Groundwater Research Report
WR10R007

TRANSPORT OF MANURE-DERIVED, TETRACYCLINE RESISTANT ESCHERICHIA COLI IN UNSATURATED SOIL

Lucia Feriancikova
Shangping Xu

(2013)

Project Number WR10R007

TRANSPORT OF MANURE-DERIVED, TETRACYCLINE RESISTANT ESCHERICHIA COLI IN UNSATURATED SOIL

Lucia Feriancikova
Department of Geosciences
University of Wisconsin-Milwaukee

Shangping Xu
Department of Geosciences
University of Wisconsin-Milwaukee

Administered by:
University of Wisconsin Water Resources Institute

Funded by:
State of Wisconsin Groundwater Research and Monitoring Program

(2013)

This project was also supported, in part, by General Purpose Revenue funds of the State of Wisconsin to the University of Wisconsin System for the performance of research on groundwater quality and quantity. Selection of projects was conducted on a competitive basis through a joint solicitation from the University and the Wisconsin Departments of Natural Resources; Agriculture, Trade and Consumer Protection; Commerce; and advice of the Wisconsin Groundwater Research Advisory Council and with the concurrence of the Wisconsin Groundwater Coordinating Council.

Transport of manure-derived, tetracycline resistant *Escherichia coli* in unsaturated soil

A Report Prepared for Wisconsin Groundwater Coordinating Council

Lucia Feriancikova, Shangping Xu

Department of Geosciences, University of Wisconsin Milwaukee

1. Introduction

As "America's Dairyland", Wisconsin is the home to ~15,000 dairy farms and 1.2 million cows, which are producing ~ 4 million tons (dry weight) of manure. Lately, the prevalence of antibiotic resistant bacteria in Wisconsin's dairy manure was reported (Ray et al. 2006, 2007, Sato et al. 2004, Sato et al. 2005, Halbert et al. 2006, Walczak and Xu 2009). Ray *et al.* (2006), for instance, compared the antibiotic-resistance of *Salmonella* isolated from conventional and organic dairy farms in four states including Wisconsin, Michigan, Minnesota and New York, and found that 1) *Salmonella* isolates from most farms were resistant to at least one of following antibiotics: amoxicillin-clavulanic acid, ampicillin, cephalothin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline, and 2) *Salmonella* isolates that were resistant to 5 or more antibiotics were occurring on 24.6% of conventional farms and 11.5% of organic farms. Halbert *et al.* (2006) reported that out of 2030 *Campylobacter* isolates from both organic and conventional dairy farms, 10% were resistant to ampicillin, and 30-60% were resistant to kanamycin, sulfamethoxazole and tetracycline. Sato *et al.* (2004) studied the antibiotic resistance of *Campylobacter* isolated from Wisconsin dairy farms and found that 45% of *Campylobacter* isolates were resistant to tetracycline and no difference was observed between organic and conventional farms. In another study, Sato *et al.* (2005) tested antibiotic resistance of *E. coli* isolated from manure samples collected from 30 organic and 30 conventional Wisconsin dairy farms, and found prevalence of antibiotic resistance to ampicillin, amoxicillin-clavulanic acid, cephalothin, cefoxitin, ceftiofur, streptomycin, kanamycin, gentamycin, apramycin, chloramphenicol, tetracycline, sulfamethoxazole and trimethoprim-sulfamethoxazole. My group recently tested the antibiotic resistance of *E. coli* isolated from lactating cows of different ages and observed that 1) 99.3%, 25.1%, 98.9%, 59.1% and 0.6% of the *E. coli* isolates were resistant to cephalothin, tetracycline, erythromycin, ampicillin and sulfamethoxazole, respectively; 2) 99.6%, 67.6%, 23.2% and 0.1% of the *E. coli* isolates were resistant to 2, 3, 4 and 5 antibiotics (Walczak and Xu 2011).

Manure produced in dairy farms is usually applied to the crop fields as a source of fertilizer. The spread of manure is performed either daily or following temporary storage in structures that are often not lined (Turnquist et al. 2006, Jackson-Smith et al. 2000). Leakage from manure storage structures and downward infiltration of water through manure-laden soil could lead to groundwater contamination by antibiotic resistant bacteria (Anderson and Sobsey 2006, Mckeon et al. 1995). Sapkota *et al.* (2007) reported that a concentrated animal feeding operation (CAFO) resulted in groundwater pollution by *Enterococci* that were resistant to erythromycin, tetracycline and clindamycin. Anderson and Sobey (2006) observed high percentages of antibiotic-resistant *E. coli* in groundwater samples collected in the vicinity of a CAFO. Makeon *et al.* (1995) tested antibiotic resistance of more than 250 coliform and noncoliform bacteria isolated from rural groundwater supplies. It was found that 100% of the noncoliforms and 87% of the coliforms were resistant to at

least one of the 16 antibiotics, with resistance most commonly directed toward novobiocin, cephalothin, and ampicillin. Approximately 60% of the coliforms were resistant to multiple drugs.

About 70% of Wisconsin's population depends on groundwater as the source of drinking water (Solley et al. 1998). In rural areas where most of the dairy farms are located groundwater often accounts for 100% of drinking water supply (Ellefson et al. 2002). Contamination of groundwater by antibiotic resistant bacteria associated with dairy manure thus poses a direct and serious public health threat (World Health Organization 2003). Additionally, the health risks associated with the spread of antibiotic resistant bacteria in soil and groundwater are amplified by the phenomenon of horizontal gene transfer, through which antibiotic resistance can be passed to a diverse group of microorganisms, including pathogens (Levy 1998).

Recently, we compared the transport of tetracycline-resistant (tet^R) and tetracycline-susceptible (tet^S) *E.* (Walczak et al. 2011a, Walczak et al. 2011b). Our results showed that the tet^R *E. coli* consistently displayed higher mobility than the tet^S *E. coli* within saturated sands (Figure 1, left panel). The surface properties of the *E. coli* isolates were characterized and compared. The profiling of cell outer membrane protein (OMPs) using sodium dodecyl-sulfate polyacrylamide gel (SDS-PAGE) suggested that different proteins existed on the outer membrane of tet^R and tet^S strains (Figure 1, right panel). Specifically, there were at least four proteins present in the outer membrane of the tet^R strains that were absent in the tet^S strains (indicated by arrows in Figure 1). These proteins had approximate molecular masses of 54, 47, 44 and 40 kDa. Additionally, there were three proteins (indicated with arrow heads in Figure 1) that were present in the tet^S strains but were absent in the tet^R strains. The OMP TolC, which has a molecular mass of 52 kDa and represents the OMP component of drug efflux pumps, was identified as an OMP that *coli* strains that were isolated from dairy manure and Lake Michigan water potentially could have enhanced the *E. coli* mobility within saturated sands (Xu et al. 2006, Alekshun and Levy 2007, de Cristobal et al. 2006).

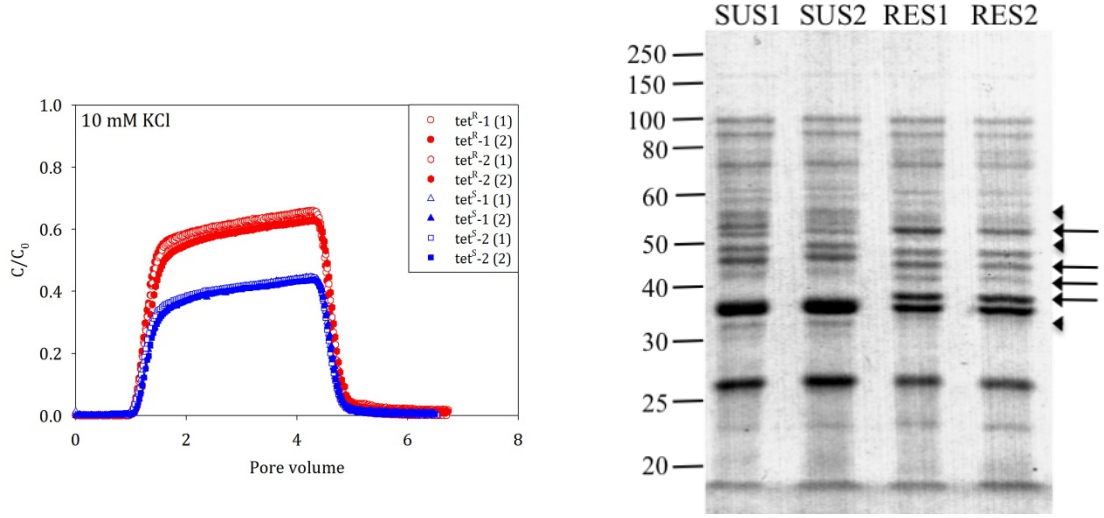

Figure 1. (Left) Comparison of the mobility of the tet^R and tet^S *E. coli* isolated from dairy manure within saturated sands. (Right) Outer membrane protein profiles of the tet^R (RES1 and RES2) and tet^S (SUS1 and SUS2) *E. coli* isolates using SDS-PAGE. Molecular masses (kDa) are indicated on the left. Proteins that are present in the tetracycline resistant strains, but absent in the tetracycline susceptible strains, are indicated with an arrow. Proteins that are present in the tetracycline susceptible strains, but absent in the tetracycline resistant strains are indicated with arrow heads.

2. Research Goals

Based on the previously published research findings, the first goal of this research is to specifically evaluate the effects of OMP TolC on the transport of *E. coli* within saturated sands using mutants that are created through genetic manipulation. The findings from this research will provide valuable insights into the relationship between cell surface structures and *E. coli* mobility.

In saturated porous media, the transport of bacterial cells is primarily controlled by the kinetics of cell deposition at the solid-water interfaces. When the soil is partially saturated, several mechanisms contribute to cell deposition. In addition to the solid-water interface, bacterial cells may adhere to the air-water interface. Bacterial cells can also be strained at the edges of pendular rings that form around adjacent grains or by the thin water film that stretches between the pendulum rings (Denovio et al. 2004). Published experimental results showed that pore water chemistry and soil moisture content are the most important parameters that control the retention of colloid-sized particles in unsaturated soil (Denovio et al. 2004, Lenhart and Saiers 2002). The second goal of this research is to evaluate the influence of pore water chemistry and soil moisture content on the transport of tet^R *E. coli* in unsaturated soil. It was also reported that chemical perturbation, drainage (i.e. drying front) and imbibitions (i.e. wetting front) could mobilize substantial quantities of colloid-sized particles in partially saturated soil (Cheng and Saiers 2009, Saiers et al. 2003).This proposed research will also examine the release of the retained bacterial cells under transient chemical and flow conditions.

3. Effects of OMP TolC on *E. coli* transport[1]

3.1. Materials and Methods

In this research, the wild type strain was *E. coli* K12 (strain W25113), which was used to make the Keio Collection of single-gene knockouts (Baba et al. 2006). The strain JW5503 (*tolC::kan*) was obtained from the Keio collection: in this strain the *tolC* open reading frame was replaced with a kanamycin cassette (amplified from plasmid pKD13) flanked by FLP recombination sites (Baba et al. 2006). To construct the markerless deletion of *tolC* (i.e., to excise kanamycin resistance), *E. coli* JW5503 was transformed with plasmid pCP20 and the kanamycin resistant colonies were selected at 30°C. pCP20 has temperature sensitive replication and thermal induction of FLP recombinase expression (Cherepanov and Wackernagel 1995, Datsenko and Wanner 2000). The transformants were cultured at 43°C, after which the loss of both pCP20 and the kanamycin resistance cassette were confirmed via polymerase chain reaction (PCR) and ampicillin/kanamycin sensitivity testing. The markerless strain lacking TolC was referred to as *ΔtolC*.

