Elimination of viruses, bacteria and protozoan oocysts by slow sand filtration

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Abstract The decimal elimination capacity (DEC) of slow sand filters (SSF) for viruses, bacteria and oocysts of Cryptosporidium has been assessed from full-scale data and pilot plant and laboratory experiments. DEC for viruses calculated from experimental data with MS2-bacteriophages in the pilot plant filters was 1.5-2 log₁₀. E. coli and thermotolerant coliforms (Coli44) were removed at full-scale and in the pilot plant with 2-3 log₁₀. At full-scale, Campylobacter bacteria removal was 1 log₁₀ more than removal of Coli44, which indicated that Coli44 was a conservative surrogate for these pathogenic bacteria. Laboratory experiments with sand columns showed 2-3 and >5-6 log₁₀ removal of spiked spores of sulphite-reducing clostridia (SSRC; C. perfringens) and oocysts of Cryptosporidium respectively. Consequently, SSRC was not a good surrogate to quantify oocyst removal by SSF. Removal of indigenous SSRC by full-scale filters was less efficient than observed in the laboratory columns, probably due to continuous loading of these filter beds with spores, accumulation and retarded transport. It remains to be investigated if this also applies to oocyst removal by SSF. The results additionally showed that the schmutzdecke and accumulation of (in)organic charged compounds in the sand increased the elimination of microorganisms. Removal of the schmutzdecke reduced DEC for bacteria by $\pm 2 \log_{10}$, but did not affect removal of phages. This clearly indicated that, besides biological activity, both straining and adsorption were important removal mechanisms in the filter bed for microorganisms larger than viruses.

Keywords Elimination of microorganisms; slow sand filtration; surrogates

Introduction

Recently the Dutch Drinking Water Decree has been revised (VROM, 2001). Drinkingwater companies should demonstrate sufficient elimination of pathogenic viruses, bacteria and (oo)cysts of *Cryptosporidium* and *Giardia* by treatment to comply with an annual infection risk lower than 10⁻⁴ per person. This requires quantitative knowledge about the elimination capacity of processes. An initial quantitative microbial risk assessment at the plants of Amsterdam Water Supply (AWS) and the Dune Water Company South-Holland (DWS) revealed a lack of knowledge about the efficacy of their slow sand filters for the removal of microorganisms. In collaboration with the National Institute of Public Health and the Environment (RIVM) and Kiwa Water Research, AWS and DWS started a project to determine the decimal elimination capacity DEC of slow sand filtration for viruses, bacteria and protozoan oocysts. Additional goals were to investigate the effect of the schmutzdecke and the sand, and to verify the validity of surrogates used to assess pathogen elimination capacities.

The project was started by evaluation of the literature for removal of pathogenic microorganisms. Simultaneously, full-scale data of the elimination of indigenous microorganisms, such as thermotolerant coliforms (Coli44), spores of sulphite-reducing clostridia (SSRC) and *Campylobacter*, were collected and reviewed. Both activities did not answer all the questions. Moreover, the low and variable removal of SSRC by full-scale filters cast

serious doubt on the use of these indicators as surrogates for protozoan oocyst removal. Therefore, further experimental research on the pilot and laboratory scale has been carried out, the results of which are presented and discussed in this paper.

Materials and methods

Full-scale data analysis

The elimination of thermotolerant coliforms (Coli44) and spores of sulphite-reducing clostridia (SSRC) by the SSF at two plants of AWS and one plant of DWS was determined from data of three years of routine sampling (n = 32 up to 5,184) and of a two-week period in winter and summer with daily large-volume sampling ($n = \le 20$). Periodically, the influent of the filters at the location of DWS contained *Campylobacter* bacteria, and concentrations before and after SSF were measured by large-volume simultaneous sampling. The decimal elimination capacity DEC, $\log_{10} C_{in} - \log_{10} C_{out}$, was calculated from the average concentration in influent and effluent. The average concentration was the total number of colony-forming units divided by the total sampled volume (number of samples × sample volume) in the selected period.

Pilot-plant experiments

Under full-scale situations, elimination of viruses could not be determined; therefore, elimination of bacteriophage MS2 by two slow sand filters of the AWS pilot plant Leiduin was determined. MS2 is an icosahedral phage with a diameter of 27 nm, and is a conservative surrogate for viruses (Schijven *et al.*, 2003). *E. coli* WR1 was co-injected as a reference to the elimination of indigenous Coli44. The influent of SSF1 was surface-water pre-treated by coagulation floc removal, rapid sand filtration 1, dune infiltration followed by an open collection reservoir, rapid sand filtration 2, ozonation, softening and granular activated carbon filtration. SSF2 was supplied with the filtrate of rapid sand filter 2. DOC, turbidity and pH of the influent of SSF1 were: 1.5 mg C/l, 0.1 FTU and 8.0 respectively, and of the influent of SSF2: 2.1 mg C/l, 0.7 FTU and 8.0 respectively.

