Cell Preparation Methods Influence *Escherichia coli* D21g Surface Chemistry and Transport in Saturated Sand

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The effect of cell preparation methods on the surface chemistry and retention of *Escherichia coli* D21 g was investigated over a range of ionic strength conditions. The cell preparation methods that were considered included filtration and centrifugation (at various speeds and for different durations). For a given ionic strength condition, it was found that cells prepared by filtration were more negatively charged and hydrophobic than cells prepared by centrifugation. Increasing the centrifugation speed (force imposed) or duration produced cells with a higher zeta potential (less negative) and a lower hydrophobicity. Column transport experiments for *E. coli* D21 g were also conducted with ultra pure quartz sand and the same solution chemistries. The first-order retention rate coefficient for *E. coli* D21 g increased with increasing speed and duration of centrifugation, and was lowest in the case of filtered cells. Moreover, the influence of cell preparation method was more pronounced in lower ionic strength solutions.

Published in J. Environ. Qual. 37:2108–2115 (2008).
doi:10.2134/jeq2008.0023
Received 15 Jan. 2008.
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Bacteria cell surfaces play a crucial role in their interaction with porous media in the environment (Pembrey et al., 1999). The ability of cells to adhere to abiotic surfaces can be attributed to the physiological state of the organism (Zvyagintsev et al., 1977; Fletcher, 1977) which has recently been associated with the physicochemical properties and metabolic behavior exhibited by their cell surface (Grasso et al., 1996, Dufrene and Rouxhet, 1996, Walker, 2005). Physicochemical interactions between the bacterial cell surface and the porous medium surface have been reported to govern the adhesion of cells (Smets et al., 1999). Most experimental procedures (e.g., cell surface analysis, bacterial adhesion experiments) require that cultures of organisms are harvested in some way before experimentation. The purposes of such procedures are to concentrate the cells and to clean the cells from their high nutrient environment.

Harvesting protocols may affect the physiochemical nature of and/or the metabolic behavior of the bacteria, creating artifacts in the measurement of adhesion and other bacterial properties (Pembrey et al., 1999). Some research has indicated that variation in the surface physiochemical properties in vitro may completely change the attachment mechanisms of an organism compared to its behavior in the natural environment (Marshall et al., 1994). Exposure to such parameters as different ionic strength (IS), temperature, centrifugation protocol, freezing, and drying are just some of the processes that need to be considered for their ability to cause experimental artifacts with bacteria (Bell et al., 2005). Specifically, the effects of centrifugation on a bacterium's capacity to attach to substrates are of particular concern because centrifugation is commonly used in cell preparation procedures (Bell et al., 2005; Bell and Camesano, 2005). It has been reported that centrifugal separation of the cells from their suspending medium subjects them to enormous centrifugal forces, which in centrifuge tubes is translated to high hydrostatic pressures (Gilbert et al., 1991). Few systematic studies of the effects of centrifugation on cell surface characteristics, however,

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Abbreviations: BTC, breakthrough curves; DLVO, Derjaguin-Landau-Verwey-Overbeek; EPS, extra-cellulaar polymeric substances; IS, ionic strength; LB, Luria–Bertani; LPS, lipopolysaccharides; MATH, microbial adhesion to hydrocarbons.

have ever been reported (Gilbert et al., 1991; Makin and Beveridge, 1996; Pembrey et al., 1999). In the work conducted by Pembrey et al. (1999), the net surface charge of *E. coli* and *S. epidermidis* was found to decrease when the cells were subjected to high speed centrifugation (15,000 × *g*). In contrast, the charge characteristics of *Psychrobacter* sp. strain SW8 remained unchanged after high speed centrifugation.

Filtration is also a separation method commonly used in the field of aquatic microbiology for a variety of objectives including bacteria harvesting (Kepner and Pratt, 1994). In contrast with centrifugation, it is reported that filtration does not greatly affect cellular behavior (Bell et al., 2005). In spite of this advantage, it is seldom used in routine studies and experiments, in part due to the relatively longer time required for filtering the cell suspension.

The aim of this research was to investigate the influence of cell harvesting methods (centrifugation vs. filtration) on the surface chemistry and retention of bacteria (*E. coli* D21 g) under well controlled solution chemistry conditions. Different speeds (i.e., 3689 and 14,000 × *g*) and duration (10 and 15 min) of centrifugation were considered in these studies. The nature of the cell surfaces was characterized using the microbial adhesion to hydrocarbon (MATH) test and measurements of the cell surface electrophoretic mobility. The transport and retention behavior of *E. coli* D21 g under various solution chemistries was quantified in packed bed column experiments using cells that had been harvested by filtration or various modes of centrifugation.

