The effect of wastewater treatment processes, in particular ultraviolet light treatment, on pathogenic virus removal: EPA Research Report 171

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The Effect of Wastewater Treatment Processes, in Particular Ultraviolet Light Treatment, on Pathogenic Virus Removal

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Comhshaol, Pobal agus Rialtas Áitiúil
Environment, Community and Local Government

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Executive Summary

Background

Municipal wastewater treatment plant discharges are a recognised source of human pathogenic viruses, of which norovirus is of great concern and the leading cause of viral gastroenteritis worldwide. Currently, no legislation (nationally or internationally) exists for the monitoring of viral loads in treated effluent. While primary and secondary treatment processes can reduce virus concentrations, they are not specifically designed for this purpose and so tertiary treatment can be required in many cases. Continuous low- and/or medium-pressure ultraviolet (UV) light systems are used in conventional wastewater treatment plants as a method of pathogen disinfection. Barrier-based systems, such as membrane filtration processes, are widely used in the drinking water sector as a pathogen removal system; however, operational challenges associated with wastewater have limited their use in this industry.

The detection of norovirus is limited to molecular methods that do not distinguish between infective and non-infective viruses. This poses a problem when evaluating certain disinfection methods, such as UV light, which does not remove the virus but rather inactivates it. Thus, in this case, overestimation of virus infectivity can occur. The use of a surrogate virus, the F-specific RNA (FRNA) bacteriophage has been suggested as it is morphologically and physiochemically similar to norovirus and it may also be cultivated, i.e. infectivity can be determined.

This project investigated the use of FRNA bacteriophage as (i) a potential surrogate for norovirus response/behaviour, and (ii) a model to determine the fate of viruses through a municipal wastewater treatment plant. The project also evaluated the efficacy of membrane filtration (microfiltration and ultrafiltration) as a pathogen removal method and two UV light-based technologies, pulsed UV light and low-pressure UV light, as pathogen disinfection methods. The potential impacts of parameters such as organic carbon, metals and suspended solids (typically present in wastewater) on the investigated pathogen removal processes were also analysed.

Key points

- Molecular methods were not sufficient to determine how effective UV light treatment was in reducing the concentrations of norovirus and bacteriophages. However, the infectivity assay (available for bacteriophage only) indicated significant reductions in concentration.
- Settlement processes within a wastewater treatment plant can play an important role in the removal of viruses from treated effluent and in improving the efficiency of subsequent disinfection processes. Thus, good ongoing facility operation is vital in ensuring that facilities can effectively remove pathogens where required.
- Wastewater quality was found to have a significant impact on UV light disinfection.
  - Bacteriophage reduction was significantly enhanced in distilled water when compared with wastewater.
  - The impact of effluent wastewater from various wastewater treatment plants on pulsed disinfection varied notably. In general, samples with increased suspended solids or organic carbon concentrations exhibited decreased pathogen removal via UV light disinfection.
- The novel pulsed UV light bench-scale system showed good potential when compared with traditional low-pressure UV light technologies. The pulsed UV light systems can confer advantages in terms of operation and potentially enhanced disinfection potential; however, this needs further research.
- Ultrafiltration membranes yielded high removal rates of FRNA bacteriophage and removal of norovirus to below the limit of detection. They can offer an alternative to disinfection systems in sensitive areas, but their widespread deployment may be limited by maintenance and operational challenges.
- Deep freezing of samples had no impact on virus copy number detection indicating that long-term sample storage and batch analysis is possible for norovirus analysis.

Findings/recommendations

- Owing to the high resistance of bacteriophages to UV light treatment and their morphological similarities to norovirus, the use of this virus as a potential surrogate has value. This option may currently offer the most effective method of determining the efficiency of disinfection processes (in particular UV light) in achieving a reduction in norovirus concentrations.
- The impact of suspended solids on pathogen removal via UV light varied widely between samples and the wastewater treatment facilities tested. The implementation of batch tests prior to installation of new UV light systems would be useful to inform design and system range requirements, such as the site-specific impacts of hydraulic residence times and wastewater characteristics (e.g. organic carbon and suspended solids). Furthermore, continuous commissioning, via periodic verification trials of installed UV light systems, may enable operators to ensure the optimal operation of existing systems.
- The operation efficiency of the treatment plant as a whole should be taken into consideration when selecting a particular tertiary treatment system, as a poorly performing plant may significantly impact UV light disinfection processes.
- Biofouling control of membrane filtrations systems poses a significant maintenance and operational challenge. Further work could focus on design, operational and cost challenges that may be encountered when such technology is deployed on site.

1 Introduction

Discharges from municipal wastewater treatment facilities continue to be one of the most prominent point source pollutions for both surface and bathing waters in Ireland today. While the extent of the pollution has decreased in recent years, numerous urban wastewater treatment plants (WWTPs) have yet to meet national standards for effluent discharges into the environment (EPA, 2012). Wastewater that has not undergone specific pathogen removal or disinfection can contain high concentrations of protozoans, bacteria and viruses; this in turn can lead to environmental pollution, economic impacts (e.g. to shellfish and tourism industries) and an increased risk to human health from contaminated shellfish consumption and bathing in contaminated areas. For these reasons, tertiary treatment designed for pathogen removal or inactivation (disinfection) of wastewater is increasingly necessary in order to comply with strict regulations designed to protect recreational water bodies and associated industries. Pathogen removal and disinfection technologies can be either physical [e.g. ultraviolet (UV) irradiation or barrier methods] or chemical (e.g. ozone or chlorine-based disinfection systems); in the wastewater sector UV irradiation is the most widely applied with ozone also being popular. Conventional UV light technologies can generally comprise either continuous low-pressure (LP) or continuous medium-pressure (MP) UV light processes. More recently, novel pulsed UV (PUV) light systems have been proposed as an alternative to conventional UV light technology with more potent antimicrobial properties associated with the high-energy pulses emitted. Barrier methods include membrane filtration, which employs membrane cassettes of varying pore sizes dependant on the size of the target microorganism to be removed.

Current legislation in Ireland and Europe does not require the monitoring of pathogenic virus concentrations in treated wastewater. Testing for viruses, in particular, has proved challenging and, while detection methods have improved in recent years, some limitations still exist, particularly in the case of norovirus (NoV). NoV is the most common cause of viral gastroenteritis globally; however, information regarding its behaviour and health significance in the environmental setting remains limited. Furthermore, owing to a lack of a cell culture system, monitoring of NoV is restricted to molecular methods, namely real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR). This method allows quantification of the virus, but it also has its drawbacks, particularly when assessing the germicidal effects of UV irradiation (Wolf *et al.*, 2009). RT-qPCR methods can overestimate the amount of infectious virus in a sample. For example, when a virus genome has been damaged by UV light (and so is presumed unviable), PCR is likely to be positive unless the damage is within the specific target of amplification and is sufficient to render the sample non-amplifiable. Moreover, molecular analysis can be expensive and technically demanding, therefore the costs of monitoring NoV are relatively high.

The use of surrogate viruses as an alternative monitoring system for NoV has been suggested. The male-specific or F-specific RNA (FRNA) bacteriophage may be an attractive candidate due to its similar physical and physiochemical characteristics (Doré *et al.*, 2000; Flannery *et al.*, 2012). Bacteriophages are viruses that infect bacteria and, as they are present in the environment all year round and are culturable, have potential as surrogates for NoV. Rapid, technically simple and cost-effective microbiological assays are available for enumeration of infectious FRNA bacteriophages.

This study investigated the use of two types of tertiary treatment technologies for virus removal from wastewater; (i) pathogen removal via membrane filtration and (ii) pathogen disinfection via PUV light. The impact of wastewater characteristics, such as suspended solids (SS), organic carbon and metals on system efficiency was evaluated. The project also investigated the use of FRNA bacteriophages as a potential surrogate indicator of NoV removal or inactivation.

2 Summary Review: State of Knowledge and Technology Reports

2.1 Wastewater Treatment System Pathways

Municipal wastewater treatment is generally achieved via a number of standard steps: preliminary treatment (e.g. screening, grit removal, oils, fats and grease removal and pH correction); primary treatment (sedimentation of settleable solid sludge); secondary treatment of the liquid waste (aerobic-/anoxic-/anaerobic-biological processes); and finally, if feasible, tertiary treatment (chemical phosphorus removal, sand filtration, microstraining, physiochemical, pathogen removal or disinfection) (Figure 2.1).

2.2 Human Pathogenic Viruses

The virological risks associated with the discharge of treated wastewater can pose a threat to a multitude of stakeholders including the food industry, public health, local and regional economies and tourism. The human pathogenic viruses of main concern include the human adenovirus (HAdV), hepatitis A virus (HAV), hepatitis E (HEV), rotaviruses, human enterovirus (EV) and NoV. Infections caused by these human pathogenic viruses include gastroenteritis, hepatitis and meningitis (Table 2.1).

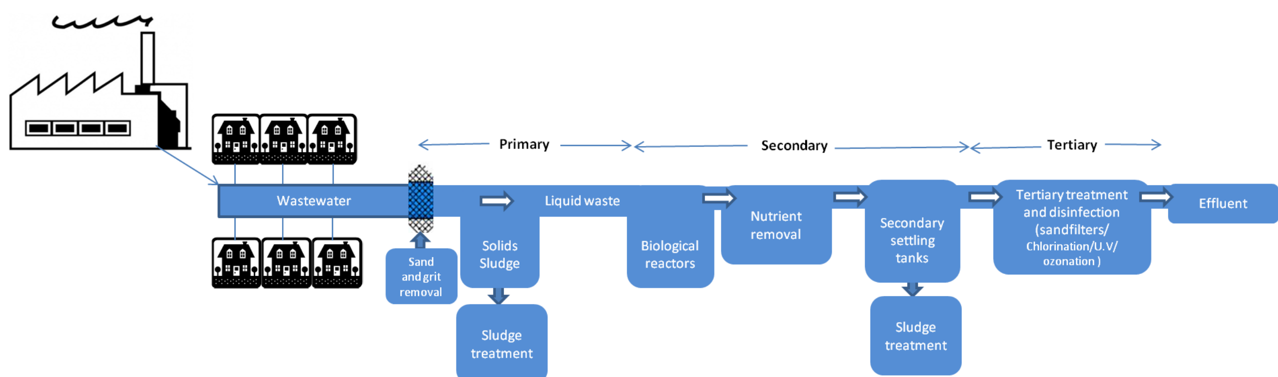

Figure 2.1. Schematic illustrating the flow of wastewater through a common WWTP.

Table 2.1. Properties and characteristics of common human pathogenic viruses (USEPA, 2006; Carter, 2007)

Name	Virus family	Abbreviation	Size (nm)	Illness	Reference
Adenovirus	*Adenoviridae*	AdV	c. 100 (dsDNA)	Diarrhoea	Russell *et al.*, 2006
Enterovirus	*Picornaviridae*	EV	c. 28 (ssRNA)	Muscle pains; nausea; viral meningitis; hand, food and mouth disease	Lee and Chang, 2010
Hepatitis A	*Picornaviridae*	HAV	c. 28 (ssRNA)	Hepatitis	Hammitt *et al.*, 2008
Hepatitis E	*Hepeviridae*	HEV	c. 34 (ssRNA)	Viral hepatitis	Shrestha *et al.*, 2007
Norovirus	*Caliciviridae*	NoV	c. 27 (ssRNA)	Gastroenteritis; vomiting; diarrhoea	Vinje, 2010
Rotavirus	*Reoviridae*	RV	c. 70 (dsRNA)	Gastroenteritis; osmotic diarrhoea	Soares-Weiser *et al.*, 2012

ds, double-stranded; ss, single-stranded.

Some human pathogenic viruses are difficult to investigate as current methods for their detection are complex and expensive when compared with traditional bacterial indicators. NoV (and indeed other enteric viruses) circulate in local communities resulting in a high abundance of NoV in influent entering WWTPs. Analysis of influent wastewater may prove more valuable than clinical records as it may inform authorities in advance of possible outbreaks. This is particularly so because many individuals do not report illness because of the self-limiting nature of the virus.

2.3 Disinfection Process and Strategies

The ability of these viruses to persist in viable form, not only through the common WWTP processes, but also in the environment, results in an increased risk to all community stakeholders. Technologies employed at all stages of treatment vary widely between facilities. While primary and secondary sewage treatment processes can contribute to the reduction of bacterial and viral loads, further treatment, such as removal/inactivation (disinfection) processes is typically required, particularly in the case of viruses (Ottoson *et al.*, 2006; Nordgren *et al.*, 2009). In the wastewater sector, disinfection strategies, where present, vary and are selected based on several factors (USEPA, 2006a; Shannon *et al.*, 2008; EPA, 2011; Table 2.2). Where a tertiary treatment is being used, its effectiveness is reliant on the continuous performance of upstream primary and secondary treatment processes.

The disinfection strategies employed vary considerably (Table 2.3) and are generally selected on a site-specific basis.

Figure 2.2 compares the unit costs of chlorine-based, UV light and ozone pathogen removal/inactivation systems; these costs are sourced from water treatment facilities, as data and comparisons are more readily available for this sector. It is clear that the volume of water treated plays a major role in the overall costs per unit volume treated. In general, at larger scales the cost differences for various technologies relative to each other are low. However, the overall costs incurred by various systems can be high and thus even small differences in costs per unit volume treated can result in large overall cost differences.

Table 2.2. Selection criteria of tertiary disinfection systems

Factor	Consideration
1	The need for an effective primary (screening/filtration) process and secondary treatment processes to enable disinfection to target the microbes successfully
2	The specific pathogen to be targeted
3	The quantity and quality of the water to be disinfected (e.g. is the water highly turbid?)
4	The formation and removal/treatment of hazardous by-products (e.g. wastewater composed of high loads of organic matter and bromide increases ozone demand and the potential for the formation of harmful by-products)
5	Ease of handling, safety and storage
6	Overall added cost to existing WWTP operation and maintenance

Table 2.3. Properties of disinfection techniques (EPA, 1999)

Consideration	Chlorine	Chloramines	Ozone	Chlorine Dioxide	UV light
Equipment reliability	Good	Good	Good	Good	Medium
Technology complexity	Low	Low	More	Medium	Medium
Safety concerns	Low–high	Medium	Medium	High	Low
Bacterial	Good	Good	Good	Good	Good
Viral	Good	Medium	Good	Good	Medium
Protozoa	Medium	Poor	Good	Medium	Good
By-product production	High	Medium	Medium	Medium	None
Intensiveness of operations and management	Low	Moderate	High	Moderate	Moderate

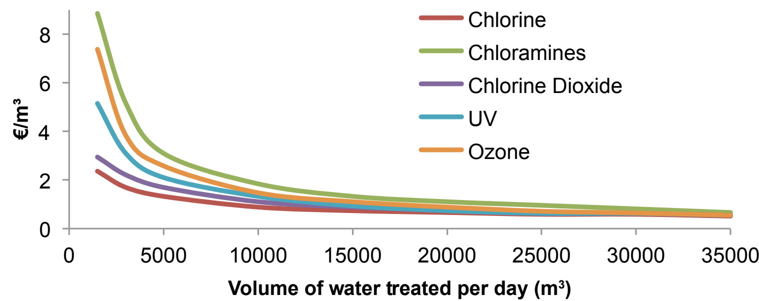

Figure 2.2. A cost comparison between chlorine-based disinfection systems, UV light and ozone (adapted from Moghadam and Doré, 2012).

2.4 Ultraviolet Light Disinfection

Ultraviolet light disinfection has proven successful in inactivating most viruses, spores and cysts and its application in the treatment of wastewater has been well recorded (Qualls *et al.*, 1984; USEPA, 1986; Darby *et al.*, 1993; Emerick *et al.*, 1999; Table 2.4). Disinfection with UV light has proven effective for a broad range of microbial species when typically employed at the germicidal monochromatic wavelength of 253.7 nm in the UV-C range. As such, UV light disinfection is now considered an acceptable process for inactivation of pathogens in drinking water. LP and/or MP UV light is the current method used in water and WWTPs (Bohrerova *et al.*, 2008). Flash or PUV light lamps are a relatively new technology that generate a broadband spectrum generally in the 100–1100-nm wavelength in short, high-intensity pulses. PUV light lamps (usually generated from xenon or krypton) differ from the LP/MP continuous mercury lamp as the high-energy pulse results in the output of a broad and powerful polychromatic spectrum of UV light, visible and infrared light (Lee *et al.*, 2009). The high energy intensity of the PUV light is theorised to be highly germicidal with the added benefit of inhibiting the photorepair ability of pathogens usually associated with LP/MP UV light treatment.

