



# Bacteriophages and clostridium spores as indicator organisms for removal of pathogens by passage through saturated dune sand

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## Abstract

In a field study on the efficiency of dune recharge for drinking water production, bacteriophage MS2 was shown to be removed 8 log<sub>10</sub> by passage through the dune sand. The question of whether pathogenic viruses would be removed as much as MS2 was studied by comparing complete breakthrough curves of MS2 with those of the human viruses Coxsackievirus B4 (CB4) and Poliovirus 1 (PV1) in laboratory columns. The columns were designed to closely simulate the field conditions: same sand, water, porewater velocity and temperature. Employing a two-site kinetic model to simulate breakthrough curves, attachment/detachment to two types of kinetic sites as well as inactivation of free and attached viruses were evaluated. It was found that attachment to only one of the sites is of significance for determining overall removal. At field scale, removal of the less negatively charged PV1 was extrapolated to be about 30 times greater than that of MS2, but removal of CB4 would be only as much as that of MS2. Also, removal of spores of *Clostridium perfringens* D10, a potential surrogate for *Cryptosporidium* oocysts, was studied. The attachment rate coefficient of the spores was 7.5 times greater than that of MS2. However, this does not imply that the removal of the spores is 7.5 times greater than that of MS2. Due to negligible inactivation in combination with detachment of previously attached spores, the actual removal rate of the spores depends on the duration of contamination and eventually all spores will break through. Provided no irreversible attachment or physical straining occurs, this may also be the case for other persistent microorganisms, like oocysts of *Cryptosporidium*.

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## 1. Introduction

Drinking water is considered to be microbiologically safe if certain maximum allowable concentrations of pathogenic microorganisms are not exceeded. Maximum allowable concentrations of pathogens in drinking water can be calculated from a maximum acceptable risk of infection of one per 10 000 persons per year, drinking

water consumption, and dose response relations of pathogens [1]. For viruses, this maximum allowable concentration is  $1.8 \times 10^{-7}$  plaque forming particles per liter (pfp l<sup>-1</sup>) which is based on the dose response relationship of rotavirus and poliovirus 3, as a worst case. This approach has formed the basis for the Enhanced Surface Water Treatment Rule (ESWTR) and was under consideration for the Ground Water Disinfection Rule (GWDR) in the USA [2]. In The Netherlands, this policy for production of safe drinking water has been incorporated into legislation in the

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beginning of 2001. Concentrations of enteroviruses in the surface waters in The Netherlands are in the range of  $0.02\text{--}10\text{ pfpl}^{-1}$  [3]. This implies that virus concentrations in surface water need to be reduced  $5\text{--}8\log_{10}$  in order to produce safe drinking water from surface water. Obviously, concentrations of pathogenic viruses are too low to allow measurement of such high removal. Instead, bacteriophages have been used to measure treatment efficiency. Bacteriophages are harmless to humans, easy to measure and can be seeded in high numbers in tracer experiments.

In The Netherlands about 14% of the total drinking water production comes from pre-treated surface water that is artificially recharged in dune area. In a field study on the effectiveness of dune recharge for virus removal in Castricum, The Netherlands, it was shown that bacteriophages MS2 and PRD1 were removed  $8\log_{10}$  within 30 m of soil passage due to a combination of attachment to the sand grains and inactivation [4]. Nevertheless, very low collision efficiencies, as a measure of electrostatic interaction, were found for MS2 and PRD1. This was due to electrostatic repulsion of the negatively charged bacteriophages by the net negatively charged sand grains. Consequently, attachment of the phages to the sand grains was slow [4]. This relatively conservative behavior suggested their suitability as indicators for pathogenic viruses. However, low collision efficiencies indicate that the soil conditions may be unfavorable for attachment of other negatively charged (pathogenic) viruses too. In most soils, attachment of MS2 was found to be relatively low compared to most other viruses [5–14]. In some of these studies, MS2 was only compared with other bacteriophages [10,11]. Some of these studies describe batch experiments, where percentage of adsorption was measured, assuming equilibrium was reached [8,9]. In several column or field studies MS2 was compared with other phages or human viruses by means of mass reduction [6,10,14], or in terms of a collision efficiency [5,7,11–13], neglecting detachment and not considering inactivation.

