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Abstract: Wastewater reclamation has been widely practiced in agriculture. When reclaimed wastewater is used for irrigation, among other requirements, it is important that the pathogenic bacteria are removed. Consequently, technologies such as immobilization or sorption barriers have been developed. To enhance the removal efficiency, biopolymers have been introduced to amend these immobilization or sorption barriers. In this study, removal of pathogenic bacteria by lipopolysaccharide (LPS)-amended barriers was investigated by means of laboratory column experiments. Two typical gram-negative pathogenic bacterial strains of *Escherichia coli* and *Pseudomonas fluoresences* and one gram-positive bacterial strain of *Streptococcus mitis* were selected as the model bacteria in this research. Bacterial adhesion to uncoated and LPS-coated silica sand was correlated to their interaction free energies. Both *E. coli*, *P. fluorescens,* and *S. mitis* had negative interaction free energies with silica

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sand, demonstrating their adhesion potentials to silica sand. After LPS coating, bacterium-sediment interaction free energies decreased (negatively increased), and consequently, bacterial retention increased. Bacterial deposition coefficient in silica sand corresponded to their interaction free energies with silica sand. This study demonstrated that bacterial retention in porous media was determined by their interactions with the sediments, which could be predicted based on independently determined bacterial and medium physicochemical surface properties.

Keywords: *Escherichia coli*, *Pseudomonas fluoresences*, *Streptococcus mitis*, silica sand, lipopolysaccharide, coating

INTRODUCTION

Wastewater reclamation has been widely practiced in agriculture (1). For instance, in California, 60% of recycled wastewater is used for irrigation (2). Usage of reclaimed wastewater for agricultural irrigation can reduce the demands on potable sources of freshwater. Most importantly, it may diminish the volume of treated wastewater discharged directly to natural water resources of streams, rivers, or lakes, resulting in a beneficial impact on the aquatic environment. In addition, nutrient-rich wastewater supplies not only water, but also plant nutrients (especially nitrogen and phosphorus) that can benefit agricultural production.

On the other hand, wastewater is rich in pathogenic microorganisms. Among these pathogenic microorganisms, bacteria are the most common ones found in wastewater and gastrointestinal infections are among the most common diseases caused by pathogenic bacteria in wastewater. Special care must be taken to monitor the fate of pathogenic bacteria when reclaimed wastewater is used for irrigation since pathogenic bacteria can negatively impact human health and the soil. In practice, when reclaimed wastewater is used for irrigation, among other requirements, it is important that the pathogenic bacteria are removed, for which technologies such as the use of immobilization or sorption barriers have been proposed (3, 4). To enhance the removal efficiency, these immobilization or sorption barriers have often been amended by different materials such as polymers (5). Among polymers commonly used for coating applications, biopolymers have drawn more and more attention owing to their biodegradability (6). According to the chemical structures, biopolymers can be classified into the following classes:

1. nucleic acids,
2. polyamides,
3. lipopolysaccharides,
4. polyoxoesters,
5. polythioesters,
6. polyanhydrides,

7. polyisoprenoides, and
8. polyphenols (7).

Of these biopolymers, lipopolysaccharide (LPS), the major outer membrane constituent of gram-negative bacteria, has been proposed as the most potential amending material. LPS has been used to solublize heavy metals from the subsurface by binding the heavy metals (e.g., uranium) into the biopolymer (8). Many studies have also demonstrated that LPS enhances the sorption and reduces the mobility of microorganisms (9–11). Most importantly, LPS is produced by intrinsic bacteria, making it abundant and easy to obtain (12, 13). Whereas, the effectiveness of LPS-coated barriers on pathogenic bacterial removal has not been verified.

In this study, the effectiveness of LPS-coated silica sand on bacterial retention was evaluated by means of column experiments. Bacterial adhesion to uncoated and LPS-coated silica sand was correlated with their interactions with the sediments. Two typical gram-negative pathogenic bacterial strains of *Escherichia coli* and *Pseudomonas fluoresences* and one gram-positive bacterial strain of *Streptococcus mitis* were used as the model bacteria in this research. So far, no reliable risk estimation associated with reclaimed wastewater usage in irrigation is available. However, it is evidenced that pathogenic bacteria exist in the reclaimed wastewater and are able to transport in aquifer systems, threating public health and the environment (3, 14, 15, 16). Results of this research will provide practical guidelines for reclaimed wastewater usage in irrigation.

MATERIALS

Bacterial Strains

Gram-negative bacterial strains used in this research, *Escherichia coli* k12 (ATCC 29181) and *Pseudomonas fluorescens* (ATCC 17559) were cultured in a minimal salt medium, which had a composition of KH_2PO_4, 160 mg/l; K_2HPO_4, 420 mg/l; Na_2HPO_4, 50 mg/l; NH_4Cl, 40 mg/l; $MgSO_4 \cdot 7H_2O$, 50 mg/l; $CaCl_2$, 50 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot 4H_2O$, 0.05 mg/l; H_3BO_3, 0.1 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.05 mg/l; $(NH4)_6Mo_7O_{24}$, 0.03 mg/l; NH_4Cl, 0.6 g/l and glucose, 2 g/l. The medium was adjusted to pH 7.4 with 1 N HCl or 1 N NaOH. Before usage, the medium was sterilized by autoclaving (121°C and 1 atm) for 20 mins. Glucose was filter-sterilized and aseptically added to the autoclaved minimal salt medium. Gram-positive strain of *Streptococcus mitis* (ATCC 9456) was cultured in Todd Hewitt broth (THB). Both *E. coli*, *P. fluorescens* and *S. mitis* were quantified using Adenosine Triphosphate (ATP) analysis (17). For column experiments, bacterial cells collected from the stationary growth stage (predetermined by ATP assay) were centrifuged at 2500 × g (Damon/IEC Divison, Needham Heights, MA) and washed twice with the sterilized buffer solution

(potassium phosphate monobasic-sodium hydroxide buffer, Fisher Scientific, Pittsburgh, PA). They were then re-suspended in sterilized nanopure de-ionized water at a concentration of 5×10^8 cells/ml and used as injectants for column experiments. After the buffer wash, exopolysaccharide (if any) was stripped off the bacteria (18). During the transport process, bacterial growth was assumed to be minimal due to the lack of substrate or nutrients.

