/v). The sucrose concentration of the fresh growth media was controlled from 40 to 300 g/L. Each inoculum was poured into a thin-walled tube (inner diameter: 71 mm), and the wet packing was proceeded to prepare 300-mm-long specimens while achieving fully liquid-saturated conditions. 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 inoculum in each sand-pack was incubated under an ambient room temperature of 22–25°C for more than 2 days. During the bacterial growth and biopolymer formation in the sands, the bacterial respiration generated carbon dioxide which could have changed the specimen volume; therefore, a vertical stress of approximately 25 kPa was applied to all specimens, minimizing the volume change. Because the model bacteria have no motility and no advective fluid flow was induced during incubation, it was presumed that the dextran produced by the model bacteria was homogeneously distributed over the specimen.

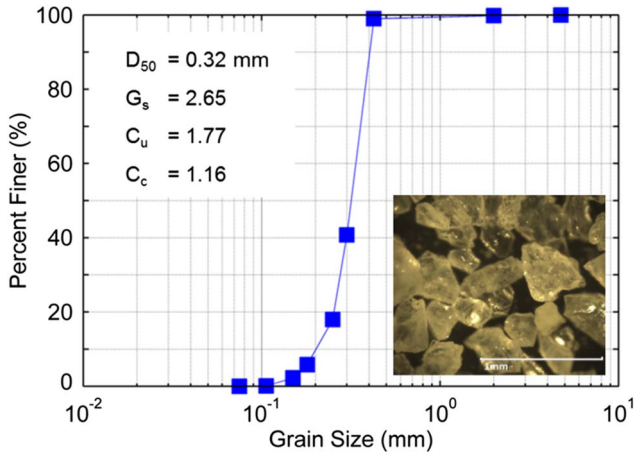

Fig. 2. Grain size distribution of the sand used. D_{50} = mean grain size; G_S = specific gravity of mineral; C_u = coefficient of uniformity; and C_c = coefficient of curvature. Inset: optical microscope image of the sand grains.

Experimental Procedure: EFA Test for Surface Erosion Resistance

The erosion function apparatus (EFA) developed by Briaud et al. (1999, 2001) and instrumented with the P-wave monitoring system by Ham et al. (2016) was used in this study to examine the surface erosion resistance of the biopolymer-grown soil specimens (Fig. S1). Following the test procedure and data reduction described in Ham et al. (2016), the erosion rate $\dot{z}$ can be plotted against the shear stress τ for the tested specimens: this plot is referred to as the erosion curve. The erosion rates were determined by averaging the values obtained from the P-wave monitoring method. We repeated the erosion test more than five times under similar flow velocity conditions to confirm the homogeneous distribution of dextran within the specimens and the consistency of the results.

Quantification of Quantity and Pore Saturation of Dextran

The mass of the produced dextran per inoculum volume at each sucrose concentration and thus the pore saturation of the produced dextran in the compacted silica sand specimens were estimated by performing the additional batch experiment in triplicates. Inoculums were prepared by mixing an aerobic culture of *L. mesenteroides* and the fresh growth media with different sucrose concentrations at a ratio of 1:10 v/v. After 2 days of culturing, the produced insoluble biopolymer in each inoculum was separated by using filter papers (GF4-90, CHMLAB Group) with a 2.7-μm pore size. This filtered biopolymer was oven-dried at 60°C for 2 days, and the mass of the produced dextran was measured. It is believed that the condition of these batch experiments for bacterial growth and biopolymer production was consistent with that of the sand-pack because the silica sand used was clean without any solute or salt, and because the pore size of the sand-pack (~20–40 μm) was much larger than the size of model bacteria (~0.6–1 μm). This is corroborated by the fact that the dextran is extracellularly produced via enzymes secreted by *L. mesenteroides* (Leathers 2005). Assuming a density of 1.50 g/cm^3 (Noh et al. 2016), the pore saturation of biopolymer dextran in each specimen was estimated from the triplicates; the results are presented in Table 1 and more details in Table S3.

