Supplementary Information for

Engineered Bioclogging in Sands: Comparison of Microbially Induced and Enzyme-induced Biopolymer

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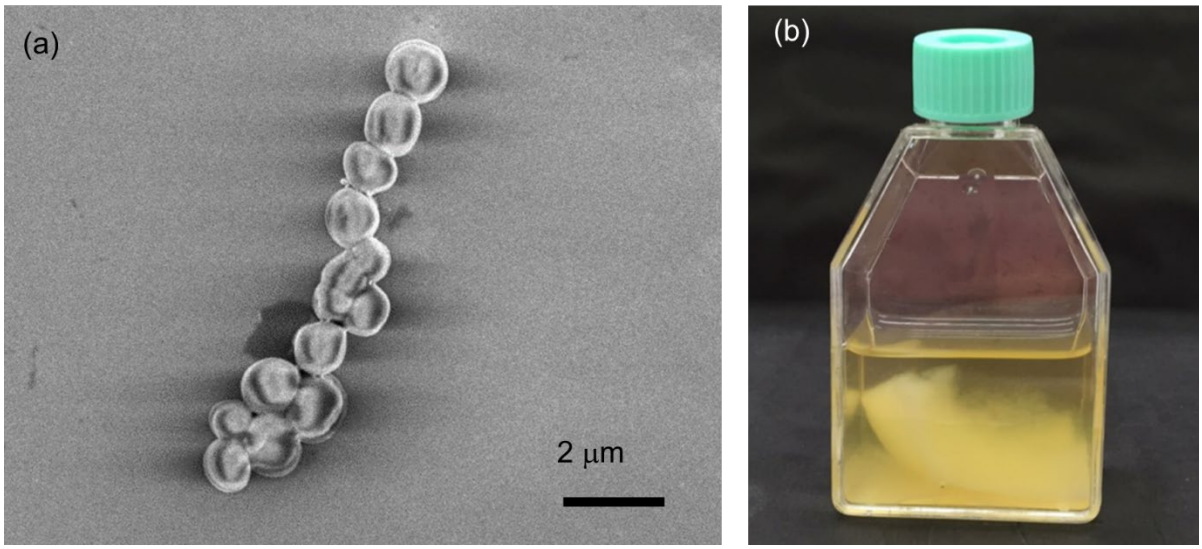

Fig. S1. (a) An SEM image of *Leuconostoc mesenteroides* aerobically grown, and (b) a digital photograph of dextran aerobically produced by the model bacteria *Leuconostoc mesenteroides.*

Table S1. Formulations of treatment solutions

Solution type	Formulation
Enzyme production media from *L. mesenteriodes* (pH = 6.9)	20 g/L Sucrose 10 g/L Yeast extract 40 mL/L 1 M monobasic (KH_2PO_4) 60 mL/L 1M dibasic (K_2HPO_4)
Treatment solution in EIBF-MFC and EIBF-column tests (or *optimized E/S solution*)	E/S ratio = 0.5 Final sucrose concentration = 40 g/L *ex*) Enzyme solution (13.3 mL) mixed with 60 g/L sucrose solution (26.7 mL) at a volume ratio of 1:2
Glucose-based growth media for *L. mesenteriodes* used in MFC test (pH = 6.9; Section 2.3.2)	20 g/L Glucose 10 g/L Yeast extract 40 mL/L 1 M monobasic (KH_2PO_4) 60 mL/L 1M dibasic (K_2HPO_4)
Sucrose-based growth media for *L. mesenteriodes* used in MIBF-MFC tests	40 g/L Sucrose 10 g/L Yeast extract 40 mL/L 1 M monobasic (KH_2PO_4) 60 mL/L 1M dibasic (K_2HPO_4)
Refilling solution in MIBF-column tests for *L. mesenteriodes*	40 g/L Sucrose 10 g/L Yeast extract 40 mL/L 1 M monobasic (KH_2PO_4) 60 mL/L 1M dibasic (K_2HPO_4)

