Science of the Total Environment 488–489 (2014) 290–296

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Relative abundance and treatment reduction of viruses during wastewater treatment processes — Identification of potential viral indicators

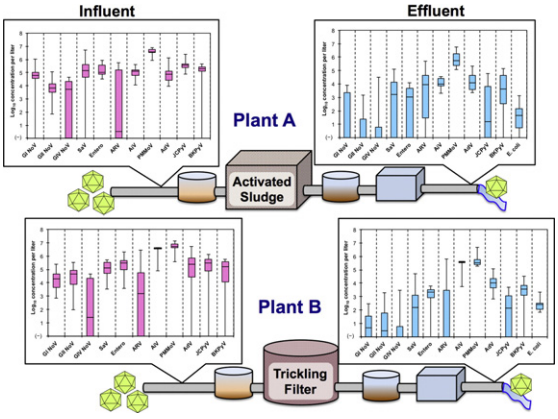
Masaaki Kitajima *, Brandon C. Iker, Ian L. Pepper, Charles P. Gerba

Department of Soil, Water and Environmental Science, The University of Arizona, 1117 E. Lowell St., Tucson, AZ 85721, USA

HIGHLIGHTS

- Occurrence of eleven types of viruses in wastewater over one-year was determined.
- Pepper mild mottle virus was most prevalent in both influent and effluent water.
- Pepper mild mottle virus showed a low reduction and no significant seasonality.
- Aichi virus showed greater abundance and lower reduction than other human viruses.
- No clear difference in virus removal was observed between the two treatment plants.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 March 2014
Received in revised form 18 April 2014
Accepted 22 April 2014
Available online 15 May 2014

Editor: Damia Barcelo

Keywords:
Viruses
Quantitative PCR
Wastewater
Treatment
Indicators
Seasonal occurrence

ABSTRACT

Waterborne pathogenic viruses discharged from wastewater treatment plants (WWTPs) pose potential public health risks. In the present study, we investigated the relative abundance, occurrence, and reduction of eleven different viruses at two WWTPs in southern Arizona over a 12-month period, from August 2011 to July 2012. Influent and effluent samples from the two WWTPs were collected monthly. Viruses were concentrated using an electronegative filter method and quantified using TaqMan-based quantitative PCR (qPCR) assays for each of the virus types (i.e., genogroup I, II and IV noroviruses, sapovirus, enterovirus, group A rotavirus, Aichi virus, pepper mild mottle virus, adenovirus, and JC and BK polyomaviruses), with murine norovirus internal control for the monitoring of extraction-RT-qPCR efficiencies. The pepper mild mottle virus, a plant virus, was found to be the most prevalent virus in both influent and effluent wastewater (annual mean concentration of $3.7–4.4 \times 10^6$ copies/L and $4.6–6.3 \times 10^5$ copies/L in influent and effluent wastewater, respectively), showing a low reduction by the treatment processes (0.76–0.99 annual mean $\log_{10}$ reduction), and no significant seasonal change in concentration. Aichi virus, a human enteric virus, was also found in greater abundance, and showed lower reduction during wastewater treatment than other human enteric viruses. Our results suggest that these viruses could be used as potential indicators of wastewater reclamation system performance, with respect to virus occurrence and removal.

Abbreviations: WWTP, wastewater treatment plant; qPCR, quantitative polymerase chain reaction; AdV, adenovirus; EV, enterovirus; PMMoV, pepper mild mottle virus; MPN, most probable number; MNV, murine norovirus; RT, reverse transcription; GI, genogroup I; NoV, norovirus; SaV, sapovirus; AiV, Aichi virus; ARV, group A rotavirus; PyV, polyomavirus.

* Corresponding author at: Center for Environmental Sensing and Modeling, Singapore-MIT Alliance for Research and Technology, 1 CREATE Way, CREATE Tower #09-03, Singapore 138602, Singapore. Tel.: +65 6516 6597; fax: +65 6684 2118.

E-mail addresses: kitajima@smart.mit.edu (M. Kitajima), ikerb@email.arizona.edu (B.C. Iker), ipepper@ag.arizona.edu (I.L. Pepper), gerba@ag.arizona.edu (C.P. Gerba).

http://dx.doi.org/10.1016/j.scitotenv.2014.04.087
0048-9697/

1. Introduction

Increased water consumption associated with exploding human population and limited precipitation within arid and semi-arid areas in the United States, as well as in other parts of the world, has perpetuated a growing shortage of reliable water supplies. To address this problem, reclaimed or recycled water derived from treated municipal wastewater is being used for various purposes, such as direct and indirect potable reuse, industrial use, agricultural irrigation, recreational use, and environmental enhancement, which if done correctly is a safe, sustainable, and feasible strategy to manage limited water resources (Levine and Asano, 2004).

The potential public health risks associated with wastewater reuse are mainly derived from insufficient removal of pathogenic viruses, which are commonly found in high concentrations in untreated wastewater and highly infectious to humans. Thus, the possibility of inadequate treatment of pathogenic viruses by wastewater treatment plants (WWTPs) that use treated wastewater for reuse purposes requires additional scrutiny (Harwood et al., 2005). In addition, the concentration of viruses in treated wastewater may vary according to the type of the wastewater treatment process, season, geographical area, and hygiene conditions within the community, which makes it difficult to generalize about the occurrence of pathogenic viruses in treated wastewater (Gerba et al., 2013).

