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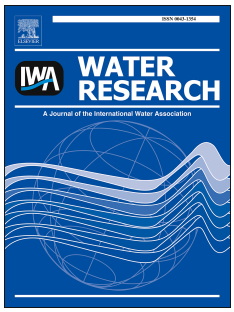

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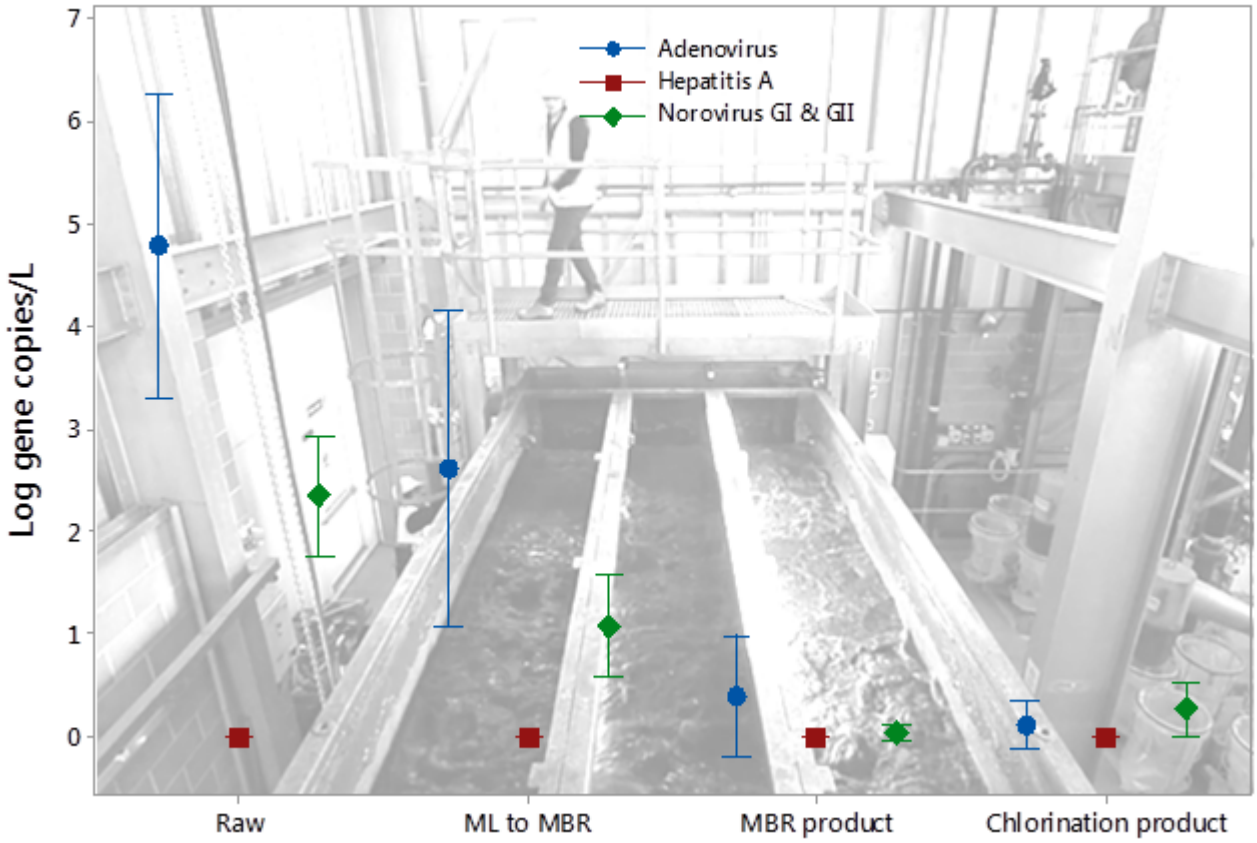

REMOVAL OF PHAGES AND VIRAL PATHOGENS IN A FULL-SCALE MBR: IMPLICATIONS FOR WASTEWATER REUSE AND POTABLE WATER

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Abstract

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).

Keywords

Bacteriophages; Health; Pathogenic virus; Removal efficacy; Wastewater reuse

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 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 over-estimates 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 et al., 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; 2011; Chaudry 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 Figure 1. The processes comprise a pre-treatment stage with gross solids removal using underground septic tanks (hydraulic retention time (HRT) 6.46 hrs) 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 hrs) and aerobic zones (HRT 2.50 hrs). 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 hrs (Siemens Water Technologies Memcor Ltd)), which have pulsated air scouring to mitigate membrane fouling and are periodically cleaned in place (1500mg/l of hypochlorite for 6-8 hrs every 90 days). In addition, maintenance washes are performed every seven days (300 mg/l of hypochlorite for 45 minutes). The reclaimed wastewater undergoes post-treatment in the form of granular activated carbon (GAC), primarily for colour removal (HRT 0.58 hrs) and chlorination (0.3 to 1.5 mg/l chlorine residual, contact time 0.51 hrs) before entering a dedicated 3.65 km distribution network.

