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Quantification of Bacterial Attachment-related Parameters in Porous Media

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Abstract

Transport of *Escherichia coli* ATCC 11105 through porous media was investigated in this study using two sets of column experiments to quantify the attachment-related parameters (sticking efficiency, attachment rate coefficient and filter factor). The first set of experiments was performed in quartz sand under different ionic strength conditions (1, 20, 100, 200 mM) while the second experiments were carried out in quartz sand mixed with metal oxyhydroxide-coated sand (0, 5, 10, 25%). The breakthrough curves of bacteria were obtained by monitoring effluent, and then bacterial mass recovery and attachment-related parameters were quantified from these curves. The first experiments showed that the mass recoveries were in the range of 13.3 to 64.7%, decreasing with increasing ionic strength. In the second experiments, the mass recoveries were in the range of 15.0 to 43.4%, decreasing with increasing coated sand content. The analysis indicated that the sticking efficiency, attachment rate coefficient and filter factor increased with increasing ionic strength and coated sand content. The value of filter factor in the first experiments ranged from 1.45 e-2 to 6.72 e-2 1/cm while in the second experiments it ranged from 2.78 e-2 to 6.32 e-2 1/cm. Our filter factor values are one order of magnitude lower than those from other studies. This discrepancy can be attributed to the size of sand used in the experiment. The analysis demonstrated that the travel distance of bacteria estimated using the filter factor can be varied greatly depending on the solution chemistry and charge heterogeneity of porous media.

Keywords: Bacterial transport, Sticking efficiency, Attachment rate coefficient, Filter factor, Travel distance

1. Introduction

Microbial contamination of groundwater resources is a widespread environmental problem, deteriorating drinking water quality and posing a great threat to human health.[1,2] Thus, an understanding of bacterial transport in porous media is important in the protection of groundwater. *Escherichia coli* is widely used as an indicator organism for bacterial contamination of water resources. Several researchers have investigated attachment and transport of *E. coli* in porous media. These studies have looked at the transport of *E. coli* through saturated soil in field experiments,[3] transport of *E. coli* K-12 through columns packed with silt loam and silty clay loam,[4] movement of *E. coli* NAR through unsaturated soil columns with macropores,[5] removal of *E. coli* ATCC 13607 in columns containing metal hydroxides coated sands,[6] adhesion of *E. coli* HCB437 and HCB137 to surface-modified quartz surfaces,[7] transport of *E. coli* ATCC 25922 through columns packed with quartz sand[8] and with various contents of goethite-coated sand,[9] and transport of *E. coli* JM109 in soil aquifer treatment system.[10] However, more quantitative approach is required in order to improve our knowledge regarding the bacteria transport in porous media and to protect the groundwater from microbial contamination.

In this study, bacterial transport through porous media was investigated using column experiments to quantify the attachment-related parameters. The first set of the experiments was performed in quartz sand under different ionic strength conditions while the second experiments were carried out under different mixtures of quartz and metal oxyhydroxide-coated sands. Bacterial breakthrough curves were obtained by monitoring effluent, and then bacterial mass recovery and attachment-related parameters were quantified from these curves.

2. Theory

In porous media, the bacterial movement is mainly controlled by advective-dispersive transport and adhesion to solid matrices.[11] The bacterial attachment to a solid matrix is influenced by solution chemistry (e.g., ionic strength and pH), properties of porous media (e.g., surface charge and grain size), and characteristics of bacteria (e.g., cell geometry and surface property).[12,13] The one-dimensional bacteria transport in saturated porous media can be described as:

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

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 attachment rate coefficient (T^{-1}). Note that bacterial growth and decay are assumed to be negligible. According to colloid filtration theory, the attachment rate coefficient (k_a) can be described by the following equation:[14]

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

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:[15]

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

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 parameters used in the calculation of η are summarized in Table 1. The sticking efficiency (α) can be determined with the following equation:[15]

Table 1. Parameters used for collision efficiency (η) of *Escherichia coli* in sand

