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Influence of Surfactants on Bacterial Adhesion to Metal Oxide-Coated Surfaces

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Abstract

The objective of this study was to investigate the bacterial adhesion to iron (hydr)oxide-coated sand (IHCS) and aluminum oxide-coated sand (AOCS) in the presence of Tween 20 (nonionic surfactant) and lipopeptide biosurfactant (anionic surfactant) through column experiments. Results show that in the presence of Tween 20, bacterial adhesion to the coated sands was slightly decreased compared to the condition of deionized water; the mass recovery (*Mr*) increased from 0.491 to 0.550 in IHCS and from 0.279 to 0.380 in AOCS. The bacterial adhesion to the coated sands was greatly reduced in lipopeptide biosurfactant; *Mr* increased to 0.980 in IHCS and to 0.797 in AOCS. Results indicate that the impact of lipopeptide biosurfactant on bacterial adhesion to metal oxide-coated sands was significantly greater than that of Tween 20. Our results differed from those of the previous report, showing that Tween 20 was the most effective while the biosurfactant was the least effective in the reduction of bacterial adhesion to porous media. This discrepancy could be ascribed to the different surface charges of porous media used in the experiments. This study indicates that lipopeptide biosurfactant can play an important role in enhancing the bacterial transport in geochemically heterogeneous porous media.

Keywords: Bacterial adhesion, Column experiment, Lipopeptide biosurfactant, Metal-oxide coated sands, Surfactants, Tween 20

1. Introduction

Contamination of subsurface environments by organic contaminants is a wide-spread environmental problem, posing a significant threat to drinking water supplies. For contaminated soils and aquifers, bioaugmentation could be practiced by introducing bacteria with specific metabolic capabilities of degrading target contaminants. In this remediation practice, the successful delivery of contaminant-degrading bacteria to the targeted area is a subject of great interest [1]. An understanding of bacterial interaction with porous media is important with respect to bacterial transport and retention in the subsurface. The deposition of bacteria on a solid matrix is affected by the properties of porous media (e.g., surface charge and grain size), characteristics of bacteria (e.g., cell size, surface charge, and hydrophobicity), and solution chemistry (e.g., pH and ionic strength) [2, 3].

The surfactant is a surface-active agent, composed of both hydrophilic and hydrophobic moieties. This amphiphilic structure gives surfactants the capability of reducing bacterial adhesion to surfaces via modification of the surface characteristics [4]. Several studies have been conducted of surfactants to examine their role in bacterial transport in geological media [5-8], including the enhanced transport of *Pseudomonas pseudoalcaligenes* in sandy clay loam in the presence of sodium dodecyl benzene sulfonate (SDBS) [9], the influences of Tween 20 (nonionic surfactant) and SDBS (anionic surfactant) on the transport of *Alcaligenes paradoxus* in borosilicate glass beads [1], the effect of monorhamnolipid (anionic biosurfactant) on the transport of *P. aeruginosa* in sterile sand [10], the significant increase of cell recovery of aquifer isolate bacteria in unsaturated sand columns under the presence of SDBS compared to no surfactant condition [11], and the release of deposited bacteria (*Lactobacillus casei* and *Streptococcus mitis*) from silica sand by flushing the sand column with rhamnolipid biosurfactant [12]. These studies have shown that bacterial transport could be enhanced in the presence of surfactants. The interaction between bacteria and metal (aluminum, iron) oxide-coated surfaces is important in the transport of bacteria in the subsurface. In geochemically heterogeneous aquifers where the metal oxides provide surface charge heterogeneities, bacteria can favorably adhere to the positively-charged surfaces of aquifer sediments [13]. However, studies on the effects of surfactants on the transport of bacteria in metal oxide-coated porous media are scarce.

The objective of this study was to investigate the bacterial

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adhesion to metal oxide-coated sands in the presence of surfactants. Column experiments were performed in duplicate with *Bacillus subtilis*. The first set of experiments was performed in iron (hydr)oxide-coated sand while the second experiments were carried out in aluminum oxide-coated sand. Bacterial breakthrough curves were obtained by monitoring the effluent, and the bacterial mass recovery and adhesion rate coefficient were then quantified from these curves. Also, the sticking efficiency was quantified from the colloid filtration theory along with the filter factor.

