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Role of Oxygen Tension on the Transport and Retention of Two Pathogenic Bacteria in Saturated Porous Media

FELIPE D. CASTRO AND
NATHALIE TUFENKJI*

Department of Chemical Engineering, McGill University,
Montreal, Quebec H3A 2B2, Canada

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To examine the influence of variations in the dissolved oxygen (DO) concentration on pathogen mobility, laboratory-scale filtration experiments were performed using the enterohemorrhagic strain *Escherichia coli* O157:H7 and the enteroinvasive organism *Yersinia enterocolitica*. Cells were incubated either in the absence (anaerobic) or in the presence (aerobic) of oxygen to understand how these two growth conditions may affect bacterial transport and retention in water-saturated granular porous media. The influence of DO during growth is found to be organism dependent, whereby *E. coli* O157:H7 exhibits decreased transport potential when grown in the presence of O₂ and *Y. enterocolitica* exhibits greater transport when grown aerobically. To understand the influence of DO changes during cell acclimation and transport, bacteria were resuspended and acclimated in either oxygen-depleted (low DO) or oxygen-rich (saturated DO) electrolytes prior to conduction of filtration experiments. The effect of DO on bacterial transport and retention is shown to be dependent on the antecedent growth conditions and on the organism studied. Measurements of the cell surface charge, shape, and size reveal some variability when the oxygen tension is changed during bacterial growth or acclimation and are linked to the observed bacterial transport behavior.

Introduction

Microbial transport and deposition in granular porous media is of interest in a broad range of environmental applications, including in situ bioaugmentation (1), riverbank filtration (2), and engineered water treatment systems (3). Adequate protection of potable water supplies and effective treatment of contaminated soils or waters necessitate predictive models of microbe migration in granular matrixes. Such mathematical models of microbial fate and transport require a mechanistic understanding of the key processes or factors controlling microbe migration, retention, and survival in varied natural and engineered aqueous environments. Consequently, over the past 30 years, more than 100 laboratory or field studies have been aimed at improving our knowledge of the chemical, physical, and biological factors influencing the migration of microorganisms in granular porous media (4, 5). More recently, with increasing concerns over contamination of potable water supplies by microbial pathogens,

studies have been conducted with toxigenic strains of bacteria such as *Escherichia coli* O157:H7 and infective parasites (e.g., *Cryptosporidium*) (6, 7). Nonetheless, there remain important gaps in our understanding of microbe behavior that limit our ability to predict microbial transport and fate in aqueous systems.

Baseflow groundwater can be anoxic or hypoxic with dissolved oxygen (DO) concentrations commonly ranging between 0 and 0.95 mg of DO/L (8). Higher DO values can be observed in shallow and unconfined aquifers, turbulent groundwater, and aquifers directly connected to aerobic surface waters (9). In environments such as alluvial aquifers used in riverbank filtration, varying DO levels are commonly observed due to facultative microbial activity through soil strata and reaeration at the soil surface (2). Aquifer recharge activity, heavy rainfall events, and stormwater infiltration may also disturb the groundwater chemistry and the composition of dissolved gases in the subsurface (10). For instance, it has been shown that cold winter stormwater can slightly reoxygenate groundwater in shallow aquifers, whereas warm summer stormwater may lower DO concentrations in groundwater at depths of 2–3 m (10). In engineered water treatment systems (e.g., deep-bed granular filtration or trickling filters), the concentration of DO can also fluctuate along the flow path of the treated water.

Although it is known that water oxygen tension can exhibit temporal and spatial variability in natural and engineered granular matrixes, studies examining the influence of oxygen tension on microbe migration in these environments are scarce. Some earlier studies have been aimed at investigating the mechanisms controlling microbial colonization of granular porous material under static conditions in an anaerobic environment most relevant to deep aquifers with very slow groundwater velocities (11, 12). Yang (13) evaluated the transport behavior of multiple *E. coli* isolates, as well as a nonpathogenic O157:H7 strain, in different granular materials when grown under anaerobic or aerobic conditions. This study demonstrated important variability in cell surface properties between the different isolates and showed how the growth conditions strongly influenced the surface properties, biofilm formation potential, and migration behavior of the organisms (13, 14). Overall, the environmental *E. coli* isolates examined exhibited increased retention when grown under aerobic conditions. Similarly, in a recent investigation of *E. coli* W3100 transport and retention in sand-packed columns, it was shown that anoxia is a negative environmental signal for attachment of this organism to sand (15). This finding was linked to the observation that growth under oxygen-limited conditions leads to increased expression of the *fliC* gene (encoding major flagellar subunit FliC) and overproduction of lipopolysaccharide (LPS). The above-mentioned studies have begun to address the effect of anaerobic growth on *E. coli* migration; however, variations in DO concentrations during bacterial transport have not been taken into consideration. In certain cases, the bacteria of interest may grow in an anaerobic environment (e.g., the intestinal tract of wildlife or farm animals), but migration can take place in oxygen-rich waters. The opposite is also true, whereby cells may proliferate in an aerobic atmosphere (e.g., the uppermost soil layers and open water bodies) but subsequently be released to an anaerobic or hypoxic environment. Although these different scenarios are likely encountered in natural and engineered aqueous systems, research examining the influence of variable DO concentrations during bacterial growth and acclimation is lacking.

