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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

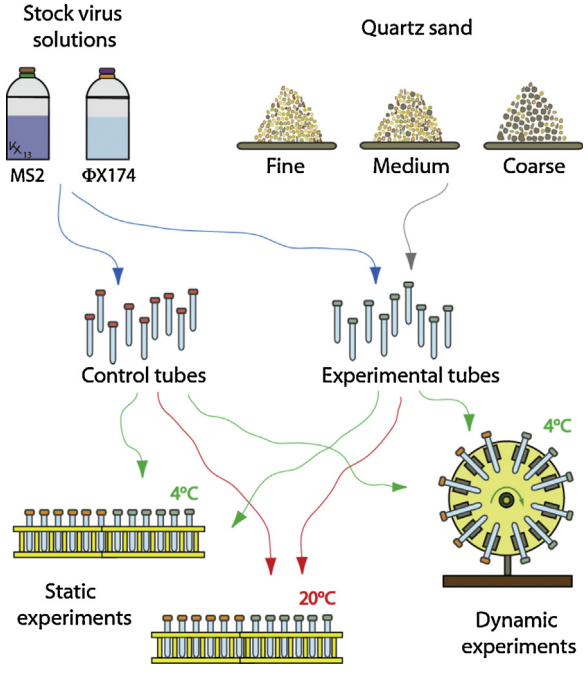

Fig. 1. Pictorial illustration of the experimental procedures. Control tubes received virus stock solution and experimental tubes received virus stock solution as well as quartz sand. Static batch experiments were conducted with all glass tubes placed in a rack at 4 °C and 20 °C; whereas, dynamic batch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

determined by the following equation:

$$C^* = \frac{C_{\text{control}} - C \cdot f}{S_m} \quad (1)$$

where C [M_v/L^3] in units of [pfu/mL] is the aqueous phase virus concentration in the experimental tube, C_{control} [M_v/L^3] in units of [pfu/mL] is the aqueous phase virus concentration in the control tube, S_m [M_s/L^3] in units of [(g sand)/mL] is the mass of quartz sand per unit volume of liquid in the experimental tube, and f [–] is a correction factor defined as:

$$f = \frac{C_{\text{corrected}}(t)}{C(t)} = \frac{C_o e^{-\lambda_{\text{control}} t}}{C_o e^{-\lambda t}} = \exp[-t(\lambda_{\text{control}} - \lambda)] \quad (2)$$

where $C_{\text{corrected}}(t)$ [M_v/L^3] is the corrected aqueous phase virus concentration in the experimental tubes at time t, C_o [M_v/L^3] is the initial aqueous phase virus concentration, λ [$1/t$] is the inactivation rate coefficient of the viruses in the experimental tubes, and λ_{control} [$1/t$] is the inactivation rate coefficient of the viruses in the control tubes. The correction factor f is necessary because Chrysikopoulos and Aravantinou [38] reported that ΦX174 and MS2 inactivation in experimental and control tubes (with and without the presence of quartz sand) is different under both static and dynamic conditions. Note that in order to simplify the notation, the various masses are indicated as follows: M_v is the mass of viruses, and M_s is the mass of solids (quartz sand).

The virus attachment onto the quartz sand was quantified by the Freundlich isotherm, which is a non-linear relationship between virus concentration in the liquid phase at equilibrium, C_{eq} [M_v/L^3] in units of [pfu/mL], and virus concentration onto the solid phase at equilibrium, C^*_{eq} [M_v/M_s] in units of [pfu/(g sand)], expressed as follows [44]:

$$C^*_{eq} = K_f C^m_{eq} \quad (3)$$

where K_f [$L^{3+m}/M_s M_v^{m-1}$] is the Freundlich constant in units of [$(mL)^m$/(g sand)$(pfu)^{m-1}$], and m [–] is the Freundlich exponent, which is equal to one for linear attachment. The Freundlich describes equilibrium attachment onto heterogeneous sorbent surfaces, and does not assume monolayer attachment. The Freundlich parameters K_f and m were estimated by fitting of the log-transformed experimental data $\log C^*_{eq}$ versus $\log C_{eq}$ with the linearized form of the Freundlich isotherm:

$$\log C^*_{eq} = \log K_f + m \log C_{eq} \quad (4)$$

It should be noted that K_f is directly proportional to the sorbent capacity for attachment, and m is a measure of the surface heterogeneity of the sorbent (the smaller its value the higher the surface heterogeneity). Therefore, m can be used to predict the adsorption characteristics [45]. When m has a value less than unity, the attachment process is favorable. Freundlich isotherms can result from overlapping of several Langmuir isotherms (combination of Langmuir isotherms) that describe sorption onto heterogeneous sorbents with surfaces consisting of several different sites [39].

