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Author(s)	Shirakawa, D.; Shirasaki, N.; Matsushita, T. et al.
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Evaluation of reduction efficiencies of pepper mild mottle virus and human enteric viruses in full-scale drinking water treatment plants employing coagulation-sedimentation–rapid sand filtration or coagulation–microfiltration

D. Shirakawa, N. Shirasaki*, T. Matsushita, Y. Matsui, R. Yamashita, T. Matsumura, S. Koriki

Division of Environmental Engineering, Faculty of Engineering, Hokkaido University, N13W8, Sapporo 060-8628, Japan

* Corresponding author. Tel.: +81-11-706-7282; fax: +81-11-706-7282.

E-mail address: nobutaka@eng.hokudai.ac.jp (N. Shirasaki)

Abstract

Here, we evaluated the reduction efficiencies of indigenous pepper mild mottle virus (PMMoV, a potential surrogate for human enteric viruses to assess virus removal by coagulation-sedimentation–rapid sand filtration [CS–RSF] and coagulation–microfiltration [C–MF]) and representative human enteric viruses in four full-scale drinking water treatment plants that use CS–RSF (Plants A and B) or C–MF (Plants C and D). First, we developed a virus concentration method by using an electropositive filter and a tangential-flow ultrafiltration membrane to effectively concentrate and recover PMMoV from large volumes of water: the recovery rates of PMMoV were 100% when 100-L samples of PMMoV-spiked dechlorinated tap water were concentrated to 20 mL; even when spiked water volume was 2000 L, recovery rates of >30% were maintained. The concentrations of indigenous PMMoV in raw and treated water samples determined by using this method were always above the quantification limit of the real-time polymerase chain reaction assay. We therefore were able to determine its

reduction ratios: $0.9\text{--}2.7\text{-log}_{10}$ in full-scale CS–RSF and $0.7\text{--}2.9\text{-log}_{10}$ in full-scale C–MF. The PMMoV reduction ratios in C–MF at Plant C ($1.0 \pm 0.3\text{-log}_{10}$) were lower than those in CS–RSF at Plants A ($1.7 \pm 0.5\text{-log}_{10}$) and B ($1.4 \pm 0.7\text{-log}_{10}$), despite the higher ability of MF for particle separation in comparison with RSF owing to the small pore size in MF. Lab-scale virus-spiking C–MF experiments that mimicked full-scale C–MF revealed that a low dosage of coagulant (polyaluminum chloride [PACl]) applied in C–MF, which is determined mainly from the viewpoint of preventing membrane fouling, probably led to the low reduction ratios of PMMoV in C–MF. This implies that high virus reduction ratios ($>4\text{-log}_{10}$) achieved in previous lab-scale virus-spiking C–MF studies are not necessarily achieved in full-scale C–MF. The PMMoV reduction ratios in C–MF at Plant D ($2.2 \pm 0.6\text{-log}_{10}$) were higher than those at Plant C, despite similar coagulant dosages. In lab-scale C–MF, the PMMoV reduction ratios increased from 1-log_{10} (with PACl [basicity 1.5], as at Plant C) to $2\text{--}4\text{-log}_{10}$ (with high-basicity PACl [basicity 2.1], as at Plant D), suggesting that the use of high-basicity PACl probably resulted in higher reduction ratios of PMMoV at Plant D than at Plant C. Finally, we compared the reduction ratios of indigenous PMMoV and representative human enteric viruses in full-scale CS–RSF and C–MF. At Plant D, the concentrations of human norovirus genogroup II (HuNoV GII) in raw water were sometimes above the quantification limit; however, whether its reduction ratios in C–MF were higher than those of PMMoV could not be judged since reduction ratios were $\geq 1.5\text{-log}_{10}$ for HuNoV GII and $2.3\text{--}2.9\text{-log}_{10}$ for PMMoV. At Plant B, the concentrations of enteroviruses (EVs) and HuNoV GII in raw water were above the quantification limit on one occasion, and the reduction ratios of EVs ($\geq 1.3\text{-log}_{10}$) and HuNoV GII ($\geq 1.5\text{-log}_{10}$) in CS–RSF were higher than that of PMMoV (0.9-log_{10}). This finding supports the usefulness of PMMoV as a potential surrogate for human enteric viruses to assess virus removal by CS–RSF.

Keywords

Coagulation, Microfiltration, Pepper mild mottle virus, Quantitative real-time PCR, Rapid sand filtration, Virus concentration method.

1. Introduction

Human enteric viruses are pathogens that mainly cause gastrointestinal illnesses, and their infective doses are typically low (1–100 particles; WHO, 2011). Because surface waters are frequently contaminated with these viruses (Health Canada, 2017; WHO, 2011), use of such contaminated waters as sources of drinking water production may lead to the spread of waterborne viral diseases if drinking water treatment processes are inadequate for virus reduction (i.e., removal, inactivation, or both). Accordingly, evaluating virus reduction efficiency in drinking water treatment processes and conducting adequate treatment for effective virus reduction are needed to prevent waterborne viral diseases and supply safe drinking water.

Lab-scale experiments using virus-spiked water have been widely used to investigate virus reduction efficiency in drinking water treatment processes (Abbaszadegan et al., 2007; Fiksdal and Leiknes, 2006; Kato et al., 2018; Matsui et al., 2003; Matsushita et al., 2013; Shirasaki et al., 2017a, 2018; Zhu et al., 2005). However, it is unclear whether virus reduction ratios (ratios of virus concentrations before and after treatment) obtained in lab-scale experiments are actually achieved in full-scale drinking water treatment plants (DWTPs). Thus, full-scale studies are needed to investigate virus reduction efficiency in drinking water treatment processes.

Evaluation of virus reduction efficiency in full-scale DWTPs requires quantification of the concentrations of indigenous viruses in raw and treated water. The concentrations of indigenous human enteric viruses in water samples, especially in treated water samples, are usually below the quantification limit of the real-time polymerase chain reaction (PCR) assay, and concentrating these viruses from water is necessary before virus quantification. The concentrations of indigenous human

76 enteric viruses in raw water can be quantified by applying an appropriate virus concentration method,
77 whereas those in treated water are still nearly always below the quantification limit even when >1000
78 L of water are concentrated to several milliliters (Sylvestre et al., 2021; Varughese et al., 2018); in
79 many cases, this makes it impossible to evaluate virus reduction efficiency in full-scale DWTPs.

80 In surface waters, including drinking water sources, the concentrations of the plant virus, pepper
81 mild mottle virus (PMMoV), are higher than those of human enteric viruses (Hamza et al., 2011;
82 Haramoto et al., 2013). Thus, the concentrations of indigenous PMMoV not only in raw but also in
83 treated water can be quantified, which makes it possible to evaluate its reduction efficiencies in full-
84 scale DWTPs. In addition, our research group has reported that the reduction ratios of PMMoV are
85 similar to or lower than those of human enteric viruses in lab-scale virus-spiking coagulation-
86 sedimentation–rapid sand filtration (CS–RSF) and low-pressure membrane (LPM) filtration
87 experiments (Shirasaki et al., 2017a, 2018). Accordingly, when the reduction ratios of indigenous
88 PMMoV in full-scale CS–RSF and LPM filtration are successfully determined by applying an
89 appropriate virus concentration method, similar or higher reduction ratios could be expected for
90 human enteric viruses.

91 CS–RSF is used worldwide in full-scale DWTPs, mainly to reduce turbidity. Virus reduction
92 efficiency in full-scale CS–RSF has been successfully determined by targeting indigenous PMMoV,
93 and reduction ratios of 1.7–2.9- $\log_{10}$, as evaluated by PCR assay, have been reported (Asami et al.,
94 2016; Kato et al., 2018). Application of LPM technology including microfiltration (MF) to drinking
95 water treatment has increased dramatically (Huang et al., 2009). A combination of coagulation
96 pretreatment and MF (coagulation–MF [C–MF]) has been widely used at full-scale DWTPs because
97 coagulation is the most successful pretreatment for MF to mitigate membrane fouling (Huang et al.,
98 2009). In previous lab-scale virus-spiking C–MF studies, including those of our research group, virus
99 reduction ratios of >4- $\log_{10}$ are achieved under appropriate coagulation conditions (Fiksdal and
100 Leiknes, 2006; Matsui et al., 2003; Matsushita et al., 2013; Shirasaki et al., 2017a; Zhu et al., 2005).

101 However, virus reduction efficiency in full-scale C–MF has not yet been investigated. Thus, it is
102 unclear whether the high virus reduction ratios obtained in lab-scale C–MF are actually achieved in
103 full-scale C–MF.

104 Although numerous virus concentration methods have been developed and used to quantify virus
105 concentrations in water, a standard method that can effectively concentrate all viruses from various
106 types of water has not yet been established (Haramoto et al., 2018). Currently, membrane-based virus
107 concentration methods are commonly applied to concentrating viruses from water (Shi et al., 2017).
108 An electronegative filter-based method is widely used to concentrate indigenous viruses from water
109 (Haramoto et al., 2018). In fact, this method has been applied to concentrating indigenous PMMoV
110 in raw and treated water in full-scale CS–RSF (Asami et al., 2016; Kato et al., 2018). However, virus
111 recovery efficiencies and the extent of inhibition of the PCR assay depend on virus concentration
112 method, virus type, sample volume, and water quality (Haramoto et al., 2018). Accordingly,
113 application of a virus concentration method other than the electronegative filter-based method is
114 needed to accumulate data on virus reduction efficiency in full-scale CS–RSF, which contributes to
115 determining how much log credit for virus reduction is allocable to CS–RSF.

