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Title	Improved virus removal by high-basicity polyaluminum coagulants compared to commercially available aluminum-based coagulants
Author(s)	Shirasaki, N.; Matsushita, T.; Matsui, Y. et al.
Citation	Water research, 48, 375-386 https://doi.org/10.1016/j.watres.2013.09.052
Issue Date	2014-01-01
Doc URL	https://hdl.handle.net/2115/54779
Type	journal article
File Information	Improved virus removal by high-basicity polyaluminum coagulants compared to commercially available aluminum-based coagulants.pdf

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Improved virus removal by high-basicity polyaluminum coagulants compared to commercially available aluminum-based coagulants

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Abstract

We investigated the effects of basicity, sulfate content, and aluminum hydrolyte species on the ability of polyaluminum chloride (PACl) coagulants to remove F-specific RNA bacteriophages from river water at a pH range of 6–8. An increase in PACl basicity from 1.5 to 2.1 and the absence of sulfate led to a reduction of the amount of monomeric aluminum species (i.e., an increase of the total amount of polymeric aluminum and colloidal aluminum species) in the PACl, to an increase in the colloid charge density of the PACl, or to both and, as a result, to high virus removal efficiency. The efficiency of virus removal at around pH 8 observed with PACl-2.1c, a nonsulfated

high-basicity PACl (basicity 2.1–2.2) with a high colloidal aluminum content, was larger than that observed with PACl-2.1b, a nonsulfated high-basicity PACl (basicity 2.1–2.2) with a high polymeric aluminum content. In contrast, although extremely high basicity PACls (e.g., PACl-2.7ns, basicity 2.7) effectively removed turbidity and UV260-absorbing natural organic matter and resulted in a very low residual aluminum concentration, the virus removal ratio with PACl-2.7ns was smaller than the ratio with PACl-2.1c at around pH 8, possibly as a result of a reduction of the colloid charge density of the PACl as the basicity was increased from 2.1 to 2.7. Liquid ^{27}Al NMR analysis revealed that PACl-2.1c contained Al_{30} species, which was not the case for PACl-2.1b or PACl-2.7ns. This result suggests that Al_{30} species probably played a major role in virus removal during the coagulation process. In summary, PACl-2.1c, which has high colloidal aluminum content, contains Al_{30} species, and has a high colloid charge density, removed viruses more efficiently (>4 log_{10} for infectious viruses) than the other aluminum-based coagulants—including commercially available PACls (basicity 1.5–1.8), alum, and PACl-2.7ns—over the entire tested pH (6–8) and coagulant dosage (0.54–5.4 mg-Al/L) ranges.

Key words: Aluminum hydrolyte species, Bacteriophages, Coagulation, Colloid charge density, Sulfate

1. Introduction

Aluminum-based coagulants such as polyaluminum chloride (PACl) and alum are commonly used in coagulation processes to destabilize suspended and dissolved materials in water and combine them into large flocs that are easily separated from the water by subsequent sedimentation or filtration. Waterborne enteric viruses, which do not settle from suspension under the influence of gravity, can also be removed with aluminum-based coagulants. For example, Nasser et al. (1995) reported that 88.4% and 47% of hepatitis A virus and poliovirus, respectively, can be removed by coagulation with 30 mg/L of alum. We have reported that a coagulation process with PACl or alum effectively removes bacteriophages, which are viruses that infect bacteria and may be indicators for waterborne enteric viruses (Matsushita et al., 2011).

The efficiency of microorganism removal by coagulation processes is strongly influenced by several factors, including the nature and dosages of the coagulant used, pH, temperature, and mixing method (Hijnen and Medema, 2010). In particular, pH control during the coagulation process is essential for optimal coagulation (Bratby, 2006). Guo and Hu (2011) reported that coagulation with alum at pH 8 does not result in significant virus removal, whereas coagulation at pH 6 and 7 does. Worldwide, the pH of various surface drinking water sources is changing from neutral to alkaline because of the excessive growth of algae (Hu et al., 2006; Matsukawa et al., 2006), and this change can be expected to reduce coagulation efficiency and thus virus removal performance if commercially available PACl or alum is used without pH adjustment. Reducing the

pH of drinking water sources with acid or increasing the coagulant dosage is sometimes required to improve coagulation efficiency (Hu et al., 2006; Yan et al., 2008a). However, both of these methods have some disadvantages, such as increasing the residual aluminum concentration in treated water (Matsukawa et al., 2006) and increasing the treatment cost (Yan et al., 2008a). Therefore, the development of new coagulation processes that effectively remove suspended and dissolved materials, including viruses, from both neutral and alkaline drinking water sources without the need for pH optimization is highly desired.

For the improvement of coagulation efficiency, PACl coagulants with various aluminum hydrolysis ratios (basicity = $[OH^-]/[Al^{3+}]$) have been produced, and the influence of PACl basicity on coagulation processes has been investigated (Wang et al., 2002; Yan et al., 2008a,b; Yang et al., 2011; Zhang et al., 2008). For example, Wang et al. (2002) reported that turbidity removal at alkaline pH is improved by increases in PACl basicity. Zhang et al. (2008) reported that the coagulation efficiency of PACl increases with increasing basicity: specifically, PACl with a basicity of 2.4 exhibits higher humic acid removal efficiency and lower residual aluminum concentration at a broader pH range and a wider PACl dosage range compared to PACls with basicities of 1.2 and 1.8. In previous work, we compared PACls with basicities of 2.1 and 1.5 in the pH range of 6.8–7.8 and found that the former, which contains a smaller percentage of monomeric aluminum species and a larger percentage of colloidal aluminum species than the latter, removes dissolved organic carbon more efficiently and with a lower residual aluminum concentration (Kimura et al., 2013).

Moreover, PACls with basicities of >2.6 yield a very low residual aluminum concentration (<0.02 mg/L), even at a wide pH range (6.5–8.5; Kimura et al., 2013). High-basicity PACls are expected to effectively remove viruses not only at neutral pH but also at weakly alkaline pH; however, virus removal during coagulation processes with high-basicity PACls has not been fully investigated. In addition, little information is available about how the small amount of sulfate (e.g. 3% w/w) that is present in commercially available PACls to improve flocculation and sedimentation efficiency (Pernitsky and Edzwald, 2003) affects virus removal during coagulation processes.

Here, we conducted batch coagulation experiments to investigate the effect of PACl basicity on virus removal by comparing a wide variety of PACls with different basicities, including commercially available PACls (basicity 1.5–1.8) and extremely high basicity PACls (basicity 2.7). In addition, we investigated the effect of sulfate in the PACls on virus removal by comparing sulfated and nonsulfated PACls. Moreover, we experimentally evaluated the aluminum species distributions and colloid charge densities of the tested coagulants to determine what caused the differences in virus removal performance.

2. Materials and methods

2.1. Source water and coagulants

River water was sampled from the Toyohira River in Sapporo, Japan, on 1 October 2010, 24 June 2011, and 4 December 2012 (water quality data are shown in Table S1, Supplementary

Information).

We conducted three sets of coagulation experiments on the river water samples. For the first set of experiments, we used five aluminum-based coagulants (Table S2). Two commercially available PACls (PACl-1.5s and PACl-1.8s, where 1.5 and 1.8 are the basicity values, and "s" stands for "sulfated"; Taki Chemical Co., Kakogawa, Japan). A trial high-basicity PACl (PACl-2.1s, which is now commercially available) was also supplied by the same company. For comparison with the commercially available PACls, we evaluated an $AlCl_3$ solution prepared by dilution of reagent-grade aluminum(III) chloride hexahydrate ($AlCl_3 \cdot 6H_2O$, Wako Pure Chemical Industries, Osaka, Japan) in Milli-Q water (Milli-Q Advantage, Millipore Corp., Billerica, MA, USA), and we also evaluated a commercially available alum (Taki Chemical Co.).

After the first set of experiments was completed, we conducted a second set of experiments with eight aluminum-based coagulants (Table S2). In addition to two of the sulfated PACls described above, we evaluated a trial nonsulfated PACl (PACl-1.5ns, where "ns" stands for "nonsulfated"), a high-basicity nonsulfated PACl (PACl-2.1ns), and an extremely high basicity nonsulfated PACl (PACl-2.7ns), all provided by Taki Chemical Co., to further investigate the effects of basicity and sulfate on virus removal. We also evaluated three PACls (PACl-2.1b, PACl-2.1c, and PACl-2.7, where "b" and "c" indicate high Al_b and Al_c content, as measured by a ferron method, described below) prepared by a base titration method in our laboratory, as described previously (Kimura et al., 2013).

Finally, we conducted a third set of experiments with eight aluminum-based coagulants (Table S2), which were provided by Taki Chemical Co. or prepared in our laboratory by the base titration method.

All the laboratory-made PACls (PACl-0.9, PACl-1.5, PACl-2.1b, PACl-2.1c, and PACl-2.7) were nonsulfated, and they are distinguished from the company-made PACls in that “ns” is not included in the name.

All the coagulants were used in batch coagulation experiments immediately after dilution with Milli-Q water.

