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Release of *E. coli* D21g with Transients in Water Content

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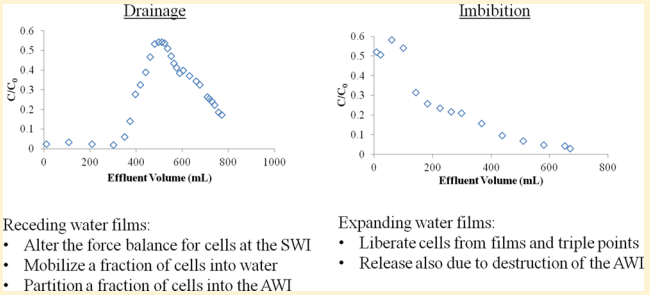
Supporting Information

ABSTRACT: Transients in water content are well-known to mobilize microorganisms that are retained in the vadose zone. However, there is no consensus on the relative importance of drainage and imbibition events on microorganism release. To overcome this limitation, we have systematically studied the release of *Escherichia coli* D21g during cycles of drainage and imbibition under various solution chemistry and initial conditions. Results from these column studies revealed the influence of imbibition and drainage on D21g release. In particular, imbibition efficiently released cells from the air−water interface (AWI) that were initially retained under steady-state unsaturated conditions by expansion of water films and destruction of the AWI. Conversely, significant release and transport of cells during drainage only occurred below a critical water saturation (water film thickness). In this case, a fraction of the cells that were initially retained on the solid−water interface (SWI) partitioned into the mobile aqueous phase and the AWI as the receding water film thickness decreased during drainage. The efficiency of cell release from the SWI during drainage was much less than for the AWI during imbibition. Cycles of drainage and imbibition removed cells from the SWI and the AWI, respectively. However, the peak concentration and amount of cells that were released increased with the number of retained cells and the amount of drainage and imbibition, and decreased with the number of drainage and imbibition cycles. Release of cells during drainage and imbibition was found to be more pronounced in the presence of a weak secondary minimum when the ionic strength (IS) was 5 mM NaCl. Increases in the solution IS decreased the influence of water transients on release, especially during drainage. Complete recovery of the retained cells could be achieved using both IS reduction and cycles of drainage and imbibition, even when the cells were retained under favorable attachment conditions. In general, cell release was more pronounced with transients in water content than transients in IS when the IS ≥ 5 mM.



INTRODUCTION

The vadose zone is an important barrier to protect groundwater from pathogenic microorganisms and other colloids that can pose a threat to the public health.[1] Transients in water content and solution chemistry triggered by infiltration and drainage events, evapotranspiration, and water table fluctuations are very common in the vadose zone. Much evidence exist that transients in water content and solution chemistry may mobilize retained microorganisms and can promote their transport through the vadose zone.[2−15] Consequently, an understanding of the release of microorganisms with transients in water content and solution chemistry is necessary to protect water resources and public health.

Microorganisms may be attached to the solid−water interface (SWI) or strained in small pores spaces that occur near grain−grain contacts and larger-scale surface roughness locations under saturated conditions.[16−27] In addition, microorganisms may also be attached to the air−water interface (AWI) or strained at the air−water solid (AWS) contact line and in thin water films under unsaturated conditions.[28−40] Release of retained microorganisms is typically low under steady water flow, water saturation, and solution chemistry conditions.[11,40] In this case, release is considered to be a slow, diffusion controlled process.[4,40−44] Conversely, transients in water content and solution chemistry can rapidly mobilize retained microorganisms and/or colloids.[2−8,12−15,34,40,45−49]

Transients in water content can promote the release of retained microorganisms by several incompletely understood mechanisms.[10,11,13,40,48−55] The AWI increases and the water film thickness decreases with a decrease in the water saturation during drainage.[33,56−58] Microbes on the SWI may partition into the AWI when the water film thickness is smaller than the microbe radius.[9,33,56] A moving AWI can potentially liberate microbes from the SWI during drainage.[50,59] The AWI is destroyed, water films expand, and the pore water velocity increases during water imbibition.[34,40] Microbes associated with the AWI, AWS contact line, and in thin water films may be released by these processes during imbibition.[10,11,13,34,40,49,50,55] Larger microbes are expected to be more efficiently released by drainage and imbibition than smaller microbes.[52,60,61]