The filters (surface area 2.56 m², bed depth 1.5 m and 0.3 mm diameter sand) were operated at a filtration rate of 0.3 m/h. The filter bed porosity determined with a tracer (NaCl) test was 0.27. The schmutzdecke of SSF1 was scraped 12 d before the experiment. SSF2 was tested with a schmutzdecke of 81 d. Then the filter was scraped and tested again after 4 d. The temperature ranged from 9.4 to 11.7° C. Before the start of the spiking experiments, MS2 bacteriophages and *E. coli* WR1 were added and well mixed in the water above the filter bed to avoid dilution effects (this was done before each test with different spiking concentrations). Spiking of a low and a high concentration of MS2 and WR1 to the influent of the filters (13 mL of suspension/min) lasted for two periods of 24 h respectively. The effluent concentrations of both microorganisms had been monitored during a 10 d period.

Column experiments

Due to the relatively large scale and possible health risk in the pilot plant, removal of oocysts of *Cryptosporidium* was determined in columns and compared with elimination of spores of *Clostridium perfringens* D10, a possible surrogate for oocyst removal in treatment (Hijnen *et al.*, 2000). MS2 and WR1 were co-injected as references with the pilot plant and full-scale results. A full-scale filter of DWS was sampled at four layers (20–40, 40–60, 60–80 and 80–100 cm) and the AWS pilot plant filter at five layers (20–40, 50–70, 80–100, 110–130 and 130–150 cm) for ripened sand. In the DWS and AWS columns (diameter 9 cm), the 40 cm sand bed was packed with 10 cm and 8 cm sand of each separate layer of sampled sand, respectively. Each separate layer was introduced into the column with water and mechanically packed by ticking against the Perspex column. Underneath

this sand bed a layer of gravel (1-2 mm) had been placed on a rough iron mesh. The influent (700 L) of SSF1 sampled in a large RVS-tank and transported to the experimental location, an unheated hall, was used. The temperature of the water varied between 8°C and 13.5°C. The columns were operated at a filtration rate of 0.08 m/h for two to three weeks before the spiking experiment by a peristaltic pump in the filtrate side of the columns. The test filtration rate was increased to 0.3 m/h 48 h before the spiking. The water was inoculated with microorganisms in a separate RVS-vessel located directly above the sand columns (no dilution effect). During 2 h this inoculated influent was supplied to the columns, and during the following 30 h the concentrations of microorganisms in the filtrate were monitored. To verify the effect of co-injection, two columns with AWS sand were tested, one spiked with MS2 and *E. coli* WR1 (AWS1) and the other two (AWS2 and DWS) spiked with a cocktail of MS2, WR1, *Clostridium perfringens* (D10) and *Cryptosporidium parvum*.

Microbiological methods

The methods used for Coli44 and SSRC in the full-scale study have been described (Hijnen *et al.*, 2003). The most probable numbers of thermophilic *Campylobacter* bacteria in the water before and after SSF of DWS were enumerated in 1 mL directly or in 10 mL to 100 L by isolation over 0.22 μ m membrane filters. The samples were pre-cultured in Preston-bouillon (Ribeiro and Price, 1984), in 3 × 3 portions of a decimal dilution, for 48 h at 42 ± 0.5°C under microaerophilic conditions. Each of these pre-cultures was incubated on solid Karmali-medium (Karmali *et al.*, 1986) plates (48 ± 2 h at 42 ± 0.5°C microaerophilic). Typical colonies were grey and glancing and the cells microscopically confirmed (*Campylobacter* cells are mobile and have a typical spiral form).

The methods of production and storage of the stock-solutions and the enumeration methods of MS2 bacteriophages and *E. coli* WR1 used in the pilot plant study have been described (Schijven, 2000). WR1 used for the column tests was pre-cultured in autoclaved tap water with 1 mg/L glucose-C. The production of the stock-solution and the enumeration method of spores of *C. perfringens* D10 were according to Hijnen *et al.* (2002). *Cryptosporidium parvum* oocysts (Moredun, Scotland; harvested by sedimentation and differential centrifugation) were spiked from a 1 mL suspension (10^8 oocysts). For oocyst counting, samples of 1–200 mL were analysed directly without concentration by the DFA technique with the Chemscan (Chemunex). Samples were filtered and prepared for scanning with the Chemscan-kit (Chemunex 200 k0009-01 with IMS) including a mounting medium (85μ L), 25 mm diameter 2.0 μ m membrane filters and support Pad. The membrane filters were labelled with 100 μ L monoclonal antibody reagents (Oxoid; diluted 1:1 in deionised water) for 30 min at 37°C. The filters were scanned and counted spots were microscopically confirmed based on colour, form and size.

