Difference in behaviors of F-specific DNA and RNA bacteriophages during coagulation–rapid sand filtration and coagulation–microfiltration processes

N. Shirasaki, T. Matsushita, Y. Matsui, T. Urasaki and K. Ohno

ABSTRACT

Difference in behaviors of F-specific DNA and RNA bacteriophages during coagulation–rapid sand filtration and coagulation–microfiltration (MF) processes were investigated by using river water spiked with F-specific DNA bacteriophage f1 and RNA bacteriophage f2. Because the particle characteristics of f1 (filamentous) and f2 (spherical) are quite different and the surface charge of f1 in the river water was slightly more negative than that of f2, the removal ratios of f1 were approximately 1-log lower than the removal ratio of f2 after any treatment process used in the present study. This result indicates that the behaviors of the two bacteriophages during the treatment processes were different, and that the removal of f1 by the combination of coagulation and filtration processes was more difficult than that of f2. The removal ratios for f1 and f2 were approximately 3-log and 4-log, respectively, in the coagulation–rapid sand filtration process, and 6-log and 7-log, respectively, in the coagulation–MF filtration process. Therefore, as expected, the coagulation–MF process appears to be more effective than the coagulation–rapid sand filtration process for the removal of not only spherical viruses but also filamentous viruses.

Key words | coagulation, F-specific DNA bacteriophage, F-specific RNA bacteriophage, microfiltration, rapid sand filtration

INTRODUCTION

Microbial safety of drinking water has been of primary interest for public health protection. Most waterborne pathogens are introduced into drinking water supplies by human or animal feces (Guillot & Loret 2010). Because fecal coliform bacteria are consistently present and often abundant in human and animal feces (Nappier et al. 2006), these bacteria have traditionally been used as surrogates for fecal contamination in source and drinking water. However, some researchers have demonstrated that fecal coliform bacteria may not be appropriate surrogates for waterborne enteric viruses, owing to the differences in their resistance to drinking water treatment processes (Payment et al. 1985; Havelaar et al. 1993). In other words, fecal coliform bacteria are less resistant than enteric viruses to physicochemical treatments such as filtration and disinfection processes. Hence, other reliable surrogates are required for enteric viruses so as to guarantee the microbial safety of drinking water.

Bacteriophages, which are viruses that infect bacteria, have been proposed as surrogate candidates for enteric viruses. This is based on the greater similarity of bacteriophages to enteric viruses than to fecal coliform bacteria, in terms of their environmental persistence and resistance to drinking water treatment processes as well as lack of pathogenicity to humans (Stetler 1984; Havelaar et al. 1993). Among bacteriophages, especially the F-specific bacteriophages, viruses that infect F+ male Escherichia coli bacteria through the F sex pilus, are considered to be better surrogates for enteric viruses (World Health Organization 2008).
F-specific bacteriophages are categorized into DNA and RNA bacteriophages, and belong to the two families, Inoviridae and Leviviridae (Cole et al. 2003). Virions in the Inoviridae family are rods or filaments containing a single molecule of circular, positive-sense, single-stranded DNA, whereas virions in the Leviviridae family are spherical and of icosahedral symmetry and contain a single molecule of linear, positive-sense, single-stranded RNA (Fauquet et al. 2005). Because these F-specific DNA and RNA bacteriophages are widely present in fecal waste (Cole et al. 2003), the presence, prevalence and population of F-specific DNA and RNA bacteriophages in surface water have been investigated to identify fecal contamination sources (Cole et al. 2003; Haramoto et al. 2009). In addition, because F-specific RNA bacteriophages are morphologically similar to hepatitis A viruses and polioviruses, the bacteriophages are used worldwide as surrogates for enteric viruses during drinking water treatment (Shelton & Drewry 1973; Matsushita et al. 2005; Zhu et al. 2005; Fiksdal & Leiknes 2006; Shirasaki et al. 2009; Pierre et al. 2010). F-specific DNA bacteriophages are not used as surrogates for enteric viruses because of their morphological differences. However, the F-specific DNA bacteriophage of *Vibrio cholerae* has been implicated in the lysogenic conversion of *V. cholerae* to its toxic form, suggesting that at least some F-specific DNA bacteriophages are indirectly involved in waterborne disease transmission (Waldor & Mekalanos 1996; Redman et al. 1999). Accordingly, removal of not only enteric viruses but also F-specific DNA bacteriophages by drinking water treatment processes is important to demonstrate the microbial safety of drinking water. However, removal of F-specific DNA bacteriophages by drinking water treatment processes has not been fully investigated in drinking water treatment processes, although the environmental persistence of F-specific DNA bacteriophages has been investigated and compared with that of F-specific RNA bacteriophages (Long & Sobsey 2004).