The *E. coli* cells preserved in 20% glycerol under -80°C was streaked onto Luria-Bertani (LB) (Fisher Scientific) agar plates. After overnight incubation at 37°C, cells from a freshly formed colony were transferred to culture tubes containing 15 ml LB broth. The culture tubes were shaken at 90 rpm and incubated at 37°C for 6 hours. The starter culture was used to inoculate LB broth (1:500 dilution ratio). Following the incubation at 37°C for 18 hours on an orbital shaker (90 rpm), the *E. coli* cells were harvested by centrifugation (4000×*g*, 10 minutes, 4°C). To remove the growth medium, the bacterial pellet was rinsed 4 times with the appropriate electrolyte solution. The

[1] This research was also supported by UWM Research Foundation through a fellowship to LF. A manuscript has been published in Environmental Science & Technology (doi: 10.1021/es400292x).

concentration of cells was then adjusted to ~4×10^7 cell/ml for the column transport experiments. The pH of the cell suspensions was ~5.7.

The silica sands used for the column experiments were purchased from US Silica and had a size range of 0.210-0.297 mm. The sands received from the manufacturer were alternately cleaned using concentrated nitric acid to remove metal hydroxides and diluted NaOH solution to remove natural clay particles (Xu et al. 2008), rinsed with deionized water and dried at 80 ºC. The porosity of the sand was 0.37 as determined using the bulk density method (Weight 2008).

A pair of glass chromatography columns (Kontes, Vineland, NJ) measuring 15 cm in length and 2.5 cm in diameter were vertically oriented and then wet-packed with the clean silica sands. Care was taken to eliminate the possibility of trapped air bubbles. Once packed, >30 pore volumes (PV) of appropriate background electrolyte (i.e., 1, 5, 20, 50 or 100 mM NaCl) was injected into the columns to equilibrate them. The downward flow was driven by gravity and the Darcy velocity was maintained at 0.31 cm/min using peristaltic pumps (MasterFlex, Vernon Hills, IL). The flow velocity that was employed in the column experiments is on the high end of natural groundwater flow, and is on the same order of magnitude as that encountered in riverbank filtration (Havelaar et al. 1995).

Upon the completion of the equilibration step, the transport experiments were initiated by injecting the *E. coli* cell suspensions to the top of the columns and concentrations of the bacterial cells in the effluent were determined through measuring the absorbance at a wavelength of 220 nm using a Shimadzu UV-1700 spectrophotometer. The injection of *E. coli* cell suspension (~3.5 PV) lasted for 60 min, after which the columns were flushed with bacteria-free background electrolyte solution until the absorbance of effluent returned to the background values.

The clean-bed deposition rate coefficients (k_d) of the *E. coli* cells within the saturated sand packs were estimated from the steady-state breakthrough concentrations in the effluent (Walker et al. 2005, Kretzschmar et al. 1997, Castro and Tufenkji 2007):

$$k_d = -\frac{v}{\varepsilon L}\ln\left(\frac{C}{C_0}\right) \qquad (1)$$

where ε is porosity, v is the specific discharge, L is the column length and C/C_0 is the normalized breakthrough concentration relevant to clean-bed conditions, which was obtained from the average bacterial breakthrough concentrations between 1.8-2.0 PV (Walker et al. 2005, Castro and Tufenkji 2007).

The retained *E. coli* cells can be remobilized when the ionic strength of the solution is lowered (Redman et al. 2004). For each *E. coli* strain, upon the completion of the column experiments using 100 mM NaCl, the 1 mM NaCl solution was injected to the columns and the concentrations of the released *E. coli* cells were monitored similarly using the spectrophotometer. The results obtained were used to evaluate the reversibility of *E. coli* retention within the sand packs.

The mobility of *E. coli* cells within the saturated sands is determined by the energy interactions between the cells and the surface of the sands. According to the XDLVO theory, the energy interactions between the *E. coli* cells and the surface of quartz sands are the summation of the Lifshitz−van der Waals (LW) interaction, the electrostatic double layer (EDL) interaction and the Lewis acid-base (AB) interaction:

$$\Phi^{\text{Total}} = \Phi^{\text{LW}} + \Phi^{\text{EDL}} + \Phi^{\text{AB}} \qquad (2)$$

The LW, EDL and AB interaction energies (Φ^{LW}, Φ^{EDL} and Φ^{AB}) for the cell-sand (sphere-plate geometry) system can be calculated using the following equations (Redman et al. 2004, Ong et al. 1999, Bayoudh et al. 2006, Bayoudh et al. 2009, Farahat et al. 2009, Elimelech 1994, Huang et al. 2010, Morrow et al. 2005a):

$$\Phi^{LW} = -\frac{Aa_b}{6h}\left[1-\frac{5.32h}{\lambda}\ln\left(1+\frac{\lambda}{5.32h}\right)\right] \qquad (3)$$

$$\Phi^{EDL} = \pi\varepsilon_0\varepsilon_w a_b\left\{2\psi_b\psi_s\ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right]+\left(\psi_b^2+\psi_s^2\right)\ln\left[1-\exp(-2\kappa h\right]\right\} \qquad (4)$$

$$\Phi^{AB} = 2\pi a_b\lambda_w\Delta G_{h_0}^{AB}\exp\left(\frac{h_0-h}{\lambda_w}\right) \qquad (5)$$

where A is the Hamaker constant; a_b is the radius of the bacterial cells; λ is the characteristic wavelength and was set as 42.5 nm; h is the separation distance between the cell and sand surface; ε_0 is the dielectric permittivity of vacuum, ε_w is the dielectric constant of water; κ^{-1} is the Debye length ($\frac{0.302}{\sqrt{I}}$ nm at 22ºC, I=ionic strength); ψ_b and ψ_s are the surface potentials of the bacterial cells and sand, respectively; λ_w (= 0.6 nm) is the characteristic decay length of AB interactions in water; h_0 represents the minimum equilibrium distance between the cell and sand surface due to Born repulsion and equals to 0.157 nm; and $\Delta G_{h_0}^{AB}$ represents the hydrophobicity interaction free energies per unit area corresponding to h_0.

The values of a_b, ψ_b, ψ_s, A and $\Delta G_{h_0}^{AB}$ were required for the interaction energy calculations. To determine cell sizes (a_b), images of the *E. coli* cells suspended in 1 and 100 mM NaCl were obtained using a Nikon Eclipse 50i microscope that was equipped with a Photometric CoolSnap ES digital camera and MetaMorph software. The length and width of ~ 30 cells were determined using the ImageJ software and the equivalent radii of the cells were calculated as $\sqrt{\frac{L_C\times W_C}{\pi}}$, where L_C and W_C represent the length and width of the cell, respectively (Haznedaroglu et al. 2008, Wang et al. 2011). In this research, zeta potential values were used in place of surface potentials for the XDLVO calculations (Walker et al. 2004). *E. coli* cell suspensions were prepared in a similar fashion as the column transport experiments and the quartz sands were pulverized and the colloid-sized quartz particles were suspended in the NaCl solutions (Porubcan and Xu 2011). The zeta potential values of the bacterial cells and quartz particles were then measured using a ZetaPALS analyzer (Brookhaven Instruments Corporation).

The Hamaker constants were calculated from the LW surface tension parameters of bacteria (γ_b^{LW}), water (γ_w^{LW}) and sand (γ_s^{LW}) (van Oss 1993):

$$A = 24\pi h_0^2\left(\sqrt{\gamma_b^{LW}}-\sqrt{\gamma_w^{LW}}\right)\left(\sqrt{\gamma_s^{LW}}-\sqrt{\gamma_w^{LW}}\right) \qquad (6)$$

The values of $\Delta G_{h_0}^{AB}$ can be obtained from the electron-accepting (γ^+) and electron-donating (γ^-) surface tension parameters (van Oss 1993):

$$\Delta G_{h_0}^{AB} = 2\left[\sqrt{\gamma_w^+}\left(\sqrt{\gamma_b^-}+\sqrt{\gamma_s^-}-\sqrt{\gamma_w^-}\right)+\sqrt{\gamma_w^-}\left(\sqrt{\gamma_b^+}+\sqrt{\gamma_s^+}-\sqrt{\gamma_w^+}\right)-\sqrt{\gamma_b^-\gamma_s^+}-\sqrt{\gamma_b^+\gamma_s^-}\right] \quad (7)$$

where the subscripts of *b*, *w* and *s* represent bacteria, water and sand, respectively. For water, the values of γ_w^{LW}, γ_w^+ and γ_w^- are 21.8, 25.5 and 25.5 mJ m^{-2}, respectively (Morrow et al. 2005b). For quartz sands, the previously reported values of γ_s^{LW} (39.2 mJ m^{-2}), γ_s^+ (1.4 mJ m^{-2}) and γ_s^- (47.8 mJ m^{-2}) were used in this research (2005a). To determine the values of γ_b^{LW}, γ_b^+ and γ_b^- for each *E. coli* strain, bacterial lawns were produced by filtering cells onto porous membrane, which was subsequently dried at room temperature for ~15 minutes. The contact angles (θ) of two polar and one non-polar probe liquids with known surface tension parameters on the bacterial lawns were measured using a Rame-Hart goniometer (Ong et al. 1999, Morrow et al. 2005a, van Oss 1993):

$$\gamma_i^L(1+\cos\theta) = 2\sqrt{\gamma_i^{LW}\gamma^{LW}} + 2\sqrt{\gamma_i^+\gamma^-} + 2\sqrt{\gamma_i^-\gamma^+} \quad (8)$$

where the subscript *i* represents water (γ^L=72.8, γ^{LW}=21.8 and γ^+=γ^- = 25.5 mJ m^{-2}), glycerol (γ^L=64.0, γ^{LW}=34.0, γ^+= 3.92 and γ^-=57.4 mJ m^{-2}) or diiodomethane (γ^L=50.8, γ^{LW}=50.8 and γ^+=γ^- = 0 mJ m^{-2}) (van Oss 1993), respectively.

