

Removal of microorganisms by deep well injection

Jack F. Schijven^{a,*}, Gertjan Medema^{b,1}, Ad J. Vogelaar^{b,2},
S. Majid Hassanizadeh^{c,3}

^a National Institute of Public Health and the Environment, Microbiological Laboratory for Health Protection, P.O. Box 1, 3720 BA Bilthoven, Netherlands

^b Kiwa Research and Consultancy, P.O. Box 1072, 3430 BB Nieuwegein, Netherlands

^c Delft University of Technology, Faculty of Civil Engineering and Geosciences, P.O. Box 5, 2600 AA Delft, Netherlands

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Abstract

The removal of bacteriophages MS2 and PRD1, spores of *Clostridium bifermentans* (R5) and *Escherichia coli* (WR1) by deep well injection into a sandy aquifer, was studied at a pilot field site in the southeast of the Netherlands. Injection water was seeded with the microorganisms for 5 days. Breakthrough was monitored for 93 days at 4 monitoring wells with their screens at a depth of about 310 m below surface. Within the first 8 m of soil passage, concentrations of MS2 and PRD1 were reduced by 6 log₁₀, that of R5 spores by 5 log₁₀ and that of WR1 by 7.5 log₁₀. Breakthrough of MS2 and R5 could also be followed at greater distances from the injection well. Concentrations of MS2 were reduced only by about 2 log₁₀ in the following 30 m, and reduction of concentrations of R5 was negligible. Apparently, attachment was greater during the first 8 m of aquifer passage.

At the point of injection, the inactivation rate coefficient of free MS2 was found to be 0.081 day⁻¹, that of free PRD1 0.060 day⁻¹, and that of *E. coli* strain WR1 0.063 day⁻¹. In injection water that had passed 8 m of soil, inactivation of MS2 phages was found to be less than in water from the injection well: 0.039 day⁻¹. Probably, the higher inactivation rate of MS2 in water from

* Corresponding author. Tel.: +31-30-274-2994; fax: +31-30-274-4434.

E-mail addresses: jack.schijven@rivm.nl (J.F. Schijven), medema@kiwaoa.nl (G. Medema), vogelaar@kiwaoa.nl (A.J. Vogelaar), majid.hassanizadeh@ct.tudelft.nl (S.M. Hassanizadeh).

¹ Tel.: +31-30-606-9653.

² Tel.: +31-30-606-9592.

³ Tel.: +31-15-278-7346.

the injection well may be ascribed to the activity of aerobic bacteria. Inactivation of the R5 spores was not significant.

From geochemical mass balances, it could be deduced that within the first 8 m distance from the injection well, ferric oxyhydroxides precipitated as a consequence of pyrite oxidation, but not at larger distances. Ferric oxyhydroxides provide positively charged patches onto which fast attachment of the negatively charged microorganisms may take place. The non-linear logarithmic reduction of concentrations with distance may therefore be ascribed to preferable attachment of microorganisms to patches of ferric oxyhydroxides that are present within 8 m distance from the injection point, but not thereafter.

Declogging of the injection well introduced hydrodynamic shear that remobilized MS2, which was then transported farther downstream. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

In this paper, results of a field study, aimed at investigating removal of viruses and bacteria from pre-treated surface water by deep well injection, are presented. In the Netherlands, about a third of the total drinking water production relies on surface water. Surface water is contaminated with pathogenic microorganisms, mainly because of discharges of domestic wastewater and manure run-off from agricultural land. To produce drinking water from surface water, these pathogens need to be removed. One effective way is found to be passage of surface water through soil, as has been shown in the case of bank filtration (Havelaar et al., 1995; Schijven and Rietveld, 1996) and dune recharge (Schijven et al., 1998, 1999). Removal of microorganisms for drinking water production by deep well injection is another effective option. When renovating or designing recharge systems, drinking water companies want to minimize ecological side effects of artificial recharge and keep the land claim for recharge projects within limits (Peters, 1996). Deep well injection offers the added advantage that the use of land as well as effects on surface ecosystems are kept to a minimum. Knowledge about the removal of microorganisms is important for the design of deep well injection systems. Therefore, a field study was carried out at a pilot location for deep well injection to investigate removal processes of bacteriophages, bacterial spores and bacteria. Highly concentrated suspensions of bacteriophages MS2 and PRD1, spores of *Clostridium bifermentans* (R5) and *Escherichia coli* (WR1), were added to the injection water for a period of 5 days. Their transport was followed in monitoring wells at different distances from the injection well. MS2 and PRD1 are considered to be relatively conservative tracer viruses because they attach less than most pathogenic viruses and are relatively persistent during transport through the subsurface, as evidenced in the literature (see Schijven and Hassinazadeh, 2000 for an extensive review). Spores of *Cl. bifermentans* were chosen as surrogates for removal of oocysts of *Cryptosporidium parvum*. Although these spores are about five times smaller in diameter (1 μm), they resemble *Cr. parvum* oocysts in being highly persistent in the aquatic environment. According to colloid filtration theory (Yao et al., 1971), the 1- μm spores will collide less frequently with the

soil grains than the 5- μ m oocysts, which is a conservative approach. Strain WR1 of *E. coli* was selected as a representative of fecal bacteria. Although MS2 was co-injected with an *E. coli* strain, it will not replicate by using this strain as a host, because the water temperature (12°C) was too low for any *E. coli* strain to produce F-pili (Woody and Cliver, 1995). Together, the injected microorganisms span a size range of about 26–2600 nm.

The goals of this field study were the following:

- (i) Measuring reduction in concentrations of microorganisms by deep well injection as a function of distance;
- (ii) Gaining insight into the contribution of attachment and inactivation to the removal of microorganisms; and
- (iii) Investigating effects of declogging of the injection well on the removal of microorganisms.

2. Site description

The deep well injection site is located near the village of Someren in the southeast of the Netherlands. It has been constructed to investigate the feasibility of deep well injection for storage and treatment of pre-treated surface water for drinking water production. The deep well injection project started in July 1996. Surface water is taken in from a nearby canal, the Zuid–Willemsvaart, at a distance of 95 m from the pilot site. Surface water is primarily taken in during nighttime, when concentrations of suspended solids are lower due to the absence of shipping traffic, and stored in a pond. Pre-treatment consists of microstraining, coagulation, rapid sand filtration and active carbon filtration. Fig. 1 shows a schematic vertical cross-section of the aquifer. The pre-treated surface water is injected at well IP2 through a screen at a depth of 280–310 m below the surface. The borehole of the injection well has a diameter of 80 cm. The standing pipe has an inner diameter of 46 cm for the first 36 m and an inner diameter of 23 cm for the next 272 m. The standing pipe is surrounded by a gravel pack of 15–28 cm. A 50-m thick layer of clay overlies this aquifer. The aquifer itself lies on top of another clay layer which is 2–3 m thick. The aquifer consists of several sandy layers (fluvial sediments) that differ in permeability. Water is pumped up at production well PP1 at a distance of 98 m from the injection point and at a depth of 278–298 m below the surface. Monitoring wells were located at distances of 8 (WP3), 12 (WP2), 22 (WP4) and 38 m (WP1) from the injection well along the line of flow symmetry connecting IP2 and PP1. They are screened with 2-m long polyvinylchloride (PVC) screens at depths of 275, 285, 297, 310 and 330 m below the surface, respectively. Such monitoring screens were also installed in the gravel pack surrounding the screen in the borehole of injection well IP2.

