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Diversity of DNA viruses in effluents of membrane bioreactors in Traverse City, MI (USA) and La Grande Motte (France)

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ABSTRACT

This study assesses diversity of DNA viruses in the effluents of two membrane bioreactor (MBR) wastewater treatment plants (WWTPs): an MBR in the United States and an MBR in France. Viral diversity of these effluents is compared to that of a conventional activated sludge WWTP in the U.S. Diversity analysis indicates *Herpesvirales* to be the most abundant order of potentially pathogenic human DNA viruses in wastewater treated effluent in all utilities. Other potentially pathogenic human viruses detected include *Adenoviridae*, *Parvoviridae*, and *Polyomaviridae*. Bacteriophage order *Caudovirales* comprises the majority of DNA virus sequences in the effluent of all utilities. The choice of treatment process (MBR versus activated sludge reactor) utilized had no impact on effluent DNA viral diversity. In contrast, the type of disinfection applied had an impact on the viral diversity present in the effluent.

1. Introduction

Viruses are potentially the most hazardous pathogens among those found in wastewater (Sidhu et al., 2008; Toze, 1997). They are also generally more difficult to detect in environmental samples. A high diversity of human viral pathogens is present in the environment (approximately 200 recognized human viral pathogen species) and is further elevated in samples affected by pollution. Moreover, additional species are continuously discovered (Bibby, 2013). It has been estimated that 2 to 12 million people die every year from waterborne diseases. While the majority of the outbreaks are caused by unidentified agents, it has been suggested that most agents in question are enteric viruses in groundwater and surface water bodies (US EPA, 2006). Despite recent advances in water and wastewater treatment technology, waterborne diseases still pose a serious threat to public health across the world (Toze, 1997).

The Contaminant Candidate List (CCL) compiled and periodically updated by the U.S. Environmental Protection Agency includes contaminants that are known or anticipated to occur in public water systems, and which may require regulation under the Safe Drinking Water Act. Included on the CCLs are numerous viruses, such as the double-stranded DNA adenoviruses. Double-stranded DNA viruses have been shown to be more resistant to UV disinfection when compared with other virus types (Gerba et al., 2002). Adenoviruses have been investigated and detected in wastewater in prior studies via conventional methods (Bofill-Mas et al., 2010, 2006; Cantalupo et al., 2011; Francy et al., 2012; Hewitt et al., 2011; Jacangelo et al., 2003; Katayama et al., 2008; Kuo et al., 2010; La Rosa et al., 2010; Petrinca et al., 2009; Prado et al., 2014, 2011; Schlindwein et al., 2010; Sedmak et al., 2005; Sima et al., 2011; Simmons et al., 2011; Simmons and Xagoraraki, 2011; Toze, 1999) and determined to be among the most abundant human viruses in WWTP effluent (Hata et al., 2013; La Rosa et al., 2010). It has been concluded that adenoviruses may serve as indicators for general viral contamination (La Rosa et al., 2010; Okoh et al., 2010).

In addition to human viral pathogens, bacteriophages may also have a significant impact on the natural water that receives treated effluent from a wastewater treatment facility. It has been shown that bacteriophages are strong regulators of microbial diversity within a WWTP (Shapiro et al., 2009). Bacteriophages can have an effect on the microbial community as well as on eukaryotic members of an ecosystem that rely on bacteria (Díaz-Muñoz and Koskella, 2014), making phages all the more important in

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maintaining ecological health of the aquatic environment. The majority of bacteriophages contain a DNA genome, making DNA bacteriophages by far the most prevalent group of viruses impacting the microbial community (McAuliffe et al., 2007).

Both conventional activated sludge (AS) WWTPs and MBRs release pathogenic viruses into surface water (Kuo et al., 2010; Simmons et al., 2011; Simmons and Xagoraraki, 2011). It has been demonstrated that MBRs can remove several viruses (including adenovirus) at higher efficiencies compared to conventional AS utilities (Francy et al., 2012; Xagoraraki et al., 2014); for example, log removal of adenovirus ranged from 1.3 to 2.4 in conventional AS utilities (Haramoto et al., 2007; Hewitt et al., 2011; Katayama et al., 2008), whereas MBRs accomplished 3.4 to 5.6 log removals (Kuo et al., 2010; Simmons et al., 2011; Simmons and Xagoraraki, 2011). Though removal of specific viruses in these utilities has been investigated, there has been little application of metagenomics in studying the viral diversity of MBR effluent and how it compares to that of a conventional AS plant.

Next-generation DNA sequencing has recently been applied to study viral metagenomes (viromes) in environmental samples (Alhamlan et al., 2013; Gomez-Alvarez et al., 2012; Kim et al., 2015; Wommack et al., 2011) as well as at different stages of the wastewater treatment (Aw et al., 2014; Bibby et al., 2011; Bibby and Peccia, 2013; Cantalupo et al., 2011; Rosario et al., 2009; Tamaki et al., 2012). These methods have been shown to provide more conservative estimates of viral occurrence compared to the rates detected by qPCR (Bibby and Peccia, 2013). The advantage of metagenomics is that it allows comprehensive characterization of microbial communities. However, metagenomic methods do not assess infectivity and the sequence annotation is only as reliable and robust as the assembly methods and database used for analysis. Metagenomic analysis is presently only capable of identifying a fraction of the viruses present in the environment (Aw et al., 2014; Cantalupo et al., 2011).