3.2 Results and Discussion

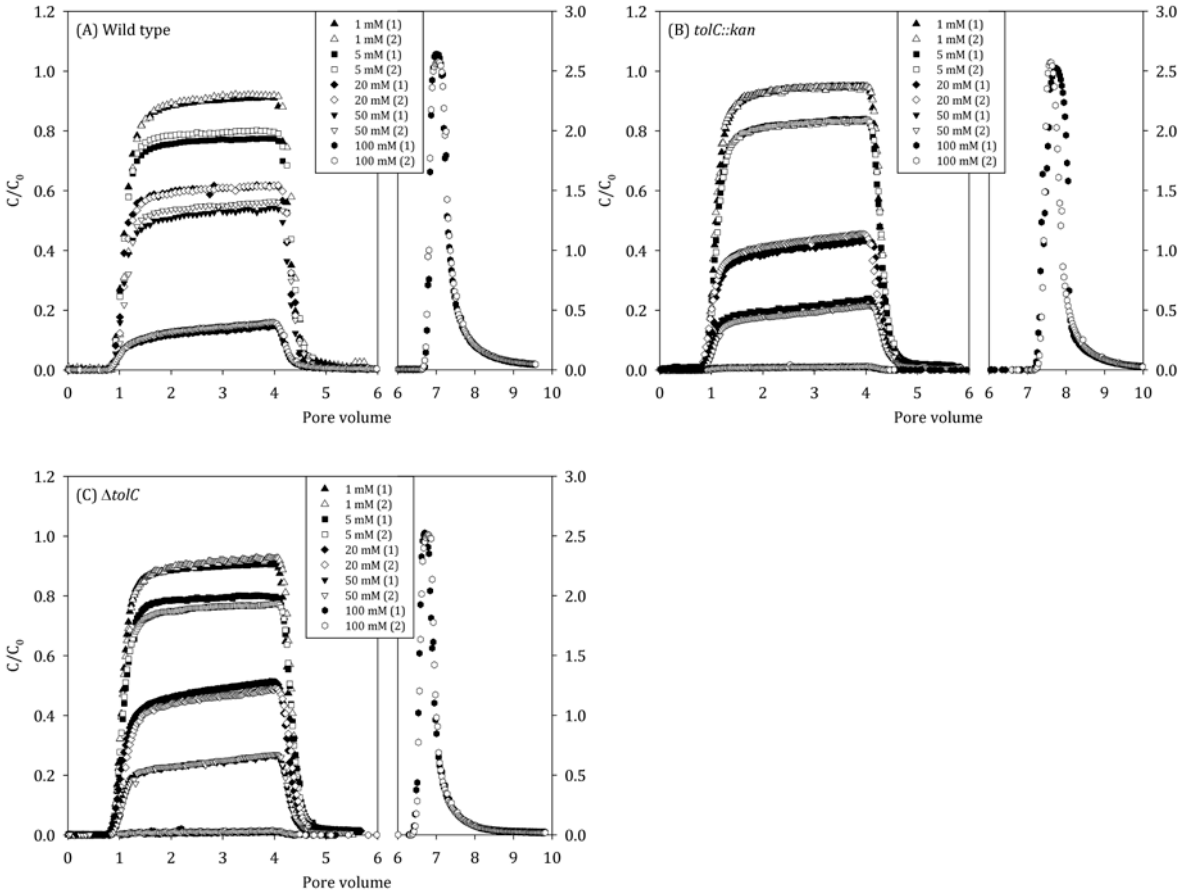

Figure 2. Breakthrough curves of (A) wild type, (B) t*olC::kan* and (C) Δ*TolC E. coli* cells suspended in 1, 5, 20, 50 and 100 mM NaCl, respectively (left panels). *C* represents *E. coli*

concentrations in the effluent and C_0 represents influent *E. coli* concentrations. Upon the completion of the 100 mM NaCl experiments, the 1 mM NaCl solution was injected to the columns and the release kinetics of the previously retained bacterial cells are shown in the right panels.

The normalized effluent *E. coli* concentrations under various ionic strength conditions are shown in Figure 2 (left panels). For all the *E. coli* strains, the increase in ionic strength led to lower breakthrough concentrations and lower recovery of bacterial cells in the effluent. For instance, the breakthrough concentrations (between 1.8-2.0 PV) of the wild type strain decreased from 87.0(±1.7)% to 11.2(±0.6)% when ionic strength increased from 1 mM to 100 mM. Accordingly, integration of the breakthrough curves showed that 86.3(±2.9)% and 12.8(±0.6)% of the wild type *E. coli* cells traveled through the sand columns under 1 and 100 mM NaCl conditions, respectively.

Upon the completion of the column experiments using 100 mM NaCl, the 1 mM NaCl solution was injected into the columns and the concentrations of the released *E. coli* cells were monitored (Figure 2, right panels). The pulse-type release of the previously retained *E. coli* cells led to extremely high cell concentrations in the effluent. Integration of the release curves showed that, at the end of the release experiments, 28.0(±2.6)%, 50.9(±7.8)% and 60.4(±0.5)% of the cells remained immobilized for the for the wild type, *tolC::kan* and Δ*tolC E. coli* strains, respectively. In comparison, the column experiments performed using 1 mM NaCl showed that ~13.7(±2.9)%, 9.4(±0.9)% and 11.7(±1.7)% of the wild type, *tolC::kan* and Δ*tolC* cells were retained, respectively. The fact that higher fractions of the cells remained retained following the release experiments suggested that cell immobilization was only partially reversible.

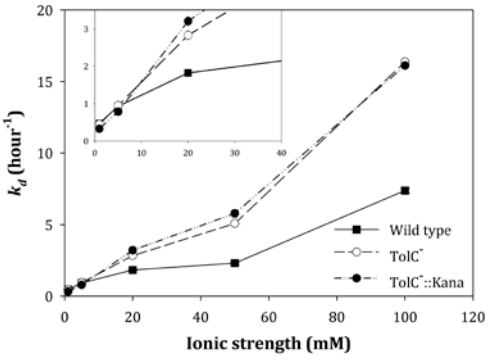

Figure 3. Average deposition rate coefficients (k_d) for the three *E. coli* strains under ionic strength conditions of 1, 5, 20, 50 and 100 mM. The error bars, which represent the standard deviations, are usually smaller than the symbols.

The clean-bed deposition rate coefficients (k_d) were calculated from the early breakthrough concentrations using equation (1) (Figure 3). Student's *t*-test performed using Microsoft Excel showed that the deposition rate coefficients were similar (p>0.05) for the *tolC::kan* and Δ*tolC* strains under all ionic strength conditions (1-100 mM NaCl). This indicated that the insertion of the kanamycin resistance cassette into *E. coli* chromosome and the gain of kanamycin resistance had little effects on the mobility of *E. coli* within saturated quartz sands. When the ionic strength was between 1-5 mM, the wild type *E. coli* strain also had similar k_d values as the TolC-deletion *E. coli* strains (Student's *t*-test, $p > 0.05$). The removal of OMP TolC, however, led to significant increase in values of k_d (i.e., decrease in *E. coli* mobility) when the ionic strength was between 20 and 100

mM (Student's *t*-test, $p < 0.05$). This finding was consistent to previously published results that suggested that OMP TolC could enhance the transport of *E. coli* isolated from various natural sources such as dairy manure (Walczak et al. 2011a, Walczak et al. 2011b). To elucidate the mechanisms behind the effects of OMP TolC on the mobility of *E. coli*, we characterized the *E. coli* cells and examined the energy interactions between the *E. coli* cells and quartz sands using the XDLVO theory.

Results from the cell size measurements under 1 and 100 mM NaCl conditions suggested that ionic strength had minimal effects (Student's *t*-test, $p > 0.05$) on the size of the *E. coli* cells. For instance, the equivalent diameter of the wild type *E. coli* cells was 2.04(±0.19) μm in 1 mM NaCl and 2.01(±0.17) μm in 100 mM NaCl, respectively. For each *E. coli* strain, all the size measurements were thus pooled together and a single diameter value was calculated. The average sizes of the wild type, *tolC::kan* and Δ*tolC* strains were 2.04(±0.19) μm, 1.98(±0.22) μm and 2.02(±0.17) μm, respectively. The results from Student's *t*-test indicated that the removal of OMP TolC and/or the presence of kanamycin resistance did not significantly alter cell size.

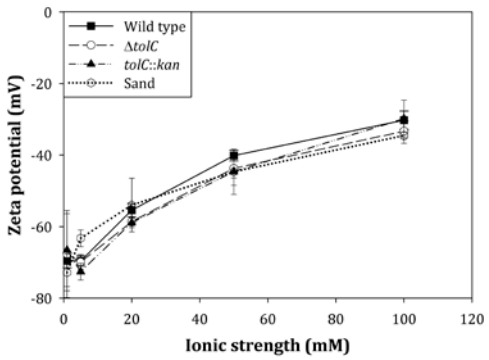

Figure 4. Zeta potential values of the *E. coli* cells and the quartz sands in 1, 5, 20, 50 and 100 mM NaCl. Error bars represent the standard deviation of a minimum of 5 measurements.

When suspended in 1, 5, 20, 50 and 100 mM NaCl (non-buffered, pH ~5.7), the zeta potential values of the *E. coli* cells were negative, suggesting that their surfaces were negatively charged (Figure 4). Given the slightly acidic pH of the cell suspensions, these negative charges should originate from the deprotonation of acid-base functional groups such as carboxylic and phosphoric groups (Hong and Brown 2006). With the increase in ionic strength, the zeta potential values of the *E. coli* cells generally became less negative due to the compression of the electric double layer (Wang et al. 2011). Additionally, all three *E. coli* strains displayed comparable zeta potential values under the same ionic strength conditions (Student's *t*-test, $p > 0.05$). The OMP TolC is a 471-residue trimer that contains an α-helical domain, a mixed α/β domain and a β domain (Koronakis et al. 2000). The α-helical domain, which forms a tunnel through the periplasm, is anchored to bacterial wall. The β-barrel, which extends to the outside of the outer membrane, has a length of ~4 nm (Koronakis et al. 2000). Because the exterior of the β domain is largely non-polar (Koronakis et al. 2000), it is expected that the deletion of TolC from the outer membrane would not significantly alter cell surface charge and zeta potential. Similarly, because the kanamycin resistance in the *tolC::kan* strain is conferred by the Tn5 neomycin phosphotransferase, an

aminoglycoside modifying enzyme that exists and functions inside the *E. coli* cell (Datsenko and Wanner 2000, Wright 2008), the kanamycin resistance had little effect on cell zeta potential.

Table 1. Contact angle measurements, surface tension components, Hamaker constant (A) and $\Delta G_{h_0}^{AB}$ for the three *E. coli* strains. Numbers in parenthesis are the standard deviations of the contact angle measurements.

Properties		**Wild type**	***tolC::kan***	**Δ*tolC***
Contact angle (°) (n≥4)	Water	18.7(±1.4)	19.0(±3.0)	19.6(±1.5)
	Glycerol	21.4(±8.4)	25.9(±2.0)	20.6(±2.8)
	Diiodomethane	67.4(±3.9)	55.4(±8.7)	54.1(±2.8)
Surface tension components (mJ m^{-2})	γ_b^{LW}	24.33	31.2	32.0
	γ_b^+	6.51	3.63	4.3
	γ_b^-	47.93	48.3	44.8
Hamaker constant, A (10^{-21} J)		0.78	2.72	2.92
$\Delta G_{h_0}^{AB}$ (mJ m^{-2})		23.8	26.4	23.8

The results of the contact angle measurements are shown in Table 1. Student's *t*-test indicated that there was significant difference ($p < 0.05$) in the diiodomethane contact angle between the wild type and TolC-deletion *E. coli* strains. It was interesting to note that the wild type, which was able to produce the non-polar OMP TolC, had a higher contact angle of the non-polar diiodomethane than the two TolC-deletion mutants. Similar trend was observed in Ong et al.(Ong et al. 1999) and Johanson et al (Johanson et al. 2012). Ong et al. observed *E. coli* D21f1, which lacks the charge-containing LPS, had a higher contact angle of diiodomethane than the LPS-producing *E. coli* D21 (Ong et al. 1999). Johanson et al. reported that *Enterococcus faecium* that produced the hydrophobic enterococcal surface protein (*esp*) had a higher contact angle of diiodomethane than the corresponding *esp*-deletion mutant (Johanson et al. 2012). The values of γ_b^{LW}, γ_b^+ and γ_b^- for each *E. coli* strain were then calculated from the contact angle measurements using equation (8) (Table 1). These values, together with the zeta potential values and cell size measurements were then used to quantify the energy interactions between the *E. coli* cells and the quartz sands.