2. Materials and Methods

2.1. Preparation of Bacteria

B. subtilis ATCC 6633 (KCCM 11316) obtained from the Korea Culture Center for Microorganisms was used in the experiment. All glassware and materials used in the study were sterilized by autoclaving at 121˚C and 17.6 psi for 20 min to prevent any interference by other microorganisms. Initially, the freeze-dried bacteria were revived in 250-mL Erlenmeyer flasks containing 100 mL of LB medium (tryptone 10 g, yeast extract 5 g, NaCl 5 g in one liter of deionized water at pH of 7.0) over a period of 84 hr at 30˚C. Then, 1 mL of culture was transferred to a volume of 500 mL LB broth, and the bacteria were incubated over a period of 84 hr at 30℃. The suspension was centrifuged at 4℃ and 10,000 rpm for 15 min. The supernatant was removed and replaced with deionized water to prevent growth of the bacteria. Then, the diluted bacteria were centrifuged again under the same conditions. The centrifuged bacteria were washed three times with deionized water and resuspended in deionized water to an optical density of 0.5 at 600 nm (OD_{600}). Transmission electron microscopy (JEM 1010; JEOL, Tokyo, Japan) was used to take images of the bacterial cells. The images were imported into an image-processing program (Image-Pro Plus; Media Cybernetics, Bethesda, MD, USA) and analyzed. The average length and diameter of *B. subtilis* were 1.67 ± 0.31 μm and 0.77 ± 0.07 μm, respectively, which corresponded to an equivalent spherical diameter of 1.18 ± 0.10 μm. The net surface electrostatic characteristics of the cells were analyzed with an electrophoretic light scattering spectrophotometer (ELS-8000; Otsuka Electronics, Osaka, Japan). Electrophoretic mobility was determined for the bacterial surface (pH 6.2, temperature 25℃, ionic strength ≈ 0 mM) and converted to zeta potentials using the Smoluchowski Equation (–31.9 ± 2.5 mV).

2.2. Metal Oxide-Coated Sands

Quartz sand (Jumunjin Silica, Gangneung, Korea) was used to prepare metal oxide-coated sand. Mechanical sieving was conducted with US Standard Sieves (Fisher Scientific, Pittsburgh, PA, USA), Nos. 35 and 10. Sand fractions with a grain size of 0.5–2.0 mm and a mean diameter of 1.0 mm were used in the experiments. Before use, the sand was washed twice using deionized water to remove impurities on the surface, and the wet sand was autoclaved for 20 min at 17.6 psi, cooled to room temperature, and oven-dried at 105˚C for 1–2 days. For the preparation of metal oxide-coated sand, $AlCl_3{\cdot}6H_2O$ (4.4 g) or $FeCl_3{\cdot}6H_2O$ (5.5 g) was dissolved in deionized water (100 mL), and the solution pH was adjusted with 6N NaOH. The quartz sand (200 g) was added to the $AlCl_3{\cdot}6H_2O$ or $FeCl_3{\cdot}6H_2O$ solution and then mixed in a rotary evaporator (90˚C, 80 rpm, 20 min) to remove water in the suspension by heating (Hahnvapor; Hahnshin Scientific Co., Bucheon, Korea). The coated sand was dried at 150˚C for 6 hr, washed with deionized water and then dried again at the same conditions. Scanning electron microscopy (SEM) analysis along with Energy Dispersive X-ray Spectrometer (EDS) analysis were performed using a scanning electron microscope (JSM 5410LV; JEOL), indicating the presence of Al- or Fe-oxides on the coated sand. SEM images and EDS patterns of coated sand were provided elsewhere [14].