Text S1. Fourier transform-infrared spectrometric (FT-IR) spectrum analysis of dextran formed by MIBF and EIBF

The dextran synthesized by two different methods (i.e., bacterial cell and extracted cell-free enzyme solution) is lyophilized and qualitatively characterized to identify its functional groups using Fourier transform-infrared spectrometric (FT-IR) spectrum analysis. The functional group of dextran, which is prepared as a powder, is compared with that of the commercial dextran sample (Sigma-Aldrich, St. Louis, MO, USA). A spectrometer (Nicolet iS50, Thermo Fisher Scientific) is used to record the FT-IR spectra in the range of 500–4000 cm^{-1}.

FT-IR spectra are used to compare the functional groups of dextran produced by two different methods (i.e., MIBF and EIBF) with standard dextran samples. The FT-IR spectra of dextran are shown in Figure S2. The results exhibit similar spectra among the three dextran. The band at 3270 cm^{-1} is attributed to the hydroxyl(-OH) stretching vibration of the polysaccharide (Güner et al. 2001; Liu et al., 2007). The band at 2930 cm^{-1} indicates the presence of $-CH_2$ stretching vibration (Cao et al., 2006). The peaks measured at ~1630 cm^{-1} are due to the -C-C stretching vibration. The bands at 100 cm^{-1} are attributed to -C-O-C-. By performing a FT-IR spectra comparison, it is confirmed that a cell-free enzyme solution can produce dextran.

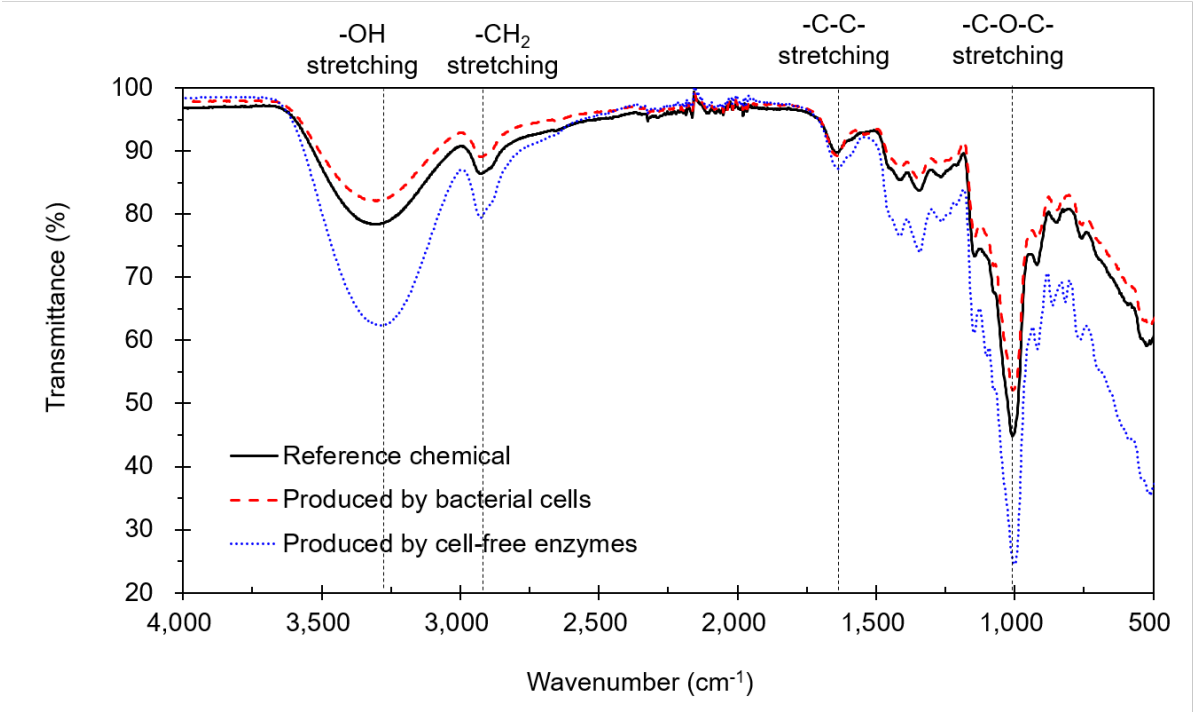

Fig. S2. FT-IR spectra of dextran produced by the model bacteria *L. mesenteroides* and by the extracted cell-free enzyme, compared with the reference dextran, respectively.