116 An electropositive filter-based method is also widely used to concentrate indigenous viruses from
117 water (Haramoto et al., 2018); unlike the electronegative filter-based method, it does not require the
118 addition of multivalent cations prior to concentration, making it easy to concentrate viruses on-site
119 from large volumes of water. The U.S. Environmental Protection Agency has proposed an
120 electropositive filter-based method to concentrate two types of human enteric viruses, enteroviruses
121 (EVs) and noroviruses (Method 1615; Fout et al., 2014). Method 1615 effectively concentrates
122 poliovirus (PV; included in EVs) and murine norovirus (MNV), a surrogate of human noroviruses,
123 from large volumes of water: the recovery rates of these viruses, as evaluated by the PCR assay, were
124 $\geq 20\%$ when 1500–1900 L of groundwater followed by 10 L of PV- and MNV-spiked groundwater
125 were filtered and concentrated to 400 μL (Cashdollar et al., 2013). However, human enteric viruses

126 are round-shaped (20–70 nm in diameter), whereas PMMoV is rod-shaped (18 nm × 300–310 nm;
127 Fauquet et al., 2005). Thus, it remains unclear whether Method 1615 can effectively concentrate
128 PMMoV along with human enteric viruses from water. In fact, Shi et al. (2017) have suggested that
129 physicochemical properties of viruses (e.g., size, shape, and surface charge) can affect virus recovery
130 efficiencies.

131 In the present study, we first tested a slightly modified version of Method 1615 to concentrate
132 PMMoV from large volume of water, but the recovery rate was very low (0.7%) when 1000 L of
133 PMMoV-spiked dechlorinated tap water was concentrated to 20 mL. To effectively concentrate and
134 recover PMMoV along with human enteric viruses from large volumes of water compared with
135 Method 1615, we developed a virus concentration method by using an electropositive filter and a
136 tangential-flow ultrafiltration (TF-UF) membrane. We then used the developed virus concentration
137 method to evaluate the reduction efficiencies of indigenous PMMoV and representative human
138 enteric viruses (adenovirus [AdV], EVs, hepatitis A virus [HAV], and human norovirus genogroups I
139 and II [HuNoVs GI and GII]) in four full-scale DWTPs, two employing CS–RSF and two employing
140 C–MF. In addition, we conducted lab-scale virus-spiking CS–RSF and C–MF experiments that
141 mimicked full-scale CS–RSF and C–MF to validate the virus reduction ratios observed in full-scale
142 CS–RSF and C–MF.

143

144 **2. Materials and methods**

145

146 *2.1. Plant viruses, human enteric viruses, MNV, and a bacteriophage*

147

148 Two plant viruses, PMMoV pepIwateHachiman1 strain (MAFF 104099) and cucumber green
149 mottle virus (CGMMV) SH strain (MAFF 260018), were obtained from the National Institute of
150 Agrobiological Sciences Genebank (Tsukuba, Japan). AdV type 40 Dugan strain (ATCC VR-931),

151 coxsackievirus (CV) B5 Faulkner strain (ATCC VR-185), HAV IB HM175/18f strain (ATCC VR-
152 1402), and MNV type 1 CW1 strain (ATCC PTA-5935) were obtained from the American Type
153 Culture Collection (ATCC, Manassas, VA, USA). F-specific RNA bacteriophage MS2 (NBRC
154 102619) was obtained from the National Institute of Technology and Evaluation Biological Resource
155 Center (Kisarazu, Japan). Details of propagation and purification of PMMoV, AdV, CV, HAV, MNV,
156 and MS2 are described in our previous reports (Shirasaki et al., 2016, 2017b, 2018). CGMMV was
157 propagated in the same way as PMMoV.

158 AdV, CV, and HAV were used as representative human enteric viruses, and MNV was used as a
159 surrogate for human caliciviruses for the assessment of the efficacy of the virus concentration method
160 to concentrate and recover human enteric viruses. In addition, CGMMV, MNV, and MS2 were used
161 as process control viruses, because CGMMV is phylogenetically and morphologically similar to
162 PMMoV (both belong to the genus *Tobamovirus* in the family Virgaviridae; Fauquet et al., 2005), and
163 because MNV and MS2 are widely used as process control viruses (Haramoto et al., 2018; Shi et al.,
164 2017).

165

166 2.2. Virus quantification by real-time PCR or real-time reverse-transcription PCR

167

168 Viral DNA of AdV was quantified by real-time PCR, and viral RNA of PMMoV, CGMMV, EVs
169 including CV, HAV, HuNoVs GI and GII, MNV, or MS2 was quantified by real-time reverse-
170 transcription PCR (real-time RT-PCR), as described in the Supplementary Information (Section S1.1,
171 Table S1).

172

173 2.3. Virus concentration method

174

Each water sample (4–2000 L) was filtered through an electropositive filter (NanoCeram cartridge filter P2.5-5DP or VS2.5-5; Argonide, Sanford, FL, USA) at an initial flow rate of 2–6 L/min by using a magnetic pump. The virus was eluted from the filter with 1.5% (w/w) beef extract (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) solution containing 0.05 M glycine (hereafter “BE solution”, pH 9.0 or 9.5) as follows. Approximately 350 mL of BE solution was added onto the filter in a designated housing to cover the filter completely, the filter was allowed to soak, and then the BE solution was passed through the filter together with newly added 150 mL of BE solution at approximately 1.5 L/min by a peristaltic pump. The eluate (500 mL) was collected into a sterilized glass beaker. Elution was performed four times: the soaking times before the four elution steps were (1) 1 min, (2) 15 min, (3) 15 or 30 min, and (4) 15 or 30 min. All eluates (2 L in total) were collected in the same beaker. The pH was adjusted to 3.5 with HCl, and the eluate was mixed with a sterilized magnetic stirrer at 400 rpm for 30 min to generate BE floc particles (organic flocculation). The floc mixture was centrifuged at $2500 \times g$ for 15 min to separate the flocs from the mixture. The supernatant (2 L) was collected in the beaker, and its pH was adjusted to 7.0 with NaOH. The supernatant was then concentrated to 20 mL by means of a TF-UF cassette (Pellicon XL; nominal molecular weight cutoff, 300 kDa; regenerated cellulose; Millipore Corp., Billerica, MA, USA), and filtered through a hydrophilic cellulose acetate membrane filter (nominal pore size, 0.45 μm ; Dismic-25CS, Toyo Roshi Kaisha, Tokyo, Japan). The resultant sample is referred to as the “supernatant concentrated sample”. The floc pellet was dissolved in 20 mL of 0.15 M sodium phosphate buffer (pH 9.0), centrifuged at $4000 \times g$ for 10 min to collect the dissolved solution completely; the dissolved solution (20 mL) was collected into another sterilized glass beaker, its pH was adjusted to 7.0 with HCl, and it was filtered through the hydrophilic cellulose acetate membrane filter. The resultant sample is referred to as the “floc dissolution sample”. Virus concentrations in both samples were quantified, and their sum was considered as virus concentration in each concentrated sample.

200 2.4. Full-scale studies

201

202 2.4.1. Sample collection and concentration in full-scale DWTPs

203

204 All four DWTPs are located in Japan. Plants A and B employ CS–RSF, and Plants C and D employ
205 C–MF. Water samples were collected and concentrated at Plant A from October 2017 to February
206 2019, Plant B from November 2017 to February 2019, Plant C from May 2018 to July 2019, and
207 Plant D from July 2020 to March 2021. Detailed information about the four DWTPs and samples is
208 provided in Section S1.2 and Table S2.

209 Water samples (4–2000 L) were concentrated on-site by filtering through the NanoCeram filter at
210 an initial flow rate of 2–6 L/min by using the magnetic pump. To dechlorinate water after chlorination
211 (samples after RSF at Plant A; after CS and primary RSF at Plant B in February 2019; after pre-
212 chlorination, manganese oxide [MnOx]-coated media filtration, and C–MF at Plant C; and after
213 coagulation and MF at Plant D), sodium thiosulfate was injected in-line by the peristaltic pump before
214 filtration of water through the NanoCeram filter to a final concentration of 50 mg/L. After on-site
215 filtration, the NanoCeram filters were transferred to our laboratory at Hokkaido University at 4 °C,
216 and stored at 4 °C. Virus elution from the filter was conducted immediately after the filter was brought
217 to 20 °C.

218 Water samples for virus-spiking experiments (see Sections 2.4.3 and 2.5) were collected in plastic
219 containers on on-site sampling days. The samples were stored at 4 °C until use, and brought to 20 °C
220 immediately prior to use.

221

222 2.4.2. Calculation of virus reduction ratio in full-scale DWTPs

223

224 The concentrations of indigenous PMMoV and representative human enteric viruses (AdV, EVs,
225 HAV, and HuNoVs GI and GII), included in the Drinking Water Contaminant Candidate List 4
226 (USEPA, 2016), were quantified in the on-site concentrated samples. Undiluted samples or samples
227 diluted 10-fold with sterilized DNase/RNase-free distilled water were used for virus quantification.
228 The virus reduction ratios were calculated by dividing the virus concentrations before (C_0) by those
229 after (C) treatment. Comparison of the virus reduction ratios between DWTPs was performed using
230 Student's t -test, with P -values less than 0.05 being considered statistically significant.