2.4.1 Ultraviolet light dose requirements

The degree to which microbes are inactivated by UV light is related to the UV dose (mJ/cm^2), which is calculated as outlined by Metcalf & Eddy (2004). The UV dose applied is site specific and is determined by the type of wastewater to be treated, the volume and the contact time. Wastewater characteristics such as SS, metals (iron and manganese) and organic carbon are all factors that may impact on UV light disinfection performance. Batch tests on the wastewater are recommended at the design and commissioning stage of the on-site UV light system. The recommended UV dose for the inactivation of microbes (mJ/cm^2; EPA, 2011) is outlined in Table 2.4.

2.4.2 Photoreactivation potential

The inactivation of pathogenic microorganisms is generally adequate. Nonetheless, the risk of microbial re-growth has led to the development of barrier tertiary treatment systems, such as membrane filtration technologies, that can ensure the complete removal of targeted pathogens. Concerns exist in relation to the photoreactivation potential of organisms; however, this occurrence is generally limited to bacteria (Guo *et al.*, 2009, 2011). Studies conducted into the photoreactivation of viruses have shown very few or no repair mechanisms (Baron, 1997), particularly in the case of ssRNA viruses such as NoV (Rodriguez *et al.*, 2014).

2.5 Membrane Filtration

Advantages of membrane filtration include the production of high-quality effluent and the physical removal of a target pathogen. The main disadvantage of membrane filtration is membrane fouling, caused by a deposition of components at the surface and inside the pores of the membrane. The application of two or more strategies may be necessary depending on the required end use of the treated wastewater (e.g. discharge, water reuse and reclaim).

Tangential flow filtration (TFF) is a type of membrane filtration that can be used as a mechanism for concentrating viruses in a sample or for separation purposes, such as pathogen removal. TFF can be suitable for wastewater mediums because of the type of filtration involved. The cross-flow filtration action across the membrane

Table 2.4. Recommended UV dose for the inactivation of microbes (mJ/cm²) (EPA, 2011)

Target	Log inactivation							
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0
Protozoa								
Giardia cysts[a]	1.5	2.1	3.0	5.2	7.7	11	15	22
Cryptosporidium oocysts[a]	1.6	2.5	3.9	5.8	8.5	12	15	22
Viruses								
"Viruses"[a]	39	58	79	100	121	143	163	186
Adenovirus type 40[b]		56		111		167		
Poliovirus[b]		7		15		22		30
Adenovirus type 41[c]								112
Hepatitis[c]								21
Coxsackievirus B5[c]								36
Poliovirus type 1[c]								27
Rotavirus SA11[c]								36
Bacteria								
Bacillus subtilis spores[a]		28						62
Escherichia coli[a]		3						8.4
Streptococcus faecalis[b]		9						30
Vibrio cholerae[b]		2						9
Enterobacter cloacae[c]								10 (33)
Enterocolitica faecum[c]								17 (20)
Campylobacter jejuni[c]								4.6
Clostridium perfringens[c]								23.5
E. coli 0157:H7[c]								6 (25)
E. coli wild type[c]								8.1
Klebsiella pneumoniae[c]								20 (31)
Legionella pneumophila[c]								9.4
Mycobacterium smegmatis[c]								20 (27)
Pseudomonas aeruginosa[c]								11 (19)
Salmonella enterica subsp. *enterica* serovar Typhi[c]								8.2
Shigella dysenteriae ATTC29027[c]								3.0
Streptococcus faecalis								11.2
Vibrio cholerae								2.9 (21)

[a]USEPA (2006b).

[b]Hijnen *et al.* (2006).

[c]Bolton and Cotton (2008) – values in brackets include photoreactivation data.

as opposed to dead-end filtration is advantageous in the medium of wastewater as it limits the amount of biomass build-up associated with poor-quality effluent. Polypropylene membrane cassettes are typically used with TFF systems and are available in various pore sizes depending on the macro solute/microorganism to be removed. The classification of membrane filtration falls into four categories; microfiltration (MF; 0.1–1 μm),

ultrafiltration (UF; 0.01–0.1 μm), nanofiltration (NF; 0.01–0.001 μm) and reverse osmosis (RO; < 0.001 μm). For pathogen removal purposes, it is generally microfiltration and ultrafiltration pore sizes that are utilised. Larger pathogens, such as protozoa and bacteria, are typically removed via MF whereas smaller UF pore sizes are required for the removal of most viruses. To date, membrane filtration is largely associated with the water treatment industry. However, with an increasing focus on the removal of pathogens (and other emerging contaminants) from treated wastewaters, membrane filtration may play a larger role in the wastewater industry in the future

2.6 Emerging Technologies

In addition to current methods of disinfection, several new and innovative approaches are currently being developed. These include e-beam irradiation, PUV light systems, peracetic acid (PAA) and photocatalytic disinfection, and are described in full in the accompanying report (Technical report – Appendix X). They offer novel solution strategies, both alone and in combination with traditional methods. Combinations such as PAA and UV light; chlorine and UV light; barrier methods (filtration) and UV light; and ozone and UV light have been investigated for their pathogen inactivation/removal efficiency (Caretti and Lubello, 2003; Montemayor *et al.*, 2008).

3 Materials and Methods

3.1 Tertiary Disinfection Systems

Both bench- and site-scale pathogen disinfection/removal systems were investigated with regards to virus inactivation/removal efficiency and impacting factors affecting inactivation/removal efficiency. Impacting factors included SS, organic carbon and metals. The particular systems tested included a bench-scale PUV light system, a site-scale low-pressure ultraviolet (LPUV) light system and a bench-scale TFF system.

3.1.1 Bench-scale ultraviolet light experiments

A bench-scale pulsed power source (PUV-1, Samtech Ltd, Glasgow, UK) was used to power an LP (60 kPa) xenon-filled flashlamp (Heraeus Noblelight XAP type NL4006 series, constructed from a clear UV light transparent quartz tube), which produced a high-intensity beam of polychromatic pulsed light. The lamp was placed 10.75 cm above an aluminium flow-through vessel, which pumped secondary effluent through the vessel at the desired flow rate (Figure 3.1).

Briefly, batches of secondary effluent (5–7 L) were seeded with a known quantity of NoV [genogroup I (GI) and genogroup II (GII)] (2 × 1-mL aliquots of approximately 10^7 copies/mL) and FRNA bacteriophage GA (GA bacteriophage) (1 × 1-mL aliquots of 10^9–10^{11} copies/mL) and pumped through the PUV light system.

The PUV light system potentially offers a number of operational advantages over conventional UV light systems. In particular, the system allows the discharge energy and thus UV dosage (measured in mJ/cm^2) to be easily controlled by varying the operating voltage of the unit or the pulse length [10 to 0.1 pulses per second (PPS)]. Table 3.1 lists the discharge voltage (V) and corresponding energy per pulse (J) input into the UV lamp. The maximum frequency (Hz) achievable at each voltage setting is also detailed. Initially, in this investigation the PUV light was operated at varying discharge

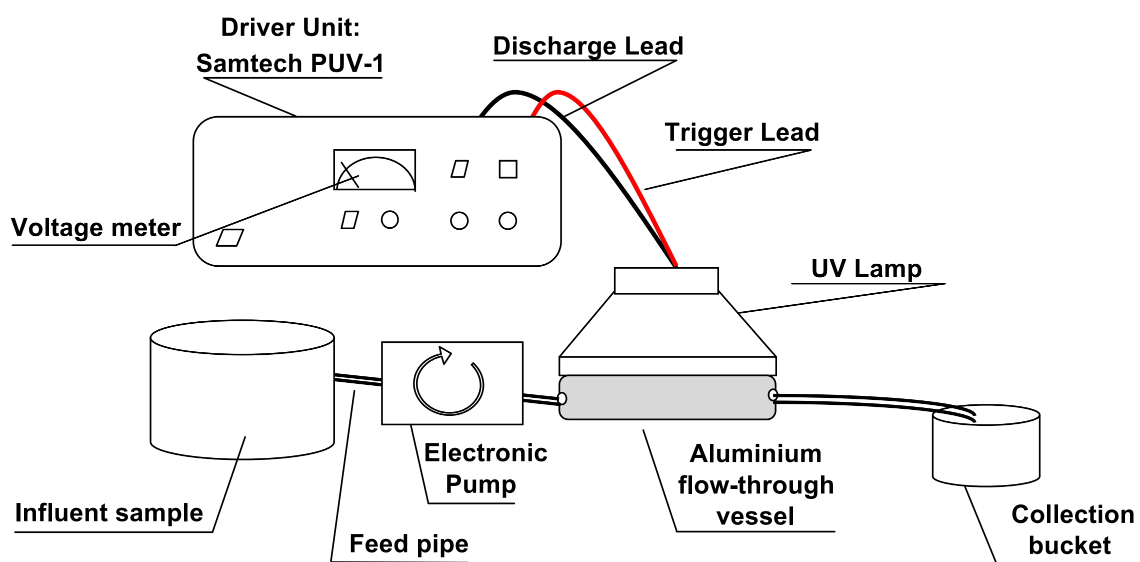

Figure 3.1. Schematic of the bench-scale PUV light experimental set-up.

Table 3.1. Discharge voltage, corresponding energy per pulse and maximum frequency capacity of PUV light power source (Samtech Ltd)

Voltage (V)	300	400	500	600	700	800	900	1000
Energy (J)	1.8	3.2	5	7.2	9.8	12.8	16.2	20
Maximum frequency (Hz)	10	10	10	10	10	7	5.6	5

energies to ascertain the impact of pulse rate and voltage on pathogen inactivation.

Pulse operating parameters of the UV lamp include peak current (A), peak power (kW), peak admittance(s) and current rise/fall time at various discharge voltages. Table 3.2 lists the said parameters from 500 V to 1000 V.

The distance of the UV lamp from the sample was also taken into account when calculating UV dose. Table 3.3 gives an overview of the spectrum output at a range of bandwidth regions (μJ/cm²) relative to the distance of the UV lamp from the sample vessel. In this study, the sample vessel lay 10.75 cm below the UV lamp, which indicated a spectrum of < 300 nm was applicable.

To determine optimum operating conditions for the PUV light system, a range of voltages and hydraulic retention times (HRTs) were compared for GA bacteriophage reduction. As the voltage meter of the system ranged from 0 to 1000 V, it was decided to test low (300 V), medium (600 V) and high (900 V) voltages at the median pulse operating time of 1 PPS. Table 3.4 outlines the resulting UV doses emitted at each voltage and HRT when the system is operated at 1 PPS at a bandwidth of < 300 nm.

The UV dose (mJ/cm²) is the product of irradiance (mW/cm²) and exposure time (s), therefore the desired dose can be produced by adjusting the pulse frequency and/or discharge voltage. The relationship between the discharge voltage and the energy per pulse is defined by Equation 1.

$$E = 0.5CV^2 \qquad \text{(Equation 3.1)}$$

Table 3.2. Pulse operating parameters of UV lamp from 500 V to 1000 V (Samtech Ltd)

Discharge voltage (V)	Peak current (A)	Peak power (kW)	Peak admittance (s)	Current rise/fall time (μs)
500	443	175	1.10	12/36
600	573	273	1.20	10/22
700	753	415	1.37	10/28
800	853	553	1.37	7/28
900	1020	757	1.40	7/28
1000	1173	985	1.46	7/27

Table 3.3. Spectrum output at various bandwidth regions at the distance from the UV lamp (μJ/cm²) (Samtech Ltd)

Distance (cm)	< 300 nm	300–400 nm	400–500 nm	500–600 nm	600–700 nm	> 700 nm
10	–	–	630	370	444	1877
15	346	222	295	156	191	778
20	166	140	168	93	112	462
25	129	84	114	67	73	302
30	76	57	83	42	54	215
35	62	43	57	38	40	158
40	40	39	48	26	30	122
45	38	27	40	20	24	98
50	34	21	33	16	20	79

Table 3.4. UV dose outputs of three HRTs and three voltages at 1 PPS for the PUV light system

Voltage (V)	300			600			900		
HRT(s)	60	75	120	60	75	120	60	75	120
PPS	1	1	1	1	1	1	1	1	1
Estimated UV dose (< 300 nm; mJ/cm²)	68.4	85.5	136.8	273.6	342.0	547.2	615.7	769.6	1231.4

where: C is the capacitance in Farads (in this case, capacitance is 40 μF) and V is the operating voltage in volts.

For the rest of the investigation, the PUV light system was operated at 900 V, 5.6 PPS at various HRTs measured (60 s, 75 s and 120 s) to ensure that the full capacity of the system was being evaluated. As previously noted, the PUV light system output may operate at a broad spectrum (100 nm–1000 nm) (see Table 3.3). Table 3.5 compares the calculated energy output from the PUV light unit based on the manufacturer's technical documentation at both the lowest bandwidth region (< 300 nm) and the broad spectrum at a voltage output of 900 V.

3.1.2 The comparison of PUV light efficiency in filtered versus unfiltered effluent

Twenty-five-litre batches of secondary treated wastewater samples were collected from three separate WWTPs – Sites 1, 2 and 3. Five litres from each batch was seeded with a known quantity of GA bacteriophage and tested by pumping through the PUV light system at two HRTs, 60 s and 120 s. Influent and effluent samples to and from the PUV light system were collected and analysed in each case. For comparison, a separate sample from each batch (5 L) of the aforementioned 25-L samples was filtered using a 0.1- to 0.2-μm filter to remove all SS. The same procedure for the unfiltered samples was then applied to the filtered samples and the results were compared.

3.1.3 Analysis of on-site LPUV light and bench-scale PUV light systems

A heavy duty, stainless steel on-site UV light treatment system (UV12GPM-HTM, AquaPRO) was used for comparison with the bench-scale PUV light system. Specifications include a working pressure of 120 pounds per square inch (psi); a lamp wattage of 39; a flow rate of 6 gallons per minute; and a transmissivity meter to measure UV light penetration through the effluent.

Samples from the on-site LPUV light system were collected from the NUI Galway/EPA Water Research Facility, Tuam, Co. Galway (municipal wastewater that had undergone secondary treatment). Grab samples (1 L) were collected from the influent and effluent points of the LPUV light system to test for pathogen removal. The LPUV light system was run at three flow rates, namely 0.2 m^3/h, 0.5 m^3/h and 0.8 m^3/h, in order to vary the HRT (and hence the total dose imparted) of the wastewater. Each of these trials was carried out on three separate days.

In each case, after a sample was collected at a given flow rate, the flow rate was adjusted to the next flow rate in turn. The LPUV light system was operated for at least 10 minutes after any change in flow before collecting the next sample.

In parallel, a grab sample (5 L) of the wastewater influent to the LPUV light system was collected for processing via the bench-scale PUV light system. The grab sample was stored in a refrigerator at 4°C and was processed via the bench-scale PUV light system within 24 hours. The PUV light was operated as described in section 3.1.2. The operating details of the LPUV light and PUV light systems during this experiment are summarised in Table 3.6. Three categories of pathogen inactivation/removal were examined following LPUV light and PUV light treatment: *E. coli*, total coliforms and FRNA bacteriophage (infectivity assay). The comparison was carried out in duplicate on three consecutive days.

3.1.4 Virus testing at various stages of a WWTP

The studied WWTP is a conventional activated sludge plant with phosphorus removal by ferric sulphate and tertiary treatment by sand filtration. The organic load entering the plant is estimated at 21,410 population equivalent (PE). The average daily flow during 2013 was

Table 3.5. Energy intensities at a given HRT for the bench-scale PUV light system at 900 V

HRT(s)	PPS	Estimated UV dose (<300nm; J/cm²)	Broad-spectrum dose (broad spectrum; J/cm²)
60	5.6	3.4	18.8
75	5.6	4.3	23.4
120	5.6	6.9	37.5

Table 3.6. Operational details of LPUV light and PUV light

Day	LPUV light			PUV light
(1)				
Flow rate (m^3/h)	0.228	0.520	0.849	0.4
UV dose (mJ/cm^2)	117	51.61	31.61	3447[a]
(2)				
Flow rate (m^3/h)	0.220	0.529	0.818	60
UV dose (J/cm^2)	121.99	50.73	32.81	3447[a]
(3)				
Flow rate (m^3/h)	0.209	0.526	0.810	60
UV dose (mJ/cm^2)	128.41	51.02	33.13	3447[a]

[a]Note for the PUV light, the energy dose indicated is the broad-spectrum dose and not directly comparable to the UV dose figures given for the LPUV light system.

3572 m^3/day with an average 5-day biochemical oxygen demand loading of 1476 kg/day. To ascertain the fate of FRNA bacteriophage through the WWTP, samples were taken from various key locations on two sampling days (Days 1 and 2). Batch samples of approximately 2 L of wastewater were collected from five sampling points at the WWTP; raw influent (R), primary treatment (PT), activated sludge reactors and secondary treatment (ST). The activated sludge reactor samples (denoted as A and AS) represented an activated sludge sample fully mixed (A) and following clarification (AS). Each sample was tested for FRNA bacteriophage presence via microbiological analysis.