References

Cao, W., Li, X. Q., Liu, L., Yang, T. H., Li, C., Fan, H. T., Jia, M., Lv, Z.G. & Mei, Q. B. (2006). Structure of an anti-tumor polysaccharide from Angelica sinensis (Oliv.) Diels. *Carbohydr. Polym.* **66**, No. 2, 149-159.

Güner, A., Akman, Ö. & Rzaev, Z. M. (2001). Crosslinking of dextran with some selective Cl-, P-and N-containing functional substances in aqueous solutions. *React. Funct. Polym.* **47**, No. 1, 55-65.

Liu, C., Lin, Q., Gao, Y., Ye, L., Xing, Y. & Xi, T. (2007). Characterization and antitumor activity of a polysaccharide from Strongylocentrotus nudus eggs. *Carbohydr. Polym.* **67**, No. 3, 313-318.

Text S2. The EIBF optimal condition determined by preliminary batch experiments

A series of preliminary batch tests enabled the determination of the optimal conditions for dextran formation with the extracted cell-free enzyme solution (EIBF). The test variables in this batch experiment program are (1) the enzyme-to-sucrose (E/S) ratio and (2) sucrose concentration. Herein, this study prepared a sucrose solution by dissolving pure sucrose to de-ionized water at pre-determined concentrations. In EIBF batch experiments, the extracted enzyme solution was mixed with the sucrose solution under various conditions (E/S solution). Upon mixing, a time for the enzymatic reaction, more than 72 h, was given to the E/S solution without shaking. After completion of the dextran synthesis reaction, the E/S solution was centrifuged at 12,000 rpm for 10 min to separate a dextran pellet from the supernatant. The separated pellet was centrifuged with deionized water three times under the same centrifugation condition to remove the remaining sucrose. The washed pellets were oven-dried at 60°C for more than 3 days until there were no further mass changes. Thereafter, the mass of the dried dextran pellet was measured. All dextran mass measurements were performed in triplicate.

S1.1 Test program

First, the effect of E/S ratio on dextran formation was examined by adding different amounts of enzyme solution to 20 mL of 60 g/L sucrose solution. The resulting final E/S ratios were 0.05, 0.1, 0.2, 0.5, and 1, which corresponded to 1, 2, 4, 10, and 20 mL of extracted cell-free enzyme solution, respectively, as shown in Table S2. Approximately 72 h was given for dextran synthesis at room temperature of 25°C.

Second, the effect of sucrose concentration on dextran formation was also investigated, in which 20 mL of the enzyme solution was added to 20 mL of sucrose solutions with four different concentrations (40, 80, 160, and 320 g/L) to achieve final sucrose concentrations of 20, 40, 80, and 160 g/L, respectively (Table S3). In addition, we varied the cultivation temperature upon preparing the E/S solution, at the room temperature of 24°C and at 30°C to see the temperature effect.

S1.2 Test result

S1.2.1 Effect of enzyme/sucrose ratio on dextran formation

First, we examined the effect of the mixing ratio between the enzyme solution and the sucrose

solution, or E/S ratio. We varied the volume of the enzyme solution from 1, 2, 4, 10, and to 20 mL to be added to 20 mL of 60 g/L sucrose solution, which corresponded to the E/S ratios of 0.05, 0.1, 0.2, 0.5, and 1.0. The results of this batch experiments are shown in Figure S3 and summarized in Table S2.

