The 2016 World Congress on
Advances in Civil, Environmental, and Materials Research (ACEM16)
Jeju Island, Korea, August 28-September 1, 2016

Measuring Elastic Moduli of Bacterial Biofilms in a Liquid Phase using Atomic Force Microscopy (AFM)

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ABSTRACT

With the increasing demands for innovative and sustainable construction materials and techniques, biological methods using bacteria are attracting huge interests nowadays. To use biological methods properly, mechanical properties of biological materials need to be identified. But mechanical properties of biological materials have rarely considered in the field of geomechanics and geotechnical engineering. In this study, atomic force microscopy (AFM) was deployed to investigate quantitatively the nanoscale Young's modulus of biofilms produced by *Shewanella oneidensis* MR-1. The indentation tests were performed on a biofilm sample grown on an agar surface using AFM with a tip having a silicon dioxide sphere. The obtained force-indentation curves were fitted to the Hertzian model and the Young's modulus of the biofilm was estimated to be ~29 kPa.

1. INTRODUCTION

With the increasing demands for sustainable and eco-friendly construction materials and techniques, biological methods have received significant interest due to their eco-friendly characteristics and potentials in geo-engineering applications, including soil improvement, hydraulic barrier installation, and microbially enhanced oil recovery (MEOR). Among numerous microbial activities and products, formation of biofilm has been used for the purposes of bioclogging (Dennis and Turner, 1998; Thullner et al., 2002) and enhancement of shear strength (Ivanov and Chu, 2008). The biofilm is known as an aggregate of microorganisms imbedded in a matrix composed of

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microbially produced extracellular polymeric substances (EPS), which consist of protein, polysaccharides, nucleic acids, and lipids, and attached to a surface (Lewandowski and Beyenal, 2013).

It is important to understand mechanical properties of soft gel-like biofilm to apply the biological methods effectively. For example, Stoodley et al. (1999) measured the elastic moduli of biofilm in the range of 17 to 40 N/m^2 by observing the structural deformations caused by changes in hydrodynamic shear stress. The elastic moduli of *Pseudomonas aeruginosa* biofilm was determined by using a uniaxial compression measurement device; and the elastic moduli was estimated to be ~6–7 kPa (Körstgens et al. 2001). In this study, Atomic force microscopy (AFM) was used to observe the elastic moduli of *Shewanella oneidensis* MR-1 biofilm in a liquid phase. The indentation tests were performed on a biofilm sample grown on an agar surface using AFM with a tip having a silicon dioxide sphere. The obtained force-indentation curves were fitted to the Hertzian model to estimate the Young's modulus of the biofilm.

2. Material and Method

2.1 Model bacteria and biofilm

Shewanella oneidensis MR-1 strain was chosen in this experiment. *Shewanella oneidensis* MR-1 is a Gram-negative facultative bacterium which is often found in marine sediments. *Shewanella* species have the ability to reduce a broad spectrum of metals and organic compounds (Venkateswaran et al., 1999). There are several reasons to select *Shewanella oneidensis* MR-1 as a model bacterium; they can grow both anaerobic and aerobic conditions and are harmless to humans.

2.2 Sample preparation

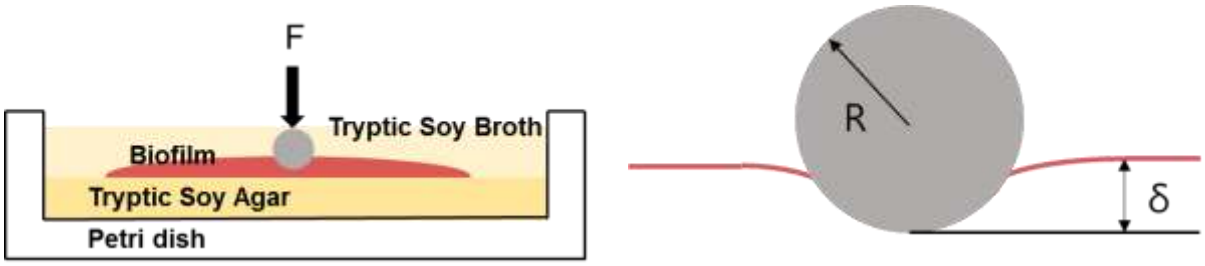
The biofilm was prepared on an agar plate. Before preparation on an agar plate, the bacteria was cultured in the prepared growth media during 24 hours. The growth media consisted of tryptic soy broth (TSB; Sigma-Aldrich Co), phosphate buffer, and sodium fumarate dibasic to stimulate the growth of the bacteria, as shown in Table 1. Using the designed growth media, *Shewanella oneidensis* MR-1 was cultured in a batch at the room temperature and atmospheric pressure.

