Virus removal in ceramic depth filters based on diatomaceous earth

Supporting Information

Benjamin Michen [1,2], Fabian Meder [1], Annette Rust [3], Johannes Fritsch [4], Christos Aneziris [2] and Thomas Graule [1,2]

[1] Laboratory for High Performance Ceramics, EMPA, Swiss Federal Laboratories for Materials Science and Technology, Ueberlandstrasse 129, 8600 Duebendorf, Switzerland.

[2] Institute for Ceramics, Glass and Construction Materials, Technical University Bergakademie Freiberg, Agricolastrasse 17, 09596 Freiberg, Germany.

[3] Bachema AG, Ruetistrasse 22, 8952 Schlieren, Switzerland.

[4] Technology and Management Faculty, University of Applied Sciences Ravensburg–Weingarten, Doggenriedstrasse, 88241 Weingarten, Germany.

Contents

Characteristics of the ceramic filter candle

Table S-1: A compendium of the diatomaceous earth based filter candle characteristics. More details can be found elsewhere (Michen et al. 2011).

Table S-1. Characteristics of the ceramic filter candle	
Type	Depth filter
Major ingredient	Diatomaceous earth (DE; amorphous and crystalline silica > 80 %)
Length (mm)	119 ± 2
Inner diameter (mm)	35 ± 2
Outer diameter (mm)	50 ± 2
Filter weight (g)	89± 3
Porosity (v%) [1]	64 ± 6
Pore volume (ml)	86 ± 5
Mean pore diameter (μm) [1]	3.2 ± 0.2
Specific surface area (m^2/g) [2]	2.1 ± 0.1
Flux at 3 bar and 25°C (m/h)	6.8 ± 1.3
Isoelectric point in 4 mM NaCl [3]	< 2 (if any)

[1] Obtained by mercury intrusion porosimetry

[2] according to the method of Brunauer-Emmett-Teller (BET)

[3] electrophoretic measurement of zeta potential as a function of pH

Propagation and enumeration of the bacteriophages

We chose two bacteriophages, namely *Enterobacteria* phage MS2 (MS2) and *Enterobacteria* phage PhiX174 (Phi) that have been frequently used to investigate virus adsorption to surfaces. Both viruses are coliphages, i.e. they infect host bacteria of the species *Escherichia coli (E. coli)*. However, the phages specifically infect two different *E. coli* strains. The bacteriophages MS2 (DSM 13767) and Phi (DSM 4497) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Germany) and some of their properties are listed in Table S-2.

Table S-2. Properties of the bacteriophages MS2 and Phi

	MS2	**Phi**
Family [1]	Leviviridae	Microviridae
Genus [1]	Levivirus	Microvirus
Species [1]	*Enterobacteria phage MS2*	*Enterobacteria phage ΦX174*
Nucleic acid [1]	ssRNA	ssDNA
Host [2]	*E. coli*; F-specific	*E.coli, Salmonella typhimurium*;
Burst size * [3]	100 - 20,000	73 - 441
Morphology [1]	Non-enveloped; icosahedral d≈25 nm	Non-enveloped; icosahedral d≈26 nm
Molecular weight (Mega Dalton) [4,5]	3.6	6.2
IEP [mean ± standard deviation] [6]	3.5 ± 0.6	6.2 ± 1.6

References: [1] ICTVdB 2009; [2] Leclerc et al. 2000; [3] Ackermann and DuBow 1987; [4] Overby et al. 1966; [5] Sinsheimer 1959; [6] Michen and Graule 2010. * Burst size: A measure of viral replication expressed as infectious units generated per infected cell. For example, a burst size of 1,000 would indicate that 1,000 progeny viruses were generated by each initially infected cell.

The enumeration took place by counting plaques on lawns of bacteria in Petri dishes. The bacteria strains used for the specific enumeration of the phages MS2 and ϕ were obtained from the American Type Culture Collection (ATCC): E. coli HS(pFamp)R/Famp (ATCC 700891) and E. coli CN-13 (ATCC 700609) for MS2 and Phi, respectively. Dilution series of the phage suspensions were performed in 0.02 M $MgCl_2$/0.15 M NaCl solutions in 10-fold steps. A sample volume of 4.5 ml containing phages was mixed at 25°C with 0.5 ml of phage-specific host culture, grown at 37°C for 4 h in 30 g l^{-1} of sterile tryptic soy broth (TSB; Oxoid, UK), and placed into a water bath at 46°C for two to three minutes.

Subsequently, 5 ml of a phage-specific nutrient agar (46°C) was added into the test tube. The ingredients were mixed prior to pouring them into the Petri dish (9 cm in diameter) and finally incubated at 37°C for 12 to 16 h. The concentration of agar may alter the enumeration results due to the change in viscosity (Abedon and Culler 2007). However, unequal concentrations of agar were chosen for the two phages as the plaque size of Phi and MS2 showed a great variance which made plaque counting impractical at identical agar concentrations. Reliable results were obtained for Phi with 80 g l^{-1} tryptic soy agar (TSA) (Oxoid, UK), revealing a turbid plaque of a diameter in the range of $3 < d < 10$ mm. MS2 produced smaller, clear plaques between $1 < d < 5$ mm at 40 g l^{-1} TSA supplemented by 15 g l^{-1} TSB to balance the nutrient concentration. Upper and lower limits of PFUs per plate were considered between 15 and 150 as well as between 8 and 80 for phage MS2 and Phi, respectively. The error of plating was investigated as relative error of numerous parallel samples and found to be 20%. Further platings were performed once and are reported using the established error. To assure the test's accuracy, the entire dilution series of the water sample was always plated and evaluated for conformity.