Our objective in the present study was to investigate the difference in behaviors of F-specific DNA and RNA bacteriophages during the coagulation–rapid sand filtration process, which is commonly used in drinking water treatment facilities, and during the coagulation–microfiltration (MF) process, which is becoming an important technology in this century for drinking water treatment.

**MATERIALS AND METHODS**

**Source water, coagulant, filter media and MF membrane**

River water was sampled from the Toyohira River (Sapporo, Japan; water quality shown in Table 1) on 12 October 2007. The coagulant used was a commercial aluminium coagulant, polyaluminium chloride (PACl) (PACl 250A; 10.5% Al2O3, relative density 1.2 at 20 °C; Taki Chemical Co., Ltd, Hyogo, Japan). Silica sand (effective size 0.6 mm, uniformity coefficient <3; Nihon Genryo Co., Ltd, Kanagawa, Japan) was used as a filter medium for rapid sand filtration. A flat type of ceramic MF membrane (nominal pore size 0.1 μm, effective filtration area 0.0007 m2; NGK Insulators, Ltd, Nagoya, Japan), which was installed in an acrylic-resin casing, was used for the MF process.

**Bacteriophages**

The F-specific DNA bacteriophage, f1 (NBRC 20015), and the F-specific RNA bacteriophage, f2 (NBRC 20011), were obtained from the NITE Biological Research Center (NBRC, Chiba, Japan). f1 is a filamentous particle that has a diameter of 6 nm and length of 800 nm (Dotto et al. 1981). In contrast, f2 is an icosahedral particle that has a diameter of 22 nm (Shelton & Drewry 1973). Each bacteriophage was propagated for 22–24 h at 37 °C in *E. coli* (NBRC 13965) obtained from NBRC. The bacteriophage culture solution was centrifuged (2,000×g, 10 min) and then passed through a membrane filter (pore size 0.45 μm, hydrophilic cellulose acetate; Dismic-25cs, Toyo Roshi Kaisha, Ltd, Tokyo, Japan). The filtrate was purified by using a centrifugal filter device (molecular weight cutoff 100,000, regenerated cellulose; CentrifuPlus-100, Millipore Corp.,

<table>
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<th>Table 1</th>
<th>Water quality of the Toyohira River</th>
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<tr>
<td>pH</td>
<td>7.5</td>
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<tr>
<td>DOC (mg/L)</td>
<td>0.90</td>
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<tr>
<td>OD260 (cm⁻¹)</td>
<td>0.027</td>
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<tr>
<td>Turbidity (NTU)</td>
<td>0.50</td>
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<tr>
<td>Alkalinity (mg-CaCO₃/L)</td>
<td>19.1</td>
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Billerica, MA, USA) to prepare the bacteriophage stock solution. The concentration of each bacteriophage stock solution was approximately \(10^{12}\) PFU/mL.