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There presently is no consensus on the relative importance of drainage and imbibition events on colloid or microorganism release.[11,13,50,51,62] Some studies have attributed release only to imbibition,[49] whereas others have found that release occurred during both drainage and imbibition.[11,13,40,50] In the latter case, the amount of release is sometimes observed to be more pronounced during drainage[11] and other times imbibition.[13] These conflicting results suggest a strong sensitivity of microorganism release to the initial conditions and saturation dynamics that has not yet been fully resolved. For example, the accessibility of microorganisms retained on the SWI to the AWI is expected to increase with decreasing water saturation.[33] Hence, the amount of release during imbibition may therefore depend on how much of the pore space was previously drained. If retained microbes on the SWI are preferentially found in the smallest regions of the pore space, then little interaction or release of microbes with the AWI is expected at higher water saturations. As noted in the preceding paragraph, release of microbes retained on the AWI, AWS contact line, and in thin water films under unsaturated conditions is expected to be very different from microbes attached or strained on the SWI. The efficiency of release from the SWI and AWI is therefore expected to be very different, but still is not qualified.

Several researchers have reported that release during drainage and imbibition is sensitive to the chemistry of the aqueous and solid phases,[4,11,13,55,59,62−64] as well as the surface properties of the colloids.[64,65] These chemical properties influence the strength of the adhesive interaction between the microbe and the SWI and/or AWI. Enhanced microbe retention and release is expected when the adhesive interaction is stronger and weaker, respectively. For example, larger amounts of colloid release have been observed during transient water content conditions when the solution ionic strength (IS) and the magnitude of the secondary minimum were lower.[11,55,59,62−64] However, the combined influence of initial conditions (e.g., amount of retained microbes) and interaction strength in determining release during drainage and imbibition has not yet been determined. In addition, chemical perturbations, such as a reduction in IS, can induce release from the SWI and AWI by shifting the net interaction forces from attractive to repulsive.[3,4,8,15,66] The relative importance of transients in water content and solution chemistry on microbe release still has not been quantified.

The overall objective of this research is to improve our understanding of microbial release during transient water content conditions. In particular, we systematically study the release of *Escherichia coli* D21g from the AWI during imbibition, from the SWI during drainage, and from the SWI and AWI during cycles of drainage and imbibition under various solution chemistry conditions. Other experiments were conducted to investigate the efficiency of cell release from the SWI and AWI for various amounts of drainage and imbibition, and the combined influence of transients in saturation and solution IS on release. Results demonstrate that consideration of saturation dynamics, initial conditions, and release efficiency can explain many of the reported discrepancies in colloid release behavior during drainage and imbibition in the literature.

MATERIALS AND METHODS

Porous Media and Electrolyte Solutions. Ottawa (quartz) sand with a median grain size (d_{50}) of 120 μm was used in the column experiments. In order to eliminate any background interference from clay particles, the sand was treated by a salt cleaning procedure described by Bradford and Kim.[14] Electrolyte solutions for the column experiments consisted of autoclaved deionized (DI) water (pH 5.8) with the IS adjusted to 0, 1, 2.5, 5, 10, 20, and 100 mM using NaCl to create a range of adhesive conditions between the bacteria and sand.

***Escherichia coli* D21g.** *Escherichia coli* D21g was employed in the transport experiments discussed below. It is a Gram-negative, nonmotile bacterial strain that produces minimal amounts of lipopolysaccharides and extra-cellular polymeric substances.[67] The surface properties of D21g have been well characterized in terms of surface charge, acidity, and hydrophobicity.[68,69] We selected D21g for our studies because it has relatively simple surface properties that helped us to isolate the influence of water transients on release.

The culture and harvest procedures used in this study have been described by Wang et al.[69] In brief, D21g was cultured overnight (12−18 h) at 37 °C in Luria−Bertani broth (LB Broth, Fisher Scientific, Fair Lawn, NJ) containing 30 mg/L gentamycin (Sigma, St. Louis, MO), transferred onto a LB media plate containing 30 mg/L gentamycin, and the plates were cultured overnight (12−18h) at 37 °C. The colonies were harvested into sterile water, and then the bacteria suspension was centrifuged and resuspended two times to remove all traces of the growth medium. A fresh cell suspension at the desired electrolyte solution was prepared right before the start of each experiment. The concentrations of D21g in influent ($C_o \approx 10^8$ cells mL^{-1}) and effluent samples were determined using a linear correlation between spectrophotometer (Unico UV-200, United Products & Instruments, Dayton, NJ) readings at 600 nm and known cell concentration. The detection limit was approximately 1% of C_o when using the spectrophotometer, whereas the standard deviation of the conventional spread plating method was 14.3% of C_o.[69]

The Supporting Information (SI) contains details pertaining to the calculation of interaction energies between D21g and the SWI for the various IS conditions (SI S1). Results from these calculations are summarized in SI Table S1. Information is also presented in SI S1 about the expected interaction of D21g with the AWI.