* Corresponding author phone: (514) 398-2999; fax: (514) 398-6678; e-mail: nathalie.tufenkji@mcgill.ca.

The general objective of this study is to investigate the sensitivity of pathogenic bacteria to variations in oxygen tension during (i) growth or (ii) acclimation and transport. The selected enteric pathogens *E. coli* O157:H7 and *Yersinia enterocolitica* were grown in the absence (anaerobic) or presence (aerobic) of O₂ and then resuspended in oxygen-rich or oxygen-depleted electrolytes to examine the effect of this environmental parameter on bacterial migration potential. Electrokinetic and physical properties of the two organisms characterized over the range of conditions examined are used in the interpretation of data obtained from the packed column experiments.

Materials and Methods

Experimental Design. Four treatments were considered that examined the influence of oxygen tension during bacteria growth and acclimation on bacterial transport. Selected bacteria were grown either anaerobically (ANA) or aerobically (AER) and then resuspended in a prepared electrolyte solution (10 mM KCl) of either low dissolved oxygen (lowDO) or saturated dissolved oxygen (satDO) concentration. Additional details regarding the preparation of the electrolyte solutions are provided below. Following a 21 h acclimation period in the lowDO or satDO electrolyte solution at 9 °C, bacterial transport was examined using column transport experiments conducted at 11 °C.

Bacteria Selection and Growth Protocols. Two pathogenic Gram-negative microorganisms were selected for this study, *E. coli* O157:H7 ATCC 700927 and *Y. enterocolitica* ATCC 23715. Pure cultures were maintained at -80 °C in Luria-Bertani (LB) Lennox broth (20 g/L, Fisher) with 15% glycerol. One day prior to inoculation, frozen cultures were streaked onto solid LB agar plates that were then incubated at 37 °C for 21 h. For each bacterial transport experiment, a colony from the starter plate was used to inoculate sterile LB broth. Aerobic (AER) growth for both strains involved incubation at 37 °C and 200 rpm for 8 h in 150 mL of LB broth (in a 500 mL baffled flask), at which point the cells were harvested. Anaerobic (ANA) growth for both strains involved incubation at 37 °C in 200 mL of LB broth in capped 250 mL centrifuge bottles (Nalgene) maintained without agitation for 20 h.

Bacterial Cell Acclimation. Following the selected growth protocol (AER or ANA), the cell culture was centrifuged (Sorvall RC6) for 15 min at 5860g in an SS-34 rotor (Kendro). The growth medium was decanted, and the pellet was resuspended in freshly prepared electrolyte (either lowDO or satDO 10 mM KCl solution). To remove all traces of the growth medium, the cells were centrifuged and resuspended in fresh electrolyte one additional time. Analytical reagent grade KCl, KHCO₃, KOH, and HCl (Fisher) and deionized (DI; Milli-Q) water were used to prepare the electrolyte solutions. The satDO electrolyte was prepared by vigorous stirring and sparging of 1.2 L of KCl solution (10 mM) using filtered (Durapore PVDF membrane, 0.22 µm, Millipore) air at 1.8 vvm in a 2 L Erlenmeyer flask for 30 min. The final DO level of the satDO solution was 8.6 ± 0.3 mg of DO/L. The lowDO electrolyte was prepared by vigorously stirring and sparging a 1.2 L KCl solution (10 mM) with prepurified N₂ at 1.8 vvm in a tightly capped 2 L Erlenmeyer flask for 30 min. The final DO level of the lowDO electrolyte was 0.4 ± 0.3 mg of DO/L. Prepared lowDO or satDO electrolytes were transferred to a 2 L low gas permeability bioprocess bag (Labtainer, Hyclone) using a glass tube positioned at the bottom of the 2 L flask. After transfer of the lowDO or satDO electrolytes to the Labtainer bag, the total equivalent dissolved CO₂ concentration was adjusted to $\sim 1.2 \times 10^{-5}$ M using 0.1 M KHCO₃ (Fisher) and the pH was adjusted to 6.7 ± 0.2 using 0.1 M HCl or 0.1 M KOH (Fisher). Washed cells were resuspended to a final concentration of $\sim 4 \times 10^7$ cells/mL