Porous Medium

The porous medium used in this research was silica sand (Fisher Scientific, 8 mesh). Silica sand was first rinsed with de-ionized water and then treated with sodium acetate, hydrogen peroxide, sodium dithionate, and sodium citrate to remove organic matters. Silica sand was then extensively flushed with sterilized nanopure de-ionized water until the electrical conductivity was less than 1 dS/m. Before experiments, silica sand was sterilized at 121°C and 1 atm for 20 mins.

Lipopolysaccharide

Lipopolysaccharide (LPS) was extracted from *P. aeruginosa* (ATCC 15152), which was cultured in a salt medium containing 5.44 g KH_2PO_4, 0.06 g NH_4Cl, 0.2 g glucose and 6 ml salt solution per 100 ml medium. The salt solution was prepared by dissolving 10 g $MgSO_4 \cdot 7H_2O$, 1.0 g $MnCl_2 \cdot 4H_2O$, 0.4 g $FeSO_4 \cdot 7H_2O$ and 0.1 g $CaCl_2 \cdot 2H_2O$ in 1 L distilled water. Initial pH of the medium was adjusted to 7.4 with 1 N HCl or 1 N NaOH. The medium was sterilized by autoclaving (121°C and 1 atm) for 20 mins. Glucose was filter-sterilized and aseptically added to the autoclaved salt medium. The extraction followed the method modified from Hancock and Poxton. (18) as described below. Collected bacterial cells were first washed free from medium components with phosphate-buffered saline (pH 7.0) and freeze-dried. The lyophilized cells were then re-suspended in distilled water at a concentration of 5% (W/V) and mixed with equal volume of 90% (W/W) aqueous phenol at 67°C for 15 mins. The mixture was transferred to centrifuge tubes and cooled in ice until phase separation occurred. The tubes were then centrifuged at 5000 × g at 0°C for 15 mins. The upper (aqueous) phase, containing the LPS, was dialyzed against running tap water for at least 18 hrs, until the smell of phenol cannot be detected. The dialyzed extract was then transferred to a round bottom flask connected to a rotoevaporator. The evaporation process was allowed to proceed until the precipitation turned to a honey-color, viscous consistency, which was then freeze dried.

LPS-Coated Silica Sand

LPS coating on silica sand was accomplished by dissolving LPS in chloroform and mixing with silica sand by vortexing. After drying at room temperature,

silica sand was further dried under vacuum (0.1 mmHg, 20°C) for 12 hrs to remove any remaining chloroform. The silica sand was separated from the excess LPS and uncoated silica sand by centrifugation in a discontinuous Percol gradient composed of 230 mM sucrose and 10 mM phosphate buffer (pH 7.4) with layers of 20, 40 and 60% Percol (V%) (19). LPS coating on silica sand was calculated based on the net weight increase of silica sand after coating.

EXPERIMENTAL PROTOCOLS

Surface Thermodynamic Property Measurement

According to the traditional and extended DLVO theory, bacterial surface thermodynamic properties are composed of apolar, or Lifshitz-van der Waals (LW) component; polar, or Lewis acid-base (AB) component; and electrical charged, or electrostatic (EL) component (20). Bacterial Lifshitz-van der Waals and Lewis acid/base components of surface thermodynamic properties were estimated using the van Oss-Chaudhury-Good equation in terms of surface tensions with contact angles measured with an apolar liquid, diiodomethane and two polar liquids, formamide, and water (20, 21).

$$(1 + \cos\theta)\gamma_L = 2\left(\sqrt{\gamma_C^{LW}\gamma_L^{LW}} + \sqrt{\gamma_C^{+}\gamma_L^{-}} + \sqrt{\gamma_C^{-}\gamma_L^{+}}\right) \qquad (1)$$

where θ is the bacterium-liquid contact angle (degree); γ_L surface tension of the liquid used for the measurement (J/m^2); γ^{LW} Liftshitz-van der Waals component of surface tension (J/m^2); γ^+ and γ^- electron-acceptor parameter and the electron-donor parameter of the Lewis acid/base component of surface tension (J/m^2). In the above equation, subscript "S" denotes bacteria and "L" denotes the liquid.

Bacterial-liquid contact angles were measured using a gomiometer (Contact-angle Meter, Tantec) following the method described by Chen and Strevett (17) with bacterial strains vacuum filtered on silver metal membrane filters (0.45 μm, Osmonic, Inc.). Uncoated and LPS-coated silica sand-liquid contact angles were measured using a Kruss K100 tensiometer (Krüss GmbH, Hamburg, Germany) by means of the wicking method and the Washburn Equation (22, 23).

