Report No. 145

epa Research
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Development of a pulsed light approach as a novel solution in drinking water treatment

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

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ENVIRONMENTAL PROTECTION AGENCY

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- Office of Environmental Enforcement
- Office of Environmental Assessment
- Office of Radiological Protection
- Office of Communications and Corporate Services

The EPA is assisted by an Advisory Committee of twelve members who meet regularly to discuss issues of concern and provide advice to the Board.

EPA Research Programme 2014–2020

Development of a Pulsed Light Approach as a Novel Solution in Drinking Water Treatment

2011-W-MS-5

Prepared for the Environmental Protection Agency

by

Athlone Institute of Technology

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ACKNOWLEDGEMENTS

This report was published as part of the EPA Research Programme 2014–2020. The programme is financed by the Irish Government under the National Development Plan 2007–2013. It is administered on behalf of the Department of the Environment, Heritage and Local Government by the Environmental Protection Agency which has the statutory function of co-ordinating and promoting environmental research.

All research was conducted in Athlone Institute of Technology in partnership with National University of Ireland, Galway. The authors wish to express their sincere gratitude to the Steering Committee Eamonn Merriman, John Gray and Ruth Barrington whose knowledge and guidance was essential to this study.

DISCLAIMER

The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

EPA RESEARCH PROGRAMME 2014–2020

Published by the Environmental Protection Agency, Ireland

Price: Free **Online version**

ISBN: 978-1-84095-582-8

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Executive Summary

The presence of parasitic species such as *Cryptosporidium* and *Giardia* in water supplies has been well documented – and in some cases before and after the disinfection processes have been implemented in water-treatment plants. The resistance of these species to chemical disinfection, such as chlorination, aids their viability and infection potential in the environment. Research is focusing on using alternative methods to remove these organisms from water supplies. Such methods include the use of ultraviolet (UV) light at certain wavelengths. UV treatment has proven effective at causing cell death in numerous microbial species. However, it does have its limitations, such as depth-of-water penetration, presence of contaminants and the repair mechanisms organisms possess to fix UV damage. New methods of delivering UV light to the treatment area – such as pulsed UV (PUV) – have been developed which may eliminate these issues. Consequently, the aim of this study was to determine if a novel PUV system provides a suitable means of disinfecting water, with particular emphasis on parasite species.

In order to achieve this goal a variety of techniques were used, including the identification of a suitable means of determining parasite viability *in vitro* using human-derived cell lines. Therefore, this project reports on the use of an *in vitro* cell culture-based polymerase chain reaction (PCR) method for the determination of inactivation rates of the pathogenic parasites *Cryptosporidium parvum* and *Giardia lamblia* using a novel PUV system. Results from this study show that the human-derived HCT-8 cell line was more suitable for supporting parasite infection and PCR amplification and was used for all viability studies. Findings described in this report highlight the effectiveness of this light system in activating a broad range of microbial test species and parasitic organisms. Findings described show that this light system can be adapted for use as a flow-through system disinfecting water flowing at varying flow rates. Moreover, these studies have identified a non-pathogenic harmless *Bacillus* endospore (*Bacillus megaterium*) which may be used as a surrogate for *Cryptosporidium* inactivation studies as it shows similar inactivation rates to that of the problematic parasite. The use of such a surrogate will allow for more in-depth studies on the removal of *C. parvum* from water supplies without causing contamination issues. It is worth noting that *G. lamblia* proved more UV resistant than *C. parvum.* An extensive range of ecotoxicological studies showed that no harmful effects were caused to the treatment liquid following UV exposure. The system is therefore deemed safe to use on water that is to be released into the natural environment.

It was also found that the PUV light system provided a more rapid and cost-effective means of inactivating test species than the standard UV lamp system currently in use. A significant increase in microbial and parasite death was achieved within a much shorter treatment time using the PUV system.

Inactivation of test species using a flow-through PUV system was achieved for all flow rates and retention times assessed. Indeed, *Bacillus megaterium* vegetative and endospore cells were repeatedly inactivated, highlighting its potential for the removal of *Cryptosporidium parvum*. Therefore, the findings of this research project conclude that the PUV system may successfully be adapted for use in water-treatment plants if used as a system with two or more lamps adjacent to each other and using operational parameters that have proven effective at bench scale.

1 Introduction

1.1 Cryptosporidium

Cryptosporidiosis is a diarrheal disease caused by the parasite *Cryptosporidium* which resides in the intestines of humans and animals and is passed in the stool of the infected host. The parasite is protected by an outer shell that allows it to survive outside the body for extended lengths of time in extreme temperatures and also makes it very resistant to chlorine-based disinfectants. Once ingested, the walls of the oocysts are softened by the digestive fluids in the stomach and small intestine. Four tiny sporozoites emerge and immediately begin to infect and reproduce in the intestinal lining of the host. This process weakens the small intestine's ability to absorb water and nutrients, which causes the infected person or animal to expel the oocysts through diarrhoea and vomiting. It is estimated that an infected person produces 100 million oocysts a day. Outbreaks of infected people have been linked to contaminated drinking water, recreational water, food and zoonotic transmission.

Cryptosporidium can be found in virtually any surface water source. Indeed, since the 1990s, *Cryptosporidium* has become recognised as one of the most common causes of waterborne disease in humans (Table 1.1). At present there are 26 known species of *Cryptosporidium* which can infect a wide range of animals and humans. Of the 26 species, 20 are known to have the capability to infect humans (Ryan *et al*., 2014). These species are identified based on differences in oocyst morphology, site of infection, vertebrate class specificity and genetic differences. The incubation period (time between being infected and having symptoms of infection) is usually up to two weeks post consumption. Symptoms can persist for two to four weeks depending on the immune status of the patient. Patients with a healthy immune system (i.e. immunocompetent patients) commonly experience illness for up to one month followed by complete recovery without complications. However, in cases involving infected immunocompromised patients, such as those on immunotherapy or suffering from Acquired Immune Deficiency Syndrome (AIDS), symptoms are usually prolonged and possibly life threatening. To date there is no treatment available for cryptosporidiosis; oral re-hydration to replace the fluid and nutrients lost during infection is essential but does not provide a mechanism of eradicating the parasite from the site of infection.

Table 1.1. Select number of *Cryptosporidium* species and their relation to human infection (adapted from WHO Guidelines 2001).

Species	Hosts	Human cases	Waterborne outbreaks
C. hominis	Humans	Frequent	Yes
C. parvum	Mammals	Frequent	Yes
C. meleagridis	Turkey, Human	Occasionally	No
C. muris	Rodents, ruminants	Occasionally	No
C. andersoni	Cattle, camel	No	No
C. felis	Cats	Very occasionally	No
C. canis	Dogs	Very occasionally	No
C. wrairi	Guinea pig	No	No
C. baileyi	Gallinaceous birds	Once	No
C. galli	Birds	No	No
C. serpentis	Snakes	No	No
C. saurophilium	Lizards	No	No
C. molnari	Sea bass	No	No

1.2 Giardia

Giardia lamblia is a flagellated protozoan parasite which causes the highly infectious disease state known as 'giardiasis' in host individuals. This problematic parasite is frequently associated with gastrointestinal infection in both developed and developing countries: routes of transmission include the consumption of contaminated food and water, person-to-person and often animal-to-person transmission via the faecal oral route. The life cycle of *Giardia* occurs in two stages: (i) the cyst and (ii) the trophozoite. After the cyst is ingested, excystation occurs in the intestines after the change in pH causes the cyst to open and release the infective trophozoites (two per cyst). These trophozoites then attach to the host intestinal cells and initiate their reproductive cycle. Symptomatic patients have diarrhoea with loose, foul-smelling stools; there are increased amounts of fat and mucus in faecal samples. Flatulence, abdominal cramps and bloating, and nausea are common, as are anorexia, malaise, and weight loss; blood is not present in stools however. Symptoms can last for weeks to months, and fever is occasionally present at the beginning of the infection. The detection of cysts in the stools from infected persons is used for diagnosis. However, cysts are shed sporadically and often unnoticed in symptomatic people.

1.3 Waterborne Transmission of Parasite Species

Waterborne transmission of parasite species occurs following the consumption of water which has been contaminated. This can occur via direct consumption of contaminated drinking water or via recreational activities such as swimming, amongst other water sports. There are numerous ways in which parasites can enter water supplies, including urban runoff, agricultural runoff, wastewater discharges, leaking septic systems, direct faecal waste from wildlife, and human faecal accidents including young children shedding protozoa while bathing. Because nearly all new-born calves become infected and excrete large numbers of oocysts in their faeces, and also because older cattle continue to excrete oocysts, cattle are thought to be an important source of waterborne *Cryptosporidium parvum* (Fayer, 2004). The outbreak of human cryptosporidiosis that occurred in San Antonio, Texas during the summer of 1984 was the first recorded waterborne outbreak of the disease (Fayer *et al.,* 2000). From then on, the number of reported water-related outbreaks (be it drinking water or recreational water) has increased worldwide. To date, the largest reported waterborne outbreak was that of the 1993 Milwaukee epidemic (Mac Kenzie *et al*., 1994). In 2007 a *Cryptosporidium* outbreak occurred in Galway, Ireland following contamination of a drinking water-treatment plant.

Waterborne giardiasis was first documented in Aspen, Colorado, USA, in 1965/1966, and the Centers for Disease Control and Prevention (CDC) began waterborne disease surveillance in 1971. Between 1979 and 1988, *Giardia* was the most frequently implicated organism in waterborne disease. Between 1965 and 1984, some 90 outbreaks with a total of 23,776 cases were reported in the United States (Shin *et al.,* 2010). Between 1992 and 1997, surveillance carried out by 43 states in the United States indicated that as many as 2.5 million cases of giardiasis occur annually in that country. Waterborne giardiasis is well known among travellers to countries in Eastern Europe and the former Soviet Union (Dawson, 2003). Waterborne transmission of *Giardia* is the most common cause of outbreaks, as *G. lamblia* cysts are abundant in surface and source waters (Shin *et al.,* 2010). Notably, 20 of the 45 European countries have reported the prevalence of *Giardia* in human and water samples, with 10 countries reporting its presence in recreational waters. Furthermore, giardiasis has recently been categorised as a 'neglected disease' by the World Health Organization (WHO) (Plutzer *et al.,* 2010). The excretion of extensive numbers of *G. lamblia* cysts by high numbers of cattle and sheep around water supplies make infected animals an important source of water contamination. Indeed, occurrence data from developed and developing countries suggest that *Cryptosporidium* and *Giardia* are commonly found in raw sewage with the latter present in higher numbers more frequently. As with parasites such as *Cryptosporidium,* the removal of *Giardia* from water supplies has proven problematic due to its resistance to current water-disinfection methods and the low number of cysts required for infection to occur. Box 1.1 lists the properties of parasites which are conducive to their waterborne transmission.

Box 1.1. Parasite properties leading to their waterborne transmission

- Extreme resistance to chemical disinfection;
- Persistence in the environment;
- Small size;
- High infectivity;
- Human and livestock sources;
- Oocyst shedding in high numbers;
- No maturation of the organism is required.

1.4 Water Policy

From 1975, the European Union has adopted eight 'primary' directives, three of which are particularly significant to the quality of water for various uses (Table 1.2). National regulations have been made in connection with all these directives, which stipulate the requirements to be followed to ensure that a safe supply of water is made readily available to consumers, with the basic standards governing the quality of drinking water intended for human consumption are set out in EU Directive 98/83/EC. This directive also covers water for human consumption, whether in its original state or after treatment, and regardless of origin. In Ireland, the Environmental Protection Agency (EPA) prepares and publishes annual reports on the results of the monitoring programmes carried out. Where the water quality does not meet the specified standards, corrective actions are outlined in S.I. No. 122 of 2014 for public and private water supplies.

1.4.1 Water Disinfection

Disinfection is the process by which an organism's viability/infectivity is destroyed, with a specific percentage of the population dying over a specific time frame defined as a 'rate'. The most common method of disinfection used for water treatment is chlorination (adding chlorine to water) the purpose of which is to eliminate the threat of microbial species present in water. With the ability of certain pathogenic species such as *Cryptosporidium* and *Giardia* to resist chlorination, a need has arisen to find an alternative means of disinfecting water supplies. Alternative disinfectants including chlorine dioxide, ozone and PUV light are now the focus of much research. UV light has shown much potential as a suitable disinfection method as it has been shown to inactivate *Cryptosporidium* at bench-scale level (Garvey *et al.,* 2010).

Table 1.2. EU water quality directives (taken from the Irish EPA *Parameters of Water Quality* manual).

No.	Official Ref. No.	Full title of directive	Short title
1	75/440/EEC	Council Directive of 16 June 1975 concerning the quality required of surface water intended for the abstraction of drinking water in the Member States.	Surface Water
2	76/160/EEC	Council Directive of 8 December 1975 concerning the quality of bathing water.	Bathing Water
3	76/464/EEC	Council Directive of 4 May 1916 on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community.	Dangerous Substances
4	78/659/EEC	Council Directive of 18 July 1978 on the quality of fresh waters needing protection or improvement In order to support fish life.	Freshwater Fish
5	79/923/EEC	Council Directive of 30 October 1979 on the quality required of shellfish waters.	Shellfish
6	80/68/EEC	Council Directive of 17 December 1979 on the protection of groundwater against pollution caused by certain dangerous substances.	Ground Water
7	80/778/EEC	Council Directive of 15 July 1980 relating to the quality of water intended for human consumption.	Drinking Water
8	98/83/EC	Council Directive of 3 November 1998 relating to the quality of water intended for human consumption.	Drinking Water

1.5 Ultraviolet Light

UV radiation/light lies between visible light and X-rays on the electromagnetic spectrum. UV light is divided into UVA (400–320 nm), UVB (320–280 nm), UVC (280–200 nm) and vacuum UV (VUV) (200–100). The inactivation of micro-organisms by UV radiation is directly related to the UV dose:

$$\text{Dose} = \frac{\text{Time (s) x Output (watts)}}{\text{Area (cm}^2\text{)}}$$

UV dose is the quantity of the energy per unit area that falls upon a surface. UV dose is written as mWs/cm^2, but UV dose is also regularly expressed as millijoules per square centimeter (mJ/cm^2), because 1 mWs = 1 mJ. For disinfection of drinking water, a minimum UV dose of 40 mJ/cm^2 has been established in at least two European countries (cited in Anderson *et al*., 2003). Guo and co-workers (2009) concluded that there is no difference between operation of low- and medium-pressure UV lamps as regards critical photoreactivation of treated total coliforms as long as the applied germicidal UV dose is greater than 40 mJ/cm^2.

1.5.1 UV- induced Cellular Damage

UV inactivates microorganisms by their absorption of light. This causes a photochemical reaction that alters the molecular components required by the organism for growth and/or reproduction. UV radiation penetrates the cell membranes to impact directly on DNA molecules. Nucleic acid absorbs light energy at 240 to 280 nm with an absorption maximum at 265 nm (UVC) (Kiefer, 2007). Dimers are formed when DNA or RNA absorbs this energy. The most regular dimers formed are cyclobutane dimers between nearby pyrimidines (CPD) on the same DNA strand. Specifically, thymine-to-thymine dimers are most common (Kiefer, 2007), so instead of pairing with adenine, a thymine base pairs with another thymine (in RNA uracil pairs with another uracil). The thymine dimer forms a four-membered cyclobutyl ring, which inhibits DNA replication and function. In total there are three types of pyrimidine dimers: (i) thymine–thymine, (ii) thymine–cytosine and (iii) cytosine–cytosine. Thymine dimers are more often produced because thymine has a greater absorbance than cytosine in the germicidal range and the quantum yield for the formation of thymine-to-thymine dimers is greater than that for the formation of the other dimer possibilities (Giese and Darby, 2000). Higher doses of UV light also cause protein damage leading to a loss of structure and function and also can result in cell lysis (McDonnell, 2007). UV energy is absorbed by proteins at 280 nm and there is some absorption by the peptide bond within protein structures at 240 nm. Additional important biological molecules with unsaturated bonds – for example, hormones, coenzymes and electron carriers – may also be vulnerable to destruction by UV. This is an important factor in larger organisms such as fungi and protozoa. Treatment of bacterial spores with UVC leads to the development of the 'spore photoproduct' 5-thyminyl-5, 6-dihydrothymine, single and double strand breakage as well as cyclobutane pyrimidine dimer (CPD) formation (Gomez-Lopez *et al.,* 2007).

Another damage type results from covalent linking between two pyrimidine bases involving the 6-position and the 4-position of the ring; this damage is referred to as '6-4-photoproducts' or 6-4 pyrimidine-pyrimidone (6-4 PPs) adducts (Kiefer, 2007). The frequency with which these 6-4-photoproducts are formed depends on the base composition of the DNA. In *Escherichia coli* lacI and lacZ genes cyclobutane pyrimidine dimers and 6-4 pyrimidine photoproducts form in a 2:1 ratio following UV exposure (Beggs, 2002).

1.5.2 Artificial Sources of UV Light

The earth's atmosphere prevents UVC (also known as germicidal UV) from reaching the earth's surface. As a result, artificial sources of generating UVC are needed for disinfection purposes. Producing UV radiation requires electricity to power UV lamps. A UV lamp consists of a quartz tube containing an inert gas (e.g. argon) and a small amount of liquid mercury. Ballasts (devices used to limit the amount of current in an electric circuit) control the power to the UV lamps. When a voltage is applied to the lamp, some of the liquid mercury vapourises. Free electrons and ions then collide with the gaseous mercury atoms, 'exciting' the mercury atoms into a higher energy state. The excited mercury atoms return to their ground (normal) energy state by discharging energy as UV light. Mercury is favourable for UV disinfection because it emits light in the germicidal wavelength range (200–300 nm). The UV light produced depends on the concentration of mercury atoms in the UV lamp, which is directly related to the mercury vapour pressure. UV disinfection uses either LP lamps at a wavelength of 253.7 nm or medium pressure (MP) lamps at wavelengths from 200 to 600

nm or lamps that emit high-intensity pulses of light. There are numerous sources of UV radiation: however, the most common is the electric arc and mercury lamp which provide continuous sources of UV light.

1.5.3 Standard UV Technologies

Inactivation of organisms with continuous wave UV light is performed by using LP mercury lamps designed to emit light at 254 nm (i.e. monochromatic light). Due to the distinct disinfection method of UV (the absorption of UV energy at 254 nm by DNA) traditional UV disinfection systems consisted of LP lamps that produce this monochromatic radiation. However, in the late 1990s MP UV lamps were introduced because they emit polychromatic light including the germicidal wavelengths (200 to 300 nm) (Gomez-Lopez et al., 2007). While there is usually no difference in the disinfection ability between these lamps, each has advantages and disadvantages. Medium pressure lamps have a higher germicidal output than LP lamps, and so require fewer lamps for disinfection. However, the LP and MP approaches to UV delivery have recognised limitations, including poor penetrability, considerable energy usage and the possibility that mercury can be leaked to the environment if the lamp is broken. Most existing proprietary UV disinfection systems are marketed and validated as units with the capability of inactivating the full spectrum of possible waterborne pathogens such as bacteria, viruses and *Cryptosporidium*. Consequently, most registered UV disinfection units are typically validated in accordance with the US Environmental Protection Agency, German Association for Gas and Water (DVGW) and Austrian (ONORM) protocols for a UV dose (fluence) of 40mJ/cm^2 (Irish EPA, Water Treatment Manual: disinfection). Box 1.2 outlines the advantages and disadvantages of UV disinfection.

1.5.4 Pulsed UV light

Pulsed light (PL) is a non-thermal method of disinfection produced by storing electrical energy in a capacitor and releasing it as a short high-intensity pulse with a duration of between 1 μs and 0.1 s (Elmnasser *et al.,* 2007). A modest energy input of a few joules (J) can result in high peak-power dissipation of about 107–108 W. The electrical energy is applied to a xenon flash-lamp in which the energy ionises the gas to create plasma that expands to fill the lamp. Outer shell electrons are stripped away and intense pulses of UV light are emitted. The efficacy of the pulse system is attributed to the unique effects of high peak power and broad spectrum UV content coupled with the ability to control pulse duration and frequency (Anderson *et al*., 2000). The light produced by the lamp includes broad spectrum wavelengths from UV to near-infrared; during each pulse the system delivers a spectrum that is 20,000 times more intense than sunlight at the earth's surface (Elmnasser *et al.,* 2007). The UV dose can be adjusted by increasing or decreasing the frequency of the pulsing. Preliminary findings from other research groups suggest that PL is effective for killing bacteria, fungi, and viruses and

Box 1.2. Advantages and disadvantages of UV disinfection

Advantages

- Environmentally friendly and does not require the use of dangerous chemicals;
- Economical as it is energy efficient providing microbial inactivation with minimal cost;
- Effectiveness is independent of factors such as pH, temperature and ionic strength;
- Does not alter the taste or other properties of the water;
- Compatible with other treatment processes;
- Effective and quick.

Disadvantages

Potential disadvantages of using UV technology for surface treatment or water decontamination relate to poor penetrability, considerable energy usage and the possibility that mercury can be leaked to the environment if the lamp is broken.

the killing effect is much higher in a much shorter time than with continuous UV treatment (Takeshita *et al.,* 2002) .

