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Measuring elastic modulus of bacterial biofilms in a liquid phase using atomic force microscopy

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Abstract. With the increasing interest in using bacterial biofilms in geo-engineering practices, such as soil improvement, sealing leakage in earth structures, and hydraulic barrier installation, understanding of the contribution of bacterial biofilm formation to mechanical and hydraulic behavior of soils is important. While mechanical properties of soft gel-like biofilms need to be identified for appropriate modeling and prediction of behaviors of biofilm-associated soils, elastic properties of biofilms remain poorly understood. Therefore, this study investigated the microscale Young's modulus of biofilms produced by *Shewanella oneidensis* MR-1 in a liquid phase. The indentation test was performed on a biofilm sample using the atomic force microscopy (AFM) with a spherical indentor, and the force-indentation responses were obtained during approach and retraction traces. Young's modulus of biofilms was estimated to be ~33–38 kPa from these force-indentation curves and Hertzian contact theory. It appears that the AFM indentation result captures the microscale local characteristics of biofilms and its stiffness is relatively large compared to the other methods, including rheometer and hydrodynamic shear tests, which reflect the average macro-scale behaviors. While modeling of mechanical behaviors of biofilm-associated soils requires the properties of each component, the obtained results provide information on the mechanical properties of biofilms that can be considered as cementing, gluing, or filling materials in soils.

Keywords: elastic modulus; biofilm; hertz contact model; AFM

1. Introduction

With the increasing demands for sustainable and environmentally low-impact construction materials and techniques, use of biological methods have received significant interests due to their eco-friendly characteristics and potentials in geo-engineering applications, such as soil improvement, hydraulic barrier installation, leakage sealing in earth structures, and microbially enhanced oil recovery. Among numerous microbial activities, formation of biofilms has garnered interest for the purposes of bioclogging (e.g., Dennis and Turner 1998, Sidik *et al.* 2014, Thullner *et al.* 2002) and enhancement of soil strength (e.g., Chang *et al.* 2016, Chang *et al.* 2015, Chang and Cho 2014,

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Ivanov and Chu 2008, Yasodian *et al*. 2012). The biofilm is an aggregate of microorganisms embedded in a matrix composed of microbially produced extracellular polymeric substances (EPS), which consist of protein, polysaccharides, nucleic acids, and lipids, and attached to a surface (Lewandowski and Beyenal 2013, Chang and Cho 2012). As bacterial adhesion onto mineral surfaces and subsequent biofilm growth contributes to the aggregate stability, mineral weathering and the fate of contaminants in soils (Huang *et al*. 2015), understanding of mechanical properties of soft gel-like biofilms is critically required for appropriate modeling and prediction of behaviors of biofilm-associated soils. The elastic moduli of biofilms are important parameters that indicate the stiffness of biofilms and that can be used for modeling the overall stiffness of biofilm-grown soils. However, it is difficult to measure their mechanical responses because biofilms are too soft and grow in an aqueous phase; elastic properties of biofilms remain poorly identified due to such difficulties in measurement.

Meanwhile, under certain conditions in various practices, including wastewater treatment systems and industrial bioreactors, biofilms are often subjected to mechanical stresses caused by fluid flows, which leads to undesired loss of biofilms (Cense *et al*. 2006). There have been a few studies aiming on minimizing such undesired loss. For an example, the mechanical properties of biofilms were estimated by observing structural deformation under shear stress caused fluid flows (Stoodley *et al*. 1999, Cense *et al*. 2006). Despite its simplicity, this method has a limitation of poor estimation of the stress exerted on a specific location on a sample. A uniaxial compression device has been developed and attempted on biofilm samples prepared on a disc of 20-mm diameter (Körstgens *et al*. 2001), however, the measured Young's modulus values were profoundly affected by the sample thickness, owing to bulging of the sample. Whereas, the atomic force microscopy (AFM) has been increasingly used to investigate mechanical properties of bio-materials because the AFM can carry out the force-indentation measurement with pico-newton sensitivity (e.g., Kundukad *et al*. 2016, Lau *et al*. 2009).

Therefore, in this study, we have attempted to obtain the elastic modulus, in particular Young's modulus, of biofilms in a liquid phase by the nano-indentation method using AFM. *Shewanella oneidensis* MR-1 was chosen as the model bacterium and its biofilm was prepared on a tryptic soy agar surface. The indentation experiments were carried out on the prepared biofilm sample using a spherical tip of AFM. The force-indentation curves were obtained during approach and retraction, and those were fitted to the Hertzian contact model to estimate the Young's modulus of the biofilm.

