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Pepper mild mottle virus as a process indicator at drinking water treatment plants employing coagulation-sedimentation, rapid sand filtration, ozonation, and biological activated carbon treatments in Japan

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ABSTRACT

To assess the potential of pepper mild mottle virus (PMMoV) as a viral process indicator, its reduction through coagulation–sedimentation (CS) and rapid sand filtration (RSF) were compared with those of *Escherichia coli*, previously used viral indicators, and norovirus genotype II (NoV GII; enteric virus reference pathogen) in a bench-scale experiment. PMMoV $\log_{10}$ reductions in CS (1.96 ± 0.30) and RSF (0.26 ± 0.38) were similar to those of NoV GII (1.86 ± 0.61 and 0.28 ± 0.46). PMMoV, the most abundant viruses in the raw water, was also determined during CS, RSF, and advanced treatment processes at two full-scale drinking water treatment plants under strict turbidity management over a 13-month period. PMMoV was concentrated from large-volume water samples (10–614 L) and quantified by Taqman-based quantitative polymerase chain reaction. The PMMoV $\log_{10}$ reduction in CS (2.38 ± 0.74, $n = 13$ and 2.63 ± 0.76, $n = 10$ each for Plant A and B) and in ozonation (1.91 ± 1.18, $n = 5$, Plant A) greatly contributed to the overall $\log_{10}$ reduction. Our results suggest that PMMoV can act as a useful treatment process indicator of enteric viruses and can be used to monitor the $\log_{10}$ reduction of individual treatment processes at drinking water treatment plants due to its high and consistent copy numbers in source water.

1. Introduction

Microbial safety management of drinking water has been changed after *Cryptosporidium* caused several outbreaks worldwide in the 1990s (Mackenzie et al., 1994) due to its tolerance to chlorine. In Japan, the Interim Guideline for *Cryptosporidium* Treatment in the Water Supply was established in 1996 and reformed in 2007, where a strict turbidity control policy was employed as a critical control parameter not to exceed 0.1° (approximately 0.14 NTU) after filtration treatment. Careful operation has been in practice, including minute control of the coagulant dosage, re-addition of a small amount of coagulant after sedimentation/before sand filtration, and initiation at a lower flow rate after backwashing of the sand filter. No outbreaks of *Cryptosporidium* via the water supply have been reported in Japan since implementation of the guideline; yet, its efficiency at protecting the drinking water supply from enteric viruses, which may more easily pass through coagulation/sedimentation and filtration process due to their small size, remains uncertain.

As a means of managing the risk of illness due to public consumption of drinking water, quantitative microbial risk assessment (QMRA) has been conducted (Masago et al., 2006; Smeets et al., 2009) and is also included in the Water Safety Plan recommended by the World Health Organization (WHO, 2011). In QMRA processes, $\log_{10}$ reduction values (LRVs) corresponding to viruses reduced in each treatment process are a key parameter in assessing the virus concentration in treated water using pathogen levels

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determined from raw water (RW) (Teunis et al., 1997). Although several studies have evaluated virus reduction in pilot or bench-scale studies using test water spiked with viruses (Abbaszadegan et al., 2007, 2008; Boudaud et al., 2012; Shin and Sobsey, 2015; Shirasaki et al., 2010), virus reduction in the laboratory may not fully represent reduction in full-scale DWTPs (Medema et al., 2006). In a QMRA in The Netherlands, plant scale data were preferred, followed by pilot plant scale data, over data from laboratory experiments (Schijven et al., 2011).

Studies associated with virus reduction at full-scale DWTPs have been limited so far due to the challenges of detecting low concentration of viruses in water (Albinana-Gimenez et al., 2009; Asami et al., 2016), where a large volume of water samples must be concentrated. During virus concentration procedures, polymerase chain reaction (PCR) inhibiting substances are co-concentrated with viruses and interfere with the following molecular methods: RNA extraction, reverse transcription (RT) and quantitative PCR (qPCR). Many researchers have dealt with this problem (Schrader et al., 2012), and PCR inhibition was found to be caused mainly by low molecular weight organic fractions (Hata et al., 2015a). Although some procedures have effectively removed or mitigated PCR inhibition (Gibson et al., 2012; Hata et al., 2011), a common strategy that mitigates PCR inhibition has not been widely established. Recently, Asami et al. (2016) quantified the reduction efficiency of viruses at a full-scale DWTP located in Bangkok, Thailand, using indigenous pepper mild mottle virus (PMMoV) and JC polyomavirus (JC PyV) as treatment process indicators. However, virus reduction during water treatment in Japan may be different from that of other countries given the previously mentioned strict turbidity control policy for *Cryptosporidium* safety management. Therefore, it is necessary to monitor virus reduction for Japanese water treatment systems specifically.