Parameter	Unit	Value
Column length	cm	30
Particle diameter of sand	mm	1.0
Particle diameter of bacteria	μm	1.21
Particle density of bacteria*	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}

*Particle density of bacteria was from Martínez-Salas[23]

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

where L is the column length, and Mr is the bacterial mass recovery in the effluent, which can be quantified by the following relationship:

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

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

The attachment rate coefficient (k_a) has the following relationship with the filter factor (f, L^{-1}):[14]

$$f = \frac{k_a}{v} \quad (6)$$

Thus, f can be expressed in terms of mass recovery (M_r) by incorporating Equations (2) and (4) into Equation (6) as:[16]

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

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

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

where the log removal denotes - $\log_{10}$ (Mr). For instance, 99% of bacterial removal is equal to 2 log removal.

3. Materials and Methods

3.1. Bacteria and Culture Preparation

E. coli ATCC 11105 obtained from the Korea Culture Center for Microorganisms was used in this experiment. All glassware and materials used in this 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 7.0) over a period of 84 h. Then one milliliter of culture was transferred to a volume of 500 mL LB broth, and the bacteria were incubated over a period of 84 h 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 (OD_{600}). Transmission electron microscopy (JEM 1010, JEOL, Japan) was used to take images of *E. coli* cells. The images were imported into an image-processing program (Image-Pro Plus) and analyzed. The average length and diameter of *E. coli* were 2.2 μm and 0.6 μm, respectively, which corresponded to an equivalent spherical diameter of 1.21 μm. The net surface electrostatic characteristics of cells were analyzed with an Electrophoretic Light Scattering Spectrophotometer (ELS-8000, Otsuka Electronics, Japan). Electrophoretic mobility was determined for the bacterial surface (pH = 6.8, temp. = 25°C, ionic strength $\approx$ 0 mM) and converted to zeta

potentials using the Smoluchowski equation. The zeta potential of *E. coli* was determined to be - 51 ± 6 mV.

3.2. Porous Media

Quartz sand (effective size: 1.0 mm, uniformity coefficient: 1.53) supplied by Jumunjin Silica was used in the experiments. Before use, sand was washed twice using deionized water to remove impurities on the surface, and 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 Al-coated sand, $AlCl_3 \cdot 6H_2O$ (4.9 g) was dissolved in deionized water (100 mL), and the solution pH was adjusted to pH 7.0 with 6N NaOH. The quartz sand (200 g) was added to the $AlCl_3 \cdot 6H_2O$ solution and then mixed in a rotary evaporator (HAHNVAPOR, Hahnshin Scientific Co., Korea) to remove water in the suspension by heating (90°C, 80 rpm, 20 min). The same procedure used for Al-coated sand was applied using $FeCl_3 \cdot 6H_2O$ (5.5 g) for Fe-coated sand. The coated sand was dried in a drying oven (J-NDS2, JISICO, Korea) at 150°C for 6 hr, washed with deionized water and then dried again at the same conditions.

3.3. Column Experiments

Two sets of column experiments were performed using a Plexiglas column (length = 30 cm; inner diameter = 2.5 cm) under the leaching solution prepared with NaCl and phosphate (KH_2PO_4, K_2HPO_4). The experimental conditions were summarized in Table 2. The first set of experiments (1a-1d) was conducted in quartz sand at different ionic strength (IS) conditions (1, 20, 100, 200 mM). A column was packed for each experiment by the tap-fill method to attain a bulk density of 1.583 ± 0.002 g/cm^3 and a porosity of 0.403 ± 0.001. The column was connected to a pump (QG400, FASCO, USA) operating at a rate of 0.5 mL/min. Prior to the experiments, the packed column was flushed upward with 8-10 pore volumes of the leaching solution until the column effluents were clear and a steady state flow condition was established. The column experiment was performed by injecting bacteria solution of 0.5 OD_{600} downward for 60 min. After completing injection of the tracer, leaching solution was introduced again. Effluent samples were collected using an auto collector (Retriever 500, TELEDYNE, USA) at a regular interval and analyzed for bacterial concentration along with pH and EC. Bacterial concentration was determined by measuring the optical density of the effluent using a UV-visible spectrophotometer (Helios, Thermo, USA) at 600 nm (OD_{600}), pH with a pH probe (9107BN, Orion, USA), and EC with an EC probe (815PDL, Istek, Korea). The second set of experiments (2a-2d) was performed (flow rate = 0.35 mL/min) in quartz sand mixed with metal oxyhydroxide-coated sand (0, 5, 10, 25%) under the ionic strength of 5.3 mM. The second experiments used the same procedure as the first. The bulk density and porosity of columns were 1.580±0.002 g/cm^3 and 0.403±0.001, respectively.