231

232 *2.4.3. Assessment of the usefulness of the developed virus concentration method for raw and treated*
233 *water in full-scale DWTPs*

234

235 The usefulness of the developed virus concentration method for raw and treated water in full-scale
236 DWTPs was assessed as follows. (1) The raw and treated water samples collected at full-scale DWTPs
237 were spiked with the known amounts of viruses, concentrated, and the total recovery rates of the
238 viruses from the spiked samples were evaluated. (2) The on-site concentrated samples were spiked
239 with the known amounts of viruses, and the quantification efficiencies (i.e., the RNA extraction, RT,
240 and real-time PCR efficiencies) of the viruses in the spiked samples were evaluated (for details, see
241 Sections S1.3 and S1.4).

242

243 *2.5. Lab-scale virus-spiking CS-RSF and C-MF experiments*

244

245 To validate the virus reduction ratios observed in full-scale CS-RSF and C-MF, we conducted lab-
246 scale virus-spiking CS-RSF and C-MF experiments with the water samples collected at Plants A, B,
247 and C. To mimic full-scale CS-RSF (Plants A and B) and C-MF (Plant C), we used the same sulfated
248 polyaluminum chloride (PACl) products with basicity ($[\text{OH}^-]/[\text{Al}^{3+}]$) 1.5 (PACl-1.5s) as used at the

three DWTPs as coagulants; they are specified in Section S1.5. To mimic full-scale CS–RSF, we used silica sand or manganese sand (effective size, 0.6 mm; uniformity coefficient, <1.3; Nihon Genryo Co., Kawasaki, Japan) as the filter medium for RSF. To mimic full-scale C–MF, where a hollow-fiber membrane module (nominal pore size, 0.1 μm ; polyvinylidene difluoride) is installed, we used a commercially available lab-scale MF membrane module (effective filtration area, 0.012 m^2 ; Microza, Asahi Kasei, Tokyo, Japan) that consists of the same hollow-fiber membrane as used at Plant C. Details of experimental procedures are described in Sections S1.6 and S1.7.

3. Results and discussion

3.1. Development of a virus concentration method

First, we filtered and concentrated 1000 L of PMMoV-spiked dechlorinated tap water by using Method 1615 with slight modifications (different filter type and four elutions instead of two; see Section S1.8 for details of experimental procedures; see legend to Table 1 for the description of Method 1615). The total recovery rate (R_t) of PMMoV (0.7%; Table 1, Method 1) was far below acceptable total virus recovery rates in Method 1615 (5%–200%; Fout et al., 2014). A retention rate (R_r) of 95% and elution rate (R_e) of 28% for PMMoV did not fully explain its low total recovery rate; therefore, organic flocculation was the most-likely cause. Accordingly, we conducted virus-spiking organic flocculation experiments to investigate the efficacy of organic flocculation in concentrating and recovering viruses from eluates (for details of experimental procedures, see Section S1.9). We found that CGMMV, human enteric viruses (AdV, CV, and HAV), and MNV were recovered mainly in floc dissolution samples, whereas PMMoV and MS2 mostly remained in the supernatant of the floc mixture (Fig. 1). This result indicated that the low total recovery rate of PMMoV was due to its poor recovery during organic flocculation. Proteins in the BE solution self-flocculate at pH around

274 3.5, and viruses are entrapped in BE flocs during organic flocculation (Katzenelson et al., 1976). The
275 isoelectric points of PMMoV (3.2) and MS2 (2.2; Shirasaki et al., 2016, 2017a) are <3.5, whereas
276 that of CGMMV (4.3; Sano et al., 1978) and those of AdV, CV, HAV, and MNV (3.6–3.8; Shirasaki
277 et al., 2016, 2017b) are >3.5. Thus, viral surface charges at pH 3.5 were probably different between
278 viruses with isoelectric points of <3.5 and >3.5, and the difference in viral surface charges at pH 3.5
279 possibly determined whether the viruses were recovered in floc dissolution samples or remained in
280 the supernatant.

281 To effectively concentrate and recover PMMoV, we further concentrated the supernatant of the floc
282 mixture using a TF-UF membrane with a nominal molecular weight cutoff of 300 kDa, which is much
283 smaller than the molecular weight of PMMoV (40×10^3 kDa; Fauquet et al., 2005). The total recovery
284 rates of PMMoV were markedly increased from 0.7% to 49%–80% by adding the TF-UF process to
285 the virus concentration processes (Table 1, Methods 2–4). In addition, the elution rate of PMMoV
286 increased as the pH of BE solution was increased from 9.0 to 9.5 (Table 1, Method 3), indicating that
287 the pH of BE solution affected the elution rate of PMMoV. In contrast, filter type, and soaking times
288 for the third and fourth elution did not affect the retention and elution rates of PMMoV, and
289 consequently did not affect its total recovery rate (Table 1, Methods 2 and 4). Details of the effects of
290 filter type and elution conditions on virus recovery rates are described in Section S2.1. On the basis
291 of these findings, we concluded that Method 4 was optimal for concentrating PMMoV in our trials,
292 and used it in subsequent experiments.

293 To investigate the effect of filtration volume on virus recovery rates, we filtered and concentrated
294 100–2000 L of PMMoV-spiked dechlorinated tap water (Fig. 2). Regardless of filtration volume, the
295 retention rates of PMMoV were very high (94%–100%), indicating that the NanoCeram filter retained
296 almost all PMMoV even at the maximum volume tested. The total recovery rate of PMMoV was
297 108% when 100 L of spiked water was concentrated, indicating that the developed virus concentration
298 method completely recovered PMMoV from 100 L of the spiked water. With increasing filtration

299 volume to 500–2000 L, total recovery rates were decreased, but maintained at 33%–42%. Whereas
300 the elution rates of PMMoV ($\geq 87\%$) and its recovery rates in the supernatant of the floc mixture
301 ($\geq 73\%$) were almost independent of filtration volume, the recovery rates of PMMoV by TF-UF were
302 decreased from 150% to 39%–56% with increasing filtration volume from 100 to 500–2000 L (data
303 not shown). This result indicates that the decrease in the total recovery rate of PMMoV was due to
304 the decrease in its recovery rate by TF-UF. We also confirmed that the developed method can
305 effectively concentrate and recover PMMoV along with MNV, which is recovered mainly in floc
306 dissolution samples as described above, from large volumes of dechlorinated tap water (discussed in
307 detail in Section S2.2). These results suggest that the developed method combining organic
308 flocculation and TF-UF can effectively concentrate not only viruses that are recovered mainly in floc
309 dissolution samples (e.g., human enteric viruses) but also viruses that mostly remain in the
310 supernatant of floc mixture (e.g., PMMoV) from large volumes of water.

311

312 *3.2. Usefulness of the developed virus concentration method for raw and treated water in full-scale* 313 *DWTPs*

314

315 As described above, the developed virus concentration method can effectively concentrate and
316 recover PMMoV along with MNV from large volumes of dechlorinated tap water. In general, water
317 quality can affect virus recovery efficiencies during virus concentration processes (Haramoto et al.,
318 2018). In addition, inhibitory substances against the PCR assay that were originally present in water
319 samples could be co-concentrated with viruses, and interfere with virus quantification. To assess the
320 usefulness of the developed method for raw and treated water in full-scale DWTPs, we evaluated the
321 total recovery rates of viruses from spiked raw and treated water samples collected at full-scale
322 DWTPs, and the quantification efficiencies of viruses in spiked on-site concentrated samples. The
323 total recovery rates were $\geq 79\%$ for PMMoV and $\geq 17\%$ for the process control viruses (CGMMV,

324 MNV, and MS2) in all samples tested (Fig. S1). This result suggests that the developed method can
325 effectively concentrate and recover not only viruses that are recovered mainly in floc dissolution
326 samples but also viruses that mostly remain in supernatant concentrated samples derived from raw
327 and treated water in full-scale DWTPs.

328 Quantification efficiencies (E_q) of the process control viruses (CGMMV, MNV, and MS2) in on-
329 site concentrated samples are shown in Fig. S2. Supernatant concentrated samples, in which PMMoV
330 mostly remained, showed quantification efficiencies of $\geq 30\%$ for the process control viruses even
331 when the samples were tested without dilution, indicating that inhibition of the PCR assay was
332 negligible when PMMoV was concentrated by using the developed virus concentration method.
333 Accordingly, we adopted the concentrations of indigenous PMMoV quantified without dilution of the
334 concentrated samples. The difference between the concentrations of indigenous PMMoV quantified
335 with 10-fold dilution and without dilution was $< 0.5\text{-log}_{10}$ (Fig. S3). On the other hand, floc dissolution
336 samples, in which human enteric viruses were recovered mainly, occasionally showed quantification
337 efficiencies of $< 10\%$ when samples were tested without dilution. Quantification efficiencies for those
338 samples increased to $\geq 42\%$ when 10-fold dilution was used. Accordingly, if the concentrations of
339 indigenous human enteric viruses quantified with 10-fold dilution were $> 0.5\text{-log}_{10}$ higher than those
340 quantified without dilution, we judged that the PCR assay was considerably inhibited, and adopted
341 the concentrations quantified with 10-fold dilution as the concentrations in the concentrated samples;
342 otherwise, we adopted those quantified without dilution. At 100-fold dilution, quantification
343 efficiencies increased to 72%–115% for supernatant concentrated samples and to 82%–132% for floc
344 dissolution samples. This result indicates that inhibition of the PCR assay can be completely mitigated
345 by 100-fold dilution. Thus, 100-fold dilution before virus quantification in virus concentration
346 experiments (described in Sections 3.1 and 3.2) was reasonable to completely mitigate inhibition of
347 the PCR assay.