The propagation of bateriophages was carried out with two different strains of *E. coli* obtained from the DSMZ. The two strains were DSM 5210 and DSM 13127 for the replication of F-specific phage MS2 and the somatic phage Phi, respectively. All bacterial cultures produced in this work were grown for 20 h in an incubator at 37°C. From these overnight cultures, 100 µl of each was inoculated in 50 ml of TSB and incubated for 4 h at 37°C in order to obtain bacterial working cultures that emerged in an exponential growth phase. Such cultures showed typical concentrations, expressed in colony forming units (CFU), of about 10^9 CFU ml^{-1}. With the goal of removing contaminating agents from the future virus stock, two principal approaches have been used with which to modify the standard propagation procedure:

- Washing: The host culture (exponential growth phase) was washed to remove low molecular agents of the complex TSB medium in which the

bacteria had been grown. To do this, the 4 h culture was centrifuged (3000g, 30 min) and the greenish-yellow supernatant containing the growth medium was discarded. The bacteria were re-suspended in 50 ml salt solution with 0.02 M $MgCl_2$ and 0.15 M NaCl and placed back into the water bath at 37°C for phage inoculation.

- Dialysis: This step was added following the actual propagation procedure. The virus suspension was dialysed using a simple ready-to-use device equipped with a 100 kDa cellulose ester membrane (Float-A-Lyzer G2, Spectrum Laboratories Inc., USA) that also enabled the removal of dissolved salts. Therefore, 10 ml of virus suspension were subsequently dialysed twice against 1000 ml of pure water for 5 days at 4°C.

The host culture contained about 10^9 CFU ml^{-1} as no loss of bacterial vitality was observed following the washing step (data not shown). Next, the bacteriophages were added at 37°C for MS2, and at 46°C for Phi. The phage to bacterium ratio was adjusted to be close to unity and any suspension was incubated for 3-4 h. Any prepared virus stock was cleared of bacterial debris by filtration through sterile 0.45 and subsequently 0.2 μm syringe filters. Finally, aliquots of virus suspensions were stored at 4°C.

Wild type phage - the ‘Siphophage’

While working with bacteriophage Phi, different plaque morphologies in a single Petri dish had been noticed, as shown in Figure S-1. The larger plaques appeared turbid whereas the smaller ones were clear. The white dots found inside the larger plaque were responsible for the turbid appearance and arose from phage-resistant bacteria colonies. As such colonies were found in a random fraction of plaques in a single Petri dish, this may have indicated that two different virus types were present which possessed diverse phage-host behaviour. Further, these two morphologies could be correlated with differential retention behaviour in filtration units which led to the hypothesis that two virus types were present in the stock.

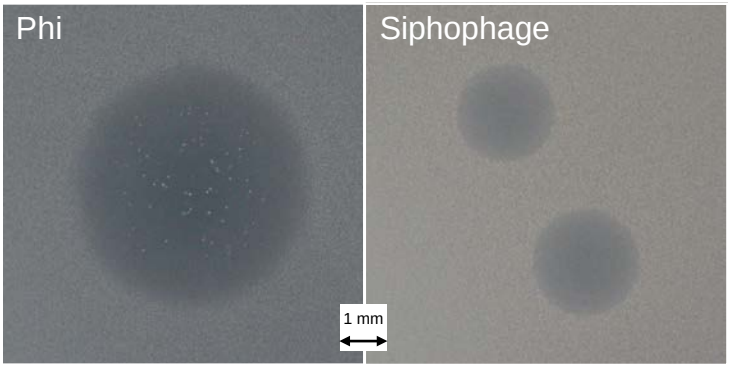


Figure S-1. Different plaque morphologies found in contaminated ϕ virus stocks.

Plaques of both morphologies were isolated from a single Petri dish and propagated independently to obtain pure virus stocks containing either of the two virus types. When the titres of these virus stocks were determined, the two plaque morphologies could be successfully separated and hence appeared to be isolated in each virus stock. Yet, the difference in retention behaviour was observed with tests using either of the two virus stocks. This provided evidence for the assumption that the two virus types co-existed in the primary virus stock. This could be due to mutations of the phages altering their properties or another phage species which had contaminated the primary virus stock. These two phage stocks were then subjected to characterisation using transmission electron

microscopy (TEM) which revealed two clearly diverse bacteriophage morphologies: the expected morphology of Phi as well as an unexpected complex morphology of a tailed bacteriophage, given in Figure S-2. As the phage's morphology differed greatly from that of Phi, this could not be explained solely by mutation and thus led to the identification of a viral contaminant.

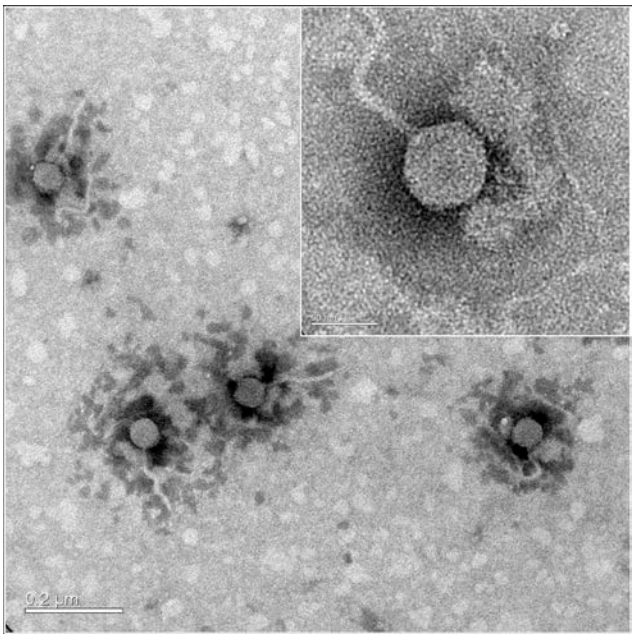


Figure S-2. TEM images of the contaminant termed Siphophage.

This unknown contaminant showed an icosahedral head with an estimated diameter of 60 nm and a non-contractile, flexible tail with a length of about 160 nm and a width of 10 nm. Due to the morphology, this phage was assigned to the T-series of bacteriophages fitting best with the dimensions of the *Enterobacteria phage T1* of the family Siphoviridae*.* This species has been known to contaminate laboratories as it remains stable there for weeks in the form of aerosols (Calendar 2006). On the other hand, this viral contaminant may have derived from a temperate phage, such as the *Enterobacteria phage λ*, which has been introduced into the genetic code of some *E.coli* strains (Calendar 2006). The prophage might have been triggered to become virulent whilst a number of propagation methods were performed and could have been established in

parallel to the phage Phi. Some properties of the phages that potentially could be assigned as the contaminant are listed in Table S-3. However, no other properties could be identified which allowed to us to distinguish further between the two candidates, and hence the viral contaminant is referred to as the 'Siphophage' as both candidates belong to the family of Siphoviridae.