2.2 Monitoring programme

Levels of a selection of faecal indicator organisms (thermotolerant coliforms (TTC), intestinal enterococci (IE), somatic coliphage (SC), F-specific RNA phages (F-RNA) and phages infecting *Bacteroides fragilis* strain (GB124)) and enteric viral pathogens of public health concern (norovirus GI & GII (NoV), adenovirus (AdV), and hepatitis A virus (HAV) were monitored at multiple points in the WRP. Figure 1 shows the nine sampling points (1-9), at which phages and faecal indicator bacteria were monitored and the four sampling points (a-d) at which viral pathogens were monitored. The lower number of sampling points for viral pathogens was a practical response to the large volumes of sample (10L) that were required for their enumeration. All samples were transported to the University of Brighton laboratory, in the dark, at 4^{o}C, for analysis within four hours of collection.

2.3 Quantification of faecal indicator organisms

TTC and IE were enumerated by membrane filtration on mFC agar, and M*Enterococcus* agar (Difco), respectively, in triplicate, according to standard methods (Anon., 2000) and expressed as colony-forming units per 100 ml (CFU/100 ml). SC, F-RNA and GB124 phages were quantified by enumerating plaque-forming units (PFU/100ml), in triplicate, on Modified Scholten's Media, Tryptone Yeast Glucose Media and *Bacteroides* Phage Recovery Media, respectively, according to standardised double-agar-layer methods (Anon, 2001[a-c]). Host strain WG5 was used for SC enumeration, *Salmonella typhimurium* WG49 was used for F-RNA enumeration, and *Bacteroides fragilis* was used for GB124 phage enumeration.

2.4 Concentration of samples for detection of pathogenic viruses

Samples were concentrated using a skimmed milk flocculation procedure, as described previously by Calgua et al. (2013). Before concentration, the conductivities of all samples were measured, and all those with a conductivity ≤1.5mS were conditioned by adding artificial sea salts (Sigma-Aldrich, UK) to obtain values ≥1.5mS. Once samples were conditioned, a pre-flocculated 1 % (w/v) skimmed milk solution (PSM) was prepared by dissolving 10 g skimmed milk powder (Difco, USA) into 1L of artificial sea water at a pH of 3.5. The pH of the sample was reduced to 3.5 by adding HCL 1N. Then, PSM was added to achieve a final concentration of 0.01 % (w/v) skimmed milk in each sample. Samples were stirred for 8 h at room temperature using a magnetic stirrer (Grant, UK) and flocs were allowed to form and to sediment by gravity for an additional 8-10 hrs. The supernatant was removed with care, so as not to disturb the sedimented floc. The final volume of 500 ml, including the sedimented floc was transferred to centrifuge tube(s) and centrifuged at 7000 x g for 30 min at 4^{o}C. The supernatant was carefully removed and the pellet was re-suspended and dissolved in phosphate buffer (1:2, v/v of Na_2HPO_4 0.2 M and NaH_2PO_4 0.2M) at pH

200 7.5, at a ratio of 1 ml of phosphate buffer per 1 L of concentrated sample. The resulting viral
201 concentrate was then stored at -80^{o}C.
202
203 **2.5 Nucleic acid extractions**
204 Nucleic acid extractions (DNA and RNA) were performed using the QIAamp Fast DNA stool
205 Mini Kit and the QIAamp Viral RNA Mini Kit (Qiagen, USA), respectively, according to the
206 manufacturer's instructions. For DNA, 220 µl of concentrated sample were extracted and
207 eluted in 200 µl of elution buffer. For RNA, 140 µlof concentrated sample were extracted
208 and eluted in 60 µl of elution buffer. Nucleic acids were stored at -80^{o}C until further analysis.
209 Following extraction, the quantities of viral nucleic acid extracts from all samples were
210 checked using a NanoDrop Lite Spectrophotometer (NanoDrop Lite®, Labtech, UK).
211
212 **2.6 Quantitative real-time PCR assays**
213 All qPCR assays were performed using a Qiagen Rotor-gene Q. 'Positive', 'no template' and
214 'internal extraction' controls were used in every assay run. Dilutions were used to decrease
215 inhibition in samples. Genesig kits were used for the detection of AdV type F and G, NoV
216 GI/II genomes and HAV virus genomes (PrimerDesign™, UK). The target sequence (hexon
217 gene) has previously been shown to be a good genetic marker for AdV in other real-time
218 PCR-based studies (Hernroth et al., 2002). The primers present 100% homology with all
219 reference sequences included in the NCBI database (as of 1st November 2007) and therefore
220 these kits are considered to have very broad detection profiles. For AdV, each sample (5 µl)
221 was prepared with a 15 µl reaction mix, containing 10 µl PrecisionPLUS^{TM} 2 x qPCR
222 MasterMix, 1 µl primer/probe mix, 1 µl internal extraction control primer/probe mix and 3 µl
223 RNAse/DNAse free water. For both NoV GI/II and HAV detection, each sample (5 µl) was
224 prepared with a 15 µl reaction mix, containing 10 µl PrecisionTM OneStep 2 x qRT-PCR

