Published in final edited form as:

Colloids Surf B Biointerfaces. 2012 February 1; 90: 129–136. doi:10.1016/j.colsurfb.2011.10.010.

The effects of starvation on the transport of *Escherichia coli* in saturated porous media are dependent on pH and ionic strength

Jacob J. Walczak¹, Lixia Wang², Sonia L. Bardy³, Lucia Feriencikova¹, Jin Li², and Shangping Xu^{1,*}

¹Department of Geosciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53201

²Department of Civil Engineering and Mechanics, University of Wisconsin-Milwaukee, Milwaukee, WI 53201

³Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI 53201

Abstract

In this research, we investigate the effects of starvation on the transport of *Escherichia coli* K12 in saturated porous media. Particularly, we examine the relationship between the starvation effects and the pH and ionic strength of the electrolyte solutions used for cell starvation. *E. coli* K12 was cultured using either Luria-Bertani Miller (LB-Miller) broth, which contained 10 g/L of NaCl, or LB-Luria broth, which contained 0.5 g/L of NaCl. As both types of broths had similar pH (~7.2) they differed in ionic strengths. The bacterial cells were harvested at late-exponential phase and resuspended in buffered (pH = 7.2) and non-buffered (pH = 5.7) electrolyte solutions that had ionic strengths of 8.4 mM or 168 mM, respectively. Column transport experiments were performed following 4, 25 and 52 hours of cell starvation to evaluate the temporal changes in cell mobility. Our results showed that starvation led to a significant increase in the mobility of *E. coli* K12, particularly between 4 and 25 hours, when *both* pH and ionic strength of the electrolyte solution were different from those of the growth media. The size, viability and surface properties (e.g., zeta potential, hydrophobicity, LPS sugar content, outer membrane protein profiles) of the bacterial cells were determined and related to the observed temporal variation patterns of cell mobility. We found that starvation in electrolyte solutions that had different pH and ionic strength from the growth media significantly lowered cell viability, which may be related to the temporal change in cell mobility under these specific conditions.

Keywords

bacterial transport; groundwater microbial contamination

1. Introduction

A thorough understanding of bacterial transport within saturated porous media is essential to the protection of groundwater resources from microbial contamination, to the design, implementation and management of *in-situ* bioremediation projects, and to the effective use

© 2011 Elsevier B.V. All rights reserved.

*Corresponding author, telephone: 414-229-6148, fax: 414-229-5452, xus@uwm.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of granular filters for the removal of pathogenic microorganisms during water treatment processes [1–4]. The transport of bacteria in saturated porous media has been studied extensively and the results suggest that bacterial mobility is strongly influenced by a wide range of factors and processes such as properties of the soil matrix, water chemistry (e.g., ionic strength, pH), temperature, flow conditions, as well as cell type, motility, morphology and surface properties [5–15].

Bacterial cells are dynamic systems and they may experience physiological changes in response to variations in environmental conditions [16–18]. Carbon starvation, for instance, can trigger protein degradation in *Escherichia coli* K12, and the amino acids produced can be used to synthesize new proteins, thereby increasing survival of the bacterial cells [19, 20]. An increase in osmotic pressure inhibits electron transfer and sugar transport, and alters the cytoplasmic volume and morphology of *E. coli* [21–23]. Changes in osmotic pressure can also affect outer membrane protein synthesis through controlling the expression of related genes [24]. Similarly, a change in pH was reported to induce the expression of a wide variety of outer membrane proteins such as OstA and TolC [16].

The physiological responses of bacterial cells to changes in environmental conditions can alter cell morphology and surface properties, which in turn may influence bacterial transport within saturated porous media [1, 25–28]. Particularly, it was reported that starvation, which is commonly experienced by bacterial cells released into the natural environment, could have significant impact on the transport of various bacterial strains. Haznedaroglu *et al.* [25] reported that starvation could cause changes in *E. coli* surface characteristics and subsequently cell attachment to quartz sands. MacLeod *et al.* [27] showed that starvation led to smaller sizes and deeper penetration of *Klebsiella pneumonia* within saturated glass bead cores.

While previous investigations provide invaluable insights into the relationship between starvation and bacterial transport within porous media, the effects of pH and ionic strength on cell starvation have been largely ignored. As a result, cell starvation experiments were often performed using electrolyte solutions that had different ionic strength and/or pH from those of the growth media [25, 27]. The observed mobility of starved bacterial cells thus likely reflected the combined effects of starvation as well as pH and/or osmotic pressure stresses. In this research, we investigated the effects of short-term (up to 52 hours) starvation on the transport of *E. coli* K12 in saturated porous media by systematically varying the pH and ionic strength of the solutions that were used for starvation. Our findings suggest that the effects of starvation on *E. coli* transport within saturated sand packs were strongly influenced by the pH and ionic strength of the solutions that were used for cell starvation.