Therefore, PL is an approach that has received considerable attention as a strategy for decontaminating food, packaging, water and air (Dunn *et al.,* 1997; Gómez-López *et al.,* 2007). Pulsed light technology is also a strong candidate for contact surface decontamination in the healthcare setting. This approach kills microorganisms by using ultrashort duration pulses of an intense broadband emission spectrum that is rich in UVC germicidal light (200–280 nm band). Pulsed light is produced using techniques that multiply power manifold by storing electricity in a capacitor over relatively long times (fractions of a second) and releasing it in a short time (millionths or thousandths of a second) using sophisticated pulse-compression techniques (Rowan *et al.,* 1999; Gómez-López *et al.,* 2007). The emitted flash has a high peak power and usually consists of wavelengths from 200 to 1100 nm broad spectrum light enriched with shorter germicidal wavelengths (MacGregor *et al.,* 1998; Gómez-López *et al.,* 2007).

The technology has received several names in the scientific literature: PUV light (Anderson *et al.,* 2000; Sharma and Demirci, 2003; Wang *et al.,* 2005), 'high intensity broad-spectrum pulsed light' (Roberts and Hope, 2003), 'pulsed light' (Rowan *et al.,* 1999), 'intense pulsed light' (Gómez-López *et al.,* 2007) and 'pulsed white light' (Marquenie *et al.,* 2003). Seminal developments pertaining to these next generation light-flashing technologies have been the subject of recent review (Elmnasser *et al.,* 2007; Gómez-López *et al.*, 2007), with a strong emphasis on decontamination efficacy for food and water applications. A strong advantage of using pulsed xenon lamps over continuous low- to medium-pressure conventional UV lamps is that the former has a characteristic high peak-power dissipation which allows for more rapid microbial inactivation. A continuous 10 W lamp needs to be operated for 10 seconds to achieve the same decontamination efficacy (supplying the same energy) as a pulsed lamp of typically 1 MW operated for just 100 µs. Otaki *et al.* (2003) also reported that adaptive microbial survival (the tailing phenomenon) occurs when samples are treated in high turbidity solutions using continuous UV sources, whereas tailing does not occur when similar samples are treated with a pulsed xenon lamp.

While current findings from the literature suggest that development of a PL approach appears promising, most studies up to now have focused on food or water applications using a limited range of electro-physical or biological parameters, such as the use of a single lamp discharge energy (J) or fluence (UV dose cm^2) and/or employing a single distance from light source to target treatment area. These landmark in vitro and *in vivo* PL studies have been recently reviewed for efficacy in terms of inactivating food-related spoilage organisms and potential microbial pathogens (Elmnasser *et al.,* 2007; Gómez-López *et al.,* 2007), and include studies carried out using lamp-discharge energies of 3 J (MacGregor *et al.,* 1998; Rowan *et al.,* 1999), 7 J/cm^2 (Gómez-López *et al.,* 2005 and Marquenie *et al.,* 2003), 0.99 J/cm^2 (Krishnamurthy *et al.,* 2004), 0.7 J/cm^2 (Takeshita et al., 2003) and 1 J/cm^2 (Wekhof, 2001). These studies demonstrated that factors such as number of light pulses applied, lamp-discharge intensity, distance from lamp to treatment surfaces, shading, microbial species, age and density affected the efficacy of PL decontamination performances.

This study focuses on a novel approach to water disinfection using a PUV system as this PUV method has the potential to provide a broader range of UV wavelengths coupled with a better penetration rate than conventional UV systems.

1.5.5 Factors Affecting UV Damage

The germicidal effects of UV are proportional to the dose of energy absorbed by the organism in question. The UV dose needed to inactivate microorganisms increases with cell size and DNA or RNA content. Although the effectiveness of UV is not hindered by chemical water quality parameters, the presence of suspended solids in the water being treated influences UV disinfection; these particles reduce the amount of UV energy reaching the organisms by absorbing or scattering light. The turbidity of the water being treated can also affect the ability of UV to penetrate the water and can shield micro-organisms from the UV energy. Another factor known to affect UV disinfection is the presence of chemical and biological films on the lamps

(e.g. a build-up of iron and manganese deposits), which will lead to staining on the UV light system and will interfere with the UV light transmittance into the water. Cell density and cell aggregation during treatment are important factors when using light energy for disinfection purposes. Further, the sensitivity of microorganisms to UV radiation may differ depending on which growth phase they are in (i.e. lag, exponential, stationary or death phase). Microorganisms are most sensitive to light-induced damage in the exponential phase.

1.6 Chlorine Reduction through UV Reactors

The addition of chlorine into waters with naturally occurring humic acids (HAs), fulvic acids or other naturally occurring organic material can lead to the formation of trihalomethane (THM) compounds. Approximately 90% of the total THMs formed are chloroform, with the remaining 10% consisting of bromodichloromethane ($CHCl_2Br$), dibromochloromethane ($CHBr_2Cl$) and bromoform ($CHBr_3$). Since THMs have been shown to be cancer causing to laboratory animals in relatively low concentrations, there is concern about limiting their presence in waters. Under Drinking Water Directive (98/83/EC), the EPA has set the maximum contaminant level in primary drinking water to be 100 parts per billion (ppb). Wavelengths between 180 nm and 400 nm produce photochemical reactions which dissociate free chlorine to form hydrochloric acid. The peak wavelengths for dissociation of free chlorine range from 180 nm to 200 nm, while the peak wavelengths for dissociation of chloramines (mono-, di-, and tri-chloramine) range from 245 nm to 365 nm. Up to 5 parts per millions (ppm) of chloramines can be successfully destroyed in a single pass through a UV reactor and up to 15 ppm of free chlorine can be removed.

2 Methods

2.1 Parasite Test Species

C. parvum oocysts (Iowa isolate derived from a bovine calf) and *G. lamblia* cysts (derived from experimental infected gerbils) were purchased from Waterborne Inc. USA. Parasites were stored in sterile phosphate buffered saline (PBS) (0.01 M phosphate buffer, containing 0.0027 M KCL and 0.137 MNaCl at a pH of 7.4) with 100 U of penicillin/ml, 100 µg of streptomycin/ml and 100 µg of gentamicin/ml and stored at 4 °C until they were used for UV treatment studies. Both parasites were chosen due to their highly infectious nature and presence in both water and wastewater-treatment plants.

2.1.1 Viability Staining of Cryptosporidium parvum *Oocysts and* Giardia lamblia *Cysts*

A combined surrogate dye-staining method comprising propidium iodide (PI) and a fluorescein-labelled mouse-derived monoclonal antibody A400FLR-1X Crypt-a-Glo™ for *Cryptosporidium* and Giardi-a-Glo™ for *Giardia* was used to confirm the viability of oocysts/cysts. The excystation rate was determined for each batch of oocysts/cysts by microscopic observation following sequential incubation at 37°C in acidified Hank's balanced salt solution (HBSS) for 1 h and in 0.8% trypsin–0.75% sodium taurocholate for 1 h (*Cryptosporidium* only), followed by incubation at room temperature for 30 min as described elsewhere (Rochelle *et al.,* 2002). For negative infection studies, oocysts were inactivated by heating at 70°C for 30 min. Standard counts were determined for all parasite stocks. Viable oocysts/cysts were enumerated by serial dilution in PBS containing the aforementioned antibiotics using both fluorescence microscopy (confocal microscopy, Leica DM 600 CS Germany). Non-viable oocysts/cysts stained bright red due to uptake of PI when viewed at an excitation wavelength of 460 to 500 nm and an emission spectrum of 510 nm to 560 nm. All viable and non-viable oocysts/cysts stained apple-green when stained with the specific dyes at an excitation wavelength of 460 to 500 nm and an emission wavelength of 510 to 560 nm. Entire slides were scanned and all oocyst suspensions were counted by fluorescence microscopy. All counts were determined in triplicate. All experiments were carried out using oocysts with greater than 95% viability.

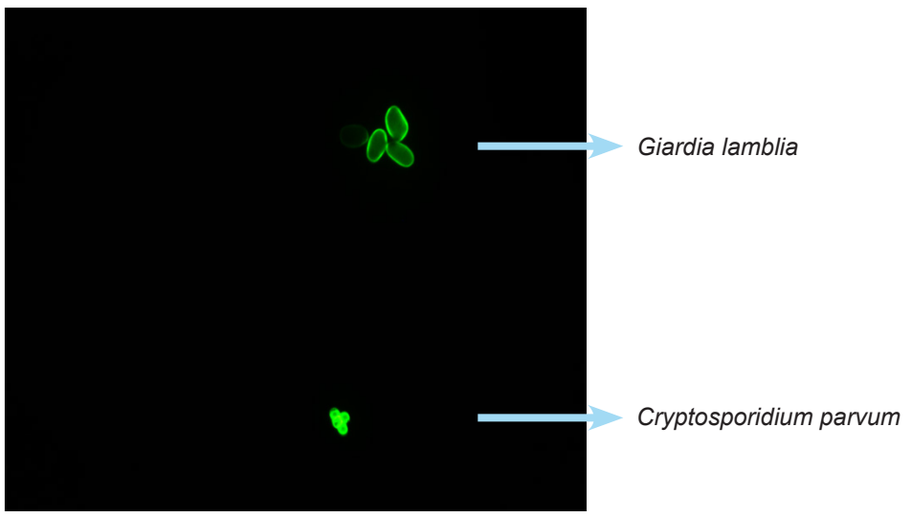

Figure 2.1. Fluorescent image of viable *C. parvum* and *Giardia lamblia* taken with a fluorescent microscope.

2.2 Mammalian Cell Culture and Maintenance of Cell Lines

Monolayers of the human ileocoecal adenocarcinoma cell line HCT-8 (ATCC CCL-244: American Type Culture Collection, Rockville, Md.) were grown with regular sub-culturing in RPMI 1640 growth media with L-glutamine and supplemented antibiotics (penicillin G, 100,000 U/L, streptomycin, 0.5 g/L and amphotericin B, 0.5 g/L), sodium bicarbonate, 2 g/L, and 10% foetal calf serum adjusted to pH7.4. Caco-2 cells (ATCC HTB-37), established from a human colon adenocarcinoma Caco-2 cells, were maintained at 37°C in Dulbecco modified Eagle's medium/Ham's F-12 medium, supplemented with 20% (v/v) foetal bovine serum, 1% 200 mM L-glutamine, 1% (v/v) non-essential amino acids, 0.5% (v/v) penicillin-streptomycin and 0.5% (v/v) amphotericin B (Sigma-Aldrich). Maintenance media was stored at 4°C and heated to 37°C before use. HCT-8 and Caco-2 cells were cultured and maintained in T75 cm^2 cell culture flasks in a humidified incubator at 37°C in an atmosphere containing 5% (vol/vol) CO_2 for ca. 24 h until 80 to 90% confluent monolayers had formed. Once confluent, cells were trypsinised to remove the cell monolayer from the flask and seeded into 6-well plates for 24 h at 37°C at a seeding density of 1 $x10^6$ cells/well for use in real-time PCR studies and at a density of $1x10^5$ cells/well for chamber slides for infectivity studies using fluorescent stains.

2.3 Viability and Infectivity Determination of Parasite Species

Cell culture infectivity was confirmed by immunofluorescent (IF) staining of treated HCT-8 and Caco-2 cell monolayers following exposure to viable oocysts/cysts. Cell monolayers were seeded into each of the 8-well chambered slides (Lab Tec II, Nunc) at a concentration ca. 1×10^5 cells per well. Parasites were stimulated to excyst by re-suspension in bovine and bile and acidified HBSS pH 2.7 for 1 h at 37°C. After one washing step with sterile PBS, oocysts/cysts were re-suspended in cell culture media containing varying concentrations of proteose and thereafter 350 µl aliquots were then added to each well. Samples were incubated for up to 48 h at 37°C in 5% (vol/vol) CO_2 atmosphere, to determine optimal conditions for cell infectivity. At set times each individual well containing a separate monolayer was air dried at room temperature until all moisture had evaporated. After which, 45 µl of Troph-o-Glo™ (Waterborne Inc., UK) which detects different life cycle stages of *Giardia in vitro* was added to each well of *Giardia* infected cells for 25 min at 37°C. Spor-a-glo was used to stain *Cryptosporidium* infected cells as per Garvey *et al.* (2010). Slides were rinsed from unbound stain by flooding with 100 µl SureRinse (Waterborne Inc., USA). The inoculated cell monolayers were then counterstained for 1 min with C101 containing Evans blue dye (Waterborne Inc., USA). All slides were examined under fluorescence microscopy (Leitz Diaplan fluorescence microscope) at an excitation wavelength of 460 to 500 nm and an emission wavelength of 510 to 560 nm for Troph-o-Glo™ and an excitation wavelength of 550 nm and emissions wavelength of 610 nm for the counterstain C101. All wells containing separate monolayers were examined and noted as positive or negative for sites of parasitic infection.

2.4 Pulsing of Parasites Samples with UV Rich Light

A bench-top pulsed power source (PUV-1, Samtech Ltd, Glasgow) was used to power a low-pressure (60 kPa) xenon-filled flashlamp (Heraeus Noblelight XAP type NL4006 series constructed from a clear UV transparent quartz tube), that produced a high-intensity diverging beam of polychromatic PL, which was used in this study as per Garvey e*t al.* (2010). The PL has a broadband emission spectrum extending from the UV to the infrared region with a rich UV content; the intensity of this also depends on the level of voltage applied. The light source has an automatic frequency control function that allows it to operate at 1 pps – the frequency used throughout this study. Light exposure was homogenous as the xenon lamp measuring 9 cm×0.75 cm was longer than the 8.5 cm diameter. Petri dishes used in the tests were placed directly below the lamp, which ensured that full coverage of the surface occurred and eliminated possible shading effects. For standard treatments the light source was mounted at 8 cm above the treatment area, as this distance was shown previously to be optimal for inactivation of *C. parvum* (Garvey *et al.,* 2010). In this study, standard treatments involved suspending predetermined numbers of *C. parvum* or *G. lamblia* and all microbial test strains in 20 ml of PBS which were transferred to petri dishes that were then subjected to lamp discharge energies of 16.2 J. Treated and untreated controls were then centrifuged at

10,000 rpm for 10 minutes and re-suspended in HBSS pH 2.7 to initiated excystation. Cell culture infectivity was then conducted as previously described (Section 2.5) followed by real-time PCR. All studies were run in triplicate.

2.5 Combined Cell Culture-quantitative PCR (CC-qPCR) Assay for Enumerating Viable Parasite Species Post-treatments

Cell culture Taqman-quantitative PCR (CC-qPCR) was conducted as per described by Garvey *et al.* (2010) using HCT-8 cell monolayers as host cells for parasitic infectivity followed by real-time PCR. Real-time PCR reactions are characterised by an increase in fluorescence emission due to probe degradation by DNA polymerase in each elongation step during PCR cycling. The higher the starting copy number of the nucleic acid target, the earlier the fluorescence will reach the predetermined threshold cycle (Ct) and the smaller the Ct value will be. The Ct value is the fractional PCR cycle number at which a significant increase in target signal fluorescence above the baseline is first detected for a sample. Quantification of test samples is performed by determining the Ct value and the use of a standard curve to deduce the starting copy number. Real-time qPCR was performed using primers (TIB MOLBIOL, Berlin, Germany) specific for the 18S region of *Cryptosporidium* following the method of Garvey *et al.* (2010) and Keegan *et al.* (2003). For *C. parvum* the sequence of the Taqman probe was based on the conserved eukaryotic probe of Amman *et al.* (1990) with the following sequence: 5-′-(6-FAM) ACC AGA CTT GCC CTC C (TAMRA).

For *G. lamblia* real-time qPCR specific for the β-giardin region of DNA was conducted. Giardins are filamentous proteins with an alpha coiled helix structure and are a component of the attachment mechanism of *G. lamblia* trophozoites to host cells. An aliquot (4 µl) of the Lightcycler Taqman® Master kit (Roche Diagnostics, West Sussex, England) comprising Taq DNA polymerase, reaction buffer, $MgCl_2$ and dNTP was used in each reaction. Cycling parameters were initial denaturation for 10 min at 95°C followed by 60 cycles of denaturation for 10 s at 95°C, annealing for 40 s at 40°C, extension for 1 s at 70°C and cooling for 30 s at 40°C on a NanoCycler® device (Roche Diagnostics, West Sussex, England). The large number of cycles was used to ensure detection of low levels of infection. On completion of each qPCR run amplification curves were analysed by LightCycler® software (Roche) and a standard curve of oo/cyst DNA concentration determined. DNA standards were prepared from fresh oocysts/cysts ranging in concentration from 10^1 to 10^7 oocysts/cysts/ml by dilution in PBS following standard viable count determinations. Aliquots of oocysts/cysts at different densities were then stimulated to infect the HCT-8 cell line that were seeded into 24-well plates (Sarstedt) at a concentration of ca. 1×10^4 cells/ml at 90% confluency as per the method of Garvey *et al.* (2010). One mililitre aliquots of each concentration range of excysted oocysts were re-suspended in RPMI cell culture growth media and added to 1 well of the 24-well plate. Following 48-h incubation at 37°C in a humidified atmosphere of 5% (vol/vol) CO_2, the cell culture media with non-adherent or internalised parasites were removed by aspiration and discarded. Mammalian cells were then washed with sterile PBS and trypsinised using 200 µl of 0.25% (vol/vol) trypsin/EDTA (Sigma) and left for 15 min at 37°C until complete detachment of the monolayer had occurred. Cells were then centrifuged at 500 rpm for 5 min and re-suspended in 200 µl sterile PBS. Thereafter, the mammalian cells and parasite cell membranes were lysed using a PCR template preparation kit (Roche Diagnostics, West Sussex, England) in order to produce DNA (template) and standard curve (Fig. 3.1). The aforementioned procedure was then repeated to determine the infectivity of oocysts/cysts subjected to varying UV parameters or heating at 70°C for 30 min (negative control) as per method of Garvey *et al.* (2010). Samples were also re-suspended in PBS containing 10% w/v HA in order to investigate the influence of organic matter on microbial inactivation as per Garvey *et al.* (2010). Log inactivation of oocysts (L) is defined by $L=\log_{10}[Nd/No]$, where No is the initial concentration of oocysts and Nd is the concentration of viable infectious oocysts post-disinfection treatments as detected by combined cell culture-qPCR assay as per method of Lee *et al.* (2008).

2.6 Preparation and Pulsed UV Treatment of Planktonic Bacterial Cells

Bacillus strains chosen for this study were *B. megaterium* ATCC 14581*, B. pumilus* ATCC 14884*, B. cereus* ATCC 11778*, B. thuringienses* ATCC 10792*, B. subtilis* ATCC 23857 *and B. lichenformis* ATCC 12759 based on their ability to grow at ambient temperature and because their large size allows for direct microscopic examination. Also, strains were chosen to represent both pathogenic and non-pathogenic *Bacillus* species. For biofilm studies UV inactivation was also conducted on 12-hour cultures of *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853. For ecotoxicological studies, *E. coli, Staphylococcus aureus* and *Saccharomyces cerevisiae* were grown in nutrient broth and malt extract broth respectively for 18 h at 37°C followed by dilution in sterile PBS to obtain a population of 6 $\log_{10}$ colony-forming units (CFU)/ml for each organism. Parasites of *C. parvum* were diluted from stock in sterile PBS to obtain 6 $\log_{10}$ oocysts/ml. All test species were then centrifuged at 10,000 rpm for 10 minutes and suspended in fresh water (as prepared for each MicroBio Test kit) for all ecotoxicity tests.

Strains were sourced from the American Type Culture Collection and grown from storage on nutrient agar at 37°C and their identity confirmed via Gram stain and standard biochemical tests. For PUV studies a single colony of the test strain was aseptically transferred to 100 ml sterile nutrient broth followed by incubation at 37°C for 24 h. Following incubation, test samples were centrifuged at 10,000 rpm for 10 min and the pellet re-suspended in sterile phosphate buffered saline (0.01 M phosphate buffer, containing 0.0027 M KCL and 0.137 MNaCl at a pH of 7.4) (PBS) to give a working stock with ca. $1x10^8$ CFU/ml. Pulsed UV studies were conducted on samples diluted from the working stock in 20 ml final volumes of sterile PBS at 8 cm from the light source. Samples were treated at 16.2 J for various time periods at a rate of 1 pps to determine the relative sensitivity of each test species. Following treatment, 100 µl of treated or untreated sample was spread on plate count agar and incubated for 24 h at 37°C, after which the number of colony-forming units (CFU/ml) of survivors was determined. For analysis purposes the levels of inactivation were determined by plotting the $\log_{10}$ ratio of survivors against UV dose (µJ/cm^2) for each experimental organism.

2.7 Preparation of *Bacillus* Species Endospores

All *Bacillus* spores were cultivated as per the method of Periago *et al.* (2006) with some modifications. Spores were prepared on petri dishes containing plate count agar which was supplemented with 3 mg/L of manganese sulphate (Sigma). The agar surface was inoculated with 400 µl of a 24-hour culture grown in nutrient broth at 37°C. Plates were then incubated at 30°C for four days to allow for bacterial sporulation due to nutrient depletion. Spores were then collected by flooding the agar plate with sterile PBS (pH7) and rubbing the surface with a sterile spatula. Samples were transferred to sterile bottles for storage. Spore samples were then heated to 90^{0}C for 25 min to kill any vegetative cells present. A control bottle was used to ensure the water inside each bottle reached 90°C. After heating, spores were washed twice by centrifugation at 10,000 rpm for 10 min and re-suspended in sterile PBS. The purity of spore suspensions was checked using malachite green spore staining and phase contrast microscopy as per Rowan *et al.* (2001). The concentration of spores in the final suspension was assessed by serial dilution of the stock culture followed by incubation on plate count agar at 37°C for 24 h.