Table1. Composition of growth media for *S.oneidensis* MR-1

Compound	**Concentration**
Tryptic soy broth	30 g/L
Sodium fumarate dibasic	16 g/L
Monobasic – KH_2PO_4	13.609 g/L
Dibasic – K_2HPO_4	17.418 g/L

Prior to culture biofilms on an agar plate, a tryptic soy agar plate was prepared in a petri dish by mixing TSB and the agar powder in 2:1 mass ratio. Thereafter, 10 μL droplet of the inoculum from *S. oneidensis* liquid culture was placed on the prepared agar plate. The inoculum was prepared after 24 h growth in the growth media. The bacteria placed on the agar plate was cultured at 30°C in an incubator for 24 h. Prior to

indentation test using AFM, the fresh growth medium was poured to submerge the cultured biofilms in a liquid phase (Fig. 1a).



(a) (b)

Fig. 1. (a) A schematic diagram of the sample, and (b) a schematic diagram of the Hertzian contact model for a spherical indentor on a half-elastic plane.

2.3 Atomic Force Microscopy (AFM)

The indentation tests were conducted on the cultured biofilm that was submerged in liquid using a commercial AFM (XE-100, Park Systems) to observe in-situ mechanical property. The tests were carried out at the room temperature of ~22–24°C. To obtain force-distance curves, a triangular shaped cantilever with the stiffness k = 0.17 N/m, made with silicon nitride, was used. At the end of the cantilever, a silicon dioxide sphereical particle (D = 1 μm) was attached to prevent stress concentration. The indentation was operated at a rate of 0.5 μm/s (Fig. 1b).

3. RESULTS AND DISCUSSION

Fig. 2 shows the typical shape of deflection-distance and force-distance curves from the nano-indentation test. As the cantilever approaches to the sample surface, the distance between sample and cantilever decreases. After contact with the sample, the deflection of the cantilever starts to increase. When the cantilever retracts from the sample, the opposite happens (Fig. 2a). The deflection of the cantilever can be converted to the force by multiplying pre-calibrated spring constant of cantilever (Fig. 2b).

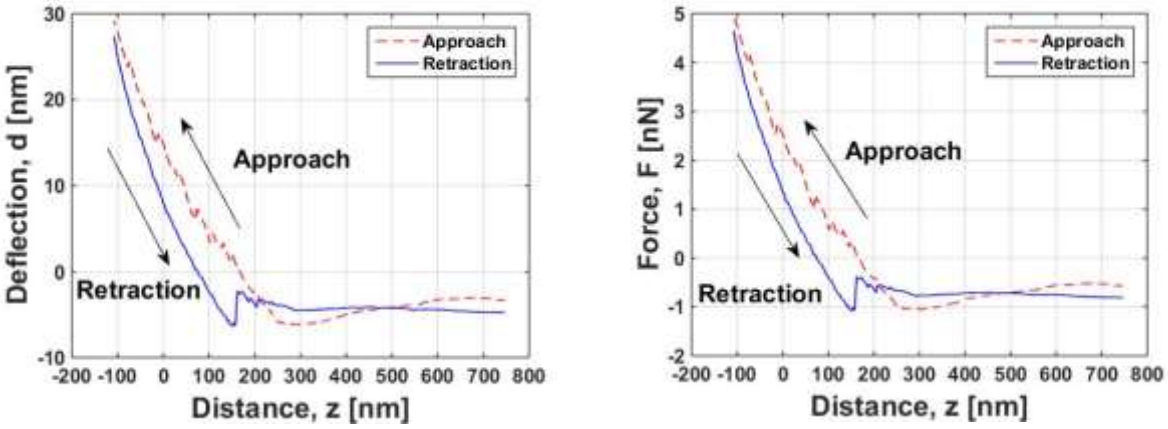
Because the tip is deflected after the contact, indentation depth of the tip into sample can be obtained by subtracting the deflection of the cantilever from the distance between sample and cantilever ($\delta = z - d$). The portion after contact with biofilm surface was extracted to calculate elastic moduli of biofilm by using Hertzian contact theory, as shown in Fig. 3(a).