Table S-3. Potential candidates for the viral contaminant

	λ	**T1**
Family [1]	Siphoviridae	Siphoviridae
Genus [1]	"λ-like viruses"	"T1-like viruses"
Species [1]	*Enterobacteria phage λ*	*Enterobacteria phage T1*
Nucleic acid [1]	dsDNA	dsDNA
Host [2,1]	*E.coli, Salmonella typhimurium;* somatic	Bacteria, genus *Escherichia*
Burst size [3,4]	50-100	180
Morphology [3]	Complex; icosahedral-head with a diameter of 60 nm having a non-contractile tail with a length of 150 nm and a diameter of 10 nm.	Complex; icosahedral-head with a diameter of 60 nm having a non-contractile, flexible tail with a length of 151 nm and a diameter of 10 nm.
Molecular weight (Mega Dalton) [5]	60	?
IEP [6]	3.8 (or 2.7 in this study ?)	(2.7 in this study ?)

References: [1] ICTVdB 2009; [2] Leclerc et al. 2000; [3] Calender 2006; [4] Mulligan 2002; [5] Ackermann and DuBow 1987; [6] Michen and Graule 2010.

The Siphophage was subjected to further characterisation in terms of size and zeta potential using light scattering techniques and electrophoresis (experimental procedures are described in section S.6). The results obtained in 4 mM NaCl are shown in Figure S-3. As can be seen from the inlet in the figure, the larger dimensions of the Siphophage were confirmed by size measurements of phage suspensions revealing a d_{Hydro}=94.4 ± 2.6 nm (three measurements at titres in the range from $1.9*10^9$ to $7.6*10^9$ PFU/ml). Due to the complex morphology of the phage, the determination of a hydrodynamic diameter was difficult. However, one would expect a diameter larger than the icosahedral head of the phage (60 nm) and smaller than the maximum length of the phage (head and tail), which was about 210 nm long. Hence, the d_{Hydro} corresponded well with the phage size determined by TEM. The zeta potential in 4 mM NaCl as a function of pH is shown in Figure S-3. Under such conditions, the zeta potential had values of about -20 mV at neutral pH. The IEP of the Siphophage was determined to be

equal to pH 2.7. In the literature, only one IEP value for phage lambda at pH 3.8 has been reported and none for the phage T1, making further assignment to either of the two species not feasible.

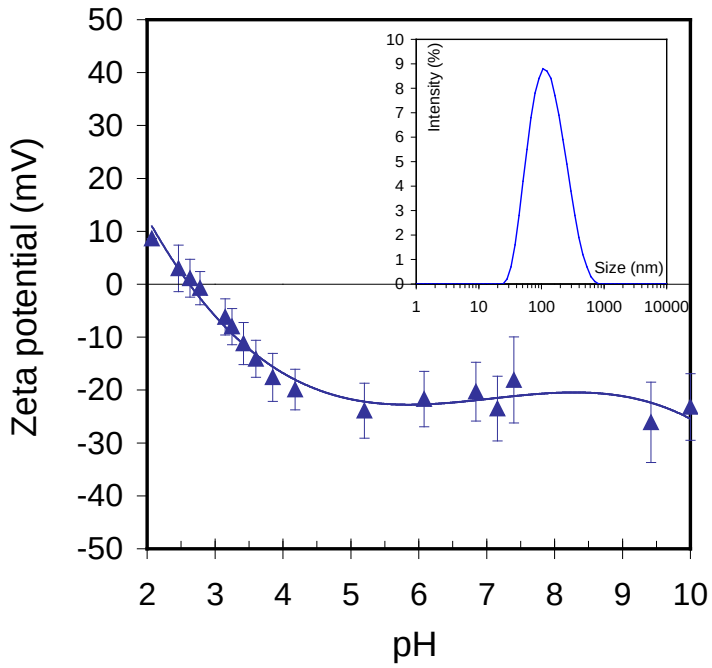


Figure S-3. Zeta potential as a function of pH of the Siphophage as well as the size distribution shown in the inlet; all conducted in 4 mM NaCl.

Tap water details

Table S-4: Ion concentrations in mg/l according to a report (24.09.2007) obtained from the water supply in Wallisellen. The table includes the conversion to ionic strength.

Table S-4 Ionic conditions found in Swiss tap water near Zurich.			
Ion [i]	**Cm (mg/l)**	**C (mol/l)**	**$C_i*z_i^2$ (mol/l)**
Mg^{2+}	17	0.0007	0.0028
Na^{+}	4.5	0.0002	0.0002
Ca^{2+}	113.8	0.0028	0.0114
Cl^{-}	16.1	0.0005	0.0005
NO_3^{-}	23.3	0.0004	0.0004
SO_4^{2-}	26.5	0.0003	0.0011
Ionic strength (I):			
I (M)	**I (mM)**		
0.0081	**8.1424**		

Calculation of Hamaker constants

Table S-5: The table lists some values of the Hamaker constant taken from the literature and calculated for systems which are of interest in this work. The upper part of the table lists values of like materials across air (vacuum) or water. In the lower section, Hamaker constants are given for asymmetric materials in water. According to equation S-1, the calculations were performed for two viruses, namely Tobacco mosaic virus (TMV) and MS2. The refractive index of bacteriophage MS2 has been determined by Balch et al. (2000) with n=1.11 and ε=4 was assumed for icosahedral viruses as done by Reddy et al. (1998). For TMV, values of n=1.57 and ε=55 were used according to Lee et al. (2008) to calculate the Hamaker constant.

Since these calculations underlie some simplifications, an average from those values available (indicated in the box in Table S-5) for viruses, or materials which are thought to have similar A_H (polystyrene, BSA; bovine serum albumin), was taken to determine a non-specific average value of A_H for viruses in air, resulting in A_H=11.4*10^{-20} J. This value was transferred to an A_H which refers to the virus-water-virus system using equation S-2.

