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Influence of sulfate on the transport of bacteria in quartz sand

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ABSTRACT

The influence of sulfate on the transport of bacteria in packed quartz sand was examined at a constant 25 mM ionic strength with the sulfate concentration progressively increased from 0 to 20 mM at pH 6.0. Two representative cell types, *Escherichia coli* BL21 (Gram-negative) and *Bacillus subtilis* (Gram-positive), were used to determine the effect of sulfate on cell transport behavior. For both examined cell types, the breakthrough plateaus in the presence of sulfate in suspensions were higher and the corresponding retained profiles were lower than those without sulfate ions, indicating that the presence of sulfate in suspensions increased cell transport in packed quartz sand regardless of the examined cell types (Gram-positive or Gram-negative). Moreover, the enhancement of bacteria transport induced by the presence of sulfate was more pronounced with increasing sulfate concentration from 5 to 20 mM. In contrast with the results for EPS-present bacteria, the presence of sulfate in solutions did not change the transport behavior for EPS-removed cells. The zeta potentials of EPS-present cells with sulfate were found to be more negative relative to those without sulfate in suspensions, whereas, the zeta potentials for EPS-removed cells in the presence of sulfate were similar as those without sulfate. We proposed that sulfate could interact with EPS on cell surfaces and thus negatively increased the zeta potentials of bacteria, contributing to the increased transport in the presence of sulfate in suspensions.

1. Introduction

Bacterial contamination of groundwater, one of the most important drinking water supplies around the world, has recently been regarded as one of the most important drinking water supply problems. To effectively protect groundwater from bacterial contamination, it is imperative to understand the fate and transport of bacteria in porous media under environmentally relevant conditions [1,2]. Therefore, in past decades, great efforts have been devoted to explore factors controlling the transport of bacteria in porous media under environmentally relevant conditions. A number of physical, chemical, and biological factors such as surface coating on grain collector [3–5], solution ionic strength and cation valence [6,7], fluid conditions [8,9], mineral crystallization structure [10], nutrient conditions [11–14], colloids such as natural organic matter and clay particles present in solutions [15–17], bacterial growth phase [18], bacterial cell type and motility [7,19–21], lipopolysaccharides (LPS) [22,23], and extracellular polymeric substances (EPS) [24,25] have been shown to have great influence on the transport and deposition kinetics of bacteria in porous media.

Similar to many cations such as Na^+, K^+, Ca^{2+}, and Mg^{2+}, which are ubiquitously present in surface water and groundwater, anions such as Cl^-, HCO_3^-, NO_3^-, SO_4^{2-}, SiO_4^{2-} and PO_4^{3-} are also widely present in natural water systems. These anions can get into surface water and groundwater systems via various approaches, including dissolved fertilizers leaching from agricultural fields through soils [26,27], groundwater recharge with reclaimed water [28,29], infiltration of stormwater with air pollutants [30,31], and leakage of sewage from decrepit sewer networks and septic tanks [32,33]. Many previous studies have investigated the effects of cations on the transport of microbe and found that comparing with monovalent cations, the presence of divalent ions such as $Ca^{2+}$and Mg^{2+} in suspensions significantly reduced the transport (or increased the deposition) of microbe [7,16,34–37]. Similar to cations, anions are also expected to affect the transport (or deposition) behavior of microbe. For example, phosphate has been proved to enhance the transport of bacteria [38–42]. Appenzeller et al. [38] found that adding phosphate to water allowed a decrease of 75% of *Escherichia coli* SH 702 adhering to corrosion products in water distribution systems. By comparing the transport behaviors of *E. coli* O157:H7 cells in the presence of phosphate with those in the absence of phosphate, Wang et al. [40] very recently showed that the addition

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of phosphate (only 0.1 mM) into NaCl solutions could significantly enhance the transport of *E. coli* O157:H7 cells in saturated quartz sand. Moreover, these authors also found that increased phosphate in the mobile aqueous phase could lead to the release of previously immobilized *E. coli* O157:H7 cells.

While several studies have conducted to investigate the effects of phosphate on the transport behavior of bacteria [38–42], to the best of our knowledge regarding the role of sulfate on the transport (deposition) behavior is very limited. By investigating the sorption of virus MS2 onto Mg-Al layered double hydroxides in the presence of different anions, You et al. [43] found the presence of SO_4^{2-} in suspensions significantly decreased the sorption of MS2 onto the surfaces of Mg–Al layered double hydroxides, whereas, the presence of NO_3^- had little effect on the sorption behavior of MS2. Park and Kim [44] recently found that the presence of sulfate in solutions slightly increased the transport of *Bacillus subtilis* in metal-oxide coated quartz sand. The presence of sulfate in background solutions may also affect the transport (adhesion) behavior of bacteria in porous media without coating by metal-oxide. However, to date, the effects of the sulfate on the transport of bacteria in bare quartz sand, the most common surfaces in environment, are not clear and thus investigation is required.

Hence, this study was designed to fully understand the influence of sulfate on the transport of bacteria in packed quartz sand. To achieve the objective, two representative cell types, *E. coli* BL21 (Gram-negative) and *B. subtilis* (Gram-positive), were employed. Packed quartz sand column experiments were performed at a constant 25 mM ionic strength (NaCl) with the concentration of sulfate progressively increased from 0 to 20 mM at pH 6.0. Additional experiments for treated cells (with the removal of EPS from cell surfaces) were also performed. Different mechanisms by which sulfate affects the transport behavior of bacteria were discussed.