In the present study, the role of MS2 as model or indicator virus was evaluated by comparing its breakthrough curves with those from human viruses, which could not be studied in the field. This was studied in column experiments that were designed to closely simulate the Castricum field conditions to allow extrapolation of virus removal relative to that of MS2 from laboratory- to field-scale [4]. The human viruses Cocksackievirus B4 (CB4) and Poliovirus 1 (PV1), were selected for these experiments. About 95% of the enteroviruses in Dutch surface waters are Cocksackieviruses B [3]. PV1 was a vaccine strain that has been used in many batch and column experiments to study its adsorption to soil [15].

To our knowledge, it is here for the first time that complete breakthrough curves, i.e. including the tails of

the breakthrough curves of human viruses were measured and analyzed. This was done in such detail that parameter values for attachment to and detachment from two types of kinetic sites were obtained, as well as parameter values for inactivation of free and attached viruses.

In addition to bacteriophages and viruses, we studied removal of spores of *Clostridium perfringens*. This is a pathogen that also has been used as an indicator of fecal contamination of drinking water sources and supplies for many years [16]. Because of its very low inactivation rate, it may be a potential surrogate for oocysts of *Cryptosporidium parvum*. According to colloid filtration theory [17], the  $1\text{-}\mu\text{m}$  spores collide less frequently with the soil grains than the  $5\text{-}\mu\text{m}$  oocysts, which would result in lower removal rates.

## 2. Materials and methods

### 2.1. Microorganisms

Portions of the highly concentrated suspension of MS2 that was used in the Castricum field study [4] were kept refrigerated at  $5\pm 3^\circ\text{C}$  for over two years. The following human enteroviruses were included in the experiments: an isolate of Cocksackievirus B4 (CB4) from recreational surface water (July 15, 1993) and the Poliovirus 1 Lsc 2ab vaccine strain (PV1). PV1 is a virus with a diameter of 23 nm and a pI of 6.6 [5], but a pI of 7.15 has also been reported by Sobsey et al. [18]. CB4 is in use at our laboratory as a positive control strain for the enumeration of enteroviruses by the plaque method on Buffalo Green Monkey (BGM) cells [16]. Highly concentrated suspensions of PV1 and CB4 were prepared by cell culture method [19]. Moreover, a highly concentrated suspension of *Clostridium perfringens* strain D10 was prepared as described for *Clostridium bifermentans* strain R5 [20].

### 2.2. Soil columns

Preparation of the dune sand columns was described in detail in an earlier paper [21]. Briefly, two 1.4-m columns of saturated dune sand were filled with a saturated soil sample that was taken near the first monitoring well at the field site. Water from the recharge canal was used. The experiments were all conducted in a cold room at the same temperature as that of the groundwater during the field study ( $5\pm 3^\circ\text{C}$ ). The same transport velocity was applied as in the field ( $1.5\text{ m day}^{-1}$ ). For both columns, a salt tracer experiment with sodium chloride was carried out in order to estimate interstitial flow velocity and medium dispersivity.

PV1 and CB4 were enumerated on the same Buffalo Green Monkey cells (see below). Therefore, two columns were needed to be able to distinguish PV1 and CB4 virus particles. MS2, PV1 and D10 were seeded together in column I, whereas MS2 and CB4 were seeded together in column II. Seeding suspensions were made in a container with Castricum canal water. Initial seeding concentrations were about  $10^7$  to  $10^8$  phage particles  $l^{-1}$  for each microorganism. Suspensions of microorganisms were seeded for 24 h and breakthrough was monitored for a period of 4–25 days. In column I, samples were taken at 0.3 m and in column II at 0.4 m as described previously by Schijven et al. [21].