2.2. Characterization of coagulants

2.2.1. Ferron method

The aluminum hydrolyte species in the coagulants were analyzed by means of a ferron method (Wang et al., 2004) after dilution with Milli-Q water to a concentration of 2.7 g-Al/L, i.e., 0.1 M-Al (analytical pH condition was approximately 4–5). On the basis of the kinetic differences between the reactions of the aluminum species and the ferron reagent (8-hydroxy-7-iodoquinoline-5-sulfonic acid, Wako Pure Chemical Industries), aluminum hydrolyte species were categorized as monomeric species, fast-reacting polymeric species, or slow-reacting colloidal species, denoted as Al_a, Al_b, and Al_c, respectively (Wang et al., 2004). After addition of the ferron reagent to the diluted coagulant, the mixture was immediately stirred magnetically for 10 s at 400 rpm, and then the absorbance at

366 nm was measured with a UV-1700 Pharma Spec spectrophotometer (Shimadzu Corp., Kyoto, Japan) at predetermined reaction times. The aluminum hydrolyte species were operationally divided into the three categories as follows: Al_a, species that reacted with ferron within 30 s; Al_b, species that reacted with ferron within 120 min (absorbance at 120 s minus the absorbance due to Al_a); and Al_c, species that did not react with ferron ($Al_c = Al_t - [Al_a + Al_b]$, where Al_t = total Al). To obtain Al_t, we adjusted the pH of the diluted coagulant to approximately 0.5 with ultrapure nitric acid (Kanto Chemical Co., Tokyo, Japan), heated it for 3 h at 85 °C in a muffle furnace, cooled it to room temperature, and then analyzed it by the ferron method as described for Al_a.

2.2.2. Liquid ^{27}Al nuclear magnetic resonance analysis

In addition to the ferron method, ^{27}Al nuclear magnetic resonance (NMR) spectrometry was also used to characterize the aluminum hydrolyte species in the coagulants after dilution with Milli-Q water to a concentration of 2.7 g-Al/L, i.e., 0.1 M-Al (analytical pH condition was approximately 4–5). On the basis of chemical shift differences, aluminum hydrolyte species were categorized into four groups: monomeric species (Al_m), dimeric and trimeric species, tridecameric species (Al_{13}), and Al_{30} species (Chen et al., 2006, 2007; Gao et al., 2005). After addition of deuterium oxide (75% v/v, Wako Pure Chemical Industries) to the diluted coagulant, the solution was placed in a 5-mm NMR tube. A 3-mm coaxial capillary filled with diluted sodium aluminate (Wako Pure Chemical Industries) solution, which was diluted with Milli-Q water to 0.01 M-Al and then added the

deuterium oxide (75% v/v). The coaxial capillary was used as an internal standard for Al content and as the deuterium lock (Chen et al., 2007; Gao et al., 2005). The NMR spectra were measured with a JEOL JNM-ECA 600 spectrometer (JEOL, Tokyo, Japan) by means of a single-pulse method (field strength 14.09 T, resonance frequency 156.39 MHz, pulse width 5.0 μs, repetition time 1.13 s, scans 8,000, X-sweep 78.25 kHz). The reference chemical shift (0 ppm) was adjusted with $AlCl_3$ solution prepared by the procedure described above.

2.2.3. 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 N potassium polyvinyl sulfate (a standard negative colloid, Wako Pure Chemical Industries) at a constant rate of 10 mL/min. The vessel contents were magnetically stirred during the titration, and the absorbance at 630 nm was recorded continuously until little change in the absorbance (i.e., subtle change in the color of the indicator from light blue to bluish-purple) was observed. The positive colloid charge was determined from the volume of potassium polyvinyl sulfate that corresponded to the half height of the descending slope of the recorded absorbance

curve.

2.3. Bacteriophages

F-specific RNA bacteriophages Qβ (NBRC 20012) and MS2 (NBRC 102619) were obtained from the NITE Biological Research Center (Kisarazu, Japan). Qβ (Boudaud et al., 2012; Matsui et al., 2003; Matsushita et al., 2011; Shirasaki et al., 2009a,b) and MS2 (Boudaud et al., 2012; Fiksdal and Leiknes, 2006; Guo and Hu, 2011; Matsushita et al., 2011; Nasser et al., 1995; Shirasaki et al., 2009a,b; Zhu et al., 2005) are widely used as surrogates for waterborne enteric viruses in coagulation processes because these bacteriophages are morphologically similar to hepatitis A viruses and polioviruses, removal of which during drinking water treatment is important. Qβ is the prototype member of the genus *Allolevivirus* in the virus family Leviviridae, and MS2 is the prototype member of the genus *Levivirus* in the Leviviridae family. The genomes of these two bacteriophages contain 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). Each bacteriophage was propagated and purified prior to the preparation of a bacteriophage stock solution as described in our previous report (Shirasaki et al., 2010).

2.4. Coagulation experiments with bacteriophage-spiked river water

Batch coagulation experiments were conducted with 1,000 mL of bacteriophage-spiked river water

in square plastic beakers at 20 °C. The bacteriophage stock solution (see section 2.3) was added to the river water in a beaker at approximately 10^8 plaque forming unit (PFU)/mL (C_0), and the spiked water was mixed with an impeller stirrer. After enough HCl or NaOH was added to the water to bring the final pH to a target value of at 6, 7, or 8, coagulant was injected into the water at a dosage of 0.54, 1.08, 1.89, 2.16, or 5.4 mg-Al/L. The water was stirred rapidly for 1 min (G = 200 s^{-1}, 136 rpm) and then slowly for 10 min (G = 20 s^{-1}, 29 rpm). The water was left at rest for 60 min to allow the generated aluminum floc particles to settle. Then the supernatant was sampled from the beaker for quantification of the bacteriophage concentrations (C_s) and turbidity. A portion of each supernatant was filtered through a membrane filter (first and second sets of experiments, nominal pore size 0.4 μm, polycarbonate, Isopore, Millipore; third set of experiments, nominal pore size 0.45 μm, polytetrafluoroethylene, Dismic-25HP, Toyo Roshi Kaisha, Tokyo, Japan) for quantification of the ultraviolet absorbance at 260 nm (an indication of natural organic matter [NOM] concentration) and for measurement of the aluminum concentration. Turbidity and UV260-absorbing NOM were quantified with a 2100AN turbidity meter (Hach Company, Loveland, CO, USA) and a UV-1700 Pharma Spec spectrophotometer, respectively. After ultrapure nitric acid (1% v/v, Kanto Chemical Co.) was added to the membrane permeate, the aluminum concentration was determined by means of inductively coupled plasma–mass spectrometry (Agilent 7700 series, Agilent Technologies, Inc., Santa Clara, CA, USA).

2.5. Bacteriophage assay

The infectious bacteriophages were quantified by determination of the number of PFUs according to the double-layer method (Adams, 1959) with *E. coli* (NITE Biological Research Center 13965) as the bacterial host. The average of the plaque counts of triplicate plates prepared from one sample was considered as the infectious bacteriophage concentration for that sample.

Bacteriophage RNA was quantified by a real-time reverse transcription–polymerase chain reaction (RT-PCR) method, which detects all bacteriophages regardless of their infectivity and the existence of aggregates. The details of the real-time RT-PCR method are described in Supplementary Information.

3. Results and discussion

3.1. First set of experiments

3.1.1. Effect of coagulant type on bacteriophage removal

The effect of coagulant type on the infectious Qβ removal ratio ($\log_{10}[C_0/C_s]$) during the coagulation process was evaluated by the PFU method after settling (Fig. 1a). Because Qβ is small and was stably dispersed in the river water (because of electrical repulsion), no removal (<0.3-$\log_{10}$) of infectious Qβ was observed in the absence of coagulant at any pH. In contrast, the coagulation process removed infectious Qβ at a pH range of 6–7 no matter what type of coagulant was used. This result indicates that the Qβ stably monodispersed in the river water was destabilized by the

addition of coagulant and became adsorbed on or entrapped in the aluminum floc particles generated during the coagulation process and that the aluminum floc particles along with the destabilized Qβ then settled out from the suspension under the influence of gravity during the settling process. The efficiency of infectious Qβ removal depended on coagulant type: whereas coagulation with $AlCl_3$ and alum resulted in approximately 2-log_{10} removal at a pH range of 6–7, approximately 6-log_{10} removal was achieved with all the PACls, regardless of their basicity. Matsushita et al. (2011) also reported that the infectious Qβ removal ratio during the coagulation process with PACl is larger than that with alum at neutral pH. Moreover, we previously reported that PACl is more effective than alum for removing norovirus particles (Shirasaki et al., 2010).

The virus removal performances of $AlCl_3$, alum, PACl-1.5s, and PACl-1.8s markedly decreased when the pH of the treated water was increased from 7 to 8 (Fig. 1a). Hu et al. (2006) reported that the aluminum species distributions of $AlCl_3$ and commercially available PACl during coagulation process were greatly changed depending on the pH: although the aluminum species distributions of those coagulants were almost same in the pH range from 6 to 7, monomeric aluminum species were increased while polymeric and colloidal aluminum species were decreased when the pH of the treated water was increased from 7 to 8. Therefore, difference in the aluminum species distributions of the $AlCl_3$, alum, PACl-1.5s and PACl-1.8s probably contribute to the difference in the virus removal performances between pH range from 6 to 7 and pH 8. In contrast, PACl-2.1s retained its high virus removal performance (~5-log_{10} removal) even at weakly alkaline pH. This result

indicates that PACl basicity affected virus removal performance during the coagulation process and that a high-basicity PACl (PACl-2.1s) effectively removed the virus not only under weakly acidic and neutral pH conditions but also at weakly alkaline pH. The total Qβ removal ratios evaluated by the PCR method were also observed to be somewhat larger with PACl-2.1s than the ratios with $AlCl_3$, alum, PACl-1.5s, and PACl-1.8s, especially at around pH 8 (Fig. 1b). In addition, the coagulation process with PACl-2.1s removed turbidity and UV260-absorbing NOM more efficiently and resulted in a lower residual aluminum concentration than did $AlCl_3$, alum, PACl-1.5s, and PACl-1.8s, especially at weakly alkaline pH (Fig. S1).

The Qβ removal ratios determined by the PFU and PCR methods differed markedly: the infectious Qβ removal ratios (Fig. 1a) were larger than the total Qβ removal ratios (Fig. 1b). This difference between the PFU and PCR methods could be explained by the formation of aggregates consisting of several infectious Qβ particles, the inactivation of Qβ during the coagulation process, or both. Matsushita et al. (2011) reported that Qβ loses its infectivity after being mixed with aluminum hydrolyte species during the coagulation process with PACl-1.5s, as indicated by a combination of filtration and particle size measurements at neutral pH. This result suggests that the virucidal activity of the aluminum-based coagulants contributed to the efficiency of infectious Qβ removal during the coagulation process.

