International Journal of Hygiene and Environmental Health xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Differential removal of human pathogenic viruses from sewage by conventional and ozone treatments

Hao Wang[a], Per Sikora[b,c], Carolin Rutgersson[a,d], Magnus Lindh[a], Tomas Brodin[e], Berndt Björlenius[f], D.G. Joakim Larsson[a,d], Heléne Norder[a,*]

[a] Department of Infectious Diseases, Institute of Biomedicine at Sahlgrenska Academy, University of Gothenburg, 41346 Gothenburg, Sweden
[b] Department of Pathology and Genetics, Institute of Biomedicine at Sahlgrenska Academy, University of Gothenburg, 41345 Gothenburg, Sweden
[c] Clinical Genomics Gothenburg, SciLife Labs, Gothenburg, Sweden
[d] Centre for Antibiotic Resistance Research (CARe) at University of Gothenburg, Sweden
[e] Department Ecology and Environmental Science, Umeå University, Umeå, Sweden
[f] Division of Industrial Biotechnology, KTH Royal Institute of Technology, AlbaNova University Center, SE-106 91 Stockholm, Sweden

ARTICLE INFO

Keywords:
Waste water
Next generation sequencing
Effluent
Adenovirus
Parvovirus

ABSTRACT

Sewage contains a mixed ecosystem of diverse sets of microorganisms, including human pathogenic viruses. Little is known about how conventional as well as advanced treatments of sewage, such as ozonation, reduce the environmental spread of viruses. Analyses for viruses were therefore conducted for three weeks in influent, after conventional treatment, after additional ozonation, and after passing an open dam system at a full-scale treatment plant in Knivsta, Sweden. Viruses were concentrated by adsorption to a positively charged filter, from which they were eluted and pelleted by ultracentrifugation, with a recovery of about 10%. Ion Torrent sequencing was used to analyze influent, leading to the identification of at least 327 viral species, most of which belonged to 25 families with some having unclear classification. Real-time PCR was used to test for 21 human-related viruses in inlet, conventionally treated, and ozone-treated sewage and outlet waters. The viruses identified in influent and further analyzed were adenovirus, norovirus, sapovirus, parechovirus, hepatitis E virus, astrovirus, pecovirus, picobirnavirus, parvovirus, and gokushovirus. Conventional treatment reduced viral concentrations by one to four log10, with the exception of adenovirus and parvovirus, for which the removal was less efficient. Ozone treatment led to a further reduction by one to two log10, but less for adenovirus. This study showed that the amount of all viruses was reduced by conventional sewage treatment. Further ozonation reduced the amounts of several viruses to undetectable levels, indicating that this is a promising technique for reducing the transmission of many pathogenic human viruses.

1. Introduction

Pathogenic human and animal viruses found in aquatic environments are usually shed from feces (enteric viruses), urine, and respiratory secretions from the infected host and enter into sewage water. The human viruses belong to different viral families. The most common viruses that are widely dispersed in sewage around the world include hepatitis A virus, hepatitis E virus, rotavirus, adenovirus, norovirus, astrovirus, parvovirus, coronavirus, poliovirus, and other enteroviruses (Hellmer et al., 2014; Laverick et al., 2004; Lodder and de Roda Husman, 2005). Not only human enteric viruses and animal pathogens, but also other viruses can be found in waters contaminated with sewage (Bosch, 1998; Cantalupo et al., 2011). If the pathogens are not removed in the treatment plants, they will be released into natural watersheds where many of them can persist for long periods (Fong and Lipp, 2005; Kotwal and Cannon, 2014). New hosts might be infected with these viruses through direct contact with contaminated water or by drinking it or by eating animals such as mollusks that have filtered and concentrated viruses from sewage-contaminated water (Nenonen et al., 2008).