3.1.5 Freezing stability test

The effect of deep-freezing wastewater samples for up to six months was investigated to evaluate its impact on viral nucleic acid. To date, no literature exists on the impacts that deep-freezing has on measurement accuracy for such environmental samples (e.g. in order to enable batch analysis of samples), although clinical samples are frequently stored for periods.

A batch of primary treated effluent (10 L) was collected and eighteen separate 50-mL samples were taken. These were separated into three batches of six 50-mL samples. The first batch of six was tested within 24 hours for norovirus GII by RT-qPCR. The remaining samples were stored at –80°C. The tests were repeated on batches of six samples at three and six months. The results from the three time points were compared.

3.1.6 Bench-scale barrier system experiments

A bench-scale TFF was employed as the barrier method for this experiment. The TFF employed cross-flow filtration action across a membrane cassette [Pall's Omega™ polyethersulfone (PES)] (Figure 3.2).

Batches of approximately 5 L of secondary treated wastewater were seeded with 1 × 1-mL aliquots of 10^9–10^{11} copies/100 mL of FRNA bacteriophage (GA strain;

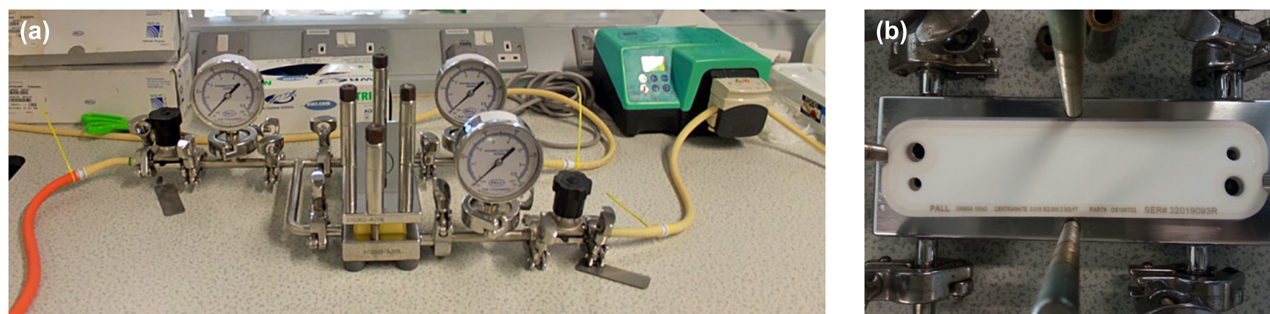

Figure 3.2. (a) Bench-scale TFF system; (b) membrane cassette.

from this point on it will be referred to as GA bacteriophage) and pumped into a bench-scale platform TFF system (Pall Life Sciences, Ireland, Carrigaline, Co. Cork) at a permeate flow rate of approximately 2 L/h. It was decided at the beginning of the study that only the membranes exhibiting significant reductions would then be spiked with NoV GI and GII (2 × 1-mL aliquots of approximately 10^7 copies/100 mL) stocks in addition to the bacteriophage because of the costly nature of the molecular assays. The microfiltration cassettes analysed had pore sizes 0.45 μm, 0.2 μm and 0.1 μm while the ultrafiltration cassettes analysed had molecular weight cut-offs of 500 kDa and 100 kDa. The microfiltration cassettes were trialled at least four times before moving on to the next pore size. For the ultrafiltration cassettes, both the 500-kDa cassette and the 100-kDa cassette clogged after two runs. A second 100-kDa cassette was used to complete an additional experiment for the ultrafiltration membrane.

3.2 Laboratory Methods

3.2.1 Virus enumeration

Samples were processed using (i) molecular analysis and (ii) microbiological analysis. NoV GI and GII analysis was carried out using molecular methods only while GA bacteriophage was processed by both molecular and microbiological analysis.

3.2.2 Molecular analysis

The virus concentration method employed a filter adsorption–elution method and was based on methods described in Flannery *et al.* (2012) modified from (Katayama *et al*., 2008). Four hundred microlitres of 2.5-M $MgCl_2$ was added to a sample of wastewater or spiked distilled water (40 mL) to give a final concentration of 25 mM. The pH was then adjusted to between 3.5 and 6.0 with 1-M HCL and mixed on a rocking platform for 30 minutes. The sample was then filtered through a glass fibre pre-filter, which was placed on a bacteriological membrane filter (filter with a pore size of 0.45 μm and diameter of 90 mm; Merck Millipore, Carrigtwohill, Co. Cork, Ireland) attached to a plastic magnetic filter holder. Once the sample had passed through, the bacteriological membrane filter was placed in 4 mL of 50-mM glycine-NaOH buffer (pH 9.5) and mixed on a rocking platform for 20 minutes. The virus eluate was transferred to a centrifugal filter tube containing 100 μL of 1-M HCL (pH 1.0), which was centrifuged at 4000 × g for 10 minutes. The filter unit was then washed in 550 μL of molecular biology-grade (MBG) water to give a virus concentrate of approximately 500 μL.

The 500-μL sample was then brought forward for RNA extraction using the NucliSENS miniMAG extraction platform and NucliSENS magnetic extraction reagents (bioMérieux, Marcy l'Etoile, France). Briefly, the sample was placed in lysis buffer to release viral RNA. Magnetic silica was then added to the lysed samples to which the RNA attached. This sample was then washed in a series of buffers to remove inhibitors and contaminants and was finally eluted off the silica using elution buffer and placed in –20°C storage until PCR analysis. RT-qPCR was carried out on samples to test for NoV GI, NoV GII (Flannery *et al.*, 2012) and GA bacteriophage (Flannery *et al.*, 2013). Aliquots (5 μL) of sample RNA were added in duplicate to a 96-well optical reaction plate. Appropriate one-step mastermix (20 μL), including specific primers and probes for the target virus genome, was also added to the well. In addition, a double-stranded DNA (dsDNA) curve was constructed for quantification, and a positive control and MBG water as a negative control were also used. The use of a non-related virus (Mengovirus; Costafreda *et al.*, 2006) was also employed as an "internal process control" to determine the extraction efficiency. PCR inhibition was also controlled for in the assay as described by Flannery *et al.* (2012). The plate was then placed in an AB7500 real-time PCR instrument (Applied Biosystems, Foster City, CA, USA) under the appropriate run conditions. Results were determined by examining cycle threshold (Ct) values and comparing them against the dsDNA curve generated by the PCR instrument to give genome copies per 100-mL of sample (copies/100 mL). The limit of quantification (LOQ) for this test was 125 detectable copies/100 mL and the limit of detection (LOD) was 25 detectable copies/100 mL. Both the LOQ and LOD have been demonstrated analytically in the laboratory.

3.2.3 Infectivity assay

A double-layer overlay plaque assay was employed for the microbiological analysis [International Organization for Standardization (ISO) 10705-1]. An appropriately diluted sample (1-mL sample) was added to 1 mL of host culture (*Salmonella enterica* subsp. *enterica* serovar Typhimurium) and 2.5 mL of molten tryptone–yeast glucose agar and held at 45°C. This mix was then

poured onto hardened tryptone–yeast glucose agar plates and left to solidify before being transferred to a 37°C incubator for 18±2 hours. Once the incubation period had passed, the plates were removed and characteristic plaques were counted where each plaque was assumed to originate from one GA bacteriophage. The results were expressed as plaque-forming units (pfu)/mL. The LOQ for this test was 1 pfu/mL.

3.2.4 **E. coli** ***enumeration in wastewater***

The standardised five-tube, three-dilution most probable number (MPN) method (ISO-TS 16649) was used to assess the presence/reduction of *E. coli* in UV light influent and UV light-treated effluent wastewater samples. Appropriate $\log_{10}$ dilutions of samples were inoculated into 10-mL volumes of minerals-modified glutamate broth (MMGB) (CM0607, Oxoid, Fisher Scientific Ireland Ltd, Dublin, Ireland) and were incubated at 37°C for 24±2 hours. The presence of *E. coli* was subsequently confirmed by sub-culturing tubes indicating acid production onto Tryptone Bile X-glucuronide (TBX) agar (CM0945, Oxoid) at 44°C for 22±2 hours. The LOD of the assay was 20 *E. coli*/100 mL.

3.2.5 Total coliforms test

Total coliforms were enumerated using the enzyme substrate coliform test, which followed the standard method 9223B enzyme substrate test (Quanti-Tray Colisure test, IDEXX). One hundred millilitres of appropriate $\log_{10}$ dilutions of the samples were mixed with commercially prepared enzyme substrates in sterile glass containers, poured into a 97-well Quanti-Tray/2000, sealed with the IDEXX tray-sealer and incubated at 35±0.5°C. Beta-galactosidase, an enzyme produced by total coliforms, was detected by hydrolysis – seen as a yellow colour after incubation for over 24 hours – confirming the presence of coliforms. The MPN method was used to quantify total coliforms presence with reference to the Quanti-Tray/2000 MPN table.

3.2.6 Suspended solids test

Two segments of 0.45-µm circular filter paper were labelled and weighed before 100-mL samples of secondary effluent were passed through each using a vacuum pump to give two replicates. The filter papers were then dried in a hot oven (105°C) for 24 hours. Once dried, the filter papers were removed and re-weighed to determine the amount of SS present. Two measurements were taken from each sample and an average value calculated.

3.2.7 Nutrient analysis

Total organic carbon (TOC) and total inorganic carbon (TIC) were measured using the Biotector TOC TN (total nitrogen) Analyser (Biotector Analytical System Ltd, Cork, Ireland). Samples (30 mL) were placed in glass cylindrical tubes and run at the appropriate cycle conditions. Deionised water was included in each run of samples analysed as a control, as well as nutrient standards to check that the system was reading the samples correctly. Results were expressed in milligrams per litre.

3.2.8 Metals analysis

Wastewater samples were tested for iron and manganese levels by the Environmental Laboratory Services (ELS) Ltd Acorn Business Campus, Mahon Industrial Park, Blackrock, Cork, Ireland. Five-litre batch samples were collected from three separate WWTPs (see section 3.1.6). Two litres of each sample was filtered through a 0.1-µm membrane cassette. One 75-mL sample of both unfiltered and filtered effluent from each site was held in a storage vial at the correct pH at 4°C. All samples were collected after two days and couriered to ELS for metals analysis. The LOQ of the tests were 5 µg/L and 1 µg/L for iron and manganese, respectively.

4 Results and Discussion

4.1 Bench-scale Ultraviolet Light Experiments

The key objectives of this study were to:

1. Evaluate the efficacy of RT-qPCR for analysing wastewater samples post UV light treatment.
2. Evaluate the use of FRNA bacteriophage as a surrogate for NoV.
3. Determine the impact of varying the operating conditions of the PUV light (i.e. voltage and pulse rate and ultimately UV dosage) on pathogen removal.
4. Compare pathogen removal in distilled water versus secondary effluent using the PUV light system.
5. Evaluate the impact of SS, TOC and TIC on PUV light performance (and by extension UV light performance) on pathogen removal (focusing on FRNA bacteriophage).

4.1.1 Comparison of RT-qPCR and infectivity assay

For this study, GA bacteriophage and NoV GI and GII were treated via PUV light and the results assessed via RT-qPCR (GA bacteriophage, NoV GI and GII) and via an infectivity assay (GA bacteriophage only). The effect of PUV light on virus removal in secondary effluent was measured at HRTs of 60 s and 120 s and at varying total SS concentrations. A total of seven effluent samples were tested for NoV GI and GII and GA bacteriophage via RT-qPCR pre- and post PUV light treatment. In parallel, these samples and a further three samples were tested for GA bacteriophage via an infectivity assay pre- and post PUV light treatment ($n = 10$). Figure 4.1 illustrates the mean $\log_{10}$ reduction of all three viruses via the molecular method (RT-qPCR) and the microbiological method (infectivity assay). Results for the infectivity assay show approximately a 2 $\log_{10}$ mean reduction/inactivation of GA bacteriophage at the maximum HRT of 120 s over the entire study period.

In contrast, the RT-qPCR results for GA bacteriophage, NoV GI and NoV GII display a mean reduction/inactivation of $< 0.5 \log_{10}$ of copy number at a maximum HRT of 120 s. These results further highlight the difficulties associated with using this type of molecular analysis when assessing the effects of UV light on NoV reduction (Figure 4.1).

4.1.2 FRNA bacteriophage as a surrogate for norovirus

Owing to the high resistance properties of bacteriophages to UV light treatment and its morphological similarities to NoV, the use of this virus as a potential surrogate has value. This option may currently offer the

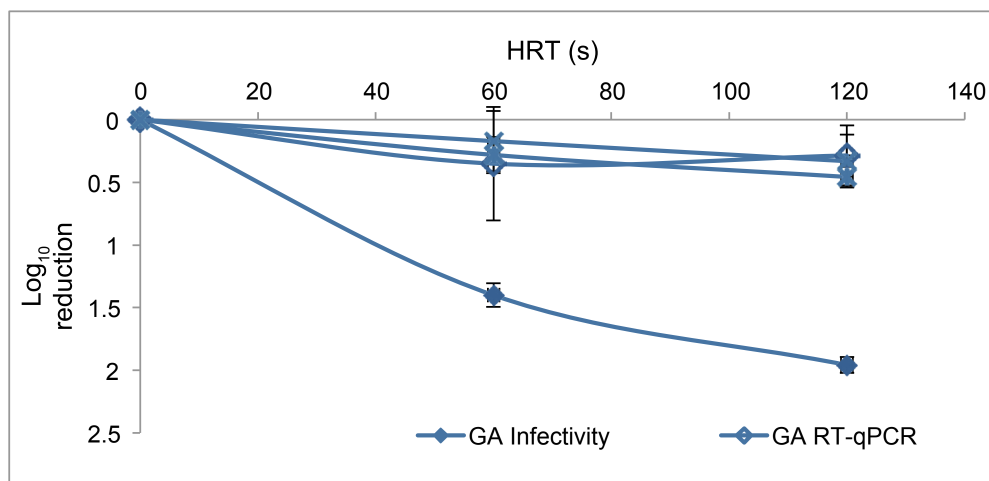

Figure 4.1. Average $\log_{10}$ reduction of viable GA bacteriophage (infectivity assay, $n = 10$) and copy number of GA bacteriophage and NoV GI and GII (RT-qPCR, $n = 7$) post PUV light treatment in wastewater.

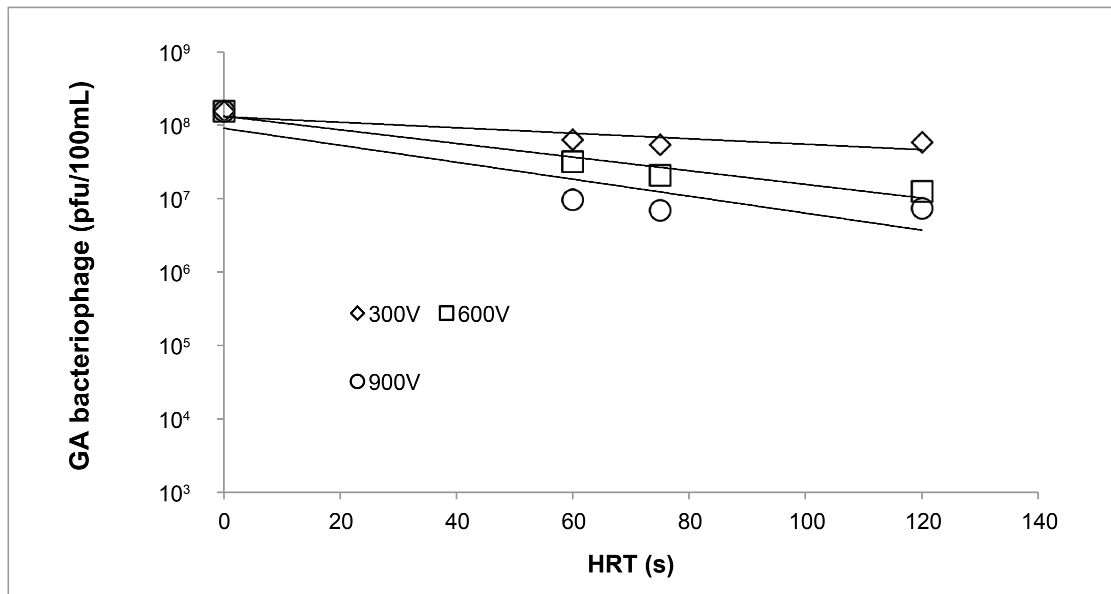

Figure 4.2. GA bacteriophage reduction at various voltages (300 V, 600 V and 900 V) and HRTs (60 s, 75 s and 120 s) at 1 PPS.

most effective method of determining the efficiency of disinfection processes (in particular UV light) in achieving NoV reduction. However, direct comparison of both viruses post UV light treatment was not possible due the current lack of a suitable methods for NoV cultivation.

