Evaluation of Modified Flow-Through Pulsed UV Technology for Bacterial Inactivation with Comparison to a Standard Continuous-Flow Low Pressure UV system.

by

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Declaration

I, the undersigned, hereby declare that this thesis, entitled, 'Evaluation of Modified Flow-Through Pulsed UV Technology for Bacterial Inactivation with Comparison to a Standard Continuous-Flow Low Pressure UV system.", is entirely my own work. The thesis has not been submitted in whole or in part to any other University or Institution. All sources used have been acknowledged and referenced in the text.

Kelly Fitzhenry

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Dedication

This thesis is dedicated to the memory of my brother James who was a huge source of inspiration to me growing up. He taught me to work hard and persevere and to not take life too seriously! He continues to inspire me to this day and for that I will always be grateful.

"Character cannot be developed in ease and quiet. Only through experience of trial and suffering can the soul be strengthened, ambition inspired, and success achieved."

- Helen Keller

"You can't just turn on creativity like a faucet. You have to be in the right mood.
What mood is that?
Last-minute panic."

- Calvin & Hobbes

"*I think I should have no other mortal wants if I could always have plenty of music. It seems to infuse strength into my limbs and ideas into my brain. Life seems to go on without effort when I am filled with music.*"

- George Bernard Shaw

Abstract

Approximately 8,000 million litres of milk were processed by the Irish dairy industry in 2017. The most recent data indicates that industry water consumption rates average 2.5m^3/m^3 of milk processed and 14.9 m^3/tonne product; therefore, significant volumes of wastewater are produced by this sector annually. Currently, water reuse practices within dairy plants remain low however, with the potential introduction of stricter legislation (owing to the abolishment of milk quotas and resulting industry expansion), the sector appears receptive to strategies to minimise their water footprint.

This study compared a modified flow-through pulsed ultraviolet (PUV) system with a continuous low-pressure UV (LPUV) system as potential technologies for (i) tertiary wastewater treatment plant effluent disinfection and (ii) disinfection systems to restore dairy wastewater to reusable levels for certain dairy plant practices. The systems were compared in terms of bacterial UV response for the inactivation of *Bacillus spp.* endospores and dairy pathogens *Staphylococcus aureus, Listeria innocua* (*Listeria monocytogenes* surrogate) and *Escherichia coli* (*E. coli*). The photoreactivation potential of dairy pathogens post PUV and LPUV inactivation was also analysed. In addition, the influence of cultivation media supplement $MnSO_4.H_2O$ on endospore UV resistance was also explored. The impact of total suspended solid (TSS) content on UV system inactivation efficiency was evaluated using *E. coli*. On-site dairy wastewater was also used to compare total coliform inactivation efficiency via both UV systems with the impact of TSS and chemical oxygen demand (COD) on UV system performance also being investigated. Finally, an energy meter and pyroelectric detector were employed to compare UV system

energy efficiency and to measure the actual PUV energy imparted onto water samples.

The key findings showed a PUV output of 2,052mJ/cm^2 was required for a 2 $\log_{10}$ inactivation of *Bacillus spp.* endospores in comparison to a LPUV output of 12 mJ/cm^2 for the same inactivation. The LPUV system completely inactivated all three dairy pathogens at a UV output of 14 mJ/cm^2 while the UV energy requirements for complete inactivation strain. No photoreactivation of dairy pathogens was found post LPUV disinfection however the photoreactivation of *S. aureus* and *E. coli* was found post PUV disinfection. Cultivation media supplement $MnSO_4.H_2O$ was shown to significantly ($P < 0.05$) enhance endospore UV resistance to both PUV and LPUV. Site-scale analysis showed the LPUV to consistently remove total coliforms at minimum UV output of 14 mJ/cm^2 while the PUV system showed a varied inactivation rate of total coliforms at a PUV output of 2,052 mJ/cm^2. Moreover, the LPUV appeared to be influenced by TSS and COD to a lesser degree in comparison to the PUV system. Energy meter analysis showed the LPUV was more efficient (77%) at converting electrical energy to UV energy in comparison to the PUV (35%) and used significantly less energy per litre of wastewater treated in comparison to the flow-through PUV.

The project findings conclude that for the PUV to be a viable technology in this application, significant enhancements would be necessary as it did not offer disadvantages over low pressure UV disinfection systems in this study. This study also highlighted the effectiveness of existing LPUV as technology suitable for dairy wastewater disinfection and reuse.

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List of Abbreviations

ATP	Adenosine triphosphate
AdV	Adenovirus
BOD	Biochemical Oxygen Demand
$CaCO_3$	Calcium Carbonate
CAP	Common Agricultural Policy
CE	Coefficient of efficiency
CFU	Colony Forming Unit
CIP	Cleaning in Place
COD	Chemical Oxygen Demand
CPS	Coagulase-positive staphylococci
CWA	Clean Water Act
DAFM	Department of Agriculture, Food and the Marine
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
FOG	Fats, Oils and Grease
HAA	Haloacetic Acid
hAdV	Human adenovirus

HPC	Heterotrophic Plate Count
HPSC	Health Protection Surveillance Centre
HRT	Hydraulic Residence Time
IASBR	Intermittent Aerating Sequencing Batch Reactor
kPa	Kilopascal
LB	Luria Broth
LED	Light Emitting Diode
LPHO	Low Pressure High-Output
LPUV	Low Pressure Ultraviolet Irradiation
$MnSO_4.H_2O$	Manganese Sulphate Monohydrate
MODW	Microbiology of Drinking Water
MPUV	Medium Pressure Ultraviolet Irradiation
MPN	Most Probable Number
MNV	Murine Norovirus
PHR	Photoreactivation
PI	Propidium Iodide
PPS	Pulses Per Second
PUV	Pulsed Ultraviolet Irradiation
RED	Reduction Equivalent Dose
RNA	Ribonucleic Acid

RO	Reverse Osmosis
ROS	Reactive Oxygen Species
SA	Surface Area
SDWA	Safe Drinking Water Act
STEC	Shiga Toxigenic *E. coli*
TBX	Tryptone Bile X-glucuronide
THM	Trihalomethane
TN	Total Nitrogen
TOC	Total Organic Carbon
TP	Total Phosphorous
TSA	Tryptic Soy Agar
TSS	Total Suspended Solids
UK	United Kingdom
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet
UVDGM	UV Disinfection Guidance Manual
VBNC	Viable But Non-Culturable
VTEC	Verocytotoxigenic *E. coli*
WHO	World Health Organisation

WWTP	Wastewater Treatment Plant

Chapter 1
Introduction

1.1 Background

The volume of milk processed by the Irish dairy industry in 2017 increased to 8,000 million litres as the sector continues to expand in the wake of the abolishment of milk quotas introduced in 2015 (CSO, 2018). Similarly, to most industries, the use of water within the dairy food production sector is essential for plant operation. Recent studies have shown average industry water consumption rates (in Ireland) are 2.5m^3/m^3 of milk processed and 14.9 m^3/tonne product (Geraghty, 2011). It follows that significant volumes of wastewater are produced by this sector annually. While the industry appears receptive to minimising water consumption in dairy plants, wastewater reuse practices remain low. In terms of methods of disinfection for wastewater reuse, physical methods e.g. membrane filtration or ultraviolet (UV) disinfection are often preferred over chemical methods which can be associated with the production of harmful disinfection by-products. Depending on the desired end use of the treated wastewater, UV disinfection can offer added advantages over membrane filtration such as fewer maintenance issues, lower cost and typically greater microbial inactivation rates (Morris et al., 2007).

Pulsed UV (PUV) disinfection is a relatively new technology that has been accepted by both the European Union (EU) and Food & Drug Administration (FDA) as a static microbial disinfection technology within the food industry (Elmnasser *et al.,* 2007). PUV has been reported as a superior disinfection technology for contact surface food disinfection along with treatment of associated packaging to that of using standard low-pressure UV (LPUV) systems (Rowan *et al.,* 2015; Kramer *et al.,* 2017).

However, the potential for PUV as an alternative approach to using standard LPUV *disinfection* in water and wastewater treatment applications has yet to be fully explored. Moreover, LPUV has been deployed by the water industry as an effective disinfection technology for the past 50 years where a successor innovation or next-generation approach offering improved UV delivery has yet to be elucidated. PUV technology differs to LPUV systems by emitting short, high-energy pulses of broadspectrum light in the range 200 nm to 1100 nm as opposed to continuous monochromatic lower energy light at fixed wavelength of 254 nm. The characteristic high peak power of PUV systems is thought to offer advantages of shorter contact times and enhanced microbial lethality due to the rapid emission of highly concentrated energy (Abida *et al.*, 2014).

1.2 Gaps in Knowledge

Information regarding the current Irish dairy industry's views towards the disinfection of dairy water/wastewater for reuse practices in/around dairy plants is lacking. Moreover, typical microbiological contamination levels of wastewater streams from dairy plants are often unknown with most studies focused on dairy farm effluents or bulk tank milk (Oliver *et al.*, 2005; Connell *et al.*, 2013; Quigley *et al.*, 2013). The impact of typical wastewater parameters on standard LPUV disinfection systems are known but have not been established for emerging pulsed UV technology, particularly as a flow-through disinfection system. Furthermore, studies investigating the reliable and repeatable performance efficacy of flow-through PUV systems in comparison to using flow-through LPUV systems are necessary given that there is a gap in this knowledge. Thus, it is imperative that this deficit be addressed

if PUV technology is to be considered as a potential wastewater treatment technology.

1.3 Research Objectives

The specific research objectives of the study were to:

1. Ascertain the presence/absence of faecal indicators and dairy pathogens of interest in water and wastewater streams for contrasting dairy plants.
2. Investigate the impact of dairy wastewater parameters namely total suspended solids (both organic and inorganic), total organic carbon and chemical oxygen demand on the inactivation efficiency of continuous-flow (flow-through) LPUV and flow-through PUV using *Escherichia coli* (*E. coli*) and total coliforms.
3. Compare both UV irradiation systems for the inactivation of robust challenge organisms (i.e. *Bacillus* endospores) and to further elucidate the degree by which supplementation of culture media may influence the UV resistance of endospores to LPUV and PUV irradiation.
4. To establish the photoreactivation potential of bacterial pathogens *E. coli*, *Staphylococcus aureus* and *Listeria innocua* post flow-through PUV and continuous-flow LPUV disinfection.
5. Compare the energy usage of the LPUV and PUV systems for each of the inactivation studies above.

1.4 Structure of Dissertation

Chapter 2 (Literature Review) describes various aspects of the Irish dairy industry and compares it to other countries worldwide. An in-depth review of the UV disinfection process and UV technologies is also detailed in addition to a description

of microorganisms of interest to the dairy industry. Gaps in knowledge are also identified and discussed. **Chapter 3** provides an overview of the nationwide dairy plant survey and sample collection for dairy wastewater microbiological analysis. A description of all laboratory analysis methods are detailed along with a technical description of both of the UV technologies used in the study. The various methods of energy measurements for the PUV system are also presented. **Chapter 4** outlines the results of the dairy plant survey and wastewater characterisation in addition to describing the impact of physical and chemical parameters on the flow-through PUV and continuous-flow LPUV systems. The impact of routine artificial enhancement of endospore culture media on the UV sensitivity of *Bacillus* endospores is examined in **Chapter 5** with a comparison of both irradiation systems for various *Bacillus spp.* endospore inactivation. **Chapter 6** describes the inactivation and potential photoreactivation of two dairy pathogens and one dairy pathogen surrogate post flow-through PUV and continuous-flow LPUV disinfection. Two fundamental methods of determining the energy output/dose from PUV systems are evaluated and compared in **Chapter 7**. Furthermore, the energy efficiency of the LPUV system and PUV systems are explored as is the UV power characteristics of both systems. Finally, **Chapter 8** lists the main conclusions from the study and lists recommendations for future work. Figure 1.1 illustrates the connection between the thesis chapters and the research objectives set out in Section 1.2.

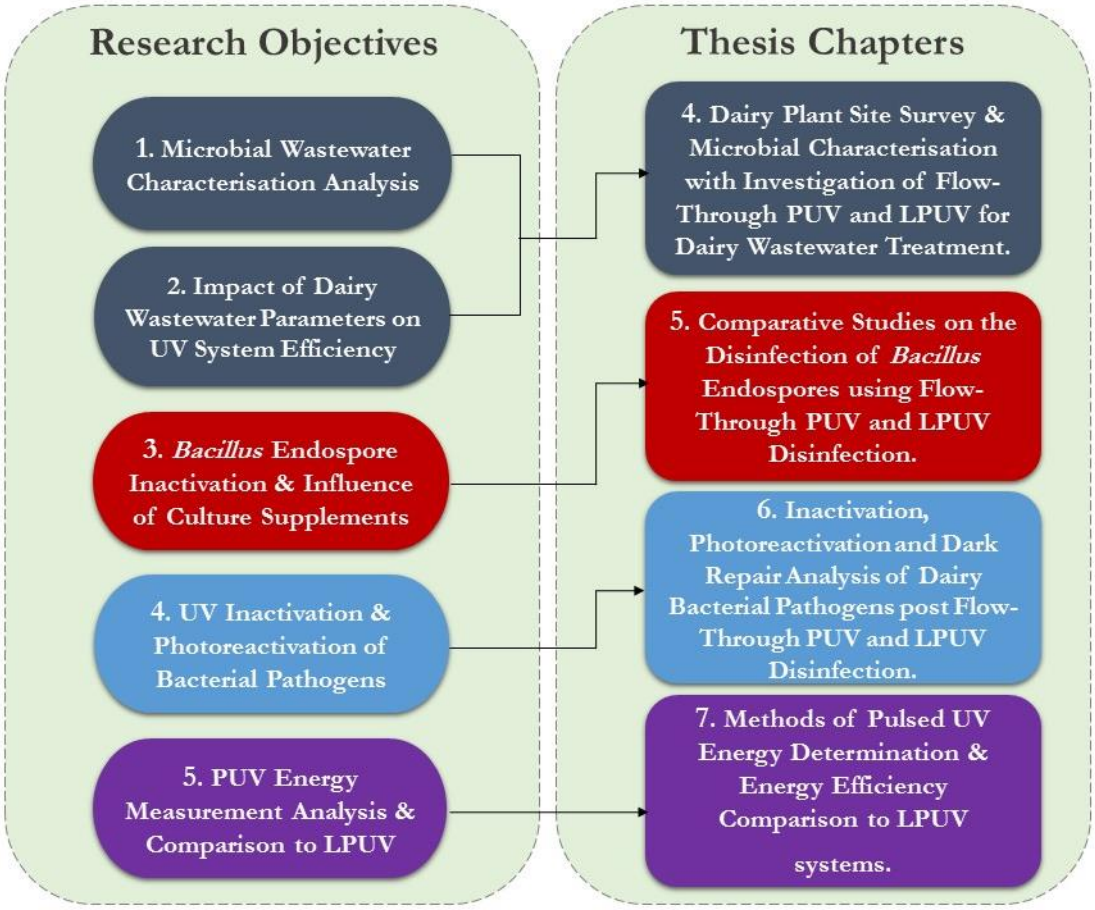


Figure 1.1. Connection between research objectives and thesis chapters.

1.5 Contribution to Existing Knowledge

1.5.1 Journal Papers (Accepted)

Fitzhenry, K., Rowan, N., Val del Rio, A., Cremillieux, A., Clifford, E. (2018). Conventional low-pressure UV-light irradiation demonstrates superior inactivation efficiency compared to similarly-treated *Bacillus* endospores using pulsed UV light in continuous flow-through water treatment systems. *Journal of Water Process Engineering.*

1.5.2 Short Communication

Fitzhenry, K., Rowan, N., Finnegan, W., Zhan, X., Clifford, E. (2018). Microbiological characterisation and impact of suspended solids on pathogen removal from wastewaters in dairy processing factories. *Journal of Dairy Research* **85** (3) 391 – 395.

1.5.3 Peer reviewed Conference Papers

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2016). Evaluation of UV disinfection technologies for water reuse and rainwater harvesting in the Irish dairy industry. Civil Engineering Research in Ireland 2016 (CERI 2016). National University of Ireland, Galway, Ireland. 29th-30th August.

1.5.4 Oral Presentations

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2018). UV Technologies for Wastewater Disinfection & Reuse in the Irish Dairy Industry. DairyWater Workshop II: Wastewater Treatment and Reuse for the Irish Dairy Processing Industry. Ballaghdereen, Co. Roscommon. 4th May.

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2017). UV Technologies for Wastewater Disinfection & Reuse in the Irish Dairy Industry. International Ultraviolet Association World Congress 2017. Dubrovnik, Croatia. 17th – 20th September.

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2016). Tertiary Treatment Technologies for Water Reuse and Rainwater Harvesting in the Irish Dairy Industry. 26th Irish Environmental Researchers' Colloquium, ENVIRON 2016. University of Limerick, Ireland. 22nd – 24th March.

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2015). Tertiary Treatment Technologies for Water Reuse and Rainwater Harvesting in the Irish Dairy Industry. 25th Irish Environmental Researchers' Colloquium, ENVIRON 2015. Institute of Technology, Sligo, Ireland. 7th – 10th April.

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2015). Tertiary Treatment Technologies for Water Reuse and Rainwater Harvesting in the Irish Dairy Industry. Ryan Institute Overview presentations. Ryan Institute, NUI, Galway, Ireland. 13th October.

1.5.5 Poster Presentations

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2016). UV Disinfection Technologies for Water Reuse and Rainwater Harvesting in the Irish Dairy Industry. Ryan Institute Research Day 2016. Ryan Institute, NUI, Galway, Ireland. 9th December. **Best Poster Award.**

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2015). Tertiary Treatment Technologies for Water Reuse and Rainwater Harvesting in the Irish Dairy Industry. 25th Irish Environmental Researchers' Colloquium, ENVIRON 2015. Institute of Technology, Sligo, Ireland. 7th – 10th April.

Fitzhenry, K., Finnegan, W., Zhan, X., Goggins, J., Rowan, N., Clifford, E. (2015). Tertiary Treatment Technologies for Water Reuse and Rainwater Harvesting in the Irish Dairy Industry. Ryan Institute Research Day 2015. Ryan Institute, NUI, Galway, Ireland. 25th September.

1.5.6 Magazine Articles

Major €1 million study aimed at increasing resource efficiency in the Irish dairy processing sector. IWRA Update: Newsletter of the International Water Resources Association, 28 (2) p3.

€1m study will increase resource efficiency in Irish dairy processing. 2014. Engineers Journal. Available at: http://www.engineersjournal.ie/2014/09/30/e1m-study-will-increase-resource-efficiency-irish-dairy-processing/.

Chapter 2
Literature Review

2.1 Introduction

This chapter describes wastewater disinfection and water reuse in the Irish dairy industry. An in-depth comparison of PUV and LPUV disinfection is presented with emphasis on the water and wastewater industry. In addition, challenge organisms and pathogens of interest in the dairy industry are reviewed and gaps in knowledge relating to this study are outlined. The chapter also identifies gaps in knowledge which formed the basis for this study.

2.2 The Dairy Industry

Ireland has a long history within the dairy industry with exports of butter and cream dating back to the early 19th century. However, it was Ireland's application to join and subsequent joining of the European Union in 1973 that instigated the rapid expansion of the dairy industry increasing milk production by approximately one billion litres from 1960 to 1973 (Breathnach, 2000). The common agricultural policy (CAP) introduced by the European Union (EU) created a framework for the food and agricultural industries by way of providing a steady income on which farmers could develop a livelihood from dairy farming. As production grew, quotas were introduced on milk production (the first in 1984) to curb excessive farming practices and alleviate the detrimental impacts on surrounding environments (Donnellan *et al.*, 2015). Nonetheless, the Irish dairy industry has continued to prosper with the value of dairy exports increasing to €3.4 billion in 2016 (National Milk Agency, 2017). A wide range of products are produced and exported annually with butter, cheese and milk powders amongst the most common products. Since the abolishment of the

CAP quotas in 2015 the volume of milk (both domestic and imported) processed by creameries and pasteurisers has risen sharply (Figure 2.1). As a consequence, there has been heightened interest in the strategic development of Ireland's agri-food sector with current governmental policies attempting to increase exports by 85% to €19 billion by 2025 (DAFM, 2015). Such an increase would increase pressure on reducing food waste and highlight the requirements for increased recycling measures to minimise environmental impact, improve efficiencies and enhance innovation.

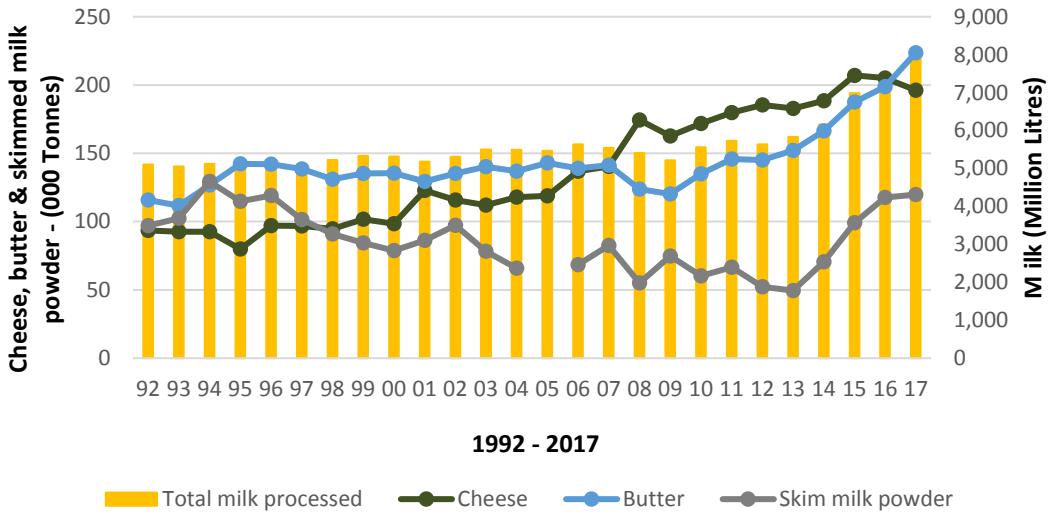


Figure 2.1.Total milk processed and production levels (tonnes) of various dairy products manufactured in Ireland from 1992 – 2017 (adapted from CSO, 2018).

2.2.1 Water Use in the Dairy Industry

Water consumption ratios in the Irish dairy sector are approximately 2.5 m^3/m^3 of milk processed and 14.9 m^3/tonne product (Geraghty, 2011). Aside from product manufacturing, water is also used for other activities such as steam generation, cooling duties and cleaning in place (CIP). Of these, studies have shown CIP duties to account for the majority of water consumption in dairy plants (Figure 2.2). The

average annual volume of water used per plant in Ireland has been estimated at 875,000 m^3 however it should be noted that the volume of water used per plant varies considerably in terms of the type of product being manufactured e.g. milk powder and the scale of the plant in question (Geraghty, 2011). With the recent rise in the volume of milk being processed in the last three years (Figure 2.1), the total amount of water being used within the industry is likely to have increased.

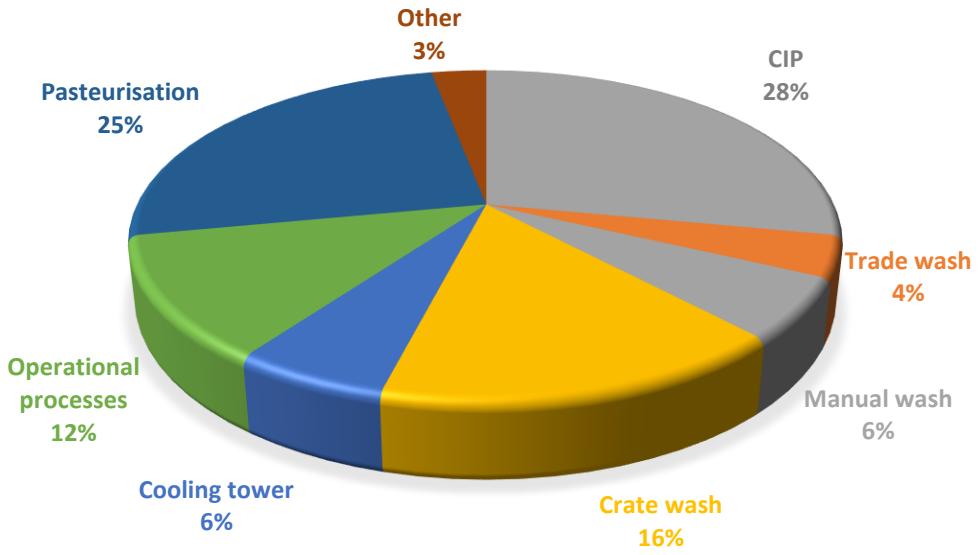


Figure 2.2. Breakdown of water use in a dairy plant by a typical market milk processor (Prasad, 2004).

In Poland, the average water consumption rate is 7 m^3/m^3 of milk processed while in Denmark water consumption rates vary from between 2.21 – 9.44 m^3/m^3 of milk processed depending on the dairy product manufactured (Wojdalski *et al.*, 2013). Recent reports of water use in the UK dairy processing industry have shown a drop in water consumption ratios from 1.37 L/tonne of input in 2008 to 1.05 L/tonne of input in 2018. This equates to a water consumption reduction of 23.4% since 2008 (Dairy UK, 2018). In contrast, the Australian dairy manufacturing industry saw a 5.7% increase in water consumption from 1.75 L/L of milk processed in 2010/2011

to 1.85 L/L of milk processed in 2017 however targets are in place to reduce this figure by 20% by 2020 (DMSC, 2018). Large variations in water consumption rates within the industry is attributed to differences in the types of products produced and the requirements for that process e.g. cheese and whey products use less water (on average) in comparison to cream and butter (EDA, 2018b; Wojdalski *et al.*, 2013).

2.2.2 The Dairy Wastewater Treatment Process

Dairy wastewater typically contains milk wastes and solids, sanitizers, cleaning water, detergents and potential pathogenic bacteria and viruses. It is generally characterised by high concentrations of nutrients, dissolved and suspended solids and organic/inorganic content and this varies depending on the food type produced. For example, cheese – producing plants may detect high levels of salinity in their wastewater due to increased levels of salts used in the production process. In addition, some plants can have a variety of waste streams entering the wastewater treatment plant (WWTP) such as cooling water, municipal wastewater or stormwater that can modify the constituents further. The defining characteristics of dairy effluent and of importance in WWTP processing include large variations in pH, biochemical oxygen demand (BOD), chemical oxygen demand (COD), increased temperatures (relative to intake and discharge points), total nitrogen (TN), total phosphorous (TP), suspended and dissolved solids and fats, oils and grease (FOG) (Slavov, 2017). Treatment often includes a combination of mechanical, biological and physicochemical methods and consequently, a multitude of dairy WWTP configurations are possible (Shete & Shinkar, 2013). A typical dairy WWTP process is outlined in Figure 2.3 – treatment strategies are proposed based on the strength of the influent wastewater. Initial mechanical treatment physically removes any

suspended solids which can lead to increased soluble COD levels. Physicochemical treatment involves protein colloids and FOG removal followed by chemical treatment which destroys soluble contaminants and corrects pH (Slavov, 2017). Most WWTPs employ aerobic biological treatment processes however problems can arise when treating highly contaminated wastewater such as limitations to oxygen transfer, sludge bulking and sludge settling (Prazeres *et al.*, 2012).

In contrast, anaerobic systems favour higher strength dairy wastewater due to their high degradability but can be hindered by high TSS and lipid content (Demirel *et al.*, 2005; Karadag *et al.*, 2015). Tertiary treatment as a final stage process may or may not be required depending on the efficiency of the WWTP and the discharge licence requirements. Tertiary treated water may however, offer the opportunity for wastewater to be recycled back in/around the dairy plant in areas where food production processes are not involved e.g. condensate or water for generic cleaning purposes (e.g. yards, trucks etc).

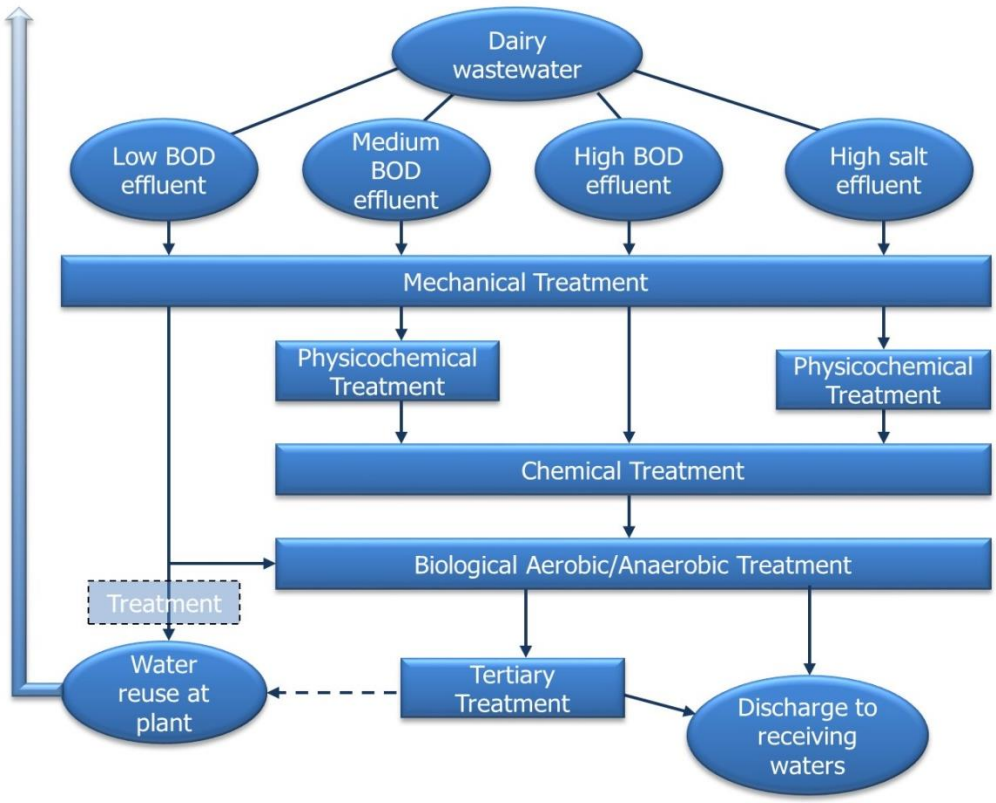


Figure 2.3. Dairy wastewater treatment strategies (adapted and modified from Slavov 2017 & EPA 1997).

2.3 Water Reuse - Legislation & Incentives

In Ireland, no guidelines/standards exist pertaining to reclaimed water use in any sector including the industrial sector (Raso, 2013). This is likely due to the country's high rainfall index and traditionally low drought risk. However, recent weather patterns in 2018 have seen unprecedented heatwaves which resulted in severe drought across the country. Water restrictions were enforced nationally and significant reductions in grass growth caused detrimental impacts on the Irish dairy farming industry in terms of milk output and the ability to build winter fodder supplies (Irish Examiner, 2018). Water stress within the EU territories is predicted to increase in the coming years owing to a combination of climate change and socio-economic factors including population growth and increasing water consumption

thus further research into this issue is warranted (EEA, 2012). Within the EU, water reuse is practiced to varying degrees depending on the country and level of water scarcity. A review of water reuse practices of 27 EU member states carried out in 2013 reported eight countries where reclaimed water use was commonplace with six of these implementing water reuse standards (Deloitte, 2015). The majority of these countries are based in the Mediterranean region e.g. Spain and Cyprus and each have established their own individual water reuse regulations modelled on criteria from the World Health Organisation (WHO), Californian regulations and Australian regulations (Angelakis & Gikas, 2014). The United States Environmental Protection Agency (USEPA) details extensive guidelines for water reuse applications in various categories including urban water reuse, agricultural water reuse and industrial water reuse with regulations delegated by different state agencies. Regulations can fall into a number of categories depending on the end use of the reclaimed water and include laws such as the Clean Water Act (CWA) and the Safe Drinking Water Act (SDWA) (USEPA, 2012).

Factors influencing the minimisation of water consumption and water reuse in the dairy industry may also include the cost of water. If the cost of process water is significant, this may incentivize water reuse measures within the plant. In Ireland, process water is typically sourced from either the public mains supply, a private well/borehole (groundwater) or nearby water bodies e.g. local river or lake (surface water). There is no fixed cost associated with groundwater or surface water extraction as a means of process water for dairy plants aside from the initial capital cost of implementation e.g. digging of borehole/well etc. However, a fee may be attached for access to the public water supply with fees per volume of water used

applied thereafter. This fee varies depending on the region and governing local authority. In Ireland, the cost of water in the county of Galway for example is €2.10/m^3 (Irish Water, 2018). Globally, the price of mains water varies depending on the country. In Germany, the cost is over €1.49/m^3, in the USA water costs are approximately €0.44/m^3 while rates in the UK can range from between €0.74/m^3 to €2.21/m^3 for process water (Rad & Lewis, 2014). The highest cost of water in Europe is charged in Denmark with an average price of €9.00/m^3 charged in 2016 (DANVA, 2017). It should also be noted that in the UK, the form of energy used to heat mains water also affects the mains price e.g. electrically-heated hot water is more expensive than gas-heated hot water (Rad & Lewis, 2014). Water prices in Australia vary slightly from €1.59/m^3 to €2.68/m^3 subject to location and the associated State Water Authority (Australian Bureau of Statistics, 2017). Research carried out on 24 major dairy processing factories in Australia found the relatively low cost of water to be a disincentive to conserve water (Wilkinson *et al.*, 2007).

2.3.1 Water Reuse Within the Dairy Industry

Due to increasing environmental awareness and changing climate, reusing water within the dairy industry is emerging both as an attractive and necessary option to minimise water consumption (Bord Bia, 2016). Dairy plant process water in Ireland is of potable standard and so is fit for purpose in any part of the dairy plant ranging from the pasteurisation process to truck washes. If a plant does decide to implement water reuse practices, they must determine to what quality the water is restored to and where it will then be reused. Guidelines set out by the European Commission list cooling water, condensate water, permeate water and cleaning water as acceptable streams for water reuse and which may be applied for specific processes e.g.

cleaning/washing, reuse, make-up for utilities and in very exceptional cases, the product itself (European Commission, 2006). Boiler feed water is often the first point of use to recycle water as it does not come into contact with either the product or internal feed piping thus a lower grade of water may be acceptable. However, even in this case, the mineral content must be low enough to avoid water hardness which can lead to scaling/corrosion of the system (EPA, 2008). The UK Environment Agency (2009) has identified a number of opportunities of potential water reuse within a plant including;

- Recycling condensate water as boiler feed water (where the quality of water is suitable).
- Counter-flow reuse; a method in which the water flows counter-current to the product so that the end product comes in contact only with potable water.
- Sequential reuse; the process by which the water stream is used for two or more processes prior to disposal.
- Recycling within a unit process or a number of processes without treatment. Water from cooling systems should not be reused.
- Recycling following treatment.

The sources of water for recycling and reuse within dairy plants are varied and plant specific however very often it is the condensate and/or permeate water that is recycled. Figure 2.4 illustrates the various available sources of recycled water in Australian dairy manufacturing plants; condensate, also known as ‘cow water’, accounted for almost 50% of recycled water in 2008.

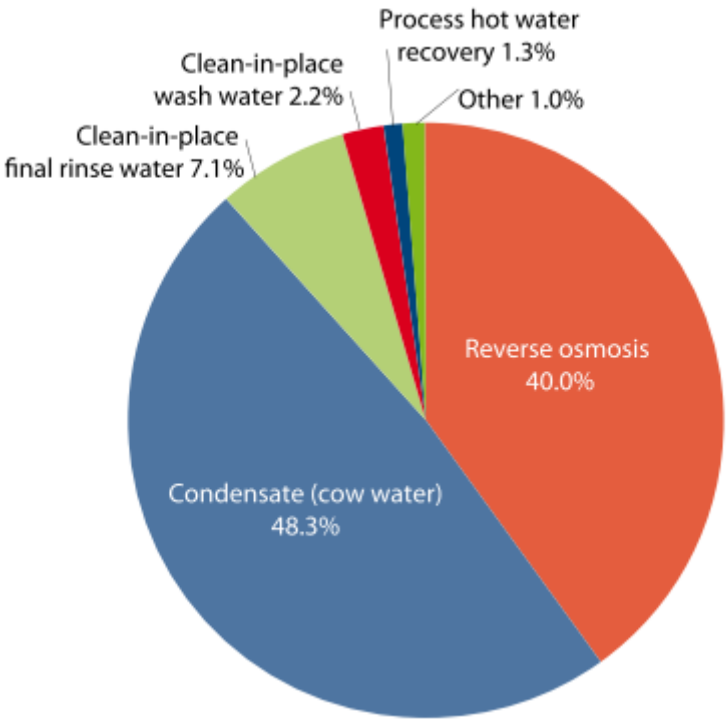


Figure 2.4. Sources of recycled water in the Australian Dairy Industry (Kershaw & Gaffe, 2008).

Countries with significant dairy industries e.g. Australia and New Zealand have made attempts to improve water sustainability within the sector. In the Australian dairy industry in 2008/2009, 26% of water consumed was recycled back into dairy plants (Kershaw & Gaffe, 2008) while in the UK, water recycling measures reduced water consumption by 20% and saved 840 million litres across the entire industry (Rad & Lewis, 2014). In Madrid, Spain, dairy food company Danone is currently investing approximately €2 million to improve water sustainability within the dairy plant with a target of 80% water autonomy by the end of 2019 (EDA, 2018a). While in the Netherlands, the multinational dairy cooperative FrieslandCampina reported a 2.4% drop in water consumption from 4.59 m^3/tonne end product to 4.48 m^3/tonne end product for 2015 and 2016 respectively with a target of 3.38 m^3/tonne end product by the year 2020 (Royal FrieslandCampina N.V., 2017).

2.3.2 Water Reuse Technologies

The selection of an appropriate technology for water reconditioning is informed by the quality of the water/wastewater being treated and the desired end-point use of the reconditioned water. In general, the technology should remove undesirable chemical, physical and microbiological components and may also be subject to other factors such as cost-effectiveness, existing standards, safety and wastewater treatment capacity (Lazarova *et al.*, 1999; Casani *et al.*, 2005). This treatment system may be either chemical e.g. chlorine, chlorine dioxide or ozone, or physical e.g. membrane processes or UV disinfection. The use of chemical treatments for water/wastewater reuse in the dairy industry does not appear to be widely implemented. The may be due to the formation of hazardous by-products which occurs when chlorine/chlorine dioxide reacts with organic matter (Casani *et al.*, 2005). Physical treatment systems, specifically membrane separation processes, appear to be more widely researched as potential treatment systems with several studies investigating the potential of ultrafiltration, nanofiltration, reverse osmosis (RO) or combinations thereof (Vourch *et al.*, 2005, 2008; Riera *et al.*, 2013; Andrade *et al.*, 2014). Of the membrane separation processes, RO has been shown to produce the highest quality reconditioned water (Vourch *et al.*, 2008).

The use of RO for recovering and reconditioning wastewater in dairy plants has increased in recent years. Research studies have shown RO to be effective at reconditioning flash cooler condensate for reuse as boiler feed water in addition to treating wastewater from milk processing for reuse in the CIP process (Suárez *et al.*, 2014; Suárez & Riera, 2015; Buabeng-Baidoo *et al.*, 2017). The volume of recycled water generated from RO systems in the whole of the Australian dairy industry rose

from 5 million litres to 1,858 million litres over a four-year period (2004-2008) (Kershaw & Gaffe., 2008). Moreover, Ireland's leading dairy food manufacturer Ornua installed a RO system at a newly built facility in Wisconsin, USA with a view to saving approximately 4.5 million litres of water annually (Ornua, 2014). The impact of climate change and increased water scarcity prompted a British dairy company to implement a RO plant to treat wastewater to reusable standards for water use in every part of the dairy plant from cleaning to pasteurisation processes. The installation currently supplies over 50% of the dairy plant's water demand which significantly reduces the withdrawal of water from surrounding water bodies (Dairy UK, 2018).

While RO is a suitable technology for restoring water to reusable standards, it can prove expensive and often a pre-treatment stage is necessary (Warsinger *et al.*, 2018). Furthermore, this intensive level of treatment may not be required for the type of wastewater to be treated in question and the desired end use. The primary drawback of this technology for water reuse purposes remains the issue of membrane fouling (Pandey *et al.*, 2012). Studies investigating the fouling of both ultrafiltration and RO membranes in the dairy industry are well established with some reporting a relationship between multispecies biofilm formation and enhanced resistance to cleaning and sanitation processes (Tang *et al.*, 2009; Hassan *et al.*, 2010; Anand & Singh, 2013; Anand *et al.*, 2014). Recent research investigating the application of RO membranes for water recovery in the dairy industry reported extensive biofouling after flushing and a transfer of microorganisms to the permeate (Stoica *et al.*, 2018). Follow up UV treatment was required in this case to ensure non-detectable microbial activity. UV treatment has long been applied in dairy plants for first point-of-use

water disinfection at the process water stage and end-point use at the WWTP tertiary treatment phase however it's applicability as a water reuse technology in dairy plants remains in its infancy. Furthermore, investigations into the potential application of emerging UV technologies for dairy wastewater reuse are extremely limited. The assurance of high microbiological quality is often a prerequisite for reconditioned wastewater, if membrane processes lack certainty in this regard, UV treatment may prove an encouraging alternative. As the focus of this study is on UV disinfection, a detailed discussion is outlined in Section 2.4.

2.4 UV Disinfection

The adoption of UV treatment as a method of tertiary disinfection for drinking water treatment and for wastewater treatment has increased significantly worldwide in recent years (Dotson *et al.*, 2012; EPA, 2015). A key driver of this uptake may be attributed to its' success in inactivating chlorine-resistant pathogens, in particular *Cryptosporidium parvum* (Craik *et al.*, 2001; Drescher *et al.*, 2001). UV treatment is also a physical disinfection method that is not associated with the formation of harmful disinfection by-products e.g., trihalomethanes (THMs) and haloacetic acids (HAAs) which are typically associated with free chlorines/chloramines disinfection applications. In addition, advanced oxidation processes that utilise UV treatment in combination with other chemical treatments (e.g. hydrogen peroxide) are effective in the degradation of a number of organic pollutants (Matilainen & Sillanpää, 2010; Sarathy & Mohseni, 2010). Typically, two types of UV lamps may be employed for use in water/wastewater disinfection applications; continuous low pressure UV (LPUV) and/or medium pressure (MPUV). LP lamps are monochromatic, emitting light at a single wavelength of 254 nm in within the UV-C range while MPUV lamps

emit light from a broader spectral range of <200 nm to >600 nm (EPA, 2011). In addition, MPUV lamps have a higher power output in comparison to LPUV lamps but have a lower energy conversion from electrical energy to germicidal UV (WERF, 2008). Typically, MPUV lamps are suited to treating significant water flows (particularly in plants with a small footprint) while LPUV is sometimes considered more suitable for both small-scale and large-scale operations (Schalk *et al.*, 2005). Newly emerging UV technologies such as pulsed UV light and UV-C light emitting diodes (LEDs) have drawn interest in the water/wastewater sector due to advantages such as shorter contact times and compact size (Würtele *et al.*, 2011; Uslu *et al.*, 2015, Uslu *et al.*, 2016). However, drawbacks exist for both technologies within the areas of uniform UV dose determination, low wall plug efficiency and diminished microbial inactivation efficiency in flow-through set-up conditions (Schaefer *et al.*, 2007; Oguma *et al.*, 2013; Gómez-López & Bolton, 2016; Song *et al.*, 2016; Yi *et al.*, 2016; Beck *et al.*, 2017). As the focus of this thesis compares and contrasts LPUV systems as the default, commonly-deployed, UV technology with PUV system (as an emerging new technology), the remainder of the literature will be limited to discussing these UV technologies.

