Virus removal vs. subsurface water velocity during slow sand filtration

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ABSTRACT

In an attempt to obtain a conservative estimate of virus removal during slow sand and river bank filtration, a somatic phage was isolated with slow decay and poor adsorption to coarse sand. We continuously fed a phage suspension to a 7-m infiltration path and measured the phage removal. In a second set of experiments, we fed the phage suspension to 1-m long columns run at different pore water velocities. Using the data obtained, a mathematical model was constructed describing removal vs. pore water velocity (PWV), assuming different statistical distributions of the adsorption coefficient λ. The bimodal distribution best fit the results for PWVs higher than 1 m/d. It predicted a removal of approximately 4 $\log_{10}$ after 50 days infiltration at 1 m/d. At PWVs below 1 m/d the model underestimated removal. Sand-bound phages dissociated slowly into the liquid phase, with a detachment constant k_{det} of 2.6×10^{-5}. This low k_{det} suggests that river bank filtration plants should be intermittently operated when viral overload is suspected, e.g. during flooding events or at high water-marks in rivers, in order for viruses to become soil-associated during the periods of standstill. Resuming filtration will allow only a very slow virus release from the soil.

Key words | groundwater recharge, phages, river bank filtration, slow sand filtration, virus-removal simulation

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INTRODUCTION

At the end of the 19th century, when the groundwater level of rapidly expanding cities began to decline due to increasing demand for potable water, replenishing groundwater by infiltrating surface water into the underground started to be implemented as a solution (Richert 1900; Schübel 1936).

In the beginning, no experimental evidence was available for setting infiltration conditions – soil quality, water velocity and residence time of the water underground – that would produce an acceptable microbial quality of the exfiltrate. Experimental studies were then set up for gaining information on microbial removal (Table 1). As a result, 40–100 days underground residence time was recommended by Gross (1929) and 60 days by Knorr (1951b). Finally, Germany adopted 50 days residence time as sufficient for removing pathogens (DVGW 2006). As of 2006, numerous countries have adopted minimum residence time for groundwater recharge and riverbank filtration (RBF), mostly around 50 days (Chave *et al.* 2006). No rules have been issued with respect to the pore water velocity (PWV), i.e. of the interstitial velocity of the water underground.

Acording to the colloid filtration theory (CFT) (Yao *et al.* 1971; Tufenkji & Elimelech 2003; Tufenkji 2006), removal of colloids by soil filtration is more efficient when a lower PWV is chosen. A number of observations corroborate this prediction for viruses. Funderburg *et al.* (1981) found that removal of the phage $\varphi\chi174$ was directly proportional to the retention time in columns. Vaughn *et al.* (1981) did not find any retention of poliovirus at velocities of 25 m/d, yet the retention at 1 m/d was substantial. In columns

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Table 1 | Early studies of riverbank filtration directed to evaluate the removal of microorganisms

Quality of the infiltrate	Soil structure	Shortest distance between infiltration and abstraction (m)	Travel time (d)	Pore water velocity in the aquifer (m/d)	Microbial indicators in the exfiltrate	Source
River water		200	60–90	1–4	From 10 to 10,000 colonies in the raw water to undetectable in 1 mL exfiltrate	Richert (1902)
Pretreated (fast sand filtration) river water	Fine sand/gravel	20	40	0.5	From several thousand colonies/mL in the river water to less than 15/mL exfiltrate	Scheelhaase (1911)
Spiked river water	'Middle sand' with some clay	167	>66 (calculated with uranine)	1.3–18	No *Escherichia coli* found in 100 mL infiltrate, but spiked phages found in the infiltrate	Schübel (1936)
Primary effluent after sedimentation (after dilution with genuine ground water the filtrate contained only 4.5–7.5% infiltrate)	'Gravel embedded in fine sand'	1,800	80 (calculated with uranine)	17.5–45	No *E. coli* in 100 mL (as titer) after 24 months infiltration	Knorr (1951a)
River water	'Middle sand' with some clay	200	64 (calculated with uranine)	1.8–7.5	No *E. coli* in 165 mL after 200 m but *E. coli* present in 50 mL after 50 m distance	Knorr (1951b)

percolated with suspensions of Poliovirus 1 and Echovirus 1, Wang *et al.* (1981) established a direct relationship between virus removal and column retention time, a relationship that fitted well with the predictions of the CFT (Schijven & Hassanizadeh 2000). Pang (2009) found a strong inverse relationship between the removal of viruses and PWV.

In a number of cases, even low PWVs yielded rather poor virus removal. At PWV of 0.33–0.56 m/d, Van der Wielen *et al.* (2008) observed adsorption-removal rates of 0.02 and 0.07 $\log_{10}$/d, respectively, for the phages MS2 and φX174 (0.06 and 0.08 for adsorption plus inactivation-removal, respectively). Pang (2009) has reviewed the removal rates ($\log_{10}$/m) of 86 viruses, including phages, reported in the literature. Among experiments conducted in sandy aquifers, two out of five would have undergone a removal of less than 5 $\log_{10}$ over 50 days. In gravel aquifers the ratio was six out of 24, and in karst aquifers one out of nine. Such removal degrees are deemed insufficient if RBF was the only pathogen-removing procedure to obtain drinking water from raw water (WHO 2008). It is therefore important to find conservative estimates of the relationship of PWV to virus removals since in many locations underground passage of surface water is now the only procedure for eliminating pathogens to a tolerable level, for example, in Berlin (Grohmann & Petersohn 2000).