3.1.2. Comparison of Qβ and MS2 removal ratios during the coagulation process

As described above, PACl-2.1s removed Qβ more efficiently than did other aluminum-based coagulants used in the present study, especially at weakly alkaline pH. To confirm that PACl-2.1s actually removed viruses more effectively than PACl-1.5s, we also evaluated the MS2 removal ratio, because MS2 is less sensitive than Qβ to the virucidal activity of PACl (Matsushita et al., 2011; Shirasaki et al., 2009a). We evaluated the MS2 removal efficiency by means of the PFU and PCR methods after settling during the coagulation process, and then compared the results with those for Qβ (Fig. 2). For both bacteriophages, the removal ratios observed with PACl-2.1s were larger than those with PACl-1.5s at around pH 8. This result means that compared to coagulation with PACl-1.5s, coagulation with PACl-2.1s more effectively removed not only a virus that is highly sensitive to the virucidal activity of the aluminum-based coagulants but also a virus that is less sensitive.

The infectious Qβ removal ratio of PACl-2.1s was approximately 3-$\log_{10}$ larger than the infectious MS2 removal ratio, partly because of the different sensitivities of Qβ and MS2 to the virucidal activity of PACl-2.1s. Because Qβ is more sensitive than MS2, the infectious Qβ concentration after settling during the coagulation process may have been less than the quantification limit of the PFU method when the other high-basicity PACl was applied. Therefore, we used MS2 in our second and third sets of experiments.

3.2. Second set of experiments

3.2.1. Effects of coagulant basicity and sulfate content on bacteriophage removal

To further investigate the effective virus removal observed with PACl-2.1s, we conducted batch coagulation experiments with various sulfated and nonsulfated PACls and evaluated the infectious MS2 removal ratios by means of the PFU method after settling (Fig. 3a). Although no removal of infectious MS2 was observed in the absence of coagulant at any pH, as was the case for Qβ (data not shown), the coagulation process with PACl did remove infectious MS2, and the removal efficiency increased with increasing PACl basicity under all pH conditions. In addition, nonsulfated PACls removed infectious MS2 more efficiently than did sulfated PACls, regardless of their basicity: the infectious MS2 removal ratios during the coagulation process with PACl-1.5ns and PACl-2.1ns were approximately 1–4-$\log_{10}$ larger than the ratios with PACl-1.5s and PACl-2.1s, although the removal ratios observed with PACl-2.1s and PACl-2.1ns were almost same at around pH 7. The total MS2 removal ratios evaluated by the PCR method were also observed to be somewhat larger with nonsulfated PACls than the ratios with sulfated PACls, especially at around pH 8 (Fig. 3b). These results indicate that the sulfate in the PACls affected virus removal performance and that a nonsulfated high-basicity PACl (PACl-2.1ns) removed the virus more effectively than PACl-1.5s, PACl-1.5ns, and PACl-2.1s, not only under weakly acidic and neutral pH conditions but also at weakly alkaline pH.

To determine why PACl-2.1ns effectively removed viruses, we used the ferron method to investigate the distribution of aluminum species in the coagulants (Fig. 4). Whereas the major

aluminum species in $AlCl_3$ and alum was monomeric aluminum species (Al_a), colloidal aluminum species (Al_c) were present in high proportions in the PACls (Fig. 4a). In addition, the Al_c content in the PACls increased and the Al_a content decreased with increasing basicity, whereas the content of polymeric aluminum species (Al_b) remained almost constant (Fig. 4a). The Al_{13} species $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}]^{7+}$ is generally believed to be the most effective aluminum species for coagulation processes, because of its strong charge neutralization capability and structural stability (Chen et al., 2006); and the amount of Al_{13} species in a coagulant is almost equivalent to the amount of Al_b measured by the ferron method (Chen et al., 2007). In the present study, the virus removal performances of PACl-1.5s, PACl-1.5ns, PACl-2.1s, and PACl-2.1ns differed markedly, especially at weakly alkaline pH, even though their Al_b contents were not substantially different (Fig. 4b). Therefore, Al_b, including Al_{13} species, may not have been the dominant species responsible for controlling virus removal performance during the coagulation process.

The Al_{30} species $[Al_{30}O_8(OH)_{56}(H_2O)_{24}]^{18+}$ is known to be an effective aluminum species for coagulation processes, and some researchers have demonstrated that PACls with a high Al_{30} content remove more turbidity and more humic acid than PACls with a high Al_{13} content (Chen et al., 2006; Zhang et al., 2008). Because Al_{30} species do not react with the ferron reagent within 120 min, they are categorized as Al_c by the ferron method (Chen et al., 2007). We found that PACl-2.1s and PACl-2.1ns had higher Al_c contents and lower Al_a contents than $AlCl_3$, alum, PACl-1.5s, PACl-1.5ns, and PACl-1.8s (Fig. 4a,b). Therefore, Al_c, including Al_{30} species, may have been the dominant

species controlling virus removal performance during the coagulation process. Our investigation of the effects of the Al_b and Al_c contents in the coagulants on virus removal are discussed in section 3.2.2.

We observed no large differences between the distributions of aluminum species in the sulfated and nonsulfated PACls. These results suggest that PACl basicity affected aluminum species distributions but that the presence of sulfate in the PACls did not.

We also determined the positive colloid charge densities of the coagulants by using a colloid titration technique (Fig. 5). The colloid charge densities of $AlCl_3$ and alum were very small and almost zero; those of the PACls increased with increasing basicity, and PACl-2.1s and PACl-2.1ns showed higher colloid charge densities than $AlCl_3$, alum, PACl-1.5s, PACl-1.5ns, and PACl-1.8s (Fig. 5a,b). In addition, the colloid charge densities of the nonsulfated PACls were higher than those of the sulfated PACls. Wang et al. (2002) reported that the presence of sulfate during the coagulation process reduces the charge neutralization capability of coagulants; this reduction is due to the moderate interaction of sulfate with aluminum hydrolyte species and aluminum hydroxide. Nevertheless, sulfate is often added to aluminum-based coagulants to broaden the pH range of optimum destabilization (i.e., acceleration of floc formation) to the acidic side (Hanna and Rubin, 1970). Therefore, the high Al_c content and the absence of sulfate in PACl-2.1ns probably led to the increased colloid charge density, which gave this coagulant its high capability to neutralize the negative charge on the viruses during the coagulation process.

3.2.2. Effect of aluminum species in coagulants on bacteriophage removal

To investigate the effect of the nature of the aluminum species in the coagulants on virus removal, we compared the MS2 removal efficiencies of nonsulfated high-basicity PACl-2.1b and PACl-2.1c, whose predominant aluminum hydrolyte species are Al_b and Al_c, respectively. PACl-2.1b and PACl-2.1c removed infectious MS2 at a pH range of 6–7 with nearly identical removal efficiencies (~6–7-log_{10} removal), as evaluated by means of the PFU method (Fig. 6a). In contrast, at around pH 8, the infectious MS2 removal ratio observed with PACl-2.1c was approximately 2-log_{10} larger than that with PACl-2.1b. The total MS2 removal ratios evaluated by means of the PCR method were also observed to be somewhat larger with PACl-2.1c than the ratios with PACl-2.1b at around pH 7 and 8 (Fig. 6b). These results indicate that the distribution of aluminum species in the PACls affected virus removal performance during the coagulation process and that Al_c-dominant PACl (i.e., PACl-2.1c) was particularly effective at removing the virus at weakly alkaline pH. Our hypothesis that coagulants with high Al_c content effectively removed viruses during the coagulation process is supported by these results.

To identify the aluminum hydrolyte species in PACl-2.1b and PACl-2.1c, we analyzed the coagulants by ^{27}Al NMR in addition to the ferron method. In the ^{27}Al NMR spectra of all the coagulants, two or three signals were observed (Fig. 7): the signals at 0, 63, and 80 ppm were attributed to monomeric species (Al_m), the central tetrahedral Al in Al_{13} species, and the internal

standard (that is, to the formation of $[Al(OH)_4]^-$), respectively (Chen et al., 2006, 2007; Gao et al., 2005; Liu et al., 2009). Whereas no signal or only a weak signal for Al_{13} species was confirmed in the spectra of $AlCl_3$ and PACl-1.5s, a strong signal for this species was observed in the spectrum of PACl-2.1b (Fig. 7). Because the MS2 removal ratios observed with PACl-2.1b were markedly larger than those with PACl-1.5s (Figs. 3 and 6), we suggest that Al_{13} species in PACl are among the important species controlling virus removal performance during the coagulation process. Although a signal for Al_{13} species was also observed for PACl-2.1c, the intensity of the signal was lower than that for PACl-2.1b. This result suggests that the Al_{13} content in PACl-2.1b was higher than that in PACl-2.1c (Fig. 7). This observation is in accord with the results obtained by the ferron method, which indicate that the predominant aluminum hydrolyte species in PACl-2.1b was Al_b and that the Al_{13} species in coagulant are almost equivalent to that of Al_b, as described above.

In addition to the signals at 0, 63, and 80 ppm, the spectrum of PACl-2.1c showed broad signals at 10–12 and 70 ppm, which were attributed to the octahedral Al of external shells in Al_{13} and Al_{30} species and the central tetrahedral Al in Al_{30} species, respectively (Chen et al., 2007). This result indicates that PACl-2.1c contained not only Al_{13} species but also Al_{30} species, which was not the case for PACl-2.1b. In addition, Al_{30} species played the major role in virus removal, as indicated by the fact that the efficiency of MS2 removal with PACl-2.1c was somewhat larger than that with PACl-2.1b (Fig. 6), even though the Al_{13} content in PACl-2.1c was smaller than that in PACl-2.1b (Fig. 7). Although the Al_m (Al_a) species contributed to virus removal—as indicated by the fact that

high-Al_m-content coagulants ($AlCl_3$ and PACl-1.5s) removed some virus at a pH range of 6–7, probably because of the formation of Al_{13} (Al_b) species in situ (Hu et al., 2006; Yan et al., 2008a)—conversion of Al_m species in the coagulant to Al_{13} species and further transformation of Al_{13} species into Al_{30} species effectively improved virus removal performance during the coagulation process.