2.3. Column Experiments

Column experiments were conducted using a Plexiglas column with an inner diameter of 2.5 cm and a height of 10 cm packed with metal oxide-coated sands (mass of medium 78.12 ± 1.47 g). All the experiments were performed in duplicate (Table 1). A column was packed for each experiment by the tap-fill

Table 1. Column experimental conditions and results for *Bacillus subtilis* in metal oxide-coated sands in the presence of surfactants

Ex.	Media	Solution	v (cm/min)	D (cm^2/min)	ka (1/min)	R^2	Mr	α	f
1a	IHCS	DW	0.147	0.0142	0.0103	0.995	0.489	0.0194	0.0715
1b	IHCS	DW	0.150	0.0174	0.0100	0.993	0.493	0.0192	0.0707
2a	IHCS	Tween 20	0.159	0.0215	0.0097	0.995	0.547	0.0164	0.0603
2b	IHCS	Tween 20	0.150	0.0162	0.0093	0.997	0.553	0.0161	0.0592
3a	IHCS	Biosurfactant	0.158	0.0234	0.0010	0.994	0.971	0.0008	0.0029
3b	IHCS	Biosurfactant	0.154	0.0176	0.0004	0.994	0.989	0.0003	0.0011
4a	AOCS	DW	0.158	0.0224	0.0229	0.990	0.253	0.0373	0.1374
4b	AOCS	DW	0.162	0.0240	0.0200	0.996	0.306	0.0321	0.1184
5a	AOCS	Tween 20	0.150	0.0191	0.0142	0.990	0.406	0.0244	0.0901
5b	AOCS	Tween 20	0.146	0.0143	0.0151	0.995	0.354	0.0282	0.1038
6a	AOCS	Biosurfactant	0.152	0.0210	0.0028	0.999	0.826	0.0052	0.0191
6b	AOCS	Biosurfactant	0.152	0.0173	0.0046	0.984	0.768	0.0072	0.0264

IHCS: iron (hydr)oxide-coated sand, AOCS: aluminum oxide-coated sand, DW: deionized water, *Mr*: mass recovery.

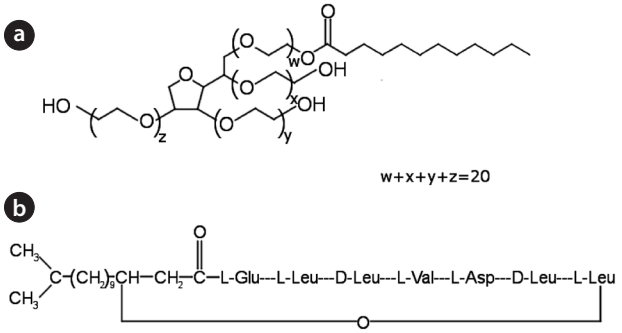

Fig. 1. Structures of surfactants used in the experiments. (a) Tween 20, (b) lipopeptide biosurfactant.

method to attain a bulk density of 1.59 ± 0.03 g/cm^3 and a porosity of 0.40 ± 0.01. The column was connected to a HPLC pump (Series II; Scientific Systems Inc., State College, PA, USA), operating at a rate of 0.5 mL/min. The surfactants used in the experiments were Tween 20 (nonionic surfactant) in Fig. 1(a) and lipopeptide biosurfactant (anionic surfactant) in Fig. 1(b) [15, 16]. Before bacterial injection, the packed column was flushed upward with 15 pore volumes of deionized water (or surfactant solution, 0.1% v/v) to achieve a steady state flow condition. The bacteria (OD_{600} = 0.5) in deionized water (or surfactant solution) were introduced downward into the column for 30 min. After completing bacterial injection, deionized water (or surfactant solution) was introduced again into the column. Effluent samples were collected using an auto collector (Retriever 500; Teledyne, Lincoln, NE, USA) at regular intervals. Effluents were analyzed for bacterial concentration. Bacterial concentration was determined by measuring the optical density of the effluent using a UV-visible spectrophotometer (Helios; Thermo, Waltham, MA, USA) at 600 nm (OD_{600}). A preliminary test indicated that the surfactants in the effluent did not interfere with the measurement of bacterial concentration.