348

349 3.3. Reduction of indigenous PMMoV in full-scale DWTPs employing CS–RSF and C–MF

350

351 Next, we evaluated the reduction efficiencies of indigenous viruses in full-scale DWTPs by
352 applying the developed virus concentration method. Indigenous PMMoV was always quantified not
353 only in raw but also in treated water (i.e., its concentrations were above the quantification limit of the
354 PCR assay in all of the raw and treated water samples tested; Fig. 3). We therefore successfully
355 determined the reduction ratios ($\log_{10}[C_0/C]$) of indigenous PMMoV in full-scale DWTPs. At Plants
356 A and B, the concentrations of indigenous PMMoV were decreased by CS and RSF (Fig. 3a and 3b).
357 The reduction ratios of PMMoV were $0.6\text{--}1.5\text{-log}_{10}$ in CS and $-0.1\text{--}1.4\text{-log}_{10}$ in RSF at Plant A, and
358 $0.4\text{--}1.0\text{-log}_{10}$ in CS and $0.5\text{--}1.2\text{-log}_{10}$ in RSF at Plant B; reduction ratios of $1.3\text{--}2.7\text{-log}_{10}$ at Plant A
359 and $0.9\text{--}2.2\text{-log}_{10}$ at Plant B were observed in CS–RSF. The reduction ratios of PMMoV in full-scale
360 CS–RSF determined by using the developed method (i.e., the electropositive filter-based method)
361 roughly corresponded to the previously-reported reduction ratios of PMMoV in full-scale CS–RSF
362 determined by using the electronegative filter-based method ($1.7\text{--}2.9\text{-log}_{10}$; Asami et al., 2016; Kato
363 et al., 2018). Thus, virus reduction ratios of $1\text{--}3\text{-log}_{10}$ could be expected in full-scale CS–RSF.

364 At Plant C, the concentrations of indigenous PMMoV in treated water after pre-chlorination and
365 MnOx-coated media filtration (including intermediate-chlorination) were almost the same as those in
366 raw water (Fig. 3c), suggesting that the contributions of these processes to reduction of PMMoV was
367 negligible. Our research group has recently reported that PMMoV has very high resistance to chlorine
368 treatment, probably due to its robust capsid structure: almost no reduction of the PCR signal (i.e.,
369 almost no degradation of the viral genetic material) was observed within CT values (chlorine
370 concentration multiplied by contact time) of approximately $<80\text{ mg-Cl}_2\cdot\text{min/L}$ (Shirasaki et al., 2020).
371 This high resistance probably resulted in almost no reduction of PMMoV by pre- and intermediate-
372 chlorination, because CT values of pre- and intermediate-chlorination are <20 and $<30\text{ mg-Cl}_2\cdot\text{min/L}$,

373 respectively. In contrast, the concentrations of indigenous PMMoV were decreased by C–MF, and the
374 PMMoV reduction ratios of $0.7\text{--}1.5\text{-log}_{10}$ were observed in C–MF.

375 At Plant D, the concentrations of indigenous PMMoV in treated water after pre-chlorination,
376 powdered activated carbon adsorption, and coagulation were almost the same as those in raw water
377 (Fig. 3d), suggesting that the contributions of pre-chlorination and powdered activated carbon
378 adsorption to reduction of PMMoV were negligible. This is consistent with the fact that a CT value
379 of pre-chlorination is $<70\text{ mg-Cl}_2\cdot\text{min/L}$, and supported by quantification of PMMoV concentrations
380 in the liquid phase of coagulated water samples before and after removing powdered activated carbon
381 (details are described in Section S2.3; Fig. S4). In contrast, the concentrations of indigenous PMMoV
382 were decreased by C–MF, and the PMMoV reduction ratios of $1.3\text{--}2.9\text{-log}_{10}$ were observed in C–MF.

383 We expected higher virus reduction ratios in C–MF than in CS–RSF, because the ability of MF for
384 particle separation is higher than that of RSF owing to the small pore size in MF. However, the
385 reduction ratios of PMMoV in C–MF at Plant C ($1.0 \pm 0.3\text{-log}_{10}$) were significantly lower ($P < 0.05$)
386 than those in CS–RSF at Plant A ($1.7 \pm 0.5\text{-log}_{10}$), and tended to be lower than those in CS–RSF at
387 Plant B ($1.4 \pm 0.7\text{-log}_{10}$). In general, coagulation conditions differ greatly between CS–RSF and C–
388 MF: the conditions used in CS–RSF are determined mainly from the viewpoint of turbidity reduction,
389 whereas the conditions used in C–MF are determined mainly from the viewpoint of preventing
390 membrane fouling because MF can completely remove turbidity without coagulation pretreatment
391 due to its high ability for particle separation. On the other hand, coagulation conditions have been
392 shown to affect virus reduction efficiency in previous lab-scale virus-spiking C–MF studies: due to
393 small diameters of viruses compared with nominal pore sizes of MF membranes, only limited virus
394 reduction ratios ($<1\text{-log}_{10}$) were obtained without coagulation pretreatment or under inappropriate
395 coagulation conditions, whereas virus reduction ratios of $>4\text{-log}_{10}$ were obtained under appropriate
396 coagulation conditions (Fiksdal and Leiknes, 2006; Matsui et al., 2003; Matsushita et al., 2013;
397 Shirasaki et al., 2017a; Zhu et al., 2005). Thus, the lower reduction ratios of PMMoV in C–MF at

Plant C than in CS–RSF at Plants A and B could be attributed to the difference in coagulation conditions. The reduction ratios of PMMoV in C–MF at Plant D ($2.2 \pm 0.6\text{-log}_{10}$) were significantly higher ($P < 0.05$) than those at Plant C. Because the nominal pore sizes of membranes installed at Plants C and D are the same ($0.1\text{ }\mu\text{m}$; see Section S1.3), the difference in coagulation conditions between these DWTPs could contribute to this difference. The reasons for the lower reduction ratios of PMMoV in C–MF at Plant C than in CS–RSF at Plants A and B, and the reasons for the higher reduction ratios of PMMoV in C–MF at Plant D than at Plant C are further discussed in Section 3.4.

3.4. Lab-scale virus-spiking CS–RSF and C–MF experiments to mimic full-scale CS–RSF and C–MF

To validate the virus reduction ratios observed in full-scale CS–RSF and C–MF, we conducted lab-scale virus-spiking CS–RSF and C–MF experiments. The reduction ratios of PMMoV at lab-scale experiments were similar to those in full-scale studies at similar coagulant dosages, except in raw water sample collected at Plant A in July 2018 (Fig. 4). This result indicated that virus reduction in lab-scale CS–RSF and C–MF successfully mimicked that in full-scale CS–RSF and C–MF, except in the above case (discussed in detail in Sections S2.4 and S2.5; Fig. S5). Accordingly, we investigated the effects of coagulation conditions on virus reduction in lab-scale CS–RSF and C–MF to elucidate why PMMoV reduction ratios were lower in C–MF at Plant C than in CS–RSF at Plants A and B, and why PMMoV reduction ratios were higher in C–MF at Plant D than at Plant C.

In lab-scale CS–RSF with raw water sample collected at Plant A in February 2019, the reduction ratio ($[\log_{10}[C_{c0}/C_{cs}] + \log_{10}[C_{r0}/C_{rf}]$) of PMMoV was 3.6-log_{10} at a coagulant dosage of 1.62 mg-Al/L (similar to 1.51 mg-Al/L used at Plant A; Fig. 4a), but decreased to 0.5-log_{10} at 0.54 mg-Al/L . In raw water sample from Plant B, the reduction ratio was 2.9-log_{10} at 1.08 mg-Al/L (similar to 0.98 mg-Al/L used at Plant B; Fig. 4b), but decreased to 1.8-log_{10} at 0.54 mg-Al/L . In lab-scale C–MF with water samples from Plant C, the reduction ratios ($\log_{10}[C_{m0}/C_{mf}]$) of PMMoV were only 0.5--

423 1.1- $\log_{10}$ at 0.27 mg-Al/L (similar to 0.33 or 0.27 mg-Al/L used at Plant C; Fig. 4c), but markedly
424 increased to 2.8–3.5- $\log_{10}$ at 0.54 mg-Al/L. These results indicate that coagulant dosage affected virus
425 reduction efficiency in CS-RSF and C-MF, and that low coagulant dosage used in C-MF at Plant C
426 (0.3–0.4 mg-Al/L on the sampling days) was a reason for the lower PMMoV reduction ratios in C-
427 MF at Plant C than in CS-RSF at Plants A and B.

428 At lab-scale experiments at 0.54 mg-Al/L, the reduction ratios of PMMoV in C-MF (2.8–3.5- $\log_{10}$)
429 were higher than those in CS-RSF (0.5–1.8- $\log_{10}$), indicating that C-MF has higher ability for virus
430 reduction than CS-RSF. On the other hand, coagulant dosages used in C-MF are typically lower than
431 those used in CS-RSF; because MF has high ability for particle separation, the aim of coagulation
432 pretreatment in MF is mainly to prevent membrane fouling, not to reduce turbidity like in RSF. This
433 implies that high virus reduction ratios compared with those achieved in full-scale CS-RSF are not
434 necessarily achieved in full-scale C-MF, despite its higher ability for virus reduction in comparison
435 with CS-RSF. Therefore, control of coagulation conditions, including coagulant dosage, is of great
436 importance for actually achieving high virus reduction ratios in full-scale C-MF.