### 2.3. Enumeration of microorganisms

MS2 was assayed as described in ISO 10705-1 using host strain WG49 [22]. PV1 and CB4 were enumerated according to the BGM monolayer assay [19]. To assay the spores of *Clostridium perfringens*, aliquots of 1 ml from sample dilutions were mixed with about 5 ml of molten Sulfito Cycloserine Agar of 45°C in 9-cm Petri dishes [23]. As soon as this agar had solidified, a layer of about 10 ml of the same agar was poured on top. The plates were incubated for 48 h at 37°C in anaerobic jars. The black colonies that had developed were counted.

### 2.4. Conceptual model

In similar column experiments as we present here, we have previously shown that a two-site kinetic model fitted the breakthrough curves of bacteriophages from column and field experiments better than a one-site kinetic model [21]. A two-site kinetic model describes two types of sites for attachment/detachment. Interaction with kinetic site 1 is characterized by relatively fast attachment and slow detachment, whereas interaction with kinetic site 2 is characterized by both fast attachment and detachment. The governing equations for one-dimensional transport are as follows:

$$\frac{\partial C}{\partial t} + \frac{\rho_B}{n} \frac{\partial S_1}{\partial t} + \frac{\rho_B}{n} \frac{\partial S_2}{\partial t} = \alpha_L v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - \mu_l C - \mu_{s1} \frac{\rho_B}{n} S_1 - \mu_{s2} \frac{\rho_B}{n} S_2, \quad (1)$$

$$\frac{\rho_B}{n} \frac{\partial S_1}{\partial t} = k_{att1} C - k_{det1} \frac{\rho_B}{n} S_1 - \mu_{s1} \frac{\rho_B}{n} S_1, \quad (2)$$

$$\frac{\rho_B}{n} \frac{\partial S_2}{\partial t} = k_{att2} C - k_{det2} \frac{\rho_B}{n} S_2 - \mu_{s2} \frac{\rho_B}{n} S_2, \quad (3)$$

where  $C$  is the concentration of free microorganisms (plaque forming particles (pfp)  $m^{-3}$  for phages, and colony forming particles for bacteria (cfp)  $m^{-3}$ ),  $S$  is the concentration of attached microorganism (pfp  $kg^{-1}$  or cfp  $kg^{-1}$ ),  $t$  is the time (days),  $x$  is the distance (m),  $\alpha_L$  is the dispersivity (m),  $v$  is the average interstitial water

velocity (m  $day^{-1}$ ),  $\rho_B$  is the dry bulk density ( $kg m^{-3}$ ),  $n$  is the porosity (dimensionless),  $k_{att}$  and  $k_{det}$  are the attachment and detachment rate coefficients, respectively ( $day^{-1}$ ),  $\mu_l$  and  $\mu_s$  are the inactivation rate coefficients of free and attached microorganisms, respectively ( $day^{-1}$ ). Subscripts 1 and 2 refer to the two different kinetic sites. These equations are subject to boundary conditions  $C = C_0$  at  $x = 0$  and  $\partial C / \partial x = 0$  at  $x = L$ . The initial conditions were zero concentration for all microorganisms.

For solving the governing equations, a numerical model called EQ2KIN was used [21]. Quantities  $v$  and  $\alpha_L$  were found from fitting the salt breakthrough curves. The values of  $\mu_l$  for the viruses were found by measuring their inactivation in column influent suspensions. Estimation of parameters  $k_{att1}$ ,  $k_{att2}$ ,  $k_{det1}$ ,  $k_{det2}$  and  $\mu_{s1}$  made through coupling EQ2KIN to the parameter estimation code PEST version 1.07 (Watermark Computing, 1994). The estimation was carried out by applying log-transformation of these parameters. The value of parameter  $\mu_{s2}$  was assumed to be equal to  $\mu_{s1}$ . Fitting of breakthrough curves was carried out using log-transformed concentrations. The justification for log-transformations was as follows. Dilutions of the samples were made to obtain an approximately constant counting range in each plate. Within each analyzed dilution, phages are approximately Poisson-distributed. Poisson distribution implies that mean and variance are the same. Since, mean counts were approximately constant, this is also the case for the variance. To obtain concentrations, counts are multiplied by their corresponding dilution factor. Due to this multiplication, the errors in the observed concentrations will be approximately constant after log-transformation. From previous column experiments by Schijven et al. [21] it was also shown that corresponding residual values were randomly scattered around zero implying that the assumption of log-normally-distributed concentrations is reasonable. As a measure of goodness of fit, the coefficient of determination  $r^2$  [24] was calculated on the basis of  $N$  logarithmically transformed observations  $C_i$ , and fitted values  $F_i$ .

