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Virus attachment onto quartz sand: Role of grain size and temperature

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ABSTRACT

Virus transport in groundwater is controlled mainly by attachment onto the solid matrix and inactivation. Therefore, understanding how the various parameters affect virus attachment can lead to improved virus transport predictions and better health risk evaluations. This study is focused on the attachment of viruses onto quartz sand under batch experimental conditions. The bacteriophages ΦX174 and MS2 were used as model viruses. Three different sand grain sizes were employed for the static and dynamic experiments. The batch sorption experiments were performed under static conditions at 4 °C and 20 °C and dynamic conditions at 4 °C. The experimental data were adequately described by the Freundlich isotherm. It was shown that temperature significantly affects virus attachment under static conditions. The attachment of both MS2 and ΦX174 onto quartz sand was greater at 20 °C than 4 °C. Higher virus attachment was observed under dynamic than static conditions, and in all cases, the affinity of MS2 for quartz sand was greater than that of ΦX174. Furthermore, in most of the cases considered, bacteriophage attachment was shown to decrease with increasing quartz sand size.

1. Introduction

Although groundwater is less susceptible to pollution than surface waters, groundwater contamination by biocolloids can easily occur. Viruses can infiltrate into the subsurface by a variety of ways: broken sewer lines, through septic tanks, improperly constructed landfills, open dumps, and intentional groundwater recharge or crop irrigation with treated municipal wastewater [1–4]. As the demand for clean water increases and water supplies decrease, artificial recharge of groundwater with treated municipal wastewater is expected to become a common practice [5]. Therefore, active human enteric viruses can be delivered to the subsurface with the recycled water and become a public health threat, unless the soil acts as final treatment step for virus retention. It should be noted that waterborne viruses, especially human enteric viruses, are often the cause of numerous outbreaks in many parts of the world [6–8].

Viruses are relatively disinfection-resistant microbial pathogens, and are known to exhibit quite conservative transport behavior in subsurface formations because they remain infective for a considerable period of time (i.e., weeks to months) [9–11]. The fate and transport of viruses in groundwater is controlled mainly by attachment onto the solid matrix, and inactivation or loss of infective capability [12–19]. Virus attachment onto aquifer sediments is affected by several factors including viral surface properties, groundwater quality, and soil surface charges [20–25]. Furthermore, the most important factors that influence virus inactivation rates are temperature, attachment/detachment to particulate matter and solid matrix, the degree of water saturation, and the presence of microorganisms [1,12,13,21,26–28].

The bacteriophage ΦX174 and MS2, which have been employed in numerous other investigations [17,30–33], were used in this study as surrogates for naturally occurring pathogenic enteric viruses because they are easy to handle, they are not pathogenic, and have similar size and as typical enteric viruses. MS2 is a F-specific, single-stranded RNA phage with 31% nucleic acid content, whose host bacterium is *E. coli* (ATTC 15597-B1). The MS2 particle diameter ranges from 24 to 26 nm, and its protein coat is hydrophobic [34] with isoelectric point (pH_{iep}) of 4.1 [29]. ΦX174 is an icosahedral, single-stranded DNA phage with 26% nucleic acid content, whose host bacterium is *E. coli* (ATTC 13706-B1). The ΦX174 particle diameter ranges from 25 to 27 nm, and has hydrophilic protein coat [34] with pH_{iep} = 4.4 [29].

The present study aims to extend the work presented by Chrysikopoulos and Syngouna [29] who have studied the attachment of MS2 and ΦX174 onto kaolinite and montmorillonite, by investigating virus attachment onto quartz sand grains of different particle sizes under static and dynamic batch conditions, at two different temperatures. Quartz sand was employed in this study because quartz is the most common mineral found on the surface

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of the Earth. To our knowledge the combined effects of grain size, and ambient temperature variability on MS2 and ΦX174 attachment onto different quartz sands has not been examined before.

2. Materials and methods

2.1. Bacteriophages

The bacteriophages ΦX174 and MS2, were suspended and diluted in phosphate buffered saline (PBS) solution (1.2 mM NaCl, 0.027 mM KCl, and 0.10 mM Na_2HPO_4) at pH = 7 to concentrations in the range of 10^3–10^8 pfu/mL. Note that the PBS solution was used to enhance virus stability by eliminating unspecified factors that could cause virus inactivation [35]. Both bacteriophages were assayed by the double-layer overlay method [36], where 0.1 mL solution containing the appropriate host bacterium and 0.1 mL of a diluted virus sample solution, were mixed in a centrifuge tube. The mixture was combined with molten soft-agar medium (4.5 mL), maintained at 45 °C in a tube, and poured onto a petri dish containing solid agar medium. The plates were solidified for 10 min and incubated overnight at 37 °C. Viable virus concentrations were determined by counting the number of plaques in each host lawn and reported as plaque-forming units per milliliter (pfu/mL). Only dilutions that resulted in 20–300 plaques per plate were accepted for quantification. All virus concentrations reported in this study represent the average of three replicate plates.