4. Results and Discussion

4.1. Bacterial Mass Recovery

The bacterial breakthrough curves (BTCs) obtained from the first experiments (1a-1d) are presented in Fig. 1. The bacterial mass recoveries in the first experiments were in the range of 13.3 to 64.7%, decreasing with increasing ionic strength (IS). In quartz sand, the mass recovery was 64.7% at IS = 1 mM (1a), decreasing to 50.5% with increasing IS to 20 mM (1b). When IS increased further to 100 and 200 mM, the mass recoveries decreased to 20.2 and 13.3% (1c, 1d), respectively. Our result is comparable with the studies of other researchers who have examined the decreasing bacterial mass recovery with increasing IS in quartz sand.[17,18] This phenomenon can be explained by the DLVO theory. Because the surface charges of both quartz sand and bacteria are negative at circumneutral pH,[19] increas-

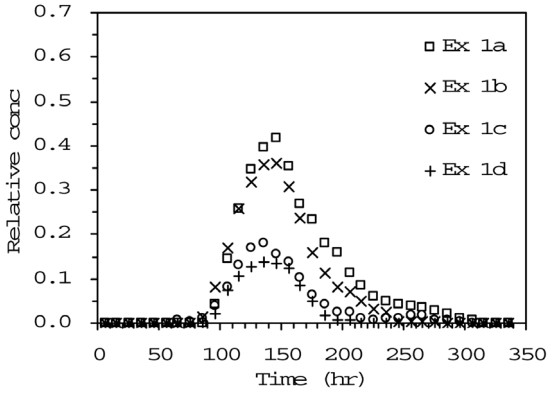

Fig. 1. Breakthrough curves of *Escherichia coli* in quartz sand at various ionic strength conditions.

Table 2. Experimental conditions for column experiments of *Escherichia coli*

Ex	Media (%)		Bulk density (g/cm^3)	porosity	Flow rate (mL/min)	pH	Is (mM)	EC (μS/cm)
	quartz	coated						
1a	100	0	1.586	0.402	0.50	7.3	1	91
1b	100	0	1.581	0.403	0.50	7.3	20	2,259
1c	100	0	1.582	0.403	0.50	7.2	100	9,856
1d	100	0	1.581	0.403	0.50	7.3	200	19,073
2a	100	0	1.582	0.403	0.35	7.5	5.3	539
2b	95	5	1.580	0.404	0.35	7.6	5.3	539
2c	90	10	1.581	0.403	0.35	7.6	5.3	538
2d	75	25	1.577	0.405	0.35	7.7	5.3	548

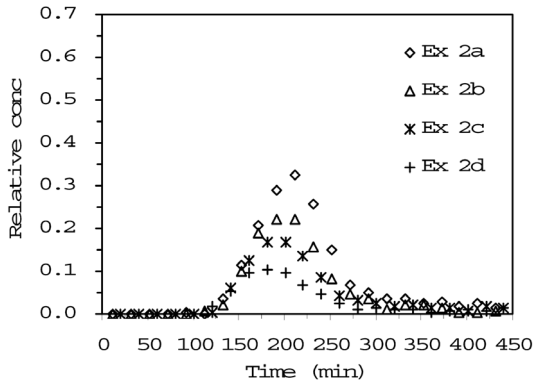

Fig. 2. Breakthrough curves of *Escherichia coli* in quartz sand mixed with metal oxyhydroxide-coated sand.

ing IS of leaching solution leads to a decrease in the thickness of the electrical double layers and in the average distance between both surfaces, resulting in the enhancement of bacterial adhesion to the surfaces of quartz sand.

