Removal of sulphite-reducing clostridia spores by full-scale water treatment processes as a surrogate for protozoan (oo)cysts removal

W.A.M. Hijnen,* J. Willemsen-Zwaagstra,** P. Hiemstra,*** G.J. Medema* and D. van der Kooij*

* Kiwa NV, PO Box 1072, 3430 BB Nieuwegein, The Netherlands
** NV PWN Water Supply Company North Holland, PO Box 5, 2060 BA Bloemendaal, The Netherlands
*** Present address: Witteveen + Bos, PO Box 233, 7400 AE Deventer, The Netherlands

Abstract At eight full-scale water treatment plants in the Netherlands the removal of spores of sulphite-reducing clostridia (SSRC) was determined. By sampling and processing large volumes of water (1 up to 500 litres) SSRC were detected after each stage of the treatment. This enabled the assessment of the removal efficiency of the full-scale unit processes for persistent micro-organisms. A comparison with literature data on the removal of Cryptosporidium and Giardia by the same type of processes revealed that SSRC can be considered as a potential surrogate. The average Decimal Elimination Capacity (DEC) of the overall treatment plants ranged from 1.3–4.3 log. The observed actual log removal of SSRC by the unit processes and the overall treatment at one of the studied locations showed that the level of variation in removal efficiency was approximately 2 log. Moreover, from the actual log removal values it was observed that a low SSRC removal by one unit process is partly compensated by a higher removal by subsequent unit processes at this location. SSRC can be used for identification of the process conditions that cause variation in micro-organism removal which may lead to process optimization. Further research is necessary to determine the optimal use of SSRC in water quality monitoring for the production of microbiologically safe drinking water.

Keywords Cryptosporidium and Giardia; drinking water; large volume sampling; spores of sulphite-reducing clostridia; surrogate parameter; removal efficiency

Introduction

Outbreaks of waterborne diarrhoea caused by persistent pathogenic protozoa (Cryptosporidium and Giardia) in the USA and the UK have increased interest in the effect of water treatment processes on these micro-organisms. Cryptosporidium and Giardia have also been detected in faecally contaminated surface waters in The Netherlands, but outbreaks via drinking water have not been observed. 80% of the drinking water is produced from groundwater and surface water after soil passage with long residence times and direct treatment of surface water consists of multiple barriers. Yet Dutch authorities and drinking water companies want to be able to determine the (im)probability of the transmission of both pathogenic protozoa via drinking water. Provisional guidelines are proposed for Cryptosporidium and Giardia based on a maximum annual infection risk of $10^{-4}$ (2.2 $10^{-5}$/l and 5.5 $10^{-6}$/l, respectively; VROM, 1995). Compliance with these low values cannot be assessed by direct monitoring of these pathogens in treated water. The proposed strategy is (i) to determine the required treatment efficiency based upon the concentration of pathogens in the raw water and (ii) to assess the removal efficiency of the involved water treatment using surrogate parameters. Spores of sulphite-reducing clostridia (SSRC) and of Clostridium perfringens have been proposed as a surrogate parameter for the assessment of the treatment efficiency for protozoan (oo)cysts (Payment and Franco, 1993; Hijnen et al., 1997). These spores show long survival in the environment and a high resistance against disinfectants. Since 1984, in addition to the coliforms and faecal streptococci, SSRC are
included in the Dutch Drinking Water Decree (1984) as part of the microbiological standards for drinking water (weekly monitoring in 100 ml samples at surface water treatment plants). These micro-organisms are measured by every laboratory for routine drinking water quality monitoring in The Netherlands. The number of zero counts in the samples is usually high due to the low concentrations in the treated water, which implicates that removal of these spores is difficult to assess. By enlarging the sampled water volume the concentrations after every treatment stage could be determined. Hence, SSRC has been used to assess the removal efficiency of full-scale water treatment plants.

**Methods**

**Treatment plants**

The removal of SSRC by processes was monitored at 8 full-scale treatment plants with different source waters (Table 1). At each location the treatment consisted of several processes in different order: coagulation/floc removal (CFR), rapid sand filtration (RSF; sand or anthracite/sand), ozonation (O$_3$), chlorination (Cl$_2$), granular activated carbon filtration (GAC), slow sand filtration (SSF), powdered activated carbon (PAC), softening (SF) and post-disinfection (PD).