Under steady-state conditions, the relative contributions of inactivation and adsorption to the removal of viruses by soil passage can be calculated analytically. Virus removal is given by [21]

$$\log_{10} \left( \frac{C}{C_0} \right) = \frac{x}{2.3} \frac{(1 - \sqrt{1 + 4\alpha_L(\lambda/v)})}{2\alpha_L}, \quad (4)$$

where

$$\lambda = \mu_l + \frac{k_{att1}}{1 + k_{det1}/\mu_{s1}} + \frac{k_{att2}}{1 + k_{det2}/\mu_{s2}}. \quad (5)$$

From Eq. (5), the relative contributions of adsorption and inactivation to virus removal rate can be deduced. The first term in Eq. (5) gives the removal rate by

inactivation of free virus. The second and last terms give the removal rate of viruses due to interaction with kinetic sites.

*2.5. Calculation of collision efficiencies*

In colloid filtration theory for attachment of colloids, collision efficiency is introduced as a measure of the intrinsic adsorption capacity of the soil [17]. The collision efficiency accounts for electrostatic interactions, in this case, between microorganisms and the porous medium. Collision efficiencies were calculated using the following equation [17]:

$$\alpha = \frac{2}{3} \frac{d_g}{(1-n)} \frac{k_{att}}{v} \frac{1}{\eta} \tag{6}$$

where  $\alpha$  is the collision efficiency and  $\eta$  is the single collector efficiency. The single collector efficiency  $\eta$  was calculated using the following relationship [25]:

$$\eta = 1.0A_s N_{Lo}^{1/8} N_R^{15/8} + 0.00388A_s N_G^{1.2} N_R^{0.4} + 4A_s^{1/3} N_{pe}^{-2/3} \tag{7}$$

Here,  $N_R = d_p/d_c$  accounts for interception,  $N_G = d_p^2(\rho_p - \rho)g/(18\mu v n)$  for gravity effects,  $N_{Lo} = 4H/(9\mu d_p^2 v n)$  for van der Waals interactions, and  $N_{pe} = d_p v n / D_{BM}$  for diffusion. In these definitions,  $d_p$  and  $d_c$  represent the microorganism particle sizes and soil grain sizes (m), respectively,  $g$  is the gravitational acceleration,  $\rho$  and  $\rho_p$  are the density of water and the microorganism particle, respectively,  $\mu = \rho * 0.000947/(T + 42.5)^{1.5}$  is the dynamic viscosity ( $kg\ m^{-1}\ s^{-1}$ ) with  $T$  the water temperature ( $^{\circ}C$ ),  $H = 6.2 \times 10^{-21}$  is the Hamaker constant (J) for the bacterium–glass–water interface [26],  $D_{BM} = K_B(T + 273)/(3\pi d_p \mu)$  is the diffusion coefficient ( $m^2\ s^{-1}$ ) with Boltzmann constant  $K_B = 1.38 \times 10^{-23}(J\ K^{-1})$ , and  $A_s = 2(1 - \gamma^5)/(2 - 3\gamma + 3\gamma^5 - 2\gamma^6)$  is Happel’s porosity-dependent parameter, with  $\gamma = (1-n)^{1/3}$ . Clostridium spores have an assumed size of 1  $\mu m$  and a buoyant density of 1270  $kg\ m^{-3}$  [27]. Because viruses are small, their transport in the immediate vicinity of the collector surface is dominated by Brownian diffusion. In this case,  $\eta$  is given approximately by the last term in Eq. (7). Eq. (6) is employed to calculate collision efficiencies  $\alpha_1$  and  $\alpha_2$ , based on  $k_{att1}$  and  $k_{att2}$ , respectively.