Materials and Methods

Sand Preparation

Ultra pure quartz sand was used as the porous media. The sand was prepared in advance to ensure that sand grain sizes were uniform and the grain surfaces were free of organic matter and metal oxides. The sieved sand (Unimin Corp., Spruce Pine, NC) had an average diameter (d_{50}) of 205 μm. To remove chemical heterogeneities that could influence the bacteria retention behavior, the sand was soaked in 12 *M* HCl (Fisher) at least 24 h. The sand grains were then thoroughly washed, baked in an oven at 800°C for 8 h, and rehydrated again by boiling in deionized water (Barnstead Thermolyne Corp., Dubuque, IA) for 1 h.

Bacterial Cell Preparation and Growth

Escherichia coli D21 g a Gram-negative, nonmotile bacterial strain was chosen for experimentation. It is reported that this bacterium has minimal lipopolysaccharides (LPS) (Gmeiner and Schlecht, 1980; Walker et al., 2004), and negligible amounts of extra-cellular polymeric substances (EPS) (Razatos et al., 1998). A preculture of bacteria was prepared by inoculating 5 mL of Luria–Bertani broth (LB Broth, Fisher Scientific, Fair Lawn, NJ) that had been supplemented with 0.03 mg/L gentamycin (Sigma, St. Louis, MO). The preculture was incubated on a rotary shaker overnight (12–18 h) at 37°C and then added to 200 mL LB liquid media containing 0.03 mg/L gentamycin and incubated at 37°C until reaching mid-exponential growth phase.

Bacterial Separation Methods

Centrifugation

Cells were separated from the growth media using a centrifuge device (Fisher accuspin * 3R centrifuge). In the standard case the cell suspension was centrifuged for 15 min at 3689 × *g* (Swing Bucket Rotor 7500–4339) to separate the cells from the growth media. The supernatant was decanted and the pellet was resuspended in 10 mmol L^{-1} KCl solution by a vortex (Auto Touch Mixer Model 231, Fisher) for about 1 min. To ensure all traces of growth medium were removed, the process of centrifuging, decanting, and resuspending in the electrolyte solution was repeated twice more, so that the cells were subjected to a total of 45 min of centrifugation. During the centrifugation process, the temperature was maintained at 4°C. The electrolyte solution used for this rinsing process was prepared with deionized water and reagent-grade KCl (Fisher Scientific) with no pH adjustment (pH 5.6–5.8). To elucidate the effect of centrifugation time on the bacterial properties, the same procedure was employed but using a centrifugation time of 10 min instead of 15 min. In this case, the total time of centrifugation was 30 min. To study the impact of centrifugation speed, the cell suspension was spun at a higher speed (14,000 × *g*) for 15 min following the same rinsing protocol.

Filtration

Cells were also separated from the LB media by filtering through a 0.45 μm membrane (Fisher Scientific, Pittsburgh, PA) and back-washing with 30 mL of electrolyte solution (10 mmol L^{-1} KCl). The back-washing procedure was repeated twice to remove any trace of the growth medium. To recapture cells retained on the membrane, the membrane was removed and placed into the centrifuge tube. Then 15 mL of KCl solution (10 mmol L^{-1}) was added to the tube. The tube was vortexed (100 rpm) a few seconds to remove the bacterial mass from the membrane. Completion of this cell separation method lasted about 1 h because filtration was a slow process as cells blocked membrane pores and reduced the flow across the membrane.

Bacterial Characterization

Electrophoretic Mobility

The zeta potential of cells was determined as follows: concentrated cell suspensions were diluted in KCl electrolyte solution to a final concentration of 10^7 to 10^8 cells/mL. Electrolyte solutions were prepared with deionized water and KCl (3.16, 10, 31.6, and 100 mmol L^{-1}) with no pH adjustment (5.6–5.8). Electrophoretic mobility measurements were conducted at 25°C using a ZetaPALS analyzer (Brookhaven Instruments Cooperation, Holtsville, NY). Briefly, the micro electrophoresis chamber was filled with a bacterial suspension. This device calculates the zeta potential from measured electrophoretic mobility's using the Smoluchowski equation (Heimenz, 1977). Zeta-potential measurements were deter-

mined in triplicate for cells in the various ionic strength solutions (3.16, 10, 31.6, and 100 mmol L^{-1}) and harvested with each cell separation method.

Hydrophobicity

Cell surface hydrophobicity was quantified using the MATH test, following a procedure described by Walker et al. (2005) and Pembrey et al. (1999). Briefly, 4-mL samples of a cell suspension (optical density of 0.2–0.25 at 546 nm in 10 mmol L^{-1} KCl) (PerkinElmer UV/VIS spectrophotometer, Irvine, CA) were transferred to individual test tubes, each of which contained 1 mL of n-dodecane (laboratory grade, Fisher Scientific). The test tubes were vortexed at full speed for 2 min and then left to stand for 15 min to allow phase separation. Partitioning of the bacterial suspension was expressed as the percentage of cells adsorbed by the hydrocarbon phase. The mean percentage of partitioning of an organism into the n-dodecane phase was calculated by using triplicate samples.