ζ potentials of bacteria and silica sand were estimated from their electrophoretic mobility measured by dynamic light scattering (Zetasizer 3000HAS, Malvern Instruments Ltd., Malvern, UK). Bacterial strains and silica sand were measured by suspending in the buffer solutions. Silica gel was ground first before being suspended in the solutions. Each measurement was repeated 5 times and the average results were reported.

Interaction Free Energy Calculation

Bacteria suffer from repulsive electrostatic interactions when getting close to the media as both the bacteria and sediments are negatively charged. Once bacteria overcome the repulsive barrier and get close to the sediments with the help of hydrodynamics forces, Lifshitz-van der Waals and Lewis acid /base interactions begin to dominate. At the equilibrium distance (1.57 Å (20)) where the physical contact occurs, Lifshitz-van der Waals and Lewis acid/base interactions are actually the driving forces that are responsible for bacteria to adhere to the sediments. At this stage, electrostatic interactions are ignored owing to the double layer superimposition (20, 24).

$$\Delta G^{LW}_{y_0 132} = -4\pi R y_0[(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_2^{LW}})(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}})] \quad (2)$$

$$\begin{aligned}\Delta G^{AB}_{y_0 132} = 4\pi R y_0[&(\sqrt{\gamma_1^{+}} - \sqrt{\gamma_2^{+}})(\sqrt{\gamma_1^{-}} - \sqrt{\gamma_2^{-}}) \\ &- (\sqrt{\gamma_1^{+}} - \sqrt{\gamma_3^{+}})(\sqrt{\gamma_1^{-}} - \sqrt{\gamma_3^{-}}) \\ &- (\sqrt{\gamma_2^{+}} - \sqrt{\gamma_3^{+}})(\sqrt{\gamma_2^{-}} - \sqrt{\gamma_3^{-}})]\end{aligned} \quad (3)$$

where $\Delta G^{LW}_{y0\,132}$ and $\Delta\, G^{AB}_{y0\,132}$ are Lifshitz-van der Waals and Lewis acid/base interaction free energies between bacteria, 1 and sediments, 2 immersed in the solution, 3 evaluated at the equilibrium distance (kT); R bacterial radius (m); and y_0 equilibrium distance of 1.57 Å.

Column Experiment

Column experiments were conducted using an acrylic column (Kimble-Kontes, 2.5 cm × 15) under water saturated conditions. The column was oriented vertically and sealed at the bottom with a custom frit to permit the flow of water and retain the medium. The bottom of the column was equipped with a single-layer nylon membrane (500 mesh, 25 μm pore opening, Gilson Company, Lewis Center, OH). Silica sand was packed in the column through CO_2 solvation to eliminate air pockets. Bacterial suspensions were introduced to the column by a peristaltic pump from the bottom at a flow rate of 0.33 ml/min. Bacterial concentrations were monitored from the elution collected on the top of the column and at locations 5 cm and 10 cm below the top. For each run, 150 ml bacteria suspended in sterilized nanopure de-ionized water (5×10^8 cells/ml as determined by ATP analysis) was pumped into the column. The column was then flushed with sterilized nanopure de-ionized water alone for up to 50 pore volumes until no bacterial cells could be detected from the elution. Elution was collected by a fraction collector and was measured for bacterial concentrations using ATP analysis. After each run, a breakthrough curve was generated and mass balance was performed.

Bacterial retention in sand columns is believed to be controlled by kinetic adsorption instead of equilibrium adsorption processes (25–29). To be more general, bacterial transport through saturated porous media can be described by the equilibrium-kinetic two-region model with bacterial deposition occurs in the kinetic region only (30). These two-site models consist of an expression to describe bacterial retention at equilibrium adsorption sites as well as an expression to describe bacterial retention at kinetic adsorption sites. Adsorption at equilibrium sites is assumed to be instantaneous and can be represented by linear adsorption isotherms; while adsorption at kinetic sites is assumed to be a time-dependent, first-order irreversible reaction (30).

$$\left[1 + \frac{f\rho_b K_d}{\theta}\right]\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} - \frac{\alpha\rho_b}{\theta}[(1 - f)K_d C - S_k] \quad (4)$$

$$\frac{\partial S_k}{\partial_t} = \alpha[(1 - f)K_d C - S_k] - \mu_{s,k} S_k \quad (5)$$

where C is the bacterial concentration in the aqueous phase (cells/m^3); S_k bacterial concentration on kinetic adsorption sites (cells/g); t elapsed time (sec); f fraction of adsorption sites that equilibrate with the bacteria in the aqueous phase (−); ρ_b bulk density (g/m^3); K_d partitioning coefficient of bacterial to the equilibrium adsorption sites of the medium (m^3/g); θ, porosity (m^3/m^3); D longitudinal dispersion coefficient (m^2/sec); x coordinate parallel to the flow (m); v pore velocity (m/sec); α first order mass transfer coefficient governing the rate of bacterial exchange between equilibrium and kinetic adsorption sites (sec^{-1}); and $\mu_{s,k}$ first order bacterial deposition coefficient on kinetic adsorption sites (sec^{-1}). Transport parameters in equations (4) and (5) were obtained by fitting experimentally obtained bacterial breakthrough data using CXTFIT 2.1 (30). The initial and boundary conditions for Equations (4) and (5) are:

$$C = C_0 \quad x = 0 \quad \text{for} \quad t \le t_{pulse} \quad (6)$$

$$C = 0 \quad x = 0 \quad \text{for} \quad t > t_{pulse} \quad (7)$$

$$C = 0 \quad x = L \quad t = 0 \quad (8)$$

All these parameters were optimized by minimizing the sum of the squared difference between observed and fitted concentrations using the nonlinear least-square method. A conservative pulse tracer (chloride) breakthrough curve was determined separately before the introduction of bacteria. The conductivity (μmhos) of the tracer was measured and fitted in equations (4) and (5) with f, K_d, α, S_k equal to 0 to estimate media porosity. The tracer (150 ml of 1 M NaCl) was introduced to the column by the peristaltic pump from the bottom at the same flow rate of 0.33 ml/min.