We further investigated the effect of the aluminum species in the coagulants on virus removal by evaluating two extremely high basicity PACls (i.e., PACl-2.7ns and PACl-2.7). The efficiencies of infectious MS2 removal at a pH range of 6–7, as evaluated by the PFU method after settling, were 6–8-log_{10} (Fig. 6a). These removal ratios were similar to the ratio obtained with PACl-2.1c. In contrast, the ratios observed with PACl-2.7ns and PACl-2.7 were approximately 1–3-log_{10} smaller than the ratio with PACl-2.1c at around pH 8. The total MS2 removal efficiencies, as evaluated by the PCR method, during the coagulation process with extremely high basicity PACls were also similar to or somewhat smaller than the ratio observed with PACl-2.1c (Fig. 6b). The effective removal of turbidity and UV260-absorbing NOM, and the very low residual aluminum concentration, observed in the coagulation process with PACl-2.7ns and PACl-2.7 were attained not only under weakly acidic and neutral pH conditions but also at weakly alkaline pH compared with other aluminum-based coagulants used in the present study, including PACl-2.1c (Fig. S2). However, increasing the PACl basicity from 2.1 to 2.7 was not effective for virus removal, even though the Al_c content in PACl-2.7ns was larger than that in PACl-2.1c (Fig. 4b). Moreover, we

observed a reduction of the colloid charge densities of the PACls when the basicity was increased from 2.1 to 2.7 (Fig. 5b,c), and no signal for Al_{30} species was observed in the ^{27}Al NMR spectrum of PACl-2.7ns (Fig. 7). The reason why the colloid charge densities of the coagulants were reduced by the increase in basicity is not clear, but these results suggest that virus removal efficiency during the coagulation process with PACls was not determined simply by the amount of Al_c in the coagulants.

3.2.3. Relationship between bacteriophage removal, aluminum species and colloid charge density

In our previous study, we found that the amount of Al_a in PACls, rather than their basicity, was a better indicator to use for minimizing residual aluminum concentration after settling at weakly alkaline pH (Kimura et al., 2013). To investigate whether the Al_a, Al_b, or Al_c content or the colloid charge density of the coagulants could be used as an indicator for the effectiveness of virus removal during the coagulation process, we determined the relationships between the MS2 removal ratio at around pH 8 and the Al_a, Al_b, and Al_c contents and the colloid charge density (Fig. 8). There was no correlation between the efficiency of infectious MS2 removal and the Al_b and Al_c contents; whereas the Al_a content, that is, $[100\% – (Al_b + Al_c)]$, and the colloid charge density were weakly correlated with the infectious MS2 removal ratios (Fig. 8). In the third set of experiments (described below), the amount of Al_a, the colloid charge density, and both together were also correlated with the efficiency of infectious or total MS2 removal at all coagulant dosages, except for the total MS2

removal ratio at a coagulant dosage of 0.54 mg-Al/L (Table S4). In addition, the removal ratios tended to increase as the amount of Al_a in the PACls decreased (that is, as Al_b + Al_c increased) and as the colloid charge density of the PACls increased. However, the virus removal efficiencies during the coagulation process with aluminum-based coagulants were not solely dependent on either Al_b + Al_c or the colloid charge density. Further investigation is needed to elucidate a completely reliable indicator for the effectiveness of virus removal during the coagulation process.

3.3. Third set of experiments

3.3.1. Effect of coagulant dosage on bacteriophage removal

Fig. 9a shows the effect of coagulant dosage on the efficiency of infectious MS2 removal from treated water at around pH 8, as evaluated by means of the PFU method after settling. The infectious MS2 removal ratio increased as the coagulant dosage was increased from 0.54 to 2.16 mg-Al/L; although the removal ratios observed with $AlCl_3$ and PACl-1.5ns were unaffected by the increase in coagulant dosage, approximately 1–2-log_{10} improvements were obtained for the other aluminum-based coagulants. For most of the coagulants, the infectious MS2 removal ratio reached a maximum at a dosage of 2.16 mg-Al/L, and retained its virus removal performance when the coagulant dosage was further increased to 5.4 mg-Al/L, except in the case of PACl-1.5s. A similar trend was observed for removal of turbidity and UV260-absorbing NOM (Fig. S3). These results indicate that re-stabilization likely did not occur at this dosage range for any of the coagulants

except PAC-1.5s.

The infectious MS2 removal ratios during the coagulation process with high-basicity PACls (i.e., PACl-2.1b, PACl-2.1c, and PACl-2.7) were larger than the ratios with the other aluminum-based coagulants used in the present study at all coagulant dosages. A similar trend was observed for the total MS2 removal ratios, as evaluated by the PCR method (Fig. 9b). Therefore, increasing coagulant basicity tended to lower the coagulant dosage required for effective removal of viruses. In addition, the coagulation process with PACl-2.1c removed MS2 more efficiently than the other aluminum-based coagulants at all coagulant dosages; PACl-2.1c was therefore useful for virus removal over a broader pH range and wider coagulant dosage range compared to commercially available aluminum-based coagulants.

3.3.2. Overall comparison of coagulation efficiency of the tested coagulants

As described above, PACl-2.1c, which contains Al_{30} species, removed viruses more efficiently than the other aluminum-based coagulants, especially at weakly alkaline pH. In contrast, at pH 8, the UV260-absorbing NOM removal and the residual aluminum concentration attained with PACl-2.7 were better than those attained with PACl-2.1c (Fig. S3). Because a low residual aluminum concentration is associated with a low content of monomeric aluminum species in the coagulant, our previously reported coagulation process with PACl-2.7 (Kimura et al., 2013), which has a low Al_a content (Fig. 4c), attained a very low residual aluminum concentration. Taken together, our

results suggest that the development of novel aluminum-based coagulants for different purposes such as efficient virus removal and low residual aluminum concentration can be achieved. The experimental results obtained in the present study will be useful for the development and investigation of highly effective aluminum-based coagulants.

4. Conclusions

(1) An increase in PACl basicity (from 1.5 to 2.1) and the absence of sulfate in the PACls improved virus removal efficiency.

(2) The efficiency of virus removal at around pH 8 observed with PACl-2.1c, a nonsulfated high-basicity PACl with a high Al_c content, was larger than that with PACl-2.1b, a nonsulfated high-basicity PACl with a high Al_b content.

(3) Although extremely high basicity PACls (PACl-2.7ns and PACl-2.7) effectively removed turbidity and UV260-absorbing NOM and resulted in a very low residual aluminum concentration, the virus removal ratios of these two PACls were smaller than the ratio with PACl-2.1c at around pH 8, possibly as a result of a reduction in the colloid charge density of the PACl due to the increase in basicity from 2.1 to 2.7.

(4) Al_{30} species probably played the major role in virus removal during the coagulation process.

(5) Among the various aluminum-based coagulants used in the present study, PACl-2.1c, which has a high Al_c content (including Al_{30} species) and a high colloid charge density, showed the highest virus removal ratio (>4 log_{10} for infectious viruses) in the pH range from 6 to 8 and a coagulant dosage range from 0.54 mg-Al/L to 5.4 mg-Al/L.

(6) The virus removal ratios tended to increase as the amount of Al_a in the coagulant decreased (that is, as Al_b + Al_c increased) and as the colloid charge density of the coagulant increased.

Acknowledgements

This research was supported in part by a Grant-in-Aid for Young Scientists A (no. 25709044, 2013), a Grant-in-Aid for Scientific Research S (no. 24226012, 2012), and a Grant-in-Aid for Scientific Research B (no. 24360211, 2012) from the Japan Society for the Promotion of Science; by a Grant-in-Aid (2013) from the Ministry of Health, Labor and Welfare of Japan; by the Kurita Water and Environment Foundation (no. 23050, 2011); and by the Japan Prize Foundation (2012).

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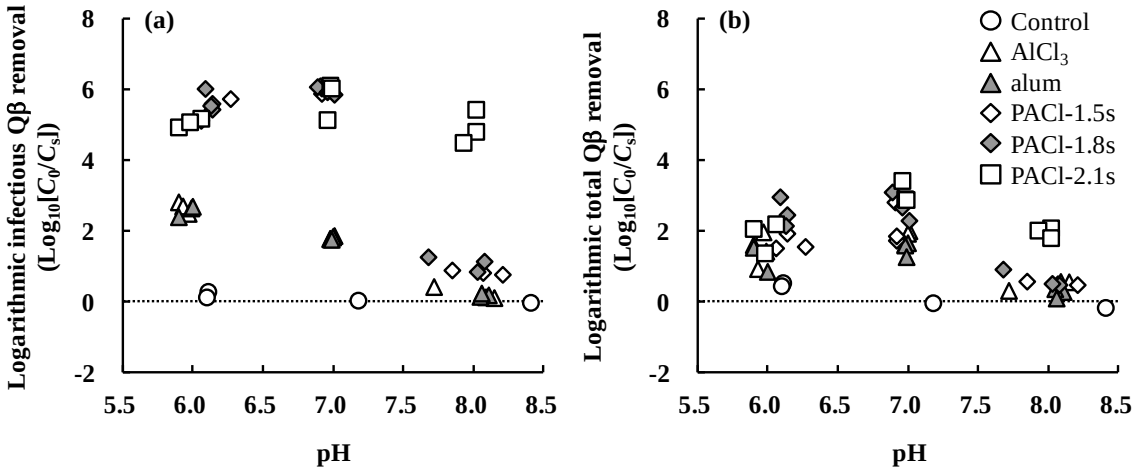


Fig. 1. Effect of coagulant type on removal of infectious Qβ as evaluated by the PFU method (a) and on total Qβ removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 1, and the coagulant dosage was 2.16 mg-Al/L.

