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Evaluation of the suitability of a plant virus, pepper mild mottle virus, as a surrogate of human enteric viruses for assessment of the efficacy of coagulation–rapid sand filtration to remove those viruses

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ABSTRACT

Here, we evaluated the removal of three representative human enteric viruses — adenovirus (AdV) type 40, coxsackievirus (CV) B5, and hepatitis A virus (HAV) IB — and one surrogate of human caliciviruses — murine norovirus (MNV) type 1 — by coagulation–rapid sand filtration, using water samples from eight water sources for drinking water treatment plants in Japan. The removal ratios of a plant virus (pepper mild mottle virus; PMMoV) and two bacteriophages (MS2 and φX174) were compared with the removal ratios of human enteric viruses to assess the suitability of PMMoV, MS2, and φX174 as surrogates for human enteric viruses. The removal ratios of AdV, CV, HAV, and MNV, evaluated via the real-time polymerase chain reaction (PCR) method, were 0.8–2.5-$\log_{10}$ when commercially available polyaluminum chloride (PACl, basicity 1.5) and virgin silica sand were used as the coagulant and filter medium, respectively. The type of coagulant affected the virus removal efficiency, but the age of silica sand used in the rapid sand filtration did not. Coagulation–rapid sand filtration with non-sulfated, high-basicity PACls (basicity 2.1 or 2.5) removed viruses more efficiently than the other aluminum-based coagulants. The removal ratios of MS2 were sometimes higher than those of the three human enteric viruses and MNV, whereas the removal ratios of φX174 tended to be smaller than those of the three human enteric viruses and MNV. In contrast, the removal ratios of PMMoV were similar to and strongly correlated with those of the three human enteric viruses and MNV. Thus, PMMoV appears to be a suitable surrogate for human enteric viruses for the assessment of the efficacy of coagulation–rapid sand filtration to remove viruses.

1. Introduction

Human enteric viruses are one of the leading causes of nonbacterial gastrointestinal illness and can be transmitted via water. Because large numbers of human enteric viruses are excreted in the feces of patients, not only raw sewage and sewage discharges, but also drinking water sources that receive sewage discharges are often contaminated with those viruses (Albinana-Gimenez et al., 2006; Bosch, 2007). The implication is that consumption of water may result in exposure to human enteric viruses, particularly when the drinking water treatment process for virus reduction is inadequate. Assessment of the extent of virus reduction during drinking water treatment is therefore important for the prevention and control of waterborne viral diseases.

Some researchers have determined the virus removal efficiency at drinking water treatment plants (DWTPs) by using quantitative real-time polymerase chain reaction (PCR), which is a rapid, highly sensitive, highly specific means of quantifying viruses (Albinana-Gimenez et al., 2006, 2009; Asami et al., 2016). Because the numbers of indigenous human enteric viruses in water samples, particularly treated water, are usually below the PCR quantification limit, large-volume water samples and concentration techniques to reduce sample volume are required to estimate the concentrations of indigenous human enteric viruses (Rames et al., 2016). However, even when more than 1000 L of water are concentrated to less than several milliliters, human enteric viruses are sometimes not detected in the treated water (Albinana-Gimenez et al., 2009; Prevost et al., 2016). Accurate assessment of the efficacy of human enteric virus removal by DWTPs has therefore been hampered by the low virus concentrations in treated water.

A metagenomic analysis has revealed that a plant virus, pepper mild mottle virus (PMMoV), an RNA virus (genus *Tobamovirus*,

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family Virgaviridae) that infects bell, hot, and ornamental peppers, is present at concentrations up to 10^9 virus particles per gram of human feces (Zhang et al., 2006). Because human feces are the most likely source of PMMoV in surface waters and because PMMoV is more frequently detected and is present at higher concentrations and with less seasonality than human enteric viruses in surface waters, including drinking water sources (Hamza et al., 2011; Haramoto et al., 2013), PMMoV has been proposed as an indicator of fecal pollution in surface water. In addition, the concentrations of PMMoV in drinking water sources are probably high enough to determine virus removal efficiency at DWTPs. In fact, Asami et al. (2016) successfully evaluated the virus removal efficiency of coagulation–sedimentation and rapid sand filtration at a DWTP in Bangkok, Thailand, by monitoring PMMoV concentrations during the treatment process. If the removal efficiencies of PMMoV and human enteric viruses are comparable, PMMoV could be a useful surrogate for evaluating the efficacy of drinking water treatment processes to remove human enteric viruses. Because coagulation–sedimentation followed by rapid granular filtration and in particular coagulation–rapid sand filtration are used worldwide in DWTPs to produce drinking water from surface water, whether PMMoV is an adequate surrogate for human enteric viruses in coagulation–rapid sand filtration is an important question. However, the relationship between the removal efficiencies of PMMoV and human enteric viruses in coagulation–rapid sand filtration has not yet been investigated.