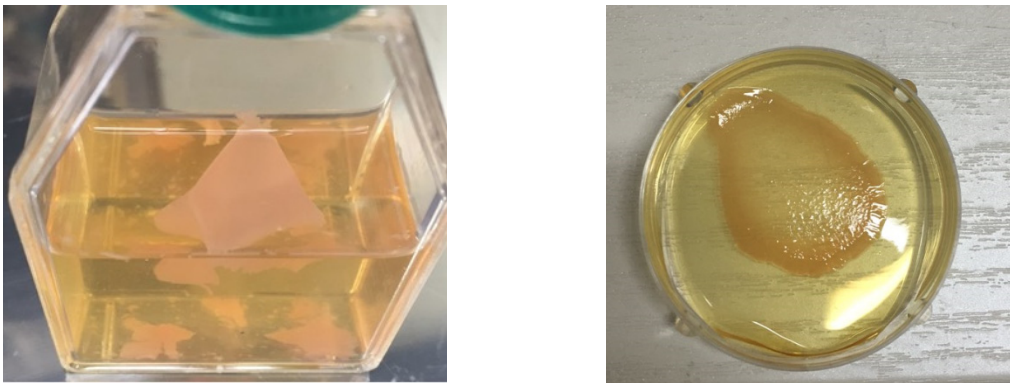
2. Materials and methods

2.1 Model bacteria and biofilm sample preparation

Shewanella oneidensis MR-1 strain was chosen as the model bacterium in this study because they can grow both anaerobic and aerobic conditions and the biosafety level is Class 1 which means harmless to humans. *Shewanella oneidensis* MR-1 is a gram-negative facultative bacterium which can be found in marine sediments. *Shewanella* species have the ability to form biofilms and reduce a broad spectrum of metals and organic compounds (Venkateswaran *et al*. 1999). The biofilm is an assembled matrix of the EPS and the microbial cells that are irreversibly attached onto a surface. The biofilms may form on a variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems. EPS may account for 50–90% of the total organic carbon of biofilms and can be considered as the primary matrix material of the biofilms. EPS may vary in chemical and physical properties, but it is

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

Compound	Concentration
Tryptic soy broth	30 g/L
Sodium fumarate dibasic	16 g/L
Potassium phosphate monobasic (KH_2PO_4)	13.609 g/L
Potassium phosphate dibasic (K_2HPO_4)	17.418 g/L



(a) (b)

Fig. 1 (a) Biofilms formed in a culture flask: and (b) biofilms formed on an agar plate

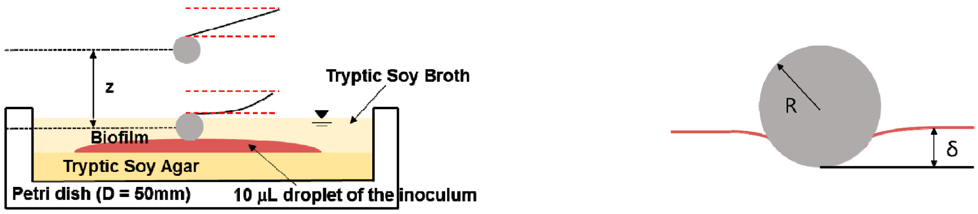
primarily composed of polysaccharides (Donlan 2002).

The bacteria inoculum of *Shewanella oneidensis* MR-1 was aerobically cultured in the prepared growth media at the room temperature of ~22–24°C (Fig. 1(a)). The growth media for *S. oneidensis* MR-1 was defined to stimulate the growth of the bacteria and their biofilms, it consisted of tryptic soy broth (TSB; Sigma-Aldrich Co), potassium phosphate monobasic, potassium phosphate dibasic, and sodium fumarate dibasic, as shown in Table 1.

A tryptic soy broth agar plate was prepared in a petri dish by mixing tryptic soy broth and the agar powder in 2:1 mass ratio. Thereafter, 10 μL droplet of the inoculum in the exponential growth phase (~24 h of growth) was placed on the prepared agar plate to culture biofilms. The bacterial biofilms were incubated at 30°C for 24 h (Fig. 1(b)). Prior to the indentation test using AFM, the fresh growth medium was poured into the biofilm-formed agar plate to fully submerge the biofilms in a liquid phase (Fig. 2(a)).