2. Materials and Methods

2.1 Bacteria preparation

Two different types of Luria-Bertani (LB) broths (LB-Miller and LB-Luria) were used to culture *E. coli* K12 (ATCC 10798). The broths contained equal amounts of tryptone (10g/L, Fisher Scientific) and yeast extract (5g/L, Fisher Scientific) but different amount of NaCl (LM-Miller: 10 g/L; LB-Luria: 0.5g/L). The pH of both broths was ~7.2, and the ionic strengths were ~170 mM and 8.5 mM, respectively. *E. coli* K12 was preserved in 20% glycerol at –80 °C. To obtain isolated colonies *E. coli* was streaked onto Muller-Hinton (MH) agar (Remel) plates, which were incubated at 37 °C overnight. These colonies were used to inoculate 15 ml LB-Miller/Luria broth cultures, which were incubated at 37 °C for 6 hours with 90 rpm shaking. The starter culture was used to inoculate LB-Miller/Luria broth (1:500 dilution), which was incubated at 37 °C for 18 hours (late exponential phase) with shaking (90 rpm).

The bacterial cells were harvested by centrifugation (4000×g, 10 minutes, 4 °C) and rinsed 4 times to remove the growth medium. Cell starvation began when the bacterial pellet was initially suspended in the electrolyte solution for rinsing purposes. In total, four different electrolyte solutions were used in this research (Table 1). Upon completion of the rinsing step, cell suspensions ($\sim 4 \times 10^7$ cell/ml) were prepared and the cells were starved for 4, 25 and 52 hours, respectively, before the initiation of column injection experiments.

2.2 Porous media

Quartz sands (US Silica) that had a size range of 0.71–0.85 mm and a porosity of 0.356 (bulk density method) were used in the transport experiments. The sands were first cleaned using hot, concentrated nitric acid (70%) to remove iron hydroxide/oxide coating. After rinsing with deionized water, the sands were soaked in diluted NaOH (~ 0.1 M) to remove fine particles that were attached to the surface of the sand [29]. Following a second cleaning step using diluted nitric acid ($\sim 10\%$ nitric acid), the sands were rinsed using deionized water and dried in an oven at 60 °C. To measure the zeta potential of the sands, the cleaned sands were pulverized in to colloid-sized particles. These colloidal particles were suspended in the electrolyte solutions used in this research and zeta potential was determined using a Brookhaven ZetaPALS analyzer [30, 31].

2.3 Column transport experiments

The column transport experiments were performed using duplicate glass columns (Kontes) measuring 2.5 cm in diameter and 15 cm in length. For all column experiments, the approach velocity was maintained at 0.31 cm/min using peristaltic pumps (Cole-Parmer). The wet-packed sand columns were first equilibrated with >40 pore volumes of background electrolyte solution. Bacterial cell suspensions were then injected into the columns. The effluents of the columns were connected to flow-through quartz cuvettes (NSG Precision) and cell concentrations were determined at a wavelength of 220nm at 30-s intervals using a spectrophotometer (Shimadzu UV-1700). The injection lasted for 60 min (~ 3.5 pore volumes), and the columns were subsequently flushed with background electrolyte solution until the absorbance of effluent returned to the pre-injection values.

For the porous media used in this research, hydrodynamic dispersion was negligible under the selected flow conditions [31]. The deposition rate coefficients under clean-bed conditions (K_d , min^{-1}) can be determined from the breakthrough concentration of bacterial cells using the following equation [32–34]

$$K_d = -\frac{v}{\theta L} \ln \left(\frac{C}{C_0} \right) \quad (1)$$

Where v is approach velocity (cm/min), L is the length of the packed bed (cm), θ is the porosity of the porous medium (cm^3/cm^3), C is concentration of bacteria cell in the effluent under clean-bed conditions (cell/mL) and C_0 is the bacteria concentration in the influent

(cell/mL). The values of $\frac{C}{C_0}$ were determined for each column experiment by calculating the average normalized breakthrough concentrations (i.e., $\frac{C}{C_0}$) measured between 1.8 and 2 pore volumes [33].

2.4 Cell size and surface properties

In general, the immobilization of bacterial cells within saturated porous media is controlled by the energy interactions between the bacterial cells and the solid matrix. These energy interactions are strongly influenced by factors such as cell size, cell surface potential as well as hydrophobicity [5]. In this research, various cell properties were determined and related to the transport of the starved *E. coli* cells.

A Nikon Eclipse 50i microscope equipped with a Photometric coolsnap ES digital camera and MetaMorph software was used to take photos of the bacterial cells suspended in the electrolyte solutions. The photos were processed using ImageJ software to determine the length and width of a minimum of 30 cells. The equivalent radius was then calculated for each cell and the average equivalent radii values were then obtained.

The zeta potential of the bacterial cells suspended in various electrolyte solutions was measured in triplicate using a Brookhaven ZetaPALS analyzer. To determine cell hydrophobicity, microbial adhesion to hydrocarbon (MATH) tests were also performed in triplicate [35]. 1 mL of *n*-dodecane was mixed with 4 mL of cell suspension, and vortexed for 2 minutes. After standing for 15 minutes, cell concentrations in the aqueous phase before and after partitioning were determined using a spectrophotometer at a wavelength of 546 nm. The fraction of bacterial cells that partitioned into the hydrocarbon phase was calculated from the difference between initial and final cell aqueous concentrations and expressed cell hydrophobicity.