**3. Results and discussion**

*3.1. MS2 and PV1*

Correlation matrices from fitting breakthrough curves showed a variable correlation coefficient between  $k_{det}$  and  $\mu_s$  (5–50%). In our case,  $k_{att} \gg k_{det}$ , also  $\mu_s > k_{det}$ . In Schijven et al. [4] showed that this implies that the height

of the breakthrough tail is mainly determined by  $k_{det}$  and the slope of the tail is mainly determined by  $\mu_s$ . This has also been shown in simulations by Schijven and Hassanizadeh [21].

Table 1 shows the estimates of all model parameters. A  $\mu_1$  value of 0.082  $day^{-1}$  was found for MS2. PV1 appeared to be less stable as indicated by the  $\mu_1$  value of 0.16  $day^{-1}$ . The latter is within the wide range of 0.01–0.18  $day^{-1}$  reported in literature for  $\mu_1$  values of PV1 in groundwater at 10 $^{\circ}C$  [28–30]. Values of  $\mu_s$  were similar to those of  $\mu_1$  for both MS2 and PV1.

Fig. 1 shows the breakthrough curves of MS2 and PV1 in column I. The breakthrough curve of MS2 shows a flattening tail, and the estimate of  $\mu_s$  is similar to that of  $\mu_1$ . PV1 attached much faster than MS2, thus a high value of the attachment rate coefficient was obtained. The values of  $\mu_s$  of PV1 were similar to that of  $\mu_1$ . The two-site model appeared to follow the skewness of the breakthrough curve of PV1 very well.

The value of  $k_{att1}$  for PV1 was found to be 35 times higher than that of MS2 (Table 1). The value of the collision efficiency,  $\alpha_1$ , for MS2 was found to lie between 0.00052 and 0.00061, which is very similar to the range of 0.00051–0.00068 that was previously reported [21]. A value of 0.018 was found for  $\alpha_1$  of PV1. A very similar value of the collision efficiency for the CHAT strain of PV1 (a value of 0.019) was reported for a sand and gravel aquifer at pH 7.2 [7]. In columns with silica beads at pH 7, much smaller values of 0.0040–0.0072 were reported [5], meaning that more sites for attachment were available for PV1 in the dune sand compared to silica beads. At pH 7.5–8.0, the dune sand is predominantly negatively charged and conditions are unfavorable for attachment to negatively charged viruses. Therefore, one may argue that the virus-grain interaction is the rate-limiting step for attachment and not molecular diffusion within pores [31]. It is known that differences in attachment rates of MS2 and PV1 to diatomaceous earths, whose zeta-potentials range from –12 to –71 mV (due to different coatings with metallic salts), increases with a more negative zeta-potential [32]. Similarly, it has been shown that at pH 7, attachment of PV1 to silica beads is 2–3 times higher than that of MS2, but about the same at pH 5–5.5 [5]. Therefore, the differences in interactions site 1 we found in our study can be explained by greater electrostatic repulsion that MS2 experiences compared to the less negatively charged PV1. A similar explanation has been given for the difference in attachment between MS2 and  $\phi X174$  [21]. The latter phage has a similar isoelectric point as PV1 [33], and is thus also less negatively charged than MS2. Because of the high reproducibility of evaluating attachment of MS2, we may directly compare attachment of  $\phi X174$  in the previous study [21] with that of PV1 in the present study. It appears that attachment of PV1 is about 7 times faster than that of  $\phi X174$ .