To observe the fate of enteric viruses in water, indicator viruses are often targeted due to their higher relative concentration compared with low concentration indigenous enteric viruses in the source and treated water (Haramoto et al., 2004; Kittigul et al., 2012). Recently, a number of studies have compared the prevalence of PMMoV to enteric viruses in feces, wastewater and environmental waters (Rosario et al., 2009; Zhang et al., 2006; Asami et al., 2016; Hamza et al., 2011; Haramoto et al., 2013; Kuroda et al., 2015; Rachmadi et al., 2016). PMMoV showed greater persistence than human enteric viruses in surface water (Symonds et al., 2016), wastewater (Kitajima et al., 2014b; Schmitz et al., 2016; Symonds et al., 2015, 2014), in wetland treatment (Rachmadi et al., 2016), and in membrane filtration (Shirasaki et al., 2017); this suggest PMMoV is useful as an indicator representing the efficiency of virus removal via a treatment process. However, the efficiency of a drinking water treatment process at reducing PMMoV in comparison with enteric viruses (including human noroviruses, one of the major causes of waterborne nonbacterial gastroenteritis [Maunula et al., 2005; Parshionikar et al., 2003]) has not yet been examined. Since PMMoV and enteric viruses are different from a morphological viewpoint, it is necessary to evaluate whether PMMoV can act as an indicator of the presence of enteric viruses.

Noroviruses, which are found in water and cause waterborne disease, are the most common cause of pediatric gastroenteritis (Katayama and Vinje, 2017); for this reason, norovirus genotype II (NoV GII) was used as a reference pathogen for comparison with PMMoV in this study instead of using other surrogates (Abbaszadegan et al., 2008; Bae and Schwab, 2008; Boudaud et al., 2012). For the bench-scale experiments in this study, several reference indicators were adopted to compare their reduction during the treatments with that of PMMoV: murine norovirus (MNV), often used as a human norovirus surrogate; aichivirus (AiV) as one of the most prevalent virus in water environment; MS2 and Qβ, the most widely used bacteriophages used as virus indicators in water treatment studies; and *E. coli,* which is used as a fecal indicator.

To overcome the difficulty in measuring low concentration of indigenous viruses in water, we needed to investigate how to measure, remove, or mitigate PCR inhibition. Cucumber green mottle mosaic virus (CGMMV), which, like PMMoV, belongs to the *Tobamovirus* group, was introduced as a molecular control due to its phylogenetical and morphological similarity to the target virus (i.e., PMMoV).

Our study was composed of two main parts: a bench-scale experiment using coagulation–sedimentation (CS) and rapid sand filtration (RSF), as well as a field survey at full-scale DWTPs. The aim of the bench-scale experiment was to evaluate the behavior of PMMoV as a treatment process indicator for enteric viruses, including human norovirus. The aims of the full-scale DWTP survey were (1) to investigate whether the indigenous PMMoV copy number in RW was high enough to evaluate the stepwise virus reduction efficiency in DWTPs; (2) to evaluate the molecular detection inhibition caused by co-concentrated organic matter and to verify methods to mitigate this inhibition; and (3) to evaluate the virus reduction efficiency of CS, ozone, biological activated carbon (BAC), and RSF process at two full-scale DWTPs under strict turbidity control in Japan.

2. Material and methods

2.1. Physicochemical water quality parameters

Water temperature, pH and electrical conductivity (EC) were measured immediately after collecting the samples using an HI 98129 water tester (HANNA, Japan). Turbidity was measured with a DR/890 portable colorimeter (HACH, Japan).

2.2. Quantification of microbes

2.2.1. Quantification of indicator bacteria

Water samples containing chlorine were collected in plastic bags in which sodium thiosulfate was added in advance for dechlorination. Concentrations of *E. coli* and total coliforms were quantified within 6 h of sample collection for all water samples collected at DWTPs using a filter unit (37-mm 192 MONITOR UNIT; 37-mm diameter, 0.45-μm pore size, ADVANTEC) and m-ColiBlue24® Broth (HACH). In bench-scale experiment, *E. coli* was quantified within 6 h of sample collection using Chromocult® Coliform Agar, (Merck Millipore, Japan) following the manufacturer's instructions.

2.2.2. Virus quantification by RT-qPCR

Viral RNA was extracted from 140 μL of concentrated samples and reverse-transcribed following previously established methods (Asami et al., 2016). The primers, probes and positive controls are shown in Table A1, further details of PCR shown in the supplemental information.

2.2.3. Evaluation of viral RNA extraction and RT-qPCR efficiency

In order to evaluate RNA extraction and RT-qPCR efficiency, CGMMV, provided by the National Institute of Agrobiological Sciences (NIAS, Japan), was added as a molecular control based on its phylogenetic and morphological similarity to the target virus (PMMoV). Although MNV has been widely introduced as a molecular control in previous studies (Asami et al., 2016; Kitajima et al., 2014a), it is phylogenetically and morphologically different from PMMoV; thus, CGMMV, which has higher similarity to PMMoV, was chosen as a more appropriate molecular control for this study.