Characterization of transport and retention of biocolloids in unsaturated soils


Cornell University, Department of Biological & Environmental Engineering, Riley-Robb Hall, Ithaca, NY 14853, USA. Email: tss1@cornell.edu

*Department of Earth & Planetary Sciences, University of Tennessee, Knoxville, TN 37996, USA

Abstract Unsaturated soils are considered excellent filters for preventing the transport of pathogenic biocolloids to groundwater, but little is known about the actual mechanisms of biocolloid retention. To obtain a better understanding of these processes, a number of visualization experiments were performed, which are briefly described here.

Keywords colloid transport, colloid retention, microbial transport, microbial retention, partially-saturated soil, unsaturated porous media, pathogens.

Pathogenic microbes including bacteria, viruses and protozoa have been implicated in waterborne disease outbreaks (Barwick et al., 2000; Macler and Merkle, 2000). Almost all studies of pathogen mobility in soils have focused on the transport and retention of bacteria in saturated soils. However, pathogens generally enter the soil environment from land-applied wastes or septic systems at or near the soil surface, where the soil is typically unsaturated (i.e. only partially saturated with water). Unsaturated systems are much more complex and more poorly understood than water-saturated groundwater systems with respect to contaminant flow and retention (McCarthy and McKay, 2004). In addition to the retention that occurs in saturated soil, bacteria may interact with two additional classes of interfaces – air-water-solid (AWS) and air-water (AW) – in unsaturated soils. The area of these interfaces is highly variable depending on changes in soil moisture, and is thus highly impacted by transient wetting and drainage events such as storms or snowmelt.

To characterize transport, retention and remobilization of biocolloids in unsaturated soil, we used small flow cells packed with translucent silica sand (cleaned by combustion and rinsing) imaged using a confocal scanning laser microscope. A syringe inlet pump and peristaltic outlet pump control the chamber moisture content and flow rate. Hydrophobic and/or hydrophilic fluorescent synthetic microsphere surrogates with sizes and properties similar to either pathogenic bacteria or Cryptosporidium parvum oocysts are injected into the flow cell as dilute suspensions. Bright field microscopy was used for detection of dyed blue or red colloids in backlighted chambers (Figures 1,2). Fluorescent colloids are detected with a Leica TCS SP2 confocal scanning laser microscope (10x 0.40 UV objective) which simultaneously records three different spectral channels: 1) fluorescent microsphere emissions (500 to 540 nm) excited at 488 nm by an argon laser; 2) the water phase emissions (555 to 650 nm) due to Rhodamine B stain excited at 543 nm by a green HeNe laser; and 3) transmitted visible light to show the location of the sand grains.

Imaging with confocal laser and bright field microscopes indicated that hydrophilic colloid retention occurred primarily at the air/water meniscus/solid (AW₃S) interface, as we termed it in Zevi et al. (2005), denoting the region where between-grain water menisci diminish to a thin water film on the grain surface in connected pores where most of the water flows (Figure 1). Conversely, isolated unconnected pendular rings did not contain microspheres, as also can be seen in Figure 1. Zevi et al. (2005) showed that microspheres were retained at the AW₃S interface

Figure 1: Isolated menisci (or pendular ring of water) between two sand grains associated with static air-water interfaces without microspheres. Blue hydrophilic microspheres can be found in pores connected with each other through which water is flowing and are retained mainly the air water solid interface.
where the water film thickness approximately equaled the microsphere diameter. The greater retention efficiency for hydrophilic microspheres at this interface (where a greater portion of the microspheres were retained compared to the remainder of the solid/water interface) can be explained by the additional surface tension capillary potentials exerted on microspheres protruding from the water film at the interface. We also observed that hydrophilic microspheres readily attached to other microspheres already present at the AWmS interface, as can be seen in the “bridge” of colloids starting at the AWmS interfaces (Figure 2) observed by Crist et al. (2005). In the experiments of Zevi et al. (2005) we also found that the mechanisms for hydrophobic microsphere retention differed slightly. Microsphere distribution in flowing water played an important role in determining contact efficiency, with many more hydrophobic microspheres found near the water-solid (WS) interface. Hydrophobic microspheres were retained not only at the AWmS interface but also at WS and AW interfaces, as hydrophobicity impelled the microspheres to avoid water. The greater contact efficiency of hydrophobic microspheres explains the greater retention of hydrophobic colloids observed in the literature. Another major factor controlling hydrophobic microsphere retention efficiencies was physical imperfections (surface roughness and irregularities) of the sand grains. While some 0.8 μm microspheres were observed being retained in thin water films, film straining played no significant role in the retention of larger microspheres.