4.1.3 Impact of varying the operating conditions of the PUV light

Figure 4.2 illustrates the reduction of GA bacteriophage at the range of UV doses listed in Table 3.4 (section 3.1.1). At 300 V, maximum virus reduction was 0.4 log_{10} at a UV dose of 137 mJ/cm^2 (120 HRT). A reduction of 1 log_{10} was achieved at maximum UV dose of 547 mJ/cm^2 (120 HRT) at 600 V. While at 900 V, GA bacteriophage concentration was reduced by 1.3 log_{10} at a UV dose output of 1231 mJ/cm^2.

4.1.4 Comparison of pathogen removal in distilled water and secondary treated wastewater

GA bacteriophage removal via PUV light was investigated in both distilled water and secondary effluent. In this study, 5-L batches of secondary treated effluent wastewater were seeded with known concentrations of GA bacteriophage. The samples were pumped through the PUV light system at HRTs of 60 s, 75 s (only used in one trial) and 120 s. In total, 10 secondary effluent samples of varying SS concentrations were tested ($n = 10$).

Similarly, during distilled water trials, samples ($n = 3$) were pumped through the PUV light system at HRTs of 60 s, 75 s and 120 s with the exception of one trial, which also included a HRT of 67.5 s. In all cases, samples were collected pre- and post PUV light treatment and assessed for virus presence/reduction at each stage.

Figure 4.3 summarises the reduction of infective GA bacteriophage following PUV light in both distilled water and secondary effluent. In distilled water, at a HRT of 60 s a 3–4 log_{10} reduction in viable GA bacteriophage was observed. At a HRT of 67.5 s GA bacteriophage was below the limit of detection (< 1 pfu/mL).

However, in the secondary wastewater effluent samples, the greatest removal of GA bacteriophage achieved was 3 log_{10} at the maximum HRT of 120 s. These data suggest that there are significant PUV light “inhibitory” factors within secondary effluent not present in distilled water. SS, organic carbon and inorganic carbon concentrations are known to impact on pathogen removal in UV light processes. Metals, such as iron and magnesium, are also known to affect UV light performance; however, this was considered unlikely given the municipal nature of the wastewater and the specific site’s commercial and residential characteristics.

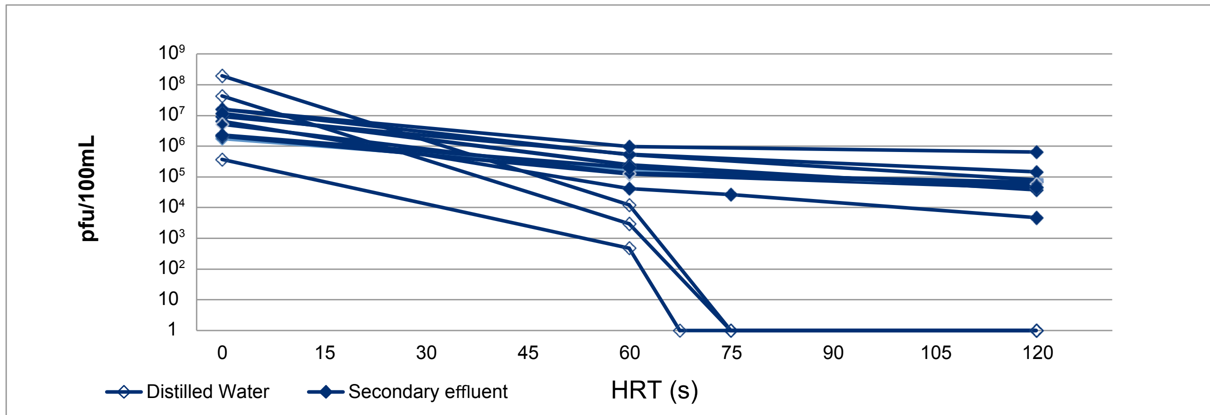

Figure 4.3. Comparison of UV light performance in both distilled water and secondary effluent at a maximum UV dose of 6895.6mJ/cm² (120 HRT); secondary effluent samples (*n*=10), distilled water samples (*n*=3).

4.1.5 Impact of suspended solids, organic and inorganic carbon on PUV light performance

The impact of SS on PUV light efficiency was investigated over a series of trials. In all cases, except where stated, wastewater samples used in the trials were taken directly from the same municipal WWTP.

Trial 1 investigated the effects of PUV light on GA bacteriophage reduction. Following on from this three further trials (Trials 2, 3 and 4) were carried out to establish the impact of SS concentration on pathogen inactivation/removal performance (Table 4.1). For each of Trials 2, 3 and 4, which were carried out on separate sampling days, a batch of secondary effluent was spiked with a known concentration of mixed liquor SS (MLSS) and seeded with a known concentration of GA bacteriophage. Samples were collected pre-treatment and post treatment at 60 s HRT and 120 s HRT. The $\log_{10}$ reduction at each HRT was then assessed for each SS concentration. The TOC and TIC concentrations were also recorded for each sample tested and compared with virus $\log_{10}$ reduction.

The virus removal efficiency at each SS concentration was compared over the four trials. Table 4.1 and Figures 4.4 and 4.5 illustrate the relationship between SS concentration and maximum $\log_{10}$ reduction (120 s HRT) of GA bacteriophage. It should be noted that the correlation for each individual trial between SS concentration and $\log_{10}$ reduction was high. However, the results also

Table 4.1. Range of sample SS concentrations (mg/L) and $\log_{10}$ reductions of infective GA bacteriophage in wastewater at two HRTs for the PUV light study period

				Log_{10} reduction HRT	
Trial	SS (mg/L)	TOC (mg/L)	TIC (mg/L)	60 (s)	120 (s)
1	57.5	8	24	2.2±0.2	3.1±0.0
2	12.0	14	41	1.3± 0.1	1.8±0.0
	18.6	20	38	1.0±0.1	1.6±0.1
	121.2	20	39	1.2±0.0	1.5±0.1
3	38.0	10	33	1.5±0.1	2.3±0.1
	105.0	8	32	1.2±0.2	1.8±0.0
	140.6	7	29	1.2±0.0	1.4±0.1
4	19.5	12	25	1.7±0.1	2.4±0.1
	72.0	16	26	1.6±0.1	1.2±0.0
	89.8	15	28	1.1±0.1	1.7±0.2

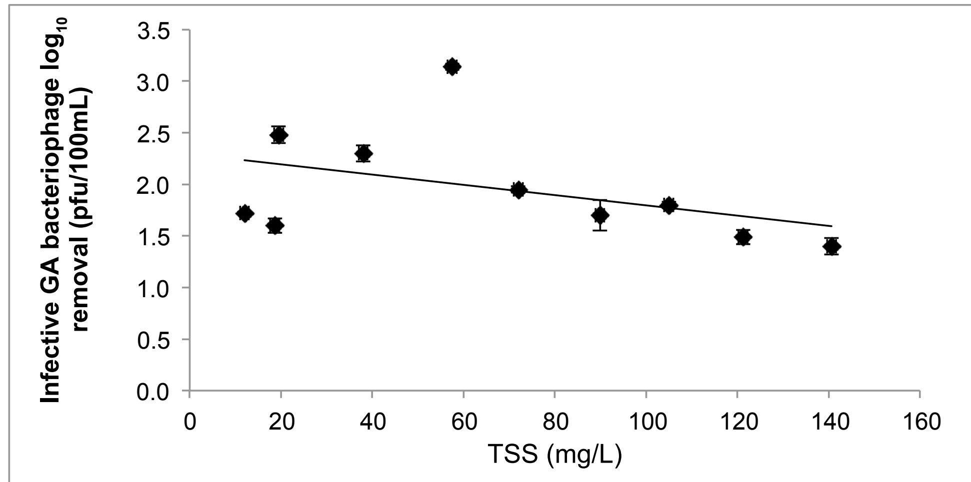

Figure 4.4. Relationship between SS and maximum log reduction (dose = 6895.6 mJ/cm²) of GA bacteriophage determined by infectivity assay over a series of trials.

indicate a relatively low statistical correlation (≈ –0.42) between higher SS concentrations and decreasing $\log_{10}$ reductions of GA bacteriophage across all samples. This indicates that while SS has a major impact on UV light performance the presence of other contaminants, such as organic carbon or certain metals, may also be important.

Figure 4.5 illustrates the maximum $\log_{10}$ reduction during Trials 2, 3 and 4 for varying SS concentrations.

The impact of TOC and TIC on $\log_{10}$ reductions of GA bacteriophage was investigated over the four trials. The results show a similar overall trend (but relatively low statistical correlations; the correlation coefficients were –0.42 and –0.59 between $\log_{10}$ removal and TOC and TIC, respectively) to the impact of SS. At higher TOC and TIC concentrations the general trend is for decreased $\log_{10}$ reductions (Figure 4.6).

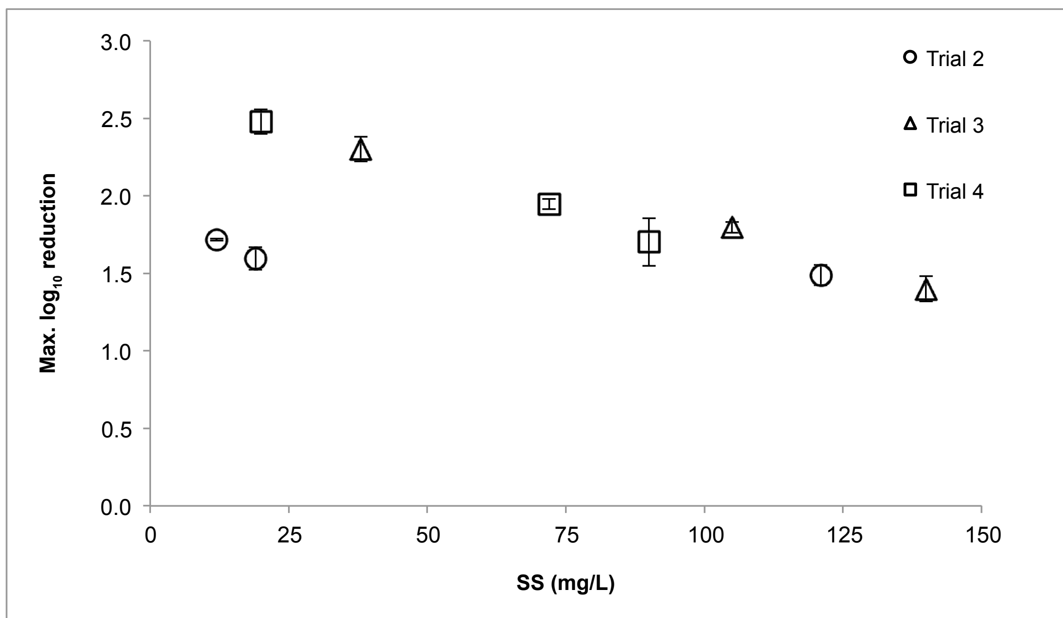

Figure 4.5. Comparison of the relationship between SS and maximum $\log_{10}$ reduction (dose = 6895.6 mJ/cm²) of GA bacteriophage determined by infectivity assay in three separate trials.

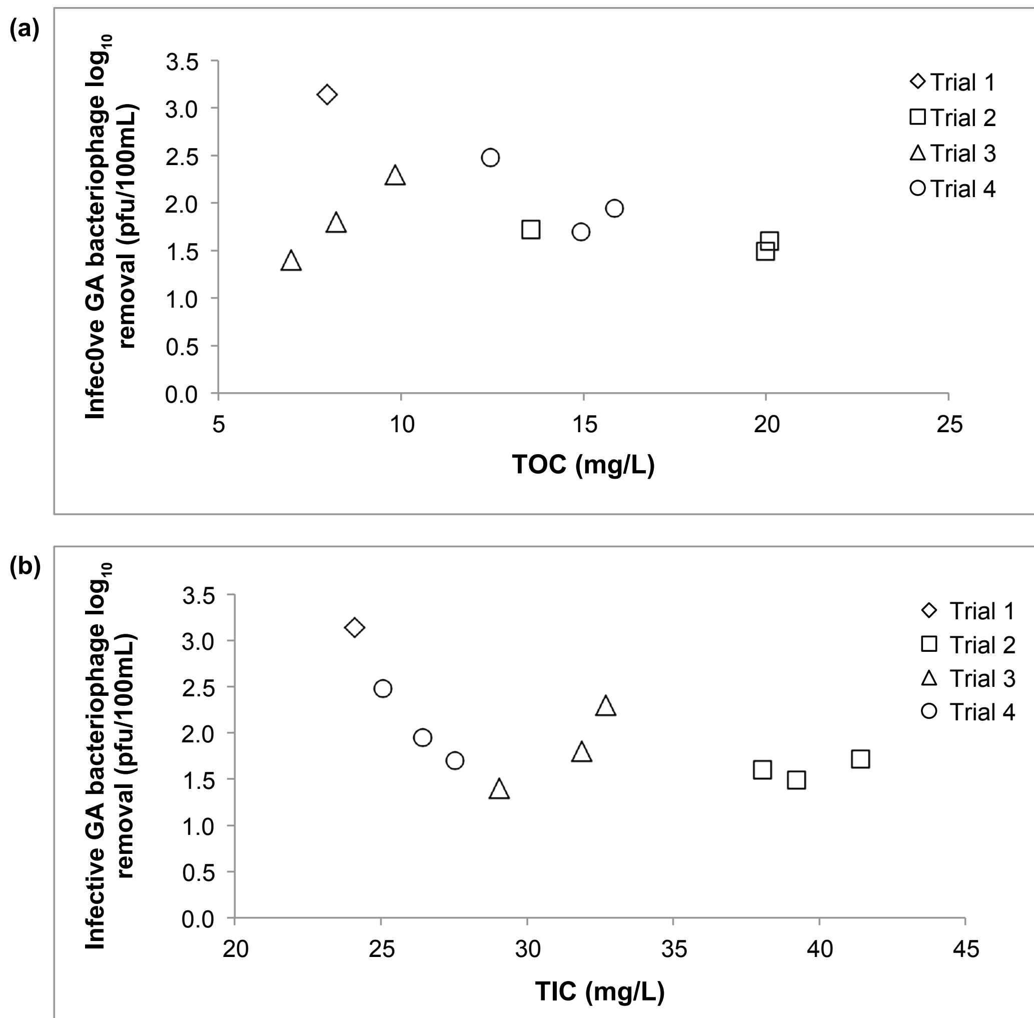

Figure 4.6. (a) The relationship between TOC and maximum log reduction (dose = 6895.6 mJ/cm²) of GA bacteriophage determined by infectivity assay over a series of trials. (b) The relationship between TIC and maximum log reduction (dose = 6895.6 mJ/cm²) of GA bacteriophage determined by infectivity assay over a series of trials.

4.1.6 The comparison of PUV light efficiency in filtered versus unfiltered effluent

To further investigate the effects of SS concentrations on PUV light performance, analysis was carried out on filtered and unfiltered secondary effluent. The SS concentrations of the treated wastewater at Sites 1, 2 and 3 were 19.5 mg SS/L, 8.7 mg SS/L and 16.0 mg SS/L, respectively.

Table 4.2 shows the comparison of GA bacteriophage $\log_{10}$ reduction in the filtered and unfiltered samples from each site. Site 1 exhibited an increased $\log_{10}$ reduction of 3.9 $\log_{10}$ when the secondary effluent was filtered. Moreover, removal to below the LOD (< 1 pfu/mL) was achieved at just 60 s HRT for GA bacteriophage. Increased virus $\log_{10}$ reduction in filtered effluent compared with unfiltered effluent was also observed for Sites 2 and 3; however, to a lesser extent with $\log_{10}$ reductions of 0.9 $\log_{10}$ for both sites.

These results indicate that while SS has a key impact on UV light performance, other wastewater characteristics also play a significant role. It also suggests that batch tests on individual sites should be carried out during the commissioning stage of the UV light system, and while the WWTP is performing at a steady state (or indeed while it is stressed) to ascertain the impact of wastewater quality on virus removal on a site-by-site basis.

