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Elimination of representative contaminant candidate list viruses, coxsackievirus, echovirus, hepatitis A virus, and norovirus, from water by coagulation processes

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Highlights

- Removal of CV, EV, HAV and NV by coagulation was evaluated.
- Hepatitis A virus was inactivated by contact with a PACl.
- A nonsulfated high-basicity PACl effectively removed CCL viruses.
- Bacteriophage MS2 is not an appropriate surrogate for CCL viruses.
- Bacteriophage φX174 may be a conservative surrogate for CCL viruses.

Abstract

We examined the removal of representative contaminant candidate list (CCL) viruses (coxsackievirus B5 [CV], echovirus type 11 [EV], and hepatitis A virus IB [HAV]), recombinant norovirus virus-like particles (rNV-VLPs), and murine norovirus type 1 (MNV) by coagulation. Water samples were subjected to coagulation with polyaluminum chloride (PACl, basicity 1.5) followed by either settling or settling and filtration. Together with our previously published results, the removal ratio order, as evaluated by a plaque-forming-unit method or an enzyme-linked immunosorbent assay after settling, was HAV > EV = rNV-VLPs ≥ CV = poliovirus type 1 = MNV > adenovirus type 40 (range, 0.1–2.7-$\log_{10}$). Infectious HAV was likely inactivated by the PACl and therefore was removed to a greater extent than the other viruses. A nonsulfated high-basicity PACl (basicity 2.1), removed the CCL viruses more efficiently than did two other sulfated PACls (basicity 1.5 or 2.1), alum, or ferric chloride. We also examined the removal ratio of two bacteriophages. The removal ratios for MS2 tended to be larger than those of the CCL viruses, whereas those for φX174 were comparable with or smaller than those of the CCL viruses. Therefore, φX174 may be a useful conservative surrogate for CCL viruses during coagulation.

Keywords: Bacteriophages, Coagulation, Contaminant candidate list virus, Enteric virus, Virus inactivation

1. Introduction

The reduction of human enteric viruses from contaminated water by means of physical and physicochemical removal processes, and chemical and photochemical disinfection processes is essential for ensuring the virological safety of drinking water. Human enteric viruses such as adenoviruses (AdVs), astroviruses, caliciviruses, enteroviruses, hepatitis A and E viruses, and rotaviruses have a devastating effect on public health all over the world and are classified by the World Health Organization as having a moderate to high health significance [1]. The U.S. Environmental Protection Agency recently published a draft fourth contaminant candidate list (CCL4) for drinking water, which includes 100 unregulated chemicals or chemical groups and 12 microbial contaminants that are known or anticipated to be present in drinking water systems and may require future regulation [2]. Four virus types are included in the draft CCL4, namely, AdVs; caliciviruses, which include noroviruses (NVs) and sapoviruses; enteroviruses, which include polioviruses (PVs), coxsackieviruses (CVs), and echoviruses (EVs); and hepatitis A viruses (HAVs).

Although virus inactivation by means of chemical or photochemical disinfection processes such as chlorination, chloramination, ozonation, and ultraviolet irradiation are effective for inactivating human enteric viruses in drinking water, some viruses (serotypes) are highly resistant to these processes. For example, CV B5 (compared with CV B3 and CV B4, AdVs, PVs, and EVs) is highly resistant to free-chlorine disinfection, EV type 11 (compared with EV type 1, AdVs, and CVs) is highly resistant to monochloramine disinfection, and AdV type 40 (compared with AdV types 1, 2, and 6, and PV type 1) is highly resistant to ultraviolet disinfection [3-5]. Therefore, multiple-barrier approaches that both remove and inactivate viruses are important for preventing the spread of diseases caused by exposure to human enteric viruses in drinking water [6]. However, data on the physical and physicochemical removal of human enteric viruses, particularly regarding the CCL viruses, are limited [7-9]. One reason for this lack of data is that bench- or pilot-scale studies of the removal of human enteric viruses in water are labor intensive and time consuming in terms of cultivating the viruses and conducting cell culture–based infectivity assays [10].

Furthermore, it is difficult to evaluate the physical and physicochemical removal of human caliciviruses through virus-spiking studies (i.e., studies using raw water spiked with artificially cultivated viruses) because human caliciviruses (i.e., human NVs and sapoviruses) cannot be cultivated using a cell culture system or propagated in small laboratory animals [11, 12]. Shin and Sobsey [9] recently examined the use of coagulation to remove human NV from water spiked with NV extracted from stool samples of NV-infected volunteers; however, the range of experimental conditions and number of experiments conducted were limited. As an alternative to human NV, our research group previously used recombinant NV virus-like particles (rNV-VLPs), which are artificially expressed NV capsid proteins that are morphologically and antigenically identical to those of native human NV [13, 14], to investigate the removal of human NV from water by physical and physicochemical processes (i.e., coagulation followed by rapid sand filtration, membrane filtration alone, and coagulation followed by membrane filtration) [15, 16]. In addition, murine NV (MNV; genus *Norovirus*, family Caliciviridae) can be cultivated by using cell culture systems [17],

and MNV has previously been used as a surrogate for human caliciviruses in investigations of the disinfection performance of chlorination [3, 18, 19], chloramination [3], ozonation [20], and ultraviolet irradiation [21, 22]. However, despite these studies, the physical and physicochemical processes and process conditions that are the most effective for removing human caliciviruses such as NV from drinking water remain unknown.

In the present study, we conducted batch coagulation experiments using water samples from 6 drinking water sources across Japan to investigate the performance of coagulation with polyaluminum chloride (PACl), alum, or ferric chloride ($FeCl_3$) followed by settling or settling and filtration for the removal of three representative CCL viruses (i.e., CV, EV, and HAV), and of rNV-VLPs and MNV as surrogates of human caliciviruses. The results of our previous study examining the removal of two other CCL viruses (i.e., human AdV type 40 and PV type 1) under identical treatment conditions are also presented here for comparison [23]. CV B5 and EV type 11 were specifically chosen for use in the present study because they are highly resistant to free-chlorine disinfection and monochloramine disinfection, respectively, as described above. The bacteriophages MS2 and φX174 are widely used as surrogates for human enteric viruses in drinking water treatment processes; therefore, we also investigated the suitability of the two bacteriophages as surrogates for the representative CCL viruses. Infectious and total (i.e., infectious + inactivated) virus concentrations were quantified by using a plaque-forming unit (PFU) method and a real-time reverse transcription polymerase chain reaction (RT-PCR) method, respectively, which also allowed examination of the mechanism of virus reduction (i.e., physical removal, inactivation, or both).

2. Materials and methods

2.1. Source water and coagulants

Water samples were collected from six raw water sources supplying drinking water treatment plants in Japan (source water quality data are shown in Table 1). All of the treatment plants use coagulation with aluminum-based coagulants (PACl or alum) followed by rapid sand filtration for the production of drinking water. The source water samples were stored at 4 °C until use and brought to 20 °C immediately prior to use. Five coagulants were used in the coagulation experiments and the characteristics of the coagulants are given in Supplementary Information.