The attachment mechanisms occurring at the AWmS are not limited to unsaturated porous media but can be seen in evaporating water drops as well. An example is shown in Figure 3 where blue microspheres were pinned in successive rings at the AWmS interface at the edge of a water droplet as it evaporated.

We used the same experimental apparatus as described above and added a range of green fluorescent protein (gfp)-expressing *E. coli* PHL 628 GFP-1 bacteria to test whether our visualization techniques could be applied to the retention of bacteria. Not only was this successful, but an initial set of experiments went on to determine the effects of various exocellular bacterial surface structures (flagella and curli) on the bacterial sticking coefficient (alpha) in the flow chamber. Unaltered bacteria (Figure 4a) were compared to those with fewer structures that encourage attachment: a sheared bacteria that experienced the loss of curli via vortexing (Figure 4b), and a flc mutant that had neither flagella nor curli (Figure 4c). Observations in Figure 5a show that the extent of exocellular structures differed substantially as a result of these treatments.

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Figure 2. Coagulated hydrophilic microspheres formed “bridges” between sand grains.

Figure 3. Blue microspheres left in rings by evaporating water drop.

Figure 4. Transmission electron microscope images of *E. coli* PHL 628 GFP-1 bacteria made with negative staining: a) untreated, with thin curli visible above and below bacteria; b) sheared to remove curli, with flagella still visible; and c) flc mutant that expresses neither curli nor flagella.
Although additional tests will be needed to confirm findings, the trend in Figure 5b indicated that while unaltered bacteria had retention characteristics similar to the microsphere surrogates, the altered bacteria were less likely to be retained.

Implicit in Figure 5 is the fact that our confocal microscope configuration makes it possible to quantify fluorescent microsphere and biocolloid retention. To do so, a sequence of confocal microscope images of acquired from the same location is saved as a stack file. Figure 6a shows a reconstructed confocal laser microscope image with false coloring of fluorescent colloids (yellow), dyed water (red) and the sand grains in grayscale. Image analysis techniques are used to transform the images to black and white (binary) images by thresholding the argon laser channel images (in which the microsphere location information is stored). This discriminates pixels as representing either particles or background. No further image enhancement is needed except where interference may encountered from spurious light reflecting from the water surface. The interfacial regions in which the counting will take place are delineated such as is shown in Figure 6b. The total number of white pixels in the selected regions are counted in each image sequence. The area of particles is then calculated as the product of the number of white pixels and the pixel size. Pixels are counted as representing attached particles when colloids are at the same location in sequential images compared using a Boolean logical operator; the difference between total and attached particles represents mobile (suspended) particles.

Figure 5. a) Flagella and curli counts in 50 bacteria investigated per sample. Unaltered bacteria is E. coli PHL 628; sheared bacteria were vortexed and centrifuged to remove curli; Flic mutant has flagellum knockout genes. b) Alpha sticking coefficient for microspheres, unaltered bacteria (curli and flagella present), sheared bacteria (most without curli) and the Flic mutant (no flagella or curli).

Figure 6. Fluorescent microsphere colloid attachment at AWmS interface: a) Left: overlay of confocal scanning laser microscope images, with false coloring of colloids (yellow) and dyed water (red) shown on grayscale image of sand grains; b) Right: interfacial regions delineated for particle counting, shown as inverted image (colloids appear black). Image size is 1024 x 1024 pixels with a resolution of 0.73 μm/pixel.
The quantification results for the colloidal retention in AW₉₅S interfacial regions R1 through R4 in Figure 6 are shown in Figure 7, where the data are fitted to a modified Langmuir form:

$$M_s = \frac{KM_wN}{1 + KM_w}$$

where $M_s$ is the cumulative amount of colloids attached on the AW₉₅S interface, $M_w$ is the amount of mobile colloids passing through the image, $N$ is the total available sites for colloid attachment at the AW₉₅S interface, and $K$ is the adsorption rate constant. The Langmuir approach assumes maximum retention capacity ($N$) that corresponds to the available sites on the AW₉₅S interfaces. As can be seen in Figure 7, this approach describes colloid attachment well ($r^2$ in this example is 0.967). Ongoing work in our group is focused on quantifying the effects of key variables – solution chemistry, dynamic flow events, soil properties and colloid/biocolloid surface characteristics – on retention and transport in order to better understand and ultimately ameliorate threats to groundwater posed by contaminant colloids and biocolloids.

![Figure 7. Langmuir fits (solid lines) of quantification results for the AW₉₅S interfacial regions indicated in Figure 6 (regions R1 through R4, as well as total of all regions). The perturbation at $M_w=170,000$ was due to reloading the input syringe pump.](image)

**References**