437 As described in Section 3.3, the reduction ratios of PMMoV in C-MF were higher at Plant D than
438 at Plant C, despite similar coagulant dosages (0.3–0.6 mg-Al/L on the sampling days) at both DWTPs.
439 On the other hand, Plant C uses PACl-1.5s as a coagulant, whereas Plant D uses high-basicity PACl
440 with basicity 2.1 (PACl-2.1s; see Section S1.3). Accordingly, we investigated the effect of coagulant
441 type on virus reduction in lab-scale C-MF; the use of PACl-2.1s instead of PACl-1.5s increased the
442 reduction ratios of PMMoV from 0.5–1.1- $\log_{10}$ to 1.6–3.5- $\log_{10}$ (Fig. S6b). This result indicates that
443 coagulant type affected virus reduction efficiency in C-MF, and that the use of high-basicity PACl at
444 Plant D was probably a reason for the higher reduction ratios of PMMoV at Plant D than at Plant C.
445 We further investigated the usefulness of high-basicity PACls, particularly a non-sulfated one, for
446 improving virus reduction efficiency in full-scale CS-RSF and C-MF. In lab-scale CS-RSF, the use
447 of high-basicity PACls increased the reduction ratios of PMMoV from 2.9- $\log_{10}$ to 3.5–4.1- $\log_{10}$ (Fig.

448 S6a). In lab-scale C–MF, the PMMoV reduction ratios of $>4\text{-log}_{10}$ were achieved by using non-
449 sulfated, high-basicity PACl at 0.27 mg-Al/L (Fig. S6b), similar to the dosage in C–MF at Plant C. In
450 addition, our research group has reported that coagulation pretreatment with high-basicity PACls
451 more effectively mitigates membrane fouling in MF than that with PACl-1.5s (Kimura et al., 2015).
452 Thus, the efficiency of virus reduction could be increased to $>4\text{-log}_{10}$ in full-scale C–MF with a more
453 effective mitigation of membrane fouling by the use of non-sulfated, high-basicity PACl even at a
454 low dosage similar to those applied in full-scale C–MF (e.g., 0.27 mg-Al/L). Details of the effect of
455 coagulant type on virus reduction are described in Section S2.6.

456 Powdered activated carbon is injected into water after pre-chlorination at Plant D, but not Plant C.
457 Our research group has reported that the size of aluminum floc particles generated in C–MF clearly
458 increases in the presence of powdered activated carbon (Matsui et al., 2009). The addition of
459 powdered activated carbon likely generated larger floc particles during coagulation at Plant D than at
460 Plant C, although floc size was not measured in the present study. In general, large floc particles are
461 more effectively removed by the membrane than small floc particles. Thus, enhanced removal of floc
462 particles that entrapped viruses may explain the higher reduction ratios of PMMoV in C–MF at Plant
463 D than at Plant C. Further study is needed to investigate whether virus reduction ratios are actually
464 improved by the addition of powdered activated carbon in C–MF.

465

466 *3.5. Reduction of indigenous human enteric viruses in full-scale DWTPs employing CS–RSF and C–* 467 *MF*

468

469 We compared the reduction ratios of indigenous human enteric viruses (AdV, EVs including CV,
470 HAV, and HuNoVs GI and GII) with those of indigenous PMMoV in full-scale CS–RSF and C–MF.
471 Indigenous PMMoV was quantified in 100% (20/20) of the raw water samples, whereas indigenous
472 EVs were quantified in 25% (5/20; 0/7 for Plant A, 1/3 for Plant B, 4/5 for Plant C, 0/5 for Plant D)

473 and HuNoV GII in 15% (3/20; 0/7 for Plant A, 1/3 for Plant B, 0/5 for Plant C, 2/5 for Plant D) of the
474 samples (Fig. S7). Concentrations of indigenous AdV, HAV, and HuNoV GI in raw water were always
475 below the quantification limit.

476 At Plant B, the concentrations of indigenous EVs and HuNoV GII in treated water after CS and
477 after RSF were always below the quantification limit (Fig. 5a). The reduction ratios in CS–RSF were
478 $\geq 1.3\text{-log}_{10}$ for EVs and $\geq 1.5\text{-log}_{10}$ for HuNoV GII; both were higher than that of PMMoV (0.9-log_{10})
479 on the same day in February 2019. On this sampling day, pre-chlorination was applied before CS. CV
480 (included in EVs) is less resistant to chlorine treatment than PMMoV (Shirasaki et al., 2020), and
481 HuNoV is less resistant than PV (included in EVs; Shin and Sobsey, 2008). Thus, not only removal
482 of viral particles by CS–RSF but also degradation of viral genetic material by pre-chlorination could
483 reduce the PCR signal of EVs and HuNoV GII in CS–RSF at Plant B.

484 At Plant C, the concentrations of indigenous EVs in treated water after MnOx-coated media
485 filtration (i.e., water just before C–MF) were always below the quantification limit (Fig. 5b), so the
486 reduction ratios of EVs in C–MF could not be determined. At Plant D, the concentrations of
487 indigenous HuNoV GII in treated water after coagulation and after MF were always below the
488 quantification limit (Fig. 5c). We could not conclude whether the reduction ratios of HuNoV GII in
489 C–MF ($\geq 1.5\text{-log}_{10}$) were higher than those of PMMoV ($2.3\text{--}2.9\text{-log}_{10}$) on the same days in January
490 and March 2021.

491 In the present study, the concentrations of indigenous human enteric viruses in treated water after
492 CS–RSF or C–MF were always below the quantification limit, because of their low concentrations in
493 raw water and low resistance to chlorine treatment. Thus, it is difficult to directly evaluate their
494 reduction efficiencies in full-scale physical and physicochemical treatment processes (i.e., particle
495 separation processes), particularly when pre- and/or intermediate-chlorination is applied. In contrast,
496 indigenous PMMoV was always quantified even in treated water, owing to its high concentrations in
497 raw water and higher resistance to chlorine treatment than that of human enteric viruses including a

498 chlorine-resistant virus, CV (Shirasaki et al., 2020). In addition, our research group has reported that
499 the reduction ratios of PMMoV are similar to or lower than those of AdV, CV, HAV, and the surrogate
500 of human noroviruses, MNV, in lab-scale virus-spiking CS–RSF and LPM filtration experiments
501 (Shirasaki et al., 2017a, 2018). Thus, PMMoV could be used as a potential surrogate for these viruses
502 to assess their removal by full-scale physical and physicochemical treatment processes, even when
503 pre- and/or intermediate-chlorination is applied. In full-scale CS–RSF and LPM filtration, reduction
504 ratios of AdV, CV, HAV, and human noroviruses could be expected to be similar to or higher than
505 those of PMMoV. This is supported by the higher reduction ratios of EVs and HuNoV GII in CS–
506 RSF at Plant B than that of PMMoV.

507

508 **4. Conclusions**

509

- 510 (1) The developed virus concentration method by using an electropositive filter and TF-UF
511 membrane effectively concentrated and recovered PMMoV from large volumes of water: total
512 recovery rates of 33%–42% were maintained even when as much as 500–2000 L of PMMoV-
513 spiked dechlorinated tap water was concentrated to 20 mL.
- 514 (2) The reduction ratios of indigenous PMMoV determined by using this method were 0.9–2.7-
515 $\log_{10}$ in full-scale CS–RSF and 0.7–2.9- $\log_{10}$ in full-scale C–MF.
- 516 (3) The reduction ratios of PMMoV in C–MF at Plant C ($1.0 \pm 0.3\text{-}\log_{10}$) were lower than those in
517 CS–RSF at Plants A ($1.7 \pm 0.5\text{-}\log_{10}$) and B ($1.4 \pm 0.7\text{-}\log_{10}$), although MF has higher ability
518 for particle separation than RSF. Lab-scale virus-spiking C–MF experiments that mimicked
519 full-scale C–MF demonstrated that the low coagulant dosages applied in C–MF, which are
520 determined mainly from the viewpoint of preventing membrane fouling, probably led to the
521 lower reduction ratios in C–MF than in CS–RSF. This implies that higher virus reduction ratios

than those achieved in full-scale CS–RSF are not necessarily achieved in full-scale C–MF, despite its higher ability for virus reduction in comparison with CS–RSF.

(4) The reduction ratios of PMMoV in C–MF at Plant D ($2.2 \pm 0.6\text{-log}_{10}$) were higher than those at Plant C, despite similar coagulant dosages. In lab-scale C–MF, the PMMoV reduction ratios were improved from 1-log_{10} to $2\text{--}4\text{-log}_{10}$ by using high-basicity PACl instead of PACl-1.5s. Thus, the use of high-basicity PACl at Plant D probably resulted in higher reduction ratios of PMMoV in C–MF at Plant D than at Plant C.