The mixed solutions became cloudy as dextran formed (Figures S3a and S3b). The mass of dextran was measured after ~108 h of cultivation, after which there was no further production of dextran. The mass of produced dextran increased with an increase in E/S ratio, and the test with the E/S ratio of 1 produced the greatest amount of dextran (Figure S3c). However, when normalizing it with the enzyme solution volume, the mass of produced dextran per 1 mL of enzyme solution showed the maximum at the E/S ratio of 0.2, which corresponded to the addition of 4 mL of enzyme solution to 20 mL of sucrose solution (Figure S3d). While the mass of dextran per 1 mL of enzyme solution reached 0.0345 g at the E/S ratio of 0.2, the mass of dextran decreased to 0.0179 g at the E/S ratio of 0.5. This study determined the optimum E/S ratio as 0.2–0.5 because this range would produce a sufficient amount of dextran while keeping the efficiency in using the enzyme solution.

S.1.2.2. Effect of sucrose concentration on dextran formation

Secondly, the effect of sucrose concentration on dextran formation was also investigated to determine the optimal sucrose concentration. We mixed 20 mL enzyme solution and 20 mL sucrose solution with the E/S ratio of 1.0, in which the final sucrose concentration varied from 20 g/L to 160 g/L. The results of this batch experiments are shown in Figure S4 and summarized in Table S3.

After 12 h of cultivation, dextran started to form and sank over time due to its self-weight (Figure S4a). The mass of dextran was measured after ~84 h of cultivation, after which there was no further dextran production (Figure S4b). The higher sucrose concentration produced more dextran, as shown in Figure S4c. By contrast, the dextran production efficiency can be assessed with the mass of dextran produced by 1 g of sucrose, which is calculated by normalizing the total dextran mass produced with the initial sucrose mass. It reveals that approximately 0.065–0.15 g of dextran was produced per gram of sucrose, which is ~6.5–15% efficiency. The result shows that the lower the sucrose concentration, the higher the dextran production efficiency (Figure S4d). This study determined the optimal sucrose concentration for the bioclogging experiment to be 40 g/L though the sucrose concentration of 20 g/L showed

the highest production efficiency. It is also important to consider the number of treatments for bioclogging and find the optimal point between the number of treatments and resource management.

In addition, the temperature appeared to affect dextran production; the EIBF reaction at 30°C showed greater dextran production than that at 24°C. This is attributable to the higher enzyme activity at higher temperatures (Santos et al., 2000). The dextran production efficiency of EIBF was also compared with that of MIBF, and the results showed that EIBF had greater efficiency than MIBF. The EIBF at 24°C showed an efficiency of ~6.5–15% and the MIBF at 24°C had an efficiency of ~5.8–11.6%

Reference

Santos, M., Teixeira, J. & Rodrigues, A. (2000). Production of dextransucrase, dextran and fructose from sucrose using Leuconostoc mesenteroides NRRL B512 (f). *Biochem. Eng. J.* **4**, No. 3, 177-188.