Results and Discussion

Results of Erosion Rates and Erosion Curves

Fig. 3(a) shows the erosion curves, which plot the erosion rates against the shear stresses for the specimens. The results are summarized in Table 2 (more detailed data are available in Table S2 and Figs. S2 and S3). REF, S40, and S80 showed no noticeable difference in the critical shear stress τ_c (~0.1 Pa) and in the erosion rate because the amount of produced biopolymer was too small, whereas the greater critical shear stresses were observed for S150 and S300 because τ_c = ~0.19 and ~0.23 Pa, respectively; these were attributed to the biopolymers formed in soils. Furthermore, S150 and S300 have slower surface erosion time for the 1-mm-thick protrusion. At the velocity of 0.22 m/s and τ = ~0.19 Pa, the soil protrusion of S150 did not easily erode for approximately 1,000 s with the surface erosion rate $\dot{z}$ of 3.3 mm/h, whereas the REF specimen eroded in approximately 52 s at a similar velocity of 0.20 m/s with the surface erosion rate of 69 mm/h (see Table S2 and Fig. S2; it also can be seen from Video S1). For S300, the 1-mm-thick soil protrusion did not erode entirely in 2,000 s at a velocity of 0.24 m/s and τ = ~0.23 Pa, resulting in the surface erosion rate $\dot{z}$ of 1.4 mm/h (Video S2). Therefore, more dextran formation in the soils was found to increase surface erosion resistance.

Effect of Biopolymers on Critical Shear Stress and Surface Erosion Resistance

In this study, following Partheniades (1965) and Hanson and Cook (1997), the experimentally obtained erosion curves were modeled with the power relation between the erosion rate $\dot{z}$ and the excess shear stress (τ–τ_c), as

$$\dot{z} = k\left(\frac{\tau - \tau_c}{1\ \text{Pa}}\right)^{\theta} \quad (1)$$

where $\dot{z}$ = erosion rate (mm/h); τ = shear stress; τ_c = critical shear stress; k = erodibility coefficient (mm/h); and θ = power exponent. The erosion resistance parameters k and θ indicate how fast the

Table 1. Description of soil specimens

Specimen	REF	S40	S80	S150	S300
Sucrose concentration (g/L)	40	40	80	150	300
Void ratio	0.77	0.80	0.75	0.76	0.77
Degree of saturation (%)	100	100	100	100	100
Water content (%)[a]	0.29	0.30	0.28	0.29	0.29
Volume of liquid inoculum (cm^3)[b]	517	528	509	513	517
Pore saturation of dextran (%)	0	0.46	0.75	0.95	1.23

[a]Water content was calculated from the void ratio by assuming the specific gravity of silica sand as 2.65 and the density of liquid as 1 g/cm^3.
[b]The diameter, height, and total volume of specimens were 7.1 cm, 30 cm, and 1,188 cm^3, respectively.

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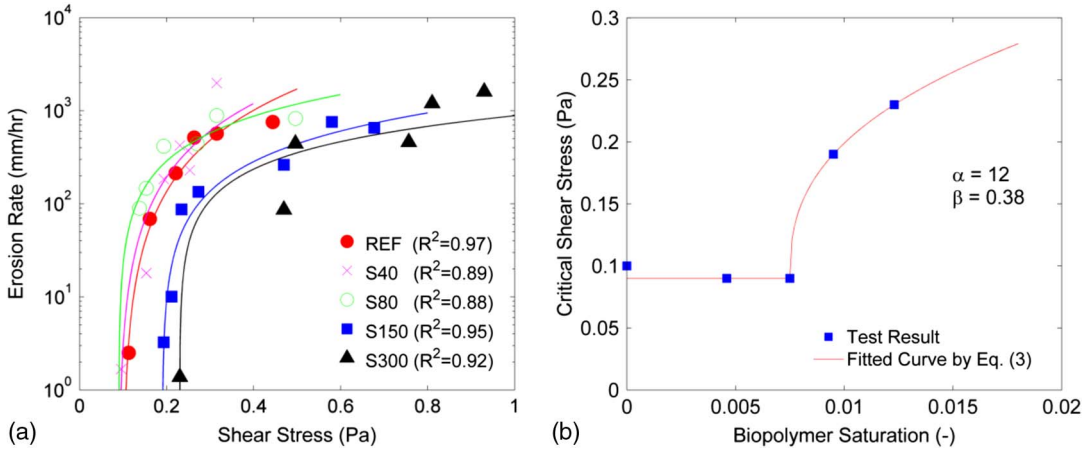