MasterMix, 1 µl primer/probe mix, 1 µl internal extraction control primer/probe mix and 3 µl RNAse/DNAse free water. Thermal conditions for AdV consisted of enzyme activation for 2 minutes at 95 ^{o}C, followed by 50 cycles of denaturation for 10 seconds at 95 ^{o}C and data collection for 60 seconds at 60 ^{o}C. NoV and HAV detection followed the same thermal conditions, with the addition of a reverse transcription stage of 10 minutes at 42 ^{o}C before enzyme activation. Results were expressed as log gene copies per 1 L.

2.7 Log removal values

Log removal values (LRV) for microbial parameters were calculated for the three treatment stages shown in Figure 1, according to equation (1). Where $C_{initial}$ is the starting concentration of microorganisms, and C_{final} is the concentration of microorganisms after the treatment stage of interest.

$$LRV = -\log_{10} (C_{initial} / C_{final}) \quad (1)$$

3. Results and discussion

3.1 Faecal indicator bacteria

Mean log levels of TTC and IE recorded at the nine sampling points through the Old Ford WRP are presented in Figure 2. Mean numbers following MBR treatment were reduced to <1 CFU/100 ml for both IE and TTC. Following chlorination, both IE and TTC were undetected (<1 CFU/100ml) in all samples. Mean removal values following the MBR stage were 7.0 log and 6.2 log for TTC and IE, respectively. Concentrations of faecal indicator bacteria throughout the treatment stages did not vary significantly according to season ($P<0.05$; Kruskal-Wallis).

3.2 Phages

The mean log number of the three phage groups somatic coliphages (SC), F-specific RNA phages (F-RNA); and *Bacteroides* GB124 phage (GB124), detected at each stage of the Old Ford WRP system are shown in Figure 3. Of the three bacteriophages monitored, SC were shown to be present at the highest concentrations throughout the system, with levels as high as 2.5×10^6 PFU/100ml recorded in the raw wastewater. Significant mean reductions in SC numbers were observed following MBR treatment (5.6 log). GB124 and F- RNA phages also demonstrated notable reductions following MBR treatment, with mean reductions of 4.0 and 3.9 log, respectively. GB124 and F-RNA phages were undetected (<1 PFU/100ml) in all post-MBR treatment samples. SC were the only group observed in MBR product. This finding is consistent with a previous study by the same authors (Purnell et al., 2015). The concentrations of the three phage groups detected at each stage of treatment did not vary significantly according to season ($P<0.05$; Kruskal-Wallis).

The detection of SC in MBR product may possibly be explained by “breakthrough” of viruses at a threshold concentration. Alternatively, Purnell et al. (2015) determined SC morphology post-MBR treatment and found only phages belonging to the family *Microviridae* suggesting that morphology and attachment characteristics could be the major factors influencing removal (as SC are a morphologically diverse group of phages compared with F-RNA and GB124 phages). Surface electrostatic charge affects the adsorptive behaviour of viruses (Michen and Graule, 2010). Miura et al. (2015) also suggest that the interfacial characteristics of viruses cause diversity of attachment behaviour to MLSS, resulting in varying removal properties in an MBR process.

3.3 Enteric viral pathogens

Four groups of viral pathogens were monitored: namely, adenovirus (AdV), hepatitis A (HAV), norovirus genogroup I and norovirus genogroup II (NoV GI/GII). The results for NoV GI/II (human-associated genogroups) were combined to demonstrate the removal of NoV as a whole. Figure 4 presents the mean levels of these viral groups at each of the four selected stages of the Old Ford WRP. Of the enteric viruses monitored, AdV were observed at the highest concentrations, with levels as high as 1.2×10^8 gene copies/L recorded in the raw wastewater. NoV GI/GII were detected in raw wastewater at a level of 6.2×10^5 gene copies/L. Log reduction values post-MBR for NoV and AdV were 2.3 and 4.4, respectively. It is important to note that both AdV and NoV were detected after the chlorination stage of the treatment system in single samples (AdV was detected in July, whereas NoV was detected in February). Conversely HAV was undetected (<2 gene copies/reaction) in all samples.

Concentrations of AdV and NoV GI/II fluctuated to a greater degree than those of the enteric phages monitored. Concentrations of viral pathogens in raw wastewater can vary because excreted loads change according to the proportion of the population infected with these viruses at any one time. Interestingly, levels of AdV in the raw wastewater were observed to increase from autumn through to winter, though this variation was not statistically significant (P= 0.085; Kruskal-Wallis). In temperate climates, NoV has been shown to be more prevalent in cooler months (de la Noue, 2014) and in this study NoV GI was detected at higher levels in autumn and winter months, a seasonal variation that was statistically significant (P=0.027; Kruskal-Wallis). Whilst levels were not significantly different across the four seasons (P=0.139; Kruskal-Wallis), recorded levels of NoV GII were highest in the summer months.