Column Experiments. An illustration of the experimental setup that was used in the transport and release experiments is given in SI Figure S1. Column experiments were conducted in a plexiglass (acrylic) column that was 22 cm long and had an inside diameter of 13.2 cm. *E. coli* D21g suspensions or eluting solution, which were mixed continuously with a magnetic stirrer, were delivered to the top of the column at a steady flow rate using a rain simulator connected to a peristaltic pump (Barnant Company, Barrington, Illinois). The capillary pressure head in the column was measured with three miniature stainless steel tensiometers (Chemiquip Products Co.,West New York, NJ) inserted at 5, 10, and 15 cm from the upper sand surface. Data from the tensiometers were registered using a CR7 datalogger (Campbell Scientific, Logan, UT). Stainless steel tubes were located on the opposite side of the column to the tensiometers to facilitate air entry and exit. These tubes were capped during saturated conditions. A polyester membrane (Saatifil PES 18/13) with an 18 μm nominal pore size was placed at the bottom of the column and connected to a hanging water column (tube) to control the bottom boundary pressure. The membrane has a bubbling pressure head of about 130 cm-H_2O. Neither the column body nor the nylon membrane

retained the cells.[69] Effluent samples were collected from the hanging water column using a fraction collector and monitored for *E. coli* D21g concentrations using a spectrophotometer as described above. An electronic balance (Sartorius Master Series, LP Models, Germany) was used to determine the total water balance in the column over time. The average water saturation was calculated from the initial balance reading under saturated conditions and the change in water storage.

Ottawa sand was wet packed into the column to a height of 20 cm. The porosity of the column was around 0.34, and the pore volume was around 930 mL under saturated conditions. Around 2 pore volumes of a selected NaCl solution was flushed through the column to achieve steady state flow (saturated/unsaturated), and to allow the sand to equilibrate with the solution. Saturated and unsaturated transport experiments were initiated by injecting about two pore volumes of cell suspension (Phase 1) and then eluting NaCl solution with the same IS (Phase 2) to the column top at a steady-state flow rate (SI Table S2). The column was flushed with eluting NaCl solution until no significant concentration of bacteria was detected. Saturated conditions were maintained in the column by keeping a fine layer of ponding at the top boundary and 0 pressure head at the bottom boundary. Unsaturated, steady-state water flow conditions at selected water saturations were achieved by reducing the inflow rate of water at the column top and by simultaneously increasing the suction at the bottom of the column. The latter was done by lowering the hanging water column until the readings of the tip tensiometers showed the same values, that is, a unit hydraulic head gradient was achieved.

Various drainage and/or imbibition conditions were implemented in the column (Phase 3) to study the release behavior of *E. coli* D21g (SI Table S2). Drainage was conducted with no flow at the top boundary while maintaining a negative pressure at the bottom. By adjusting the height of the hanging water column, the sand column was drained to different levels. Imbibition was initiated by increasing the flow rate at the top of the column and/or by elevating the bottom boundary pressure. The imbibition process was considered finished when steady-state flow was reached and the concentration in the effluent went down back to background levels.

Additional details about the column packing (SI S2), replication of transport and release experiments (SI S3, Table S2, and Figure S2−S3), and a summary of experimental conditions and mass balance information (Table S2) are given in the SI.

RESULTS AND DISCUSSION

Cell Release From the AWI. Figure 1 shows breakthrough and release curves for D21g as a function of effluent volume when the solution IS = 5 mM NaCl. Breakthrough curves (BTCs) are shown for both saturated (S_w = 1) and unsaturated (S_w = 0.64) conditions. Enhanced retention was observed when retention occurred under unsaturated (80.7%) than saturated (58%) conditions (SI Table S2). This finding is consistent with the reported literature and has primarily been attributed to attachment to the AWI, film straining, or retention at the air−water−solid triple point.[28−39,64] Cell release from the unsaturated sand was initiated during phase 3 using two imbibition steps that are shown in the figure. Step increases in water saturation each produced a cell release pulse with similar peak concentrations that were equal to the maximum value of the unsaturated BTC. Cell release during imbibition has previously been attributed to destruction of the AWI and expansion of water films which liberate cells that were retained at the AWI, triple point, or in thin water films. Interestingly, the amount of cell release during the two imbibition steps (21.9%) was similar to the enhancement of cell retention caused by unsaturated (in comparison with saturated) conditions during phases 1 and 2 (22.7%). This suggests that the extra cell retention under unsaturated conditions can be released by imbibition, and that this cell release was very efficient.