RESULTS AND DISCUSSION

Marshall et al. (31) described bacterial adhesion to solid surfaces as a two-step process. Bacterial adhesion begins with long-range, non-specific, reversible interactions between bacterial cells and substrate, which is unstable and bacterial cells at this stage can be removed from adsorbed surfaces by fluid shear before firm adhesion can occur. These long-range interactions are dependent upon physicochemical properties of bacterial cells and substratum surfaces, as well as the intervening medium. Once bacterial cells are in close proximity to a surface, they can establish short-range, irreversible interactions, which is also dependent upon physicochemical properties of bacterial cells and substratum surfaces, as well as the intervening medium. These two processes together refer to initial adhesion. When bacteria stabilize on a surface, they may turn down their metabolism and grow slowly as microcolonies, starting to secrete an exopolysaccharide matrix in order to cement themselves to the surface, which is a time-dependent biological process. This slimy layer of bacteria embedded in a polysaccharide matrix is known as biofilm, which may change an essentially homogeneous surface into a chemically active heterogeneous surface (32). Physico-chemical processes of bacterial adhesion to abiotic surfaces have been extensively studied and described by the traditional and extended DLVO theory (21, 33, 34). It has been proven that the initial adhesion of bacteria to substrate plays an important role in bacterial adhesion (34–36). Meinders et al. (33) further concluded that initial bacterial adhesion could be explained in terms of bacterial physicochemical surface properties.

Surface Thermodynamic Property of Bacteria, Silica Sand, and LPS-coated Silica Sand

Both bacterial strains, silica sand and LPS-coated silica sand were negatively charged as demonstrated by their negative ζ potential values. Bacterial and silica sand surface thermodynamic properties were calculated according to equation (1) based on their contact angles measured with diiodomethane, formamide and water (Table 1). *E. coli*, *P. fluorescens*, and *S. mitis* had γ^{LW} values of 39.1 mJ/m^2, 35.7 mJ/m^2, and 44.7 mJ/m^2, respectively. Besides, they exhibited a monopolar surface, i.e., their γ_l^- was at least one magnitude order greater than γ_l^+ (58.4 mJ/m^2, 56.8 mJ/m^2 and 23.5 mJ/m^2 as compared to 0.58 mJ/m^2, 1.29 mJ/m^2 and 0.74 mJ/m^2) (20). Gram-negative bacteria, e.g., *E. coli* and *P. fluorescence* had greater γ^- values than that of gram-positive bacteria, e.g., *S. mitis* (58.4 mJ/m^2 and 58.6 mJ/m^2 as compared to 23.5 mJ/m^2). Silica sand had a γ^{LW} of 22.7 mJ/m^2, γ^+ of 1.57 mJ/m^2, and γ^- of 15.4 mJ/m^2. After LPS coating, its γ^{LW} and γ^+ increased to 28.6 mJ/m^2 and 2.67 mJ/m^2; while γ^- decreased to 12.7 mJ/m^2,

Table 1. Contact angles and surface thermodynamic properties

	θ^{DII} (°)	θ^{F} (°)	θ^{W} (°)	γ^{LW} (mJ/m^2)	γ^{+} (mJ/m^2)	γ^{-} (mJ/m^2)	ζ (mV)
E. coli	41.0 ± 0.7	24.5 ± 0.4	14.6 ± 0.5	39.1	0.58	58.4	−15.8 ± 0.5
P. fluorescens	47.4 ± 0.8	22.2 ± 0.5	14.2 ± 0.4	35.7	1.29	56.8	−18.9 ± 0.4
S. mitis 357	28.8 ± 0.8	28.5 ± 0.6	49.5 ± 0.5	44.7	0.74	23.5	−25.4 ± 0.6
Silica sand	70.3 ± 1.2	59.9 ± 0.4	70.8 ± 0.4	22.7	1.57	15.4	−36.4 ± 0.8
LPS coated silica sand	59.5 ± 1.0	45.7 ± 0.6	65.8 ± 0.6	28.9	2.67	12.7	−40.6 ± 0.7

[DII]Contact angles measured with diiodomethane.
[F]Contact angles measured with formamide.
[W]Contact angles measured with water.

respectively. Silica sand also exhibited a monopolar surface and its monopolarity decreased after LPS coating.

Bacterial Interaction with Uncoated and LPS-coated Silica Sand

As both *E. coli*, *P. fluorescens*, *S. mitis*, and silica sand were negatively charged, electrostatic interactions between bacteria and silica sand and between bacteria and LPS-coated silica sand were positive, which served as the barrier to prevent bacteria to get close to the medium (34). These repulsive electrostatic interactions operated in the range of several tens of nanometers (37). Once bacteria overcome the repulsive barrier and get close to the medium surface with the help of hydrodynamics forces, Lifshitz-van der Waals and Lewis acid/base interactions begin to dominate. At the equilibrium distance of 1.57 Å where physical contacts occur, electrostatic interactions can be ignored owing to the superimposition of the double layers (20). Moreover, interactions evaluated at the equilibrium distance are often used to interpret bacterial adhesion in porous media (17).