2.5 Outer membrane polysaccharides and proteins

The surface properties of Gram-negative bacteria (e.g., *E. coli*) are closely related to the molecular structure of their outer membrane, which contains phospholipids, lipids, polysaccharides as well as proteins [36, 37]. The lipids and polysaccharides are linked to form the lipopolysaccharide (LPS) complex, which is exposed to the environment and can affect cell mobility [37, 38]. The proteins are anchored within the membrane and several recent studies have shown that outer membrane proteins (OMPs) could have strong influences on bacterial transport through altering cell surface properties (e.g., hydrophobicity) [34, 39, 40].

To examine the potential impact of cell starvation on cell LPS polysaccharide quantity, triplicate cell suspension samples (5 mL) were sonicated (50W, 20s) to extract LPS polysaccharides [41]. The resulting samples were centrifuged ($10\,000\times g$, 40min, 4°C) and filtered through 0.2 μm cellulose acetate filters. The LPS polysaccharides present in the filtrates were measured using the phenol-sulfuric acid method [42]. Calibration curves were prepared separately using xanthan gum in each of the four electrolyte solutions used in this research. No difference was observed between the calibration curves.

Starvation, pH, and osmotic stresses can lead to changes in OMP profiles, which in turn may affect cell transport [16, 18–20, 24]. In this research, the OMPs of the *E. coli* cells were extracted and profiled using sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [43–45]. Briefly, starved bacterial cells (as described above) were collected by centrifugation ($4000\times g$, 10 min, 4 °C), resuspended in 5 mL sterile 0.15 M NaCl, and subsequently disrupted by sonication (50W, $8\times 15\text{s}$, VirTis Virsonic). Unlysed cells and cellular debris were removed through centrifugation ($5000\times g$, 40 min). The supernatant was centrifuged at $100\,000\times g$ for 40 minutes at 4°C to pellet the membranes. To solubilize the OMPs, the membranes were resuspended in 2% sodium lauryl sarcosinate (Sigma) at room temperature for 1 hour. Centrifugation at $100\,000\times g$ (40 min, 4°C) was performed again to pellet the OMPs. The resulting OMP pellet was resuspended in 0.25 mL sterile 0.15 M NaCl solution and the OMPs were separated by SDS-PAGE. The proteins were fixed in 4%

perchloric acid, then stained using 0.1% Coomassie G250, 4% perchloric acid [47]. Equal amounts of outer membrane proteins were loaded on the gel, as determined by the Bradford assay [46].

2.6 Cell viability

Environmental stresses could reduce cell viability, which could alter cell surface properties and mobility within saturated porous media [18, 48–51]. Cell viability was assayed using Invitrogen Live/Dead BacLight Viability Kit. For each column experiment, duplicate effluent samples were collected (~1000 μL) and stained. Cell viability was determined from the fluorescence readings using a FACS flow cytometer (Becton Dickinson).

3. Results and Discussion

3.1 Transport of starved *E. coli* K12 cells

The breakthrough concentrations of *E. coli* cells following various starvation periods are shown in Figures 1 and 2. When *E. coli* K12 cells cultured in LB-Miller broth were resuspended and starved in solutions that had a pH of 7.2, there was little change in cell breakthrough concentrations with starvation time (Figure 1A and 1B), suggesting that starvation under these water chemistry conditions had minimal impact on cell mobility. Cell transport behavior also displayed little change when the starvation was performed using the 168 mM NaCl, which had a pH of 5.7 (Figure 1D). The 8.4 mM NaCl solution (pH = 5.7), however, led to significant increase in cell mobility (i.e., decrease in cell retention) over time (Figure 1C).

Consistent patterns were observed for *E. coli* cells grown in LB-Luria broth. Starvation had a small impact on cell mobility when the cells were starved in solutions that had a pH of 7.2 or ionic strength of 8.4 mM (Figure 2). There was significant increase in cell mobility especially between 4 and 25 hours when *E. coli* was starved in 168 mM NaCl (Figure 2D). Overall, it was observed that starvation led to significant changes in cell mobility when the solution used for starvation differed from growth media in *both* pH and ionic strength. It is worth noting that the negligible change in *E. coli* mobility under buffered (pH = 7.2) conditions was unlikely related to phosphate, the buffer selected for this research, because similar pattern (i.e., negligible change in cell mobility) was also observed under the absence of phosphate.