Currently, the microbiological safety of reclaimed water is indirectly assessed through routine monitoring of bacterial indicators in the disinfected effluent water. However, human pathogenic viruses are more resistant to the wastewater treatment than bacterial indicators such as *Escherichia coli*, total coliforms, and fecal coliforms (Gerba et al., 2013). Traditional bacterial indicators are not always appropriate predictors of the occurrence and fate of viral pathogens during wastewater treatment (Baggi et al., 2001). Bacteriophages have also been proposed as indicators of viral contamination (IAWPRC Study Group on Health Related Water Microbiology, 1991), but their presence does not always correlate with the occurrence of human enteric viruses (Hot et al., 2003). Accordingly, several types of viruses, such as adenoviruses (AdVs), polyomaviruses (PyVs), enteroviruses (EVs), and pepper mild mottle virus (PMMoV), have recently been suggested as potential indicators of the presence of viruses in water (Albinana-Gimenez et al., 2009; Hamza et al., 2011; Hot et al., 2003; Silva et al., 2011; Wong et al., 2012).

Recent advancements in molecular techniques, especially quantitative PCR (qPCR), have enabled the detection and quantification of a wide range of pathogenic and indicator viruses, including emerging and non-culturable ones, in water (Girones et al., 2010). As a result, data on the concentration of virus genomes in wastewater has been rapidly accumulated in recent years. Seasonal changes of the concentration of viruses have been seen throughout the year, since some virus infections are seasonal (e.g., norovirus (NoV), rotavirus (RV), EV, etc.). Recent studies have also determined the occurrence of viruses in wastewater of different origins. For example, human enteric viruses propagate in the human enteric tract and thus originate from human feces, while PyVs that are associated with human carcinomas and excreted in feces as well as in urine (Fratini et al., 2013); PMMoV, a plant virus that infects various pepper species, is of dietary origin and excreted in high numbers in human feces and not propagated in the human enteric tract (Zhang et al., 2006). However, there is no previous study comprehensively determining the concentration of these viruses with diverse properties and origins in wastewater over the year.

In the present study, we investigated the relative abundance, occurrence, and reduction of eleven different viruses at two WWTP in southern Arizona, throughout a one-year period, with the goal of identifying a conservative viral indicator of human fecal contamination for tracing the fate and transport of viruses in wastewater reuse schemes. The criteria that we used to identify optimal indicator viruses for the purposes stated above were: no observable seasonal changes in abundance, low removal during wastewater treatments, high relative abundance to well-studied enteric viruses such as AdVs and EVs, and considered to be specific to human fecal contamination.

2. Materials and methods

2.1. Collection of wastewater samples

Between August 2011 and July 2012, wastewater sampling was conducted monthly at two WWTP (Plants A and B) located in southern Arizona. Plant A utilized a conventional activated sludge process and plant B utilized a biological trickling filter process (biotower). In addition, both plants used chlorination for disinfection. The characteristics of each plant are described in Table S1 in the Supplementary materials. A total of 48 grab samples were collected, which consist of 12 influent (after screening and before primary sedimentation) and 12 effluent (after chlorination and dechlorination) wastewater samples each from two plants. All samples were collected in sterile plastic bottles, stored on ice, and transported to the laboratory, where they were processed within 12 h of collection. To determine whether the microbiological water quality of effluent water met the criteria for recreational water (USEPA, 1986), *E. coli* in 100 mL of the effluent water sample was assayed by the Colilert method (SM 9223B), and expressed as most probable number (MPN)/100 mL (American Public Health Association, 2005).

2.2. Concentration of viruses in wastewater samples

The wastewater samples were concentrated using an electronegative filter method as described previously (Katayama et al., 2002) with slight modification. Briefly, 2.5 M $MgCl_2$ was added to the wastewater samples to obtain a final concentration of 25 mM. The samples (100 mL influent and 1000 mL effluent) were subsequently passed through the electronegative filter (cat. no. HAWP-090-00; Millipore, Billerica, MA) attached to a glass filter holder (Advantec, Tokyo, Japan). Magnesium ions were removed by passing 200 mL of 0.5 mM H_2SO_4 (pH 3.0) through the filter, and the viruses eluted with 10 mL of 1.0 mM NaOH (pH 10.8). The eluate was recovered in a tube containing 50 μL of 100 mM H_2SO_4 (pH 1.0) and 100 μL of 100× Tris–EDTA buffer (pH 8.0) for neutralization, followed by further centrifugal concentration using a Centriprep YM-50 (Millipore) to obtain a final volume of approximately 650 μL. The concentrates were stored at −80 °C until further analysis.

2.3. Sample process control for extraction-RT-qPCR

Murine norovirus (MNV, S7-PP3 strain), kindly provided by Dr. Y. Tohya (Nihon University, Kanagawa, Japan) and propagated in RAW 264.7 (ATCC TIB-71) cells (American Type Culture Collection, Manassas, VA, USA), was used as a sample process control to determine the efficiency of extraction-reverse transcription (RT)-qPCR, as previously described (Hata et al., 2013). Briefly, 2.0 μL of MNV stock (4.0×10^4 copies/μL) was spiked into 200 μL of concentrated wastewater samples, and pure water (as a control). MNV-RNA was co-extracted with other indigenous viral nucleic acids from the water samples, and the MNV-RNA yield was determined by RT-qPCR (Kitajima et al., 2010). The % extraction-RT-qPCR efficiency (E) was calculated as follows:

$$E = C/C_0 \times 100;$$

where C represents the observed MNV-cDNA copy numbers per qPCR tube in a wastewater sample, and C_0 represents copy numbers in the control. The MNV process control was used to identify the viral nucleic acid loss during extraction and/or the occurrence of RT-qPCR inhibition, if any, and the actual concentration of indigenous viruses in the