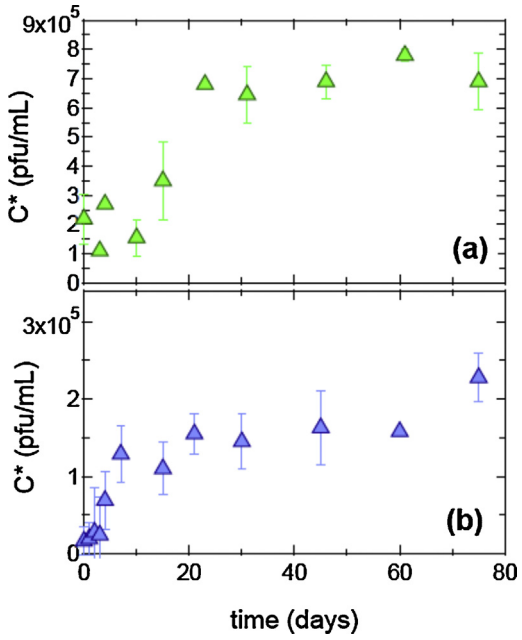

Fig. 2. Attachment kinetics of (a) ΦX174 and (b) MS2 onto CQS for $C_o = 10^7$ pfu/mL under static conditions at 4 °C.

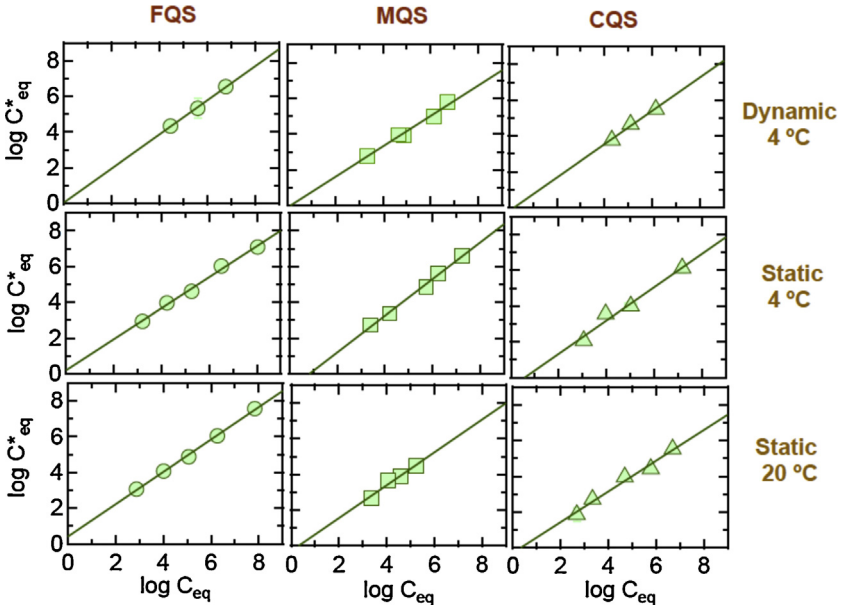

Fig. 3. Log-transformed equilibrium data (symbols) of ΦX174 attachment onto three different size distributions of quartz sand (FQS, MQS, and CQS) under dynamic conditions at 4 °C, and static conditions at 4 °C and 20 °C, together with the fitted linearized Freundlich isotherms (lines).

3. Results and discussion

The experimental data suggested that bacteriophage attachment onto quartz sand is a relatively slow process. As shown by the selected experimental data presented in Fig. 2, both bacteriophages (ΦX174 and MS2) in the presence of CQS reached equilibrium after approximately 20 days. Similar results were also observed with the other two size distributions of quartz sand and the various experimental conditions examined in this study.