Based on this value, together with the Hamaker constant of the filter media (SiO_2), the calculation of the asymmetric A_H between the virus and the potential adsorbent in water was performed with equation S-3. Those values that are of interest in this work are listed in Table S-5 and marked blue. Previously reported A_H values, e.g. such that have been applied to account for viral sorption processes, are given for comparison.

Equation S-1:

$$A_H = \frac{3}{4}kT\left(\frac{\varepsilon_{r(b)} - \varepsilon_{r(m)}}{\varepsilon_{r(b)} + \varepsilon_{r(m)}}\right)^2 + \frac{3h\nu_e}{16\sqrt{2}}\left(\frac{\left(n_b^2 - n_m^2\right)^2}{\left(n_b^2 + n_m^2\right)^{\frac{3}{2}}}\right)$$

A widely used model capable of calculating A_H for like bodies (b) in a medium (m) according to Israelachvili (1992). Thereby, only the refractive index (n) and the relative permittivity (ε_r) of the like bodies and the media, the Plank's constant (*h*) and the main electronic absorption frequency in the UV region (v_e) of the media are essential to estimate the Hamaker constant. In this work, the relation always refers to the aquatic system (w), hence $v_e=3*10^{15}$ 1/s. However, the A_H can be easily transformed with the help of equation S-2 in order to obtain the A_H of the like bodies in a vacuum (or air) (v) (Elimelech et al. 1995). This is of interest when the Hamaker constants of two different materials #1 and #2 in water are to be determined using the relation in equation S-3 (Israelachvili 1992).

Equation S-2:

$$A_{1w1} = \left(\sqrt{A_{1v1}} - \sqrt{A_{wvw}}\right)^2$$

Equation S-3:

$$A_{1w2} = \left(\sqrt{A_{1v1}} - \sqrt{A_{wvw}}\right)\cdot\left(\sqrt{A_{2v2}} - \sqrt{A_{wvw}}\right)$$

Table S-5. Hamaker constants (A_H)

Material#1 across air or water	**A_H (10^{-20}J) air/water**	**Reference**
Water	5.7/-	Roth and Lenhoff 1996
Polystyrene (=virus)[+]	6.15 to 6.6/-	Murray and Parks 1980
Polystyrene latex particles	8.4/0.9	Roth and Lenhoff 1996
BSA	9.6/1.28	Roth and Lenhoff 1996
TMV	13/1.48	this study
MS2	13.7/1.71	this study
Virus average value	11.4/0.97	this study
SiO_2 (silica, amorphous)	6.5/0.46	Bergström 1997
SiO_2 (quartz, trigonal)	8.9/1.02	Bergström 1997
Material#1 – Water – Material#2	**A_H (10^{-20}J) system**	**Reference**
Virus-water-SiO_2*	0.37	this study
Virus-water-quartz	0.33 to 0.49	Murray and Parks 1980
BSA-water-SiO_2 (quartz)	0.68	Roth and Lenhoff 1996

[+] Murray and Parks assumed that poliovirus possesses a comparable H_A to polystyrene;
* assuming a SiO_2 composition of half amorphous and half trigonal;
Bovine serum albumin (BSA), Tobacco mosaic virus (TMV).

Colloidal characterisation of bacteriophages

Experimental

The hydrodynamic diameter (d_{Hydro}) of the virus particles in suspension was investigated by dynamic light scattering (DLS). We used a Zetasizer Nano ZS (Malvern Instruments, UK), equipped with a laser light source (He-Ne; λ=633 nm) to illuminated the sample suspension. Thanks to the Brownian motion of the particles, the intensity of the scattered light fluctuated with time. The DLS equipment recorded an intensity correlation curve from which the diffusion coefficient (D) as well its distribution could be calculated. Knowing D, the d_{Hydro} could be computed with the help of the Stokes-Einstein equation (equation S-4), where η refers to the viscosity of the solution (water). Experiments were driven by Dispersion Technology Software (Version 5.03), which gave particle size distributions (PSD) expressed as percentage fraction of intensity as a function of particle size. Additionally, the software calculated the d_{Hydro} based on the assumption that the sample showed a monomodal distribution.

Equation S-4:

$$D = \frac{k \cdot T}{3 \cdot \pi \cdot \eta \cdot d_{Hydro}}$$

The surface charge of the phages in terms of the zeta potential (ζ) was determined using a microelectrophoresis system which was incorporated into the Zetasizer Nano ZS. The movement of charged particles in an alternating electrical field was determined by the frequency shift between a reference laser beam and the light which was scattered by the moving particles (Doppler Effect). Based on the frequency shift, the particle's velocity (ν) could be determined. The electrophoretic mobility (μ_E) of the particles could be calculated under consideration of the applied electrical field (E), equation S-5. The μ_E could be converted into the ζ with the help of the equation derived by Henry (Shaw 1992) (equation S-5, term on the right hand side). This takes into account the electric

permittivity of a vacuum (ε_0), the relative permittivity of the suspension media (ε_r), the viscosity of the suspension (η) as well as the Henry function (f(κa)), with *a* as the particle radius and κ as the Debye-Hückel parameter, given in equation S-6 (Adamczyk 2003). f(κa) has a value between 1 (Hückel approximation; for $\kappa a \ll 1$) and 1.5 (Smoluchowski approximation; for $\kappa a \gg 1$) (Shaw 1992). The Debye-Hückel parameter takes into account the ionic strength (I), elementary charge (e_-) as well as Avogadro's number (N_A). Further, ε_0 is the electric permittivity in vacuum and ε_W is the dielectric constant of water, k refers to the Boltzmann constant and T to the absolute temperature. The inverse κ is also used to describe the thickness of the EDL. The software supplied by Malvern also drove the experiments performed here. Clear disposable capillary cells (DTS 1060, Malvern Instruments, UK) were used and connected with the autotitrator MPT-2 (Malvern Instruments, UK) in order to determine zeta potential (ζ) as a function of titrant (e.g. acid: HCl and base: NaOH to adjust pH). The measurements were done in salt solutions of NaCl with I = 4 mM.