2.8 Pulsed UV Inactivation of *Bacillus* Endospores

The spore suspensions were stored frozen at –18°C until used. Spore viability was observed to be unchanged after storage under these conditions for the duration of the experiments. For PUV studies 2 ml of the spore working stock suspension was added to 18 ml of sterile PBS in a petri dish and placed under the light source at a distance of 8 cm to give a final concentration of $1x10^8$ spores/ml in 20 ml volumes. Studies were also conducted on 100 ml samples (volume depth 8.8 cm) both with and without the presence of 10 ppm HA for comparative analysis. Humic acid (HA) constitutes the major portion of organic material present in surface waters, deriving from living or decaying vegetation or

microbial decomposition processes. They are usually quantified in terms of total organic carbon (TOC). Typical source waters for drinking water treatment frequently have organic carbon concentrations less than 10 mg/L (ppm) (Cantwell *et al.,* 2008). Therefore, for this study it was decided to use HA at a concentration of 10 mg/L (pH 7.1) to assess the effect of organic matter on PUV disinfection. Inactivation rates were determined as previously described.

2.8.1 Scanning Electron Microscopy Studies on Bacillus *endospores*

Scanning electron microscopy (SEM) studies were conducted on *Bacillus* spores pre- and post-UV treatment to determine if such treatment had any effect on the surface of the organism. Samples were prepared for SEM as per method of Periago *et al.* (2006) with some modifications. One millilitre of untreated and UV-treated (21.6 μJ/cm^2) samples were placed in sterile centrifuge tubes and centrifuged at 3000 rpm for 10 min, after which the supernatant was removed and the spores re-suspended in 1 ml of 50% ethanol for 15 min. Samples were again centrifuged and re-suspended in 70% and finally 100% ethanol. Once complete sample dehydration had occurred, 100 μl of each sample was placed on a SEM disk and placed in the oven until all ethanol had evaporated. Samples were then gold coated using a Bal-Tec SCD sputter coater for 110 seconds at a current of 25 milliamps and flushed with argon before coating. Samples were then viewed at various magnifications using a Tescan Scanning Electron Microscope.

2.9 Comparative Low-pressure UV Inactivation of Test Species

Low-pressure UV studies were conducted on selected microbial strains on agar surfaces and in 20 ml suspensions using a lab scale UV lamp at 254 nm (UVGL-55 handheld UV lamp). For surface treatment 100 μl of sample was spread on the agar surface and exposed to an LP UV lamp at a distance of 8 cm from the light source. Bacterial suspensions were prepared by adding 2 ml of the stock broth to 18 ml of sterile PBS followed by exposure to the LP UV lamp. The UV dose (mJ/cm^2) was varied by altering the time of exposure as required.

2.10 Biofilm Studies

2.10.1 Centers for Disease Control Biofilm Reactor

The Centers for Disease Control (CDC) reactor (Biosurface Technologies Corp, Bozeman, MT) is composed of a glass vessel which holds the reactor media. Placed into this vessel is a polyethylene top which holds eight removable polypropylene rods. Each rod in turn has three inserts for holding the coupons on which the biofilms form. Therefore, each biofilm reactor has space for 24 coupons equivalent to 24 separate biofilms which makes it an ideal apparatus for inactivation studies. In the centre of the reactor a magnetic stirrer is present which provides a continuous flow of nutrients over the colonised surface of the coupons. The CDC reactor is a recognised method for the growth of *P. aeruginosa* biofilms under high shear and continuous flow (Coenye and Nelis, 2010) and is the standard method in use by the American Society for Testing and Materials (ASTM).

2.10.2 Preparation of Microbial Biofilms

Pseudomonas aeruginosa ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were sourced, cultured and maintained in nutrient broth. The CDC biofilm reactor (BioSurface Technologies Corp. USA) was used for the formation of microbial biofilms as per the recommended procedure for continuous fluid shear flow-through biofilm formation (ASTM, E2562-12). Specifically, a selected number of polyvinyl chloride (PVC) coupons were inserted into the coupon holes in each reactor rod and firmly secured. To initiate *P. aeruginosa* biofilm formation, 350 ml of 300 mg/L tryptone soya broth (TSB) was added to the reactor ensuring each coupon was completely submerged. This low concentration of TSB provided a nutrient-depleted environment within the reactor. The reactor was sterilised at 121^{o}C for 15 min, after which 1 ml of a 12-hour microbial culture of *P. aeruginosa* was added to the reactor chamber to ensure cells were in the log phase of reproduction. Bacterial counts were also conducted to determine the seeding density of the reactor which was ca. 3.4x10^6 CFU/ml. The reactor was incubated at 20^{o}C for 24/48/72 h as required under rotatory conditions (125 rpm). For the formation of *S. aureus* biofilms, 350 ml of TSB broth supplemented with 0.2% glucose was added to the reactor vessel. Following sterilisation as previously

described 1 ml of a 12 h culture of known population density of *S. aureus* was added to the reactor media which was then incubated for 48 h at 37°C under rotatory conditions. To allow for comparative analysis a 48-hour biofilm of *P. aeruginosa* which was formed at 37°C was also treated. To allow for the enumeration of CFU per microbial biofilm all coupons were removed from each reactor rod aseptically and rinsed with sterile phosphate buffered saline (PBS) to remove any planktonic cells. Biofilms were removed from each coupon by scraping the coupon using a sterile cell scraper into 10 ml of sterile PBS. Serial dilutions were then made and the standard plate count technique was used to determine the CFU/ml bacterial population in the biofilm as per the recommended procedure (ASTM, E2562-12).

2.10.3 PUV inactivation of bacterial biofilms

Polyvinyl chloride coupons (representative of the piping used for water distribution) were aseptically removed from the reactor and transferred to a sterile petri dish. Samples were exposed to pulses of UV light at 16.2 J at 8 cm from the light source at varying UV doses which were obtained by increasing the pulse number. Once treated, coupons were submerged in 10 ml of sterile PBS and surface scraped using a sterile cell scraper to remove the treated biofilms and to allow for the determination of inactivated rates. The liquid was then transferred to a sterile 20 ml container and centrifuged at 3000 rpm for 10 min. to pellet the cells. The sample was then re-suspended and agitated to ensure biofilm dispersion. Serial dilutions were made from the biofilms suspension and 100 µl spread on triplicate agar plates to determine the CFU/ml of treated samples. This process was repeated for coupons at varying UV doses to determine the Log_{10} reduction obtained with increasing UV dose. A cell count was also conducted on the media present in the reactor vessel by spread plate techniques.

2.11 Flow-through Pulsed UV Inactivation Studies

Flow-through PUV inactivation studies were conducted using a flow-through UV light system adapted for use by Dr Eoghan Clifford, NUIG. The flow-through system consisted of a UV lamp placed above a treatment chamber which held water for a variable amount of time (retention time) depending on the flow rate used.

2.11.1 Pulsed UV Inactivation of Flowing Test Species

Flow-through inactivation of test species was conducted on both planktonic and bacterial endospores. Twelve-hour cultures of microbial species or pre-cultured endospores (Section 2.7) prepared in sterile PBS were suspended in a 5-litre sterile duran. Liquid was then pumped from the duran via a peristaltic pump to the sterile treatment chamber at set flow rates. The two flow rates chosen for this study (24 L/hour and 30 L/hour) where achieved by setting the pump to 200 and 375 rpm respectively, giving a retention time of 60 and 120 s. Liquid was pulsed at a rate of 1 pps and treated samples were collected at the exit of the treatment chamber. All waste liquid was collected and autoclaved before disposal.

2.11.2 Effect of the Presence of Inorganic Matter in the Treatment Liquid

To determine the effect of inorganic contaminants on the inactivation rates of test species, PUV studies were conducted in the present of manganese (II) sulfate and iron and also in the presence of both. In drinking-water supplies, iron salts are unstable and are precipitated as insoluble iron (III) hydroxide, which settles out as a rust-coloured silt. Anaerobic groundwaters may contain iron (II) at concentrations of up to several milligrams per litre without discolouration or turbidity in the water when directly pumped from a well, although turbidity and colour may develop in piped systems at iron levels above 0.05 to 0.1 mg/litre (WHO, 1993). Manganese is abundant in the natural environment and is also a product of much industrial applications. Although the WHO limits for manganese in drinking water are 400 to 500 µg/L, concentrations of this element can increase or decrease depending on location – for example, down-stream of industrial settings or natural geological conditions.

Therefore, concentrations of both these inorganic compounds were chosen to represent high levels of contaminants commonly found in surface waters (0.3 mg/L Fe II [powder], 10 mg/L manganese [II] sulfate). Five-litre volumes of sterile water containing the set concentrations of contaminants were prepared and inoculated with bacterial test species. Treatment was then carried out as in Section 2.11.1.

2.12 Ecotoxicological Testing of Pulsed UV Treated Water

2.12.1 Ecotoxicological Test Battery

A battery of tests was conducted to assess the effect of these different disinfection methods on species present in the environment (Table 2.1). The tests which were chosen are representative of different trophic levels present in the aquatic environment which may come into contact with pulse-powered treated discharge. Table 2.1 details the tests used, their target species, trophic level and toxic endpoints. All test kits were sourced through MicroBio Tests Inc., Belgium and required the use of freshwater as a media for the growth and maintenance of each test species. Freshwater was prepared by filling a 1 litre volumetric flask with 800 ml of distilled water (dH_2O). Each vial supplied with the test kit was uncapped and added to the dH_2O in order of labelling: (i) vial 1 containing $NaHCO_3$, (ii) vial 2 containing CaSO, (iii) vial 3 containing $MgSO_4$ and (iv) vial 4 containing KCl. The flask was subsequently filled to the mark with dH_2O and stored at 5°C in the dark until required. Before use, the freshwater was aerated for 15 min by bubbling air through a tube connected to an aquarium air pump. The standard freshwater was used as a hatching medium for the cysts and as dilution medium for the test solutions for all tests used. For each test kit a positive control was used as a reference toxicant, potassium dichromate ($K_2Cr_2O_7$) was the recommended chemical and therefore used for each test species. The positive control phenol was used for the Microtox® assay as recommended by the manufacturer. For each test kit test samples were prepared as per previously described. Procedures were followed precisely as instructed by the kit manufactures for all kits i.e. Thamnotoxkit F^{tm} (test species *Thamnocephalus platyurus*), Algaltoxkit F^{tm} (test species *Pseudokirchneriella subcapitata*), Artoxkit M^{Tm} (test species *Artemia franciscana*), Protoxkit F^{Tm} (test species *Tetrahymena thermophila*), Daphtoxkit F^{tm} (test species *Daphina magna*) and the Microtox^{Tm} Assay (test species *Vibrio fischeri*).

2.12.2 Cytotoxicological Analysis

Cytotoxicological analysis of treated samples was conducted at the highest ecotoxicological trophic level using a rainbow trout fish cell line. For these studies the RTH-149 (ATCC CRL-1710) was cultured and maintained *in vitro*. Cell culture media for the RTH-149 cell line consisted of 87% (v/v) Eagles Minimal Essential Media, 10% (v/v) foetal bovine serum, 1g/L 200 mM L-glutamine and supplemental antibiotics (100,000 U/L penicillin G; 0.5 g/L streptomycin; 0.5 g/L amphotericin B), 1% non-essential amino acids and 1mM Hepes. Cells were grown and maintained in a 20°C incubator in standard T75 cm^2 cell culture flasks and subcultured as required when cell confluency was reached. The *in vitro* cytotoxicity assays chosen were the MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and the Neutral Red (NR) assay. The MTT assay determines the activity of mitochondria in viable cells by measuring the ability of mitochondrial succinate dehydrogenase to convert the tetrazolium salt MTT to a dark blue/purple formazan product. The amount of formazan produced can be taken as a direct measure of cell viability. The cleavage of MTT has

Table 2.1. Test kits and associated test species used in the test battery.

Test kit	Test species	Trophic level	Origin	Exposure period and endpoint
Algaltoxkt F™	*Pseudokirchneriella subcapitata*	Primary producer	Algae	72 h inhibition
Protoxkit F™	*Tetrahymena thermophilia*	Trophic intermediary	Protozoan	2 h
ThamnotoxkitF™	*Thamnocephalus platyurus*	Primary consumer	Crustacean	24 h lethality
Daphtoxkit F™	*Daphnia magna*	Primary consumer	Crustacean	24 h immobilisation 48 h immobilisation
Artoxkit M	*Artemia franciscana*	Primary consumer	Crustacean (saline environment)	24 h immobilisation
Mictrotox™	*Vibrio fischeri*	Decomposer	Bacteria	Bioluminescence inhibition
Cytotoxicity	RTH-149	Consumer	Fish cell line	24 h lethality

several desirable properties for assaying cell survival and proliferation as MTT is cleaved by all living, metabolically active cells but not dead cells. The NR assay is based on the ability of viable cells to incorporate and accumulate the weakly cationic supravital dye NR (Borenfreund and Puerner, 1985). The dye readily penetrates the cell membranes of viable cells by non-ionic diffusion and binds with anionic sites in the intracellular lysosomal matrix (Lullmann-Rauch, 1979). In general, the dye is not incorporated in damaged or dead cells, but may appear, however, as a diffuse stain throughout the cytoplasm.

2.12.3 Preparation of a Representative Liquid Test Sample

For the toxicological analysis of PUV-treated samples, test liquid samples were prepared as above, using the same organisms with the exception of the treatment media which was dH_2O. Twenty milliliters of the suspension was placed in a sterile petri dish using a septic technique. The sample was placed in the treatment chamber and given up to 100 pulses (10.2 $\mu J/cm^2$) to ensure microbial inactivation as confirmed by counting survival colonies for bacterial species and qPCR for *C. parvum*. The treated sample was transferred to a sterile universal and centrifuged at 10000 rpm for 10 min. The supernatant was discharged and the pellet re-suspended in sterile PBS.

2.12.4 MTT Assay

A 5mg/ml MTT stock solution was prepared by adding 50 mg MTT to 10 ml PBS in a sterile universal, and the solution stored in the dark at 4°C. Before use, this mixture was pre-heated by incubation at 37°C for 1 h. The MTT solution was filter sterilised using a 0.2 µm pore sized syringe filter to remove any crystal which had formed. Dimethyl sulfoxide (DMSO) was used to extract and solubilise the formazan precipitate. Cytotoxic effects on cell growth and viability was determined using 96-well microplates seeded with 1–1.5x10^4 cells in 200 µl of complete culture medium. The plates were then incubated for 12–24 h at 20°C until the desired percentage of confluency (80%) was observed. Cells were exposed to samples of PUV-treated *C. parvum* seeded water by adding 20 µl of sample with 180 µl of cell culture media to the cells in triplicate for 24 h. Studies were also conducted on 100 µl treated samples with 100 µl cell media to determine if cytotoxicity occurred with a higher amount of PUV-treated sample. (It is not practical to expose cells to pure treated samples without the presence of cell culture media as the cells would die from starvation or from the change in pH.) A range of potassium dichromate concentrations were also studied for comparative analysis, as potassium dichromate was the positive control used in all the environmental toxicology studies. The concentration range chosen was 10, 20, 30, 40 and 50 µM. Subsequently, after 24-hour incubation the medium containing test chemicals was discarded and the wells were washed twice with pre-heated PBS. After the washing step 180 µl of fresh media and 20 µl of MTT solution was added to each well to obtain a final MTT concentration of 0.5 mg/ml. Following an additional 4-hour incubation period at 20ºC, the MTT-containing medium was carefully aspirated off and resulting blue formazan crystals were solubilised by adding 100 µl of DMSO to each well. To support solubilisation the plates were shaken gently and the optical densities of each well recorded using a multiwell plate reader (BioTek® EL 800 attached to a Dell PC with Gen5™ software for analysis of absorbance readings at set wavelengths) at a test wavelength of 540 nm and a reference wavelength of 690 nm. Results are presented as a percentage of untreated controls against test chemical concentrations.

2.12.5 Neutral Red Assay

A 1 mg/ml NR stock solution was prepared and the solution acidified with two drops of glacial acetic acid, autoclaved and stored in the dark at 4°C. Immediately before use, a working solution was prepared by diluting the NR stock solution 1:10 with pre-heated PBS. The solution was filter sterilised, using a 0.2 µm pore-sized syringe filter to remove any crystal formation. A 1% glacial acetic acid/50% ethanol (v/v) solution was used to extract the dye and fix the cells. Cells were seeded in 96-well microplates at a density of 1–1.5x10^4 cells in 200 µl of complete culture medium and then further incubated for 12–24 h at 20°C until the desired percentage of confluency (80%) was observed. Cells were exposed to samples of PUV-treated *C. parvum* seeded water by adding 20 µl of sample with 180 µl of cell culture media to the cells in triplicate for 24 h. Studies were also conducted on 100 µl treated samples with 100 µl cell media to determine

if cytotoxicity occurred with a higher amount of PUV-treated sample. Test plates were incubated at 20°C for 24 hr. After incubation the medium containing test chemicals was discarded and the wells were washed twice with warmed PBS. Subsequently, 200 µl of the pre-warmed NR working solution (100 µg/ml) was added to each well and further incubated for 80 min at 20ºC. The NR-containing solution was carefully aspirated of the cells which were washed twice with 200 µl of pre-warmed PBS. The dye was extracted by adding 100 µl of the NR extract solution to each well. To support solubilisation the plates were shaken gently and the optical densities recorded using a multiwell plate reader (BioTek® EL 800 attached to a Dell PC with Gen5™ software for analysis of absorbance readings at set wavelengths) at a test wavelength of 540 nm. Wells exposed to NR solution without cells were used as blanks and were subtracted from measured optical densities. Results are presented as percentage of untreated controls against test samples.

2.13 Statistical Analysis

The $\log_{10}$ reduction for UV-treated cysts was calculated as the $\log_{10}$ of the ratio of the concentration of the non-treated (N_0) and UV treated (N) samples [$\log_{10}$ (N_0/N)]. Student's t-tests and ANOVA one-way model (MINITAB software release 16; Mintab Inc., State College, PA) were used to compare the effects of the relationship of independent variables on UV treatments. Student t-tests were used to compare infectivity in both cell lines and parasite types. For ecotoxicology testing median lethal concentrations (LC_{50}) and other lethal concentrations (LC_{20}) were determined by the trimmed Spearman-Karber method, using TOXSTAT software, version 3.4 (Azur Environmental, 1995; West Inc. and Gulley, 1994). Statistical analysis for the Microtox™ test was performed using the Microtox 'Chronic Toxicity Testing DOS software', in accordance with guidelines provided by the US Environmental Protection Agency (1994). All experiments were conducted in triplicate in three separate experiments.

3 Results

3.1 Development of a Cell Culture Assay for Parasite Infectivity

All oocysts/cysts were less than two weeks old and 100% viable before studies were conducted as determined by viability staining using specific IF dyes. Initial excystation methods were conducted as per the methods of Garvey *et al.* (2010) for the excystation of C*ryptosporidium* and included exposing parasite preparations to HBSS pH 2.7 (stomach-like environment) followed by re-suspension in 1% bovine bile. However, it was found that the rate of excystation of *G. lamblia* decreased significantly with an increase in the exposure times to bovine bile as determined by microscopic trophozoite count. Findings showed that maximal cyst excystation occurred in the absence of bile with a 1 h suspension in HBSS pH 2.7 proving sufficient to cause cyst excystation and trophozoite release as determined by trophozoite count via microscopy. For the excystation of *Cryptosporidium* sporozoites the second step consisting of incubation in 1% bovine bile at 37°C for 15 min was required for optimal excystation.

For cell culture infectivity it was found that optimal infectivity of the HCT-8 and Caco-2 cell lines occurred in media which contained 2% proteose for *G. lamblia*. These findings are based on the observation of multiple sites of infection via fluorescent microscopy and the lower Ct readings obtained following PCR amplification of parasitic DNA. Furthermore, for parasitic cell line infectivity it was found that infection was optimal when cells were exposed to parasites at a ratio of 1:1. The Ct readings for parasitic DNA extracted from cell culture were consistently higher than those extracted from cysts/oocysts without cell culture, indicating that a loss of parasite DNA occurred during this step. There was a significant difference ($p<0.05$) in the amplification values for target DNA from parasites which were stimulated to infect mammalian cells and those which were only stimulated to excyst. Notably, for both cell lines the limit of detection was 10 cysts and oocysts per monolayer for *Giardia* and *Cryptosporidium* respectively with a detection limit of 1 oocyst/cyst, without passage through cell culture for both species.

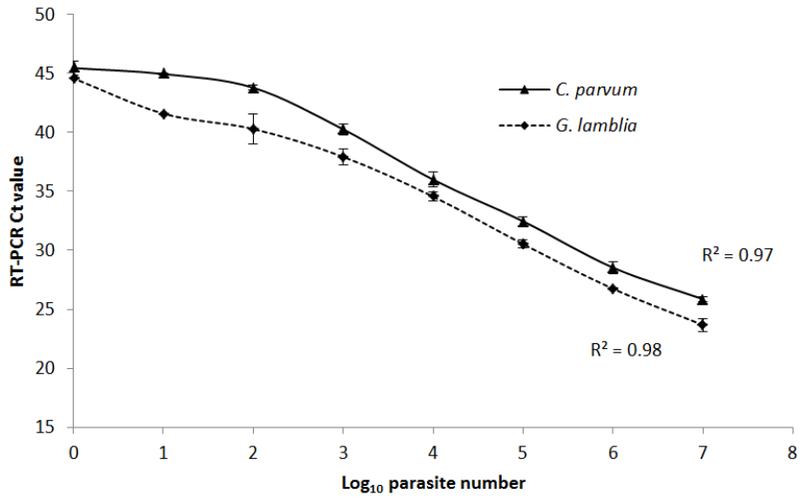

Figure 3.1. Standard curve for *C. parvum* and *G. lamblia* parasite numbers vs. Ct value as determined by real-time PCR (+/-S.D) using species specific primers. Standard curve obtained without cell culture infectivity.