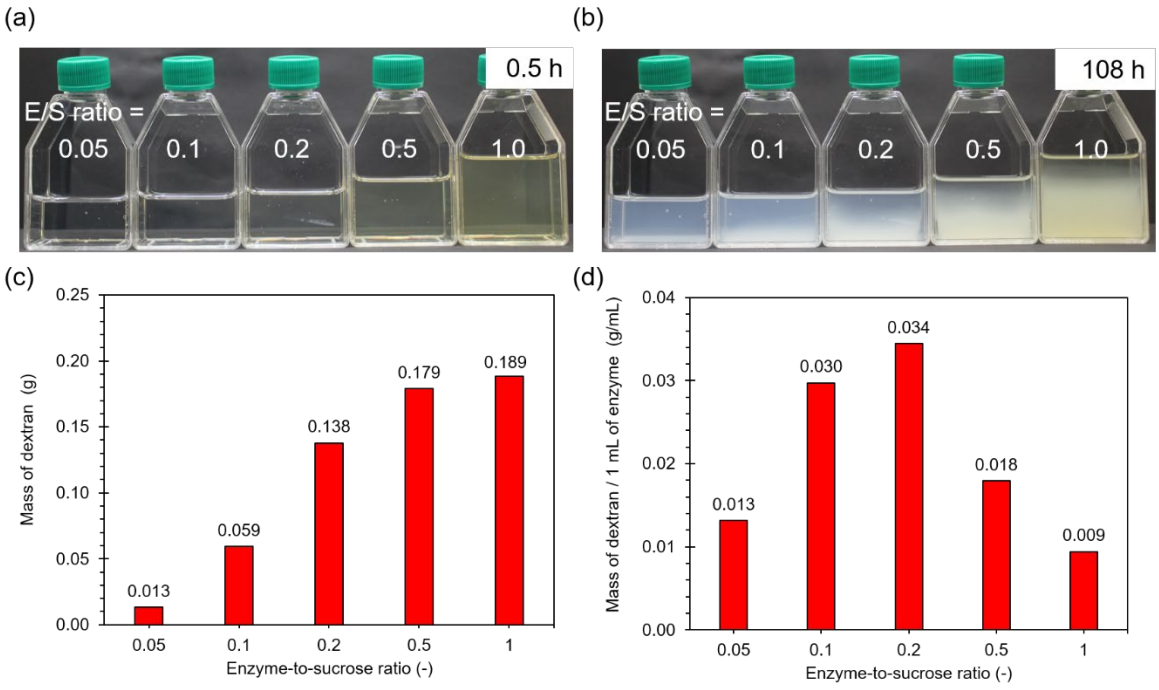

Fig. S3. The effect of enzyme to sucrose ratios (E/S ratio) on the dextran formation: (a) and (b) the digital images of dextran formed by EIBF at 0.5 h and 108 h, respectively, (c) the total mass of dextran, and (d) the mass of dextran produced per 1 mL of enzyme solution