Coagulation experiments

Batch coagulation experiments were conducted with 200 mL of bacteriophage-spiked river water in glass beakers at 20°C. The bacteriophage stock solution (see section on Bacteriophages) was added to the beaker at approximately \(10^6\) or \(10^8\) PFU/mL, and the spiked water was mixed with an impeller stirrer. Because around 1 mg-Al/L of PACl is usually dosed for the treatment of Toyohira River water, which is the source water in the present study, in the actual drinking water treatment plant (Moiwa drinking water treatment plant, Sapporo, Japan), PACl was injected into the water at a coagulant dose of 0.54, 1.08 or 1.62 mg-Al/L. The pH of the water was immediately adjusted to, and maintained at, 6.8 with HCl. The water was stirred rapidly for 2 min \(\left( G = 200 \text{ s}^{-1}, 61 \text{ rpm} \right) \) and then slowly for 28 min \(\left( G = 20 \text{ s}^{-1}, 13 \text{ rpm} \right) \). The water was then left at rest for 20 min to settle the generated aluminium floc particles. Samples were taken from the beaker \(c_0\) and after settling \(c_s\) for quantification of the bacteriophage concentrations.

The suspended aluminium floc particles that had not settled by gravity were separated from the floc mixture by centrifugation \(\left( 2,000 \times \text{g}, 10 \text{ min} \right) \) to quantify the bacteriophage concentration in the liquid phase of the floc mixture.

Rapid sand filtration experiments

After the coagulation experiments (without centrifugal separation), rapid sand filtration experiments were carried out with a glass column \(\left( \text{diameter } 0.8 \text{ cm, length } 20 \text{ cm} \right)\) packed with silica sand washed with Milli-Q water and dried at 105°C for 1 h. The cleaned silica sand was gradually added into the glass column to achieve a filter depth of 10 cm. This column was connected to another such column to achieve a total filter depth of 20 cm. Subsequently, Milli-Q water was pumped through the column with the help of a peristaltic pump for 15 min to saturate the filter medium, and the excess Milli-Q water was drained from the column just before the filtration experiment. Approximately 170 mL of the supernatant of the settling sample (see section on Coagulation experiments) was withdrawn from the beaker by the peristaltic pump and transferred to another glass beaker to be considered as raw water for rapid sand filtration experiments. During the filtration experiments, the raw water was continuously mixed with a magnetic stirrer at 200 rpm and fed into the column at a constant flow rate \(\left( 120 \text{ m/day} \right)\) by the peristaltic pump. Samples were taken from the beaker \(c_0\) and from the first \(10\) cm and second \(20\) cm column filtrates \(c_{rf}\) at 5, 15 and 30 min after the initiation of filtration for the quantification of bacteriophage concentrations.

MF experiments

After the coagulation experiments (without centrifugal separation), MF filtration experiments were carried out with a flat type of ceramic MF membrane. Approximately 170 mL of the supernatant of the settling sample (see section on Coagulation experiments) was withdrawn from the beaker by the peristaltic pump and transferred to another glass beaker to be considered as raw water for MF filtration experiments. During the filtration experiment, the raw water was continuously mixed with a magnetic stirrer at 200 rpm and fed into a ceramic MF membrane at a constant flux \(\left( 83 \text{ L/} \left( \text{m}^2 \text{ h} \right) \right) \) by the peristaltic pump. Samples were taken from the beaker \(c_{m0}\) and from the MF permeate \(c_{mf}\) at 15, 30, 60 and 120 min after the initiation of filtration for the quantification of bacteriophage concentrations.

Bacteriophage assay

The infectious bacteriophages were enumerated by the determination of the number of plaque-forming units (PFU) according to the double-layer method \(\left( \text{Adams, 1959} \right)\) with the bacterial host \(\textit{E. coli} \left( \text{NBRC 13965} \right)\). The average of the plaque counts of triplicate plates prepared from one sample was considered as the infectious bacteriophage concentration for that sample.