in either lowDO or satDO electrolyte contained in a separate 500 mL Labtainer bag. The Labtainer bags holding the washed cells (bacteria suspension, 500 mL bag) and the bacteria-free electrolyte (background solution, 2 L bag) were then maintained at 9 °C for 21 h. This cold temperature acclimation was used to replicate conditions that may be encountered by cells following release into groundwater representative of the Canadian climate. Actual DO levels of the background solution and bacteria suspension were verified using a micro flow-through polarographic DO probe (model DO-166FT, Lazar Laboratories) connected to each Labtainer bag. In all four treatments, the cells were exposed to the same temperature and chemistry shifts when transferred from the incubation phase to the acclimation/transport phase; namely, the cells were all incubated at 37 °C in LB and all acclimated at 9 °C in KCl solution.

Loss of cell viability during acclimation in the conditioned electrolytes at 9 °C was verified using the BacLight viability kit (Molecular Probes, Eugene, OR). This technique involves direct counting of stained live and dead cells using a fluorescent microscope (IX-71, Olympus). Cell viability during acclimation for the four treatments examined was found to be greater than 92% and 83% for ATCC 700927 and 23715, respectively.

Bacterial Cell Characterization. The shape and size of the bacteria were determined by optical microscopy. Cell electrophoretic mobility was measured and potentiometric titrations of bacterial cells were conducted for each of the four treatments. Details regarding the bacterial cell characterization are provided in the Supporting Information.

Bacterial Transport and Deposition Experiment. Column experiments were conducted to evaluate the transport of the two organisms separately after acclimation in lowDO or satDO KCl solution. The column apparatus was placed inside a cold chamber maintained at 11 °C. Preparing cell suspensions at a slightly colder temperature (9 °C; see above) facilitated conducting the experiment at 11 °C (due to slight heat gain by the suspension during transfer to the chamber). Experiments were conducted by pumping a bacterial suspension contained in a Labtainer bag through a glass column packed with clean electrolyte-saturated sand. An adjustable-height column (C 16/40, Amersham) with an inner diameter of 1.6 cm was used. The sand was wet-packed to a height of 89 mm with vibration. Standard gravimetric methods were used to determine the sand density (2.62 g/cm³) and a column packing porosity of 0.35.

The conditions of the four treatments considered (ANALow, ANAsat, AERlow, and AERsat) were maintained for the transport phase of the experiment conducted in the packed column. Prior to each experiment, the packed column was equilibrated by allowing 40 pore volumes (PV) of the background electrolyte of interest held inside a 2 L Labtainer bag to flow through the column. The solution flow rate was maintained by a syringe pump (model 200, KD Scientific) downstream of the column to retain an approach velocity of 1.7×10^{-4} cm/s. Next, a bacterial suspension of concentration (C_0) 4×10^7 cells/mL of the same background electrolyte composition was flowed through the column for at least 4 PV followed by a bacteria-free electrolyte solution (4 PV) using the same pumping method described above. The bacterial cell concentration at the column outlet was monitored online with a UV/vis spectrophotometer (Hewlett-Packard model 8453) using a 1 cm flow-through cell (at 600 nm). Each experiment was conducted at least twice, and good reproducibility was observed in the measured bacteria breakthrough curves. The DO level in the column effluent was monitored online using a flow-through DO probe to verify gas impermeability of the experimental apparatus. No significant changes in the DO level were observed for all conditions.