Recorded log reduction of the enteric viral pathogens was comparable with those of the enteric phages. However, AdV and NoV were detected in chlorinated product. Pathogenic viruses were detected using qPCR, a molecular technique that does not distinguish between viable (potentially infectious) and non-viable (non-infectious/damaged) virus particles. So, whilst this result does suggest AdV and NoV GI/II passed through the membrane, the data do not reveal whether these organisms were intact and infectious particles of public health concern after the chlorination stage. Despite evidence of very occasional viral "breakthrough", log reduction values suggested that the system is capable of acting as an effective physical barrier to the transmission of human viral pathogens. This observation is also supported by recent research from other similar full-scale systems that have demonstrated viral pathogen removal rates of greater than 4 log (Chaudhry et al., 2015; Kuo et al., 2010; Simmons et al., 2011).

3.4 Enteric bacteriophages as surrogates for viral pathogens

Figures 3 and 4 present mean concentrations for all the studied viral pathogens and enteric phages throughout the Old Ford WRP.. Levels of all three phage groups were found to correlate positively with levels of both AdV and NoV GI/II ($P<0.05$; Spearman's Rank) when data from all seasons were analysed together, as shown in Table 1. However, levels of phage indicators did not correlate with those of NoV genogroups during the winter months or with AdV during the summer months ($P>0.05$; Spearmans Rank). The lack of correlation between phage indicators and NoV GI/II during the winter may be because there is increased prevalence of NoV in the human community (and consequently its wastewater) at this time of the year, whereas concentrations of the three phage groups remain relatively stable throughout the year. Correlation between indicators and AdV is not evident during the summer months. This may be the result of potential differential inactivation of the phage

groups and AdV, but further investigation is warranted before definitive conclusions are drawn. Of the three phage groups, SC demonstrated the strongest positive correlation with AdV and NoV across all four seasons and the log reductions of the phages did not differ significantly from those of the viral pathogens ($P>0.05$, Kruskal-Wallis). A number of instances of phage detection in samples post-MBR can be considered to be 'false positive' sample results (in terms of using phages as a surrogate for viral pathogens), because phage groups were detected when viral pathogens were not. More importantly for the protection of public health, there were also a number of 'false negative' sample results, in which viral pathogens were detected in post-MBR samples but phages were not. For both FRNA and GB124 phages there were no false positive sample results post-MBR throughout the length of this study, as these groups were absent in MBR and chlorinated product. However, on the four occasions viral pathogens on which detected post-MBR treatment, neither phage group were present (4/4 false negatives sample results). Somatic coliphages were recorded on a single occasion on which viral pathogens were also detected in MBR product, but again were absent on the three other occasions on which viral pathogens were detected (three false negatives sample results). Somatic coliphages were also detected on five occasions on which viral pathogens were not (five false positives sample results). These results suggest that these phage groups may not be suitable surrogates of viral pathogens in this type of treatment system. However, viral pathogens were detected using qPCR, whilst phages were detected using culture-based methods. Therefore there is some uncertainty as to the viability/infectivity of these viral pathogen particles. In order to be confident that phages are suitable surrogates of the viral pathogens monitored in this investigation, it will first be necessary to establish the infectivity of the virus particles, to ensure that they do indeed pose a risk to the human health of end users. Therefore future studies should ideally involve a

blend of cell culture infectivity assays, run in parallel with culture dependant and culture independent detection of phages and viral pathogens (where possible).

3.5 Physico-chemical conditions

The physico-chemical conditions at each stage of the Old Ford WRP system are shown in Table 2. As shown in Table 2, suspended solids, BOD and COD were efficiently reduced by the MBR treatment system. Statistically significant relationships were observed between levels of viruses and concentrations of BOD, COD and suspended solids (P=<0.05; Spearman's Rank). However, no statistical relationships were observed between the concentration of mixed-liquor suspended solids and numbers of viral indicators and pathogens present in the MBR product (P=>0.05; Spearman's Rank).

3.6 Contribution of treatment stages to log removal values

Figure 5 presents the percentage contribution of treatment processes (Treatment Stages 1, 2 and 3, illustrated in Figure 1) to viral log removal values at the Old Ford WRP. The results show that for AdV and NoV the largest percentage contribution to removal was the membrane itself (Treatment Stage 2), whilst for FRNA and GB124 phages over 80% of recorded log removal occurs in the initial stages of treatment (Treatment Stage 1), in which settling, screening, and activated sludge treatments take place. Somatic coliphage appear to be more resistant to Treatment Stage 1 with percentage log removal split fairly evenly between Treatment Stages 1 and 2. Adenovirus, which, perhaps unsurprisingly, was detected most often in MBR, demonstrated the largest percentage log removal during Treatment Stage 3 (GAC and chlorination). These results suggest that although post-disinfection would be needed to achieve the higher removal requirement of existing reuse standards for drinking water (California Department of Public Health, 2014), MBR treatment could reduce the

chemical doses required to meet these standards in comparison to conventional treatment processes.