The clean-bed deposition rate coefficients (K_d) were calculated from the breakthrough concentrations using equation (1) (Figure 3). In general, the values of K_d displayed minimal variations with starvation time, except when *E. coli* was starved in a solution that had different pH and ionic strength from the growth media. For *E. coli* grown in LB-Miller broth and starved in the 8.4 mM NaCl solution (pH ~5.7), calculated K_d value equaled to $0.012(\pm 0.0015) \text{ min}^{-1}$ at 4 h and dropped to $0.0072(\pm 0.0007) \text{ min}^{-1}$ at 25 h and $0.0047(\pm 0.0002) \text{ min}^{-1}$ at 52 h. When *E. coli* was cultured in LB-Luria broth and starved in the 168 mM NaCl solution, the values of K_d measured at 4 h, 25 h and 52 h were $0.062(\pm 0.001) \text{ min}^{-1}$, $0.037(\pm 0.001) \text{ min}^{-1}$ and $0.038(\pm 0.001) \text{ min}^{-1}$, respectively. The results presented in Figure 3 also show that for the same pH, the *E. coli* mobility was significantly higher under low ionic strength conditions; when the ionic strength was similar, higher pH led to higher *E. coli* mobility.

It was found that under similar water chemistry conditions, the calculated K_d values for *E. coli* cells grown in LB-Miller and LB-Luria broths generally were scattered along the 1:1 line, suggesting that different NaCl concentrations in the growth broths in general did not cause significant differences in *E. coli* mobility (Figure 4). It is interesting to note that, for the 8.4 mM and 168 mM NaCl solutions, although the K_d values measured at 4 hours were

comparable, increasing deviations from the 1:1 line occurred at 25 and 52 hours. This was caused by the different responses of the *E. coli* cells grown in different growth media to starvation when suspended in the two electrolyte solutions (indicated by the two arrows in Figure 4). For instance, when suspended in 8.4 mM NaCl, a significant drop in K_d (i.e., increase in mobility) occurred with the increase in starvation time for *E. coli* grown in LB-Miller broth but not for *E. coli* cultured in LB-Luria broth. As a result, although the calculated K_d values were comparable at 4 hour, the K_d values for *E. coli* grown in LB-Miller broth became lower than those of *E. coli* grown in LB-Luria broth at 25 and 52 hours.

3.2 Size and surface properties of *E. coli* K12 cells

The measured sizes of the *E. coli* cells that were grown in two different types of broths and starved in various electrolyte solutions are shown in Figure 5. The results showed that cell sizes did not vary with starvation time under all water chemistry conditions. Cell size was thus unlikely a major cause of the observed increase in the mobility of *E. coli* cells that were starved under pH and ionic strength conditions that were different from the growth media.

The measured zeta potential values of the quartz sand were $-58.8(\pm 7.2)$ mV (ionic strength: 8.4 mM, pH: 7.2), $-36.4(\pm 3.4)$ mV (ionic strength: 8.4 mM, pH: 5.7), $-21.0(\pm 1.6)$ mV (ionic strength: 168 mM, pH: 7.2), and $-17.7(\pm 2.4)$ mV (ionic strength: 168 mM, pH: 5.7), respectively. Zeta potential measurements of the *E. coli* cells showed that their surface was also negatively charged under all conditions (Figure 6), suggesting repulsive electrostatic interactions between *E. coli* and the surface of quartz sands. It was found that, for all water chemistry and growth medium conditions, there was little temporal change in the zeta potential values of *E. coli*. The observed increase in *E. coli* mobility with starvation time under certain chemistry conditions was thus not caused by changes in cell zeta potential values. Our results also showed that, under the same pH, the zeta potential values of both the quartz sands and the *E. coli* cells were more negative when the ionic strength was lower. The more negative zeta potential values resulted in stronger repulsive electrostatic interaction between *E. coli* and the quartz sands. This was consistent with the observation that, given a specific pH, the mobility of *E. coli* was generally higher when the ionic strength of the solution was lower. For the same ionic strength, while little difference was observed for the zeta potential values of the *E. coli* cells under different pH conditions (Figure 6), the zeta potential values of the sands were more negative when the pH was higher. Overall, the response of sand zeta potential to water pH suggested that the repulsive cell-sand interactions became stronger when pH increased from 5.7 to 7.2. This could explain the observed increase in *E. coli* mobility with pH when the ionic strength was maintained constant (Figure 3).

The hydrophobicity of the *E. coli* cells was determined through the MATH test and the results are presented in Figure 7. In general, cell hydrophobicity decreased with the increase of starvation time (lower percentages of *E. coli* cells partitioned into the hydrocarbon phase). It was previously reported that a decrease in cell hydrophobicity could reduce the attachment of bacterial cells to clean quartz sands and thus enhance their mobility within saturated sand packs [33, 51–53]. This was particularly true when the zeta potential values of the cells were similar [33, 51, 52]. Walker *et al.* [33], for instance, reported that while the stationary- and exponential-phase *E. coli* D21g cells had similar zeta potential values, the less hydrophobic exponential-phase cells (16% of cells partitioned into the hydrocarbon phase) displayed slightly higher mobility than the more hydrophobic stationary-phase cells (34% of cells partitioned into the hydrocarbon phase). Walczak *et al.* (2011c) observed that desiccation did not affect the zeta potential but significantly reduced the hydrophobicity of *E. coli* K12 cells and the reduction in hydrophobicity in turn led to increased cell mobility (a decrease in cell attachment efficiency). It is thus likely that the reduction in *E. coli* hydrophobicity was partly responsible for the increase in *E. coli* mobility when the cells were starved in