2.4.1 LPUV Technology

The generation of UV light within a standard UV lamp occurs when a voltage is applied across a gas mixture which results in the discharge of photons. Almost all LPUV lamps designed for water treatment contain a mixture of mercury and one other inert gas – predominantly argon (Masschelein, 2002). The type of UV light produced is dependent on the concentration of mercury within the lamp and the mercury vapour pressure. In LPUV lamps a low vapour pressure of between 6.9 x

10^{-5} and 6.9 x 10^{-3} kilopascal (kPa) is applied (at a moderate temperature of 40ºC) to achieve monochromatic UV light at 253.7 nm. Hence the term, 'low pressure'. In comparison, MPUV lamps are exposed to a higher vapour pressure of between 14 and 1379 kPa (and a higher operating temperature of 600 – 900ºC) which produces a broad spectrum of polychromatic UV light at a higher intensity (USEPA, 2006).

LPUV lamps are the de facto UV disinfection systems used for water/wastewater disinfection applications. Aside from the dairy industry, LPUV disinfection is applied in many other sectors such as aquaculture, ballast water treatment, municipal wastewater, agriculture and the beverage industry (Chmiel *et al.,* 2002; Summerfelt *et al.*, 2009). Depending on the volume of water to be treated, LPUV systems can be either large-scale or small-scale systems. Large-scale systems may be 'open channel' whereby modules of lamps submerged into flowing water in banks, as the water passes the banks it is disinfected. The water is exposed to the air in this system set-up. Large-scale systems may also be 'closed channel' whereby water flows parallel to LPUV lamps housed within a closed metal cylinder; both longitudinal and cross-flow systems are available in this configuration (Metcalf & Eddy, 2004). Finally, small-scale LPUV systems are designed to treat lower water flows and may be either smaller closed channel configurations or bench-top size for domestic water use.

Currently, there are three main types of LPUV lamps used; standard low pressure lamps, low pressure high-output (LPHO) lamps and amalgam lamps, all of which are typically housed in a quartz glass tube (sodium-barium glass is sometimes used but quartz glass is preferred) (Wright *et al.,* 2007; WERF, 2008). In some cases, amalgam lamps are referred to as LPHO lamps however this is not strictly correct as the UVC-flux of LPHO lamps is significantly lower than that of amalgam lamps

(Schalk *et al.,* 2005). Amalgam lamps are alloys of mercury and other solid phase metals such as indium or gallium (USEPA, 2006). In comparison to standard low pressure lamps, amalgam lamps can operate at higher wall temperatures (up to 100ºC) and produce a much higher UVC-flux for the same lamp length making them a more attractive option for disinfection applications (Schalk *et al.,* 2005; USEPA, 2013). Table 2.1 describes the key characteristics of the various LPUV lamp types.

Table 2.1. LPUV lamp types and key physical characteristics (modified from Schalk *et al.*, 2005).

Characteristic	Standard Low Pressure	Low Pressure High Output	Amalgam
Wall Temperature (ºC)	30 - 50	>50	90-120
Electrical Power (W)	5 - 80	10 - 150	40 - 500
Current (A)	0.3 – 0.4	0.8 – 1.3	1.2 – 5.0
Specific Electrical Power* (W/cm)	0.3 – 0.5	0.5 – 1.0	1.0 – 3.0
Specific UVC-Flux* (W/cm)	<200	<350	<1000
% UVC efficiency (254 nm)	30 - 40	25 - 35	35
Influence of ambient temp.	High	High	Low

*per unit arc length

The typical advantages of standard UV disinfection include the following (WERF, 2008);

- Effective against most viruses, spores and cysts including chlorine resistant *Cryptosporidium.*
- User-friendly and safe for operators due to;
- No handling/transport of toxic or hazardous chemicals.
- The lamp being typically housed within a stainless steel frame limiting UV exposure.
- No residual effect which may be toxic to aquatic or human life.
- Little formation of disinfection by-products.
- Shorter contact time in comparison to other disinfection methods.
- A contact time of between 1 and 10 minutes is typical for chlorine treatment whereas a contact time of 20 – 30 seconds is typical with low pressure lamps (EPA, 2011; USEPA, 1999).
- Capital and operating costs are low compared to alternative disinfection methods.
- No contact tanks are required for UV treatment in comparison to chlorine treatment.

2.4.2 PUV Technology

Flash-output or pulsed UV light differs to mercury vapour-based LPUV light by utilising xenon (or sometimes krypton) gas to generate a high energy electron pulse which lasts microseconds. The system typically comprises of three parts; the power supply, the pulse configuration system and the flash lamp (Elmnasser *et al.*, 2007). An alternating current is stored in a capacitor where energy is discharged to create

an intense pulse of light which spans across the polychromatic broadspectrum of UV, visible and infrared light (Wang *et al.,* 2005). Thus, the emission wavelength range of PUV lamps is significantly larger at 200-1100 nm in comparison to monochromatic LPUV at 253.7nm (Figure 2.5). The conversion efficiency of electrical to UV irradiation is lower for xenon lamps at approximately 10-15% in comparison to LPUV lamps which are reported to have a coefficient of efficiency (CE) of approximately 35-50% (Schaefer *et al.,* 2007; Vasilyak, 2009). The high peak power stored in the capacitor is a trademark of this system which, it is has been proposed, offers the advantage of shorter treatment times (Oms-Oliu *et al.,* 2010). PUV light is thought to exhibit stronger germicidal/inactivation properties due to a greater photon flux which can target both the cell membrane and protein structures rather than the deoxyribonucleic acid (DNA) structure alone. In addition, the use of xenon gas is considered more environmentally friendly than the standard mercury vapour LPUV lamps (Gómez-López *et al.,* 2007).

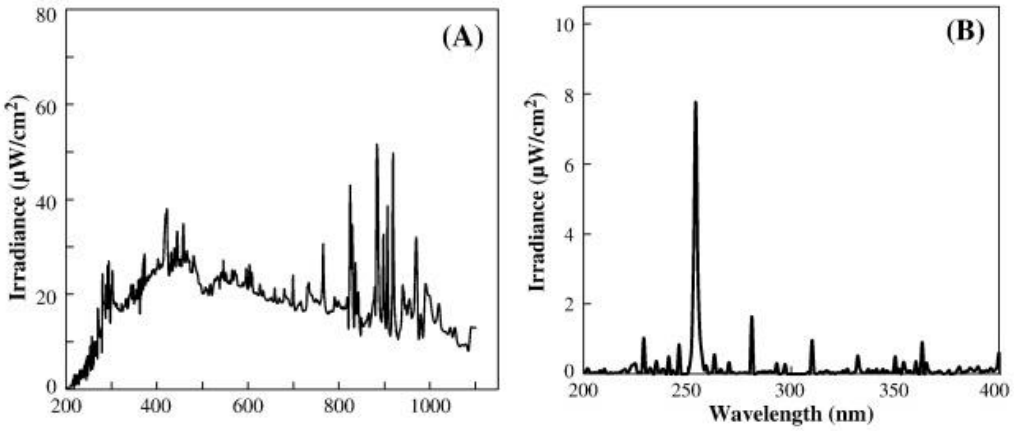


Figure 2.5. Wavelength emission spectrum of (A) pulsed light system (B) and conventional low pressure UV light system (Cheigh *et. al.,* 2013).

Flow-through PUV systems are relatively unexplored in the water/wastewater disinfection sector but are noted as a promising alternative to LPUV as studies

suggest high energy outputs may decrease contact times and inhibit photoreactivation (Uslu *et al.,* 2016). However, questions remain as to whether the intensity and/or peak power of the applied PUV fluence for water treatment applications has a considerable impact on microbial inactivation in comparison to LPUV applications. The application of PUV as a microbial disinfection method within the food industry is widely accepted with the technology being approved by both the EU and the United States Food & Drug Administration (FDA) (Elmnasser *et al.,* 2007). This decontamination process is considered a favourable alternative to conventional thermal/chemical disinfection processes however currently, it is predominantly applied as a disinfection method for food packaging and to a lesser extent as a decontamination method for food products themselves (Heinrich *et al.,* 2016). The vast majority of the published literature pertaining to PUV studies describe experimentation analysis performed under static conditions i.e. samples are fixed below the lamp and so little information has been published regarding the disinfection potential of the system using a continuous 'flow-through' experimental set-up *(*Krishnamurthy *et al.,* 2007; Uslu *et al.,* 2016).

2.4.3 The UV Disinfection Process

The ultraviolet light spectrum is subdivided into four categories; vacuum UV (100-200 nm) UV-C (200-280 nm), UV-B (280-315 nm) and UV-A (315-400 nm) (USEPA, 2006). While it is the UV-C range which is generally considered to be the most germicidal (as it is within this wavelength that peak germicidal efficiency coincides with peak UV absorption by bacterial DNA), microbial disinfection can also occur within the UV-A and UV-B range and has been shown to produce a different mechanism of inactivation in comparison to UV-C light (Harm, 1980; Lee

& Ko, 2013). Studies investigating the inactivation potential of violet-blue light, comprised of UV-A and visible light wavelengths of between 380-500 nm, report the destruction of microorganisms to be brought about by photodynamic inactivation. This method of inactivation employs exogenous photosensitizers e.g. porphyrins which, in the presence of oxygen become excited when exposed to violet-blue wavelengths. Once excited, the photosensitizers produce reactive oxygen species (ROS) such as hydroxyl radicals which can lead to cellular damage in a wide variety of microorganisms including bacteria, viruses, fungi, yeasts, and parasites (Tomb *et al.*, 2018). Studies comparing the lethal effects of UV-A and UV-B light found a similar mechanism of inactivation for both wavelength ranges but concluded that UV-B light was the most germicidal of the two (King *et al.*, 2008; Lee & Ko, 2013). Despite microbial inactivation being possible within both the UV-A and UV-B wavelength ranges, the energy required to achieve microbial inactivation via these wavelengths has been shown to be significantly higher in comparison to that of UV-C light thus very often it is the UV-C wavelength range that is applied in standard low pressure UV disinfection in water/wastewater systems (Tomb *et al.*, 2018).

Peak germicidal efficiency can be described as the wavelength at which a UV system delivers the most energy to a medium, for example low pressure mercury vapour lamps radiate approximately 85% of total UV intensity at 253.7 nm within the UV-C range (Masschelein, 2002). Figure 5.6 illustrates the peak absorbance of UV light for *E. coli* at approx. 260 nm in addition to the emission wavelengths for monochromatic low pressure UV light and polychromatic medium pressure UV light. The optimum wavelength or 'action spectrum' for the inactivation of microorganisms varies as not all DNA absorbs UV light at the same wavelength.

This is due to differences in nucleic acid base ratios (Jin *et al.,* 2007). For instance, the wavelength at which *E. coli* is most susceptible to UV radiation is 265 nm while *Bacillus subtilis* (*B. subtilis*) spores have been found to be most vulnerable at 270 nm (Chen *et al.,* 2009). The notoriously UV resistant adenovirus (AdV) has been shown to be readily inactivated at both 220 nm and 228 nm to a significantly higher degree than when exposed to wavelengths of 239 nm and 260 nm (Linden *et al.,* 2007). More recently, a similar study found the loss of AdV viral infectivity to be 16 times greater at 210 nm in comparison to 254 nm (Beck *et al.,* 2014). The spectral sensitivity of another virus, *MS2* coliphage was also found to be three times higher near a wavelength of 214 nm using a polychromatic light than when exposed to a LPUV lamp at the typical 254 nm output (Beck *et al.,* 2015a; Mamane-Gravetz *et al.,* 2005). Thus, one could argue in this case that targeted polychromatic light e.g. pulsed UV could be a more effective inactivation method in comparison to LPUV (particularly for viruses) due to a variation in UV-C spectrum output.

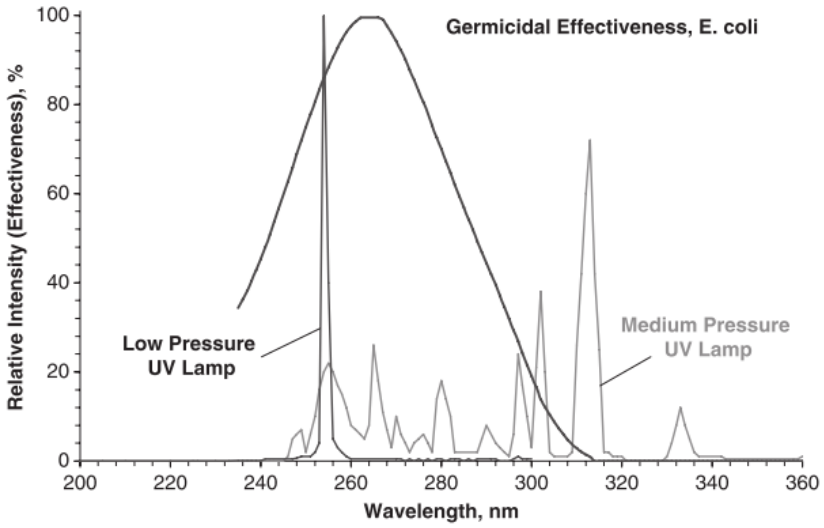


Figure 2.6. Comparison of the germicidal efficiency of low pressure and medium pressure UV lamp outputs with % effectiveness for *E. coli* (Kowalski, 2009).

2.4.4 Methods of Microbial Inactivation

To date, studies investigating the various types of UV disinfection systems have identified three main mechanisms in which UV rays can inactivate microbial cells; (i) photochemical (Cheigh *et al.,* 2012; Rowan *et al.,* 1999; Sinha & Häder, 2002; Wang *et al.,* 2005) (ii) photothermal (Dunn, 1989; Wekhof, 2000) and to a lesser extent (iii) photophysical (Wekhof *et al.,* 2001; Takeshita *et al.,* 2003). It should be noted that there tends to be an overlap between the photothermal and photophysical inactivation mechanisms whereby the former may sometimes be a precursor to the latter. Hence, the majority of studies have also reported evidence of cell death attributable to a combination of either two or all three mechanisms (Cheigh *et al.,* 2012; Gómez-López *et al.,* 2007).

Microbial inactivation via a standard LPUV system is a photochemical process. UV photons are absorbed by either the DNA (protozoa, bacteria and some viruses) or

ribonucleic acid (RNA) (some viruses) which create molecular lesions rendering the organism incapable of reproduction. Various molecular lesions can be produced, however the two major classes are described as pyrimidine adducts and cyclobutane-pyrimidine dimers (Sinha & Häder, 2002). Pyrimidines refer to three of the five nucleic bases; thymine, cytosine and uracil while guanine and adenine are defined as purines. Purines are ten times more resistant to photochemical radiation in comparison to pyrimidines and thus less susceptible to DNA damage as it is the pyrimidines which will be damaged in the first instance of UV exposure (Bintsis *et al.,* 2000). In DNA, thymine is considered the most sensitive pyrimidine while in RNA it is uracil. However fewer studies have focused on the effects of UV radiation on RNA genomes in comparison to DNA genomes (Simonet & Gantzer, 2006). Thus, the primary dimers formed in DNA by UV disinfection are known as thymine dimers (Kowalski, 2009).

The photothermal effects of UV radiation are typically associated with pulsed UV systems due to the high density radiation emitted from the light pulse. It is recognised that shorter pulse durations (at any given system output) result in increased energy deliverance and in turn, increased germicidal effect be it photochemical or otherwise (Dunn, 1989; Heinrich *et al.,* 2016). Increases in the temperature of agar, food and liquid mediums post pulsed light treatment have been reported (Krishnamurthy *et al.,* 2007; Farrell *et al.,* 2010; Cheigh *et al.,* 2013). The thermal resistance of microorganisms vary i.e. a bacterial spore is generally more resilient than a vegetative cell, however it is estimated that for stable disintegration to occur, a temperature of at least 130ºC is required to bring about irreversible changes (Vasilyak, 2009). Wekhof (2000) analysed previous research studies for the potential

thermal effects of flash lamps on *E. coli* in an aqueous solution. It was deduced in this study, mathematically, that the heating of bacteria could indeed be the dominant physical process during UV radiation using a high intensity flash lamp (Wekhof, 2000). Further studies have confirmed this; *A. niger* spores were found to severely rupture after exposure to intense PUV irradiation in the absence of UV-C light where a fluence energy of 1 J/cm^2 resulted in a 4.8 $\log_{10}$ inactivation (Wekhof *et al.*, 2001). Extensive cell wall damage of *Listeria monocytogenes* (*L. monocytogenes*) was also reported when the pathogen was exposed to pulsed UV at a total UV fluence of approx. 1.6 J/cm^2, however the authors did not confirm a direct relationship between cell damage and microbial inactivation (Cheigh *et al.,* 2013). The photophysical effects of pulsed light has been observed during the inactivation of yeast cells whereby cell membrane damage induced by PUV was thought to contribute to yeast inactivation independent of molecular disruption (Takeshita *et al.,* 2003). Severe cellular destruction including mesosome rupture, cell wall damage and content leakage of *S. aureus* have been reported after just five seconds of pulsed UV light treatment (total energy of 4.95 J/cm^2). Results also concluded an insignificant temperature increase thus indicating the cause of inactivation to be photophysical (Krishnamurthy *et al.*, 2010). A comparative study between continuous UV and pulsed light for photothermal/photophysical effects have found cells treated with continuous UV to remain fully intact (Cheigh *et al.*, 2012).

It should be noted that there have been very limited studies published on holistic cell and molecular activities surrounding the UV destruction of spoilage or microbial pathogens. However, Farrell *et al.,* (2011) investigated the relationship between increased PUV fluence on the simultaneous and sequential occurrence of

lethal effects of the yeast *Candida albicans* (Farrell *et al.,* 2011). Specifically, this study highlighted that PUV differs from LPUV in that the former causes irreversible cell death through a multi-hit biocidal process when yeast were treated on agar surfaces or in PBS suspension. Moreover, Farrell and co-workers (2011) used protein leakage and propidium iodide (PI) uptake stains to demonstrate that the increases in cell membrane permeability in PUV-treated yeast depended on the amount of UV pulses applied. This finding correlated well with the measurement of increased levels of lipid hydroperoxidation in the yeast cell membrane. PUV-treated yeast cells also displayed a specific pattern of intracellular ROS generation, where ROS were initially localised in the mitochondria after low levels of pulsing (UV dose 0.82 $\mu J/cm^2$) before more wide-spread cytosolic ROS production occurred with enhanced pulsing. The measurement of the corresponding fluence rate at each applied pulse was determined using chemical actinometry. Intracellular ROS levels were measured using specific mitochondrial stains (dihydrorhodamine 123 and dichloroflurescin diacetate). Moreover, during the initial phases of PUV treatment, cell membrane damage also includes the production of toxic free radicals within the cytosol. Where the cells have been exposed to a very strong biocidal fluence then additional cell damage occurs in macromolecular constitutes such as DNA and at the cell membrane leaks or bursts. The latter process is referred to as irreversible permeabilisation. Cells treated at this level of high fluence also transition to what is known as an 'apoptotic state (or programmed cell death)' where interestingly, detection of these apoptosis markers in these cells coincides with failure to grow on agar plates and irreversible cell death (Farrell *et al.,* 2011). This study also highlighted that the inactivation inflicted by PUV on targeted microorganisms is

caused by a multi-hit cellular and molecular approach and this may explain why complex microorganisms such as the waterborne enteroparasites *Cryptosporidium parvum* or *Giardia lamblia* are sensitive to broad spectrum light encompassing 200 nm to 1100nm range (Rowan *et al.,* 2015). A similar multi-hit process was not evident using LPUV where the focus was predominantly on DNA destruction (Farrell *et al.,* 2011; Rowan *et al.,* 2015).

2.4.5 Susceptibility of Microorganisms to PUV

Literature regarding the groups and susceptibility of various microorganisms to static pulsed UV is well established (Elmnasser *et al.,* 2007; Gómez-López *et al.,* 2007; Oms-Oliu *et al.,* 2010). Studies have reported gram-positive bacteria to be more resistant to pulsed light in comparison to Gram-negative bacteria (Anderson *et al.,* 2000; Farrell *et al.,* 2010; Rowan *et al.,* 1999) while fungal spores were shown to be more resistant than either Gram-negative or Gram-positive bacteria. (Anderson *et al.,* 2000). The authors postulate that Gram-negative enteropathogens e.g. *E. coli* may be more PUV susceptible as they typically exist and proliferate in the absence of light (Rowan *et al.,* 1999). In contrast, Gómez-López *et al.,* (2005) found no clear pattern in the sensitivities of a range of microorganisms citing high inactivation levels as a limiting factor for statistical analysis.

Pigmentation in the cell walls of spores is thought to play a role in UV resistance. Dark i.e. black pigments in cell wall layers of *A. niger* spores were found to enhance protection from pulsed light whereas *Fusarium culmorum* (a fungal pathogen) spores lacked such pigments and thus were more readily inactivated via PUV (Anderson *et al.,* 2000). However, a separate study concluded green pigmentation in *Aspergillus repens* to exhibit increased resistance properties to intense pulsed light in comparison

to *A.niger* and *Aspergillus cinnamomeus* which produce black and brown spore pigmentation respectively (Turtoi & Nicolau, 2007). Structure or coat proteins have also been found to contribute significantly to the resistance of bacterial spores. Studies have shown *B. subtilis* spores to be more resistant to pulsed irradiation when expressing proteins linked to coat formation in comparison to mutated strains where said proteins were absent (Esbelin *et al.,* 2016). The cellular damage of *Listeria innocua* (*L. innocua*) and *E. coli* via PUV was also compared via conventional plate counts and viability staining. Results indicated a loss of bacterial cultivability at low UV doses which did not correspond to the loss of cellular functions indicating that cells may have entered a dormant phase also referred to as a 'viable but non-culturable' (VBNC) state post PUV disinfection (Kramer & Muranyi, 2013; Kramer *et al.,* 2015).

2.4.6 Photoreactivation & Dark Repair

A primary drawback of UV disinfection is that of microbial DNA damage repair post disinfection via either photoreactivation (PHR) or dark repair. Lethal dimers caused by UV photons may be repaired or replaced by the microorganism either by (i) using enzymes that require light to repair DNA; PHR or (ii) employing enzymes that replace damaged DNA with undamaged nucleotides; excision or dark repair (Goosen & Moolenaar, 2008; Sinha & Häder, 2002).

The recovery of UV damaged nucleic acids in the absence of light (dark repair) is possible via a complex multienzymatic process that requires energy. Typically, an undamaged strand of DNA can act as a copy for complementary-strand replication resulting in new DNA (Harm, 1980). Two types of dark repair pair pathways are possible; base excision repair (BER) and nucleotide excision repair (NER). BER

utilizes glycosylases to detect damged nucleotides, the nucleotide is then removed using endonuclease and replaced with new synthesised DNA via DNA polymerase. NER, on the other hand, employs a protein complex (UvrA-UvrB complex) to scan for damaged DNA and does not require endonucleases for damage detection and nucleotide repair (Sinha & Häder, 2002).

Photoreactivation or light repair is predominatly associated with bacteria and endospores with little evidence of PHR occurring in animal viruses. This may be due to viruses lacking enzymes, however viral photoreactivation has been reported to occur via host-cell repair mechanisms (Kowalski, 2009). DNA repair enzymes, photolyases, are highly efficient at reversing the damaging effects of UV irradiation by utilizing near-UV or blue light (320-500 nm) to cleave cyclobutane-pyrimidine dimers (see section 2.4.4) allowing the cell to recover damaged DNA (Figure 2.7). (Essen & Klar, 2006). In terms of UV disinfection, studies have shown a relationship between UV energy and relative numbers of dimers whereby the restoration of DNA is unattainable i.e. a high enough UV dose can cause irreversible DNA damage however this is dependant upon the microorganism, the UV wavelength applied and the experimental conditions (Guo *et al.,* 2009; Kowalski, 2009).

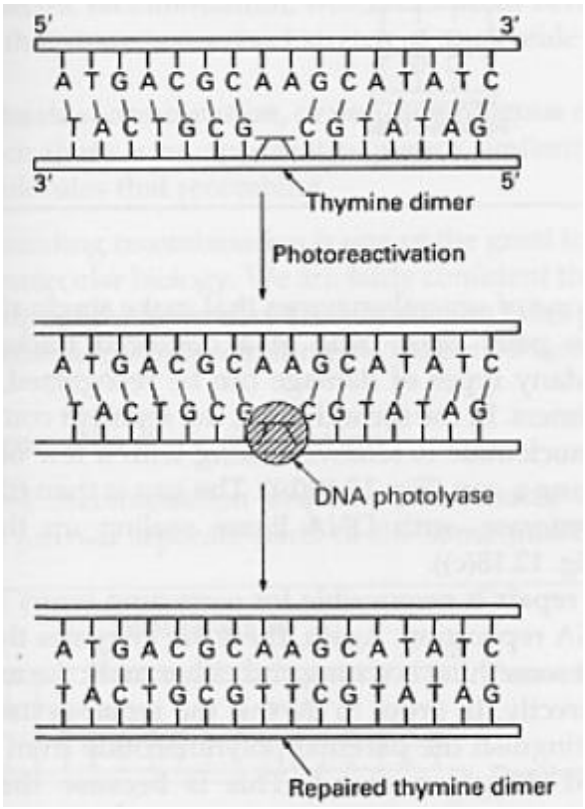


Figure 2.7. Mechanism of photoreactivation using DNA photolyase (Ingraham & Ingraham, 1995).

Research involving PHR of viruses post LPUV treatment in wastewater reported little or no reactivation particularly in the case of single stranded RNA viruses (Baron, 1997; Rodriguez *et al.*, 2014). However, single stranded DNA viruses such as adenovirus have exhibited photoreactivation in cell culture infectivity assays (Eischeid *et al.*, 2009). Studies investigating photorepair potential of protozoan parasites is limited. It has been reported that the infectivity of *Cryptosporidium parvum* was not restored post LPUV treatment for both PHR and dark repair conditions (Oguma *et al.*, 2002; Zimmer, Slawson & Huck, 2003). Li *et al.*, (2008) found *Giardia lamblia* (*G. lamblia*) trophozoites (the feeding/motile stage of life cycle, non-infective) to survive or be reactivated following exposure to a LPUV dose of up to 10 mJ/cm^2 while at an increased dose of 100 mJ/cm^2 there was no evidence

of survival or reactivation. Further studies have investigated both the PHR and dark repair potential of *G. lamblia* cysts (infective stage) post LPUV exposure and have reported restoration of infectivity at low UV doses (1 mJ/cm^2) but not at high UV doses (16 to 40 mJ/cm^2) (Shin & Linden, 2015).

At low UV doses (5 mJ/cm^2) Guo *et al.*, (2009) reported photoreactivation rates for *E. coli* exposed to LPUV in wastewater however no photoreactivation was detected for *E. coli* after treatment via LPUV at the higher UV dose of 15 mJ/cm^2. Investigations of PHR potential of *Mycobacterium terrae* post LPUV disinfection in drinking water yielded evidence of photoreactivation within 30 minutes of treatment (Bohrerova & Linden, 2006). Research carried out on *L. monocytogenes* disinfected via PUV (UV dose not given) on agar plates confirmed photoreactivation of the bacteria after 4 hours of light exposure post treatment (Gómez-López *et al.*, 2005). More recently, PHR was observed for both *E. coli* and *L. innocua* post PUV treatment on gel mediums with an *E. coli* recovery rate of up to 2 log; the PUV dose applied was 0.45 – 1 J/cm^2 with relative recovery being energy dependant (Kramer *et al.*, 2015). A similar study also concluded *L. Innocua* to exhibit photoreactivation post PUV treatment (UV dose 0.16 – 0.4 J/cm^2) in the presence of daylight and cited temperature as an important factor post PUV treatment (Lasagabaster & de Maranon, 2014). Comparison of photoreactivation rates between *E. coli*, total coliforms and *B.subitilis* in reclaimed wastewater showed *E. coli* to have the highest level of PHR while no detectable PHR was reported for *B. subtilis* strains post LPUV disinfection (UV dose 5 mJ/cm^2). The authors also concluded a good correlation between PHR and UV dose (R^2 = 0.963) and dark repair to be of less significance in comparison to PHR (Guo *et al.*, 2011).

Therefore, previous research suggests that for the same microorganism, photoreactivation capabilities are strongly influenced by the type of UV treatment applied i.e. the UV dose/output and the conditions of the photoreactive light used e.g. exposure time and temperature. In the water/wastewater disinfection sector PHR is of primary concern when considering incorporating UV systems in the disinfection process as microbial re-growth can influence system effectiveness (Bohrerova & Linden, 2007). Investigations into whether PHR occurs in bacteria disinfected via a flow-through PUV set-up has yet to be determined and would prove valuable if the system were considered to be scaled up as a full-scale UV disinfection technology.

2.4.7 UV Dose Determination

UV dose refers to the energy of the radiation per unit area (mW/cm^2) multiplied by exposure time (s) and is typically presented in units of mJ/cm^2 or $mW.s/cm^2$ (Eq. (2.1)).

$$D = I \text{ x } t \tag{2.1}$$

Where: I = intensity, mW/cm^2 or $mJ/s.cm^2$ and t = exposure time, s.

In a laboratory setting, a collimated beam apparatus can be used to deliver a controlled amount of UV energy to receiving samples, thus the UV dose may be accurately determined. In contrast, LPUV reactors installed at drinking water/wastewater treatment plants may be subject to varying water flow dynamics, varying influent water quality and varying UV intensities within the reactor (due to lamp aging and fouling etc) (Barrett *et al.,* 2016; C. Farrell *et al.,* 2018; Metcalf &

Eddy, 2004; USEPA, 2006). These variations can in turn lead to inaccuracies when determining the exact UV dose delivered. For this reason, each UV reactor should undergo validation testing whereby biodosimetry is used to determine a 'reduction equivalent dose' (RED) from the reactor (Austrian Standards Institute (ÖNORM), 2001; DVGW, 2006; Fitzhenry *et al.*, 2016; USEPA, 2006). The process involves measuring the inactivation of a robust or 'challenge' microorganism after it has been exposed to UV light within the reactor and then comparing the inactivation level to a UV dose response curve of the same microorganism ascertained under controlled laboratory conditions using a collimated beam apparatus - from this a RED may be determined (NWRI, 2012).

Methods of PUV fluence measurement are generally carried out in one of the two following ways; (i) calculation of energy delivered by the power system via theoretical mathematics i.e. energy output (ii) measurement of energy received by the sample via a detector or chemical actinometry i.e. absorbed dose. In the case of the latter approach, the high energy intensity, short pulse duration and polychromatic spectrum associated with PUV systems create difficulties in measuring UV irradiance via a traditional radiometer (as it is designed to measure monochromatic light). Variances in the spectral emittance over the entire wavelength range of PUV coupled with the fact that not all wavelengths are considered 'germicidally active' deem the direct reading of PUV fluence from a radiometer unsuitable (Jin *et al.*, 2007). Instead, pyroelectric detectors have, more recently, been used as an alternative method of measuring the exceptionally high energies emitted from PUV systems (Vimont *et al.*, 2015; Uesugi *et al.*, 2016; Uslu *et al.*, 2016; Xu & Wu, 2016; Kramer *et al.*, 2017; Rajkovic *et al.*, 2017). The sensors have a higher threshold of maximum

measurable energy however caution should be noted that it is the 'total broadspectrum dose' that is being measured and not the UV dose alone. Previous researchers have incorporated the use of UV filters in combination with the detector to determine the proportion of UV content emitted from the PUV system in question (Kramer & Muranyi, 2013).

As pulsed UV is a relatively new research area in comparison to continuous wave LPUV, authors have warned that precautions should be taken when reporting energies received (absorbed dose) by the sample as they differ significantly to the energy delivered (system output) (Gómez-López *et al.,* 2007). Recent studies have shown variables such as PUV voltage, treatment time and distance from the lamp to be significant factors in microbial inactivation, adding to the issues that should be considered when reporting on PUV systems (Pollock *et al.,* 2017). The literature surrounding PUV experimental data is varied with some authors choosing to report experimental conditions not as PUV fluence but as the number of pulses or flashes applied (Anderson *et al.,* 2000; Gómez-López *et al.,* 2005; Rajkovic *et al.,* 2009; Rowan *et al.,* 1999), the lamp discharge energy applied (Farrell *et al.,* 2009; Farrell *et al.,* 2010), the exposure time applied (Takeshita *et al.,* 2003) or the broadspectrum energy per pulse applied (Uslu *et al.,* 2016). The lack of a standard protocol for PUV fluence measurements hinders the opportunity for comparative analysis and reproducible tests. Gómez-López & Bolton (2016) expressed significant concern regarding the experimental design of research in the PUV field alluding to the fact that fundamental laws governing pulsed light action have sometimes been overlooked. The authors attempted to address the challenges associated with determining the exact PUV dose delivered by suggesting a standard method of PUV

dose determination in water by way of chemical actinometry methods (Gómez-López & Bolton, 2016). Chemical actinometry or dosimetry is a well-established method of irradiance measurement whereby the chemical compound i.e. the actinometer undergoes a light-induced reaction at a particular wavelength with a known quantum yield (Kuhn *et al.*, 2004). The photoproducts created from the reaction may then be measured via absorbance readings and further quantified to give the absorbed irradiance in $W.cm^2$. The revised protocol for PUV fluence measurements calls for energy calculations to be reported in terms of photon irradiance using the units einstein/(m^2/s) and further suggests the fluence rate be weighted by the germicidal action spectrum of the target microorganism. Specific instrumentation is required which includes a collimated beam apparatus, a spectrophotometer and a spectroradiometer. Thus, while the method may be the most precise measurement of absorbed PUV dose, challenges remain in terms of resources and experimental capacity. In addition, the reporting of UV fluence as einstein/(m^2/s) and not mJ/cm^2 is in contrast to existing convention in the wastewater industry which has consistently employed the latter units and thus conversions would be necessary.

2.4.8 Comparison of LPUV and PUV UV Dose/Output Requirements

As the power emitted from pulsed UV lamps is generally at least an order of magnitude higher than the power emitted from LPUV lamps this is reflected in the respective UV dose requirements of the system for microbial inactivation. Table 2.2 compares the UV dose requirements of PUV and LPUV systems for the $\log_{10}$ inactivation of various microorganisms. The UV dose/output measured or calculated for the PUV systems typically includes the full polychromatic broadspectrum energy in comparison to monochromatic energy output for LPUV systems at 254 nm. Some

studies investigating PUV disinfection efficiency include only the energy emitted from wavelengths in the UV range (approximately 200-400 nm) and calculate the UV dose/output of the system accordingly while others include both the 'total broadspectrum fluence/dose' and the 'UV fluence/dose' (Bohrerova & Linden, 2007; Lee *et al.*, 2009; Vimont *et al.*, 2015). A lack of clarity regarding how the UV dose for PUV systems is determined can hinder replication studies and does not allow for direct comparisons (Gómez-López & Bolton, 2016). For example, in Table 2, Kramer *et al.,* (2015) reported a PUV energy requirement of 450 mJ/cm^2 for a 3-4 $\log_{10}$ inactivation of *E. coli* DSM 498 whereas Uslu *et al.,* (2015) reported sixteen times that amount of PUV energy for a 5.6 $\log_{10}$ inactivation rate of the same strain. Incidentally, while Uslu *et al.,* (2015) reported the energy as 'UV dose' in the paper, in fact it was the energy output of the system that was used to calculate a final broadspectrum energy output. Hence, a lack of uniformity in PUV energy reporting methods clearly inhibits comparison between studies.

Table 2.2 UV dose/output (mJ/cm^2) requirements of PUV and LPUV systems for the log$_{10}$ inactivation of microorganisms.

Microorganism	Medium	UV System	UV dose/output (mJ/cm^2)*	Log inactivation	Reference
E. coli DSM 498	Polysaccharide gel	PUV	450[1]	3-4	(Kramer *et al.*, 2015)
E. coli DSM 787	Saline suspension	LPUV	8[2]	4.2	(Zimmer & Slawson, 2002)
E. coli DSM 498	Synthetic wastewater	PUV	7,200[3]	5.6	(Uslu *et al.*, 2015)
B. subtilis DSM 10	Spore suspension	PUV	1,000[1]	2.7	(Artíguez & Martínez de Marañón, 2015)
B. subtilis ATCC 6633	Synthetic wastewater	PUV	8,800[3]	5.3	(Uslu *et al.*, 2015)
B. subtilis ATCC 6633	Spore suspension	LPUV	40.4[2]	5.0	(Bolton *et al.*, 2010)
L. monocytogenes 10493S	Culture liquid medium	PUV	3,200[1]	3.10	(Uesugi *et al.*, 2016)
L. monocytogenes 10493S	Culture liquid medium	LPUV	33[2]	3.14	(Uesugi *et al.*, 2016)
Murine Norovirus	PBS solution	Static PUV	1,450[3]	3.77	(Yi *et al.*, 2016)
Murine Norovirus	Groundwater	Flow-through PUV	4,300[3]	3.35	(Yi *et al.*, 2016)
Murine Norovirus (CW3)	PBS	LPUV	29[2]	4.0	(Park *et al.*, 2011)

[1]broadspectrum dose
[2]UV dose
[3]system output
*where energies presented in Table 2.2 were calculated by the authors or based on system output the term "output" has been applied. If the energies were measured at the sample surface the term "dose" has been applied.

2.4.9 LPUV and PUV Comparative Studies

A comparative study has shown static PUV to inactivate both *L. monocytogenes* and *E. coli* O157:H7 by 7 $\log_{10}$ at treatment times of 180 and 150 seconds, respectively, in comparison to static LPUV which required a treatment time of 1200 seconds to achieve 4 and 5 $\log_{10}$ inactivations, respectively; however the output applied by the PUV system was two orders of magnitude higher than that of the LPUV lamp (Cheigh *et al.,* 2013). Recently, Holck *et al*., (2018) compared pathogen inactivation via LPUV and PUV and observed LPUV to require less energy for the inactivation of *Salmonella*, *L. monocytogenes* and *E. coli* on eggs (Holck *et al.,* 2018). Limitations remain when attempting to compare LPUV and PUV systems for the inactivation of pathogens as the UV dose/output of the PUV system is often not clarified, or experimental conditions are not fully detailed. For example, studies which list only exposure times/number of flashes create difficulty in determining the exact broadspectrum dose/output (Montgomery & Banerjee, 2015; Rajkovic e*t al.,* 2009). In addition, studies regarding the comparative inactivation efficiency of flow-through PUV and continuous-flow LPUV system have yet to be established.

2.4.10 Factors Affecting UV Disinfection

The performance of a standard UV system is dependent on a number of factors which include contact time, lamp fouling and influent water quality. The presence of organic compounds in a water body is thought to have a negative effect on UV efficiency as cations such as iron and manganese can cause fouling on the lamp sleeve, which decreases UV transmission through the water column. Moreover, high concentrations of iron in water can absorb UV light before it reaches the target microorganism. Iron may also adsorb onto suspended solids particles preventing UV

light penetrating these particles and thus protecting entrapped microorganisms (Das, 2001). Lee *et al.,* (2009) demonstrated decreasing *E. coli* inactivation efficiency with increasing concentrations of humic acids using a novel PUV system (Lee *et al.,* 2009). Furthermore, a study by Lazarova *et al.,* (1999) reported a significantly improved inactivation rate of MS2 bacteriophage via LPUV using filtered effluent compared to unfiltered effluent (Lazarova *et al.,* 1999).

It is widely accepted that UV disinfection processes are negatively affected by the presence of suspended particles as they can either decrease UV transmittance through the water/wastewater or shield the target microorganism from UV irradiation (Christensen & Linden, 2003; Emerick *et al.,* 1999; Loge *et al.,* 1999; Mamane, 2008; Templeton *et al.,* 2009). Studies have shown the particle association of coliform bacteria to wastewater colloids to enable bacterial persistence through UV disinfection processes (Emerick *et al.,* 1999). Madge & Jensen (2006) investigated the impact of organic suspended solids particle size on UV disinfection efficiency. Wastewater samples from two different WWTPs were processed via a fractionation technique to separate particle-associated bacteria into three size fractions (<5 µm, 5 - 20 µm and >20 µm) after which they were exposed to LPUV light. Results showed the smallest size fraction to produce a significantly faster disinfection rate in comparison to the largest fraction indicating that particle size influences UV disinfection (Madge & Jensen, 2006). Similarly, Carré *et al.,* (2018) reported large particles (>25 µm) to both scatter UV light and shield bacteria inside the particles (Carré *et al.,* 2018). Other studies have cited particle sizes of >7 µm and >10 µm to impact upon coliform LPUV inactivation rates in filtered secondary effluent (Jolis *et al.,* 2001; Wang *et al.,* 2006). While previous studies have investigated the efficiency

of flow-through PUV disinfection in synthetic and real wastewater, the impact of suspended solids present in the sample wastewater on PUV system inactivation efficiency was not evaluated by Uslu *et al.,* (2016).

2.4.11 UV Disinfection Regulations

There are three primary UV reactor validation guidelines/protocols that are observed internationally; the US Environmental Protection Agency (US EPA) UV Disinfection Guidance Manual (UVDGM), the German Association for Gas & Water (DVGW W294) and the Austrian Standards Institute (ÖNORM M 58373-1) (Austrian Standards Institute (ÖNORM), 2001; DVGW, 2006; USEPA, 2006). The UVDGM is typically applied for UV reactor validation testing in North America however as a guideline document it does not set out one standard protocol but rather recommends validation protocols based on the type of water source to be treated and the target pathogen in question. The RED is determined using the biodosimetry of a surrogate challenge microorganism with a similar UV sensitivity to the specific target pathogen requiring inactivation e.g. MS2 bacteriophage may be used as a surrogate organism for the inactivation of *Cryptosporidium* (USEPA, 2006). In Europe, the primary accepted protocols for UV reactor validation tend to be the ÖNORM and the DVGW protocols. Both protocols stipulate that all UV reactors are required to supply a constant minimum UV dose of 40 mJ/cm^2 with the DVGW specifying *Bacillus subtilis* endospores to be used as the challenge microorganism for biodosimetry analysis (Austrian Standards Institute (ÖNORM), 2001; DVGW, 2006).