2.2. Quartz sands

Three different size distributions of quartz sand (Filcom Filterzand & Grind) were used in the experiments: fine quartz sand (FQS) with grain diameter ranging from 0.150 to 0.212 mm (sieve No. 70/100), medium quartz sand (MQS) with grain diameter ranging from 0.425 to 0.600 mm (sieve No. 30/40), and coarse quartz sand (CQS) with grain diameter ranging from 1.180 to 1.700 mm (sieve No. 12/16). Particle size distribution values obtained by sieve analysis were used to calculate the coefficient of uniformity, $C_u = d_{60}/d_{10}$ (were d_{10} and d_{60} are the diameter of a sand grain that is barely too large to pass through a sieve that allows 10%, and 60%, respectively, of the material (by weight) to pass through). The coefficient of uniformity for each sand distribution was determined as: $C_u = 1.19$, 1.21, 1.2 for FQS, MQS, CQS, respectively. Note that the sand fractions employed were relatively uniform because the smaller the C_u number the more uniform the sand fraction. Thus, $C_u = 1$ corresponds to a sand fraction with only one grain size [43]. The chemical composition of the quartz sand as reported by the manufacturer (Filcom, Netherlands) was: 96.2% SiO_2, 0.15% Na_2O, 0.11% CaO, 0.02% MgO, 1.75% Al_2O_3, 0.78% K_2O, 0.06% SO_3, 0.46% Fe_2O_3, 0.03% P_2O_5, 0.02% BaO, 0.01% Mn_3O_4, and 0.28% loss on ignition. The three quartz sand distributions were thoroughly cleaned with 0.1 M HNO_3 (70%) for 3 h to remove surface impurities (e.g., iron hydroxide and organic coatings) that could promote physicochemical deposition of the viruses, rinsed with distilled deionized water (ddH_2O), then soaked in 0.1 M NaOH for 3 h, and rinsed with ddH_2O again [10]. Subsequently, the sand distributions were sterilized and dried in an oven at 105 °C for 24 h.

2.3. Static and dynamic batch experiments

The static batch experiments were performed under controlled conditions at 4 °C and 20 °C, and the dynamic experiments at 4 °C. Several virus stock solutions with concentrations ranging from 10^3 to 10^8 pfu/mL were used for both static and the dynamic experiments. At least 5 different virus stock concentrations were used for the static experiments, and 3 different virus stock concentrations were used for the dynamic experiments. A total of 78 experiments (39 for each bacteriophage) were performed in 20 mL Pyrex glass screw-cap tubes (Fisher Scientific). The glass tubes were washed with detergent, soaked in 6 N HCl, rinsed thoroughly in ddH_2O, autoclave sterilized, and oven dried at 105 °C overnight. The specific conductance of the final virus suspension was 212 μS/cm, which corresponds to ionic strength $I_s \approx 2$ mM.

For each experiment, 30 glass tubes were employed, which were divided into two groups. Each group consisted of 15 glass tubes. The glass tubes of the first group (experimental tubes) contained 14 mL of virus suspension with 14 g of sand, and the glass tubes of the second group (control tubes) contained virus suspension without sand. All glass tubes were filled to the top. However, the tubes were not completely free of air because a small air bubble was always trapped within the tubes when the caps were screwed onto the tubes. Both groups were treated in the same manner. The static batch experiments were conducted in a constant-temperature dark room at 4 °C, and in an incubator at 20 °C. The dynamic batch experiments were performed in the constant-temperature dark room at 4 °C, with all the tubes attached to a tube rotator (Selecta, Agitador orbit), operated at 12 rpm, in order to allow the sand to mix within the virus solution. All the experiments were conducted in a dark room to eliminate the possibility of inactivation by sunlight [37]. One tube of each group was chosen at random at pre-determined time intervals during the experiment. A sample of the PBS solution (2.0 mL) was removed from each selected glass tube and assayed for bacteriophage ΦX174 and MS2. Then, the used glass tubes were discarded. Fig. 1 presents an illustration of the batch experimental procedures employed in this work.

2.4. Theoretical considerations

The concentration of viruses attached onto quartz sand in the experimental tubes (C^* [M_v/M_s] in units of [pfu/(g sand)]) was

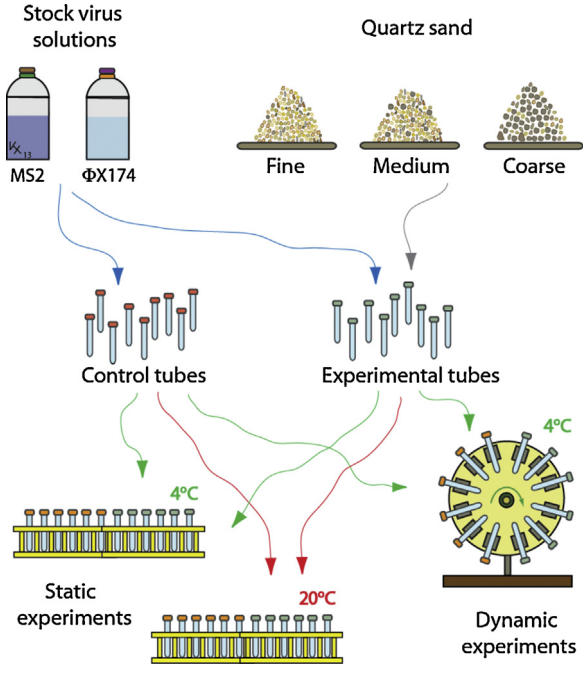

Fig. 1. Pictorial illustration of the experimental procedures. Control tubes received virus stock solution and experimental tubes received virus stock solution as well as quartz sand. Static batch experiments were conducted with all glass tubes placed in a rack at 4 °C and 20 °C; whereas, dynamic batch experiments were performed with all glass tubes attached to a tube rotator at 4 °C.