The values of $\Delta G_{h_0}^{AB}$ were 23.8 mJ m^{-2}, 26.4 mJ m^{-2} and 23.8 mJ m^{-2} and the Hamaker constants were 0.78×10^{-21} J, 2.72×10^{-21} J and 2.92×10^{-21} J for the wild type, *tolC::kan* and Δ*tolC E. coli* strains, respectively (Table 1). The values $\Delta G_{h_0}^{AB}$ showed that the AB force between the *E. coli* cells and the surface of quartz sands was repulsive. Equation (6) suggests that the Hamaker constant is a function of the LW surface tension components of the bacterial cell, water and quartz sands. Because the electron-accepting (γ^+) and electron-donating (γ^-) surface tension components for diiodomethane are both zero, the LW surface tension parameter for each *E. coli* strain was calculated from equation (8) that corresponded to diiodomethane. The difference in the Hamaker constants thus was the result of the difference in the diiodomethane contact angles measured on the bacterial lawns of the three *E. coli* strains. The results obtained in this research indicated that variations in cell surface structures such as OMPs could alter cell surface tension components and subsequently the energy interactions between the cells and the surface of quartz sands. Similar relationship was reported in several previous publications (Ong et al. 1999, Johanson et al. 2012). Ong et al. (1999), for instance, observed that the wild type *E. coli* D21 and its LPS-deletion mutant

(strain D21f2) had different surface tension values. As a result, for four different types of surfaces (i.e., mica, glass, polystyrene and Teflon), the Hamaker constants for strain D21 were approximately two times the magnitude of the Hamker constants for strain D21f2. Johanson et al. (2012) reported that for *Enterococcus faecium*, the removal of the surface protein *esp* increased the Hamaker constant for the cell-water-quartz system from 4.2×10^{-21} J to 4.8×10^{-21} J and the value of $\Delta G_{h_0}^{AB}$ from 24.1 to 31.4 mJ m^{-2}.

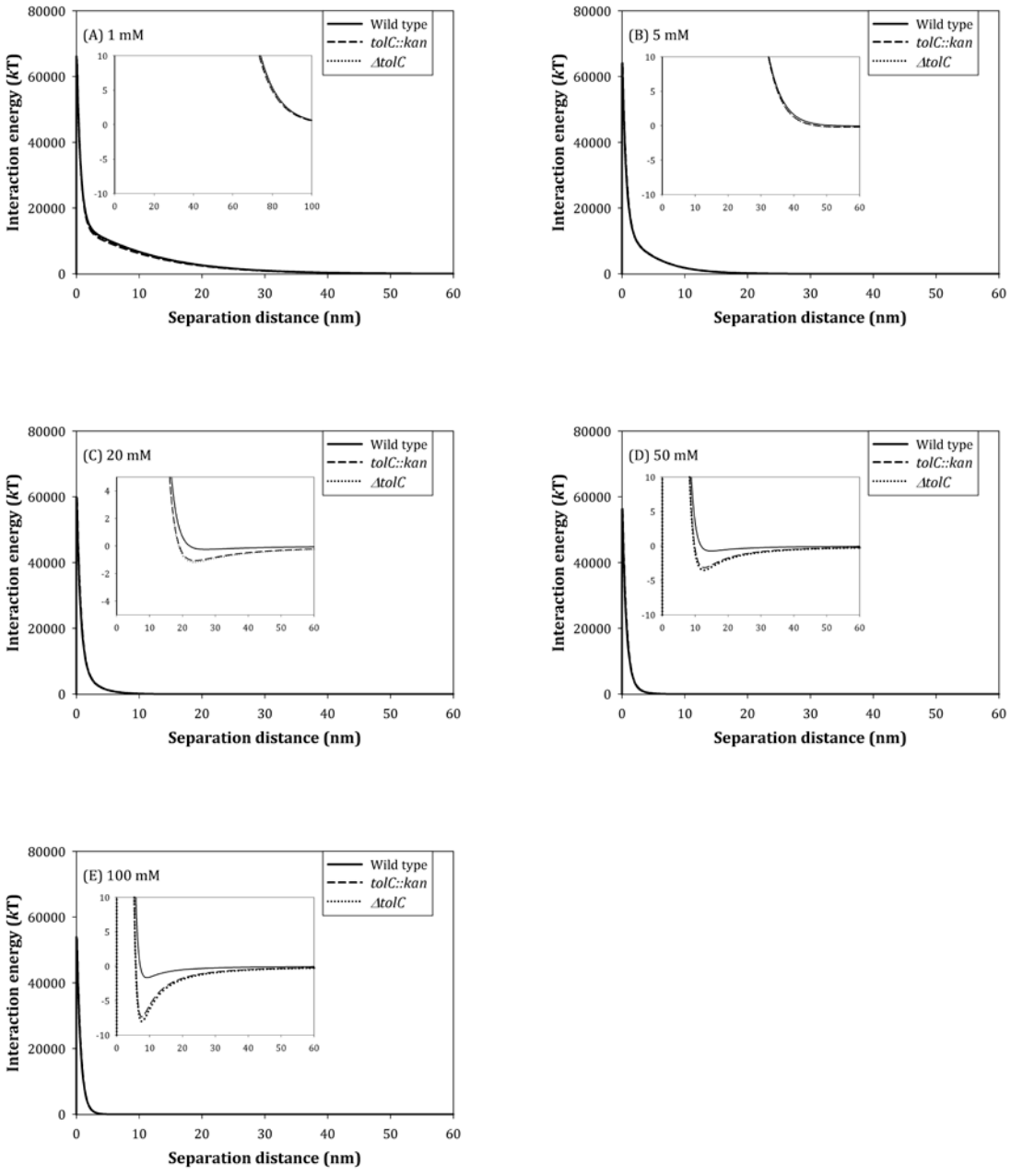

Figure 5. The calculated XDLVO interaction energy profiles as a function of separation distance for (A) 1 mM, (B) 5 mM, (C) 20 mM, (D) 50 mM and (E) 100 mM ionic strength conditions.

The XDLVO energy interactions between the *E. coli* cells and the surface of quartz sands were calculated and shown in Figure 5. For all the ionic strength conditions, there existed sizeable energy barriers for the attachment of *E. coli* cells to quartz sands. The magnitude of the energy barrier was comparable for all three *E. coli* strains under the same ionic strength condition, but decreased from ~6.4×10^4 *k*T to ~5.4×10^4 *k*T when the ionic strength increased from 1 mM to 100 mM. The presence of the substantial energy barriers made it difficult for the *E. coli* cells to be deposited into the primary energy minimum and the immobilization of the *E. coli* cells should thus occur primarily through their entrapment within the secondary energy minimum (Redman et al. 2004, Wang et al. 2011, Morrow et al. 2005b, Azeredo et al. 1999).

When the ionic strength was within 1-5 mM, the three *E. coli* strains shared similar XDLVO energy interaction profiles (Figures 5A and 5B). For the higher ionic strength range (20-100 mM), the XDLVO calculations showed that the two TolC-deletion strains had similar interaction energy profiles; and their secondary energy minimum was always deeper than the wild type strain. For each *E. coli* strain, the depth of the secondary energy minimum also increased with ionic strength. The XDLVO calculations were thus consistent to the experimental observations that i) the three *E. coli* strains had similar mobility under the low ionic strength conditions; ii) the TolC-deletion strains had higher deposition rates than the wild type strain under high ionic strength conditions; and iii) the deposition of the *E.coli* cells increased with ionic strength (Figure 3). Overall, our data suggested that the deposition rate coefficients (k_d) were negatively related to the depth of the secondary energy minimum.

The XDLVO calculations predicted the absence of the secondary minimum for the ionic strength of 1 mM NaCl (Figure 5A). Inspection of the XDLVO interaction energy profiles revealed that the lowest point was ~0.5 *k*T. In theory, this indicated that there should be no cell immobilization at the secondary energy minimum. The experimental results showed that the transport of the *E. coli* cells was not conservative and ~12% of the *E. coli* cells were immobilized. Similar discrepancies between the interaction energy calculations and particle transport results were previously reported (Farahat et al. 2009, Sharma and Rao 2003). For instance, Farahat et al. observed that *E. coli* cells could attach to quartz under pH > 4.5 when the XDLVO energy calculations showed the absence of secondary energy minimum (Farahat et al. 2009). A wide range of factors such as heterogeneity in cell properties (e.g., the zeta potential of some cell might be less negative than the average values), charge heterogeneity and roughness on the surface of quartz sands, XDLVO forces as well as flow hydrodynamics could have contributed to the deposition of a fraction of *E. coli* cells when the average XDLVO profile predicted the absence of secondary energy minimum (Torkzaban et al. 2008, Dong 2002, Bhattacharjee et al. 1998, Wang and Keller 2009).

The XDLVO calculations were also consistent to the results obtained from the release experiments. When the ionic strength was lowered from 100 mM NaCl to 1 mM, the depth of the secondary energy minimum decreased and a fraction of the *E. coli* cells that were previously retained was remobilized (Redman et al. 2004).

Compared to the DLVO theory, the extra force that is considered by the XDLVO theory is the AB force (equation 5). In this research, the AB force was repulsive and the inclusion of this force significantly increased the magnitude of the energy barrier. However, because the AB force decreased more rapidly with the separation distance than the LW and EDL forces, and because the secondary energy minimum was generally located 7 nm or further away from the sand surface, the AB force has very small effects on the depth of the secondary energy minimum. For *tolC::kan*, the

depth of the secondary energy minimum was -7.34 *k*T and -7.57 *k*T for the XDLVO theory and DLVO theory, respectively.

For Gram-negative bacteria such as *E. coli*, outer membrane structures such as LPS and proteins can exert steric interactions for the cell-surface system (Wang et al. 2011, Strauss et al. 2009). Such steric interactions are not considered by the XDLVO calculations and can significantly influence the transport behavior of bacterial cells within porous media (Wang et al. 2011). The *E. coli* K12 strain that was used in this study did not express the O-antigen of the LPS and the length of the LPS was estimated as 3±2 nm (Strauss et al. 2009). The length of OMP TolC that is extended to the outside of the membrane is ~4 nm (Koronakis et al. 2000). The XDLVO calculations showed that the secondary energy minimum was usually located >7 nm from the surface of the sands (Figure 4). Therefore, the steric forces could increase the magnitude of the energy barrier, but should have negligible impact on the location and depth of the secondary minimum. Wang et al. (2011) derived the steric force formula from the deGennes equation (Butt et al. 2005, Israelachvili 1991) using the Derjaguin's approximation:

$$\Phi^{steric} = \frac{4}{385h}\pi a_b \frac{kT}{s^3}\left[-20D^3\left(\frac{h}{L}\right)^{\frac{3}{4}} + 308L^3\left(\frac{h}{L}\right)^{\frac{3}{4}} - 420hL^2 + 132h^2L\right] \text{ (if } h<L\text{) (9)}$$

where Φ^{Steric} is the steric interaction energy, L is the length of the brush (e.g., LPS or protein), h is separation distance, a_b is the radius of the bacterial cell, k is Boltzmann constant, T is absolute temperature in Kelvin, s average distance between anchoring sites.