2.5 Pathogens of Interest in the Dairy Industry

The microbiological contamination of cow's milk can occur when it comes in to contact with the external surfaces of the cow (teats, faeces and hides), cow feed and bedding as well as milking and farm equipment (Vacheyrou *et al.,* 2011). The concentration and diversity of microorganisms that enter milk via the various routes is significant and while some microorganisms can contribute to human health by aiding digestion etc., others can cause severe illness (Quigley *et al.,* 2013; Verdier-Metz *et al.,* 2012). Human pathogens of interest in the dairy industry include *Staphylococcus aureus*, *Campylobacter spp.*, *Salmonella spp.*, *Listeria monocytogenes* and *Escherichia coli*, all of which have been detected in 'bulk tank milk' i.e. tankers which transport milk from dairy farms to processing plants (Muraoka *et al.,* 2003; Van Kessel *et al.,* 2004; Karns *et al.,* 2005, 2007; Oliver *et al.,* 2005; Antognoli *et al.,* 2009; Haran *et al.,* 2012; Weber *et al.,* 2014; Sonnier *et al.,* 2017). Each of the above pathogens are listed as notifiable diseases to the national Health Protection Surveillance Centre (HPSC) and are the causative agents of enterotoxigenic *Staphylococcus aureus*, campylobacteriosis, listeriosis and *E.coli* infection respectively (HPSC, 2016). While the pasteurisation process (the heat treatment of milk to remove pathogenic microorganisms) destroys most foodborne pathogens, some have been known to persist in biofilms while others can produce thermo-resistant enzymes which allow them to remain active after pasteurisation (Teh *et al.,* 2011). Moreover, some milk products do not undergo the pasteurisation process e.g. cheese which heightens the risk of food-related outbreaks (Carrique-Mas *et al.,* 2003; Rosengren *et al.,* 2010; Hennekinne *et al.,* 2012). Information regarding dairy pathogens of interest is described further below.

2.5.1 *Staphylococcus aureus*

Staphylococcus is a genus of Gram positive, facultative aerobe/anaerobe, nonsporulating bacterium which are ubiquitous in the environment and may be found in dust, air, water, sewage, environmental surfaces, animals and humans (Hennekinne *et al.,* 2012). Currently, there are 77 recognised species and subspecies however it is the coagulase-positive staphylococci (CPS) which produce enterotoxins responsible for food poisoning outbreaks (Hennekinne *et al.,* 2012; Peters *et al.,* 2017). Among the CPS, it is *Staphylococcus aureus* enterotoxins which are associated with outbreaks related to processed meat and dairy products *(*Argudín *et al.,* 2010). *S. aureus* is commonly detected in bulk tank milk and is the cause of both animal and human illnesses such as bovine mastitis and toxic shock syndrome (Fox & Gay, 1993; Peters *et al.,* 2017). Heat-stable enterotoxins can withstand pasteurisation processes and persist in dairy products causing gastrointestinal illnesses which include symptoms of nausea, diarrhoea and vomiting (Argudín *et al.,* 2010; Quigley *et al.,* 2013). Food poisoning outbreaks of *S. aureus* have been linked to a variety of contaminated foods including milk, cheese and beef (Asao *et al.*, 2003; Hennekinne *et al.,* 2012).

2.5.2 *Campylobacter spp.*

Campylobacter spp. are spiral-shaped, Gram negative, microaerophilic, non-spore-forming, fastidious, commensal organisms whose normal habitat is the gastrointestinal tract of warm-blooded domestic and wild animals, predominantly birds (Bolton, 2015). Although bacterial concentrations as high as 10^8 CFU/g have been detected, colonisation does not cause harmful effects to the animal but can have major consequences for human health (Meade et al., 2009). Most human infections

are caused by *Campylobacter jejuni* (*C. jejuni*) and transmission to humans is often associated with the handling and/or consumption of poultry contaminated during the processing stage (Humphrey *et. al.,* 2007). Campylobacteriosis outbreaks are also associated with contaminated water and dairy products, primarily unpasteurised or raw milk (Del Collo *et al.,* 2017; Pitkänen, 2013). Dairy cows are reported to shed *C. jejuni* in high numbers which can lead to faecal contamination of the milk and further transference to milk tankers (Oliver *et al.,* 2005; Rapp *et al.*, 2012). The onset of disease symptoms (abdominal pain, fever, headache, nausea, and/or vomiting) usually occurs 2-5 days post infection and typically last 3-6 days (WHO, 2018a). Campylobacteriosis is the most common cause of bacterial gastroenteritis in Ireland and Europe and is a notifiable disease to the HPSC with 2,513 cases being reported in Ireland in 2016 (HPSC, 2016). *Campylobacter spp.* are sensitive to heat treatments thus the thorough cooking of meats and avoidance of unpasteurised milk is encouraged (Silva *et al.,* 2011).

2.5.3 *Salmonella*

Salmonella spp. are responsible for 298,000 or 41% of global diarrheal disease-associated deaths annually (Besser, 2018). *Salmonella* typhimurium and *Salmonella* enteritiditis are the two primary etiological agents of salmonellosis that infect humans in Ireland and the EU (Carrasco *et al.,* 2012; HPSC, 2017). Of the family Enterobacteriaceae, *Salmonella* is a Gram negative, rod-shaped, facultative aerobic bacteria which is typically transmitted via food-producing animals such as poultry, swine and cattle (Eng *et al.,* 2015). Thus, foodborne outbreaks typically involve uncooked animal food products and dairy products, particularly raw milk and unpasteurised cheese (Verraes *et al.,* 2015). Food poisoning may also be brought

about by cross-contamination of raw/cooked food during the preparation process e.g. lettuce may become contaminated with bacteria on a surface where raw meat had been previously prepared. The detection of *Salmonella spp.* in bulk tank milk is frequently reported, with faecal contamination during milking being the most likely transmission route (Karns *et al.,* 2005; Oliver *et al.,* 2005; Sonnier *et al.,* 2017). Clinical manifestations of the pathogen include enteric fever, gastroenteritis and bacteraemia depending on the strain and geographical region. Enteric fever (typhoid) is much more prevalent in underdeveloped countries while serotrypes responsible for gastroenteritis are the dominant strain reported in Europe and North America (Eng *et al.,* 2015). Preventative measures to avoid *Salmonella* outbreaks related to the dairy industry include milk pasteurisation however studies have shown pasteurised milk to be linked to salmonellosis outbreaks where the heat-treatment process was adequate (Olsen *et al.*, 2004).

2.5.4 *Listeria monocytogenes*

Listeria monocytogenes poses a serious health risk in the food industry as it can proliferate in a wide range of challenging environments e.g. broad pH ranges, in both aerobic and anaerobic conditions, at high salt concentrations and at low temperatures (psychotrophic) i.e. refrigerated foods (Lundén *et al.,* 2004; Barba *et al.*, 2017). *L. monocytogenes* also has the capacity to form biofilms which can prolong bacterial survival periods of up to ten years in some instances (Swaminathan & Gerner-Smidt, 2007). This Gram positive bacterium is ubiquitous in nature and can be isolated from environmental, raw and processed foods and has been linked to foodborne outbreaks involving soft cheeses, raw milk, fish, meat and even fruits (Barba *et al.,* 2017; Carrique-Mas *et al.,* 2003; Hunt *et al.,* 2012). The associated disease Listeriosis has

can be categorised as either invasive or non-invasive. Non-invasive listeriosis is considered a mild form of the disease which affect otherwise healthy people, symptoms are typical of gastroenteritis and include diarrhoea, fever, headache and muscle pain (WHO, 2018b). Invasive listeriosis is a much more severe form of the disease (e.g. meningitis, encephalitis and sepsis) and has a high mortality rate of 20-30% which is much more pronounced in people belonging to the YOPI group (young, old, pregnant, immunodeficient) (Lundén *et al.*, 2004; Verraes *et al.*, 2015; WHO, 2018b). While heat treatment can effectively destroy *L. monocytogenes*, their ability to adapt and survive in a variety of environments increases the risk of contamination, particularly in low temperature, ready to eat foods.

2.5.5 Shiga toxigenic *E. coli* (STEC)

E. coli O157:H7 is a strain of *E. coli* which produces a toxin called shiga toxin. Because of this it is known as shiga toxigenic *E. coli* (STEC) also known as verocytotoxigenic *E. coli* (VTEC). STEC are considered highly pathogenic to humans and can cause severe diarrhoeal and systemic illness which can progress to hemolytic uremic syndrome in some cases (Karmali *et al.*, 2010). The primary reservoir for STEC are ruminants, specifically cattle, and so food products of bovine origin remain the predominant vehicle for *E. coli* O157:H7 outbreaks e.g. ground beef, raw milk and raw milk products (Rangel *et al.*, 2005). However, STEC is becoming increasingly linked to other domestic animals including sheep and goats where studies have detected STEC in bulk tank ewes' milk, goat milk and goat farm environments (Álvarez-Suárez *et al.*, 2016; Ercoli *et al.*, 2016). In 2016, 6,378 confirmed cases of STEC infections were reported to the EU with the highest notification rates observed in Ireland (Brehony *et al.*, 2018; EFSA, 2017). The

zoonotic disease is not only transmitted to humans via animals but also through person-to-person contact and the ingestion of contaminated water and foodstuffs (Kintz *et al.,* 2017). The pathogen exhibits a very low infectious dose of <100 cells which heightens the risk of contracting the disease and the frequency of outbreaks. Garvey *et al.,* (2016) reviewed STEC outbreaks in Ireland over an 8 year period and reported poorly treated private water supplies as a significant transmission route (Garvey *et al.,* 2016). In July, 2018, the HPSC advised the public to be extra cautious following a rise in the number of reported STEC cases which was over three times as high as those reported in July 2017 (HPSC, 2018). Thus, caution should be observed when considering the treatment of water/wastewater for reuse purposes in a dairy environment to ensure adequate microbial disinfection is achieved.

2.5.6 *Bacillus cereus*

Bacillus cereus (*B. cereus*) is a human pathogen which causes two different types of food poisoning in humans; diarrhoeal syndrome (diarrhoea and abdominal pain) and emetic syndrome (nausea and vomiting) (Granum & Lund, 1997). It can be found in marine and fresh waters, decaying organic matter, vegetables and fomites and the gut microflora of invertebrates and from which milk and foodstuffs may become contaminated (Borge *et al.,* 2001; Bottone, 2010). *B. cereus* endospores are particularly problematic for the dairy industry as pasteurisation temperatures are insufficient at destroying spores and there is evidence of psychotrophic behaviour which allows for growth at low temperatures (4-6ºC) (Andersson *et al.,* 1998; Soni *et al.,* 2016). Moreover, they also have the ability to form biofilms on stainless steel surfaces and areas within processing plants that are difficult to access and clean (Soni *et al.,* 2016). *B. cereus* infection is a notifiable disease to the Health Protection

Surveillance Centre (HPSC) in Ireland however no cases were reported between 2010-2014 (FSAI, 2016). It has also been suggested that incidences of the illness may be under-reported as symptoms are generally self-limiting and do not require visits to doctors. An outbreak in 2014 in 12 EU countries resulted in 89 hospitalisations and no deaths (FSAI, 2016).

2.6 *Bacillus* Endospores

As mentioned in Section 2.5.6, *B. cereus* endospores are a problem for the dairy industry due to their human pathogenicity and resulting food poisoning outbreaks within the global food sector. Studies have shown bacterial spores to be up to two orders of magnitude more resistant to UV-C irradiation in comparison to their vegetative counterparts with endospore UV resistance to be a product of molecular interactions that occur during the stages of sporulation, dormancy and germination (Slieman & Nicholson, 2001). Malayeri *et al.,* (2016) rank *Bacillus* endospores as one of the most UV-C resistant organisms alongside protozoan cysts and the highly UV resistant adenovirus (Malayeri *et al.,* 2016). It is *Bacillus pumilus* (*B. pumilus*) endospores which exhibits comparable UV resistance to AdV while *Bacillus subtilis* endospores have long been deployed as a challenge organism for on-site UV reactor validation. Therefore, for the purposes of this literature review, this section will also provide details on *B. pumilus*, *B. subtilis* and *B. cereus* endospores due to their importance within the food, water and wastewater industries.

Bacillus endospores are Gram positive, rod shaped, aerobic or facultative anaerobic, spore forming bacteria that are ubiquitous in soils but can inhabit a wide range of environments (Checinska *et al.,* 2015). Sporulation of bacteria occurs in response to adverse environmental conditions e.g. desiccation, nutrient limitation, pH and

temperature wherein the bacteria enter a metabolically inert, dormant state (Soni *et al.,* 2016). The components of an endospore include a core, enveloped in a cortex layer which is then surrounded by a proteinaceous shell or 'spore coat' (Henriques & Moran, Jr., 2007). Some bacterial endospores contain a second outer layer called an exosporium while others lack the structure; *B. cereus* endospores exemplify the former while *B. subtilis* endospores are defined as exosporium-less (Stewart, 2015). Endospores in their dormant state are resistant to environmental stresses which include acidity, heat, radiation, oxygen/water depletion and lack of nutrient availability (Wells-Bennik *et al.,* 2016). Once endospores sense favourable changes in their environment, the germination process begins and the bacteria return to their vegetative state (Figure 2.7).

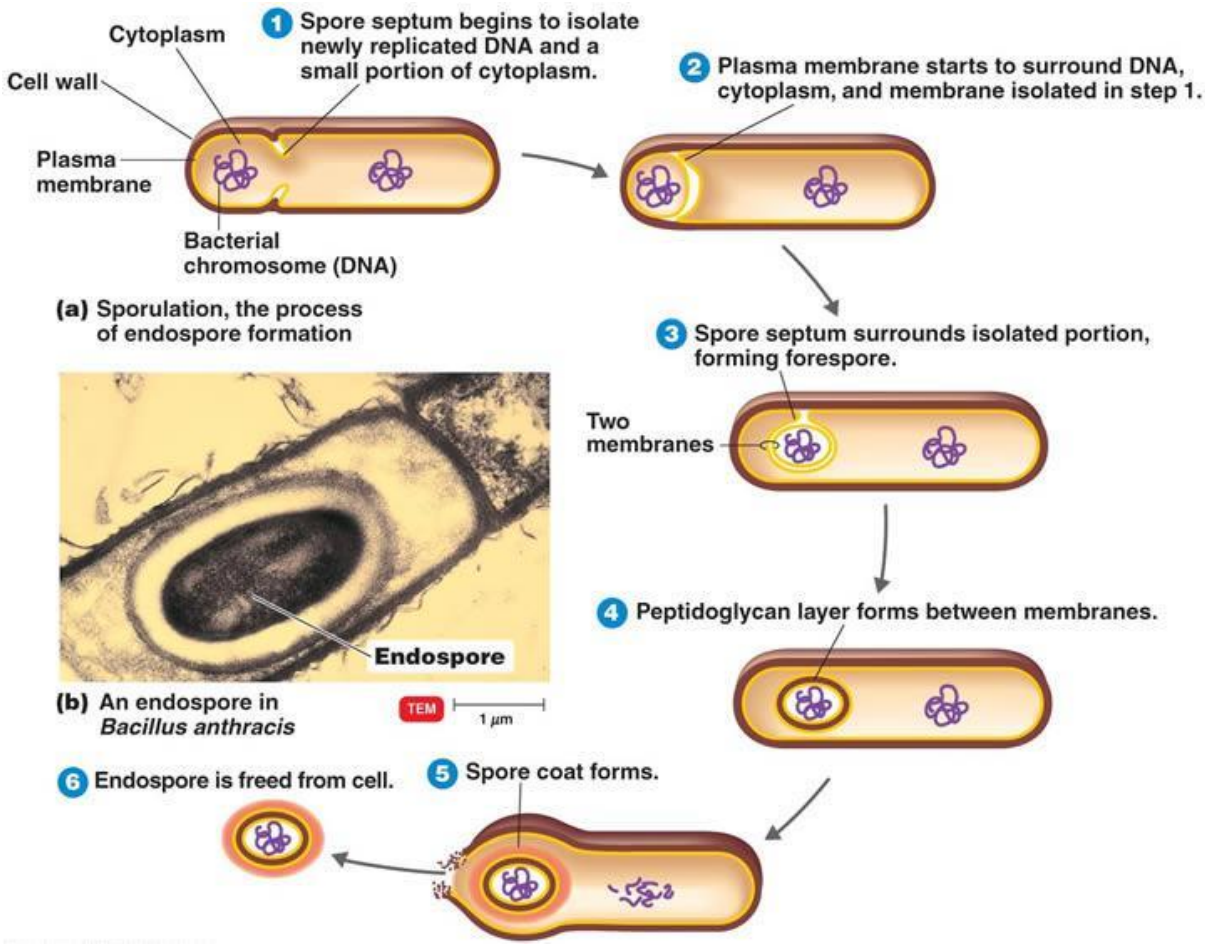


Figure 2.7. (a) Stages of endospore formation and (b) Microscopic image of *Bacillus anthracis* endospore (Pearson Education Inc., 2010).

2.6.1 *Bacillus pumilus* Endospores

Bacillus pumilus, formerly *Bacillus intermedius*, is ubiquitous in the environment and resistant to extreme environmental conditions owing to its phylogenetic diversity and spore dispersal (Han *et al.*, 2017; Liu *et al.*, 2013; Sharipova *et al.*, 2011). The bacterium is recognised as non-pathogenic however exceptional cases of human infection have been reported (Tena *et al.*, 2007). *B. pumilus* endospores are noted to be the most UV resistant *Bacillus* endospores with a reported UV dose requirement of 272 mJ/cm^2 for a 4 $\log_{10}$ inactivation credit (Boczek *et al.*, 2016). In recent years *B. pumilus* endospores have been suggested as a potential surrogate for human

adenovirus (hAdV) in UV reactor validation trials due to their comparable UV resistance (Boczek *et al.*, 2016; Rochelle *et al.*, 2010; Verhoeven *et al.*, 2012). Moreover, the spectral response of *B. pumilus* also matches that of hAdV whereby it is shown to be more sensitive to UV wavelengths below 254 nm, specifically those wavelengths around 220 nm (Beck *et al.*, 2015). To date, fewer studies have investigated *B. pumilus* endospores' response to UV inactivation in comparison to *B. subtilis* and *B. cereus* endospores and the majority of those involved UV-C inactivation only using LPUV light (Boczek *et al.*, 2016; Rochelle *et al.*, 2010; Verhoeven *et al.*, 2012). Inactivation of the endospores via pulsed UV light in static experimental conditions has also been reported however flow-through PUV studies have yet to be established (Garvey *et al.*, 2013; Levy *et al.*, 2012).

2.6.2 *Bacillus subtilis* Endospores

Like other members of the *Bacillus* genus, *B. subtilis* may be found in both terrestrial and aquatic environments. This is, in part, influenced by the endospore stage which can become aerosolised and further dispersed by wind (Jaenicke, 2005; Merrill *et al.*, 2006). *B. subtilis* endospores boast a long history in scientific research having been the model organism for investigating fundamental properties and characteristics of Gram positive spore forming bacteria as well as the study of biofilm formation (Chu *et al.*, 2006; Earl *et al.*, 2008; Stanley & Lazazzera, 2005; Vlamakis *et al.*, 2013). In the water sector, the endospores are commonly used as challenge microorganisms for UV reactor validation at drinking water treatment plants in Europe (Austrian Standards Institute (ÖNORM), 2001, 2003; DVGW, 2006). They exhibit increased resistance to UV irradiation in comparison to most other waterborne pathogens and are sensitive to wavelengths around 265 nm (Mamane-Gravetz *et al.*, 2005). Other

characteristics conducive to their use as a biodosimeter include them being non-pathogenic, easily cultivated to high concentrations and easily enumerated in an infectivity/growth assay. Thus, studies investigating the disinfection of *B. subtilis* endospores via LPUV disinfection are numerous (Malayeri *et al.,* 2016). The disinfection of *B. subtilis* endospores in wastewater effluent via a flow-through PUV system has previously been studied (once to the best knowledge of this author), however the system energies were not indicated. Instead, the $\log_{10}$ inactivation at various flow rates was reported (Uslu *et al.,* 2016). PUV flow-through inactivation studies of *B. subtilis* endospores with corresponding energy values is thus an obvious research gap.

2.6.3 Influence of $MnSO_4.H_2O$ Media Supplemention on *Bacillus* Endospore UV Resistance

The cultivation of *Bacillus spp.* endospores in a laboratory typically involves the addition of supplement manganese sulphate monohydrate ($MnSO_4.H_2O$) for rapid endospore propagation (Oh & Freese, 1976). A concentration of approximately 0.05 mM $MnSO_4.H_2O$ is recommended by the German National Resource Center (DSMZ) for the sporulation of *Bacillus spp.* strains while a concentration of up to 0.1 mM $MnSO_4.H_2O$ has been applied in a number of research studies (Artíguez & Martínez de Marañón, 2015; Beck *et al.,* 2017, 2015; Boczek *et al.,* 2016; Garvey & Rowan, 2015; Garvey *et al.,* 2013; Garvey *et al.,* 2014; Linden *et al.,* 2015; Periago *et al.,* 2006). Recent studies have shown a relationship between *Bacillus spp.* endospores cultivated on agar supplemented with $MnSO_4.H_2O$ and increased UV resistance to LPUV disinfection (Boczek *et al.,* 2016; Rochelle *et al.,* 2010; Verhoeven *et al.,* 2012). However, little information is available regarding *Bacillus spp.* endospores cultivated on agar supplemented with $MnSO_4.H_2O$ and enhanced

UV resistance to PUV disinfection. The inadvertent modification of *Bacillus spp.* endospore UV resistance may be an important consideration in laboratory scale analysis particularly as *Bacillus subtilis* endospores are currently the challenge organism of choice for UV system validation under DVGW regulations (DVGW, 2006).

2.7 Gaps in knowledge

2.7.1 The Irish Dairy Industry

The Irish dairy sector is currently showing trends of expansion following the abolishment of the CAP, the prospect of increased stringent legislation may be realised. Therefore, research on new technologies or the use of existing technologies in this field that can adapt to mitigate stricter discharge standards is timely. Furthermore, details surrounding the concentration and profile of the microbiological contamination of dairy wastewater effluent streams are lacking with the majority of studies focusing on microbial contamination at dairy farms or in milk at stages preceding dairy plant processing e.g. bulk tank milk. Knowledge of the characterisation of dairy effluent streams would be of benefit if water/wastewater reuse measures were being considered at plant level.

2.7.2 UV Technologies

The potential of PUV technology as a flow-through disinfection system is in its infancy and requires further analysis to establish whether it could be a suitable alternative to standard LPUV/MPUV systems for water/wastewater treatment. Additionally, flow-through PUV has yet to be evaluated for the treatment of dairy wastewaters with regard to investigating the impact of typical inhibitory factors such as TSS and organic carbon on system inactivation efficiency. A comparison between

LPUV and flow-through PUV systems for the inactivation of robust challenge microorganisms is merited in addition to investigating the potential for bacterial photoreactivation post flow-through PUV as opposed to prior studies which involved static PUV analysis only.

The lack of a standard 'UV dose' reporting method for PUV systems in the literature is a significant challenge in this field of research. While a standard method (Gómez-López & Bolton, 2016) has recently been proposed, issues remain around the capacity and the resources to employ such a complex (albeit necessary) approach. The implementation of basic guidelines for PUV energy reporting e.g. whether it is the UV dose or UV output of system that is being described, whether it is the broadspectrum energy and UV portion alone and simpler factors such as lamp distance would all strengthen the method by which PUV energies are reported.

2.7.3 Aims of Present Study

In the context of the gaps in knowledge identified in the literature review, the key aims of this study were as follows;

- Establish the presence/absence of dairy pathogens in water and wastewater streams from Irish dairy plants.
- Evaluate the impact of dairy wastewater parameters, in particular TSS and organic carbon on the inactivation efficiency of LPUV and flow-through PUV.
- Compare both LPUV and PUV systems for the inactivation of robust *Bacillus* endospores and to investigate whether the supplementation of culture media may influence the UV resistance of endospores to LPUV and PUV irradiation.

- Determine whether bacterial pathogens *E. coli*, *Staphylococcus aureus* and *Listeria innocua* exhibit the potential for dark repair/photoreactivation post flow-through PUV and continuous-flow LPUV disinfection.
- Compare the LPUV and PUV systems in terms of energy use and efficiency for each of the inactivation studies above.

Chapter 3
Materials & Methods

3.1 Introduction

Chapter 3 describes the experimental procedures and analysis approaches used in this study. The chapter also outlines the dairy processing plants from which wastewater and water samples were collected. The technical specifications of the UV systems deployed in this research are also discussed.

3.2 Wastewater Characterisation Analysis

Three dairy processing factories were selected for water/wastewater stream analysis ranging from factories which process milk from 50 million litres per year (Site 1) to those which process up to 1,300 million litres per year (Site 3). See Chapter 4, Section 4.3 for further information. A variety of ingredients were produced at each Site and are outlined below.

- Site 1 – Fresh products (fluid milk, cream etc.)
- Site 2 – Butter & milk powder production
- Site 3 – Cheese, whey powder, milk powder & concentrate production

Grab samples (1 – 2 L) were collected at various sampling points of the dairy processing factory which included cooling water, condensate water, wastewater treatment plant (WWTP) influent and WWTP effluent (Figure 3.1).

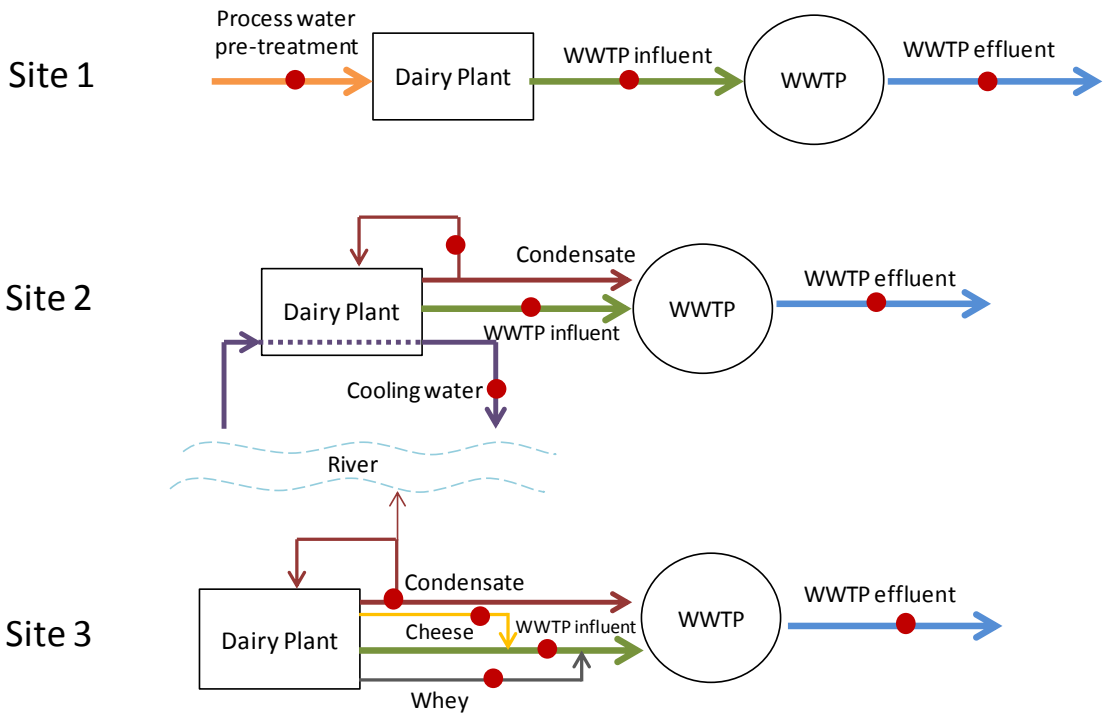


Figure 3.1. Sampling points (red circles) at the three dairy plant sites.

The samples were analysed microbiologically for heterotrophic bacteria, total coliforms and *E. coli* at the Environmental Engineering Laboratories, NUI, Galway (see Section 3.10.1 and 3.10.2 for analytical methods). One hundred mL aliquots of these samples were also sent externally for specific pathogen target analysis at an accredited laboratory, Complete Lab Solutions (CLS), Rosmuc, Co. Galway, for the analysis of *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Campylobacter spp*. and *Salmonella spp*. detection and/or enumeration (see Section 3.10.3 for further information). While NUI, Galway Environmental Engineering Laboratories are equipped to work to Biosafety level 2 standards i.e. the ability to work with pathogenic or infectious organisms, it was decided to use an external validated laboratory for the analysis of these particular samples. This was due to

concerns around threats to the health of other personnel (without microbiological laboratory practice experience) also utilizing the laboratory work space in addition to a low infectious dose associated with some of the bacterial pathogens e.g. *Listeria monocytogenes* thus further increasing the contamination risk. Each site was surveyed twice with the exception of Site 1 which was surveyed three times due to inconclusive results. As the external pathogen analysis testing varied between enumeration and/or detection tests, further details of each test carried out are outlined in Table 3.1.

Table 3.1. Test method applied to each of the water/wastewater samples from all three sampling sites.

Site	Day	Sample Type	HPC - abundance (CFU/100mL) 37°C	HPC - abundance (CFU/100mL) 22°C	Total coliforms (MPN/100mL)	*E. coli* (MPN/100mL)	*Salmonella* detection (100mLs)	*Listeria monocytogenes* detection & enumeration (CFU/100mL)	*Campylobacter spp* detection (100mL)	*S. aureus* (CFU /100mL)	*B. cereus* (CFU /100mL)
1	1	Process water pre-treatment	A	A	A	A	B	B	B	A	A
		WWTP influent	A	A	A	A	B	B	B	A	A
		WWTP effluent	A	A	A	A	B	B	B	A	A
	2	Process water	A	A	A	A	D	A	D	A	A
		WWTP influent	A	A	A	A	D	A	D	A	A
		WWTP effluent	A	A	A	A	D	A	D	A	A
	3	Process water	A	A	A	A	B	C	B	A	A
		WWTP influent	A	A	A	A	B	C	B	A	A
		WWTP effluent	A	A	A	A	B	C	B	A	A
1	1	WWTP influent	A	A	A	A	B	B	B	A	A
		WWTP effluent	A	A	A	A	B	B	B	A	A
		Condensate	A	A	A	A	B	B	B	A	A
		Cooling water	A	A	A	A	B	B	B	A	A
	2	WWTP influent	A	A	A	A	B	C	B	A	A
		WWTP effluent	A	A	A	A	B	C	B	A	A
		Condensate	A	A	A	A	B	C	B	A	A
		Cooling water	A	A	A	A	B	C	B	A	A
3	1	Cheese process effluent	A	A	A	A	B	B	B	A	A
		Mixed process effluent excluding whey	A	A	A	A	B	B	B	A	A
		Whey process effluent	A	A	A	A	B	B	B	A	A
		Condensate	A	A	A	A	B	B	B	A	A
		WWTP effluent	A	A	A	A	B	B	B	A	A
	2	Cheese process effluent	A	A	A	A	B	B	B	A	A
		Mixed process effluent excluding whey	A	A	A	A	B	B	B	A	A
		Whey process effluent	A	A	A	A	B	B	B	A	A
		Condensate	A	A	A	A	B	B	B	A	A
		WWTP effluent	A	A	A	A	B	B	B	A	A

HPC – Heterotrophic plate count, MPN – Most probable number, A – enumeration test, B – detection test, C – detection & enumeration, test D – not tested.

3.3 PUV System Analysis

The PUV system used throughout this study comprised a bench–scale pulsed power unit (PUV-01, Samtech Ltd., Glasgow) connected to a low pressure (60 kPa) xenon-filled flashlamp (Heraeus Noblelight XAP type NL4006 series encased in a clear UV transparent quartz tube) which produced a high intensity beam of broadspectrum polychromatic pulsed light (200-1100 nm) (Figure 3.2).

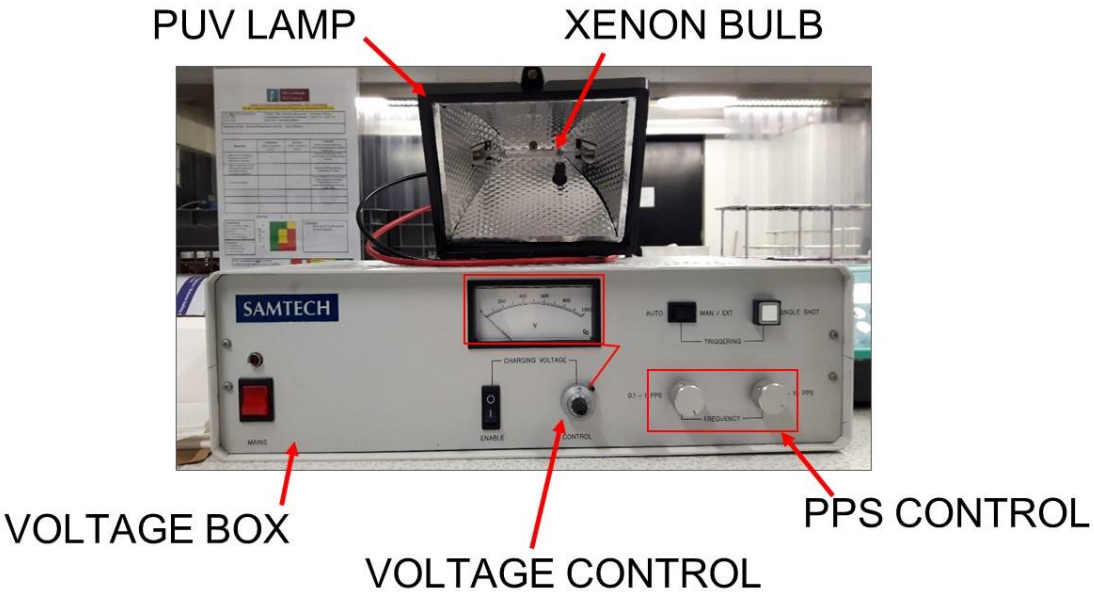


Figure 3.2. PUV-01 system with xenon flash lamp (Samtech Ltd., Glasgow).

3.3.1 Pulse Operation

PUV systems differ from conventional UV systems used in the water/wastewater industry as they produce a significantly higher intensity of UV light within a short time period. Briefly the system operates by storing electrical energy in a capacitor which is rapidly

released into the flash lamp producing the high current and peak power necessary to emit the intense UV light. On activation of a trigger circuit a high pulse trigger voltage activated the flash lamp and released the stored energy. A schematic of this process is shown in Figure 3.3.

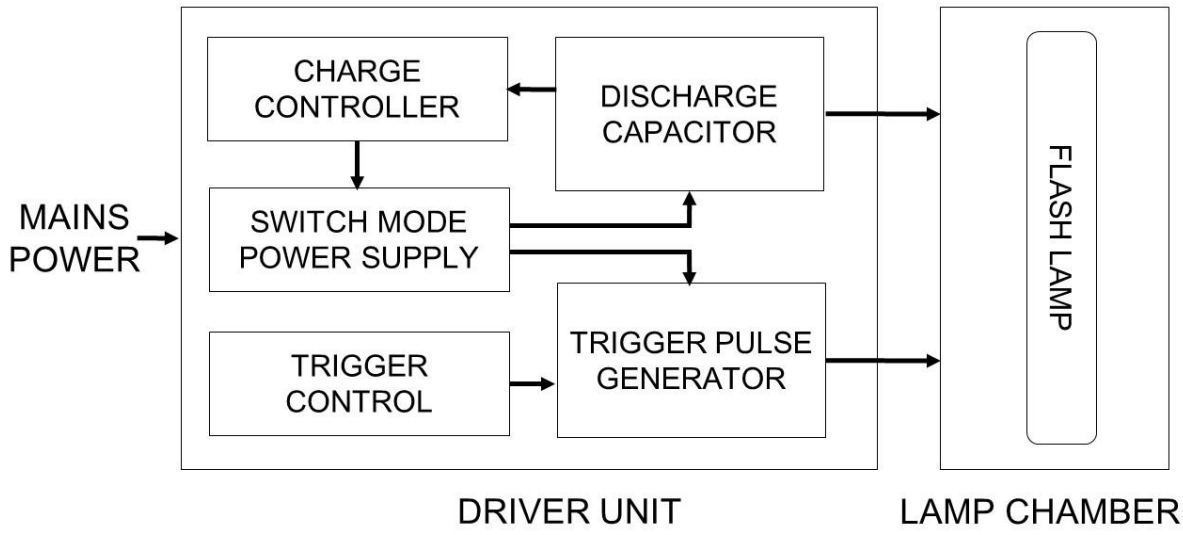


Figure 3.3. Block diagram of internal workings of the PUV system.

The pulse operating parameters of the PUV system include peak current (A), peak power (kW), peak admittance (s) and current rise/fall time for each pulse emitted at the various discharge voltages. Table 3.2 details the parameters from 500 V to 1000 V for the PUV system.

Table 3.2. Pulse operating parameters of the PUV system from 500V to 1000V (Samtech Manual; Pulsed UV System PUV-01 – available in Appendix A).

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

3.3.2 Discharge Voltage and Pulse Energy

The UV output/dose per pulse varied with the discharge voltage applied. The relationship between the two variables is shown in equation 3.1:

$$E = 0.5CV^2 \quad (3.1)$$

Where: C = 40 µF

The discharge voltage of between 400 V and 1000 V had a corresponding increasing energy output per pulse of 3.2 joule (J) to 20 J. A pulse frequency of between 0.1 and 10 pulses per second (PPS) was available (though this range was limited at various voltage settings). The discharge voltage, the corresponding energy output per pulse (J) and the maximum

PPS frequency (Hz – where 1 PPS = 1 Hz) achievable at each voltage setting are listed in Table 3.3.

Table 3.3. Discharge voltage, corresponding energy per pulse and maximum PPS frequency capacity of PUV power unit (Samtech Manual; Pulsed UV System PUV-01).

Voltage (V)	**400**	**500**	**600**	**700**	**800**	**900**	**1000**
Energy (J)	3.2	5	7.2	9.8	12.8	16.2	20
PPS max. Frequency (Hz)	10	10	10	10	7	5.6	5

3.3.3 Emission Spectrum of the PUV Lamp

The spectrum emitted by the PUV flash lamp is shown in Figure 3.4. The recorded spectra at three different voltage settings (600 V, 800 V and 1000 V) are illustrated and all follow a similar trend. Spectral peaks are visible in the UV-C region at 229 nm, 247 nm and 260 nm.

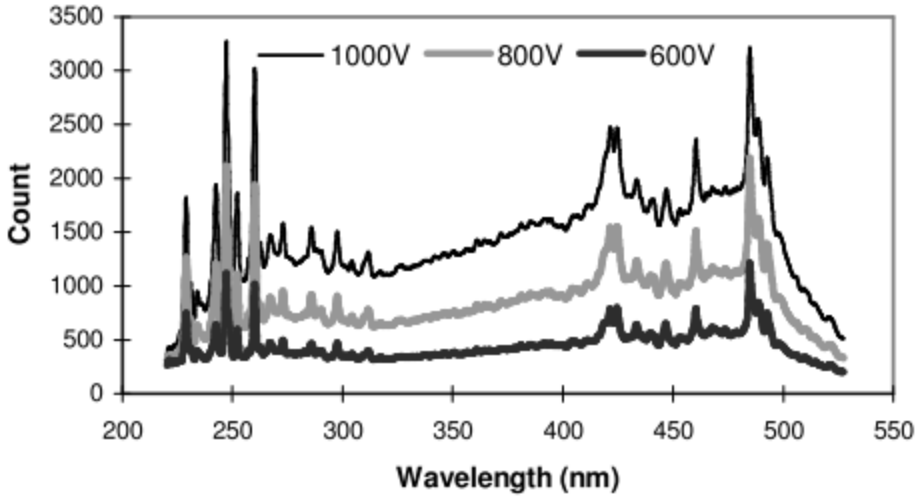


Figure 3.4. Emission spectrum of the PUV lamp at different discharge voltages (Samtech Manual; Pulsed UV System PUV-01).

The distance between the PUV lamp and the sample impacted the UV dose received by the sample. Table 3.4 outlines the portion of energy ($\mu J/cm^2$) emitted from the system (spectrum output) at a range of bandwidth regions relative to the distance between the PUV lamp and the sample. In this study, the sample was placed at a distance of 10.75 cm from the PUV lamp as this was the shortest distance achievable between the lamp and the sample vessel.

Table 3.4. Spectrum output at various bandwidth region at the distance from the UV lamp (µJ/cm^2) (Samtech Manual; Pulsed UV System PUV-01).

Distance (cm)	<300	300-400	400 - 500	500 - 600	600 - 700	> 700	Ratio < 300nm
10 cm	-	-	630	370	444	1877	-
15 cm	346	222	295	156	191	778	0.21
20 cm	166	140	168	93	112	462	0.17
25 cm	129	84	114	67	73	302	0.20
30 cm	76	57	83	42	54	215	0.17
35 cm	62	43	57	38	40	158	0.18
40 cm	40	39	48	26	30	122	0.15
45 cm	38	27	40	20	24	98	0.18
50 cm	34	21	33	16	20	79	0.20
						Average	0.1837

3.3.4 PUV Experimental Set-up

The PUV lamp was placed 10.75 cm above a sterilised aluminium flow-through vessel (with a plan surface area (SA) of 290 cm^2, sample depth of 5.5 cm and hold-up volume of 750 mL) through which water samples were pumped at various flow rates. The aluminium

vessel consisted of two influent tube ports (6.4 mm) and four effluent tube ports (6.4 mm). The flow rates corresponded to hydraulic residence times (HRTs) which also influenced final UV output determination. HRTs ranged between 60 s and 120 s (equivalent to flow rates of between 0.75 L/min and 0.375 L/min respectively) depending on the desired UV output (Barrett *et al.*, 2016). Figures 3.5(a) and 3.5(b) demonstrate the experimental set-up. Samples were collected at the influent and effluent points in duplicate for bacterial analysis.