Column Experiments

Bacterial transport experiments were performed in glass chromatography columns packed with ultra pure quartz grains. An adjustable bed height column (Omnifit USA, Toms River, NJ) which had a 1.5 cm inner diam. was wet packed by allowing the quartz grains to settle in deionized water while the column was agitated. Sodium nitrate (0.2 mmol L^{-1}, Fisher Scientific) was used as a conservative tracer to characterize the hydrodynamic properties of the porous medium. Solutions were pumped (Model 200 syringe pump, KD Scientific Inc., New Hope, PA) to the column at a constant flow rate of 1.16 mL/min. The approach (superficial) velocity (*U)* and the column length *(L)* in the column experiments were 0.66 cm/min and 10 cm, respectively. Porosity values were determined gravimetrically and ranged from 0.43 to 0.45 cm^3/cm^3. Before each experiment, the packed column was flushed with several pore volumes of deionized water followed by 6 pore volumes of the background electrolyte solution. The electrolyte solution, with the ionic strength ranging from 30 to 100 mmol L^{-1} KCl and the pH between 5.6 to 5.8, was maintained at room temperature (22–25°C). Bacterial cells (10^7–10^8 cell/mL) harvested with a given separation method (i.e., low speed centrifugation with 15 min duration, low speed centrifugation with 10 min duration, high speed centrifugation with 15 min duration, and filtration) were injected into the column for approximately 4 pore volumes (equivalent to 24 min) followed by background solution of the same ionic strength for an additional 5 to 6 pore volumes. Nitrate (NO_3) and the cells were detected in the effluent using a spectrophotometer (PerkinElmer UV/VIS spectrophotometer, Irvine, CA) at a wavelength of 204 and 240 nm, respectively.

Numerical Modeling

The HYDRUS-1D code (Simunek et al., 2005) is a finite element model for simulating the one-dimensional movement of water, heat, and multiple solutes in variably saturated media. The code numerically solves the Richards' equation for saturated–unsaturated water flow and advection–dispersion equations for the nonlinear equilibrium and kinetic reactions between solutes (here bacteria cells) and porous media. The model parameters can be obtained by optimization of the breakthrough curve and/or retention profile information to experimental data. The transport of bacteria through the sand column is described using the one-dimensional form of the advection–dispersion equation that accounts for bacteria retention in the column:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial z^2} - v\frac{\partial C}{\partial z} - r \qquad [1]$$

where C is the number of bacteria per unit volume of the aqueous phase (NL^{-3}), D is the dispersion coefficient (L^2T^{-1}), v is the average pore water velocity (LT^{-1}), z (L) is the vertical direction, t (T) is time, and r is the retention rate of bacteria ($NL^{-3}T^{-1}$) given by:

$$\frac{\rho_b}{n}\frac{\partial S}{\partial t} = r = k\,\psi\,C \qquad [2]$$

Where ρ_b is the soil bulk density (ML^{-3}), n is the porosity (-), k is the bacterial retention rate coefficient (T^{-1}), ψ is a dimensionless retention function for retained bacteria (-), and S is the solid phase concentration of retained bacteria in the column ($N_c m^{-1}$).

A simple and flexible form for ψ is used in the model to account for time-dependent retention behavior according to the Langmuirian blocking approach (Adamczyk et al., 1994) as:

$$\psi = 1 - \frac{S}{S_{\max}} \qquad [3]$$

Here S_{max} is the maximum solid phase concentration of retained bacteria ($N_c m^{-1}$). When the value of S_{max} is large, then the ψ term approaches a value of 1 and time-dependent retention behavior becomes irrelevant.

Results and Discussion

Bacteria Transport and Retention

The effects of the various cell harvesting methods on the transport and retention of *E. coli* D21 g in porous media was studied by conducting a series of column experiments at several solution ionic strengths (30, 50, and 100 mmol L^{-1}). The transport experiments were repeated twice in each of the considered solution chemistries. Figures 1 to 4 present representative measured and simulated bacteria breakthrough curves (BTC) obtained from the column experiments. Figures 1, 2, 3, and 4 depict the breakthrough data (for one of the duplicate experiments) for cells harvested using high-speed centrifugation (14,000 × *g* for 15 min), 15 min centrifugation (3689 × *g*), 10 min centrifugation (3689 × *g*), and filtration, respectively. In these figures the normalized effluent concentration (C/C_0) is plotted vs. the number of pore volumes passed through the column. After about 1 pore volume, the introduced bacteria break through the column and are detected in the effluent. Subsequently, the normalized effluent concentration gradually increases toward steady-state conditions, except for the IS = 100 mmol L^{-1} experiments. After about four pore volumes, the influent was switched to a bacteria-free solu-