Assuming that L = 5 nm, and s = 2.2 nm (Strauss et al. 2009, Neidhardt 1996), the steric force between the *E. coli* K12 cell and the surface of the sands was calculated (Figure S1A). Because the steric force was 0 beyond 5 nm, the inclusion of the steric force did not alter the location or magnitude of the secondary energy minimum (Figure 6).

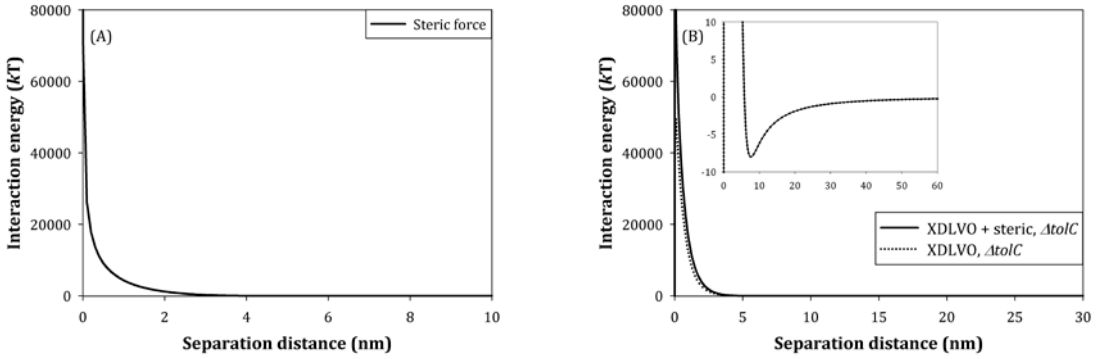

Figure 6. (A) The steric force profile between *E. coli* K12 cells and the surface of sand. (B) The inclusion of the steric force into the XDLVO calculation did not change the location and magnitude of the secondary energy minimum because the steric force diminished beyond 5 nm (i.e., the length of the LPS or protein).

Findings from this research confirmed that OMP TolC, which was firstly identified by Walczak et al. (2011a, 2011b), could enhance the mobility of *E. coli* within saturated sands. Drug efflux pumps represent one of the most effective and widespread mechanisms for antibiotic resistance in bacterial cells (Walsh 2003). Particularly, efflux pumps that span the cytoplasm, periplasm and outer membrane could transport antibiotics to the outside environment and therefore lead to bacterial resistance against high levels of antibiotics. The OMP TolC was reported to be the outer membrane component of several common efflux pumps (e.g., the AcrAB-TolC pump) that are responsible for bacterial resistance to various antibiotics such as tetracycline, macrolide and ampicillin (de Cristobal et al. 2006, Chollet et al. 2004, Fralick 1996, Nishino et al. 2003). It was observed that the total dissolved solid (TDS) of groundwater that was influenced by manure storage and application was often 1000 mg/L or higher (20 mM NaCl is equivalent to a TDS of 1170 mg/L) (Harter et al. 2002). Findings from this research suggested that antibiotic resistant bacteria with the OMP TolC could have higher mobility and display wider spread within sandy aquifers influenced by manure.

4. Transport of tet^R and tet^S *E. coli* isolated from manure within unsaturated soil

4.1 Materials and Methods

The tet^R and tet^S *E. coli* isolates used in this research were from Walczak et al. (2011b), where their mobility within saturated quartz sands were investigated. The results from the *saturated* experiments showed that the tet^R *E. coli* strain exhibited higher mobility than the tet^S *E. coli* strain. For this research, one tet^R and one tet^S *E. coli* isolates were selected for the experiments. The *E. coli* isolates stored in 20% glycerol under -80^{0}C were streaked onto Muller-Hinton (MH) agar plates. After overnight incubation at 37^{0}C, cells from the freshly formed colonies were transferred to culture tubes containing 15 ml Luria-Bertani (LB) broth. The culture tubes were incubated at 37^{0}C for 6 hours with 90 rpm shaking. The starter culture were used to inoculate LB broth (dilution ratio 1:500), which was then be incubated at 37^{0}C with 90 rpm shaking. After 18 hours of incubation, the bacterial cells were harvested using centrifuge (5000*g*, 10 minutes, 4^{0}C). To remove the growth medium, the bacterial pellet was rinsed 4 times with the appropriate electrolyte solution. The concentration of cells was adjusted to ~10^7 cells/ml and the suspension is ready for column transport study.

All column transport experiments were run in duplicates. Custom-built acrylic columns, measuring 7.6 cm in diameter and 7.6 cm in length, were used for the transport experiments. Two venting holes were drilled on opposite sides at the top of the vertically oriented column. The venting holes were covered with gas-permeable PTFE membranes which allowed for free air exchange and prevented loss of water from the column. On top of the PTFE membrane, the venting holes could be sealed using PVC tapes when air exchange was not desired (e.g., when a freshly packed column is equilibrated with background electrolyte solution). A moisture probe (ML2x Thetaprobe, Delta-T Devices) were inserted at the middle of the column to determine volumetric moisture content. Readings from the moisture probe were recorded using a datalogger (Delta-T Devices, Model GP1). Plates made from perforated stainless steel sheet were placed at the bottom and top of the column to support the sands within the column and to distribute the influent solution. Nylon membranes with 20 μm openings (Spectrum Laboratories) were placed on top of the stainless steel plates to hold the sand and to maintain capillary pressure inside the column. Both ends of the columns were sealed using butyl rubber O rings. The inflow (top) and outflow (bottom) rates were regulated using two peristaltic pumps (Masterflex, ColeParmer).

The columns were wet-packed by adding natural sands (US Silica, 595-841 μm in diameter) in 50 ml increments into electrolyte solution standing in the column. The porosity of the sand was 0.369. The newly added sands were stirred and mixed with the previously added sands. After each increment, the columns were tapped with hands to allow the newly added sands to settle. The packed sand columns were equilibrated with >4 pore volumes of appropriate electrolyte solution.

The transport of the *E. coli* isolates within the unsaturated soil was investigated under two different soil moisture content levels: ~12(±1)% (~30% saturation level) and ~25(±1)% (~68% saturation level), respectively. Following the equilibration step, the saturated sand columns were slowly drained by setting the outflow rate to be ~50% higher than the inflow rate. It was observed that by setting the inflow rate at 0.3 mL/min and 1.0 mL/min, we were able to achieve the ~12% and ~25% of soil moisture contents, respectively. Once the drying front reached the bottom of the column and the desired moisture content was achieved, the inflow and outflow rates were set to equal. The columns were further stabilized for ~0.5 hour before they were used for the *E. coli* transport experiments.

The *E. coli* cells suspended in the NaCl solutions (1 mM, 5 mM or 10 mM) were introduced to the top of the column. The concentrations of bacterial cells in the effluent was determined by measuring the light extinction at a wavelength of 220 nm with a UV/Vis spectrophotometer (Shimadzu UV-1700) equipped with 1-cm flow-through cuvettes at 30-second intervals (Walczak et al. 2011a, Walczak et al. 2011b). The injection of the cell suspension usually lasted ~ 2 pore volumes (PV) and then influent was switched to the background, cell-free NaCl solution. The experiments were completed when the cell concentration in the effluent approached zero. During the injection experiments, the soil moisture content remained stable.

The transport of the *E. coli* cells within the unsaturated soil was described by the two-region, dual-porosity model that assumes that the liquid phase could be separated into mobile and immobile zones (Pang et al. 2008). The exchange of *E. coli* cells between θ_m (flowing, inter-aggregate) and immobile θ_{im} (stagnant, intra-aggregate) zones was modeled as a first order process (Note: $\theta = \theta_m + \theta_{im}$). Additionally, we considered the potential detachment of *E. coli* cells that were previously immobilized. The general governing equations for the transport of the tracer and *E. coli* cells within the unsaturated soil were:

$$\theta_{im}\frac{\partial c_{im}}{\partial t} = \omega(c_m - c_{im}) \qquad (10)$$

$$\frac{\partial(\theta_m c_m)}{\partial t} + \rho\frac{\partial S}{\partial t} = \frac{\partial}{\partial x}\left(\theta_m D\frac{\partial c_m}{\partial x}\right) - \frac{\partial(q c_m)}{\partial x} - \omega(c_m - c_{im}) \qquad (11)$$

$$\rho\frac{\partial S}{\partial t} = \theta_m k_a c_m - k_d \rho S \qquad (12)$$

where c_m is the cell ($N_c\ L^{-3}$) concentration in the mobile region, c_{im} is the cell ($N_c\ L^{-3}$) concentration in the immobile region, ω (T^{-1}) is the first-order mass transfer rate between the mobile and immobile regions, ρ ($M\ L^{-3}$) is soil bulk density, S ($N_c\ M^{-1}$) is the quantity of immobilized *E. coli* cells in the solid phase, D (L^2T^{-1}) is the dispersion coefficient, k_a is the first-order attachment rate coefficient (T^{-1}), and k_d is the first-order detachment rate coefficient (T^{-1}).

The values of θ_m, θ_{im}, ω, D, k_a and k_d were estimated by inversely solving equations (10) to (12) using HYDRUS-1D. In other words, the HYDRUS-1D solutions were fitted to the breakthrough data using the least-squares algorithm to obtain the best-fit values.

The ionic strength of the soil solution may drop and this change in pore water chemistry could lead to the release of previously retained bacterial cells in unsaturated soil (Jensen et al. 1998).

Such release of the bacterial cells was previously identified as a major mechanism that can lead to groundwater contamination. For this purpose, the transport experiments were firstly carried out using 10 mM NaCl. The 1mM NaCl solution was then introduced to the columns. Once a new steady state was reached (i.e., effluent bacterial cell concentration returns to zero), the experiments was ended.

Soil moisture content constantly changes as a result of rainfall, snowmelt, irrigation and evapotranspiration. It was previously reported that the wetting (imbibitions) fronts can mobilize substantial quantities of colloid-sized particles in unsaturated soil (Cheng and Saiers 2009). To study the mobilization of retained bacterial cells during imbibition, a saturated column was firstly drained to a moisture content of ~12% of soil moisture content using a flow rate of 0.3 mL/min. Bacterial cell suspension was injected into the column under steady flow conditions. Following the flushing step under the same flow rate (i.e., 0.3 mL/min), the inflow rate was increased to 1 mL/min. Once the wetting front reached the bottom of the column and the soil moisture content rose to 25%(±2%), the outflow rate was set at 1 mL/min to maintain steady flow condition. As the moisture content inside the column increased, a fraction of the previously retained bacterial cells were mobilized. The experiment was terminated following the pulse-type release of bacterial cells.