Equation S-5:

$$\mu_E = \frac{v}{E} = \frac{2 \cdot \varepsilon_0 \cdot \varepsilon_r \cdot \zeta \cdot f(\kappa a)}{3 \cdot \eta}$$

Equation S-6:

$$\kappa = \left(\frac{2 \cdot e_-^2 \cdot N_A \cdot I}{\varepsilon_0 \cdot \varepsilon_W \cdot k \cdot T} \right)^{\frac{1}{2}}$$

Results

The d_{Hydro} of MS2 was determined by DLS measurements of subsequently in NaCl (I=4mM) diluted suspensions. Three dilutions led to final titres of $1.4*10^{10}$, $7*10^9$ and $3.5*10^9$ PFU ml^{-1}. Further dilutions below $1*10^9$ PFU ml^{-1} gave

unreliable results accompanied by deficient measurement quality reports given by the operating software. However, measurements of these three different concentrations yielded a mean value with a standard deviation of d_{Hydro}(MS2)=33.4 ± 1.3 nm. In contrast, a d_{Hydro}(Phi)=87.9 nm was determined for the phage Phi in a suspension with a titre of $2.8*10^{9}$ PFU ml^{-1}. Further dilution of the suspensions gave also unreliable results indicating that the light detector was working in the range of the detection limit (i.e. not sufficient light is scattered at suspended matter in the sample). In Figure S-4, we show the PSD of the two phages. While the scattered intensity of MS2 suspensions revealed an almost monomodal PSD, the Phi suspension showed a bimodal distribution (the relatively small peaks, in terms of intensity, at the micron scale found in both samples are neglected in this context as those are probably due to insignificant amounts of contaminants).

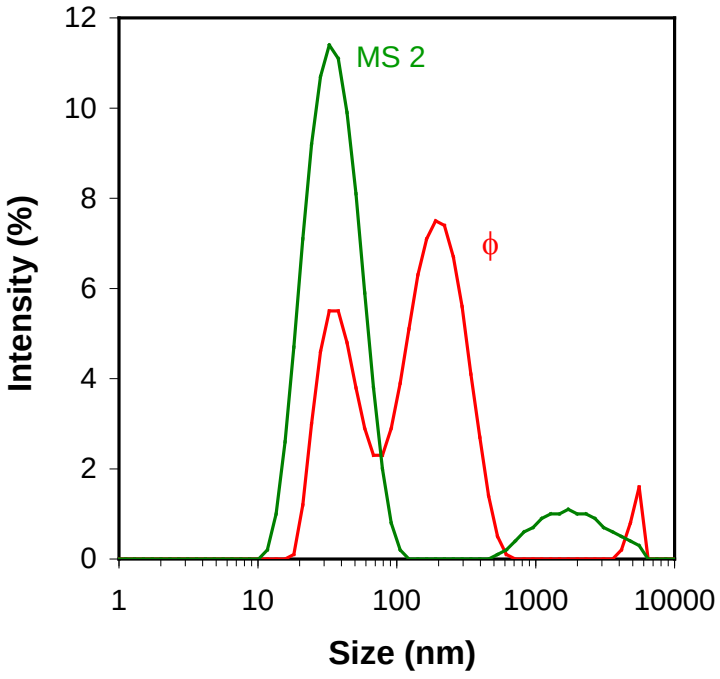


Figure S-4. Particle size distributions (PSD) determined by laser scattering for MS2 and Phi(ϕ) in NaCl, I = 4 mM at pH 7.

The ζ as a function of pH were measured using MS2 and Phi samples taken from the same stock as used for the size measurements. The results are presented in Figure S-5. In the case of MS2, the surface charge in terms of the ζ carried a net negative charge in the pH region of most naturally-occurring water sources (5 < pH < 9). The IEP of bacteriophage MS2 was determined at pH 3.5. The sample containing phage Phi showed a net negative ζ in the pH range under investigation (3 < pH < 10) and no IEP could be detected.

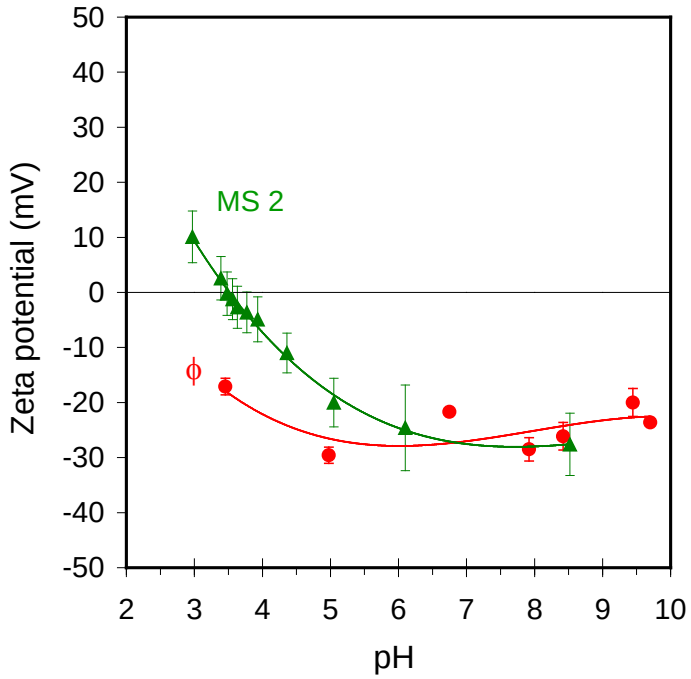


Figure S-5. Zeta potential (ζ) of suspensions containing bacteriophages MS2 and ϕ as a function of pH in NaCl; I = 4 mM.