Free energies of interactions between bacteria and silica sand and between bacteria and LPS-coated silica sand evaluated at the equilibrium distance were calculated according to equations (2) and (3) (Table 2). Both *E. coli*, *P. fluorescens* and *S. mitis* had attractive Lifshitz-van der Waals and Lewis acid/base interactions with silica sand. ΔG_{132}^{TOT}, sum of Lifshitz-van der Waals and Lewis acid/base interaction free energies, was negative for all the cases, demonstrating adhesion potentials of *E. coli*, *P. fluorescens*, and *S. mitis* to silica sand. Among these three bacteria strains, *S. mitis* had the smallest (negatively greatest) ΔG_{132}^{TOT} value, followed by *E. coli* and *P. fluorescens*. After LPS coating, Lifshitz-van der Waals and Lewis acid/base interactions between bacteria and silica sand negatively increased (Table 2).

Table 2. Interaction free energies between bacteria and uncoated and LPS-coated silica sand

Uncoated silica sand	ΔG_{132}^{LW} (kT)[a]	ΔG_{132}^{AB} (kT)	ΔG^{TOT} (kT)
E. coli	−137.1	−230.4	−367.5
P. fluorescens	−67.8	−105.2	−173.0
S. mitis 357	−174.5	−1014.0	−1188.5
LPS-coated silica sand	ΔG_{131}^{LW} (kT)	ΔG_{131}^{AB} (kT)	ΔG^{TOT} (kT)
E. coli	−268.5	−666.8	−935.3
P. fluorescens	−132.8	−348.8	−481.6
S. mitis 357	−341.7	−1267.3	−1609

[a] k is the Boltzmann constant (1.38×10^{-23} J/K) and T is absolute temperature (K). At 25°C, 1 kT = 4.11×10^{-21} J.

Bacterial Retention

Bacterial breakthrough curves are plotted as a function of pore volumes that were estimated from the tracer experiments. Bacterial strains displayed symmetric-shaped breakthrough curves, which became broader and diffuser with the increase of the column length. Among the three bacterial strains used in this research, *S. mitis* had the most retention as manifested by the broader and diffuser and smaller peak-valued breakthrough curves (Fig. 1). Changes in breakthrough curves with LPS coating manifested enhanced bacterial retention. By integrating bacterial breakthrough curves over the range to 50 pore volumes, it was found that 34.1%, 29.6% and 13.8% of

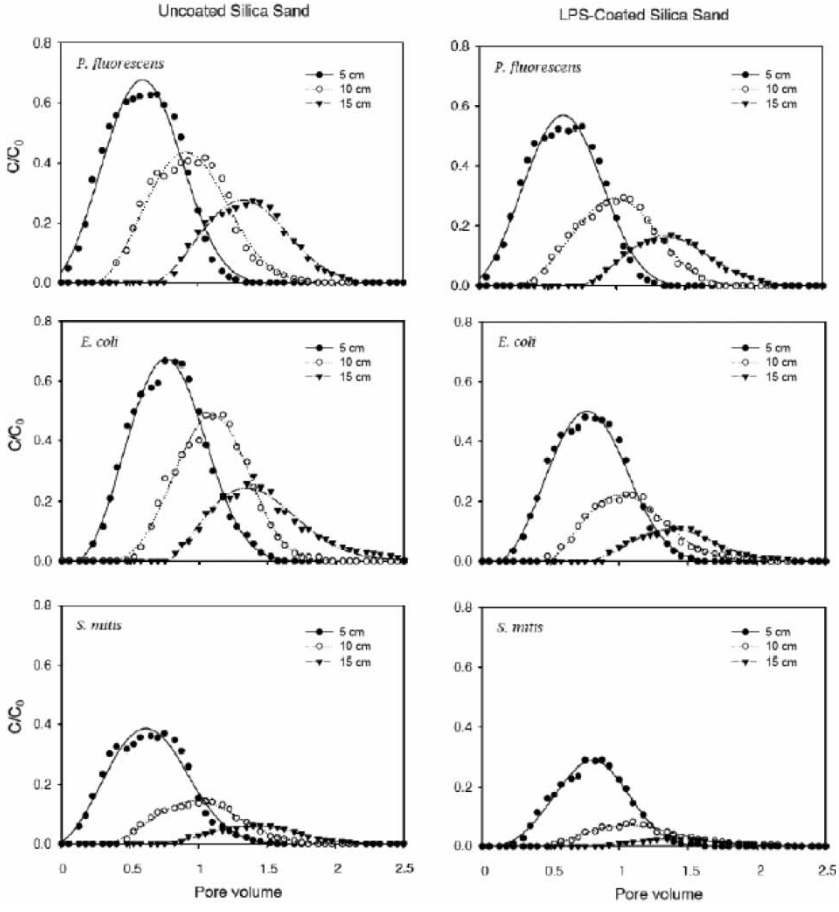

Figure 1. Bacterial breakthrough curves in uncoated and LPS-coated silica sand columns.

P. fluorescens, *E. coli*, and *S. mitis* were recovered in uncoated silica sand and 23.9%, 19.8% and 6.8% in LPS-coated silica sand. Bacterial retention was not even along the length of the column. For instance, around 63.3%, 47.5%, and 29.6% of *E. coli* was recovered when collected at a length of 5 cm, 10 cm and 15 cm below the top, respectively. After LPS-coating, the percentage recovery decreased to 58.3%, 38.6%, and 19.8%, respectively. For *P. fluorescens* and *S. mitis*, similar observations were made.