solutions that had different ionic strength and pH from the growth media. The trend of reduced hydrophobicity due to cell starvation, however, was observed for all the water chemistry conditions. It was unclear why similar changes in hydrophobicity did not cause comparable increases in cell mobility when starvation was performed using other electrolyte solutions (i.e., solutions that had similar pH and/or ionic strength of the growth media) and further investigations are warranted. Overall, our observation was consistent with findings reported in Haznedaroglu *et al.* [25], which suggested that 1) starvation could reduce the hydrophobicity of *E. coli* cells, and 2) no clear relationship between cell hydrophobicity and mobility could be identified.

3.3 Characterization of LPS and outer membrane proteins

The measured outer membrane LPS sugar contents (Figure 8) did not display significant temporal changes, suggesting that the observed increase in the mobility of *E. coli* cells under starvation conditions of different pH and ionic strength was not related to LPS sugar quantities. It is well known that *E. coli* K12 cells lack O-antigen (the repeating polysaccharide component of LPS) [54], as a result, the values obtained in this research (2–6 $\mu\text{g}/10^8$ cells) were significantly lower than those measured for many environmental *E. coli* strains [25, 34].

The OMPs of the starved cells were extracted and the protein profile was determined using SDS-PAGE. We specifically focused on OMP profiles of *E. coli* cells that were suspended in unbuffered (pH 5.7) electrolyte solutions because significant changes in cell mobility occurred only under this pH condition (Figure 9). When *E. coli* cells grown in LB-Miller broth were starved in the 8.4 mM NaCl solution, there was no discernible change in OMP profiles over time, although a significant increase in cell mobility was observed between 4 and 25 hours. In contrast, when the LB-Luria broth cultured *E. coli* cells were starved in 168 mM NaCl solution and displayed increase in mobility between 4 and 25 hours, there were noticeable decreases in the quantity of a few OMPs (e.g., the OMP that has a size of 80 and 100 kDa). Similar variations in OMP profiles were observed when *E. coli* cells grown in LB-Luria broth were starved in 8.4 mM NaCl although cell mobility remained stable between 4 and 52 hours. Overall, the OMP profiling data indicated that starvation could lead to changes in OMP profiles but no distinct and consistent patterns could be identified to explain the increase in cell mobility due to starvation under certain water chemistry conditions.

3.4 Cell viability

In general, when suspended in solutions that did not cause significant changes in cell mobility over time, the viability of *E. coli* cells was higher than 85% (Figure 10). In contrast, when suspended in solutions that led to increase in cell mobility over time, cell viability was generally lower than 65%. The cell viability data suggested that starvation combined with pH and ionic strength changes represented severe stress to *E. coli* and a significant portion of the cells did not survive. It is possible that the mechanisms used by *E. coli* to maintain viability during the stressful starvation period led to increased mobility. Assuming that the viability of fresh *E. coli* cells was >85%, the drop in cell viability to ~65% for the selected cases occurred within the initial 4 hours. It is thus likely that, along with the loss of cell viability within the first 4 hours under selected conditions, significant changes in cell mobility could have occurred. Future research should address the effects of <4 hours starvation on cell mobility.

4. Conclusions

In this research, we observed that starvation led to a significant increase in *E. coli* mobility (decrease in *E. coli* retention) and a decrease in cell viability when the solution used for starvation differed from the growth media in *both* pH and ionic strength. Besides cell viability, however, we could not identify any other cell properties (e.g., size and zeta potential) that can be consistently related to the effects of starvation on cell mobility. We found that the zeta potential values of both *E. coli* cells and quartz sands became less negative when ionic strength increased. Additionally, the zeta potential values of the quartz sand were less negative when pH decreases. As a result, the mobility of *E. coli* cells increased as pH became higher and decreased with increasing ionic strength. Results obtained in this research also indicated that *E. coli* cells cultured in growth media with different NaCl concentrations exhibited similar mobility. Combined with previous studies on the relationship between starvation and bacterial transport behavior in saturated porous media [1, 25, 27], our findings suggested that the complex relationship between cell starvation under various water chemistry conditions and cell mobility within saturated porous media should be accounted for in the understanding and modeling of the transport of bacteria within the groundwater environment.