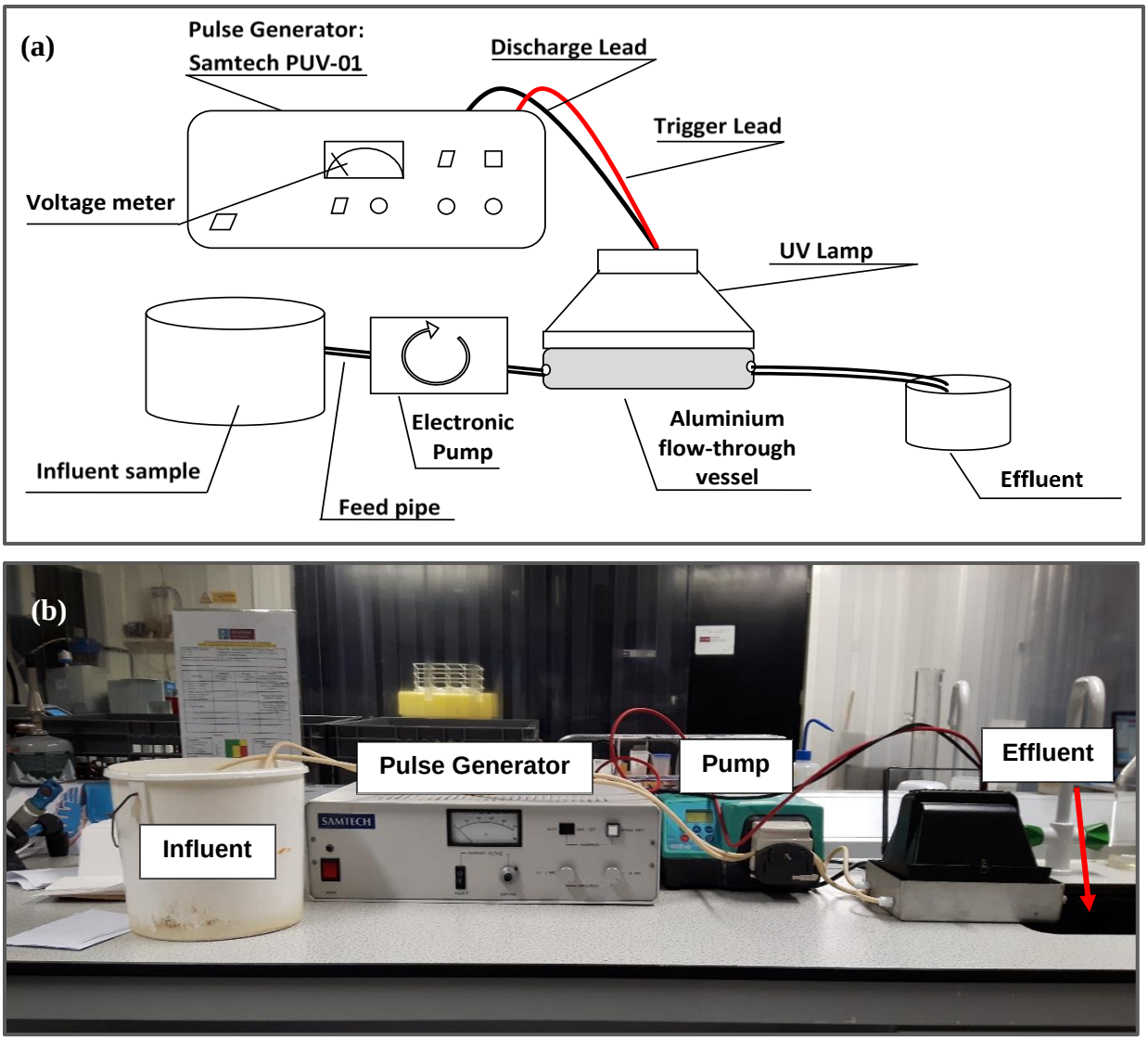


Figure 3.5 (a) Labelled schematic of bench-top experimental set-up of PUV system (b) bench-top experimental set-up of PUV system.

3.3.5 PUV Static Experiment

While not the focus of the study, a short experiment for the comparison of static PUV disinfection to flow-through PUV disinfection (using *E. coli* as a measure) was carried out for reference (Chapter 6, Section 6.2). Experimental conditions were the same as the flow-through set-up with the exception that water was not pumped through the aluminium vessel. Rather, sample water was pumped into the vessel and once full (750 mL), the pump

was stopped and the water remained static in the vessel and was pulsed for exposure times of 50 and 100 seconds at 900V corresponding to UV outputs of 513 mJ/cm^2 and 1026 mJ/cm^2 (see Section 3.3.6 for UV output determination). After the experiment was completed, samples were aseptically collected from the centre of the vessel after gentle agitation with sterile pipette and analysed as per standard methods described in Section 3.6.1. *E. coli* inactivation at each UV output was performed three times.

3.3.6 UV Dose/Output Determination

As described in Chapter 2, Section 2.4.7, there are various ways the PUV energy may be reported depending on the measurement or estimation method. In this study, it was decided to report the energy output of the lamp as the **'UV output'** of the system for experimental analysis as opposed to the PUV energy absorbed by the sample i.e. the UV dose. Thus in general, and unless stated otherwise, energy intensities are generally presented in mJ/cm^2 and describe the UV output of the lamp at the given settings (and accounting for the distance of the lamp from the sample). In Chapter 8 the results of a study of various methods of measuring and estimating the energy output of the lamp and the energy received by the sample as well as the total electrical energy used by the device are further analysed. The methods by which energy was calculated or measured are described in the following sections.

3.3.6.1 UV Output

The UV energy output (per unit area at any given sample residence time) was determined by calculating the output energy of the system (at less than 300 nm) divided by the plan area of the vessel exposed to the lamp for any given residence time. It is important to note

that the adjustable factors listed previously (voltage, pulse frequency) as well as the type of sample medium influences the penetration of the PUV light thus the energy output of the lamp can be significantly higher than the energy received by the sample. This was achieved by dividing the total broadspectrum dose by the average energy ratio (at <300 nm) of 0.1837 calculated from the spectrum output energies in Table 3.4 An example of how the UV output was calculated is shown in Table 3.5.

Table 3.5. Example of UV output calculation for PUV energy determination

Discharge voltage (V)	**Joules per pulse (J)**	**J per unit time - J/s (W)**	**E_A (W/cm^2)**	**E_A (mW/cm^2)**	**$E_{A < 300\ nm}$ (mW/cm^2)**	**HRT/ Exp. time (s)**	**UV Output (mJ/cm^2)**
900	16.2	**32.4**	**0.112**	112	**20.5**	100	**2052**

At a setting of 2 PPS the energy output per second is (16.2 J × 2 PPS) **32.4 J/s. The plan surface area of the vessel enclosing the lamp casing was** 290 cm^2 and thus the energy intensity is **0.112 J/s/cm^2. Using the ratio of energy at < 300 nm to broadspectrum energy of** 0.1837 the output of the lamp per unit time and plan area at less than 300 nm is **20.5 mW/cm^2 (0.1837 × 112 mW).** At a hydraulic residence time of 100 s (for example) the energy of the lamp per unit area would equate to **2052** mJ/cm^2 (20.5 mW/cm^2 × 100 s).

3.3.6.2 PUV Broadspectrum Dose

The total broadspectrum dose received by the sample was analysed using a thermopile power detector (Model: XLP12-3S-H2-IN, Gentec-EO, Quebec, Canada) in conjunction with Integra software. The detector was situated at the bottom of a metal cylinder 5cm in

depth through which the light was directed downwards towards the cylindrical detector surface (Figure 3.6). The detector (12 mm aperture) was placed on the aluminium vessel 10.75 cm below the xenon lamp. Pulse energies were measured three times at each voltage and each voltage setting was analysed on three separate occasions (to determine consistency/deviation of detector readings) to give nine energy readings in total after which an average value was obtained. The energy was measured in irradiance units (mW/cm^2).

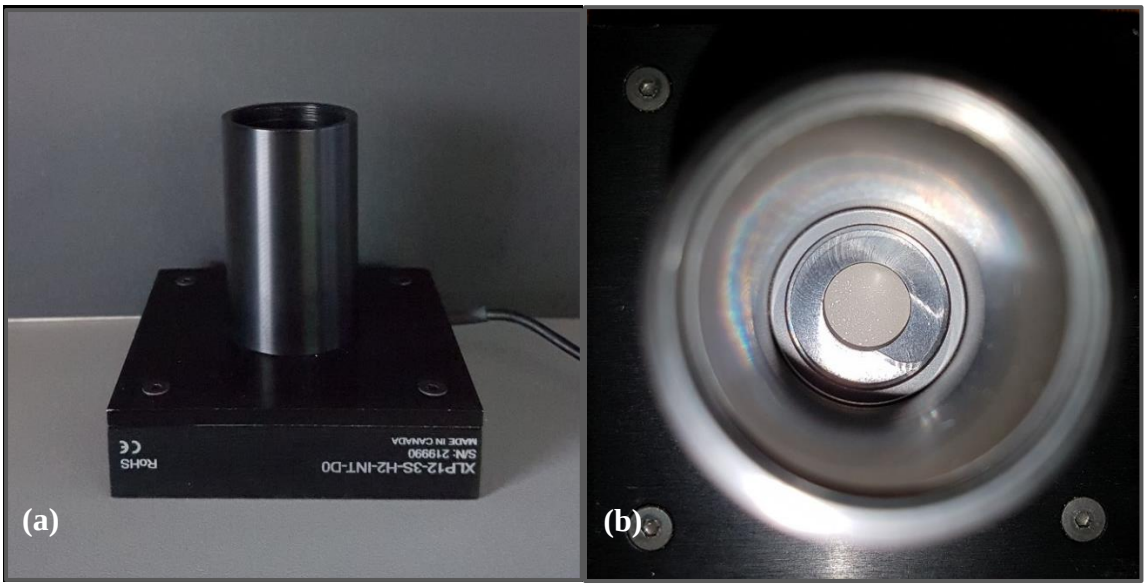


Figure 3.6. Thermopile detector for PUV energy measurements (a) Outside view of detector with 5 cm metal tube and (b) view of detector at the base of metal tube (Gentec-EO, Quebec, Canada).

In addition, two longpass colour glass filters; FGL 400 and FGL 280 (Thorlabs GmbH, Dachau, Germany) were also used to filter out the wavelengths emitted from the lamp below the UV range and below the UV-C range respectively (Figure 3.7). The filters were placed separately directly on top of the detector at the base of the metal tube and the power

measurements were taken as previously described. The differences in the power values obtained with and without the filters was used to calculate the power emitted from the lamp within the UV (Eq. 3.2) and the UV-C (Eq. 3.3) range.

$$\text{UV}\ Irradiance = Broadspectrum\ irradiance - irradiance > 400nm \quad \text{Eq. [3.2]}$$

$$\text{UV} - \text{C}\ Irradiance = Broadspectrum\ irradiance - irradiance > 280\ nm \quad \text{Eq. [3.3]}$$

The % transmission of both filters was approximately 90%.

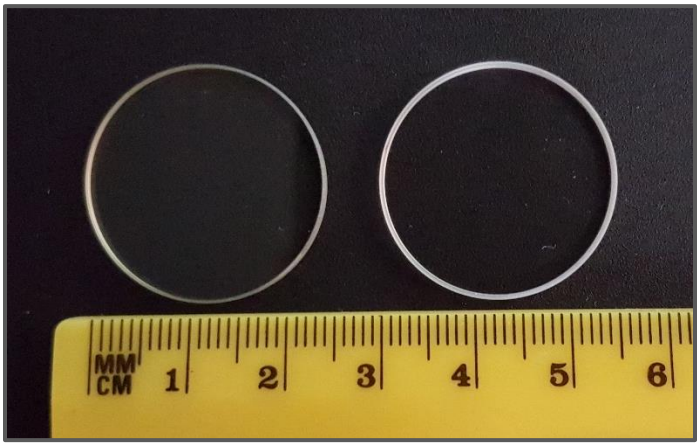


Figure 3.7. Long pass filters FGL 400 (left) and FGL 280 (right) (Thorlabs, GmbH, Dachau, Germany).

Power measurements were also measured at a number of distances from the PUV lamp with and without the longpass filters to analyse further the relationship between energy intensities at various sample distances. The distances analysed were 10.75 cm, 15.75 cm, 20.75 cm and 30.75 cm. Figure 3.8 shows the sensor placement at various distances from the PUV lamp.

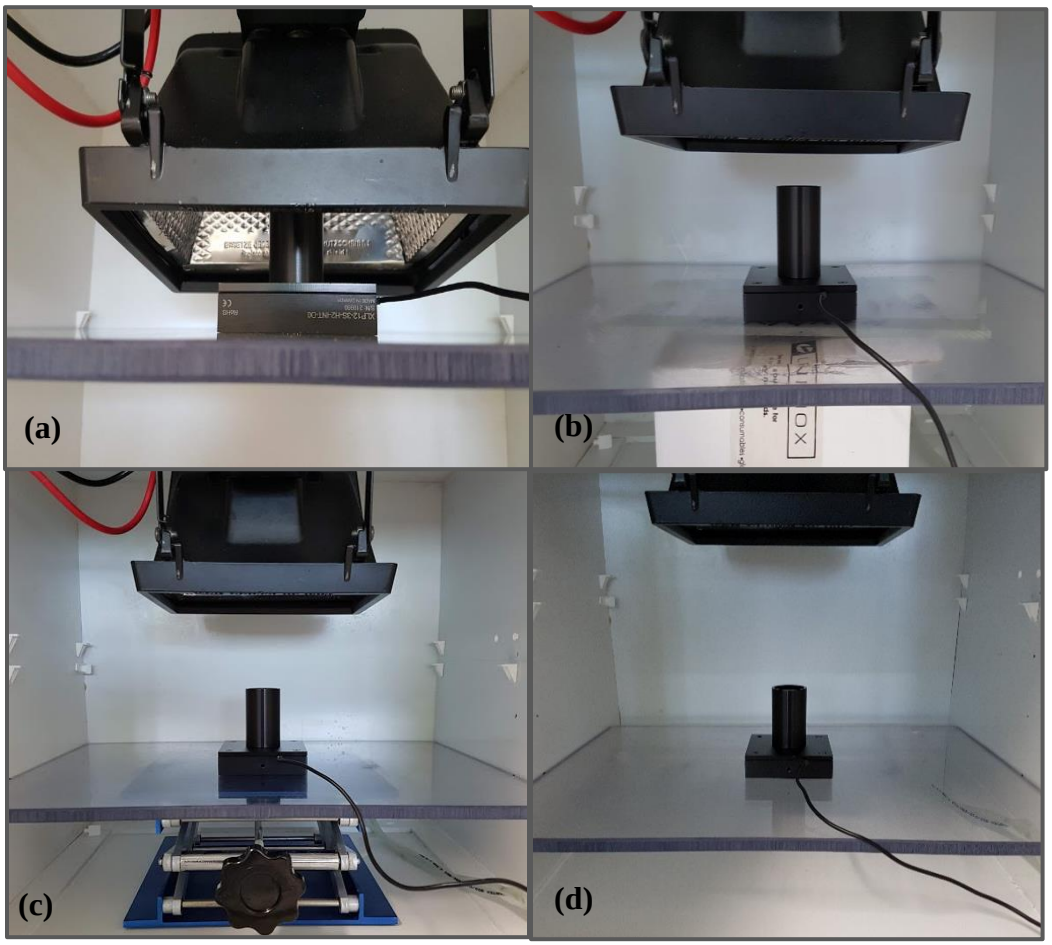


Figure 3.8. PUV broadspectrum dose measurements using thermopile detector at (a) 10.75 cm (b) 15.75 cm (c) 20.75 cm and (d) 25.75 cm.

3.4 LPUV System Analysis

The continuous-flow monochromatic LPUV system (LCD 412 Plus, S.I.T.A., Halpin & Hayward Ltd.) had a fixed power output of 40 W at a UV-C wavelength of 254 nm. The UV output could be altered by varying the influent flow rate thereby altering the HRT which equated to exposure time. The system had a volume capacity of 0.95 L with chamber

dimensions of 95 cm x 6 cm. The lamp dimensions were 84.3 cm in length x 1.6 cm diameter. The flow rates ranged between 11 L/min and 27 L/min which corresponded to HRTs of between 1.07 s and 0.42 s to give a UV output range between 31.9 mJ/cm^2 and 12.6 mJ/cm^2 respectively. The UV output was calculated using the rated energy of the system. In addition, a transmissivity sensor was also used to monitor % UV transmittance which remained above 80% at all times. A schematic figure of the system and laboratory photo is shown in Figure 3.9.

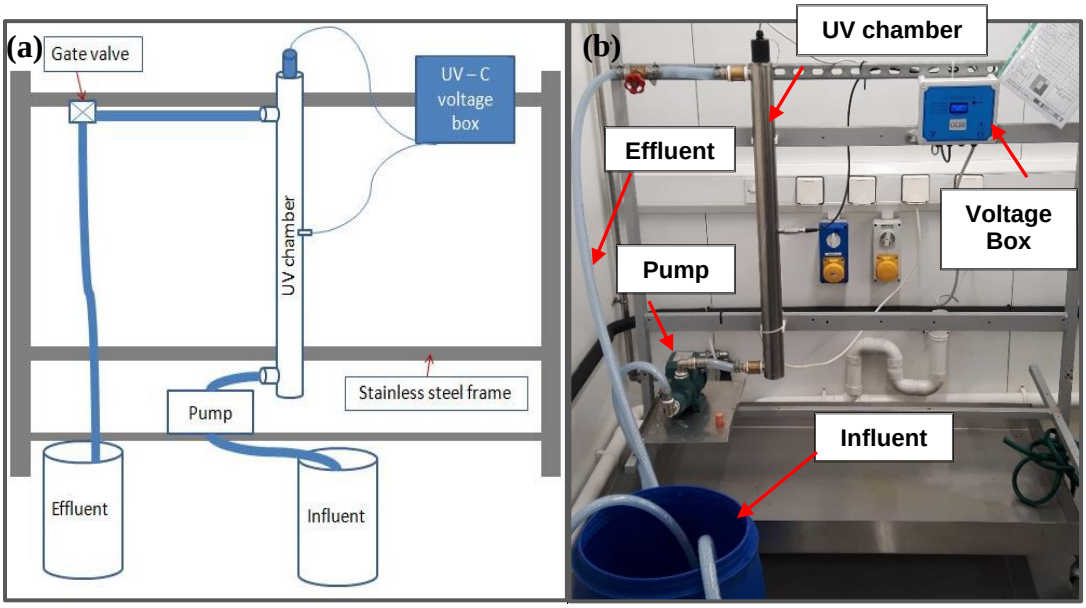


Figure 3.9. (a) Labelled schematic of laboratory-scale LPUV system (b) experimental set-up of laboratory-scale LPUV system.

3.5 UV System Wall Plug Efficiency Analysis

A standard plugin energy monitor (Gadget Plug-In Electricity Cost Monitor, Maplin Electronics, Yorkshire, UK) was used to measure the electrical energy being drawn from the wall for the PUV and LPUV systems. Energy meter readings of power (W), voltage

(V) and current (I) were collected from the meter when the systems were switched on and drawing power from the wall plug socket. In the case of the LPUV system, only one set of readings was obtained as the system had only one fixed setting available, either on or off. For the PUV system, energy meter readings were collected at (i) 400V, 600V, 800V and 1000V when the system was turned on but not pulsing (ii) 400V, 600V, 800V and 1000V when the system was turned on and pulsing at 1 PPS (iii) 800V when the system was turned on and pulsing at 7 PPS. The voltage settings were chosen to show maximum and minimum power and increments of energy in between. Energy readings at a higher PPS frequency of 7 was collected only for the 800 V setting as the energy meter was not capable of measuring reliably at higher pulse rates (or at lower pulses for higher voltages).

3.6 Propagation of *Bacillus* Endospores for UV Inactivation Studies

Pre-determined endospore populations of different *Bacillus* species were propagated on artificial laboratory-based media as a means of comparing and contrasting UV disinfection efficacy of PUV and LPUV systems. Bacterial test strains DSM 492 *Bacillus pumilus*, DSM 347 *Bacillus subtilis* and DSM 4490 *Bacillus cereus* were sourced from the German National Resource Center (DSMZ, Braunschweig, Germany). Vegetative cells of all three *Bacillus* strains were cultivated aerobically on nutrient agar (Biolab, Lennox, Ireland) at 35°C for 24 hours to produce pure colonies. The identity of these aerobic endospore formers was previously confirmed by establishing characteristic physiological and morphological properties as reported previously by (Haughton *et al.,* 2010). This confirmation process also included use of API 50 CHB and 20 E biochemical galleries (API Biomerieux, France). One pure isolated colony was transferred into 100 mL tryptic

soy broth (Fisher Scientific, Dublin, Ireland) and placed on a rotary shaker at 90 rpm for 24 hours at 30-35°C. After incubation 0.5 mL of the 24-hour culture was spread onto nutrient agar (Biolab, Lennox, Ireland) plates and incubated for up to and including four days at 35°C to encourage endospore production. *Bacillus* test strains were cultivated in the presence of $MnSO_4.H_2O$ to artificially stimulate and to expedite endospore formation for UV disinfection kinetic studies. *Bacillus* test strains were spread across surfaces of nutrient agar plates, which were supplemented with various $MnSO_4.H_2O$ (Honeywell, Sigma-aldrich, Wicklow, Ireland) concentrations ranging from 0.005 mM to 0.5 mM. After a 4-day incubation period at 35°C, a sporulation rate of approximately 90% was achieved for *B. pumilus* and *B. subtilis* which was confirmed by endospore staining (Schaeffer and Fulton spore stain kit, Sigma-Aldrich, Wicklow, Ireland) using light microscopy at 1000X (oil immersion) magnification (Figure 3.10). Sporulation rates for *B. cereus* were not as visible in comparison to *B. pumilus* and *B. subtilis* i.e. proportion of green endospores matched that or was lower than comparative pink vegetative cells.

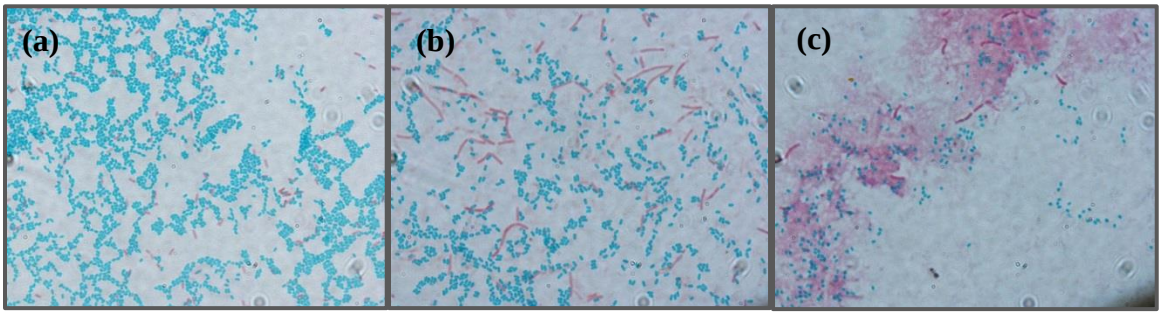


Figure 3.10. Light microscopy images of stained endospores (green) and vegetative cells (pink) of (a) *B. pumilus* endospores (b) *B. subtilis* endospores and (c) *B. cereus* endospores using a spore staining kit (Schaeffer and Fulton spore stain kit, Sigma-aldrich, Wicklow, Ireland).

Respective endospores populations for different *Bacillus* species were collected by flooding the plate with 8 mL distilled water after which a sterile spreader was used to gather endospores from the seeded plates. The water/endospore emulsion was then aseptically transferred to a sterile 30 mL sealed glass vial before submerging into a water bath at 80°C for 20 minutes to kill any remaining vegetative cells. Post heat-treatment, endospores suspended in water were used in UV trials within 4 hours. *B. pumilus* and *B. subtilis* endospores were exposed to a range of PUV outputs from 0 to 2052 mJ/cm^2 and LPUV outputs from 12 to 32 mJ/cm^2. For PUV experiments, batches of distilled water (2.5 L) were spiked with 1 mL of the heat-treated endospores to give a starting concentration of 6.6 $\log_{10}$ ± 0.7, 6.4 $\log_{10}$ ± 0.8 and 4.8 $\log_{10}$ ± 1.0 for *B. pumilus*, *B. subtilis* and *B. cereus* endospores respectively. For LPUV experiments, 25 L of tap water was spiked with 5 mL of the heat-treated endospores to give an average starting influent concentration 5.66 $\log_{10}$ ± 0.9, 6.4 $\log_{10}$ ± 0.7 and 4.0 $\log_{10}$ ± 0.55 for *B. pumilus*, *B. subtilis* and *B. cereus* endospores respectively. Efforts to augment the number of *Bacillus cereus* endospores on artificially-seeded nutrient agar plates involved adjusting relative humidity to enhance stress. It is desirable to have starting pre-determined populations of ca. ≥4 $\log_{10}$ CFU/ml in order to investigate kinetic data and to compare disinfection performances as reported previously by (Rowan *et al.,* 2015). Appropriately diluted influent and effluent samples were analysed in triplicate for each run pre and post UV treatment via pour plate technique (1 mL) using non-selective nutrient agar (Biolab, Lennox, Dublin, Ireland) and maximum recovery diluent (MRD, Sigma-aldrich, Wicklow, Ireland) as buffer solution for serial dilutions. Pour plate technique was carried out as per standard methods (APHA-AWWA-

WEF, 2012). Each UV run was performed at least three times. Log_{10} inactivation was determined as per equations in Section 3.7.

3.7 Photoreactivation Analysis

3.7.1 Dairy Pathogen Culture & UV Disinfection Analysis

Escherichia coli ATCC 25922 (sourced from LGC Standards, Middlesex, UK), *Staphylococcus aureus* DSM 1104 and *Listeria innocua* DSM 20649 (both sourced from DSMZ, Braunschweig, Germany) were used to investigate the photoreactivation and dark repair potential of dairy pathogens post PUV and LPUV disinfection. The strain *L. innocua* DSM 20649 was used as a non-pathogenic surrogate for *Listeria monocytogenes*. All three freeze-dried cultures were reconstituted using tryptic soy agar (TSA, Sigma-aldrich, Wicklow, Ireland) and tryptic soy broth (Fisher Scientific, Dublin, Ireland) at 37ºC for 18-24 hours. Fresh cultures were then inoculated aseptically on to cryobeads (Pro-Lab Microbank, Cruinn Diagnostics, Dublin Ireland) for long-term storage at -80°C. The fresh cultures were also cultured on TSA slopes as working culture stocks and stored at 4°C in the fridge. Working stock cultures were discarded every three months and replaced with fresh working cultures to avoid contamination issues.

Prior to the photoreactivation experimentation, the bacteria were exposed to both PUV and LPUV disinfection as a prerequisite to photoreactivation. For experimental analysis, one colony of each strain was inoculated into 80 mL of Luria Broth (LB) (Sigma-aldrich, Wicklow, Ireland) for *E. coli* culture and tryptic soy broth (Fisher Scientific, Dublin, Ireland) for *L. innocua* and *S. aureus*. The broth(s) were cultured on a rotary shaker at 90 rpm for 24 hours at 37°C. For LPUV runs, batches of tap water (20 L) were spiked with 10

mL (6 $\log_{10}$ ± 0.5) of broth strains (previously determined via plate count methods), while for PUV runs 2.5 L of distillate water was spiked with 1 mL (6 $\log_{10}$ ± 0.5) of broth strains. The distillation system in the laboratory produced a limited volume of water daily and could not meet the volumes required for LPUV analysis thus tap water was used instead. Experimental analysis was carried out to ensure there were no differences in inactivation rates of bacteria (*E. coli* was used as the test strain) via LPUV between both mediums (data not shown). Influent and effluent samples were analysed in duplicate pre and post UV treatment via pour plate technique (1 mL) as per standard methods (APHA-AWWA-WPCF, 2012) using Tryptone Bile X-glucuronide (TBX) Agar (VWR, Dublin, Ireland) for *E. coli* and TSA (Sigma-aldrich, Wicklow, Ireland) for *S. aureus* and *L. innocua*. Each UV inactivation experiment was performed at least three times.

3.7.2 Photoreactivation Experiment & Analysis

Immediately following UV treatment, effluent samples were placed under light and dark experimental conditions to investigate the potential for bacterial photoreactivation/dark repair. For photoreactivation (light) experiments, duplicate sample aliquots (40 mL) were placed into open petri dishes (diameter of 90 mm, surface area of 58 cm^2) and placed at a distance of 9 cm under two compact fluorescent lamps (23W power, luminous flux (Lm) 1450) which emitted light in the 300 nm to 700 nm spectral range (OSRAM model DPRO MITW 23 W/840 E27) for a maximum of 120 minutes (Figure 3.11).

Figure 3.11. Experimental set-up for photoreactivation analysis using compact fluorescent lamps (OSRAM model DPRO MITW 23 W/840 E27).

For dark repair experiments, duplicate sample aliquots (20 mL) were aseptically transferred to 60 mL tubes covered with aluminium foil and placed into a sealed box in the dark for the same duration of time as the photoreactivation experiments. Both the light and dark experimental analysis was carried out in the same incubator (Velp Scientifica) at 20 ± 1°C. Sample volumes of 1 mL were collected aseptically from both the light and dark sample experiments at a series of time intervals ranging from 0 to 120 min post UV treatment. The samples were analysed in duplicate via pour plate technique (1 mL) as per standard methods (APHA-AWWA-WEF, 2012) using TBX agar (VWR, Dublin, Ireland) for *E. coli* and tryptic soy agar (Sigma-aldrich, Wicklow, Ireland) for *S. aureus*.

3.8 Quantitative Analysis

Bacterial inactivation via UV treatment using logarithmic scale and CFU/mL was determined according to Eq. [3.3], where N_0 and N are the concentrations (CFU/mL) in the influent and the effluent, respectively.

$$\text{Inactivation (UV)} = log_{10}(N_0 / N) \qquad \text{Eq. [3.3]}$$

Bacterial inactivation post UV treatment followed by photoreactivation/dark repair analysis was determined similarly using Eq. [3.4], where N_t is the concentration at time "*t*" after the beginning of the photoreactivation/dark repair experiment. Note that at time zero, N_t is equal to N.

$$\text{Inactivation (UV + repair)} = log_{10}(N_0 / N_t) \qquad \text{Eq. [3.4]}$$

The photoreactivation/dark repair was calculated using only those microorganisms inactivated during the UV disinfection step according to Eq. [3.5].

$$\text{Repair } (\log_{10} CFU/mL) = log_{10}(N_0 / N) - log_{10}(N_0/N_t) \qquad \text{Eq. [3.5]}$$

Bacterial repair expressed as a percentage was determined with Eq. [3.6] adapted from methods used in Maclean *et al.*, (2008) and Shafaei *et al.*, (2017) .

$$\text{Repair } (\%) = \frac{N_t - N}{N_0 - N} \times 100 \qquad \text{Eq. [3.6]}$$

3.8.1 Statistical Analysis

As some of the data sets were not normally distributed and small in sample size it was decided to apply non-parametric analysis. The Mann-Whitney U test was used to test for differences between two independent groups; the null hypothesis states the two populations to be equal. Rejection of the null hypothesis at 95% confidence interval ($P < 0.05$) concluded the two populations to be unequal i.e. significantly different from each other. The Kruskal-Wallis test (one-way non-parametric ANOVA) was used to determine significant differences ($P < 0.05$) between sample populations. If the null hypothesis (samples are from identical populations i.e. there is no difference between samples) for the Kruskal-Wallis test was rejected, this was then followed by a post-hoc Dunn Test for pairwise multiple comparisons to determine significant differences between sample populations. Analysis was carried out using IBM SPSS Statistics 23.

3.9 Physical & Chemical Analysis

3.9.1 TSS – UV Disinfection Analysis

To investigate the impact of TSS on the inactivation efficiency of the PUV and LPUV systems, both organic and inorganic material was used to simulate TSS in water. Two types of clay, bentonite and calcium carbonate ($CaCO_3$) (McGraths Limestone, Mayo, Ireland) were used to simulate inorganic TSS. The bentonite clay was separated into four particle sizes 150 µm, <100 µm, <75 µm and <63 µm using sieves of respective pore size (Endecotts Limited, London, UK) and a sieve shaker (model no. 1132-2-A, Pascall

Engineering, Crawley, UK) after which a sufficient quantity of clay was collected for experimental analysis. The $CaCO_3$ clay was kindly provided by McGraths Limestone, Mayo, Ireland in batches pre-separated into particle sizes of <150 µm, <75µm and <10µm. Organic TSS analysis was investigated using dairy wastewater sludge sourced from a secondary treatment system (an intermittent aerating sequencing batch reactor (IASBR)) located in the Environmental Engineering Laboratories, NUI, Galway. One grab samples of activated sludge were collected from the centre of the mixed IASBR reactor for appropriate dilution with distilled water. Various concentrations of between 0 and 200mg/L of bentonite and $CaCO_3$ were added to the influent sample water of the PUV (2.5 L distilled water) to simulate inorganic TSS. For organic TSS analysis, various concentrations of dairy wastewater activated sludge were added to the influent sample water of both the PUV (2.5 L distilled water) and the LPUV (30 L tap water) to give a range of concentrations that varied between 0 and 155 mg/L for the PUV system and 0 to 260 mg/L for the LPUV system**.** Higher TSS concentrations were analysed for the LPUV system as initial experimental results suggested concentrations > 155 mg/L were required to evaluate the impact of increased TSS concentrations on LPUV efficiency. The 0 mg/L sample consisted of distilled water which was initially spiked to approximately 10 mg/L and then filtered through 1.2 µm filter to give 0 mg/L. This was done to ensure that the 0 mg/L sample was as representative as possible to organic TSS wastewater without the solids present. Subsequently the samples were spiked with *E. coli* to give an initial concentration, prior to UV treatment, of 6 $\log_{10} \pm 0.5$. Samples were then processed through the LPUV and PUV systems. Influent and effluent samples were analysed for *E. coli* inactivation using the

standard pour plate technique (1 mL) using non-selective nutrient agar (Biolab, Lennox, Ireland). Log_{10} inactivation was determined as per section 3.7.

3.9.2 TSS Determination

Duplicate 60-100 mL samples were passed through two pre-weighed glass-fibre circular filter paper (Fisherbrand, Fisher Scientific, Dublin, Ireland) (pore size 1.2 µm) under vacuum pressure. The filter papers were then placed on aluminium trays and dried in a hot oven (105°C) for 24 hours. Once dried, the filter papers were removed and re-weighed to determine suspended solids content. A mean of two measurements was taken for each sample tested. Method as per APHA-AWWA-WEF (2012).

3.9.3 COD Determination

COD analysis was carried out as per standard Standard Method 5220 D (APHA-AWWA-WEF, 2012) using medium range (0 – 1500 mg/L) COD vials (Lovibond, Lennox, Dublin, Ireland) with a Hach DR1900 spectrophotometer (Hach, Co. Cork, Ireland). Briefly, 2 mL of sample was added to a COD vial and heated at 150ºC in heating block (DRB 200, Hach, Co. Cork, Ireland)) for 2 hours. Once complete, the vial(s) were left to cool for a minimum of 20 minutes before being placed into the spectrophotometer for reading at 620 nm. A standard curve was also generated for each test with a series of COD standard solutions (Hach, Co. Cork, Ireland) and distilled water blanks. Absorbance readings were extrapolated with the standard curve to give sample concentrations in mg/L units.

3.10 Site-scale Dairy Wastewater for UV Disinfection Analysis

On-site dairy WWTP effluent was sourced from Aurivo Co-Operative Society Ltd., Ballaghdereen, Co. Roscommon and from a pilot-scale secondary treatment system, an

Intermittent Aerated Sequencing Batch Reactor (IASBR) also based at Aurivo dairy plant for site-scale analysis detailed in Chapter 4. In addition, effluent from a lab-scale IASBR (based at the Environmental Engineering Laboratories, NUI, Galway) for dairy wastewater treatment was also collected as another source of dairy WWTP effluent. Increasing the sources of dairy effluent enabled greater variability in the physical and chemical characteristics of the wastewater which aided the UV disinfection analysis of 'on-site' dairy wastewater. Twenty litre batches of WWTP effluent from Aurivo dairy plant and the pilot-scale IASBR were available for collection, sixty litres was collected in three batches weekly and stored at 10ºC, all three batches were used within 3 days. Each batch was split into 2.5L for flow-through PUV analysis and 17.5L for LPUV analysis thus each batch collected from the full-scale WWTP and pilot-scale IASBR was treated via both PUV and LPUV disinfection and compared. As the lab-scale IASBR produced a maximum of 9L of effluent per day this wastewater was analysed via flow-through PUV only. Duplicate influent and effluent samples were collected for each UV disinfection trial and tested for *E. coli* and total coliforms (method as per Section 3.10.2), *Listeria monocytogenes* and *Staphylococcus aureus* enumeration (see Section 3.10.3) and TSS and COD analysis (see section 3.8).

3.11 Site-scale Microbiological Analysis

3.11.1 Heterotrophic Plate Count Analysis

Heterotrophic plate count (HPC) analysis of dairy plant samples was carried out at 37°C and 22°C as per blue book method "The Microbiology of Drinking Water (2012) - Part 7 - Methods for the enumeration of heterotrophic bacteria by pour plate technique". The HPC

test is a simple measure to monitor aerobic bacterial density in a sample. Briefly, appropriately diluted (repeated 1:10 dilution to allow for colony count between 25 and 250 colonies) duplicate samples (1mL) were analysed via pour plate technique using yeast extract agar at two separate incubation temperatures of 22 °C and 37 °C. Samples at 22 °C were incubated aerobically for 68 ± 4 hours while samples at 37 °C were incubated for 44 ± 4 hours. After incubation, plates with a colony range of between 25 and 250 colonies were counted to give a final sample concentration in CFU/mL.

3.11.2 Total Coliforms & *E. coli* Enumeration

Total coliform and *E. coli* enumeration were performed using the IDEXX Quanti-tray/2000 colilert-18 test (IDEXX Water, Suffolk, UK) based on the MPN standard method (APHA-AWWA-WEF, 2012). Briefly, appropriately diluted (dilution to allow for accurate bacterial enumeration e.g. colour change in < 97 wells and within the limit of detection) 100 mL samples were mixed with commercially prepared enzyme substrates in sterile Schott bottles and then poured into 97-well Quanti–Trays/2000 (which counts 1-2,419 bacteria per tray). The trays were labelled and sealed in an IDEXX tray-sealer (based in the Microbiology Dept. NUI, Galway, Ireland) and incubated aerobically at 35 ± 0.5 °C for 18 hours. After the incubation period, results were read by counting colour changes to yellow in individual wells that inferred probable presence of total coliforms due to conversion of substrate to acid and conversion of indicator dye. The tray was then placed under UV light to determine if *E. coli* were present in the sample (Figure 3.12). Any yellow well that showed fluorescence under UV light was deemed positive for *E. coli*. Once all of the yellow/fluorescent wells were counted, an MPN table was used to determine the final

colony count based on the number of positive large and small wells with results expressed as MPN/100mL. Distilled water was used as a negative control and stock cultures of *E. coli* (ATCC 25922) were used as a positive test control.

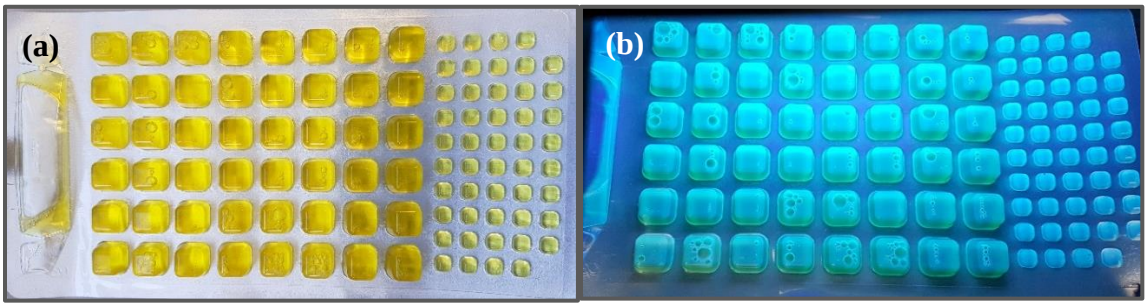


Figure 3.12. Positive Quanti-Tray/2000 test for (a) total coliforms and (b) *E. coli*.

3.11.3 External Pathogen Analysis

Dairy wastewater samples (100 mL) were sent to an INAB accredited laboratory (ISO 9001:2008 and ISO 17025), CLS, located in Rosmuc, Co. Galway. Specific target analysis of the samples was performed for the detection/enumeration of dairy bacterial pathogens *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Campylobacter spp*. and *Salmonella spp*. The following tests were carried out;

- Enumeration of Coagulase Positive *Staphylococcus aureus* in water as per method ISO 6888-1 AI
- Enumeration of *Bacillus cereus* in water as per method ISO 7932
- Detection of *Salmonella* spp. in water Microbiology of Drinking Water (MODW) Part 9 – accredited under ISO 17025

- Detection and enumeration of *Listeria monocytogenes* in water as per method 11290-1/2
- Detection of *Campylobacter* spp. in water as per method ISO 10272-1

3.12 Summary

This chapter describes the experimental conditions for PUV and LPUV system analysis. In addition, methods of dairy wastewater microbial characterisation were outlined as well as methods for investigating bacterial behaviour pre and post UV disinfection.

Chapter 4
Dairy Plant Site Survey, Microbial Characterisation and Investigation of Flow-through PUV and Continuous-Flow LPUV for Dairy Wastewater Treatment

4.1 Introduction

Chapter 4 describes the preliminary analysis carried out to inform the experimental design and further develop the focus and rationale for this study. Results of a nationwide dairy plant site survey are presented and microbiological characterisation of various water and wastewater streams from three contrasting Irish dairy plants are described. Chapter 2 reviewed microbial pathogens that may contaminate food and which are of general interest for the dairy sector and similar challenges in relation to water use. However, given the specific focus on the testing and development of PUV irradiation technology for wastewater processing in the dairy sector, it was felt this aspect needed to be studied in greater detail in an Irish context. Thus, results presented in this chapter informed key research areas of importance in terms of water and wastewater treatment from the dairy industry perspective. In addition, key bacterial pathogens of interest were identified which informed requirements for microbiological disinfection of wastewater within this dairy sector. Results for UV system analysis for on-site wastewater disinfection are also presented in this Chapter. This Chapter also includes research findings which were carried out after the work presented in Chapters 5 and 6 but were included for continuity and context.