In most western wastewater treatment plants, raw sewage is treated with combined mechanical, biological, and chemical processes such as screening, flocculation, sedimentation, and filtration. Gross pollutants and most organic and inorganic solids are removed during these steps. The effluent is thereafter either discharged into a receiving water system or reused for other purposes. Little is known, however, about the efficiency of removal of human viral pathogens from sewage by conventional treatment. Several studies have shown that such treatments

* Corresponding author at: Department of Clinical Microbiology/Virology, Sahlgrenska University Hospital, Guldhedsg 10B, 413 45, Gothenburg, Sweden.
E-mail address: Helene.norder@gu.se (H. Norder).

https://doi.org/10.1016/j.ijheh.2018.01.012
Received 13 November 2017; Received in revised form 24 January 2018; Accepted 24 January 2018

are efficient for the reduction of the parasites Giardia and Cryptosporium and for bacteria but have little effect on adenoviruses and enteroviruses (Li et al., 2015; Ottoson et al., 2006a; Rodriguez-Manzano et al., 2012). Additional disinfection after conventional treatment is applied in some treatment plants to further remove pathogens, such as treatment with peracetic acid, chlorination, and ultraviolet irradiation (Das, 2001; Kitis, 2004). These treatments are efficient for inactivating and removing bacteria and protozoa, but not for most enteric viruses (Freese and Nozaic, 2004; Shannon et al., 2008).

Ozone treatment is an alternative for removing microcontaminants in sewage because ozone is an extremely reactive oxidant and thereby a powerful disinfectant. It has been used for disinfection of drinking water in Europe since 1906 (Rice et al., 1981) and has also been installed in some sewage treatment plants (Oh et al., 2007; Rakness et al., 1993). The disinfecting ability of ozone treatment has been shown to be efficient for bacteria and parasites in clean water (Kim et al., 1999; Peeters et al., 1989), and this treatment also has been shown to reduce the concentrations of enteric viruses and bacteriophages (Burleson et al., 1975; Kim et al., 1980). However, the ability of ozonation to inactivate pathogens in wastewater might be hampered due to the high contents of organic materials in sewage (Burleson et al., 1975). One mechanism for reducing viable viruses in water by ozone treatment is assumed to be due to a conformation change of the viral capsid proteins by oxidation that either destroys the capsid or suppresses the virus/host cell receptor binding by changing the viral capsid proteins (Shannon et al., 2008). Previous studies conducted in wastewater treatment plants have shown that ozone disinfection might be highly efficient in inactivating bacteria and bacteriophages after conventional sewage treatments (Kim et al., 1999; Tyrrell et al., 1995), but knowledge regarding its effect for reducing human enteric viruses is relatively scarce.

We used next-generation sequencing (NGS) technology and real-time PCR to investigate the efficiency of virus removal in sewage by conventional treatment and to evaluate the effect of additional ozone treatment at a full-scale pilot plant in Sweden.

2. Materials and methods

2.1. Ozone treatment of conventionally treated sewage

The investigated sewage treatment plant in Knivsta, which is situated 50 km north of Stockholm, Sweden, uses traditional activated sludge treatment and receives primarily household waste from up to 12,000 population equivalents with a hydraulic design flow of 300 m^3/h. The initial treatment is mechanical with two parallel screens and an aerated grit chamber. The subsequent biological treatment includes activated sludge and reactors with carriers of active biofilms, and this is followed by a chemical treatment step where ferric chloride is added prior to the sewage entering the two final parallel sedimentation basins. Before release into the recipient river (Knivstaån), the effluent passes through a pond for the removal of phosphorus-containing fine particles. In 2015 an additional ozonation step treating the entire wastewater flow was added at the end of this process line. The full-scale ozonation step is divided into two parallel lines with a total maximum capacity of 560 m^3 effluent wastewater per hour. The ozonation step includes lifting pumps, the production of ozone in generator units, the injection of ozone by static mixers, contact tanks, and final contact filters. Each line contains two lifting centrifugal pumps (APEX ISF C, Bristol, UK), one static mixer (NR Mixer, Statiflo International Ltd, UK), one 50 m^3 stain-less steel contact tank with 5 m water depth and two compartments, one ozone destructor for off-gas (Primozone, Sweden), and two contact filters with a total area of 25 m^2 filled with 1 m light-expanded clay aggregates (Leca, Saint-Gobain Linköping, Sweden) for potential stripping or quenching of ozone residues in ozonated wastewater. The ozone is produced from evaporated liquid oxygen with > 99.5% O_2 (YaraPraxair, Sweden) diluted to 98% O_2 by addition of air in an ozone generator with a maximum production capacity of 2.4 kg O_3/h (GM48, Primozone, Sweden). An ozone dose of around 6 mg/L is added to the effluent wastewater through static mixers that transfer more than 98% of the added ozone to the wastewater. Most of the ozone reacts or degrades rapidly after the addition to the wastewater. Analysis of the water samples in the inlets and outlets of the contact tanks show ozone concentrations of 1–3 mg O_3/L and 0.1–0.3 mg O_3/L respectively. To verify that adequate amounts of ozone are transferred, the removal of pharmaceutical residues was calculated based on frequent inlet and outlet samples, and the results showed a typical removal efficiency for an ozone dose of 6–7 mg O_3/L, which is also reported in other studies and is related to total organic carbon (TOC) concentrations (Beijer et al., 2017). The hydraulic retention time in the contact tanks was on average 46 min and the minimum and maximum retention time was 15 and 180 min, respectively, during the period of ozonation that began in August 2015 and ended in February 2016.