2.4. Data Analysis

Assuming that bacterial growth and decay are negligible, the one-dimensional bacteria transport can be described as:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - k_a C \tag{1}$$

where C is the bacterial concentration in the aqueous phase, D is the hydrodynamic dispersion coefficient, v is the pore-water velocity, and K_a is the adhesion rate coefficient (T^{-1}). The parameters in the transport models were obtained by fitting the CXTFIT code [17] to the breakthrough data. According to colloid filtration theory, the adhesion rate coefficient (K_a) can be described by the following equation [18]:

$$k_a = \frac{3}{2}\frac{(1-n)}{d_c}v\eta\alpha; \qquad v = \frac{U}{n} \tag{2}$$

where n is the porosity, d_c is the particle diameter of porous media, η is the collision efficiency, α is the sticking efficiency, and U is the flow approach velocity (Darcy velocity).

The collision efficiency (η) can be calculated using the following equation [19]:

$$\eta = 2.4A_S^{1/3}N_R^{-0.081}N_{Pe}^{-0.715}N_{vdW}^{0.052} + 0.55A_S N_R^{1.675}N_A^{0.125} + 0.22N_R^{-0.24}N_G^{1.11}N_{vdW}^{0.053} \tag{3}$$

where A_S is the porosity–dependent parameter, N_R is the aspect ratio, N_{Pe} is the Peclet number, N_{vdW} is the van der Waals number, N_A is the attraction number, and N_G is the gravity number. The sticking efficiency (α) can be determined with the following equation [19]:

$$\alpha = -\frac{2}{3}\frac{d_c}{(1-n)L\eta}\ln(Mr) \tag{4}$$

where L is the column length, and Mr is the bacterial mass recovery in the effluent. The parameters used in the calculation of η and α are summarized in Table 2. Mr can be quantified by the following relationship:

$$Mr = \left(\frac{\int_0^\infty C dt}{C_0 t_0}\right) \tag{5}$$

where C_0 is the initial concentration of bacteria, and t_0 is the duration of bacteria injection (injection time, 30 min).

The adhesion rate coefficient (K_a) has the following relationship with the filter factor (f, L^{-1}) [18]:

$$f = \frac{k_a}{v} \tag{6}$$

Thus, f can be expressed in terms of Mr by incorporating Equations (2) and (4) into Equation (6) as [21]:

$$f = -\frac{1}{L}\ln(Mr) = -\frac{2.3025}{L}\log_{10}(Mr) \tag{7}$$

From Equation (7), the relationship between the log removal and travel distance (T_d) of bacteria can be described as:

Table 2. Parameters used in the calculation of collision efficiency (η) and sticking efficiency (α) for *Bacillus subtilis* in metal oxide-coated sands

Parameter	Unit	Value
Column length	cm	10
Particle diameter of collector grain (sand)	mm	1.0
Particle diameter of colloidal particle (bacteria)	μm	1.18
Particle density of colloidal particle (bacteria)[a]	g/cm^3	1.105
Fluid absolute temperature	K	298
Fluid density	g/cm^3	0.997
Fluid viscosity	g/cm/s	8.91×10^{-3}
Hamaker constant	J	6.5×10^{-21}
Boltzman constant	J/K	1.38×10^{-23}
Bulk diffusion coefficient	cm^2/sec	4.05×10^{-9}

[a]Particle density of bacteria was from Martínez-Salas et al. [20].

$$T_d = \frac{2.3025}{f} * (\log \text{ removal}) \quad (8)$$

where the log removal is denoted by $-\log_{10}(Mr)$ For example, 99.9% of bacterial removal is equal to 3 log removals.