During the first 2 years of its performance, the deep well injection site was studied extensively in order to assess its injection capacity and to investigate factors that affect

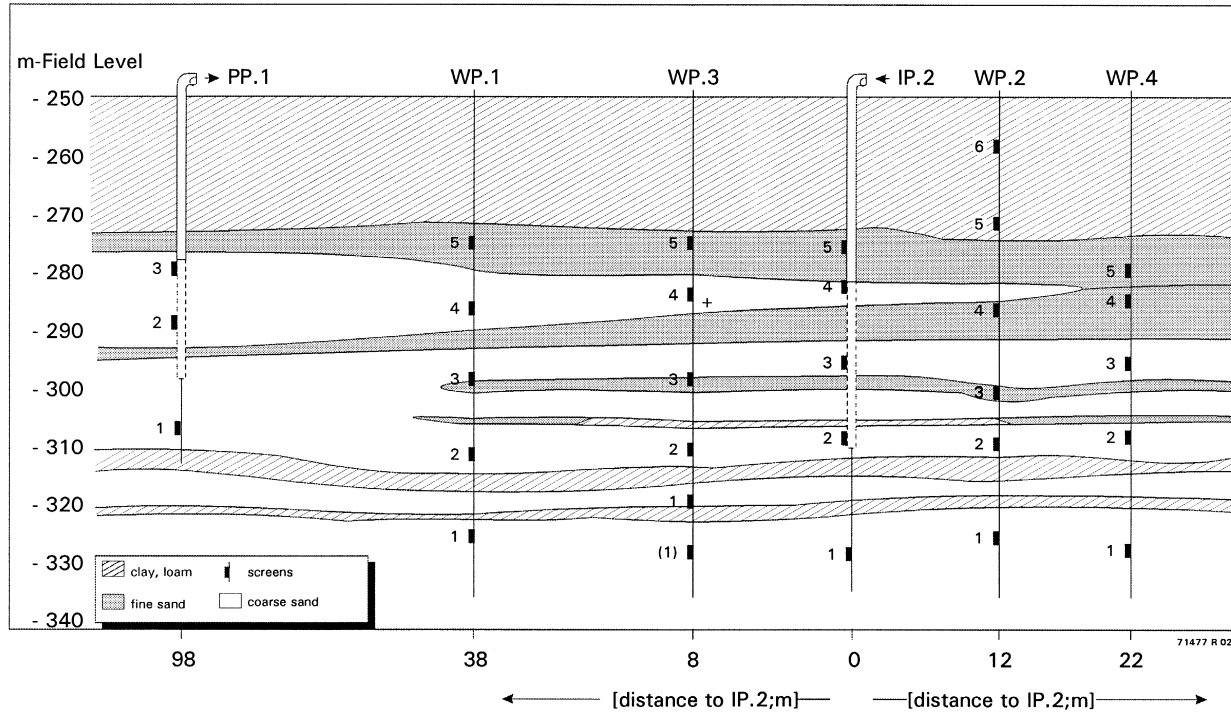


Fig. 1. Schematic cross-section of field site with injection well IP2, monitoring wells WP1 to WP4 and production well PP1. Samples were taken from the number 2 screens.

Table 1

Granular and chemical analysis of soil from production well PP1 at the time of construction (Stuyfzand, 1999)

Depth	m	295
Grain size ^a	μm	272
Clay ($\leq 2 \mu\text{m}$)	%	0.9
Silt (> 2 and $\leq 53 \mu\text{m}$)	%	1.1
Sand ($> 53 \mu\text{m}$)	%	98
Porosity		0.32
f_{oc}	%	0.17
CEC	$\text{mEq (kg dry weight)}^{-1}$	10
Fe-(hydr)oxides	$\text{mg (kg dry weight)}^{-1}$	< 1
Mn-(hydr)oxides	$\text{mg (kg dry weight)}^{-1}$	< 1

^aGrain size is geometric mean.

this capacity. In particular, travel times and quality changes of the injected water (Stuyfzand, 1999) and frequency and causes of clogging have been studied (Vogelaar, 1999).

The study on the removal of microorganisms started in October 1998. During this study, the injection rate of recharge water was kept constant at $960 \text{ m}^3 \text{ day}^{-1}$. Samples from all the wells were taken from the number 2 screens at 310 m below the surface. These screens are all situated within the same layer of sand. This sand layer has the highest permeability as compared to the other sand layers within this aquifer. Its permeability is 25 m day^{-1} (Stuyfzand, 1999). Table 1 shows the results of granular and chemical analyses of soil core samples from this sand at a depth of 295 m. The groundwater in this sand is anoxic (oxygen-, nitrate- and sulfate-free) and alkaline. The sand has low calcium content ($\text{Ca} \leq 0.3\%$). Cation exchange capacity (CEC) is moderate. More than 80% of the adsorbed cations are calcium ions. The sulfide content is high ($655\text{--}4600 \text{ mg S kg}^{-1}$) and is largely present as FeS_2 (pyrite) (Stuyfzand, 1999).

3. Experimental and modeling methods

3.1. Salt tracing

Prior to the injection of water with microorganisms, sodium chloride was injected as a conservative salt tracer to estimate interstitial flow velocity of the injected water and dispersivity of the porous medium. For a period of exactly 5 days, 265 kg NaCl in 880 l was injected each day into the main injection pipeline through which pre-treated surface water was injected at a rate of $960 \text{ m}^3 \text{ day}^{-1}$. This resulted in a salt concentration of 0.275 kg m^{-3} in injection water and the electrical conductivity (EC) of the injection water increased by one step from $450 \mu\text{S cm}^{-1}$ to about $1000 \mu\text{S cm}^{-1}$.

In each monitoring well, 300 m of polyethylene (PE) tubing with an inner diameter of 10 mm was installed. The end of the tubing was 1 m above the bottom of the screens. Three submersed pumps were connected in series along the tubing. During the first 2

weeks of the salt tracing, EC was measured automatically from the number 2 screens of IP2, WP3 (8 m) and WP2 (12 m). Water was pumped up continuously at a rate of 0.1 l min^{-1} from the top of the water column in the monitoring wells in order to refresh stagnant water at the level of the screens. EC measurements at the level of the number 2 screens were carried out at 4-h intervals. To that aim, water was pumped up at a rate of 1.51 l min^{-1} for 30 min and EC was measured at the end of this pumping period by means of fixed EC sensors with a data logger.

EC measurements in samples from the number 2 screens of WP1 (38 m) and WP4 (22 m) were carried out manually at daily and weekly intervals. Prior to the manual sampling for EC measurements, a mobile pump was used to pump up 25 l of water from the top of the water column in order to refresh stagnant water at the level of the screens, and the content of the tubing was refreshed first with twice its volume (50 l) at a pumping rate of 1.5 l min^{-1} .

3.2. Microorganisms, preparation and dosage

Highly concentrated bacteriophage suspensions were prepared as described in Schijven et al. (1999). A highly concentrated suspension of spores of *Cl. bifermentans* R5 was prepared by culturing R5 on Perfringens agar base medium (Oxoid) for 18 h at 37°C . One to two milliliters of sterile water was pipetted onto the plates after cultivation and the colony material was suspended by swabbing the colonies with a sterile swab. The colony material from several plates was combined. To prevent alteration of the surface properties of the spores, the suspension was not pasteurized and contained vegetative cells. These vegetative cells were not included in the analysis, since the samples were pasteurized before analysis. *E.coli* WR1 was grown in buffered peptone water for 18 h at 37°C . Cells were harvested by centrifugation and washed in sterile water. All suspensions were pre-diluted, distributed over five 1-l bottles, and kept at $5 \pm 3^\circ\text{C}$. All microorganisms were kept in separate bottles to prevent interaction. Concentrations were 7×10^{13} plaque-forming particles per liter (pfp l^{-1}) of MS2, 5×10^{11} pfp l^{-1} of PRD1, 2×10^{10} colony-forming particles per liter (cfp l^{-1}) of R5 and 10^{12} pfp l^{-1} of WR1.

At the pilot site, 1-l bottles with suspensions of the microorganisms were emptied into 96 l of fresh injection water in a PVC container that was stirred gently. The seeding of the aquifer with microorganisms started by pumping the contents of the 100-l container into the main injection pipeline at constant rate within 24 h. Just before the container was empty, it was filled again with 96 l of fresh injection water and 1 l of each of the highly concentrated suspensions of the microorganisms. Microorganisms were injected in this fashion for a period of exactly 5 days.

3.3. Sampling

To avoid cross-contamination, seeding activities were kept strictly separated from sampling activities and were performed by different persons. Samples of 100 ml were taken from each 100-l container just after filling of the container and after 24 h, when the container was almost empty.

The scheme for taking samples from the monitoring wells was based on the assumption that the microorganisms traveled equally fast as the salt tracer. Prior to each sampling, 40 l was pumped out from the top of the water column in the monitoring well at a rate of about 2 l min^{-1} in order to refresh stagnant water at the screen level. Also, prior to each sampling, 40 l of water was pumped out from the level of the number 2 screen in order to rinse the content of the sampling tubing and pumps with about twice their volume. Depending on the expected breakthrough concentrations, 4-l or 400-l samples were taken by filling 20-l PVC containers at a rate of 2 l min^{-1} . Temperature and pH were immediately measured on site in an additional (small) sample. All samples were kept under refrigeration and analyzed for tracer microorganisms within 18 h.

