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...
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°C. The suspension was centrifuged at 4°C 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 (OD600). 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].

2.2. 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, AlCl3·6H2O (4.4 g) or FeCl3·6H2O (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 AlCl3·6H2O or FeCl3·6H2O solution and then mixed in a rotary evaporator (90°C, 80 rpm, 20 min) to remove water in the dispersion 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. 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

<table>
<thead>
<tr>
<th>Ex.</th>
<th>Media</th>
<th>Solution</th>
<th>ν (cm/min)</th>
<th>D (cm/min)</th>
<th>ka (1/min)</th>
<th>R²</th>
<th>Mr</th>
<th>α</th>
<th>f</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>IHCS</td>
<td>DW</td>
<td>0.147</td>
<td>0.0142</td>
<td>0.0103</td>
<td>0.995</td>
<td>0.489</td>
<td>0.0194</td>
<td>0.0715</td>
</tr>
<tr>
<td>1b</td>
<td>IHCS</td>
<td>DW</td>
<td>0.150</td>
<td>0.0174</td>
<td>0.0100</td>
<td>0.993</td>
<td>0.493</td>
<td>0.0192</td>
<td>0.0707</td>
</tr>
<tr>
<td>2a</td>
<td>IHCS</td>
<td>Tween 20</td>
<td>0.159</td>
<td>0.0215</td>
<td>0.0097</td>
<td>0.995</td>
<td>0.547</td>
<td>0.0164</td>
<td>0.0603</td>
</tr>
<tr>
<td>2b</td>
<td>IHCS</td>
<td>Tween 20</td>
<td>0.150</td>
<td>0.0162</td>
<td>0.0093</td>
<td>0.997</td>
<td>0.553</td>
<td>0.0161</td>
<td>0.0592</td>
</tr>
<tr>
<td>3a</td>
<td>IHCS</td>
<td>Biosurfactant</td>
<td>0.158</td>
<td>0.0234</td>
<td>0.0010</td>
<td>0.994</td>
<td>0.971</td>
<td>0.0008</td>
<td>0.0029</td>
</tr>
<tr>
<td>3b</td>
<td>IHCS</td>
<td>Biosurfactant</td>
<td>0.154</td>
<td>0.0176</td>
<td>0.0004</td>
<td>0.994</td>
<td>0.989</td>
<td>0.0003</td>
<td>0.0011</td>
</tr>
<tr>
<td>4a</td>
<td>AOCs</td>
<td>DW</td>
<td>0.158</td>
<td>0.0224</td>
<td>0.0229</td>
<td>0.990</td>
<td>0.253</td>
<td>0.0373</td>
<td>0.1374</td>
</tr>
<tr>
<td>4b</td>
<td>AOCs</td>
<td>DW</td>
<td>0.162</td>
<td>0.0240</td>
<td>0.0200</td>
<td>0.996</td>
<td>0.306</td>
<td>0.0321</td>
<td>0.1184</td>
</tr>
<tr>
<td>5a</td>
<td>AOCs</td>
<td>Tween 20</td>
<td>0.150</td>
<td>0.0191</td>
<td>0.0142</td>
<td>0.990</td>
<td>0.406</td>
<td>0.0244</td>
<td>0.0901</td>
</tr>
<tr>
<td>5b</td>
<td>AOCs</td>
<td>Tween 20</td>
<td>0.146</td>
<td>0.0143</td>
<td>0.0151</td>
<td>0.995</td>
<td>0.354</td>
<td>0.0282</td>
<td>0.1038</td>
</tr>
<tr>
<td>6a</td>
<td>AOCs</td>
<td>Biosurfactant</td>
<td>0.152</td>
<td>0.0210</td>
<td>0.0028</td>
<td>0.999</td>
<td>0.826</td>
<td>0.0052</td>
<td>0.0191</td>
</tr>
<tr>
<td>6b</td>
<td>AOCs</td>
<td>Biosurfactant</td>
<td>0.152</td>
<td>0.0173</td>
<td>0.0046</td>
<td>0.984</td>
<td>0.768</td>
<td>0.0072</td>
<td>0.0264</td>
</tr>
</tbody>
</table>

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

http://dx.doi.org/10.4491/eer.2011.16.4.219
method to attain a bulk density of 1.59 ± 0.03 g/cm³ 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 (OD₂₀₀ = 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.

