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# Measuring microrheological properties of porous biopolymers in liquid

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## ABSTRACT

With growing interests in utilizing soft biological materials for soil improvement, it is crucial to understand the mechanical characteristics biopolymers to predict the behavior of the biopolymer-treated soils. This study presents rheological parameters of insoluble and porous polysaccharidic biopolymer, which has a potential to be used for soil enrichment. Due to the internal porosity and high heterogeneity of dextran, the traditional rheometer would be inadequate to measure representative properties. Instead, particle-tracking microrheology technique was employed to obtain reliable rheological properties of dextran in liquid where Brownian motions of small fluorescent beads trapped in porous dextran structure were recorded at 100 Hz. The frequency-dependent complex shear moduli were found; the storage modulus ranged ~0.01–0.1 Pa and the loss modulus was in the range of ~0.001–0.1 Pa in the frequency of ~1–100 Hz.

## 1. INTRODUCTION

Formation of bacterial biopolymers or biofilms is considered as an efficient way to reduce permeability, cause bioclogging, and treat cracks or leakage (Mitchell and Santamarina 2005, Pintelon et al. 2012, Noh et al. 2016). While identification of interactions between soil particles and bacterial biopolymers and biofilms is important,

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as the first step, it is necessary to measure the rheological properties of biopolymer itself.

However, a traditional rheometer that measures bulk properties of materials has limitation in measuring micro-scale local properties of biopolymers that are inherently porous and inhomogeneous. Therefore, in this study, particle-tracking microrheology (PTM) was used to obtain local and microscale rheological properties of bacterial biopolymers while overcoming limitation of the rheometer. PTM uses the Brownian motions of thermally excited small particles. The trajectory of these particles can be converted to the diffusion coefficient of surrounding materials using the Stokes-Einstein relation (Mason and Weitz 1995, Wirtz 2009, Dasgupta and Weitz 2005). The movement of particles are only affected by surroundings of particle, hence the obtained results represent the local properties of the surrounding materials. In this study, we presents the frequency-dependent shear modulus of *in situ* insoluble dextran by PTM.

## 2. MATERIALS AND METHODS

### *2.1 Model bacteria and biopolymers*

*Leuconostoc mesenteroides* NRRL-B523 (ATCC 14935) was used to produce insoluble bacterial biopolymer, dextran. These model bacteria are gram-positive and facultative anaerobes. In the presence of sucrose, the bacteria produce not only soluble dextran but also insoluble dextran (Padmanabhan and Kim 2002). Dextran, a polysaccharide having $\alpha$-1, 6 glycosidic bonds, is produced by dextransucrase, the enzyme converting sucrose to dextran and fructose. The growth medium consisted of 40 g/L sucrose, 10 g/L yeast, and 1M phosphate buffer (1M monobasic phosphate 41 ml/L and 1M dibasic phosphate 59 ml/L).

### *2.2 Sample preparation*

The model bacteria and the biopolymer were grown on an agar plate of 2 mm thickness at 30°C. The agar medium was prepared by mixing the growth medium and 2% agar (Difco Bacto agar). A droplet of 200 $\mu$L inoculum in an exponential phase was placed on the agar plate. The fluorescent bead solution (0.5 $\mu$m diameter; F8812, Thermo Fisher) was diluted with the growth medium (1:10000 v/v). After 12 hours, when small amounts of dextran were produced, the diluted fluorescent bead solution of 100 $\mu$L was spread every two hours on the produced dextran surface for 12 hours. The addition of the beads were carefully carried out to evenly distribute them in the dextran structure.