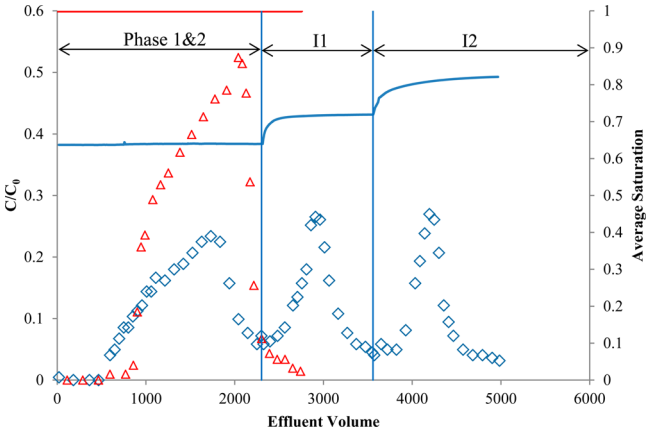

Figure 1. An example of cell release from the AWI during imbibition when the IS = 5 mM NaCl (Phases 1, 2, and 3). Initial deposition occurred under unsaturated conditions (around 0.64 water saturation), and then the sand was saturated using two imbibition sequences (denoted by I1 and I2). Also included in the figure is a breakthrough curve for D21g under saturated conditions. See SI Table S2 for experimental details and mass balance information. Legends: blue diamond as breakthrough and release concentrations for unsaturated conditions, red triangle as breakthrough concentration for saturated conditions, blue line as average saturation for unsaturated conditions, red line as average saturation for saturated conditions.

Cell Release From the SWI During Drainage. Figure 2 presents an example of D21g release from the SWI during drainage when the IS=5 mM NaCl. In this case, phases 1 and 2 occurred under saturated conditions, and then the sand was drained during phase 3 by lowering the bottom boundary condition to −120 cm. No cell release was observed when the

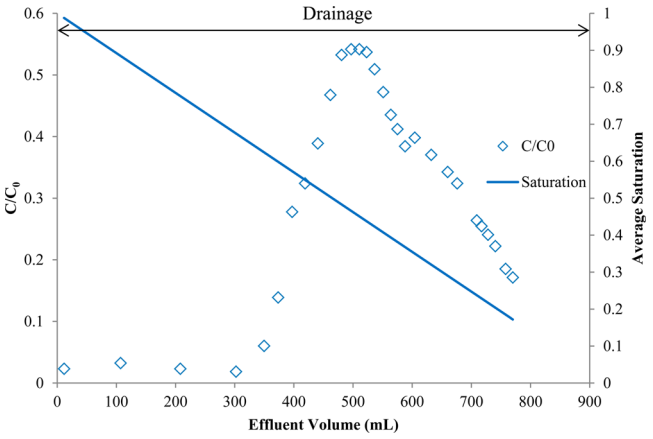

Figure 2. An example of D21g release from the SWI during drainage when the IS = 5 mM NaCl (Phases 1, 2, and 3). Phases 1 and 2 were conducted under saturated conditions, and then the sand was drained during phase 3 by lowering the bottom boundary condition. See SI Table S2 for experimental details and mass balance information.

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water saturation was greater than 0.6. Conversely, significant amounts of cell release occurred when the water saturation was less than 0.6. This observation suggests that release during drainage was triggered when a critical saturation was reached. This behavior was further confirmed by replicate information in the SI (Table S2 and Figure S3). The critical saturation concept may help explain the divergence of findings in the literature on the reported effects of drainage on colloid release.[11,13,51] It should be noted that a critical water saturation is likely to be media specific, but it is theoretically related to capillary pressure and/or water film thickness terms that are more general.[34,51] However, it is possible that the critical water saturation will also depend on the solution chemistry and properties of the microbe, and additional studies are needed to fully resolve this issue.

