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Removal of phages and viral pathogens in a full-scale MBR: Implications for wastewater reuse and potable water

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A B S T R A C T

The aim of this study was to demonstrate how seasonal variability in the removal efficacy of enteric viral pathogens from an MBR-based water recycling system might affect risks to human health if the treated product were to be used for the augmentation of potable water supplies. Samples were taken over a twelve month period (March 2014–February 2015), from nine locations throughout a water recycling plant situated in East London and tested for faecal indicator bacteria (thermotolerant coliforms, intestinal enterococci n = 108), phages (somatic coliphage, F-specific RNA phage and *Bacteroides* phage (GB-124) n = 108), pathogenic viruses (adenovirus, hepatitis A, norovirus GI/GII n = 48) and a range of physico-chemical parameters (suspended solids, DO, BOD, COD). Thermotolerant coliforms and intestinal enterococci were removed effectively by the water recycling plant throughout the study period. Significant mean log reductions of 3.9–5.6 were also observed for all three phage groups monitored. Concentrations of bacteria and phages did not vary significantly according to season ($P < 0.05$; Kruskal-Wallis), though recorded levels of norovirus (GI) were significantly higher during autumn/winter months ($P = 0.027$; Kruskal-Wallis). Log reduction values for norovirus and adenovirus following MBR treatment were 2.3 and 4.4, respectively. However, both adenovirus and norovirus were detected at low levels (2000 and 3240 gene copies/L, respectively) post chlorination in single samples. Whilst phage concentrations did correlate with viral pathogens, the results of this study suggest that phages may not be suitable surrogates, as viral pathogen concentrations varied to a greater degree seasonally than did the phage indicators and were detected on a number of occasions on which phages were not detected (false negative sample results).

1. Introduction

Treated wastewater is increasingly recognised to be a valuable and sustainable resource, particularly as greater affluence, population growth and climate change are projected to increase the demand on limited conventional freshwater supplies. Reuse of treated wastewaters is already used to supplement water supplies for non-potable uses in many parts of the world and the practice has the potential to provide potable water as long as the risks to human health associated with the consumption of wastewater contaminants, including pathogenic microorganisms, are comprehensively and continuously controlled. Currently, there is no consensus as to what standards are appropriate to govern wastewater reuse (Paranychianakis et al., 2015). However, to date the most stringent regulations have been issued in the United States (US) by the California Department of Public Health (2014), which relate to indirect reuse of wastewaters as a source of raw drinking water through groundwater recharge. These regulations require a 12 log reduction in enteric virus concentrations, a 10 log *Giardia* cyst reduction and a 10 log *Cryptosporidium* oocyst reduction.

Membrane bioreactor (MBR) technology has been proposed as being highly suitable for water reuse (Hai et al., 2014). A membrane bioreactor is a treatment process that achieves separation of solids by combining a permselective membrane with a biological process (Judd, 2011; De Luca et al., 2013). Solids are therefore removed by the membrane, rather than a secondary settling process. Membranes have relatively small pore sizes (0.03–0.40 μm), resulting in the physical exclusion of a wide variety of microorganisms (Ottoson

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et al., 2006; Simmons et al., 2011). The majority of viruses are smaller than the membrane pore sizes present in MBR treatment systems. Nevertheless studies have reported virus removal, as reviewed by (Hai et al., 2014). Studies, performed at both pilot-scale and within full-scale municipal wastewater plants, have demonstrated that microbial removal in MBR systems is more effective than in conventional activated sludge treatment systems (Ottoson et al., 2006; Marti et al., 2011).

There is some disagreement as to the most important mechanisms for virus removal in MBR. Although removal is thought to be primarily influenced by the development of a biofilm on the membrane, and by virus adsorption to this biomass (Da Silva et al., 2007; Wong et al., 2009; Hirani et al., 2014; Van den Akker et al., 2014), recent research has reported that virus removal is ensured by a smaller membrane pore size (0.04 μm), even after chemically-enhanced membrane backwashes (Chaudhry et al., 2015). In contrast, Miura et al. (2015) found that virus adsorption to mixed-liquor suspended solids (MLSS) made an important contribution to virus removal in a pilot-scale 'anoxic-oxic' (AO) MBR process with a nominal membrane pore size of 0.4 μm.

Other workers have monitored pathogenic viruses in MBR directly using quantitative polymerase chain reaction (qPCR) methodologies, which are based on the detection of nucleic acids, rather than of complete, infectious particles (virions). In a range of studies, MBR treatment systems have recorded log reductions of between 3.9 and 5.5 log units for adenovirus (Adv), 1.3 and 4.1 log units for sapovirus (SaV), 0.2 and 5.7 log units for norovirus genogroup II (NoV GII), 0.3 and 3.6 log units for enterovirus (EnV), and 3.3 and 6.8 log units for calcivirus (CaV) (Chaudhry et al., 2015; Kuo et al., 2010; Miura et al., 2015; Ottoson et al., 2006; Sima et al., 2011; Simmons et al., 2011). Whilst qPCR allows for the detection of unculturable pathogens, such as NoV GI/II, the detection of nucleic acids from damaged particles in treated product, may lead to overestimates of the risk to human health of reuse water. The method also remains prohibitively expensive as a means to monitor routinely the wide range of pathogens of public health concern that may be present in waters and wastewaters, as a component of regulatory practise. Therefore, the enumeration of viruses capable of infecting bacteria (bacteriophages or phages) has been proposed as a way to model the removal of enteric viruses in treatment systems (IAWPRC, 1991).