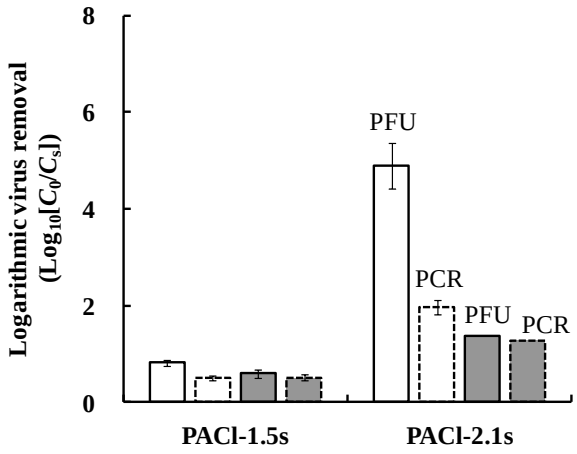


Fig. 2. Comparison of Qβ (white) and MS2 (gray) removal ratios from treated water at around pH 8 after settling during the coagulation process. The source water was river water 1, and the coagulant dosage was 2.16 mg-Al/L. Values are means (n = 2–3), and the error bars indicate standard deviations.

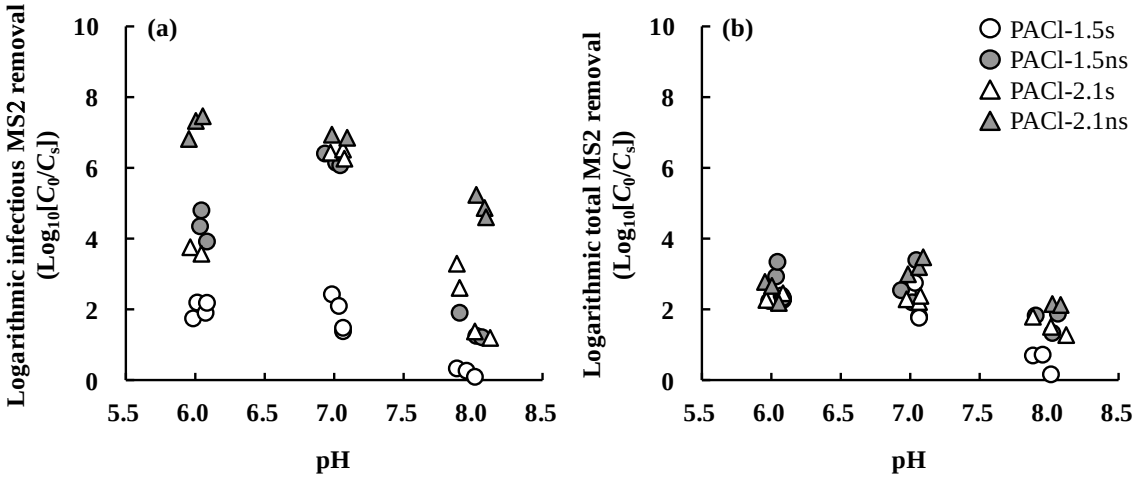


Fig. 3. Effects of coagulant basicity and sulfate content on infectious MS2 removal as evaluated by the PFU method (a) and on total MS2 removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 2, and the coagulant dosage was 1.89 mg-Al/L.

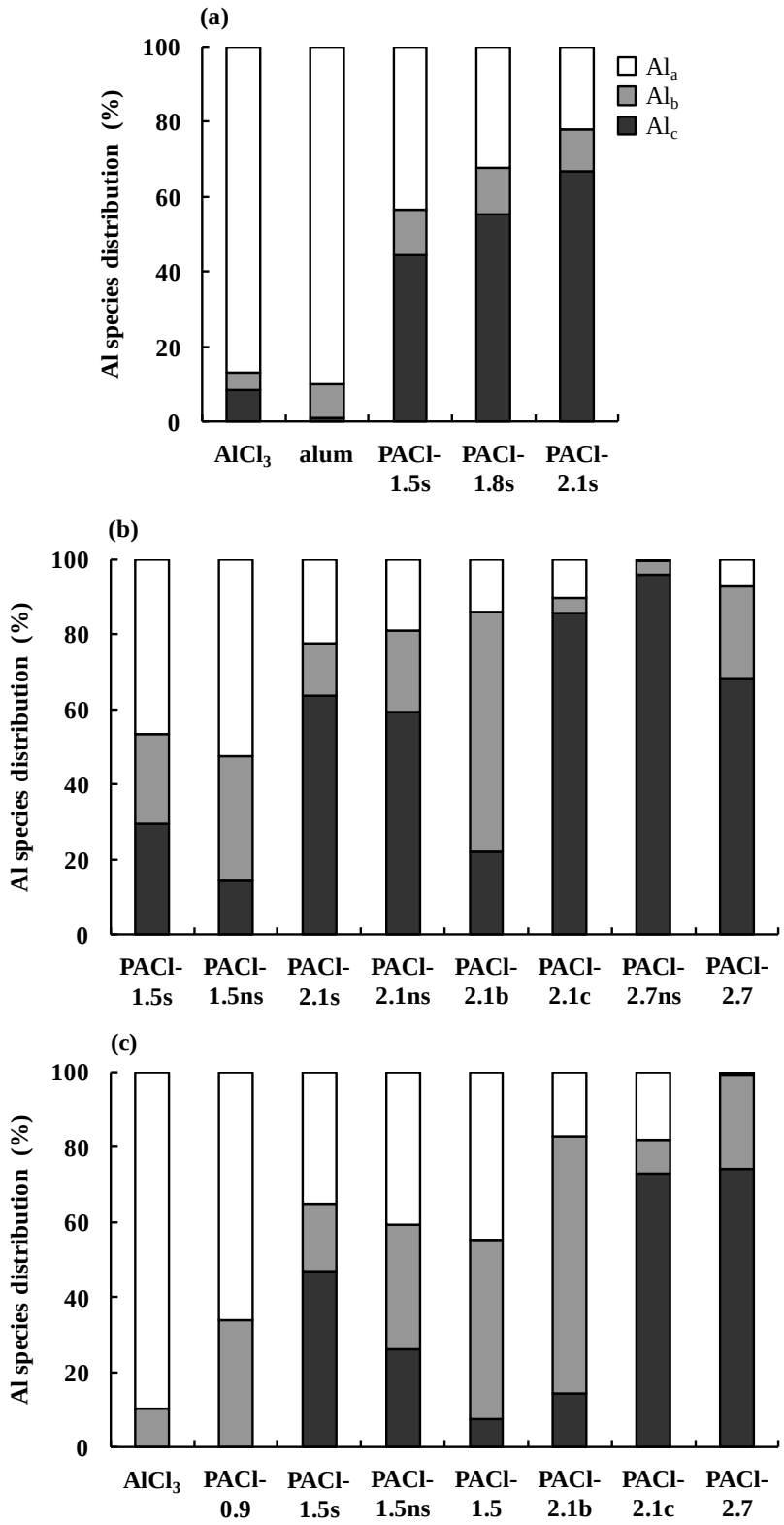


Fig. 4. Distribution of aluminum species in the coagulants used in the first (a), second (b), and third (c) sets of experiments, as evaluated by the ferron method.