**Microbiological sampling**

In a two-week period in the winter and in the summer of 1997 the concentration of SSRC in source water and the water after the different treatment stages was measured daily, except for the weekends, (n=10) by the laboratories of the participating Water Companies. Sample volumes from 100 ml up to 1 litre were filtered using the standard membrane filtration method (0.45 µm, Ø47 mm membrane filters). For volumes larger than 100 ml it was sometimes necessary to use more than one membrane filter to process the total sample volume. Larger volumes (50 up to 500 litres) of finished water before post-disinfection were tested with a specially constructed filtration device, the MF-sampler (Hijnen et al., 1999).

**Microbiological analysis**

The SSRC determination was conducted as described in Dutch standard methods (NEN6567, 1985). The spores were isolated from the water by membrane filtration and black colonies developed during anaerobic cultivation at 37°C in sulphite-iron agar. Instead of pasteurising the water samples before filtration as described in the standard method, in this study the membrane filters were pasteurised in the liquified medium in an oven at 70±1°C for 30 minutes. Perfringens-Agar-Base medium (PAB; Oxoid CM587), Shahidi-Ferguson-Perfringens-Agar-Base (Difco 0811-17-0), Trypton-Sulphite-Cycloserine-Agar (TSC; Merck and Biokar BK031) were used as media by the participating water companies.

**Table 1** The successive processes at the 8 full-scale treatment plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>Source water</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>River Meuse after impoundment reservoirs</td>
<td>CFR, O$_3$, RSF, GAC, PD</td>
</tr>
<tr>
<td>2</td>
<td>Lake IJssel</td>
<td>Cl$_2$, CFR, RSF, GAC, PD</td>
</tr>
<tr>
<td>3</td>
<td>River Meuse after impoundment reservoirs</td>
<td>CFR, RSF, O$_3$, GAC</td>
</tr>
<tr>
<td>4</td>
<td>River Meuse after impoundment reservoirs</td>
<td>CFR, O$_3$, RSF, GAC, PD</td>
</tr>
<tr>
<td>5</td>
<td>River Drentse Aa</td>
<td>CFR, RSF, GAC, RSF, SSF</td>
</tr>
<tr>
<td>6</td>
<td>River Rhine after CFR, RSF and soil passage$^a$</td>
<td>RSF, O$_3$, SF, GAC, SSF</td>
</tr>
<tr>
<td>7</td>
<td>River Rhine after reservoir and RSF</td>
<td>O$_2$, SF, GAC, SSF</td>
</tr>
<tr>
<td>8</td>
<td>River Meuse after RSF and soil passage$^a$</td>
<td>PAC, SF, RSF, SSF, PD</td>
</tr>
</tbody>
</table>

$^a$ faecally recontaminated by wildlife in open water after soil passage
The incubation procedure of the membrane filters for SSRC analysis was carried out as described previously (Hijnen et al., 1997).

**Calculation of the removal efficiency**

From the average (avg) concentration of SSRC in the inlet \( C_{in} \) and the outlet \( C_{out} \) of a treatment process or an overall treatment the removal efficiency or decimal elimination capacity \( DEC \) was calculated using the following equation:

\[
DEC = \log(\text{avg } C_{in}) - \log(\text{avg } C_{out})
\]  

(1)

The average concentration is the arithmetic mean concentration (n/l) calculated from the sum of SSRC counts (Colony Forming Units) of all samples, divided by the total sampled volume (\( \sum \text{CFU}/(n \times v) \), where \( n \) is the number of samples and \( v \) the sample volume in litres.

The variation in \( DEC \) of the different unit processes and treatment plants is described by presenting the minimum and maximum \( DEC \). Variation in \( DEC \) of an individual unit process or a treatment plant is described by presenting the observed actual log removal calculated from the actual concentrations in the inlet and the outlet of the process or treatment:

\[
\text{Actual log removal} = \log(C_{in}) - \log(C_{out})
\]  

(2)

The variation coefficient (\( V_c \%; \)) of the average actual log removal was calculated with the standard deviation (SD) and \( n \) the number of observations (\( V_c = (SD/\sqrt{n})/\text{avg actual log removal} \times 100\% \)).