2.2. Human enteric viruses and MNV

CV B5 Faulkner strain (ATCC VR-185) and EV type 11 Gregory strain (ATCC VR-41) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), propagated in buffalo green monkey kidney epithelial cells (kindly supplied by Dr. Daisuke Sano, Hokkaido University, Sapporo, Japan), and maintained in 1× Eagle's minimum essential medium (with phenol red; Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Life Technologies, Carlsbad, CA, USA), 2 mM L-glutamine (Life Technologies), 100 U/mL penicillin, 100 μg/mL streptomycin (Pen Strep; Life Technologies), and 1.125 g/L sodium hydrogen carbonate. HAV IB strain HM175/18f (ATCC VR-1402), which is a cytopathic strain, was

obtained from ATCC, propagated in fetal rhesus monkey kidney epithelial cells (FRhK-4 cells; ATCC CRL-1688) obtained from ATCC, and maintained in 1× Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. MNV type 1 strain CW1 (ATCC PTA-5935) was obtained from ATCC, propagated in murine macrophage cells (RAW264.7 cells; ATCC TIB-71) obtained from ATCC, and maintained in 1× DMEM. Further details of the propagation and purification of CV, EV, HAV, and MNV are given in Supplementary Information.

2.3. rNV-VLPs

rNV-VLP strain Chiba (GI.4) was produced via a baculovirus–silkworm expression system (ProCube; Sysmex Corp., Kobe, Japan), as described in our previous report [15].

2.4. Bacteriophages

MS2 (NBRC 102619), an F-specific RNA bacteriophage, φX174 (NBRC 103405), a somatic DNA bacteriophage, and the *Escherichia coli* bacterial hosts in which the bacteriophages were propagated (NBRC 13965 for MS2, NBRC 13898 for φX174) were obtained from the National Institute of Technology and Evaluation Biological Research Center (Kisarazu, Japan). Details of the propagation and purification of the bacteriophages were given in our previous report [23].

2.5. Batch coagulation experiments

Batch coagulation experiments were conducted with 300 mL of virus-spiked source water in square plastic beakers at 20 °C. The experimental procedures are given in Supplementary Information.

2.6. CV, EV, HAV, and MNV assay

Infectious CV, EV, HAV, or MNV concentrations were determined by means of plaque assays. Infectious HAV was quantified by using the plaque assay developed by Richards and Watson [24] with some modifications. Details of the plaque assays are given in Supplementary Information. CV, EV, HAV, or MNV viral RNA concentrations, as an index of total (infectious + inactivated) virus concentration, were quantified by means of real-time RT-PCR. Details of the real-time RT-PCR method are given in Supplementary Information and Table S1.

2.7. rNV-VLP assay

rNV-VLPs were quantified by using a commercially available enzyme-linked immunosorbent assay kit (NV-AD (III); Denka Seiken Co., Tokyo, Japan). The assay was conducted in accordance with the manufacturer's instructions. Optical densities at wavelengths of 450 nm and 630 nm in a 96-well microplate were measured with a microplate reader (Infinite 200 PRO; Tecan Japan Co., Kawasaki, Japan).

2.8. Bacteriophage assay

Infectious bacteriophage concentrations were determined by using the double-layer plaque assay [25] with *E. coli* (NBRC 13965 for MS2, NBRC 13898 for φX174) as the bacterial host. The average plaque count of triplicate plates prepared from one sample was considered the infectious bacteriophage concentration for that sample. MS2 viral RNA concentrations, as an index of total (infectious + inactivated) virus concentration, were quantified by means of real-time RT-PCR. φX174 viral DNA concentrations, as an index of total (infectious + inactivated) virus concentration, were quantified by means of real-time PCR. Details of the real-time RT-PCR and real-time PCR methods were given in our previous report [23].

2.9. Electrophoretic mobility and hydrophobicity

The electrophoretic mobilities of the viruses and virus-like particles were measured in Milli-Q water and in filtered samples of source water. The hydrophobicities of the viruses and virus-like particles were estimated by using the bacterial adherence to hydrocarbon method [26], with some modifications. Details of the electrophoretic mobility and hydrophobicity measurements are given in Supplementary Information.

3. Results and discussion

3.1. Virus removal by coagulation with PACl-1.5s

Figure 1a shows the removal ratios ($\log_{10}[C_0/C_s]$) after coagulation with PACl-1.5s followed by settling under gravity for infectious CV, HAV, and rNV-VLPs in samples of six different raw water sources. The removal ratios of CV and HAV were determined by using the PFU method, and those of rNV-VLP were determined by using an enzyme-linked immunosorbent assay. CV and HAV particles, and rNV-VLPs are small and they formed a stable monodispersion as a result of the electrical repulsion in the source water samples used in the present study (see Section 3.2); therefore, in the absence of coagulant, no removal of the viruses or virus-like particles (<0.1-$\log_{10}$) was observed after settling at pH 6 or 7. When the water was subjected to coagulation with PACl-1.5s followed by settling (coagulant dosages and pH were identical to those applied at the corresponding drinking water treatment plant on the day the source water was sampled, i.e., 40, 100, or 110 μM-Al; pH 7), the removal ratios for CV, HAV, and rNV-VLPs were 0.6–1.3-$\log_{10}$, 2.0–2.7-$\log_{10}$, and 0.4–1.3-$\log_{10}$, respectively. These results indicated that the virus monodispersion in the source water samples was destabilized by the addition of PACl-1.5s and that the viruses became adsorbed to or entrapped within the aluminum floc particles generated during the coagulation process before settling out from suspension under gravity during the settling process.

Figure 1b shows the removal ratios ($\log_{10}[C_0/C_f]$) after coagulation with PACl-1.5s followed by settling and membrane filtration for infectious CV, HAV, and rNV-VLPs. Again, in the absence of a coagulant, settling followed by membrane filtration resulted in no removal (<0.1-$\log_{10}$) of the viruses or virus-like particles. This is because the diameters of CV, HAV, and rNV-VLPs (20–30 nm, 20–30 nm, and 30–40 nm, respectively) are smaller than the pore size of the membrane filter (0.45 μm). However, when the water was subjected to coagulation with PACl-1.5s followed by settling

and membrane filtration, there was approximately 1 to 2-$\log_{10}$ improvement in the removal ratios of the viruses and virus-like particles compared with coagulation followed by settling alone (Figure 1a). The removal ratios for CV, HAV, and rNV-VLPs were 1.4–2.8-$\log_{10}$, 2.3–>3.5-$\log_{10}$, and 1.6–2.3-$\log_{10}$, respectively. These results indicate that viruses entrapped or adsorbed in the aluminum floc particles that did not settle out from the suspension under gravity were effectively removed by the membrane filtration.

3.2. Effects of coagulant type and dosage on virus removal

To investigate whether the efficacy of virus removal was dependent on coagulant type or dosage, the efficacy of PACl-1.5s followed by settling was compared with that of PACl-1.5s at a 2-fold increased dosage and those of four other coagulants (i.e., alum, $FeCl_3$, PACl-2.1s, and PACl-2.1ns) followed by settling using source water F (Figure 2). In addition, the removal ratios for infectious CV, HAV, and rNV-VLPs were compared with those for EV and MNV, and with those for AdV and PV, which we reported in a previous study [23]. The removal ratios for CV were improved by using a 2-fold increased dosage of PACl-1.5s, whereas those for rNV-VLP were not, indicating that the effect of PACl-1.5s dosage on virus removal depended on virus type. The removal ratios for CV, HAV, and rNV-VLPs obtained with alum and $FeCl_3$ were comparable with those obtained with PACl-1.5s, with the exception of the removal ratio for HAV after coagulation with alum. When another source water sample was examined (i.e., source water D), similar removal ratios for CV, HAV, and rNV-VLPs were observed when PACl-1.5s and alum were used (Figure S1).