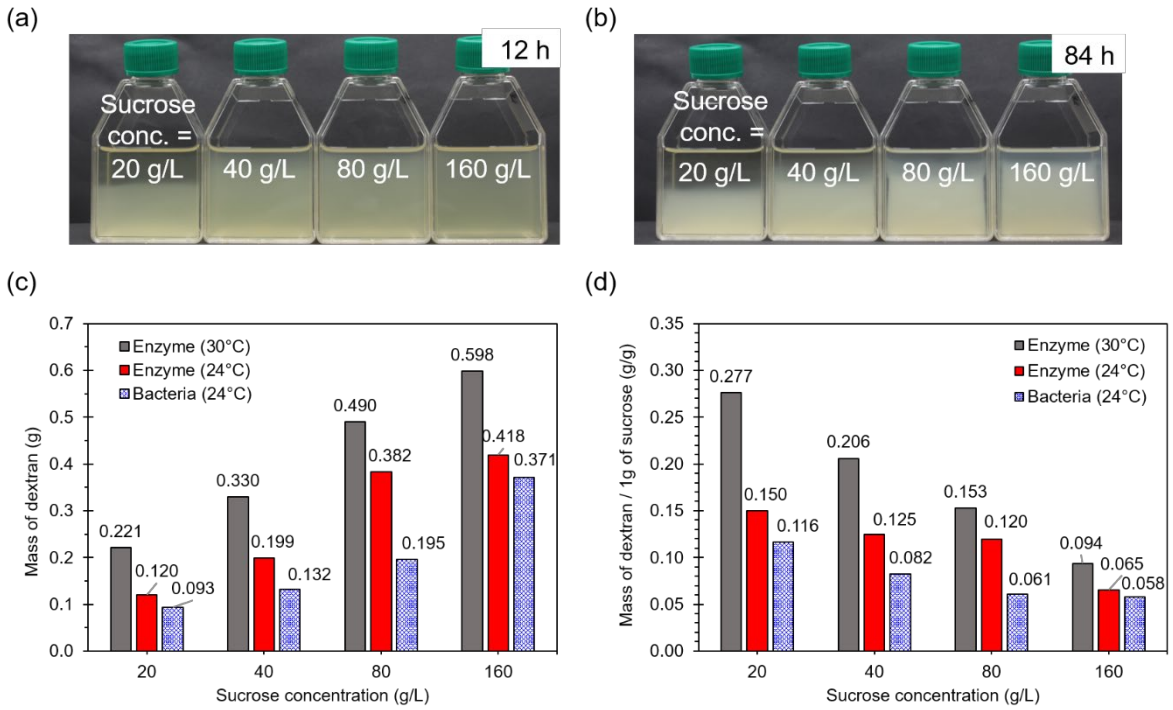

Fig. S4. The effect of sucrose concentration on the dextran formation: (a) and (b) the digital images of dextran formed by EIBF at 12 h and 84 h, respectively, (c) the total mass of dextran, and (d) the mass of dextran produced per 1 g of sucrose. All the tests herein were conducted with the E/S ratio of 0.5.

Table S2. The batch test result (I): the effect of enzyme to sucrose solution ratio (E/S ratio)

		Volumetric ratio of enzyme solution to sucrose solution (or E/S ratio)				
		0.05	0.1	0.2	0.5	1
Volume of enzyme solution (mL)		1	2	4	10	20
Volume of 60 g/L sucrose solution (mL)				20		
Sucrose mass in the mixed fluid (g)				1.2		
Total mixed fluid volume (mL)		21	22	24	30	40
Mass of dextran produced (g)	Sample #1	0.012	0.060	0.132	0.179	0.191
	Sample #2	0.013	0.058	0.131	0.181	0.185
	Sample #3	0.015	0.060	0.151	0.178	0.190
Average mass of dextran produced (g)		0.013	0.059	0.138	0.179	0.189
Average mass of dextran produced per 1 mL of enzyme solution (g/mL)		0.013	0.030	0.034	0.018	0.009

Table S3. The batch test result (II): the effect of sucrose concentration

		Sucrose concentration (g/L)			
		40	80	160	320
Volume of enzyme solution (mL)			20		
Volume of sucrose solution (mL)			20		
Total volume of mixture (mL)			40		
Final sucrose concentration in mixture (g/L)		20	40	80	160
Mass of sucrose in 40 mL mixture (g)		0.8	1.6	3.2	6.4
Mass of dextran produced (g)	Sample #1	0.119	0.196	0.396	0.451
	Sample #2	0.121	0.198	0.373	0.470
	Sample #3	0.120	0.203	0.379	0.333
Average mass of dextran produced (g)		0.120	0.199	0.382	0.418
Average mass of dextran produced per 1 g of sucrose (g/g of sucrose)		0.150	0.125	0.120	0.065

Text S3. Imaging tools and image analysis in microfluidic chip experiments

With the continued fluid feeds, the biopolymer "dextran" was formed and accumulated in the pore space of the microfluidic chips. Digital photographs were acquired every 10 min, and the biopolymer formation patterns and biopolymer pore occupancy were monitored until the pore occupancy reached a pseudo-steady state. The experiments with large microfluidic chip used an optical microscope with 40× and 100× magnifications which acquired local images within a chip. Whereas, the experiments with small microfluidic chip used a high-resolution digital single-lens reflex (DSLR) camera (Canon EOS 100D, Tokyo, Japan) equipped with a 100-mm micro-lens (Canon 100 mm 2.8f macro lens, Tokyo, Japan) which enabled acquisition of a whole microfluidic chip image at a frame. This allowed the quantitative estimation of the biopolymer pore occupancy.