4. Conclusions

- This study, investigated for the first time the seasonal variability in the removal of viral pathogens, traditional indicators and potential phage surrogates in a full-scale MBR treatment system over a full twelve months, thus providing valuable new information on the potential risks to human health associated with the reuse of MBR effluents for the augmentation of potable water supplies.
- In light of the findings of this study, it can be concluded that membrane bioreactor technology appears to have considerable potential to protect human health in wastewater reuse systems, potentially including the augmentation of potable water supplies.
- However, the results of this study do not currently support the use of phages as surrogates for viral pathogens, because viral pathogen concentrations varied to a greater degree seasonally and were detected on a number of occasions on which phages were not detected (false negative sample results).
- Future studies therefore need to ascertain the infectivity of viral particles to determine whether the presence of viral pathogens in chlorinated product during this study presents a real risk to human health.
- Finally, the relatively low levels of suspended solids, organics and microbial contaminants observed in the MBR product of this study suggest that the previously perceived need for post-treatment disinfection (certainly prior to non-potable reuse) is not supported by the evidence of treatment efficacy. The work therefore supports the further development of reuse systems that are able to protect human health

without the need for chemical disinfection, thereby avoiding the production of potentially toxic disinfection by-products in recycled water.

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Table 1. Correlation between phage indicators and pathogenic viruses

	Somatic coliphages	FRNA phages	GB124 phages	Adenovirus	Norovirus GI
FRNA phages	0.808[a]				
GB124 Phages	0.829[a]	0.945[a]			
Adenovirus	0.675[a]	0.620[a]	0.619[a]		
Norovirus GI	0.646[a]	0.621[a]	0.604[a]	0.404[a]	
Norovirus GII	0.627[a]	0.590[a]	0.613[a]	0.614[a]	0.814[a]

[a] Two tailed significance, $p<0.01$

Table 2. Physcio-chemical conditions at each treatment stage in the Old Ford WRP

Parameter	Treatment Stage	N	Minimum (mg/l)	Mean (mg/l)	Maximum (mg/l)
Biological oxygen demand	Raw sewage	37	79.6	210.5	355.9
	Settled sewage	26	58.7	141.8	271.6
	Screened sewage	29	46.2	133.3	262.1
	Mixed Liquor	6	266.1	419.5	554.5
	MBR product	37	<1.9	<1.9	<1.9
	GAC product	29	<1.9	7.2	7.2
	Reclaimed wastewater	185	<1.9	<1.9	<1.9
Chemical oxygen demand	Raw sewage	37	198.0	699.0	5930.0
	Settled sewage	30	154.0	329.3	401.0
	Screened sewage	29	142.0	270.6	512.0
	Mixed Liquor	74	644.0	175654.0	778000.0
	MBR product	39	<1.9	15.9	22.8
	GAC product	33	<1.9	13.9	22.3
	Reclaimed wastewater	185	<1.9	15.0	48.3
Suspended solids	Raw sewage	37	96.0	512.0	6720.0
	Settled sewage	26	60.0	138.7	172.0
	Screened sewage	29	47.5	94.3	240.0
	Mixed Liquor	76	116	42363.0	433200.0
	MBR product	37	<1.9	3.0	5.5
	GAC product	29	<1.9	2.5	3.0
	Reclaimed wastewater	189	<1.9	2.9	7.2