4.2 Results and Discussions

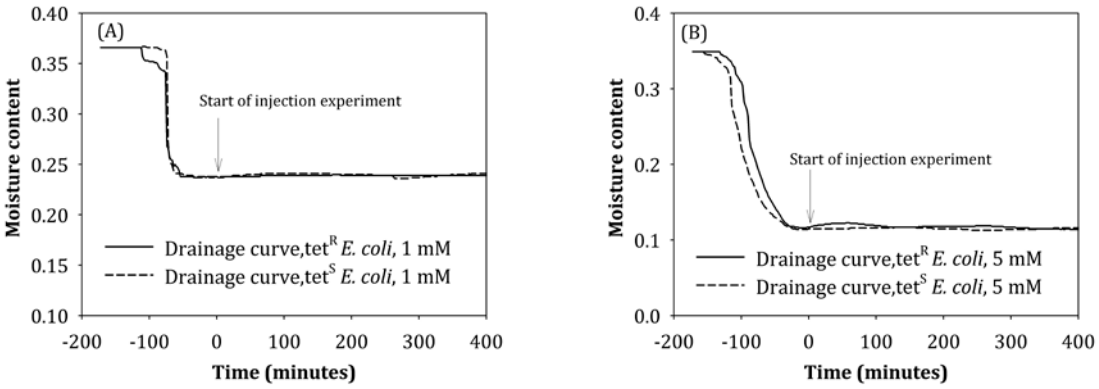

Figure 7. Representative soil drainage curve (soil moisture content vs. time) for the steady-state (i.e., constant soil moisture content) experiments. (A) drainage to ~25% of soil moisture content; (B) drainage to ~12% of soil moisture content. The injection of cell suspensions started at time 0.

From the saturated sand columns, the soil moisture content was lowered to ~12% and ~25% through controlling the inflow and outflow rates (Figure 7). Figure 7 also showed that once the target soil moisture content was reached, it can be maintained steady during the *E. coli* transport experiments.

The breakthrough curves of the tet^R and tet^S *E. coli* strains under high soil moisture content conditions (i.e., ~25%) are shown in Figure 8. When the ionic strength was 1 mM, the breakthrough concentrations of the tet^S *E. coli* strain were slightly higher than those of the tet^R *E. coli* strain. When the ionic strength was either 5 mM or 10 mM, the tet^R *E. coli* strain consistently displayed higher mobility than the tet^S *E. coli* strain (i.e., higher breakthrough concentrations for the tet^R *E. coli* strain). The mobility of the tet^S *E. coli* strain also decreased more rapidly with increasing ionic strength than the tet^R *E. coli* strain. Overall, the results suggested that, consistent to the findings

obtained under saturated conditions, the manure-derived tet^R *E. coli* strain could spread more easily than the corresponding tet^S *E. coli* strain that was isolated from the same source.

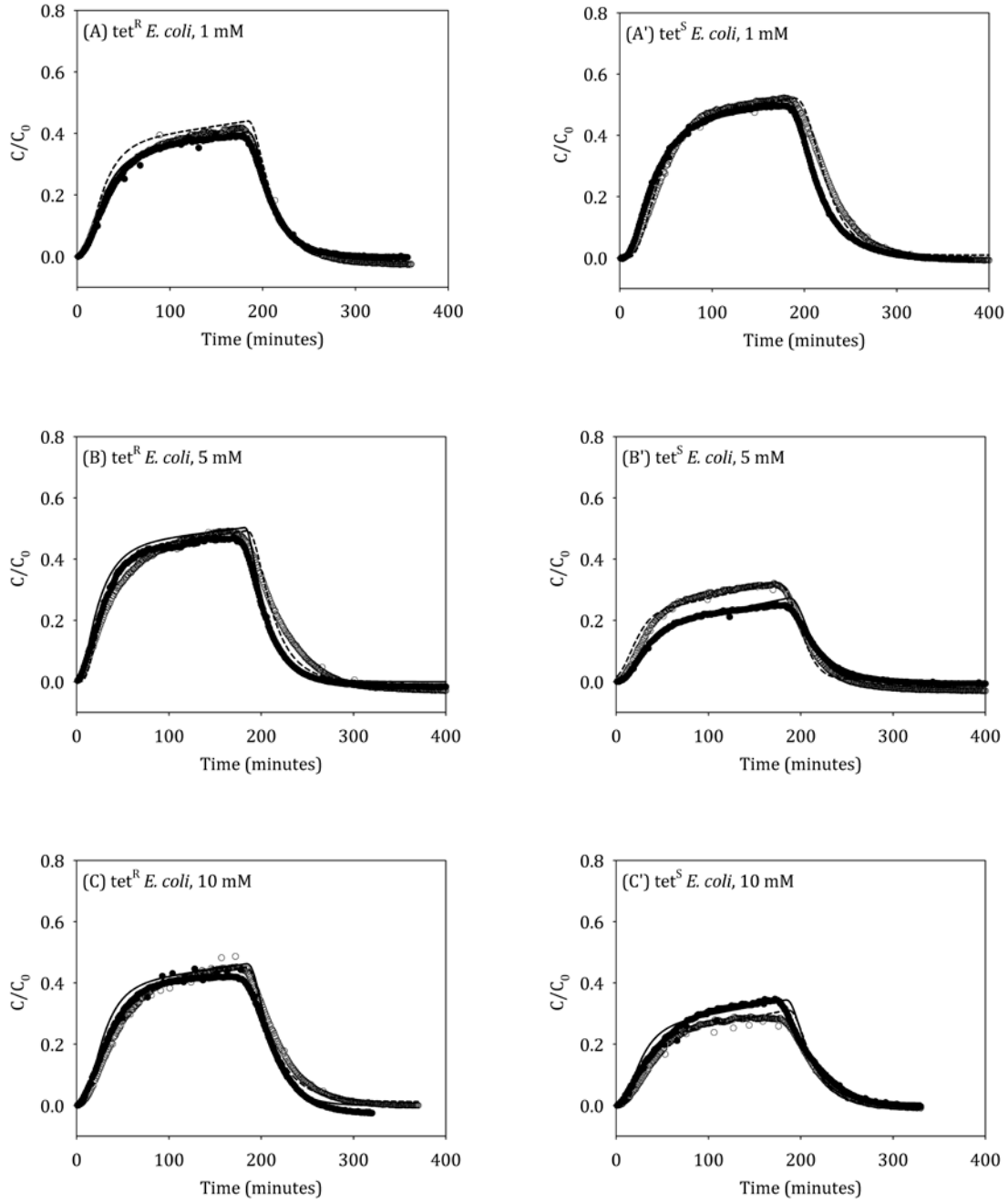

Figure 8. Breakthrough concentrations of the tet^R (A-C) and tetS (A′-C′) *E. coli* strains under ~25% of soil moisture content. Symbols represent experimental observations and lines represent model simulation results.

The mathematical model of equations (10) to (12) was fitted to the breakthrough data using the computer program HYDRUS-1D (Pang et al. 2008). Overall, the dual porosity model could

satisfactorily describe the transport behavior of the tracer under both soil moisture conditions with R^2 values greater than 0.95 (Figure 8). The best-fit values of θ_{im}, D, k_a and k_d were shown in Table 2. The simulation results indicated that the immobile fraction of the aqueous phase is usually less than 3%, suggesting that the aqueous phase was predominantly mobile. Because the values of θ_{im} is relatively small compared to θ, the effects of immobile zone on *E. coli* transport was negligible. Additionally, it was found that the values of k_d were usually two orders of magnitude or more lower than k_a, suggesting that cell detachment was negligible.

Table 2. The best-fit values of θ_{im}, ω, D, k_a and k_d that were estimated from the *E. coli* breakthrough curves using HYDRUS-1D. Note that $\theta = \theta_m + \theta_{im}$. Because the values of θ_{im} is relatively small compared to θ, the effects of immobile zone on *E. coli* transport was negligible.

Moisture content (θ)	*E. coli* strain	Ionic strength (mM)	θ_{im}	D (cm^2/min)	k_a (min^{-1})	k_d (min^{-1})
25%	tet^R	1	2.85(±0.93)%	3.39(±1.67)	0.0273(±0.0014)	$<10^{-4}$
		5	3.99(±1.24)%		0.0222(±0.0020)	$<10^{-4}$
		10	2.03(±2.87)%		0.0234(±0.0015)	$<10^{-4}$
	tet^S	1	<1%	2.79(±1.65)	0.0166(±0.0016)	$<10^{-4}$
		5	<1%		0.0346(±0.0087)	$<10^{-4}$
		10	<1%		0.0347(±0.0017)	$<10^{-4}$
12%	tet^R	1	<1%	5.05(±4.25)	0.0175(±0.0055)	$<10^{-4}$
		5	<1%		0.0169(±0.0084)	$<10^{-4}$
		10	<1%		0.0228(±0.0020)	$<10^{-4}$
	tet^S	1	<1%	2.27(±1.38)	0.0200(±0.0022)	$<10^{-4}$
		5	<1%		0.0319(±0.0076)	$<10^{-4}$
		10	<1%		0.0300(±0.0097)	$<10^{-4}$

Figure 9 showst breakthrough concentrations of the tet^R and tet^S *E. coli* strains under low soil moisture content conditions (i.e., θ = ~12%). In general, the breakthrough concentrations of both *E. coli* strains were lower than those measured under the high soil moisture content conditions. Within partially saturated porous media, the water-air interface provides an important adsorption sites for the immobilization of colloidal sized particles such as *E. coli* cells and the area of the water-air interface usually increases when soil moisture content decreases (Denovio et al. 2004).The decrease in *E. coli* mobility under lower soil moisture content thus reflected the increased adsorption of *E. coli* cell at the water-air interface. The size of *E. coli* cells was found to be 1 μm or greater (Walczak et al. 2011b), *E. coli* cells could also be immobilized as a result of straining within the thin water films that coat the surface of the solid matrix (Wan and Tokunaga 1997). It is also expected that the thin-film straining of *E. coli* cells would increase as soil moisture content was lowered.

Consistent to the observations from the high soil moisture content conditions, the mobility of the tet^R *E. coli* strain was higher than the mobility of the tet^S *E. coli* strain under all three ionic strength conditions, suggesting that the tet^R *E. coli* strain tend to spread more easily within the partially saturated soil. The values of θ_{im}, D, k_a and k_d estimated using HYDRUS-1D showed that, even under the low soil moisture conditions, the fraction of immobile aqueous phase was small and the cell detachment from the solid matrix was slow and negligible (Table 2).