Results and discussion

Elimination of bacteria and bacterial spores by full-scale filters

The process conditions of SSF at the three locations of AWS and DWS showed no large differences (Table 1).

From the concentrations of Coli44 in influent and effluent observed in three separate periods, DEC-values of >1.5 up to $3.2 \log_{10}$ were calculated (Table 1). The average DEC for these indicator bacteria was $2.6 \pm 0.5 \log_{10}$ higher than the average DEC of $1.9 \pm 0.5 \log_{10}$ calculated from a number of studies in the literature (Poynter and Slade, 1977; Slade, 1978; Cleasby *et al.*, 1984; Bellamy *et al.*, 1985; Ellis, 1985). Removal of *Campylobacter* bacteria by SSF at the DWS location Scheveningen was $3.4 \pm 0.6 \log_{10}$, 1-log more than Coli44 removal by these filters (Table 1). This indicated that *E. coli* was a conservative surrogate parameter for *Campylobacter* removal by SSF.

Table 1 Decimal elimination capacity (DEC) of full-scale slow sand filters

Location	Weesperkarspel AWS	Leiduin AWS	Scheveningen DWS	
Filtration rate (m/h)	0.4	0.25	0.25	
Bed length (m)	1.5	1.5	1.0	
Surface cleaning frequency (year)	1	2	4–5	
Grain size (mm)	0.15-0.6	0.13-0.37	0.3–1.8	
DEC (log ₁₀) Coli44	2.2ª; >1.5 ^b ; >3.7 ^c	3.2 ^a ; nd ^d ; nd	2.9ª; 2.3 ^b ; 2.2 ^c	
DEC (log ₁₀) Campylobacter DEC (log ₁₀) SSRC	nd 1.6 ^a ; 1.5 ^b ; 1.0 ^c	nd (1.8) ^{a,e} ; -0.2 ^{b,e} ; -0.1 ^{c,e}	4.1 ^a ; 3.0 ^b ; 3.2 ^c 1.8 ^a ; 0.0 ^b ; –0.2 ^c	

^a three years routine monitoring in small volumes; ^{b,c} daily large volume sampling in 2 weeks in winter (^b) and summer (^c); ^d not determined; ^e 1st DEC high (45/L) and 2nd and 3rd DEC low (0.16/L) SSRC level in influent C_{in}

Elimination of SSRC by SSF varied significantly. The maximum removal was $1.8 \log_{10}$, but at locations Leiduin (AWS) and Scheveningen (DWS), the data also showed little to no elimination and even a small increase in the SSRC numbers (negative DEC). Multiplication in anaerobic zones cannot be excluded. However, accumulation and retarded transport seemed the most plausible explanations for this phenomenon. Spores not retained irreversibly by straining or attachment to the sand grains, may persist long enough to pass the sand filter eventually in concentrations that may even be higher than in the influent at the time of monitoring. This phenomenon for these spores has also been described by Schijven et al. (2003), and indicated that DEC depended on the duration of the contamination. Hence, it explained the positive correlation between elimination and the influent concentration observed at Leiduin. DEC of 1.8 and -0.2 log₁₀ was observed at an average SSRC influent concentration of 45 and 0.16 spores/L respectively. These results raised serious doubts about the use of these spores as a quantitative surrogate for Cryptosporidium oocyst removal by SSF. On the other hand, provided that no irreversible attachment or physical straining had occurred, the same phenomenon of breakthrough in the long run may also be the case for protozoan oocysts removal by SSF.

Pilot-plant study

Figure 1 shows the breakthrough curves of MS2 and WR1. The difference between the average influent and maximum effluent concentration was used to estimate DEC. The tailing after the spiking period of 48 h was caused by slow detachment of the attached



Figure 1 The concentrations of bacteriophage MS2 and *E. coli* WR1 in the influent and effluent of SSF2 during two separate spiking experiments with 24 h of low and 24 h of high concentrations; between these experiments, on d 11, the filter bed surface was cleaned (schmutzdecke was scraped off)

microorganisms (Schijven, 2000). The filters SSF1 and SSF2 reduced the concentration of both organisms by 1.7 and up to $4.2 \log_{10}$ (Table 2).