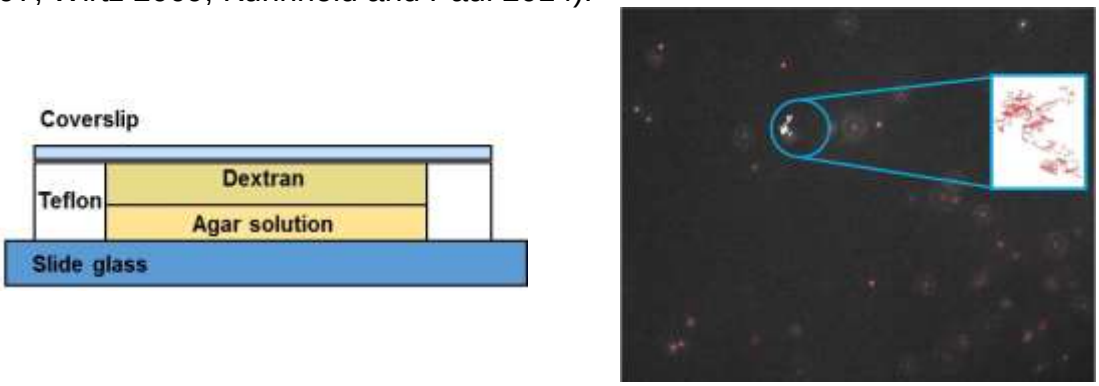
### *2.3 Experiment methods*

Two-dimensional trajectories of fluorescent microspheres were captured by an inverted fluorescent microscope (Axio Lab.A1) with 100x and high-speed CCD (Zyla sCMOS 5.5; Andor) at 100 frame per seconds. Duration of recording was 10 s, thus total 1000 images were acquired. From these images, two-dimensional trajectories of the selected bead were obtained, as shown in Fig. 1 (b), and the corresponding mean square displacements (MSD) were calculated by using Eq. (1) with the assumption of the isotropic and homogeneous sample. From the Stokes-Einstein relationship shown in Eq. (2), the complex modulus can be obtained:

$$< \Delta r(\tau)^2 >=< (r(t+\tau)-r(t))^2 >=< (x(t+\tau)-x(t))^2 > + < (y(t+\tau)-y(t))^2 >= 4D\tau\,, \quad (1)$$

$$\tilde{G}(s)=\frac{k_B T}{\pi a s < \tilde{r}^2(s) >}\,, \quad (2)$$

where *r(t), x(t),* and *y(t)* are the coordinates at time *t*, *D* is the diffusion coefficient, $\tau$ is the lag time, *s* is the Laplace frequency, $k_B$ is Boltzmann constant, *T* is the absolute temperature and *a* is the radius of a spherical bead (Mason and Weitz 1995, Mason et al. 1997, Wirtz 2009, Kuhnhold and Paul 2014).



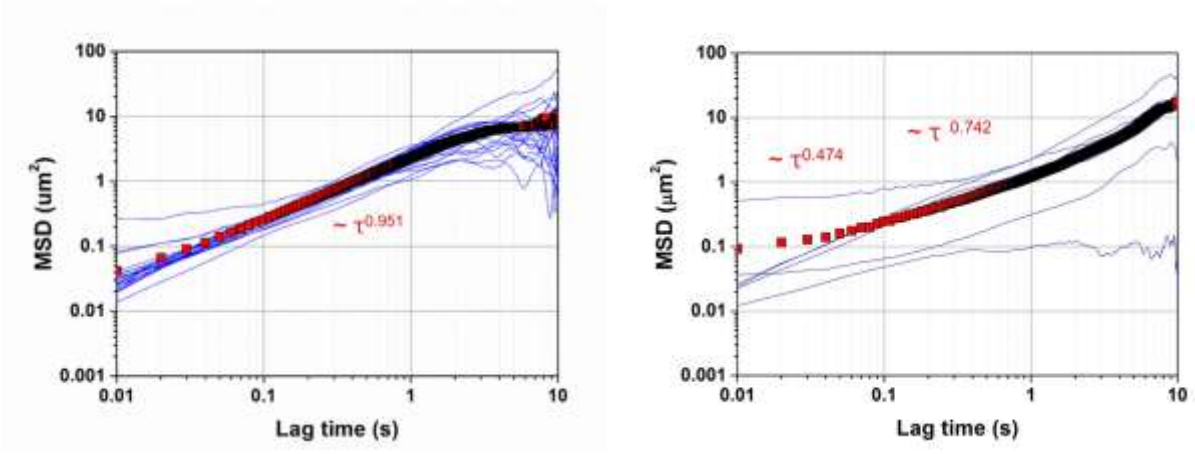

(a) (b)

Fig. 1. (a) A diagram of a dextran sample, and (b) an image of fluorescent beads and one representative trajectory.