The experimental data from the equilibrium attachment experiments of ΦX174 onto each of the three size distributions of quartz sand used in this study (FQS, MQS, and CQS), under static conditions at 4 °C and 20 °C, and dynamic conditions at 4 °C, are shown in Fig. 3. The experimental data were plotted ($\log C^*_{eq}$ versus $\log C_{eq}$). The Freundlich parameters K_f and m were estimated by linear regression of the log-transformed experimental data using Eq. (4). The fitted Freundlich parameter vales together with the corresponding coefficients of determination, R^2, which ranged between 0.98 and 1.00, are listed in Table 1. All concentrations of viruses attached onto sand were determined with Eq. (1), using appropriate inactivation rate coefficients previously reported by Chrysikopoulos and Aravantinou [38]. Note that the values of attached virus concentrations were adjusted with a correction factor (see Eq. (2)), to account for differences in virus inactivation rate coefficient observed in the presence and absence of quartz sand. Based on the R^2 values estimated in Fig. 3, the Freundlich isotherm successfully fitted the ΦX174 experimental data. The values for the Freundlich exponent m ranged from 0.85 to 1.03.

Similarly, the experimental data from the equilibrium attachment experiments of MS2 onto each of the three size distributions of quartz sand used in this study (FQS, MQS, and CQS), under static conditions at 4 °C and 20 °C, and dynamic conditions at 4 °C, are shown in Fig. 4. The experimental data were successfully represented with the Freundlich isotherm and the fitted Freundlich parameters K_f and m, together with the corresponding R^2 values were are listed in Table 1. Note that the Freundlich exponent m ranged from 0.71 to 0.96, suggesting that the attachment process is favorable; whereas, the R^2 values ranged between 0.91 and 1.00, indicating that the selected Freundlich isotherm describes the adsorption process well.

Table 1
Fitted Freundlich parameter values.

	Conditions	K_f [(mL)^m/g pfu)$^{m-1}$]	m	R^2
ΦX174				
FQS	Static 4 °C	1.55 ± 0.58	0.87 ± 0.04	1.00
	Static 20 °C	2.57 ± 0.74	0.91 ± 0.02	1.00
	Dynamic 4 °C	1.15 ± 1.08	0.95 ± 0.04	1.00
MQS	Static 4 °C	0.14 ± 0.57	1.03 ± 0.04	1.00
	Static 20 °C	0.52 ± 0.29	0.92 ± 0.12	0.98
	Dynamic 4 °C	0.79 ± 2.15	0.85 ± 0.06	0.99
CQS	Static 4 °C	0.30 ± 0.25	0.93 ± 0.11	0.99
	Static 20 °C	0.52 ± 0.46	0.86 ± 0.07	0.99
	Dynamic 4 °C	0.63 ± 0.30	0.94 ± 0.10	0.99
MS2				
FQS	Static 4 °C	2.06 ± 0.63	0.85 ± 0.04	1.00
	Static 20 °C	3.54 ± 0.33	0.71 ± 0.09	0.98
	Dynamic 4 °C	3.55 ± 0.12	0.89 ± 0.17	0.98
MQS	Static 4 °C	1.54 ± 0.31	0.96 ± 0.12	0.97
	Static 20 °C	2.22 ± 0.21	0.85 ± 0.14	0.96
	Dynamic 4 °C	8.51 ± 0.50	0.85 ± 0.07	0.99
CQS	Static 4 °C	0.57 ± 0.13	0.86 ± 0.18	0.91
	Static 20 °C	2.12 ± 0.25	0.75 ± 0.12	0.96
	Dynamic 4 °C	3.08 ± 0.07	0.79 ± 0.20	0.97

The experimental results for all three quartz sands used, under static conditions, clearly show that K_f values increased with increasing temperature. This result is in agreement with those presented previously in the literature. It should be noted that Syngouna and Chrysikopoulos [42] have also reported that the attachment of ΦX174 and MS2 onto kaolinite and bentonite increase with increasing temperature. The compiled results in Table 1 indicate that, with the exception of ΦX174 with FQS, the attachment of ΦX174 and MS2 onto quartz sand was greater under dynamic than static conditions. This is an intuitive result because under dynamic conditions more contacts occurred between the bacteriophages and the quartz sand. It is worthy to note that, in most of the cases considered in this study, the K_f values for both bacteriophages (ΦX174 and MS2) decreased with increasing quartz size (FQS > MQS > CQS). This is attributed to the increasing surface area available for attachment with decreasing quartz sand size. Although the morphology of the different quartz sand size