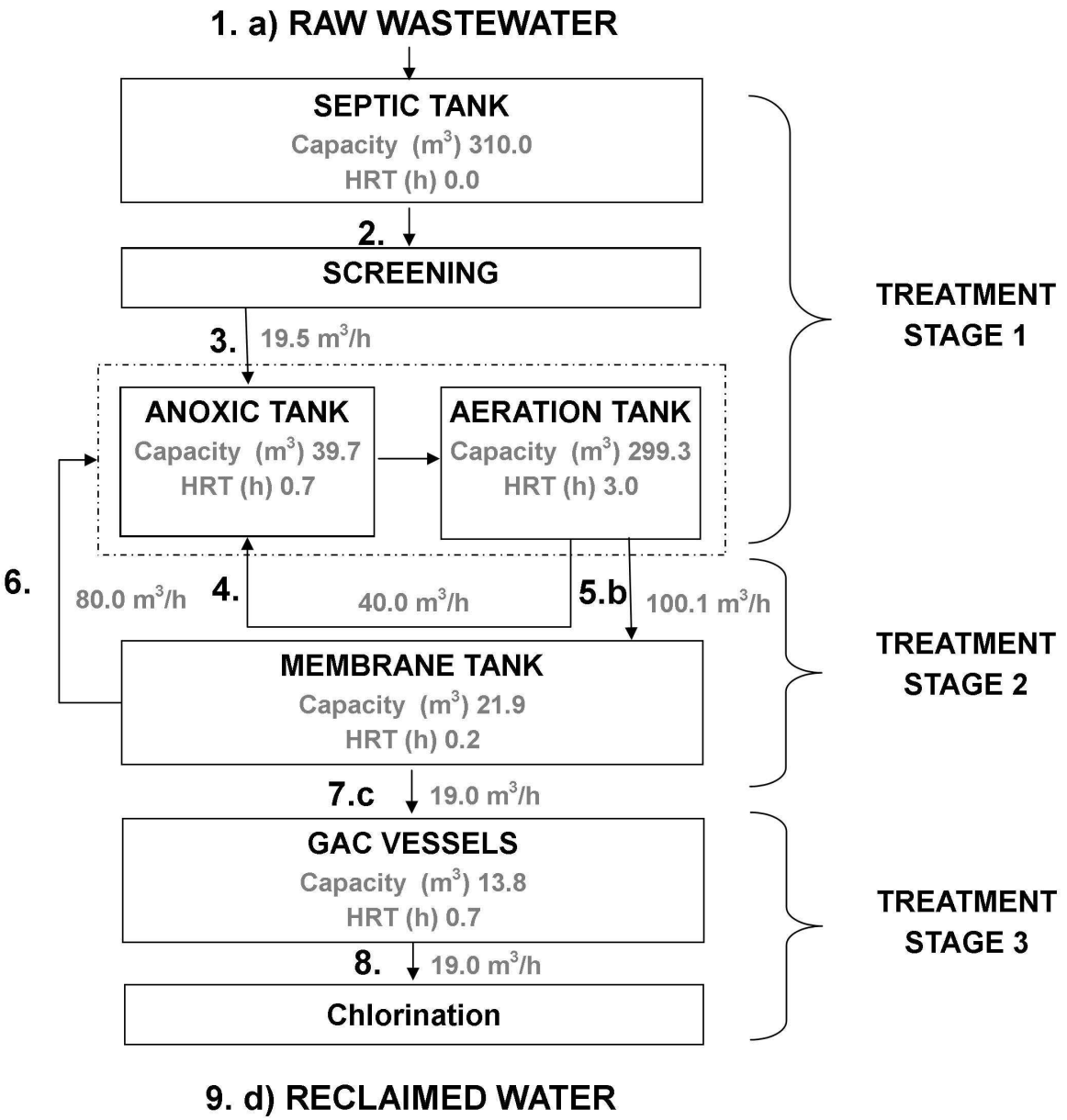

Figure 1. Sampling locations for four weekly monitoring of faecal indicator organisms (annotated 1-9) and viral pathogens (annotated a-d) at the Old Ford WRP. HRT = hydraulic retention time/h.

