Virus removal by an in-line coagulation–ceramic microfiltration process with high-basicity polyaluminum coagulation pretreatment

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

ABSTRACT

The ability of in-line coagulation pretreatment with high-basicity polyaluminum chloride (PACl) coagulants to enhance virus removal by ceramic microfiltration (MF) was examined by comparing virus removal efficiencies from water pretreated with PACl-2.2 (basicity 2.2) and PACl-2.5 (basicity 2.5) versus alum, a synthetic aluminum chloride (AlCl3) solution, and two commercially available PACls, PACI-1.5 and PACI-1.8. The virus removal ratios for AlCl3, alum, PACI-1.5, and PACI-1.8 decreased markedly when the pH of the treated water shifted from 6.8 to 7.8, but was high at both pHs for PACI-2.2 and PACI-2.5. PACI-2.5 contains Al13 species and possibly Al30 species, and has a high colloid charge density. It removed viruses more efficiently than the other aluminum-based coagulants, not only at neutral pH, but also under weakly alkaline conditions. Moreover, the in-line coagulation–ceramic MF process with PACI-2.5 pretreatment removed not only viruses but also dissolved organic carbon and UV260-absorbing natural organic matter more efficiently and resulted in a lower residual aluminum concentration than did commercially available PACls, especially under weakly alkaline conditions. A combination of coagulation pretreatment with a high-basicity PACI and ceramic MF can provide effective treatment of drinking water over a broader pH range than is possible with commercially available aluminum-based coagulants.

Key words | aluminum hydrolyte species, bacteriophage, ceramic microfiltration, colloid charge density, in-line coagulation

INTRODUCTION

Low-pressure membrane (LPM) filtration, including microfiltration (MF) and ultrafiltration, is widely used for drinking water treatment because of its ability to produce high-quality water, its small footprint, and its relatively low costs (Huang et al. 2009). To improve the filtration efficiency of LPM, integration of pretreatment with LPM filtration has been widely employed in actual drinking water treatment plants. Pretreatment methods include adsorption, coagulation, and oxidation. Among these methods, coagulation is the most successful pretreatment for controlling membrane fouling (Huang et al. 2009), which reduces membrane permeability and increases the frequency of hydrodynamic or chemical cleaning. In addition, coagulation pretreatment is also useful for improving the quality of treated water. Enhancement of removal of dissolved organic carbon (DOC) and natural organic matter (NOM) can be expected with a combination of pre-coagulation and membrane filtration (Lee et al. 2000). Moreover, effective removal of waterborne enteric viruses, those having diameters of 20–100 nm, is possible by this hybrid process. For example, Tanneru et al. (2013) have reported that a coagulation–MF process with a coagulant dosage of more than 20 mg-Al/L produced 5.5–6.0 log reduction of viruses when the pH of the treated water was about 6.4, whereas MF alone with a 0.22-μm-pore-size, hydrophilic polyvinylidene difluoride filter produced only a 0.2-log
removal of viruses. Our research group has also reported the effectiveness of the coagulation–MF process: an approximately 6-log reduction of viruses was achieved at around pH 6.8 with a combination of in-line coagulation and a 0.1 μm-pore-size ceramic membrane filter (Shirasaki et al. 2009). This means that the coagulation–MF process has the potential to effectively mitigate the public health risk posed by virus contamination of drinking water.

Many factors affect the virus removal performance of the coagulation–MF process. Matsushita et al. (2005) have reported the effects of coagulant dosage, coagulation time, and MF membrane pore size on virus removal. They concluded that coagulant dosage strongly affected virus removal compared with two other factors when the pH was near 7. In addition, Zhu et al. (2005) have investigated the effect of pH on virus removal and reported a significant reduction of virus removal as the pH increased from 6.3 to 8.3 when a solution of ferric chloride (FeCl₃) was used as the coagulant. Adjustment of the pH during coagulation pre-treatment is therefore one of the important steps that must be taken to control virus removal in the coagulation–MF filtration process. However, an increase in the pH of drinking water sources from neutral to alkaline conditions because of excessive algal growth has been reported throughout the world (Hu et al. 2006; Matsukawa et al. 2006). Under these circumstances, reducing the pH of the drinking water source with acid or adding more coagulant is sometimes required to improve coagulation efficiency when commercially available aluminum-based coagulants such as polyaluminum chloride (PACl) and alum are used (Hu et al. 2006; Yan et al. 2008). However, both of these methods have some disadvantages, including an increase of the residual aluminum concentration in treated water (Matsukawa et al. 2006) and treatment cost (Yan et al. 2008).