The removal ratios for CV, HAV, and rNV-VLPs obtained with the high-basicity PACls, particularly PACl-2.1ns, were larger than those obtained with the other coagulants, including PACl-1.5s at a 2-fold increased dosage. A similar trend was also observed for EV and MNV. As shown in Figure 2, we previously reported that PACl-2.1ns removed infectious AdV and PV more efficiently than did the other coagulants [23]. These results indicated not only that the degree of virus removal was dependent on coagulant type, but also that PACl-2.1ns was more effective than the other coagulants at removing the free-chlorine-resistant virus CV B5, the monochloramine-resistant virus EV type 11, and the ultraviolet irradiation-resistant virus AdV type 40. We previously reported that PACl-2.1ns has a higher colloid charge density compared with PACl-1.5s, PACl-2.1s, and alum, which is attributable to the high colloidal aluminum content and absence of sulfate in PACl-2.1ns [27]. Because a high colloid charge density confers a high capacity to neutralize negatively charged viruses during coagulation, PACl-2.1ns removed the representative CCL viruses more effectively than did the other coagulants.

In the present study, AdV was removed to a lesser extent than the other viruses examined (Figures 2 and S1). Abbaszadegan *et al.* [7] reported that the removal ratios for infectious AdV type 4 after coagulation with $FeCl_3$ were lower than those of feline calicivirus, which, like MNV, is also a widely used surrogate for human caliciviruses. Our observations agree with those of Abbaszadegan *et al.* [7], although the virus type (AdV type 40, rNV-VLPs, and MNV type 1 vs. AdV type 4 and feline calicivirus) and coagulation conditions differed between our study and theirs.

In contrast, infectious HAV was removed to a greater extent than the other viruses examined. Nasser *et al.* [28] showed that the removal ratios of infectious HAV are larger than those of infectious PV after coagulation with alum, which is consistent with the present results (the same virus type was used both in our study and that of Nasser *et al.* [28]). In the present study, the removal ratios for infectious PV were comparable with those of infectious CV and were smaller than those of infectious EV. In addition, the removal ratios for infectious MNV were also comparable with or smaller than those of rNV-VLPs. These results suggest that studies of the removal of PV and MNV by means of coagulation could be useful for the estimation of the removal ratios of other enteroviruses and human NVs, respectively. To summarize, the order of the removal ratios obtained in the present study was as follows: HAV > EV = rNV-VLPs ≥ CV = PV = MNV > AdV (range, 0.1–2.7-$\log_{10}$).

The surface charge of virus particles is often used to explain virus removal by physicochemical water treatment processes such as coagulation [7, 8, 15, 29]. Indeed, in a previous study we confirmed that the main mechanism for virus removal via coagulation was coprecipitation into a growing matrix of aluminum hydroxide during charge neutralization [30], suggesting that the surface charge of virus particles is related to the efficiency of virus removal by coagulation. To further examine why differences in removal performance were observed among the viruses and virus-like particles examined in the present study, we compared the electrophoretic mobilities of the viruses and virus-like particles in Milli-Q water at various pH values (Figure S2) and in filtered samples of source water at pH 6 or 7 (Figure 3). In Milli-Q water, the electrophoretic mobilities of the virus particles were positive at pH values less than 4 for CV, EV, HAV, and MNV, and pH values less than 3 for rNV-VLPs, whereas the electrophoretic mobilities were negative at pH values larger than 4 to 5 for all viruses (Figure S2). The pH values at which the electrophoretic mobilities of CV, EV, HAV, rNV-VLPs, and MNV were equal to zero (i.e., the isoelectric points) were 3.6, 3.5, 3.8, 3.3, and 3.8, respectively. These values are close to the isoelectric points of AdV type 40 (3.7) and PV type 1 (4.1) [23]. These results suggest that the isoelectric points of the CCL viruses are similar and that the viral surface is negatively charged at around neutral pH since the isoelectric points of these viruses were less than 4.1. Indeed, CV, EV, HAV, MNV, and rNV-VLPs showed negative electrophoretic mobilities in the filtered source water samples at pH 6 and 7 (Figure 3). In addition, the magnitudes of the electrophoretic mobilities of these viruses were also comparable, except for that of rNV-VLPs at pH 6 (Figure 3), although the virus removal ratios differed depending on the virus. Therefore, the differences in the removal ratios of the viruses and virus-like particles by coagulation was not solely a result of the differences in electrophoretic mobility between the viruses in the source water samples used in the present study.

Because hydrophobic forces contribute to the adsorption of protein on aluminum phosphate adjuvant [31] and because the hydrophobicities of virus particles have been shown to influence virus adsorption on a variety of materials [32, 33], we next estimated the hydrophobicities of CV, EV, HAV, rNV-VLPs, and MNV by using the bacterial adherence to hydrocarbon test (Figure 4). The percentage of CV and EV remaining in the water phase was almost unchanged after mixing

with the tested solvents, whereas a large percentage of HAV, rNV-VLPs, and MNV were transferred to the solvent phase when *n*-octane or *p*-xylene was used as the solvent. These results indicate that these viruses have different surface hydrophobicities, and that HAV, rNV-VLPs, and MNV are more hydrophobic than are CV and EV. However, the order of the surface hydrophobicities of the viruses was not consistent with the order of the removal ratios by coagulation (HAV > EV = rNV-VLPs ≥ CV = MNV). Therefore, the virus removal efficiencies of the coagulants examined in the present study cannot be explained by the differences in electrophoretic mobility or hydrophobicity between the viruses. The reason why infectious HAV was removed to a greater extent than the other viruses examined is further discussed in Section 3.4.

3.3. Relationships between enteric virus removal and bacteriophage removal

To investigate whether the bacteriophages MS2 and φX174 are suitable surrogates for CV, EV, HAV, and NV during coagulation, we examined the relationships between the removal ratios of the enteric viruses and virus-like particles and the removal ratios of the bacteriophages after coagulation with PACl-1.5s, alum, or $FeCl_3$ followed by either settling or settling and filtration (Figures 5 and 6). Removal ratios that were below the detection limit were excluded from the analysis. Data obtained from the experiments involving coagulation with PACl-2.1s and PACl-2.1ns were also excluded because the number of samples was limited. Analysis of covariance was used to examine the relationships between the removal ratios of the enteric viruses and virus-like particles and the removal ratios of the bacteriophages for the various coagulants and additional separation processes, and then to determine which data could be combined for a regression analysis. The statistical analyses were conducted by using Excel Toukei 2012 software (Social Survey Research Information Co., Tokyo, Japan). Because the number of removal ratio data for EV and MNV were limited, we conducted the analyses with only the data obtained for CV, HAV, and rNV-VLPs.

The relationship between the infectious removal ratios of CV and MS2 was determined by means of the PFU method and was comparable among the three coagulants examined, irrespective of whether or not coagulation and settling were followed by filtration (analysis of covariance, $P \geq 0.05$), except for when coagulation with alum was followed by settling and filtration and when coagulation with $FeCl_3$ was followed by settling and filtration ($P = 0.03$). However, the relationships were significantly different between after settling and after settling and filtration for all three coagulants ($P < 0.02$) (Table S2).

The relationship between the total removal ratios of CV and MS2, as determined by means of the PCR method, was also comparable among the three coagulants, irrespective of whether or not coagulation and settling were followed by filtration (analysis of covariance, $P > 0.06$), except for when coagulation with alum was followed by settling and filtration and when coagulation with $FeCl_3$ was followed by settling and filtration ($P = 0.01$). However, no significant differences were observed in the total removal ratios obtained after settling or after settling and filtration for any of the coagulants ($P > 0.41$), which is in contrast to the relationships between the removal ratio for infectious CV and the removal ratio for infectious MS2 (Table S2). Likewise, the results of the

statistical analyses differed by dataset (Table S2). In addition, because the data for the removal ratios for HAV and part of the data for the removal ratios of rNV-VLPs did not satisfy several key assumptions that underlie the use of analysis of covariance, we could not analyze these data. Accordingly, further regression analyses were not conducted.