The equilibrium-kinetic two-region concept model was successful in describing bacterial transport (Fig. 1). Two-site models provide better descriptions of bacterial transport to account for the complications arising from the potential of bacteria to react with different components of the medium matrices. It should be noted that kinetically controlled bacterial adsorption dominates over equilibrium adsorption. It was hypothesized that bacterial deposition occurred in the kinetic adsorption region only and was limited by mass transfer between the equilibrium region and the kinetic region. *S. mitis* had the greatest dispersion coefficient, equilibrium fraction, mass transfer coefficient, and partitioning coefficient values among the three bacterial strains studied, followed by *E. coli* and *P. fluorescens* (Fig. 2). After LPS coating, dispersion coefficient, equilibrium fraction, mass transfer coefficient, and partitioning coefficient increased for these three strains.

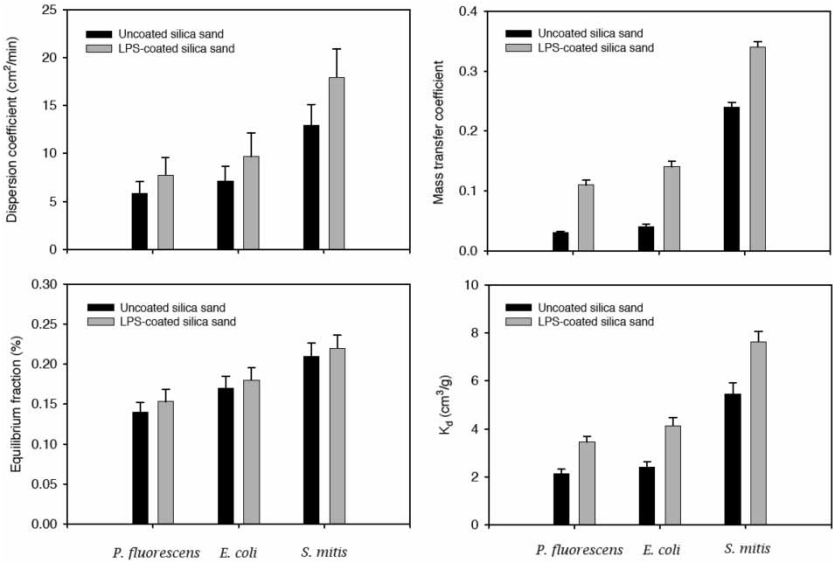

Figure 2. Dispersion coefficient and equilibrium fraction of bacterial transport in uncoated and LPS-coated silica sand columns.

Bacterial-Sediment Interaction and Bacterial Retention

The underlying principles behind bacterial retention in porous media resulted from forms of bonding between bacterial strains and adsorption receptor sites on silica sand. The amount of bacterial adsorption that occurred was dependent on the surface characteristics of bacterial cells and silica sand. More generally, bacterial adsorption was thought to be determined by interactions between bacteria and silica sand. To develop a surface thermodynamic explanation of the phenomena, free energies of interactions between bacteria and silica sand were investigated. Bacterial retention in silica sand was found to correspond to free energies of interactions between bacteria and silica sand (ΔG_{132}^{TOT}) evaluated at the equilibrium distance (Fig. 3). *S. mitis* had greater retention in silica sand (deposition coefficient of 0.124 min^{-1} in uncoated silica sand and 0.168 min^{-1} in LPS-coated silica sand) than *P. fluorescens* and *E. coli* (0.054 min^{-1} and 0.055 min^{-1} in uncoated silica sand and 0.079 min^{-1} and 0.097 min^{-1} in LPS-coated silica sand) because it had smaller ΔG_{132}^{TOT} values (-1188.5 kT as compared to -173.0 kT and -367.5 kT in silica sand and -1609.0 kT as compared to -481.6 kT and -953.3 kT in LPS-coated silica sand). After LPS coating, γ^{LW} and γ^{+} increased and γ^{-} decreased for silica sand, resulting in a decrease in ΔG_{132}^{TOT} (negatively increase). Consequently, more bacterial cells were retained. Dispersion coefficient exponentially increased with the increase of free energies of interactions between bacteria and silica sand (Fig. 4). On the other hand, equilibrium fraction, mass transfer coefficient, and partitioning coefficient increased linearly with the increase of free energies of interactions between bacteria

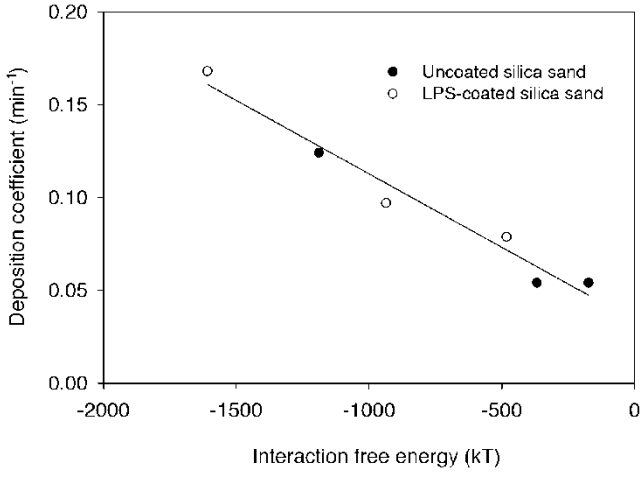

Figure 3. Bacterial deposition coefficient as a function of interaction free energies between bacteria and uncoated and LPS-coated silica sand.