In numerous studies, phages have been shown to be the most suitable available indicator of the presence of enteric viruses in water and wastewaters (Jofre et al., 1986; Gantzer et al., 1998; Purnell et al., 2011; Ebdon et al., 2012; Jofre et al., 2014), because they have a similar structure, morphology, size and resistance to inactivation to the viral pathogens of concern. Membrane bioreactor systems with varying nominal pore sizes (0.04–0.4 μm), have been shown to remove up to 7.1 log units of various indigenous and artificially introduced 'spiked' phages (Chaudhry et al., 2015; Hirani et al., 2012; Marti et al., 2011; Ueda and Horan, 2000; Wong et al., 2009; Zanetti et al., 2010; Zhang and Farahbakhsh, 2007). The lowest log removal values were most frequently attributed to 'spiked' phages with clean membranes, rather than indigenous phages that were more likely to be associated with solids (Shang et al., 2005). Purnell et al. (2015) studied the removal of somatic coliphages (SC), F-specific RNA (F-RNA) phages and *Bacteroides fragilis* (GB-124) phages through a full-scale MBR system with submerged aerated ultra-filtration membranes (nominal pore size of 0.04 μm) and posited that SC may represent a potential conservative model by which to assess the efficacy of viral pathogen removal in MBR systems, although they did not elucidate the relationships between candidate phages and specific enteric viral pathogens of public health significance.

Globally, as freshwater resources become more stressed, wastewater reuse is increasingly considered to be an acceptable way to provide non-potable and more recently to augment potable water supplies. However, the primary concern of wastewater reuse is to ensure that the potential public health consequences are properly understood and effectively minimized. Unfortunately, the lack of uniform water quality guidelines and uncertainties about the removal efficacy of the available wastewater reuse technologies has adversely affected the development, public perception, and economic viability of wastewater reuse projects. Membrane bioreactor technology (MBR) has the potential to provide treated wastewater of a sufficient quality to augment potable water supplies, but knowledge gaps with regard to the removal of viruses in MBR and the relationship between pathogenic viruses and their potential surrogates remain. This has meant that the full potential of such MBR technologies to treat wastewater for different reuse purposes is only now beginning to be recognised. In addition, whilst research has shown encouragingly high removal values for viruses, studies of full-scale MBR's remain limited (Da Silva et al., 2007; Kuo et al., 2010; Chaudhry et al., 2015), and to date, no full-scale study has investigated the removal of potential viral surrogates and pathogenic viruses over the course of an entire year.

In light of this, the aim of this study was to demonstrate how seasonal variability in the removal efficacy of enteric viral pathogens from an MBR-based water recycling system might affect risks to human health if the treated product were to be used for the augmentation of potable water supplies. This was achieved by; a) monitoring the virus removal efficacy of a full-scale MBR treatment system over an entire year (using traditional faecal indicators, enteric phages, and viral pathogens), b) identifying seasonal fluctuations in system performance over this period and c) assessing whether enteric phages may be used as surrogates for the presence of enteric viral pathogens, which could in turn inform future monitoring and regulation practises.

2. Materials and methods

2.1. The membrane bioreactor water recycling plant

The Old Ford Water Recycling Plant (WRP) in London, UK, treats raw municipal wastewater, taken from the Northern Outfall Sewer, to provide 574 m^3 of non-potable water per day to the Queen Elizabeth Olympic Park for the purposes of parkland irrigation, venue toilet flushing and to supplement rain water harvesting systems (Hill and James, 2014). The raw wastewater is predominantly domestic and light commercial in origin with additional surface drainage inputs from a relatively large catchment that has a population of approximately 360,000. The Old Ford WRP receives a relatively small proportion of the flow from the Northern Outfall Sewer. The series of unit processes that constitutes full treatment of the wastewater are summarised in Fig. 1. The processes comprise a pre-treatment stage with gross solids removal using underground septic tanks (hydraulic retention time (HRT) 6.46 h) followed by 1 mm fine screens to remove large debris (hair and fibres that could potentially damage the membrane). Screened wastewater flows to an above-ground activated sludge tank (that operates at a mixed-liquor suspended solids concentration of 7 g/L), which is segregated into anoxic (HRT 0.52 h) and aerobic zones (HRT 2.50 h). The wastewater then flows to a cross-flow membrane tank that holds three racks of ultra-filtration membranes (with a nominal pore size of 0.04 μm and a HRT of 0.18 h (Siemens Water Technologies Memcor Ltd)), which have pulsated air scouring to mitigate membrane fouling and are periodically cleaned in place (1500 mg/l of hypochlorite for 6–8 h every 90 days). In addition, maintenance washes are performed every seven days (300 mg/l of hypochlorite for 45 min). The reclaimed wastewater undergoes post-treatment