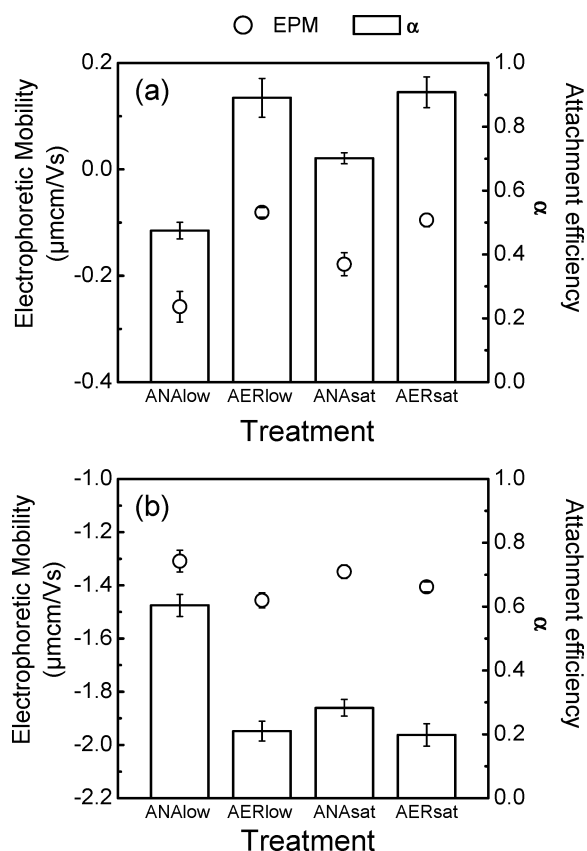


FIGURE 1. Measured bacterial EPMs shown as open symbols (left y-axis) and bacterial attachment efficiencies (α) shown as a bar plot (right y-axis) for (a) *E. coli* O157:H7 and (b) *Y. enterocolitica*. Four treatments were considered: (i) ANA_low (cells grown anaerobically and then acclimated in lowDO electrolyte); (ii) AER_low (cells grown aerobically and then acclimated in lowDO electrolyte); (iii) ANA_sat (cells grown anaerobically and then acclimated in satDO electrolyte); (iv) AER_sat (cells grown aerobically and then acclimated in satDO electrolyte). Data represent the mean \pm 95% confidence interval.

Interpretation of Bacterial Column Experiments. Classical colloid filtration theory (CFT) was used to quantitatively compare the bacterial attachment behavior to quartz sand in the four treatments examined. The attachment efficiency (α) in CFT is a useful parameter to compare bacterial transport and deposition behavior observed under different conditions and was calculated from each breakthrough curve as follows (16):

$$\alpha = -\frac{2}{3} \frac{d_c}{(1-\varepsilon)L\eta_0} \ln(C/C_0) \quad (1)$$

where d_c is the diameter of the sand grains, ε is the bed porosity, L is the bed length, and η_0 is the theoretical single-collector contact efficiency evaluated using a correlation equation developed by Tufenkji and Elimelech (16). Development of eq 1 assumes that the system under study is at steady state and that bacterial detachment from the grain surface is negligible. The value of C/C_0 in eq 1 was obtained from the experimental breakthrough curves (data not shown) during the initial (clean-bed) phase of bacterial elution.

Results and Discussion

Characterization of Bacterial Cells. Measured electrophoretic mobilities (EPMs) of the two bacterial species for the four treatments considered in this study are presented in Figure 1 (open symbols). These data demonstrate the

influence of the cell growth protocol (AER vs ANA) and cell acclimation protocol (lowDO vs satDO) on the cell surface charge. Both cell types have a negatively charged surface in all four conditions examined. For *E. coli* O157:H7 (Figure 1a), the cells exhibit a greater absolute charge when grown in the absence of oxygen versus those grown aerobically. This observation is in agreement with measurements previously reported by Landini and Zehnder (15) showing more negative EPMs for *E. coli* K12 mutants grown anaerobically in comparison to those grown aerobically. In contrast, Yang et al. (14) found no significant differences in the EPMs for 14 *E. coli* isolates (including an O157:H7 strain) when grown anaerobically or aerobically. In this latter study, however, the growth medium and incubation temperature were also different for the anaerobic and aerobic growth protocols. These differences in experimental protocols between the study of Yang et al. (14) and the current study could have contributed to the observed different trend in the EPM. The data shown in Figure 1a reveal that the cell acclimation process influences the cell surface charge only for the *E. coli* grown anaerobically, where the average measured EPMs are -0.26 and -0.18 ($\mu\text{m cm})/(\text{V s})$ for lowDO and satDO conditions, respectively. When *E. coli* O157:H7 is grown aerobically, the cell acclimation protocol does not affect the measured cell surface charge (at a 95% confidence level).