Table 1  
Parameter values from column experiments with microorganisms

Column	I			II	
Length (m)	0.27			0.43	
$v$ (m day <sup>-1</sup> )	1.9			2.0	
$\alpha_L$ (m)	0.00074			0.0015	
Microorganism	MS2	PV1	D10	MS2	CB4
$\mu_l$	0.082	0.16	0	0.082	0.079
$k_{att1}$	1.7 (0.36–8.1)	59 (51–68)	15 (6–40)	2.0 (1.8–2.3)	2.1 (1.6–2.7)
$k_{det1}$	0.0037 (0.00079–0.017)	0.0093 (0.00067–0.093)	0.023 (0.0014–0.40)	0.068 (0.054–0.077)	0.054 (0.020–0.15)
$k_{att2}$	0.28 (0.075–1.1)	11 (0.033–2600)	25 (12–49)	12 (10–15)	39 (23–65)
$k_{det2}$	2.7 (1.1–6.4)	17 (0.19–2400)	0.34 (0.067–1.7)	77 (65–90)	46 (23–93)
$\mu_{s1} = \mu_{s2}$	0.089 (0.011–0.73)	0.14 (0.0031–6.2)	0	0.37 (0.34–0.40)	0.23 (0.097–0.56)
$\alpha_1$	0.00052	0.018	0.037	0.00061	0.00063
$\alpha_2$	0.000086	0.0034	0.059	0.0037	0.012
$r^2$	98%	55%	74%	98%	85%
$\lambda$	1.7	56	—	1.9	2.0
% $\mu_1$	4.8%	0.3%	—	4.4%	4.0%
% $s_1$	94.7%	99.6%	—	92.4%	86.1%
% $s_2$	0.5%	0.2%	—	3.2%	9.9%
$k_{att1}/\lambda$	0.99	1.1	—	1.1	1.1
$-\log_{10}(C/C_0)/x$	0.40	13	—	0.41	0.44
$-\log_{10}(C/C_0)/t$	0.75	24	—	0.81	0.86

Dimension of parameters is day<sup>-1</sup>. 95%-confidence intervals are given between brackets. Porosity is 0.35.

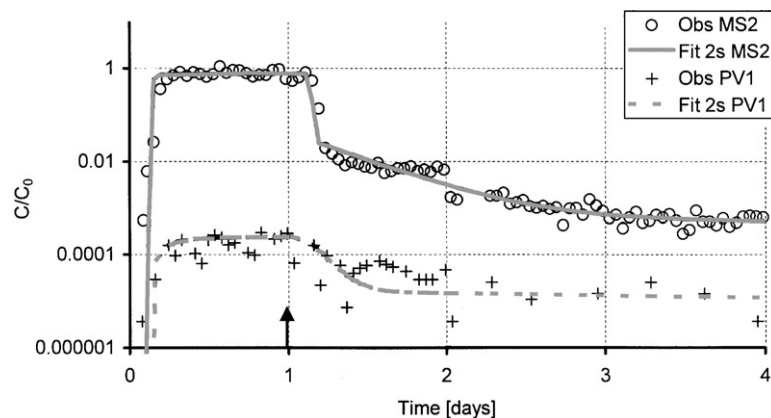


Fig. 1. Breakthrough curve of MS2 and PV1 in column I. Obs = observations, Fit 2s = two-site kinetic model fit. Virus was seeded from 0 to 24 h. The bold arrow denotes the time that virus-free water was introduced.

Column experiments were carried out at low temperature ( $5 \pm 3^\circ\text{C}$ ), at which inactivation rates are low. As can be seen in Table 1, interaction with site 1 accounts for 92–96% of removal of MS2 and almost for

100% for removal of PV1. Because  $k_{att1} \gg k_{det1}$ , it follows from Equation 5 that  $\lambda \approx k_{att1}$ , meaning that  $k_{att1}$  is the most important parameter for removal of MS2 and PV1 viruses at low temperatures. The same

conclusion was previously drawn for removal of bacteriophages MS2, PRD1 and  $\phi$ X174 [21]. Since  $\lambda \approx k_{\text{att1}}$ , removal rates as a function of travel time or distance are about 30 times higher for PV1 than for MS2.

### 3.2. MS2 and CB4

CB4 appeared to be similarly stable as MS2 (Table 1). In literature, a value of 0.012–0.04 day<sup>-1</sup> in groundwater at 10°C of  $\mu_1$  for Coxsackievirus B1 has been reported [28], which is lower than the value of 0.079 day<sup>-1</sup> that was found for CB4 in the present study.