4.2 Nationwide Dairy Plant Survey

There are almost two hundred registered milk and dairy establishments in the Republic of Ireland. However, it is estimated that approximately 83% of milk produced is processed by seven main dairy processors (Finnegan *et al.*, 2017). The nationwide plant survey conducted during this study focused on four of the seven main processors taking in two plants of two of the processors bringing the total number of participating plants to six (designated and encoded as DP 1, DP 2, DP 3, DP 4, DP 5 and DP 6). Qualitative and quantitative information was collected on various topics reflecting inter-related objectives of this study that encompassed water/wastewater reuse practices, on-site rainwater harvesting and effluent discharge standards. Table 4.1 describes the products manufactured at each of the surveyed dairy plants and the volume of milk processed annually which is indicative of the scale of each plant.

Table 4.1. Description of products manufactured at each of the six dairy plants that participated in the site survey and volume of milk processed annually per plant.

Plant ID	Plant Products	Volume of milk Processed Annually (litres x10^6)
DP 1	Casein, whey powder, butter and milk powder	243
DP 2	Milk and cream	50
DP 3	Butter and milk powder	580
DP 4	Casein, UHT milk and milk powder	90
DP 5	Butter and milk powder	210

DP 6	Powders, butter, spreads, cheese, casein and whey derivatives	486

Key staff at each dairy plant completed the questionnaire outlined in Figure 4.1 that specifically focused on process information and did not seek any personal or private staff details. The anonymised questionnaire was part of a larger survey (combined for two projects; DairyWater & Waternomics) which focused on other aspects of dairy manufacturing such as energy use within the plant, nutrient removal efficiency at WWTP level and overall costs at various parts of the dairy plant and WWTP. Those questions relating to this study are shown in the highlighted sections of Water Use and Wastewater & Waste Management.

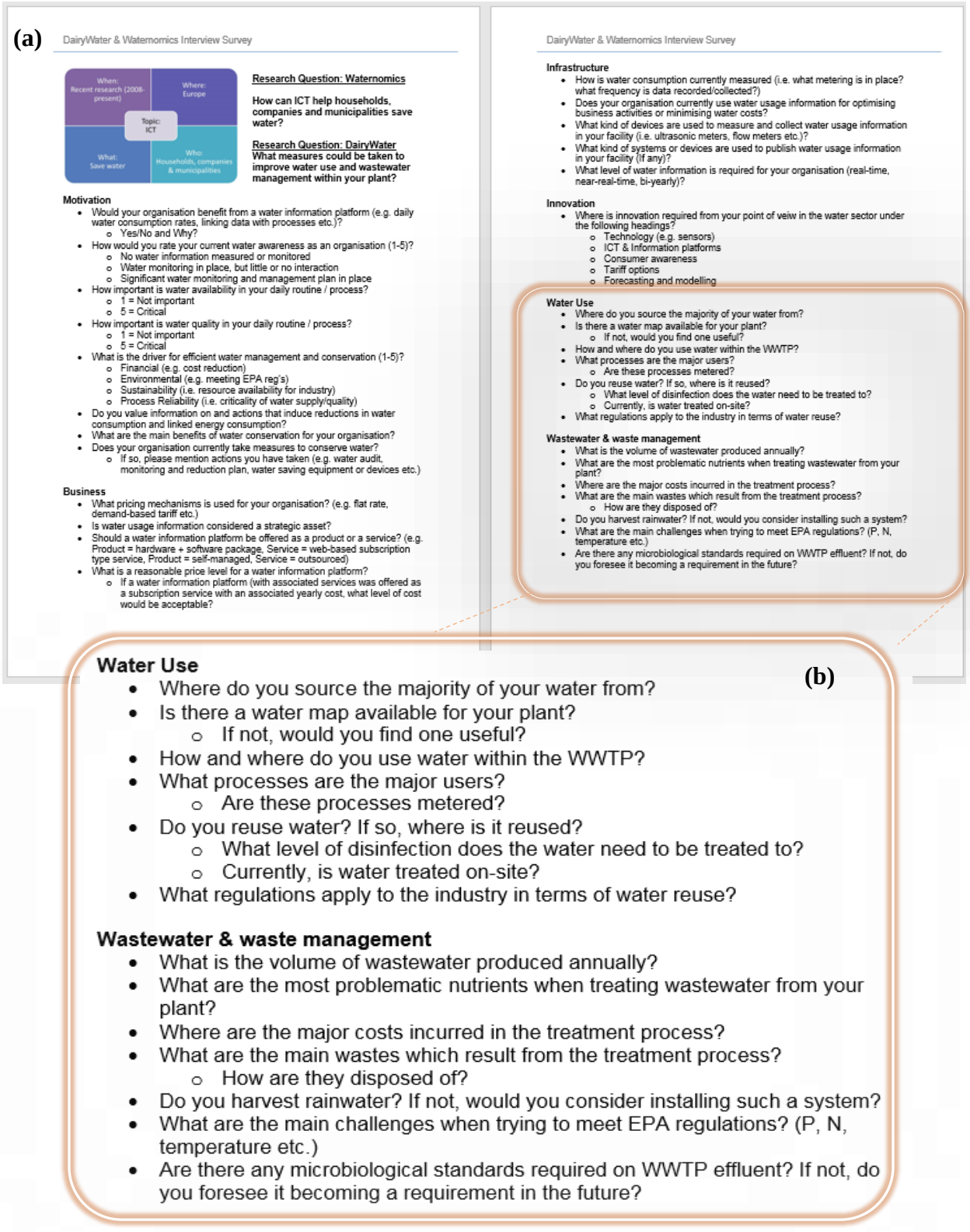


Figure 4.1. (a) Full survey questionnaire for Irish dairy processing plants for Dairywater & Waternomics projects and (b) survey questions as part of this study.

4.2.1 Wastewater Discharges & Water/Wastewater Reuse

Wastewater discharges in Ireland are regulated by the Urban Wastewater Treatment Directive (91/271/EEC) and the Water Framework Directive **(**2000/60/EC) as designated by the European Commission. The Directives are designed to protect receiving water bodies such as rivers, lakes groundwater etc by monitoring the water quality for harmful contaminants through discharge regulations. These regulations are enforced by either the Environmental Protection Agency (EPA) or governing local authority (depending on the receiving water body in question) who issue discharge licences to WWTP operators for compliance standards. In addition, sensitive water bodies may fall under the Shellfish Waters Directive (2006/113/EC) which aims to improve the quality of shellfish waters through the enforcement of strict physical, chemical and microbiological discharge standards. As outlined in Chapter 2, Section 2.3, no water/wastewater reuse standards currently exist in Ireland however with recent changes in climate and extreme weather events, in addition the increasing environmental awareness, water reuse in the Irish dairy industry may become necessary in the future.

Four out of the six plants surveyed agreed that reporting on microbial discharge limits could be a strong possibility in the near future, with the other two participating plants choosing not to comment. Of the plants that agreed on possible future reporting of microbial discharge limits, they added that a change in which state organisation will regulate their discharges may also influence the stringency regarding wastewater discharges. As some plants report to local government (county councils) and others are under the jurisdiction of the Environmental Protection Agency (EPA), consensus would be necessary before deciding on a reporting framework. One of the respondent plants added

that they have carried out ‘spot checks’ for the presence of faecal contaminants, typically *E. coli* and *streptococci* in dairy WWTP effluent. Information regarding the annual (data representative of the year 2013) volume of process water used and wastewater generated was provided by each dairy plant aside from DP 4 where only wastewater volumes were available (Figure 4.2). In addition, the volume of wastewater generated per litre of milk processed (using the data in Table 4.1) is also illustrated in Figure 4.2. Results show dairy plants DP 1, DP 2, DP 4 and DP 5 to produce approximately 1.5 to 2.2 litres of wastewater for every litre of milk processed. The largest contrast in ratios of wastewater generation are between that of DP 3 and DP 5. DP 3 appeared to be the most efficient plant of all six surveyed in terms of minimising wastewater generation. It was also the only plant out of all surveyed that produced less wastewater in comparison to the volume of process water used that year (aside from DP 4 where process water volumes were not provided). During the survey it was revealed that DP 4 reused wastewater from processes within the plant by treating it via ultrafiltration and then reusing it in the drying system within the plant. Moreover, condensate water was also reused as boiler feed water. However, crucially, any excess condensate water produced was discharged into a nearby river (providing it met the discharge limits) thus minimising the volume of wastewater to be treated at the WWTP. In contrast, DP 6 illustrated the lowest efficiency with 5L of wastewater produced/L of milk processed in the dairy plant. This plant produces a variety of ingredients including cheese, casein and whey derivatives (Table 4.1). Therefore, while DP 4 and DP 6 process a similar volume of milk annually (580 million litres and 486 million litres respectively), the processes within DP 6 are likely to be more varied and this may explain why more process water is used and in turn, more wastewater is produced. The results highlight that the type

and number of products manufactured at a dairy plant can impact upon the volume of process water required and the methods by which wastewater may be disposed of.

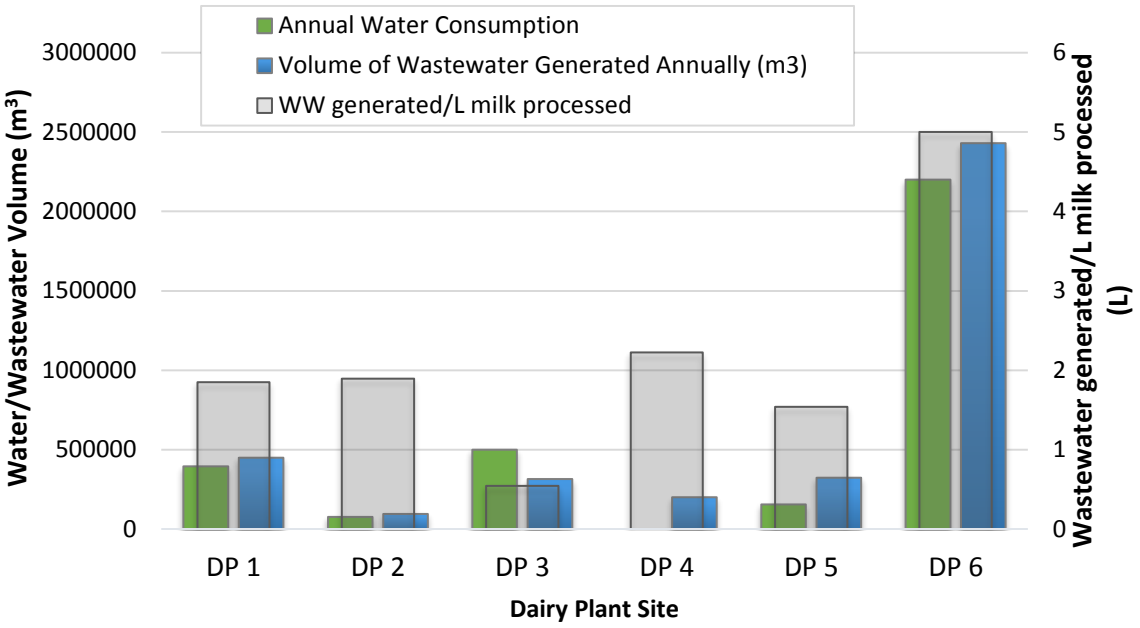


Figure 4.2. Annual water consumption, volume of wastewater generated and ratio of wastewater generated per litre of milk processed at six Irish dairy plants.

Five out of the six plants reported wastewater reuse to varying degrees within dairy plants with DP 4 choosing not to comment. Volumes of reused wastewater in relation to total water consumption was not reported, rather a more general overview was given. Of the five plants that answered yes to wastewater reuse, DP 1 described the wastewater reuse as minimal with a small fraction of their condensate water (vapour condensate from evaporation processes in the plant) being reused (the point of reuse was not specified). This plant expressed concern over a stigma attached to reusing water extracted from milk citing potential microbial contamination inherent in the water. DP 4 also expressed similar concerns. Nevertheless, DP 3, DP 5 and DP 6 reported the reuse of condensate water for boiler feed make-up with DP 5 utilising 50% of their condensate water for this purpose.

DP 6 stated that if the condensate water is contaminated, heat recovery is still an option with the heat being re-used within the plant. Furthermore, a project was currently being developed at that plant for the recovery of contaminated condensate water. As stated previously, DP 3 implemented two methods of water reuse within the plant by treating and reusing process water and recirculating condensate water. Other water reuse projects included the modification of a water chilling system to recycle 50% of the process water used at DP 2. Overall, while the majority of water used within a dairy plant is process water for CIP use, there does appear to be an incentive within the Irish dairy industry to reuse water and increase water efficiency within the plant.

4.2.2 Rainwater Harvesting at Irish Dairy Plants

Notably, the survey results showed that all six plants were not in favour of rainwater harvesting to increase water efficiency at their respective dairy plant for various reasons. DP2 commented that there was potential for a rainwater harvesting installation at the plant with the current positioning of roof catchments; however, no plans were in place to do so. DP3 mentioned that the generation of condensate water from evaporation processes within the plant would be the first point of use for wastewater disinfection and reuse. Moreover, it was also noted that there would be no necessity for rainwater collection as reusing the condensate water would have an added benefit of decreasing costs of the treatment and disposal of the condensate water. Finally, DP6 also highlighted that the cost of extracting water was so inexpensive that they would not consider the instalment of a rainwater harvesting system. Since this survey was completed in 2014, a rainwater harvesting system has been installed at a dairy plant in Co. Cork, Ireland. However, survey results show that in general there appears little incentive to harvest rainwater at this time.

4.3 Wastewater Characterisation Analysis

Two dairy plants from the six surveyed (Table 4.1) were selected for water/wastewater stream analysis. In addition, another one of largest of the seven primary milk processors was also selected for wastewater characterisation analysis. The sites were selected based on the size of the plant e.g. small-scale (Site 1) to large-scale (Site 3) and the type of products manufactured at the plant. For further information on sample collection see Chapter 3 Section 3.2.

Table 4.2. Identification of the three dairy plants (highlighted in red) selected for wastewater characterisation analysis from the six dairy plants that took part in the site survey as per Table 4.1.

Plant ID	Plant Products	Volume of milk Processed Annually (litres x10^6)	
DP 1	Casein, whey powder, butter and milk powder	243	
DP 2	Milk and cream	50	Site 1
DP 3	Butter and milk powder	580	
DP 4	Casein, UHT milk and milk powder	90	
DP 5	Butter and milk powder	210	Site 2
DP 6	Powders, butter, spreads, cheese, casein and whey derivatives	486	
New Plant	Cheese, butter, infant formula, casein, various milk powders	1,300	Site 3

In total, 12 dairy water and wastewater samples from the three contrasting dairy plants were profiled for total abundance of aerobic bacteria (HPC at 22ºC and at 37ºC), total coliforms, *E. coli*, *Listeria monocytogenes*, *Salmonella spp.*, *Campylobacter spp.*, *Bacillus*

cereus and *Staphylococcus aureus.* Table 3.1 in Chapter 3 describes in detail all of the tests carried out on the samples from each site.

4.3.1 Heterotrophic Bacteria and Faecal Indicator Analysis

Figure 4.3 (a) illustrates the concentration of aerobic bacteria (HPC 22 and HPC 37) in the samples collected from Site 1 on three separate days. Untreated process water had the lowest concentration of total aerobic bacterial (HPC) with concentrations of 1×10^3 and 3×10^3 CFU/mL for the HPC 37 and HPC 22 tests respectively. The concentration of faecal indicators (total coliforms and *E. coli*) decreased by approximately 1 $\log_{10}$ from WWTP influent to WWTP effluent which was expected as the wastewater treatment system in place at the dairy plant may not have been optimised for microbial disinfection (Figure 4.3 (b)).

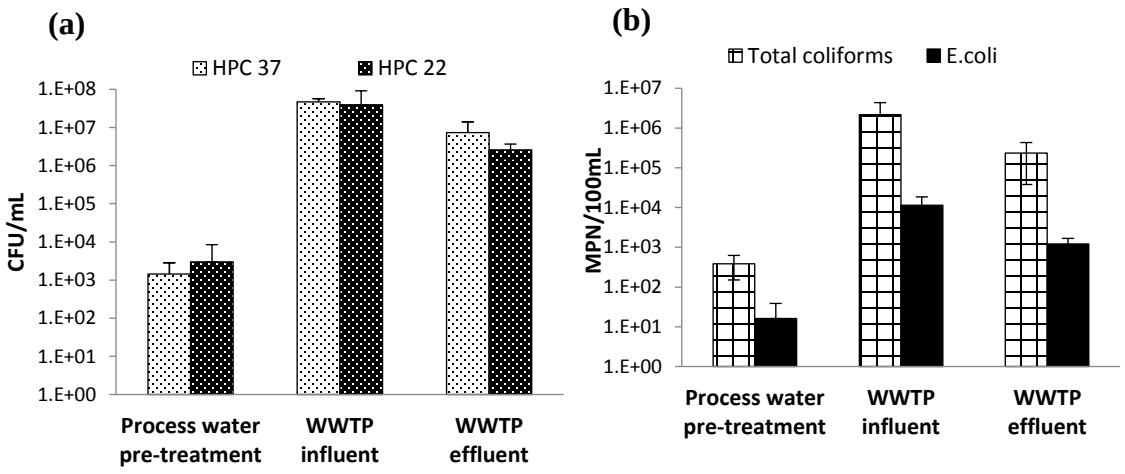


Figure 4.3. (a) Aerobic bacterial abundance as per HPC tests at 22ºC and 37 ºC and (b) faecal contamination indicators total coliforms and *E. coli* in water/wastewater samples – Site 1 (n=3 ± SD).

Wastewater streams 'condensate water' and 'cooling water' were also available for collection at Site 2 (Figure 4.4). Results show the condensate water to be the least contaminated of the wastewater streams with no faecal indicators present (Figure 4.4(b)) and no aerobic bacteria growth at 37ºC (Figure 4.4(a)). A lack of aerobic bacterial growth at 37ºC (HPC 37) indicated bacteria of animal origin may not be present while the presence of aerobic bacteria grown at 22ºC (HPC 22) suggested the presence of bacteria from water/soil environments (Allen *et al.,* 2004).

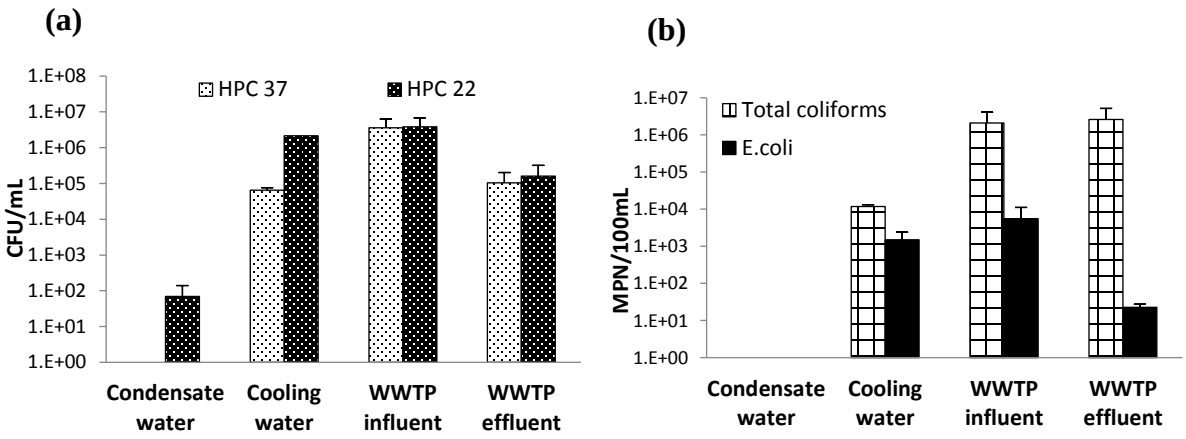


Figure 4.4. (a) Aerobic bacterial abundance as per HPC tests at 22ºC and 37 ºC and (b) faecal contamination indicators total coliforms and *E. coli* in water/wastewater samples – Site 2 (n=2). Error bars represent minimum and maximum values.

Site 3 constituted the largest sample collection out of the dairy plant sites with three influent WWTP streams analysed, one condensate wastewater stream and one WWTP effluent stream. This plant produced the most dairy products out of all three sites including cheese, butter and infant formula (demineralised whey powder). WWTP influent samples from the cheese production process showed the highest concentration of heterotrophic bacteria, total

coliforms and *E. coli* with almost 1×10^8 CFU/mL of aerobic bacterial abundance (HPC 22 and HPC 37) detected (Figure 4.5 (a)). Similarly, *E. coli* concentration were also found to be the highest in the cheese process WWTP influent at 2.19×10^4 CFU/mL in comparison to 1.12×10^3 CFU/mL and 2.53×10^3 CFU/mL for the mixed process WWTP influent and whey process WWTP influent respectively. These findings suggest a higher level of contaminated wastewater may arise from cheese processes as opposed to butter and infant formula production. (Figure 4.5 (b)). The condensate stream appeared to be the least contaminated of all samples with *E.coli* being undetected however the presence of total coliforms is indicative of potential pathogenic contamination.

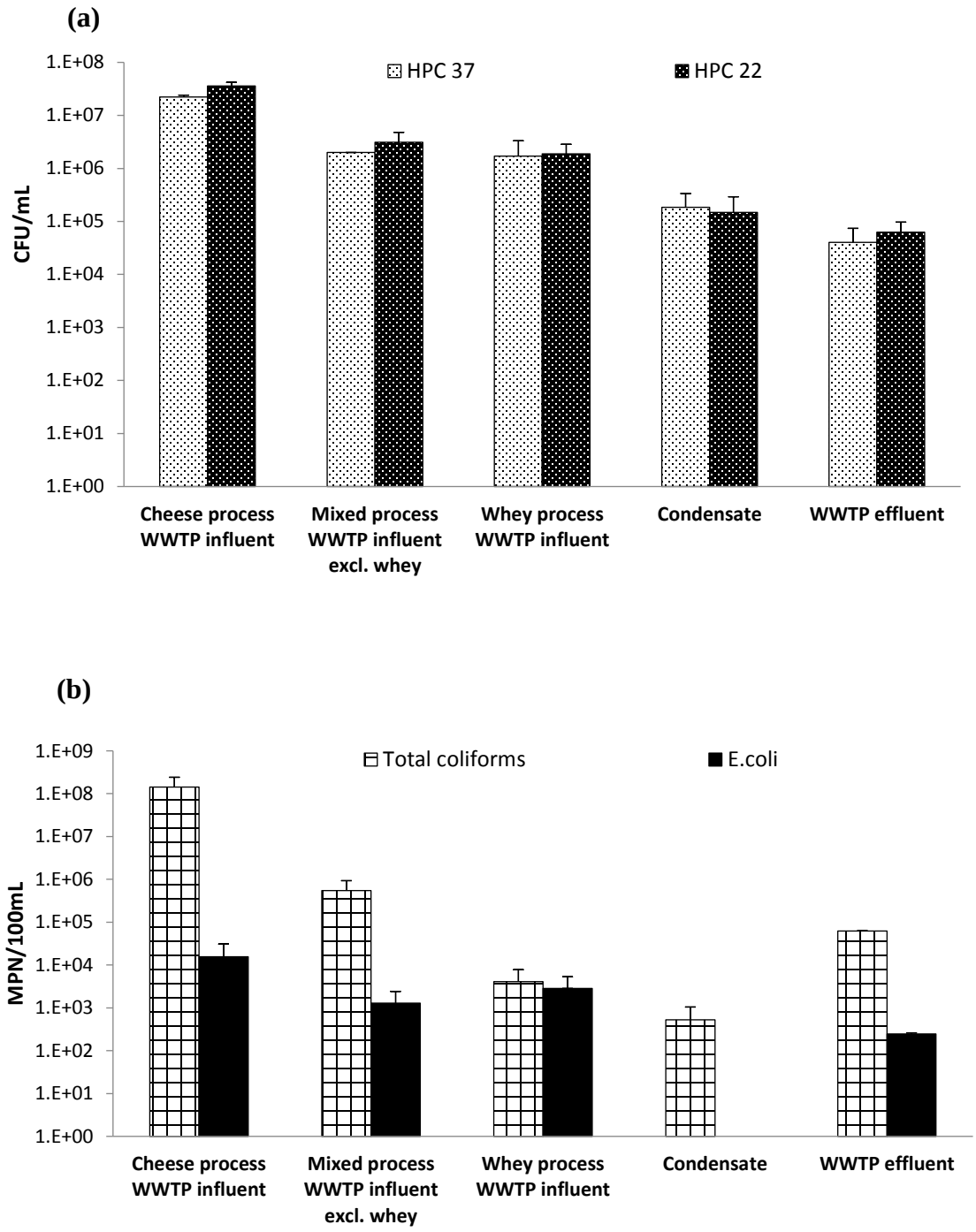


Figure 4.5 (a) Aerobic bacterial abundance as per HPC tests at 22ºC and 37 ºC and (b) faecal contamination indicators total coliforms and *E. coli* in water/wastewater samples –Site 3. n=2. Error bars represent minimum and maximum values.

4.3.2 Specific Dairy Pathogen Analysis

Table 4.3 outlines the presence/abundance of five targeted pathogens in the dairy water samples. For further information on all of the tests performed during this analysis see Table 3.1 in Chapter 3.2. *Salmonella spp.* was found to be absent in all 12 samples while *Campylobacter spp*. was detected in just two samples at Site 2 on sample Day 1; WWTP effluent and cooling water. *Campylobacter spp*. were not detected in WWTP influent. As the test involved pathogen detection only, bacterial concentration levels were not measured, thus postulating why *Campylobacter spp*. was present in effluent but not influent is challenging. When the plant was surveyed on the second sample day, the pathogen was not detected. This may be due to the variability of the wastewater, and specifically, the build-up of microbial biofilms which often occur within wastewater treatment systems. For example, the 'breaking away' of biofilms within the system and resultant wash-out could cause the sporadic presence and detection of bacteria which would explain inconsistent findings. *Listeria monocytogenes* was initially detected in all samples at Sites 1 and 2. Enumeration tests were then carried out on a second set of samples taken from each Site. If wastewater streams were to be considered for reuse purposes e.g. cooling water at Site 2, UV disinfection would be essential, even in the case of low-level water reuse in/around the dairy plant to ensure a sufficient reduction in pathogenic bacteria and remove the threat of potential human infection. Food poisoning bacteria *Bacillus cereus* was found to be consistently present in all samples at a concentration of approximately 1×10^3 CFU/100mL. What isn't clarified with the enumeration test is whether the bacteria were in a vegetative or endospore state. If the bacteria were in the dormant endospore lifecycle stage, the level of disinfection required for removal/inactivation would be higher and thus

persistence through the dairy plant/WWTP is more likely. In this case, a higher level of disinfection would be necessary to remove the more robust pathogenic microorganisms. Results showed *Staphylococcus aureus* to be most prevalent at Site 1. Table B.1 in Appendix B outlines all of the data collected for heterotrophic bacteria, faecal indicator and pathogenic bacterial analysis in all dairy wastewater samples.

Table 4.3. Pathogenic bacterial analysis of various water & wastewater streams at three Irish dairy plants.

Site	Sample Day	Sample Type	*Salmonella* detection (cfu/100mL)	*Listeria monocytogenes* detection & enumeration (cfu/100mL)	*Campylobacter spp* detection (100mL)	*S. aureus* (cfu/100mL)	*B. cereus* (cfu/100mL)
1	1	Process water pre-treatment	*ND	Detected	ND	4.40E+03	4.48E+03
		WWTP influent	ND	Detected	ND	4.32E+03	5.04E+03
		WWTP effluent	ND	Detected	ND	4.08E+03	5.26E+03
	2	Process water	*N/A	<1 cfu/mL	N/A	1.63E+03	1.04E+03
		WWTP influent	N/A	<1 cfu/mL	N/A	1.63E+03	9.60E+02
		WWTP effluent	N/A	<1 cfu/mL	N/A	1.85E+03	1.07E+03
	3	Process water	ND	8.40E+03	ND	<1	9.80E+02
		WWTP influent	ND	7.90E+03	ND	<1	9.40E+02
		WWTP effluent	ND	6.20E+03	ND	<1	9.23E+02
2	1	WWTP influent	ND	Detected	ND	1.46E+03	1.99E+03
		WWTP effluent	ND	Detected	Detected	1.25E+03	1.67E+03
		Condensate	ND	Detected	ND	1.10E+03	1.84E+03
		Cooling water	ND	Detected	Detected	1.16E+03	1.96E+03
	2	WWTP influent	ND	3.60E+02	ND	<1	1.05E+03
		WWTP effluent	ND	6.40E+02	ND	<1	1.06E+03
		Condensate	ND	<1	ND	<1	1.05E+03
		Cooling water	ND	1.10E+02	ND	<1	9.60E+02
3	1	Cheese process effluent	ND	ND	ND	<1	1.08E+03
		Mixed process effluent excl. whey	ND	ND	ND	<1	1.06E+03
		Whey process effluent	ND	ND	ND	<1	1.05E+03
		Condensate	ND	ND	ND	<1	9.67E+02
		WWTP effluent	ND	ND	ND	<1	1.02E+03
	2	Cheese process effluent	ND	ND	ND	<1	1.04E+03
		Mixed process effluent excl. whey	ND	Detected	ND	<1	1.01E+03
		Whey process effluent	ND	ND	ND	<1	1.06E+03
		Condensate	ND	ND	ND	<1	9.84E+02
		WWTP effluent	ND	ND	ND	<1	1.06E+03

*ND – Not detected *N/A – test not performed

4.4 Discussion

4.4.1 Dairy Plant Survey

The purpose of the dairy plant site survey was to act as a guidance measure for the direction of the experimental analysis carried out in this study. The findings of the site survey confirmed an interest in water reuse within dairy plants with the majority of plants implementing water reuse to a certain degree. In the case of the dairy plants with extensive evaporation processes, it was noted that the production of large volumes of condensate water incentivised the plant to practice water reuse as this had a dual effect of alleviating wastewater treatment pressures at the WWTP and minimising process water costs. However, the survey also highlighted the concerns of some dairy plants that reusing water (particularly internally within the plant) may not always be favourable due to public opinion and perceptions that recovered water or 'cow water' remains unsanitary. In addition, the low cost of process water also contributed to some dairy plants choosing not to reuse water.

Feedback relating to rainwater harvesting practices at dairy plants was consistent, all plants showed limited interest in the installation of such a system. The primary reasons for the lack of interest included low water tariffs in Ireland (or no fixed charge if a private source was used) and the availability of alternative water sources produced on-site i.e. condensate water. As a consequence of this dairy industry feedback, it was decided not to carry out the experimental analysis of a rainwater harvesting system with regards to dairy wastewater.

With regard to future legislation of microbiological discharge standards for WWTP effluent, the majority of the dairy plants surveyed agreed that such standards may be implemented in the future owing to the abolishment of the CAP in 2015 and the resulting

expansion of a number of dairy plants across the country. In addition to this observation, findings from the wastewater characterisation analysis confirmed the presence of faecal indicators in the effluent of all three WWTPs investigated thus if standards were to be introduced pertaining to microbiological contamination, the tertiary treatment of WWTP effluent would be a necessity. Accordingly, the experimental analysis of the study concentrated on evaluating the efficacy of pulsed UV (with LP UV as a comparator) in disinfecting process water and wastewater in terms of (i) dairy specific pathogens, (ii) faecal indicators and (iii) investigating how the composition of the dairy wastewater may impact the working efficiency of LPUV and PUV.

4.4.2 Dairy Wastewater Characterisation

Faecal indicators of total coliforms and *E. coli* were present in all WWTP influent & effluent samples. *E. coli* was detected in all samples apart from the condensate water samples from Site 2 and Site 3. Thus, if effluent discharge regulations were extended to microbiological monitoring in addition to current regulations, it is likely that disinfection of wastewater effluent would be required at all three WWTP sites tested. Separate wastewater streams emerging directly from the dairy processing factories were analysed to determine bacterial contamination levels and suitability for potential low-level water reuse in/around the dairy processing factory. A cooling water waste stream was analysed at Site 2 while condensate wastewater was available for collection at both Site 2 and Site 3. Analysis of the cooling water stream yielded the presence of both faecal indicators and four out of the five targeted pathogens (thus disinfection may be required depending on the desired water reuse purpose). Condensate water from Site 2 appeared relatively uncontaminated as aerobic bacterial loads were low and faecal indicators absent. However

pathogenic *Listeria monocytogenes* was still detected on both sampling days highlighting the importance of rigorous microbiological analysis of dairy wastewater streams if they are to be considered for reuse purposes. Studies have shown these bacteria to survive post-pasteurisation in dairy processing environments and further poses problems in end products due to the ability to grow at 4º. Therefore, particular attention may be warranted for this strain in terms of water reclamation in the dairy environment (Oliver *et. al.,* 2005). *Listeria monocytogenes* was also detected in all samples at Site 1 and Site 2 and after a further enumeration test the highest levels were detected in Site 1. *Salmonella spp.* went undetected in all 12 samples tested while *Bacillus cereus* was consistently detected in all 12 samples at low concentrations. *Staphylococcus aureus* was found to be most prevalent at Site 1 where process water (pre-treatment) WWTP influent and WWTP effluent streams were tested.

Overall, results from the wastewater characterisation analysis indicated that the majority of wastewater streams from different dairy processing factories were contaminated with either faecal indicators or foodborne pathogens or a mixture of both. The condensate wastewater streams appeared to be the most suitable to utilise in terms of water reuse and indeed was the stream most utilised in plants that re-used process water.

4.4.3 Conclusions from Dairy Plant Surveys and Site Characterisation

- The dairy plant site survey indicated that water reuse practices are favoured in the industry however reservations remain relating to a stigma attached to wastewater reuse. In addition, the low cost of water may also be a disincentive to reuse water.
- Rainwater harvesting was not favoured by any of the plants surveyed.

- A majority agreed that the legislation of microbiological discharge standards in WWTP effluent may be introduced in the near future.
- Faecal indicators, total coliforms and *E. coli* were detected in all samples apart from the condensate water samples collect at Site 2. *E. coli* was also absent from condensate water at Site 3 in contrast to total coliforms which were detected in this sample.
- Dairy pathogen *Salmonella spp*. was absent from all samples while *Campylobacter spp*. was detected in just two samples at Site 2.
- *Listeria monocytogenes* appeared to be most prevalent at Site 1 as it was present in all samples and in the highest concentration in comparison to the other two sites.
- Results for the presence of *Staphylococcus aureus* varied from 1×10^3 detection levels at Site 1 to minimal detection levels at Site 3.
- *Bacillus cereus* was found to be consistently present in all samples.

4.5 Impact of Dairy Wastewater Constituents on Flow-Through PUV Efficiency with Comparison to Continuous-flow LPUV.

The impact of suspended solids on the working efficiency of LPUV systems has been thoroughly investigated however the impact of TSS on flow-through PUV disinfection systems has yet to be established (Chapter 2, Section 2.4.9). Thus, this section initially describes the impact of inorganic and organic total suspended solids on the working efficiency of the flow-through PUV systems. Following on from this analysis, the efficiency of the flow-through PUV in treating dairy wastewater from a dairy processor (DP5) is also described. While the impacts of TSS and organic carbon on LPUV system

efficiency has previously been described, for comparative purposes the LPUV was also analysed.

4.6 Laboratory-Scale TSS Analysis

4.6.1 Impact of Inorganic TSS on PUV efficiency

Wastewater can contain inorganic and organic particles and studies from the literature have shown both types of particle to impact upon UV disinfection efficiency (Wu *et al.*, 2005; Carré *et al.*, 2018) It was decided to analyse inorganic material as well as organic as (i) it was possible to analyse individual particle sizes and (ii) particular waste streams from dairy plants e.g. stormwater may contain a higher degree of inorganic TSS in comparison to organic TSS particles. Two types of clay were used to evaluate the impact of inorganic suspended solids on the bacterial inactivation efficiency of the PUV system; bentonite and calcium carbonate ($CaCO_3$). Analysis began with bentonite whereby the clay was the separated into four particle sizes (see Chapter 3, Section 3.8.1 for further details); <150 µm, <100 µm, <75 µm and <63 µm. As the <63 µm was the smallest particle size available, this size was trialled first at various individual TSS concentrations of between 0 and 200 mg/L and a UV output of 1946 mJ/cm^2 for *E. coli* inactivation. As mentioned in Chapter 2, Section 2.3.10, TSS concentration of greater than 30 mg/L are considered to have a negative impact on the optimum performance of UV systems. Log_{10} inactivation rates varied from a minimum of 3.8 log_{10} to a maximum of 5.4 log_{10} with no clear relationship found between increasing TSS concentrations and decreasing *E.coli* inactivation efficiency. Rather, *E.coli* log_{10} inactivation rates tended to vary considerably at any one TSS concentration. For example, at 15 mg/L *E.coli* log_{10} inactivation varied between 3.8 and 4.6 and at 200 mg/L log_{10} inactivations of 4.0 and 5.1 were found. (Figure 4.6). Larger

particle sizes (<100 µm) were then analysed to determine whether the particle size might be a factor in *E. coli* inactivation (particles above 100 µm could not be kept in suspension in the flow-through vessel even with magnetic stirrers probably due to the relatively high residence time). Inorganic individual TSS concentrations ranging from 60 to 200 mg/L were analysed; Figure 4.6 illustrates the impact of both bentonite particle sizes (<63 µm and <100 µm) on *E. coli* inactivation. Analysis of *E.coli* inactivation efficiency using the <100 µm particle size illustrated a lower variance between inactivation rates (4.7 to 5.3 log_{10}) in comparison to <63 µm particle size and results were found to be consistent whereby increasing TSS concentrations did not inhibit PUV inactivation efficiency of *E.coli*. Thus, neither particle size appeared to influence *E. coli* log_{10} inactivation at inorganic TSS concentrations above 30 mg/L.

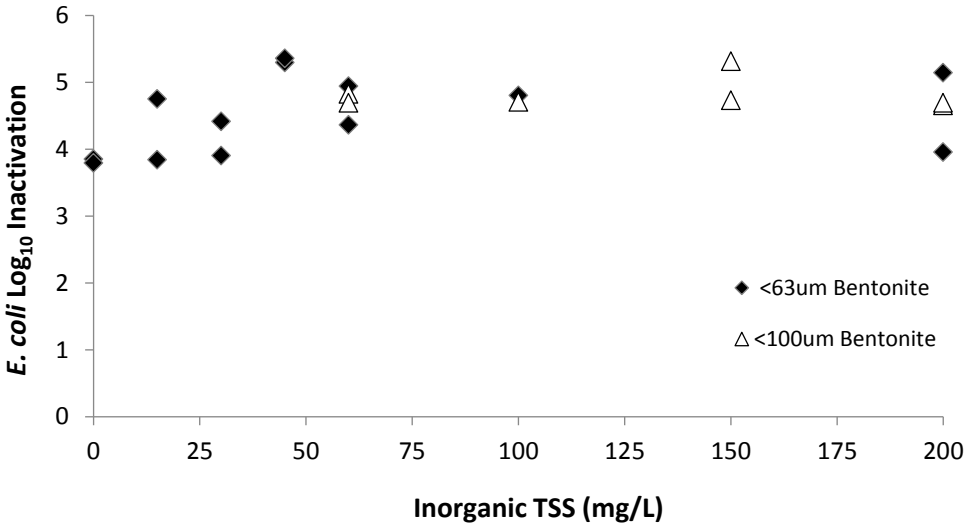


Figure 4.6. Impact of inorganic TSS (bentonite <63 µm and <100 µm particle size) on *E. coli* inactivation via PUV treatment at a PUV output 1946 mJ/cm^2.

Three particle sizes of $CaCO_3$ were sourced for comparative analysis; 0 - 150 µm, 0 – 75 µm and 0 – 10 µm. As with the bentonite analysis, the <150 µm particle size would not remain suspended in water samples and so was eliminated from experimental analysis. Both the <75 µm and <10 µm particle sizes were evaluated under the same experimental conditions as that of the bentonite analysis. However, for the <75 µm particle size, magnetic stirrers were added to the PUV aluminium vessel to aid particle suspension. In addition, turbidity measurements of the water samples analysed during each experiment were also carried out. Figures 4.7 (a) and (b) illustrate *E. coli* inactivation at five TSS concentrations; 0mg/L, 25mg/L, 50mg/L, 100mg/L and 200mg/L for the <10 µm particle size and at 25mg/L, 50mg/L, 100mg/L and 200mg/L for <75 µm particle size respectively. Limited differences in the level of *E. coli* inactivation via PUV at any of the inorganic TSS concentrations tested. Notably, the turbidity of the water sample with <75 µm $CaCO_3$ particle size did not increase in a linear fashion in comparison to the <10 µm $CaCO_3$ particle size indicating that even with the addition of magnetic stirrers for particle agitation, the particles were not remaining suspended in the water sample. Furthermore, the overall level of *E. coli* inactivation in the <75 µm $CaCO_3$ particle size experiment was approximately one $\log_{10}$ lower in comparison to the bentonite and <10 µm $CaCO_3$ particle size experiments. The addition of the magnetic stirrers in this case may have decreased the overall bacterial inactivation efficiency. It should be noted, however, that sample agitation/stirring typically has the reverse effect in experimental analysis whereby the addition of magnetic stirrers would generally increase the overall inactivation efficiency.

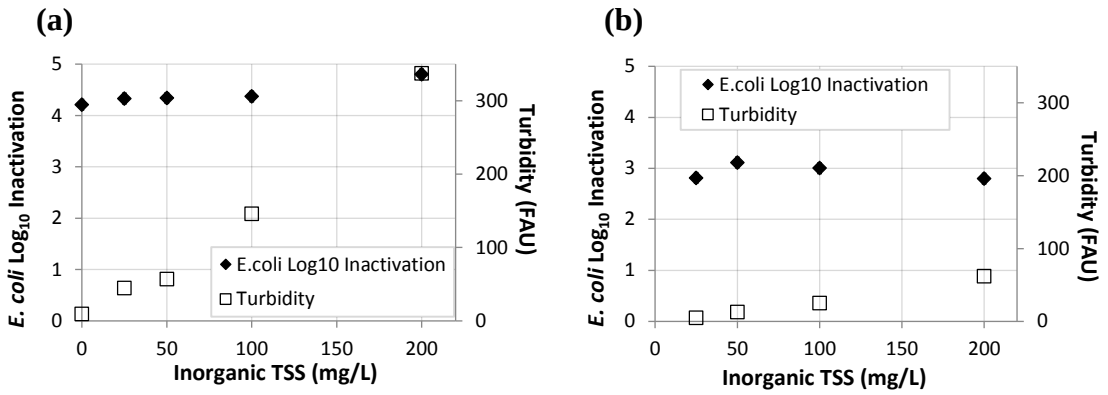


Figure 4.7. Impact of inorganic TSS $CaCO_3$ particle sizes (a) < 10 µm and (b) < 75 µm on *E. coli* log_{10} inactivation and sample turbidity via flow-through PUV treatment at 1946 mJ/cm^2.