References

1. Cunningham AB, Sharp RR, Caccavo F, Gerlach R. Effects of starvation on bacterial transport through porous media. *Advances in Water Resources*. 2007; 30:1583–1592.
2. Gross MJ, Logan BE. Influence of Different Chemical Treatments on Transport of *Alcaligenes paradoxus* in Porous-Media. *Applied and Environmental Microbiology*. 1995; 61:1750–1756. [PubMed: 7646012]
3. Cleasby, JL.; Logsdon, GS. Granular bed and percoat filtration. In: Letterman, RD., editor. *Water Quality and Treatment: A Handbook of Community Water Supplies*. McGraw-Hill, Inc; 1999. p. 8.1-8.99.
4. Tufenkji N, Ryan JN, Elimelech M. The promise of bank filtration. *Environmental Science & Technology*. 2002; 36:422a–428a.
5. Foppen JWA, Schijven JF. Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions. *Water Research*. 2006; 40:401–426. [PubMed: 16434075]
6. Bolster CH, Cook KL, Marcus IM, Haznedaroglu BZ, Walker SL. Correlating Transport Behavior with Cell Properties for Eight Porcine *Escherichia coli* Isolates. *Environmental Science & Technology*. 2010; 44:5008–5014. [PubMed: 20540542]
7. Bolster CH, Haznedaroglu BZ, Walker SL. Diversity in Cell Properties and Transport Behavior among 12 Different Environmental *Escherichia coli* Isolates. *Journal of Environmental Quality*. 2009; 38:465–472. [PubMed: 19202016]
8. Kim HN, Walker SL. *Escherichia coli* transport in porous media: Influence of cell strain, solution chemistry, and temperature. *Colloids and Surfaces B-Biointerfaces*. 2009; 71:160–167.
9. Torkzaban S, Tazehkand SS, Walker SL, Bradford SA. Transport and fate of bacteria in porous media: Coupled effects of chemical conditions and pore space geometry. *Water Resources Research*. 2008; 44:W04403.
10. Becker MW, Collins SA, Metge DW, Harvey RW, Shapiro AM. Effect of cell physicochemical characteristics and motility on bacterial transport in groundwater. *Journal of Contaminant Hydrology*. 2004; 69:195–213. [PubMed: 15028391]
11. Gannon JT, Manilal VB, Alexander M. Relationship between Cell-Surface Properties and Transport of Bacteria through Soil. *Applied and Environmental Microbiology*. 1991; 57:190–193. [PubMed: 16348394]
12. van Loosdrecht MCM, Lyklema J, Norde W, Zehnder AJB. Bacterial Adhesion - a Physicochemical Approach. *Microbial Ecology*. 1989; 17:1–15.

13. van Loosdrecht MCM, Norde W, Lyklema J, Zehnder AJB. Hydrophobic and Electrostatic Parameters in Bacterial Adhesion. *Aquatic Sciences*. 1990; 52:103–114.
14. Lawrence JR, Hendry MJ. Transport of bacteria through geologic media. *Canadian Journal of Microbiology*. 1996; 42:410–422.
15. Mills AL, Herman JS, Hornberger GM, Dejesus TH. Effect of Solution Ionic-Strength and Iron Coatings on Mineral Grains on the Sorption of Bacterial-Cells to Quartz Sand. *Applied and Environmental Microbiology*. 1994; 60:3300–3306. [PubMed: 16349383]
16. Wu L, Lin XM, Peng XX. From Proteome to Genome for Functional Characterization of pH-Dependent Outer Membrane Proteins in *Escherichia coli*. *Journal of Proteome Research*. 2009; 8:1059–1070. [PubMed: 19132928]
17. Beney L, Gervais P. Influence of the fluidity of the membrane on the response of microorganisms to environmental stresses. *Applied Microbiology and Biotechnology*. 2001; 57:34–42. [PubMed: 11693931]
18. Siegle DA, Kolter R. Life after Log. *Journal of Bacteriology*. 1992; 174:345–348. [PubMed: 1729229]
19. Reeve CA, Amy PS, Matin A. Role of protein synthesis in the survival of carbon-starved *Escherichia coli* K-12. *The Journal of Bacteriology*. 1984; 160:1041–1046.
20. Reeve CA, Bockman AT, Matin A. Role of protein degradation in the survival of carbon-starved *Escherichia coli* and *Salmonella typhimurium*. *The Journal of Bacteriology*. 1984; 157:758–763.
21. Houssin C, Eynard N, Shechter E, Ghazi A. Effect of Osmotic-Pressure on Membrane Energy-Linked Functions in *Escherichia-Coli*. *Biochim Biophys Acta*. 1991; 1056:76–84. [PubMed: 1984787]
22. Hajmeer M, Ceylan E, Marsden JL, Fung DYC. Impact of sodium chloride on *Escherichia coli* O157 : H7 and *Staphylococcus aureus* analysed using transmission electron microscopy. *Food Microbiology*. 2006; 23:446–452. [PubMed: 16943036]
23. Record MT, Courtenay ES, Cayley DS, Guttman HJ. Responses of *E. coli* to osmotic stress: Large changes in amounts of cytoplasmic solutes and water. *Trends Biochem Sci*. 1998; 23:143–148. [PubMed: 9584618]
24. Pratt LA, Hsing WH, Gibson KE, Silhavy TJ. From acids to *osmZ*: Multiple factors influence synthesis of the OmpF and OmpC porins in *Escherichia coli*. *Molecular Microbiology*. 1996; 20:911–917. [PubMed: 8809744]
25. Haznedaroglu BZ, Bolster CH, Walker SL. The role of starvation on *Escherichia coli* adhesion and transport in saturated porous media. *Water Research*. 2008; 42:1547–1554. [PubMed: 18037467]
26. Sanin SL, Sanin FD, Bryers JD. Effect of starvation on the adhesive properties of xenobiotic degrading bacteria. *Process Biochem*. 2003; 38:909–914.
27. MacLeod FA, Lappinscott HM, Costerton JW. Plugging of a Model Rock System by Using Starved Bacteria. *Applied and Environmental Microbiology*. 1988; 54:1365–1372. [PubMed: 16347647]
28. Wrangstadh M, Conway PL, Kjelleberg S. The production and release of an extracellular polysaccharide during starvation of a marine *Pseudomonas* sp. and the effect thereof on adhesion. *Archives of Microbiology*. 1986; 145:220–227. [PubMed: 3767571]
29. Xu SP, Gao B, Saiers JE. Straining of colloidal particles in saturated porous media. *Water Resources Research*. 2006; 42:W12S16.10.1029/2006WR004948
30. Liu YY, Janjaroen D, Kuhlenschmidt MS, Kuhlenschmidt TB, Nguyen TH. Deposition of *Cryptosporidium parvum* Oocysts on Natural Organic Matter Surfaces: Microscopic Evidence for Secondary Minimum Deposition in a Radial Stagnation Point Flow Cell. *Langmuir*. 2009; 25:1594–1605. [PubMed: 19133757]
31. Porubcan AA, Xu SP. Colloid straining within saturated heterogeneous porous media. *Water Research*. 2011; 45:1796–1806. [PubMed: 21185052]
32. Kretschmar R, Borkovec M, Grolimund D, Elimelech M. Mobile subsurface colloids and their role in contaminant transport. *Advances in Agronomy*. 1999; 66:121–193.
33. Walker SL, Redman JA, Elimelech M. Influence of growth phase on bacterial deposition: Interaction mechanisms in packed-bed column and radial stagnation point flow systems. *Environmental Science & Technology*. 2005; 39:6405–6411. [PubMed: 16190193]