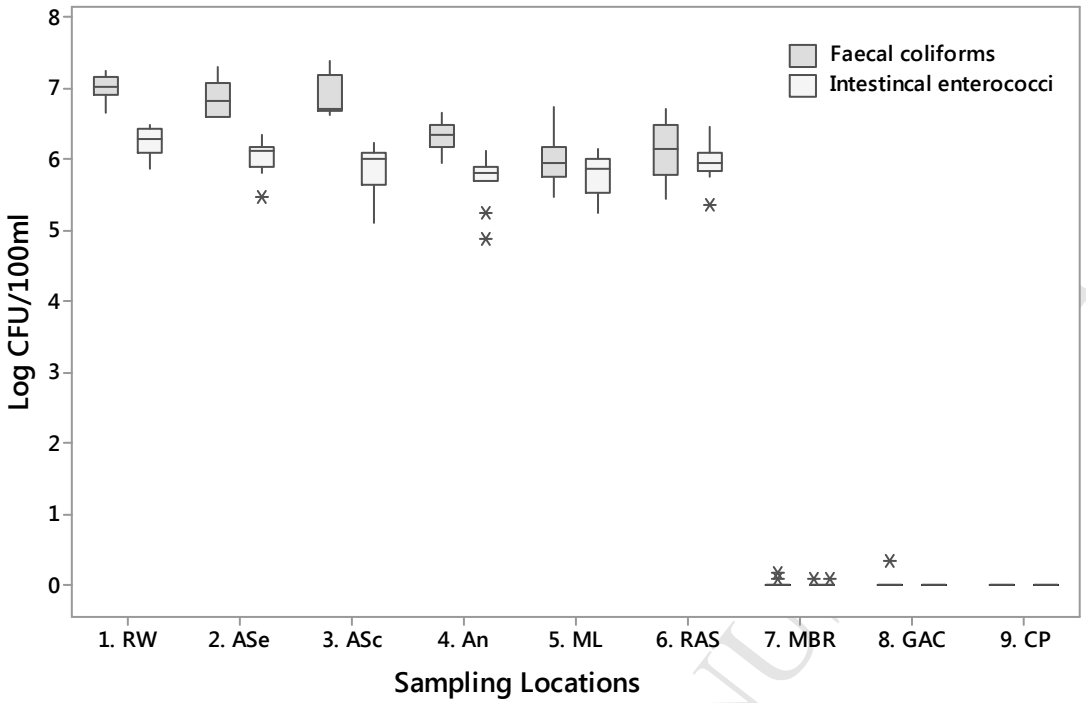

Figure 2. Mean log numbers of faecal thermotolerant coliforms and intestinal enterococci at each sampling location in the Old Ford WRP (cultured). Outliers (observations >1.5 times the interquartile range) are represented by a *. Boxes represent the interquartile range. (n=108). RW= raw sewage, Ase= after septic, Asc = after screening, An= after anoxic, ML= mixed liquor, RAS= returned activated sludge, MBR= membrane bioreactor product, GAC= granular activated carbon product, and CP = chlorinated product.

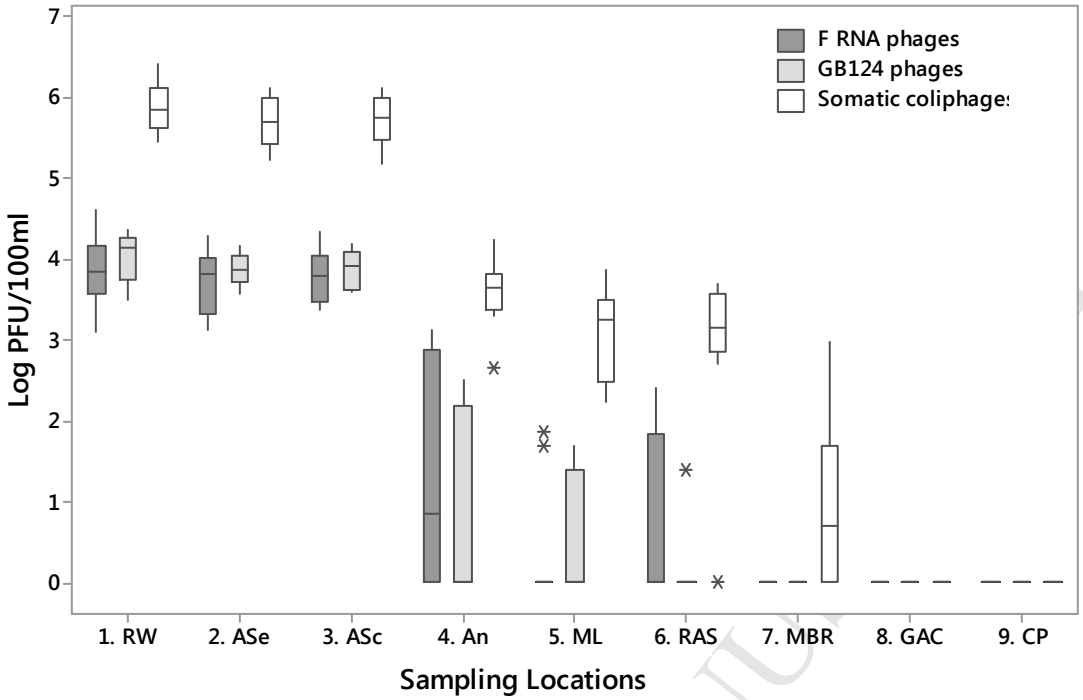

Figure 3. Mean log numbers of bacteriophages at each sampling location in the Old Ford WRP (cultured-based detection methods). Outliers (observations >1.5 times the interquartile range) are represented by a *. (n=108). Boxes represent the interquartile range. RW= raw sewage, Ase= after septic, Asc = after screening, An= after anoxic, ML= mixed liquor, RAS= returned activated sludge, MBR= membrane bioreactor product, GAC= granular activated carbon product, and CP = chlorinated product.