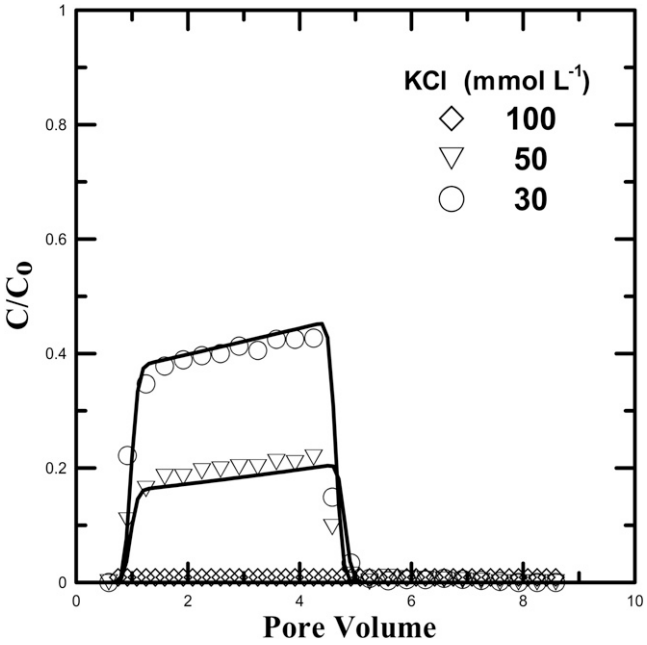

Fig. 1. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mmol L^{-1}) as a function of pore volumes. The cell separation method consisted of applying 15 min of high-speed centrifugation at 14,000 × *g* (repeated three times). Key experimental conditions were as follows: approach velocity, *U* = 0.66 cm/min; porosity, *n* = 0.43; pH = 5.6–5.8; and temperature = 20–22°C.

tion with the same ionic strength for an additional 5 to 6 pore volumes. The breakthrough concentrations decreased after about one pore volume from the end of cell suspension injection.

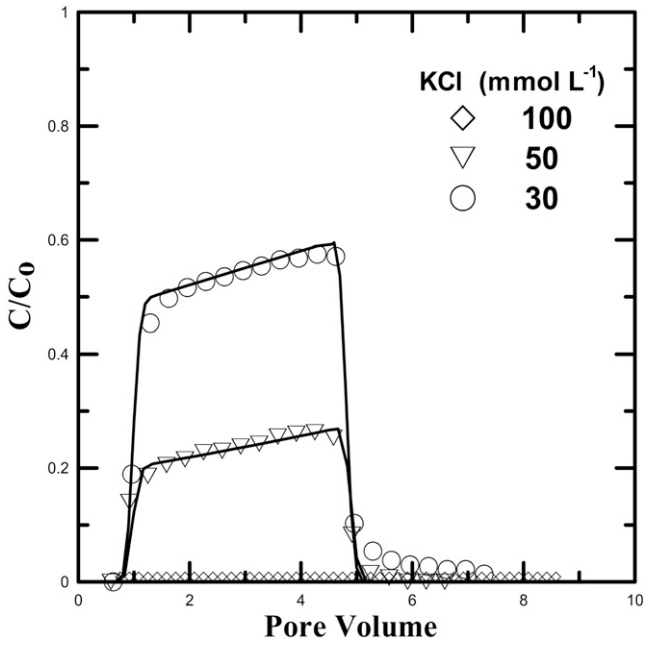

Fig. 2. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mmol L^{-1}) as a function of pore volumes. The cell separation method consisted of applying 15 min centrifugation at 3689 × *g* (repeated three times). Key experimental conditions were as follows: approach velocity, *U* = 0. 66 cm/min; porosity, *n* = 0.43; pH = 5.6–5.8; and temperature = 20–22°C.

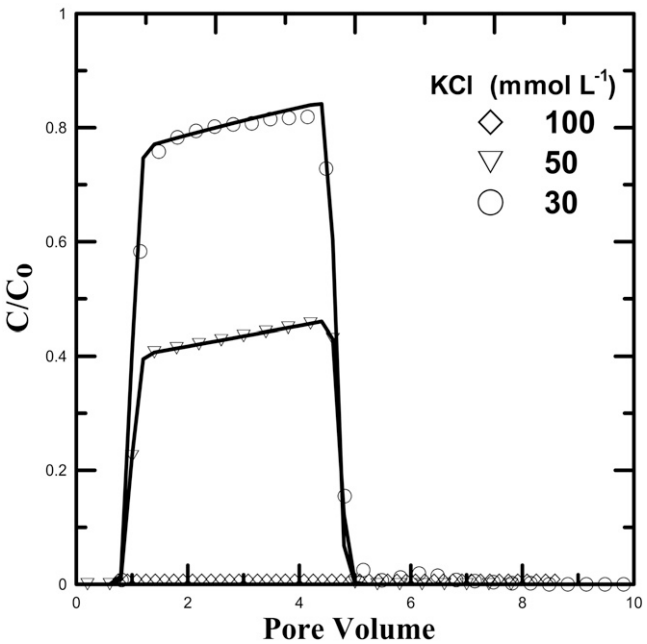

Fig. 3. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mmol L^{-1}) as a function of pore volumes. The cell separation method consisted of applying 10 min centrifugation at 3689 × *g* (repeated three times). Key experimental conditions were as follows: approach velocity, *U* = 0. 66 cm/min; porosity, *n* = 0.43; pH = 5.6–5.8; and temperature = 20–22°C.