3. Results and Discussion

3.1. Bacterial Breakthrough Curves and Mass Recovery

The bacterial breakthrough curves (BTCs) obtained from the column experiments in the metal-oxide coated sand are presented in Fig. 2. In iron (hydr)oxide-coated sand (Ex. 1-3 in Fig. 2), the BTCs showed different relative peak concentrations depending on the solution conditions. The relative peak concentrations ranged from 0.417 to 0.782, with the lowest obtained for deionized water (Ex. 1), and the highest obtained for the biosurfactant (Ex. 3). The transport parameters (v and D) obtained from the model fit for the bacterial BTCs were 0.153 ± 0.005 cm/min and 0.018 ± 0.003 cm^2/min, respectively. The bacterial BTCs from the experiments in aluminum oxide-coated sand are given in Fig. 2 (Ex. 4-6). The BTCs had relative peak concentrations ranging from 0.206 to 0.684, with the highest obtained for biosurfactant (Ex. 6). The values of v and D determined from the BTCs were 0.153 ± 0.006 cm/min and 0.020 ± 0.004 cm^2/min, respectively.

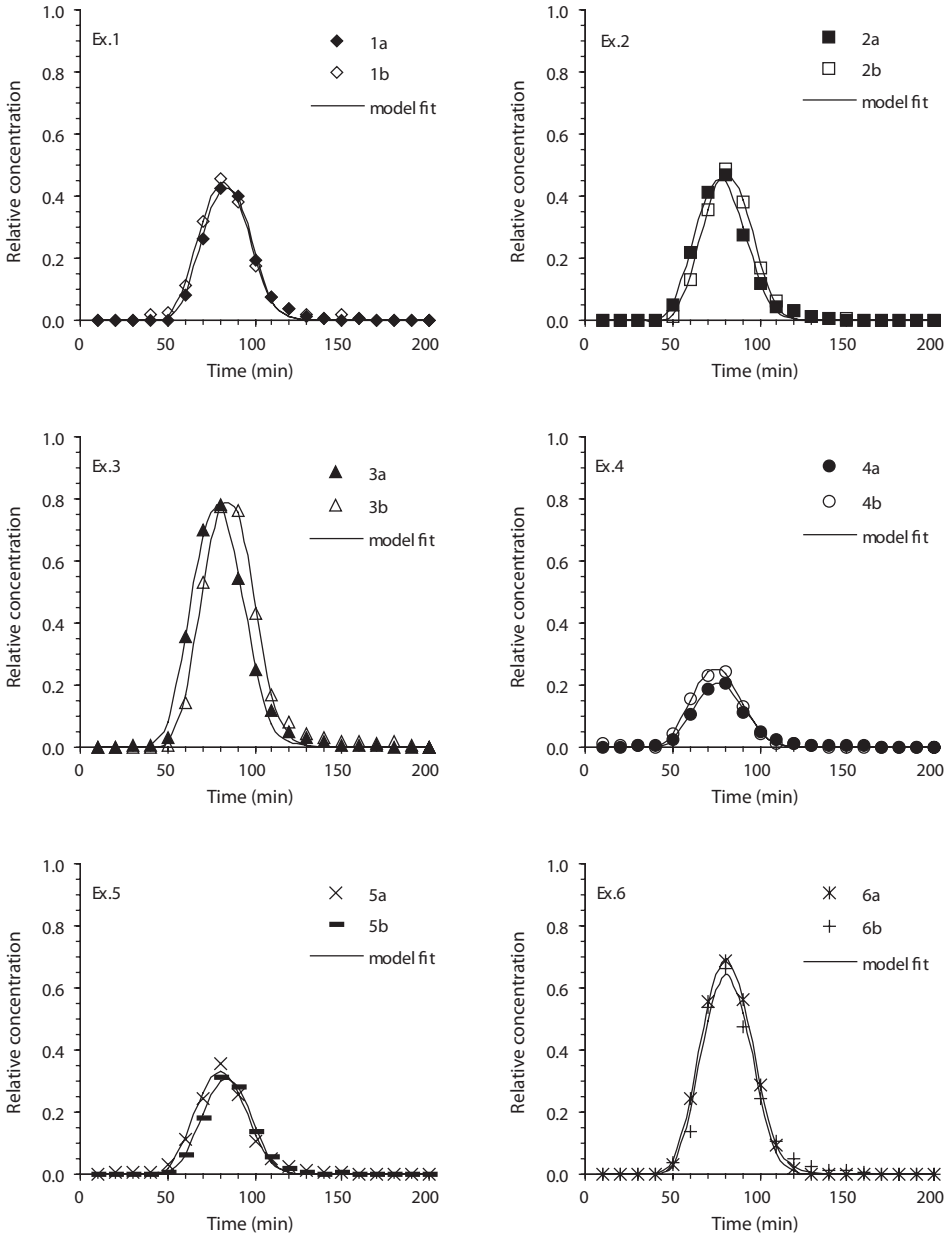

Fig. 2. Breakthrough curves and model fit of *Bacillus subtilis* obtained from column experiments in iron (hydr)oxide-coated sand (Ex. 1–3) and aluminum oxide-coated sand (Ex. 4–6) under different solution conditions. The experimental conditions are provided in Table 1.

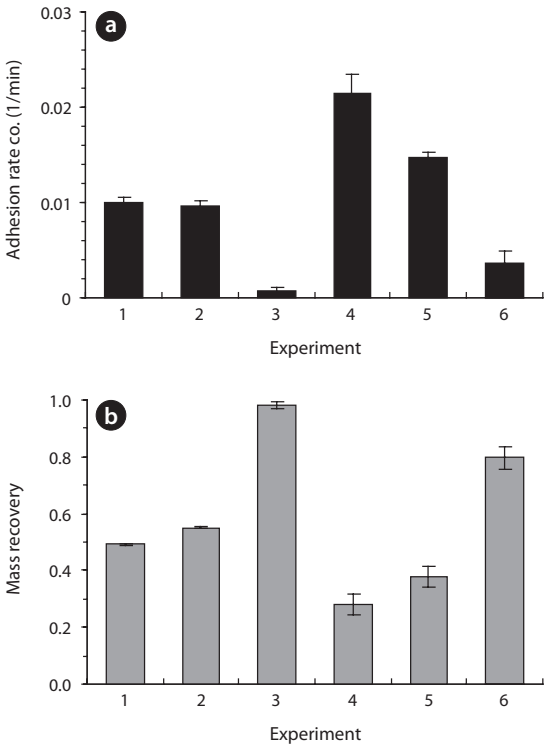

Fig. 3. Comparison of (a) adhesion rate coefficient (k_a) and (b) bacterial mass recovery (*Mr*) obtained from column experiments under different conditions (see Table 1).

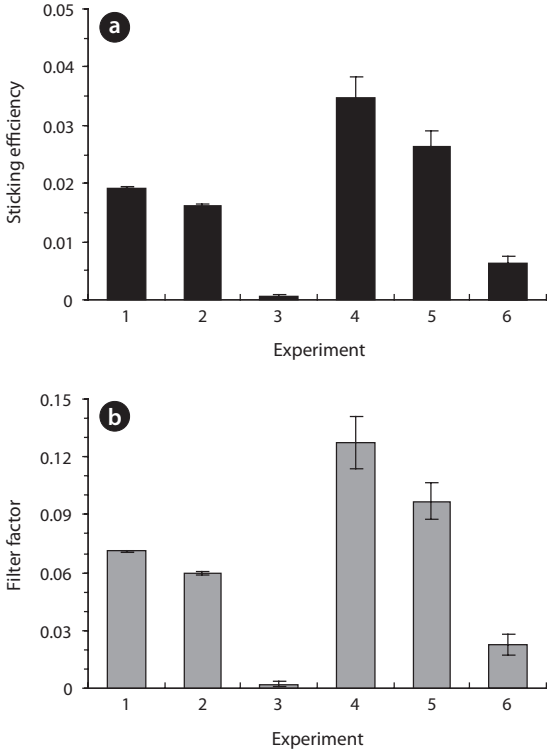

Fig. 4. Comparison of (a) sticking efficiency (α) and (b) filter factor (*f*) obtained from column experiments under different conditions (see Table 1).