**Results and discussion**

**The concentration of SSRC in source and finished water**

The average concentration of SSRC in the raw water observed at the different locations in the winter and in the summer periods varied between 10 and 1500 CFU/l (Figure 1). The SSRC-concentration in the finished water after all treatment processes and before post disinfection (if applied; Table 1) could be assessed accurately using the MF-sampler. At six of the eight locations the percentage of zero counts (ZC) in the finished water was less than 10% and at two locations the ZC-values were 25 and 30%, respectively.

In Figure 2 an example of the decrease of the SSRC-concentration during water treatment at one location is presented. After coagulation/floc-removal (CFR), ozonation \( (\text{O}_3) \) and rapid sandfiltration \( (\text{RSF}) \) a reduction of the SSRC-concentration was observed. In winter a small reduction of the SSRC-concentration was observed after GAC filtration,
whereas in summer the average concentration after this process was higher. The overall treatment reduced the concentration in winter and in summer with 3.5 and 3.3 log units, respectively.

Removal efficiency of unit processes
The average removal efficiency of the different full-scale unit processes for SSRC showed a large variation (Table 2). For disinfection with chlorine applied at one full-scale plant (CT-value >50 mg/l.min) an inactivation of 2.2 log was observed.

In winter the average actual log removal was 1.5 (SD=0.25) and lower than the removal observed in summer (3 log; SD=0.15). Ozone disinfection at the other locations was less effective in the SSRC inactivation. This may be due to the lower applied CT-values (1–4) or to a higher resistance of SSRC against ozone. More effectively than by ozonation was the SSRC removal by individual coagulation/floc-removal and rapid sand filtration processes as well as by the combination of both processes.

Slow sand filtration (SSF) and GAC-filtration are the final processes in water treatment in the Netherlands (Table 1). The SSRC concentration after these processes was accurately determined with the MF-sampler (Figure 1) and the removal efficiency could be assessed. A relatively low average DEC value was observed for the SSF and also the GAC processes (Table 2). Moreover, the observed negative average removal efficiencies (increase instead of decrease of the concentration) for some SSF and GAC processes indicate that the removal of the persistent SSRC by these processes can not always be guaranteed. The question if accumulation, survival and reintroduction of spores is the most important cause for a negative removal efficiency or whether growth of SSRC may also occur in these filters remains to be answered. An important difference with RSF is that both filtration processes (SSF and GAC) have a low intensity of filter bed cleaning. The low die-off rate of clostridia spores (Medema et al., 1997) may lead to accumulation of SSRC in filter beds and low removal efficiencies or even breakthrough. SSRC breakthrough after GAC filters with a low backwash frequency was previously described (Hijnen et al., 1997) and high SSRC counts (35 CFU/g) were observed in the top layer of two full-scale slow sand filters (Hijnen et al., 1999).

No direct comparison of the removal efficiency of treatment processes for SSRC and for the protozoan (oo)cysts of Cryptosporidium and Giardia, respectively, was made because (oo)cysts concentrations in the treated water were too low to measure. SSRC can be regarded as a potential surrogate for protozoan (oo)cysts when the removal efficiency of a process for these spores is similar or to some extent lower than the removal of (oo)cysts. This counts for conventional treatment with CFR/RSF when properly designed and operated under
full-scale conditions. The 2 log removal of protozoan (oo)cysts determined by LeChevallier et al. (1991) for these processes is a little higher than the 1.7 log removal observed for SSRC removal by the full-scale CFR/RSF processes in the Netherlands (Table 2). For disinfection the SSRC data can only be compared with lab-scale experiments for protozoan (oo)cysts. Korich et al. (1990) showed that Cryptosporidium was hardly inactivated by high dosages of chlorine, whereas a 4 log Giardia inactivation was achieved at CT-values of 180–530 mg/L.min chlorine (Hibler et al., 1987). The observed effect of chlorine on SSRC (Table 2) indicates that for Cryptosporidium oocysts, the spores are too sensitive to chlorine, but for Giardia cysts SSRC seem to be a useful surrogate. For ozone disinfection a two log inactivation of Cryptosporidium and Giardia (oo)cysts was achieved at CT-values of 3.5 and 0.65 mg/L.min (22ºC), respectively (Finch et al., 1993a;1993b). Lab-scale experiments with spores of C. perfringens revealed a comparable resistance with Cryptosporidium oocysts for ozone (preliminary and unpublished results). However, SSRC showed a higher resistance against ozone under full-scale conditions with CT-values of 1–4 and temperatures of 2–23ºC (Table 2). The low removal efficiency of SSRC by SSF indicates that these spores are not a good surrogate for SSF; Cryptosporidium and Giardia (oo)cysts removal by SSF determined in seeding experiments were >3.3 (Hall et al., 1994) and >3.7 log (Bellamy et al., 1985), respectively. The significance of the observed accumulation and breakthrough of SSRC in full-scale SSF and GAC processes for the protozoan (oo)cysts removal by these processes under full-scale conditions is not clear. Literature data on the removal of both protozoan (oo)cysts by GAC are not available. For a more accurate comparison more information is needed, but these preliminary results indicate that SSRC is a potential surrogate for protozoan (oo)cyst removal.