The removal ratios obtained by coagulation followed by settling and filtration for infectious MS2, as determined by means of the PFU method, tended to be larger than those for infectious CV (Figure 5a). A similar trend was observed for the relationship between the infectious virus removal ratios for the other enteric viruses and virus-like particles and the removal ratios of infectious MS2 (Figure 5b–e). The total removal ratio for MS2, as evaluated by means of the PCR method, was also comparable with or larger than the total virus removal ratios of the other enteric viruses and virus-like particles (Figure 6a–e). These results indicate that the removal ratios of MS2 and of the enteric viruses and virus-like particles were not comparable. In a previous study, we reported that the removal ratios of MS2 tended to be larger than those of AdV and PV during coagulation [23]. Taken together, our results suggest that MS2 is not an appropriate surrogate for the CCL viruses examined here during coagulation because its use would result in overestimation of the CCL virus removal ratio.

In contrast, the removal ratios of infectious φX174 obtained by coagulation combined with settling and filtration were comparable with or smaller than those of infectious CV (Figure 5f), and a similar trend was observed for the relationship between the infectious virus removal ratios of the other enteric viruses and virus-like particles and the infectious removal ratios for φX174 (Figure 5g–j). Total removal ratios for φX174, as determined by means of the PCR method, were also comparable with or smaller than the total virus removal ratios of the other enteric viruses and virus-like particles (Figure 6f–j). In a previous study, we reported that the removal ratios of φX174 were comparable with or smaller than those of AdV and PV [23]. Therefore, φX174 has the potential to be a conservative surrogate for the CCL viruses examined here during coagulation.

3.4. Effect of virucidal activity of coagulant on virus removal

As described in Section 3.3, the removal ratios of infectious MS2 tended to be larger than those of the enteric viruses and virus-like particles (Figure 5a–e), whereas the removal ratios of infectious φX174 tended to be comparable with or smaller than those of the enteric viruses and virus-like particles (Figure 5f–j). Several research groups, including ours, have shown, by comparing the removal ratios of infectious viruses evaluated by means of the PFU method and the total virus removal ratios evaluated by means of the PCR method after dissolution or exclusion of aggregates, that the bacteriophages MS2 and Qβ lose their infectivity after contact with PACl [34, 35]. In our previous study, because the removal ratios determined by the two methods were comparable for AdV, PV, and φX174, but statistically different for MS2 [23], it is likely that the former three retain their infectivity after contact with PACl, whereas MS2 does not. These results indicate that the virucidal activity of PACl contributed to the removal of infectious MS2, and accounts for why the infectious MS2 removal ratio was higher than those of infectious AdV, PV, and φX174. However,

the possibility that CVs, EVs, HAVs, and NVs are inactivated during coagulation has not yet been fully investigated.

We next compared the removal ratios obtained by means of the PFU method and the total virus removal ratios obtained by means of the PCR method for infectious CV, EV, HAV, and MNV (Figure 7). The removal ratios determined by the two methods did not significantly differ for CV, EV, or MNV, regardless of the type of coagulant used (PACl-1.5s, alum, or $FeCl_3$; two-tailed *t*-test, $P > 0.73$; Figure 7a, b, d), indicating that these viruses were not inactivated by the coagulant. Ryu *et al.* [10] reported that the removal ratios of CV B6 and EV type 12, evaluated by using a plaque-based assay and a PCR-based assay, were comparable after coagulation with $FeCl_3$, which is consistent with our result, even though the coagulation conditions were quite different between the two studies.

However, although there was no significant difference between the removal ratios of HAV obtained with the two methods when alum or $FeCl_3$ was used as a coagulant ($P > 0.09$), the removal ratios differed significantly when PACl-1.5s was used as the coagulant ($P = 0.00$). The removal ratios of infectious HAV were significantly larger than those of total HAV (Figure 7c), indicating that, unlike the other viruses examined, HAV was inactivated by the coagulant. Therefore, the virucidal activity of PACl-1.5s likely contributed to the removal of infectious HAV and accounts for why infectious HAV was removed to a greater extent than the other viruses examined (see Section 3.2).

We reported previously that the hydrophobicity and sensitivity of viruses to the virucidal activity of coagulant are likely important determinants of the efficiency of virus removal by means of coagulation because the order of the hydrophobicities of the viruses was consistent with their removal ratios by coagulation (i.e., MS2 > PV > AdV > φX174; [23]). However, in the present study (see Section 3.2), the order of the removal ratios of the enteric viruses and virus-like particles (HAV > EV = rNV-VLPs ≥ CV = MNV) was not consistent with the order of the hydrophobicities of the viruses. Therefore, further investigation is needed to elucidate a completely reliable index of the effectiveness of coagulation for virus removal.

In the present study, the removal ratios obtained by means of the PFU method and the PCR method had an approximate 1:1 correlation for CV, EV, and MNV (Figure 7a,b,d). A similar correlation was observed for AdV and PV in our previous study [23]. Therefore, the PCR method, which is a rapid, highly sensitive, and highly specific means of quantifying viruses, may be a viable alternative to the PFU method, which is labor intensive and time consuming, for evaluating the efficiency of coagulants for the removal of CCL viruses from water, except for when assessing the reduction of HAV when a PACl is used.

4. Conclusions

(1) The removal ratios of infectious CV, HAV, and rNV-VLPs after coagulation with PACl-1.5s were 0.6 to 1.3-$\log_{10}$, 2.0 to 2.7-$\log_{10}$, and 0.4 to 1.3-$\log_{10}$, respectively.

(2) Because the removal ratios of PV tended to be similar to those of CV and smaller than those of

EV, and the removal ratios of MNV were comparable with or smaller than those of rNV-VLPs during coagulation, studies on the removal of PV and MNV by coagulation could be useful for estimating the removal ratios of other enteroviruses and human NVs, respectively.

(3) Taken together with our previous results, the order of the removal ratios by coagulation was as follows: HAV > EV = rNV-VLPs ≥ CV = PV = MNV > AdV (range 0.1–2.7-$\log_{10}$).

(4) HAV likely lost its infectivity after contact with the PACl, unlike the other viruses examined, which resulted it its greater removal compared with the other viruses.

(5) A nonsulfated high-basicity PACl (PACl-2.1ns) removed enteric viruses and virus-like particles more efficiently than did the other coagulants (PACl-1.5s, alum, and $FeCl_3$).

(6) Because the removal ratios of the bacteriophage MS2 were larger than those of the CCL viruses examined, MS2 is not an appropriate surrogate for CCL viruses during coagulation. However, since the removal ratios of φX174 were comparable with or smaller than those of the CCL viruses examined, φX174 is potentially a conservative surrogate for CCL viruses during coagulation.

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References

[1] World Health Organization, Guidelines for Drinking-water Quality, fourth ed., World Health Organization, Geneva, 2011.

[2] U.S. Environmental Protection Agency, Draft Drinking Water Contaminant Candidate List 4, EPA-HQ-OW-2012-0217, Office of Water, U.S. Environmental Protection Agency, Washington, DC, 2015.

[3] T.L. Cromeans, A.M. Kahler, V.R. Hill, Inactivation of adenoviruses, enteroviruses, and murine norovirus in water by free chlorine and monochloramine, Appl. Environ. Microb., 76 (2010) 1028-1033.