In addition, a drainage release pulse was observed in Figure 2 that initially increases and then decreases with increasing amounts of drained water. The above observations are not consistent with a constant diffusion controlled release process, which predicts that the effluent concentration would continuously increase with decreasing water saturation because of the smaller water volume and lower flow rates. Rather, the results suggest that drainage below a critical saturation level (or film thickness) can remove a fraction of the cells from the SWI, and transport these cells with the aqueous phase. Microbes on the SWI experience different forces and torques as the water film thickness decreases during drainage.[70,71] For example, microbes will be subjected to strong capillary forces and can be directly incorporated into the AWI when the water film thickness is smaller than the microbe radius.[9,34,50] Eventually, the microbes will begin to experience interaction energies arising from both the SWI and AWI, which will alter the force and torque balance at a particular location, and may mobilize some of the microbes from the SWI to the aqueous phase and/or cause a fraction of the cells to partition into the AWI. The rate of transport of a released cell pulse during drainage will be very slow because the water velocity rapidly decreases with decreasing water content.[72]

Cell Release by Drainage and Imbibition. Figure 3 presents examples of D21g release during a drainage and imbibition cycle for different amounts of initial cell retention when the IS = 5 mM NaCl. Short and long input pulse durations (1.5 and 3.1 PVs) were employed during phase 1 to obtain different initial conditions. Phases 1 and 2 were again conducted under saturated conditions, and then the sand was drained and imbibed during phase 3 by adjusting the boundary conditions at the bottom (constant −90 cm) and top (water flux was changed from 0 to 23.5 mL min^{-1}) of the column. All of the retained cells were initially on the SWI. Release of cells from the SWI was initiated by water drainage. As noted in the section above, this occurred due to the presence of thin receding water films that partition some of the cells from the SWI to the aqueous phase and/or the AWI. The relative importance of the drainage and imbibition release pulses depended on the initial conditions during phases 1 and 2. In particular, the release pulse became more pronounced with larger input pulse durations because greater amounts of cells were initially retained on the SWI. Cells that are directly associated with the AWI will be released during imbibition when the AWI is destroyed (Figure 1). Imbibition will also produce an expansion of water films (and flow rates) that will enhance the mobility of cells that were released from the SWI during drainage. Hence, imbibition may complete the release

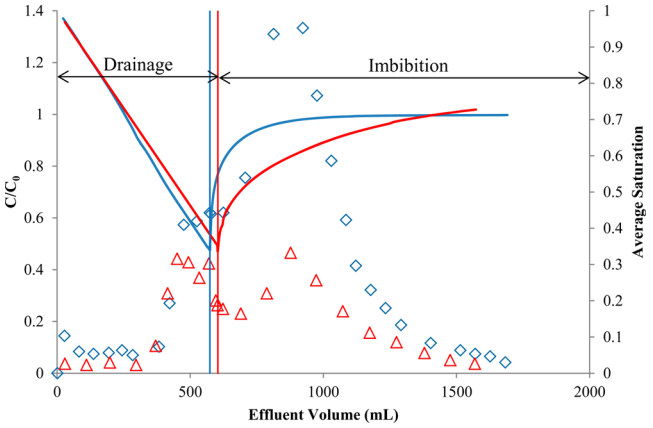

Figure 3. An example of D21g release during a drainage and imbibition cycle for different initial conditions when the IS = 5 mM NaCl (Phases 1, 2, and 3). Short and long input pulse durations (1.5 and 3.1 PVs) were employed during phase 1 to obtain different initial conditions. Phases 1 and 2 were conducted under saturated conditions, and then the sand was drainage and imbibed during phase 3 by adjusting the boundary conditions at the bottom and top of the column. See SI Table S2 for experimental details and mass balance information. Legends: blue diamond as release concentration for long deposition, red triangle as release concentration for short deposition, blue line as average saturation for long deposition, red line as average saturation for short deposition.

pulse initiated during drainage. Higher release pulses during imbibition than drainage reflect differences in the efficiency of release from the SWI and AWI that will be discussed below.

Figure 4 presents an example of D21g release with repeated cycles of similar amounts of water drainage and imbibition

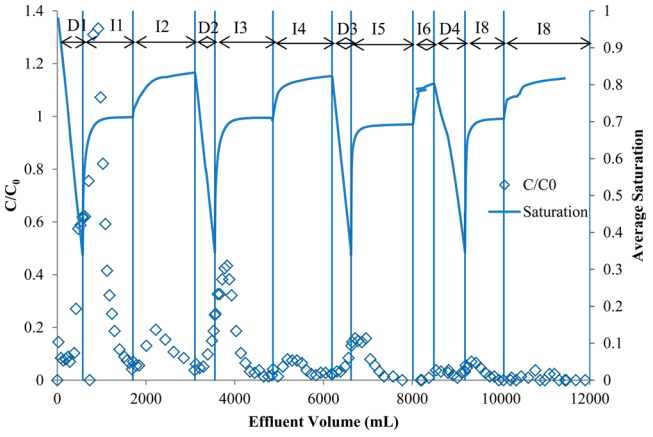

Figure 4. An example of D21g release with repeated cycles of similar amounts of water drainage and imbibition when the solution IS = 5 mM NaCl (Phases 1, 2, and 3). Phases 1 and 2 were conducted under saturated conditions, and then the sand was repeatedly drained and imbibed during phase 3 by adjusting the boundary conditions at the bottom and top of the column. D# and I# denote the drainage and imbibition number (#), respectively. See SI Table S2 for experimental details and mass balance information.