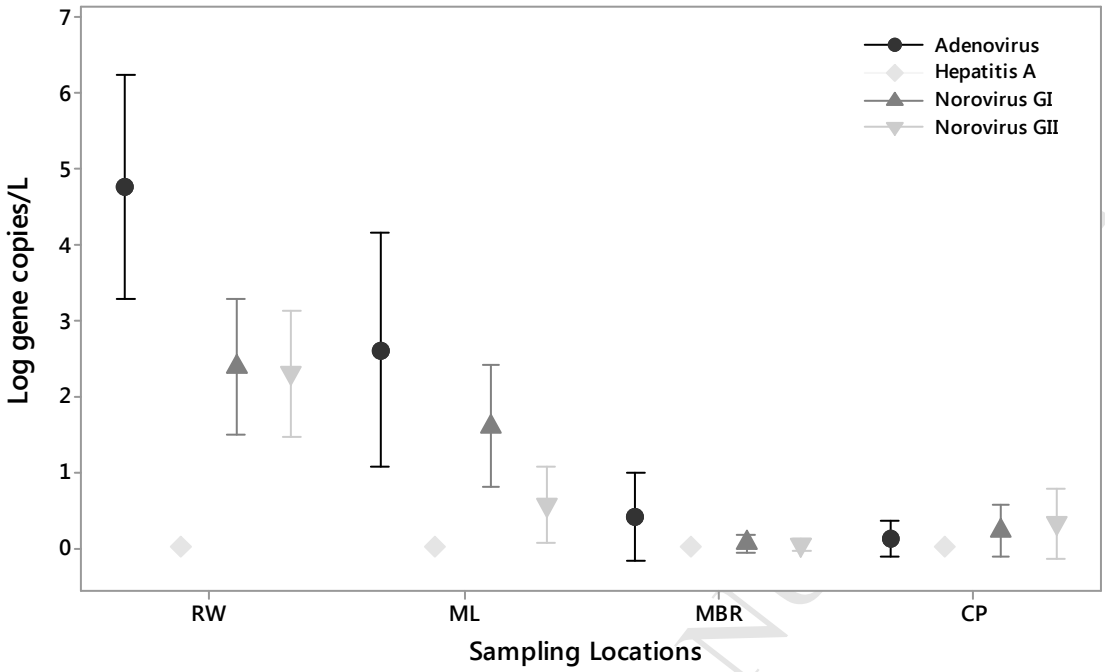

Figure 4. Confidence intervals (95%) for mean log numbers of select enteric viruses determined using qPCR. Individual standard deviations were used to calculate the intervals. (n=48)

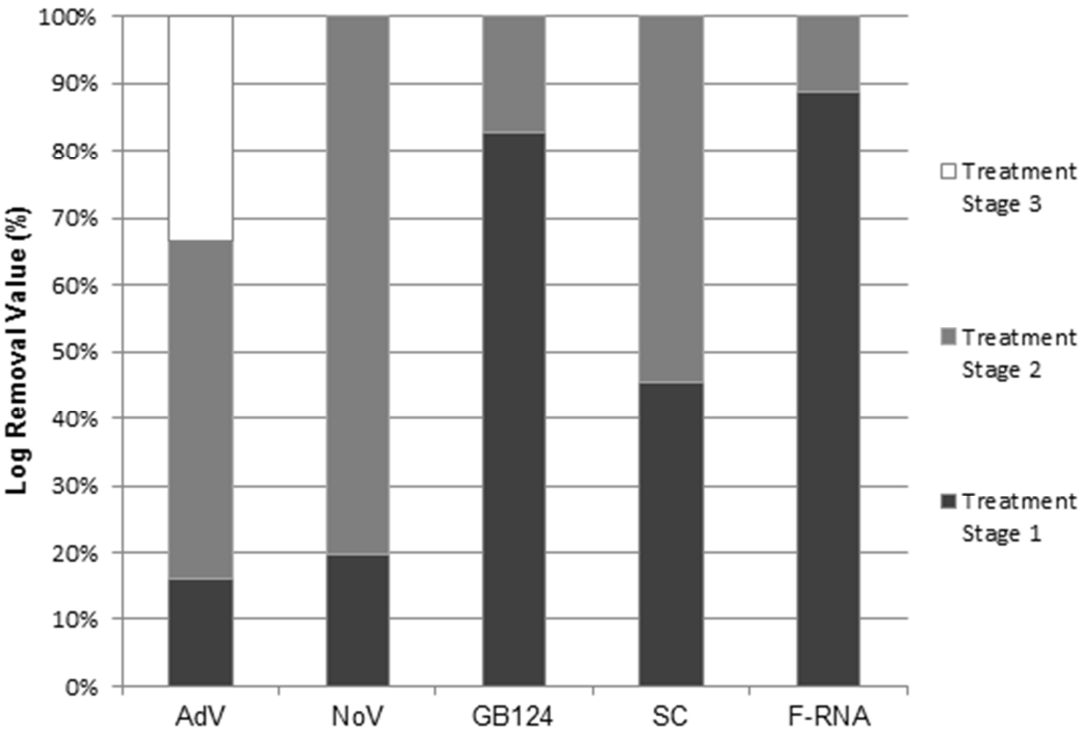

Figure 5. Percentage log removal value (LRV) contributions from treatment stage 1 (septic, screening and anoxic), treatment stage 2 (mixed liquor to MBR) and treatment stage 3 (GAC and chlorination) of the Old Ford WRP for adenovirus (AdV), norovirus GI and GII (NoV), phages of GB124 (GB124), somatic coliphages (SC), and F-RNA phages (F-RNA). N= 48.

Highlights

1. Seasonal variability of removal of viruses in a full-scale MBR is investigated.
2. Log reductions of NoV GI/II and AdV of between 2.3 and 4.4 were achieved.
3. AdV and NoV were both detected at low levels post chlorination in single samples.
4. The findings do not support the use of phages as surrogates for viral pathogens.
5. Future studies need to ascertain the infectivity of viral particles to determine risk.