The present study had the following goals:

- To isolate a phage with a long survival time and a small adsorption tendency to coarse sand as a poor adsorbent

matrix. This phage was to be used to establish a quantitative relationship between its removal by filtration in coarse sand and the PWV used during infiltration.

- Using observed removal data, a mathematical model was to be constructed to estimate PWV-dependent virus removal expected after underground passage of 50 days.

MATERIAL AND METHODS

Properties of the sand and of the percolated water

Clay-free, coarse sand used in all experiments has been described elsewhere (Bauer *et al.* 2011). Tap water of the city of Berlin was used for the percolation. This water is not chlorinated and had a conductivity of 705 μS/cm and a pH of 7.3.

Isolation and characterization of the phage strain 241

Among many others phages, the phage strain 241 was isolated from the Teltow River, a fecally polluted stream in Berlin. One of the two criteria for its selection was that it should have long survival in a water-saturated sandy environment. To test for survival, the isolates, raised in *Escherichia coli* strain WG5, were suspended in sterilized water-saturated sand, 50 mL of the slurry was placed in a column, and the column outlet plugged. At different times, up to 1 mL of gravity-obtained eluate was collected and the phage concentration determined. The second criterion, low adsorption to coarse sand, was estimated by sorption isotherm experiments. A 10 mL phage suspension containing approximately 10^3 phages/mL was incubated with 1 g coarse sand at room temperature in a shaker. After 2 hours incubation, the phages remaining in the liquid phase were determined. The sand type used was identical to the one used in the column experiments presented in this paper. Among 61 isolates tested for survival and adsorption, coliphage 241 was chosen for its long survival in the sandy environment and low adsorption to coarse sand. The long survival should mean that the removal observed after its passage through sand columns was attributable mostly to adsorption to the sand particles. The low adsorption tendency should ensure that the results and conclusions obtained with this phage were conservative with respect to other viruses.

Detection of coliphages

For the analysis of coliphage 241, USEPA Method 1601 (2001) with the *E. coli* strain ATCC700078 as indicator strain was used, but instead of taking the agar formula recommended there, double-concentrated modified Scholtens-Soft-Agar (DIN-EN-ISO-10705-2 2002) was added to the sample before plating. The detection limit of the method was 1 plaque-forming unit (pfu)/100 mL.

Column experiments

Three different series of column experiments were carried out. The experiment series, which differ with respect to column length, PWV, and phage input, are outlined below.

Filtration of phage 241 at a PWV of 1 m/d along a 7 m filtration path

Six acrylic glass columns with a diameter of 15 cm and a length of 120 cm were filled with a slurry of the sand described above. After upwards saturation, tap water was percolated top down at a filtration velocity of 0.30 m/d corresponding to a pore velocity of 1 cm/d for 2 days to stabilize the hydraulic conditions. The columns were connected in series with silicon tubing of an internal diameter of 4 mm, so as to allow top down flow in all columns. Hollow needles perpendicularly placed into the column walls and ending at the length axis of the column allowed sampling thanks to small faucets located at the outer extreme of the needles. Sampling was also carried out at the outlet of each column. In this way, the following filtration distances were monitored daily: 30, 50, 70, 90, 120, 180, 240, 300, 360, 480, 600, and 720 cm. Sampling volumes of 100 mL were taken slowly, each sampling taking 30–40 minutes in order to avoid too great a disturbance of the columns' flow rate. The reservoir containing the phage suspended in tap water was located approximately 4 m above the entrance of the columns and was connected with a silicon hose with the first column. The PWV of 1 m/d was maintained by the hydraulic pressure exerted by the reservoir and was regulated by a peristaltic pump at the outlet of the last column. The pump's function was not to increase, but to slow down the flow rate imposed by the

hydraulic pressure. The phage concentration in the reservoir was monitored daily and a concentration of 10^5–10^6 pfu/100 mL was maintained, adding more phages if required.

Statistical analysis

The normality of the groups of values for each filtration distance was checked using three measures: skewness and two normality tests – Kolmogorov–Smirnov (K–S) and Shapiro–Wilk (S–W). In addition, the $\log_{10}$ transformed values were checked in the same manner. The K–S and S–W test results showed a statistically significant departure from normality using both the raw and transformed data ($p < 0.05$). However, the log transformed values showed greatly reduced skewness (0.052 vs. 5.622). This indicates that log transformation does improve the normality, which was also evident in corresponding normal-probability plots. Thus, the log transformed data showed a better approximation to normality than the untransformed data. The subsequent analysis for significance was carried out with the log transformed values using the Tukey multiple comparison test.

Filtration experiments with phage 241 at different PWVs

The inlets of acrylic glass columns with a length of 120 cm as described for the previous experiment, filled and treated in the same manner, were connected to a reservoir containing a phage suspension. The columns were run in parallel. Different PWVs (4, 2, 1, 0.5, 0.25, and 0.1 m/d) were adjusted with peristaltic pumps at the outlets of the columns, and sampling was carried out daily. In the first series of the experiment, the duration was 15 days (Experiment A in Table 2 for PWVs of 4, 2, and 1 m/d). In the second series, the duration was 28 days (Experiment B in Table 2 for PWVs of 1, 0.5, 0.25, and 0.1 m/d). To keep the same sample size for all PWVs within one series of experiments, we took the single observations from days 4 to 15 in Experiment A (12 values) and from days 14 to 28 in Experiment B (15 values) for calculation of the average removals.