Certain metals (e.g. iron and manganese) can have an impact on UV light performance and so “spot checks” of both metals were carried out on all three sites (USEPA,

Table 4.2. Comparison of maximum log_{10} reduction of GA bacteriophage in filtered and unfiltered effluent at three separate WWTP sampling sites

	Unfiltered effluent		Filtered effluent	
	HRT (s)	GA bacteriophage (pfu/100 mL)	HRT (s)	GA bacteriophage (pfu/100 mL)
Site 1	0	$1.2\times10^7\pm6.4\times10^5$	0	$2.3\times10^6\pm1.8\times10^5$
	60	$2.5\times10^5\pm4.4\times10^4$	60	< 1 pfu/mL
	120	$3.9\times10^4\pm4.9\times10^3$	120	< 1 pfu/mL
Log_{10} reduction		**2.5±0.1**		**6.4±0.0**
Site 2	0	$1.5\times10^7\pm9.7\times10^5$	0	$1.5\times10^7\pm1.1\times10^6$
	60	$2.7\times10^5\pm6.7\times10^4$	60	$9.5\times10^4\pm2.3\times10^4$
	120	$6.4\times10^4\pm2.0\times10^4$	120	$7.0\times10^3\pm1.4\times10^3$
Log_{10} reduction		**2.4±0.1**		**3.3±0.1**
Site 3	0	$1.1\times10^6\pm7.1\times10^4$	0	$1.1\times10^7\pm4.6\times10^6$
	60	$3.7\times10^4\pm7.5\times10^3$	60	$8.1\times10^4\pm3.8\times10^4$
	120	$2.4\times10^4\pm7.8\times10^3$	120	$3.8\times10^4\pm3.4\times10^4$
Log_{10} reduction		**1.7±0.2**		**2.5±0.3**

2006b). Ferric iron (Fe^{3+}) and permanganate ($MnO4^-$) have high absorption coefficients causing them to absorb UV light significantly in the 200–300-nm region, which, in turn, decreases UV light transmittance (Bolton *et al.*, 2001). The acceptable limit for iron concentrations in wastewater is about 0.3 mg/L (Das, 2001). Figure 4.7 illustrates the concentrations of both metals (µg/L) in filtered and unfiltered samples from all three sites. Manganese concentrations were low in all sites and samples, and were unlikely to cause inhibition. While iron concentrations in Sites 2 and 3 were higher in unfiltered effluent than in Site 1, wastewater is a complex media thus the role of any one contaminant (e.g. SS, TOC, Fe) in inhibiting UV light was difficult to ascertain and may indeed be site specific.

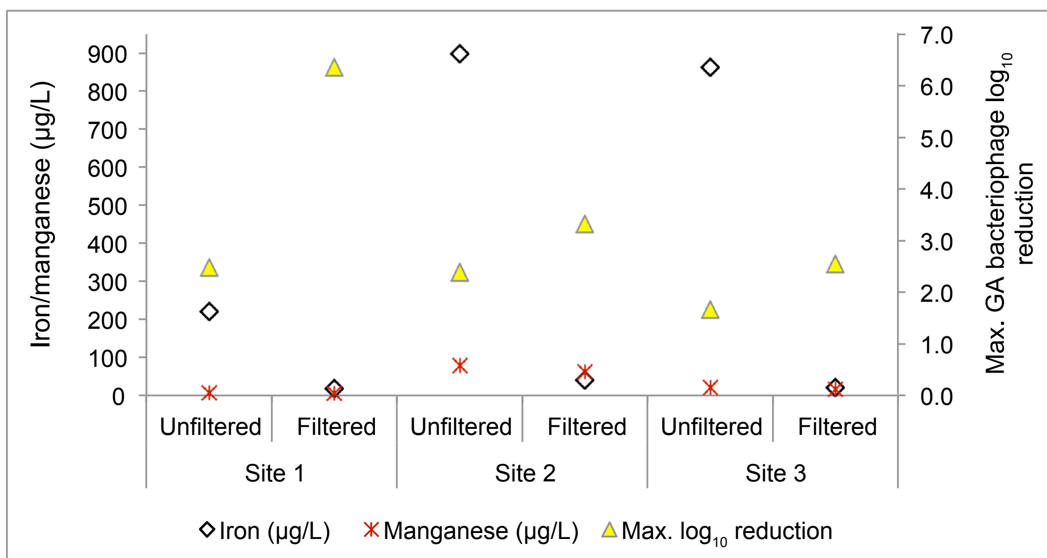

Figure 4.7. Iron and manganese concentrations and GA bacteriophage log_{10} reduction via PUV light in both filtered and unfiltered effluent from three separate WWTP sites.

4.2 Performance of On-site LPUV Light and Bench-scale PUV Light Systems

The performance of the bench-scale PUV light and an on-site LPUV light was analysed by comparing the effects of a UV dose (mJ/cm²) on the reduction of *E. coli*, total coliforms and FRNA bacteriophages over three consecutive sampling days (Figure 4.8a, b and c, respectively). For further information see section 3.1.3. As expected, for each organism, a decreasing reduction was observed with increasing UV dose.

Table 4.3 outlines the removal of *E. coli, Bacillus subtilis (B. subtilis)* and GA bacteriophage via PUV light both in this study and in recent work by Uslu *et al.* (2015). To

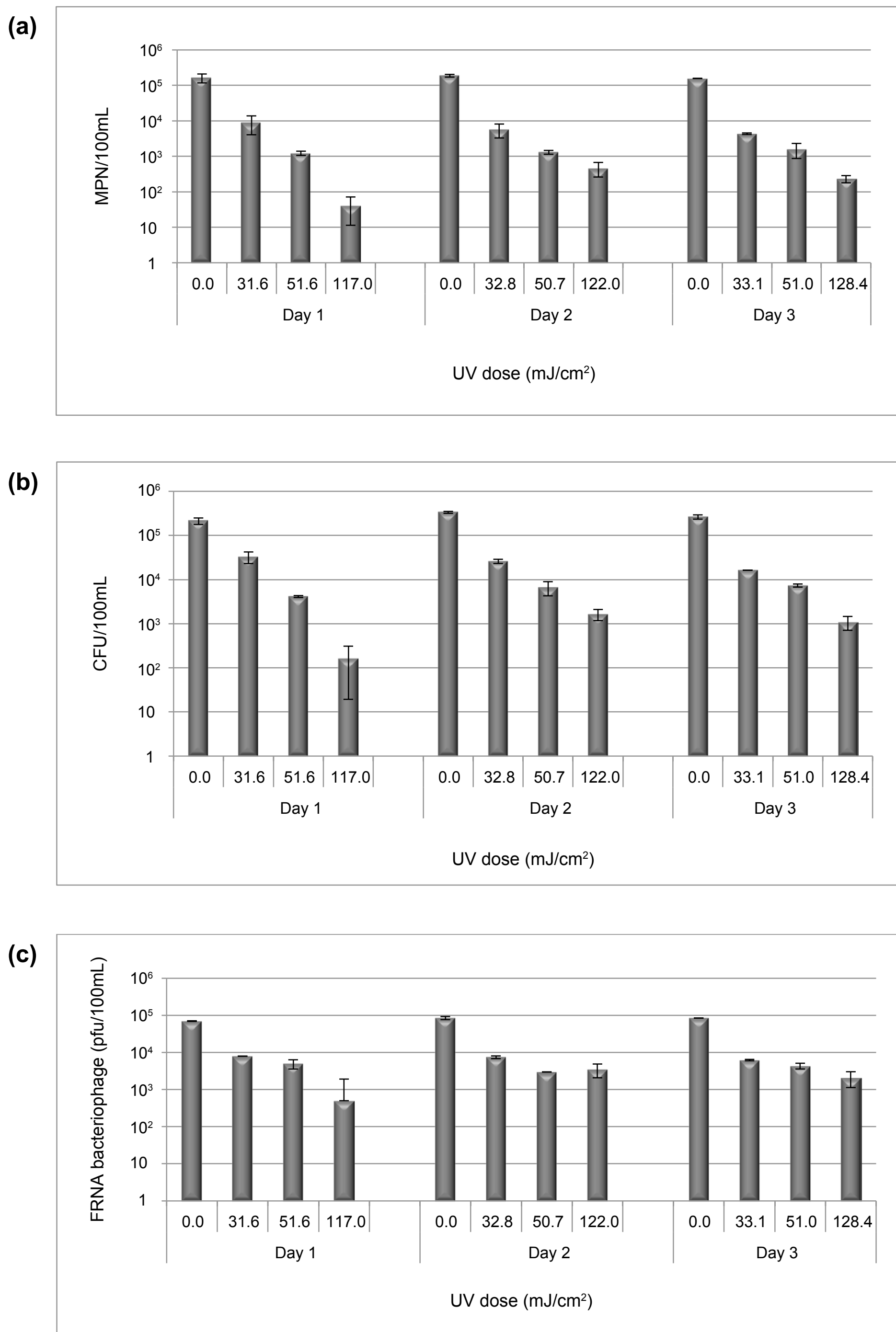

Figure 4.8. (a) *E. coli* reduction via LPUV light over three separate trials. (b) Total coliforms reduction via LPUV light over three separate trials. (c) FRNA bacteriophage reduction via LPUV light over three separate trials.

Table 4.3. Microorganism reduction via PUV light treatment

Results from Uslu *et al.* (2015)						
PUV light operating conditions	**Voltage: 3800 V** **Capacitance: N/A** **System set-up: static**			**Lamp distance from sample: 8 cm** **Discharge time: 360 µs** **Medium: treated municipal wastewater**		
Microorganism	*E. coli*			*B. subtilis*		
Initial concentration	10^8 CFU/mL			10^9 CFU/mL		
SS (mg/L)	4			4		
Exposure time (s)	5	10	15	5	10	15
Broad-spectrum dose (J/cm^2)	3.6	7.2	10.9	3.6	7.2	10.9
Log_{10} removal (CFU/mL)	≈7 log	≈7 log	Complete reduction (undetected)	≈4 log	≈4 log	≈6 log
Results from this study						
PUV light operating conditions	**Voltage: 900 V** **Capacitance: 40 µF** **System set-up: flow-through**			**Lamp distance from sample: 10.75 cm** **Discharge time: 28 µs** **Medium: treated municipal wastewater**		
Microorganism	*E. coli*			GA bacteriophage		
Initial concentration	10^5 MPN/100 mL			10^7 pfu/mL	10^7 pfu/mL	10^6 pfu/mL
SS content (mg/L)	28			20	38	72
Exposure time (s)	60			120	120	120
Broad-spectrum dose (J/cm^2)	18.8			37.5	37.5	37.5
Log_{10} removal	2 $\log_{10}$ MPN/100 mL			2.48 $\log_{10}$ pfu/100 mL	2.30 $\log_{10}$ pfu/100 mL	1.95 $\log_{10}$ pfu/100 mL

CFU, colony-forming units.

the knowledge of the authors this study is the first on the use of flow-through PUV light systems in the wastewater sector. Unlike this study, in which a flow-through system as investigated, Uslu *et al.* (2015) operated the PUV light as a static system (as yet there have been no publications on flow-through systems for wastewater applications to the knowledge of the authors). It should be noted that, although the systems are somewhat comparable, issues such as the distance from the sample to the lamp, operational differences and sample differences mean that direct comparison between these systems is not yet possible. However, Table 4.3 does provide a first of this kind comparison for indicative purposes. In general, the broad-spectrum dose emitted via the PUV light systems is considerably higher than the energy emitted by LPUV light systems; however, as stated in section 2.4, this includes wavelengths other than UV light. Further research is required in these areas to determine system capabilities, the efficacy of flow-through systems (which would be required in the wastewater sector) and methods to enable better comparison between LP and PUV light systems.

4.3 Fate of FRNA Bacteriophage Through a WWTP

Batch samples of approximately 2 L of wastewater were collected from five sampling points at the WWTP; raw influent (R), primary treatment (PT), activated sludge treatment (A) and (AS) and secondary treatment (ST). The "activated sludge treatment" sample was tested twice; (A) and (AS). (A) represented the sample when fully mixed; (AS) represented the same sample with total suspended solid settlement. Suspended solid settlement was achieved by transporting the mixed sample back to laboratory and allowing it to settle for at least 2 hours before the supernatant was collected and tested. At each point, samples were tested for FRNA bacteriophage presence via microbiological analysis. This analysis was carried out on two separate occasions; Days 1 and 2.

Figure 4.9 represents the fate of infectious FRNA bacteriophage through a municipal WWTP. Activated sludge samples (A) and (AS) are MLSS samples that are mixed and settled, respectively – i.e. AS is the clarified

supernatant after sample A was left to settle. Greater than 2 $\log_{10}$ reduction of FRNA bacteriophage was observed in the clarified wastewater (AS) after settling occurred.

A sample of secondary effluent was collected during a period of heavy rainfall and tested as both mixed and clarified (Figure 4.10 – sample 3). Results were compared with both activated sludge samples collected on Days 1 and 2 at points in the municipal WWTP (Figure 4.9). A 1.75 $\log_{10}$ removal FRNA bacteriophage was achieved via settling in the secondary treated effluent sample.

While this was a mini-study, information indicating that virus removal may be achieved via "settling" processes is useful and would warrant further investigations. Moreover, if virus attachment to solids is indeed occurring, this may have implications for combined sewer overflows or emergency overflows from pump stations, which can release untreated effluent into the environment during periods of heavy rainfall. These results also highlight the impact of the importance upstream settlement processes, i.e. primary and secondary treatment on downstream effluent quality.

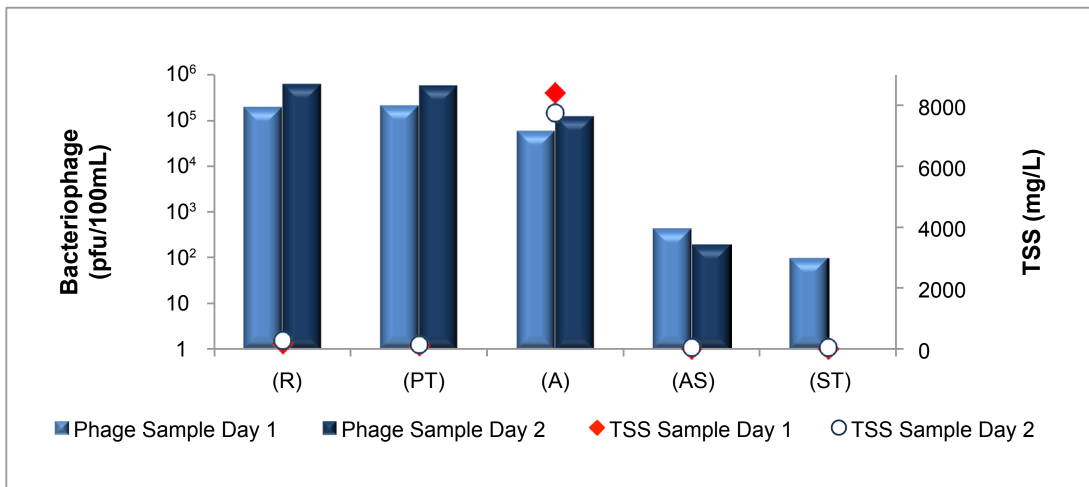

Figure 4.9. FRNA bacteriophage abundance (pfu/100 mL) at points in a WWTP. R, raw influent; PT, primary treatment; A, activated sludge; AS, activated sludge "settled"; ST, secondary treatment.

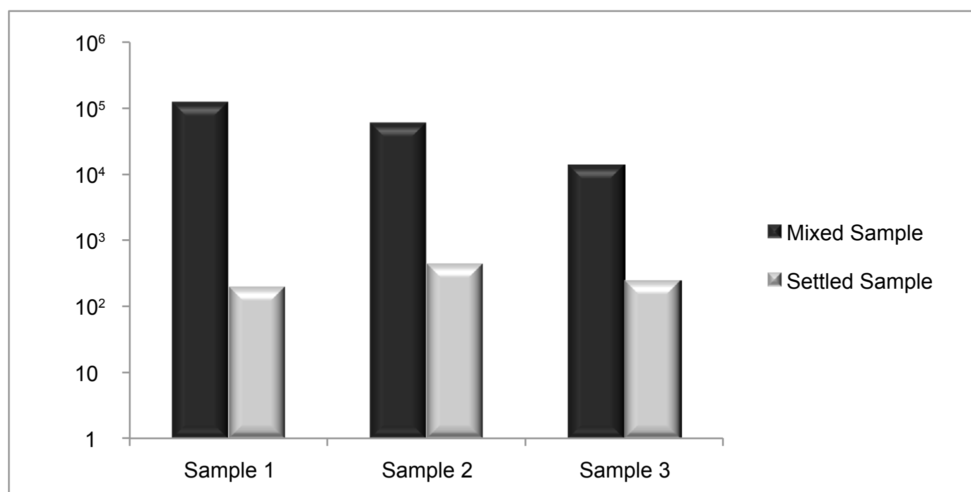

Figure 4.10. Comparison of FRNA bacteriophage in a wastewater sample; tested as both mixed and settled (*n* = 3).