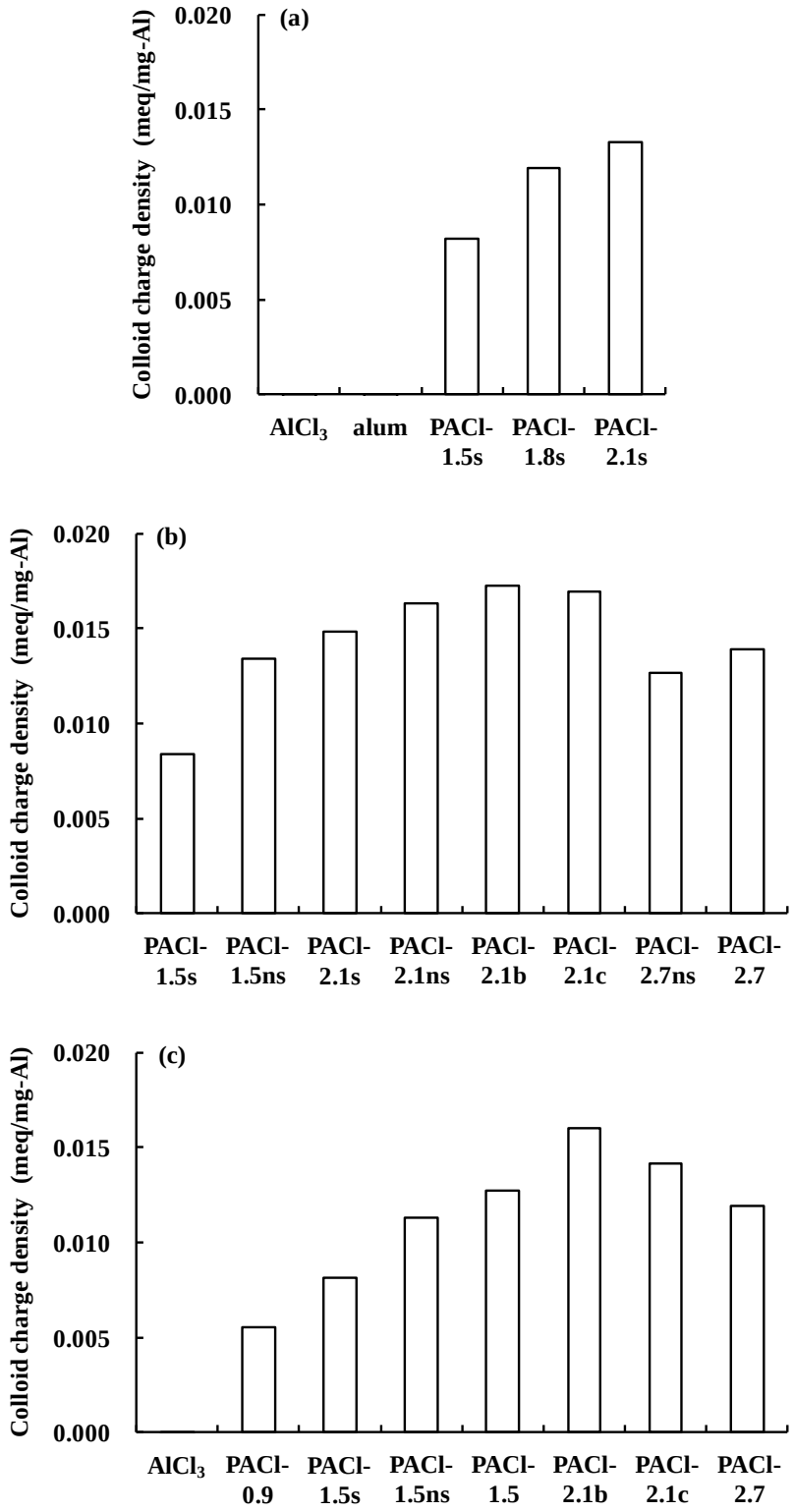


Fig. 5. Colloid charges densities of the coagulants used in the first (a), second (b), and third (c) sets of experiments, as evaluated by a colloid titration technique.

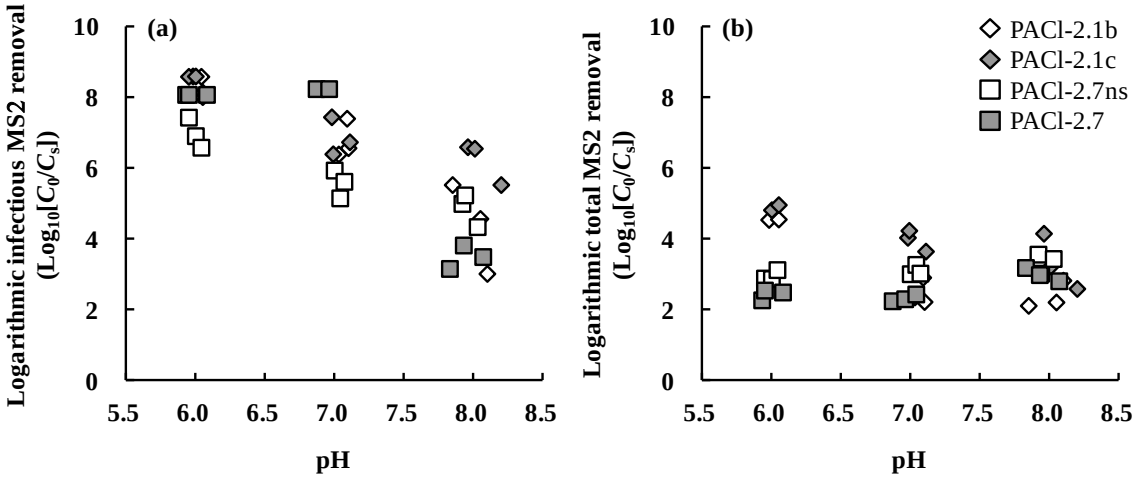


Fig. 6. Effect of the aluminum hydrolyte species in the coagulants on infectious MS2 removal as evaluated by the PFU method (a) and on total MS2 removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 2, and the coagulant dosage was 1.89 mg-Al/L.

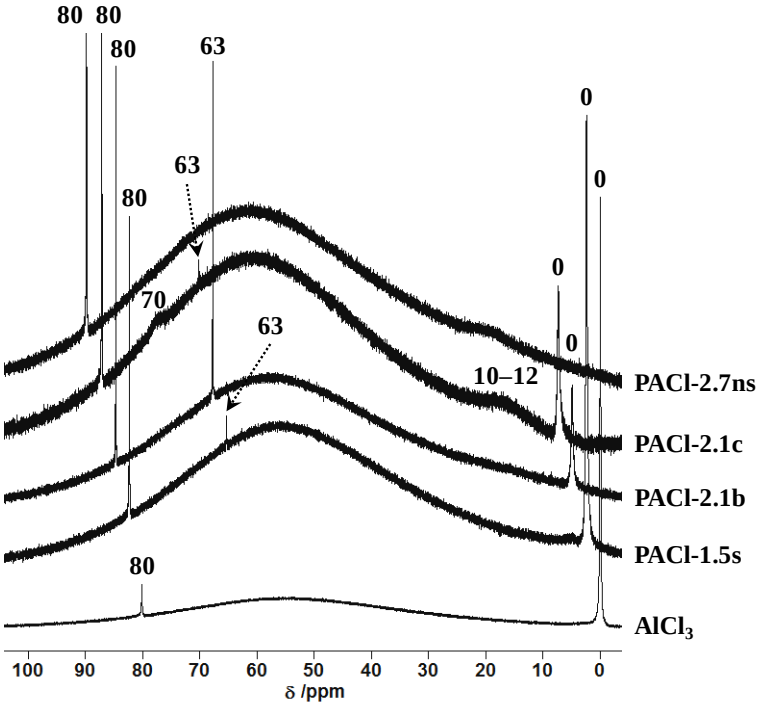


Figure 7. ^{27}Al-NMR spectra of coagulants used in the second set of experiments.

Fig. 7. ^{27}Al NMR spectra of coagulants used in the second set of experiments.

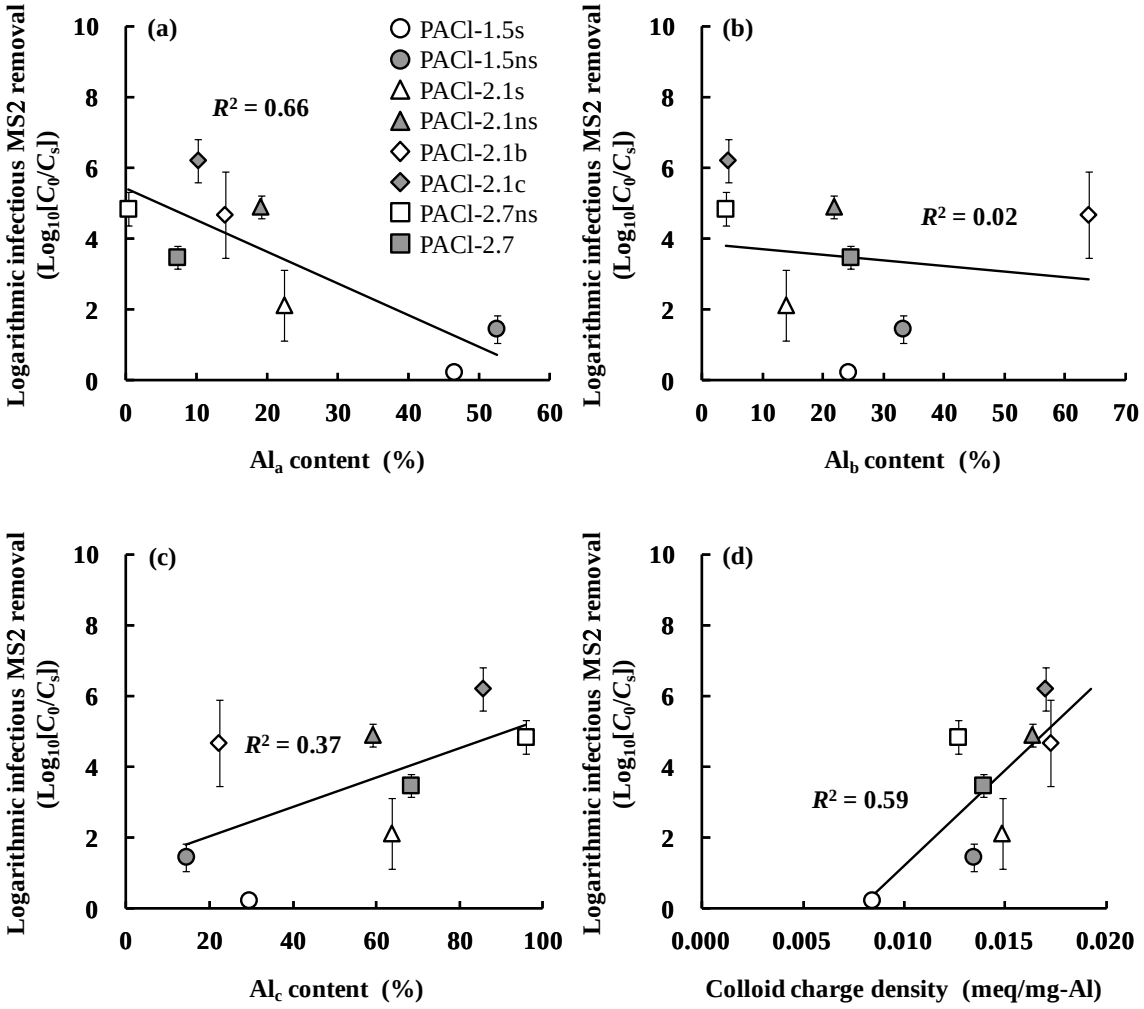


Fig. 8. Relationship between infectious MS2 removal ratios and Al_a (a), Al_b (b), Al_c (c), and colloid charge density (d). The source water was river water 2, and the coagulant dosage was 1.89 mg-Al/L. The pH of the treated water was approximately 8. Values are means (n = 3–4), and the error bars indicate standard deviations.