Release of phage 241 from sand columns after increasing PWV

After the experiment for estimating the dependence of the phage removal on the PWV was finished, the columns were run without phage input at a PWV of 2.6 m/d with tap water over 26 days. The exfiltrates were collected in a pool every 24 hours, and the phage concentration in these pools was determined. The number of plaque-forming units eluted was calculated from the concentration in the 12 L pools collected daily. During Saturdays and Sundays the rinsing was stopped and the columns were held under water-saturated conditions, so that the active rinsing period was 18 days.

Table 2 | Average phage removals at different PWVs after 1 m infiltration (same experiment as in Figure 2)

	PWV (m/d)	Averages of the concentrations in Figure 2 (pfu/100 mL)		Removal/m $-\log_{10}(c/c_0)$
Experiment A	Input	38,987	± 47,715	–
	4	9,705	± 18,760	0.6
	2	5,203	± 4,635	0.87
	1	4,055	± 5,210	0.98
Experiment B	Input	1,289,545	± 905,813	–
	1	125,237	± 114,989	1.01
	0.5	5,788	± 4,911	2.35
	0.25	533	± 427	3.38
	0.1	1.3 (mostly under detection limit)		6

Modeling of phage removal during sand filtration

The removal of biogeochemical components during bank filtration has been the subject of several modeling studies. An example study is presented by Holzbecher (2006) and Holzbecher *et al.* (2006) showing the interaction between dispersion and degradation processes. Here we focus on adsorption as the major process for the removal of phages during sand filter passage.

Numerical models for transport, ad- and desorption as well as removal processes are based on the following set of differential equations:

$$\theta \frac{\partial c}{\partial t} = \nabla \alpha_L v \nabla c - v \nabla c - \theta \lambda_f c - \theta \kappa_f c + \kappa_s s$$
$$\rho_b \frac{\partial s}{\partial t} = -\lambda_s s + \theta \kappa_f c - \kappa_s s \quad (1)$$

with population concentration c in the fluid phase per unit volume, and s at the solid phase per unit mass; v the Darcy velocity, α_L the longitudinal dispersion length, θ

volumetric moisture, ρ_b the bulk density, λ_f and λ_s the degradation/deactivation rates connected with the different phases, κ_f and κ_s the transfer coefficients between the fluid phase on one side and the solid phase on the other side. An equivalent formulation concerning bacteriophage migration in saturated dune sand was given by Schijven & Šimunek (2002). An extended model with two sorption-sites was presented by Holzbecher & Dizer (2006).

Chatterjee & Gupta (2009) have presented evidence for nonlinear removal if the colloid is present in aggregates of different sizes. According to Redman *et al.* (2001), even mono disperse suspensions of colloids may consist of different populations with sufficient heterogeneities, in size or electrostatic charge, to cause different adsorption kinetics (Redman *et al.* 2001). In these cases the filter coefficient λ of the colloid suspension is not a fixed value, but follows a distribution. Tufenkji & Elimelech (2003) have analyzed several distribution alternatives and found that the power law distribution fits best to many published results of adsorption kinetics.

In the description below we follow Tufenkji & Elimelech (2003); that is, we consider the steady state under neglect of the solid phase and also neglect dispersion. The system (Equation (1)) then reduces to:

$$-v\nabla c - \theta\lambda c = 0 \tag{2}$$

using $\lambda = \lambda_f$; or in terms of the real velocity $u = v/\theta$ for a single space variable x:

$$u\frac{\partial c}{\partial x} + \lambda c = 0 \tag{3}$$

with the solution:

$$c(x) = c_0 \exp(-\lambda x/u) \tag{4}$$

for the typical boundary value problem. Equation (4) shows that an exponential decrease of concentrations with distance from the inlet has to be expected with a 'decay constant' λ/u; that is, for lower velocities the removal of phages is higher than for higher velocities. We will examine the validity of this theoretical expectation using the observations from the laboratory experiments.

Following Tufenkji & Elimelech (2003) further, we examine the situation of a heterogeneously distributed deactivation rate coefficient $p(\lambda)$. For the fluid phase concentration the following formula results:

$$c(x) = c_0 \int_0^\infty \exp(-\lambda x/u)p(\lambda)\,d\lambda \tag{5}$$

For $p(\lambda)$ we use the following functions:

- normal distribution with mean value $\bar{k}$ and standard deviation σ;
- bimodal distribution with a low value k_{low} and a high value k_{high}, and corresponding standard deviations σ_{low} and σ_{high}, and fractions f_{low} and f_{high};
- power law distribution with coefficient A and exponent $-b$: $p(k) = Ak^{-b}$.

The computations, described in the following 'Results and discussion' section, based on Equation (5), were performed using MATLAB®.

RESULTS AND DISCUSSION

Electron microscopic characterization and survival of phage 241 in coarse sand

Phage 241 was chosen from 61 strains isolated following the procedure mentioned in 'Material and methods'. This phage had the most appropriate combination of long survival and poor adsorption to coarse sand. The particles of strain 241 were round, with a diameter of *c.* 25 nm, and had no tail (Figure 1, inset). When suspended in a slurry of sterile sand and tap water, the phage concentration slightly increased during the first day presumably due to a disintegration of clumps, and afterwards started to decay. The decay rate then became approximately linear on a log scale, and the decay time (T_{90}), expressed as the time elapsed for a decrease of one $\log_{10}$ unit, was calculated to be 40 days. This phage was used for all further experiments to obtain conservative estimates of removal by sand filtration.