2.2 Indentation test using Atomic Force Microscopy (AFM)

The indentation tests were conducted on the cultured biofilms submerged in liquid using a commercial AFM (XE-100, Park Systems). To obtain force-distance responses during indentation, a triangular shaped, pre-calibrated silicon nitride cantilever tip with the stiffness k = 0.17 N/m was used. A silicon dioxide spherical particle of 1 μm diameter was attached at the end of the cantilever tip, and this sphere made an indent on biofilms, preventing stress concentration. When the tip is far enough not to interact with the sample surface, the cantilever is flat and not deflected. But as the cantilever approaches and indents the sample surface, the tip starts to be deflected. This



(a) (b)

Fig. 2 (a) A schematic diagram of the AFM indentation test on a biofilm sample; and (b) a schematic of a indented surface with a spherical indentor

vertical indentation at the cantilever tip is detected by the position-sensitive photo-detector. The force F applied to the sample surface can be estimated by using this deflection and the pre-calibrated spring constant. The indentation was operated at a rate of 0.5 μm/s (Fig. 2). The tests were carried out at the room temperature of ~22–24°C.

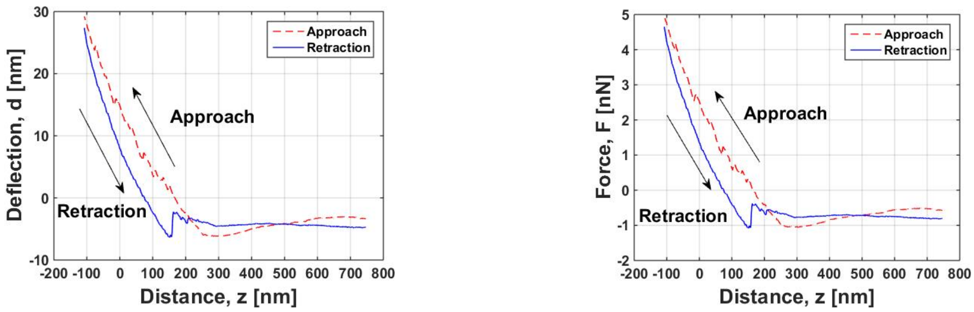
3. Results and discussion

3.1 Estimation of elastic modulus of biofilm produced by Shewanella oneidensis

We followed the analysis method suggested by Touhami *et al*. (2003). Fig. 3 shows the typical curves of deflection-distance and force-distance responses from the indentation test. As the cantilever approached to the sample surface, the distance between the sample and the cantilever decreased. After the contact, the deflection of the cantilever started to increase. When the cantilever retracted from the sample, the opposite occurred (Fig. 3(a)). The deflection of the cantilever can be converted to the force by multiplying the pre-calibrated spring constant k and the deflection d (Fig. 3(b))

$$F = k \cdot d \,. \tag{1}$$

Because the tip is deflected after the contact (see Fig. 2), the actual indentation depth of the tip into the sample (δ) can be calculated by subtracting the deflection of the cantilever (d) from the



(a) (b)

Fig. 3 (a) Deflection-distance responses; and (b) force-distance responses of *S. oneidensis* MR-1 biofilm sample during approach and retraction traces

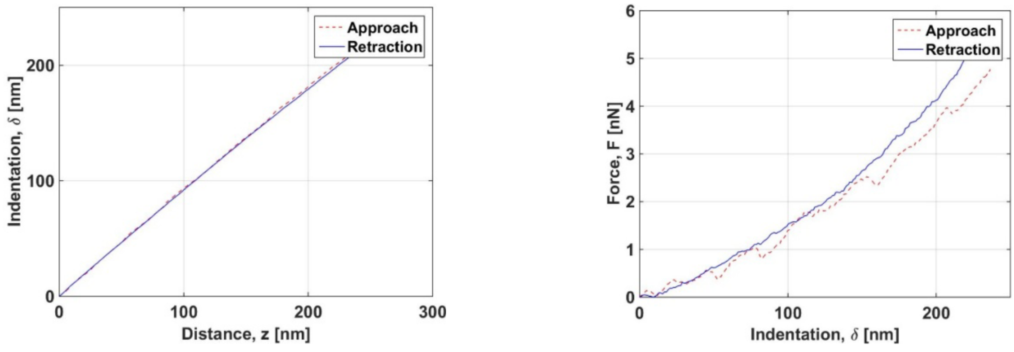


Fig. 4 (a) Indentation-distance relations; and (b) the force-indentation responses during approach and retraction traces