No significant BTC tailing was observed in Fig. 1 to 4, which indicates that release or detachment of cells was negligible in these experiments. For a given cell harvesting method,

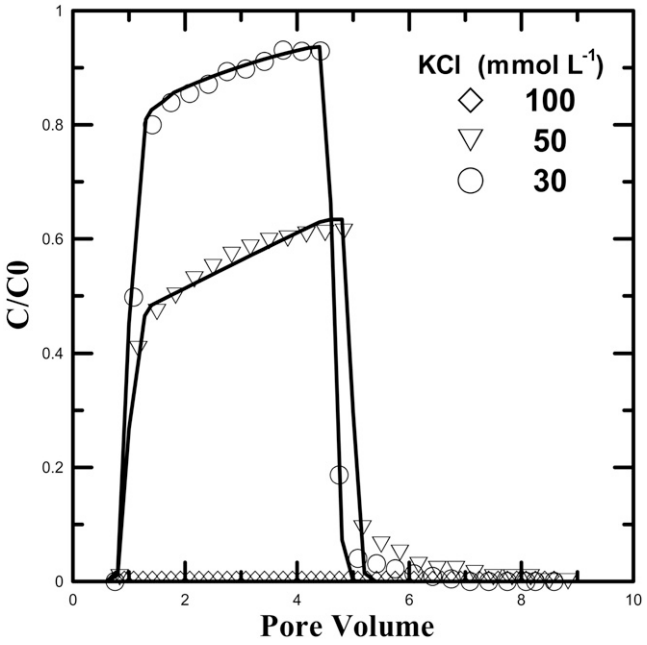

Fig. 4. Representative breakthrough curves (observed and simulated) at three different solution ionic strengths (30, 50, 100 mmol L^{-1}) as a function of pore volumes. The cell separation method consisted of filtration. Key experimental conditions were as follows: approach velocity, *U* = 0. 66 cm/min; porosity, *n* = 0.43; pH = 5.6–5.8; and temperature = 20–22°C.

Table 1. Model parameters and the percentage of bacteria mass that were recovered in the effluent (M_{eff}) that was obtained for different suspension ionic strength (IS) and separation methods. The fitted model parameters were the bacterial retention rate (k) and the normalized maximum solid phase concentration of retained bacteria ($S_{max}^* = S_{max}/N_{ic}$; where N_{ic} is the number of cells in a unit volume of influent suspension).

Cell preparation method	IS	k (min^{-1})	S_{max}^* (g^{-1})	M_{eff}
	mmol L^{-1}			
High-speed	30	0.16 ± 0.08	4 ± 0.2	38.5
		0.19 ± 0.04	3.7 ± 0.5	33.3
	50	0.31 ± 0.05	7 ± 0.9	17.4
		0.42 ± 0.06	7.4 ± 0.7	13.3
	100	1. 0 ± 0.09	–	< 1
		0.95 ± 0.08	–	< 1
15 min	30	0.121 ± 0.07	1.7 ± 0.06	59.6
		0.09 ± 0.02	1.4 ± 0.03	64.7
	50	0.29 ± 0.01	3.9 ± 0.7	27.4
		0.256 ± 0.01	4.1 ± 0.3	23.8
	100	0.904 ± 0.13	–	<1
		0.83 ± 0.002	–	<1
10 min	30	0.04 ± 0.001	0.5 ± 0.02	71.3
		0.07 ± 0.07	0.4 ± 0.06	74.6
	50	0.15 ± 0.02	3.6 ± 0.08	43.7
		0.19 ± 0.08	3.2 ± 0.01	34.9
	100	0.85 ± 0.1	–	<2
		0.9 ± 0.02	–	<2
Filtration	30	0.03 ± 0.01	0.16 ± 0.03	84.3
		0.03 ± 0.01	0.12 ± 0.09	79.6
	50	0.13 ± 0.05	1.1 ± 0.06	59.5
		0.11 ± 0.04	0.9 ± 0.02	65.3
	100	0.83 ± 0.01	–	<2
		0.89 ± 0.05	–	<2

increasing the IS resulted in lower peak effluent concentrations and greater mass retention of the bacteria in the column as expected due to compression of electrostatic double layer (EDL) (Redman et al., 2004).