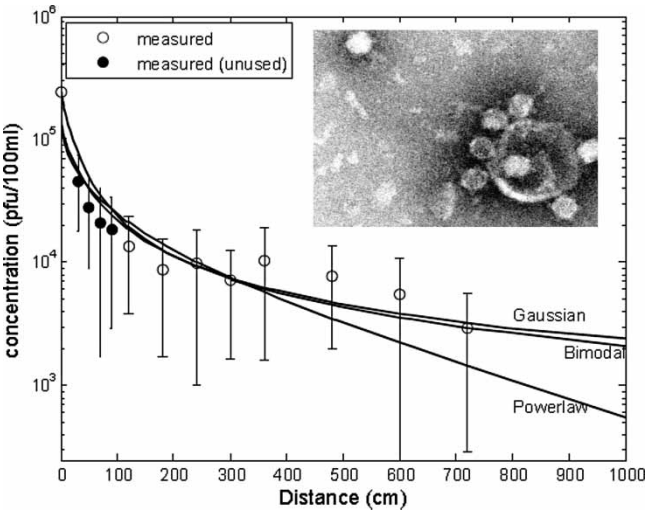

Figure 1 | Removal of the bacteriophage 241 during infiltration through six columns connected in series over 39 days at 1 m/d PWV. The circles, open, and filled are the averages of 32 observations, with the standard deviations depicted as bars. The continuous lines correspond to the simulated values obtained with the models mentioned below. Only the open circles were used for the calibration of the model in order to obtain a conservative simulation. Inset: electron micrograph of the bacteriophage 241. Lower right corner: seven viral particles attached to a larger debris particle; upper left corner: single virion.

Removal of the phage 241 along a filtration path of 7.2 m in coarse sand at a PWV of 1 m/d

A suspension of phage 241 was applied to the inlet of six columns connected in series at a PWV of 1 m/d over 39 days and samples were taken daily. The average concentrations of phage 241 found at different infiltration distances are shown in Figure 1 (circles). The average concentrations were corrected for the decay of the phages during the infiltration period, assuming the T_{90}-value of 40 days obtained in the survival experiment. To make sure that the concentration measurements at all distances had the same number of single observations, the averages of Figure 1 were built starting with the values obtained 7 days after initiation of the experiment. Consequently, each average consists of 32 single observations (30 at distance of 240 cm).

The phage showed an initial removal of approximately 1.2 $\log_{10}$ during the first 120 cm (corresponding to approximately 1 $\log_{10}$/m) of infiltration. During further infiltration the removal was much lower, amounting to approximately 0.72 $\log_{10}$ between 120 and 720 cm, or 0.12 $\log_{10}$/m (Figure 1). However, according to the statistical analysis outlined above, the averages at filtration distances greater than 90 cm were not significantly different among themselves ($p > 0.05$), with the exception of the value at 120 cm vs. 300, 600, and 720 cm, respectively ($p < 0.05$).

A fast initial rate of viral removal, followed by a slower rate at larger distances of the filtration path, has been frequently reported (Schijven & Hassanizadeh (2000) and the literature therein). Work by Schijven (2001) showed that soil heterogeneities might be responsible for this effect, but in our case the columns were used only 2 days after they were packed and it is therefore very improbable that build-up of heterogeneities in the columns was the cause of the nonlinear behavior of the phage removal. The removal results of this experiment were used for constructing the model below.

Estimation of the relationship between PWV and virus removal

The removal of the phage 241 in columns run at different PWVs is shown in Figure 2. Removal after 1 m infiltration was 0.6 $\log_{10}$, at a PWV of 4 m/d. Decreasing the PWV to 1 m/d did increase the removal degree, but only moderately, from 0.6 to 0.98 $\log_{10}$ units. Further decrease of the PWV from 1 to 0.1 m/d caused a considerable increase of the removal from 1.01 to 6 $\log_{10}$ units (Table 2). As the columns were 1 m long, the column run at a PWV of 0.1 m/d yielded phage concentrations no sooner than 10 days after the start of the experiment. Even after this time, the exfiltrate concentrations of the phage were below the detection limit (1 pfu/100 mL) in more than half of the samples. Therefore, at 0.1 m/d only a tentative $\log_{10}$-removal, averaging 6 $\log_{10}$ was estimated, by considering all concentrations below the detection limit as 1 pfu/100 mL, compounding all concentration values between days 14 and 28 and dividing them by the total volume of all samples. This calculation probably underestimates the true removal.