distance that cantilever moved down (z)

$$\delta = z - d\,. \tag{2}$$

When the tip moved down by ~250 nm, the tip deflected only by ~30 nm, thus the indentation depth was ~220 nm, as shown in Fig. 3(a). Fig. 4(a) shows the relation between indentation δ and distance z. It was found that the biofilm was fairly soft. The force at the maximum indentation was ~5 nN, as shown in Fig. 4(b). The force-indentation responses after the contact with biofilm surface was clipped to extract the Young's modulus of biofilms, as shown in Fig. 4(b).

The Hertz theory for the sphere-flat surface contact is expressed as follows (Hertz 1882)

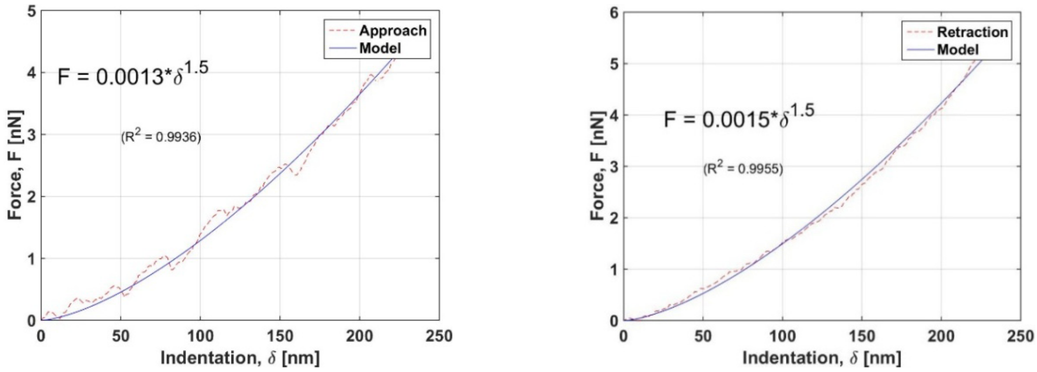
$$F = \frac{4}{3} E^{*} R^{1/2} \delta^{3/2}\,. \tag{3}$$

where F is the indentation force, E^* is the surface elastic modulus of the combination of spherical tip and sample, and R is the sphere radius. Herein, the radius of the sphere tip R was 0.5 μm. Then, the surface elastic modulus E^* is defined as follows (Baniasadi *et al*. 2014)

$$\frac{1}{E^{*}} = \frac{(1-{\nu_{tip}}^{2})}{E_{tip}} + \frac{(1-{\nu_{biofilm}}^{2})}{E_{biofilm}} \cong \frac{(1-{\nu_{biofilm}}^{2})}{E_{biofilm}}\,. \tag{4}$$

where E is the Young's modulus and ν is the Poisson's ratio. Because the elastic moduli of the silicon dioxide spherical tip E_{tip} is much bigger than that of biofilm E_{biofilm}, the first term with E_{tip} is assumed negligible. Biofilm materials are expected to behave like a polymer, which expected to have Poisson's ratio within the range of 0.4–0.5. So, the Poisson's ratio ν_{biofilm} of the biofilm sample is assumed to be 0.49 (Laspidou and Aravas 2007). Hence, the experimentally obtained force-indentation (F-δ) curve can be fitted to by the Hertz contact model using the following relation

$$F = \frac{4}{3} \frac{E_{biofilm}}{(1-{\nu_{biofilm}}^{2})} R^{1/2} \delta^{3/2}\,. \tag{5}$$



(a) (b)

Fig. 5 The force-indentation curves obtained from tests and the model fitting result

Then, the Young's modulus of the biofilm sample E_{biofilm} was estimated from the least-square regression method. Fig. 5 shows that the test results agreed well with the Hertz contact model for the approach and retraction processes. The elastic moduli of biofilm was determined to be ~38 kPa from the retraction trace and ~33 kPa from the approach trace.

3.2 Discussion – Comparison with previous studies

Table 2 lists the elastic moduli of biofilms reported in previous studies. Various methods have been used for determination of elastic moduli of biofilms. Stoodley *et al*. (1999, 2002) measured the shear and Young's moduli of bacterial biofilms by observing the structural deformations caused by hydrodynamic shear stress by water flows; however, the variation in elastic modulus values was significantly large, often more than one order of magnitude, even though the biofilm was produced by the single strain *Pseudonomas aeruginosa* (e.g., 1–280 Pa for shear modulus of biofilms).