To account for possible loss of injected microorganisms by attachment to the 300-m-long sampling tubing, the following experiment was carried out. At the end of the transport experiment with microorganisms, the PE tubing, including the three small, submersed pumps at monitoring well WP1 (38 m), was taken out. First, the tubing was emptied. Next, a 20-l suspension containing about 10^6 MS2 and 10^6 R5 was pumped through the tubing and recollected. Subsequently, the tubing was rinsed with three portions of 10 l of tap water, which were also collected. Finally, 20 l of a beef extract, with pH 9, was pumped through the tubing and recollected at the end. All samples were analyzed for MS2 and R5 concentrations. These analyses showed that no significant loss of MS2 and R5 occurred during the passage of microorganisms through 300 m of sample tubing. Therefore, the measured concentrations may be considered to represent those in the water at 300 m below surface.

3.4. Microorganism enumeration

All samples were split into four parts. MS2 was assayed as described in ISO 10705-1 (1995) using host strain, WG49 (Havelaar et al., 1984). PRD1 was assayed according to ISO 10705-1 using *S. typhimurium* LT2 as the host, omitting nalidixic acid on the top agar layer. For enumeration of spores of *Cl. bifermentans*, samples of up to 1 l were pasteurized for 15 min at 75°C and filtered through a 47-mm $0.45\text{-}\mu\text{m}$ nitrocellulose filter. Larger samples were filtered through 143-mm $0.45\text{-}\mu\text{m}$ filters, and then these filters were pasteurized in a humid chamber for 15 min at 75°C (Hijnen et al., 2000). The filters were placed in the lid of a 9-cm or 15-cm petri dish and embedded in Perfringens Agar Base medium (Oxoid) of 45°C . The medium was sealed with the base of the petri dish and incubated at 44°C . After 24 and 48 h, the number of black colonies was recorded. Both large and small colonies were observed in the samples from the observation wells. Between 0% and 93% of the colonies were small colonies (average 36%). Small and large colonies were typed using API20A (Biomérieux). Only the larger colonies were found to be *Cl. bifermentans*. Hence, only the counts of the large colonies were used in this study. The smaller colonies could not be classified by API20A.

For enumeration of *E.coli* WR1, samples of up to 1 l were filtered through a 47-mm $0.45\text{-}\mu\text{m}$ nitrocellulose filter, but larger samples were filtered through 143-mm $0.45\text{-}\mu\text{m}$ filters. Filters were placed onto Lauryl Sulphate agar and incubated for 5 h at 25°C and 14 h at 44°C . Yellow colonies were counted and all colonies from the observation well samples were confirmed by incubation at 44°C in brilliant green bile lactose broth.

3.5. Declogging

Clogging is one of the most troublesome phenomena when applying artificial recharge. Clogging may be caused by accumulation of suspended particles, bacterial growth, chemical precipitation, gas generation, and compaction of the clogging layer (Pyne, 1995). Because the injection rate was kept constant, an increased hydraulic head was measured at the injection well due to the clogging that took place. In the course of this field study, declogging of the injection well was carried out twice. The first declogging was carried out 31 days after the start of the injection with microorganisms. At day 93, pumping was stopped and the site was left undisturbed to simulate Aquifer Storage Recovery (ASR). ASR is a cost-effective technology that is widely used for underground storage of large volumes of water (Pyne, 1995). After a period of 4 months, a second declogging was carried out.

The declogging was carried out as follows. First, injection of water was stopped. Then, the hydraulic head was lowered 20 m by air pressure, followed by immediate release of air pressure. This induced a high rate of flow of water towards the injection well for a few seconds. The aim was to cause the release of clogged material in the vicinity of the injection well. This procedure was carried out 25 times. Next, water was pumped up from the injection well at a flow rate of $200 \text{ m}^3 \text{ h}^{-1}$. During the first declogging, 68 m^3 of water was pumped up and during the second declogging, 1100 m^3 of water. The volume of the standing pipe is 17 m^3 . Samples were taken to analyze the suspended sediment content. The sediment was investigated for its iron, manganese and aluminum content by shaking 2.5 g of the sediment in 0.1 l of ammonium oxalate/oxalic acid, pH 3, for a period of 2 h. Subsequently, the extract was analyzed for iron, manganese and aluminum by atomic adsorption spectrometry as described in Houba et al. (1989) and Stuyfzand and van der Jagt (1997). To analyze the chemical processes that have taken place during ASR, water samples were also analyzed for turbidity, iron, and total organic carbon (TOC). This was also an opportunity to investigate where most of the injected microorganisms were retained. Therefore, during backpumping samples, of 1 l (first declogging) and 200 l (second declogging) were taken to analyze for MS2 and R5.

3.6. Conceptual model

Major transport processes included in the mathematical model of our field experiments are advection, hydrodynamic dispersion, attachment, detachment and inactivation. The flow field is assumed to be at steady state. The monitoring wells WP1–WP4 lie along the line of flow symmetry connecting IP2 and PP1. The number 2 screens of all wells are located at the same depth within a 30-m-thick high-permeable sand layer. Therefore, the flow is assumed to be one-dimensional. Furthermore, calculations of the actual flow velocity profile show that after a distance of about 8 m, the variations in velocity magnitude remain limited. Thus, for our modeling purposes, the velocity is assumed to be constant. Transverse dispersivity was neglected. Attachment and detachment are modeled as first-order processes. Inactivation is modeled as first-order decay with different rate coefficients for free and attached microorganisms. With these

assumptions, the governing equations of a one-dimensional, one-site kinetic transport model apply:

$$\frac{\partial C}{\partial t} = \alpha_L v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - k_{\text{att}} C - \mu_1 C + k_{\text{det}} \frac{\rho_B}{n} S, \quad (1)$$

$$\frac{\rho_B}{n} \frac{\partial S}{\partial t} = k_{\text{att}} C - k_{\text{det}} \frac{\rho_B}{n} S - \mu_s \frac{\rho_B}{n} S. \quad (2)$$

Subject to boundary conditions $C = C_0$ at $x = 0$ and $(\partial C / \partial x) = 0$ at $x = \infty$. Here, C is the concentration of free microorganisms, i.e. microorganisms in the aqueous phase [number m^{-3}]; S is the concentration of attached microorganisms [number kg^{-1}]; t is the time [days]; x is the distance from the injection well towards the production well [m]; α_L is the longitudinal dispersivity [m]; v is the average interstitial water velocity [m day^{-1}]; ρ_B is the aquifer bulk density [kg m^{-3}]; n is the porosity [–]; k_{att} and k_{det} are the attachment and detachment rate coefficients, respectively [day^{-1}]; μ_1 and μ_s are the inactivation rate coefficients of the free and attached microorganisms, respectively [day^{-1}]. The computer code CXTFIT (Toride et al., 1995) contains the analytical solution of these equations, including the code for inverse parameter estimation.

Dispersion of both MS2 and PRD1 and the salt tracer has been shown to be similar in columns with sandy soil (Bales et al., 1989) and silica beads (Bales et al., 1991). In field studies where preferential flow was absent, PRD1 was transported at about the same rate as a conservative salt tracer (Bales et al., 1995; Pieper et al., 1997; Schijven et al., 1999). Therefore, the quantities v and α_L that were found from fitting the salt breakthrough curves using CXTFIT (Toride et al., 1995) were assumed to apply to the microorganisms, too.

Attachment of MS2 and PRD1 has been shown to be reversible and kinetically limited (Bales et al., 1991, 1993, 1997; Kinoshita et al., 1993; Schijven et al., 1999). Therefore, phage transport may be simulated using a one-site kinetic model. Moreover, in many field studies (see, e.g. Bales et al., 1991; McCaulou et al., 1994; Schijven et al., 1999), the level of breakthrough was found to be mainly determined by attachment, with detachment being much slower. The same applies for bacterial transport. In several column studies (Hornberger et al., 1992; Camper et al., 1993; McCaulou et al., 1994, 1995; Rijnaarts et al., 1995; Hendry et al., 1997) and a field study (Harvey and Garabedian, 1991), it has been shown that the level of breakthrough of bacteria is mainly determined by attachment, and that detachment is much slower. The temperature of the water in the aquifer was about 12°C and, therefore, bacterial growth was not considered. The average grain size diameter of the aquifer sand of the present field study was 0.27 mm. The size of the studied bacteria is less than 5% of the average grain size and, therefore, straining can be neglected (Harvey and Garabedian, 1991).