An alternative investigated by some researchers has been the effect of adjusting the aluminum hydrolyte ratio (basicity = [OH⁻]/[Al³⁺]) in PACl on DOC removal and residual aluminum concentration during the coagulation process. They have reported that high-basicity PACls (basicity 2.1–2.7) yield higher removal of DOC and lower residual aluminum concentrations than commercially available PACls with basicities of 1.5–1.8, especially under weakly alkaline conditions (Yan et al. 2008; Kimura et al. 2013). Accordingly, effective removal of viruses as well as DOC is possible not only under neutral pH conditions but also under weakly alkaline conditions when the MF process is combined with a coagulation pretreatment with high-bASICITY PACl instead of commercially available PACl or alum. However, there is no report about the effectiveness of coagulation pretreatment with high-basicity PACl for virus removal during the coagulation–MF process. Our objective in the present study was to investigate the effects of coagulant type and PACl basicity on virus removal during the coagulation–MF process by comparing four PACls with different basicity, a synthetic aluminum chloride (AlCl₃) solution, and commercially available alum.

## MATERIALS AND METHODS

### Source water, coagulants, and MF membrane

On 17 July 2009 and 10 November 2009, river water was sampled from the Toyohira River (Sapporo, Japan), the water quality of which is shown in Table 1. The coagulants used for the coagulation process were six aluminum-based coagulants, the specifications of which are shown in Table 2. Two commercially available PACls with normal basicities of 1.5 (PACl-1.5) and 1.8 (PACl-1.8) were provided by the Taki Chemical Co., Ltd (Kakogawa, Japan). For experimental purposes, the same company also supplied high-basicity PACls with basicities of 2.2 (PACl-2.2, presently available commercially) and 2.5. To provide a comparison of coagulation efficiency with PACls, a synthetic AlCl₃ solution, which was prepared by dilution of reagent-grade aluminum (III) chloride hexahydrate (AlCl₃·6H₂O, Wako Pure Chemical Industries, Ltd, Osaka, Japan) dissolved in Milli-Q water

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Water quality of the Toyohira River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sampling date</td>
<td>River water 1</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>2.0</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>0.9</td>
</tr>
<tr>
<td>UV260 (cm⁻¹)</td>
<td>0.031</td>
</tr>
<tr>
<td>Alkalinity (mg-CaCO₃/L)</td>
<td>14.5</td>
</tr>
</tbody>
</table>
Table 2 | Specifications of aluminum-based coagulants used in the present study

<table>
<thead>
<tr>
<th>Coagulants</th>
<th>Basicity</th>
<th>Aluminum concentration</th>
<th>Sulfate concentration</th>
<th>Relative density at 20°C</th>
<th>Aluminum species distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlCl3</td>
<td>0.0</td>
<td>2.7 g-Al/L</td>
<td>0.0 g/L</td>
<td>1.0</td>
<td>75.8 4.6 19.6</td>
</tr>
<tr>
<td>Alum</td>
<td>0.0</td>
<td>8% (w/w) as Al2O3</td>
<td>23% (w/w)</td>
<td>1.3</td>
<td>73.3 9.4 17.3</td>
</tr>
<tr>
<td>PAC1-1.5</td>
<td>1.5</td>
<td>10% (w/w) as Al2O3</td>
<td>3% (w/w)</td>
<td>1.2</td>
<td>46.2 15.5 38.3</td>
</tr>
<tr>
<td>PAC1-1.8</td>
<td>1.8</td>
<td>10% (w/w) as Al2O3</td>
<td>3% (w/w)</td>
<td>1.2</td>
<td>42.2 11.6 46.3</td>
</tr>
<tr>
<td>PAC1-2.1</td>
<td>2.2</td>
<td>10% (w/w) as Al2O3</td>
<td>3% (w/w)</td>
<td>1.2</td>
<td>36.4 6.3 57.3</td>
</tr>
<tr>
<td>PAC1-2.5</td>
<td>2.5</td>
<td>23% (w/w) as Al2O3</td>
<td>0% (w/w)</td>
<td>1.3</td>
<td>24.6 3.8 71.6</td>
</tr>
</tbody>
</table>