[4] N. Nwachuku, C.P. Gerba, A. Oswald, F.D. Mashadi, Comparative inactivation of adenovirus serotypes by UV light disinfection, Appl. Environ. Microb., 71 (2005) 5633-5636.

[5] P. Payment, M. Tremblay, M. Trudel, Relative resistance to chlorine of poliovirus and coxsackievirus isolates from environmental sources and drinking water, Appl. Environ. Microb., 49 (1985) 981-983.

[6] M.A. Shannon, P.W. Bohn, M. Elimelech, J.G. Georgiadis, B.J. Marinas, A.M. Mayes, Science and technology for water purification in the coming decades, Nature, 452 (2008) 301-310.

[7] M. Abbaszadegan, B.K. Mayer, H. Ryu, N. Nwachuku, Efficacy of removal of CCL viruses under enhanced coagulation conditions, Environ. Sci. Technol., 41 (2007) 971-977.

[8] B.K. Mayer, H. Ryu, M. Abbaszadegan, Treatability of U.S. Environmental Protection Agency contaminant candidate list viruses: removal of coxsackievirus and echovirus using enhanced coagulation, Environ. Sci. Technol., 42 (2008) 6890-6896.

[9] G.A. Shin, M.D. Sobsey, Removal of norovirus from water by coagulation, flocculation and sedimentation processes, Water Sci. Technol.: Water Supply, 15 (2015) 158-163.

[10] H. Ryu, B. Mayer, M. Abbaszadegan, Applicability of quantitative PCR for determination of removal efficacy of enteric viruses and Cryptosporidium by water treatment processes, J. Water Health, 8 (2010) 101-108.

[11] S.M. Karst, C.E. Wobus, A working model of how noroviruses infect the intestine, PLoS Pathog., 11 (2015) e1004626.

[12] T. Oka, Q.H. Wang, K. Katayama, L.J. Saif, Comprehensive review of human sapoviruses, Clin. Microbiol. Rev., 28 (2015) 32-53.

[13] X. Jiang, M. Wang, D.Y. Graham, M.K. Estes, Expression, self-assembly, and antigenicity of the Norwalk virus capsid protein, J. Virol., 66 (1992) 6527-6532.

[14] A.K. da Silva, O.V. Kavanagh, M.K. Estes, M. Elimelech, Adsorption and aggregation properties of norovirus GI and GII virus-like particles demonstrate differing responses to solution chemistry, Environ. Sci. Technol., 45 (2011) 520-526.

[15] N. Shirasaki, T. Matsushita, Y. Matsui, A. Oshiba, K. Ohno, Estimation of norovirus removal performance in a coagulation-rapid sand filtration process by using recombinant norovirus VLPs, Water Res., 44 (2010) 1307-1316.

[16] T. Matsushita, N. Shirasaki, Y. Tatsuki, Y. Matsui, Investigating norovirus removal by microfiltration, ultrafiltration, and precoagulation-microfiltration processes using recombinant norovirus virus-like particles and real-time immuno-PCR, Water Res., 47 (2013) 5819-5827.

[17] C.E. Wobus, S.M. Karst, L.B. Thackray, K.O. Chang, S.V. Sosnovtsev, G. Belliot, A. Krug,

J.M. Mackenzie, K.Y. Green, H.W. Virgin, Replication of norovirus in cell culture reveals a tropism for dendritic cells and macrophages, PLoS Biol., 2 (2004) 2076-2084.

[18] D. Sano, T. Ohta, A. Nakamura, T. Nakagomi, O. Nakagomi, S. Okabe, Culture-independent evaluation of nonenveloped-virus infectivity reduced by free-chlorine disinfection, Appl. Environ. Microb., 81 (2015) 2819-2826.

[19] M.Y. Lim, J.M. Kim, G. Ko, Disinfection kinetics of murine norovirus using chlorine and chlorine dioxide, Water Res., 44 (2010) 3243-3251.

[20] M.Y. Lim, J.M. Kim, J.E. Lee, G. Ko, Characterization of ozone disinfection of murine norovirus, Appl. Environ. Microb., 76 (2010) 1120-1124.

[21] J. Lee, K.D. Zoh, G. Ko, Inactivation and UV disinfection of murine norovirus with TiO_2 under various environmental conditions, Appl. Environ. Microb., 74 (2008) 2111-2117.

[22] J.E. Lee, G. Ko, Norovirus and MS2 inactivation kinetics of UV-A and UV-B with and without TiO_2, Water Res., 47 (2013) 5607-5613.

[23] N. Shirasaki, T. Matsushita, Y. Matsui, T. Marubayashi, K. Murai, Investigation of enteric adenovirus and poliovirus removal by coagulation processes and suitability of bacteriophages MS2 and φX174 as surrogates for those viruses, Sci. Total Environ., 563-564 (2016) 29-39.

[24] G.P. Richards, M.A. Watson, Immunochemiluminescent focus assays for the quantitation of hepatitis A virus and rotavirus in cell cultures, J. Virol. Methods, 94 (2001) 69-80.

[25] M.H. Adams, Bacteriophages, Interscience Publishers, New York, 1959.

[26] M. Rosenberg, D. Gutnick, E. Rosenberg, Adherence of bacteria to hydrocarbons - a simple method for measuring cell-surface hydrophobicity, FEMS Microbiol. Lett., 9 (1980) 29-33.

[27] N. Shirasaki, T. Matsushita, Y. Matsui, A. Oshiba, T. Marubayashi, S. Sato, Improved virus removal by high-basicity polyaluminum coagulants compared to commercially available aluminum-based coagulants, Water Res., 48 (2014) 375-386.

[28] A. Nasser, D. Weinberg, N. Dinoor, B. Fattal, A. Adin, Removal of hepatitis A virus (HAV), poliovirus and MS2 coliphage by coagulation and high rate filtration, Water Sci. Technol., 31 (1995) 63-68.

[29] T. Matsushita, Y. Matsui, T. Inoue, Irreversible and reversible adhesion between virus particles and hydrolyzing-precipitating aluminium: a function of coagulation, Water Sci. Technol., 50 (2004) 201-206.

[30] N. Shirasaki, T. Matsushita, Y. Matsui, T. Marubayashi, Effect of aluminum hydrolyte species on human enterovirus removal from water during the coagulation process, Chem. Eng. J., 284 (2016) 786-793.

[31] R.H. Alshakhshir, F.E. Regnier, J.L. White, S.L. Hem, Contribution of electrostatic and hydrophobic interactions to the adsorption of proteins by aluminum-containing adjuvants, Vaccine, 13 (1995) 41-44.

[32] K. Farkas, A. Varsani, L.P. Pang, Adsorption of rotavirus, MS2 bacteriophage and surface-modified silica nanoparticles to hydrophobic matter, Food Environ. Virol., 7 (2015) 261-268.

[33] C. Dika, J.F. Duval, G. Francius, A. Perrin, C. Gantzer, Isoelectric point is an inadequate descriptor of MS2, Phi X 174 and PRD1 phages adhesion on abiotic surfaces, J. Colloid Interface Sci., 446 (2015) 327-334.

[34] K. Kreissel, M. Bosl, M. Hugler, P. Lipp, M. Franzreb, B. Hambsch, Inactivation of F-specific bacteriophages during flocculation with polyaluminum chloride—a mechanistic study, Water Res., 51 (2014) 144-151.

[35] T. Matsushita, N. Shirasaki, Y. Matsui, K. Ohno, Virus inactivation during coagulation with aluminum coagulants, Chemosphere, 85 (2011) 571-576.