Figure 4.8 compares the impact of both bentonite and $CaCO_3$ (all particle sizes) on *E. coli* inactivation. Bacterial inactivation does not appear to be impacted as inorganic TSS increases up to 200 mg/L.

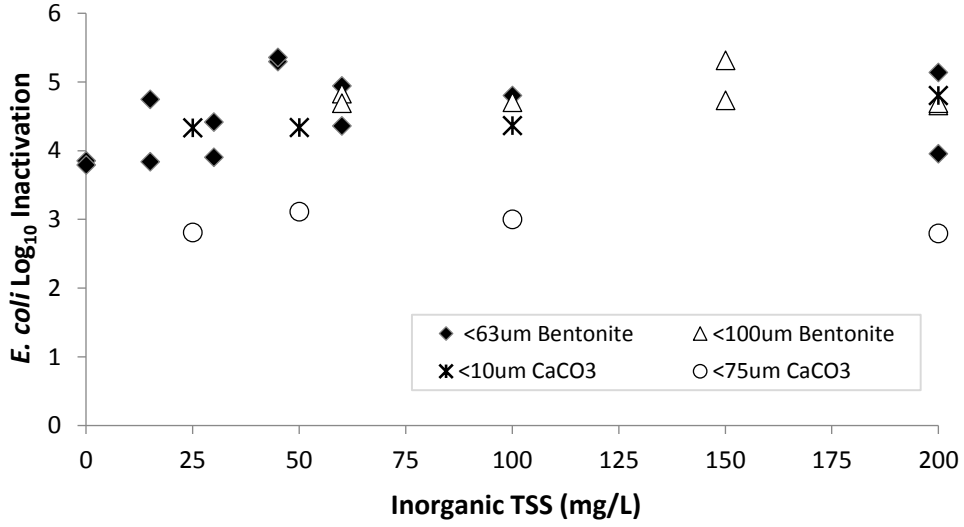


Figure 4.8. Impact of inorganic TSS (bentonite <63 µm and <100 µm particle size and $CaCO_3$ <10 µm and <75 µm particle size) on *E. coli* inactivation via PUV treatment at a PUV output 1946 mJ/cm^2.

4.6.2 Impact of Organic TSS on PUV System Efficiency

Sludge from a secondary treatment lab-scale IASBR reactor (Chapter 3, Section 3.8.1) was spiked into distilled water to give TSS concentrations ranging from 0 mg/L to 155 mg/L. Figure 4.9 illustrates the impact of various organic TSS concentrations on the efficiency of the PUV system for *E. coli* inactivation. Between 0 and 20 to 35 mg/L limited impact on inactivation was observed – thereafter PUV inactivation efficiency decreased significantly. While a relatively good linear trend was observed across the sample range between increasing TSS and inactivation this trend was more notable between 20 and 155 mg/L.

In addition to TSS analysis, the concentration of total organic carbon (TOC) present in the organic TSS samples was also evaluated (Figure 4.9). Results show a similar trend with increasing TOC concentrations resulting in lower levels of *E. coli* inactivation at the same PUV energy output. This is to be expected as diluting or increasing TSS concentrations would have the dual effect of diluting or increasing TOC concentrations; while the relative impact of either TSS or TOC could not be analysed in this study it is clear that TSS concentrations (and by association organic carbon concentrations) above typical discharge limits (typically maximum TSS discharges of 25 mg/L are permitted; the actual concentration would be site specific) could significantly impact PUV system performance. The trend line for TSS data in Figure 4.8 is from 20 mg/L (as it was the closest data point to permissible 25 mg/L concentration) to the maximum 155 mg/L for TSS data and from 0 mg/L to 158 mg/L for TOC data.

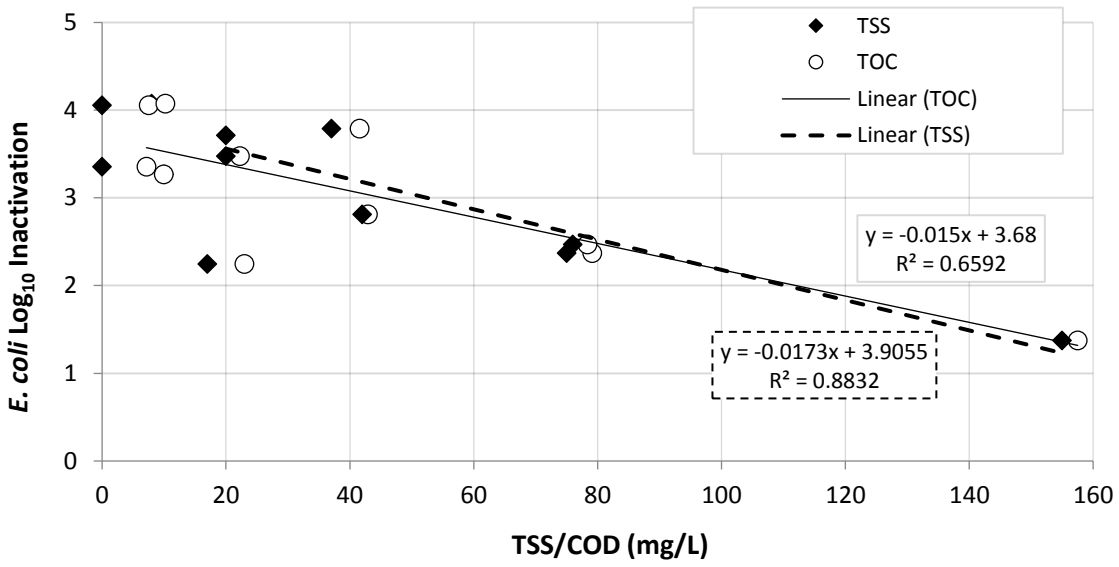


Figure 4.9. Relationship between organic TSS (IASBR sludge), TOC and *E. coli* inactivation via PUV treatment at an energy output of 1946 mJ/cm^2.

4.6.3 Impact of Organic TSS on LPUV System Efficiency

Sludge from a lab-scale IASBR reactor at NUI, Galway environmental labs (Chapter 3, Section 3.8.1) was spiked into tap water to give TSS concentrations ranging from 0 mg/L to 260 mg/L for LPUV analysis. Figure 4.10 illustrates the impact of organic TSS on LPUV system efficiency in terms of *E. coli* inactivation. LPUV system efficiency appeared to be impacted by organic TSS content to a lesser extent in comparison than the PUV system. *E. coli* inactivation appeared to remain relatively steady at 5.6 ± 0.8 $\log_{10}$ (shaded in grey) up to TSS concentrations of approximately 75 mg/L at a UV output 11.5 mJ/cm^2.

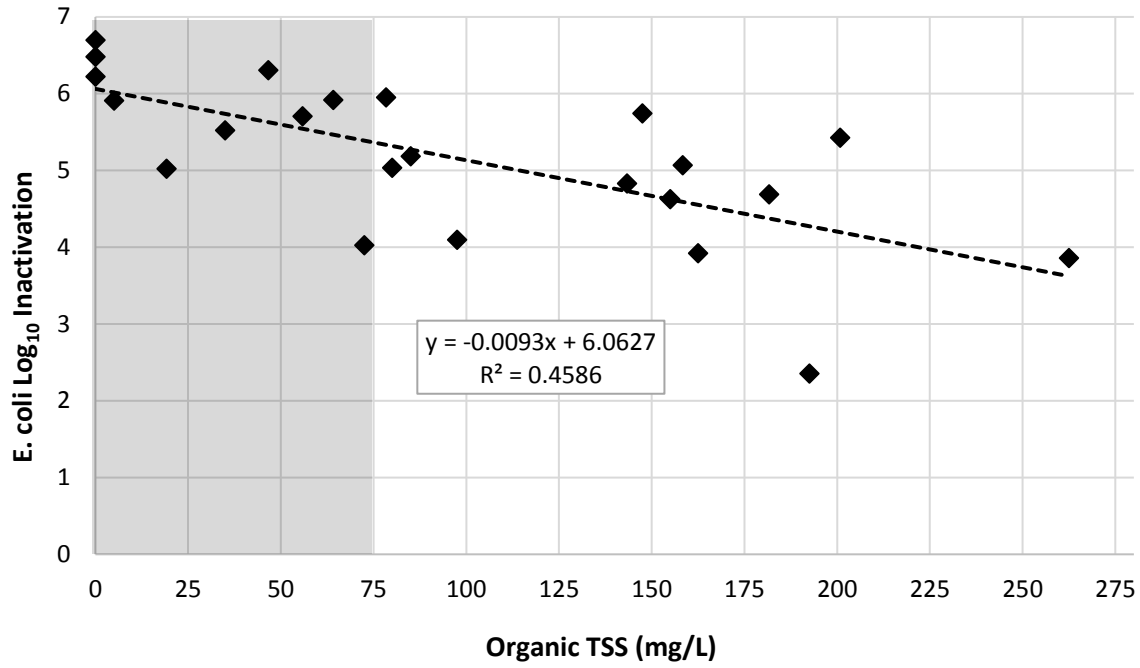


Figure 4.10. Impact of organic TSS (IASBR sludge) on *E. coli* inactivation via LPUV treatment at UV output 11.5 mJ/cm^2.

The data suggests that the LPUV system may prove to be a more robust option for tertiary wastewater disinfection as it appears to be less sensitive to increasing TSS concentrations and achieves a higher disinfection yield in terms of *E. coli* inactivation.

4.7 Site-Scale Analysis

As outlined in Chapter 3, Section 3.9, dairy wastewater samples were collected to compare the inactivation of a variety of different indigenous microorganisms in the wastewater. Results showed dairy wastewater pathogens were not detected in any of the site-scale or lab-scale wastewater samples during the experimental period and so could not be used as a comparative measure in the site-scale analysis (Table 4.4). It should be noted that as described in Section 4.3, this site (DP5) was one of those where dairy pathogens had been found during scoping studies.

Table 4.4. Concentration of dairy pathogens *L. monocytogenes* and *S. aureus* in dairy wastewater samples collected for UV system site-scale analysis.

Sample Type	*Listeria monocytogenes* (CFU/mL)	*Staphylococcus aureus* (CFU/mL)
IASBR Pilot-scale effluent (n = 6)	<1	<1
Full-scale WWTP effluent (n = 3)	<1	<1
IASBR Lab-scale effluent (n = 7)	<1	<1

The concentration of faecal indicators *E. coli* and total coliforms present in the dairy wastewater samples collected from the site-scale and lab-scale systems is illustrated in Figure 4.11. An average total coliform concentration of 4×10^5 MPN/100 mL and an average *E. coli* concentration of 2×10^2 MPN/100 mL were found in the dairy wastewater samples. Therefore, it was decided to use total coliforms as a comparative measure of UV system inactivation efficiency as the concentration of *E. coli* in the samples was insufficient to compare bacterial inactivation levels between the systems.

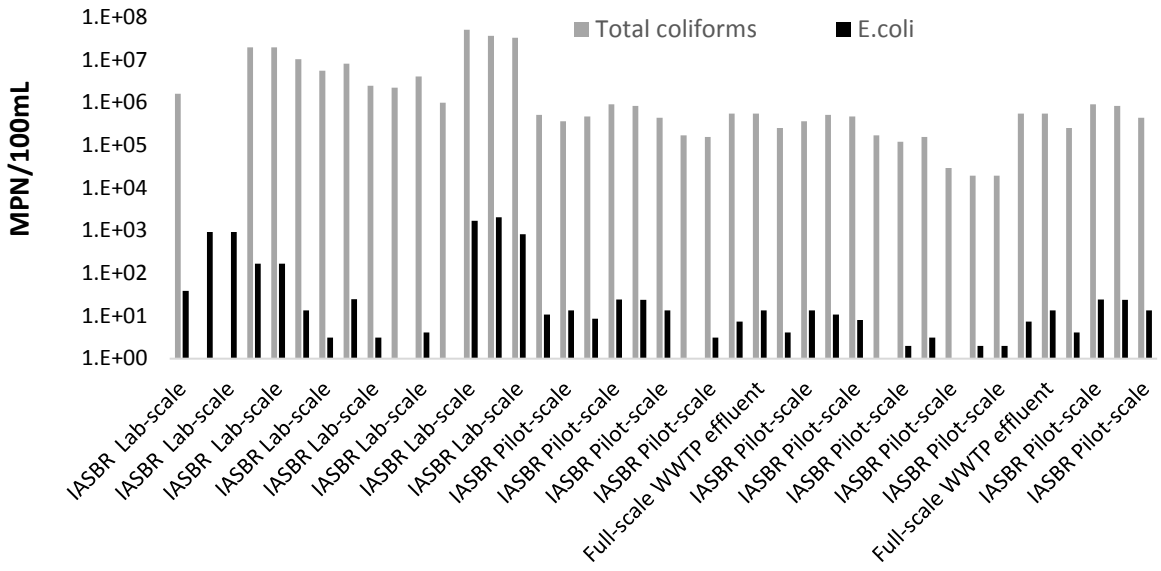


Figure 4.11. Concentration of *E. coli* and total coliforms in wastewater samples for site-scale analysis.

The impact of TSS on coliform inactivation efficiency of the flow-through PUV system is shown in Figure 4.12. The laboratory-scale IASBR effluent allowed for the analysis of samples with higher TSS concentrations in comparison to the sample wastewater retrieved from site where TSS concentrations were lower than 30 mg/L. The TSS concentrations of all of the samples tested during PUV experimental analysis ranged from 5 mg/L to 70 mg/L. The findings illustrate levels of total coliform inactivation via PUV treatment to be quite varied among similar TSS concentrations for the laboratory-scale wastewater with a difference of approximately 1.5 – 2.0 between $\log_{10}$ inactivation. For the site-scale wastewater used in the analysis, the TSS concentrations were all below 30 mg/L however the total coliform $\log_{10}$ inactivation rates at those concentrations were markedly lower in comparison to lab-scale wastewater samples. There appeared to be a slight trend of high TSS concentrations impacting upon PUV system efficiency however the large range

between total coliform inactivation rates at similar TSS concentrations creates difficulties in drawing conclusions.

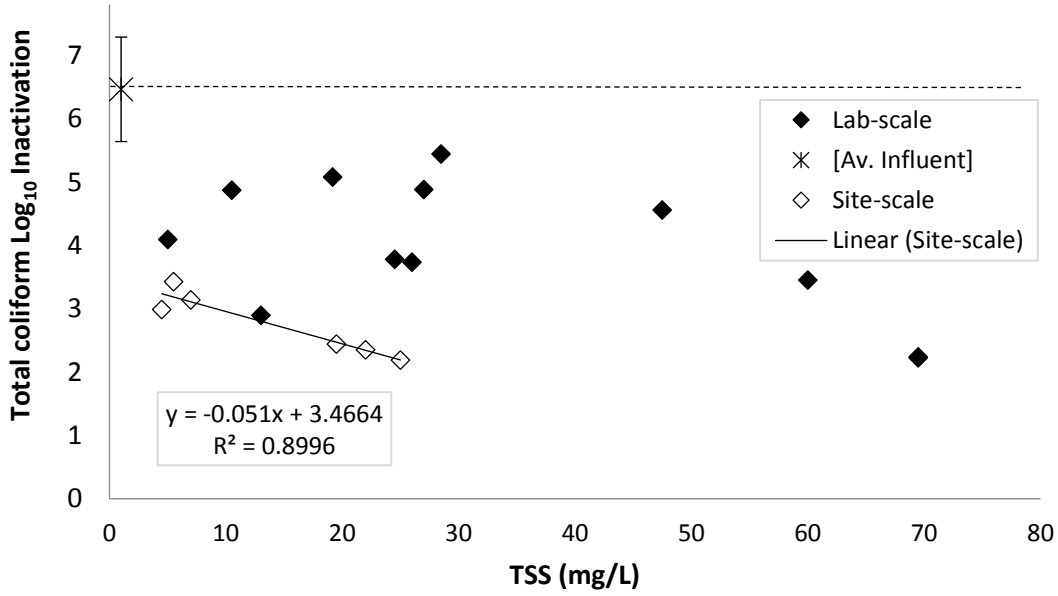


Figure 4.12. Impact of TSS on total coliform inactivation via flow-through PUV treatment (output at 2052 mJ/cm^2) using site-scale and laboratory-scale dairy wastewater. The dashed line represents the average total coliform influent concentration with standard deviation.

Total coliform inactivation in site-scale dairy wastewater via LPUV treatment at various TSS concentrations is shown in Figure 4.13. Total coliform inactivation was evaluated at a UV output of 14 mJ/cm^2 with the addition of three runs at 20 mJ/cm^2 for comparison of inactivation rates. Overall, the presence of TSS at the range of concentrations analysed did not impact on total coliform inactivation via LPUV treatment however it is acknowledged that this is to be expected as all TSS concentrations fell below the 30 mg/L cut-off point for system efficiency as per standard wastewater regulations. Average total coliform

inactivation rates of 4.6 ± 0.44 and 5.16 ± 0.09 were achieved at LPUV outputs of 14 mJ/cm^2 and 20 mJ/cm^2 respectively. The same site-scale wastewater was also analysed via PUV disinfection at three system energy outputs; 1026 mJ/cm^2, 1539 mJ/cm^2 and 2052 mJ/cm^2 (Figure 4.13). Results showed lower total coliform inactivation rates overall for the PUV system at each of the energy outputs tested in comparison to those found with the LPUV system.

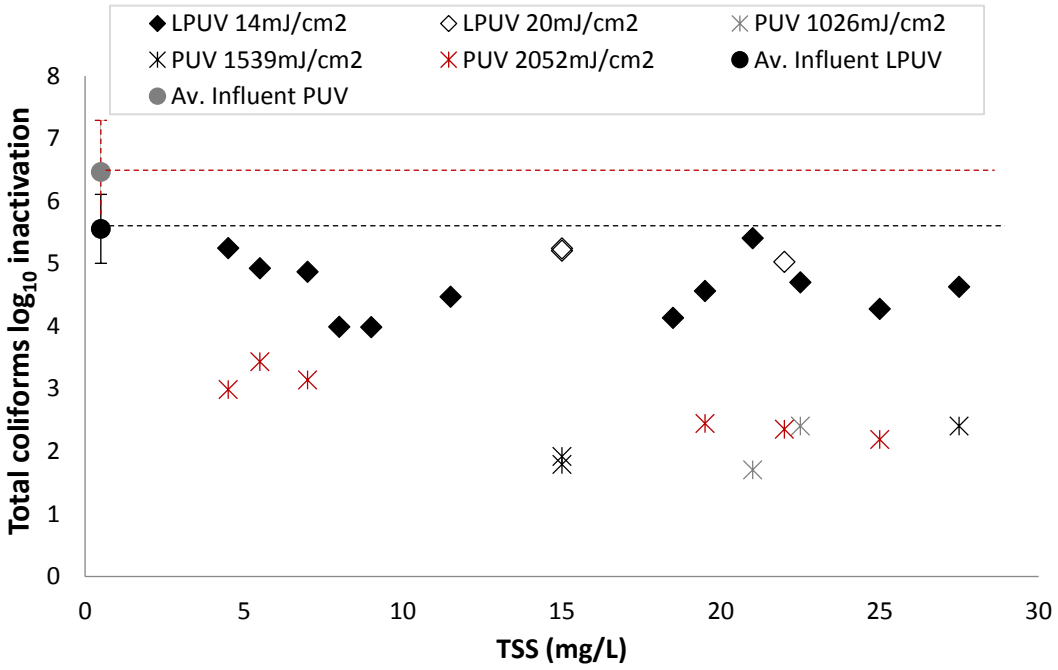


Figure 4.13. Comparative inactivation of total coliforms in site-scale dairy wastewater via both PUV and LPUV disinfection at various energy outputs. The dashed line (red for PUV and black for LPUV) represents the average total coliform influent concentration with standard deviation.

COD concentrations measured in the site-scale dairy wastewater ranged from 54 mg/L to 860 mg/L. Figure 4.14 illustrates COD concentrations appeared to have had minimal

impact on the LPUV inactivation efficiency of total coliforms (as would be expected given the results presented in 4.13).

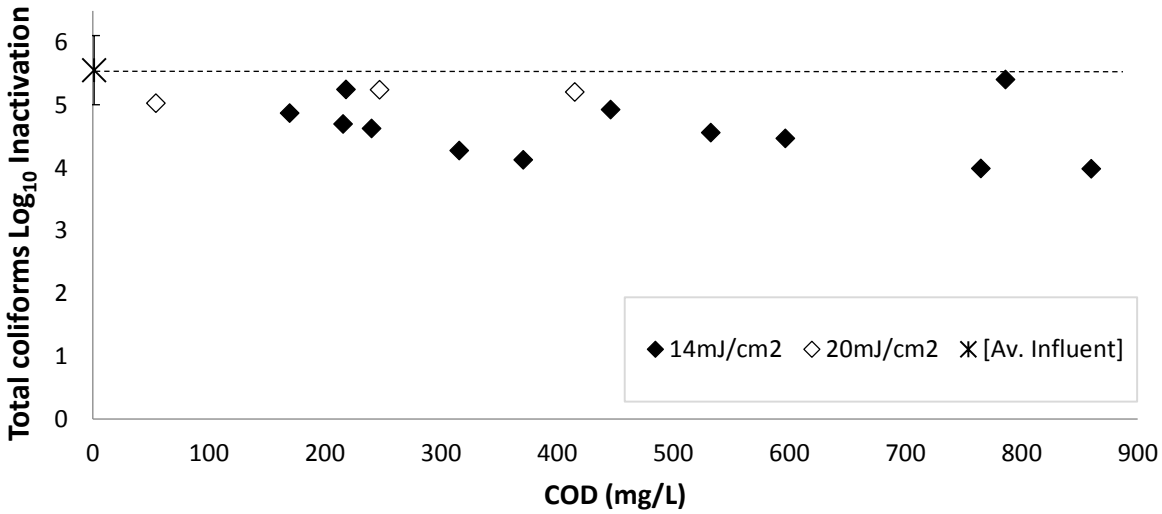


Figure 4.14. Impact of COD on total coliform inactivation via continuous-flow LPUV system at UV outputs of 14 and 20 mJ/cm^2 using site-scale dairy wastewater.
The dashed line represents the average total coliform influent concentration with standard deviation.

Analysis of varying concentrations of COD (from 103 mg/L to 572 mg/L) in dairy wastewater and its potential impact on PUV treatment efficiency yielded similar results to that of the LPUV analysis, total coliform inactivation did not appear to be influenced by increasing levels of COD. Results are presented in Figure 4.15 in addition to the impact of TSS (from 3 mg/L to 57 mg/L) on total coliform inactivation via PUV treatment. Similar to the TSS data, the range of total coliform inactivation rates via PUV treatment is quite large at any given concentration of COD.

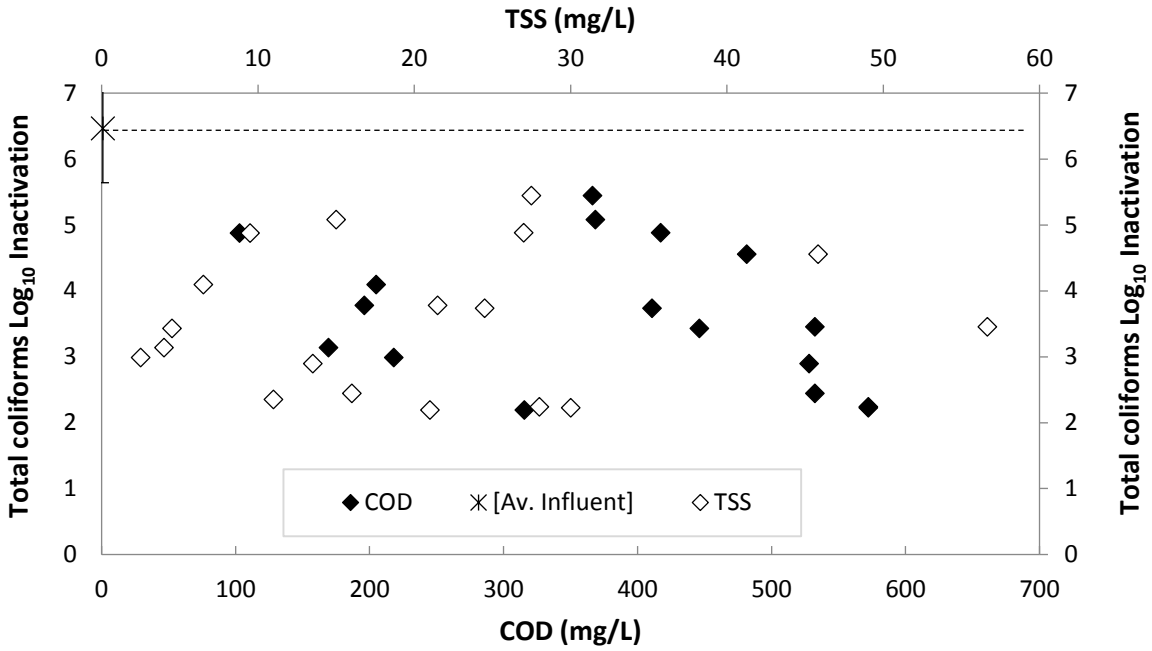


Figure 4.15. Impact of COD and TSS on total coliform inactivation via flow-through PUV treatment (output at 2052 mJ/cm^2) using site-scale and laboratory-scale dairy wastewater. The dashed line represents the average total coliform influent concentration with standard deviation.

4.8 Conclusions

The aims of this chapter included investigating the impact of dairy wastewater parameters, specifically TSS, TOC and COD on the inactivation efficiency of both the LPUV and flow-through PUV systems. The key findings are as follows;

- Inorganic TSS (bentonite and $CaCO_3$) had no impact on the working efficiency of the flow-through PUV system for *E. coli* inactivation.

- Laboratory analysis of the impact of organic TSS on UV system efficiency indicated TSS concentrations of >25-30 mg/L and >75 mg/L impacted on the PUV and LPUV systems respectively.
- The inactivation of total coliforms in site-scale dairy wastewater via PUV treatment was highly variable. This may be related to the test method which targets a population of bacteria rather than one specific species, thus variation in results could occur. No clear impact of increasing TSS or COD concentrations was detected.
- Continuous-flow LPUV consistently inactivated total coliforms in dairy wastewater at TSS concentrations of up to 28 mg SS/L (240 mg COD/l measured in that sample) and COD concentrations of up to 860 mg/L (9 mg SS/L measured in that sample): These were highest concentrations of these contaminants measured during this study. Average total coliform inactivation rates of 4.6 ± 0.44 and 5.16 ± 0.09 were achieved at LPUV outputs of 14 mJ/cm^2 and 20 mJ/cm^2 respectively.

Chapter 5
Comparative Studies on the Disinfection of *Bacillus* Endospores using Flow-Through PUV and Continuous-Flow LPUV Disinfection.

5.1 Introduction

Bacillus endospores are more UV resistant than vegetative bacterial cells and have been used as surrogates for assessing UV reactor performance for the water industry. *B. subtilis* endospores are currently the challenge microorganism of choice for UV reactor validation biodosimetry as per the DVGW drinking water standards protocol (DVGW, 2006). *B. pumilus* endospores are reported to exhibit a similar UV wavelength sensitivity to the highly resistant pathogen hAdV and have been suggested as potential surrogate challenge organisms for hAdV in UV reactor validation trials (Boczek *et al.*, 2016; Rochelle *et al.*, 2010; Verhoeven *et al.*, 2012). As discussed in Chapter 2, Section 2.4.4, previous research has shown a relationship between *Bacillus spp.* endospores cultivated on agar supplemented with $MnSO_4.H_2O$ and increased UV resistance to LPUV disinfection. Researchers adopted artificial supplementation with $MnSO_4.H_2O$ to expedite the endospore formation process and increase population densities. However, little information is available regarding *Bacillus spp.* endospores cultivated on agar supplemented with $MnSO_4.H_2O$ and enhanced UV resistance to pulsed UV (PUV) disinfection. This important factor is typically not considered when reporting on *Bacillus* endospore inactivation efficiencies of UV disinfection systems.

This chapter outlines the UV output requirements for *B. pumilus*, *B. subtilis* and *B. cereus* endospore inactivation via flow-through PUV and continuous flow LPUV systems. The

impact of varying concentrations of agar supplement $MnSO_4.H_2O$ on the enhanced resistance of *Bacillus* endospores to both PUV and LPUV is also presented. *Bacillus* endospores were subjected to PUV lamp outputs of between 486 mJ/cm^2 and 2052 mJ/cm^2 and LPUV lamp outputs of between 12 mJ/cm^2 and 32 mJ/cm^2. The PUV output range was selected as little or no inactivation was found below the 486 mJ/cm^2 (data not shown) and system limitations did not allow for PUV outputs above 2052 mJ/cm^2. For LPUV analysis, the lowest output achievable was 12 mJ/cm^2 and complete inactivation was typically found at 32 mJ/cm^2 (data not shown). Manganese sulphate concentrations of between 0 mM and 0.5 mM were applied to endospore cultivation agar to analyse the potential of enhanced UV resistance due to cultivation on agar supplemented with $MnSO_4.H_2O$.

5.2 Influence of Varying $MnSO_4.H_2O$ Concentration in *Bacillus pumilus* Sporulation Media

The impacts of increasing $MnSO_4.H_2O$ concentrations (0 mM, 0.05 mM, 0.25 mM and 0.5 mM) in culture agar media on the $\log_{10}$ inactivation of *B. pumilus* endospores using a PUV output of 2,052 mJ/cm^2 are summarised in Figure 5.1 (a). The starting population of endospores was 6.6 $\log_{10} \pm 0.7$. Results showed that 1.5 $\log_{10}$ inactivation of *B. pumilus* endospores was observed using a concentration of 0.05 mM $MnSO_4.H_2O$. In comparison, endospores cultivated in the absence of $MnSO_4.H_2O$ (or 0 mM) were inactivated by almost 2 $\log_{10}$ orders when treated at the same UV output. It should be noted that for the PUV system, the increasing UV output and exposure time are not directly related i.e. a higher UV output does not equate to a longer exposure time which is typical of LPUV systems. This is due to the variety of system settings that contribute to the final system output e.g. voltage settings, pulse frequency HRT etc. The $\log_{10}$ inactivation at 0 mM $MnSO_4.H_2O$

was found to be significantly different (P<0.05) to the two highest $MnSO_4.H_2O$ concentrations analysed; 0.25 mM and 0.5 mM. There appeared to be a saturation point for $MnSO_4.H_2O$ supplementation at 0.25mM as no significant difference was found between log_{10} inactivations at 0.25 mM and 0.5 mM $MnSO_4.H_2O$ (P>0.05).

The impact of the $MnSO_4.H_2O$ agar supplementation for *B. pumilus* endospores inactivation using LPUV at a single output of 20 mJ/cm^2 is shown in Figure 5.1 (b). Five concentrations of $MnSO_4.H_2O$ were analysed, namely 0 mM, 0.005 mM, 0.05 mM, 0.25 mM and 0.5 mM. The starting population of endospores was 5.66 log_{10} ± 0.9. Endospores cultivated in the absence of supplement $MnSO_4.H_2O$ were inactivated by 4.8 log_{10}. In comparison, the same endospores cultivated with the maximum concentration of 0.5 mM were inactivated by 2.2 log_{10} (UV output of 20 mJ/cm^2). Statistical analysis confirmed a significant difference was observed between endospore log_{10} inactivations at 0 mM and the three highest concentrations of 0.05 mM, 0.25 mM and 0.5 mM $MnSO_4.H_2O$ (P<0.05) while no significant difference was found between the log_{10} inactivations at the two highest $MnSO_4.H_2O$ concentrations of 0.25 mM and 0.5 mM. Therefore, in this case, endospores inactivated via LPUV exhibited a significantly higher UV resistance at a lower $MnSO_4.H_2O$ agar supplementation concentration of 0.05 mM in comparison to the PUV. It was also found that for both UV systems, the 0.25mM concentration appeared to be saturation point for $MnSO_4.H_2O$ supplementation and UV resistance therefore this concentration was selected for use in the following experiments.

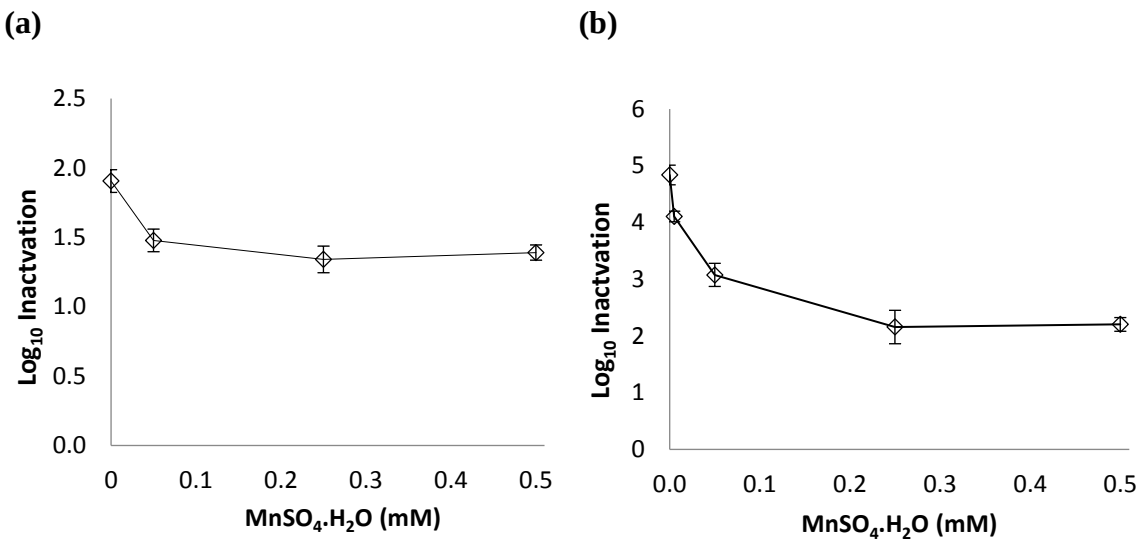


Figure 5.1. Impact of agar supplement $MnSO_4.H_2O$ concentration on *B. pumilus* inactivation via (a) PUV output at 2,052 mJ/cm^2 and (b) LPUV output at 21 mJ/cm^2 (points are averages of at least three runs and error bars are standard deviations).

The inactivation profiles of *B. pumilus* endospores cultivated in the absence of $MnSO_4.H_2O$ supplement is shown in Figure 5.2 (a) and 5.2 (b). The results illustrate a PUV output of approximately 2,052 mJ/cm^2 was required to inactivate *B. pumilus* endospores by almost 2 $\log_{10}$ in a flow-through system while a LPUV output of 12 mJ/cm^2 was required for a similar inactivation level of approximately 2 $\log_{10}$. Complete inactivation of the endospores via LPUV disinfection was achieved at a UV output of 31 mJ/cm^2.

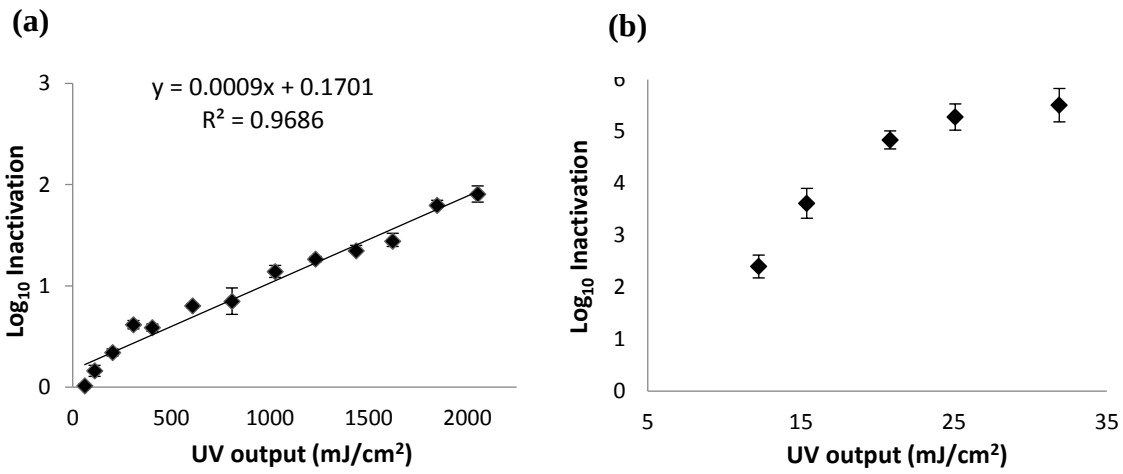


Figure 5.2. *B. pumilus* endospore inactivation via (a) PUV and (b) LPUV in the absence of $MnSO_4.H_2O$ supplement (points are averages of at least three runs and error bars are standard deviations).

Figures 5.3 (a) and 5.3 (b) compare the UV output response of *B. pumilus* endospores post PUV and LPUV treatment respectively, in the absence of $MnSO_4.H_2O$ and those cultivated with agar supplemented with $MnSO_4.H_2O$ (0.25 mM). It was noted that the impact of $MnSO_4.H_2O$ supplement on endospore UV resistance was observed to increase with increased PUV output whereas the LPUV results did not appear to follow the same trend. Taking Figure 5.3 (a) as an example, as the UV output from the PUV system increases, the difference in $\log_{10}$ inactivation between 0 mM $MnSO_4.H_2O$ and 0.25 mM $MnSO_4.H_2O$ increases i.e. the gap widens. This may suggest that the higher the UV output, the stronger the influence of $MnSO_4.H_2O$ on endospore UV resistance. In the case of the LPUV, endospores appeared to be influenced to a higher degree by $MnSO_4.H_2O$ at 21 mJ/cm^2 (a difference of 2.8 $\log_{10}$ inactivation was found between $MnSO_4.H_2O$ concentrations) in comparison to the maximum UV output (31 mJ/cm^2) where a difference of only 2 $\log_{10}$ was found between 0 mM $MnSO_4.H_2O$ and 0.25 mM $MnSO_4.H_2O$. Statistical analysis was

carried out to investigate whether the addition of $MnSO_4.H_2O$ to cultivation agar significantly enhanced log_{10} inactivation rates at comparative UV outputs for both the PUV and LPUV. For the PUV system, results showed the log_{10} inactivation of endospores cultivated without $MnSO_4.H_2O$ to be significantly different ($P < 0.05$) to endospores cultivated with 0.25mM $MnSO_4.H_2O$ at each of the five UV outputs analysed (111 mJ/cm^2, 403 mJ/cm^2, 1026 mJ/cm^2, 1622 mJ/cm^2 and 2052 mJ/cm^2). In addition, analysis of the same log_{10} inactivation rates at comparable LPUV outputs of 21 mJ/cm^2, 25 mJ/cm^2 and 32 mJ/cm^2 also found the log_{10} inactivation of endospores cultivated without $MnSO_4.H_2O$ to be significantly different ($P < 0.05$) to those cultivated with 0.25mM $MnSO_4.H_2O$. Thus, the addition of manganese sulphate monohydrate significantly enhanced the UV resistance of *B.pumilus* endospores to both flow-through PUV and LPUV treatment.

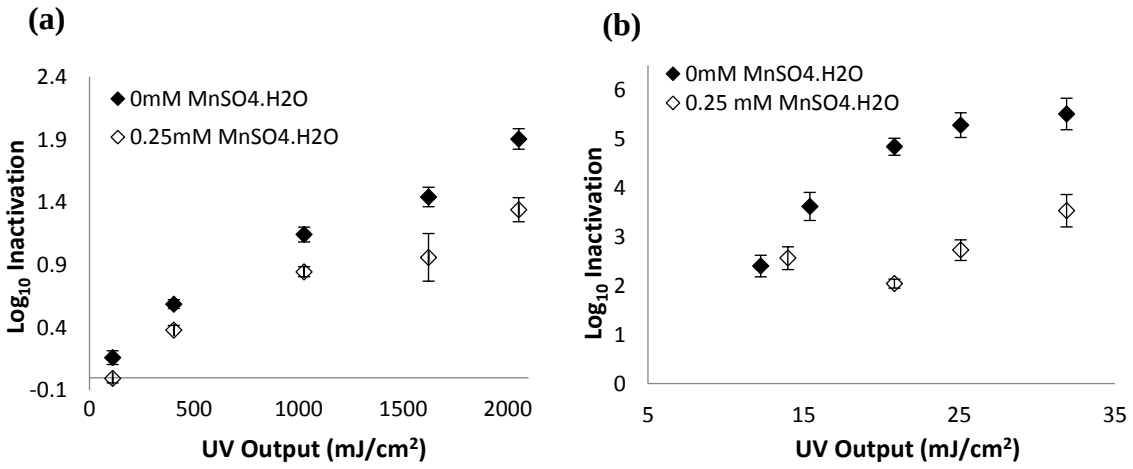


Figure 5.3. UV response curve of *B. pumilus* endospores to (a) PUV and (b) LPUV with (0.25 mM) and without (0 mM) $MnSO_4.H_2O$ supplement (points are averages of at least three runs and error bars are standard deviations).

Figure 5.4 (a) shows the relative increase in log_{10} inactivation of *B. pumilus* endospores for a given increase in UV output for the PUV system. This has been calculated as the increase

in log_{10} inactivation that resulted from a relative increase in PUV output from the lowest applied PUV output. For example, a PUV output of 111 mJ/cm^2 resulted in 0.16 log_{10} inactivation. When this output was increased by a multiple of 18.5 (i.e. to 2052 mJ/cm^2) the result was an additional 1.35 log_{10} inactivation. The same data analysis is presented in Figure 5.4 (b) for *B. pumilus* endospores inactivation via LPUV. In this case, it should be noted that the "baseline" output for the 0 mM and 0.25 mM $MnSO_4.H_2O$ were 12.3 mJ/cm^2 and 14 mJ/cm^2 respectively. Results illustrate an irregular trend for the relative increase in the log_{10} inactivation of *B. pumilus* endospores at 0.25 mM $MnSO_4.H_2O$. This trend is also reflected in Figure 5.3 (b).