2. Materials and methods

2.1. Cell culture and preparation

Two bacterial cell types, a Gram-negative strain *E. coli* BL21 and a Gram-positive strain *B. subtilis*, were used in this study. *E. coli* cells were grown in LB Broth growth medium while *Bacillus* cells were grown in Tryptic Casein Soy Agar growth medium. Both strains were grown to early stationary growth phase (16 h at 37 °C while shaking at 200 rpm for *E. coli* and 32 h at 30 °C while shaking at 200 rpm for *Bacillus*). The LB growth medium consisted of 10 g Tryptic Casein, 5 g bacto-yeast extract, and 10 g NaCl dissolved in 1000 mL distilled water, while the Tryptic Casein Soy Agar growth medium consisted of 15 g Tryptic Casein, 5 g bacto-soytone, and 5 g NaCl dissolved in 1000 mL distilled water. Both growth media were autoclaved at 121 °C for 30 min.

Cells were harvested by centrifugation ($4000 \times g$ for 8 min at 4 °C) upon reaching a stationary phase. Following centrifugation, the growth medium was decanted, and the pellets were washed three times in sterilized NaCl electrolyte solution (0.1 mM) to remove any residual growth medium [45]. The cell pellets were then re-suspended in NaCl electrolyte solution (0.1 mM). The centrifugation and re-suspension process was repeated one more time, and the concentrated bacterial solution was diluted in desired solution which was used for transport experiments. One portion of bacteria was used to prepare stock of treated bacteria, from which EPS was removed. Cation exchange resin (CER) technique was used to release EPS from cell surfaces [46]. CER (Dowex Marathon C, 20–50 mesh, sodium form, Fluka 91973), which was soaked in Milli-Q water overnight prior to use, was added to the bacteria cells with a dosage of 2.5 g/g bacterial mass. The bacteria-CER suspension was then stirred at 600 rpm for 2.5 h at 4 °C, which followed by a settlement of the suspension for 3 min to separate CER. The cell suspension was then transferred to centrifugation tube and the CER-treated bacteria were collected by centrifugation at $8000 \times g$ for 20 min at 4 °C. The CER-treated bacterial pellets were then re-suspended in Milli-Q water. The effectiveness of the EPS removal from cell surfaces via CER treatment has been clearly demonstrated in our previous studies [16,24,25].

The prepared stock cell concentration was determined using a counting chamber (Buerker-Tuerk Chamber, Marienfeld Laboratory Glassware, Germany) with an inverted fluorescent Ti-E microscope under bright field. The stock concentration was approximately 10^9–10^{10} cells per mL, which was diluted to obtain the target influent concentration of $5.0 \times 10^7 \pm 25\%$ cells mL^{-1}.

2.2. Porous media

The porous media used for bacteria transport experiments were quartz sand (Hebeizhensheng Mining Ltd., Shijiazhuang, China) with sizes ranging from 417 to 600 μm (the median diameter of 510 μm). The chemical composition of ultrapure sand reported by the manufacturer was: >99.80% SiO_2, <0.025% Fe_2O_3, and <0.2% other minerals (e.g. clay and mica). The quartz sand was cleaned by soaking in concentrated HCl for at least 24 h, followed by repeated rinsing with Milli-Q water, drying at 105 °C overnight, and baking at 850 °C for 8 h. The cleaned sand was stored under vacuum until use.

2.3. Column experiments

Ten-centimeter long cylindrical Plexiglass columns with an inner diameter of 2 cm were wet-packed with cleaned quartz sand. Prior to packing, the cleaned quartz sand was rehydrated by boiling in Milli-Q water for at least 1 h. After the rehydrated quartz sand was cooled, the columns were packed by adding wet quartz sand in small increments (~1 cm) with mild vibration of the column to minimize any layering or air entrapment. One 140 mesh stainless steel screen was placed at each end of the column. To spread the flow upon entry into the column, ~3.0 g of quartz sand was added to the top of the influent screen, forming a ~0.5-cm-thick layer that was covered by another screen. The porosity of the packed column was approximately 0.42.

After packing, the columns were pre-equilibrated with 10 pore volumes of Milli-Q water and at least 15 pore volumes of bacteria-free salt solutions at desired ionic strength and sulfate concentrations. Following pre-equilibration, 3 pore volumes of suspended bacteria were injected into the column, followed by elution with 3 pore volumes of salt solution (without bacteria) at the same ionic strength and sulfate concentration. The suspensions and solutions were injected into the columns in up-flow mode using a syringe pump (Harvard PHD 2000, Harvard Apparatus Inc., Holliston, MA). The transport experiments were conducted at a constant 25 mM ionic strength with different sulfate concentrations (adjusted with NaCl and Na_2SO_4). The sulfate concentration varied as 0 mM sulfate (25 mM NaCl), 5 mM sulfate (5 mM Na_2SO_4 + 20 mM NaCl), 10 mM sulfate (10 mM Na_2SO_4 + 15 mM NaCl) and 20 mM sulfate (20 mM Na_2SO_4 + 5 mM NaCl), respectively. All the solutions and suspensions were adjusted with 0.1 M HCl or 0.1 M NaOH to give a final pH of 6.0. The pore water velocity of all experiments was set to be 4 m day^{-1} (0.366 mL min^{-1}) to represent fluid velocities in coarse aquifer sediments, forced-gradient conditions, or engineered filtration systems. All the water used in the experiments was autoclaved for sterility.

Samples from the column effluent were collected continuously in sterile 5 mL glass culture tubes. The collected bacteria samples and reservoir samples were preserved using formaldehyde (2%), and were kept in a refrigerator at 4 °C until the cell concentration