Table 2 Elastic moduli of biofilms in previous studies

References	Species	Methods	G (Pa)	E (Pa)
Stoodely *et al*. (1999)	Mixed cuture	Structural deformation by water flows	27 ± 1	40 ±8
Stoodely *et al*. (2002)	*Pseudomonas aeruginosa*	Structural deformation by water flows	1–280	
Körstgens *et al*. (2001)	*Pseudomonas aeruginosa*	Rheometer based on uniaxial compression		6500 ± 500
Aggrawal and Hozalski (2010)	*Staphylococcus epidermidis*	Micro-cantilever (Tensile)		1270 ± 280
Baniasadi *et al*. (2014)	*Pseudomonas aeruginosa*	Atomic force microscopy (AFM)		~40000–45000
This study	*Shewanella oneidensis MR-1*	Atomic force microscopy (AFM)		~33000–38000

On the contrary to that, Körstgens *et al*. (2001) carried out a uniaxial compression tests on *Pseudomonas aeruginosa* biofilms using a rheometer, and the Young's modulus was estimated to be ~6–7 kPa. Whereas, the AFM indentation test resulted in the Young's modulus of ~40–45 kPa for the same strain (Baniasadi *et al*. 2014). Our results on *S. oneidensis* MR1 biofilms are consistent with the values obtained by Baniasadi *et al*. (2014), in the range of ~tens of kPa. It was found that the AFM indentation test led to the larger modulus values than the rheometer tests. It is because the AFM indentation captures the local characteristics as the indentation is conducted at a point while the rheometer captures the average macroscale behaviors.

Accordingly, the stiffness value tends to decrease with an increase in the internal length scale, such as indentation depth, deformation magnitude, or sample size, mainly because of inherent inhomogeneity of biofilms at different scale. Given the indentation of ~0.2 μm and the tip diameter of 1 μm used in this study, it is worth noting that the Young's modulus obtained from this AFM test may have a limited applicability to the microscale mechanical behavior of biofilms.

4. Conclusions

This study presented the AFM indentation test results and microscale elastic modulus of biofilms produced by *Shewanella oneidensis* MR-1 in a liquid phase. The indentation was conducted with a spherical tip to avoid stress concentration, and the force-indentation responses were obtained during approach and retraction traces for the indentation of ~200 nm. The Young's modulus of biofilms was estimated to be ~33–38 kPa from these the force-indentation curves and Hertz contact theory. It was found that our results were consistent with the previous AFM indentation results on bacterial biofilms. It appears that the AFM indentation test captures the microscale local characteristics of biofilms and the stiffness determined by the AFM indentation test is typically larger than that by the other previous methods, including rheometer and hydrodynamic shear tests, which reflects the average macro-scale behaviors. While modeling of mechanical behaviors of biofilm-formed soils requires the properties of each component, the obtained results are expected to provide information on the mechanical properties of biofilms that can be considered as cementing, gluing, or filling materials in the pores of soils.

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References

Aggarwal, S. and Hozalski, R.M. (2010), "Determination of biofilm mechanical properties from tensile tests performed using a micro-cantilever method", *Biofouling*, **26**(4), 479-486.

Baniasadi, M., Xu, Z., Gandee, L., Du, Y., Lu, H., Zimmern P. and Minary-Jolandan, M. (2014), "Nanoindentation of Pseudomonas aeruginosa bacterial biofilm using atomic force microscopy", *Mater.*

Res. Express, **1**(4), 045411.

Cense, A.W., Peeter, E.A.G., Gottenbos, B., Baaijens, F.P.T., Nuijs, A.M. and Dongen, M.E.H. (2006), "Mechanical properties and failure of *Streptococcus mutans* biofilms, studies using a microindentation device", *J. Microbiol. Methods*, **67**(3), 463-472.

Chang, I. and Cho, G.-C. (2012), "Strengthening of Korean residual soil with *β*-1,3/1,6-glucan biopolymer", *Constr. Build. Mater.*, **30**, 30-35.

Chang, I. and Cho, G.C. (2014), "Geotechnical behavior of a *β*-1,3/1,6-glucan biopolymer-treated residual soil", *Geomech. Eng., Int. J.*, **7**(6), 633-647.