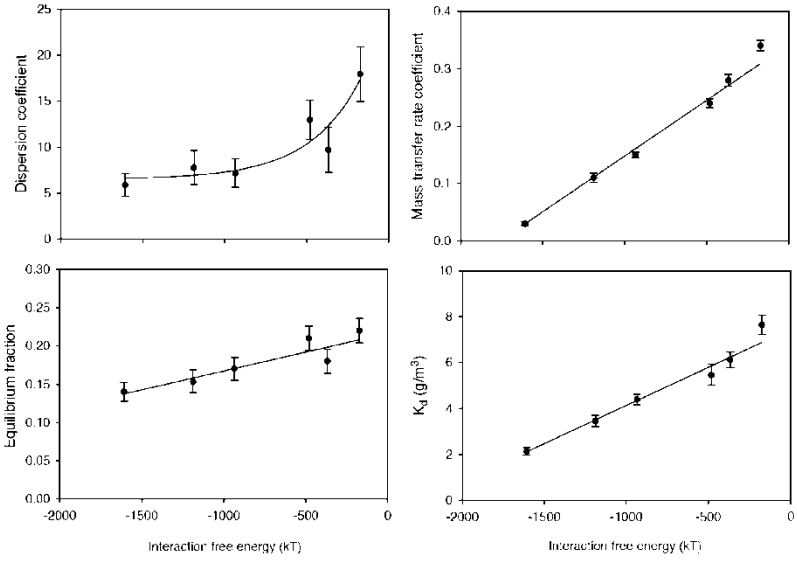

Figure 4. Dispersion coefficient, equilibrium fraction, mass transfer coefficient, and partitioning coefficient as a function of interaction free energies between bacteria and uncoated and LPS-coated silica sand.

and silica sand. Hendry et al. (38, 39) proposed that bacterial adsorption characteristics were bacteria specific and likely related to their surface chemistry. In this research, bacterial retention is directly linked to the free energy of their interactions with the porous media.

CONCLUSION

Increased demand for domestic water supplies is promoting the development of reclaimed domestic wastewater reuse projects, which is associated with concerns of the possible contamination of groundwater. So far, no reliable estimation of the risks associated with reclaimed wastewater usage in irrigation has been made. However, pathogenic bacteria in aquifer systems are a threat to public health. There is no doubt that the risk of contamination by pathogenic bacteria is somewhat higher for soils that are repeatedly receiving reclaimed wastewater for irrigation due to the persistence of pathogenic bacteria. It has been recommended to monitor fate and transport of pathogenic bacteria when reclaimed domestic wastewater is used for irrigation, especially in the areas that receive repeated applications. Results of this research accomplish this task by providing a conceptual model that can be used to predict the fate and transport of these pathogenic bacteria in soils.

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REFERENCES

1. Crook, J. and Surampalli, R.Y. (1996) Water reclamation and reuse criteria in the US. *Water Sci. Technol.*, 33 (10–11): 451–462.
2. Asano, T., Leong, L.C.Y., Rigby, M.G., and Sakija, R.H. (1992) Evaluation of the California wastewater reclamation criteria using enteric virus monitoring data. *Water Sci. Technol.*, 26 (7–8): 1513–1524.
3. Gerba, C.P. and Goyal, S.M. (1985) Pathogen removal from wastewater during groundwater recharge. In *Artificial Recharge of Groundwater*; Asano, T. (ed.), Butterworth Publishers: Boston, MA, pp. 283–317.
4. Reneau, R.B., Jr., Hagedorn, C., and Degen, M.J. (1989) Fate and transport of biological and inorganic contaminants from on-site disposal of domestic wastewater. *J. Environ. Qual.*, 18 (2): 135–144.
5. Gavaskar, A.R., Gupta, N., Sass, B.M., Janosy, R.J., and O'Sullivan, D. (1998) *Permeable Barriers for Groundwater Remediation*; Battelle Press: Columbus, Ohio.
6. Kiser, P.F., Wilson, G., and Needham, D. (2000) Lipid-coated microgels for the triggered release of doxorubicin. *J. Control. Release*, 68 (1): 9–22.
7. Steinbüchel, A. (2001) Perspectives for biotechnological production and utilization of biopolymers: metabolic engineering of polyhydroxyalkanoate biosynthesis pathways as a successful example. *Macromol. Biosci.*, 1 (1): 1–24.
8. Scharer, J.M. and Byerley, J.J. (1989) Aspects of uranium adsorption by microorganisms. *Hydrometallurgy*, 21 (3): 319–329.
9. Makin, S.A. and Beveridge, T.J. (1996) The influence of A-band and B-band lipopolysaccharide on the surface characteristics and adhesion of *Pseudomonas aeruginosa* to surfaces. *Microbiol.*, 142 (Pt 2): 299–307.
10. Rivera, M. and McGroarty, E.J. (1989) Analysis of a common-antigen lipopolysaccharide from *Pseudomonas aerugonisa*. *J. Bacteriol.*, 171 (4): 2244–2248.
11. Peterson, A.A., Hancock, R.E.W., and McGroarty, E.J. (1985) Binding of poly-cationic antibiotics and polyamines to lipopolysaccharides of *Pseudomonas aerugonisa.*. *J. Bacteriol.*, 164 (3): 1356–1261.
12. Okabe, S., Nielsen, P.H., Jones, W.L., and Characklis, W.G. (1994) Estimation of cellular and extracellular carbon contents in sulfate-reducing bacteria biofilms by lipopolysaccharide assay and epifluorescence microscopic technique. *Wat. Res.*, 28 (11): 2263–2266.
13. Walker, S.L., Redman, J.A., and Elimelech, M. (2004) Role of cell surface lipopolysaccharides (LPS) in *Escherichia coli* K12 adhesion and transport. *Langmuir*, 20 (18): 7736–7746.
14. Bitton, G. and Gerba, C.P. (1984) *Groundwater Pollution Microbiology: The Emerging Issue*; John Wiley and Sons: New York, NY.
15. Dowd, S.E. and Pillai, S.D. (1997) Survival and transport of selected bacterial pathogens and indicator viruses under sandy aquifer conditions. *J. Environ. Sci. Health.*, 32A (8): 2245–2258.