The BTCs from the second experiments (2a-2d) are presented in Fig. 2. In the second experiments, the mass recoveries ranged from 15.0 to 43.4%, decreasing with increasing coated sand content in mixed media. The mass recovery in quartz sand (2a) was 43.4%, which was lower than that of 1a (64.7%). This can be attributed to the fact that the column experiment in 2a was conducted at lower flow rate and higher IS than 1a (Table 1). As the coated sand content increased from 5 to 25% (2b-2d), the mass recoveries decreased from 30.1 to 15.0%. Our results are consistent with the work of other researchers[9] who have shown that the mass recovery of *E. coli* decreased with increasing goethite-coated sand content in column experiment. It is well known that the metal (Fe, Al) oxyhydroxides carry positive surface charges at circumneutral pH conditions. Therefore, negatively-charged bacteria can favorably adhere to the positively-charged surfaces of porous media.

4.2. Attachment-Related Parameters

In the first experiments (1a-1d), the sticking efficiencies (α) were in the range of 0.149 to 0.694, increasing with increasing IS in quartz sand. The value of α in the second experiments (2a-2d) increased from 0.213 to 0.484 with increasing coated sand content from 0 to 25% in mixed media. In colloid filtration theory, the removal of colloid by attachment to a collector (e.g. sand) can be described by collector efficiency ($C = \eta\alpha$), which denotes the probability that a colloidal particle approaching a collector both collides with and sticks to the collector.[19] The collision efficiency (η) is the probability that a colloidal particle approaching a collector collides with the collector while the sticking efficiency (α) is the probability that the particle colliding with the collector sticks to that collector. Our result indicates that the collision efficiency of *E. coli* in quartz sand increases with increasing IS and coated sand content, resulting in the enhancement of bacteria removal.

The attachment rate coefficient (k_a) in the first experiments (1a-1d) ranged from 3.68 e-3 to 1.70 e-2 1/min, increasing with increasing IS in quartz sand. In the second experiments (2a-2d), the value of k_a ranged from 4.92 e-3 to 1.11 e-2 1/min, increasing with increasing coated sand content in mixed media. The attachment rate coefficient is the temporal coefficient, which can be converted to the spatial coefficient (filter factor) using Equation (6). As presented in Equation (7), the filter factor (f) is log-linearly related to the mass recovery. The value of f in the first experiments (1a-1d) increased with increasing IS in quartz sand. It ranged from 1.45 e-2 to 6.72 e-2 1/cm. The value of f in the second experiments (2a-2d) increased with increasing coated sand content. It ranged from 2.78 e-2 to 6.32 e-2 1/cm. Our f values are one order of magnitude lower than those from other studies. The f values of *E. coli* calculated from Foppen and Schijven[9] were ranged from 4.46 e-1 to 6.97 e-1 1/cm, increasing with increasing coated sand content from 0 to 20% in mixed media (sand size = 0.235 mm). The f value of *E. coli* determined from Bolster et al.[21] was 2.21 e-1 1/cm (sand size = 0.25-0.35 mm). This discrepancy can be attributed to the size of sand used in the experiment. The grain size used in their studies was about four times larger than ours (1.0 mm).