For both parasite species the HCT-8 cell line proved significantly more susceptible to infection than the Caco-2 ($p<0.05$). This is evident from the lower Ct values obtained for PCR amplification following HCT-8 infection, indicating that a larger amount of target DNA was present (Figs 3.2 and 3.3). Fluorescent staining and imaging also indicated that infection of Caco-2 cells was not as prominent with fewer sites of infection present. Infectivity was graded on a scale of 1 to 4 based on the amount of fluorescent structures present which were not quantifiable due to the high numbers present. It was also observed that the negative effect of parasitic infection on host cell lines was more noticeable in Caco-2 cells where cell death occurred more rapidly following exposure to parasites. Also, cells detached from the culture flask with greater ease than the infected HCT-8 cells. This suggests that the Caco-2 host cell monolayer was unable to support infectivity with large numbers of parasites and the resulting loss of cell viability and attachment influenced the rate of infection. This was not observed with HCT-8 cells as they continued to proliferate with monolayer overgrowth occurring in both infected and non-infected cells.

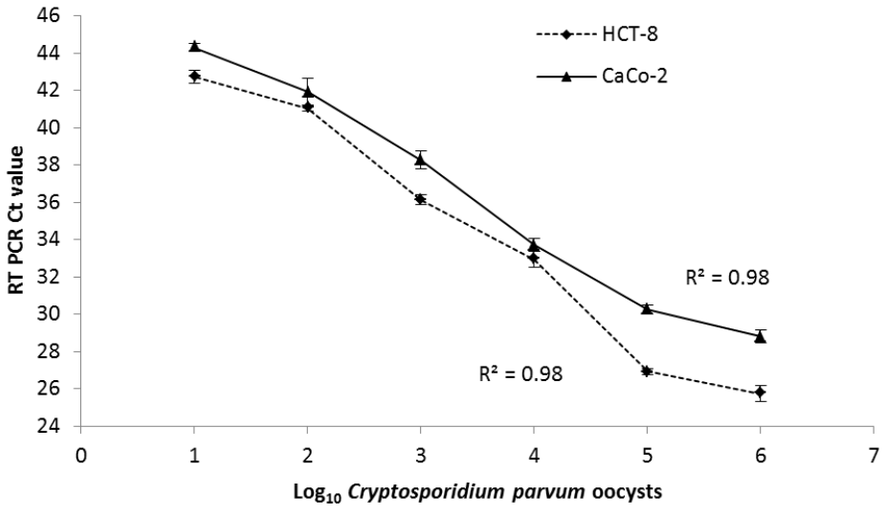

Figure 3.2. Standard curve for *C. parvum* infected HCT-8 and CaCo-2 cell as detected via real-time PCR following 48 hours incubation at 37°C (+\-S.D).

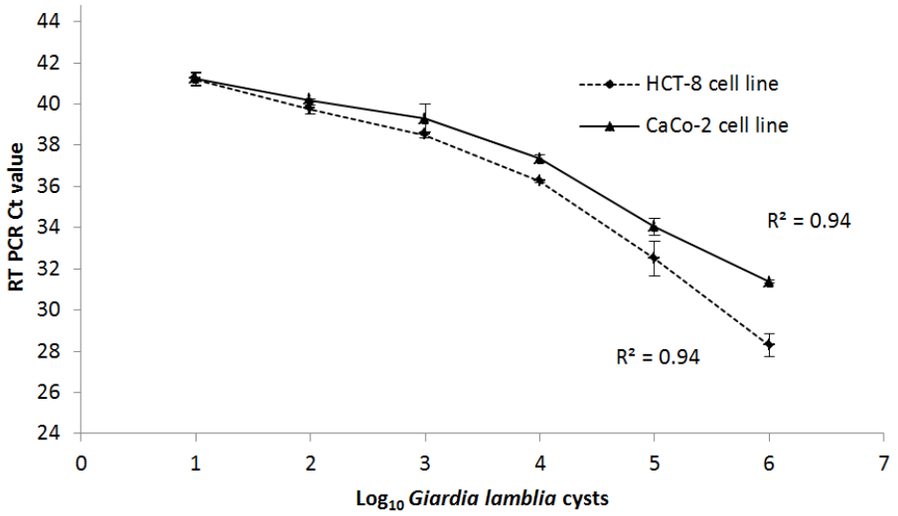

Figure 3.3. Standard curve for *G. lamblia* infected HCT-8 and Caco-2 cell as detected via real-time PCR following 48 hours incubation at 37°C (+\-S.D).

3.2 Quantitative PCR for Determination of Parasite Infectivity

The use of vital staining techniques on UV-treated cysts proved insufficient at determining viability/inactivation following UV treatment (Table 3.1). Even at maximal UV doses all treated parasites showed levels of dye uptake similar to untreated controls, indicating that no inactivation had occurred. These findings correspond to that of Campbell and Wallis (2002) where vital dye viability assays repeatedly underestimated inactivation. As with Campbell and Wallis (2002) this confirms that vital dye viability assays should not be used to define inactivation during testing of novel disinfection techniques.

The sensitivity of the real-time RT-PCR assay was examined by performing RT-PCR assays in triplicate with tenfold serial dilution of purified oocysts/cysts with and without cell culture infection. For primer amplification, a linear response ($r^2 = 0.98$) was observed from 1,000,000 to 10 oocysts/cysts per reaction for both excysted and cell culture PCR assays (Figs 3.2 and 3.3). The detection of target DNA was quicker following excystation with the absence of the cell culture step as evident by the smaller Ct values obtained. However, excystation alone does not give information on the infectivity of the parasite in a host cell environment. Indeed, excystation occurred for both treated and untreated parasite species at a similar rate, suggesting that no UV inactivation had occurred (Tables 3.1 and 3.2). However, with CC-qPCR there was a significant ($p<0.05$) loss in viability detected with an increase in UV dose, indicating a reduction in infective oocysts/cysts present. This was not achieved with vital dye staining or PCR following excystation alone.

Table 3.1. Determination of viability of UV-treated and untreated *C. parvum* ($1x10^5$ cysts/ml) via staining with vital dyes DAPI (4',6-diamidino-2-phenylindole) and propidium iodide (PI) compared to the excystation and cell culture RT-PCR assays.

LP-UV dose mJ/cm²	Viability			PUV dose µJ/cm²	Viability		
	DAPI/PI (%)¥	Excystation PCR* Log_{10}	CC-PCR^ Log_{10}		DAPI/PI (%)¥	Excystation PCR* Log_{10}	CC-PCR^ Log_{10}
0	100	5.1(+/-0.2)	5.1(+/-0.01)	0	100	5.2(+/-0.2)	5.2(+/-0.3)
10.8	99	5.0(+/-0.1)	5.0(+/-0.1)	1.08	100	5.1(+/-0.2)	3.7(+/-0.1)
32.1	99	4.9(+/-0.05)	5.1(+/-0.3)	2.15	99	5.2(+/-0.08)	3.2(+/-0.1)
65.4	100	4.9(+/-0.4)	5.0(+/-0.02)	3.24	100	5.2(+/-0.1)	2.7(+/-0.3)
130.8	100	5.1(+/-0.1)	4.7(+/-0.3)	6.48	100	5.2(+/-0.3)	2.1(+/-0.05)
261.6	100	5.1(+/-0.1)	2.1(+/-0.2)	9.72	97	5.0(+/-0.2)	1.3(+/-0.2)
392.4	100	5.0(+/-0.3)	0.31(+/-0.1)	16.2	100	5.0(+/-0.01)	0
490.5	98	4.8(+/-0.2)	0.2(+/-0.1)	22.68	100	4.6(+/-0.1)	0

¥ Viability measured by vital staining of *C. parvum* pre and post UV exposure, score is the mean of 3 separate replicates of individual experiments. 100 parasite cysts were counted and marked as positive or negative for dye uptake.

* Log_{10} viability as determined PCR amplification of *C. parvum* UV treated and untreated following excystation without passage through a cell line.

^ Log_{10} viability as determined by PCR amplification of *C. parvum* UV treated and untreated following excystation and cell culture infectivity.

Table 3.2. Determination of viability of UV-treated and untreated *G. lamblia* (1x10^5 cysts/ml) via staining with vital dyes DAPI and PI compared to the excystation and cell culture RT-PCR assays.

LP-UV dose mJ/cm^2	Viability			PUV dose µJ/cm^2	Viability		
	DAPI/PI (%)¥	Excystation PCR* Log_{10}	CC-PCR^ Log_{10}		DAPI/PI (%)¥	Excystation PCR* Log_{10}	CC-PCR^ Log_{10}
0	100	4.98 (+/-0.1)	5 (+/-0.3)	0	100	5.2(+/-0.2)	5 (+/-0.05)
5.4	100	5.1(+/-0.01)	3.52 (+/-0.05)	1.08	100	5.1(+/-0.2)	4.48 (+/-0.3)
10.8	99	5.1(+/-0.1)	3.05(+/-0.2)	2.15	100	5.2(+/-0.08)	4.48 (+/-0.1)
16.35	100	5.1(+/-0.08)	2.9(+/-0.1)	3.24	100	5.2(+/-0.1)	4.4 (+/-0.3)
32.7	100	5.0(+/-0.2)	3.08(+/-0.5)	6.48	99	5.2(+/-0.3)	4.38 (+/-0.1)
49.0	99	5.0(+/-0.1)	3.16(+/-0.4)	9.72	100	5.0(+/-0.2)	3.8 (+/-0.02)
81.7	100	5.1(+/-0.3)	3.12(+/-0.2)	16.2	100	5.0(+/-0.01)	2.98 (+/-0.2)
113.4	100	5.1(+/-0.1)	3.26(+/-0.3)	22.68	98	4.6(+/-0.1)	2.18 (+/-0.01)

¥ Viability measured by vital staining of *G. lamblia* pre- and post-UV exposure, score is the mean of 3 separate replicates of individual experiments. 100 parasite cysts were counted and marked as positive or negative for dye uptake.

* Log_{10} viability as determined PCR amplification of *G. lamblia* UV treated and untreated following excystation without passage through a cell line.

^ Log_{10} viability as determined by PCR amplification of *G. lamblia* UV treated and untreated following excystation and cell culture infectivity.

3.3 UV Inactivation of Parasite Test Species

The variance in the Ct values obtained for qPCR following UV exposure allows for the determination of UV inactivation by comparing treated to untreated controls. The equation of the line obtained for the HCT-8 CC-qPCR standard curve was used to calculate log_{10} reduction of each UV-treated batch. Findings show that both LP-UV and PUV light successfully inactivated the test species, albeit with significantly different rates of inactivation.

3.3.1 Cryptosporidium parvum

The waterborne enteroparasite *C. parvum* showed a greater PUV resistance compared to other microbial test organisms with only a 2.5 log_{10} reduction in viability observed using a UV dose of 4.32 µJ/cm^2. However, a 5 log_{10} inactivation of *C. parvum* oocysts was achieved with as much as 12.96 µJ/cm^2 of PUV light. *Cryptosporidium* was inactivated at a faster rate with PUV than with the LP-UV system where a treatment time of 15 minutes was required to give a 5 log_{10} loss in viability compared to 2.5 minutes with PUV (Table 3.3).

Table 3.3. Log_{10} reduction and infectivity of treated *C. parvum* via real-time PCR and cell culture infectivity IF staining of the HCT-8 cell line following UV treatment (+/- S.D.).

Exposure time (seconds)	LP-UV			PUV		
	Dose mJ/cm²	RT-PCR Log_{10} reduction	Cell culture infectivity*	Dose x10^{-3} mJ/cm²	RT-PCR Log_{10} reduction	Cell culture infectivity*
0	0	0	4	0	0	4
10	5.4	0	4	1.08	1.4(+/- 0.01)	4
20	10.8	0	4	2.15	1.9(+/- 0.1)	3
30	16.35	0	4	3.24	2.4(+/- 0.3)	3
60	32.7	0	4	6.48	3.1(+/- 0.05)	2
90	49	0	3	9.72	3.6(+/- 0.4)	1
120	65.4	0	4	12.96	4.8(+/- 0.1)	0
150	81.7	0	4	16.2	≥5(+/- 0.2)	0
180	98.1	0	4	19.44	≥5(+/- 0.3)	0
210	113.4	0	4	22.68	≥5(+/- 0.1)	0
240	130.8	0.1(+/- 0.1)	4			
480	261.6	2.9(+/- 0.1)	1			
900	490.5	5(+/- 0.3)	0			

*Infectivity of cells as determined by immune fluorescent (IF) staining of 3 separate HCT-8 monolayers exposed to UV treated and untreated *C. parvum* oocysts. Presence of infectivity indicates the presence of viable *Cryptosporidium*. Numbers indicate level of infectivity 4 = high, 3 = medium, 2 = low, 1 = very low

3.3.2 Giardia lamblia

With 5.4 mJ/cm² of LP-UV dose, a 1.48 log_{10} reduction in viability was achieved for *G. lamblia* as detected via CC-qPCR. With an increase in exposure the rate of inactivation also increased significantly ($p<0.05$) up to a maximum of 1.88 log_{10}. However, after a UV dose of 65.4 mJ/cm² no further increase in *Giardia* inactivation was detected (Table 3.4). Therefore, a ca. 2 log_{10} inactivation rate was obtained with a UV dose of 113.4 mJ/cm² equivalent to a treatment time of 210 s (3.5 min). These findings correspond to that of Craik *et al.* (2000) and Campbell and Wallis (2002) where studies show that 99% of *Giardia* inactivation was achieved below 8 mJ/cm² and 10 mJ/cm² respectively using an MP lamp and *in vivo* infectivity in mice and gerbil modes. At doses exceeding 8 mJ/cm² and as high as 130 mJ/cm² there was no significant increase in the inactivation of *Giardia* cysts (Craik *et al.*, 2000). The similarities in these findings using a cell-culture-based assay to that of animal infectivity suggest that the former *in vitro*-based approach may indeed show levels of infectivity comparable to that of the *in vivo* test system.

The PUV system resulted in levels of inactivation of *G. lamblia* which were greater than the LP-UV system (Table 3.4) with significantly less applied UV dose. Indeed, a 1.48 log_{10} inactivation was achieved with as little as 12.96x10^{-3} mJ/cm² of PUV compared to 5.4 mJ/cm² of LP-UV. What is noteworthy is that the inactivation plateau which was observed with LP-UV did not appear with PUV up to a dose of 22.68x10^{-3} mJ/cm² equivalent to a treatment time of 210 s. A ca. 3 log_{10} (99.9%) loss in cyst viability was determined by CC-qPCR with a PUV dose of 22.68x10^{-3} mJ/cm². Previously published work of Garvey *et al.* (2010) detailing the inactivation of *C. parvum* reported a 3.3 log_{10} inactivation following a PUV dose of 9.72x10^{-3} mJ/cm². This report also showed the correlation between mice infectivity and an *in vitro* HCT-8 CC-qPCR assay for the disinfection kinetics of *C. parvum* with PUV light. *G. lamblia* appears more UV resistant than *C. parvum* at doses up to 9.72x10^{-3} mJ/cm² with a 1.2 log_{10} and < 4 log_{10} inactivation obtained for *G. lamblia* and *C. parvum* (Garvey *et al.,* 2010) respectively. A similar trend where *C. parvum* showed increased sensitivity to MP UV than *G. lamblia* was reported by Belosevic *et al.* (2001).

Table 3.4. Log_{10} reduction and infectivity of treated *G. lamblia* via real-time PCR and cell culture infectivity IF staining of the HCT-8 cell line following UV treatment (+/- S.D.).

Exposure time (seconds)	LP-UV			PUV		
	Dose mJ/cm^2	RT-PCR Log_{10} reduction	Cell culture infectivity*	Dose $x10^{-3}$ mJ/ cm^2	RT-PCR Log_{10} reduction	Cell culture infectivity*
0	0	0	4	0	0	4
10	5.4	1.48(+/- 0.1)	4	1.08	0.52(+/- 0.1)	4
20	10.8	1.92(+/- 0.2)	4	2.15	0.52(+/- 0.5)	4
30	16.35	2.04(+/- 0.2)	3	3.24	0.6(+/- 0.2)	4
60	32.7	1.92(+/- 0.1)	3	6.48	0.62(+/- 0.1)	4
90	49	1.84(+/- 0.1)	3	9.72	1.2(+/- 0.1)	4
120	65.4	1.84(+/- 0.3)	4	12.96	1.48(+/- 0.5)	3
150	81.7	1.88(+/- 0.2)	4	16.2	2.02(+/- 0.4)	3
180	98.1	1.74(+/- 0.1)	4	19.44	2.4(+/- 0.1)	3
210	113.4	1.74(+/- 0.2)	4	22.68	2.82(+/- 0.1)	2

*Infectivity of cells as determined by immune-fluorescent (IF) staining of 3 separate HCT-8 monolayers exposed to UV treated and untreated *G. lamblia* cysts. Presence of infectivity indicates the presence of viable *Giardia*. Numbers indicate level of infectivity: 4 = high, 3 = medium, 2 = low, 1 = very low.

3.4 UV Inactivation of Planktonic Vegetative Cells

3.4.1 Pulsed UV

Substantial rates of inactivation were obtained following the PUV exposure of *P. aeruginosa, S. aureus* and *E. coli* whereby a clear pattern emerged showing an increase in cell death with an increase in UV dose ($\mu J/cm^2$). Findings also show a significant difference ($p<0.05$) in the sensitivities of each strain tested (Fig. 3.4) when treated in 20 ml suspensions up to a UV dose of 3.24 $\mu J/cm^2$. At this dose, *P. aeruginosa* proved more UV resistant at discharge energy of 16.2 J followed by *E. coli* with *S. aureus* proving most UV sensitive. However, exposure to 4.32 $\mu J/cm^2$ resulted in a 5.6 log_{10} inactivation of both *P. aeruginosa* and *E. coli* and a marginally increased 7.5 log_{10} inactivation of *S. aureus.* Results show with doses exceeding 6.48 $\mu J/cm^2$ the level of resistance shown at lower UV doses was obtained again, whereby the order of resistance (from highest to lowest) was as follows: *P. aeruginosa, E. coli* and *S. aureus* ($p<0.05$).

The findings of this study clearly show a significant difference in the rate of inactivation achieved for these test species when exposed to pulses of UV light on agar surfaces to that of microbial suspensions. Gram negative test species proved to be significantly ($p<0.05$) more sensitive to UV inactivation when treated on agar surfaces (Fig. 3.5) as opposed to treatment while in suspension. *E. coli* proved most sensitive to UV inactivation when treated on agar surfaces with a 6.6 log_{10} reduction achieved with a dose of 1.08 $\mu J/cm^2$ while a 5.4 and 5.5 log_{10} inactivation was obtained for *P. aeruginosa* and *S. aureus* respectively at this setting. Indeed, *E. coli* proved most UV sensitive when exposed on surfaces with *P. aeruginosa* and *S. aureus*, consistently showing similar rates of inactivation at all exposure regimes (Fig. 3.5). With a UV dose of 10.8 $\mu J/cm^2$ a 6.7 log_{10} inactivation of the gram negative species *E . coli* was obtained when treated in suspension compared to an 8.4 log_{10} inactivation on agar surfaces. This significant difference in inactivation rates was evident for all treatment doses, suggesting that *E. coli* is more susceptible to UV inactivation when treated on surfaces. This was also the case for the gram negative species *P. aeruginosa* where an increased level of inactivation was achieved when the bacteria was treated on agar surfaces. Interestingly, for the gram positive test species *S. aureus*, the inactivation achieved for the surface and suspension studies showed the opposite effect. *S. aureus* appears more UV sensitive when treated in suspension, whereby

a significant difference ($p<0.05$) in susceptibility was obtained at each dose (Fig. 3.4). Indeed, a PUV dose of 3.24 µJ/cm^2 provided a 6.4 and 7.1 $\log_{10}$ inactivation of surface spread and microbial suspensions respectively (Fig. 3.4). This pattern was evident for all applied doses for this test species. However, with 10.8 µJ/cm^2 maximal inactivation was obtained for both treatment methods with a ca. 8.1 and 8.3 $\log_{10}$ inactivation of *S. aureus* at 16.2 J per UV pulse for microbial suspension and surface treated samples respectively.