Simulation of the phage removal vs. PWV by a mathematical model

First we examined the 6 m column experiment, in which a velocity of 1 m/d was used. Using all averages (circles) shown in Figure 1, removal curves were simulated after applying all three distributions assayed – Gaussian, bimodal,

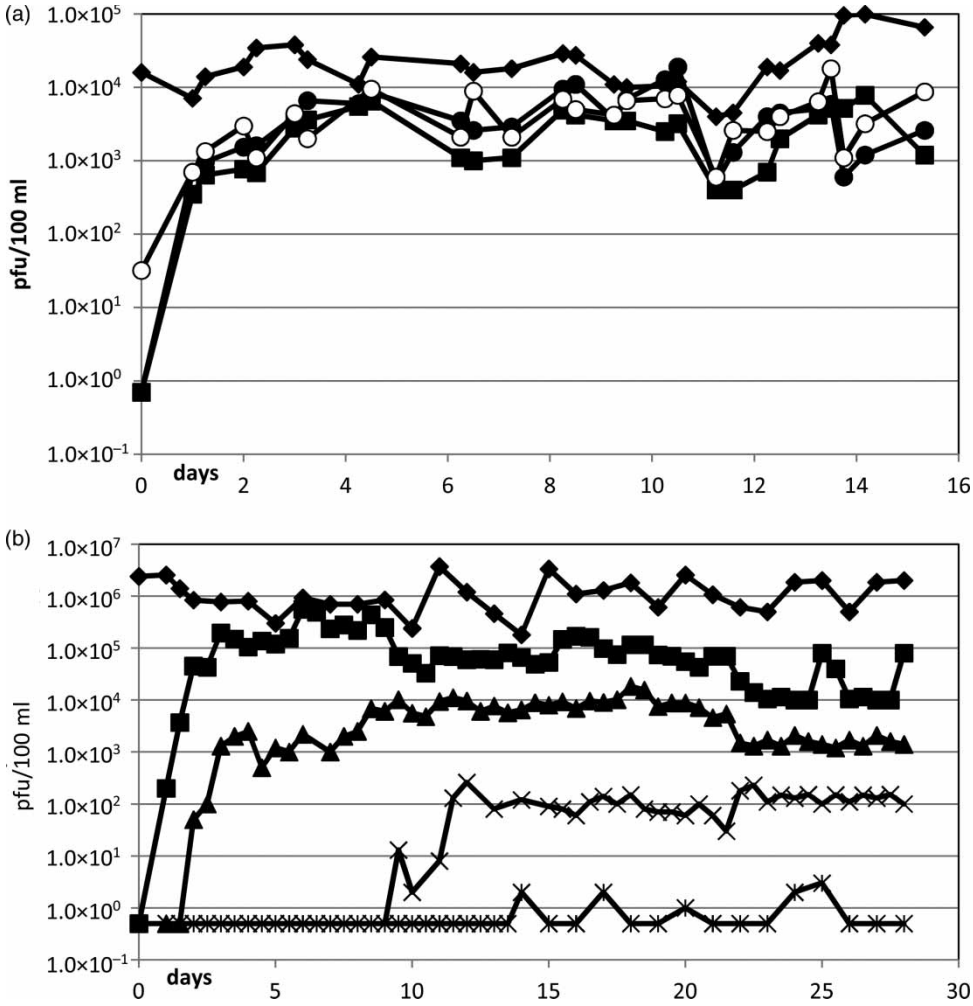

Figure 2 | Phage removal after 1 m infiltration distance in columns run at different PWVs; symbols in (a) (○) 4; (●) 2; (■) 1 m/d. Symbols in (b) (■) 1; (▲) 0.5; (×) 0.25; (✳) 0.1 m/d. In (a) and (b) the input symbol is (♦).

power law – to $p(\lambda)$. All three combinations used showed overestimations of the removal after 2 m infiltration (not shown) when compared with the empirical results. To obtain more conservative removal predictions at infiltration distances beyond 2 m, we fitted the parameters using the points symbolized with empty circles in Figure 1, i.e. the measurements obtained at larger infiltration distances. The results of the parameter estimation from this calibration are given in Table 3. The coefficient A in the power law distribution can be calculated from s by $A = 0.455s^{0.2}$. The best-fit curves for the three distributions are plotted against measurements in Figure 1. The least squares residuals, listed in the last column of Table 3, show that the bimodal distribution is the nearest approach.

The calibrated model was first used for a theoretical evaluation of velocity-dependent removal vs. distance. Velocities were selected in the range between 0.25 and 8 m/d. We show the results for the bimodal distribution in Figure 3.

In a second step, the model, calibrated for the velocity of 1 m/d, was used to compare modeled results at different PWVs with the results experimentally shown in Figure 2 and Table 2. Figure 4 shows the results for the bimodal distribution of the removal. The model makes acceptable

Table 3 | Parameters for distribution functions estimated from the 6 m column experiment; rate constants in physical unit (1/s)

Distribution function	Parameters	Residual
Gaussian	$\lambda_{avg} = 4.00 \times 10^{-6}$	54.37
Bimodal	$\sigma = 4.94 \times 10^{-5}$ $\lambda_{low} = 7.7 \times 10^{-6}$ $\lambda_{high} = 8.729 \times 10^{-4}$ $\sigma_{high} = 2.55 \times 10^{-4}$	15.13
Power law	$\sigma_{low} = 5 \times 10^{-5}$ $f_{low} = 0.3$ $f_{high} = 0.7$ $\lambda_{min} = 4 \times 10^{-6}$ $\lambda_{max} = 0.441$ $b = 0.981$ $s = 3.56 \times 10^{-3}$	86.39