In this study, we conducted laboratory-scale coagulation–rapid sand filtration experiments with water samples from eight drinking water sources across Japan to investigate the efficacy of removal of human enteric viruses via coagulation with aluminum-based coagulants, including commercially available polyaluminum chloride (PACl) and alum, followed by settling and rapid sand filtration with virgin silica sand and in-use silica sand collected from a rapid sand filter in a DWTP. We then compared the results with PMMoV and human enteric viruses to assess the suitability of PMMoV as a surrogate for human enteric viruses. The fourth contaminant candidate list (CCL4) for drinking water, published by the U.S. Environmental Protection Agency, includes four types of human enteric viruses: adenoviruses (AdVs); enteroviruses, which include polioviruses, coxsackieviruses (CVs), and echoviruses; hepatitis A viruses (HAVs); and caliciviruses, which include noroviruses and sapoviruses (USEPA, 2016). For our study, we chose three representative CCL viruses, AdV, CV, and HAV, and a surrogate of human caliciviruses, the murine norovirus (MNV). Among AdVs and CVs, AdV type 40 and CV B5 were specifically chosen for use in this study because they are highly resistant to ultraviolet (UV) disinfection (Nwachuku et al., 2005) and free-chlorine disinfection (Cromeans et al., 2010), respectively. For comparative purposes, the removal efficiencies of bacteriophages MS2 and φX174 were also investigated because they are widely used as surrogates for human enteric viruses to evaluate virus removal via coagulation–sedimentation followed by rapid granular filtration (Nasser et al., 1995; Gerba et al., 2003; Abbaszadegan et al., 2007; Boudaud et al., 2012).

2. Materials and methods

2.1. Source water, coagulants, and filter media

The water samples used in the present study were collected from eight water sources for DWTPs in various areas of Japan. Table 1 shows the water quality data for the sources. All of the treatment plants employed 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 (up within one year after sampling) and brought to 20 °C immediately prior to use. We have confirmed that water quality parameters, i.e., turbidity, dissolved organic carbon (DOC) concentration, and UV absorbance at 260 nm (UV260, an indication of natural organic matter [NOM] concentration), of the samples were stable during sample storage period.

To investigate the effects of coagulant basicity ($[OH^-]/[Al^{3+}]$) and sulfate content on virus removal via coagulation–rapid sand filtration, we used five aluminum-based coagulants (Taki Chemical Co., Kakogawa, Japan). Specifications of the coagulants are shown in Table 2 and described in the Supplementary Information.

To investigate the effect of the age of silica sand on virus removal via coagulation–rapid sand filtration, we used virgin silica sand (effective size, 0.6 mm; uniformity coefficient, <1.3; Nihon Genryo Co., Kawasaki, Japan) and in-use silica sand (>6 years of use with hydraulic backwashing every 8 days) collected from a rapid sand filter in a DWTP as the filter media for the rapid sand filtration. Table 3 shows the specifications of the sands.

2.2. Characterization of filter media

The effective size and uniformity coefficient of the silica sands were determined by sieve analyses.

The zeta potentials of the silica sands were determined with a Zetasizer Nano ZS (50 mW, 532-nm green laser; Malvern Instruments, Malvern, Worcestershire, UK) equipped with a surface zeta potential cell kit (ZEN1020, Malvern Instruments). The silica sand was attached to the sample holder by using double-faced adhesive tape. The sample was inserted into a disposable plastic square cuvette containing prepared Milli-Q water with a 0.01% (v/v) suspension of latex microspheres (mean diameter, 0.5 μm; 5050 A, Thermo Fisher Scientific Inc., Waltham, MA, USA) as tracer particles for measurements. The alkalinity of the Milli-Q water was brought to 20 mg-$CaCO_3$/L by the addition of 0.4 mM $NaHCO_3$, and the pH was adjusted to 7 with HCl. Measurements were conducted at 25 °C and a 17° measurement angle at five different distances from the sample surface to calculate the zeta potential of the silica sand.

2.3. Human enteric viruses, MNV, bacteriophages, and PMMoV

AdV type 40 Dugan strain (ATCC VR-931), CV B5 Faulkner strain (ATCC VR-185), HAV IB HM175/18f strain (ATCC VR-1402), and MNV type 1 CW1 strain (ATCC PTA-5935) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and propagated in human lung carcinoma epithelial cells (A549 cells; ATCC CCL-185, obtained from ATCC), buffalo green monkey kidney epithelial cells (BGM cells; kindly supplied by Dr. Daisuke Sano, Hokkaido University, Sapporo, Japan), fetal rhesus monkey kidney epithelial cells (FRhK-4 cells; ATCC CRL-1688, obtained from ATCC), and murine macrophage cells (RAW264.7 cells; ATCC TIB-71, obtained from ATCC), respectively. Details of the propagation and purification of AdV, CV, HAV, and MNV have been described in previous reports (Shirasaki et al., 2016, 2017a). The concentrations of AdV, CV, HAV, and MNV in the purified solutions were approximately 10^{5-6}, 10^7, 10^{5-6}, and 10^6 plaque-forming units (PFU)/mL, respectively, based on the results of plaque assays (Shirasaki et al., 2016, 2017a).

F-specific RNA bacteriophage MS2 (NBRC 102619) and somatic DNA bacteriophage φX174 (NBRC 103405) were obtained from the National Institute of Technology and Evaluation Biological Research Center (Kisarazu, Japan), as were the *Escherichia coli* bacterial hosts in which the bacteriophages were propagated (NBRC 13965 for MS2, NBRC 13898 for φX174). Details of the propagation and purification of the bacteriophages have been described by Shirasaki et al. (2016). The concentrations of MS2 and φX174 in the purified solutions were approximately 10^{10} and 10^{7-8} PFU/mL, respectively,