Discussion

With our measurements we could define a benchmark of $1*10^{9}$ PFU ml^{-1} as a minimum phage concentration necessary for the detection by light scattering techniques. It is suspected that this minimum titre will be needed for any virus type of similar size and molecular weight. In contrast, lower minimum concentrations would be expected for viruses with larger physical dimensions

and mass. Measuring the hydrodynamic diameter of phage MS2 resulted in a relatively low standard deviation that indicated high reproducibility of the measurement. The d_{Hydro}(MS2) of 33.4 ± 1.3 nm was significantly larger than the viral diameter of 25 nm as reported in Table S-2. The size of particular matter in the aquatic environment may differ from the dry state which is often investigated by transmission electron microscopy (TEM). This is because of the EDL at the interface of the particle. Hence, the virus size plus the EDL thus make up the d_{Hydro}. Our results are in accordance with this formation of an EDL. Its thickness κ^{-1} under the experimental conditions could be calculated to be equal to 4.8 nm (equation S-6). This resulted in a theoretical hydrodynamic diameter, $d_{Th\text{-}Hydro}$(MS2), of 34.6 nm that was within the standard deviation of the experimental result. In contrast, the d_{Hydro} of bacteriophage Phi measured with 87.9 nm was out of the range of expectation as the phage's diameter of 27 nm was confirmed by TEM investigation, see Table S-2. The DLS measurement was conducted in the same electrolyte as the phage MS2 (I=4 mM NaCl), thus a comparable κ^{-1} was expected. Clarification was given with the PSD of the two phages in Figure S-4. While the scattered intensity of MS2 suspensions revealed an almost monomodal PSD, the Phi suspension showed a bimodal distribution. The smaller peak of the bimodal PSD could be attributed to monodisperse ϕ bacteriophages, as the peak position corresponded well with the $d_{Th\text{-}Hydro}(\phi)$=36.6 nm based on TEM investigation. The second, and more pronounced peak, however, may have arisen from either contamination or viral aggregation. Suspended colloidal matter having an IEP near the pH of the suspension media tends to form aggregates. This has been shown for the phage MS2 which forms aggregates with sizes of a few micrometres for pH ≤ IEP (Langlet et al. 2007). Also for Phi, reversible aggregation behaviour at close to neutral pH has been reported by Sinsheimer (1959) which is in agreement with the reported mean IEP of 6.2 in Table S-2. wever, Sinsheimer observed these aggregates in suspensions with high phage concentrations (>9*1013 PFU ml-1) only. Moreover, Sinsheimer reported complete dispersion of virus aggregates upon dilution or increased pH. Although the pH of the virus suspensions produced in this work was in the neutral region

(pH 6-7) and thus was close to the reported IEP of Phi, the titres obtained were orders of magnitude smaller than those at which aggregation had been observed in the literature. Also, it was presumed here that bacteriophage Phi might be sterically stabilised thanks to the knobs that protrude from the viral capsid (Tromans and Horne 1961). Such a steric stabilization mechanism was suggested by Penrod et al. (1996) for the phage MS2.

For these reasons, we suggest the presence of an organic contaminant rather than the formation of aggregates of PhiX174. Contaminants, possibly originating from bacterial debris, might have been too small to be removed in the microfiltration using 0.2 μm syringe filters, and too large to diffuse through the dialysis membrane with a 100 kDa cut-off. It was thus suspected that the largest peak at around 200 nm of ϕ in Figure S-4 could be attributed to contaminants, possibly arising from host bacteria.

The IEP of MS2 was determined at pH 3.5, which is equal to the average IEP value reported in the literature (Michen and Graule 2010) and confirmed the accuracy of our work. However, the sample containing phage Phi showed a net negative ζ in the pH range under investigation ($3 < pH < 10$). This was in contradiction with an expected IEP at neutral pH as has been frequently reported (Sinsheimer 1959, Aach 1963, Horká et al. 2007, Brorson et al. 2008), which would give net positive ζ at pH < IEP. However, this confirmed the previous assumption of the presence of a contaminant which may carry a different surface charge. As the scattered intensity of Phi, recorded in the size distribution shown in Figure S-4, was largest in magnitude for the potential contaminant, this contaminant was expected to determine the signal read for the ζ measurement as well. It is worth mentioning that, although this negative charge of phage Phi was in contradiction with the abovementioned literature, it was in agreement with other studies which determined the ζ=-35 mV at pH 6.8 (Chattopadhyay and Puls 1999) and ζ=-31.3 mV at pH 7.5 (Attinti et al. 2010). However, these studies lack details on the purification of the phage suspensions prior to measurement. An

additional study, which also did not address the purification of the Phi suspension, investigated the ζ as well as the d_{Hydro} as a function of pH resulting in an IEP(ϕ)≈2 and d_{Hydro} ranging from 70 to 100 nm (Aronino et al. 2009). It is thus supposed that the suspension with phage Phi produced in the recent study and those, which are in agreement with the results obtained in this work, suffer from significant contamination. However, reliable zeta potential values of phages are needed in order to quantify the electrostatic interactions in sorption processes involving viruses. Due to the fact that no reliable measurements on the surface charge of bacteriophage Phi could be obtained in this work, the most trustworthy work of Aach was used in order to obtain ζ values of the phage. In the work of Aach, the bacteriophages were extensively purified and demonstrated an IEP at pH 6.6 in 40 mM acetate buffer (Michaelis), which had been determined by the electrophoretic velocity. This velocity can be converted to the ζ using equation S-5 and assuming f(κa)=1.4, as estimated graphically from the Henry equation (Shaw 1992) with κa=17. The resulting ζ as a function of pH is displayed in Figure S-6.

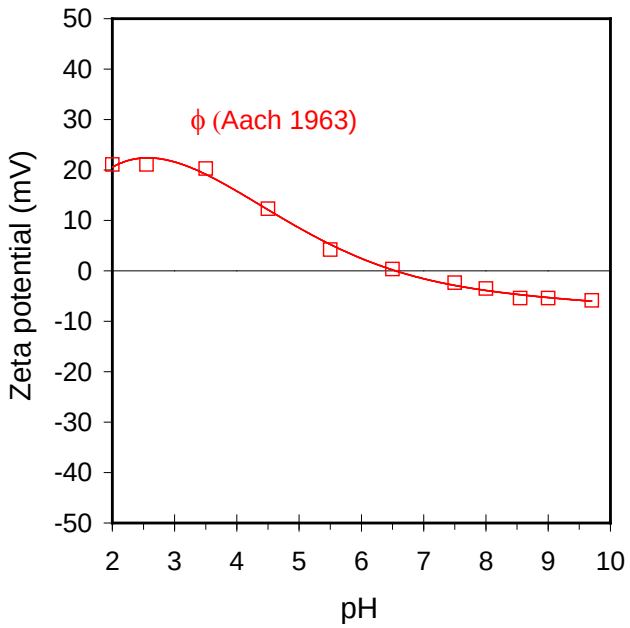


Figure S-6. Zeta potential values calculated for bacteriophage Phi (ϕ) as a function of pH, based on experimental data by Aach 1963.