Findings from this study show that at the lower PUV doses there was a significant difference in the sensitivities of the different *Bacillus* strains tested. The pathogenic *B. cereus* proved most UV resistant ($P<0.05$) at UV doses ≤ 7.56 µJ/cm^2 after which it showed similar inactivation rates to that of *B. licheniformis* and *B. subtilis* (Table 3.5). However, the pathogenic strain *B. subtilis* showed similar rates of inactivation to the non-pathogenic species *B. thuringiensis* up to a UV dose of 12.96 µJ/cm^2. *B. licheniformis* and *B. pumilus* and

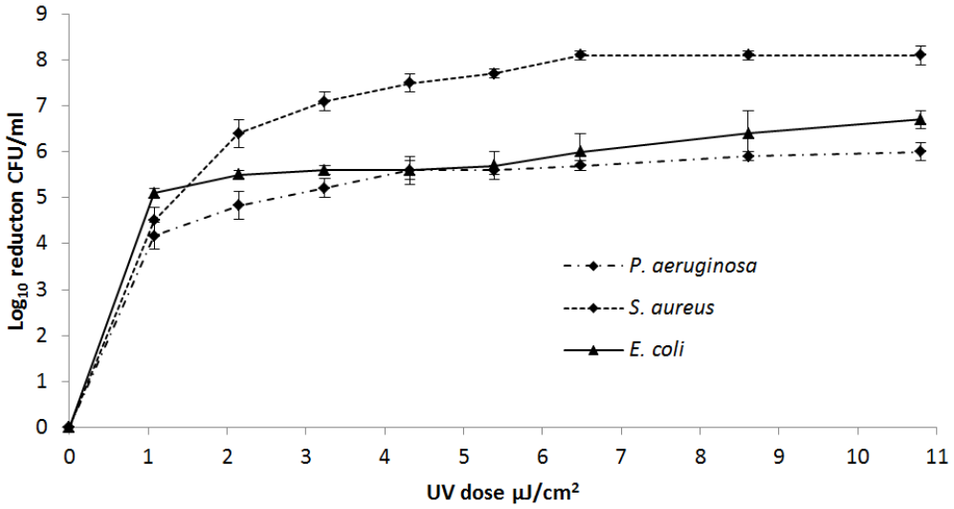

Figure 3.4. Pulsed UV inactivation given as the $\log_{10}$ reduction in viability of microbial test species suspended in 20 ml volumes at varying UV dose at a discharge energy of 16.2 J per pulse (+/-SD).

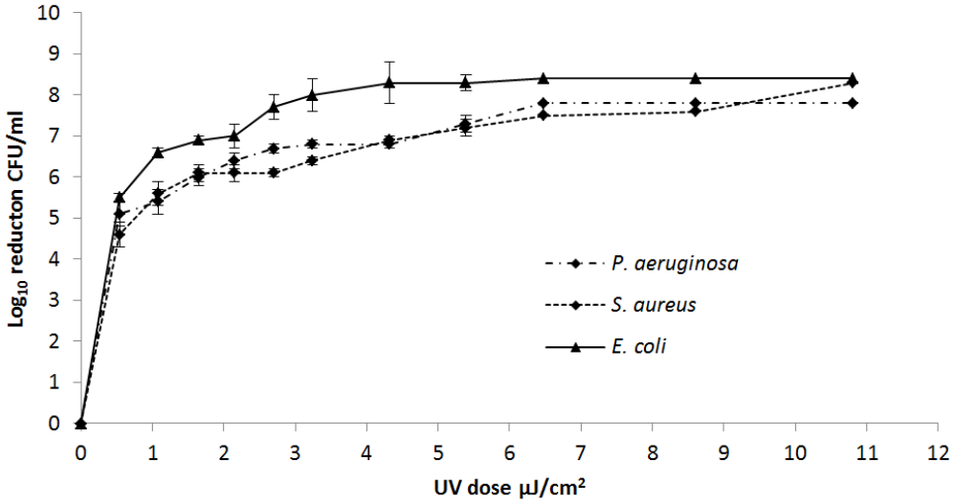

Figure 3.5. Pulsed UV inactivation given as the $\log_{10}$ reduction in viability of microbial test species spread on agar surfaces at varying UV dose at a discharge energy of 16.2J per pulse (+/-SD).

B. megaterium proved the most UV sensitive tested, with a 5 log_{10} loss in viability obtained for *B. megaterium* with a treatment of 2.1 µJ/cm^2 compared to a 4.4 log_{10} for *B. pumilus* and *B. licheniformis*, a 4.8 log_{10} for *B. thuringiensis* and *B. subtilis* and a 2.8 log_{10} for *B. cereus*. As with LP-UV these findings also suggest that *Bacillus* resistance to PUV light is not pathogenicity-related. At doses exceeding 17.28 µJ/cm^2 there was no significant difference between the various *Bacillus* strains tested to UV pulses, suggesting that with higher exposure times strain sensitivity within this species is eradicated. A ca. 7 log_{10} inactivation rate was obtained for all *Bacillus* test species with 21.6 µJ/cm^2 at 16.2J per pulse using the PUV delivery method. Pulsed UV also provided a significant level of inactivation of *E. coli* and *P. aeruginosa* test species with a 6 and 6.7 log_{10} inactivation obtained for surface-spread organisms and a 7.8 and 8.4 log_{10} for 20 ml suspensions respectively at 10.8 µJ/cm^2 .

There was a remarkable decrease in time needed to obtain significant inactivation rates following treatment with PUV light compared to LP-UV (Table 3.5). With 30 seconds of LP-UV (16.35 mJ/cm^2) a 2.6 log_{10} loss in viability was obtained for *B. megaterium* compared to 5.31 log_{10} with the PUV system for microbial suspension of 20 ml volume. This trend was evident for each strain tested, suggesting that with PUV a shorter treatment time is needed to achieve significantly greater inactivation rates than the LP system, which also highlights the energy efficiency of the pulsed approach over standard delivery methods.

The results obtained here demonstrate that the xenon flash lamp used in this study is an efficient UV source for the inactivation of the organisms both for agar surface inoculated and liquid suspensions at these set operational parameters. However, the findings have demonstrated that PUV treatment is more effective for the gram negative species studied on solid agar surfaces with a faster rate of cell death than in liquids. The results also reveal the variations in susceptibility of these test species to pulses of UV-light, whereby, gram-negative species *E. coli and P. aeruginosa* appear more UV resistant than the gram positive *S. aureus* when treated in liquid and more UV sensitive following surface exposure. Similar studies conducted by Rowan *et al.* (1999) on surface-treated organisms showed a similar order of resistance of these species to UV light.

Table 3.5. Showing log_{10} reduction of various *Bacillus* species (pathogenic and non-pathogenic) following exposure to pulsed UV light at a discharge energy of 16.2J (+/- S.D.).

Treatment time (sec)	UV dose µJ/cm^2	Log_{10} reduction (Non-pathogenic)				Log_{10} reduction (Pathogenic)	
		B. megaterium	*B. pumilus*	*B. licheniformis*	*B. thuringiensis*	*B. cereus*	*B. subtilis*
0	0	0	0	0	0	0	0
10	1.08	3.38 (+/-0.3)A	3.39 (+/-0.2) A	4.0 (+/-0.1) B	3.8 (+/-0.3) A	1.77 (+/-0.3) C	3.8 (+/-0.1) A
20	2.15	5.18 (+/-0.3) A	4.4 (+/-0.07) B	4.4 (+/-0.2) B	4.8 (+/-0.05) C	2.81 (+/-0.2) D	4.8 (+/-0.3) C
30	3.24	5.31 (+/-0.1) A	4.6 (+/-0.2) B	4.5 (+/-0.3) B	4.9 (+/-0.1) C	3.1 (+/-0.1) D	4.9 (+/-0.1) C
40	4.32	5.91 (+/-0.1) A	4.6 (+/-0.1) B	4.5 (+/-0.1) B	5.0 (+/-0.1) C	4.1 (+/-0.1) D	5.1 (+/-0.2) C
50	5.39	6.15 (+/-0.1) A	4.9 (+/-0.1) B	4.4 (+/-0.02) D	5.6 (+/-0.1) C	4.2 (+/-0.3) D	5.3 (+/-0.08) C
70	7.56	6.48 (+/-0.1) A	5.9 (+/-0.2) B	5.2 (+/-0.02) C	5.9 (+/-0.1) B	4.9 (+/-0.1) C	5.6 (+/-0.1) B
90	9.72	6.61 (+/-0.1) A	6.5 (+/-0.1) A	5.9 (+/-0.1) B	6.3 (+/-0.2) C	5.9 (+/-0.1) B	5.9 (+/-0.2) B
120	12.96	7.01 (+/-0.2) A	6.6 (+/-0.2) B	6.1 (+/-0.07) C	6.6 (+/-0.2) B	6.5 (+/-0.4) B	6.1 (+/-0.1) C
160	17.28	7.12 (+/-0.2) A	6.8 (+/-0.09)A	6.9 (+/-0.3) A	6.9 (+/-0.2) A	7.1 (+/-0.1) A	7.2 (+/-0.2) A
200	19.44	7.18 (+/-0.1) A	6.9 (+/-0.1) A	7.1 (+/-0.1) A	7.2 (+/-0.1) A	7.1 (+/-0.1) A	7.2 (+/-0.08) A
220	21.6	7.21 (+/-0.2) A	7.02 (+/-0.2) A	7.1 (+/-0.05) A	7.2 (+/-0.1) A	7.2 (+/-0.2) A	7.2 (+/-0.1) A

*A, B, C, D denotes significance difference ($p<0.05$) of each strain following exposure to PUV.

3.4.2 Low-pressure UV

Throughout this study a pattern emerged whereby with an increase in UV dose a clear increase in inactivation of each test species occurred for both surface spread and microbial suspensions (Figs 3.6 and 3.7). However, a significant difference was obtained for LP-UV studies for each strain when treated on agar surfaces as opposed to microbial suspensions (Figs 3.6 and 3.7). A LP-UV dose of 16.35 mJ/cm^2 equivalent to 30 s treatment time resulted in a 3.1, 5.4, 1.5 and ca. 2.6 $\log_{10}$ reductions of *E. coli, P. aeruginosa, B. cereus and B. subtilis, B. megaterium* and *B. thuringiensis* respectively with the pathogenic and non-pathogenic *Bacillus* strains showing similar levels of inactivation when treated on agar surfaces. The same dose resulted in a 1.6, 5, 1.5, 0.7 and 2.3 $\log_{10}$ inactivation of *E. coli, P. aeruginosa, B. cereus, B. subtilis, B. megaterium* and *B. thuringiensis* respectively when organisms were exposed in suspension. This pattern of increased resistance to LP-UV for microbial suspensions continued for all treatment doses for *P. aeruginosa* and *B. megaterium*. However, for *E. coli* and *B. subtilis* higher levels of inactivation were obtained at doses >32.7 mJ/cm^2 for microbial suspensions. Also, *B. thuringiensis* appears more resistant to UV when surface inoculated as opposed to in suspension (Figs 3.6 and 3.7) at doses exceeding 32.7 mJ/cm^2. This was also the case for *B. cereus* at doses exceeding 65.4 mJ/cm^2.

Notably, there was also a significant difference in strain sensitivity to LP-UV light. The order of decreasing resistance was: *B. thuringiensis, B. subtilis, B. cereus, E. coli* and *P. aeruginosa* at UV doses below 16.35 mJ/cm^2 for surface-spread organisms. *P. aeruginosa* consistently proved most UV sensitive with *B. thuringiensis* proving most UV resistant with continued exposure up to a UV dose of 163.5 mJ/cm^2 equivalent to 5 min treatment time. For microbial suspension inactivation *P. aeruginosa* again proved most UV sensitive up to a dose of 49 mJ/cm^2 after which a tailing affect occurred where an increase in UV dose did not lead to a significant increase in cell death. However, this tailing affect was eradicated with doses exceeding 147.1 mJ/cm^2. *B. thuringiensis* proved most resistant followed by *B. subtilis* for this study, indicating that UV sensitivity is not pathogenicity related for the *Bacillus* strains tested. The findings also indicate that for LP-UV inactivation the gram-negative species (*E. coli* and *P. aeruginosa*) appear more UV sensitive than the gram-positive strains tested (*Bacillus*) at lower doses when treated on agar surfaces at <16.35 mJ/cm^2. However, this pattern did not continue with sustained treatment above this dose for surface spread or microbial suspensions.

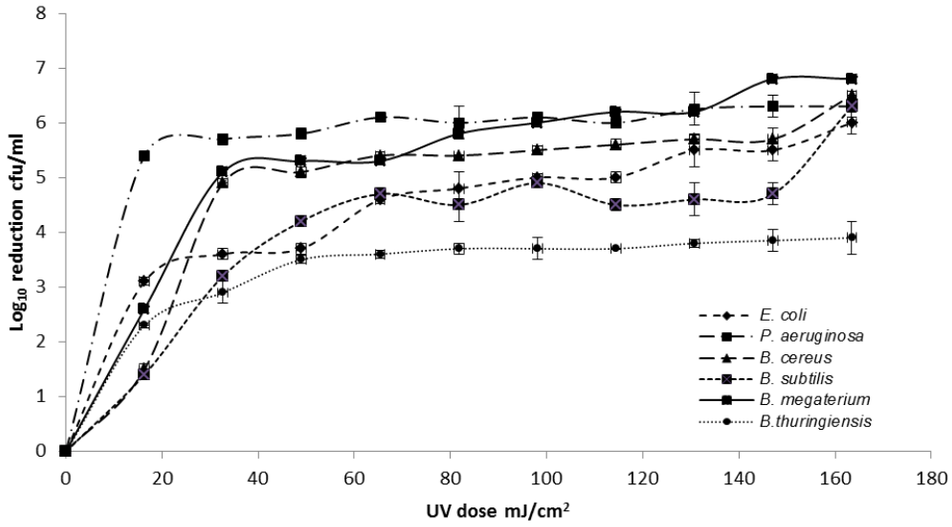

Figure 3.6. Low-pressure UV inactivation of various test species spread on agar surfaces at a wavelength of 254 nm.

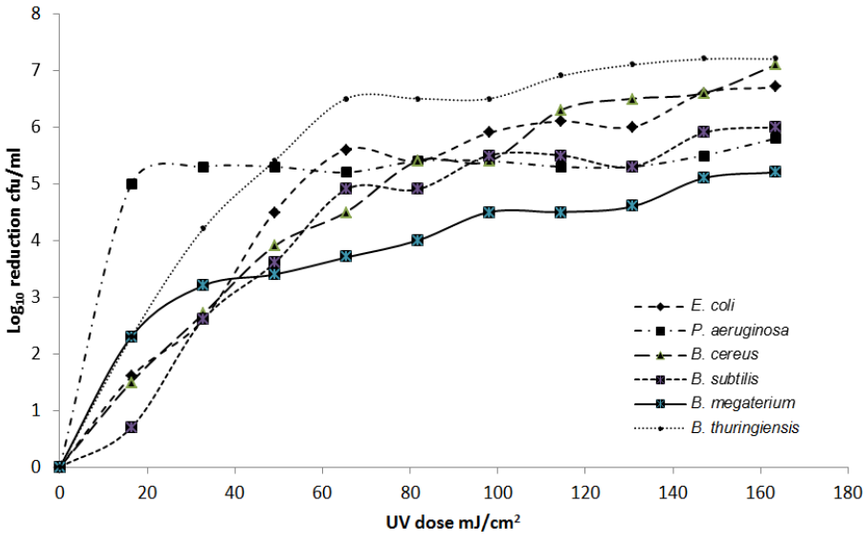

Figure 3.7. Low-pressure UV inactivation of various test species suspended in 20 ml volumes at a wavelength of 254 nm.

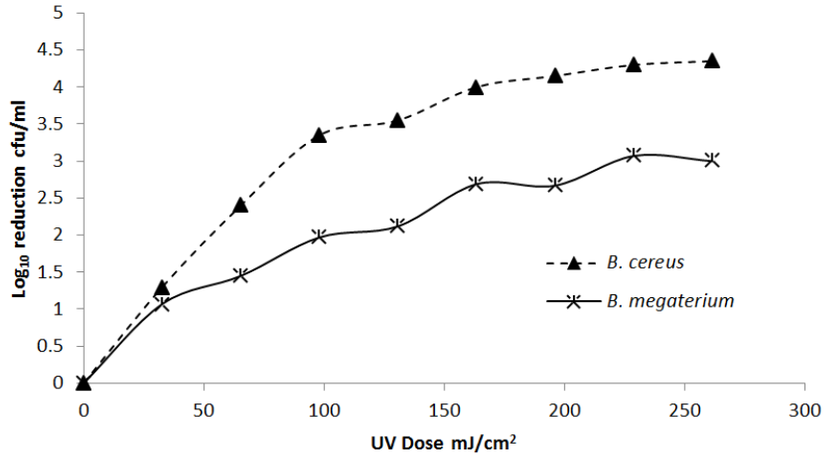

Figure 3.8. Low-pressure UV inactivation of *B. cereus* and *B. megaterium* suspended in 20 ml volume containing 10 ppm humic acid at a wavelength of 254 nm.

Studies conducted in the presence of 10 ppm HA show a significant difference ($p < 0.5$) in the inactivation rates of *B. cereus* and *B. megaterium* where an increased UV dose was required to achieve similar inactivation rates (Fig. 3.8). A treatment time of 60 s (32.7 mJ/cm^2) resulted in a 2.7 $\log_{10}$ inactivation of *B. cereus* in the absence of HA and a 1.3 $\log_{10}$ in the presence on HA, showing a clear increase in the survival of the test organism. This pattern was evident for each UV dose with as much as 8 min treatment time (261.6 mJ/cm^2) required to achieve a ca. 4.3 $\log_{10}$ inactivation of *B. cereus* in 10 ppm organic matter, with a treatment time of 2 min (65.4 mJ/cm^2) giving the same inactivation in the absence of HA. A similar trend was observed for *B. megaterium* with significantly higher UV doses required to achieve inactivation with LP UV. Also complete 7 $\log_{10}$ inactivation of *B. cereus* and *B. megaterium* occurred with 163.5 mJ/cm^2 without the presence of HA and a maximal 4 and 2.7 $\log_{10}$ inactivation in the presence of HA for *B. cereus* and *B. megaterium* respectively.

3.5 Pulsed UV Inactivation of *Bacillus* Endospores

Studies show that *Bacillus* endospores in suspension proved more UV resistant to that of *Bacillus* vegetative cells. Treatment with a UV dose of 2.1 µJ/cm^2 resulted in a ca. 1.5 $\log_{10}$ reduction in the viability of *B. megaterium* and *B. licheniformis* and a 0.84 $\log_{10}$ inactivation of *B. thuringiensis* (Table 3.6). Interestingly *B. cereus* and *B. pumilus* proved more UV sensitive at this lower dose with a ca. 2 $\log_{10}$ loss in viability obtained. Endospores of *B. thuringiensis* and *B. subtilis* consistently proved the most UV resistant for all treatment parameters, with *B. subtilis* showing levels of resistance greater than *B. thuringiensis* at doses exceeding 2.1 µJ/cm^2 ($p<0.05$). *B. pumilus* appears the most UV sensitive of the strains tested with significantly higher levels of inactivation for each treatment dose above 3.24 µJ/cm^2. As with vegetative cells the strain sensitivity of the *Bacillus* test species was affected with an increase in UV dose, whereas at a dose of 21.6 µJ/cm^2 there was no significant difference in the sensitivity of *B. megaterium, B. pumilus, B. licheniformis* and *B. cereus* to pulses of UV light. As with the findings of Sharifi-Yazdi and Darghahi (2005) the spores of *B. megaterium* proved more UV resistant than that of *B. cereus* at lower doses $\geq$ 4.32 µJ/cm^2. As expected, there was a significant difference in inactivation rates of *Bacillus* endospores and their vegetative counterpart.

The data displayed in Fig. 3.9 shows the inactivation of *B. cereus* and *B. megaterium* endospores suspended in 100 ml volumes both in the absence and presence of 10 ppm HA. At this volume depth (8.8 cm) findings show that inactivation of *Bacillus* endospores requires more UV exposure to achieve similar levels of inactivation to that of the 20 ml suspensions. Specifically, 4.32 µJ/cm^2 (treatment time 40 s) of PUV resulted in a ca. 3.44 $\log_{10}$ inactivation of *B. megaterium* and *B. cereus* respectively in 20 ml suspensions and a 0.3 and 0.4 $\log_{10}$ inactivation of *B. megaterium* and *B. cereus* in 100 ml suspensions. This trend continued over the treatment range applied (Table 3.6), suggesting that with larger volume depth a greater UV dose is required. Also in the presence of organic matter at doses $\leq$ 4.32 µJ/cm^2 there was significantly less inactivation for both strains. However, at doses exceeding this there was not a significant difference in the inactivation of endospores in the presence of HA, indicating that with sufficient treatment the presence of 10 ppm organic matter did not interfere with endospore inactivation following PUV treatment. In comparison, for LP-UV the presence of organic matter consistently reduced the inactivation rate for these species at each applied dose for vegetative cells in 20 ml suspensions (Fig. 3.8).

Table 3.6. Showing $\log_{10}$ reduction of various *Bacillus* endospores both pathogenic and non-pathogenic following exposure to pulsed UV light at a discharge energy of 16.2 J (+/- S.D.).