4.3. Log Removal and Travel Distance

The travel distance (T_d) of bacteria can be estimated with the filter factor (f) determined from the experiment using Equation (8) (Fig. 3). In the estimation of T_d, the concentration of *E. coli* is assumed to be 10^6 cfu/100 mL (cfu: colony forming unit) based on the report of Pang et al.[22] As shown in Fig. 3, the values of f used in the estimation were 1.45 e-2 (1a), 6.72 e-2 (1d), 2.78 e-2 (2a), and 6.32 e-2 (2d). At f = 1.45 e-2 (1a: quartz sand,

Table 3. Experimental results for column experiments of *Escherichia coli*

Ex	U (cm/min)	Mr (%)	η	α	ka (1/min)	f (1/cm)
1a	0.102	64.7	0.0108	0.149	3.68 e-3	1.45 e-2
1b	0.102	50.5	0.0108	0.235	5.76 e-3	2.28 e-2
1c	0.102	20.2	0.0108	0.550	1.35 e-2	5.33 e-2
1d	0.102	13.3	0.0108	0.694	1.70 e-2	6.72 e-2
2a	0.071	43.4	0.0146	0.213	4.92 e-3	2.78 e-2
2b	0.071	30.1	0.0146	0.306	7.06 e-3	4.01 e-2
2c	0.071	24.4	0.0146	0.360	8.30 e-3	4.70 e-2
2d	0.071	15.0	0.0146	0.484	1.11 e-2	6.32 e-2

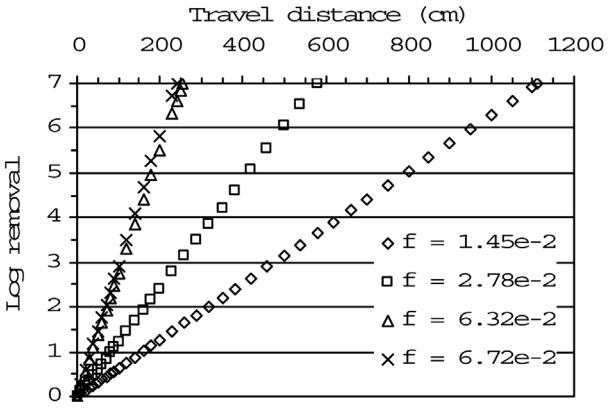

Fig. 3. Travel distance and log removal of *Escherichia coli* based on the laboratory-determined filter factor.

IS = 1 mM), T_d of 476 cm is required to achieve 3-log removal (99.9% removal) and T_d of 1112 cm for 7-log removal (complete removal of 10^6 cfu/100 mL). At 6.72 e-2 (1d: quartz sand, IS = 200 mM), T_d is 103 cm for 3-log removal and 240 cm for 7-log removal. This indicates that the travel distance of bacteria can be changed considerably due to solution chemistry (e.g. ionic strength). At f = 2.78 e-2 (2a: quartz sand 100%, IS = 5.3 mM), T_d is 248 cm for 3-log removal and 580 cm for 7-log removal. At 6.72 e-2 (2d: quartz sand 75%, coated sand 25%, IS = 5.3 mM), T_d is 109 cm for 3-log removal and 255 cm for 7-log removal. This demonstrates that the travel distance of bacteria can be reduced markedly due to the presence of positively-charged metal oxyhydroxide-coated sand. As mentioned previously, the values of f determined at smaller sand size by other researchers were one order of magnitude larger than ours. Thus, it is expected that the travel distance of bacteria can be influenced greatly by particle size of porous media.

5. Conclusions

The attachment-related parameters for *E. coli* were quantified from column experiments in porous media. The first experiments show that the mass recovery in quartz sand decreased while the sticking efficiency increased with increasing ionic strength. In addition, the attachment rate coefficient and filter factor increased with increasing ionic strength. The second experiments indicate that the mass recovery decreased while the sticking efficiency, attachment rate coefficient and filter factor increased with increasing metal oxyhydroxide-coated sands. The analysis demonstrates that the travel distance of bacteria can be varied greatly depending on the solution chemistry and charge heterogeneity of porous media. Further research should focus on examining the influence of physical heterogeneity of porous media on the attachment-related parameters and travel distance of bacteria.

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