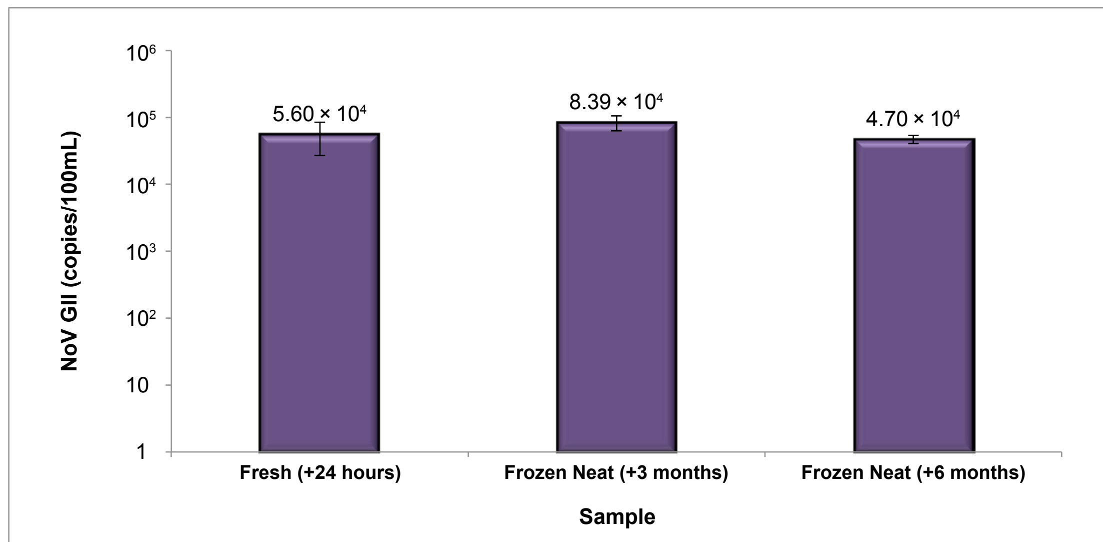

Figure 4.11. Comparison of NoV GII counts via PCR assay in primary treated wastewater (average of *n* = 6 longer than samples for each of fresh, frozen for >3 months and frozen for >6 months).

4.4 Freezing Stability Test

Little information is available on the stability of viral nucleic acids in environmental samples during long periods of storage; thus, a short study was carried out to determine whether or not freezing would be a viable option for storing samples. The ability to freeze samples could reduce workloads and enable intensive studies that may result in large numbers of samples.

Figure 4.11 summarises the effects of long-term freezing storage (–80°C) on virus copy number for NoV. There was no significant reduction in virus copy number during storage. Results indicate that deep freezing would be an option for large batch analysis of NoV decreasing the costs and requirements for immediate testing.

4.5 Bench-scale Membrane Barrier System Experiments

In this study, microfiltration cassettes (pore sizes 0.45 μm, 0.2 μm and 0.1 μm) and ultrafiltration cassettes consisting of 500 kDa and 100 kDa (0.05 μm and 0.01 μm, respectively) were investigated for their efficiency in removing NoV and FRNA bacteriophage from secondary treated wastewater.

Owing to the limitations of working with NoV, secondary effluent was seeded with GA bacteriophage only until significant virus removal was achieved using either a micro- or a ultrafiltration cassette. Cassettes that were successful in removing bacteriophage were then dosed with wastewater samples seeded with both GA bacteriophage and NoV GII.

4.5.1 *Microfiltration results*

Batches of secondary treated effluent of approximately 5 L were seeded with GA bacteriophage and pumped into a bench-scale platform TFF system (Minimate TFF capsule system, PALL Life Sciences) at a permeate flow rate of approximately 2 L/h. Each microfiltration cassette was trialled at least four times (or until it fully clogged – see section 3.1.3).

GA bacteriophage reduction varied between cassette pore sizes. However, average $\log_{10}$ reductions per cassette did not exceed 1 $\log_{10}$ (Table 4.4). The largest pore size cassette (0.45 μm) exhibited virus removal rates from 0.26 to 0.85 $\log_{10}$ reduction. GA bacteriophage reduction improved slightly with the 0.2-μm pore size cassette. The final microfiltration cassette (0.1 μm) exhibited the smallest reduction

Table 4.4. Log_{10} reduction of infective GA bacteriophage via microfiltration

Cassette	Trial	Feed	Permeate	Removal	Log_{10} reduction	Average log_{10} reduction
0.45 µm	1	5.40×10^3	7.50×10^2	4.65×10^3	0.85	0.62 ± 0.26
	2	6.50×10^4	3.60×10^4	2.90×10^4	0.26	
	3	1.65×10^4	7.00×10^3	9.50×10^3	0.38	
	4	5.65×10^3	1.80×10^3	3.85×10^3	0.49	
0.2 µm	1	9.75×10^7	7.20×10^6	9.03×10^7	1.13	0.93 ± 0.28
	2	1.17×10^8	3.49×10^7	8.21×10^7	0.52	
	3	1.17×10^8	1.18×10^7	1.05×10^8	1.00	
	4	1.17×10^8	1.01×10^7	1.07×10^8	1.06	
0.1 µm	1	9.35×10^7	4.08×10^7	5×10^7	0.36	0.15 ± 0.15
	2	8.38×10^7	6.58×10^7	2×10^7	0.11	
	3	3.68×10^7	2.75×10^7	9×10^6	0.13	
	4	3.45×10^7	3.40×10^7	5×10^5	0.01	

in GA bacteriophage. Log_{10} reductions varied from 0.01 to 0.36, and, overall, removal rates were poor. Contributing factors may include potential damage to the cassette membrane, but this could not be verified during the experiment.

The effect of SS loading on cassette performance was also investigated. The cumulative solids loading (g SS/m^2/h) on the membranes were recorded for each trial to establish whether or not clogging may have an effect on membrane performance and virus removal. The results show that cumulative solids loading had limited effect on virus reduction for microfiltration cassettes (Figure 4.12).

While each of the cassettes exhibited signs of clogging this did not have an impact on virus reduction; however, it significantly impacted on the flux through the membrane.

4.5.2 Ultrafiltration results

For the ultrafiltration cassettes, both the 500-kDa cassette and the 100-kDa cassette clogged after two trials. Given that the 100-kDa cassette was most successful in removing both viruses a second 100-kDa cassette was purchased to complete four trials for this pore size.

The 500-kDa cassette

Initial removal rates of GA bacteriophage, using the 500-kDa cassette were low (Trial 1, Table 4.5). In Trial 2, influent concentrations of GA bacteriophage and NoV GII were similar and removal of both viruses was achieved to below the LOD (< 1 pfu/mL and < 25 copies/100 mL). During this trial, the cassette ports clogged and the trial was stopped.

The 100-kDa cassette

Trial 1 consisted of batch seeding with GA bacteriophage only (NoV not included) continuing on from microfiltration analysis. The results showed a log_{10} reduction of 5.27 indicating that this pore size was sufficient for comparison analysis of NoV reduction via ultrafiltration (Table 4.6). Trial 2 showed similar log_{10} reductions for both FRNA bacteriophage and NoV GII; however, it should be noted that because of limitations regarding the concentration of NoV seeding material, the influent concentration of NoV GII for each trial is lower than that of GA bacteriophage. During Trial 2 clogging of the 100-kDa cassette occurred and the run was stopped. A second cassette was then purchased and Trials 3 and 4 were completed. Overall, results show removal of NoV GII to below the LOD while removals rates for GA bacteriophage are between approximately 3 and 5 log_{10}.

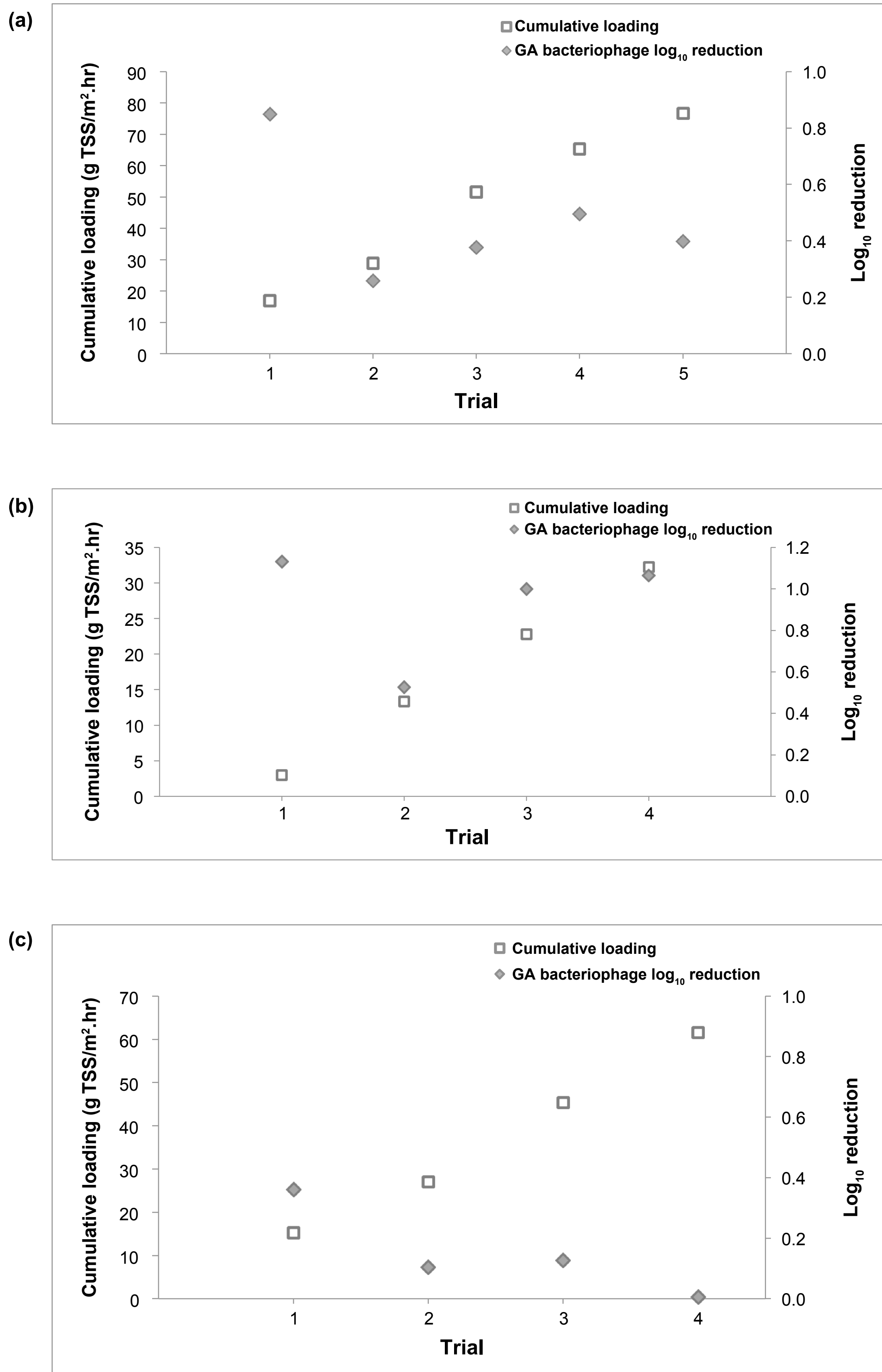

Figure 4.12. The effects of cumulative solids loading on GA bacteriophage log_{10} removal for microfiltration cassettes: (a) 0.45-µm pore size cassette, (b) 0.2-µm pore size cassette and (c) 0.1-µm pore size cassette.

Table 4.5. Log$_{10}$ reduction of GA bacteriophage and NoV GII via ultrafiltration; 500-kDa cassette

	Trial			
	1		2	
	GA	NoV GII	GA	NoV GII
Feed	2.50×10^5	4.58×10^2	2.05×10^3	1.21×10^3
Permeate	8.36×10^4	<25 copies/100 mL	<1 pfu/mL	<25 copies/100 mL
Removal	1.66×10^5	4.33×10^2	2.03×10^3	1.18×10^3
Log$_{10}$ reduction	**0.47**	**1.26**	**1.91**	**1.68**

Table 4.6. Log$_{10}$ reduction of GA bacteriophage and NoV GII via ultrafiltration; 100-kDa cassette

	Trial							
	1		2		3		4	
	GA	NoV GII	GA	NoV GII	GA	NoV GII	GA	NoV GII
Feed	5.55×10^7	–	1.07×10^8	9.67×10^3	8.18×10^6	1.92×10^4	1.01×10^7	3.21×10^3
Permeate	3.00×10^2	–	1.20×10^5	<25 copies/100 mL	3.50×10^2	<25 copies/100 mL	8.25×10^2	<25 copies/100 mL
Removal	5.55×10^7	–	1.07×10^8	2.59×10^0	8.17×10^6	2.89×10^0	1.00×10^7	2.11×10^0
Log$_{10}$ reduction	**5.27**	**–**	**2.95**	**2.59**	**4.37**	**2.89**	**4.09**	**2.11**

The effect of cumulative SS loading (g SS/m^2/h) was also investigated for the ultrafiltration cassettes with regard to both GA bacteriophage and NoV GII removal. Results followed the same trends as that of the microfiltration cassettes; cumulative solids loading had no overall effect on virus reduction for microfiltration cassettes (Figure 4.13).

Final comparisons of micro- and ultrafiltration cassettes with regard to virus removal could be completed only by comparing removal rates for GA bacteriophage. Figure 4.14 illustrates the average log removal of GA bacteriophage for each cassette. As expected, the 100-kDa pore size cassette achieved the greatest virus removal rates with an average of 4 log$_{10}$ over both cassette trials. The ultrafiltration cassettes also exhibited the greatest variation in virus reduction. The 0.1-µm cassette exhibited poor removal rates overall and results do not appear to follow the trend of the other microfiltration cassettes; it has been excluded from the trend shown in Figure 4.14.

Results generated in this section of the study highlight the challenges associated with the implementation of this type of barrier method as an on-site virus removal system. Filtration devices, particularly those of smaller pore sizes, may prove labour intensive and troublesome. As secondary effluent generally contains SS (the concentrations of which can vary widely), a pre-treatment, such as coagulation or sand filtration, would perhaps be necessary to avoid clogging of the membranes.

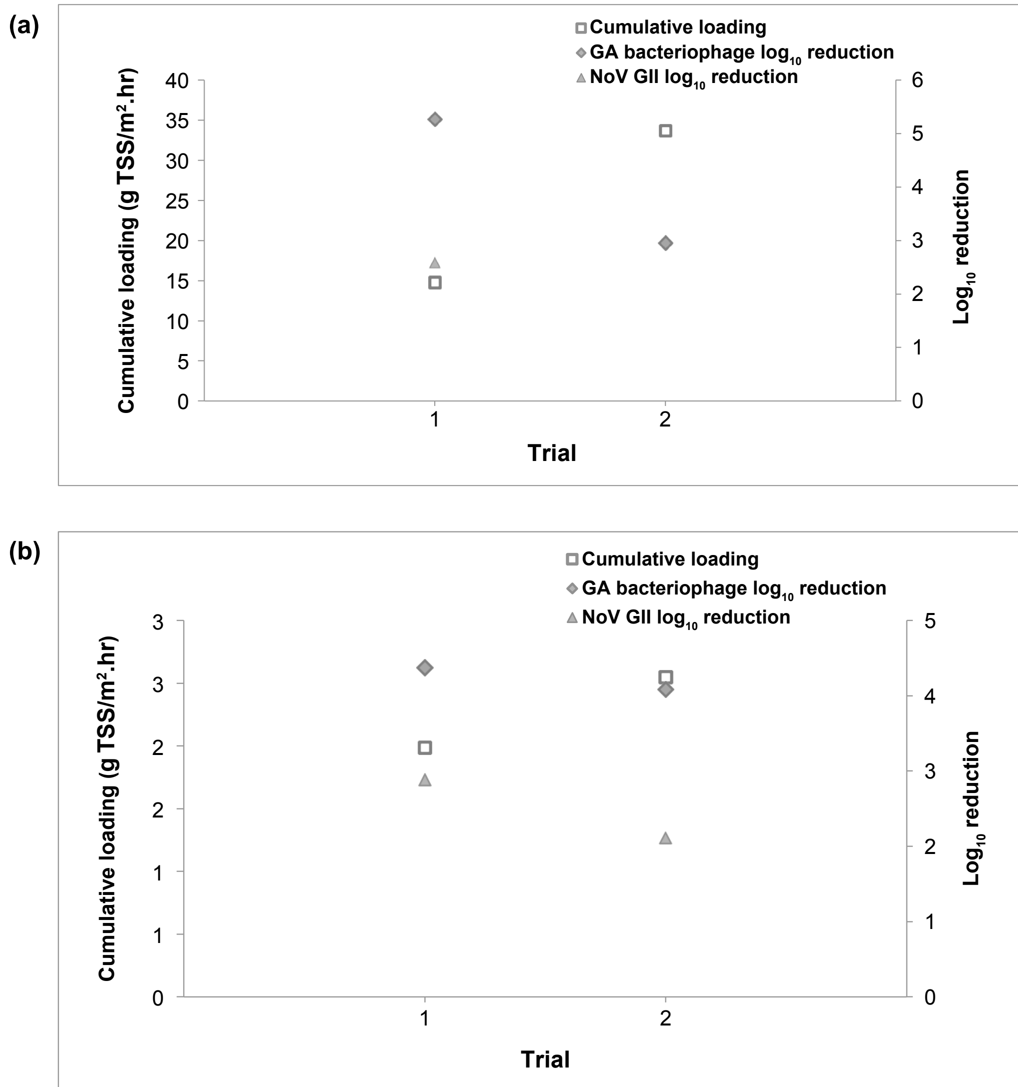

Figure 4.13. The effects of cumulative solids loading on GA bacteriophage and NoV GII $\log_{10}$ removal for ultrafiltration cassettes: (a) 100-kDa pore size cassette and (b) 500-kDa pore size cassette.