We found that the tails of the breakthrough curves of the microorganisms could not be fitted satisfactorily using CXTFIT. This was ascribed to the limited number of measurements and considerable variations in the concentrations along the tails of the breakthrough curves. As an approximate alternative, it is assumed that the detachment rate is much smaller than the inactivation rate of attached microorganisms. Therefore,

under steady-state conditions (reached during maximum breakthrough concentration), Eq. (1) reduces to:

$$\alpha_L v \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - (k_{\text{att}} + \mu_1) C = 0. \quad (3)$$

Eq. (3) has the following solution:

$$\log\left(\frac{C_{\text{max}}}{C_0}\right) = \frac{x}{2.3} \frac{\left(1 - \sqrt{1 + 4\alpha_L \frac{k_{\text{att}} + \mu_1}{v}}\right)}{2\alpha_L}, \quad (4)$$

where $\log(C_{\text{max}}/C_0)$ is the logarithmic reduction in concentration. By rearranging Eq. (4), the value of k_{att} can be calculated (Schijven et al., 1999):

$$k_{\text{att}} = \frac{\left[1 - 2\alpha_L \frac{2.3}{x} \log\left(\frac{C_{\text{max}}}{C_0}\right)\right]^2 - 1}{4\alpha_L} v - \mu_1. \quad (5)$$

The value of μ_1 was obtained by measuring the inactivation rate in water from IP2 and WP3 (8 m) at the laboratory as described below.

3.7. Inactivation rate of free microorganisms in water

To measure inactivation of the microorganisms in the water phase during the experiment, a 4-l sample was taken from well IP2 on the third day of injection of the microorganisms. A 4-l sample was also taken from monitoring well WP3 (8 m) during maximum breakthrough. The bottles with these samples were stored in a cold room at $11.8 \pm 0.5^\circ\text{C}$ and analyzed regularly for a period of about 4 months. In this manner, inactivation rates of free microorganisms just before and after 8 m of aquifer passage were determined.

3.8. Estimates of collision efficiencies

From the values of k_{att} , collision efficiencies, α , were calculated. The collision efficiency is an empirical constant that accounts for electrostatic interactions, in this case, between microorganisms and the porous medium. Collision efficiencies were calculated using the following equation (Yao et al., 1971):

$$\alpha = \frac{2}{3} \frac{d_c}{(1-n)} \frac{k_{\text{att}}}{v} \frac{1}{\eta}, \quad (6)$$

where α is the collision efficiency and η is the single collector efficiency. The single collector efficiency η was calculated using the following relationship due to Martin et al. (1992):

$$\eta = 1.0 A_s N_{\text{Lo}}^{1/8} N_{\text{R}}^{15/8} + 0.00388 A_s N_{\text{G}}^{1.2} N_{\text{R}}^{0.4} + 4 A_s^{1/3} N_{\text{Pe}}^{-2/3}. \quad (7)$$

Here, $N_R = d_p/d_c$ accounts for interception, $N_G = d_p^2(\rho_p - \rho)g/(18\mu vn)$ for gravity effects, $N_{Lo} = 4H/(9\mu d_p^2 vn)$ for van der Waals interactions, and $N_{Pe} = d_p vn/D_{BM}$ for diffusion. In these definitions, d_p and d_c represent the microorganism particle and grain sizes [m], respectively, g is the gravitational acceleration, ρ and ρ_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 s)^{-1}] with T as the water temperature [$^{\circ}\text{C}$], $H = 6.2 \times 10^{-21}$ is the Hamaker constant [J] for the bacterium–glass–water interface (Rijnaarts et al., 1995), $D_{BM} = K_B(T + 273)/(3\pi d_p/\mu)$ is the diffusion coefficient [$\text{m}^2 \text{s}^{-1}$] 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 $\gamma = (1 - n)^{1/3}$. R5 spores have an assumed size of 1 μm and a buoyant density of 1270 kg m^{-3} (Tisa et al., 1982).

Because MS2 is small ($d_p = 26 \text{ nm}$; Bales et al., 1991), its transport in the immediate vicinity of the collector surface is dominated by Brownian diffusion. In this case, η is given approximately by the last term in Eq. (7).

4. Results

4.1. Description of the breakthrough curves

Table 2 shows the injected concentrations of the microorganisms, C_0 , as calculated from the concentration in the 100-l container and the injection rate of surface water. C_0 was not found to be significantly different from the concentration, C_{IP2} , that was measured in the gravel pack of the injection well. At the end of 5 days of the injection with microorganisms, C_{IP2} did not go to zero immediately but showed a considerable tailing (Fig. 2). This tailing is most probably caused by gradual release of microorganisms retained in the borehole of the injection well by attachment. Compared to the number of injected microorganisms, the numbers of microorganisms that formed the tail part at IP2, i.e. that were slowly released, were 2.4% for MS2, 1.7% for PRD1, 12% for R5 and 1.5% for WR1.

PRD1 could only be detected at well WP3 (8 m) and WP2 (12 m), and *E. coli* strain WR1 only in one sample at WP3. Transport of MS2 and R5 could be followed at all monitoring wells. Fig. 3 shows the breakthrough curves of the salt tracer, MS2 and R5. Due to some technical problems with the intake of surface water and during the declogging, injection of water was stopped a few times varying in length from 16 to 57 h. Time axes of the breakthrough curves were adjusted accordingly so that only the time that pumping was on is given.

In Table 3, the estimates of pore water velocity and longitudinal dispersivity are given. The dispersivity values are low, which indicates that the sandy layer at the depth of 280–310 m is relatively homogeneous. The total mass of salt tracer under the breakthrough curves measured at the monitoring wells was found to be almost constant. This indicates that there is no lateral loss of mass. In other words, transverse dispersivity is much smaller than longitudinal dispersivity. This justifies a one-dimensional modeling approach.

Table 2
Initial and maximum concentrations and removal of the injected microorganisms

		MS2	PRD1	R5	WR1
C_0^a [$N\ l^{-1}$]		7.1×10^7 (1.4×10^7)	4.3×10^5 (1.4×10^5)	1.6×10^4 (5.7×10^3)	1.1×10^6 (2.6×10^5)
C_{IP2}^b [$N\ l^{-1}$]	IP2	6.1×10^7 (1.2×10^7)	5.1×10^5 (2.8×10^5)	3.0×10^4 (1.7×10^4)	1.2×10^6 (5.6×10^5)
C_{well}^{0c} [$N\ l^{-1}$]	WP3 (8 m)	95 (3.7)	0.89 (0.42)	0.29 (0.10)	0.03 (–)
	WP1 (38 m)	0.26 (0.13)	–	0.16 (0.18)	–
	WP2 (12 m)	4.6 (2.0)	0.038 (–)	0.70 (0.60)	–
	WP4 (22 m)	0.73 (–)	–	0.097 (0.016)	–
$-\log(C_{well}^0 / C_0)$	WP3 (8 m)	5.9	5.7	4.7	7.5
	WP1 (38 m)	8.4	–	5.0	–
	WP2 (12 m)	7.2	7.1	4.4	–
	WP4 (22 m)	8.0	–	5.2	–

^a C_0 was calculated from the concentration in the 100-l container and the volume of injected water.

^b C_{IP2} concentration was measured in the gravel pack surrounding the standing pipe in the injection well IP2.

^c C_{well}^0 concentration was calculated from average breakthrough concentration over the period of maximum breakthrough of salt. Standard deviations are given between parentheses.

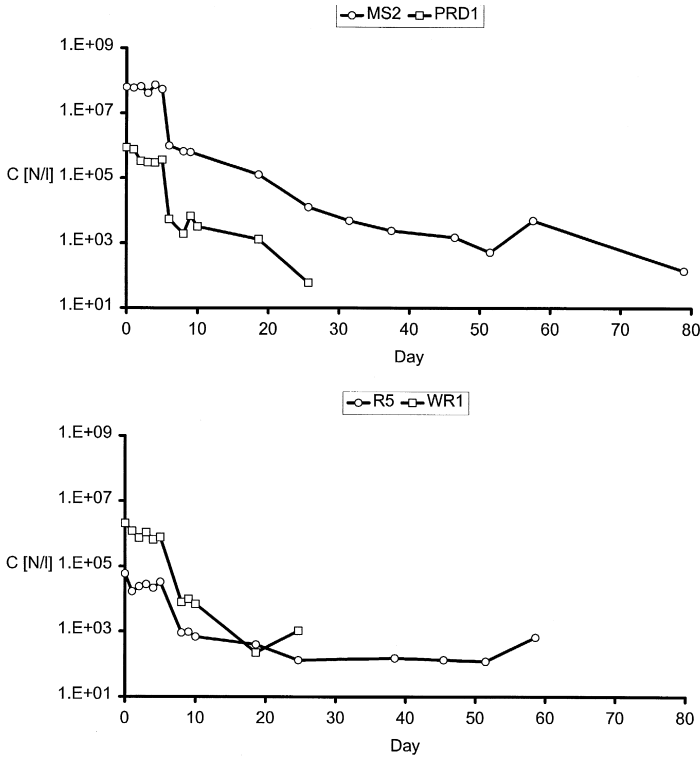


Fig. 2. Observed concentrations of the injected microorganisms at injection well IP2.