16. Herbold-Paschke, K., Straub, U., Hahn, T., Teutsch, G., and Botzenhart, K. (1991) Behavior of pathogenic bacteria, phages and viruses in groundwater during transport and adsorption. *Water Sci. Technol.*, 24 (2): 301–304.
17. Chen, G. and Strevett, K.A. (2001) Impact of surface thermodynamics on bacterial transport. *Environ. Microbiol.*, 3 (4): 237–245.
18. Hancock, I. and Poxton, I. (1988) *Bacterial Cell Surface Techniques*; John Wiley & Sons: New York.
19. Lee, C. and Tsai, C. (1999) Quantification of bacterial lipopolysaccharides by the purpald assay: measuring formaldehyde generated from 2-keto-3-deoxyoctonate and heptose at the inner core by periodate oxidation. *Anal Biochem.*, 267 (1): 161–168.
20. van Oss, C.J. (1994) *Interfacial Forces in Aqueous Media*; Marcel Dekker: New York.
21. Sharma, P.K. and Rao, K.H. (2002) Analysis of different approaches for evaluation of surface energy of microbial cells by contact angle goniometry. *Adv. Colloid Interface Sci.*, 98 (3): 341–463.
22. Washburn, E.W. (1921) The dynamics of capillary flow. *Phys. Rev.*, 17 (3): 273–283.
23. Wålinder, M.E. and Gardner, D.J. (1999) Factors influencing contact angle measurements on wood particles by column wicking. *J. Adhesion Sci. Technol.*, 13 (12): 1363–1374.
24. Dong, H., Onstott, T.C., Ko, C., Hollingsworth, A.D., Brown, D.G., and Mailloux, B.J. (2002) Theoretical prediction of collision efficiency between adhesion-deficient bacteria and sediment grain surface. *Colloids Surf. B.*, 24 (3): 229–245.
25. Bolster, C.H., Hornberger, G.M., Mills, A.L., and Wilson, J.L. (1998) A method for calculating bacterial deposition coefficients using the fraction of bacteria recovered from laboratory columns. *Environ. Sci. Technol.*, 32 (9): 1329–1332.
26. Gannon, J.T., Mingelgrin, U., Alexander, M., and Wagenet, R.J. (1991) Bacterial transport through homogeneous soil. *Biol. Biochem.*, 23 (12): 1155–1160.
27. Harvey, R.W. and Garabedian, S.P. (1991) Use of colloid filtration theory in modeling movement of bacteria through a contaminated sandy aquifer. *Environ. Sci. Technol.*, 25 (1): 178–185.
28. Hornberger, G.M., Mills, A.L., and Herman, J.S. (1992) Bacterial transport in porous media: evaluation of a model using laboratory observations. *Water Resour. Res.*, 28 (3): 915–938.
29. Lindqvist, R., Soo Cho, J., and Enfield, C.G. (1994) A kinetic model for cell density dependent bacterial transport in porous media. *Water Res.*, 30 (12): 3291–3299.
30. Toride, N., Leij, F.J., and van Genuchten, M.Th. (1995) The CXTFIT code for estimating transport parameters from laboratory or field tracer experiments, CA. Res. Ep. No. 137, U.S. Salinity Lab., USDA, ARS, Riverside.
31. Marshall, K. (1990) Biofilms, biofouling and biocorrosion. *Trends in Biotechnol.*, 8 (12): 337–337.
32. Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., and Lappin-Scott, H.M. (1995) Microbial biofilms. *Annu. Rev. Micribiol.*, 49: 711–746.
33. Meinders, J.M., van der Mei, H.C., and Busscher, H.J. (1995) Deposition efficiency and reversibility of bacterial adhesion under flow. *J. Colloid Interface Sci.*, 176 (2): 329–341.

34. Bos, R., van der Mei, H.C., and Busscher, H.J. (1999) Physico-chemistry of initial microbial adhesive interactions—its mechanisms and methods for study. *FEMS Microbiol. Rev.*, 23 (2): 179–230.
35. Gottenbos, B., van der Mei, H.C., and Busscher, H.J. (1999) Models for studying initial adhesion and surface growth in biofilm formation on surfaces. *Method Enzymol.*, 310: 523–534.
36. Gottenbos, B., van der Mei, H.C., and Busscher, H.J. (2000) Initial adhesion and surface growth of *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* on biomedical polymers. *J. Biomed. Mater Res.*, 50 (2): 208–214.
37. Smets, B.F., Grasso, D., Engwall, M., and Machinist, B. (1999) Surface physio-chemical properties of *Pseudomonas fluorescens* and impact on adhesion and transport through porous media. *Colloid Surf. B.*, 14 (1): 121–139.
38. Hendry, M.J., Lawrence, J.R., and Maloszewski, P. (1999) Effects of velocity on the transport of two bacteria through saturated sand. *Ground Water*, 37 (1): 103–112.
39. Hendry, M.J., Lawrence, J.R., and Maloszewski, P. (1997) The role of sorption in the transport of Klebsiella oxytoca through saturated silica sand. *Ground Water*, 35 (4): 574–584.