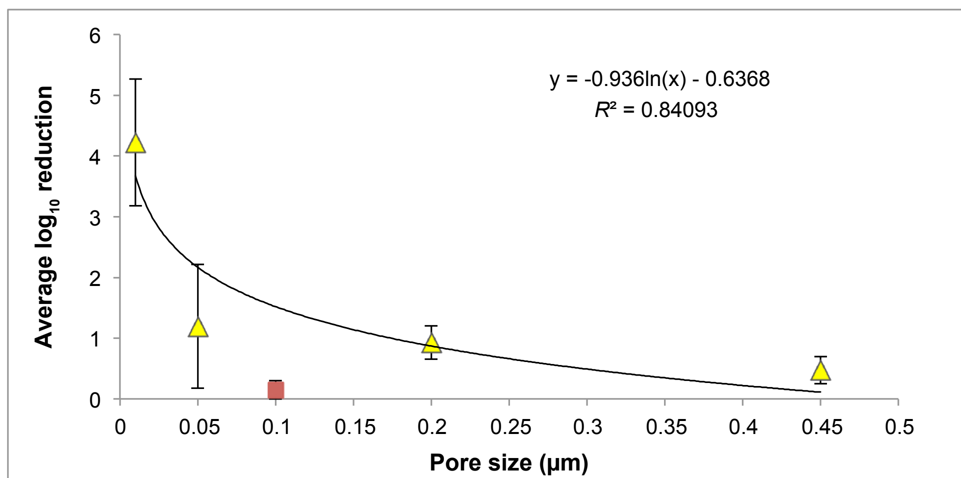

Figure 4.14. Average $\log_{10}$ reduction of GA bacteriophage for microfiltration and ultrafiltration cassettes.

5 Conclusions

Primary and secondary sewage treatment processes alone may not adequately reduce viral loads and their release can, in certain cases, pose a threat to public health and sensitive aquatic areas. It is a growing concern for many community stakeholders, such as the tourism sector, the shellfish industry and the health care sector. The employment of tertiary treatment (e.g. pathogen removal/inactivation) of municipal wastewater is increasingly becoming a requirement to protect public health and sensitive marine bodies and to comply with legislation.

This study investigated the fate of NoV and bacteriophage within typical activated sludge wastewater treatment processes (bacteriophage only) including two UV light-based technologies – PUV light and LPUV light (NoV and bacteriophage). The project also investigated the efficacy of a barrier-based pathogen removal systems – namely micro- and ultrafiltration systems – on removal of NoV and bacteriophage from treated wastewaters. The impact of key wastewater parameters, such as organic carbon and SS, on the performance of these technologies was also analysed. Finally, the use of FRNA bacteriophage as a potential surrogate indicator for NoV was investigated.

1. Use of GA bacteriophage as a surrogate for NoV (section 4.1.1)
   - (a) As judged using GA bacteriophage, RT-qPCR overestimated the concentration of infectious virus in UV light-treated effluent and is therefore an unsuitable tool for measuring virus reduction during UV light treatment.
   - (b) As RT-qPCR was found to be an unsuitable tool to assess virus reductions, the impact of UV light on viable NoV concentrations could not be measured directly.
   - (c) Direct comparison between GA bacteriophage and NoV removals was not possible with current molecular methods. However, owing to the high resistance properties of FRNA bacteriophages to PUV light (and UV light) treatment and their morphological similarities to NoV, the use of this virus as a potential surrogate has value and would warrant further analysis.
2. Virus removal using ultraviolet disinfection

Impact of various wastewater characteristics on PUV light efficiency (sections 4.1.3 and 4.1.4)

- (a) GA bacteriophage reduction following PUV light disinfection was greater in distilled water at significantly lower doses when compared with wastewater. Thus, while spiking distilled water for use as a test media offers many advantages, it is important that subsequent tests are carried out on the media (in this case wastewater) to be disinfected.
- (b) Increased SS concentrations notably decreased maximum reductions of GA bacteriophage. Thus, SS removal prior to UV light disinfection should be prioritised. However, the magnitude of this trend was found to vary significantly between tested samples. Therefore, the study was unable to recommend allowable limits for SS concentrations for efficient UV light disinfection as this appears to be site specific, as other characteristics, such as organic carbon, should to be considered.
- (c) The study indicated that, while SS concentrations should be minimised for optimal operation of disinfection processes, there may be other contributing factors present in effluent that also impact on disinfection efficiency. Further detailed studies are needed to ascertain the relative impacts of various wastewater parameters on disinfection and to determine whether or not these impacts are site specific. In the samples analysed for this study, metals that could impact on UV light performance (specifically manganese and iron) were found to be present in only relatively low concentrations.

Comparison of the PUV light and conventional LPUV light systems (section 4.2)

The germicidal effects of PUV light and LPUV light systems on various microorganisms were compared using secondary treated wastewater.

- (d) The study found that, while the PUV dose rates were high compared with standard low-/

medium-pressure UV light systems, they were comparable with recent studies of novel flow-through PUV light systems in which complete inactivation of bacteria was achieved as well as a reduction in organic carbon and SS content (Uslu *et al*., 2015).

(e) Both systems were found to be susceptible to the presence of SS and other wastewater contaminants (e.g. organic carbon)

3. Fate of FRNA bacteriophages in WWTPs (section 4.3)

(a) An average of $3\log_{10}$ reduction of FRNA bacteriophage was observed across the WWTPs (which comprised primary and secondary treatment). The majority of this reduction was observed to be due to secondary clarification. Indeed, removals of FRNA bacteriophages across the WWTP were generally higher than those subsequently achieved by UV light disinfection, although it should be noted that the removals across the WWTP were measured using only two sample days.

(b) An EPA research report (Doré *et al*., 2013) also recorded a mean reduction of greater than $2\log_{10}$ for FRNA bacteriophages post primary and secondary treatment (combined) over a 1-year period. These data suggest that settlement processes (particularly secondary clarification) within the WWTP itself can play a significant role in virus removal and further emphasise the importance of upstream processes. The successful operation of all of the stages within treatment plants is therefore important and indeed conducive to the removal of pathogens from treated effluent.

(c) It should be noted that the use and verification of additional pathogen removal or disinfection systems remain vital where further reduction or complete removal of pathogens is required.

(d) Efficient WWTP operation prior to pathogen removal/inactivation is essential. Settlement processes are fundamental to the reduction of suspended solid concentrations and can play a significant role in viral load reductions. Solids settlement performs a dual role of partial virus removal (those adhered to the solids) and reducing particular wastewater characteristics (e.g. SS, carbon and metals) that impact negatively on the tertiary treatments in question.

4. Membrane filtration system (section 4.5)

A TFF system (using both micro- and ultrafiltration cassettes) was investigated for virus removal efficiency for both GA bacteriophage and NoV. From the results generated in this study, the membrane filtration system appears to be the most effective for removal of NoV at the laboratory scale when compared with the PUV light system.

(a) Limited removal (up to $1\log_{10}$) was achieved using microfiltration cassettes (0.45 μm, 0.2 μm and 0.1 μm).

(b) As expected, virus removal using ultrafiltration cassettes (500 kDa and 100 kDa) was better than microfiltration cassettes. Maximum removals (mean of $4\log_{10}$ removal of NoV GII – to below the LOD) were observed using a 100-kDa cassette.

In general, membrane filtration can achieve high NoV removal rates if cassettes with appropriate pore sizes are deployed. Furthermore, the effectiveness of membrane systems can be measured through molecular methods as they simply “block” the passage of viruses and remove them entirely from the effluent, thus the viruses are either present or not in samples. However, significant membrane fouling was caused in all cases by the deposition of solid material at the surface and inside the pores of the membrane. Such fouling would pose a significant challenge to the deployment of membrane filters at unmanned sites or poorly performing WWTPs.

5. Sample handling (section 4.4)

The effect of bulk storage at –80°C on viral analysis of WWTP samples was investigated in order to assess whether long-term freezing was a realistic option for on-site analysis. Results indicated that freezing to –80°C did not impact sample composition and viral load. In the case of research projects in particular, the option of sample storage could allow more samples to be analysed, leading to improved efficiency and robust data sets.

6 Recommendations

The findings of this study suggest that while virus reduction (GA bacteriophage) is indeed possible via the investigated pathogen removal/disinfection systems, there are other significant factors to be considered when selecting a method of tertiary treatment.

The variation in effluent quality and characteristics between WWTPs can significantly impact the working efficiency of tertiary systems. When choosing a particular treatment system it is likely that factors such as effluent quality and the level of pathogen reduction required will influence choice. Aspects relating to these factors are outlined below.

1. GA bacteriophage as a potential NoV surrogate

Future work should continue to focus on whether or not FRNA bacteriophages could serve as potential indicators of NoV. Research could focus on the comparative physical removal of both viruses throughout a WWTP, for example in order to detect any trends/similarities between them.

2. Wastewater treatment facility operation

   (a) The continuous monitoring of plant performance should be undertaken to ensure that effluent discharge levels for any given site do not inhibit the efficient operation of pathogen removal/disinfection systems.

   (b) Alarm systems and sensors (e.g. SS or transmissivity sensors) that warn of high SS concentrations in effluent wastewaters should be used to rapidly indicate poorly performing disinfection systems. Timely mitigation measures could then be taken, although these would depend on the site in question, available infrastructure on site and the reasons behind poor performance. In general, it is likely that measures that reduce SS influent to the disinfection system would be of primary importance.

   (c) Periodic verification of on-site pathogen removal/disinfection systems should be used to ensure that the system is performing optimally. Such verification could comprise batch studies (on- or off-site) or periods of intensive monitoring on site. The frequency of these could vary between plants depending on changes in effluent wastewater quality, major process design changes or the input of new loads to the WWTP. For viruses, such verification could be performed using FRNA bacteriophage as a pathogenic virus surrogate.

   (d) Validation trials could also be performed on disinfection/removal systems prior to installation. Pilot-scale systems could be trialled on site to determine optimal operating conditions for the system on site and to ensure that the system can handle expected peaks in hydraulic loads and variations in wastewater characteristics. For viruses, such verification could be performed using FRNA bacteriophage as a pathogenic virus surrogate.

3. UV light systems

   (a) Further research focusing on the PUV light systems is desirable, particularly as the technology could address concerns in relation to the photo-reactivation potential of organisms and growing resistance of bacterial endospores compared with vegetative cells, when treated with standard LPUV light systems. As PUV light contains a broader range of wavelengths than standard UV lamps it can potentially target more cellular targets, such as cell membranes and protein structures, than DNA alone producing irreparable cellular damage.

   (b) The relative impacts of contaminants including (a) SS, (b) the level of organic (and inorganic) carbon, and (c) the presence of metals on UV light system performance in relation to pathogen removal (in particular viruses) should be investigated. This could lead to the development of real-time decision-making tools allowing for UV light system operation to be optimised. Further investigations that compare PUV light technology with current LPUV light systems and MP UV light systems are warranted.

4. Membrane filtration systems

Membrane filtration can be particularly effective for virus removal as, when properly designed and operated, it prevents passage of the targeted pathogen. However, owing to practical issues, particularly at small- and medium-scale wastewater treatment facilities, such systems may be prohibitive due to cost and maintenance issues. Remote monitoring/alarm capability could provide information on membrane performance by measuring flux or transmembrane pressure (for example).

Thus, where membranes are installed, designers should also carefully consider means of alleviating such maintenance issues (e.g. via the use of a pre-treatment to further treat wastewater prior to filtration). Sand filtration is one such pre-treatment. While not a focus of this study, the use of sand filtration as a means of tertiary treatment (often prior to UV light, ozone, chlorination or other barrier methods) to remove SS and organic material can significantly improve the performance of the pathogen removal processes. It is worth noting that sand filtration in itself can be effective in removing pathogens to varying extents.

5. Recommendations from the state-of-knowledge report

   (a) Currently, there is no legislation specifying requirements regarding the release of effluent containing potentially harmful human pathogenic viruses into marine bodies and freshwater. It is recommended that key challenges, such as the identification of appropriate and effective guidelines (or legalisation) regarding the removal of human pathogenic viruses from treated wastewaters, be addressed.

   (b) Some viruses (e.g. NoV) cannot currently be detected by cell culture methods and the use of molecular methods cannot distinguish between infective and non-infective viral particles. It is recommended that future research should focus on developing methods to differentiate infective and non-infective viral particles.

   (c) While xenon-based UV lamps (i.e. PUV light) could offer significant advantages due to the use of high-energy pulses, issues regarding their long-term performance and system design remain. A required area of research will include heat dissipation from pilot and large-scale units.

   (d) Pharmaceutical compounds/micropollutants found in wastewater is an emerging area of research and this study recommends the following:

      (i) future research should include studies on those pathogen removal processes shown to have potential in removing emerging contaminants, such as pharmaceutical compounds/micropollutants;

      (ii) a study into how pharmaceutical compounds and emerging contaminants (e.g. endocrine disrupters) impact on overall WWTP performance, public health (when discharged) and the local aquatic environment.

   (e) The use of combined disinfection processes is likely to gain attention in coming years. Recommended research in this area includes:

      (i) the study of the potential synergistic effects on pathogen removal efficiency, production of disinfection by-products and the impact on overall water quality;

      (ii) minimising operational and design complexity caused by having a number of technologies in place;

      (iii) the overall impact on the life cycle costs and the likely benefits.

References

Baron, J., 1997. Repair of wastewater microorganisms after ultraviolet disinfection under seminatural conditions. *Water Environment Research* 69: 992–998.

Bohrerova, Z., Shemer, H., Lantis, R. *et al.*, 2008. Comparative disinfection efficiency of pulsed and continuous-wave UV irradiation technologies. *Water Research* 42: 2975–2982.

Bolton, J.R. and Cotton, C.A., 2008. *The Ultraviolet Disinfection Handbook*. American Water Works Association (AWWA), Denver, CO, USA.

Bolton, J.R., Stefan, M.I., Cushing, R.S. *et al.*, 2001. The importance of water absorbance/transmittance on the efficiency of ultraviolet disinfection reactors. Proceedings of the First International Congress on Ultraviolet Technologies, Washington, DC, June.

Caretti, C. and Lubello, C., 2003. Wastewater disinfection with PAA and UV combined treatment: a pilot plant study. *Water Research* 37: 2365–2371.

Carter, M.J., 2007. Enterically infecting viruses: pathogenicity, transmission and significance for food and waterborne infection. *Journal of Applied Microbiology* 98: 1354–1380.

Costafreda, M.I., Bosch, A. and Pintó, R.M., 2006. Development, evaluation and standardisation of real-time TaqMan reverse transcription-PCR assay for quantification of Hepatitis A virus in clinical and shellfish samples. *Applied and Environmental Microbiology* 72: 3846–3855.

Darby, J.L., Snider, K.E. and Tchobanoglous, G., 1993. Ultraviolet disinfection for wastewater reclamation and reuse subject to restrictive standards. *Water Environmental Research* 65: 169–180.

Das, T.K., 2001. Ultraviolet disinfection application to a wastewater treatment plant. *Clean Products and Processes* 3: 69–80.

Doré, W.J., Henshilwood, K. and Lees, D.N., 2000. Evaluation of F-specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. *Applied and Environmental Microbiology* 66: 1280–1285.

Doré, W.J., Flannery, J., Keaveney S. *et al.*, 2013. *Assessing the Impact of Wastewater Treatment Plant Effluent on Norovirus Contamination in Shellfisheries*. STRIVE Report Series No. 109. Environmental Protection Agency, Johnstown Castle, Wexford, Ireland.

Emerick, R.W., Loge, F., Thompson, D. *et al.*, 1999. Factors influencing ultraviolet disinfection performance part II: Association of coliform bacteria with wastewater particles. *Water Environmental Research* 71: 1178–1187.

EPA (Environmental Preotection Agency), 1999. *Wastewater Technology Fact Sheet. Ozone Disinfection*. EPA 832-F-99-063. EPA, Johnstown Castle, Wexford, Ireland.

EPA (Environmental Preotection Agency), 2011. *Water Treatment Manual: Disinfection*. EPA, Johnstown Castle, Wexford, Ireland.

EPA (Environmental Preotection Agency), 2012. *Focus on Urban Waste Water Discharges in Ireland*. EPA, Johnstown Castle, Wexford, Ireland.