The adhesion rate coefficient and bacterial mass recovery obtained from the column experiments in metal oxide-coated sand are presented in Fig. 3. In iron (hydr)oxide-coated sand (Ex. 1-3), the average adhesion rate coefficient (k_a) was highest (0.0099 1/min) for deionized water (Ex. 1) and lowest (0.0007 1/min) for the biosurfactant (Ex. 3). In aluminum oxide-coated sand (Ex. 4-6), the value of k_a was also highest (0.0215 1/min) for deionized water (Ex. 4) and lowest (0.0037 1/min) for the biosurfactant (Ex. 6). Overall, the values of k_a were lowest in the presence of the biosurfactant in metal oxide-coated sand in Fig. 3(a). The average *Mr* was highest (0.980) for the biosurfactant (Ex. 3) and lowest (0.491) for deionized water (Ex. 1) in iron (hydr)oxide-coated sand. With aluminum oxide-coated sand, the value of *Mr* was highest (0.797) for the biosurfactant (Ex. 6) and lowest (0.280) for deionized water (Ex. 4). In the metal oxide-coated sand, the values of *Mr* were highest in the presence of the biosurfactant in Fig. 3(b).

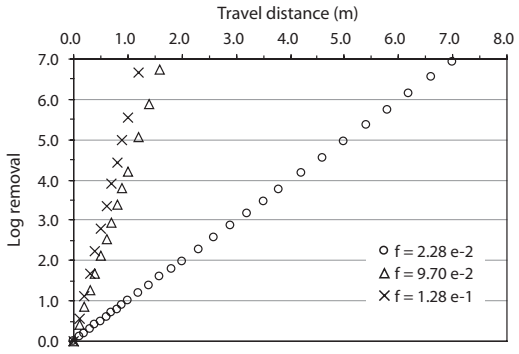

Fig. 5. Estimation of travel distance versus log removal of *Bacillus subtilis* based on the filter factor determined from column experiments.

3.2. Adhesion-Related Parameters and Travel Distance

The adhesion-related parameters (sticking efficiency and filter factor) obtained from the column experiments are compared in Fig. 4. As shown in Fig. 4(a), the average values of sticking efficiency (α) were lowest in the presence of the biosurfactant for both iron (hydr)oxide-coated sand (0.0005) and aluminum oxide-coated sand (0.0062). In deionized water, the values of α were highest with iron (hydr)oxide-coated sand (0.0193) and aluminum oxide-coated sand (0.0347). In Fig. 4(b), the average values of the filter factor (*f*) are presented. The adhesion rate coefficient (the temporal coefficient), was converted to the filter factor (spatial coefficient) using Equation (6). Note that the filter factor is log-linearly related to the bacterial mass recovery. In the deionized water, the average values of *f* were highest with iron (hydr)oxide-coated sand (0.0711) and aluminum oxide-coated sand (0.1279). The values of *f* were lowest in the presence of the biosurfactant for both iron (hydr)oxide-coated sand (0.0020) and aluminum oxide-coated sand (0.0228).

The travel distance (T_d) of bacteria was estimated with the filter factor (*f*) determined from the experiment using Equation (8) in Fig. 5. In the estimation of T_d, the bacterial concentration was

assumed to be 10^6 cfu/100 mL (cfu: colony forming unit), and the values of f obtained from the experiments in aluminum oxide-coated sand were used. At f = 1.28 e-1 (deionized water), 3-log removal (99.9% removal) was achieved at T_d = 0.54 m. At f = 9.70 e-2 (Tween 20), T_d was at 0.71 m for 3-log removal. In addition, T_d = 3.05 m for 3-log removal at f = 2.28 e-2 (biosurfactant). This indicates that the travel distance of bacteria could be altered due to surfactants. In metal oxide-coated sands, the travel distance of bacteria could be enhanced considerably in the presence of the biosurfactant.