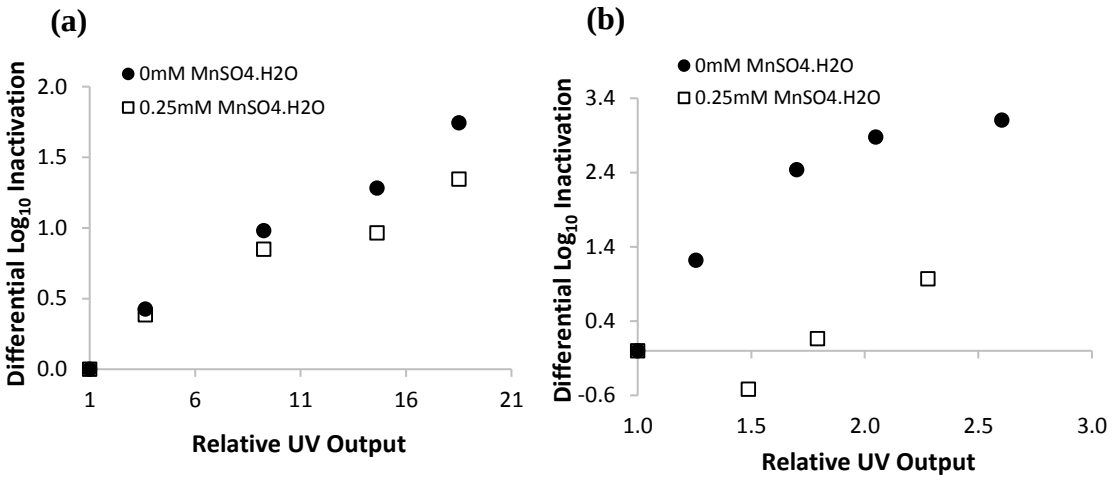


Figure 5.4. Relative increase in log_{10} inactivation of *B. pumilus* endospores for a given increase in UV output for the (a) PUV and (b) LPUV with (0.25 mM) and without (0 mM) $MnSO_4.H_2O$ supplement.

5.3 Influence of Varying $MnSO_4.H_2O$ Concentration in *Bacillus subtilis* Sporulation Media

The propagation of *B. subtilis* endospores in the absence of $MnSO_4.H_2O$ was not possible in this study and the minimum concentration of $MnSO_4.H_2O$ that could be applied while still cultivating *B. subtilis* endospores was 0.005 mM. This is likely to be attributed to the short propogation of endospores within ca. 4 days on media supplemented with $MnSO_4.H_2O$ at above ambient temperatures in contrast to natural aging process in agar that occurs after typically 7 to 10 days due to conversion of vegetative bacteria to endospores as a consequence of nutrient deprivation, build up of microbial waste products and reduction in water activity values. There is limited knowledge on the specific role of media formulation on triggering conversion to endospore state. However, recent studies using Raman spectroscopy has shown that maintaining levels of Ca2+ and pyridine-2,6-dicarboxylic acid (DPA) at 10% in the spore cortex appears critical as was shown to influence resistance environmental resistance and in spore stability (Huang *et al.*, 2007). Thus, it could be postualted that manganese sulphate in part may influence the formation of this Ca-DPA and concentrations thereof in spores, but this needs to be substantiated. One function of CA-DPA in spore resistance is to lower the core water content, probably by removing some core water. It is also thought it may make spore DNA more resistant to a variety of damaging agents, including UV radiation (Slieman & Nicholson, 2001).

In this study, a similar $\log_{10}$ inactivation (with a starting concentration of 6.4 $\log_{10} \pm 0.8$) of *B. subtilis* endospores (by the PUV unit) was found at the two lowest $MnSO_4.H_2O$ concentrations (no significant difference; $P > 0.05$) (Figure 5.5 (a)), but a decrease in $\log_{10}$ inactivation efficiency of approximately 0.6 $\log_{10}$ was observed when supplemented with

0.05 mM – a similar result was found for *B. pumilus* inactivation via PUV (Figure 5.1 (a)). A significant difference was found between the $\log_{10}$ inactivations at the two lowest concentrations in comparison to the two highest concentrations ($P<0.05$). For the LPUV system, *B. subtilis* (Figure 5.5(b)) did not appear to be as readily affected by $MnSO_4.H_2O$ supplementation when compared to *B. pumilus.* The starting concentration of endospores in this trial was 6.4 $\log_{10} \pm 0.7$. Four concentrations of $MnSO_4.H_2O$ were investigated; 0.005 mM, 0.05 mM, 0.25 mM and 0.5 mM. A $\log_{10}$ inactivation difference of 0.7 was observed between 0.005 mM $MnSO_4.H_2O$ concentration and the 0.25 mM $MnSO_4.H_2O$ concentration whereas a difference of 2 $\log_{10}$ was found between the same ranges for the *B. pumilus* experiments. Statistical analysis confirmed a significant difference ($P<0.05$) in endospore inactivation at the lowest $MnSO_4.H_2O$ concentration (0.005 mM) and the highest concentration $MnSO_4.H_2O$ concentration (0.5 mM) but not at 0.25 mM indicating a decreased sensitivity of *B. subtilis* endospores to $MnSO_4.H_2O$ supplementation in comparison to *B. pumilus* for LPUV inactivation.

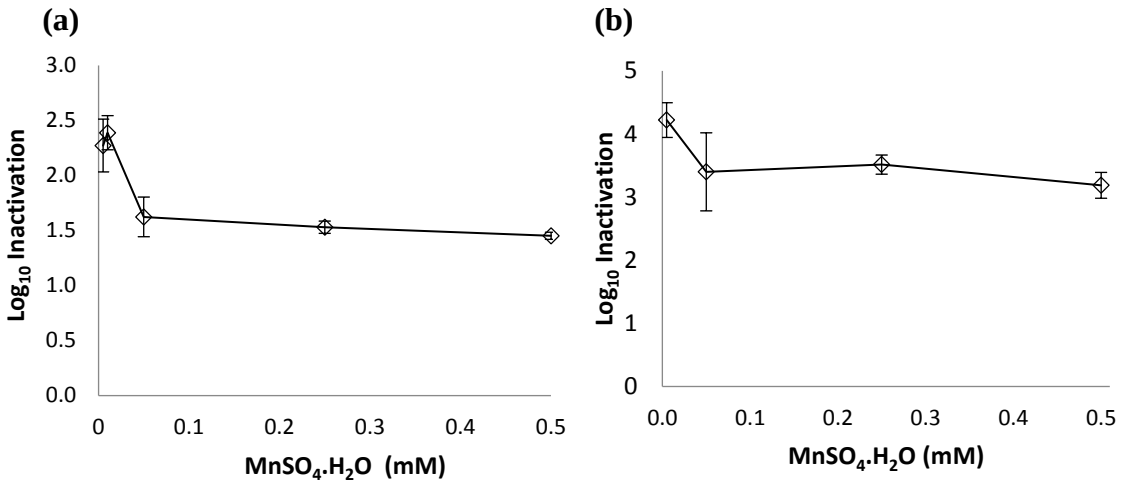


Figure 5.5. Impact of agar supplement $MnSO_4.H_2O$ concentration on *B. subtilis* inactivation via (a) PUV output (< 300 nm) at 2052 mJ/cm^2 and (b) LPUV output at 21 mJ/cm^2 (points are averages of at least three runs and error bars are standard deviations).

A UV response curve was generated for *B. subtilis* endospore inactivation via both flow-through PUV and LPUV (Figures 5.6 (a) and 5.6 (b)). The results of the PUV experiments followed a similar trend to the *B. pumilus* curve (Figure 5.3 (a)) whereby as the UV output increased, so too did the impact of the $MnSO_4.H_2O$ concentration on PUV inactivation efficiency. However, overall, *B. subtilis* endospores appeared to be more readily inactivated via PUV in comparison to *B. pumilus* endospores. This result is in keeping with previous studies which show *B. pumilus* to be more resistant to UV irradiation in comparison to *B. subtilis* (Malayeri et al., 2016), At the maximum UV output of 2,052 mJ/cm^2 *B. subtilis* endospores were inactivated by 2.27 $\log_{10}$ and 1.53 $\log_{10}$ at 0.005 mM and 0.25 mM $MnSO_4.H_2O$ concentrations respectively. In comparison, *B. pumilus* endospores were inactivated by 1.91 $\log_{10}$ and 1.34 $\log_{10}$ at 0.005 mM and 0.25 mM

$MnSO_4.H_2O$ concentrations respectively. The UV response curve of *B. subtilis* endospores to LPUV disinfection at both low (0.005 mM) and high (0.25 mM) $MnSO_4.H_2O$ concentrations (Figure 5.6 (b)) was notably different to the same analysis with *B. pumilus* endospores (Figure 5.3 (b)). *B. subtilis* endospores were inactivated by 5.91 log_{10} at 0.005 mM $MnSO_4.H_2O$ at 25 mJ/cm^2, at the same UV output the endospores were inactivated by 5.06 log_{10} when supplemented with 0.25 mM $MnSO_4.H_2O$. In contrast, a difference of 2.56 log_{10} inactivation was found for *B. pumilus* endospores when analysed at the same $MnSO_4.H_2O$ concentrations and UV outputs. These results indicate *B. subtilis* endospores are less affected by $MnSO_4.H_2O$ supplementation in terms of UV resistance when compared to *B. pumilus* endospores.

Using the Mann-Whitney U test, significant differences ($P < 0.05$) were found between log_{10} inactivation rates of endospores cultivated at low (0.005 mM) and high (0.25 mM) $MnSO_4.H_2O$ concentrations at each of the UV outputs for both the PUV and LPUV (Figure 5.6 (a) and 5.6 (b)). Analyses of endospore log_{10} inactivation rates in Figure 5.5 (b), whereby pairwise comparisons (Kruskal-Wallis and post-hoc Dunn test) were completed for the four data groups (0.005 mM, 0.05 mM, 0.25 mM and 0.5 mM $MnSO_4.H_2O$), demonstrated no significant difference between log_{10} inactivation rates of endospores cultivated in 0.005 mM and 0.25 mM $MnSO_4.H_2O$ ($P > 0.05$, $P = 0.113$). This may be attributable to the Bonferonni Correction factor which is automatically applied to the post hoc Dunn test to reduce the chance of false-positives arising when multiple pairwise comparisons are made.

A Mann-Whitney U test returned a P-value of 0.013 when comparing log_{10} inactivation rates of endospores cultured in 0.005 mM and 0.25 mM $MnSO_4.H_2O$ concentrations at a

LPUV output of 21 mJ/cm^2 i.e. a significant difference ($P < 0.05$). When the same data set was compared in conjunction with endospores cultivated in 0.05 mM and 0.5 mM $MnSO_4.H_2O$ using the Kruskal-Wallis test, an original P-value of 0.019 was found for the difference in log_{10} inactivation rates of endospores cultured in 0.005 mM (3.52 log removal) and 0.25 mM $MnSO_4.H_2O$ (4.2 log removal). This which would typically signify a significant difference between samples. However, when the Bonferonni Correction factor was then applied to the data, the original P-value of 0.019 was adjusted to 0.113.

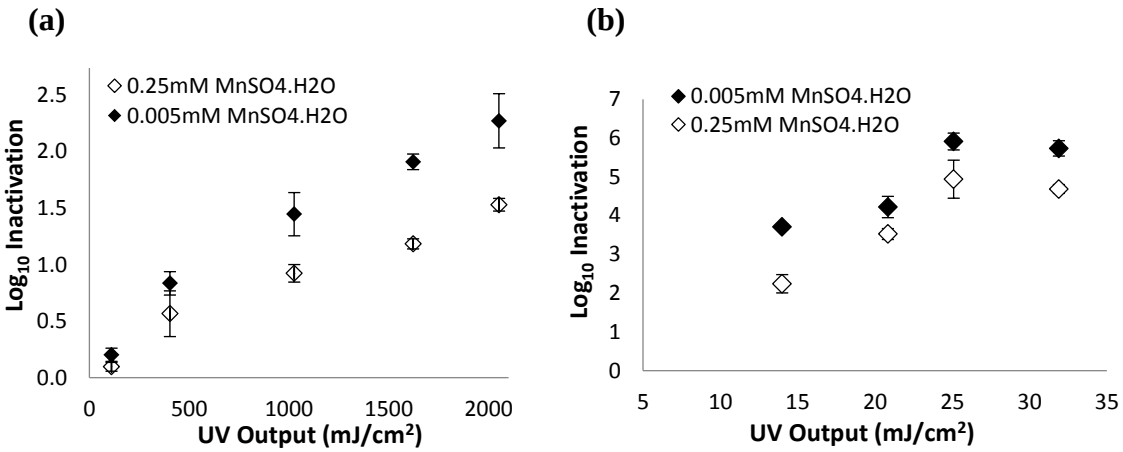


Figure 5.6. UV response curve of *B. subtilis* endospores to (a) PUV and (b) LPUV when cultivated with a high $MnSO_4.H_2O$ concentration (0.25 mM) versus a low (0.005 mM) $MnSO_4.H_2O$ concentration (points are averages of at least three runs and error bars are standard deviations).

The relative increase in the log_{10} inactivation of *B. subtilis* endospores for a given increase in UV output for the PUV system is illustrated in Figure 5.7 (a). As highlighted previously, the trends in the graph suggest a relationship between increasing UV output and stronger UV resistance when *B. subtilis* endospores are supplemented with 0.25 mM $MnSO_4.H_2O$. Figure 5.7 (b) shows the relative increase in log_{10} inactivation for *B. subtilis* endospores

for a given increase in UV output for the LPUV system. A sharp increase in the differential log_{10} inactivation (1.5 log_{10}) is notable between increased UV outputs of 50% (LPUV output of 20.8 mJ/cm^2) and 80% (UV output of 25.1 mJ/cm^2) respectively. This result may be indicative of the sensitivity of *B. subtilis* endospores to LPUV irradiation whereby a relatively minor increase in the LPUV system output results in a markedly higher endospore inactivation.

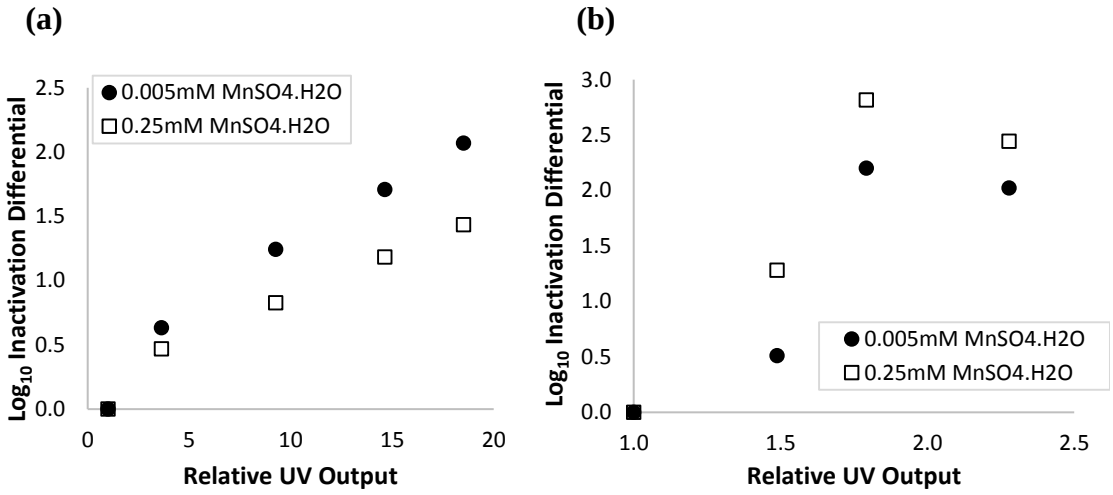


Figure 5.7. Relative increase in log_{10} inactivation of *B. subtilis* endospores for a given increase in UV output for the (a) PUV and (b) LPUV with (0.25 mM) and without (0mM) $MnSO4.H_2O$ supplement.

5.4 Influence of Varying $MnSO_4.H_2O$ Concentration in *Bacillus cereus* Sporulation Media

As with the propagation of *B. subtilis* endospores, it was not possible in this study to propagate *B. cereus* endospores in the absence of agar supplement $MnSO_4.H_2O$. The minimum concentration applied that resulted in successful cultivation was 0.005 mM $MnSO_4.H_2O$. However, limitations occurred when attempting to achieve a similar starting influent concentration of approximately 6 log_{10} (see Chapter 3, Section 2.3). For *B. cereus*

endospores, a starting influent concentration of 4.8 $\log_{10}$ ± 1.0 was the maximum concentration achievable. Nonetheless, investigations into the impact of $MnSO_4.H_2O$ supplement on the enhanced UV resistance of *B. cereus* endospores were carried out. Four concentrations of $MnSO_4.H_2O$ agar supplementation were analysed; 0.005 mM, 0.05 mM, 0.25 mM and 0.5 mM at a PUV output of 2052 mJ/cm^2. Findings were consistent with similar analysis for *B. pumilus* and *B. subtilis* endospores; the addition of agar supplement $MnSO_4.H_2O$ to cultivation agar significantly ($P<0.05$) enhanced the UV resistance of *B. cereus* endospores to PUV irradiation. Figure 5.6 (a) illustrates a decrease in $\log_{10}$ inactivation efficiency from 1.59 $\log_{10}$ at 0.005 mM to 1.32 $\log_{10}$ at 0.05 mM $MnSO_4.H_2O$. Statistical analysis confirmed a significant difference ($P<0.05$) between *B. cereus* endospore $\log_{10}$ inactivation at 0.005 mM and the three higher $MnSO_4.H_2O$ concentrations of 0.05 mM, 0.25 mM and 0.5 mM. Figure 5.6 (b) compares the UV response of *B. cereus* endospores when cultivated with low (0.005 mM) and high (0.25 mM) $MnSO_4.H_2O$ concentrations and exposed to the same PUV output energy ranging from 403 mJ/cm^2 to 2052 mJ/cm^2. Results show similar endospore $\log_{10}$ inactivation values for both of the $MnSO_4.H_2O$ concentrations (0.005 mM and 0.25 mM) at the two lowest PUV outputs of 403 mJ/cm^2 and 1026 mJ/cm^2. A significant difference ($P < 0.05$) was found between endospore $\log_{10}$ inactivations at high and low $MnSO_4.H_2O$ concentrations for endospores inactivated at 1622 mJ/cm^2 and 2052 mJ/cm^2 but not at the two lower UV outputs as expected.

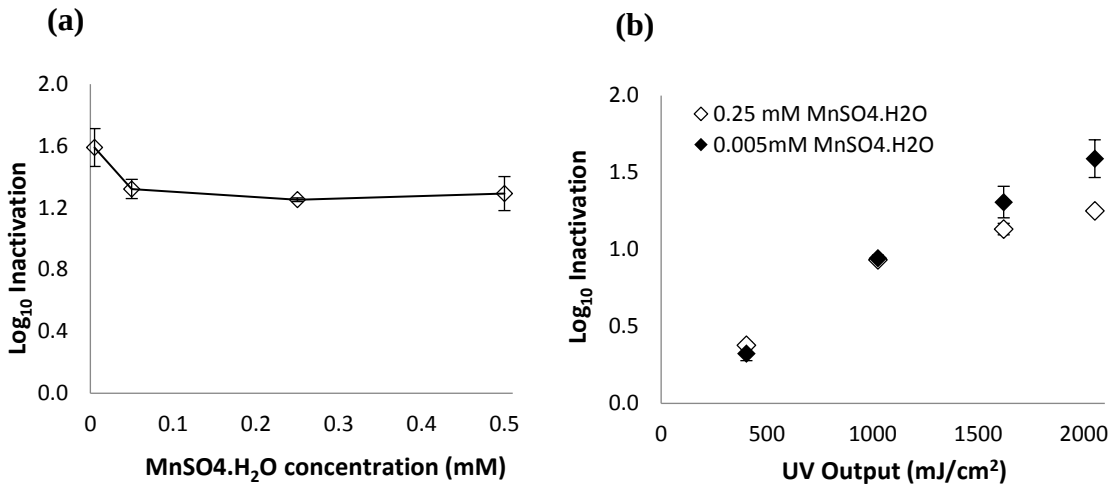


Figure 5.8. (a) Impact of agar supplement MnSO4.H2O concentration on *B. cereus* inactivation via PUV output (< 300 nm) at 2052 mJ/cm2 and (b) UV response curve of *B. cereus* inactivation via PUV when cultivated with a high $MnSO_4.H_2O$ concentration. (0.25 mM) versus a low (0.005 mM) $MnSO_4.H_2O$ concentration (points are averages of at least three runs and error bars are standard deviations).

Due to lower yields of *B. cereus* endospores at the cultivation phase, analysis via LPUV disinfection was limited to one trial (three replicate runs) which investigated *B. cereus* endospore inactivation at the lowest LPUV output available – 14 mJ/cm^2. Table 5.1 compares the average $\log_{10}$ inactivation of *B. cereus* to the $\log_{10}$ inactivations of *B. pumilus* and *B. subtilis* at similar LPUV outputs and $MnSO_4.H_2O$ cultivation concentrations. The average initial concentration of *B. cereus* endospores for this trial was 4.0 $\log_{10} \pm 0.55$. Results showed findings to be consistent with the other two strains tested, in particular *B. subtilis* analysis where experimental conditions were identical.

Table 5.1. Comparison of *Bacillus* endospores inactivation via continuous-flow LPUV.

Endospore strain	$MnSO_4.H_2O$ (mM)	LPUV Output (mJ/cm^2)	Log_{10} Inactivation
B. pumilus	0.000	15.4	3.71±0.08
B. subtilis	0.005	14.0	2.40±0.22
B. cereus	0.005	14.0	2.19±0.37

5.5 Discussion

5.5.1 Inactivation of *Bacillus pumilus* via UV disinfection and Influence of $MnSO_4.H_2O$ Sporulation Media

Table 5.2 outlines the relevant literature to date investigating the inactivation of *B. pumilus* endospores via both PUV and LPUV disinfection in comparison to results found in this study. To the best of our knowledge the inactivation of *B. pumilus* endospores via pulsed UV disinfection in a flow-through system has not previously been published. Previous research involving static PUV disinfection reported a UV dose of 500 mJ/cm^2 to achieve a 3 log_{10} inactivation of the endospores on agar using the same strain of *B. pumilus* endospores utilised in this study (Levy *et al.*, 2012). The received UV dose of 500 mJ/cm^2 achieved a higher endospore log_{10} inactivation in comparison to this research however PUV energy was measured with a sensor to give the absorbed UV dose, not the energy output of the system (see Chapter 2, Section 2.3.7 and Chapter 8, Section 8.3). Differences in log_{10} inactivation rates may also be attributed to PUV system set-up (static) and/or the

disinfection medium (e.g. agar, water or hard surfaces). Findings reported by Levy *et al.,* (2012) showed higher endospore inactivation rates on polystyrene, glass and aluminium surfaces in comparison to agar surfaces (Levy *et al.,* 2012). In addition, recent research has shown an increased PUV output requirement for the inactivation of viruses in water via flow-through PUV in comparison to static PUV while other studies have illustrated a difference in the inactivation mechanism of vegetative *B. subtilis* cells when exposed to PUV in either a dry or liquid state (Nicorescu *et al.*, 2013; Yi *et al.,* 2016). As mentioned previously, the method of UV dose/output measurement is an important consideration when comparing the literature. Garvey *et al.,* (2013) also investigated the inactivation of *Bacillus* endospores with PUV light and used chemical actinometry method was used to determine the UV dose. Therefore direct comparisons to this study in relation to UV dose/output and endospore inactivation levels was not possible (Garvey *et al.,* 2013).

For LPUV disinfection using *B. pumilus* endospores, previous research was carried out in batch analysis whereby a collimated beam apparatus was used to disinfect continuously stirring water in a petri dish (Table 5.2). The LPUV doses required for endospore inactivation are higher in comparison to this study however the flow-through system used in this study may have aided endospore inactivation. Nevertheless, despite the experimental set-up, the results for all of the LPUV studies in Table 5.2 comparing the impact of $MnSO_4.H_2O$ supplement on the enhanced UV resistance of *B. pumilus* are in agreement with the results found in this study whereby the addition of $MnSO_4.H_2O$ supplement aids endospore UV resistance (Boczek *et al.,* 2016; Rochelle *et al.,* 2010; Verhoeven *et al.,* 2012). It is also clear from data presented in Table 5.2 that the LPUV dose/output response of *B. pumilus* endospores tends to vary by strain. Previous studies have illustrated similar

findings whereby a variety of *Bacillus* species were found to demonstrate varying sensitivities to electron beam irradiation (McFadden *et al.*, 2016).

Table 5.2. Comparison of research studies for the inactivation of *B. pumilus* endospores cultivated in $MnSO_4.H_2O$ via PUV and LPUV disinfection.

UV system & set-up	Strain	$MnSO_4.H_2O$ (mM)	System output/dose (mJ/cm^2)	Log_{10} inactivation	Reference
PUV static	ATCC 27142	FNA[1]	500[2]	3.0	(Levy *et al.*, 2012)
PUV static	ATCC 14884	0.02	0.00432[3]	4.0	(Garvey *et al.*, 2013)
PUV flow-through	ATCC 27142	0	1,026[4] (1,440[2])	1.4	This study
		0.25	2,052[4] (2,880[2])	1.3	
LPUV static	ATCC 27142	0.1	138[2]	2.0	(Boczek *et al.*, 2016)
LPUV static	ASFUVRC	0.1	348[2]	2.0	(Boczek *et al.*, 2016)
LPUV static	ASFUVRC	0.1	174.7[2]	2.0	(Beck *et al.*, 2015)
LPUV static	ASFUVRC	0	77[2]	3.0	(Rochelle *et al.*, 2010)
		1	>150[2]	2.0	
LPUV static	ASFUVRA	0	59[2]	3.0	(Rochelle *et al.*, 2010)
		1	200[2]	2.0	
LPUV static	SAFR-032	0	76[2]	3.0	(Rochelle *et al.*, 2010)
		1	250[2]	2.0	
LPUV flow-through	ATCC 27142	0	12[5]	2.4	This study
		0.25	25[5]	2.2	

[1]FNA – fortified nutrient agar used – typically pre-supplemented with 30mg/L $MnSO_4.H_2O$

[2]Broadspectrum dose measured with a joulemeter or radiometer

[3]UV dose measured as per actinometry method i.e. the amount of UV energy absorbed

[4]Calculated UV output (< 300nm) based on system specifications at 0 cm from lamp

[5]UV output based on system specifications

5.5.2 Inactivation of *Bacillus subtilis* via UV disinfection and Influence of $MnSO_4.H_2O$ Sporulation Media

B. subtilis endospores were less resistant to PUV irradiation in comparison to *B. pumilus* endospores when cultivated with both low and high $MnSO_4.H_2O$ concentrations. This may be due to differences in coat structure and/or proteins; it has been shown *B. subtilis* spores are more resistant to pulsed irradiation when expressing proteins linked to coat formation in comparison to mutated strains where said proteins were absent (Esbelin *et al.*, 2016). The spectral sensitivity of both strains differ with *B. subtilis* endospores being most sensitive to wavelengths around 265 nm while *B. pumilus* endospores are most sensitive to wavelengths at approximately 220 nm (Beck *et al.*, 2015; Mamane-Gravetz *et al.*, 2005). Spectral peaks of the PUV system used in the study were measured at 247 nm and 260 nm (see Chapter 3, Section 3.3.3) therefore the UV-C portion of the lamp output may favour inactivation of *B. subtilis* endospores over *B. pumilus* endospores. The disinfection of *B. subtilis* endospores in wastewater effluent via a flow-through PUV system has previously been reported, however the applied UV dose/output was not calculated. Rather, the $\log_{10}$ reduction per flow rate was given in synthetic municipal wastewater effluent (e.g. a flow rate of 8 L/min resulted in a 3.89 $\log_{10}$ reduction) (Uslu *et al.*, 2016). The authors completed a similar study investigating *B. subtilis* endospore inactivation via static PUV and in that study a broadspectrum PUV output of 2,900 mJ/cm^2 was reported to give a 1.72 $\log_{10}$ inactivation (Uslu *et al.*, 2015). Information on whether $MnSO_4.H_2O$ supplement was added to cultivation agar for both studies was not provided. A similar study investigating the static PUV disinfection of *B. subtilis* endospores in suspension did report agar supplementation of 0.006 mM $MnSO_4.H_2O$. Samples were subjected to PUV doses of 500 mJ/cm^2 and 1000 mJ/cm^2 which reduced endospore counts by 1.1 $\log_{10}$ and 2.7 $\log_{10}$

respectively (Artíguez & Martínez de Marañón, 2015). The evaluation of PUV disinfection efficiency of *B. subtilis* endospores on a number of static surfaces; agar, polystyrene and glass was also investigated and reported a UV dose requirement of 500 mJ/cm^2 to achieve a 3 $\log_{10}$ inactivation of the endospore on all three surfaces (Levy *et al.*, 2012). Moreover, in-depth analysis of the germicidal effectiveness of the PUV broadspectrum applied confirmed the PUV system to be almost completely ineffective against *B. subtilis* endospores when UV wavelengths of <300 nm were removed from the treatment (Levy *et al.*, 2012). This result has been corroborated in other studies for the PUV disinfection of *B. pumilus* endospores, *Listeria monocytogenes* and *Listeria innocua* (Wang *et al.*, 2005; Woodling & Moraru, 2007; Esbelin *et al.*, 2016; Uesugi *et al.*, 2016). Thus, differences between inactivation results and PUV dose/output measurements within the literature underline the necessity to consider the method by which the UV dose/output is determined for each system.

A LPUV output of 25 mJ/cm^2 yielded a $\log_{10}$ inactivation of 5.91 when *B. subtilis* endospores were cultivated with 0.005 mM $MnSO_4.H_2O$ supplement. In contrast, endospores cultivated in 0.25 mM $MnSO_4.H_2O$ illustrated a greater UV resistance with a $\log_{10}$ inactivation of 5.06 at the same UV output. The inactivation of *B. subtilis* endospores via LPUV disinfection has been widely reported, perhaps due to its routine use as a challenge microorganism in UV reactor validation trials. The strain ATCC 6633 was chosen for this study as it has been widely analysed in the literature and hence allowed for greater intercomparisons. A peer review of the literature by Malayeri *et al.*, (2016) cited a total of 16 studies which met specific selection criteria for the investigation of *B. subtilis* endospore inactivation via LPUV disinfection (Malayeri *et al.*, 2016). To achieve a 2 $\log_{10}$

inactivation, a UV dose range of between 15 mJ/cm^2 and 48 mJ/cm^2 was reported for all studies. The majority of the studies did mention the use of sporulation mediums (which would typically contain the supplement $MnSO_4.H_2O$) however the addition of $MnSO_4.H_2O$ to cultivation agar or broth used in the study was not specified. In comparison, this study observed that at a UV output of 14 mJ/cm^2, $\log_{10}$ inactivations of 3.71 and 2.24 were achieved when cultivation agar was supplemented with 0.005 mM and 0.25 mM $MnSO_4.H_2O$ respectively. Thus, results consistent with those cited in the literature though in this study $MnSO_4.H_2O$ UV outputs are indicated. Ghosh *et al.*, (2011) on the other hand, carried out an extensive study which investigated the relationship between increased manganese content during the endospore formation stage of *Bacillus megaterium* (*B. megaterium*) (QM B1551) and *B. subtilis* (PS533 wild type) endospores and their response to LPUV irradiation (Ghosh *et al.*, 2011). The complex used in this study was manganese chloride ($MnCl_2$) as opposed to $MnSO_4.H_2O$. Nonetheless, results showed increased Mn levels to enhance the UV resistance of *B. subtilis* endospores and significantly so for *B. megaterium* endospores.

Differences in endospore $\log_{10}$ inactivation depending on whether the endospores were cultivated in liquid or from the surface of agar have been reported previously. Bohrerova *et al.*, (2006) demonstrated that *B. subtilis* endospores (ATCC 6633) cultivated in liquid broth required a LPUV dose 30 mJ/cm^2 to yield a 1 $\log_{10}$ inactivation while those cultivated on agar required a lower dose of approximately 10 mJ/cm^2 for the same $\log_{10}$ inactivation (Bohrerova *et al.*, 2006). As appears to be the case with *B. pumilus* spores, the LPUV sensitivity of *B. subtilis* may also vary depending on the cultivation method applied.

5.5.3 Inactivation of *Bacillus cereus* via UV disinfection and Influence of $MnSO_4.H_2O$ Sporulation Media

Analysis investigating the impact of agar supplement $MnSO_4.H_2O$ on the enhanced UV resistance of *B. cereus* endospores proved to be consistent with results found for both *B. pumilus* and *B. subtilis* endospore analysis; the addition of the supplement to cultivation agar significantly ($P < 0.05$) enhanced endospore UV resistance to PUV flow-through disinfection. At the maximum PUV output (2052 mJ/cm^2) tested, *B. cereus* endospores were inactivated by 1.59 $\log_{10}$ with 0.005 mM $MnSO_4.H_2O$ supplementation in comparison to 1.25 $\log_{10}$ inactivation with 0.25mM $MnSO_4.H_2O$ supplementation. However, as the starting concentration of the bacteria was lower in comparison to *B. pumilus* and *B. subtilis* endospores starting concentrations, a direct comparison of all three endospore's resistance to PUV is not made in this study. *B. cereus* endospore inactivation via flow-through PUV disinfection has been carried out previously. Garvey & Rowan (2015) reported a $\log_{10}$ inactivation of 1.67 when a UV dose of 25.9 µJ/cm^2 was applied (Garvey & Rowan, 2015). However, as the UV dose was measured via chemical actinometry method (see Chapter 2 Section 2.3.7) a comparison to results found in this study was not possible. Research investigating the response of *B. cereus* endospores (LMG 6924) to pulsed light in a static experimental set-up documented an inactivation of >3 $\log_{10}$ when 50 pulses of light were applied to samples in petri dishes (Gómez-López *et al.*, 2005). No UV dose was described in the study. The inactivation of *B. cereus* endospores (KBAB4) via static PUV on agar reported a UV dose requirement of 0.35 J/cm^2 for a 1 $\log_{10}$ inactivation (Levy *et al.*, 2012). Differences in methods of PUV energy measurement used in that research and the present study again highlight the difficulties around intercomparisons within the literature for PUV studies.

A similar study which investigated the effect of increasing levels of $MnCl_2$ on the resistance of *B. cereus* endospores to UV-C or LPUV radiation found the addition of the magnesium complex to have no significant impact on endospore UV resistance (Klobutcher, 2013). Rowan *et al.*, (1998) have previously studied the influence of variations in media formulation on bacterial physiology and subsequent tolerance to environmental stresses (Rowan and Anderson, 1997, 1998; Rowan, 2004; Bradley *et al.*, 2012; Rowan *et al.*, 2015). It has been reported that the supplementation of growth media with food additive maltodextrin augmented the diarrhoeal enterotoxin production in *Bacillus cereus* (Rowan & Anderson, 1997). Moreover, variations in media formulation and storage affected the virulence and pathogenesis of *B. cereus* in hospitalised HIV patients. Enhancing the osmotic environment of culture media with increasing concentrations of glucose has also been proven to affect the tolerance of *Listeria monocytogenes* to PUV treatments (Bradley *et al.*, 2012). In this study, only one LPUV output of 14 mJ/cm^2 was trialled for *B. cereus* endospore inactivation which resulted in a 2.19 log_{10} inactivation. Similar trials of *B. cereus* inactivation via LPUV in an aqueous (sodium bicarbonate) solution reported a UV dose requirement of 20 mJ/cm^2 for approximately 2 log_{10} inactivation (Blatchley *et al.*, 2005). Thus, results in this study for *B. cereus* inactivation via LPUV are in line with those found in the literature.

5.6 Conclusions

The main aims of this chapter included a comparison of both UV systems for the inactivation of *Bacillus* endospores and to investigate whether media culture supplementation influenced the UV resistance of *Bacillus* endospores to PUV/LPUV disinfection. The continuous flow LPUV system achieved complete inactivation of both *B.*

pumilus and *B. subtilis* endospores while the flow-through PUV system demonstrated incomplete inactivation at the UV outputs analysed. The addition of agar supplement $MnSO_4.H_2O$ significantly ($P<0.05$) enhanced the UV resistance of all endospore strains to both LPUV and PUV disinfection. The main findings are as follows;

- A PUV output of 2,052 mJ/cm^2 yielded 1.91 log_{10} inactivation of *B. pumilus* endospores (0mM $MnSO_4.H_2O$) while a LPUV output of 32 mJ/cm^2 achieved a 5.51 log_{10} inactivation of *B. pumilus* endospores (0mM $MnSO_4.H_2O$).
- *B. subtilis* endospores could not be cultivated in the absence of $MnSO_4.H_2O$. At the lowest $MnSO_4.H_2O$ concentration (0.005mM) *B. subtilis* endospores were inactivated by 2.27 log_{10} and 5.73 log_{10} when exposed to a PUV output of 2,052 mJ/cm^2 and a LPUV output of 32 mJ/cm^2 respectively.
- *B. cereus* also required a minimum of 0.005mM $MnSO_4.H_2O$ supplementation for endospore propagation which yielded a lower starting concentration in comparison to *B. pumilus* and *B. subtilis*. At the maximum PUV output of 2052 mJ/cm^2, endospores were inactivated by 1.59 log_{10} while a LPUV output of 14 mJ/cm^2 achieved 2.19 log_{10} inactivation.
- The addition of $MnSO_4.H_2O$ supplement significantly ($P<0.05$) enhanced the UV resistance of *B. pumilus*, *B. subtilis* and *B. cereus* endospores to PUV irradiation (UV output of 2,052 mJ/cm^2).
- The addition of $MnSO_4.H_2O$ supplement had a greater impact on the enhanced UV resistance of *B. pumilus* endospores in comparison to *B. subtilis* endospores when exposed to LPUV irradiation at 21 mJ/cm^2. Results showed a significantly different

($P<0.05$) *B. pumilus* inactivation when 0.05mM $MnSO_4.H_2O$ was added to cultivation agar at the same LPUV output. In contrast, a concentration of 0.5mM was required to achieve a significant difference ($P<0.05$) between log_{10} inactivations of *B. subtilis* via LPUV at 21 mJ/cm^2.

- The addition of key information such as whether sporulation media was supplemented with $MnSO_4.H_2O$ and at which concentration, the UV dose and/or output and method of energy measurement and the HRT (if flowing water is used) should be clearly stated in future studies to enable better inter-comparison within this field.

Chapter 6
Inactivation, Photoreactivation and Dark Repair Analysis of Dairy Bacterial Pathogens post Flow-Through PUV and Continuous-Flow LPUV Disinfection

6.1 Introduction

A drawback of UV disinfection includes that of potential bacterial photoreactivation whereby certain microorganisms can repair UV-induced DNA damage and restore cellular functions through light or dark repair processes. Such repair processes may prove problematic for the application of UV treatment for water/wastewater disinfection as treated effluents exposed to light post UV disinfection are at risk of microbial re-growth. The inactivation of three dairy pathogens via continuous-flow LPUV and flow-through PUV disinfection is described in this chapter in addition to the photoreactivation analysis of *S. aureus* and *E. coli* post PUV treatment.

6.2 Dairy Pathogen Inactivation via Continuous-Flow LPUV and Flow-Through PUV Disinfection.

Comparative UV studies focused on disinfection of pre-determined populations of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* DSM 1104 (two recognised bacterial pathogens occurring in dairy environment) along with *Listeria innocua* DSM 20649 that was incorporated as surrogate test strain to represent *Listeria* genus (as *L. monocytogenes* was not studied for health and safety reasons). Previous studies have identified *Listeria innocua* as a suitable surrogate for *Listeria monocytogenes* for pulsed light inactivation analysis (Lasagabaster & de Marañón, 2012, 2017).

Results for the LPUV system analysis showed almost complete inactivation of all three named bacterial strains at the minimum UV output of 14 mJ/cm^2, therefore only the minimum UV output was investigated (Figure 6.1). Log_{10} removals of 5.3±0.3, 6.0±0.2 and 5.9±0.1 were observed from a starting bacterial populations of 6.0 log_{10} ±0.5 for *S. aureus*, *E. coli* and *L. innocua* respectively at 14 mJ/cm^2. Sample effluent from these trials was then directly used for photoreactivation analysis immediately following continuous-flow LPUV disinfection.

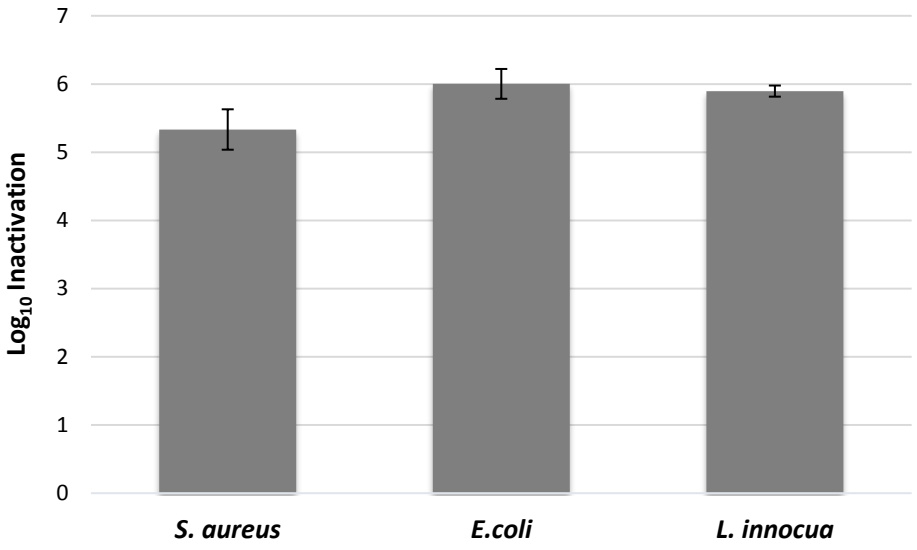


Figure 6.1. Log_{10} inactivation of *S. aureus, E. coli* and *L. innocua* via continuous-flow LPUV disinfection at a UV output of 14 mJ/cm^2. Standard error bars shown (n = 3).

In contrast to the LPUV system analysis, the results of bacterial inactivation via flow-through PUV disinfection suggested that inactivation was linearly dependent on energy with *E. coli* being the most sensitive to PUV irradiation and *L. innocua* exhibiting the most PUV resistance (Figure 6.2). The starting bacterial population of all three test bacteria was 6.0 log_{10} ±0.5. The maximum inactivation observed for *E. coli* was 5.3±0.3 log_{10} that was achieved using a PUV system output of 1539 mJ/cm^2 while a higher PUV output of 2052

mJ/cm^2 inactivated the Gram positive bacteria *S. aureus* and *L. innocua* by 5.2±0.6 $\log_{10}$ and 4.3±0.3 $\log_{10}$ respectively. Bacterial effluent concentrations from these trials were then directly used for photoreactivation analysis immediately following flow-through PUV disinfection.