Electron micrograph

Negative-stain electron microscopy was used to analyze the morphology of the bacteriophages. Ten microliters of f1 or
f2 stock solution (see section on Bacteriophages) was placed on a 400-mesh copper grid with collodion membrane (Nissin EM Corp., Tokyo, Japan) and adsorbed onto the grid for 1 min. Excess solution was drained from the side of the grid with filter paper, and f1 or f2 was negatively stained with 10 μL of 2% phosphotungstic acid (pH 7.0) for 45 s. After the excess strain was drained off, the grid was examined with a transmission electron microscope (TEM, JEM-1210, Jeol Ltd, Tokyo, Japan).

**Electrophoretic mobility**

The electrophoretic mobility of bacteriophages was measured in prepared Milli-Q water and in filtered river water. Alkalinity was adjusted to 20 mg-CaCO₃/L with 0.4 mM NaHCO₃, and HCl was used to adjust the pH to 6.8. River water was filtered through a stirred ultrafiltration cell (Model 8400, Millipore Corp.) with an ultrafiltration membrane (molecular weight cutoff 100,000, regenerated cellulose; Ultrafiltration Disks, YM-100, Millipore Corp.) to exclude the large particles, and the pH was adjusted to 6.8 with HCl. The Milli-Q and river water samples were kept for 1 day at 20°C to stabilize the pH. Just before the measurement of the electrophoretic mobility, bacteriophage stock solutions (see section on Bacteriophages) were used to suspend each bacteriophage at approximately 10¹⁰ PFU/mL in the prepared Milli-Q water or filtered river water. The electrophoretic mobility of the bacteriophages was measured 25 times for each sample at 25°C and at a 15° measurement angle with an electrophoretic light-scattering spectrophotometer (ELS-6000, Otsuka Electronics Co., Ltd).

**RESULTS AND DISCUSSION**

**Particle diameter and electrophoretic mobility**

Figure 1 shows the electron micrographs of f1 and f2. Filamentous particles that were approximately 6–10 nm in diameter and 600–1,000 nm in length were observed for f1, whereas icosahedral particles approximately 25 nm in diameter were observed for f2. These particle characteristics are in agreement with previous reports (Shelton & Drewry 1973; Dotto et al. 1981). As seen in sandy aquifer treatment processes (Dowd et al. 1998), the differences in the particle sizes of f1 and f2 also probably influence their behavior during drinking water treatment processes.

Figure 2 shows a comparison in electrophoretic mobility (i.e. surface charge) between f1 and f2 in prepared Milli-Q water and filtered river water. When an electric field is applied to a suspension of charged particles, the particles move toward one of the electrodes. Negatively charged particles move toward the positive electrode and vice versa. The velocity of the particles is directly proportional to the applied field strength, and the ratio of these two quantities is known as the electrophoretic mobility (Gregory 2006). Accordingly, surface charge of particles can be evaluated by electrophoretic mobility measurements. Both bacteriophages were negatively charged in the prepared Milli-Q water, with f1 having a slightly more negative surface charge than f2. This difference in electrophoretic mobility is probably due to the differences in the types of amino acids (Weber & Konigsberg 1967; Beck & Zink 1981) on the surfaces of f1.
and f2. The same trend in electrophoretic mobility seen in prepared Milli-Q water was observed in filtered river water, although the electrophoretic mobility of both bacteriophages was shifted to the neutral owing to the differences in the ionic strength of the river water or to the influence of multivalent cations, such as calcium and magnesium ions. In general, the surface charge on virus particles strongly affects their behavior during various physicochemical water treatment processes, including coagulation processes (Matsushita et al. 2004). The more negatively charged viruses may resist aggregation, making it more difficult to destabilize and aggregate them by charge neutralization during coagulation in comparison to less negatively charged viruses. Thus, removal of f1 from river water by coagulation is expected to be more difficult than removal of f2.