$$F = \frac{4}{3} E^* R^{1/2} \delta^{3/2} \quad , \qquad (1)$$

where F is the nanoindentation force, E^* is the surface elastic modulus of the nanoindenter tip and the sample, and R is the radius of tip. The equation of surface elastic modulus is as follows (Baniasadi et al, 2014):

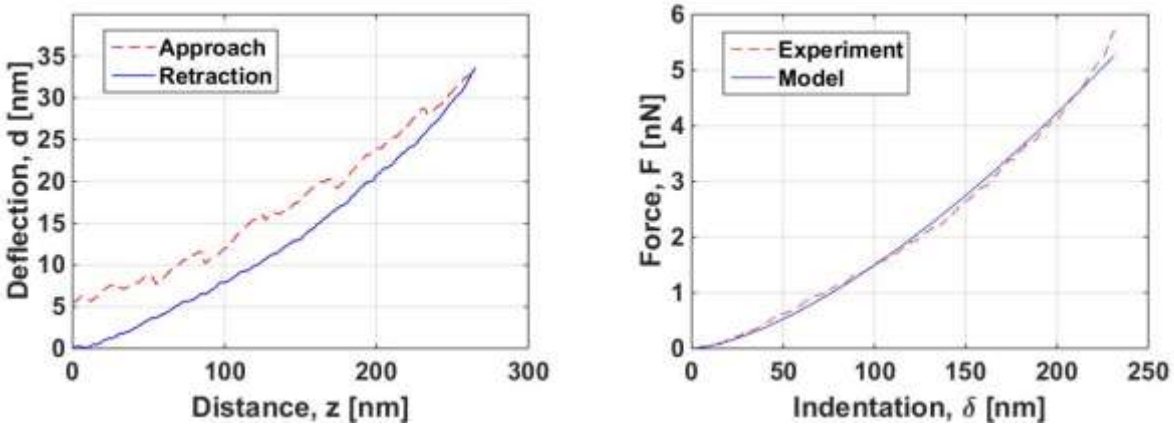
$$\frac{1}{E^*} = \frac{(1-\nu_t^{\,2})}{E_t} + \frac{(1-\nu_s^{\,2})}{E_s}, \qquad (2)$$

where t, s mean tip and sample. Because elastic moduli of tip, which is made with silicon nitride is much bigger than that of biofilm, the first term with E_t is negligible. The poisson's ratio of sample, ν_s, is assumed to be 0.49, which is a common assumption for hydrated materials. Hence, the elastic moduli of sample can be estimated by the least square method. Elastic moduli of biofilm was obtained by fitting retraction curve. Fig.3. (b) shows that the experiment result was fitted well with the Hertzian contact model (R^2 = 0.99) and the elastic moduli of biofilm was determined to be ~29 kPa.



(a) (b)

Fig. 2. (a) Deflection-distance result, and (b) force-distance result.



(a) (b)

Fig. 3. (a) The deflection-distance curve extracted, and (b) The force-indentation curve for model fitting.

ACKNOWLEDGEMENT

The research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of science, ICT Future Planning (2014R1A1003419).

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