Colloid titration analysis

The positive colloid charges of the coagulants were determined by colloid titration with a COM-555 potentiometric titrator (Hiranuma Sangyo Co., Mito, Japan). Each coagulant was diluted with Milli-Q water to 1–2 mg-Al/L (analytical pH condition was approximately 4–5), and then 150 mL of diluted coagulant was transferred to a titration vessel. After addition of 0.3 mL of toluidine blue indicator (Wako Pure Chemical Industries) to the vessel, the solution was titrated by means of a pump with 0.001 mol/L potassium polyvinyl sulfate (a standard negative colloid, Wako Pure Chemical Industries) at a constant rate of 10 mL/min. The vessel contents were homogenized with a magnetic stirrer during the titration, and the absorbance at 630 nm was recorded continuously until there was little change in the absorbance (i.e. subtle change in the color of the indicator from light blue to bluish-purple). The positive colloid charge was determined from the volume of potassium polyvinyl sulfate that corresponded to the half-height of the descending side of the recorded absorbance curve.

Bacteriophage

F-specific RNA bacteriophage Qβ (NITE Biological Research Center (NBRC) 20012) was obtained from the NBRC (Kisarazu, Japan). The bacteriophage Qβ is widely used as a surrogate for waterborne enteric viruses in the membrane filtration process (Matsushita et al. 2005; Shirasaki et al. 2009) because of its morphological similarities to hepatitis A viruses and polioviruses, which are important to remove during drinking water treatment. The bacteriophage Qβ is the
prototype member of the genus *Allolevivirus* in the virus family Leviriviridae. The genome of this bacteriophage contains a single molecule of linear, positive-sense, single-stranded RNA, which is encapsulated in an icosahedral protein capsid with a diameter of 24–26 nm (Fauquet *et al.* 2005). Bacteriophage was propagated and purified as described in our previous report (Shirasaki *et al.* 2010) prior to the preparation of a bacteriophage stock solution.

**In-line coagulation–ceramic MF experiments**

The river water, placed in a raw water tank, was spiked with Qβ at approximately 10^8 PFU/mL. Throughout the experiments, the raw water was mixed constantly with an impeller stirrer. The raw water was fed into the experimental system at a constant flow rate (83.3 L/(m² h) = 2.0 m/d) by a peristaltic pump. To maintain the MF filtrate at pH 6.8 or 7.8, hydrochloric acid or sodium hydroxide was added to the water before it reached the first in-line static mixer (hydraulic retention time (HRT) 1.8 s; 1/4-N40-172-0, Noritake Co., Ltd, Nagoya, Japan). Because about 1 mg-Al/L of PACl is usually dosed for the treatment of Toyohira River water (the source water in the present study) in the actual drinking water treatment plant (Moiwada drinking water treatment plant, Sapporo, Japan), coagulant was injected after the first in-line static mixer and before the second in-line static mixer at a constant dose rate (1.08 or 2.16 mg-Al/L). To obtain a coagulation time of 1 min, a combination of the in-line static mixer (G value 260 s⁻¹, HRT 1.8 s) and a subsequent Tygon tube reactor (inside diameter 1.6 mm, total HRT 1 min) was used as the second in-line static mixer. After the coagulant had been admixed with the water, the water was fed into the ceramic MF module in dead-end mode. Filtration was performed for 4 h without any backwashing. Bacteriophage concentrations in the raw water were measured every hour. In addition, DOC concentrations and UV260-absorbing NOM were quantified with a SIEVERS 900 laboratory TOC analyzer (GE Analytical Instruments, Boulder, CO, USA) and a UV-1700 Pharma spectrophotometer (Shimadzu Corp., Kyoto, Japan), respectively. After adding the nitric acid (1% (v/v), ultrapure, Kanto Chemical Co., Inc., Tokyo, Japan) into the MF filtrate, the aluminum concentration was analyzed with an HP4500 inductively coupled plasma–mass spectrometer (Yokogawa Analytical Systems Inc., Tokyo, Japan).