The measured EPM for *Y. enterocolitica* is presented in Figure 1b (open symbols) for the four treatments considered. Overall, *Y. enterocolitica* exhibits a greater absolute charge than *E. coli* O157:H7, with average measured EPMs ranging between -1.5 and -1.3 ($\mu\text{m cm})/(\text{V s})$ for the former, in comparison to values ranging between -0.26 and -0.08 ($\mu\text{m cm})/(\text{V s})$ for the latter. In contrast to the results observed for *E. coli* O157:H7 (Figure 1a), *Yersinia* exhibits a greater absolute charge when grown in the presence of oxygen than when grown anaerobically. Moreover, when *Y. enterocolitica* is grown anaerobically, the cell acclimation protocol does not affect the measured cell surface charge (at a 95% confidence level). The data in Figure 1 provide insight into the influence of the dissolved oxygen concentration on the cell surface charge. The noted variations in the cell EPM shown in Figure 1 are likely linked to physiological response of the cells resulting in different overall cell membrane compositions (17, 18).

To examine the effect of variations in the DO during cell growth and acclimation on the cell size and shape, microscope images of cells adhered to aminosilanized slides were analyzed. The mean equivalent spherical diameter of the cells is presented in Figure 2a,b, whereas the mean cell aspect ratio (length/width) is shown in Figure 2c,d. *E. coli* O157:H7 has an average diameter of $0.69 \mu\text{m}$ and does not exhibit any measurable differences in cell size for three of the treatments (ANA_low, ANA_sat, and AER_low) (i.e., the values are within the 95% confidence intervals of each other) (Figure 2a). The shape of this organism becomes more elongated when incubated under anaerobic conditions (Figure 2c). Specifically, the average cell aspect ratio increases from 1.3 (AER) to 1.8 (ANA).

Y. enterocolitica is larger in size and considerably more elongated than *E. coli* O157:H7 (at a 95% confidence level). The aspect ratio of *Y. enterocolitica* does not exhibit any detectable differences for three of the treatments (ANA_sat, AER_sat, AER_low) (Figure 2d). The data in Figure 2 demonstrate how changes in oxygen tension during cell culturing and acclimation protocols may sometimes alter the physical properties of the organisms. The shape and size of the bacteria can play an important role in their transport and deposition in saturated granular porous media (19, 20), and the measurements presented here are used later in the paper to link bacterial cell properties to their migration behavior.

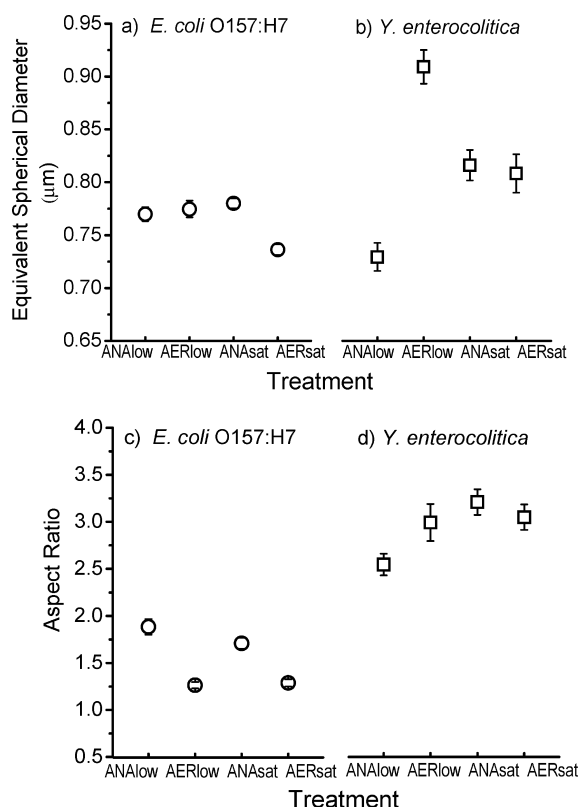


FIGURE 2. Shape descriptors for both cell types obtained by image analysis: (a, b) equivalent spherical diameter based on the volume of a spheroid (spheroid length and width determined from object projection); (c, d) cell aspect ratio (cell length divided by cell width). Data represent the mean \pm 95% confidence interval.