Treatment time (sec)	UV dose µJ/cm^2	$\log_{10}$ reduction (Non pathogenic)				$\log_{10}$ reduction (pathogenic)	
		B. megaterium	*B. pumilus*	*B. licheniformis*	*B. thuringiensis*	*B. cereus*	*B. subtilis*
0	0	0	0	0	0	0	0
10	1.08	0.54 (+/-0.1) A	1.02 (+/-0.2) B	0.98 (+/-0.2) B	0.42 (+/-0.3) A	0.4 (+/-0.1) A	0.46 (+/-0.1) A
20	2.159	1.49 (+/-0.5) A	1.90 (+/-0.1) B	1.57 (+/-0.2) A	0.84 (+/-0.2) C	2.1 (+/-0.2) D	1.34 (+/-0.3) A
30	3.24	2.09 (+/-0.4) A	2.56 (+/-0.4) B	2.3 (+/-0.2) B	1.3 (+/-0.1) C	3.5 (+/-0.09) D	1.6 (+/-0.1) E
40	4.32	3.44 (+/-0.7) A	3.95 (+/-0.1) B	3.3 (+/-0.2) A	2.2 (+/-0.2) C	3.4 (+/-0.1) A	2.1 (+/-0.1) C
50	5.39	4.16 (+/-0.3) A	4.55 (+/-0.06) B	3.7 (+/-0.1) C	3.45 (+/-0.3) C	3.9 (+/-0.3) A	2.4 (+/-0.1) D
70	7.56	4.55 (+/-0.1) A	5.2 (+/-0.2) B	4.7 (+/-0.05) A	3.81 (+/-0.1) C	4.3 (+/-0.1) A	3.0 (+/-0.1) D
90	9.72	4.6 (+/-0.4) A	5.91 (+/-0.1) B	5.1 (+/-0.1) C	4.26 (+/-0.2) A	4.8 (+/-0.1) A	3.4 (+/-0.2) D
120	12.96	5.65 (+/-0.6) A	7.40 (+/-0.1) B	5.3 (+/-0.1) C	5.1 (+/-0.3) C	5.41 (+/-0.1) A	3.63 (+/-0.1) D
160	17.28	6.31 (+/-0.2) A	7.31 (+/-0.3) B	6.34 (+/-0.3) A	5.65 (+/-0.2) C	6.75 (+/-0.3) D	4.35 (+/-0.3) E
220	21.6	7.49 (+/-0.5) A	7.51 (+/-0.1) A	7.6 (+/-0.1) A	6.7 (+/-0.1) B	7.43 (+/-0.2) A	5.4 (+/-0.1) C

*A, B, C, D, E denotes significance difference ($p<0.05$) of each strain following exposure to PUV.

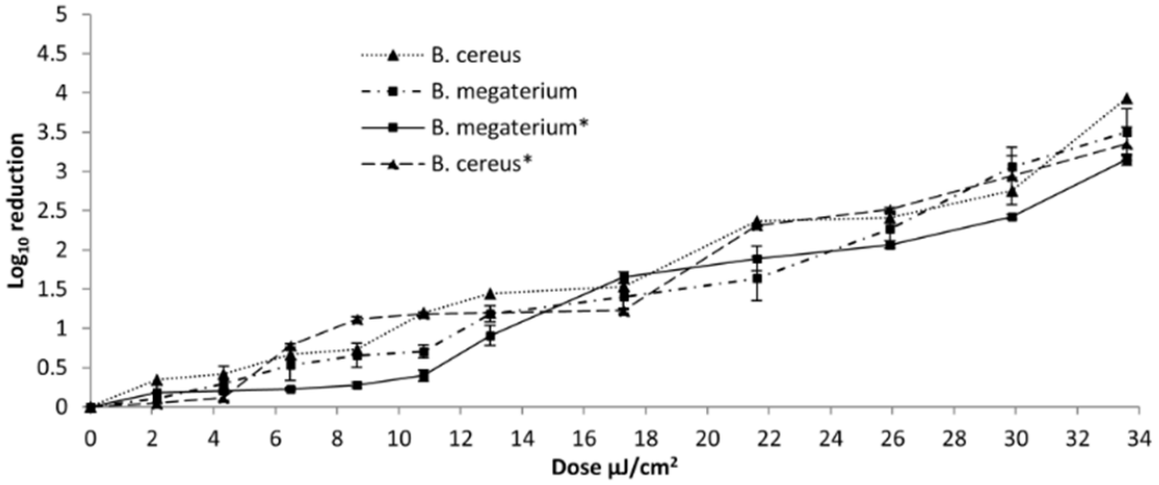

Figure 3.9. Log_{10} reduction of *Bacillus* endospores suspended in 100 ml volumes following exposure to PUV light at 16.2J per pulse. *Samples suspended in 10ppm humic acid (organic matter).

For the treatment of wastewater, it is recommended that a UV dose 40 mJ/cm^2 is used when using an LP lamp (Guo *et al.,* 2009). Based on the findings of this study, 40 mJ/cm^2 is equivalent to a treatment LP-UV time of ca. 70 s, resulting in an inactivation rate of ca 3 log_{10} for *E. coli, B. cereus, B. subtilis, B. megaterium, B. thuringiensis* and ca. 5 log_{10} for *P. aeruginosa* in suspension. In comparison, exposure to 70 s of PUV exposure gave a 6.48 log_{10} inactivation *of B. megaterium, E. coli, P. aeruginosa* and a ca. 5 log_{10} reduction in all other test species was achieved. Furthermore, with PUV a 70-second treatment time (7.65 µJ/cm^2) resulted in similar inactivation rates of *Bacillus* endospores to that of the LP-UV inactivation of their vegetative counterpart (Fig. 3.7 and Table 3.6) with between 3 and 5 log_{10} loss in viability obtained. Such findings suggest that the PUV approach which operates on a lower energy demand (UV dose expressed as µJ/cm^2 as opposed to mJ/cm^2 for LP-UV systems) provides a more rapid and energy-efficient rate of microbial inactivation than the standard LP systems.

3.6 Pulsed UV Inactivation of Microbial Biofilms

The formation of biofilms in the water distribution system raises concerns about public health, as these biofilms can harbour pathogenic bacteria and other problematic species such as viruses and parasites. Therefore, the inactivation of these resilient microbial communities in water-treatment systems is important in order to ensure a safe supply of pathogen-free water for public use. Studies by Paquin *et al.* (1992) showed that the type of piping material and residual chlorine concentrations of up to 0.05 ppm did not affect the formation of biofilms in water-distribution systems. Studies by Emtiazi *et al.* (2004) found the formation of *P. aeruginosa* biofilms in distribution systems and in their downstream networks concluded that this source of infection (by these opportunistic pathogens or other species which may be entrapped within the biofilm structure) is of concern in the water-treatment setting. Irish EPA guidelines for supplies using chlorine as disinfectant require 0.1 mg/l free residual chlorine at the end of the network to provide adequate secondary disinfection. Table 3.7 illustrates the biofilm formation for both strains tested at 20 and 37°C on PVC coupons which are representative of these distribution networks. For *P. aeruginosa* a seeding density of ca. 1x10^6 CFU/ml resulted in 1x10^7 CFU/ml biofilm following 24-hour incubation at 20°C. The cell density increased as the incubation period was extended, resulting in CFU/ml of 1x10^8 following a 48- and 72-hour incubation. Notably, there was a change in the viability of the planktonic cells which did not form the biofilm community with a 1 log_{10} loss in viability following 72-hour incubation at 20°C in the reactor broth. This may have occurred due to nutrient depletion of the reactor media. Up to this point the bacterial count of cells present in the biofilm

Table 3.7. Densities of microbial test species at seeding at time 0 hours (T0) and following biofilm production at different time points in a Centers for Disease Control (CDC) biofilm reactor at 20 and 37$_o$C (+/-S.D).

Reactor temperature	20°C			37°C	
Microbial strain		***P. aeruginosa***		***P. aeruginosa***	***S. aureus***
Biofilm age (hr)	**24**	**48**	**72**	**48**	
Initial reactor cell density (T0)	3 x10^6 CFU/ml (+/-0.1)	3 x10^6 CFU/ml (+/-0.1)	9 x10^6 CFU/ml (+/-0.1)	4 x10^6 CFU/ml (+/-0.3)	2 x10^6 CFU/ml (+/-0.1)
Biofilm per coupon cell density	1 x10^7 CFU/ml (+/-0.2)	1 x10^8 CFU/ml (+/-0.1)	1 x10^8 CFU/ml (+/-0.2)	1 x10^8 CFU/ml (+/-0.1)	7 x10^6 CFU/ml (+/-0.2)
Final reactor cell density	1 x10^7 CFU/ml (+/-0.1)	1 x10^8 CFU/ml (+/-0.1)	1 x10^7 CFU/ml (+/-0.1)	6 x10^8 CFU/ml (+/-0.05)	1 x10^8 CFU/ml (+/-0.1)

appeared equal to the planktonic cells present in the reactor (Table 3.7). It is worth noting that biofilms of similar cell density were formed for both 20 and 37°C incubation temperatures for *Pseudomonas*. Although the gram-positive *S. aureus* formed a relatively densely populated biofilm structure it did not show a population density comparable to that of *P. aeruginosa*. Following 48-hour incubation in suitable media a ca. 7x10^6 CFU/ml biofilm was formed as opposed to a 3x10^8 CFU/ml structure of *Pseudomonas*. With an initial seeding density of 2x10^6 CFU/ml there was a 1x10^8 CFU/ml planktonic cell density in the reactor at this 48-hour time point for *S. aureus*.

Staphylococcus biofilms are known to be problematic in the medical setting, especially in cystic fibrosis patients where they form in lung tissue, and central venous catheters (Gotz, 2002). Although by no means extensive, results obtained for these initial studies suggest that for this *Staphylococcus* species at these settings the formation of biofilms was not as typical on this type of material (i.e. PVC) as that of *Pseudomonas.*

For PUV inactivation studies the cupons were exposed to varying UV doses on both sides to ensure complete biofilm exposure. Replicate results indicate that this PUV system was effective at inactivating both test species at 16.2 J per pulse. Indeed, substantial amounts of inactivation were achieved with as a little as 2.15 µJ/cm^2 for biofilms of varying ages (Table 3.8). For this study it was found that the relatively younger biofilm (24 h) appeared more resistant to UV treatment than the other time frames studied. Table 3.8 illustrates the inactivation rates for each biofilm and a clear pattern is evident where biofilms required less exposure with greater inactivation for each 24 h increment. Specifically, a 1.1, 2.8 and 3.3 log$_{10}$ inactivation was obtained for 24, 48 and 72 h biofilms of *P. aeruginosa* respectively with 6.48 µJ/cm^2 of PUV light at 20°C and a 2.3 and 2.6 log$_{10}$ inactivation of *P. aeruginosa* and *S. aureus* at 37°C. This pattern remained consistent for each treatment dose where a significant difference in inactivation rates was obtained for *Pseudomonas* with increasing age where the biofilm cell density increased or remained the same ca. 1x10^7 to 1x10^8 CFU/ml. These findings indicate that the initial biofilm structure is more resistant to PUV exposure and this resistance gradually decreases with age. Indeed maximal biofilm inactivation (7.2 µJ/cm^2) occurred with a dose of 19.44 µJ/cm^2 for the 72-hour biofilm where a 3.8 and 4.7 log$_{10}$ reduction was achieved for 24 and 48 h respectively. Findings also demonstrate that the temperature of the biofilm formation did not affect the inactivation kinetics of the *Pseudomonas* test species where similar levels of inactivation were achieved for each UV dose applied (Table 3.8). Initially, levels of inactivation for 48-hour biofilms at 37°C of *P. aeruginosa* and *S. aureus* were obtained where *Pseudomonas* proved slightly more resistant up to a UV dose of ≤ 6.48 µJ/cm^2. However, at doses exceeding this and less than 15.12 µJ/cm^2 (≥ 6.48 and ≤15.12 µJ/cm^2) there was no significant difference ($p<0.05$) in sensitivities of these test species.

Table 3.8. Log_{10} reduction of *P. aeruginosa* and *S. aureus* biofilms of varying ages at 20 and 37°C following exposure to pulsed UV light at different doses at a discharge energy of 16.2 J per pulse (+/-SD).

Uv dose ($\mu J/cm^2$)	Temperature 20°C			Temperature 37°C	
	P. aeruginosa			*P. aeruginosa*	*S. aureus*
	24 h	48 h	72 h	48 h	48 h
0	0	0	0	0	0
2.15	0.56 (+/-0.4)	1.3 (+/-0.3)	1.7 (+/-0.2)	1.0 (+/-0.07)	1.4 (+/-0.2)
4.32	0.87 (+/-0.2)	1.9 (+/-0.1)	1.96 (+/-0.3)	2.1 (+/-0.02)	2.6 (+/-0.1)
6.48	1.1 (+/-0.3)	2.8 (+/-0.2)	3.33 (+/-0.1)	2.3 (+/-0.07)	2.6 (+/-0.1)
8.61	2.7 (+/-0.5)	3.5 (+/-0.5)	4.06 (+/-0.06)	2.6 (+/-0.1)	2.8 (+/-0.06)
10.8	3.3 (+/-0.6)	3.7 (+/-0.2)	4.8 (+/-0.1)	3.7 (+/-0.1)	3.6 (+/-0.01)
12.96	3.4 (+/-0.4)	3.8 (+/-0.3)	5.86 (+/-0.2)	3.9 (+/-0.1)	3.8 (+/-0.1)
15.12	3.6 (+/-0.4)	4.1 (+/-0.4)	5.86 (+/-0.2)	4.0 (+/-0.2)	4.0 (+/-0.3)
17.28	3.5 (+/-0.4)	4.1 (+/-0.3)	6.63 (+/-0.06)	4.2 (+/-0.2)	5.4 (+/-0.2)
19.44	3.8 (+/-0.3)	4.7 (+/-0.2)	7.2 (+/-0.1)	5.25 (+/-0.2)	5.9 (+/-0.02)
21.6	3.8 (+/-0.1)	5.4 (+/-0.2)	-	5.8 (+/-0.2)	5.9 (+/-0.2)
25.92	4.2 (+/-0.2)	6.2 (+/-0.1)	-	6.4 (+/-0.06)	-

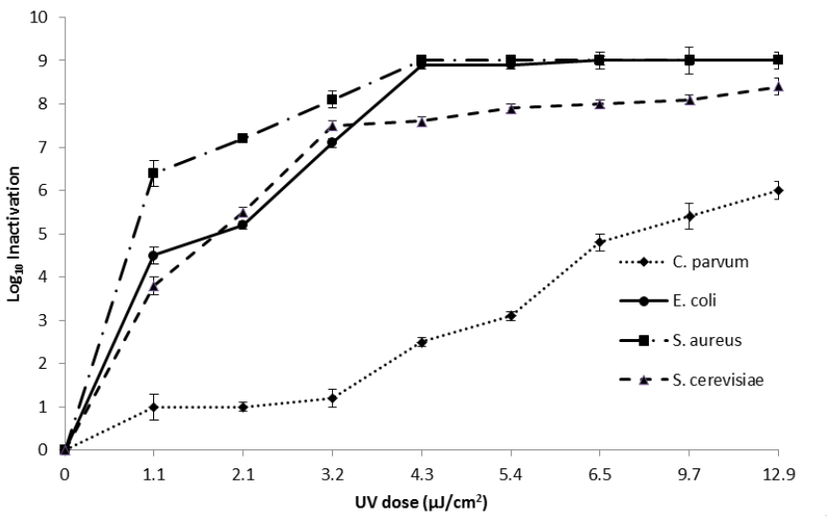

Figure 3.10. Pulsed UV inactivation of test species at a discharge energy of 16.2 J at 8 cm from the light source (+/- S.D.).

3.7 Ecotoxicological Assessment

A range of ecotoxicological tests was conducted to verify that PUV-treated samples were safe to release to the environment.

3.7.1 Pulsed UV Inactivation of Test Species

Water samples containing test organisms irradiated at the uppermost UV dose of 12.96 μJ/cm^2 were used for subsequent ecotoxicological analysis (Fig. 3.10).

3.7.2 Ecotoxicological Analysis

The EC_{50} for *Daphina magna,* and *Vibrio fischeri* respectively, LC_{50} for *Thamnocephalus platyurus, Artemia franciscana* and IC_{50} for *Tetrahymena thermophila and Pseudokirchneriella subcapitata* were determined for each test as recommended by the microbiotest kit manufacturer. Data for the ecotoxicological analysis of PUV-treated test samples are presented as per Slabbert and Venter (1999). A 10% effect in the *Daphnia* test and 5% in the protozoan test indicate toxic activity. Effective concentrations of samples (e.g. LC_{50} and EC_{50}) are statistically derived (linear regression) using dose-response curves (% effect versus log concentration). When toxicity is too low to use linear regression, endpoints are presented as LC_{50}: >100% or LC_{50}: 50–100%. For all test kits the positive control was found to be within the toxic concentration range provided by the kit manufacturers, indicating that assay conditions were correct for each assay (Table 3.9).

Table 3.9. Reference chemical ecotoxicity data for test species and endpoints included in the test battery.

Test species and test kit	Reference chemical	Exposure period and endpoint	EC/LC_{50} (mg/L)	Reference
Vibrio fischeri	C_6H_6O	5 mins inhibition	5 mins: 17.45 ± 0.140	13–26 mg/L
Microtox™			15 mins:18.25 ± 0.106	(Azur Environmental, 1998)
Pseudokirchneriella subcapitata	$K_2Cr_2O_7$	72 h inhibition	0.32 ± 1.016	0.1–1 mg/L (Algaltoxkit F., 1996)
Algaltoxkit™				
Thamnocephalus platyurus	$K_2Cr_2O_7$	24 h lethality	0.115 ± 0.015	0.1–0.2 mg/L, 0.11 mg/L
Thamnotoxkit F™				(Centeno *et al*., 1995; Thamnotoxkit F., 1995)
Daphnia magna	$K_2Cr_2O_7$	24 h immobilisation	24 h: 0.997 ± 0.048	0.6–1.7 mg/L (24h)
Daphtoxkit F™			48 h: 1.244 ± 0.209	(BS EN ISO 6341, 1996)
Thetrahymena thermophila	$K_2Cr_2O_7$	24 h inhibition	20.693 ± 2.502	15–24 mg/L
Protoxkit F™				(Protoxkit F., 1998)
Artemia franciscana	$K_2Cr_2O_7$	24 h lethality	27.63 ± 1.264	Artoxkit F™
RTH-149 fish cell	$K_2Cr_2O_7$	24 h lethality	MTT assay: 17.5 μM	NA
			NR assay: 20 μM	

Table 3.10. Comparison of the EC_{50} / LC_{50} / IC_{50} values of PUV treated water for a battery of ecotoxicity tests.

Ecotoxicity Test	PUV-treated sample EC_{50} / LC_{50} / IC_{50} (% v/v)	PUV-treated sample with 10ppm HA EC_{50} / LC_{50} / IC_{50} (% v/v)
Thamnotoxkit FTM	>100%	>100%
AlgaltoxkitTM	>100%	>100%
Daphtoxkit FTM	>100%	>100%
Protoxkit FTM	>100%	>100%
MicrotoxTM	5 min: >100%	5 min: >100%
	15 min: >100%	15 min: >100%
Artoxkit MTM	>100%	>100%

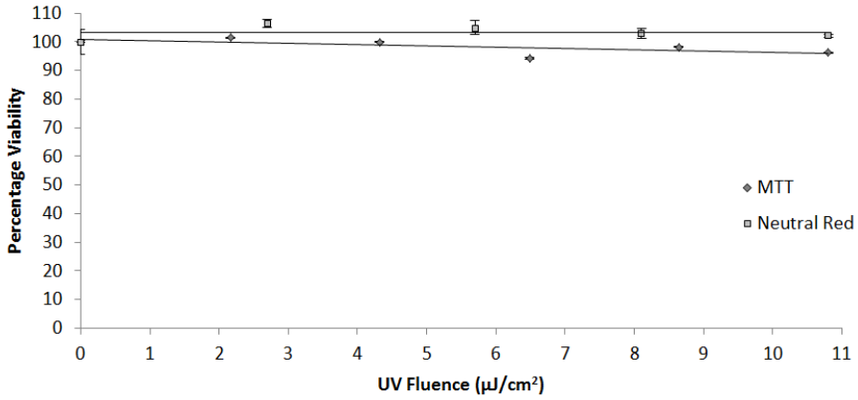

Figure 3.11. Cytotoxicity (MTT and N R assays respectively) of PUV-treated dH_2O on RTH-149 fish cell line at discharge energy of 16.2 J per pulse (+/- S.D.).

Extensive ecotoxicology testing of PUV-treated water samples with and without *C. parvum* and *E. coli* clearly showed that PUV applied at a UV dose of 12.96 µJ/cm² does not elicit any measurable toxicity at all trophic levels studied, namely *Vibrio fischeri* (bacteria), *Tetrahymena thermophila* (protozoa), *Pseudokirchneriella subcapitata* (planktonic green algae), *Artemia franciscana* (Brine shrimp), *Thamnocephalus platyurus* (Fairy shrimp), *Daphnia magna* (water flea). In addition, the presence of organic matter (10 ppm HA) in these PUV-treated samples did not affect the level of toxicity following treatments (Table 3.10).

3.7.3 Cytotoxicological Analysis

Figure 3.11 illustrates that PUV-treated water containing *C. parvum* and *E. coli* did not exert any cytotoxic effects on the cells. With the MTT and NR assay an LC_{50} of 17.5 µM and 20 µM was found for $K_2Cr_2O_7$ respectively, indicating good correlation between both assays. Results also show that both assays were operating at optimal conditions.

3.8 Flow-through PUV Inactivation Studies

Studies were conducted on organisms suspended in liquid which were pumped under a PUV light system to determine the inactivation rates of organism at set flow rates and retention times representative of water-treatment plants.