34. Walczak JJ, Bardy SL, Feriencikova L, Xu SP. Influence of tetracycline resistance on the transport of manure-derived *Escherichia coli* in saturated porous media. *Water Research*. 2011; 45:1681–1690. [PubMed: 21211813]
35. Pembrey RS, Marshall KC, Schneider RP. Cell surface analysis techniques: What do cell preparation protocols do to cell surface properties? *Applied and Environmental Microbiology*. 1999; 65:2877–2894. [PubMed: 10388679]
36. Foppen JW, Lutterodt G, Roling WFM, Uhlenbrook S. Towards understanding inter-strain attachment variations of *Escherichia coli* during transport in saturated quartz sand. *Water Research*. 2010; 44:1202–1212. [PubMed: 19765793]
37. Madigan, MT.; Brock, TD. Brock biology of microorganisms. 12. Pearson/Benjamin Cummings; San Francisco, CA: 2009.
38. Walker SL, Redman JA, Elimelech M. Role of cell surface lipopolysaccharides in *Escherichia coli* K12 adhesion and transport. *Langmuir*. 2004; 20:7736–7746. [PubMed: 15323526]
39. Lutterodt G, Basnet M, Foppen JWA, Uhlenbrook S. The effect of surface characteristics on the transport of multiple *Escherichia coli* isolates in large scale columns of quartz sand. *Water Research*. 2009; 43:595–604. [PubMed: 19042002]
40. Walczak JJ, Bardy SL, Feriencikova L, Xu S. Transport of *Escherichia coli* isolated from Lake Michigan in saturated porous media: Influence of tetracycline resistance. *Water Air and Soil Pollution*. 2011; 1007/s11270-011-0825-6
41. Liu Y, Yang CH, Li J. Influence of extracellular polymeric substances on *Pseudomonas aeruginosa* transport and deposition profiles in porous media. *Environmental Science & Technology*. 2007; 41:198–205. [PubMed: 17265948]
42. DuBois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*. 1956; 28:350–356.
43. Xu CX, Lin XM, Ren HX, Zhang YL, Wang SY, Peng XX. Analysis of outer membrane proteome of *Escherichia coli* related to resistance to ampicillin and tetracycline. *Proteomics*. 2006; 6:462–473. [PubMed: 16372265]
44. Gatewood DM, Fenwick BW, Chengappa MM. Growth-Condition Dependent Expression of *Pasteurella-Haemolytica* a1 Outer-Membrane Proteins, Capsule, and Leukotoxin. *Veterinary Microbiology*. 1994; 41:221–233. [PubMed: 7975148]
45. Ben Abdallah F, Kallel H, Bakhrouf A. Enzymatic, outer membrane proteins and plasmid alterations of starved *Vibrio parahaemolyticus* and *Vibrio alginolyticus* cells in seawater. *Archives of Microbiology*. 2009; 191:493–500. [PubMed: 19373459]
46. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*. 1976; 72:248–254. [PubMed: 942051]
47. Faguy DM, Bayley DP, Kostyukova AS, Thomas NA, Jarrell KF. Isolation and characterization of flagella and flagellin proteins from the thermoacidophilic archaea *Thermoplasma volcanium* and *Sulfolobus shibatae*. *Journal of Bacteriology*. 1996; 178:902–905. [PubMed: 8550530]
48. Mille Y, Beney L, Gervais P. Viability of *Escherichia coli* after combined osmotic and thermal treatment: a plasma membrane implication. *Bba-Biomembranes*. 2002; 1567:41–48. [PubMed: 12488036]
49. Mille Y, Beney L, Gervais P. Compared tolerance to osmotic stress in various microorganisms: Towards a survival prediction test. *Biotechnology and Bioengineering*. 2005; 92:479–484. [PubMed: 16116658]
50. Poirier I, Marechal PA, Evrard C, Gervais P. *Escherichia coli* and *Lactobacillus plantarum* responses to osmotic stress. *Applied Microbiology and Biotechnology*. 1998; 50:704–709. [PubMed: 9891930]
51. Walczak, JJ.; Wang, L.; Feriencikova, L.; Li, J.; Xu, S. Influence of desiccation on deposition kinetics of *Escherichia coli* within saturated sand packs. 2011. In review
52. Bolster CH, Walker SL, Cook KL. Comparison of *Escherichia coli* and *Campylobacter jejuni* transport in saturated porous media. *Journal of Environmental Quality*. 2006; 35:1018–1025. [PubMed: 16738386]