(5) In contrast to human enteric viruses, indigenous PMMoV could always be quantified even in treated water, owing to its high concentrations in raw water and high resistance to chlorine treatment. Together with our previous findings of similar or lower reduction ratios of PMMoV in comparison with those of human enteric viruses in lab-scale virus-spiking CS–RSF and LPM filtration experiments, PMMoV could be used as a potential surrogate for human enteric viruses to assess virus removal by full-scale physical and physicochemical treatment processes, even when pre- and/or intermediate-chlorination is applied. This possibility is supported by higher reduction ratios of EVs ($\geq 1.3\text{-log}_{10}$) and HuNoV GII ($\geq 1.5\text{-log}_{10}$) than that of PMMoV (0.9-log_{10}) in CS–RSF at Plant B, although it could not be judged whether the reduction ratios of HuNoV GII ($\geq 1.5\text{-log}_{10}$) were higher than those of PMMoV ($2.3\text{--}2.9\text{-log}_{10}$) in C–MF at Plant D.

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547

548 **References**

549

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637

638

Table 1

Effects of filter type, pH of beef extract solution, and soaking times before the third and fourth elution on retention rate of PMMoV on NanoCeram filters, its elution rate from the filters with beef extract solution, and total recovery rate of PMMoV from 1000 L of PMMoV-spiked dechlorinated tap water. The experimental conditions of Method 1615 are as follows: filter type, VS2.5-5; pH of beef extract solution, 9.0; number of virus elution procedures, two; soaking times before the first and second elution, 1 and 15 min, respectively; tangential-flow ultrafiltration, not included.

Method	Filter type	pH of beef extract solution	Soaking time (min)				Tangential-flow ultrafiltration	Retention rate R_r (%)	Elution rate R_e (%)	Total recovery rate R_t (%)
			First	Second	Third	Fourth				
1	P2.5-5DP	9.0	1	15	15	15	–	94.8	27.5	0.7
2	P2.5-5DP	9.0	1	15	30	30	+ ^a	99.6	32.1	48.8
3	P2.5-5DP	9.5	1	15	30	30	+	98.8	59.6	78.4
4	VS2.5-5	9.5	1	15	30	30	+	96.8	74.1	80.3

^a + indicates that tangential-flow ultrafiltration was added to the virus concentration processes.

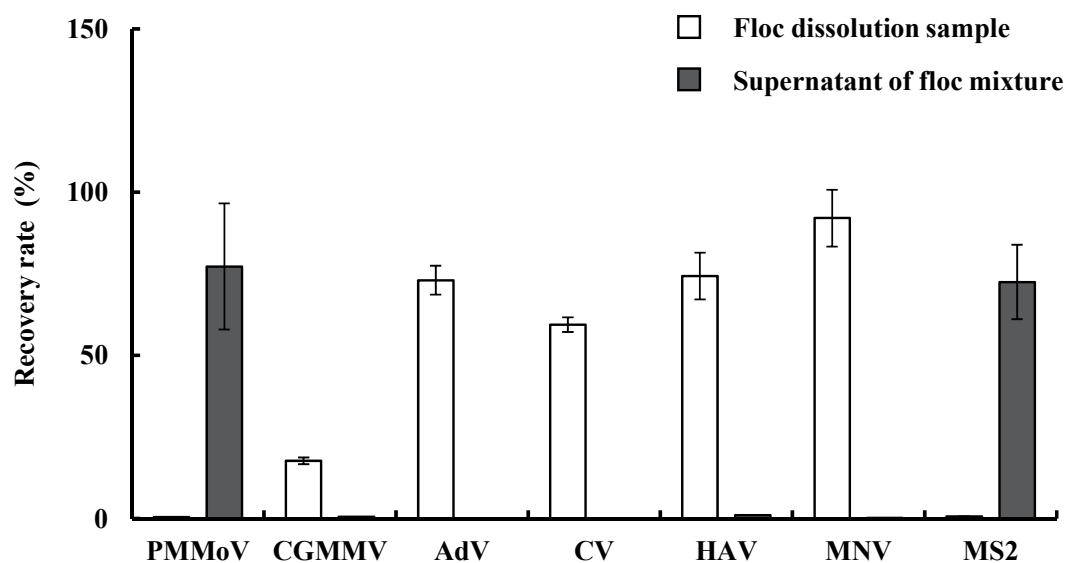
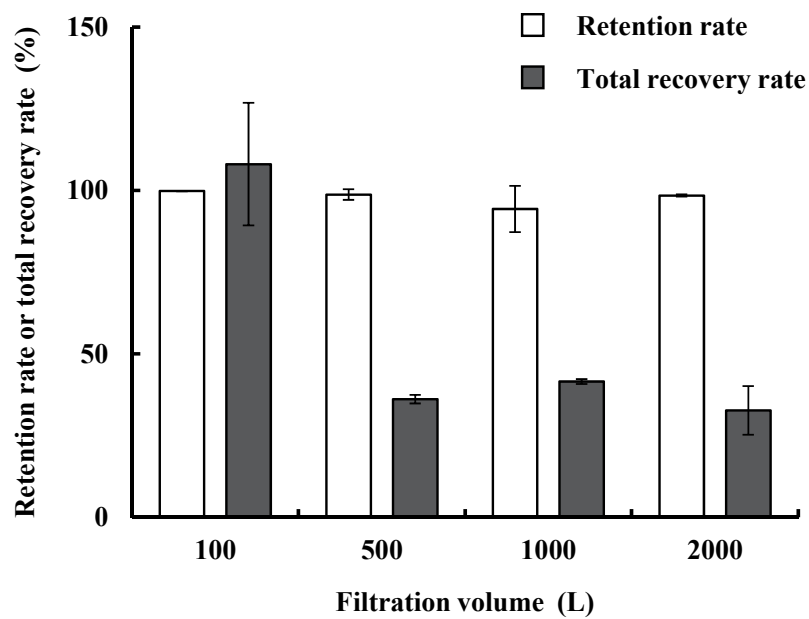


Fig. 1 – Behavior of the viruses during organic flocculation. Viruses were spiked to the BE solution (pH 9.5) at initial concentrations of 10^{8-9} copies/mL for PMMoV, 10^{7-8} copies/mL for CGMMV, CV, and MNV, 10^7 copies/mL for AdV, 10^6 copies/mL for HAV, and 10^{10} copies/mL for MS2. Values are means and error bars indicate standard deviations ($n = 3$).