To quantitatively compare the cell transport behavior the bacteria retention parameters in Eq. [1] and [2] (k and S_{max}) were optimized to the measured BTC data shown in Fig. 1 to 4. The dispersion coefficients for these simulations were obtained by fitting to the tracer (nitrate) data (not shown). Application of the advection–dispersion equation that accounts for first-order kinetic bacteria retention in the column produced a satisfactory fit to all the breakthrough data. The values of linear regression (R^2), which is a measure of the goodness of model fit, were always >0.95. The values of k and S_{max} which are presented in Table 1 can also be used for comparing the effect of each harvesting method and IS on the retention behavior of the bacteria. Table 1 also presents the percentages of bacteria mass that were recovered in the effluent for all the experiments (M_{eff}).

For a given IS, filtered cells always resulted in the highest breakthrough levels than the experiments conducted with centrifuged cells. As a result, filtered cells also showed the lowest values of the retention rate coefficient and the highest values of M_{eff} (Table 1). In the experiments conducted with cells harvested by centrifugation the following trends can be observed at each solution IS. The lowest value of M_{eff} and highest values of k and S_{max} were obtained for the high-speed centrifugation, followed by low speed centrifugation with a duration of 15 min, and then low speed centrifugation with a duration of 10 min. Decreasing the centrifugation speed and duration produced higher effluent concentrations and lower values of k and S_{max}. It should be noted that the influence of cell harvesting method on cell retention behavior was most pronounced at lower ionic strengths.

Electrophoretic Mobility

Table 2 presents the average zeta potentials of cells harvested with the different separation methods at different solution ionic strengths. Regardless of harvesting method the zeta potential of the cells became less negatively charged with increasing in IS due to compression of the electrostatic double-layer (Ryan and Elimelech, 1996). Cells with the least negative zeta potential were those prepared by centrifugation, while the most negative zeta potential was found for filtered cells. Cells became less negatively charged as the speed and duration of centrifugation increased (Table 2). These results are consistent with the trend of cell retention in the column experiments.

The zeta-potential measurements are in agreement with previous studies in which it was demonstrated that centrifugation can alter the surface charge of bacteria. For example, Pembrey et al. (1999) reported that harvesting by high-speed centrifugation (15,000 × g) generally reduced the net surface charge of *E. coli*. This effect was attributed to removal of materials from the cell surface and thus generating a new and very different microenvironment interface. It is hypothesized herein that centrifugation causes some negatively charged macromolecules such as LPS or EPS to be removed from (leave exposed surface) or folded on the cell surface due to the shear forces during centrifugation. The presence of macromolecules like LPS, and trace amounts of EPS on the outer membrane, generate the net negative environment on the cells (Walker et al., 2004). For cells prepared by the filtration method, the cells are more negatively charged presumably because of the presence of a greater amount of intact macromolecules (e.g., LPS).

Hydrophobicity

Escherichia coli has previously been reported to be hydrophilic (Noda and Kanemasa, 1986). However, our results show that cell hydrophobicity is also sensitive to preparation methods. Results for the MATH test, which was used to determine cell surface hydrophobicity, in 10 mmol L^{-1} KCl solution for the various cell separation methods are as follows: 25.9 ± 0.39 for the 15 min high-speed centrifugation at 14,000 × g, 31.6 ± 2.34 for the 15 min centrifugation at 3689 × g, 45.4 ± 1.27 for the 10 min centrifugation at 3689 × g, and 52.87 ± 0.83 for the filtration. Cells harvested by filtration were observed to be more hydrophobic (i.e., greater partitioning into dodecane) than the cells harvested by centrifugation. Cell hydrophobicity also depended on the centrifugation speed and duration, and tended to increase with decreasing speed and duration of centrifugation. Overall, our results show that centrifugation may cause alterations in the hydrophobicity of the cell. This implies that, an increase in the length of time and speed which cells are exposed to centrifugation cause the cell to become more hy-

drophilic, probably due to changes in conformation of loosely bound surface macromolecules.

The roll of hydrophobicity in cell attachment has been recognized in previous research (Noda and Kanemasa, 1986; van Loosdrecht et al., 1987b). It is reported that there is a positive relation between the bacterial hydrophobicity of some strains and their adhesion to negatively charged substrate. Despite this fact, Gilbert et al. (1991) refined the general hypotheses by van Loosdrecht et al. (1987a) and arrived at an important conclusion that for relatively hydrophilic organisms, such as *E. coli*, the hydrophobicity does not have a dominant effect on adhesion relative to the charge properties. Scholl et al. (1990) and Scholl and Harvey (1992) also concluded that for relatively hydrophilic organisms, the major factor controlling the initial adhesion of bacteria is the surface charge of the minerals in the aquifer. Therefore, based on these findings and support from the literature, the role of hydrophobicity on cell retention in the column experiments is likely to be negligible and the observed retention behavior is attributed to the surface charge of the cells and quartz.