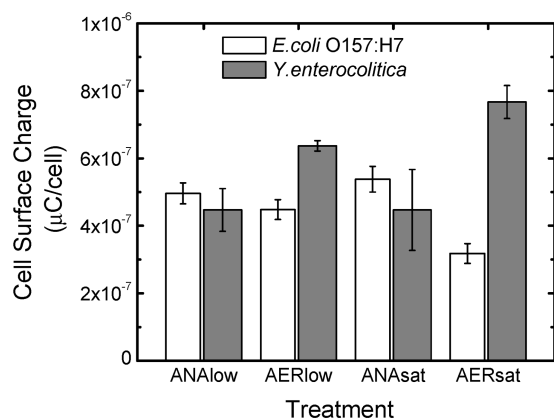


FIGURE 3. Surface charge per cell determined from the amount of NaOH consumed during titration of bacteria (suspended in 10⁻² M KCl) from pH 4.0 to pH 9.5. Data represent the mean \pm 95% confidence interval.

The results of potentiometric titrations of cell suspensions are presented in Figure 3. When the cells are grown anaerobically, *E. coli* and *Y. enterocolitica* exhibit comparable titratable cell surface charge. In the case of *E. coli* O157:H7, the number of dissociable functional groups on the cell surface is greater when the cells are grown in the absence of oxygen (for the satDO treatment only). This observation is in qualitative agreement with the measured EPM for this treatment (Figure 1a), which indicates a greater absolute charge when the cells are grown anaerobically. *Y. enterocolitica* also exhibits differences in cell surface charge when grown aerobically or anaerobically (Figure 3). The surface charge per cell measured by potentiometric titration is greater

for *Y. enterocolitica* grown in the presence of O₂ (i.e., AER_{sat}DO > ANA_{sat}DO, and AER_{low}DO > ANA_{low}DO, at a 95% confidence level). Likewise, the absolute EPM is greater for *Y. enterocolitica* grown aerobically (Figure 1b). Inspection of Figure 3 also reveals that the acclimation of the bacteria in lowDO or satDO electrolyte influences the measured titratable charge for cells grown aerobically (at a 95% confidence level). In an earlier study, Haas (21) noted significant differences in functional group acid dissociation constants and site concentrations when culture conditions were changed from aerobic to anaerobic in the Gram-negative organism *Shewanella putrefaciens*.

Effect of the Oxygen Tension during Growth on Bacterial Transport and Retention. Column transport experiments were conducted to investigate the effect of AER versus ANA growth conditions on the migration behavior of the two selected pathogenic Gram-negative strains. Bacterial attachment efficiencies (α) were calculated from the measured breakthrough curves (not shown) using eq 1 and are presented in Figure 1 in the form of bar graphs (right y-axis). *E. coli* O157:H7 exhibits a high extent of retention on the clean sand grains ($\alpha \approx 0.90$) when grown in the presence of oxygen (Figure 1a). In contrast, incubation of *E. coli* O157:H7 under anaerobic conditions results in decreased retention on the granular medium, with an average α of 0.59. These results are in agreement with a previous study (15) examining the influence of the oxygen tension during cell growth on the transport and adhesion of the nonpathogenic *E. coli* K12 in sand-packed columns. These researchers also observed decreased bacterial adhesion when cells were grown under anaerobic conditions and demonstrated clear links between the observed reduction in adhesion and increased production of LPS and flagella (15). In an investigation of *E. coli* adhesion to various candidate biobarrier materials, Yang (14) also found decreased attachment when the cells were grown in the absence of oxygen. Cell metabolism and gene expression will be affected by changes in oxygenation (22, 23). In the case of *E. coli*, it has been estimated that the expression of over one-third of the genes expressed during growth under aerobic conditions is altered when *E. coli* cells transition to an anaerobic growth state (24). Landini and Zehnder (15) demonstrated that the global regulatory *hns* gene plays a key role in controlling the adhesion behavior of *E. coli*. Anoxia has also been reported to hinder biofilm formation in *E. coli* K12 strains (25). However, further metabolic studies are required to better understand the global effect of oxygen deprivation on bacterial adhesion to surfaces.