53. Park SJ, Kim SB, Kim KW. Analysis of bacterial cell properties and transport in porous media. *J Environ Sci Heal A*. 2010; 45:682–691.
54. Boman HG, Monner DA. Characterization of lipopolysaccharides from *Escherichia coli* K-12 mutants. *Journal of Bacteriology*. 1975; 121:455–464. [PubMed: 1089628]

Figure 1.

Breakthrough concentrations of *E. coli* K12 cells that were starved under various water pH (5.7 or 7.2) and ionic strength (8.4 mM or 168 mM) conditions for 4, 25 and 52 hours. The *E. coli* cells used for the experiments were grown in LB-Miller broth. For one column experiment (pH = 7.2, ionic strength = 8.4 mM, starvation time = 52 hours), the injection time was slightly longer than the rest. For clarity purposes, only one in five data points were shown.

Figure 2.

Breakthrough concentrations of *E. coli* K12 cells that were starved under various water pH (5.7 or 7.2) and ionic strength (8.4 mM or 168 mM) conditions for 4, 25 and 52 hours. The *E. coli* cells used for the experiments were grown in LB-Luria broth. For clarity purposes, only one in five data points were shown.

Figure 3.

Variation of *E. coli* clean-bed deposition rate coefficients (K_d) as a result of starvation under various water chemistry conditions. (A) *E. coli* grown in LB-Miller broth and (B) *E. coli* grown in LB-Luria broth. The values of the clean-bed deposition rate coefficients were calculated using equation (1). Error bars reflect the variations of duplicate experiments.

Figure 4.

Comparison of calculated K_d values for *E. coli* cultured using LB-Miller and LB-Luria broths. In general, the data points are scattered along the 1:1 line, suggesting that *E. coli* grown in the two types of LB broths displayed similar mobility. The arrows indicate that significant drops in K_d values for two conditions (*E. coli* grown in LB Miller resuspended in 8.4 mM NaCl solution and *E. coli* grown in LB Luria broth resuspended in 168 mM NaCl solution) led to deviations from the 1:1 line.

Figure 5. Equivalent radius of starved *E. coli* K12 cells under various water chemistry conditions. Error bars represent the standard deviation of a minimum of 30 measurements.

Figure 6.

Zeta potential of the starved *E. coli* cells under various water chemistry conditions. Error bars present the standard deviation of triplicate measurements.

Figure 7. Hydrophobicity of the starved *E. coli* cells as measured by the MATH test. Error bars represent standard deviation of triplicate measurements.

Figure 8.

LPS sugar contents of *E. coli* starved in various electrolyte solutions. The error bars represent standard deviation of triplicate extractions.

Figure 9. Outer membrane protein profiles of *E. coli* resuspended in unbuffered solutions (pH = 5.7). Molecular masses are indicated by lane M (25, 30, 40, 50, 60, 80, 100, 150 and 250 kDa).

Figure 10.
Viability of starved *E. coli*. Error bars reflect the variations of duplicate measurements.

Table 1

The electrolyte solutions that were used for the starvation experiments. The total ionic strength was adjusted using NaCl.

Total Ionic Strength (mM)	pH	Buffer (mM)	
		Na ₂ HPO ₄	NaH ₂ PO ₄
168	7.2	17.61	4.88
8.4		0.88	0.24
168	5.7	0	0
8.4		0	0