Implications of Bacteria Preparation Methods on Transport and Retention

As noted previously *E. coli* is a Gram-negative bacterium with an outer membrane wall containing membrane-bound protein, LPS, and trace amounts of EPS. It is well documented that the net surface charge of *E. coli* in natural aquatic systems is negative (Foppen and Schijven, 2006). The negative charge of *E. coli* D21 g originates from the exposed surface molecules which are primarily LPS (Coughlin et al., 1983; Gmeiner and Schlecht, 1980). The LPS contains two parts. The first part includes a lipid containing three fatty acids and a Glycerol, and the second part or core region contains polysaccharide attached to lipid A by Ketodeoxyoctonate (KDO) (Rietschel et al., 1994). The second part of LPS is responsible for the negative charge of *E. coli* D21 g due to the presence of three additional phosphate groups in the core region (Coughlin et al., 1983; Gmeiner and Schlecht, 1980). These functional groups can easily ionize and provide the net negative charge on the membrane surface under the different solution chemistry conditions (Walker et al., 2005).

Experimental evidence in this study demonstrates that cell preparation protocols have a significant impact on cell surface properties. Our results show that the time and speed of centrifugation cause the cells to become less negatively charged, leading to more retention in the porous media. This conclusion is drawn from the results obtained from column experiments and numerical modeling which indicate an increase in k and S_{max} as the time and speed of centrifugation increased (Table 1). If the separation methods did not have an effect on cell surface charge, the column BTC should have been the same in the experiments conducted under similar solution IS. The zeta-potential measurements also demonstrate the effect of centrifugation on the cell charge characteristic (Table 2).

The impact of centrifugation on the cell surface properties has also been observed by Gilbert et al. (1991) and Pembrey et al. (1999). Gilbert et al. (1991) showed that harvesting cells by centrifugation subjects cells to huge centrifugal forces, which in centrifuge tubes produces high hydrostatic pressures. Moreover, the cells are subject to high shear stresses as they are propelled within the solution during the centrifugation. It is hypothesized herein that when cells are exposed to centrifugal forces, some macromolecules on the cell surface are removed from or folded on the surface due to this stress.

Table 2. Zeta-potential (mV) measurements of *Escherichia coli* D21g at different ionic strength (IS) and separation methods (High-speed, 15 min, 10 min centrifugation, and Filtration).†

IS	Sand	High-Speed	15 min	10 min	Filtration
mmol L^{-1}					
3.16	−30.8	−46.3 ± 1.4	−51.3 ± 1.8	−69.0 ± 0.4	−86.5 ± 0.6
10	−22.2	−41.6 ± 0.5	−45.9 ± 4.1	−57.0 ± 1.6	−66.4 ± 1.5
31.6	−13.6	−29.1 ± 1.6	−35.7 ± 1.7	−49.8 ± 1.8	−51.4 ± 0.2
100	−11.6	−19.1 ± 0.6	−21.0 ± 1.6	−22.0 ± 1.0	−24.4 ± 1.1

† Note: Zeta-potential measurements of sand were estimated from Redman et al. (2004).

It is worth noting that under all solution conditions and preparation methods examined, both the bacterial cells and the quartz grains have a net negative zeta potential. Therefore, repulsive electrostatic interactions should inhibit cell retention on the sand surface. However, a clear trend of increasing retention with increasing IS, time, and speed of centrifugation is observed (Fig. 3). To gain further insight into the mechanism responsible for cell retention, DLVO theory (Derjaguin, 1954; Verwey and Overbeek, 1948) was used to calculate the total interaction energy as a bacterial cell approaches a quartz grain. The total interaction energy, that is, the sum of attractive van der Waals and repulsive electrostatic interactions, was calculated by modeling the bacteria-quartz grain system with a sphere-plate interaction. Repulsive electrostatic double layer interaction energies were determined using the constant surface potential interaction expression of Hogg et al. (1966) with zeta potentials used in place of surface potentials. The retarded van der Waals attractive interaction energy was calculated using the formula given by Gregory (1981). A value of 6.5×10^{-21} J was used for the Hamaker constant (Simoni et al., 2000; Rijnaarts et al., 1995). It should be mentioned that this value for Hamaker constant has been reported by Rijnaarts et al. (1995) for *Pseudomonads* bacteria which are Gram-negative organisms.