Attachment efficiencies calculated for *Y. enterocolitica* migrating through electrolyte-saturated sand columns are reported in Figure 1b. Interestingly, the results observed for *Y. enterocolitica* show a trend opposite that of the results noted for *E. coli* O157:H7. Specifically, for *Y. enterocolitica*, the average attachment efficiency is greater when the cells are grown in the absence of oxygen ($\alpha_{av} \approx 0.44$) in comparison to when they are grown under aerobic conditions ($\alpha_{av} \approx 0.20$). A previous study examining the adhesion of strictly anaerobic bacteria onto glass coverslips under anaerobic and aerobic incubation periods demonstrated similar behavior (26). Specifically, it was shown that incubation in the presence of air significantly decreased adhesion of *Syntrophomonas wolfei* and *Desulfovibrio* sp. strain G11 onto glass in comparison to completely anaerobic incubation (26). Hence, our results and those of previous studies show that our understanding of the influence of the oxygen tension during growth on bacterial transport is still too limited to allow generalization. Further studies examining the influence of this environmental parameter on the migration behavior of a broader selection of organisms are needed. The causes of the bacterial deposition behavior observed in Figure 1 will be discussed in more detail later in the paper.

Effect of the Oxygen Tension during Acclimation on Bacterial Transport in Packed Columns. In this section, we examine the effect of the DO concentration during cell acclimation on the transport and deposition behavior of the two selected pathogenic bacteria. For both *E. coli* and *Y. enterocolitica*, the attachment efficiency is not affected by the DO content of the acclimation solution when they are grown aerobically (at a 95% confidence level) (Figure 1). For instance, in the case of *E. coli*, $\alpha \approx 0.90$ when the cells are acclimated in either lowDO (AERlow) or satDO (AERSat) electrolyte solutions (Figure 1a). Similarly, for *Y. enterocolitica*, α is 0.20 whether the organism is acclimated in lowDO (AERlow) or satDO (AERSat) electrolyte (Figure 1b). In contrast, the bacterial deposition behavior is affected by the DO concentration during acclimation when the cells are grown under anaerobic conditions. *E. coli* O157:H7 experiences an increase in α (from 0.47 to 0.70) when suspended in electrolyte that is saturated with oxygen (ANAsat) (Figure 1a). Here again, *Y. enterocolitica* shows a behavior that is opposite that observed for *E. coli* O157:H7. Namely, when the cells are grown anaerobically, this organism exhibits a decrease in α (from 0.60 to 0.28) when acclimated in electrolyte that is oxygen-rich (ANAsat) in contrast to electrolyte that is oxygen-deficient (ANAlow) (Figure 1b). These data suggest that the effect of the oxygen tension during cell acclimation on bacterial transport and deposition in granular porous media cannot yet be readily generalized. The results shown in Figure 1 are particularly interesting as they highlight the importance of the cell type (and respective surface characteristics) in the potential influence of this environmental parameter on bacterial adhesion.

Linking Bacterial Transport Behavior to Cell Properties.

The image analysis data presented in Figure 2 clearly demonstrate that both *E. coli* O157:H7 and *Y. enterocolitica* are rod-shaped organisms, with *Y. enterocolitica* being more elongated. Others have shown that rod-shaped particles and more elongated bacteria are retained more readily in granular porous media than spherical colloids (19, 20). However, inspection of the data in Figures 1 and 2 does not reveal greater retention of bacteria with higher aspect ratios. Furthermore, the results do not show increased retention of larger cells as would be expected if physical straining were an important mechanism (27). In fact, the data in Figures 1 and 2 show that the larger cells (*Y. enterocolitica*) generally exhibit lower retention rates than the smaller cells (*E. coli*). Hence, a physical retention mechanism is likely not controlling the extent of bacterial migration in this system.

In Figure 1, the bacterial cell EPM (scatter plot) is presented on the same graph as the measured attachment efficiency (bar plot). For *E. coli* O157:H7, the trend in α from the leftmost treatment to the rightmost treatment is the same as that of the EPM (Figure 1a). Specifically, the conditions with the highest attachment efficiency (AER) exhibit the lowest absolute charge. In a similar manner, the condition with the lowest α value is linked with the greatest absolute charge (ANAlow). A simple linear regression of α against EPM for *E. coli* O157:H7 (which includes all four treatments) reveals a significant correlation in the two sets of measurements ($R^2 = 0.98$, with a slope of 2.5). Such direct links between the cell surface charge (e.g., EPM or ζ potential) and bacterial transport in granular porous media have previously been shown by several researchers (6, 28, 29). Indeed, these results are generally in qualitative agreement with the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability for the interaction of a negatively charged particle (i.e., the bacterial cell) with a negatively charged surface (i.e., the quartz sand surface) (30, 31). According to this theory, cells with a lower absolute potential (or EPM) will experience a lower repulsive force as they approach the sand grain surface (assuming all other properties remain

constant). The data shown in Figure 1a suggest that the cell EPM (or ζ potential) is the key factor controlling the transport and deposition behavior of *E. coli* O157:H7 when grown or acclimated at two different DO levels.