Chang, I., Im, J., Prasidhi, A.K, and Cho, G.C. (2015), "Effects of Xanthan gum biopolymer on soil strengthening", *Constr. Build. Mater.*, **74**, 65-72.

Chang, I., Im, J. and Cho, G.C. (2016), "Geotechnical engineering behaviors of gellan gum biopolymer treated sand", *Can. Geotech. J.*, **53**(10), 1658-1670.

Dennis, M. and Turner, J. (1998), "Hydraulic conductivity of compacted soil treated with biofilm", *J. Geotech. Geoenviron. Eng.*, **124**(2), 120-127.

Donlan, R.M. (2002), "Biofilms: Microbial Life on Surfaces", *Emerg. Infect. Diseases*, **8**(9), 881-890.

Huang, Q., Wu, H., Cai, P., Fein, J.B. and Chen, W. (2015), "Atomic force microscopy measurements of bacterial adhesion onto clay sized particles", *Scientific Reports*, **5**, 16587.

Hertz, H. (1882), "Über die Berührung fester elastischer Körper", *Journal für die reine und angewandte Mathematik*, **92**, 156-171.

Ivanov, V. and Chu, J. (2008), "Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ", *Rev. Environ. Sci. Bio/Technology*, **7**(2), 139-153.

Körstgens, V., Flemming, H., Wingerder, J. and Borchard, W. (2001), "Uniaxial compression measurement device for investigation of the mechanical stability of biofilms", *J. Microbiol. Methods*, **46**(1), 9-17.

Kundukad, B., Sevious, T., Liang, Y., Rice, S.A., Kjelleberg, S. and Doyle, P.S. (2016), "Mechanical properties of the superficial biofilm layer determine the architecture of biofilms", *Soft Matter*, **12**, 5718-5726.

Laspidou, C. and Aravas, N. (2007), "Variation in the mechanical properties of a porous multi-phase biofilm under compression due to void closure", *Water Sci. Technol.*, **55**(8-9), 447-453.

Lau, P.C.Y., Dutcher, J.R., Beveridge, T.J. and Lam, J.S. (2009), "Absolute Quantitation of bacterial biofilm adhesion and viscoelasticity by microbead force spectroscopy", *Biophys. J.*, **96**(7), 2935-2948.

Lewandowski, Z. and Beyenal, H. (2013), *Fundamentals of Biofilm Research*, (2nd Ed.), CRC press, Taylor & Francisco Group, New York, NY, USA.

Sidik, W.S., Canakci, H., Kilic, I.H. and Celic, F. (2014), "Applicability of biocementation for organic soil and its effect on permeability", *Geomech. Eng., Int. J.*, **7**(6), 649-663.

Stoodley, P., Lewandowski, Z., Boyle, J.D. and Lappin-Scott, H.M. (1999), "Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: An in situ investigation of biofilm rheology", *Biotech. Bioeng.*, **65**(1), 83-92.

Stoodley, P., Cargo, R., Rupp, C.J., Wilson, S. and Klapper, I. (2002), "Biofilm material properties as related to shear-induced deformation and detachment phenomena", *J. Indust. Microbiol. Biotechnol.*, **29**(6), 361-367.

Thullner, M., Zeyer, J. and Kinzelbach, W. (2002), "Influence of microbial growth on hydraulic properties of pore networks", *Transp. Porous Media*, **49**(1), 99-122.

Touhami, A., Nysten, B. and Dufrêne, Y.F. (2003), "Nanoscale mapping of the elasticity of microbial cells by atomic force microscopy, *Langmuir*, **19**(11), 4539-4543.

Venkateswaran, K., Moser, D.P., Dollhopf, M.E., Lies, D.P., Saffarini, D.A., MacGregor, B.J., Ringelberg, D.B., White, D.C., Nishijima, M., Sano, Hiroshi., Burghardt, J., Stackebrandt, E. and Nealson, K.H. (1999), "Polyphasic taxonomy of the genus Shewanella and description of Shewanella oneidensis sp", *Int. J. System. Bacteriol.*, **49**, 705-724

Yasodian, S.E., Dutta, R.K., Mathew, L., Anima, T.M. and Seena, S.B. (2012), "Effect of microorganism on engineering properties of cohesive soils", *Geomech. Eng., Int. J.*, **4**(2), 135-150.