Values for (X-)DLVO calculations

Table S-6: All values used for the calculation of the energy-distance curves according to (X-)DLVO theory are summarised in Table S-6.

* in hard water no measurements for phages could be obtained thus values at comparable pH value in soft water were used.

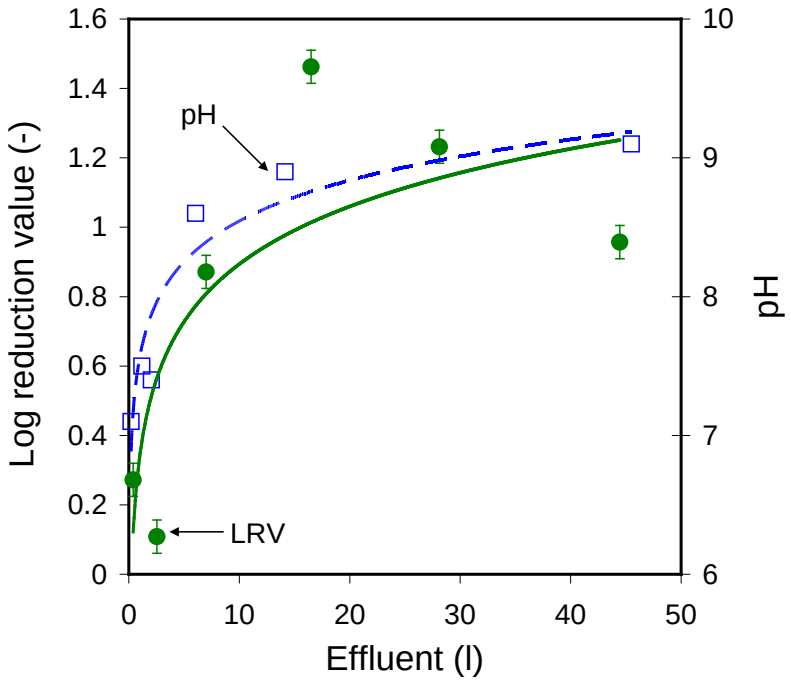
Table S-6. Values used for (X-)DLVO calculations

	Radius	Water-contact angle	Test water conditions				
			soft water pH 5	soft water pH 6.2	soft water pH 7 (7.3)	soft water pH 9	hard water* pH 7
	r (nm)	θ (°)	ζ/U_{Stern} (mV)	ζ/U_{Stern} (mV)	ζ/U_{Stern} (mV)	ζ/U_{Stern} (mV)	ζ/U_{Stern} (mV)
MS2	13[1]	50[2]	-18/-20.1[1]	-24.8/-27.8[1]	-27/-30.2[1]	-27/-30.2[1]	-27/-30.2[1]
Phi	14[1]	42[2]	9/10[1]	1/1.1[1]	-2/-2.2[1]	-5/-5.6[1]	-2/-2.2[1]
Siphophage	42[1]	96[2] (82)[1]	-22/-24.1[1]	-	-22/-24.1[1]	-23/-25.2[1]	-22/-24.1[1]
Filter	-	68[3]	-62/-67.4[3]	-69/-75[3]	-72/-78.2[3]	-79/-85.8[3]	-24.6/-27.7[3]

References:[1] this study; [2] Chattopadhyay and Puls (1999); [3] Michen et al. 2011.

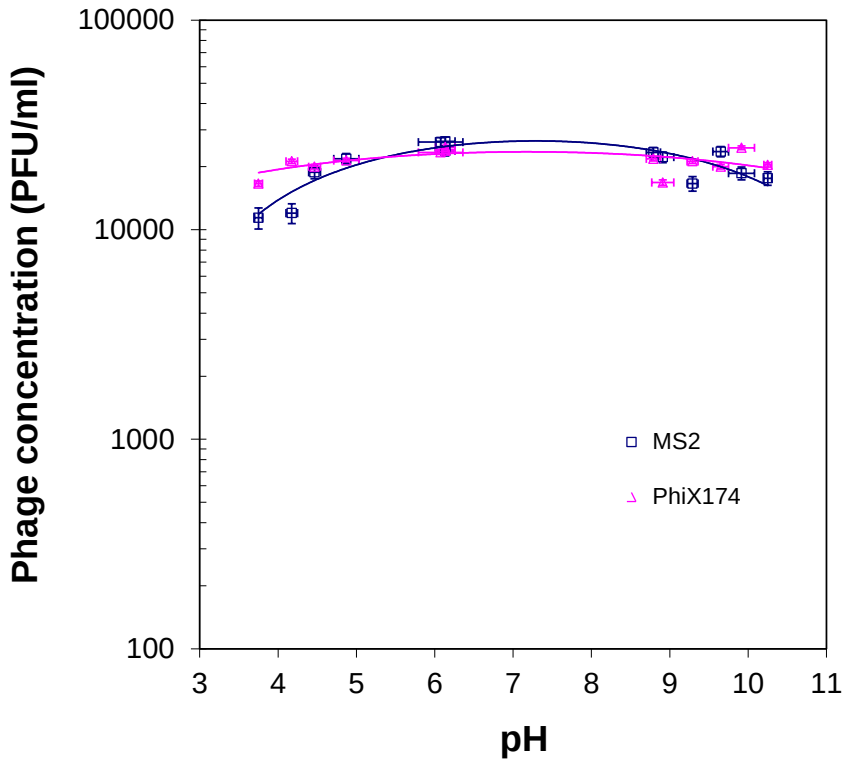
MS2 removal at pH 9

Figure S-7: Retention of bacteriophage MS2 in soft water at pH 9 and pH change in the effluent.