Table 1. Source water quality data with coagulant type and dosage.

	Sampling date	pH	Turbidity (NTU)	DOC (mg/L)	UV260 (cm^{-1})	Alkalinity (mg-$CaCO_3$/L)	Coagulant type	Coagulant dosage at sampling day (μM-Al)
A	15-Jan-14	7.6	1.0	0.4	0.009	49.8	PACl	40
B	14-Jan-14	7.9	1.2	0.4	0.011	55.7	PACl	40
C	15-Aug-13	7.3	2.9	1.4	0.028	32.5	alum	40
D	15-Aug-13	7.2	1.3	0.8	0.025	14.3	PACl	40
E	17-Nov-14	8.6	40.5	2.2	0.052	84.1	PACl	100
F	23-Jul-14	8.1	4.8	2.7	0.074	103.0	PACl	110

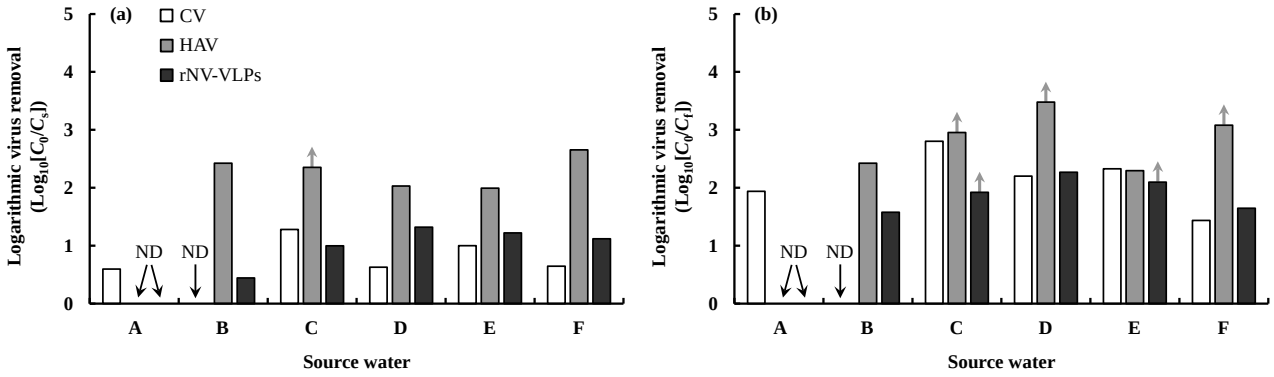


Figure 1. Efficacy of coagulation with PACl-1.5s followed by settling (a) or settling and filtration (b) for the removal of infectious CV, HAV, or rNV-VLPs from samples of raw drinking water collected from various locations in Japan (A–F). Virus concentrations were determined by means of the PFU method (CV and HAV) or an enzyme-linked immunosorbent assay (rNV-VLPs). Arrows indicate that the virus concentration was below the limit of quantification. ND, not determined.

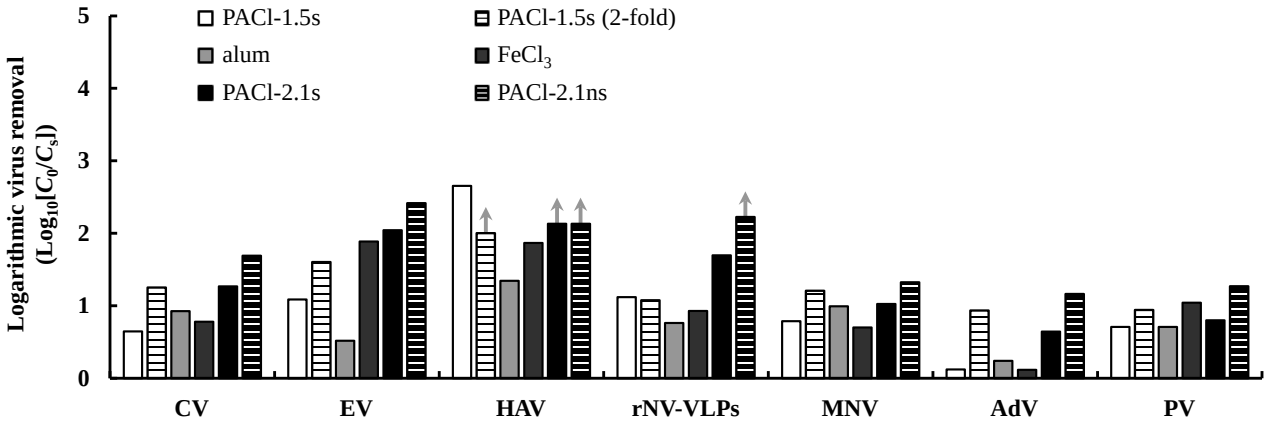


Figure 2. Effects of coagulant dosage and type on infectious virus removal as evaluated after settling by using the PFU method (CV, EV, HAV, MNV, AdV, and PV) or an enzyme-linked immunosorbent assay (rNV-VLPs). Source water, F. Arrows indicate that the virus concentration was below the quantification limit.

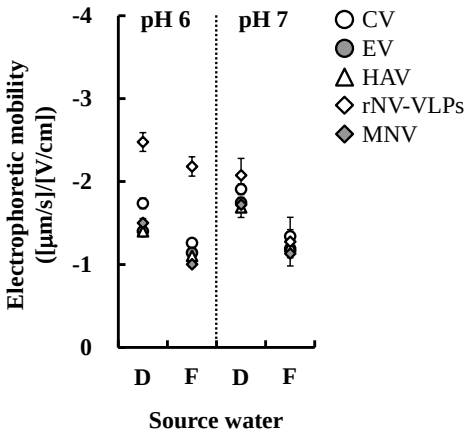


Figure 3. Electrophoretic mobilities of CV, EV, HAV, rNV-VLPs, and MNV in filtered samples of source water at pH 6 and 7. Values are means and error bars indicate standard deviations (*n* = 9).