Fig. 2 shows the breakthrough curves of MS2 and CB4 in column II. CB4 appeared to attach to a similar extent as MS2. The rising limb of the breakthrough curve is much more skewed to the right, but the declining limb and the tails of the breakthrough curves of CB4 and MS2 are almost parallel. A slightly higher removal rate is predicted for CB4 than for MS2 (Table 1). The tail of the breakthrough curve of MS2 in column I appeared to be very straight. This was probably the reason that relatively high estimates for  $\mu_s$  were obtained. The same can be said for CB4. In previous column experiments [21], it was also observed that the bending of the tail of the MS2 breakthrough curve was not always discernable, as was now also the case in column II.

The attachment rate of CB4 was found to be the same as that of MS2. The value of  $\alpha_1$  for CB4 was 0.00063, which was very similar to that of MS2, meaning that conditions for attachment were similarly unfavorable for both viruses. A similar result was found by others in batch experiments with different soils at pH 7–8 for different strains of CB4 (strains V216 and V240) [34,9]. They, however, found a larger attachment rate coefficient than in our experiments. This suggests that the

CB4 strain used in our study is a strongly negatively charged virus like MS2. Interaction with site 1 accounts for 86% of removal of CB4 and  $k_{\text{att1}}$  is, therefore, also the most important parameter for removal of CB4 at low temperatures. In addition, removal rates as a function of travel time or distance are similar for MS2 and CB4.

### 3.3. Spores of *Clostridium perfringens* D10

Fig. 3 shows the breakthrough curve of spores of *Clostridium perfringens* D10 in column II. The D10 spores attach very fast. The collision efficiency  $\alpha_1$  for the *Clostridium* spores was much higher than of the viruses: 0.037. This, however, does not mean a high removal. Because inactivation of clostridium spores is negligible, actual removal could only be due to irreversible adsorption. However, the high detachment rate, evidenced by the relatively high position of the tail of the breakthrough curve, suggests that attachment of the spores is mainly reversible. The detachment rate is high, due to the high number of previously attached spores. Although D10 spores attach relatively fast, all spores will eventually break through because of reversible adsorption and negligible inactivation of the spores. Actual removal with travel time or distance depends on the time period of seeding or contamination. By simulating breakthrough of the D10 spores at a given travel distance, it can be shown that, after approximately 3 years of continuous seeding, a value of  $C/C_0 = 1$  will be reached. A similar situation may be the case for other persistent microorganisms like oocysts of *Cryptosporidium*. Although oocysts may not be as persistent as clostridium spores, they may persist long enough to allow significant subsurface transport. Therefore, it is important to identify and quantify the possible

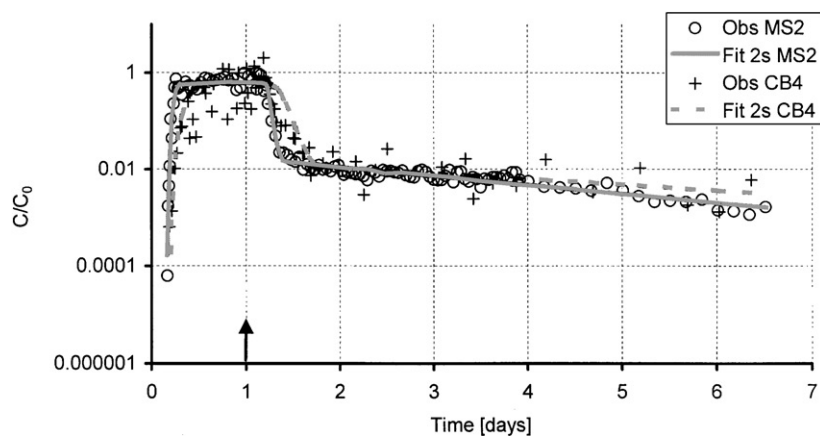


Fig. 2. Breakthrough curves of MS2 and CB4 in column II. Obs = observations, Fit 2s = two-site kinetic model fit. Virus was seeded from 0 to 24 h. The bold arrow denotes the time that virus-free water was introduced.