3.8.1 Flow-through Inactivation of Test Species

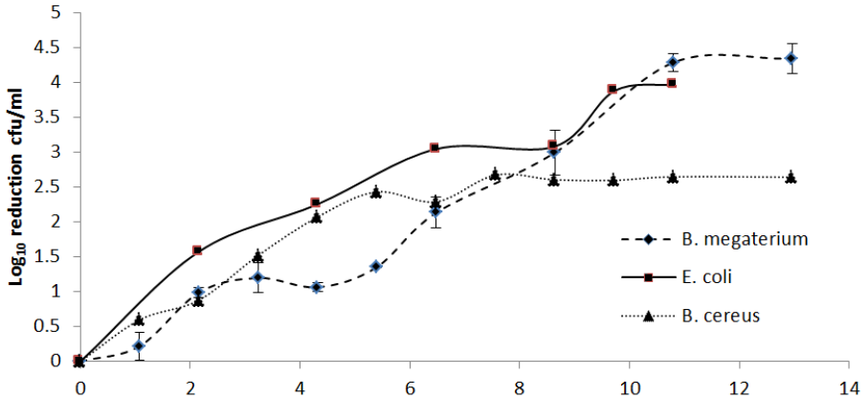

Figure 3.12. Pulsed UV inactivation of planktonic microbial test species suspended in sterile liquid at 200 rpm (flow rate of 24 L/hour and retention time of 120 s).

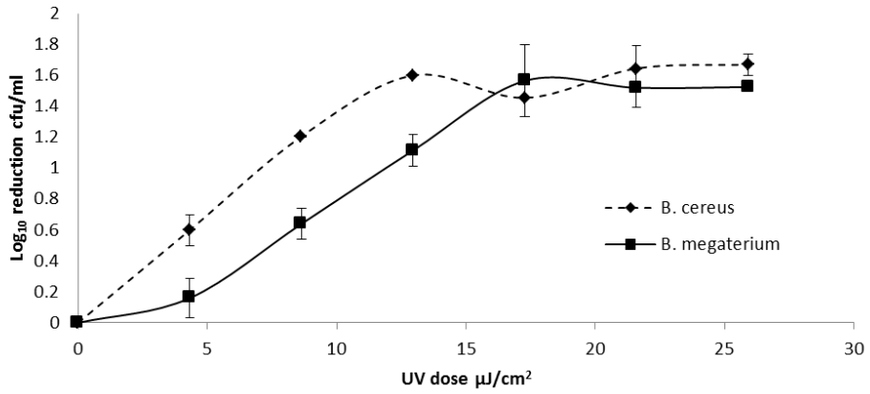

Figure 3.13. Pulsed UV inactivation of microbial endospores suspended in sterile liquid at 200 rpm (flow rate of 24 L/hour and retention time of 120 s).

3.8.2 Flow-through inactivation of test species suspended in inorganic matter

Pulsed UV successfully inactivated the test species both in vegetative and spore form at both flow rates (Figs 3.12 to 3.15). As expected, there was a significant ($p \leq 0.05$) difference in the inactivation rates for flow-through and stationary treatment. With a retention time of 120 s (200 rpm) greater levels of inactivation were achieved as organisms were exposed to UV pulsed for a greater period of time. With 90 s exposure (9.72 µJ/cm^2) a 2.6, 3.1 and 3.88 $\log_{10}$ inactivation rate was achieved for vegetative *B. cereus, B. megaterium* and *E. coli* respectively. With up to 120-second treatment time (12.96 µJ/cm^2) a 2.65, 4.34 and ca. 4 $\log_{10}$ rate of inactivation was obtained for these same strains. For the inactivation of *Bacillus* endospores a 0.16 and 0.6 $\log_{10}$ inactivation was obtained for *B. megaterium* and *B. cereus* with as little as 4.32 µJ/cm^2 (40 s) and a 1.1 and 1.6 $\log_{10}$ inactivation with up to 12.96 µJ/cm^2 or a treatment time of 120 s. At a retention time of 120 s (flow rate 375 rpm) a 2.3, 1.45 and 4.4 $\log_{10}$ inactivation of *B. megaterium, B. cereus* and *E. coli* respectively with a UV dose of 6.48 µJ/cm^2. For a UV dose of 9.72 µJ/cm^2 equivalent to a 90-second treatment time a 2.19, 1.28 and 3.5 $\log_{10}$ inactivation of *B. megaterium, B. cereus* and *E. coli* was achieved, significantly less for the slower flow rate and longer retention time of 120 s. At retention time of 60 s there was also a significant decrease in the inactivation of *Bacillus* endospores. With a UV dose of 12.96 a 1.44 and 0.79 $\log_{10}$ inactivation of *B. cereus* and *B. megaterium* respectively was achieved at a flow rate of 375 rpm, significantly lower than with a

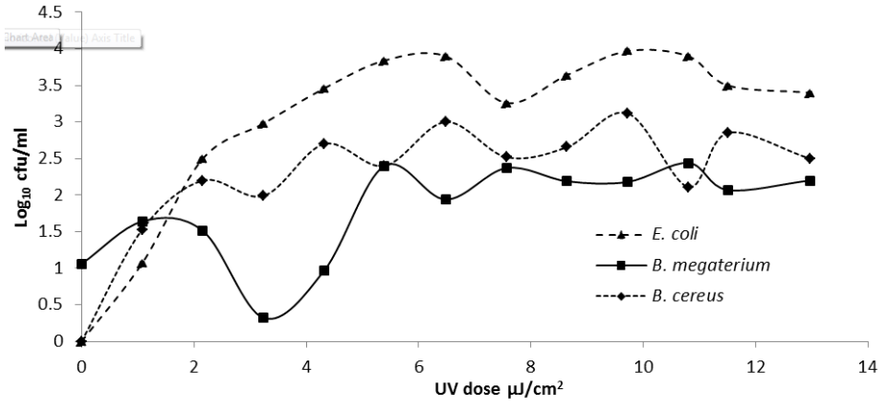

Figure 3.14. Pulsed UV inactivation of planktonic microbial test species suspended in sterile liquid at 200 rpm (flow rate of 30L/hour and retention time of 60 seconds).

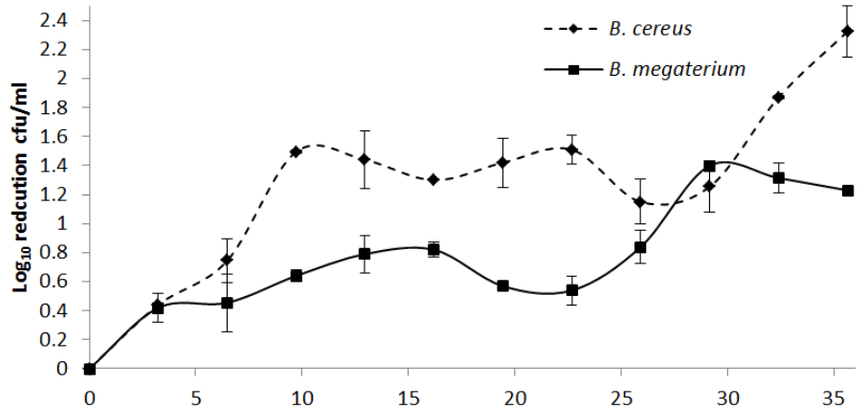

Figure 3.15. Pulsed UV inactivation of microbial endospores suspended in sterile liquid at 375 rpm.

flow rate of 200 rpm. The same reduction in inactivation was observed with an increase in flow rate for each applied UV dose. Furthermore, there was a significant difference in the inactivation rates of vegetative and endospore cells at all treatment regimens as expected (Figs 3.14 and 3.15).

The presence of inorganic contaminants also affected the inactivation rates of all test species (Tables 3.11 to 3.14) for both flow rates/retention times. At a flow rate of 200 rpm (retention time of 120 s) for vegetative *B. megaterium* cells there was an increase in inactivation up to a UV dose of 6.48 in the presence of manganese (II) sulfate (10 mg/L) and iron (0.3 mg/L). Also in the presence of both contaminants together there was an increase in inactivation at all UV doses. For *B. megaterium* endospores at this flow rate the presence of manganese salt does not appear to influence the inactivation rates; however, the presence of iron resulted in a decrease in microbial inactivation as did the presence of both contaminants together (Tables 3.11 and 3.12). At a flow rate of 30 L/hr for vegetative *B. megaterium* cells, manganese and iron appear to cause an increase in inactivation rates where a combination of the two did not seem to affect the inactivation of the test species. The presence of the manganese salt did not seem to greatly affect the inactivation of *B. megaterium* endospores at this flow rate; however, iron increased the observed effect. The presence of both inorganic contaminants together did not affect the inactivation of *B. megaterium* endospores at this flow rate.

Table 3.11. Flow-through pulsed UV inactivation of *B. megaterium* vegetative cells at 200 rpm in the presence of inorganic contaminants.

UV dose µJ/cm²	Log_{10} inactivation of *B. megaterium* No contaminant	Log_{10} reduction of *B. megaterium*		
		Inorganic contaminant		
		Manganese*	Iron^	Manganese*and Iron^
0	0	0	0	0
1.08	0.2 (+/-0.1)	0.8 (+/-0.3)	1 (+/-0.03)	2.39 (+/-0.1)
2.15	0.99 (+/-0.07)	1.1 (+/-0.01)	2.01 (+/-0.3)	2.9 (+/-0.1)
3.24	1.2 (+/-0.2)	1.2 (+/-0.07)	1.91 (+/-0.1)	3.3 (+/-0.4)
4.32	1.06 (+/-0.06)	2.2 (+/-0..2)	2.1 (+/-0.2)	3.13 (+/-0.1)
5.39	1.35 (+/-0.01)	1.75 (+/-0.4)	2.11 (+/-0.3)	3.11 (+/-0.1)
6.48	2.13 (+/-0.2)	1.6 (+/-0.3)	1.77 (+/-0.3)	3.39 (+/-0.05)
8.64	2.99 (+/-0.3)	1.8 (+/-0.1)	2.04 (+/-0.1)	3.9 (+/-0.2)
10.8	4.29 (+/-0.1)	1.5 (+/-0.2)	2.11 (+/-0.2)	3.87 (+/-0.07)
12.96	4.34 (+/-0.2)	2.2 (+/-0.2)	2.62 (+/-0.1)	3.35 (+/-0.03)
15.12	4.34 (+/-0.3)	2 (+/-0.1)	1.88 (+/-0.1)	4.23 (+/-0.04)

*Concentration of manganese (II) sulfate 10 mg/L.

^Concentration of iron was 0.3 mg/L.

Table 3.12. Flow-through pulsed UV inactivation *of B. megaterium* endospores at 200 rpm in the presence of inorganic contaminants.

UV dose µJ/cm²	Log_{10} inactivation of *B. megaterium* No contaminant	Log_{10} reduction of *B. megaterium*		
		Inorganic contaminant		
		Manganese*	Iron^	Manganese*and Iron^
0	0	0	0	0
3.24	0.05 (+/-0.1)	0.02 (+/-0.02)	0.29 (+/-0.02)	0.05 (+/-0.09)
6.48	0.56 (+/-0.2)	0.57 (+/-0.09)	0.42 (+/-0.2)	0.14 (+/-0.1)
9.72	0.74 (+/-0.05)	0.97 (+/-0.01)	0.93 (+/-0.1)	0.46 (+/-0.1)
12.96	0.85 (+/-0.08)	0.81 (+/-0.01)	1.25 (+/-0.1)	0.57 (+/-0.1)
16.2	0.87 (+/-0.2)	1.09 (+/-0.08)	1.19 (+/-0.07)	0.71 (+/-0.1)
19.44	1.62 (+/-0.6)	0.94 (+/-0.1)	1.19 (+/-0.07)	0.79 (+/-0.08)
22.68	1.53 (+/-0.3)	1.53 (+/-0.1)	1.08 (+/-0.01)	0.78 (+/-0.2)
25.92	1.52 (+/-0.2)	1.23 (+/-0.2)	1.05 (+/-0.01)	0.92 (+/-0.1)
29.16	1.66 (+/-0.01)	1.73 (+/-0.01)	0.71 (+/-0.2)	0.62 (+/-0.2)
32.4	1.99 (+/-0.1)	1.19 (+/-0.07)	1.03 (+/-0.09)	1.01 (+/-0.01)
35.64	1.1 (+/-0.02)	0.98 (+/-0.07)	1.48 (+/-0.02)	1.06 (+/-0.01)

*Concentration of manganese (II) sulfate 10 mg/L.

^Concentration of iron was 0.3 mg/L.

Table 3.13. Flow-through pulsed UV inactivation *of B. megaterium* vegetative cells at 375 rpm in the presence of inorganic contaminants.

UV dose µJ/cm²	Log_{10} inactivation of *B. megaterium* No contaminant	Log_{10} reduction of *B. megaterium*		
		Inorganic contaminant		
		Manganese*	Iron^	Manganese*and Iron^
0	0	0	0	0
1.08	1.06 (+/-0.2)	0.27 (+/-0.01)	1.01 (+/-0.1)	0.28 (+/-0.1)
2.15	1.6 (+/-0.1)	1.05 (+/-0.2)	1.97 (+/-0.1)	1.42 (+/-0.02)
3.24	1.3 (+/-0.1)	1.29 (+/-0.1)	1.58 (+/-0.1)	1.33 (+/-0.1)
4.32	1.26 (+/-0.04)	1.63 (+/-0.2)	2.32 (+/-0.09)	1.43 (+/-0.3)
5.39	1.96 (+/-0.2)	1.86 (+/-0.06)	1.84 (+/-0.1)	1.47 (+/-0.2)
6.48	2.36 (+/-0.2)	1.01 (+/-0.2)	1.55 (+/-0.3)	1.96 (+/-0.1)
8.64	2.37 (+/-0.3)	1.16 (+/-0.1)	1.49 (+/-0.01)	2.09 (+/-0.2)
10.8	2.14 (+/-0.1)	1.84 (+/-0.2)	1.17 (+/-0.2)	2.28 (+/-0.1)
12.96	2.21 (+/-0.1)	1.53 (+/-0.4)	2.22 (+/-0.3)	1.81 (+/-0.2)
15.12	2.1 (+/-0.01)	1.64 (+/-0.07)	1.90 (+/-0.1)	2.45 (+/-0.06)

*Concentration of manganese (II) sulfate 10 mg/L.

^Concentration of iron was 0.3 mg/L.

Table 3.14. Flow-through pulsed UV inactivation *of B. megaterium* endospores at 375 rpm in the presence of inorganic contaminants.

UV dose µJ/cm²	Log_{10} inactivation of *B. megaterium* No contaminant	Log_{10} reduction of *B. megaterium*		
		Inorganic contaminant		
		Manganese*	Iron^	Manganese*and Iron^
0	0	0	0	0
3.24	0.42 (+/-0.1)	0.2 (+/-0.01)	0.8 (+/-0.4)	0.1 (+/-0.1)
6.48	0.45 (+/-0.2)	0.33 (+/-0.1)	1.1 (+/-0.3)	0.63 (+/-0.1)
9.72	0.64 (+/-0.04)	0.30 (+/-0.03)	0.75(+/-0.1)	0.74 (+/-0.01)
12.96	0.79 (+/-0.1)	0.78 (+/-0.03)	0.63 (+/-0.01)	0.79 (+/-0.1)
16.2	0.82 (+/-0.05)	1.2 (+/-0.04)	1.4 (+/-0.1)	0.88 (+/-0.1)
19.44	0.57 (+/-0.01)	0.95 (+/-0.1)	1.51 (+/-0.1)	0.77 (+/-0.1)
22.68	0.54 (+/-0.1)	0.77 (+/-0.01)	1.88 (+/-0.1)	1.01 (+/-0.1)
25.92	0.84 (+/-0.1)	0.96 (+/-0.07)	2.05 (+/-0.5)	1.1 (+/-0.04)
29.16	1.4 (+/-0.01)	1.73 (+/-0.01)	2.16 (+/-0.2)	1.14 (+/-0.2)
32.4	1.31 (+/-0.1)	0.84 (+/-0.1)	2.51 (+/-0.02)	1.71 (+/-0.5)
35.64	1.23 (+/-0.0)	1.1 (+/-0.1)	2.01 (+/-0.02)	1.6 (+/-0.01)

^Concentration of iron was 0.3 mg/L.

*Concentration of manganese (II) sulfate 10 mg/L.

4 Discussion

According to the European Water Framework Directive, a 'good ecological and chemical status of surface water' must be achieved by 2015. In this context of meeting this important directive, the detection and destruction of emerging recalcitrant pathogens such as chlorine-resistant *Cryptosporidium* and *Giardia* parasites and related issues such as the occurrence of unwanted anthropogenic micropollutants in surface waters via discharges from wastewater-treatment plants has come under the spotlight of scientists as well as politicians (Rowan, 2011). We recently reported that PUV light is a superior candidate for water-treatment technology to that of pulsed-plasma gas-discharge and ozonation as PUV does not elicit any unwanted geno-, cyto- and ecotoxicological end-points, yet effectively and efficiently destroys *C. parvum* oocysts (Hayes *et al.,* 2013). This study also built on our earlier work which demonstrated that *in vitro* cell culture can replace use of the current gold-standard *in vivo* mice model for assessing the efficacy of *C. parvum* detection and disinfection post-UV treatment, thus simplifying and standardising processes for parasite destruction (Garvey *et al*., 2010). However, no prior study had focused on the efficacy of using new innovative disinfection technologies for the destruction of the waterborne *G. lamblia.* This is due to the highly complex culture requirements of this species (i.e. using *in vivo* rodent models) to determine infectivity and disinfection efficacy thus limiting developments in this important area. This present study describes the effectiveness of using pulsed light as a novel innovative technology to destroy *C. parvum* and *G. lamblia* while also reporting for the first time on the use of combined cell culture – qPCR. Application of the findings from this study will also assist the water industry in identifying effective innovations for disinfection of established and emerging pathogens along with underpinning future risk-management, toxicological and ecotoxicological studies.

4.1 Cell Culture PCR for Parasite Viability (CC-PCR)

While there is growing interest in the use of *in vitro* cell culture assays to study the pathogenesis and infectivity of waterborne *Cryptosporidium* parasite infection post-disinfection treatments (Hijjawi, 2010; Garvey *et al*., 2010), there is currently a dearth of critical information on the detection and efficacy of these emerging enabling technologies for removing or destroying *G. lamblia*. The core factor governing the choice of the appropriate human cell line to address the needs of this study was the representativeness of the target area or site of infection. Therefore, epithelial cells of intestinal origin were selected as they provide a likely matrix for *Giardia* infection.

During this study, HCT-8 cells were found to be superior to that of using Caco-2 cells for studying infectivity. Hijiawi (2010) also reported that HCT-8 was superior to that of 11 other human cell lines tested for studying infectivity in *Cryptosporidium* as these particular cells supported all life cycle stages of *Cryptosporidium* growth (Hijjawi, 2010). This present study demonstrated that the mechanism of *Cryptosporidium* cellular infectivity differed to that of *Giardia* where the latter was observed to be not cell membrane invasive but only requiring attachment to epithelial surfaces to initiate and enable infection. An increase or decrease in the amount of host cells available for *Giardia* significantly affected its infection and subsequent intracellular proliferation, which is mainly attributed to the surface area available for parasite attachment. Loss of viability and detachment which occurred with the Caco-2 cells may have led to the decrease in infectivity that was observed for this cell line following exposure to viable *Giardia* cysts. Exposure of Caco-2 cells to *Giardia* cysts consistently resulted in apoptotic or early phase cell death in this monolayer, which was not observed with similarly challenged HCT-8 cells. Previous studies

conducted by Cotton *et al*. (2011) showed that genes associated with apoptosis are up-regulated in cells exposed to *Giardia* and heightened rates of epithelial apoptosis occur shortly after exposure to *Giardia* trophozoites, which was observed both *in vitro* and *in vivo*. This marked difference in pathogenesis between these two waterborne parasites may be attributed to *C. parvum* inhibiting apoptosis at the trophozoite stage or promoting apoptosis at sporozoite and merozoite stages in HCT-8 cells (Panaro *et al.,* 2007). This may support parasite growth in the early stages after epithelial cell infection, and may explain why greater levels of infectivity were evident for this parasite for each cell line studied. *G. lamblia* predominantly colonises the proximal small intestine and this is the primary site of infection following consumption of the parasite. Therefore, the HCT-8 cell line which is of ileum origin is an ideal candidate for *in vitro* infectivity studies. The Caco-2 cell line has its origin in the colon and this difference in gastrointestinal location may contribute to the ability of the cells to withstand parasitic infection for both the *Giardia* and *Cryptosporidium* species.

Different assays have been used to compare viability and infectivity of *Cryptosporidium* oocysts and *Giardia* cysts. Traditionally, neonatal mouse infectivity has been considered the gold standard and the most sensitive assay for determining the infectivity of these intestinal parasites. Furthermore, the use of vital dye-staining techniques has consistently proven insufficient at determining UV inactivation of these species (Craik *et al.,* 2000). Indeed, for many years it was believed that conventional LP generated UV light could not sufficiently inactivate *G. lamblia* based on viability as measured via excystation or vital dye staining (Mofidi *et al.,* 2002). The development of a real-time PCR assay for the viability assessment of treated parasites allows for a rapid means of assessing the efficiacy of all disinfection techniques without the need for *in vivo* studies on live hosts. Previously published studies by this and other research groups have shown the strong correlation between mouse infectivity and *in vitro* cell culture infectivity for *C. parvum* (Garvey *et al.,* 2010; Rochelle *et al.,* 2002). This present bench-scale study demonstrated that PUV effectively destroys *Giardia* and *Cryptosporidium* parasites when suspended in water and therefore offers an exciting opportunity to investigate this approach as a candidate emerging innovation at water-treatment plant level. This has future implications in terms of critical data generation as guiding parameters to include water light transmission (presence of suspended or dissolved solids), flow-rates, volume, energy requirements and so forth merit attention as well as the related yet underappreciated area of risk management. Findings of this study can impact positively on potable water quality and safety and have broad ramifications and/or implications for the water industry moving forward.