The wastewater chemistry as well as effects on the recipient river were studied in parallel research projects before, during, and after the ozonation trial. For the present study, flow-proportional 24 h composite samples of influent (5 L per sample), effluent (10 L), effluent after ozonation (10 L), and effluent after the dam (outlet; 10 L) were collected on three occasions in 2015 (November 30 until December 4 (week 49); December 8 until December 12 (week 50); and December 19 until December 22 (week 51/52)), with time adjustment for the flow rate to represent the “same” water. All samples were cooled during sampling and then frozen at −20 °C until further processing.

2.2. Concentration of viruses in water

The water samples were first centrifuged at 8000 × g for 15 min before filtration twice through Nano-Ceram cartridge filters (Argonide, Sanford, Florida, USA) at an average flow rate of 2.5 L/min. The viruses were electrostatically attached to the filter from which they were eluted by 330 mL of 0.2 M phosphate buffer containing 0.05 M glycine (pH 9.5). The eluate was collected, and the pH was adjusted to 7.5 by the addition of 1 M HCl. The eluate was thereafter filtered through a 0.65/0.45 µm Sartobran Capsule filter (Sartorius, Göttingen, Germany) to remove remaining debris and most bacteria. The filtrate was then ultracentrifugated in eight tubes at 50,000 rpm for 4 h at 4 °C. The pellet in each tube was resuspended in 300 µL 10 mM Tris-HCl (pH 8.0) overnight, pooled, and stored at −80 °C until analysis.

2.3. Evaluation of the efficiency of the viral concentration

A fixed amount of human mastadenovirus 2 (HAdV-2) was added to 3.5 L raw sewage, and the sewage was concentrated by the method above. One milliliter of unconcentrated water and one mL from each concentration step was collected and analyzed for adenovirus by quantitative real-time PCR (qPCR) and isolation on cell culture.

Nucleic acids in the water samples were extracted from 200 µL concentrated sample using the QIAGEN DNA Blood and Tissue kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. qPCR for adenovirus was performed in a 20 µL reaction mix containing 2 µL extracted nucleic acids, 1 × universal DNA Master Mix (ThermoFisher, Waltham, MA, USA), 0.5 µM of forward and reverse primer, and 0.4 µM of probe (Table S1). The cycling conditions were 50 °C for 2 min and 95 °C for 10 min followed by two-step cycling 45 times at 95 °C for 15 s and 60 °C for 1 min on an ABI 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, CA, USA).

For isolation of HAdV-2 on cell cultures, 100 µL of 10-fold serial dilutions (1/10 to 1/10,000) in Eaglés minimal essential medium (MEM, Gibco, Waltham, MA, USA) from each concentration step were inoculated in duplicate into wells in 48-well plates (ThermoFisher) containing confluent monolayers of A549 cells. The plates were incubated at 37 °C in an atmosphere of 5% CO_2 for 2 h, after which the medium containing virus was removed from each well, followed by addition of 500 µL MEM containing 4% fetal calf serum and 1% L-