A concentration increase of about 3 \log_{10} (Figure 1) did not significantly affect DEC. The schmutzdecke of 81 d at SSF2 showed a significant effect on the elimination of WR1. Before cleaning of the filter bed, DEC was 3.9–4.2 and after cleaning 2–2.8 \log_{10} MS2 elimination, however, was hardly influenced by the filter bed cleaning and was 2.1 ± 0.6 \log_{10} . This value was similar to the average removal of indigenous enteroviruses by SSF of 1.9 \log_{10} observed by others (Slade, 1978; Ellis, 1985). A 3 \log_{10} removal of polioviruses by SSF was found by Windle-Taylor (1969), while Poynter and Slade (1977) reported a DEC of 3.5 and 2.8 \log_{10} for MS2 phages and polioviruses. Without a schmutzdecke, WR1 removal was 2.3 ± 0.4 \log_{10} . This value was close to MS2 removal by the same filters and *E*. *coli* removal observed in full-scale filters and in the literature.

Column experiments

Due to the relative large scale and possible health risk in the pilot plant, removal of oocysts of *Cryptosporidium* was determined in columns packed with ripened sand sampled 20 cm from the top of the filter bed (without schmutzdecke; worst case). The main objective of this part of the study was to compare the elimination of bacteria, viruses and protozoan oocysts under the same conditions; thus MS2 bacteriophages, *E. coli* WR1 and spores of *Clostridium perfringens* D10 were co-injected with *C. parvum*. The influent concentrations ranged from 1.0×10^3 to 1.7×10^5 /mL. Oocyst levels of 10^3 – 10^4 /mL were removed completely (<1/200 mL) by both columns with AWS and DWS sand. This resulted in a DEC of at least 5– $6 \log_{10}$ (Table 3).

DEC of SSF for protozoan (oo)cysts, compared with reports of spiking experiments in the literature, was of the same order of magnitude. For *Giardia* cysts 4–6 \log_{10} and for *Cryptosporidium* oocysts >4.5–>6.5 \log_{10} have been described (Bellamy *et al.*, 1985; Schuler *et al.*, 1991; Timms *et al.*, 1995). Fogel *et al.* (1993) determined concentrations of indigenous *Giardia* and *Cryptosporidium* (oo)cysts in the influent and effluent of a full-scale plant over a period of 1.5 years where much lower DEC values (1.2 and 0.3 \log_{10} removals respectively) were calculated. Figure 1 shows that MS2 phages and *E. coli* were

Table 2Decimal elimination capacity ($log_{10}(C_{in}/C_{max,out})$) of MS2 bacteriophage and *E. coli* (WR1) ofSSF1 and SSF2 of the pilot plant of AWS at 10°C

Filter	Time (d) after	MS2		E. coli	
	schmutzdecke removal	Low C _{in} *	High C _{in}	Low C _{in}	High C _{in}
SSF1	12	1.7	1.8	2.1	2.3
SSF2	81	1.8	2.2	3.9	4.2
SSF2	4	1.7	1.9	2.0	2.8

* influent concentrations

Table 3 Decimal Elimination Capacity ($\log_{10}(C_{in}/C_{max out})$) of the columns compared to DEC assessed for full-scale filters

	<i>E. coli</i> or Coli44 ^a	MS2	C. perfringens or SSRC ^a	C. parvum	
Column AWS1; 0.4 m	0.2	0.4	ns	ns	
Column AWS2; 0.4 m	0.3	0.2	2.3	>5.3	
Full-scale AWS; 1.5 m	2.0 ^b	1.7	–0.2–1.8 ^c	nd	
Column DWS; 0.4 m	0.4	0.1	3.2	>6.5	
Full-scale DWS; 1.0 m	2.5 ^d	nd	-0.2-1.8 ^c	nd	

ns – no spiking; nd – not determined; ^a indigenous Coli44 and SSRC; ^b average DEC for Coli44 from pilot plant filters in Table 2; ^c range of values for SSRC from Table 1; ^d average DEC for Coli44 from full-scale filters in Table 1

hardly removed by the AWS2 column (Figure 2), a result also observed in column AWS1 and DWS (Table 3). MS2 and WR1 results of the AWS columns showed that co-injection with the other organisms did not affect the elimination of MS2 and WR1. The columns removed spores of *C. perfringens* $2-3 \log_{10}$ more efficiently than MS2 and WR1, but less efficiently than *C. parvum*. The latter result demonstrated that SSRC was not a suitable quantitative surrogate for *Cryptosporidium* oocyst removal by SSF. A similar conclusion for aerobic spores as a surrogate for oocyst removal by rapid filtration was drawn by Emelko (2001).