Measured EPMs and attachment efficiencies for *Y. enterocolitica* are plotted together in Figure 1b. Although not as strong as in the case of *E. coli*, the trend in the EPM is generally in agreement with the observed changes in the α values. For instance, the conditions with the lowest attachment efficiency (aerobic growth) are associated with the greatest absolute charge (at a 95% confidence level), demonstrating qualitative agreement with DLVO theory. A linear regression of α against EPM for *Yersinia* (including four treatments) reveals a weaker correlation in the two sets of measurements ($R^2 = 0.68$) but, interestingly, with a slope (2.4) comparable to that observed for *E. coli* (2.5).

Lower α values are generally measured with *Yersinia* in comparison to *E. coli* (with the exception of the ANAlow condition). This trend in the behavior of the two organisms is also in qualitative agreement with DLVO theory because the absolute EPM of *Yersinia* is nearly 10 times greater than that of *E. coli* O157:H7. Generally, the data shown in Figure 1 demonstrate that DLVO interactions control the migration of the two cell types when grown or acclimated at different oxygen tensions. However, it should be noted that the ANAlow treatment for *Yersinia* exhibits non-DLVO behavior. Specifically, even though this condition is linked with a relatively high attachment efficiency (~ 0.6) which is comparable to the α value measured for *E. coli* for the ANAsat condition (~ 0.7), the absolute charge of *Yersinia* (absolute EPM of $1.3 (\mu\text{m cm})/(\text{V s})$) is much greater than that measured for *E. coli* (absolute EPM of $0.18 (\mu\text{m cm})/(\text{V s})$ for ANAsat). Clearly, a number of non-DLVO interactions could also contribute to the observed transport and deposition behavior of *Yersinia* in this study; including, but not limited to, electrosteric interactions and Lewis acid–base interactions (32).

Environmental Implications. This study highlights the variability in bacterial cell surface properties and deposition behavior in granular porous media as a result of changes in the water oxygen tension that may occur during cell growth or acclimation. To our knowledge, a well-controlled laboratory investigation of the effect of the oxygen tension during growth and acclimation of waterborne bacterial pathogens on their migration behavior in granular porous media has not been previously reported. Our results show how the influence of DO on bacterial transport and deposition is strongly dependent on the species examined. Significantly different results were obtained when experiments were conducted with *E. coli* O157:H7 or *Y. enterocolitica*. For instance, *E. coli* exhibits the least extent of migration when grown aerobically, whereas *Yersinia* exhibits the greatest migration potential when grown in the presence of O_2 . Such variations in bacterial transport behavior may have important implications for predictions of groundwater contamination potential or of pathogen removal efficiencies in engineered water treatment facilities.

Our study also reveals significant variations in cell surface charge (measured by potentiometric titration), electrophoretic mobility, and cell size and shape when DO concentrations are changed during bacterial growth or acclimation. It should be noted that potentiometric titrations alone cannot be used to conclusively determine the types of dissociable functional groups or biomolecules present on the bacterial cell surface. A better understanding of the composition of the outer cell membrane can be obtained by coupling potentiometric titrations with another characterization technique (e.g., ATR-FTIR). Ongoing research in our laboratory is aimed at improving our understanding of the influence of DO on the composition of the bacterial cell membrane using this technique. As evidenced by this

investigation, an improved characterization of the bacterial cell wall will lead to a better understanding of bacteria–surface interactions in a broad range of environmentally relevant applications.

In this work, we have focused on the influence of extremes in DO levels during bacterial growth and acclimation on bacterial migration and cell surface properties. Bacterial transport and deposition were examined at two DO levels (high and low) using columns packed with clean high-purity quartz sand. However, in natural and engineered granular matrixes, variations in DO concentration can also influence the chemical composition of granular collector surfaces. For instance, in shallow groundwater aquifers, aerobic conditions can lead to the formation of metal (e.g., iron or manganese) oxides on the surface of soil grains that give rise to important charge heterogeneities. Such “patches” of surface charge heterogeneity have previously been shown to control particle–surface or microbe–surface interactions (33). However, additional studies are needed to better understand the potential competing or synergetic effects of varying DO levels during bacterial growth, acclimation, and transport in geochemically heterogeneous systems.

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Supporting Information Available

Details regarding preparation of granular porous media and bacterial cell characterization. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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