4.2 Treatment of Parasite Species

In order to comprehensively investigate scale-up of PUV at water-treatment plants one must also consider identifying a surrogate harmless indicator organism of matched or greater resistance to that of *Giardia* or *Cryptosporidium* in order to conduct important disinfection and monitoring studies. We have recently reported on the use of the harmless bacterial endospore *B. megaterium*, which was shown to be of comparable PUV sensitivity to *C. parvum*, suggesting that it may potentially be applied as an alternative surrogate organism to *C. parvum* for monitoring disinfection performances of this complex enteroparasite at drinking and wastewater-treatment plants (Garvey *et al.,* 2012). Implementation of a PUV system at water-plant level will need to reflect on data presented in this study to ensure that lamps are pulsed at their uppermost UV dose or fluence settings to disinfect this recalcitrant parasite. Plant engineers should also take cognisance of other variable parameters including potential concentration of *C. parvum* oocysts present, flow rates, UV transmission and depth of water to be treated to ensure efficacy of parasite destruction. All of these factors can influence the disinfection efficiency of the system and need to be assessed. For this study it was found that treatment at 8 cm distance from the light source with 16.2 J was sufficient for achieving substantial inactivation of test species: this may therefore serve as a guide for operational parameters. It should be noted that the biocidal light spectrum emitted by pulsed medium pressure and high-voltage xenon light sources are significantly different and this must be factored into optimised disinfection studies at water-treatment plants (Rowan, 2011). Pulsed UV systems deliver a broader range of wavelengths to the treated area than the standard UV approaches and therefore potentially have a broader range of cellular targets (Farrell *et al.,* 2011). To date most published data on inactivating *C. parvum* use low- and medium-

pressure UV light sources. Craik *et al.* (2001), Keegan *et al.* (2003) and Mofidi *et al.* (2001) reported that UV doses of 6, 5.8 and 10 mJ/cm^2 respectively provided an average 2 $\log_{10}$ reduction in the viability of *C. parvum* (Lee *et al.,* 2008). The results of this study indicate that with a PUV approach greater inactivation occurs with a much reduced applied UV dose, for example, 12.96 µJ/cm_2 gave a 5 $\log_{10}$ inactivation of *C. parvum.* Also for *G. lamblia* a 2.8 $\log_{10}$ inactivation rates was obtained with a UV dose of 22 µJ/$cm_{2.}$

4.3 Ecotoxicology Testing of Treated Water

Ecotoxicological testing of PUV–treated water is of paramount importance as it is critical to show that samples treated with this novel disinfection approach are environmentally safe to release into the environment. Anything in the wrong place, in damaging quantities, can be a pollutant (Jeffries and Mills, 1990). The treatment of toxic wastewater is one of the most critical and urgent topics for water-pollution control, with pulsed power technologies being in use for wastewater treatment for many years (Shi *et al.,* 2009). The use of UV light for disinfecting drinking water or wastewater in the presence of microbial pathogens and/or organic matter may lead to the formation and release of potentially harmful substances to the aquatic environment. Currently there is no data on the possible toxic effects of PUV-disinfected water on species found in the marine environment. Our studies report that PUV using an xenon light exhibits potential for the treatment of water containing microorganisms as well as parasite species and consequently should be fully studied to ensure its suitability for use in water-treatment plants (Garvey *et al.,* 2010).

This study focused on the ecotoxicological assessment of PUV-treated water using a xenon light source both in the absence and presence of 10 ppm HA (organic matter) on a variety of test species using microbiotests at different trophic levels in an aquatic environment. The presence of organic matter allows us to determine the effect of treatment of dirty water on test species. These studies were carried out to determine if the presence of PUV-treated water containing bacterial and parasite species had an impact on the survival of these test species. The test species showed no sign of sensitivity to freshwater containing organisms following disinfection by PUV. For all studies the reference toxicant potassium dichromate (phenol for the microtox assay) was within the satisfactory range stipulated by the various kit manufacturers. The bacterium *Vibrio fischeri* proved most sensitive to the presence of PUV-treated water in the absence and presence of HA using the 82% test. Although there was no significant level of toxicity these findings highlight the sensitivity of the test system. Studies conducted by Mankiewicz-Boczek *et al.* (2008) using microbiotests to assess the quality of river and surface waters concluded that there was a need of a microbiotest battery application consisting of acute tests using organisms that represent different trophic levels. The authors stated that the number of proposed microbiotests for surface-water analysis can be reduced if the acute test Microtox, based on the decomposer bacteria *Vibrio fischeri* is used (Mankiewicz-Boczek *et al.,* 2008).

The potential of the PUV-treated test liquids to induce adverse effects on rainbow trout RTH-149 fish cells grown in culture was evaluated via the direct contact assay using both the MTT and NR endpoints. Two cytotoxic endpoints were chosen to assess a broader range of potential toxic effects. The MTT endpoint measures mitochondrial function as an indicator of cytotoxicity (Fotakis and Timbrell, 2006), while the NR endpoint assesses the ability of viable cells to incorporate and accumulate the supravital dye NR within lysosomes (Borenfreund and Puerner, 1985). Also studies by Fent (2001) concluded that the NR and MTT assays were better suited as a measure of cytotoxicity for fish cell lines than alternative assays. Pulsed UV treated water did not exert any cytotoxic effects on the rainbow trout RTH-149 cells, using either cytotoxicological endpoint. These *in vitro* studies conducted on the rainbow trout cell line completed the ecotox test battery. In ecotoxicological testing, *in vitro* cytotoxicity studies are as important as *in vivo* studies using live fish as the primary interaction between chemicals and biota occurs at the surface of or in cells. Therefore, cellular responses are not only the first manifestation of toxicity, but also suitable tools for the early and sensitive detection of chemical exposure (Fent, 2001).

4.4 Pulsed UV Inactivation of Bacterial Biofilms

Studies by Ben Said *et al.* (2011) on the effects of UVC on *Pseudomonas* biofilm production showed that biofilm formation was not inhibited after irradiation of *P. aeruginosa*, by a recommended dose for water disinfection (40 mJ/cm^2). This result suggests that this dose is insufficient to inhibit the cell-to-cell communication system and therefore biofilm formation.

Studies described herein suggest that the PUV light system offers high rates of inactivation of biofilm structures at lower energy input to standard UV methods.

The authors believe studies are merited to assess the potential of these biofilm structures to harbour other pathogenic organisms such as viruses or parasites within their matrix. Pathogens, even present below the analytical detection limit in water, can accidentally attach to biofilms which then can act as their environmental reservoir and represent a potential source of treated water contamination. Detachment from biofilms can occur by continuous erosion (Wingender and Flemming, 2011). Such studies would allow for a better understanding of the source of these pathogens in drinking water supplies and the ability of this PUV approach to eliminate such problematic species.

4.5 Inactivation of *Bacillus* Endospores

The findings of this study showed a general trend where endospores of each *Bacillus* strain tested proved more UV resistant than their corresponding vegetative form. There was also a significant difference in individual strain sensitivity to UV treatment for both culture forms at lower treatment doses of < 10.8 µJ/cm^2. Endospores of *B. pumilus* consistently showed lower levels of resistance to PUV compared to *B. megaterium* and *B. cereus*. In the vegetative state the order of increasing sensitivity to PUV was as follows: *B. cereus*, *B. pumilus* with *B. megaterium* being least sensitive. This may be related in part to the larger size of *B. megaterium* cells relative to the other *Bacillus* species tested (Rowan *et al.,* 2001). However, for endospore-inactivation studies, the order of increased sensitivity was observed to be *B. megaterium, B. cereus* with *B. pumilus*.

This study also corroborates the findings of Sharifi-Yazdi and Darghahi (2006) who reported that *B. megaterium* endospores proved more resistant to UV irradiation compared to similarly treated *B. cereus* endospores. These researchers achieved a 2 $\log_{10}$ CFU/ml reduction of *B. megaterium* with 50 pulses (7.1 µJ/cm^2) and a 5 $\log_{10}$ following 25 pulses (3.2 µJ/cm^2) for *B. cereus* at 20 J per pulse. While the mechanisms underpinning destruction of spores differs to that of similarly UV-treated vegetative cells, DNA remains the principal target with the formation of a lethal adjunct of a thymine dimer in spores as opposed to formation of cyclobutane pyrimidine dimers in vegetative cells. Repair of these photoproducts is an important factor when treating bacterial endospores with UV disinfection techniques. Currently, it is known that endospores possess three types of repair mechanisms for repair of UV damage to DNA: (i) recombinational repair (RR), (ii) nucleotide excision repair (NER) which is similar to the damage repair system present in vegetative cells, and (iii) spore photoproduct lyase (SP lyase), and the importance of these three systems varies depending on the damage to be repaired. At present, there is a lack of data on the UV repair potential of PUV-treated endospores. Research indicates that the presence of certain proteins (α/β-type SASP) within the spore structure also result in the resistance of bacterial spores to many methods of disinfection including heat, chemical and monochromatic UV (Setlow, 2001). There are high levels of this protein present in the *Bacillus* species, including *B. cereus* and *B. megaterium*, and binding of this protein to DNA has been linked to disinfection resistance (Setlow, 2001). The findings of this study also suggest that endospore resistance to disinfection with PUV light is not affected by the pathogenicity of the *Bacillus* species studied. Indeed, similar levels of inactivation were obtained for the non-pathogenic and pathogenic strains studied.

Studies have shown that once microbes are entrapped in particles or absorbed to surfaces, they can be shielded from disinfection (Verhille, 2003). Therefore, it is reasonable to assume that bacterial endospores would be more resistant in an actual water-treatment facility because of their aggregation properties. At present, studies focusing on the UV inactivation of organisms are primarily based on bench-scale inactivation kinetics derived from laboratory studies on suspensions of organisms. This

highlights the importance of conducting plant-scale studies on problematic pathogenic organisms such as *Cryptosporidium.* However, there are limitations to the extent of PUV studies which can be conducted at plant level due to the pathogenic nature of *Cryptosporidium* species. This has prevented more in-depth studies focusing on the implementation of the PUV light system at operational plant level.

5 Conclusion

In conclusion, the findings of this study showed that PUV light effectively eliminated high levels of waterborne parasite oocysts and recalcitrant endospores after extended pulsing, suggesting that it is a potential application for use at water-treatment plant level. These data also suggest that the non-pathogenic *B. megaterium* have similar inactivation rates to the Iowa strain of *C. parvum* ($p<0.05$) following exposure to PUV light at 16.2 J. Therefore, *B. megaterium* may allow for future inactivation studies on the optimisation of the PUV system at water-treatment plant level by providing a surrogate organism for the PUV inactivation of *C. parvum.* This would allow for full-scale inactivation studies at plant level using a non-pathogenic organism which is representative of the pathogenic *C. parvum.* Furthermore, it is recommended that studies into the potential of *Bacillus* endospores to repair genetic damage following PUV treatment be investigated.

Medium pressure (MP) UV lamps are also used on occasion for the treatment of water; however, studies conducted by Bohrerova *et al.* (2008) concluded that there was no significant difference between the LP and MP UV inactivation technologies with regards to microbial disinfection. The LP and MP inactivation of *E. coli* was not significantly different according to the previously published work of Bohrerova *et al.* (2008), Sommer *et al.* (2000) and Zimmer and Slawson (2002). Therefore, the findings of this study suggest that PUV offers a more rapid, energy-efficient and feasible alternative for the disinfection of water than the current LP and MP systems in use. Pulsed UV contains a broader range of wavelengths than standard UV lamps, and this broad spectrum may potentially target more cellular targets (such as cell membranes and protein structures) than DNA alone, producing irreparable cellular damage. Furthermore, previously published studies by McDonald and Curry (2001) postulate that the high proton flux from a PL source overwhelms the cellular repair mechanisms of treated organisms.

Successful inactivation of *B. megaterium* vegetative and endospore cells was also achieved using a flow-through PUV system. Treatment liquid containing the test species was pumped under the UV lamp at set flow rates and inactivation rates determined. It was found that significant levels of inactivation were achieved both in the absence and presence of organic contaminants. However, it is worth noting that the presence of the inorganic contaminants manganese, iron or a combination of both did affect the levels of inactivation. In-depth studies on the exact method of interference were not conducted as they were deemed outside the scope of this project. It was found that with a faster flow rate and decreased retention time (time under the light source) there was also a marked decrease in inactivation rates. For optimal disinfection of microbial and parasite species it is believed that a series of two or more PUV lamps operating adjacent to each other would allow for significantly increased levels of inactivation. Implementation of PUV systems in conjunction with UV or as a stand-alone method may be achieved by placing lamps along the piping in sequence to ensure exposure of the treatment area. Operators must guarantee that pulsed lamps are pulsed at their uppermost UV dose or fluence settings to disinfect the water at the lamp surface. This study found that pulsing at a rate of 1 pulse per second with 16.2 J per pulse was sufficient to achieve high levels of inactivation for all test species. There are other considerations which were not addressed in this study which need to be considered such as varying the concentration of microbial species present and the depth of water to be treated. However, this study used high levels of microbial species for all studies to represent extreme microbial pollution of test waters. It is to be noted that these factors can influence the disinfection efficiency of the system and need to be assessed.

The findings of this study highlight the potential of this system for use in water-treatment plants as a means of disinfecting water.

5.1 Deductions

1 PUV successfully inactivated *C. parvum* and *G. lamblia,* with 99.999% and 99.9% loss in viability respectively when operated as a non-flowing system.

2 PUV successfully and repeatedly inactivated a broad range of microbial species, namely *E. coli and Bacillus* spp., when operated as a flow-through system in the absence and presence of inorganic contaminates.

3 PUV successfully inactivated vegetative and endospore cells of *Bacillus* species when used as a flow-through system at flow rates of 24 and 30 L/hour (Tables 3.11–3.14).

4 PUV successfully inactivated gram-positive (*S. aureus*) and gram-negative (*P. aeruginosa*) biofilm structures of varying age and cell density (Table 3.8).

5 PUV provided a higher level of inactivation for all microbial and parasite species than the standard low pressure ultraviolet system when used as a flow-through system.

6 The findings of this study showed a general trend where endospores of each *Bacillus* strain tested proved more UV resistant than their corresponding vegetative form. There was also a significant difference in individual species sensitivity to UV treatment for both culture forms at lower treatment doses of <10.8 μJ/cm^2 for both flow-through and stationary treatment.

7 *B. megaterium* can be used for future full-scale inactivation studies on the optimisation of PUV at water/wastewater-treatment plants by providing a surrogate organism for studies of *C. parvum.*

8 The presence of the inorganic contaminants manganese and iron singly or in combination affected the levels of inactivation.

9 Ecotoxicological testing of water samples indicated that PUV did not cause any deleterious effects on water contaminated with the range of species tested (Table 3.10).

7 The findings of this study suggest that PUV offers a rapid and energy-efficient method for the disinfection of water and wastewater.

5.2 Recommendations

1 This study showed the efficacy of PUV for the inactivation of an array of test species both as a static and flow-through system. For future studies it is recommended that this system be implemented as a bolt-on system at treatment plants.

2 Operational settings of 1 pps at 16.2 J per pulse at a distance of 8 cm from the light to the treatment surface achieved high levels of inactivation of *C. parvum* and *G. lamblia*. Therefore, it is recommended that such operational parameters be taken as an initial guide for plant operation.

3 For optimal disinfection of water, it is recommended that two or more PL lamps are operated sequentially to provide significantly increased levels of inactivation.

4 For installations of PUV, plant engineers should take cognisance of other variable parameters, including potential concentration of *C. parvum* oocysts present, flow rates, UV transmission and depth of water to be treated to ensure efficacy of parasite destruction. All of these factors can influence the disinfection efficiency of the system.

5 Initial studies have shown that PUV disinfects microbial biofilms. It is recommended that further studies are carried out to assess the potential of biofilm structures to harbour other pathogenic organisms such as viruses or parasites within their matrix.

6 *B. megaterium* shows similar levels of inactivation to *C. parvum* and is recommended as a surrogate future inactivation studies on the optimisation of PUV at treatment plants.

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Acronyms and Annotations

ASTM	American Society for Testing and Materials
CC-qPCR	Cell culture Taqman-quantitative PCR
CDC	Centers for Disease Control
CFU	Colony forming units
CPD	Cyclobutane pyrimidine dimer
Ct	Threshold cycle
DMSO	Dimethyl sulfoxide
HA	Humic acid
HBSS	Hank's Balanced Salt Solution
IF	Immunofluorescent
NR	Neutral Red
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
ppb	parts per billion
PI	Propidium iodide
PL	Pulsed light
PUV	Pulsed UV
PVC	Polyvinyl chloride
TOC	Total organic carbon
THM	Trihalomethane
TSB	Tryptone soya broth
WHO	World Health Organization

Appendix

List of Publications

Development of a combined *in vitro* cell culture – quantitative PCR assay for evaluating the disinfection performance of pulsed light for treating the waterborne enteroparasite *Giardia lamblia.*

Mary Garvey, Alessia Stocca and Neil Rowan. Submitted to the Journal of Experimental Parasitology (under review)

A comparative study on the Pulsed UV and the Low Pressure UV inactivation of waterborne microorganisms.

Mary Garvey, Nikhil Tokala and Neil Rowan. Water and Environmental Research (under review)

Cyto-, geno- and ecotoxicological assessment of pulsed-plasma gas-discharge treated-water containing the waterborne protozoan enteroparasite *Cryptosporidium parvum*.

Jennifer Hayes, **Mary Garvey**, Dominik Kirf, Neil Rowan. Journal of Microbiological Methods 94, 325–37

A study assessing the pulsed UV inactivation of *Pseudomonas aeruginosa* and *Staphylococcus aureus* biofilms.

Mary Garvey, Danny Rabbit, Alessia Stocca and Neil Rowan. Water and Environment Journal DOI: 10.1111/wej.12088

Ecotoxicological assessment of pulsed UV-light treated water containing microbial species and the enteroparasite *Cryptosporidium parvum*.

Mary Garvey, Jennifer Hayes, Eoghan Clifford and Neil Rowan. Water and Environment Journal. DOI: 10.1111/wej.12073

Efficacy of Using Harmless Bacillus Endospores to Estimate the Inactivation of Cryptosporidium parvum Oocysts in Water.

Mary Garvey, Eoghan Clifford, Edmond O'Reilly, Neil Rowan. Journal of Parasitology 99(3) 448–52

Efficacy of measuring cellular ATP levels to determine the inactivation of Pulsed UV treated Cryptosporidium parvum oocysts suspended in water.

Mary Garvey, Jennifer Hayes, Eoghan Clifford, Dominik Kirf and Neil Rowan. Journal of Water Science and Technology: Water Supply 13 (2), 202–13

Inactivation of recalcitrant protozoan oocysts and bacterial endospores in drinking water using high-intensity pulsed UV light irradiation

Jennifer Hayes, **Mary Garvey**, Andrew. Fogarty, Eoghan Clifford and Neil Rowan. Journal of Water Science and Technology: Water supply 2012 12 (4), 513–22

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 Aeráide, Ceadúnaithe agus Úsáide Acmhainní
- An Oifig Forfheidhmithe i leith cúrsaí Comhshaoil
- An Oifig um Measúnú Comhshaoil
- 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.

EPA Research Report 145

Development of a pulsed light approach as a novel solution in drinking water treatment

Authors: Mary Garvey, Neil Rowan

The presence of recalcitrant parasites, such as *Cryptosporidium* and *Giardia*, as contaminants in drinking water after disinfection processes remains a significant health concern. In this report, a two year study was undertaken to 1) to determine if a novel pulsed UV (PUV) light system provides a suitable means for disinfecting drinking water with particular emphasis on killing parasites, 2) to establish reliable and repeatable methods for PUV when operated under both static and flow-through conditions, 3) to develop a novel combined *in vitro* cell culture-based polymerase chain reaction (PCR) method to determine the inactivation rates of these parasites post PUV treatments, 4) to identify a non-pathogenic harmless Bacillus endospore that may be used as surrogate for less complex parasite inactivation studies, 5) to investigate the influence (if any) of biofilm development of PUV disinfection efficacy, and 6) to conduct an extensive range of ecotoxicological studies post PUV treatments.

Identifying Pressures

There is increasing pressure of Ireland's drinking water treatment plants which do not sufficiently remove parasites that can cause human illness. The overriding objective of this report is to develop PUV as a complementary or alternative method for the destruction of harmful waterborne parasites that are not effectively eliminated or removed using traditional drinking water treatment processes. These parasites are resistant to chemical water disinfection, such as chlorination.

Informing Policy

The EU Drinking Water Directive (DWD) concerns the quality of water intended for human consumption. Its objective is to protect human health from adverse effects of any contamination of water intended for human consumption by ensuring that it is wholesome and clean. This DWD specifically states that high quality, safe and sufficient drinking water is essential for our daily life, for drinking and food preparation. This ensures that water intended for human consumption can be consumed safely on a life-long basis, and this represents a high-level of health protection. The combined microbiological, toxicological and parasitology analysis undertaken in this study provides an integrated beyond state-of-the-art approach to assess the efficacy of using PUV as a novel means for the destruction of harmful parasites in contaminated drinking water.

Developing Solutions

Drinking water is an invaluable resource for human health and food security. The development of pulsed UV light along with new tangential biological assessment methods enhances Ireland's capacity towards an alternative approach for effectively treating drinking water contaminated with harmful parasites thus protecting human health. During this study new techniques and protocols were developed to aid the disinfection and monitoring of recalcitrant contaminants for Irish water.

Comhshaol, Pobal agus Rialtas Áitiúil
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