when the solution IS=5 mM NaCl. Phases 1 and 2 were conducted under saturated conditions, and then the sand was repeatedly drained and imbibed during phase 3 by adjusting the boundary conditions at the bottom (−90 cm) and top (water flux was changed from 0 to 23.5, and then 41.2 mL min^{-1} during each cycle) of the column. In contrast to Figure 3, two

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imbibition steps were used to release the retained cells. Each imbibition step produced a corresponding pulse of released cells, but the second pulse was much smaller than the first. The first and second imbibition steps both released cells by destruction of the AWI and water film expansion. However, cell release is expected to be more pronounced at lower water saturations because the rate of change in the air–water interfacial area is greater[56] and small amounts of water film expansion may be sufficient to release cells that were pinned by the AWI.[34] The total amount of colloid release has been reported to increase with the amount of imbibition.[48]

It is interesting to note that one drainage and imbibition cycle in Figure 4 did not remove all of the retained cells. Each drainage and imbibition cycle released additional cells. However, the peak concentration and the amount of released cells diminished as the number of drainage and imbibition cycles increased. Similar to information presented in Figure 3, this observation suggests that the efficiency of cell release from the SWI is low in comparison to the AWI (Figure 1). This inefficiency is likely due to spatial variability in the adhesive force and water film thickness. In particular, the adhesive force can vary as a result of chemical heterogeneity, surface roughness, and differences in the strength of primary and secondary minimum interactions.[73] The water film thickness will also vary with the water saturation, the surface roughness, the pore-scale geometry, and the solid phase wettability.[33,74]

Figures 3 and 4 demonstrate that differences in initial conditions and release efficiencies from solid–water and air–water interfaces can help explain the wide variety of reported transient release behavior under unsaturated conditions.[11,13,34,40,48,51,59]

Cell Release with Different Amounts of Drainage. Figure 5 presents an example of D21g release during three continuous drainage and imbibition cycles when the column was successively drained to lower water saturations of 0.57, 0.36, and 0.27 by lowering the bottom boundary pressure. Similar peak concentrations and release amount were observed for each drainage and imbibition cycle. In contrast, decreasing amounts of cell release were observed for each drainage and imbibition cycle when draining to the same water saturation (Figure 4). Apparently the amount of cell release was a function of the lowest water saturation during drainage, with drainage to lower water saturations promoting greater amounts of cell release from the SWI. The water film thickness decreases and the air–water interfacial area increases as the water saturation decreases.[33,56−58] Both of these factors suggest that greater amounts of cells will be incorporated into the AWI at lower water saturations during drainage. Imbibition will subsequently release these cells to the water phase because of destruction of the AWI and expansion of water films.

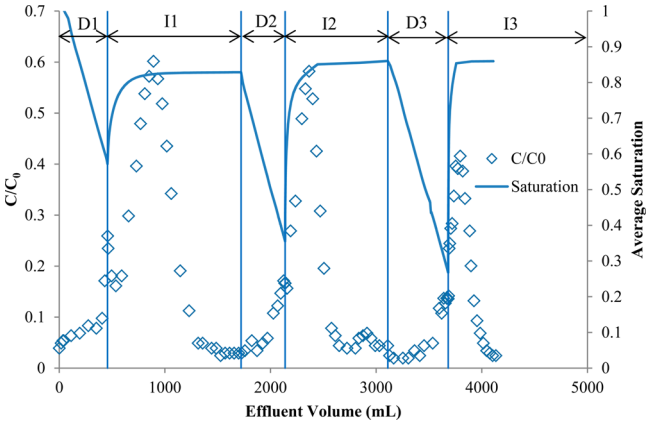

Figure 5. Examples of D21g release (IS = 5 mM during phases 1, 2, and 3) during three continuous drainage and imbibition cycles when the column was successively drained to lower water saturations of 0.57, 0.36, and 0.27 by lowering the bottom boundary pressure. D# and I# denote the drainage and imbibition number (#), respectively. See SI Table S2 for experimental details and mass balance information.