Effects of coagulant dose on bacteriophage removal

Figure 3 shows the removal ratios, \( \log(\frac{C_{CO}}{C_{cell}}) \), for f1 and f2 after settling, with and without centrifugal separation, during coagulation. Because the diameters of f1 and f2 were small (as seen by electron microscopy) and they were stabilized by electrical repulsion in the river water, neither bacteriophage was removed (<0.2-log) from river water in the absence of coagulant. In contrast, both bacteriophages were removed by coagulation by the addition of any dose of the coagulant. The addition of coagulant destabilized the stably monodispersed bacteriophages in the river water, which got adsorbed onto/entrapped in the aluminium floc particles generated during coagulation. The aluminium floc particles were settled from suspension by gravity along with the adsorbed/entrapped bacteriophages during the settling process. Removal ratios of f1 and f2 were only approximately 1-log at 0.54 mg-Al/L of the coagulant, and the ratios increased to approximately 2-log with 1.08 mg-Al/L or more. Hijnen & Medema (2010) reviewed literature for viral removal performance in different coagulation processes (full-scale systems, pilot-plant studies and laboratory experiments) and estimated that the micro-organism elimination credit (MEC) of coagulation was 1.8±0.7-log for viruses (Hijnen & Medema 2010). Although the efficacy of coagulation for the removal of viruses is influenced by several conditions, such as raw water quality, the nature and concentrations of chemicals, pH, temperature and the type of mixing (Hijnen & Medema 2010), a similar removal performance (approximately 2-log) was observed for both bacteriophages in the present coagulation process.

The removal ratios of both bacteriophages increased after centrifugal separation. This indicates that centrifugal separation was able to remove the bacteriophages that were adsorbed onto/entrapped in the floc particles that were unable to settle by gravity.

However, regardless of centrifugal separation, the removal ratios of f1 were approximately 1-log lower than the ratio of f2 at any dose of the coagulant. This difference could be partly due to the difference in the electrophoretic
mobility of f1 and f2 in river water, as described above. Thus the removal of f1 from river water by coagulation was more difficult than that of f2.

Comparison of removal ratios of f1 and f2 during the coagulation–rapid sand filtration process

Figure 4 shows the removal ratios, \((\log(C_{c0}/C_{cs}) + \log(C_{r0}/C_{rd}))\), for f1 and f2 in the coagulation–rapid sand filtration process. Based on the results of the coagulation experiments, 1.08 mg-Al/L of coagulant was used in the coagulation–rapid sand filtration experiments. Although the DOC concentration of bacteriophage-spiked river water increased with the bacteriophage feed concentration, owing to the unavoidable uptake of the residual component of the culture medium, the removal ratios did not differ between initial concentrations of \(10^6\) and \(10^5\) PFU/mL for both bacteriophages (data not shown). This finding suggests that the DOC component from the bacteriophage culture solution did not affect bacteriophage removal during the coagulation process. Approximately 1-log improvement was seen in the removal ratios for f1 and f2 by the addition of the rapid sand filtration process in comparison to the coagulation process alone (Figure 3). This means that the bacteriophages entrapped in the suspended aluminium floc particles were effectively removed by the rapid sand filtration process after coagulation. In addition, no differences were observed in the removal ratios for f1 or f2 for filter depths of 10 and 20 cm. Thus, the effect of filter depth on bacteriophage removal was negligible, and most of the suspended aluminium floc particles were retained in the surface layer of the silica sand filter in the coagulation–rapid sand filtration process. Moreover, the removal ratios for both bacteriophages increased slightly with filtration time, which was probably due to the surface clogging of the silica sand filter by the deposition of suspended aluminium floc particles on the silica sand. In general, virus removal performance in the filtration process is increased with filtration time by pore clogging. In other words, the virus removal performance in the beginning of filtration time or just after the hydraulic backwashing can be regarded as a worst case scenario. Accordingly, the virus removal performances observed in the present study are assumed as their worst cases for elimination of virus in the filtration process since filtration time applied here is shorter than that of actual cases.

Based on literature reports describing the viral removal performance by coagulation–rapid granular filtration processes, MEC was estimated to be 3.0±1.4-log for viruses (Hijnen & Medema 2010). A similar removal ratio of approximately 3–4-log was also observed for both bacteriophages after 30 min of filtration time in the present coagulation–rapid sand filtration process. However, the removal ratios of f1 were approximately 1-log lower than those of f2 for any duration of filtration. Thus, the behavior of both bacteriophages was different not only during the coagulation process but also in the coagulation–rapid sand filtration process.