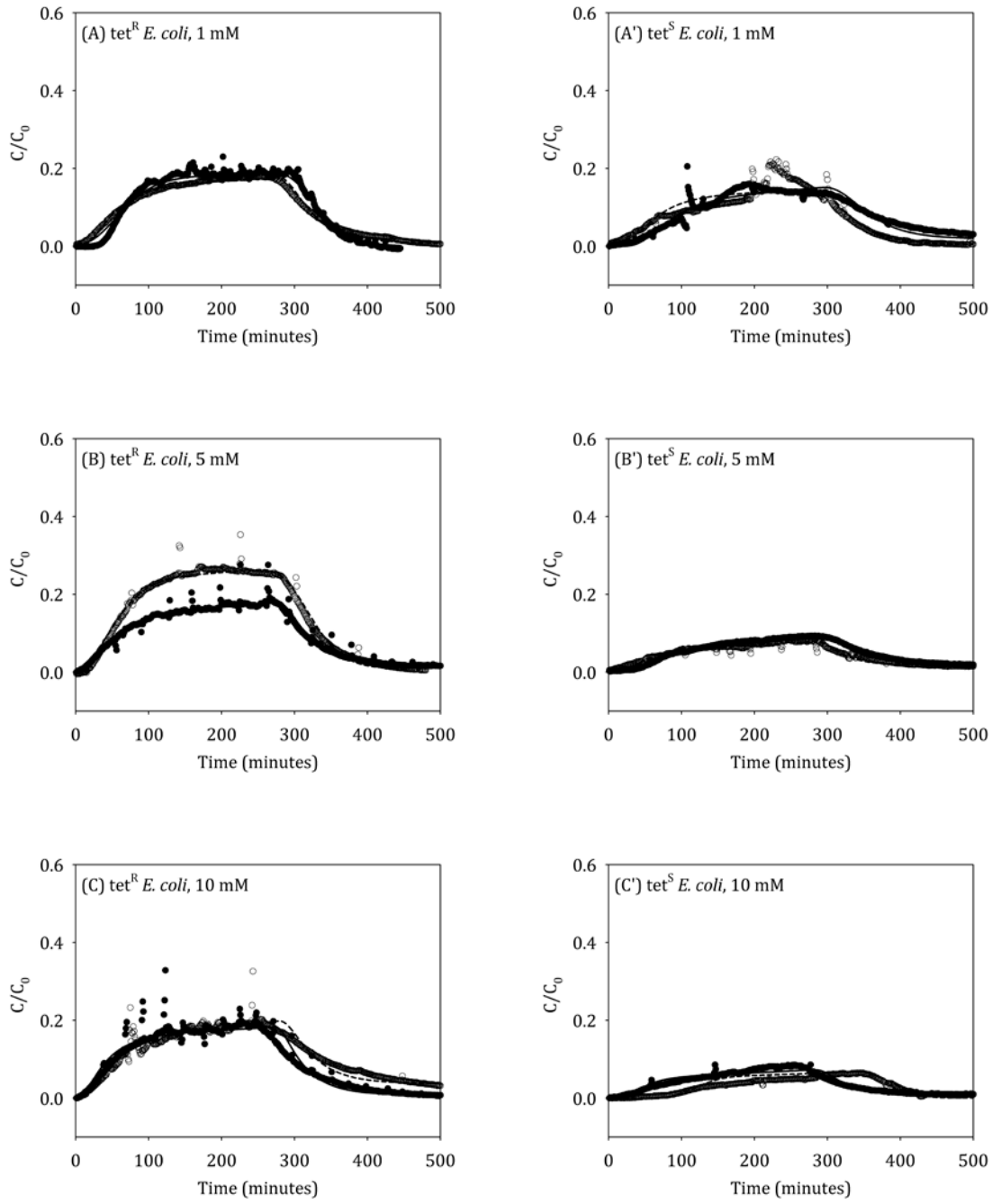

Figure 9. Breakthrough concentrations of the tet^R (A-C) and tetS (A′-C′) *E. coli* strains under 12% of soil moisture content. Symbols represent experimental observations and lines represent model simulation results.

Results from previous studies suggested that change in water chemistry conditions (e.g., a drop in ionic strength) could release the previously immobilized colloid sized particles and this process represents a major mechanism that can transport large quantities of colloids to the underlying groundwater (Denovio et al. 2004). In this research, following a few selected

experiments performed using 10 mM NaCl, the ionic strength was lowered to 1 mM NaCl while the soil moisture content was maintained constant. The effects of chemical perturbation on the transport of *E. coli* cells within partially saturated soil could thus be evaluated.

In general, our results suggested that a small fraction of the immobilized *E. coli* cells under high ionic strength conditions could be remobilized as a result of the drop in NaCl concentration (Figure 10), suggesting that the immobilization of the *E. coli* cells within the unsaturated cells was largely irreversible.

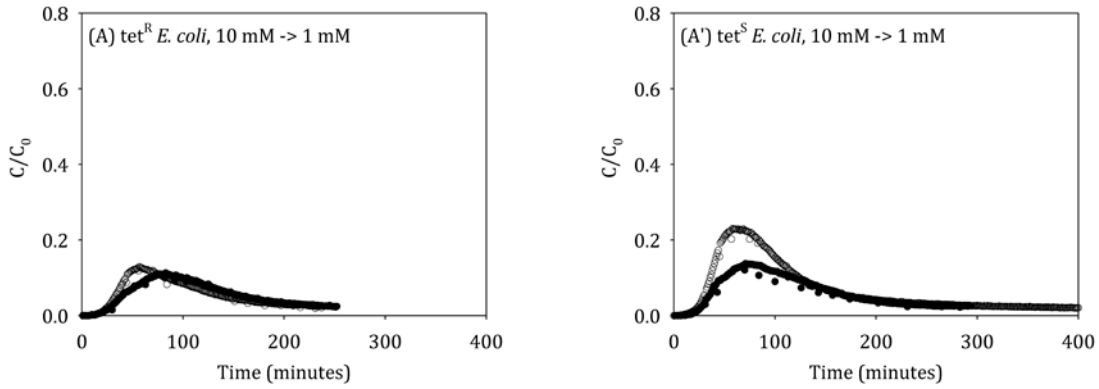

Figure 10. Typical results of the release of tet^R (A) and tetS (A′) *E. coli* cells when the ionic strength was lowered from 10 mM NaCl to 1 mM NaCl. The soil moisture content was maintained at 25%. Time 0 represents the start of the injection of the 1 mM NaCl solution. Lower effluent *E. coli* concentrations were observed during chemical perturbation experiments performed under other ionic strength and/or soil moisture conditions.

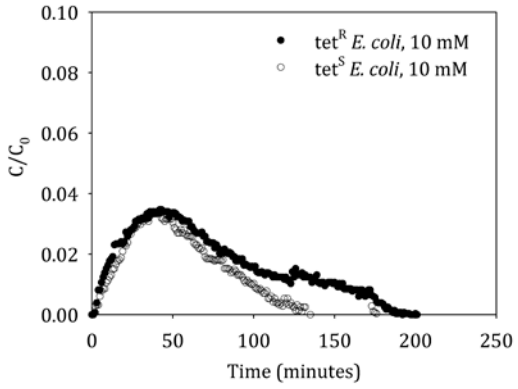

Figure 11. Typircal results for the release of tet^R and tetS *E. coli* cells when the soil moisture content was raised from ~12% to ~25% while the ionic strength was maintained at 10 mM NaCl. Time 0 represents the time when the initiation of the soil moisture content increase. start of the injection of the 1 mM NaCl solution.

It was also suggested that changes in soil moisture contents (i.e., transient flow conditions) could lead to the release of previously immobilized colloid-sized particles (Denovio et al. 2004,

Cheng and Saiers 2009). To evaluate the potential effects of transient flow on the transport of manure-derived *E. coli*, the soil moisture content was raised from 12% to 25% following selected steady-state transport experiments. Similar to the observations from the chemical perturbation experiments, our results indicated that only a small fraction of the *E. coli* cells could be remobilized as a result of imbibition (i.e., increase in soil moisture content) (Figure 11). The effluent concentration of the *E. coli* cells during the imbibition experiments was generally less than 4% of the *E. coli* concentration that was used for the transport experiments (Figure 11).

5. Conclusions

1. The OMP TolC, which is commonly involved in high-level, multi-drug bacterial resistance, could enhance the spread of *E. coli* within the subsurface system.
2. The tet^R *E. coli* strain isolated from dairy manure exhibited higher mobility than then tet^S *E. coli* strain isolated from the same source.
3. The mobility of manure-derived *E. coli* was lower when the ionic strength increased or when the soil moisture content decreased.
4. Only a small fraction of the previously immobilized *E. coli* cells were re-mobilized due to chemical perturbation or transient flow.

References

Ray, K.A., Warnick, L.D., Mitchell, R.M., Kaneene, J.B., Ruegg, P.L., Wells, S.J., Fossler, C.P., Halbert, L.W. and May, K. (2006) Antimicrobial susceptibility of Salmonella from organic and conventional dairy farms. Journal of Dairy Science 89(6), 2038-2050.

Ray, K.A., Warnick, L.D., Mitchell, R.M., Kaneene, J.B., Ruegg, P.L., Wells, S.J., Fossler, C.P., Halbert, L.W. and May, K. (2007) Prevalence of antimicrobial resistance among Salmonella on midwest and northeast USA dairy farms. Preventive Veterinary Medicine 79(2-4), 204-223.

Sato, K., Bartlett, P.C., Kaneene, J.B. and Downes, F.P. (2004) Comparison of prevalence and antimicrobial susceptibilities of Campylobacter spp. isolates from organic and conventional dairy herds in Wisconsin. Applied and Environmental Microbiology 70(3), 1442-1447.

Sato, K., Bartlett, P.C. and Saeed, M.A. (2005) Antimicrobial susceptibility of Escherichia coli isolates from dairy farms using organic versus conventional production methods. Journal of the American Veterinary Medical Association 226(4), 589-594.

Halbert, L.W., Kaneene, J.B., Ruegg, P.L., Warnick, L.D., Wells, S.J., Mansfield, L.S., Fossler, C.R., Campbell, A.M. and Geiger-Zwald, A.M. (2006) Evaluation of antimicrobial susceptibility patterns in Campylobacter spp isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern United States. Journal of the American Veterinary Medical Association 228(7), 1074-1081.

Walczak, J. and Xu, S. (2009) Antimicrobial susceptibility of *Escherichia coli* from lactating cows of different ages. Canadian Journal of Microbiology in review.

Walczak, J.J. and Xu, S.P. (2011) Manure as a Source of Antibiotic-Resistant Escherichia coli and Enterococci: a Case Study of a Wisconsin, USA Family Dairy Farm. Water Air and Soil Pollution 219(1-4), 579-589.

Turnquist, A., Foltz, J. and Roth, C. (2006) Manure management on Wisconsin farms, p. 23, Program on Agricultural Technology Studies, College of Agricultural and Life Sciences, University of Wisconsin-Madison.

Jackson-Smith, D., Moon, S., Ostrom, M. and Barham, B. (2000) Farming in Wisconsin at the end of the century: results of the 1999 Wisconsin farm poll, p. 18, Program on Agricultural Technology Studies, University of Wisconsin, Madison.

Anderson, M.E. and Sobsey, M.D. (2006) Detection and occurrence of antimicrobially resistant E-coli in groundwater on or near swine farms in eastern North Carolina. Water Science and Technology 54(3), 211-218.

Mckeon, D.M., Calabrese, J.P. and Bissonnette, G.K. (1995) Antibiotic-resistant Gram-negative bacteria in rural groundwater supplies. Water Research 29(8), 1902-1908.

Sapkota, A.R., Curriero, F.C., Gibson, K.E. and Schwab, K.J. (2007) Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated swine feeding operation. Environmental Health Perspectives 115(7), 1040-1045.

Solley, W.B., Pierce, R.R. and Perlman, H.A. (1998) Estimated use of water in the United States in 1995, U.S. Geological Survey circular 1200, p. 79.

Ellefson, B.R., Mueller, G.D. and Buchwald, C.A. (2002) Water use in Wisconsin, 2000. U. S. Geological Survey open-file report 02–356.

World Health Organization (2003) 1st Joint FAO/OIE/WHO Expert Workshop on Non-human Antimicrobial Usage and Antimicrobial Resistance: Scientific assessment, http://www.who.int/foodsafety/publications/micro/en/amr.pdf, Geneva.

Levy, S.B. (1998) The challenge of antibiotic resistance. Scientific American 278(3), 46-53.

