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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.

## Materials and Methods

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).

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<sub>2</sub>HPO<sub>4</sub>) at pH=7 to yield the desired bacteriophage concentration. Bacteriophage concentrations were measured by using the Double-agar-layer assay method.

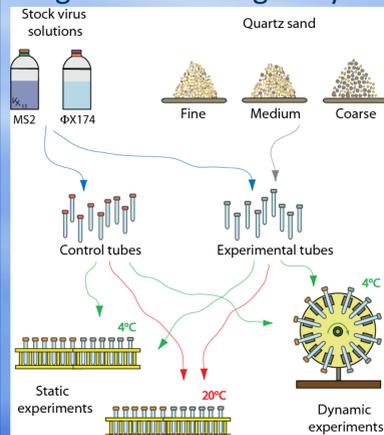


Fig.1: Pictorial illustration of the experimental procedures.

Several virus stock solutions with concentrations ranging from 10<sup>3</sup> to 10<sup>8</sup> pfu/mL were used for both static and the dynamic experiments. Batch experiments were performed in 20-mL Pyrex glass screw-cap tubes. Each experiment consisted of a 30 tubes set which 15 of them contained virus suspensions with sand and the other 15 tubes contained virus suspensions without sand. For the static batch experiments, one set of tubes was placed in a constant-temperature dark room at 4 °C, and another one set was placed in an incubator at 20 °C. The dynamic batch experiment was performed with all the tubes attached to tube rotator (Selecta, Agitador orbit) placed in the constant-temperature dark room at 4 °C.

## Virus attachment Theory

The concentration of viruses attached onto quartz sand in the experimental tubes ( $C^*$  [ $M_v/M_s$ ] in units of [pfu/(g sand)]) was 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,  $[M_v/L^3]$  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:

$$f = \frac{C_{\text{corrected}}(t)}{C(t)} = \frac{C_0 e^{-\lambda_{\text{control}} t}}{C_0 e^{-\lambda t}} = \exp[-t(\lambda_{\text{control}} - \lambda)] \quad (2)$$

where  $C_{\text{corrected}}(t)$  [ $M_v/L^3$ ] is the corrected aqueous phase virus concentration in the experimental tubes at time  $t$ ,  $C_0$  [ $M_v/L^3$ ] is the initial aqueous phase virus concentration,  $\lambda$  [1/t] is the inactivation rate coefficient of the viruses in the experimental tubes, and  $\lambda_{\text{control}}$  [1/t] is the inactivation rate coefficient of the viruses in the control tubes.

### Isotherm theory

The virus attachment onto the quartz sand was quantified by the Freundlich isotherm,  $C_{\text{eq}}$  [ $M_v/L^3$ ] in units of [pfu/mL], and virus concentration onto the solid phase at equilibrium,  $[M_v/M_s]$  in units of [pfu/(g sand)], expressed as follows:

$$C_{\text{eq}}^* = K_f C_{\text{eq}}^m \quad (3)$$

where  $K_f$  is the Freundlich constant in units of [(mL)<sup>m</sup>/(g sand)(pfu)<sup>m-1</sup>], and  $m$  is the Freundlich exponent, which is equal to one for linear attachment.

## Results

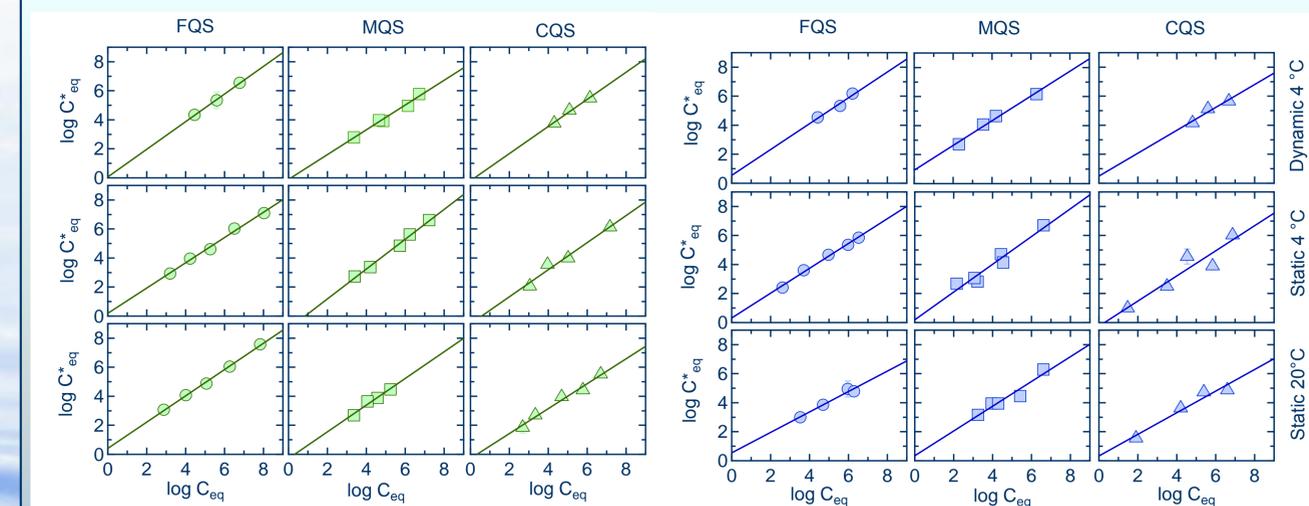


Fig.2: Log-transformed equilibrium data of ΦX174 (green symbols) and MS2 (blue symbols) attachment onto three different size distributions of quartz sand (FQS, MQS, and CQS), together with the fitted linearized Freundlich isotherms (lines).

1	Conditions	$K_f$	$m$	$R^2$	
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	

Table 1: Fitted Freundlich parameter values for ΦX174.

Table 2: Fitted Freundlich parameter values for MS2.

2	Conditions	$K_f$	$m$	$R^2$	
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	

## Conclusions

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 X174. Furthermore, in most of the cases considered, bacteriophage attachment was shown to decrease with increasing quartz sand size.

## Acknowledgements

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