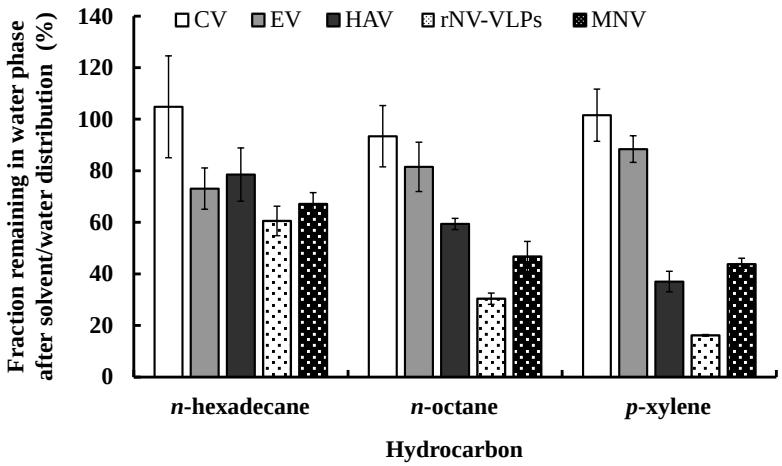
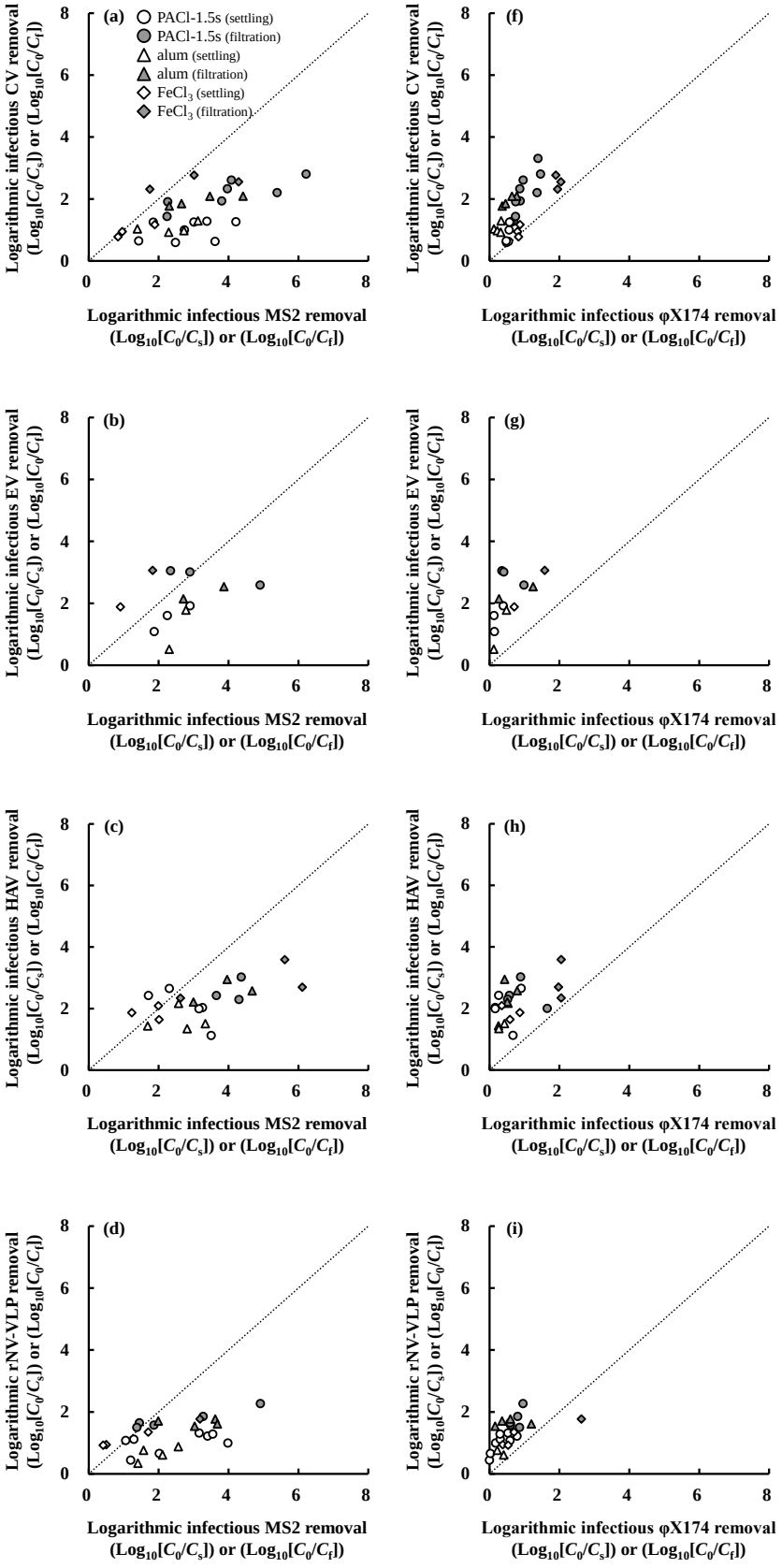


Figure 4. Hydrophobicities of CV, EV, HAV, rNV-VLPs, and MNV. Values are means and error bars indicate standard deviations (n = 3).


(a)
PACl-1.5s (settling)
PACl-1.5s (filtration)
alum (settling)
alum (filtration)
FeCl3 (settling)
FeCl3 (filtration)
(b)
(c)
(d)
(f)
(g)
(h)
(i)
Logarithmic infectious CV removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic infectious EV removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic infectious HAV removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic rNV-VLP removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic infectious MS2 removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic infectious φX174 removal (Log10[C0/Cs]) or (Log10[C0/Cf])
0
2
4
6
8

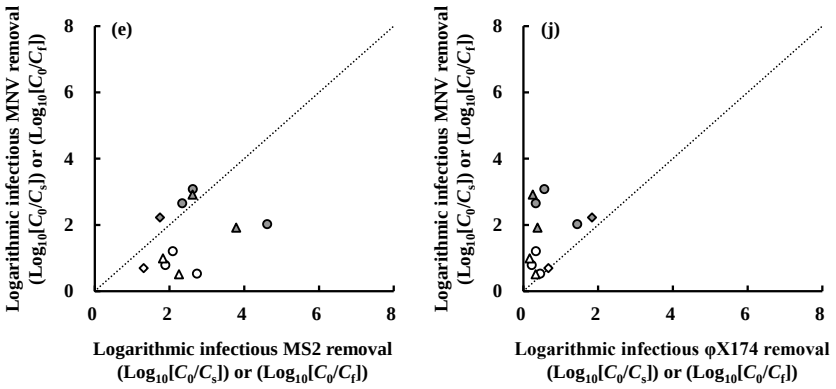
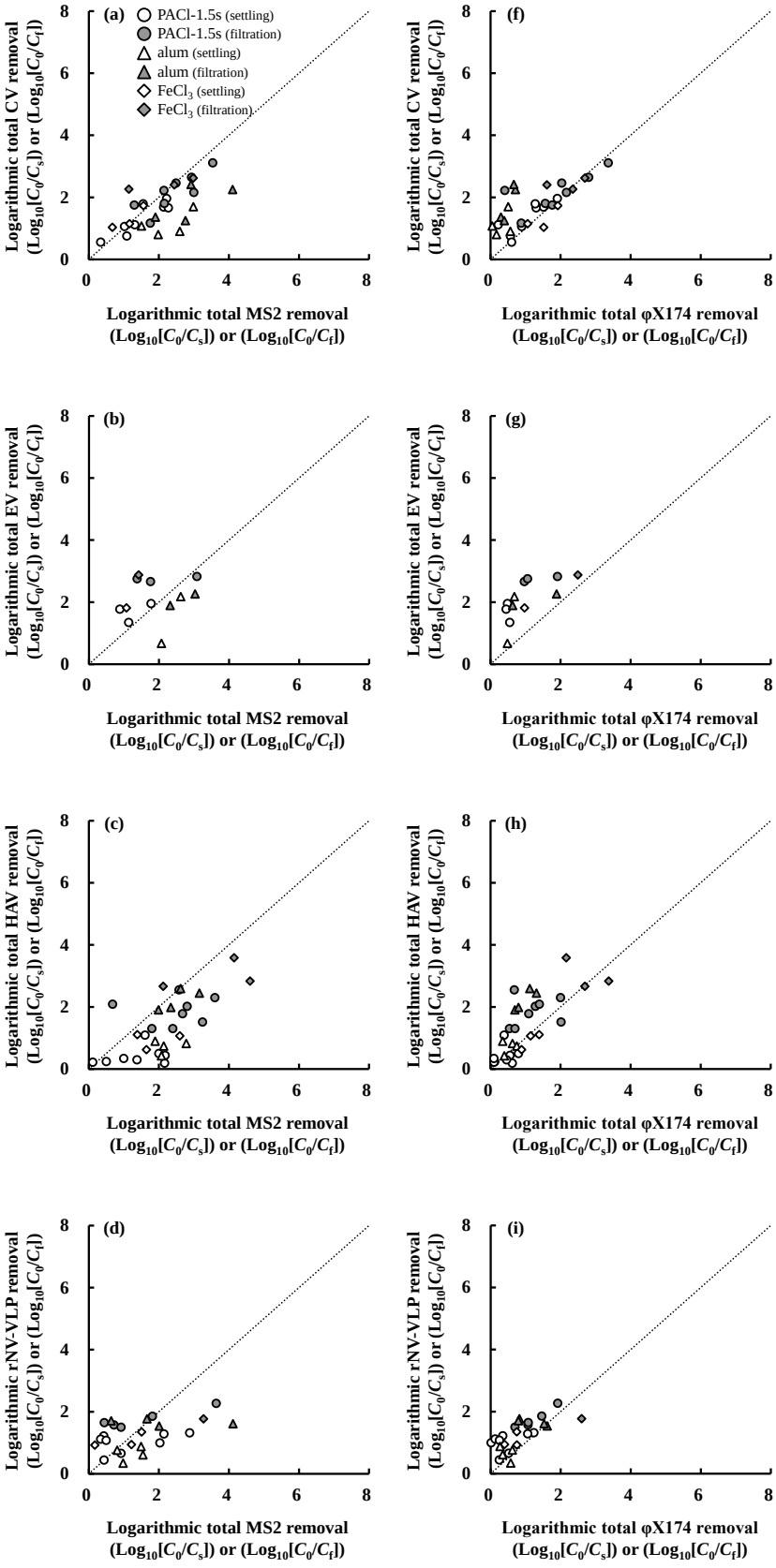