## 3. RESULTS AND DISCUSSION

### *3.1 MSD results of beads*

In a homogeneous medium, MSDs from each bead are almost the same. However, it was found that the obtained MSDs were categorized into two groups according to its slope, as shown in Fig. 2. For the first type (type A), the average MSD was correlated to the lag time with a power function and its exponent of ~0.95 (MSD ~ $\tau^{0.951}$). Because the MSD of pure viscous liquid typically shows the exponent close to 1, the result of type A is presumed to be a viscous liquid. For the second type (type B) the MSD was related to $\tau^{0.474}$ at 10–100 Hz and to $\tau^{0.742}$ at 1–10 Hz, of which the exponents were less than 1. These different results of the MSD against lag time are presumably caused by the heterogeneity of dextran, in particular internal porosity. The presence of internal porosity was also confirmed from the scanning electron microscopy (SEM) images of dried dextran samples, in which porous dextran was observed with various pore sizes, as shown in Fig. 3. Therefore, it was thought that the tracked beads of type A were in large pores of dextran, indicating the beads floating in a viscous liquid growth medium. Whereas, we concluded that the beads of type B were properly trapped in dextran structures, and their MSD results were selected for calculation of complex moduli of insoluble dextran.




(a) (b)

Fig. 2. MSD results of type A (a) and type B (b). Red squares indicate the averaged values.

*3.2 Frequency-dependent shear moduli of insoluble dextran*

The complex shear moduli were estimated by fitting the obtained MSDs of type B with the generalized Stokes-Einstein equation (Dasgupta et al. 2002), which is as follows:

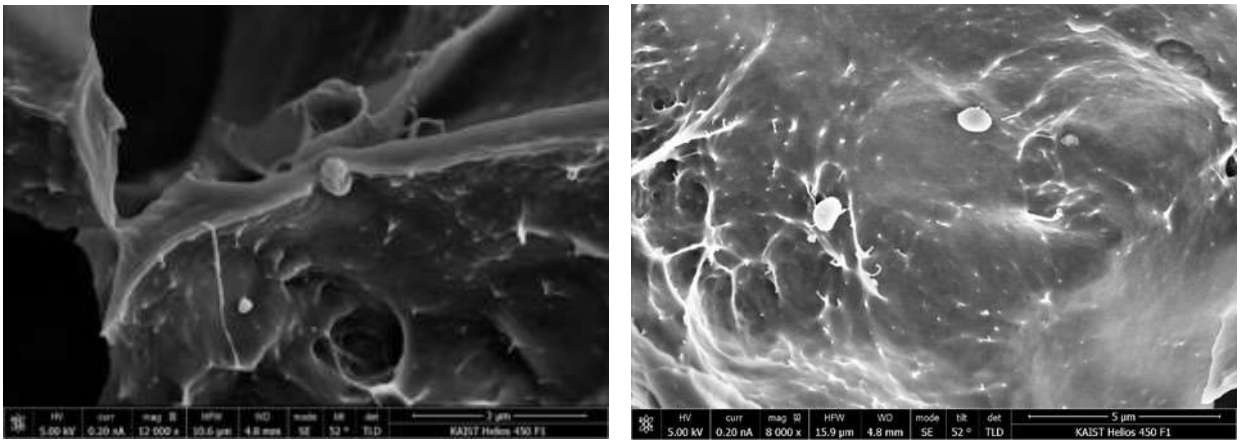
$$G(\omega) = \frac{k_B T}{\pi a < \Delta r^2(1/\omega) > \Gamma(1+\alpha(\omega))[1+\frac{\beta(\omega)}{2}]}, \quad (3)$$