Fig. 3. (a) Erosion curves of the tested specimens; and (b) the variations in critical shear stress with biopolymer saturation.

Table 2. Results of EFA tests

Specimen	Flow velocity, v (m/s)	Shear stress, τ (Pa)	Erosion rate, $\dot{z}$ (mm/h)	Critical shear stress, τ_c (Pa)	Erodibility coefficient, k (mm/h)	Power exponent, θ
REF	0.15	0.10	0.06	0.1	9,132	1.83
	0.16	0.11	2.5			
	0.20	0.16	69			
	0.24	0.22	214			
	0.27	0.26	514			
	0.29	0.32	571			
	0.35	0.44	750			
S40	0.15	0.09	1.7	0.09	9,349	1.76
	0.19	0.15	18			
	0.22	0.19	185			
	0.24	0.23	424			
	0.25	0.25	367			
	0.26	0.26	563			
	0.29	0.32	2,000			
S80	0.14	0.086	0.3	0.09	3,072	1.07
	0.18	0.14	90			
	0.19	0.15	147			
	0.22	0.19	414			
	0.26	0.27	444			
	0.29	0.32	878			
	0.37	0.50	818			
S150	0.22	0.19	3.3	0.19	1,657	1.13
	0.23	0.21	10.1			
	0.25	0.23	87			
	0.27	0.27	134			
	0.36	0.47	261			
	0.40	0.58	750			
	0.44	0.68	655			
S300	0.24	0.23	1.4	0.23	1,110	0.88
	0.36	0.47	86			
	0.37	0.50	444			
	0.47	0.76	462			
	0.49	0.81	1,200			
	0.53	0.93	1,565			

erosion rate increases when the soil is subjected to shear stress increase or the sensitivity of the erosion rate $\dot{z}$ to the excess shear stress (τ–τ_c). The critical shear stress for each specimen was estimated from the erosion curve by following the procedure proposed by Briaud et al. (2001). Then, the erosion resistance parameters were estimated using the test results for which the shear stress was greater than the critical shear stress and the erosion rate was greater than 1 mm/h.

The biopolymer formation is seen to increase the surface erosion resistance as well as the critical shear stress, as shown in Table 2.

The more biopolymer formed, the greater the critical shear stress observed; the lower values for the parameters k and θ imply that a soil is more resistant to shear stress and erosion. In our biopolymer-grown soils, the same tendency of decreasing the erosion resistance parameters was confirmed as more dextran was produced; the power exponent θ decreased from 1.83 for REF to 0.88 for S300 and the erodibility coefficient k was reduced from 9,132 mm/h for REF to 1,110 mm/h for S300 (Table 2).