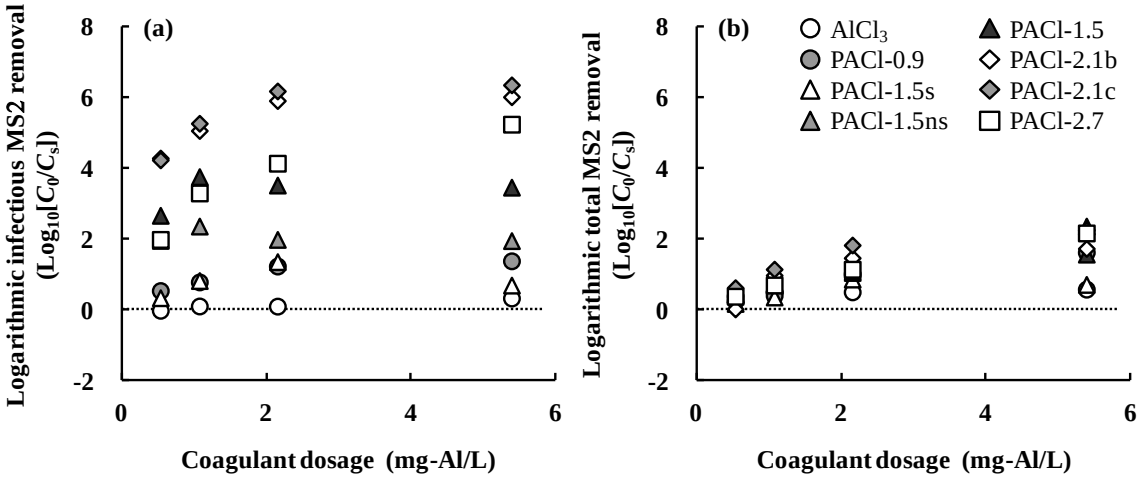


Fig. 9. Effect of coagulant dosage on infectious MS2 removal as evaluated by the PFU method (a) and on total Qβ removal as evaluated by the PCR method (b) after settling during the coagulation process. The source water was river water 3, and the pH of the treated water was approximately 8.

Supplementary Information

Improved virus removal by high-basicity polyaluminum coagulants compared to commercially available aluminum-based coagulants

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Table S1. Water quality data for Toyohira River samples.

	River water 1	River water 2	River water 3
Sampling date	01-Oct-10	24-Jun-11	04-Dec-12
pH	7.5	7.4	7.3
Turbidity (NTU)	2.6	7.2	2.5
DOC (mg/L)	1.3	0.8	1.0
UV260 (cm^{-1})	0.037	0.029	0.026
Alkalinity (mg-$CaCO_3$/L)	21.0	17.1	16.0

652 **Table S2. Characteristics of aluminum-based coagulants used in the present study.**

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Experiments	Coagulants	Basicity	Aluminum concentration	Sulfate concentration	Relative density at 20ºC
First set	$AlCl_3$	0.0	2.7 g-Al/L	0.0 g/L	1.0
	Alum	0.0	8% (w/w) as Al_2O_3	23% (w/w)	1.3
	PACl-1.5s	1.5	10% (w/w) as Al_2O_3	3% (w/w)	1.2
	PACl-1.8s	1.8	10% (w/w) as Al_2O_3	3% (w/w)	1.2
	PACl-2.1s	2.1	10% (w/w) as Al_2O_3	2% (w/w)	1.2
Second set	PACl-1.5s	1.5	10% (w/w) as Al_2O_3	3% (w/w)	1.2
	PACl-1.5ns	1.5	10% (w/w) as Al_2O_3	0% (w/w)	1.2
	PACl-2.1s	2.1	11% (w/w) as Al_2O_3	2% (w/w)	1.2
	PACl-2.1ns	2.1	10% (w/w) as Al_2O_3	0% (w/w)	1.2
	PACl-2.1b	2.2	2.9 g-Al/L	0.0 g/L	1.0
	PACl-2.1c	2.2	8.8 g-Al/L	0.0 g/L	1.0
	PACl-2.7ns	2.7	1% (w/w) as Al_2O_3	0% (w/w)	1.0
	PACl-2.7	2.7	2.5 g-Al/L	0.0 g/L	1.0
Third set	$AlCl_3$	0.0	2.7 g-Al/L	0.0 g/L	1.0
	PACl-0.9	0.9	5.4 g-Al/L	0.0 g/L	1.0
	PACl-1.5s	1.5	10% (w/w) as Al_2O_3	3% (w/w)	1.2
	PACl-1.5ns	1.6	11% (w/w) as Al_2O_3	0% (w/w)	1.2
	PACl-1.5	1.5	3.9 g-Al/L	0.0 g/L	1.0
	PACl-2.1b	2.1	3.0 g-Al/L	0.0 g/L	1.0
	PACl-2.1c	2.1	20.3 g-Al/L	0.0 g/L	1.1
	PACl-2.7	2.7	2.5 g-Al/L	0.0 g/L	1.0

654

Table S3. Oligonucleotide sequences of the primers and probes used in the present study.

Viruses		Oligonucleotide sequences	Positions	References
Qβ	Forward primer	5'-TCA AGC CGT GAT AGT CGT TCC TC-3'	49-71	Katayama *et al.* , 2002
	Reverse primer	5'-AAT CGT TGG CAA TGG AAA GTG C-3'	187-208	
	TaqMan probe	5'-CGA GCC GCG AAC ACA AGA ATT GA-3'	147-169	
MS2	Forward primer	5'-GTC GCG GTA ATT GGC GC-3'	632-648	O'Connell *et al.* , 2006
	Reverse primer	5'-GGC CAC GTG TTT TGA TCG A-3'	690-708	
	TaqMan probe	5'-AGG CGC TCC GCT ACC TTG CCC T-3'	650-671	

660 **Table S4. Coefficients of determination between MS2 removal ratios at around pH 8 and Al_a, Al_b, Al_c, and colloid charge density.**

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Experiments	Coagulant dosage	Logarithmic removal ratios	Al_a	Al_b	Al_c	Colloid charge density
Second set	1.89 mg-Al/L	PFU	0.66	0.02	0.37	0.59
		PCR	0.71	0.08	0.50	0.31
Third set	0.54 mg-Al/L	PFU	0.46	0.21	0.11	0.78
		PCR	0.00	0.25	0.14	0.01
	1.08 mg-Al/L	PFU	0.56	0.18	0.18	0.83
		PCR	0.34	0.05	0.15	0.56
	2.16 mg-Al/L	PFU	0.64	0.13	0.26	0.78
		PCR	0.52	0.05	0.27	0.70
	5.4 mg-Al/L	PFU	0.64	0.11	0.28	0.69
		PCR	0.39	0.05	0.19	0.50

