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UV inactivation of human infectious viruses at two full-scale wastewater treatment plants in Canada

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ABSTRACT

Ultraviolet (UV) disinfection is widely used to inactivate microorganisms prior to release of treated municipal wastewater. However, limited data are available for *in situ* inactivation of infectious enteric viruses by UV treatment at full-scale. In this study, a total of 51 pre-UV and 50 post-UV samples were collected over a two-year period from two wastewater treatment plants (WWTPs) and analyzed for noroviruses, rotavirus, reovirus, sapovirus, astrovirus, enteroviruses, adenoviruses and JC virus. Both pre-UV and post-UV samples had relatively high concentrations of these viruses determined by qPCR. Infectious viruses were also observed in 98% of pre-UV samples and 76% of post-UV samples by cell culture, using either cytopathic effect (CPE) or integrated cell culture with qPCR (ICC-qPCR). Reovirus was the most common virus detected by ICC-qPCR, present in 92% of pre-UV and 48% of post-UV samples. Infectious enterovirus and adenovirus were detected by ICC-qPCR in 33% and 31% of pre-UV samples, 14% and 20% of post-UV samples, respectively. Mean $\log_{10}$ reduction estimates for infectious reovirus was 1.2 and 1.8 log for the two WWTPs as assessed by ICC-qPCR, which was similar to the reduction of total infectious viruses (1.5 and 1.7 log) as assessed by CPE in cells culture. Overall, quantification of infectious reovirus appears to provide a useful index of enteric virus inactivation during wastewater treatment at full-scale. To our knowledge, this is the first comprehensive study to assess UV inactivation of human enteric viruses at full-scale in WWTPs using both molecular and cell culture techniques, providing important information for quantitative microbial risk assessment of UV inactivation of human viruses in municipal wastewater.

1. Introduction

Ultraviolet (UV) radiation has been increasingly used for pathogen inactivation in wastewater effluents. Compared to chemical disinfection, such as chlorination and ozonation, UV disinfection has numerous advantages, including no chemical addition or residual, minimal disinfection by-products, non-corrosive, simple installation and ease of operation (Zhang et al., 2016). UV radiation damages nucleic acids (DNA/RNA) as well as proteins, inhibiting genome replication/transcription and altering surface protein structure, and hence preventing microbes from replicating and establishing infections (Wigginton et al., 2012). The sensitivity of microorganisms (viruses, bacteria and protozoa) to UV light varies based on a number of biotic and abiotic factors. Biotic factors include microbial mechanisms such as DNA repair enzymes, genome thymidine content, and even the synthesis of UV-absorbing proteins (Hijnen et al., 2006; Rastogi et al., 2014), with adenoviruses representing some of the most UV resistant enteric pathogens described (Rodriguez et al., 2013). Abiotic factors that affect UV efficacy include UV transmissivity of the water (the higher transmissivity, the less quenching of the UV light), dose intensity (mJ/cm^2), hydraulic retention time and the spectrum of UV light output (Hijnen et al., 2006; Zhang et al., 2016).

Human enteric viruses are common microbial contaminants in

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wastewater and require the highest removal of all pathogen classes for reuse applications (Soller et al., 2018). The most notable ones include noroviruses, rotaviruses, sapoviruses, astroviruses, adenoviruses and enteroviruses, all of which are commonly detected in municipal wastewater worldwide (Montazeri et al., 2015; Qiu et al., 2015; Kaas et al., 2016). Although prevalent in wastewater, these viruses are rarely tested for, or monitored in, finished effluents at full-scale facilities. It is known that inactivation of bacterial indicators does not correlate well with viruses because of their differential susceptibility to UV radiation (Harwood et al., 2005; Hijnen et al., 2006). To help address these knowledge gaps, monitoring and understanding the efficiency of UV inactivation of enteric viruses during wastewater treatment is important.

The majority of studies regarding UV inactivation of viruses in water have been conducted at the laboratory or bench-scale, which cannot adequately reflect the performance of UV disinfection during wastewater treatment at full-scale (Gerba et al., 2002; Shin et al., 2005; Calgua et al., 2014). There are limited studies of UV inactivation of viruses carried out at full-scale in wastewater treatment plants (WWTPs) (Oppenheimer et al., 1997; Francy et al., 2012; Lee et al., 2018). Since many enteric viruses are not easily cultured, most studies have used molecular techniques, such as qPCR, to estimate virion concentrations in water samples. However, since molecular methods detect both infectious and non-infectious viruses, the public health impact regarding treatment efficacy is a concern (Francy et al., 2012). In the past decade, several attempts have been made to distinguish infectious and non-infectious viruses by qPCR using pre-treatment with Rnase or using intercalating dyes, such as propidium monoazide (PMA), which can penetrate damaged or compromised virus particles and bind to the genome (Lamhoujeb et al., 2008; Parshionikar et al., 2010). However, Karim et al. suggest that PMA-qPCR was unable to differentiate infectious and UV-inactivated viruses due to the inability of UV light to damage the viral capsid (Karim et al., 2015). Conversely, traditional viral cell culture using cytopathic effects (CPE) for measuring infectious viruses are relatively insensitive, due to the fact that not all infectious viruses induce a measurable CPE in cell culture (Dilnessa, 2017). Consequently, traditional viral culture methods that incorporate qPCR as a measure on infectivity (known as integrated cell culture qPCR [ICC-qPCR]), have been used as an alternative tool for estimating infectious viruses in water (Qiu et al., 2015).