**Bacteriophage assay**

**PFU method**

The infectious bacteriophages were enumerated according to the double-layer method (Adams 1959) by using the bacterial host *Escherichia coli* (NBRC 13965). Serially diluted raw water or MF filtrate (1 mL) was poured onto a solid-bottom agar plate followed by 0.3 mL of host *E. coli* culture mixed with 3 mL of molten top agar. The plates were incubated for 16–24 h at 37 °C. To measure the concentrations of infectious bacteriophage in the water samples, we calculated the average plaque counts of triplicate plates prepared from one sample on plates with 50 to 300 PFU, which we considered a countable number of plaques, and determined the number of plaque forming units per millilitre.

For quantification of low infectious bacteriophage concentrations (i.e. <30 PFU/mL) in the MF filtrate, 50 mL of MF filtrate was mixed with 5 mL of bacterial host *E. coli* culture and 50 mL molten agar, and the mixture was then poured into 10 plates (without bottom agar). The plates were incubated for 16–24 h at 37 °C. We calculated the number of plaque forming units per millilitre by dividing the total plaque counts for the 10 plates by the sample volume (50 mL).

**Real-time reverse-transcription polymerase chain reaction method (RT-PCR)**

The viral RNA of bacteriophages was quantified by the real-time RT-PCR method. This method detects all bacteriophages, regardless of their infectivity and the existence of aggregates. The detailed procedure for the real-time RT-PCR method has been described in our previous study (Shirasaki *et al.* 2010).

**RESULTS AND DISCUSSION**

**Effect of coagulant type on bacteriophage removal**

Figure 1(a) shows the effect of coagulant type on the removal of infectious Qβ, assessed by the PFU method, in the in-line coagulation–ceramic MF process (source water was river water 1). Because the diameter of Qβ
(approximately 25 nm) was smaller than the nominal pore size of the ceramic MF membrane (0.1 μm), no removal of infectious Qβ was observed in the absence of coagulation pretreatment (data not shown). In contrast, in-line coagulation pretreatment dramatically enhanced the removal ratios (log\(\frac{C_0}{C_f}\)) of infectious Qβ compared with ceramic MF alone at around pH 6.8 of treated water. Greater than 6-log removals were achieved by a combination of in-line coagulation pretreatment and ceramic MF filtration, regardless of the type of coagulant, because during the coagulation pretreatment, Qβ was effectively incorporated into the aluminum flocs, which were larger than the nominal pore size of the ceramic MF membrane, and the flocs with incorporated Qβ were removed by ceramic MF filtration.

The infectious Qβ removal performances of AlCl₃, alum, and PACI-1.5 were markedly decreased when the pH of the treated water shifted from 6.8 to 7.8. Even when the coagulant dosage was increased from 1.08 to 2.16 mg-Al/L, <1-log removals were observed for those coagulants (Figure 1(b)). In contrast, PACI-2.2 continued to remove viruses with high efficiency (>6-log removal of infectious Qβ) even under weakly alkaline pH conditions (Figure 1(a)). This result indicates that the type of coagulant affects virus removal performance in the in-line coagulation–ceramic MF process, especially under weakly alkaline pH conditions, and that a high-basicity PACI (e.g. PACI-2.2) can remove viruses more effectively than the other commercially available aluminum-based coagulants used in the present study.