Comparison of removal ratios of f1 and f2 during the coagulation–MF process

Figure 5 shows the removal ratios, \((\log(C_{c0}/C_{cs}) + \log(C_{m0}/C_{md}))\), for f1 and f2 during the coagulation–MF process. Based on the results of the coagulation experiments, the coagulant dose used in the coagulation–MF experiments was 1.08 mg-Al/L. The coagulation–MF process effectively removed both bacteriophages: approximately 5-log increments were observed in the removal ratios for both bacteriophages by the addition of the MF process in comparison to the coagulation process alone (Figure 3), and time-averaged removal ratios of 5.6-log and 6.7-log were obtained for f1 and f2, respectively. This indicates that almost all suspended aluminium floc particles with adsorbed/entrapped bacteriophages exceeded the pore size of

![Figure 4](https://iwaponline.com/ws/article-pdf/12/5/666/416939/666.pdf)
the MF membrane and were removed by the MF process. Other researchers have also demonstrated the effectiveness of the coagulation–MF process: virus removal ratios of >4-log were achieved by the combination of aluminium/iron-based coagulation and organic/inorganic MF processes (Matsushita et al. 2008; Zhu et al. 2008; Fiksdal & Leiknes 2006).

The removal ratios for both bacteriophages were almost constant during filtration. In contrast, removal ratios for the F-specific RNA bacteriophages Qβ and MS2 gradually increased with filtration time in an in-line coagulation–MF process, probably due to the accumulation of (i) a cake layer on the membrane surface and (ii) viruses as irreversible foulants in the internal structure of the membrane pores (Shirasaki et al. 2009). One reason for this discrepancy is the difference in the coagulation processes (batch coagulation vs. in-line coagulation): most of the aluminium floc particles generated during the coagulation process were settled out from the suspension by gravity, and the floc particles remaining in the supernatant were fed into the MF membrane in the batch coagulation process. On the other hand, almost all the floc particles generated were fed into the MF membrane in the in-line coagulation process. Thus, the effects of the cake layer and of the irreversible foulants on bacteriophage removal during batch and in-line coagulation were quite different, leading to the differences in the trends of the removal ratios for bacteriophages.

As seen in the coagulation and coagulation–rapid sand filtration processes, the removal ratios for f1 were approximately 1-log lower than those for f2 for any duration of filtration during the coagulation–MF process. Thus, the removal of f1 by the combination of coagulation and filtration processes is more difficult than that of f2 from river water.

The US Environmental Protection Agency (USEPA) National Primary Drinking Water Standards (USEPA 2001) require 4-log removal or inactivation of enteric viruses from source water by filtration, disinfection or a combination of these technologies. This 4-log removal was achieved in the present study by the coagulation–MF process, regardless of bacteriophage characteristics (filamentous vs. spherical). Thus, the coagulation–MF process is effective for the removal of both filamentous and spherical viruses, and has the potential to effectively mitigate the public health risk posed by virus contamination in drinking water.

CONCLUSIONS

1. The removal performances of coagulation, coagulation–rapid sand filtration, and coagulation–MF processes were different for f1 and f2: the removal ratios for f1 were approximately 1-log lower than those for f2, probably due to the differences in the particle characteristics and the surface charges of f1 and f2.
2. The coagulation–MF process was more effective than the coagulation–rapid sand filtration process for the removal of both bacteriophages: the removal ratios for f1 and f2 in the coagulation–MF process were approximately 6-log and 7-log, respectively, and these values were approximately 3-log higher than those observed for the coagulation–rapid sand filtration process.
3. The public health risk posed by virus contamination in drinking water will be effectively mitigated by the replacement of the coagulation–rapid sand filtration process with the coagulation–MF process.

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