As expected, MS2 was transported with the same velocity as the sodium chloride. At WP3 (8 m), breakthrough of R5 seemed to be retarded relative to that of sodium chloride, but at WP4 (22 m), it appeared to be faster, while at the other two wells, its breakthrough was more or less concurrent with that of the sodium chloride. It is believed that R5 was transported at the same rate as MS2 and the sodium chloride, but large variations in concentrations in combination with a limited sampling frequency give a misleading picture.

4.2. Logarithmic concentration reduction with distance

The maximum breakthrough concentration at a monitoring well, C_{well}^0 , was calculated as the mean of breakthrough concentrations over the period of maximum breakthrough; this period was determined on the basis of the sodium chloride experiment. The degree of removal was calculated as the logarithmic reduction in concentration, $-\log_{10}(C_{well}^0/C_0)$ (Table 2). For the sake of comparison, we also calculated the degree of reduction as the ratio of the total number passing a monitoring well and the total number of injected microorganisms; there was no difference with the logarithmic reduction in concentration. Fig. 4 shows a plot of the logarithmic reductions vs. traveled

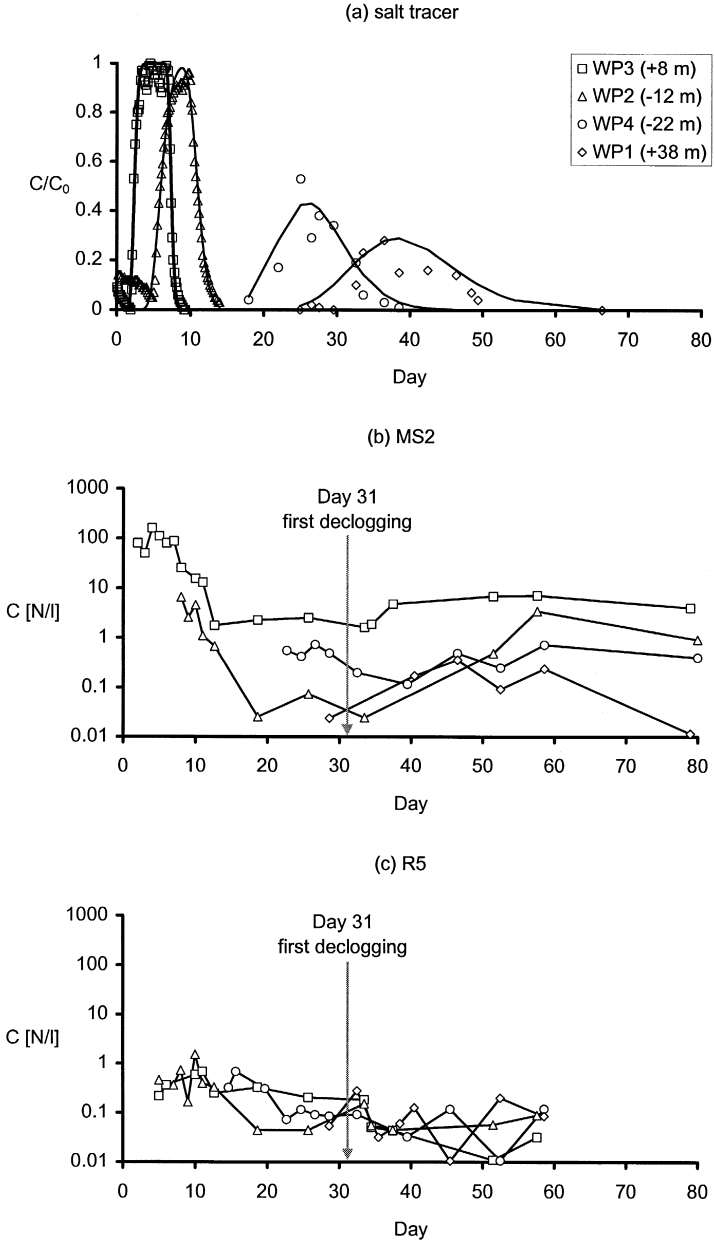


Fig. 3. Breakthrough curves of (a) salt tracer (NaCl), (b) MS2 and (c) R5.

distance. Concentration reductions of MS2 and PRD1 appear to be very similar. MS2 and PRD1 concentrations were attenuated by almost 6 log₁₀ at WP3 (8 m). Reduction of concentrations at WP3 was the smallest for R5 (4.7 log₁₀) and the largest for WR1

Table 3
Values of model parameters

	Parameter ^a	IP2	WP3	WP1	WP2	WP4
NaCl	x	0.1	+8	+38	-12	-22
	v	-	3.33	1.01	1.98	0.897
	x/v	-	2.4	38	6.1	25
	α_L	-	0.11	0.66	0.19	0.38
	α_L/x	-	0.014	0.017	0.016	0.017
	Total mass (%) ^b	-	96%	97%	92%	100%
MS2	μ_1	0.081	0.039			
	k_{att}	-	6.7	0.65	3.4	0.95
	α	-	1.4×10^{-3}	2.0×10^{-4}	8.0×10^{-4}	2.9×10^{-4}
PRD1	μ_1	0.060				
R5	μ_1	n.s. ^c				
	k_{att}	-	5.1	0.33	1.9	0.55
	α	-	8.0×10^{-3}	6.1×10^{-4}	3.2×10^{-3}	1.0×10^{-3}
WR1	μ_1	0.083				

^aDimensions: x [m], v [m day⁻¹], α_L [m], μ_1 and k_{att} [day⁻¹]. Collision efficiency α is dimensionless.

^bTotal mass of salt tracer under breakthrough curve, relative to total injected mass.

^cn.s. = not significantly different from zero.

($7.5 \log_{10}$). It can be seen that MS2 concentrations are gradually reduced at a lower rate as the distance from the injection well increases. R5 also showed a non-linear reduction in concentration with distance. At WP3, reduction was about $4.7 \log_{10}$, but from that point on, it was negligible.

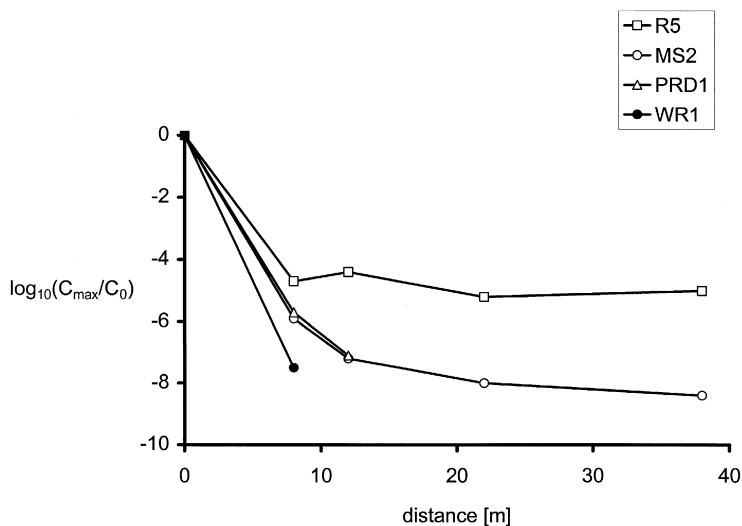


Fig. 4. Logarithmic reduction of concentrations of microorganisms with distance.

4.3. Inactivation of free microorganisms

Results of inactivation experiments for free microorganisms are shown in Fig. 5. Evidently, the inactivation rates are first order under all conditions. Inactivation rate coefficients are given in Table 3. R5 showed no significant inactivation.

The μ_1 values for MS2 and WR1 in the water from the injection well were about the same, but was a little less for PRD1. MS2 was found to inactivate at a rate of 0.039 day^{-1} in water from monitoring well WP3 (8 m) — two times slower than in water from the injection well and also slower than PRD1 in water from the injection well.