**Effect of PACI basicity on bacteriophage removal**

As described above, PACI-2.2 removed infectious Qβ more efficiently than did other aluminum-based coagulants, including PACI-1.5, especially under weakly alkaline pH conditions. To precisely quantify the effect of PACI basicity on virus removal, we compared the removal of Qβ by in-line coagulation pretreatment with four PACIs with different basicities followed by ceramic MF filtration within the pH range 6.8–7.8 (Figure 2, source water was river water 2). More than 8-log removals of infectious Qβ, assessed by the PFU method, were attained by the in-line coagulation–ceramic MF process with all the PACIs when the pH of the treated water was about 6.8, regardless of their basicity (Figure 2(a)). In contrast, the removal efficiencies of infectious Qβ depended on the basicity of the PACIs when the pH of the treated water was about 7.8. The in-line coagulation–ceramic MF process with PACI-1.5 and PACI-1.8 achieved approximately a 1-log removal of infectious Qβ, but approximately 6-log and >8-log removals were achieved with PACI-2.2 and PACI-2.5, respectively. A similar trend was observed for removal ratios of total Qβ, assessed by the RT-PCR method. Although high removal efficiencies of total Qβ, at least
6-log removals, were achieved for all PACls at around pH 6.8, marked differences in removal ratios were observed among the four PACls when the pH of the treated water was about 7.8 (Figure 2(b)). These results suggest that the basicity of PACls affects virus removal performance in the in-line coagulation–ceramic MF process, especially under weakly alkaline conditions.

As shown Figure 2, the in-line coagulation–ceramic MF process with PACl-2.5 effectively removed viruses compared to the performance of the other PACls used in the present study. In addition, this process removed DOC and UV260-absorbing NOM more efficiently and resulted in a lower residual aluminum concentration than did the processes with PACI-1.5, PACI-1.8, and PACI-2.2, especially under weakly alkaline conditions (whereas approximately 20–30% and 40–50% removals for DOC and UV260-absorbing NOM, respectively, and 0.08–0.15 mg-Al/L residual aluminum concentrations were observed for PACI-1.5, PACI-1.8, and PACI-2.2 at a pH of about 7.8, approximately 50% and 70% removals for DOC and UV260-absorbing NOM, respectively, and <0.01 mg-Al/L residual aluminum concentrations were attained for PACI-2.5). Accordingly, the combination of coagulation pretreatment with high-basicity PACI and ceramic MF is effective in treating drinking water, including virus removal, over a broader pH range compared to commercially available aluminum-based coagulants.

The Qβ removal ratios determined by the PFU and RT-PCR methods differed markedly, depending on the coagulation conditions. The infectious Qβ removal ratios (Figure 2(a)) were larger than the total Qβ removal ratios (Figure 2(b)). Because our previous study demonstrated that Qβ loses its infectivity after being mixed with aluminum hydrolyte species during coagulation with PACI-1.5 (Matsushita et al. 2011), the virucidal activity of the PACls contributed to the removal efficiency of infectious Qβ during the in-line coagulation–ceramic MF process.

Characterization of coagulants

To elucidate why PACI-2.5, which has a higher basicity, was more effective in removing viruses, we used ESI-TOF-MS to analyze the aluminum hydrolyte speciation in PACI-2.5. In the ESI-TOF-MS spectra of all the coagulants (Figure 3), we found the most intense peak at m/z 97, which is assigned to the monomeric aluminum species \([\text{Al(OH)}_2(H_2O)_2]^{3+}\) (Urabe et al. 2007). In addition, fragment ions of tridecameric species – i.e. Al13 species such as \([\text{AlO}_4(OH)_{24}(H_2O)_{12}]^{7+}\) with peaks at m/z 303 \([\text{AlO}_4(OH)_{28}(H_2O)]^{5+}\) and 454 \([\text{AlO}_4(OH)_{29}]^{2+}\) (Stewart et al. 2009) – were observed for...
PACl-2.2 (the relative intensities of these two peaks were 0.7–0.9%) and PACl-2.5. In contrast, no peak for Al13 species was confirmed in the spectra of AlCl3, alum, PACl-1.5, and PACl-1.8. Moreover, other strong peaks of Al13 species at m/z 297 [Al13O4(OH)28]3+, 309, 315, 321, 327, and 333 [Al13O4(OH)28(H2O)2-6]3+ (Stewart et al. 2009) were observed in the spectrum of PACl-2.5. These results suggest that high-basicity PACls such as PACl-2.2 and PACl-2.5 contain Al13 species, but these species are not present in AlCl3, alum, PACl-1.5, and PACl-1.8.