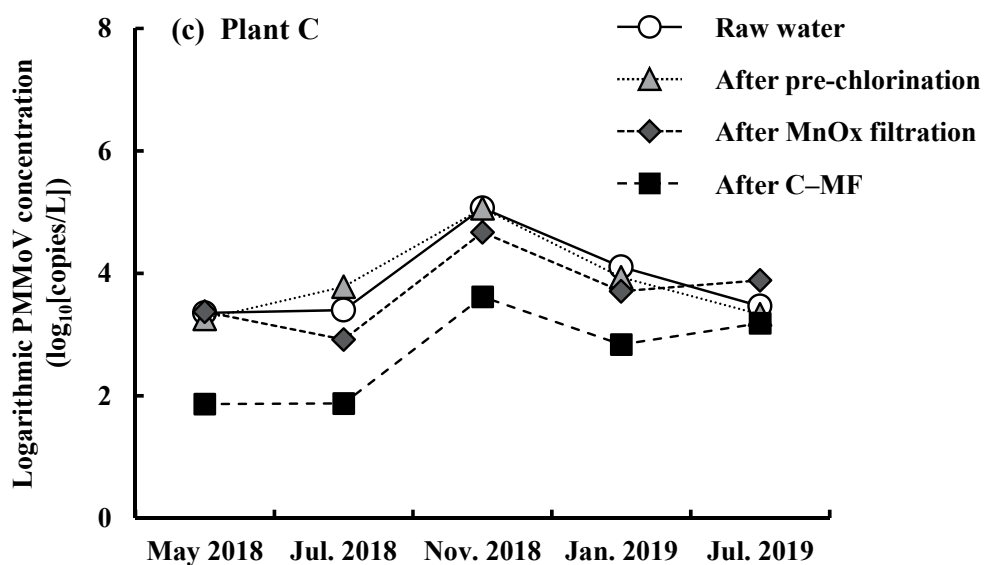
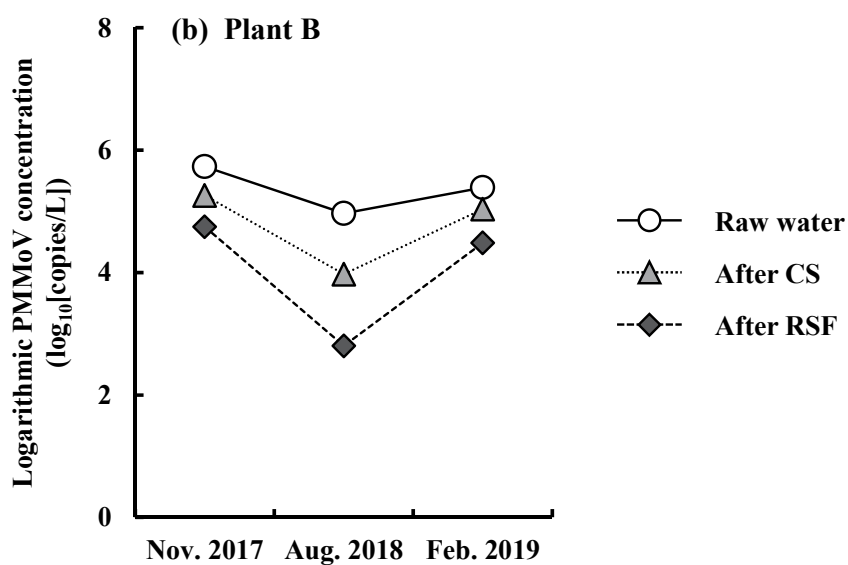
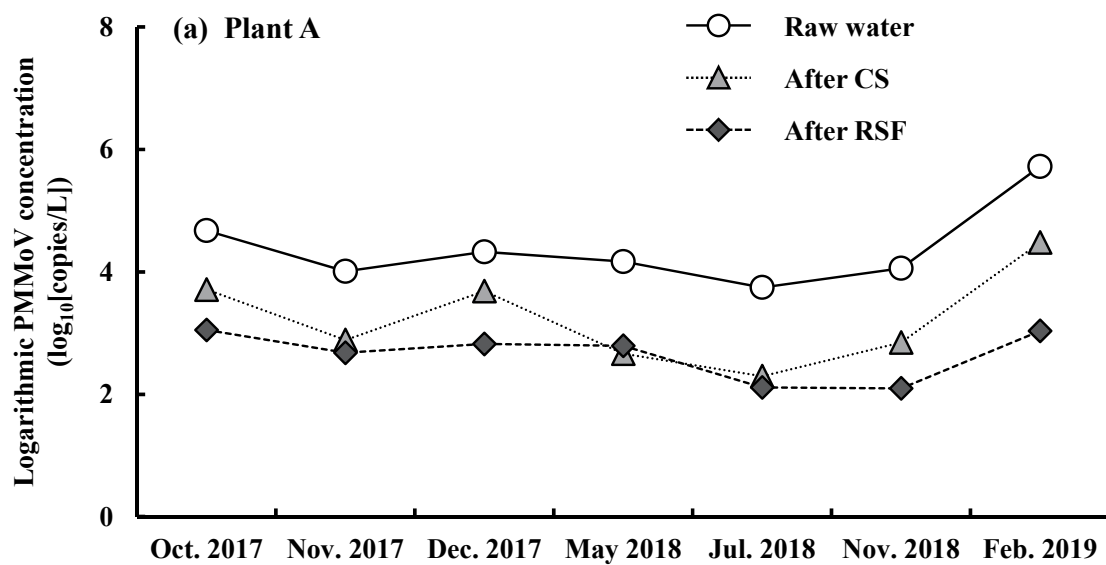
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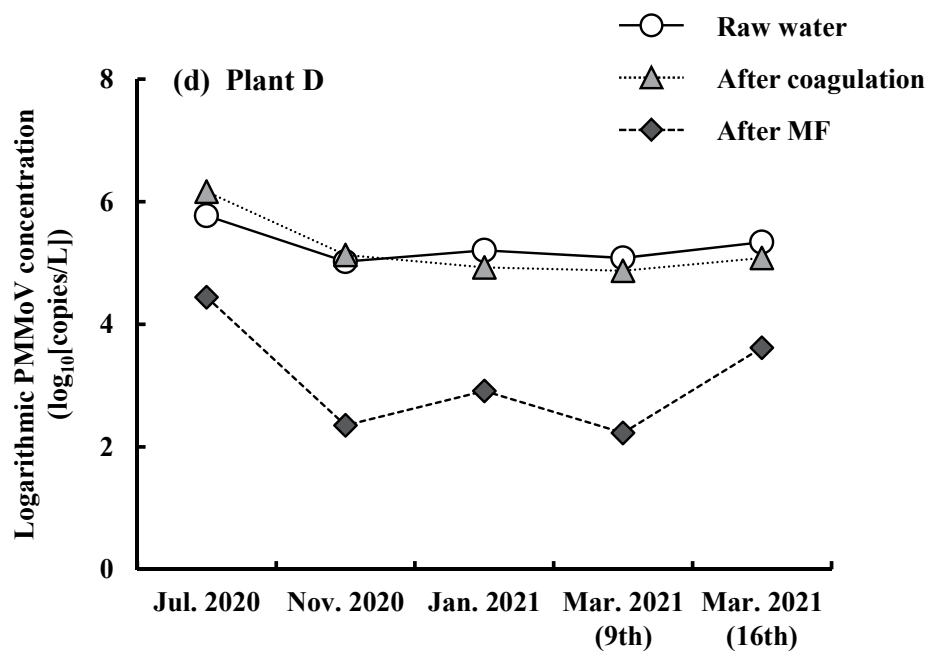
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658 **Fig. 2 – Effect of filtration volume on retention rate of PMMoV on NanoCeram filters and total**
 659 **recovery rate of PMMoV from PMMoV-spiked dechlorinated tap water.** Initial concentration of
 660 PMMoV, 10^8 copies/mL. Values are means and error bars indicate standard deviations ($n = 2$ or 3).

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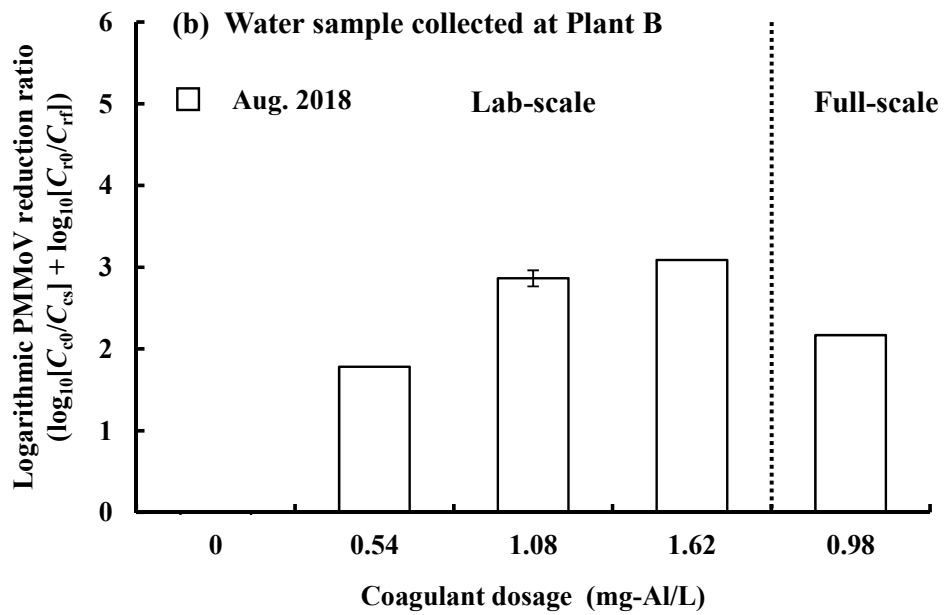
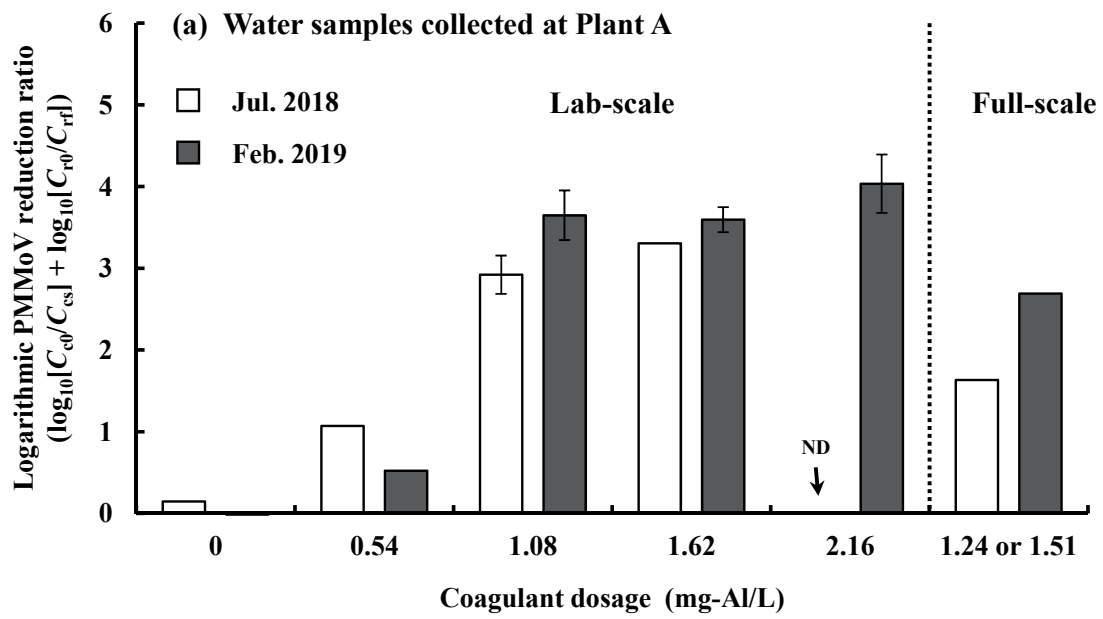
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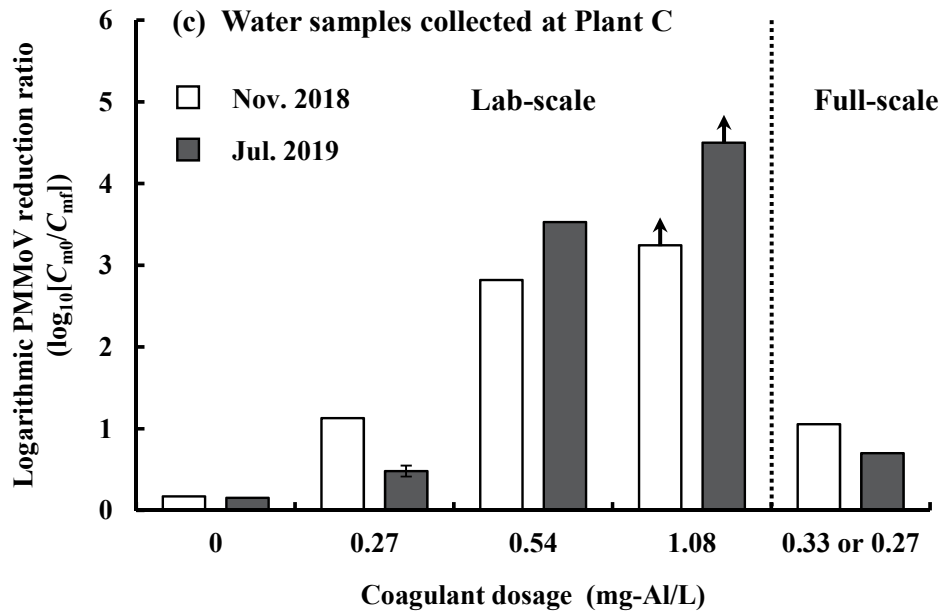
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668 **Fig. 3 – Concentrations of indigenous PMMoV in raw and treated water at Plants A (a), B (b),**
 669 **C (c), and D (d).** Samples of raw water and treated water after coagulation (4 or 20 L) collected at
 670 Plant D in July 2020 were concentrated in our laboratory, not on-site.