4.4. Estimates of attachment rate coefficients and collision efficiencies

The calculated attachment rate coefficients, k_{att} , and collision efficiencies, α , are given in Table 3. These values were only calculated for MS2 and R5, because these two microorganisms could be measured at all 4 monitoring wells. Collision efficiencies for MS2 are low; this indicates relatively unfavorable conditions for attachment. The values of α are very similar to the ones that were found in our previous study on removal of bacteriophages by dune recharge (Schijven et al., 1999). In that study, the collision efficiency of the bacteriophages decreased with travel distance from 1.4×10^{-3} at 2.4 m to 2.4×10^{-4} at 30 m. Pieper et al. (1997) found slightly higher values for PRD1 in a sewage-contaminated aquifer (1.4×10^{-3} – 2.6×10^{-3}) at a similar pH (6.0–6.7) as in the present field study. Bacterial spores are larger than bacteriophages and have therefore a lower single collector efficiency, η (Yao et al., 1971). Therefore, although

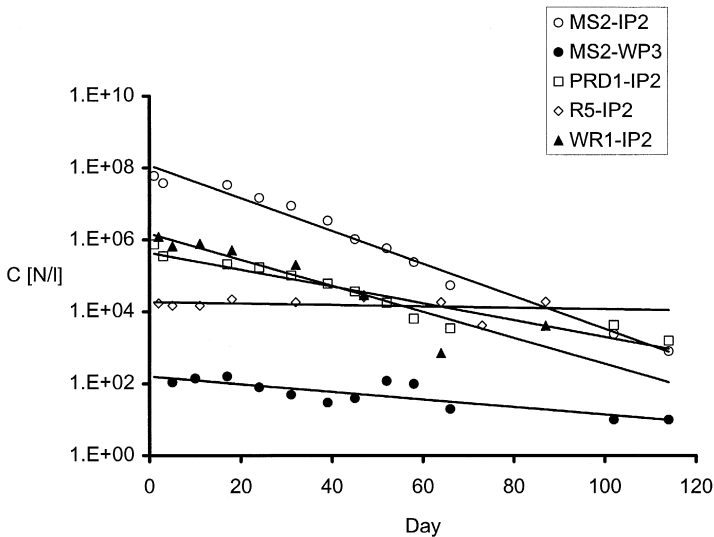


Fig. 5. Inactivation of MS2, PRD1, R5 and WR1 in water from IP2 and WP3 (8 m). Lines are regression lines. See Table 3 for inactivation rate coefficients.

R5 concentration was reduced less than that of MS2, its collision efficiency appears to be a few times higher. In a previous study, the collision efficiency for spores of sulfite-reducing clostridia after 2 m of passage through fine dune sand was found to be 9.7×10^{-3} (Schijven et al., 1998). This value is only slightly higher than the one found for R5 spores at WP3 (8 m). Also, similar collision efficiencies (5.4×10^{-3} – 9.7×10^{-3}) were found for small, indigenous bacteria (0.2–1.4 μm) in a field study by Harvey and Garabedian (1991) in the same sewage-contaminated aquifer as in the field study of Pieper et al. (1997). For an inventory of more collision efficiencies, see Schijven and Hassinazadeh (2000).

4.5. Analysis of declogging results

In this section, the data obtained after the first and second declogging operations are analyzed. Table 4 shows the analysis of sediments in the water that was pumped up during the first declogging operation. Visual inspection by naked eye and under the microscope showed that in the 11-min sample, 80% of the sediment consisted of sand, but in the sample after 15 min of pumping, the sediment consisted mainly of detritus from copepods. Sediment in the 19-min sample consisted mainly of sand again. Comparison of changes in hydraulic head in IP2, in the monitoring well in the gravel pack of IP2 and in WP3 (8 m), indicated that 95% of the collected sediment was located near or at the wall of the borehole and 5% at the openings of the injection screen (Vogelaar, 1999). Apparently, the organic detritus is too large to enter the pores of the aquifer. In all samples, 5% of the sediment consisted of oxidized iron and manganese particles. Possibly some of these are the residual iron that originate from the coagulation treatment prior to injection. The first declogging reduced the increase in hydraulic head to a level lower than after the previous declogging prior to this study. However, the hydraulic head was still higher than at the start of the deep well injection in July 1996, indicating that not all of the clogging materials were removed.

Fig. 6 shows the concentrations of MS2 and R5 measured in the water that was pumped up from the injection well plotted as a function of time. It took about 6 min to pump up the water content of the standing pipe of IP2; after that, water from the aquifer was being sampled. In the water that was pumped up from the standing pipe, concentrations of all MS2, PRD1 and R5 were at a similar level as that measured in samples from the gravel pack of IP2 just before declogging. Apparently, the declogging did not cause extra release of microorganisms within a meter distance from the injection well. About 2.5 days after declogging, i.e. the travel time of water from IP2 to WP3 (8 m), a rise in concentration of MS2 by about a factor of about 3 was measured at WP3 (Fig. 3). Also at WP2 (12 m), a rise in concentrations was observed for MS2, this time by about a factor of 100, almost to the same level as at WP3. This rise in concentration was measured more than 6 days after declogging IP2. Probably, the exact time of rise in concentration was missed. Also a rise in concentration of MS2 was observed at WP4 (22 m), 26 days after declogging. No concentration change in relation to the declogging was evident from WP1 (38 m). These observations suggest that at a few meters distance from the injection well, extra release of MS2 had occurred as a consequence of the declogging, and that a pulse of released MS2 was transported along with the water. Colloids may be mobilized by hydrodynamic shear over a wide size range, whereby

Table 4
Results from analysis of water and sediment during declogging

First declogging							
Time [min]	Sediment [mg l^{-1}] ^a	Sand [%] ^b	Debris of plants [%] ^b	Detritus [%] ^b	Fe_{ox} and $\text{Mn}_{\text{ox}}^{\text{c}}$ [%] ^b		
11	5.0×10^{-4}	80	5	10	5		
15	7.1×10^{-4}	10	5	80	5		
19	2.9×10^{-4}	95	0	0	5		
Second declogging							
Time [min]	Turbidity [FTE]	Dissolved Fe [mg l^{-1}]	TOC [mg l^{-1}]	Sediment ^a [mg l^{-1}]	$\text{Al}_{\text{ox}}^{\text{c}}$ [%] ^b	$\text{Fe}_{\text{ox}}^{\text{c}}$ [%] ^b	$\text{Mn}_{\text{ox}}^{\text{c}}$ [%] ^b
4	1.89	12	4.3	1.4×10^{-3}	0.14	0.32	0.0024
8	1.87	11	3.3	6.2×10^{-3}	0.077	0.13	0.0011
16	0.47	8.7	2.2	8.5×10^{-4}	0.070	0.15	0.00090
32	0.25	5.1	1.8	1.3×10^{-4}	0.034	0.11	0.00050
64	0.19	3.0	1.6	1.4×10^{-4}	0.17	0.41	0.00020
128	0.17	1.8	1.3	9.0×10^{-5}	0.071	0.15	0.00080
256	0.16	0.99	1.5	4.0×10^{-5}	0.057	0.16	0.00090

^aSediment with particles $> 50 \mu\text{m}$.

^bPercentage of dry weight of sediment.

^cOxidized Al/Fe/Mn.

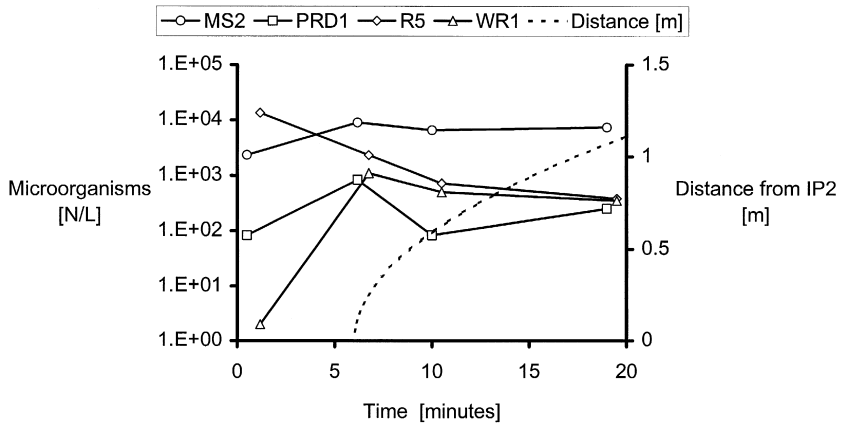


Fig. 6. Analysis of water during first declogging.

larger particles will be mobilized before small particles as velocity increases (Ryan and Elimelech, 1996). Hofmann and Schöttler (1998) showed in a study on artificial recharge of groundwater that by shockwise increases in water velocities, the size of suspended colloidal particles may increase by a factor of 10 to an average of 1.7 μm . In the case of R5, no rises in concentrations were evident, possibly due to the relatively large variations in the tail concentrations of the R5 breakthrough curves and because concentrations of attached R5 were about 10^4 times lower than concentrations of attached MS2.