Walczak, J.J., Bardy, S.L., Feriancikova, L. and Xu, S. (2011a) Comparison of the Transport of Tetracycline-Resistant and Tetracycline-Susceptible Escherichia coli Isolated from Lake Michigan. Water Air and Soil Pollution 222(1-4), 305-314.
Walczak, J.J., Bardy, S.L., Feriancikova, L. and Xu, S.P. (2011b) Influence of tetracycline resistance on the transport of manure-derived Escherichia coil in saturated porous media. Water Research 45(4), 1681-1690.
Xu, C.X., Lin, X.M., Ren, H.X., Zhang, Y.L., Wang, S.Y. and Peng, X.X. (2006) Analysis of outer membrane proteome of Escherichia coli related to resistance to ampicillin and tetracycline. Proteomics 6(2), 462-473.
Alekshun, M.N. and Levy, S.B. (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128(6), 1037-1050.
de Cristobal, R.E., Vincent, P.A. and Salomon, R.A. (2006) Multidrug resistance pump AcrAB-TolC is required for high-level, Tet(A)-mediated tetracycline resistance in Escherichia coli. Journal of Antimicrobial Chemotherapy 58(1), 31-36.
Denovio, N.M., Saiers, J.E. and Ryan, J.N. (2004) Colloid movement in unsaturated porous media: recent advances and future directions. Vadose Zone Journal 3, 338-351.
Lenhart, J.J. and Saiers, J.E. (2002) Transport of silica colloids through unsaturated porous media: Experimental results and model comparisons. Environmental Science & Technology 36(4), 769-777.
Cheng, T. and Saiers, J.E. (2009) Mobilization and transport of in situ colloids during drainage and imbibition of partially saturated sediments. Water Resources Research 45, W08414, doi:08410.01029/02008WR007494.
Saiers, J.E., Hornberger, G.M., Gower, D.B. and Herman, J.S. (2003) The role of moving air-water interfaces in colloid mobilization within the vadose zone. Geophysical Research Letters 30(21), 2083.
Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K.A., Tomita, M., Wanner, B.L. and Mori, H. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Molecular Systems Biology, -.
Cherepanov, P.P. and Wackernagel, W. (1995) Gene Disruption in Escherichia-Coli - Tcr and Km(R) Cassettes with the Option of Flp-Catalyzed Excision of the Antibiotic-Resistance Determinant. Gene 158(1), 9-14.
Datsenko, K.A. and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proceedings of the National Academy of Sciences of the United States of America 97(12), 6640-6645.
Xu, S.P., Liao, Q. and Saiers, J.E. (2008) Straining of nonspherical colloids in saturated porous media. Environmental Science & Technology 42(3), 771-778.
Weight, W.D. (2008) Hydrogeology field manual, McGraw-Hill, New York.
Havelaar, A.H., Vanolphen, M. and Schijven, J.F. (1995) Removal and inactivation of viruses by drinking-water treatment processes under full-scale conditions. Water Science and Technology 31(5-6), 55-62.
Walker, S.L., Redman, J.A. and Elimelech, M. (2005) Influence of growth phase on bacterial deposition: Interaction mechanisms in packed-bed column and radial stagnation point flow systems. Environmental Science & Technology 39(17), 6405-6411.
Kretzschmar, R., Barmettler, K., Grolimund, D., Yan, Y.D., Borkovec, M. and Sticher, H. (1997) Experimental determination of colloid deposition rates and collision efficiencies in natural porous media. Water Resources Research 33(5), 1129-1137.

Castro, F.D. and Tufenkji, N. (2007) Relevance of nontoxigenic strains as surrogates for Escherichia coli O157 : H7 in groundwater contamination potential: Role of temperature and cell acclimation time. Environmental Science & Technology 41(12), 4332-4338.
Redman, J.A., Walker, S.L. and Elimelech, M. (2004) Bacterial adhesion and transport in porous media: Role of the secondary energy minimum. Environmental Science & Technology 38(6), 1777-1785.
Ong, Y.L., Razatos, A., Georgiou, G. and Sharma, M.M. (1999) Adhesion forces between E-coli bacteria and biomaterial surfaces. Langmuir 15(8), 2719-2725.
Bayoudh, S., Othmane, A., Bettaieb, F., Bakhrouf, A., Ben Ouada, H. and Ponsonnet, L. (2006) Quantification of the adhesion free energy between bacteria and hydrophobic and hydrophilic substrata. Materials Science & Engineering C-Biomimetic and Supramolecular Systems 26(2-3), 300-305.
Bayoudh, S., Othmane, A., Mora, L. and Ben Ouada, H. (2009) Assessing bacterial adhesion using DLVO and XDLVO theories and the jet impingement technique. Colloids and Surfaces B-Biointerfaces 73(1), 1-9.
Farahat, M., Hirajima, T., Sasaki, K. and Doi, K. (2009) Adhesion of Escherichia coli onto quartz, hematite and corundum: Extended DLVO theory and flotation behavior. Colloids and Surfaces B-Biointerfaces 74(1), 140-149.
Elimelech, M. (1994) Particle Deposition on Ideal Collectors from Dilute Flowing Suspensions - Mathematical Formulation, Numerical-Solution, and Simulations. Separations Technology 4(4), 186-212.
Huang, X.F., Bhattacharjee, S. and Hoek, E.M.V. (2010) Is Surface Roughness a "Scapegoat" or a Primary Factor When Defining Particle-Substrate Interactions? Langmuir 26(4), 2528-2537.
Morrow, J.B., Stratton, R., Yang, H.H., Smets, B.F. and Grasso, D. (2005a) Macro- and nanoscale observations of adhesive behavior for several E-coli strains (O157 : H7 and environmental isolates) on mineral surfaces. Environmental Science & Technology 39(17), 6395-6404.
Haznedaroglu, B.Z., Bolster, C.H. and Walker, S.L. (2008) The role of starvation on Escherichia coli adhesion and transport in saturated porous media. Water Research 42(6-7), 1547-1554.
Wang, L., Xu, S. and Li, J. (2011) Effects of Phosphate on the Transport of Escherichia coli O157:H7 in Saturated Quartz Sand. Environmental Science & Technology 45(22), 9566-9573.
Walker, S.L., Redman, J.A. and Elimelech, M. (2004) Role of cell surface lipopolysaccharides in Escherichia coli K12 adhesion and transport. Langmuir 20(18), 7736-7746.
Porubcan, A.A. and Xu, S.P. (2011) Colloid straining within saturated heterogeneous porous media. Water Research 45(4), 1796-1806.
van Oss, C.J. (1993) Acid-Base Interfacial Interactions in Aqueous-Media. Colloids and Surfaces a-Physicochemical and Engineering Aspects 78, 1-49.
Morrow, J.B., Stratton, R., Yang, H.H., Smets, B.F. and Grasso, D. (2005b) Macro- and nanoscale observations of adhesive behavior for several E. coli strains (O157:H7 and environmental isolates) on mineral surfaces. Environmental Science & Technology 39(17), 6395-6404.
Hong, Y. and Brown, D.G. (2006) Cell surface acid-base properties of Escherichia coli and Bacillus brevis and variation as a function of growth phase, nitrogen source and C : N ratio. Colloids and Surfaces B-Biointerfaces 50(2), 112-119.
Koronakis, V., Sharff, A., Koronakis, E., Luisi, B. and Hughes, C. (2000) Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. Nature 405(6789), 914-919.

Wright, G.D. (2008) Bacterial Resistance to Antimicrobials. Wax, R.G., Lewis, K., Salyers, A.A. and Taber, H. (eds), pp. 71-101, CRC Press, Boca Raton, FL.
Johanson, J.J., Feriancikova, L. and Xu, S.P. (2012) Influence of Enterococcal Surface Protein (esp) on the Transport of Enterococcus faecium within Saturated Quartz Sands. Environmental Science & Technology 46(3), 1511-1518.
Azeredo, J., Visser, J. and Oliveira, R. (1999) Exopolymers in bacterial adhesion: interpretation in terms of DLVO and XDLVO theories. Colloids and Surfaces B-Biointerfaces 14(1-4), 141-148.
Sharma, P.K. and Rao, K.H. (2003) Adhesion of Paenibacillus polymyxa on chalcopyrite and pyrite: surface thermodynamics and extended DLVO theory. Colloids and Surfaces B-Biointerfaces 29(1), 21-38.
Torkzaban, S., Tazehkand, S.S., Walker, S.L. and Bradford, S.A. (2008) Transport and fate of bacteria in porous media: Coupled effects of chemical conditions and pore space geometry. Water Resources Research 44(4), W04403.
Dong, H.L. (2002) Significance of electrophoretic mobility distribution to bacterial transport in granular porous media. Journal of Microbiological Methods 51(1), 83-93.
Bhattacharjee, S., Ko, C.H. and Elimelech, M. (1998) DLVO interaction between rough surfaces. Langmuir 14(12), 3365-3375.
Wang, P. and Keller, A.A. (2009) Natural and Engineered Nano and Colloidal Transport: Role of Zeta Potential in Prediction of Particle Deposition. Langmuir 25(12), 6856-6862.
Strauss, J., Burnham, N.A. and Camesano, T.A. (2009) Atomic force microscopy study of the role of LPS O-antigen on adhesion of E. coli. Journal of Molecular Recognition 22(5), 347-355.
Butt, H.J., Cappella, B. and Kappl, M. (2005) Force measurements with the atomic force microscope: Technique, interpretation and applications. Surface Science Reports 59(1-6), 1-152.
Israelachvili, J.N. (1991) Intermolecular and surface forces, Academic Press, London ; San Diego.
Neidhardt, F.C. (1996) Escherichia coli and Salmonella : cellular and molecular biology, ASM Press, Washington, D.C.
Walsh, C. (2003) Antibiotics : actions, origins, resistance, ASM Press, Washington, D.C.
Chollet, R., Chevalier, J., Bryskier, A. and Pages, J.M. (2004) The AcrAB-TolC pump is involved in macrolide resistance but not in telithromycin efflux in Enterobacter aerogenes and Escherichia coli. Antimicrobial Agents and Chemotherapy 48(9), 3621-3624.
Fralick, J.A. (1996) Evidence that TolC is required for functioning of the Mar/AcrAB efflux pump of Escherichia coli. Journal of Bacteriology 178(19), 5803-5805.
Nishino, K., Yamada, J., Hirakawa, H., Hirata, T. and Yamaguchi, A. (2003) Roles of TolC-dependent multidrug transporters of Escherichia coli in resistance to beta-lactams. Antimicrobial Agents and Chemotherapy 47(9), 3030-3033.
Harter, T., Davis, H., Mathews, M.C. and Meyer, R.D. (2002) Shallow groundwater quality on dairy farms with irrigated forage crops. Journal of Contaminant Hydrology 55(3-4), 287-315.
Pang, L., McLeod, M., Aislabie, J., Simunek, J., Close, M. and Hector, R. (2008) Modeling Transport of Microbes in Ten Undisturbed Soils under Effluent Irrigation. Vadose Zone Journal 7(1), 97-111.
Jensen, M.B., Hansen, H.C.B., Hansen, S., Jorgensen, P.R., Magid, J. and Nielsen, N.E. (1998) Phosphate and tritium transport through undisturbed subsoil as affected by ionic strength. Journal of Environmental Quality 27(1), 139-145.
Wan, J.M. and Tokunaga, T.K. (1997) Film straining of colloids in unsaturated porous media: Conceptual model and experimental testing. Environmental Science & Technology 31(8), 2413-2420.