Relation between Biopolymer Saturation and Critical Shear Stress

The critical shear stress was found to be affected by the amount of dextran, and the correlation between the critical shear stress and the pore saturation of biopolymer (or biopolymer saturation S_{BP}) was examined. As shown in Fig. 3(b), the *critical biopolymer saturation*, which indicates the minimum biopolymer saturation for critical shear stress to increase, was required to model our test results. Despite the limited amount of data, the effect of the biopolymer saturation S_{BP} on the resulting critical shear stress τ_c can be correlated with a more generalized power function as follows:

$$\tau_c = \begin{cases} \tau_{c0} & \text{if } S_{BP} \le S_{BP\_c} \\ \tau_{c0}[1 + \alpha(S_{BP} - S_{BP\_c})^{\beta}] & \text{if } S_{BP} > S_{BP\_c} \end{cases} \quad (2)$$

where τ_{c0} = baseline critical shear stress before biopolymer formation; S_{BP} = biopolymer saturation; S_{BP_c} = critical biopolymer saturation above which the strengthening by biopolymer begins to reveal; and α and β = fitting parameters. In this experiment, the critical biopolymer saturation S_{BP_c} was determined as 0.0075 (or 0.75%), and α and β were estimated to be 12.11 and 0.38, respectively.

Possible Mechanisms of Biopolymer to Enhance Erosion Resistance

Improvement of surface erosion resistance of sands by microbial biopolymer formation was observed in this study, and this is presumably associated with several mechanisms. It is first presumed that the grain coating and bridging by film-like biopolymers increased the apparent cohesion and erosion resistance, as reported in previous literature (e.g., Dunsmore et al. 2004; Chang and Cho 2012; Iltis et al. 2011; Ta et al. 2018). These observations are also consistent with Tolhurst et al. (2002) and Indraratna et al. (2008) who used xanthan, lignosulfonate, and portland cement to increase erosion rate. Another possible hypothesis is that the increased erosion resistance may be attributed to the reduced seepage flow due to the pore-clogging by biopolymer because in addition to the drag force by water flow the contribution of shallow but horizontal seepage force to surface erosion may be nontrivial for the soils with high permeability. In fact, the formation of insoluble dextran produced by *L. mesenteroides* has been reported to significantly reduce soil permeability by more than one order of magnitude even with a small amount of dextran, less than 10% pore saturation (e.g., Lappan and Fogler 1996; Kwon and Ajo-Franklin 2013; Noh et al. 2016). Furthermore, it was consistently observed that the biopolymer-grown sands clearly underwent more severe heterogeneous erosion than the ones without biopolymers (Fig. S4). These observations are consistent with the results observed by Ham et al. (2016), who showed that a higher clay fraction (or fine content) resulted in greater erosion resistance and more heterogeneous erosion behavior. Therefore, both biopolymers and clays as inclusion in sands played a similar role in erosion behavior, increasing the apparent cohesion of the soils and reducing the permeability and void ratio.

Implication and Limitation

The results presented in this study shows that the use of in situ microbial biopolymer formation is promising to improve the erosion resistance of soils. Meanwhile, it is found that the in situ microbial biopolymer formation appears to be less effective in enhancing the erosion resistance, compared to the other biochemical agents, because the quantity of biopolymer produced by bacteria was fairly small (less than 2% pore saturation). Increasing the sucrose concentration in the nutrient solution to be injected or increasing the injection volume of nutrient solution, whether it is periodic or continuous injection, can be considered as a way to increase the amount of microbial biopolymer formation in situ.

For field application, a nutrient solution with a high population of model bacteria can be continuously or periodically injected into ground that needs treatment, where the injected bacteria are expected to produce EPS and enhance the erosion resistance. This is referred to as a bioaugmentation strategy. Alternatively, using a biostimulation strategy, we can design the nutrient composition to selectively stimulate certain groups of indigenous bacteria in the ground to produce EPS, and the designed nutrient solution can be injected. However, further investigation is warranted to find a suitable and efficient method to be applicable for various field conditions including the ground with fast fluid flows. In addition, because dextran is known to be biodegradable by a particular class of enzymes known as dextranases, which are produced by a variety of fungi species (Pleszczyńska et al. 1997; Khalikova et al. 2005), the long-term durability of the biopolymer of particular interest should also be examined with various environmental factors prior to field applications.

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Supplemental Data

Tables S1–S3, Figs. S1–S4, and Videos S1 and S2 are available online in the ASCE Library (www.ascelibrary.org).

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