From Table 4, it can be seen that during the second declogging, turbidity, TOC and the concentration of sediment were initially high. This indicates that most of these materials came from within a radius of 1 m around the injection well. Also, the concentration of dissolved iron was initially higher, starting at a level of two magnitudes higher than in the pre-treated surface water before injection (Table 5). During the 4 months of ASR, the injection well had turned anoxic. The high concentration of dissolved iron, together with the low oxidized iron content of the sediment, suggests that oxidized iron has dissolved under these anoxic conditions. The oxidized iron content of the sediment from the second declogging was 20–50 times lower than from the first declogging.

Fig. 7 shows the measurements of MS2 and R5 in the water that was pumped up from the injection well during the second declogging. Initially, high concentrations of MS2 of up to 2.6×10^4 pfp l^{-1} were measured in water coming from a distance of 0–1.5 m from IP2. Then, a quick decrease in concentration was seen to a level of 5×10^2 pfp l^{-1} in water coming from a distance of 2.5–5.5 m. At the beginning of the ASR experiment, the concentration of MS2 measured at IP2 was 100 pfp l^{-1} but at the end of the ASR period, concentrations that were 260 times higher were measured. Clearly, MS2 had been released by the declogging procedure. During the first 5 days of the transport experiment, a total of 4.3×10^{14} MS2 was injected. During the 4 months of ASR, equilibrium partitioning between free and attached bacteriophages was easily achieved. Because $k_{\text{att}} > k_{\text{det}}$, most of the remaining MS2 phages were attached to the

Table 5

Average concentration (μM) and concentration change (μM) of major redox parameters of injection water during first year of deep well injection (Stuyfzand, 1999) and temperature and pH of injection water during the transport experiment with microorganisms

	Average concentration		Change in concentration			
	IP2	PP1	WP3 (8 m)	WP1 (38 m)	WP2 (12 m)	WP4 (22 m)
Dissolved O ₂	310	< 16	- 300	- 330	- 300	- 320
NO ₃ ⁻	310	< 8	- 87	- 340	- 190	- 270
SO ₄ ²⁻	650	400	+ 220	+ 350	+ 250	+ 130
Fe _r	2.1	88	+ 9.6	+ 8.8	+ 1.6	0
Mn _r	0.11	3.8	+ 0.91	+ 2.0	+ 0.91	+ 4.4
Ca ²⁺	1700	2000				
Mg ²⁺	360	530				
Cl ⁻	2300	170				
Temperature ^a [°C]	11.2 (1.8)		12.2 (0.44)	12.5 (0.66)	12.9 (1.0)	12.1 (0.56)
pH ^a	7.0 (0.13)		6.5 (0.094)	6.7 (0.11)	6.8 (0.11)	6.6 (0.068)

^aStandard error between brackets.

soil. In water from the monitoring screen just outside IP2, a high μ_1 value of 0.081 day⁻¹ for MS2 was found (Table 3). If all MS2 phages were retained within this area, i.e. the aquifer was not reached, and if they were inactivated at this high rate, their numbers would have been reduced to 6.8×10^6 in the 218 days that had passed since their injection. This is much less than the number of 1.7×10^9 that was recovered within 60 min in water coming from up to 2 m distance from IP2. It is reasonable to assume that μ_s of MS2 is less than or equal to the value measured in water from WP3 (0.039 day⁻¹; see Table 3) because the breakthrough tails were flat, and also because the water has turned anaerobic during the 4 months of ASR. In that case, their numbers would have decreased less than or equal to 6.8×10^{10} in 218 days since injection. This implies that less than or equal to 2.5% of the injected MS2 phages was recovered from a

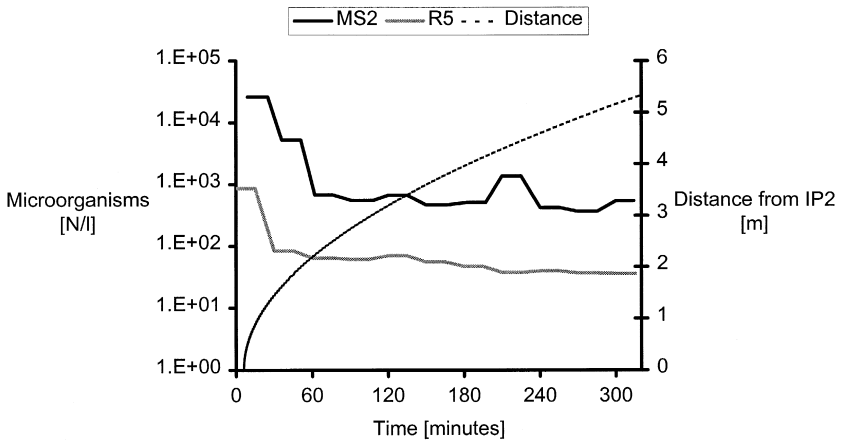


Fig. 7. Analysis of water during second declogging.

distance of up to 2 m from the injection well. Although it is not clear whether all MS2 phages within this area were detached by the declogging, these calculations suggest that most MS2 phages did penetrate the aquifer farther than 2 m from the injection well.

Concentrations of R5 were also initially high (860 l^{-1}) in water pumped up from within 1.5 m of the injection well and then stabilized at about $50\text{--}60 \text{ l}^{-1}$. During the first 5 days of the transport experiment, 7.5×10^{10} spores were injected that were subsequently reduced by a factor of 3 due to inactivation, but then remained stable (Fig. 5). After the second declogging, a total of 4.3×10^7 was recovered in water from the first meter around the injection well, which is about 0.1%. This indicates that also the majority of R5 spores had entered the aquifer farther than 1.5 m.

5. Discussion and conclusions

Removal of microorganisms from pre-treated surface water by deep well injection was studied at a pilot site in the Netherlands. After dosage of the injection water with bacteriophages (MS2 and PRD1), spores of *Cl. bifermentans* (R5) and *E. coli* (WR1), their breakthrough was followed at different monitoring wells. Within the first 8 m of soil passage, concentrations of MS2 and PRD1 were reduced by $6\log_{10}$, that of R5 spores by $5\log_{10}$ and that of WR1 by $7.5\log_{10}$. Breakthrough of MS2 and R5 could also be followed at larger distances from the injection well. Concentrations of MS2 were reduced only by about $2\log_{10}$ in the following 30 m, but the reduction of concentrations of R5 was negligible. Apparently, logarithmic reduction of concentrations decreased non-linearly with distance.

For the purpose of production of safe drinking water, these reductions are more than adequate. Nevertheless, it is important for the design of water treatment systems to understand the non-linear character of concentration reduction with distance. There are several indications that the high initial reduction takes place gradually within the first 8 m of soil passage, but not in the borehole of the injection well.

(i) The maximum concentrations of the microorganisms, as measured in the gravel pack at IP2, were not significantly different from the seeding concentration. Only a few percentages were retained in the borehole.

(ii) Concentration reduction of MS2 in the range of 8–12 m from the injection well was still 10 times more efficient than at greater distances.

(iii) During the first declogging, no significant release of microorganisms at a distance of 1–1.5 m from the injection well was observed, but after a period equal to the travel time, a rise in concentrations was observed for MS2 at WP2 (12 m), WP3 (8 m) and WP4 (22 m). This suggests extra release of MS2, probably attached to other colloidal particles, at a distance of more than 1.5 m that was induced by mechanical forces during the declogging. Consequently, these released phages were transported farther downstream.

(iv) During the second declogging, high numbers of MS2 and R5 were recovered from the injection well, coming from a distance of less than 2 m from the injection well. Still, these numbers represented only a small fraction of the originally injected numbers of microorganisms. This indicates that the majority of injected microorganisms were transported farther than 1.5 m.

During the first 2 years of its operation, the quality changes of injected water at the pilot site were studied extensively (Stuyfzand, 1999). The clogging of the injection well did not affect the chemical quality of the water during soil passage. Table 5 summarizes some of the major redox parameters and the change in their values during soil passage. It shows that dissolved oxygen disappears completely within the first 8 m of aquifer passage. Within that area, the decrease of the nitrate concentration is also the strongest, accompanied by a strong increase of the sulfate concentration. Based on geochemical mass balances, Stuyfzand (1999) concluded that dissolved oxygen is completely used up by the oxidation of pyrite (see also Stuyfzand, 1993, 1998). The following redox zones that are also visualized in Fig. 8 could be distinguished:

- (i) The O₂ zone, up to 10 m, where the water contains oxygen and nitrate, both decreasing with distance;
- (ii) The NO₃ zone, with no oxygen, but with nitrate, up to 15 m at the level of the number 2 screens; and
- (iii) The SO₄ zone, without oxygen and nitrate, but raised sulfate concentration as compared to that of the injection water.