Cell Release For Different IS Conditions. Cell release with drainage and imbibition was discussed above for a solution IS of 5 mM NaCl. Solution IS effects the height of the energy barrier and the depth of the secondary minimum between D21g and the SWI. In particular, the energy barrier decreases and the depth of the secondary minimum increases for an increase in solution IS (SI Table S1). It is therefore not surprising that the solution IS has also been reported to influence the amount of colloid release from the SWI during drainage and imbibition.[4,11,59,63,64] Repulsive electrostatic and van der Waals interactions are expected between D21g and the AWI for the various IS conditions (SI S1). Conversely, microbes that partition into the AWI are subjected to strong capillary forces.[9,34,50] Additional studies were conducted to better understand the coupled effects of IS on cell release from the SWI during drainage and imbibition.

Figure 6 presents examples of D21g release during drainage and imbibition when the solution IS = 100, 10, 5, and 2.5 mM NaCl during phases 1, 2, and 3. Phases 1 and 2 were conducted under saturated conditions, and during phase 3 the sand was first drained by lowering the bottom boundary condition to −90 cm, and then imbibed by changing the top flux from 0 to 23.5 mL min^{-1}. The amount of cell retention during phases 1 and 2 increased with the solution IS (99%, 97%, 62%, and 46% when the IS equals 100, 10, 5, and 2.5 mM NaCl, respectively) because of its effect on the interaction energy (SI Table S1). Conversely, the amount of cell release with drainage and imbibition did not simply increase with a decrease in IS. Rather cell release during drainage and imbibition depended on both the initial amount of cell retention during phases 1 and 2, and the solution IS. When the IS = 100 mM, no significant release was observed for either drainage or imbibition due to the strong secondary minimum interaction between the cells and the sand surface (SI Table S1). At an IS = 10 mM NaCl, cell release was not observed during drainage, but a small release pulse was seen during imbibition. Conversely, significant amounts of cell release were observed with both drainage and imbibition when the IS = 2.5 and 5 mM NaCl. This observation indicates that drainage and imbibition is effective at releasing cells from the SWI when they are interacting in a shallow secondary minimum.[11,55,59,62−64] The largest amount of cell release occurred when the IS = 5 mM NaCl because greater amounts of cell retention initially occurred in 5 than 2.5 mM NaCl during phases 1 and 2. Similar to Figure 3, greater amounts of cell release tended to occur during imbibition than drainage. Differences in the arrival time of the release pulses during imbibition likely reflect the influence of the initial distribution of retained cells and/or the strength of interaction with the SWI (SI Table S1).

Figure 7 present an example of D21g release with transients in solution IS and water content. Phases 1 and 2 were conducted in 100 mM NaCl. Phase 3 was conducted by successively lowering the solution IS from 100, to 20, to 5, to 0

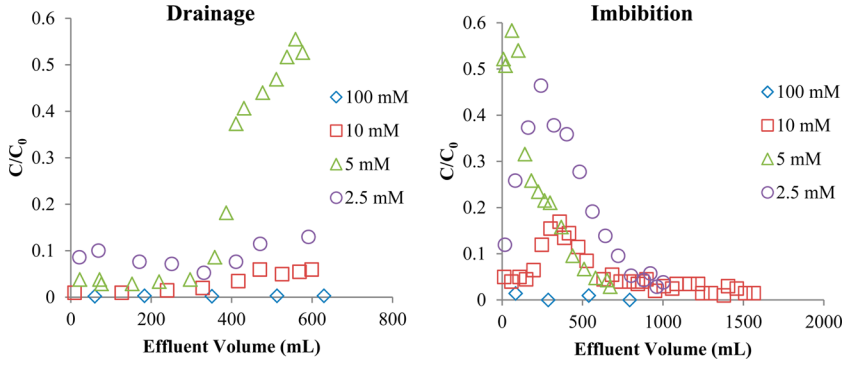

Figure 6. An example of D21g release during drainage (left) and imbibition (right) when the solution IS = 100, 10, 5, and 2.5 mM NaCl during phases 1, 2, and 3. Phases 1 and 2 were conducted under saturated conditions, and then the sand was drained during phase 3 by lowering the bottom boundary condition to −90 cm. The water flux at the top of the column was 20.5 to 24.3 mL min^{-1} for imbibition events, and the final saturation ranged from 0.70 to 0.75. See SI Table S2 for experimental details and mass balance information.