Flannery, J., Keaveney, S., Rajko-Nenow, P. *et al.*, 2012. Concentration of norovirus during wastewater treatment and its impact on oyster contamination. *Applied and Environmental Microbiology* 78: 3400–3406.

Flannery, J., Keaveney, S., Rajko-Nenow, P. *et al.*, 2013. Norovirus and FRNA bacteriophage determined by RT-qPCR and infectious FRNA bacteriophage in wastewater and oysters. *Water Research* 47: 5222–5231.

Guo, M., Hu, H., Bolton, R.J. *et al.*, 2009. Comparison of low- and medium-pressure ultraviolet lamps: Photoreactivation of *Escherichia coli* and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research* 43: 815–821.

Guo, M., Huang, J., Hu, H. *et al.*, 2011. Growth and repair potential of three species of bacteria in reclaimed wastewater after UV disinfection. *Biomedical Environmental Science* 24: 400–407.

Hammitt, L.L., Bulkow, L., Hennessy, T.W. *et al.*, 2008. Persistence of antibody to Hepatitis A virus 10 years after vaccination among children and adults. *The Journal of Infectious Diseases* 198: 1776–1782.

Hinjen, W.A., Beerendonk, E.F. and Medema, G.J., 2006. Inactive credit of UV radiation for viruses bacteria and protozoan (oo)cysts: a review. *Water Research* 40: 3–22.

Katayama, H., Haramoto, E., Oguma, K. *et al.*, 2008. One-year monthly quantitative survey of noroviruses, enteroviruses, and adenoviruses in wastewater collected from six plants in Japan. *Water Research* 42: 1441–1448.

Lee, M.S. and Chang, L.Y., 2010. Development of enterovirus 71 vaccines. *Expert Review of Vaccines* 9: 149–156.

Lee, E., Lee, H., Jung, W. *et al*., 2009. Influences of humic acids and photoreactivation on the disinfection of *Escherichia coli* by a high-power pulsed UV irradiation. *Korean Journal of Chemical Engineering* 26: 1301–1307.

Metcalf & Eddy, Inc., 2004. *Wastewater Engineering: Treatment and Reuse*. Fourth Edition. The McGraw-Hill Companies. Inc., New York, NY.

Moghadam, A.K. and Dore, M., 2012. Cost and efficacy of water disinfection practices: Evidence from Canada. *Review of Economic Analysis* 4: 209–223.

Montemayor, M., Costan, A., Lucena, F. *et al*., 2008. The combined performance of UV light and chlorine during reclaimed water disinfection. *Water Science and Technology* 57: 935–940.

Nordgren, J., Matussek, A., Mattsson, A. *et al*., 2009. Prevalence of norovirus and factors influencing virus concentrations during one year in a full-scale wastewater treatment plant. *Water Research* 43: 1117–1125.

Ottoson, J., Hansen, A., Björlenius, B. *et al*., 2006. Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. *Water Research* 40: 1449–1457.

Qualls, R.G., Chang, J.C., Ossoff, S.F. *et al*. 1984. Comparison of methods of enumerating coliforms after UV disinfection. *Applied Environmental Microbiology* 48: 699–701.

Rodriguez, R.A., Bounty, S., Beck, S. *et al*., 2014. Photoreactivation of bacteriophages after UV disinfection: Role of genome structure and impacts of UV source. *Water Research* 15: 143–149.

Russell, K.L., Broderic, M.P. and Franklin, S.E., 2006. Transmission dynamics and prospective environmental sampling of adenovirus in a military recruit setting. *The Journal of Infectious Diseases* 194: 877–885.

Shannon, M.A., Bohn, P.W., Elimelech, M. *et al*., 2008. Review: science and technology for water purification in the coming decades. *Nature* 452: 301–310.

Shrestha, M.P., Scott, R.M. and Joshi, D.M., 2007. Safety and efficacy of a recombinant hepatitis E vaccine. *New England Journal of Medicine* 56: 895–903.

Soares-Weiser, K., Maclehose, H. and Bergman, H., 2012. Vaccines for preventing rotavirus diarrhoea: vaccines in use. *Cochrane Database of Systematic Reviews* 11: CD008521.

USEPA (United States Environmental Protection Agency), 1986. *Design Manual – Municipal Wastewater Disinfection*. EPA/625/1-86/021. USEPA, Washington, DC.

USEPA (United States Environmental Protection Agency), 2006a. *Technology and Cost Document for the Final Ground Water Rule*. EPA 815-R-06-015. USEPA, Washington, DC.

USEPA (United States Environmental Protection Agency), 2006b. *Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule*. EPA 815-R-06-007. USEPA, Washington, DC.

Uslu, G., Demirci, A. and Regan, J.M., 2015. Efficacy of pulsed UV-light treatment on wastewater effluent disinfection and suspended solid reduction. *Journal of Environmental Engineering* 141: 04014090.

Vinjé, J., 2010. A norovirus vaccine on the horizon? *Journal of Infectious Diseases* 202: 1623–1625.

Wolf, S., Rivera-Aban, M. and Greening, G.E., 2009. Long-range reverse transcription as a useful tool to assess the genomic integrity of norovirus. *Food and Environmental Virology* 1: 129–136.

Abbreviations

dsDNA	Double-stranded DNA
ELS	Environmental Laboratory Services
FRNA	F-specific RNA
GI	Genogroup I
GII	Genogroup II
HRT	Hydraulic retention time
ISO	International Organization for Standardization
LOD	Limit of detection
LOQ	Limit of quantification
LP	Low-pressure
LPUV	Low-pressure ultraviolet
MBG	Molecular biology-grade
MF	Microfiltration
MLSS	Mixed liquor suspended solids
MP	Medium pressure
MPN	Most probable number
NF	Nanofiltration
NoV	Norovirus
PAA	Peracetic acid
PCR	Polymerase chain reaction
pfu	Plaque-forming units
PPS	Pulses per second
PUV	Pulsed ultraviolet
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SS	Suspended solids
TFF	Tangential flow filtration
TIC	Total inorganic carbon
TN	Total nitrogen
TOC	Total organic carbon
UF	Ultrafiltration
UV	Ultraviolet
WWTP	Wastewater treatment plant

AN GHNÍOMHAIREACHT UM CHAOMHNÚ COMHSHAOIL

Tá an Ghníomhaireacht um Chaomhnú Comhshaoil (GCC) freagrach as an gcomhshaol a chaomhnú agus a fheabhsú mar shócmhainn luachmhar do mhuintir na hÉireann. Táimid tiomanta do dhaoine agus don chomhshaol a chosaint ó éifeachtaí díobhálacha na radaíochta agus an truaillithe.

Is féidir obair na Gníomhaireachta a roinnt ina trí phríomhréimse:

Rialú: *Déanaimid córais éifeachtacha rialaithe agus comhlíonta comhshaoil a chur i bhfeidhm chun torthaí maithe comhshaoil a sholáthar agus chun díriú orthu siúd nach gcloíonn leis na córais sin.*

Eolas: *Soláthraímid sonraí, faisnéis agus measúnú comhshaoil atá ar ardchaighdeán, spriocdhírithe agus tráthúil chun bonn eolais a chur faoin gcinnteoireacht ar gach leibhéal.*

Tacaíocht: *Bímid ag saothrú i gcomhar le grúpaí eile chun tacú le comhshaol atá glan, táirgiúil agus cosanta go maith, agus le hiompar a chuirfidh le comhshaol inbhuanaithe.*

Ár bhFreagrachtaí

Ceadúnú

Déanaimid na gníomhaíochtaí seo a leanas a rialú ionas nach ndéanann siad dochar do shláinte an phobail ná don chomhshaol:

- saoráidí dramhaíola *(m.sh. láithreáin líonta talún, loisceoirí, stáisiúin aistrithe dramhaíola)*;
- gníomhaíochtaí tionsclaíocha ar scála mór *(m.sh. déantúsaíocht cógaisíochta, déantúsaíocht stroighne, stáisiúin chumhachta)*;
- an diantalmhaíocht *(m.sh. muca, éanlaith)*;
- úsáid shrianta agus scaoileadh rialaithe Orgánach Géinmhodhnaithe *(OGM)*;
- foinsí radaíochta ianúcháin *(m.sh. trealamh x-gha agus radaiteiripe, foinsí tionsclaíocha);*
- áiseanna móra stórála peitril;
- scardadh dramhuisce;
- gníomhaíochtaí dumpála ar farraige.

Forfheidhmiú Náisiúnta i leith Cúrsaí Comhshaoil

- Clár náisiúnta iniúchtaí agus cigireachtaí a dhéanamh gach bliain ar shaoráidí a bhfuil ceadúnas ón nGníomhaireacht acu.
- Maoirseacht a dhéanamh ar fhreagrachtaí cosanta comhshaoil na n-údarás áitiúil.
- Caighdeán an uisce óil, arna sholáthar ag soláthraithe uisce phoiblí, a mhaoirsiú.
- Obair le húdaráis áitiúla agus le gníomhaireachtaí eile chun dul i ngleic le coireanna comhshaoil trí chomhordú a dhéanamh ar líonra forfheidhmiúcháin náisiúnta, trí dhíriú ar chiontóirí, agus trí mhaoirsiú a dhéanamh ar leasúchán.
- Cur i bhfeidhm rialachán ar nós na Rialachán um Dhramhthrealamh Leictreach agus Leictreonach (DTLL), um Shrian ar Shubstaintí Guaiseacha agus na Rialachán um rialú ar shubstaintí a ídíonn an ciseal ózóin.
- An dlí a chur orthu siúd a bhriseann dlí an chomhshaoil agus a dhéanann dochar don chomhshaol.

Bainistíocht Uisce

- Monatóireacht agus tuairisciú a dhéanamh ar cháilíocht aibhneacha, lochanna, uiscí idirchriosacha agus cósta na hÉireann, agus screamhuiscí; leibhéil uisce agus sruthanna aibhneacha a thomhas.
- Comhordú náisiúnta agus maoirsiú a dhéanamh ar an gCreat-Treoir Uisce.
- Monatóireacht agus tuairisciú a dhéanamh ar Cháilíocht an Uisce Snámha.

Monatóireacht, Anailís agus Tuairisciú ar an gComhshaol

- Monatóireacht a dhéanamh ar cháilíocht an aeir agus Treoir an AE maidir le hAer Glan don Eoraip (CAFÉ) a chur chun feidhme.
- Tuairisciú neamhspleách le cabhrú le cinnteoireacht an rialtais náisiúnta agus na n-údarás áitiúil *(m.sh. tuairisciú tréimhsiúil ar staid Chomhshaol na hÉireann agus Tuarascálacha ar Tháscairí).*

Rialú Astaíochtaí na nGás Ceaptha Teasa in Éirinn

- Fardail agus réamh-mheastacháin na hÉireann maidir le gáis cheaptha teasa a ullmhú.
- An Treoir maidir le Trádáil Astaíochtaí a chur chun feidhme i gcomhair breis agus 100 de na táirgeoirí dé-ocsaíde carbóin is mó in Éirinn.

Taighde agus Forbairt Comhshaoil

- Taighde comhshaoil a chistiú chun brúnna a shainaithint, bonn eolais a chur faoi bheartais, agus réitigh a sholáthar i réimsí na haeráide, an uisce agus na hinbhuanaitheachta.

Measúnacht Straitéiseach Timpeallachta

- Measúnacht a dhéanamh ar thionchar pleananna agus clár beartaithe ar an gcomhshaol in Éirinn *(m.sh. mórphleananna forbartha).*

Cosaint Raideolaíoch

- Monatóireacht a dhéanamh ar leibhéil radaíochta, measúnacht a dhéanamh ar nochtadh mhuintir na hÉireann don radaíocht ianúcháin.
- Cabhrú le pleananna náisiúnta a fhorbairt le haghaidh éigeandálaí ag eascairt as taismí núicléacha.
- Monatóireacht a dhéanamh ar fhorbairtí thar lear a bhaineann le saoráidí núicléacha agus leis an tsábháilteacht raideolaíochta.
- Sainseirbhísí cosanta ar an radaíocht a sholáthar, nó maoirsiú a dhéanamh ar sholáthar na seirbhísí sin.

Treoir, Faisnéis Inrochtana agus Oideachas

- Comhairle agus treoir a chur ar fáil d'earnáil na tionsclaíochta agus don phobal maidir le hábhair a bhaineann le caomhnú an chomhshaoil agus leis an gcosaint raideolaíoch.
- Faisnéis thráthúil ar an gcomhshaol ar a bhfuil fáil éasca a chur ar fáil chun rannpháirtíocht an phobail a spreagadh sa chinnteoireacht i ndáil leis an gcomhshaol (*m.sh. Timpeall an Tí, léarscáileanna radóin).*
- Comhairle a chur ar fáil don Rialtas maidir le hábhair a bhaineann leis an tsábháilteacht raideolaíoch agus le cúrsaí práinnfhreagartha.
- Plean Náisiúnta Bainistíochta Dramhaíola Guaisí a fhorbairt chun dramhaíl ghuaiseach a chosc agus a bhainistiú.

Múscailt Feasachta agus Athrú Iompraíochta

- Feasacht chomhshaoil níos fearr a ghiniúint agus dul i bhfeidhm ar athrú iompraíochta dearfach trí thacú le gnóthais, le pobail agus le teaghlaigh a bheith níos éifeachtúla ar acmhainní.
- Tástáil le haghaidh radóin a chur chun cinn i dtithe agus in ionaid oibre, agus gníomhartha leasúcháin a spreagadh nuair is gá.

Bainistíocht agus struchtúr na Gníomhaireachta um Chaomhnú Comhshaoil

Tá an ghníomhaíocht á bainistiú ag Bord lánaimseartha, ar a bhfuil Ard-Stiúrthóir agus cúigear Stiúrthóirí. Déantar an obair ar fud cúig cinn d'Oifigí:

- An Oifig um Inmharthanacht Comhshaoil
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Fianaise is Measúnú
- An Oifig um Cosaint Raideolaíoch
- An Oifig Cumarsáide agus Seirbhísí Corparáideacha

Tá Coiste Comhairleach ag an nGníomhaireacht le cabhrú léi. Tá dáréag comhaltaí air agus tagann siad le chéile go rialta le plé a dhéanamh ar ábhair imní agus le comhairle a chur ar an mBord.

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The Effect of Wastewater Treatment Processes, in Particular Ultraviolet Light Treatment, on Pathogenic Virus Removal

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Municipal wastewater treatment plant discharges are a recognised source of human pathogenic viruses. Of concern within this group is norovirus the leading cause of viral gastroenteritis worldwide.

This project investigated the use of FRNA bacteriophage as (i) a potential surrogate for norovirus response/behaviour and (ii) a model to determine the fate of viruses through a municipal wastewater treatment plant. The project also evaluated the efficacy of membrane filtration (microfiltration and ultrafiltration) as pathogen removal method and two ultraviolet-based technologies; pulsed UV and low pressure UV as pathogen disinfection methods. The potential impacts of parameters such as organic carbon, metals and suspended solids, (typically present in wastewater) on the investigated pathogen removal processes were also analysed.

Identifying pressures

Currently, there exists no legislation (nationally or internationally) for the monitoring of viral loads in treated effluent. While primary and secondary treatment processes can reduce virus concentrations, they are not specifically designed for this purpose and so tertiary treatment can be required in many cases. Continuous low pressure and/or medium pressure ultraviolet (UV) systems are used in conventional wastewater treatment plants as a method of pathogen disinfection. Barrier based systems such as membrane filtration processes are widely used in the drinking water sector as pathogen removal systems. However, operational challenges associated with wastewater have limited their use in this industry.

Informing policy

The introduction of the Water Framework Directive (WFD - 2000/60/EC) called for all European countries to achieve a 'good status' for ground and surface waters by 2015 and has played a significant role in the implementation of more stringent discharge limits for wastewater treatment facilities. This study provides critical information that can aid engineering, scientific and policy stakeholders provide for improved design and management of wastewater treatment plants. This in turn can impact on policy and technological solutions that can ensure pathogen removal mechanisms are efficiently deployed and monitored where required.

Developing solutions

Due to the high resistance properties of bacteriophages to UV treatment and its morphological similarities to norovirus, the use of this virus as a potential surrogate for norovirus has value. This may currently offer the most effective method of indirectly determining the efficiency of disinfection processes (in particular UV) in achieving norovirus reduction. However further work should investigate the development of efficient analytical mechanisms for determining norovirus infectivity in wastewater samples.

The study demonstrates that efficient and careful management of wastewater treatment plants, in particular existing settlement processes can, in itself, have a significant and positive impact on pathogen (including norovirus) removal and also on the subsequent operation of disinfection systems. The report recommends that continuous commissioning, via periodic verification trials of installed UV systems, can enable operators to ensure optimal operation of existing systems.

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