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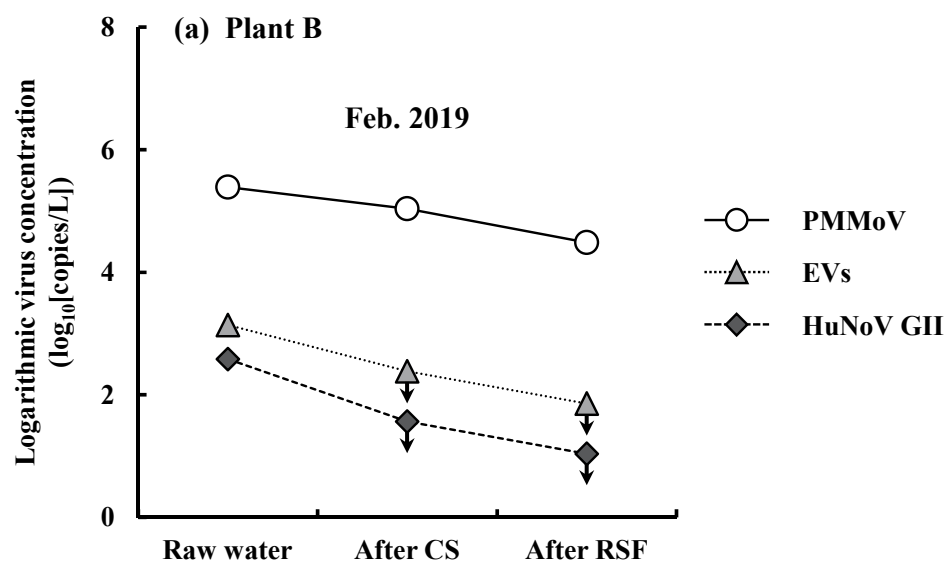
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677 **Fig. 4 – Effect of coagulant dosage on reduction ratios of PMMoV in lab-scale CS–RSF (a, b)**
 678 **and C–MF (c).** Raw water samples collected at Plant A in July 2018 and February 2019 (a), and Plant
 679 B in August 2018 (b), and treated water samples after MnOx-coated media filtration collected at Plant
 680 C in November 2018 and July 2019 (c) were spiked with PMMoV at an initial concentration of 10^{7-8}
 681 ⁸ copies/mL, and then used as experimental raw water. Coagulant, PAC1-1.5s. Manganese sand (a) or
 682 silica sand (b) was used. Values are means and error bars indicate standard deviations ($n = 1-3$).
 683 Arrows indicate that the virus concentrations were below the limit of quantification of the real-time
 684 RT-PCR. ND, not determined.

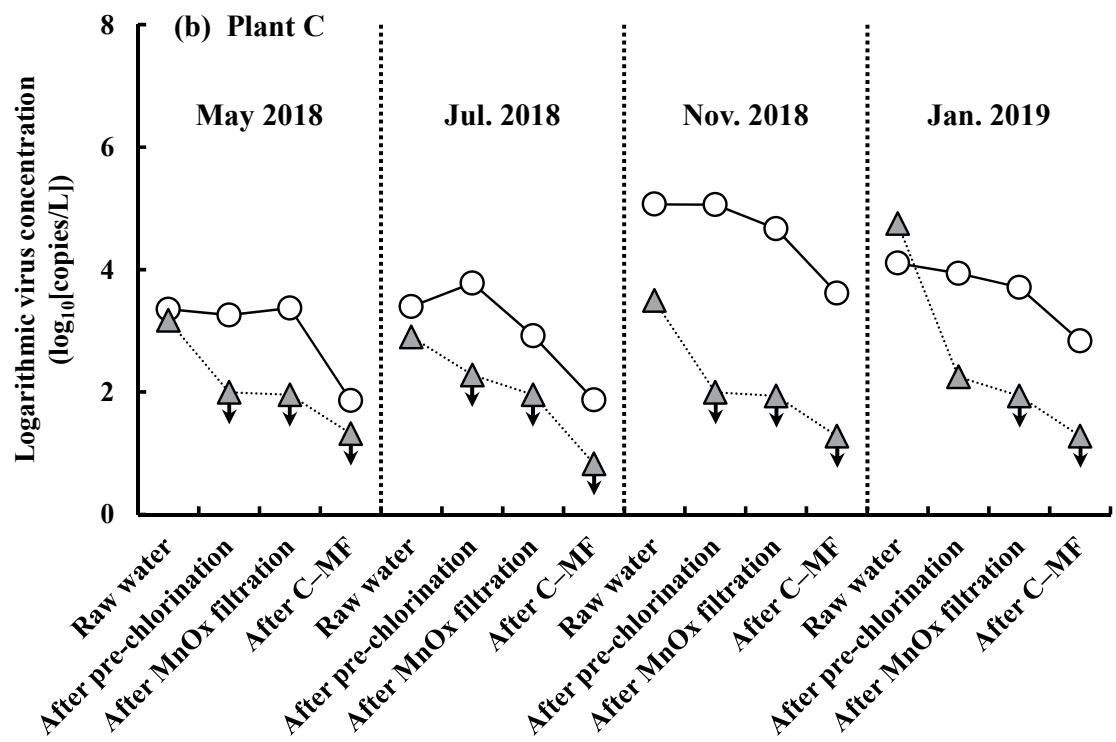
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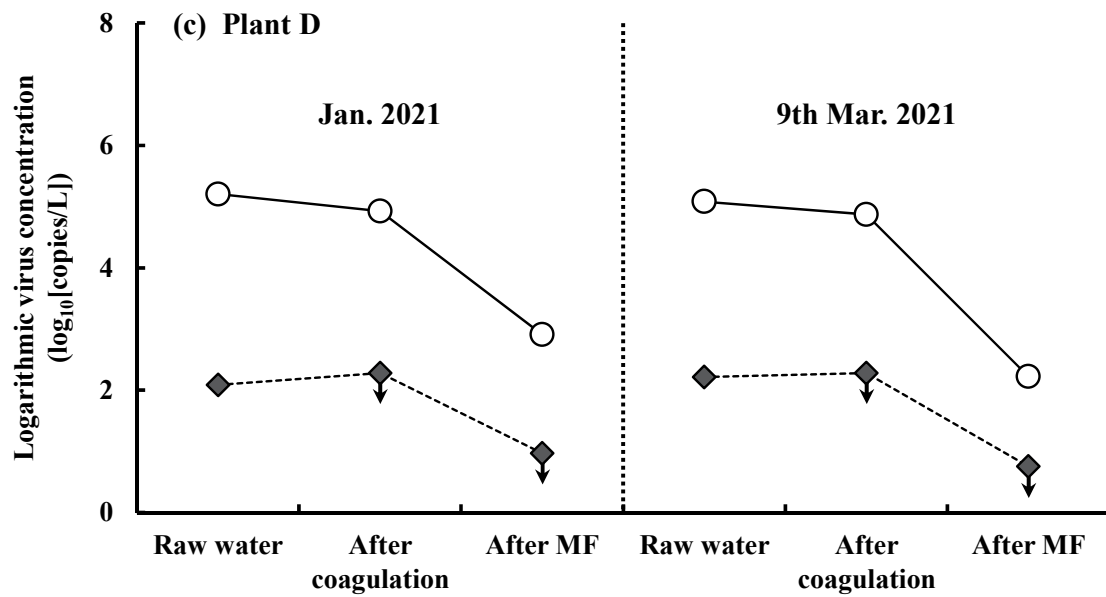


Fig. 5 – Concentrations of indigenous PMMoV and human enteric viruses in raw and treated water at Plants B (a), C (b), and D (c). Treated water samples were tested only when at least one indigenous human enteric virus could be quantified in raw water samples. Arrows indicate that the virus concentrations were below the limit of quantification of the real-time RT-PCR.