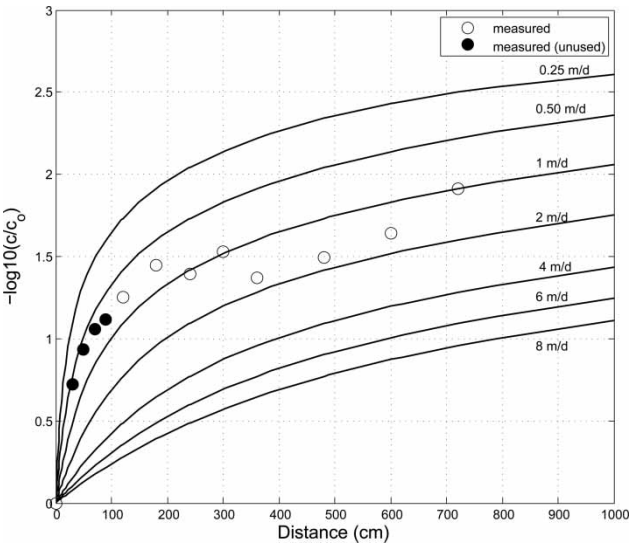

Figure 3 | Lines: simulated removals (as $-\log_{10}(c/c_0)$) for different PWVs when a bimodal distribution of $p(\lambda)$ is assumed, calculated with the parameters of Table 3. The numbers adjacent to the lines indicate the PWV. Circles: averages of the experimental removals shown in Figure 1.

predictions for velocities equal or higher than 1 m/d, but for velocities lower than 1 m/d the model grossly underestimates the removal. While this situation suggests that the model needs refining in order to explain the underestimation of removal at PWVs smaller than 1 m/d, the model's predictions are conservative and confer an extra margin of safety.

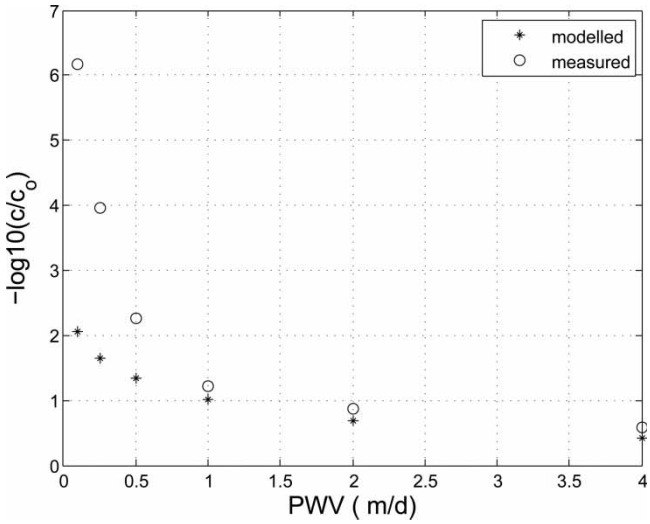

Figure 4 | Simulated (*) and measured (○) phage removal after 1 m filtration, expressed as $-\log_{10}(c/c_0)$. The measured removals are those contained in Table 2; the simulated removals were calculated on the basis of a bimodal distribution with the parameters of Table 3.

The removal at a given PWV after a particular infiltration period t can now be estimated by calculating the distance s covered during the infiltration period t chosen

$$s = \mathrm{PWV}t$$

Figure 5 depicts the phage removal (as $-\log_{10}(c/c_0)$) expected after 50 days infiltration at several PWVs. The curves are the result of the addition of both the removal by adsorption, as yielded by the model, and the removal due to the decay of a T_{90}; i.e. 1.25 $\log_{10}$ units in 50 days, observed with the incubation of the phage in water-saturated sterile sand. The results for two distributions of $p(\lambda)$ have been plotted, the one for the bimodal and the other for the power law distribution. The curve corresponding to the Gaussian distribution is similar to the bimodal one (not shown).

The curve for the bimodal distribution in Figure 5 shows that for a PWV of 1 m/d, the infiltration would remove viruses by a factor of approximately 10^{-4}. Higher PWVs result in less removal.

This result is approximately in line with the findings of Van der Wielen *et al.* (2008) if their results are converted as removal degree per day and extrapolated to a 50-day residence time.

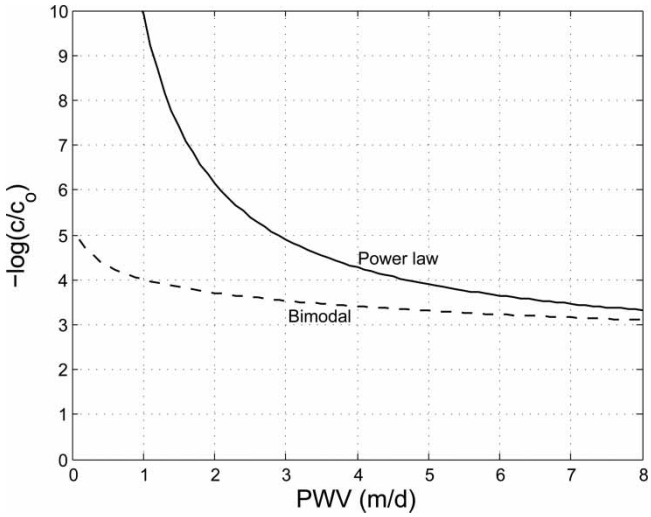

Figure 5 | Simulated removals, as $\log_{10}(c/c_0)$, of the phage 241 after infiltration at different PWVs over 50 days. Solid line: removal according to a power law distribution of $p(\lambda)$. Dashed line: removal according to a bimodal distribution of $p(\lambda)$. Distribution parameters of Table 3 were used. To the removals due to adsorption, 1.25 $\log_{10}$ was added due to the decay of the phages after 50 days in a sterile sandy environment ($T_{90} = 40$ days).