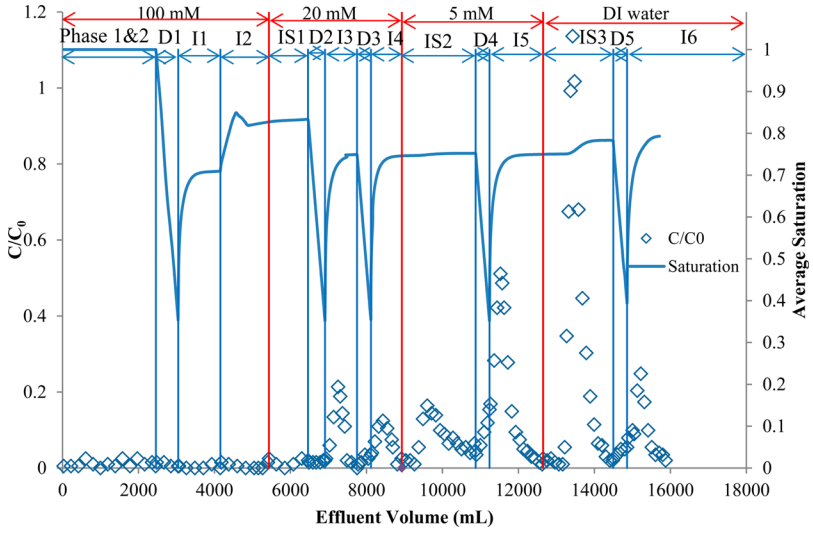

Figure 7. An example of D21g release with transients in solution IS and water saturation. Phases 1and 2 were conducted in 100 mM NaCl. Phase 3 was conducted by successively lowering the solution IS from 100, to 20, to 5, to 0 mM NaCl and initiating drainage and imbibition cycle(s) as indicated in the figure. D#, I#, and IS# denote the drainage, imbibition, and IS number (#), respectively. See SI Table S2 for experimental details and mass balance information.

mM NaCl and initiating drainage and imbibition cycle(s) at each IS as indicated in the figure. Cell release was observed directly after switch to IS = 5 and 0 mM due to reduction and/or elimination of the secondary minimum (SI Table S1). Release during drainage and imbibition for each step IS level was very similar to that shown in Figure 6. In general, cell release by drainage and imbibition was greater than by a reduction in solution IS when the IS ≥ 5 mM. After switching to DI water, an additional 10% of the retained cell mass could be recovered by drainage and imbibition. The total recovered cell mass balance was close to 100%. Collectively, the results indicate that step reductions in solution IS and/or multiple drainage and imbibition cycles can mobilize all of the retained cells, even when the initial conditions produced a deep secondary minimum that was favorable for cell retention (SI Tables S1 and S2).

Environmental Implications. Most microbial transport studies have been conducted under steady-state water content and solution chemistry conditions. Results typically suggest limited mobility of micoorganisms in the subsurface because of retention to the SWI and/or AWI, especially under unsaturated conditions. Consequently, the vadose zone is commonly viewed as an effective barrier to minimize the risks of microbial contamination of groundwater. However, this belief does not consider the confounding effects of transients in water content and solution chemistry that are ubiquitous in the vadose zone as a result of infiltration, drainage, evapotranspiration, water table fluctuations, and/or changes in surface water levels. Data presented in this manuscript demonstrate that D21g transport and release will be highly sensitive to transient conditions. In particular, drainage and imbibition may both mobilize large numbers of D21g, especially at low solution IS. The amount of release strongly depends on the initial conditions (number and location of retained cells, the solution chemistry, and the distribution of cells on the SWI and/or AWI) and the saturation dynamics (the initial water saturation when the cells were retained, and the amount of drainage and imbibition). Release of cells from the AWI was much more efficient than from SWI. Consequently, greater amounts of microbe release and transport are expected during water imbibition when microbes are added to the soil under unsaturated conditions. In addition, drainage of soil to lower water contents will facilitate the release of more microbes from the SWI during imbibition. Both of these factors will increase the risks of microorganism

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transport through the vadose zone to contaminate groundwater resources. Additional studies and model development are needed to extend the findings of this study to other microorganisms, soil types, and transient conditions that occur in natural environments.

■ ASSOCIATED CONTENT

Supporting Information

This Supporting Information section contains details pertaining to (i) calculation of interaction energies between D21g and the SWI, and D21g and the AWI for the various IS conditions (S1); (ii) a summary of interaction energy results for D21g and the SWI (Table S1); (iii) column packing (S2) and an illustration of the experimental setup (Figure S1); (iv) replication of transport and release experiments (S3 and Figures S2, S3); and (v) a summary of experimental conditions and mass balance information (Table S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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