As listed in Table 3 for solutions with ionic strength less than or equal to 50 mmol L^{-1}, DLVO calculations predict the presence of a substantial repulsive energy barrier to bacterial retention ranging from 120 k_BT_K (where k_B is the Boltzmann constant and T is the absolute temperature) at 50 mmol L^{-1} for the high-speed centrifugation, to over 400 k_BT_K at 10 mmol L^{-1} for the filtration method. Because surface chemical heterogeneities are probably negligible for the highly pure quartz sand, the huge energy barriers suggest that it is unlikely that the bacterial cells will deposit in the primary energy minimum at the quartz surface. However, at solution ionic strength of 100 mmol L^{-1}, the interaction energy calculations indicate no energy barrier to deposition, suggesting that cells, regardless of harvesting method, may be

Table 3. Total interaction energy parameters (secondary minima, energy barrier, and the separation distance) at different suspension ionic strength (IS) obtained from Derjaguin-Landau-Verwey-Overbeek calculations assuming planar quartz surfaces and spherical bacteria cells.

Cell preparation method	IS	Secondary minima	Energy barrier	Separation distance
	mmol L^{-1}	k_BT_K		nm
High	3.16	−0.203	745	56
	10	−0.845	359	25.5
	31.6	−3.99	67.4	9.5
	100*	NB†	NB	0.5
15 min	3.16	−0.199	798	57
	10	−0.583	385	35.5
	31.6	−3.69	88.6	10
	100	NB	NB	0.5
10 min	3.16	−0.187	902	60
	10	−0.778	430	27
	31.6	−3.29	118.47	11
	100	NB	NB	0.5
Filtration	3.16	−0.178	946	62
	10	−0.749	451.51	27.5
	31.6	−3.25	120	11.5
	100	NB	NB	0.5

† NB: There are no energy barriers and secondary energy minimum in this IS and the DLVO theory predicts the existence of a primary minimum.

deposited in the primary minimum. Calculations of the total interaction energy predict the presence of a secondary energy minimum at a greater separation distance than that of the energy barrier. Therefore, bacteria approaching a quartz grain would first experience an attractive force before encountering the significant repulsive energy barrier. Therefore, cells unable to overcome the energy barrier may remain associated with the quartz grain within the secondary energy minimum unless they had sufficient diffusive or hydrodynamic forces to escape (Hahn and O'Melia, 2004). Table 3 shows that the magnitude of the secondary energy minimum increases with ionic strength and speed and duration of centrifugation. In particular, the depth of the secondary minimum ranges from 0.07 k_BT_K at 10 mmol L^{-1} for the filtration method, to 12 k_BT_K at 50 mmol L^{-1} for high-speed centrifugation (Table 3).

As indicated in Table 3, sizable energy barriers exist to inhibit bacterial retention at most of the examined ionic strengths. However, experimental evidence shows a clear trend of the retention rate increasing with ionic strength and time and speed of centrifugation. The increasing k and S_{max} parallels with the increase in calculated secondary energy minimum depths with increasing ionic strength and time and speed of centrifugation. It is therefore possible that bacterial retention is influenced by the secondary energy minimum. Indeed, the significant contribution of the secondary energy minimum to the retention of colloidal particles in saturated porous media is now established (Tufenkji and Elimelech, 2004, 2005; Redman et al., 2004; Franchi and O'Melia, 2003; Hahn and O'Melia, 2004; Torkzaban et al., 2007). Therefore, it is proposed that as a result of centrifugation bacteria cells become less negatively charged and this results in an increase in the magnitude of the secondary energy minimum interactions. Consequently, centrifuged cells are retained in porous media to a greater extent than those harvested by filtration. However, it is worth noting that other bacteria with different types of surfaces might be affected differently by centrifugation and filtration conditions.

Conclusions

The effects of cell preparation methods on bacteria surface characteristics and transport and fate were studied. Multiple-step centrifugation increased the retention of cells in the sand, presumably due to decreasing the repulsive force between the bacteria and sand and the increase in secondary energy minimum. Cells isolated with high speed centrifugation had the greatest retention in sand than that of the other methods. The enhancement of cell retention that was caused by centrifugation could be attributed to changes in LPS conformation on the cell surface due to shear forces during centrifugation. The filtration method appeared to have no and/or the least impact on changes of cell surface characteristics.

The results of this systematic study illustrate that protocols for the preparation and harvesting of microbial cells, used for experiments or cell analysis, will have a significant impact on surface characteristics and retention behavior. Hence, it is essential that the effects of cell preparation protocols on microbes be ascertained in investigations pertaining to laboratory experiments and that protocols remain consistent to achieve comparable experiments. This may guarantee that cells are subjected to the least disruptive preparation methods and that the results reflect the true nature of the microbial cell surface.

Acknowledgments

This research was supported by the 206 Manure and Byproduct Utilization Project of the USDA-ARS, by a grant from NRI (NRI #:2006-02541), and an interagency agreement with the EPA (IAG # DW-12-92189901-0). Mention of trade names and company names in this manuscript does not imply any endorsement or preferential treatment by the USDA or the EPA.

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