WHO (2008), under the assumption of a concentration of 10 virus/L in the raw water (with rotavirus as a model organism), recommends (for high income countries) drinking water treatments reduce the viral concentration by a factor of $10^{-6.495}$. The resulting concentration of $10^{-5.495}$ virus/L corresponds to a disease burden of 10^{-6} disease adjusted life years per year, if 1 L water is consumed per day (WHO 2008). According to the results of the bimodal distribution shown in Figure 5, slow sand filtration (SSF) would not meet WHO requirements if carried out as the sole water treatment procedure. Either the underground residence time of the water would have to be extended beyond 50 days, or additional water treatment procedures would have to be implemented. Alternatively, the concentration of viruses in the surface water taken for infiltration would have to be reduced below 0.1 virus/L.

However, the removal degrees shown in Figure 5 have to be considered as a conservative estimate because other causes of removal (e.g. content of clay materials of the soil, known to bind viruses much better than sand (Sobsey *et al.* 1980), predation or enzymatic degradation by fungi or bacteria present in the soil) have been purposely avoided in the experimental design of this paper, and therefore have not been considered in the model. The removal of viruses by RBF carried out in a natural environment is probably much more efficient than that observed here with coarse sand free of clay, and with water poor in nutrients taken for elution. Sprenger et al. (2014) has followed the removal of indigenous somatic coliphages during bank filtration in the river Yamuna in Delhi (India). They found that the phages were removed by 3.3 $\log_{10}$ after only 1 m infiltration and by 4.31 $\log_{10}$ after 3.8 m. After approximately 119 days infiltration to a distance of 50 m the removal factor was higher than 10^6. In that study the soil contained 1% of clay minerals. Natural microbial flora in the river bank presumably also had a positive influence on the removal of the phages. However, when artificial sand filters are operated for the first time, in which a soil flora is not yet present, the limitations of removal shown in Figure 3 should be taken into account.

Estimation of the detachment constant k_{det} of the phages from the sand

An important question during SSF and RBF concerns the detachment of viruses associated to the soil particles, especially when the PWV increases and re-adsorption of the released virus is slowed down or absent. A fraction of

Table 4 | Washing down of phages adsorbed to the columns of Figure 6

	Column in Figure 6				
	Column A	**Column B**	**Column C**	**Column D**	**Average**
No.[a]	9.7×10^8	5.1×10^8	2.5×10^8	1.0×10^8	–
N/day[b]	6.9×10^4	6.8×10^3	2.1×10^3	1.0×10^3	–
N/day (in % of no.)[b]	0.0071%	0.0013%	0.0008%	0.0010%	0.0026%
k_{det} (day^{-1})	7.1×10^{-5}	1.3×10^{-5}	8.3×10^{-6}	1.0×10^{-5}	2.56×10^{-5}

[a]Number of phages present in the column at time 0.
[b]Average number of phages washed down from the column per day.