Figure 5. Relationships between the removal ratios of infectious MS2 and infectious CV (a), infectious EV (b), infectious HAV (c), rNV-VLPs (d), and infectious MNV (e); and between infectious φX174 and infectious CV (f), infectious EV (g), infectious HAV (h), rNV-VLPs (i), and infectious MNV (j) after coagulation with the indicated coagulants followed by either settling or settling and filtration.


(a)
PACl-1.5s (settling)
PACl-1.5s (filtration)
alum (settling)
alum (filtration)
FeCl3 (settling)
FeCl3 (filtration)
(b)
(c)
(d)
(f)
(g)
(h)
(i)
Logarithmic total CV removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic total EV removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic total HAV removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic rNV-VLP removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic total MS2 removal (Log10[C0/Cs]) or (Log10[C0/Cf])
Logarithmic total φX174 removal (Log10[C0/Cs]) or (Log10[C0/Cf])
0
2
4
6
8

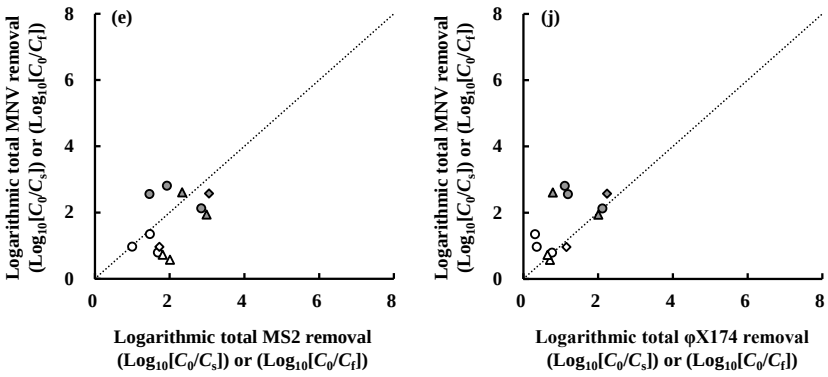


Figure 6. Relationships between the removal ratios of total MS2 and total CV (a), total EV (b), total HAV (c), rNV-VLPs (d), and total MNV (e); and between total φX174 and total CV (f), total EV (g), total HAV (h), rNV-VLPs (i), and total MNV (j) after coagulation with the indicated coagulants followed by either settling or settling and filtration.

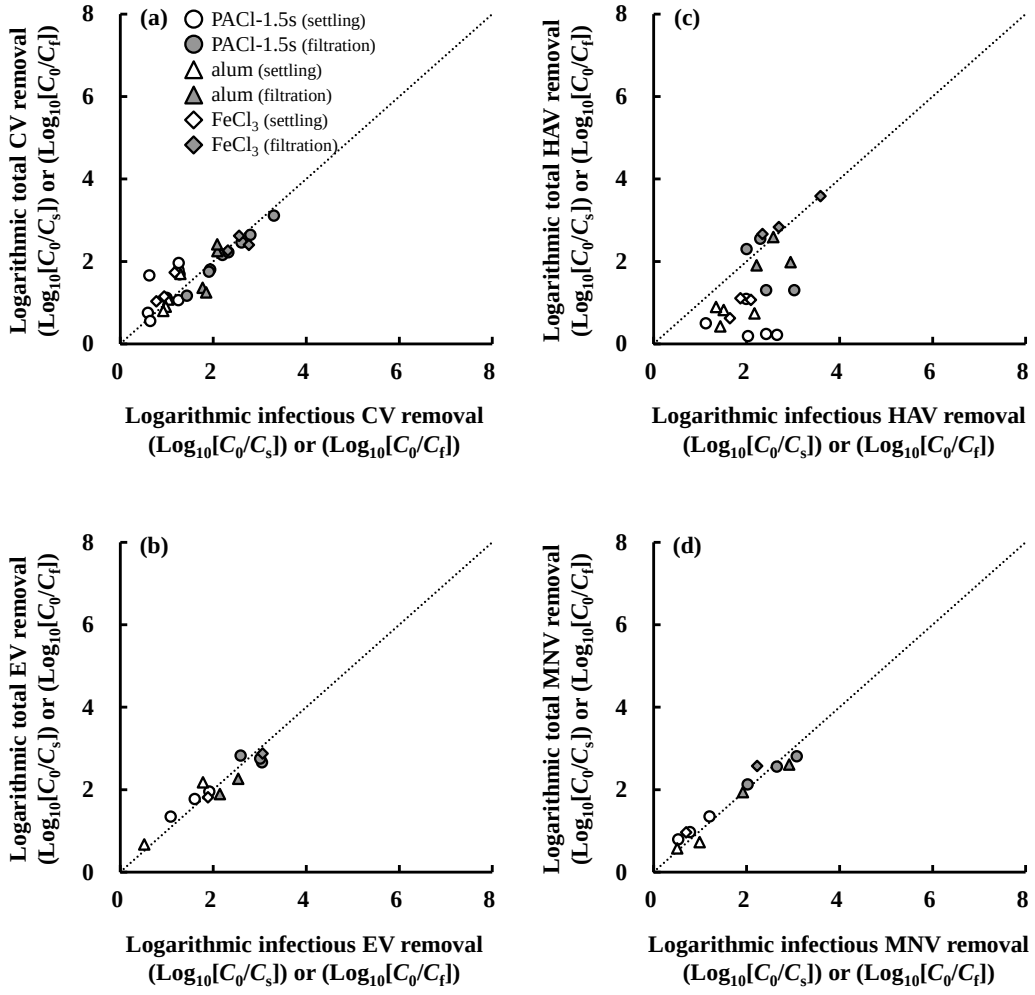


Figure 7. Relationships between the infectious virus removal ratios obtained by means of the PFU method and total virus removal ratios obtained by means of the PCR method for CV (a), EV (b), HAV (c), and MNV (d) after coagulation with the indicated coagulants followed by either settling or settling and filtration.