Some researchers have demonstrated that the content of Al13 species in a coagulant is approximately equal to that of Alb species categorized by the Ferron method (Chen et al. 2009). In the present study, whereas strong peaks related to Al13 species were observed for PACl-2.5, the content of Alb in PACl-2.5 was very similar to those in other
aluminum-based coagulants used in the present study (Table 2). In contrast, the content of Alc varied as a function of the coagulant type and was highest in PACl-2.5 (Table 2). Because the Al_{30} species [(Al_{30}O_{4}(OH)_{26}(H_{2}O)_{24})]^{18+} does not react with Ferron reagents within 120 min, it is categorized as Alc by the Ferron method (Chen et al. 2007). In addition, the Al_{30} species is known to consist of two Al_{13} species connected by four monomeric aluminum species (Chen et al. 2007), the implication being that the peaks of monomeric aluminum species and fragment ions of Al_{13} species will also be observed when the Al_{30} species in a coagulant is decomposed by fragmentation in the ESI-TOF-MS analysis. Actually, these peaks were clearly confirmed in the ESI-TOF-MS spectrum of PACl-2.5, as shown in Figure 3. Therefore, PACl-2.5 possibly contained not only Al_{13} species but also Al_{30} species.

The positive colloid charges of the coagulants were also determined by using a colloid titration technique (Figure 4). The colloid charge densities of the AlCl_{3} and alum were very small and almost constant, regardless of the aluminum concentration. In contrast, the colloid charge densities of the PACls increased with increasing concentrations of aluminum. In addition, the colloid charge density of PACl-2.5 was the highest among the aluminum-based coagulants used in the present study. Because Al_{13} species and Al_{30} species are known to be effective coagulants because of their strong charge neutralization capability and high structural stability (Chen et al. 2006; Zhang et al. 2008), the presence of Al_{13} species, Al_{30} species, or both in PACl-2.5 is possibly associated with the large amount of positive colloid charge. In addition, the absence of sulfate in PACl-2.5 probably also contributed to its high colloid charge density compared with that of other sulfated PACls such as PACl-1.5, PACl-1.8, and PACl-2.2, because the presence of sulfate during the coagulation process reduces the charge neutralization capability of coagulants (Wang et al. 2002). These characteristics of PACl-2.5 account for its high capability to neutralize the negative charges on viruses during coagulation pretreatment. Actually, the in-line coagulation–ceramic MF process with PACl-2.5 could effectively remove viruses not only under neutral pH conditions but also under weakly alkaline conditions, as described above.

We have shown through this study that coagulation pretreatment with PACl-2.5 followed by ceramic MF has a variety of advantages, including effective removal of viruses, DOC, and UV260-absorbing NOM, and the process results in very low residual aluminum concentrations compared with other aluminum-based coagulants, even under weakly alkaline conditions. To elucidate whether the in-line coagulation–ceramic MF process with high-basicity PACl is actually useful for drinking water treatment, further investigations using a wide variety of water sources are needed, because the concentration of viruses in the treated water may be affected by the characteristics of the source water.

CONCLUSIONS

1. The basicity of PACls affects the virus removal performance of the in-line coagulation–ceramic MF process. The high-basicity PACls, PACl-2.2 and PACl-2.5, could effectively remove viruses not only under neutral pH conditions but also under weakly alkaline conditions.
2. Among the various aluminum-based coagulants used in the present study, PACl-2.5, which has a high Alc content (including Al_{13} species and possibly Al_{30} species) and a high colloid charge density, removed viruses more efficiently (>8-log for infectious viruses; >6-log for total viruses) than the other aluminum-based coagulants in the pH range 6.8 to 7.8.
3. The in-line coagulation–ceramic MF process with PACl-2.5 removed not only viruses but also DOC and
UV260-absorbing NOM more efficiently and resulted in a lower residual aluminum concentration than did commercially available PACs, especially under weakly alkaline conditions.

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