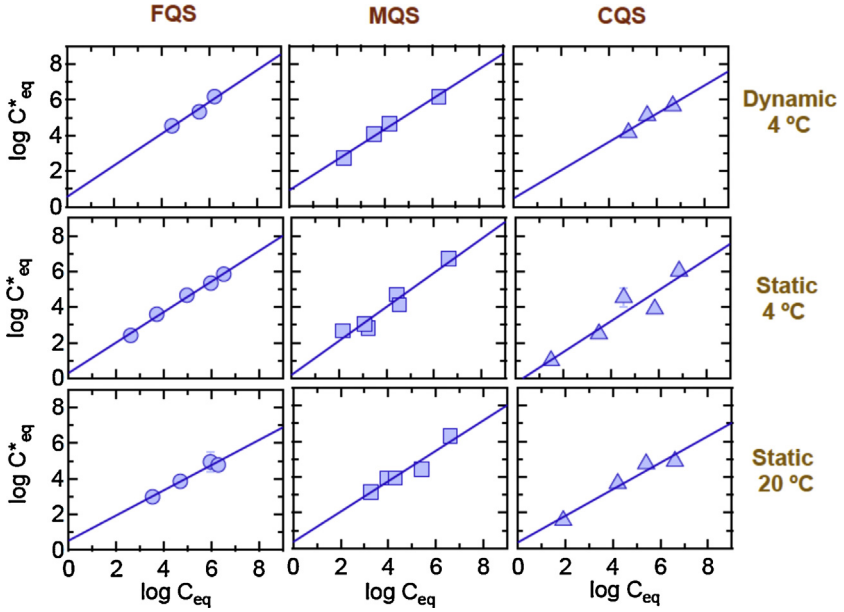

Fig. 4. Log-transformed equilibrium data (symbols) of MS2 attachment onto three different size distributions of quartz sand (FQS, MQS, and CQS) under dynamic conditions at 4 °C, and static conditions at 4 °C and 20 °C, together with the fitted linearized Freundlich isotherms (lines).

distributions is expected to be relatively similar, because all three quartz sand distributions employed in this study originated from the same batch of sand, the number of attachment sites per unit volume of sand is increasing with decreasing quartz sand size.

Furthermore, the attachment of MS2 onto all three different quartz sands (FQS, MQS, and CQS) employed in this study was greater than that of ΦX174. This result is in agreement with the findings presented by Herbold-Paschke et al. [40], and Dowd et al. [41], Syngouna and Chrysikopoulos [42], and Chrysikopoulos and Syngouna [29], who have investigated the affinity of both ΦX174 and MS2 for various clay minerals and alluvial sediments. Note that the Freundlich isotherm was also used to describe the attachment of ΦX174 and MS2 onto kaolinite and montmorillonite [29].

4. Conclusions

The attachment of MS2 and ΦX174 onto quartz sand is adequately described by the Freundlich isotherm equation. Under static conditions, both MS2 and ΦX174 were attached in greater amounts onto all three different quartz sands (FQS, MQS, and CQS) employed in this study at 20 °C than 4 °C. Furthermore, the attachment of MS2 and ΦX174 onto quartz sand was found to be dependent on the size of the quartz sand. The experimental data suggested that bacteriophage attachment was inversely correlated with quartz sand size.

Nomenclature

C	virus concentration in the aqueous phase, M_v/L^3
C_o	initial virus concentration in the aqueous phase, M_v/L^3
C_{eq}	virus concentration in the aqueous phase at equilibrium, M_v/L^3
$C_{control}$	aqueous phase virus concentration in the control tube, M_v/L^3
$C_{corrected}$	corrected aqueous phase virus concentration in the experimental tube, M_v/L^3
C^*	concentration of viruses attached onto the sand, M_v/M_s
C^*_{eq}	concentration of viruses attached onto the sand at equilibrium, M_v/M_s
C_u	coefficient of uniformity (–)
d_{10}	sand grain diameter size that can barely pass through a sieve, which allows 10% of the material (by weight) to pass through, L
d_{60}	sand grain diameter size that can barely pass through a sieve, which allows 60% of the material (by weight) to pass through, L
f	correction factor (–)
I_s	ionic strength, M/L^3
K_f	Freundlich constant, $L^{3+m}/M_sM_v^{m-1}$
m	Freundlich exponent (–)
M_s	mass of solids, M
M_v	mass of viruses, M
R^2	coefficient of determination (–)
S_m	mass of sand per unit volume liquid, M_s/L^3
λ	inactivation rate coefficient of the viruses in the experimental tubes, $1/t$
$\lambda_{control}$	inactivation rate coefficient of the viruses in the control tubes, $1/t$

Abbreviations

CQS	coarse quartz sand
ddH_2O	distilled deionized water
FQS	fine quartz sand
MQS	medium quartz sand
PBS	phosphate buffered saline
pfu	plaque forming units

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