**Removal efficiency of the overall treatment**

The removal efficiency of the overall full-scale treatment plants for SSRC, as calculated from the concentration in the source water and the finished water (Figure 1), ranged from 1.3 to 4.3 log units (Table 2). Higher treatment performance was observed by Payment and Franco (1993) for the removal of C. perfringens by three full-scale treatment plants with conventional treatment (CFR/RSF) with or without an additional ozonation and GAC filtration; the DEC ranged from 3.8–5.6 log units.

The results of our study revealed more quantitative information on the variation in the actual removal of persistent microorganisms by unit processes and the overall treatment plant operated under full-scale conditions (Figure 3).

The actual removal efficiency of individual processes may vary by 2 log units. Identification of the process conditions responsible for this variation may result in an optimization of the removal efficiency. Furthermore, the results indicate that low SSRC

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**Table 2** Range of observed removal efficiencies of full-scale unit processes in drinking water production plants in The Netherlands for SSRC

<table>
<thead>
<tr>
<th>Processes</th>
<th>Number of locations</th>
<th>DEC (log)(avg)</th>
<th>DEC (log)Min. - Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disinfection chlorine</td>
<td>1</td>
<td>2.2</td>
<td>1.5–3.0</td>
</tr>
<tr>
<td>Disinfection ozone</td>
<td>5</td>
<td>0.7</td>
<td>0–1.2</td>
</tr>
<tr>
<td>Coagulation/floc-removal (CFR)</td>
<td>5</td>
<td>1.3</td>
<td>0.8–1.7</td>
</tr>
<tr>
<td>Rapid sand filtration (RSF)</td>
<td>4</td>
<td>1.2</td>
<td>0.8–1.7</td>
</tr>
<tr>
<td>CFR/RSF</td>
<td>2</td>
<td>1.7</td>
<td>1.2–2.2</td>
</tr>
<tr>
<td>Slow sand filtration (SSF)</td>
<td>4</td>
<td>0.7</td>
<td>–0.3–2.3</td>
</tr>
<tr>
<td>GAC filtration (GAC)</td>
<td>6</td>
<td>0.3</td>
<td>–0.6–1.0</td>
</tr>
<tr>
<td>Total treatment plant</td>
<td>8</td>
<td>2.9</td>
<td>1.3–4.3</td>
</tr>
</tbody>
</table>
A cumulative frequency distribution of the actual log removal values (log $C_{in}/C_{out}$) for SSRC of the individual unit processes and of the overall full-scale treatment plant.

Conclusions

The results of a survey on the removal of spores of sulphite-reducing clostridia (SSRC) in the Netherlands revealed that these anaerobic spores can be used to assess removal efficiency of full-scale treatment processes and plants for persistent micro-organisms. The microbiological procedure for determining the SSRC concentrations in large sample volumes after every treatment stage is simple and cheap and can easily be implemented in routine microbiological laboratories for water quality control. The presented results show that with data on SSRC removal, the variation in micro-organism removal by unit processes or a treatment plant can be assessed which enables process optimization and control. Moreover, from a comparison of these results with experimental data on inactivation and removal of Cryptosporidium and Giardia by the same type of processes described in literature it was concluded that this parameter is a potential surrogate parameter for protozoan (oo)cysts. Further research will be necessary to determine how SSRC can be used in water quality control to produce microbiologically safe drinking water.

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