In the present study, we evaluated UV inactivation against an array of enteric viruses at two full-scale municipal WWTPs by monitoring the occurrence of human enteric viruses before and after UV treatment using qPCR, cell culture and ICC-qPCR. Of particular importance was the evaluation of infectious virus reduction by low-pressure UV treatment and the potential of reovirus as a useful viral indicator to assess UV disinfection efficacy (Betancourt and Gerba, 2016).

2. Materials and methods

2.1. Wastewater treatment plants and water sample collection

Two municipal wastewater treatment plants (WWTP 1 and WWTP 2), located in Calgary, Canada, were evaluated in this study. WWTP 1 and WWTP 2 have treatment capacities to serve a population of 250,000 and 1,000,000, respectively. The average daily discharge for WWTP 1 and WWTP 2 were 90,900 m^3 and 344,889 m^3, respectively. Both facilities provide tertiary treatment of wastewater through: i) 6-mm screening of raw sewage, ii) grit removal, iii) primary clarification, iv) biological nutrient removal through an activated sludge process, v) secondary clarification, and vi) UV disinfection of final effluent. WWTP 1 also has an effluent filtration step prior to UV disinfection, where the secondary effluent is filtered through an AquaDisk filter disc made of synthetic pile fabric with a 10-μm nominal pore size.

At WWTP 1, the filtered secondary effluent is UV disinfected (Trojan3000PLUS™) at an average capacity of 100 ML/d and a peak capacity of 240 ML/d. The system is designed to provide a UV dose of 24 mJ/cm^2 at peak flow from a total of 672 low-pressure, high-output, amalgam, 250 W lamps arranged in 4 lamp banks across 2 UV channels. At WWTP 2, the secondary effluent is disinfected in a Fisher & Porter UV disinfection system with an average capacity of 500 ML/d and a peak capacity of 1020 ML/d. The system is designed to provide an applied UV dose of 30 mJ/cm^2 from a total of 11,520 low-pressure, low-output, mercury, 65 W lamps arranged in 30 lamp banks across 10 UV channels.

Wastewater samples were collected monthly before UV treatment (pre-UV) and post-UV treatment from both WWTPs. The samples were collected in a reverse order of treatment processes (post-UV prior to pre-UV) in order to minimize potential cross contamination of samples with higher pathogen concentrations. The samples were collected upstream (pre-UV) and downstream (post-UV) of the light banks directly through access hatches from the continuously flowing streams of wastewater within the UV channels. The channels sampled varied between sampling events based on those in service at the time of sampling as part of the normal WWTP operations.

Samples were collected in 10 L plastic containers using a peristaltic pump (ISCO, 150 Portable pump). Tubing and connectors were sterilized by autoclaving prior to use in the pumping system and in advance of each sampling event. The sample was flushed through the pumping system for approximately 1 min prior to sample collection and the containers were rinsed three times with the sample matrix prior to sample collection. The flow rate of the pump was maintained at 4 L/min during sampling. The samples were typically collected approximately 3 h prior to the peak daytime flow at WWTP 1 and 2 h prior to peak daytime flow at WWTP 2. Samples were placed in coolers with ice packs, and transported to the laboratory for processing within 24 h.

Samples were collected from WWTP 1 for 21 months (Oct 2014–Jun 2016) and WWTP 2 for 14 months (Nov 2014–Dec 2015). A total of 51 pre-UV samples (WWTP 1 = 29, WWTP 2 = 22) and 50 post-UV samples (WWTP 1 = 28, WWTP 2 = 22) were analyzed for viruses. Additionally, 32 and 21 pre-UV samples from WWTP 1 and 2, respectively, were used for spiking studies to monitor method recoveries for viruses.

2.2. Virus concentration from water samples

The adsorption-elution method using NanoCeram 90-mm laminated disc filters (Argonide Corp, Sanford, FL, USA) was used to concentrate the virus from water samples, as previously described (Pang et al., 2012; Qiu et al., 2015). Briefly, 10 L of water sample were filtered and eluted with 1.5% beef extract buffer (pH 9.75), followed by flocculation with 0.5 mol/L $FeCl_3$ and centrifugation. The final volume of the concentrated viral suspension was adjusted to 15 mL using MEM medium (Invitrogen, Burlington, ON, Canada) with the pH at 7.2 ± 0.2. A flow chart outlining the sample processing procedure is provided in Fig. 1. The concentrated samples were aliquoted and stored at −70 °C until further processed.

2.3. Viral nucleic acid extraction and qPCR

Total viral nucleic acids were extracted from 200 μL of viral concentrates and eluted in 50 μL RNase-free water using the MagaZorb total RNA Prep kit (Promega, WI, USA) according to the manufacturer's instructions. Two step reactions including reverse