As a consequence of pyrite oxidation, ferric oxyhydroxides precipitated in the O₂ zone. This was also the case in the NO₃ zone at the level of the number 2 screens, but to a lesser extent, because oxidation of pyrite by nitrate proceeded slower than by oxygen. After 2.3 years of deep well injection, there still is reactive pyrite present in the O₂ zone. Because oxygen reacts fast with pyrites, the oxygen will not reach the production well before 1000 years (Stuyfzand, 1999). In addition to the oxidation of pyrite, residual iron flocs originating from the coagulation treatment prior to injection may also be filtrated and thus add to the accumulation of ferric oxyhydroxides.

At the ambient pH of 6.8, precipitated ferric, manganese and aluminum oxyhydroxides form positively charged patches on the soil grains and provide favorable attachment sites for negatively charged viruses (Loveland et al., 1996). Higher attachment of viruses due to the presence of such positively charged patches of ferric oxyhydroxides has been shown in column experiments by Loveland et al. (1996) and has been suggested in a column study by Jin et al. (1997)42. It has also been found in two field studies (Pieper et al., 1997; Ryan et al., 1999).

Similar to the approach of Ryan et al. (1999), the surface fraction f of positively charged patches can be calculated from the observed collision efficiencies for MS2 and R5 (Table 3):

$$f = \frac{\alpha_{\text{apparent}} - \alpha_0}{\alpha_{\text{patch}} - \alpha_0}, \quad (8)$$

where, α_{apparent} is the apparent value of α as calculated from the value of k_{att} . We can assume that the collision efficiency for MS2 and R5 to these patches, α_{patch} , approximates the value of 1 owing to electrostatic attraction. The collision efficiency for attachment to the sand without these positively charged patches, α_0 , can be assumed to be similar to the estimate of α at WP1 (38 m). This leads to a value of f for MS2 of

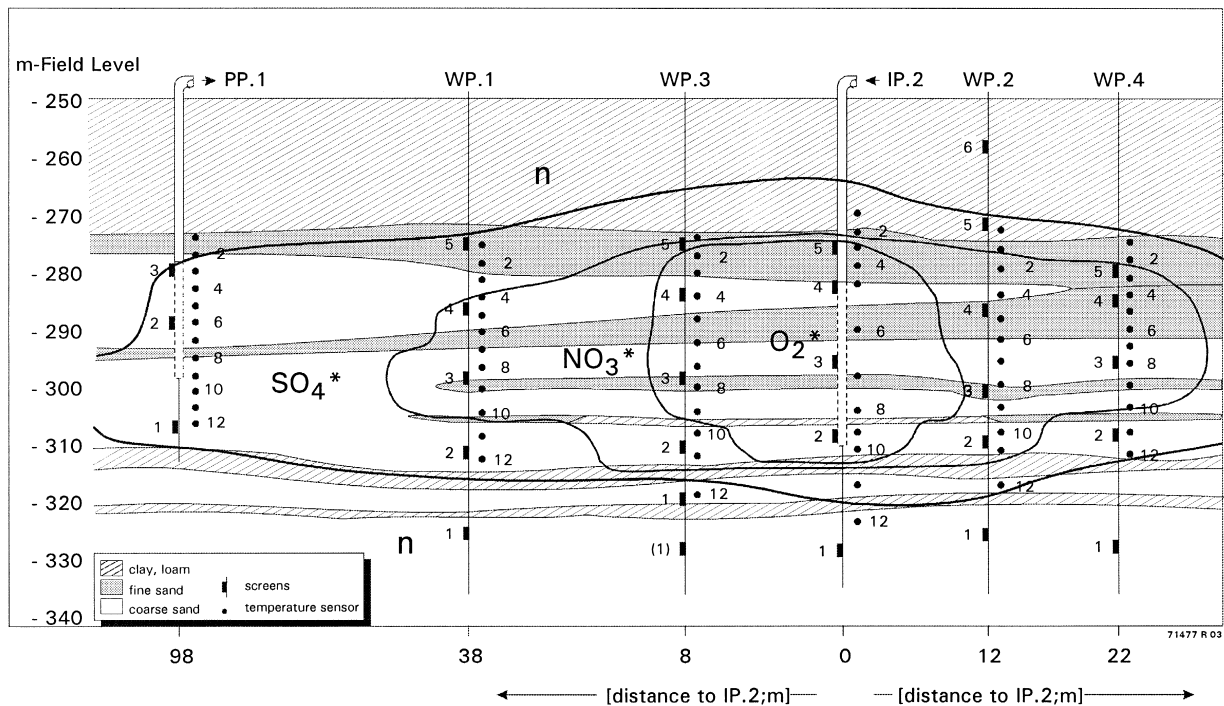


Fig. 8. Redox zones in the aquifer (Stuyfzand, 1999). *O_2 = dissolved oxygen concentration declining with distance from 10 to 0 mg l⁻¹. NO_3 = nitrate concentration declining with distance from 20 to 0 mg l⁻¹. SO_4 = sulfate concentration higher than pre-treated surface water due to pyrite oxidation.

0.12% and for R5 of 0.74% at WP3 (8 m), indicating that surface coverage of the soil grains with positively charged patches is still very low. The values given by Ryan et al. (1999) for a 12,000-year-old glacial outwash formation were one order of magnitude higher.

The removal rates of MS2 and PRD1 were almost equal. The very low collision efficiencies found in this study reflect relatively conservative behavior of both MS2 and PRD1 and suggest they are suitable indicators for virus transport. R5 was found to behave even more conservatively. The low estimated values of the collision efficiencies for MS2 and R5 show that conditions for attachment were generally unfavorable. This is consistent with the concept that in sandy soils with relatively high pH, electrostatic repulsion inhibits attachment (e.g. Goyal and Gerba, 1979; Bales et al., 1991, 1993).

A higher inactivation rate of MS2 was found in water from IP2 compared to that in water from WP3 (8 m). This difference cannot be explained by selection of more stable phages as they are transported through soil. The presence of sub-populations of phages that are inactivated at different rates should be reflected in a non-linear inactivation rate. But this was not the case and inactivation was found to be first order. The water that is injected is oxic (Table 4). It is possible that aerobic bacteria in the vicinity of the injection screen are responsible for the higher inactivation rate as compared to that at the more anaerobic monitoring well at 8 m distance. During a declogging that was carried out before this field experiment, the injection water pumped up during the first 10 min from IP2 was analyzed for colony counts at 22°C and 37°C and for *Aeromonas* (Vogelaar, 1999). Colony counts at 22°C were in the order of 10^4 – 10^5 cfp l⁻¹ and colony counts at 37°C were in the order of 10^3 cfp l⁻¹. *Aeromonas* concentrations were in the order of 10–100 cfp l⁻¹. This confirms the presence of (facultatively) aerobic bacteria in the vicinity of the injection well. Their presence may have enhanced inactivation of bacteriophages. It has been reported that inactivation of viruses may be enhanced by microbial activity (Yates et al., 1987). Jansons et al. (1989) found that the inactivation rate of poliovirus 1 in boreholes at a mean dissolved oxygen concentration of 5.4 mg/l was three times higher than at a mean dissolved oxygen concentration of 0.2 mg/l. Also, Jansons et al. (1989) found *Pseudomonas maltophilia* in large numbers only in the borehole with a high dissolved oxygen concentration. Inactivation of poliovirus 1 may have been affected directly by a higher dissolved oxygen concentration or, indirectly by microbiological activity due the higher dissolved oxygen concentration. Actively growing bacteria may excrete enzymes that are detrimental to other microorganisms. Also for oocysts of *Cryptosporidium*, microbial activity may result in increased inactivation due to production of chitinases by bacteria (Zuckerman et al., 1997).

This study has increased our understanding of the removal processes for microorganisms at field scale. Attachment of the microorganisms was shown to decrease non-linearly with distance. Within the first 8 m of soil passage, attachment was found to be efficient due to the presence of ferric oxyhydroxides. However, at larger distances, under anoxic conditions, attachment of microorganisms may be very low or negligible. This implies that deep well injection for the removal of microorganisms is adequate due to the existence of a zone where ferric oxyhydroxide deposits actually form. However, reduction of concentrations of microorganisms that incidentally contaminate an anoxic aquifer may be little.

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