The column with DWS sand (grain size 0.3-1.8 mm) showed more elimination of the bacteria and bacterial spores than the column AWS2 with finer sand (0.13-0.37 mm; Table 3). This difference was possibly caused by a higher carbon and iron oxyhydroxide content of 0.055% (dry weight) and 2.4 mg/g in the DWS sand compared to <0.002% and 0.027 mg/g, respectively, in the AWS sand. Thus DWS sand had a larger adsorption capacity, which indicated that attachment played a significant role in the elimination of bacteria.

Table 3 compares the DEC of the column experiments (0.4 m of ripened sand) with the DEC assessed in the pilot and full-scale filters of AWS (1.5 m of filter bed) and DWS (1.0 m of filter bed). This revealed that the removal of MS2-phages and WR1 per unit length in the columns was much less than observed in the slow sand filters. This may largely have been ascribed to the fact that ripened sand and schmutzdecke material had not been included in the columns (worst-case conditions). Moreover, also the higher porosity of the fresh packed columns of about 0.40, compared to the porosity of the ripened filter bed SSF1 of the pilot plant of 0.27, may have contributed to the observed difference in removal capacity.

Although scaling up of the column results to full-scale conditions was not possible, the resemblance of the breakthrough curves of MS2 and WR1 under both conditions substantiated extrapolation of the relative results to full-scale filters. It was concluded that SSF must be very effective in eliminating peak concentrations of persistent oocysts of *C. parvum* and spores of *C. perfringens*. However, the low and variable DEC of full-scale filters for SSRC and also for oocysts (as found by Fogel *et al.*, 1993) suggested that overall elimination of both biological particles by SSF assessed over a long period of time was influenced by accumulation and retarded transport. Destructive sampling of the columns for analysis of retained microorganisms (Schijven *et al.*, 2003) demonstrated that the most significant removal mechanism for the small MS2 bacteriophage was adsorption. The larger *C. perfringens* spores and *E. coli* were removed both by adsorption and physical straining, while, for *Cryptosporidium* oocysts, physical straining was the main removal mechanism. From the latter observation one can conclude that elimination in the filter bed was irreversible, and retarded transport, as proposed for SSRC, was not possible. However, unpublished data have shown that oocysts might be transported deeper into sandy soil, due to changes in



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pH and conductivity. Also, changes in hydraulic conditions may contribute to transport of oocysts through sand beds (Emelko, 2001) with the same author emphasising the need for further research to elucidate the significance of seeding experiments with high concentrations as a tool to assess DEC of full-scale filters for *Cryptosporidium*.

Conclusions

Based on the results of this study with E. coli (Coli44) and MS2-bacteriophages, DEC of slow sand filters for bacteria and viruses was quantified at 2-3 and 1.5-2 log₁₀, respectively. One log₁₀ higher was the elimination of indigenous *Campylobacter* bacteria by fullscale filters, which indicated that Coli44 was a conservative surrogate for these pathogenic bacteria. MS2-bacteriophages could be regarded as a conservative surrogate for virus removal (low attachment and survival rate). Moreover, viruses were the most critical microorganisms for the performance of slow sand filters, because they were retained the least. The column experiments showed that SSF will have a high efficacy in eliminating peak concentrations of persistent microorganisms, such as spores of sulphite-reducing clostridia (>2–3 log₁₀) and (oo)cysts of *Cryptosporidium* and *Giardia* (>5 log₁₀). This may be more in full-scale filters with a schmutzdecke. Therefore, SSRC was not a good quantitative surrogate for protozoan oocyst removal by SSF in practice. Further research will be needed to elucidate if and to what extent accumulation and retarded transport, the suggested phenomena for the low and variable removal of indigenous SSRC by full-scale filters, will affect elimination of oocysts of Cryptosporidium in practice in the long run. Additionally, this study showed that filters with a schmutzdecke had 1–2 log greater capacity to eliminate bacteria, whereas elimination of viruses was not affected by the schmutzdecke. This positive effect of the schmutzdecke on elimination would also count for protozoan oocysts, because straining was an important removal mechanism for these organisms. Furthermore, results also suggested that charged (in)organic components accumulated in the sand might have a significant effect on the removal efficiency of organisms removed by adsorption.

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