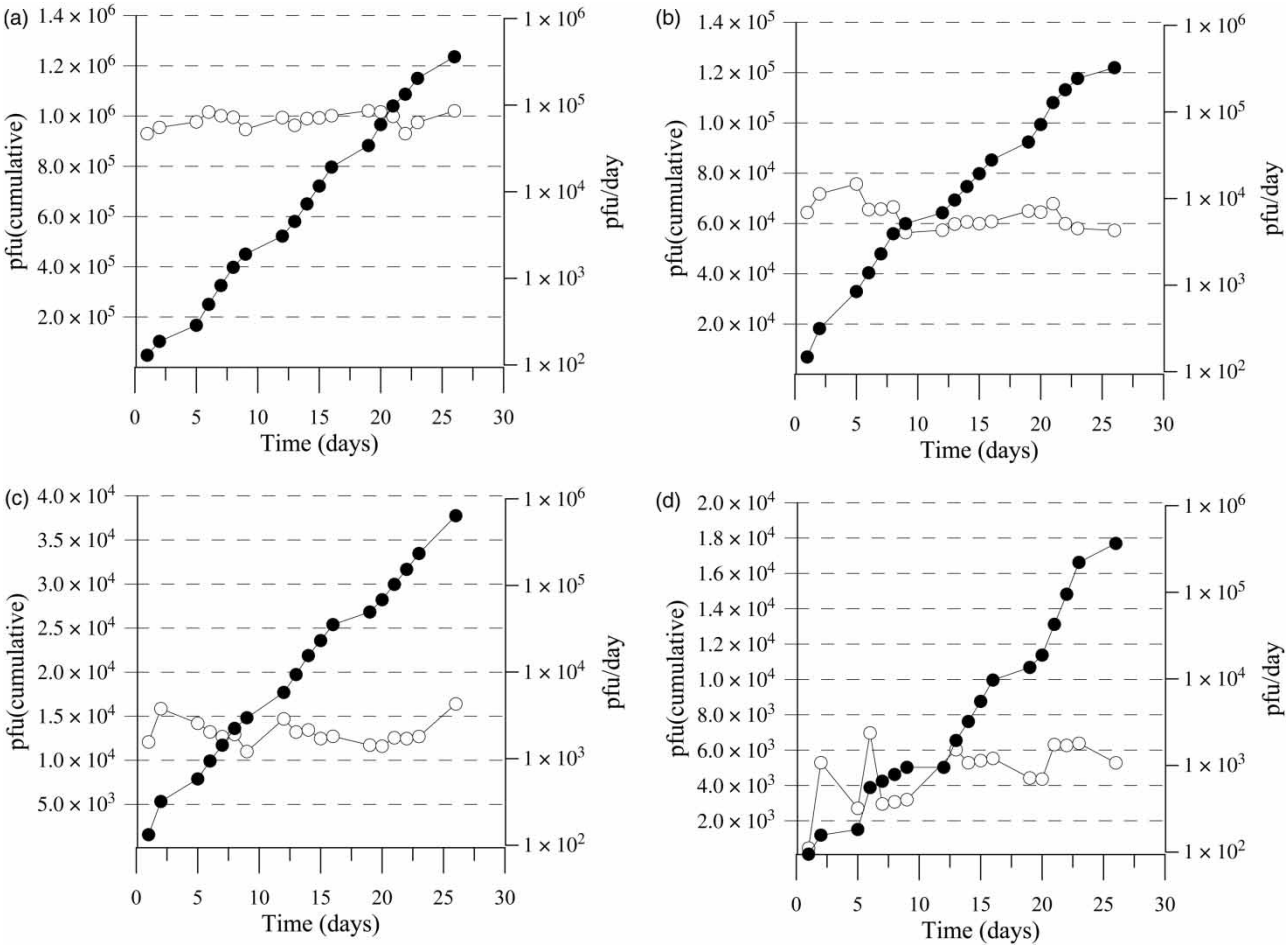

Figure 6 | Phages recovered during rinsing the columns shown in Figure 2(b) (which had been percolated with phages at 1 (a), 0.5 (b), 0.25 (c), and 0.1 (d) m/d over 28 days) with tap water free of phages at a PWV of 2.6 m/d. Each column was connected to a reservoir, and PWV in all four columns adjusted to 2.6 m/d. The rinsing was interrupted at weekends, so that over the 26 days of the measuring period the columns were running for only 18 days. Symbols: (○) number of pfus eluted per day; (●) number of eluted pfus cumulated up to the time of the measurement. The concentrations of the phages in the exfiltrates have been corrected for die-off assuming a T_{90} of 40 days.

the viruses hitherto adsorbed to soil might then be released and be eluted with the filtrate. Derx *et al.* (2013) have simulated PWV in river banks during raising of the river water level due to flooding. They concluded that removal of the viruses might be seriously impaired during flooding due to higher PWV, which might rise to 25 m/d.

We have quantified the escape of phage 241 into the liquid phase if PWV increases above 1 m/day, a PWV above which phage attachment is only moderate (Table 2). To achieve this, the columns of Figure 2(b), which had been run with a phage suspension over 18 days, were subsequently run at a constant PWV of 2.6 m/d without addition of phages over another 18 days, and the escaped phages in the exfiltrate were determined.

The average percentage of phage 241 released per day was 0.0026% of the phages attached to the sand (Table 4). According to this small percentage, the number of phages attached to, and the daily rate of phages detached from, the columns remained practically constant over the 18 days of the experiment (Figure 6).

The data allow estimation of the detachment constant governing the release of the phages from the sand, applying the equation: $dn/dt = k_{det} \times No$, where dn/dt represents the number of phages released per day and No the total number of phages adsorbed to the sand. Values for k_{det} of 8.3×10^{-6} to 7.1×10^{-5} were obtained, depending on the column, yielding a mean for the four columns of 2.6×10^{-5} (Table 4). Consequently, if a sand

filter, or a river bank, hosts 10^5 sand-associated viruses, only approximately 2.6 viruses/day will be released into the liquid phase.

Other studies have also found attached viruses to be very slowly released (Bales *et al.* 1997) and generally the attachment constants to be much higher than the detachment ones, at least in the first meters of infiltration (Schijven & Šimunek 2002; Schijven *et al.* 2002). This situation allows the design of strategies for minimizing viral breakthrough during sudden increases of PWV or even for the operation routinely followed in RBF water works. Generally it will consist of halting the abstraction of water from the wells for a certain period of time in order to reduce or stop altogether the PWV, allowing even viruses with a low attachment constant to become associated with the soil particles. Resuming the abstraction rate will allow only a very small percentage of the viruses to reach the wells.

CONCLUSIONS

Conservative estimates predict virus removal by approximately a factor 10^4 after 50 days infiltration in sand void of clay at a PWV of 1 m/d. At higher PWVs the removal degree is lower. Removal can be acceptably simulated by a mathematical model assuming that the adsorption coefficient of the viruses follows a probability distribution. At PWVs lower than 1 m/d, virus removal might increase dramatically, but simulation by the model underestimates the removal. While the results presented indicate that the model needs refining in order to explain the underestimation of removal at PWVs lower than 1 m/d, the model's predictions are conservative and confer an extra margin of safety.

As the detachment constant k_{det} has been found for this particular phage – and has been found by others for other viruses – to be very low, an intermittent operation of the water abstraction in RBF or SSF plants, with temporal interruptions of water abstraction, is expected to effectively allow attachment of viruses during the standstill periods. Resuming water abstraction will allow detachment of only a very small fraction of the viruses.

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