The image analyses with the images acquired from the small microfluidic chip tests allowed to delineate the accumulated biopolymers in the chip and estimate the size of the flocs and the variation in pore occupancy. First, the object detection method via color contrast, embedded in Photoshop 2021, delineated the biopolymer edges from a raw image. We then labelled the pixels inside the biopolymer edges as the biopolymer pixels, and the image was binarized into the biopolymer pixels and the background pixels. As a result, the pore occupancy was calculated by dividing the number of biopolymer pixels by the number of pore pixels and the area of individual biopolymer flocs was also assessed by using ImageJ. Note that this approach provides only an apparent indicator to the biopolymer formation rate because the biopolymer may not occupy the entire aperture height and because the density of the accumulated biopolymer may vary between pixels.

Text S4. Estimation of biopolymer saturation from effluent analysis

Sucrose consumption between refilling indicates the amount of dextran produced in sand columns. In three column experiments (i.e., MIBF_R2, MIBF_R3, and EIBF_R1), the sucrose concentration in the effluent fluid which flowed out during refilling was measured using a standard colorimetric assay kit (Abcam, Cambridge, UK). The effluent fluid samples were deproteinized with acetonitrile (ACN) and filtered with a 0.2-μm PVDF syringe filter. Then, the optical density was measured using a UV spectrometer at a wavelength of 570 nm and compared with the calibrated values of known standards. The pore volume of the sand pack in column experiments was approximately 12.5 mL, and the refilling volume was 60 mL which was approximately 4–5 times of the pore volume. Therefore, it can be assumed that the pore fluid's sucrose concentration almost reaches that of the fresh refilling media after refilling 4–5 times of the pore volume. The fresh refilling media in those runs contain 40 g/L sucrose, and the consumed sucrose concentration (g/L) can be calculated. Thereafter, the amount of consumed sucrose (g) is calculated by multiplying the pore fluid volume (~12.5 mL) for every refilling period. The consumed sucrose mass $M_{sucrose}$ is converted to the mass of biopolymer M_{bp} through the sucrose-to-dextran conversion ratios of EIBF and MIBF. These ratios were determined as 0.153 for EIBF and 0.085 for MIBF from the batch experiments. Finally, the pore saturation of biopolymer S_{bp} is estimated as follows:

$$S_{bp} = \frac{V_{bp}}{V_{pore}} = \frac{(M_{bp}/\rho_{bp})}{V_{pore}}, \quad (1)$$

where V_{bp} and V_{pore} are the biopolymer volume and pore volume, respectively. M_{bp} and ρ_{bp} are the biopolymer mass and biopolymer density, which is assumed to be 1.5 g/cm^3 based on the literature values (Noh et al., 2016). Note that the biopolymer saturation estimation for MIBF_R2 takes into account the pore fluid volume displaced by biogenic gas generation.

Reference

Noh, D.H., Ajo-Franklin, J.B., Kwon, T.H., & Muhunthan, B. (2016) P and S wave responses of bacterial biopolymer formation in unconsolidated porous media, *J. Geophys. Res.-Biogeosci.* **121**, No. 4, 1158–1177.

Text S5. Sampling and pretreatment for SEM

Soil particle samples were obtained by inserting a thin-walled cylinder, made of a copper sheet, to prevent structural disturbance in biopolymer formed between grains when collecting the sand grains for SEM. Both ends of the cylinder were then capped and secured with short segment of silicone tubing blocked with fine nylon mesh (Vandeviver and Baveye, 1992). The cylinder was immersed overnight in 3% (v/v) of glutaraldehyde solution at 4 °C. The cylinder was then rinsed with phosphate buffer saline solution at pH 7.4 for 1 h. Thereafter, the rinsed sample was dehydrated with an ascending series of ethanol-water mixtures (25%, 50%, 75%, 95%, and 100% for 10 min each, v/v) at ambient room temperature.

Reference

Vandevivere, P., & P. Baveye. 1992. "Sampling method for the observation of microorganisms in unconsolidated porous media via scanning electron microscopy." *Soil Sci.* **153**, No. 6, 482-485.