Control batch experiment

Figure S-8: The graph shows the titre of phages MS2 and PhiX174 as a function of pH in a control batch experiment. Both phages were subjected to a control experiment in which no potential adsorbent material has been added to the system. Instead the experiments were done at different pH values in order to investigate inactivation or aggregation of the phages due to pH change. The experiments were run for one hour before the sample has been filtrated and virus concentration in triplicates has been determined by enumeration.



Reference list

Aach, H.G. (1963) Elektrophoretische Untersuchungen an Mutanten des Phagen PhiX174. *Z Naturforsch B* 18, 290-293.

Abedon, S.T. and Culler, R.R. (2007) Bacteriophage evolution given spatial constraint. *J Theor Biol* 248, 111-119.

Ackermann, H. W. and DuBow M. S., (1987) Viruses of prokaryotes volume 2: Natural groups of bacteriophages, CRC-Press.

Adamczyk, Z. (2003) Particle adsorption and deposition: role of electrostatic interactions. *Adv Colloid Interfac* 100-102, 267-347.

Aronino, R., Dlugy, C., Arkhangelsky, E., Shandalov, S., Oron, G., Brenner, A. and Gitis, V. (2009) Removal of viruses from surface water and secondary effluents by sand filtration. *Water Res* 43, 87-96.

Attinti, R., Wei, J., Kniel, K., Sims, J. T. and Jin, Y. (2010) Virus' (MS2, ϕX174, and Aichi) attachment on sand measured by atomic force microscopy and their transport through sand columns. *Environ Sci Technol* 44, 2426-2432.

Balch, W. M., Vaughn, J., Novotny, J., Drapeau, D. T., Vaillancourt, R., Lapierre, J. and Ashe, A. (2000) Light scattering by viral suspensions. *Limnology and Oceanography*, 45(2), 492-498.

Bergström, L. (1997) Hamaker constants of inorganic materials. *Advances in Colloid and Interface Science*, 70, 125-169.

Brorson, K., Shen, H., Lute, S., Perez, J.S. and Frey, D.D. (2008) Characterization and purification of bacteriophages using chromatofocusing. *J Chromatogr A* 1207, 110-121.

Calender, R. (2006) The bacteriophages; Chapter 7: Allan Campbell – General aspects of lysogeny; Chapter 8: John W. Little – Gene regulatory circuitry of phage Lambda; Chapter 17: G. J. German, R. Misra, A. M. Kropinski - The T1-like bacteriophages. Oxfrod University Press.

Chattopadhyay, S. and Puls, R.W. (1999) Adsorption of bacteriophages on clay minerals. *Environmental Science and Technology*, 33, 3609-3614.

Elimelech, M., Gregory, J., Jia, X. and Williams R.A. (1995) Particle deposition and aggregation: Measurement, modeling and simulation. Butterworth-Heinemann, Oxford UK.

Horká, M., Kubícek, O., Ruzicka, F., Holá, V., Malinovská, I. and Slais, K. (2007) Capillary isoelectric focusing of native and inactivated microorganisms. *J Chromatogr A* 1155, 164-171.

ICTVdB – The Universal Virus Database (2009). http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/index.htm

Israelachvili, J.N. (1992) Intermolecular and surface forces, 2nd ed., Academic Press, London.

Langlet, J., Gaboriaud, F. and Gantzer, C. (2007) Effects of pH on plaque forming unit counts and aggregation of MS2 bacteriophage. *J Appl Microbiol* 103, 1632-1638.

Leclerc, H., Edberg, S., Pierzo, V. and Delattre, J. M. (2000) Bacteriophages as indicators of enteric viruses and public health risk in groundwaters. *Journal of Applied Microbiology*, 88, 5-21.

Lee, S. Y., Lim, J. S., Culver, J. N. and Harris, M. T. (2008) Coagulation of tobacco mosaic virus in alcohol–water–LiCl solutions. *Journal of Colloid and Interface Science*, 324, 92-98.

Michen, B. and Graule, T. (2010) Isoelectric points of viruses. *Journal of Applied Microbiology*, 109(2), 388-397.

Michen, B., Diatta, A., Fritsch, J., Aneziris, C. and Graule, T. (2011) Removal of colloidal particles in ceramic depth filters based on diatomaceous earth. *Separation and Purification Technology* 81(1), 77-87.

Mulligan, M. E. (2002) Biochemistry. Lecture notes from http://www.mun.ca/biochem/courses/3107/Lectures/Topics/bacteriophage.html

Murray, J.P. and Parks, G.A. (1980) Poliovirus adsorption on oxide surfaces. American Chemical Society, Washington, D.C.

Overby, L.R., Barlow, G.H., Doi, R.H., Jacob, M. and Spiegelman, S. (1966) Comparison of two serologically distinct ribonucleic acid bacteriophages. II. Properties of the nucleic acids and coat proteins. *J Bacteriol* 92, 739-745.

Penrod, S. L., Olson, T.M. and Grant, S. B. (1996) Deposition kinetics of two viruses in packed beds of quartz granular media. *Langmuir* 12, 5576-5587.

Reddy, V. S., Giesing, H. A., Morton, R. T., Kumar, A., Post, C. B., Brooks, C. L. and Johnson, J. E. (1998) Energetics of quasiequivalence: Computational analysis of protein-protein interactions in icosahedral viruses. *Biophysical Journal*, 74, 546-558.

Roth, C. M. and Lenhoff, A. M. (1996) Improved parametric representation of water dielectric data for Lifshitz theory calculations. *Journal of Colloid and Interface Science*, 179, 637-639.

Shaw, D. J. (1992) Introduction to Colloid and Surface Chemistry. Butherworth-Heinemann, Oxford, UK.

Sinsheimer, R.L. (1959) Purification and properties of bacteriophage PhiX174. *J Mol Bio* 1, 37-42.

Tromans, W.J. and Horne, R.W. (1961) The structure of bacteriophage PhiX174. *Virology* 15, 1-7.