$$G'(\omega) = G(\omega)\frac{1}{1+\beta'(\omega)}\cos\left[\frac{\pi}{2}\alpha'(\omega) - \beta'(\omega)\alpha'(\omega)\left(\frac{\pi}{2}-1\right)\right], \text{ and} \quad (4)$$

$$G''(\omega) = G(\omega)\frac{1}{1+\beta'(\omega)}\sin\left[\frac{\pi}{2}\alpha'(\omega) - \beta'(\omega)\left[1-\alpha'(\omega)\right]\left(\frac{\pi}{2}-1\right)\right], \quad (5)$$

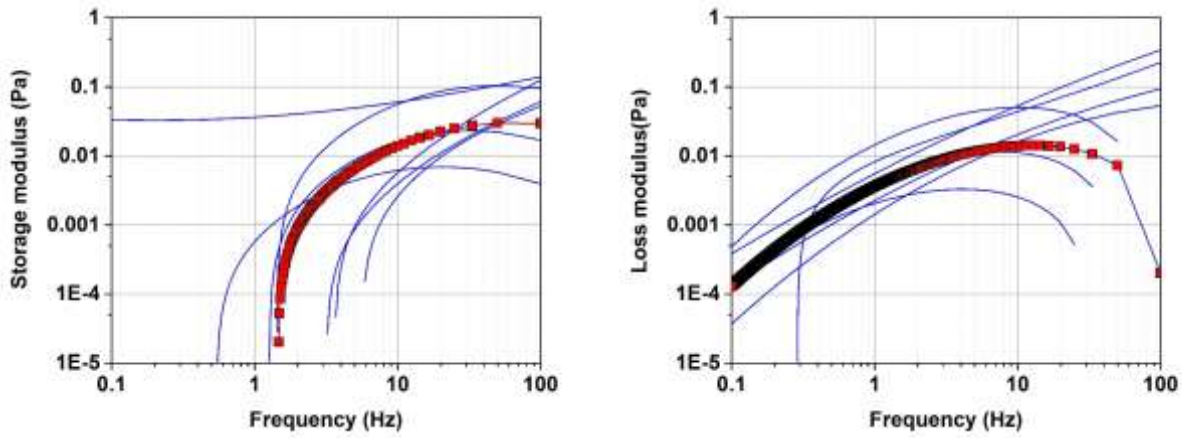
where $\Gamma$ denotes the gamma function, $\alpha(\omega)$ is the first-order logarithmic time derivatives of MSD, $\beta(\omega)$ is the second-order logarithmic time derivatives of MSD, $\alpha'(\omega)$ is the local first-order logarithmic derivatives of G($\omega$), and $\beta'(\omega)$ is the local second-order logarithmic derivatives of G($\omega$). G'($\omega$) is the storage modulus and G"($\omega$) is the loss modulus.

Fig. 4 shows the calculated complex shear moduli of dextran. The storage modulus was found to range from 0.01–0.1 Pa in the frequency range of 1–10 Hz, showing a slightly increasing pattern with frequency. The loss modulus also increased with the frequency and ranged ~0.001–0.1 Pa at the same frequency range. The presented PTM method revealed that the *in situ* insoluble dextran in liquid showed a frequency-dependent complex shear moduli in the studied frequency range of 0.1–100 Hz.



(a) (b)

Fig. 3. SEM images of freeze-dried insoluble dextran with fluorescent beads. (a) dextran with large pores, and (b) beads attached on the dextran surface.




(a) (b)

Fig. 4. Results of type B: (a) storage moduli and (b) loss moduli. Red squares were calculated from the average MSD.

## ACKNOWLEDGEMENT

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