Nonetheless, metagenomic methods are an effective tool for analyzing the microbial diversity of environmental samples. Though wastewater has been investigated with metagenomic methods, there has been little use of metagenomics to evaluate the microbial diversity of wastewater effluents, and to the authors' knowledge none comparing different types of wastewater utilities or the impact of disinfection. The specific objectives of this study are: 1) To investigate the diversity of human DNA viruses detected in effluents of MBR WWTPs equipped with membranes of different pore sizes; 2) To assess the diversity of DNA bacteriophages in MBR WWTP effluents; 3) To compare the diversity of DNA viruses in MBR WWTP effluents with that in a conventional WWTP effluent and a natural water; and 4) To investigate the impact of disinfection on DNA virus diversity in WWTP effluent.

2. Materials and methods

2.1. Sampling locations

The selected MBR WWTPs were facilities located in Traverse City (Michigan, USA) and La Grande Motte (Languedoc-Roussillon-Midi-Pyrénées, France), which are both tourism destinations. Sampling was performed during the warmer seasons when the population of each location is increased due to the large number of vacationers. Effluent samples were collected at three wastewater treatment utilities. In Spring 2013, sampling was performed at the East Lansing WWTP (East Lansing, MI), which is a conventional activated sludge plant employing hypochlorite disinfection, and the Traverse City WWTP (Traverse City, MI), which employs MBR technology with ultrafiltration membranes of 0.04 μm nominal pore size and UV disinfection. A sample was taken from the treated effluent both before disinfection and after disinfection at each of the Michigan utilities. In summer 2015, sampling was performed at La Grande Motte WWTP (La Grande Motte, France), which is also an MBR plant but employs microfiltration membranes of 0.45 μm nominal pore size and does not have an additional disinfection step. A sample was also taken from the treated effluent at this utility. Main operational parameters for these utilities are summarized in Table 1.

2.2. Sample collection

Approximately 300 L of sampled treated effluent was passed through a NanoCeram Virus Sampler filter (Argonide Corporation) at a rate of 11–12 L/min using a previously described method (Kuo et al., 2010). Samples from the two Michigan WWTPs were kept on ice and transported to Michigan State University (East Lansing, MI), while samples from France were kept on ice and transported to Université de Montpellier (Montpellier, France) for further processing.

2.3. Sample processing

All NanoCeram filters used to concentrate the treated effluent samples were eluted according to the standard method (US EPA, 2001) within 24 h of initial sampling. Briefly, a 1.5% w/v beef extract (0.05 M glycine, pH 9.0–9.5) solution was used as the eluent. The filters were submerged for a total of 2 min (two separate 1 min elutions) in filter housings with 1 L of beef extract added to the pressure vessel. After the beef extract was passed through each filter, pH of the 1 L of the eluate was adjusted to 3.5 ± 0.1 using 1 M HCl and flocculated for 30 min. Further concentration of the solution was performed by two stages of centrifugations for 15 min at 2500 g and 4 °C. The supernatant was then decanted and the process was repeated until all the beef extract solution was centrifuged. The accumulated pellets were resuspended using 30 mL of 0.15 M sodium phosphate (pH 9.0–9.5) and mixed until the pellet was mostly dissolved. The pH was then adjusted to 9.0–9.5 using 1 M HCl. The solution was placed into a 50 mL centrifuge tube and centrifuged for 10 min at 4 °C at 7000 g. The supernatant was poured off into a separate 50 mL centrifuge tube, the pH was adjusted to 7.0–7.5 for stabilization of the virus particles, and the pellet was discarded. The supernatant was loaded into a 60 mL syringe and passed through a 0.22 μm sterilized filter for removal of bacteria, fungi and other contaminants. All samples were completely mixed and placed into 2 mL cryogenic tubes. Samples from France were shipped on dry ice to Michigan State University, where all samples were stored at −80 °C until further analysis.

2.4. Nucleic acid extraction

Viral DNA was extracted using a MagNA Pure Compact Instrument (Roche Applied Science) and a MagNA Pure Compact Nucleic Acid Isolation Kit according to the manufacturer's instructions. A 400 μL sample was loaded in the instrument and yielded an elution volume of 100 μL. DNase treatment is performed by the MagNA Pure Compact prior to extraction. The extracts were stored in a freezer at −20 °C. Following extraction the quantity of viral DNA extracts from all samples were verified for quality control purposes using the NanoDrop spectrophotometer (NanoDrop® ND-1000, Wilmington, DE).

2.5. Metagenomic analyses

Viral DNA extracts were sequenced on an Illumina platform