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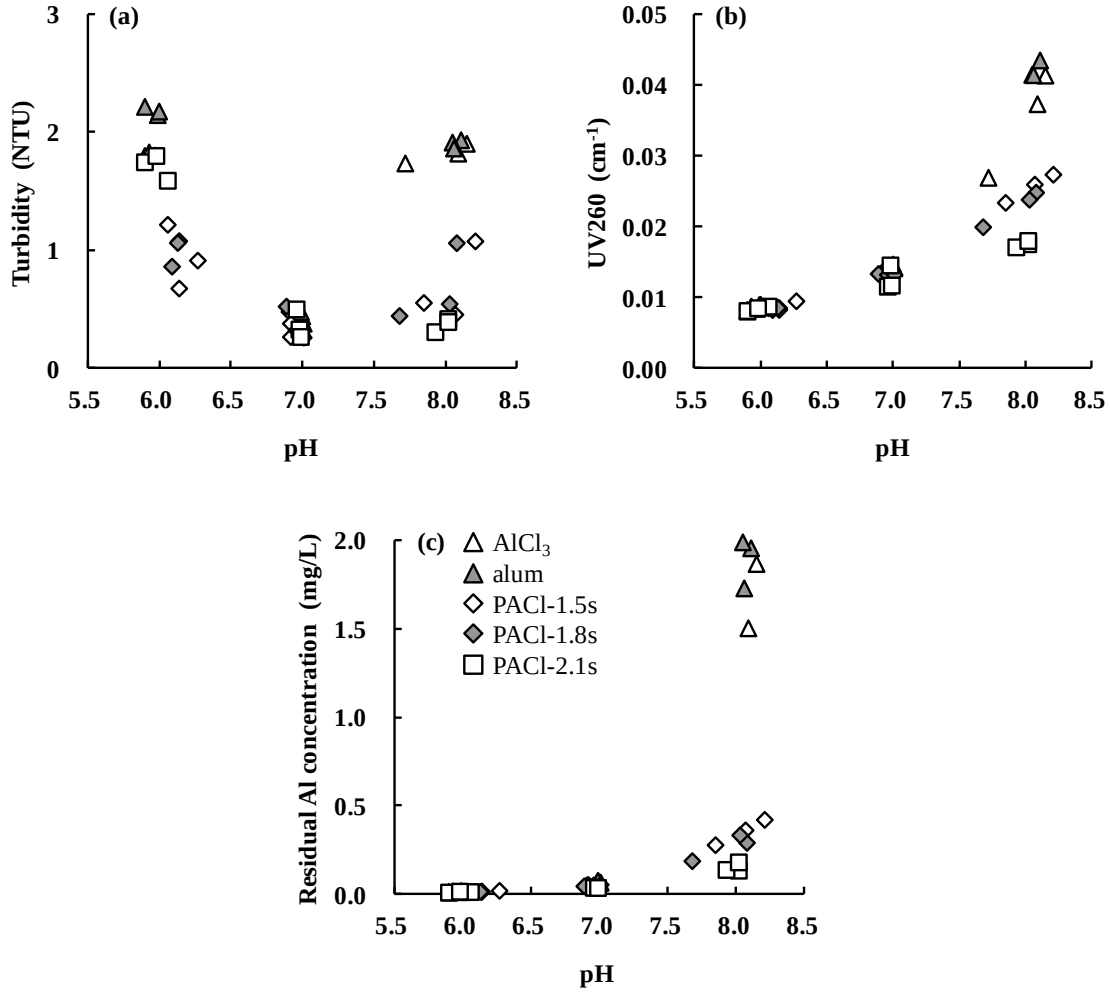
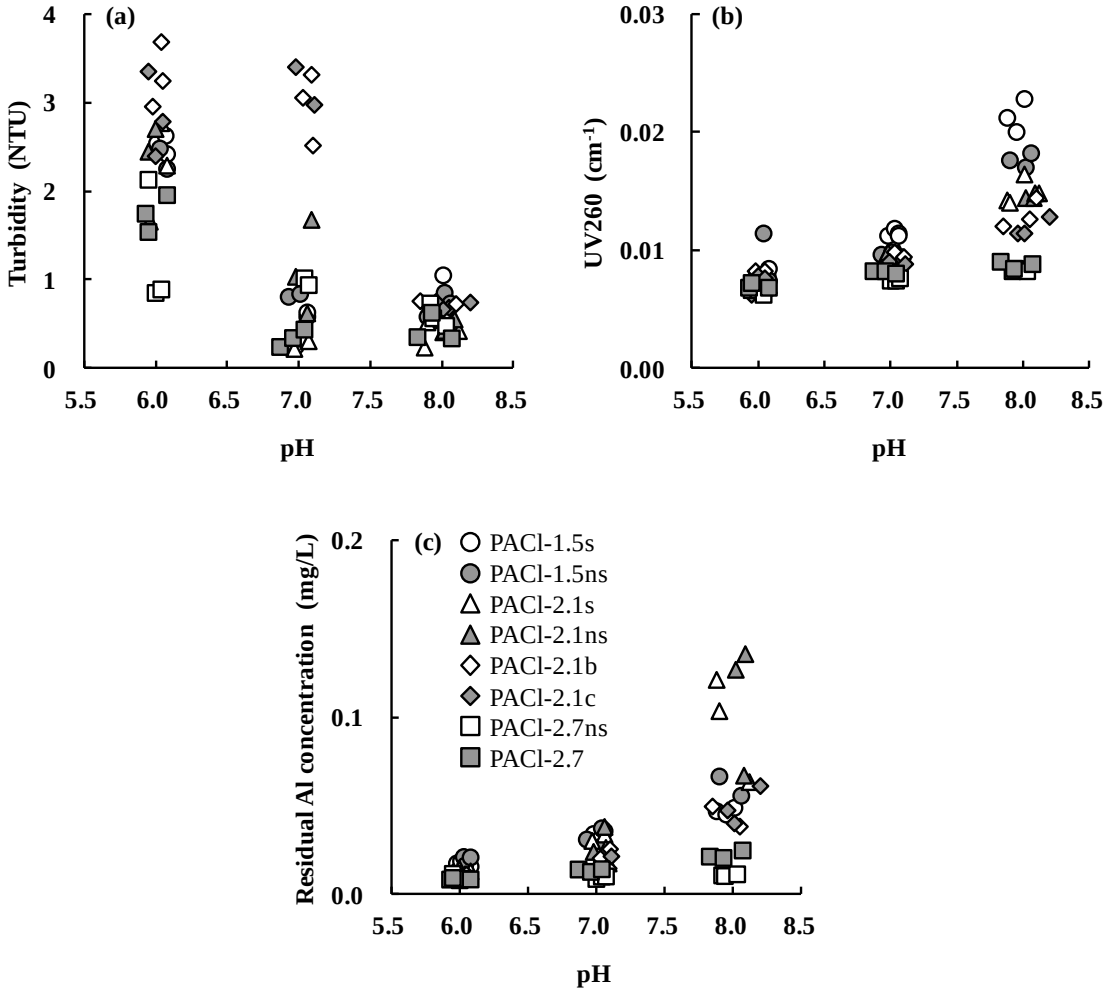


Fig. S1. Turbidity (a), UV260-absorbing NOM (b), and residual Al concentration (c) after settling during the coagulation process. The source water was river water 1, and the coagulant dosage was 2.16 mg-Al/L.



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676 **Fig. S2. Turbidity (a), UV260-absorbing NOM (b), and residual Al concentration (c) after**
677 **settling during the coagulation process.** The source water was river water 2, and the coagulant
678 dosage was 1.89 mg-Al/L.

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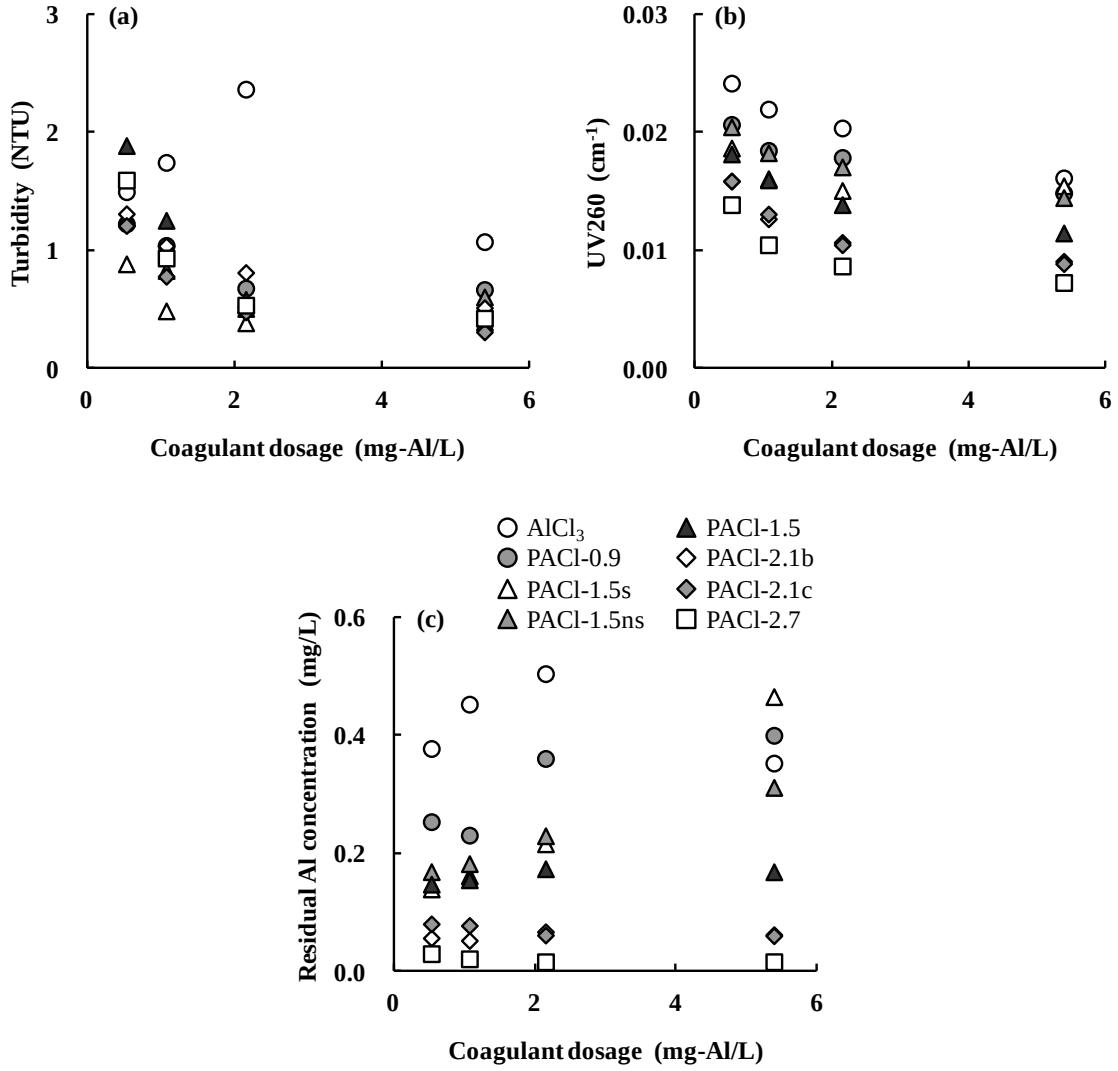


Fig. S3. Turbidity (a), UV260-absorbing NOM (b), and residual Al concentration (c) after settling during the coagulation process. The source water was river water 3, and the pH of the treated water was approximately 8.

689 Real time-RT-PCR method

690 For quantification of bacteriophages in the samples, viral RNA was extracted from 200 μL of
691 sample with a QIAamp MinElute Virus Spin Kit (Qiagen K. K., Tokyo, Japan) to obtain a final
692 volume of 20 μL. The extracted RNA solution was added to a High Capacity cDNA Reverse
693 Transcription Kit with RNase Inhibitor (Applied Biosystems Japan, Tokyo, Japan) for the RT
694 reaction, which was conducted at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 s and
695 subsequent cooling to 4 °C in the thermal cycler (Thermal Cycler Dice Model TP600, Takara Bio
696 Inc., Otsu, Japan). The cDNA solution was then amplified with a TaqMan Universal PCR Master
697 Mix, No AmpErase UNG (Applied Biosystems Japan) with 400 nM primers (HQ-SEQ grade,
698 Takara Bio Inc.) and 250 nM TaqMan probe (Applied Biosystems Japan). The oligonucleotide
699 sequences of the primers and the probes are shown in Table S3. Amplification was conducted at
700 50 °C for 2 min, 95 °C for 10 min, and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min in an
701 Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems Japan). The standard curve
702 for the real-time RT-PCR method was based on the relationship between the concentrations of
703 cDNA standards, which were known from their optical densities at 260 nm, and the number of
704 cycles (C_t value) of PCR amplification.

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706 **References**

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