3.3. Surfactant and Bacterial Adhesion to Metal Oxide-Coated Sands

In our experiments, the bacterial mass recovery in metal oxide-coated sand under deionized water was considerably less than that in quartz sand (0.989, BTC not shown), indicating that bacterial transport was greatly reduced in metal oxide-coated sand. In a neutral pH condition, the coated sand is positively charged [22, 23], such that the electrostatic interaction between the coated sand and bacteria becomes attractive. Note that bacteria are negatively charged above pH 2–3 [2, 3]. Therefore, the surface modification of quartz sand through the metal oxide coating could provide favorable adhesion sites for bacteria, resulting in the reduction of bacterial transport in porous media.

In the presence of Tween 20, the bacterial transport in metal oxide-coated sand was slightly enhanced (5–10% increase of mass recovery). This result could be explained by the expansion of the electric double layer between the bacteria and coated sand due to Tween 20, a nonionic surfactant [4, 5]. That is, Tween 20 adheres to the surface of bacteria, causing the displacement of the counterions and consequently expanding the electric double layer between bacteria and coated sand. This results in the reduction of bacterial adhesion to coated sand. Brown and Jaffé [5] observed the transport of *Sphingomonas pacilimobilis* through aquifer sand in the presence of Brij 30 and Brij 35 (nonionic surfactants). They mentioned that Brij 30 and Brij 35 could enhance the transport of bacteria by changing the structure of the electric double layer.

The transport of bacteria in metal oxide-coated sand was greatly enhanced in the presence of lipopeptide biosurfactant (about 50% increase of mass recovery). The sharp increase of bacterial transport in the coated sand in the presence of the biosurfactant could be attributed to the preoccupation of favorable adhesion sites on the coated sand by the biosurfactant along with competitive adhesion between the biosurfactant and bacteria. The lipopeptide biosurfactant is anionic; therefore, in our experiments, the biosurfactant injected into the column before bacterial injection could preoccupy the adhesion sites and mask the positively charged surfaces on the coated sand, resulting in the reduction of favorable sites for bacterial adhesion. Furthermore, the biosurfactant simultaneously injected during bacterial injection could compete for the sites with bacteria. Our result indicated that lipopeptide biosurfactant could play a similar role to that of humic acid in bacterial adhesion to metal oxide-coated sand. Foppen et al. [24] have shown that the mass recovery of *E. coli* increased in the presence of humic acid in goethite-coated sand columns. Other studies [13, 25, 26] have also reported that the bacterial adhesion to iron-coated sand or sediment was reduced in the presence of humic acid or natural organic matter.

In our experiments, the impact of the biosurfactant on bacterial transport in metal oxide-coated sand was significantly greater than that of Tween 20. This result differed from the study of Li and Logan [27], who used various nonionic surfactants (Tween 20, Tween 80, etc.) and an anionic biosurfactant (monorhamnolipid) to examine the transport of *A. paradoxus* and subsurface isolate bacteria in porous media (glass bead, sand, and two soils) in the presence of surfactants. They reported that Tween 20 was the most effective in the reduction of bacterial adhesion to porous media while the biosurfactant was the least effective among the surfactants tested. This discrepancy could be attributed to the different porous media used in the experiments. That is, the metal oxide-coated sands with positively-charged surface sites were used in our experiments, while the porous media with negatively-charged surfaces were used in Li and Logan's [27] experiments. Therefore, the influence of the biosurfactant was more prominent in our experiments compared to the study of Li and Logan [27].

4. Conclusions

Column experiments were performed to examine the effect of surfactants on bacterial adhesion to metal oxide-coated sands. Results show that the impact of lipopeptide biosurfactant on bacterial adhesion to metal oxide-coated sands was considerably greater than that of Tween 20. Our results differed from those of the previous study, reporting that Tween 20 was the most effective while the biosurfactant was the least effective in the reduction of bacterial adhesion to porous media. This discrepancy could be ascribed to the different surface charges of porous media used in the experiments. This study indicates that impacts of surfactants on bacterial adhesion to porous media largely depend on the surface charges of porous media. Also, in geochemically heterogeneous porous media, lipopeptide biosurfactant can play an important role in enhancing the transport of bacteria.

Acknowledgments

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