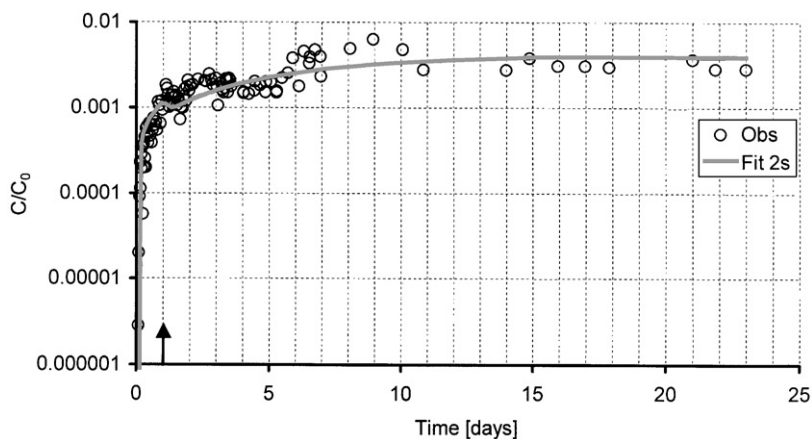


Fig. 3. Breakthrough curve of spores of *Clostridium perfringens* D10 in column II. Obs=observations, Fit 2s=two-site kinetic model fit. Spores were seeded from 0 to 24 h. The bold arrow denotes the time that spore-free water was introduced.

contribution of irreversible attachment and physical straining because those are actual removal mechanisms.

#### 4. Conclusion

The question of whether pathogenic viruses would be removed as much as MS2 by dune recharge was studied by comparing complete breakthrough curves of MS2 with those of the human viruses Coxsackievirus B4 (CB4) and Poliovirus 1 (PV1) in laboratory columns. In all cases, very satisfactory fits of the breakthrough curves of MS2, CB4, PV1 and D10 spores were obtained with the two-site kinetic model. As was already shown for bacteriophages MS2, PRD1 and  $\phi$ X174 [21], attachment of CB4 and PV1 to kinetic site of type 1 mainly determined removal, detachment from this site was slow, and interaction with kinetic sites of type 2 contributed little to virus removal. Because removal of MS2 has been measured at both laboratory and field scale and was also compared with that of other viruses at laboratory scale, it is possible to extrapolate removal of these viruses at the field site for dune recharge. Where removal of MS2 was found to be  $8 \log_{10}$  after 30 m of travel distance, that of CB4 would also be about  $8 \log_{10}$ , that of  $\phi$ X174 would be about  $33 \log_{10}$  and that of PV1  $280 \log_{10}$ . How this will relate to the removal of other human pathogenic viruses such as the waterborne hepatitis E and A viruses remains to be investigated.

Attachment rates of MS2 and PRD1 were shown in previous experiments to be very similar [4,21]. Thus, it may be concluded that both MS2 and PRD1 represent the low adsorptive behavior of negatively charged viruses and may therefore be considered as (relatively) worst case viruses, as is supported by many studies [4,5–14,35–37]. Furthermore, removal of naturally occurring

F-specific RNA bacteriophages at a nearby dune recharge site has been shown to be similar to that of MS2 [38]. We may therefore, conclude from the removal of MS2 that dune recharge adequately removes viruses. Regarding MS2 to be a relatively conservative indicator for virus removal is not an exaggeration, because we now have shown that there are pathogenic viruses that are removed by soil attachment as poorly as MS2.

Also, removal of spores of *Clostridium perfringens* D10 was studied. The attachment rate coefficient of the spores was 7.5 times greater than that of MS2. However, this does not imply that the removal of the spores is 7.5 times greater than that of MS2. Due to negligible inactivation, the actual removal rate of the spores depends on the duration of contamination.

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