2.4. Data Analysis

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

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - k_a C
\]  

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]:

\[
k_a = \frac{3}{2} \frac{(1-n)}{d_p} v \eta \alpha; \quad v = \frac{U}{n}
\]  

where \( n \) is the porosity, \( d_p \) is the particle diameter of porous media, \( \eta \) is the collision efficiency, \( \alpha \) is the sticking efficiency, and \( U \) is the flow approach velocity (Darcy velocity).

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

\[
\eta = 2.4 A_g^{13} N_d^{0.0815} N_{pe}^{0.715} N_{sd}^{0.052} + 0.55 A_g^{1.67} N_d^{1.25} N_{pe}^{2.24} N_{sd}^{1.11} N_{sd}^{0.03}
\]  

where \( A_g \) is the porosity–dependent parameter, \( N_d \) is the aspect ratio, \( N_{pe} \) is the Peclet number, \( N_{sd} \) is the van der Waals number, \( N_{sd} \) is the attraction number, and \( N_{sd} \) is the gravity number. The sticking efficiency (\( \alpha \)) can be determined with the following equation [19]:

\[
\alpha = -\frac{2}{3} \frac{d_i}{(1-n)L} \ln(Mr)
\]

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

\[
Mr = \left[ \frac{C_{dt}}{C_{d0}} \right]^{1/3}
\]

Table 2. Parameters used in the calculation of collision efficiency (\( \eta \)) and sticking efficiency (\( \alpha \)) for Bacillus subtilis in metal oxide-coated sands

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column length</td>
<td>cm</td>
<td>10</td>
</tr>
<tr>
<td>Particle diameter of collector grain (sand)</td>
<td>mm</td>
<td>1.0</td>
</tr>
<tr>
<td>Particle diameter of colloidal particle (bacteria)</td>
<td>μm</td>
<td>1.18</td>
</tr>
<tr>
<td>Particle density of colloidal particle (bacteria) (^a)</td>
<td>g/cm³</td>
<td>1.105</td>
</tr>
<tr>
<td>Fluid absolute temperature</td>
<td>K</td>
<td>298</td>
</tr>
<tr>
<td>Fluid density</td>
<td>g/cm³</td>
<td>0.997</td>
</tr>
<tr>
<td>Fluid viscosity</td>
<td>g/cm³/s</td>
<td>8.91 × 10⁻²</td>
</tr>
<tr>
<td>Hamaker constant</td>
<td>J</td>
<td>6.5 × 10⁻²¹</td>
</tr>
<tr>
<td>Boltzman constant</td>
<td>J/K</td>
<td>1.38 × 10⁻²¹</td>
</tr>
<tr>
<td>Bulk diffusion coefficient</td>
<td>cm²/sec</td>
<td>4.05 × 10⁸</td>
</tr>
</tbody>
</table>

\(^a\)Particle density of bacteria was from Martínez-Salas et al. [20].
\[ T_d = \frac{2.3025 \times \log \text{removal}}{f} \]  \hspace{1cm} (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 \( (\nu \text{ and } D) \) obtained from the model fit for the bacterial BTCs were 0.153 ± 0.005 cm/min and 0.018 ± 0.003 cm²/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 \( \nu \) and \( D \) determined from the BTCs were 0.153 ± 0.006 cm/min and 0.020 ± 0.004 cm²/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.
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).

### 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 \(\alpha\) 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 \(\alpha\) were higher 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_f = 0.54 m. At f = 9.70 e-2 (TWEEN 20), T_f was at 0.71 m for 3-log removal. In addition, T_f = 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 paucimobilis 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 surfactant 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 geochromatically heterogeneous porous media, lipopeptide biosurfactant can play an important role in enhancing the transport of bacteria.

Acknowledgments

This work was supported by a grant from the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology, Korea (NRF-2008-359-C00045).

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