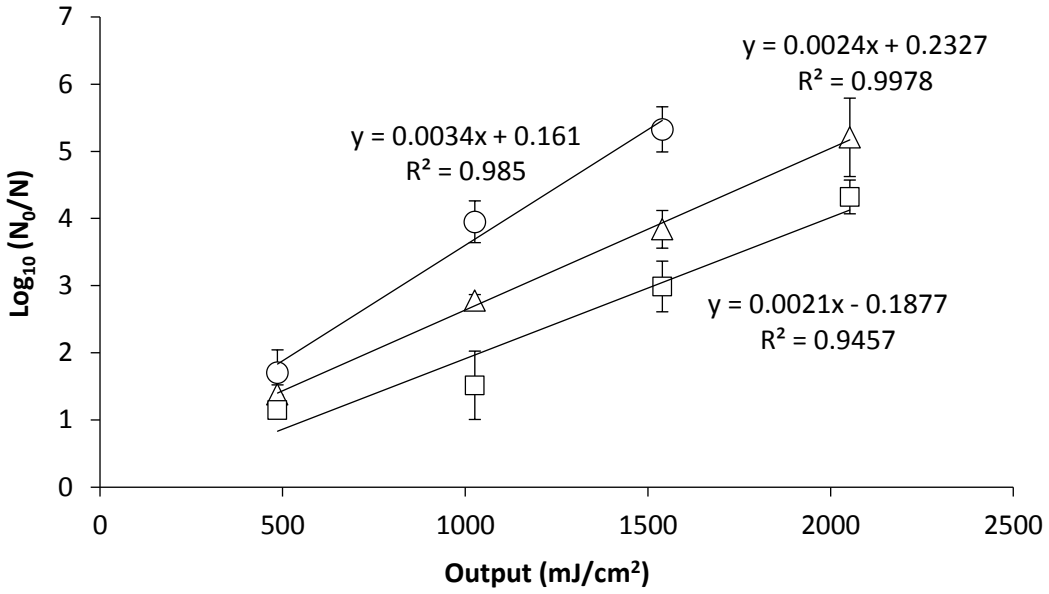


Figure 6.2. Dairy pathogen inactivation via flow-through PUV at various UV outputs for *E. coli* (○), *S. aureus* (△) and *L. innocua* (□) (points are averages of at least three runs and error bars are standard deviations).

Experiments involving *E. coli* inactivation via PUV in a static experimental set-up (Chapter 3, Section 3.3.5) were performed for a brief comparison to the inactivation efficiency of the PUV in the flow-through experimental set-up. Figure 6.3 illustrates *E. coli* inactivation efficiency via static PUV at 513 mJ/cm^2 (50 pulses) and 1026 mJ/cm^2 (100 pulses) in comparison to *E. coli* inactivation rates via flow-through PUV at UV outputs of 486 mJ/cm^2, 1026 mJ/cm^2 and 1539 mJ/cm^2 (flow-through data also presented in Figure 6.2). Results show the static experimental set-up achieved a higher inactivation of *E. coli* at both

UV outputs tested in comparison to equivalent UV outputs for the flow-through PUV set-up. For example, at a UV output of 1026 mJ/cm^2, an average *E. coli* $\log_{10}$ inactivation of 5.34 was found via static PUV analysis. In comparison, for the same UV output, an average *E. coli* $\log_{10}$ inactivation of 3.95 was found via flow-through PUV conditions. Notably, the standard deviation of the three experiments of *E. coli* inactivation via static PUV at 513 mJ/cm^2 was larger in comparison to other PUV test runs.

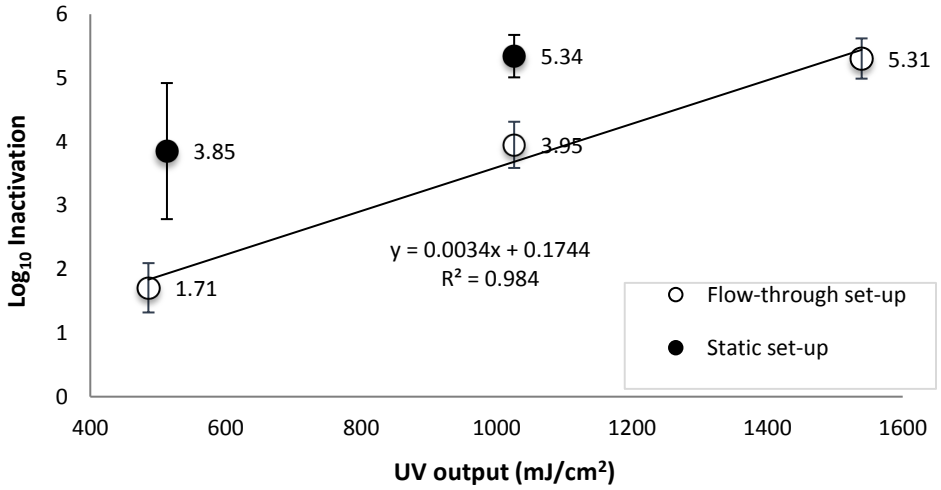


Figure 6.3. Comparison of *E. coli* inactivation via static PUV disinfection and flow-through PUV at various UV outputs (points are averages of at least three runs and error bars are standard deviations).

6.3 Photoreactivation and Dark Repair post UV Treatment

6.3.1 Photoreactivation and Dark Repair post LPUV Treatment

Experimental results for the potential photoreactivation (PHR) and dark repair of the three dairy pathogens following LPUV disinfection at 14 mJ/cm^2 are illustrated in Figure 6.4. The LPUV system achieved near complete inactivation of all three test bacteria at 14 mJ/cm^2 – the lowest output available. Therefore, the potential for photoreactivation/dark repair after LPUV treatment at this output was analysed. The results showed bacterial

concentrations after photoreactivation and dark repair to be minimal. A slight photoreactivation of *S. aureus* was seen after 120 minutes and limited *L. innocua* growth was detected in dark repair conditions at 60 minutes. However overall, the potential for PHR and dark repair post LPUV disinfection appeared negligible. As previous studies have shown, photoreactivation/dark repair typically decreases as the UV dose/output increases; thus, it was decided not to test for PHR or dark repair at higher LPUV outputs (Li *et al.*, 2008; Shin & Linden, 2015).

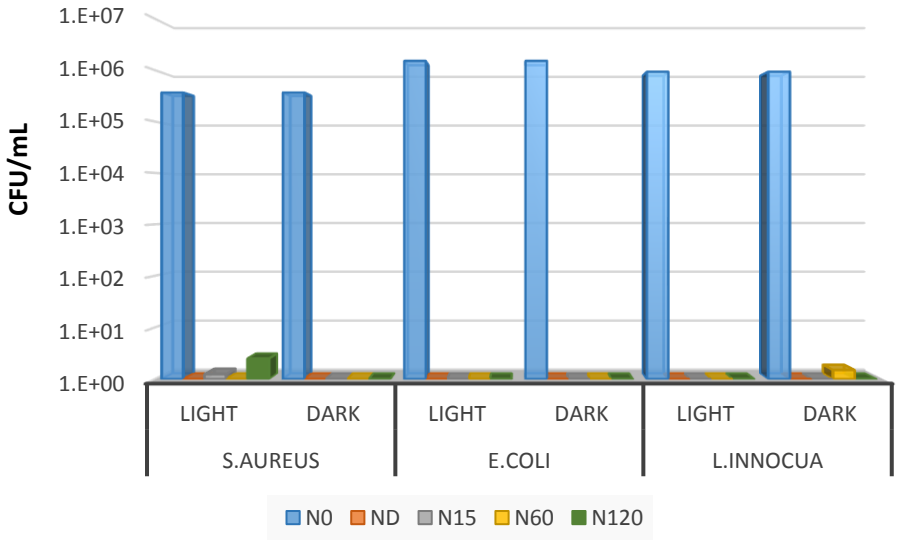


Figure 6.4. Photoreactivation and dark repair of *S. aureus*, *E. coli* and *L. innocua* post LPUV disinfection (output 14 mJ/cm^2) where N0 is bacterial concentration pre-LPUV, ND is bacterial concentration post LPUV and N15, N60 and N120 are bacterial concentrations (CFU/mL) after exposure to photoreactivation and dark repair conditions for 15, 60 and 120 minutes respectively.

6.3.2 Photoreactivation and Dark Repair post PUV Treatment

The response of the dairy test bacteria to PHR and dark repair experimental analysis post flow-through PUV treatment varied. The particular strain of *L. innocua* (DSM 20649) selected for this study appeared to be impacted by the photoreactivation experimental conditions. Figure 6.5 shows the results of the photoreactivation and dark repair experiments for *L. innocua* post flow-through PUV treatments at PUV outputs of 486, 1026 and 2052 mJ/cm^2. Minimal dark repair was observed for *L. innocua*. However, populations of *L. innocua* survivors post PUV irradiation was observed to decrease in number during the period of photoreactivation conditions. This finding was not consistent with *E. coli* and *S. aureus* analysis whereby PHR and dark repair analysis showed an increase in bacterial concentration, or bacterial concentrations remained the same i.e. no growth. Upon further investigation, it was confirmed that *L. innocua* was inactivated by the compound fluorescent lamp with no prior UV exposure. Thus, it was decided to eliminate it from the photoreactivation analysis (Figure 6.5). Despite the findings in this study, previous research has reported photoreactivation of this particular strain of *L. innocua* under artificial light conditions (Kramer *et al.*, 2015).

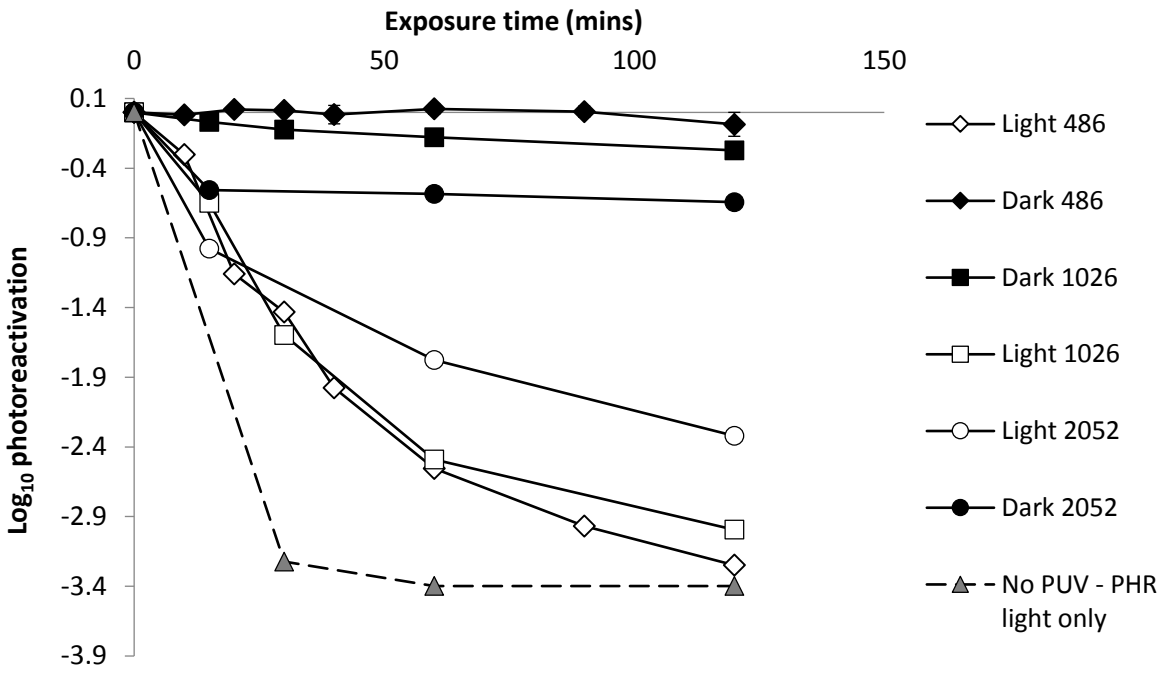


Figure 6.5. Photoreactivation and dark repair of *L. innocua* without prior PUV disinfection (PHR light only) and post PUV disinfection at PUV outputs 486, 1026, 2052 mJ/cm^2.

For confirmation purposes, similar analysis was carried out on *E. coli* and *S. aureus* whereby the strains were exposed to the compound light only (without prior exposure to PUV) to confirm that the photoreactivation experimental conditions were not impacting upon cell viability as was the case with *L. innocua*. Results illustrated in Figure 6.6 confirmed this was not the case and so photoreactivation analysis continued with *E. coli* and *S. aureus* bacterial strains only.

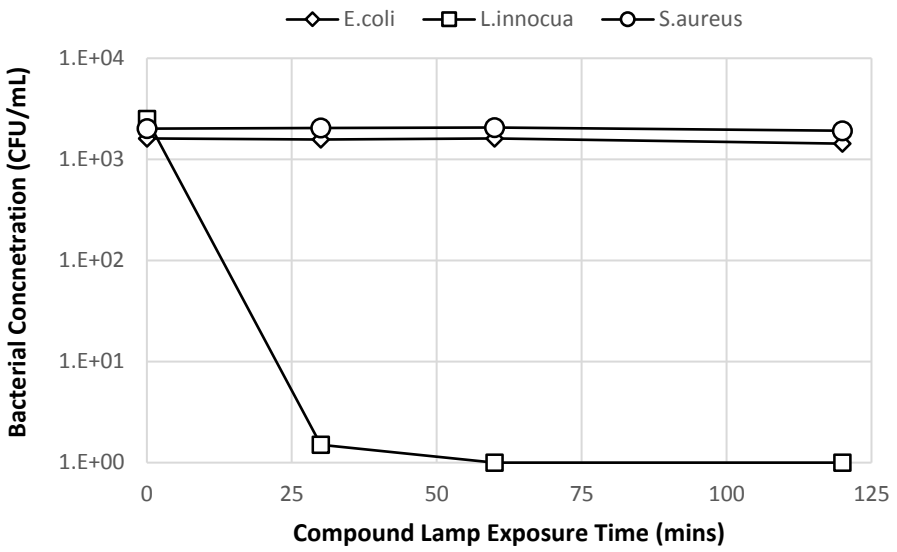


Figure 6.6. Bacterial concentration of *E. coli*, *L. innocua* and *S. aureus* after 30 minutes, 60 minutes and 120 minutes of exposure to the compound lamp used in the photoreactivation experimental analysis.

Photoreactivation was observed for both *E. coli* and *S. aureus* post flow-through PUV treatment in addition to some dark repair. The maximum pathogen photoreactivation and dark repair after 120 minutes following flow-through PUV treatment is shown in Figure 6.7. The results plotted as $\log_{10}$ units (Figure 6.7 (a)) indicate that the potential for photoreactivation and dark repair is similar for *S. aureus* and *E. coli* at similar PUV outputs (between 500 and 1500 mJ/cm^2). Dark repair for both bacteria appears minimal in comparison with bacterial photoreactivation as bacterial concentrations only increased slightly at the applied PUV output of 1026 mJ/cm^2. For comparative purposes, the results are expressed as the percentage of colonies reactivated from those inactivated via PUV in Figure 6.7 (b). and show contrasting results in comparison to Figure 6.7 (a). For example, photoreactivation rates of *S. aureus* are illustrated to be the greatest at the PUV energy output of 2052 mJ/cm^2 when plotted as $\log_{10}$ PHR (Figure 6.7 (a)). However, when the data

is plotted as % photoreactivation, *S. aureus* shows the least photoreactivation potential at 2052 mJ/cm^2 (Figure 6.7 (b)).

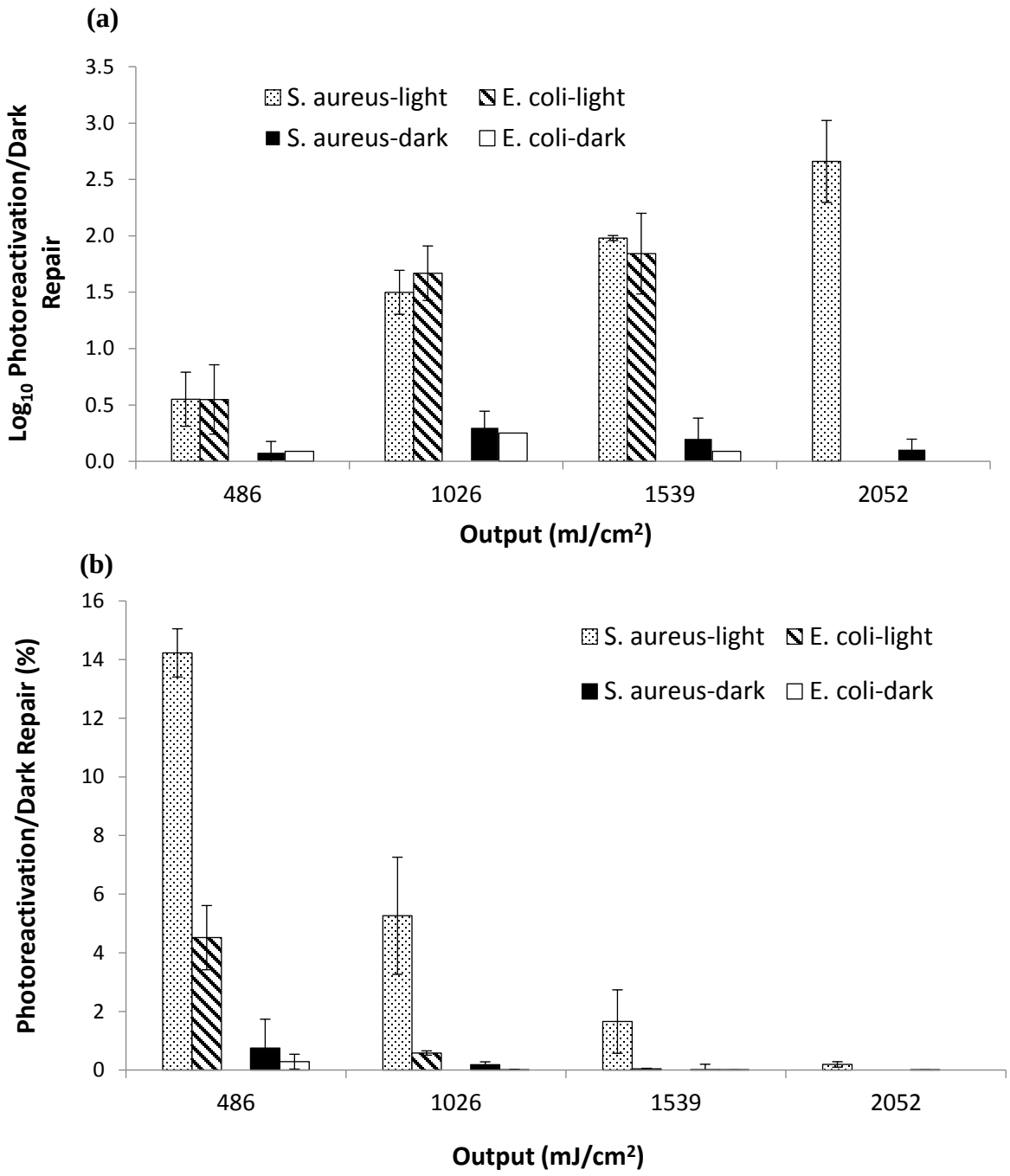


Figure 6.7. Maximum (120 minutes) photoreactivation (light) and dark repair (dark) of *S. aureus* and *E. coli* post flow-through PUV treatment at various UV outputs. (a) data expressed as log_{10} and (b) data expressed as percentage of colonies reactivated from those inactivated.

The contrast in the findings illustrated in Figure 6.7 (a) and 6.7 (b) are likely to be attributable to a difference in bacterial populations post UV treatment at various energy outputs i.e. bacterial concentrations are not uniform at the beginning of the photoreactivation analysis thus comparing photoreactivation in terms of $\log_{10}$ concentration may be misleading. To elaborate, the concentration of *S. aureus* pre (influent) and post (effluent) PUV treatment and the maximum photoreactivation concentration of *S. aureus* at the three UV outputs is outlined in both logarithmic and CFU/mL form in Figure 6.8 (a) and 6.8 (b). Figure 6.8 (a) shows a $\log_{10}$ photoreactivation of 0.55 at the lowest PUV output (489 mJ/cm^2). If this is compared to the equivalent data set in Figure 6.8 (b) it corresponds to a population growth from approximately 30,000 CFU/mL to approximately 115,000 CFU/mL. In comparison, a $\log_{10}$ photoreactivation of 2.66 was observed at the highest PUV output (2052 mJ/cm^2) (Figure 6.8(a)). However, this is equivalent to a population growth of approx. 10 CFU/mL up to 1,500 CFU/mL (Figure 6.8 (b)). Thus, caution should be exercised when presenting such data sets in logarithmic form for comparative purposes.

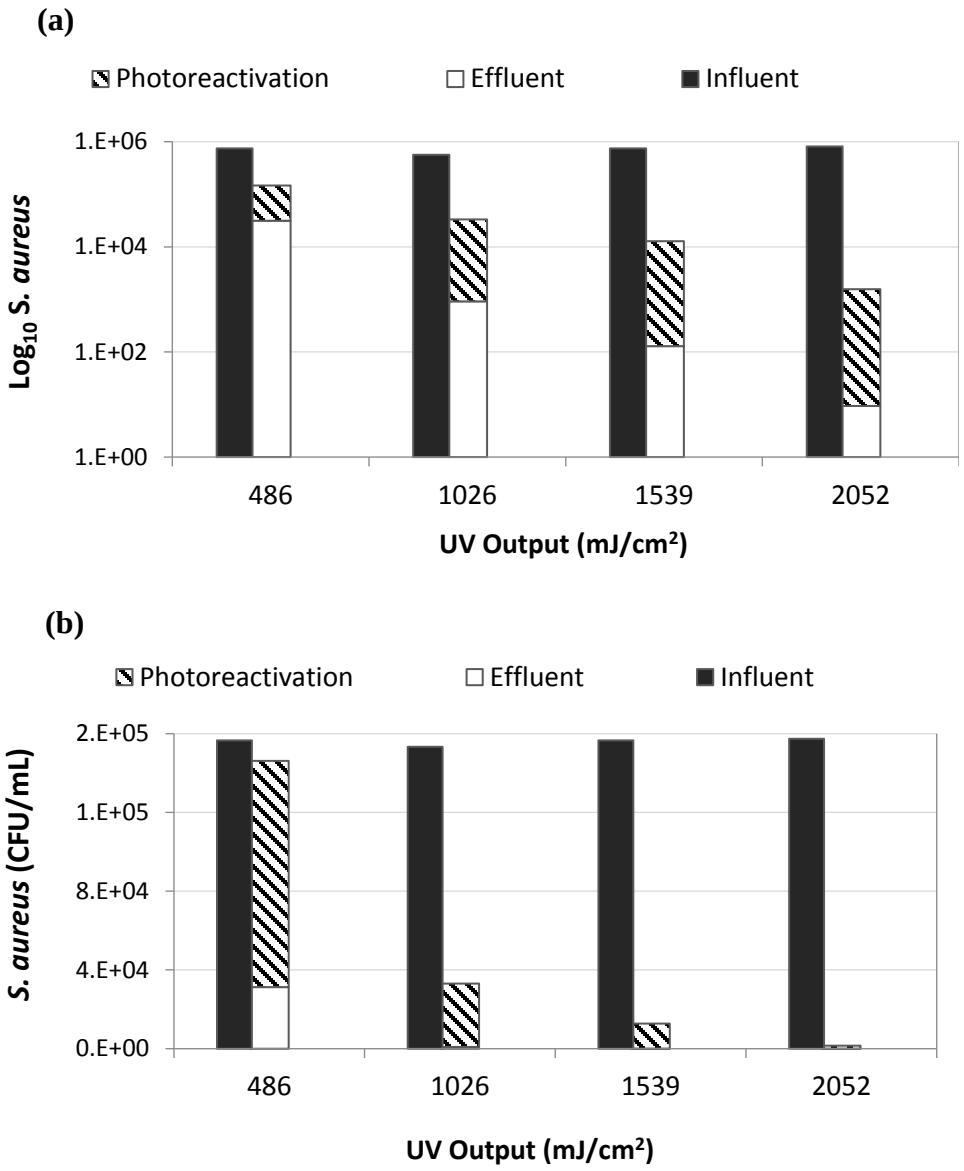


Figure 6.8. Influent and effluent concentration of *S. aureus* pre and post flow-through PUV treatment and photoreactivation concentration (maximum exposure time of 120 minutes) of *S. aureus* at various UV outputs. (a) data expressed as log_{10} and (b) data expressed as CFU/mL.

The preferred method of analysis for photoreactivation results therefore is by comparison of the percentage photoreactivation from initial populations of bacteria post UV treatment.

Table 6.1 compares the % photoreactivation and dark repair of *E. coli* and *S. aureus* post flow-through PUV disinfection at various PUV outputs. Results show *S. aureus* exhibited approximately 10 percentage points more photoreactivation in comparison to *E. coli* at the lowest UV output of 486 mJ/cm^2 and approximately 5 and 1.5 percentage points more at 1026 mJ/cm^2 and 1539 mJ/cm^2 respectively. Analysis for *E. coli* photoreactivation/dark repair at 2052 mJ/cm^2 was not carried out as the complete inactivation of *E. coli* took place at a PUV output of 1539 mJ/cm^2 (see Figure 6.2). Very little difference was found between the % dark repair of *E. coli* and *S. aureus* at the UV outputs of 486 mJ/cm^2, 1026 mJ/cm^2 and 1539 mJ/cm^2 with minimal dark repair demonstrated for both strains.

Table 6.1. Percentage photoreactivation and dark repair of *E. coli* and *S. aureus* post flow-through PUV disinfection at various UV outputs.

PUV Output (mJ/cm^2)	% Photoreactivation		% Dark Repair	
	E. coli	***S. aureus***	***E. coli***	***S. aureus***
486	4.52±1.10	14.23±0.82	0.29±0.25	0.75±0.98
1026	0.58±0.07	5.26±2.00	0.01±0.02	0.18±0.10
1539	0.04±0.02	1.65±1.08	0.00±0.00	0.02±0.18
2052	-	0.19±0.09	-	0.00±0.00

Figure 6.9 (a) and 6.9 (b) illustrate the percentage photoreactivation of *S. aureus* and *E. coli* over time post flow-through PUV treatment at various energy outputs. Results for *S. aureus* at the lowest output of 486 mJ/cm^2 showed a sharp increase of PHR between 10

and 60 minutes after which a slight tailing effect was seen between 60 and 90 minutes. However, this was not constant and the % PHR increases slightly again after this time point. Increases in the percentage photoreactivation of *S. aureus* were less pronounced at the higher UV system outputs with negligible PHR occurring at any time point after PUV treatment at 2052 mJ/cm^2 (Figure 6.9 (a)). Analysis of *E. coli* photoreactivation over time shows a slightly different trend to that of *S. aureus* post PUV treatment (Figure 6.9 (b)). At 486 mJ/cm^2, PHR of *E. coli* steadily increases between 15 and 90 minutes before a tailing effect is observed. At the two higher UV system outputs of 1026 mJ/cm^2 and 1539 mJ/cm^2 the percentage of photoreactivation *E. coli* is low.

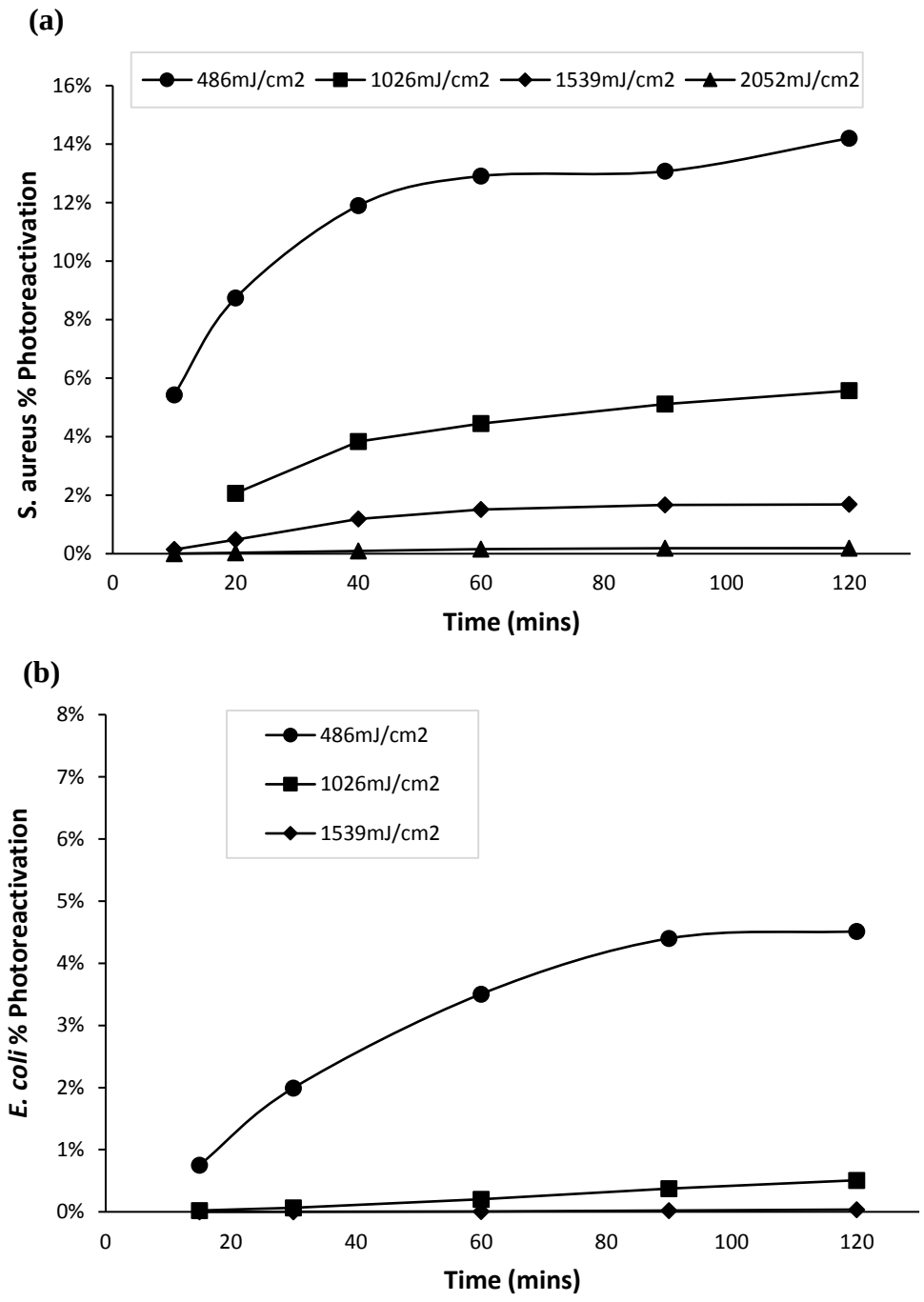


Figure 6.9. Percentage photoreactivation over time of (a) *S. aureus* and (b) *E. coli* at various UV outputs following flow-through PUV treatment.

6.4 Discussion

6.4.1 Dairy Pathogen Inactivation via LPUV and PUV Disinfection

Dairy pathogen inactivation via continuous-flow LPUV yielded complete inactivation of all three strains; *E. coli*, *S. aureus* and *L. innocua* at a UV output of 14 mJ/cm^2 with bacterial $\log_{10}$ inactivations of 6.0, 5.3 and 5.9 respectively. Table 6.2 compares the inactivation rates of the three strains reported in this study to similar research in the literature. Pereira *et al.,* (2014) investigated the treatment of *E. coli, S. aureus* and *L. innocua* in milk via LPUV continuous-flow disinfection (Pereira *et al.,* 2014). The experimental conditions included inoculated batches of milk (4 L) being passed through the unit for 2.5 minutes which resulted in $\log_{10}$ inactivations of 2.8 ± 0.2, 3.4 ± 0.3 and 3.2 ± 0.3 for *E. coli, S. aureus* and *L. innocua* respectively. The UV-C lamp had an energy output of 45 J/cm^2 however no UV dose was calculated or measured. Previous studies have shown *E. coli* and *S. aureus* to be inactivated by 5 - 6 $\log_{10}$ in water via batch LPUV disinfection at UV doses of < 10 mJ/cm^2 which is in line with findings in this study (Malayeri et al., 2016). Holck *et al.,* (2018) studied the inactivation of *E. coli* and *L. monocytogenes* on the surface of eggs via LPUV disinfection (Holck *et al.,* 2018). For *L. monocytogenes* analysis, it was observed that a LPUV dose of 50 mJ/cm^2 was required for a 2.8 $\log_{10}$ inactivation which is a higher LPUV energy requirement in comparison to LPUV inactivation studies in water.

Table 6.2 Comparison of microbial inactivation via batch and continuous-flow LPUV disinfection.

Microorganism	Medium	UV System	UV output/dose (mJ/cm^2)	Log_{10} inactivation	Reference
S. aureus DSM 1104	Water	Continuous-flow LPUV	14[1]	5.3	This study
E. coli ATCC 25922	Water	Continuous-flow LPUV	14[1]	6.0	This study
L. innocua DSM 20649	Water	Continuous-flow LPUV	14[1]	5.9	This study
S. aureus ATCC 27708	Milk	Continuous-flow LPUV	ND[2]	3.4	(Pereira *et al.*, 2014)
L. innocua DSM 20649	Milk	Continuous-flow LPUV	ND[2]	3.2	(Pereira *et al.*, 2014)
E. coli 33405-1	Milk	Continuous-flow LPUV	ND[2]	2.8	(Pereira *et al.*, 2014)
E. coli ATCC 25922	Water	LPUV	10[3]	6.0	(Malayeri *et al.*, 2016)
S. aureus DSM 1104	Water	LPUV	10[3]	4.0	(Chang *et al.*, 1985)
L. monocytogenes	Eggs	LPUV	50[3]	2.8	(Holck *et al.*, 2018)

[1]system output
[2]Not described – inactivation detailed in terms of flow rate of system.
[3]UV dose

The inactivation of *E. coli*, *S. aureus* and *L. innocua* via flow-through PUV was found to be output energy dependant with Gram-negative *E. coli* being more sensitive to PUV light

in comparison to Gram-positive *S. aureus* and *L. innocua* (Table 6.3). This finding is in agreement with findings by Farrell *et al.,* (2010) who also found Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) to be more resistant to PUV light in comparison to Gram-negative bacteria (*E. coli*) (Farrell *et al.,* 2010). Rajkovic *et al.,* (2017) investigated the inactivation of *E. coli*, *S. aureus* and *L. monocytogenes* via PUV light on fermented salami samples under static experimental conditions (Rajkovic *et al.,* 2017). Starting from a concentration of 6.3 $\log_{10}$ CFU/g, the authors obtained bacterial $\log_{10}$ inactivations of 2.29, 2.12 and 2.24 for *E. coli*, *S. aureus* and *L. monocytogenes* respectively at a PUV broadspectrum dose (See Chapter 7, Section 7.3) of 3,000 mJ/cm^2. These previously reported findings indicate that all three strains appeared to exhibit similar sensitivities to PUV irradiation which is in marked contrast to those found in this study where varying sensitivities of the three strains to PUV irradiation was observed. The inactivation of *E. coli* and *L. monocytogenes* on the surface of eggs via static PUV disinfection reported a PUV system energy output of 1,250 - 18,000 mJ/cm^2 yielded *L. monocytogenes* inactivation rates of between approximately 1.6 and 3.2 $\log_{10}$ (Holck *et al.,* 2018). In this study, an experimental comparison of *E. coli* inactivation via static and flow-through PUV experimental conditions at similar UV doses illustrated static PUV to achieve higher bacterial inactivation rates. Yi *et al.,* (2016) performed a similar study whereby a laboratory-scale static PUV system was compared to a pilot-scale flow-through PUV system for murine norovirus (MNV) inactivation (Yi *et al.,* 2016). The authors concluded higher inactivation rates at lower UV outputs for the static laboratory-scale system in comparison to the flow-through pilot-scale system with 3.77 MNV $\log_{10}$ inactivation at a UV output of 1.45 J/cm^2 for the static set-up and a 3.35 MNV $\log_{10}$ inactivation at a UV

output 4.3 J/cm^2 for the flow-through set-up. Thus, findings in this study are in agreement with those found in the literature.

Research investigating bacterial inactivation in water/wastewater via flow-through PUV systems are limited. Uslu *et al.,* (2016) analysed the inactivation of *E. coli* via flow-through PUV disinfection in synthetic wastewater with the addition of 'multiple pass' analysis whereby the sample was recirculated back under the PUV lamp for a second disinfection step (Uslu *et al.,* 2016). In the aforementioned study, the UV dose/system output was not described rather the flow rate and broadspectrum energy (J) per litre was given. Results showed complete inactivation of *E. coli* in synthetic wastewater at flow rates of 10 L/min for a two-pass treatment. A similar study investigated the inactivation of *L. innocua* via flow-through PUV for water treatment (Artíguez *et al.,* 2011). A PUV dose of 4,000 mJ/cm^2 was required for 2 $\log_{10}$ *L. innocua* inactivation in water. Notably, for any given output energy, the study reported higher inactivation rates at lower PUV system voltages (when compared to higher system voltages) and at higher flow-rates which is typically indicative of a decreased UV dose/output. Krishnamurthy *et al.,* (2007) tested the inactivation of *S. aureus* in milk under flow-through PUV experimental conditions and also adopted a multi-pass disinfection method akin to Uslu *et al.,* (2016). Log_{10} reductions were reported as function of flow-rate, lamp distance and number of sample passes. Complete inactivation of *S. aureus* was obtained at 8 cm sample distance, single pass and 20 mL/min flow rate however no UV dose/energy output was reported. Interestingly, it was also noted that a decrease in PUV energy was a polynomial function of the lamp distance i.e. inactivation rates were higher at 8 cm lamp distance than those achieved at 5 cm lamp distance (for further information see Chapter 7 section 7.3.1) (Krishnamurthy *et al.,* 2007).

Table 6.3 Comparison of microbial inactivation via static and flow-through PUV disinfection.

Microorganism	Medium	UV System	UV output/dose (mJ/cm^2)*	Log_{10} inactivation	Reference
S. aureus DSM 1104	Water	Flow-through PUV	1,539[1]	3.8	This study
E. coli ATCC 25922	Water	Flow-through PUV	1,539[1]	5.3	This study
L. innocua DSM 20649	Water	Flow-through PUV	1,539[1]	3.0	This study
E. coli K12	Wastewater	Flow-through PUV	ND[2]	2.8 - 7.2	(Uslu *et al.*, 2016)
L. innocua	Water	Flow-through PUV	4,000[1]	2	(Artíguez *et al.*, 2011)
S. aureus DSM 1104	Milk	Flow-through PUV	ND[2]	0.6 – 7.3	(Krishnamurthy *et al.*, 2007)
E. coli O157:H7	Salami	Static PUV	3,000[3]	2.3	(Rajkovic *et al.*, 2017)
S. aureus LFMFP 282	Salami	Static PUV	3,000[3]	2.1	(Rajkovic *et al.*, 2017)
L. monocytogenes LFMFP 034	Salami	Static PUV	3,000[3]	2.2	(Rajkovic *et al.*, 2017)
L. monocytogenes	Eggs	Static PUV	1,250[1]	1.6	(Holck et al., 2018)

[1]system output
[2]Not described – inactivation detailed in terms of flow rate of system.
[3]broadspectrum dose
*where energies presented in Table 6.3 were calculated by the authors or based on system output the term "output" has been applied. If the energies were measured at the sample surface the term "dose" has been applied. This is because measured energies dissipate quickly the further a sample is from the PUV light source.

6.4.2 Pathogen Photoreactivation and Dark Repair post LPUV Treatment

The photoreactivation/dark repair of all three strains post LPUV treatment at 14 mJ/cm^2 was found to be minimal. Previous research has been reported PHR for *E. coli* post LPUV treatment whereby water samples exposed to UV dose of 5 mJ/cm^2 were subsequently placed under sunlight lamps for 4 hours before photoreactivation was observed (Guo *et al.*, 2009). While photoreactivation of *E. coli* was reported after LPUV treatment at 5 mJ/cm^2, the same study reported a lack of photoreactivation when the bacteria were exposed to a higher UV dose of 15 mJ/cm^2 (Guo *et al.*, 2009). Sanz *et al.*, (2007) reported a similar conclusion when investigating the photorepair potential of total coliforms post LPUV treatment in a wastewater treatment facility. Results showed a significant decrease of photoreactivation potential at high UV doses indicating the severity of the UV damage at high doses can prohibit DNA lesion repair (Sanz *et al.*, 2007). Other studies which analysed PHR rates of *E. coli* and *B. subtilis* in reclaimed water concluded *E. coli* to photoreactivate post LPUV treatment at 5 mJ/cm^2 with no detectable PHR for *B. subtilis* at the same UV dose (Guo *et al.*, 2011). The authors also reported a strong correlation between photoreactivation and UV dose (R^2 = 0.963). Therefore, perhaps in the case of the experimental analysis carried out in this study, the applied LPUV output of 14 mJ/cm^2 may have inhibited the ability of the bacteria to photoreactivate/dark repair.

6.4.3 Pathogen Photoreactivation and Dark Repair post PUV Treatment

Analysis of the PHR/dark repair potential of *L. innocua* was not possible in this study due to an adverse reaction of the bacteria to the light used for the photoreactivation experiment. During the experimental design phase a review of literature was compiled pertaining to the

type of lamps/bulbs used in PHR experiments and general characteristics of interest such as the spectrum output, the power, the temperature (which influences sunlight simulation) and the colour of the light. It was noted that the photolyase enzyme for bacterial photoreactivation responds significantly to 'blue light' in the 360 – 500 nm range and that *L. monocytogenes* and *S. aureus* exhibit maximum photoreactivation potential between 360 – 380 nm (Maclean *et al.,* 2008). The bulbs that were eventually chosen for the analysis (See Chapter 3, section 3.6.2 for details) were compact fluorescent lamps which emitted white light in the 300 – 700 nm range at 23W power. As noted in section 6.3.2, a previous study reported photoreactivation of the same strain of *L. innocua* used in this study. Kramer *et al.,* (2015) investigated the photoreactivation of PUV treated *L. innocua* (DSM 20649) and used a 30 W fluorescent lamp with a spectrum output of between 400 and 650 nm for PHR analysis (Kramer *et al.,* 2015). Results showed photoreactivation rates of between 10^2 and 10^6 CFU/mL after 24 hours of lamp exposure on tryptic soy agar at 37ºC depending on the PUV fluence applied. The reasons for *L. innocua* becoming inactivated under the compound lamp in this study are not entirely clear. Perhaps the slightly higher spectrum output of the lamp and experimental conditions (illumination on agar and not water as was the case in this study) in the Kramer *et al.,* (2015) study was more conducive to *L. innocua* photoreactivation analysis. However, in this study it was considered important to use a lamp with a spectrum below 400 nm due to reasons stated above i.e. photolyase activity and favoured photoreactivation potential at the wavelengths between 360 – 380nm. Moreover, *S. aureus* and *E. coli* were not negatively affected by the lamp/photoreactivation conditions. While photoreactivation analysis of *L. innocua* could not be completed in this study, details surrounding the specifics of the experimental design and the particular bulbs

used may be noteworthy for other studies of a similar nature. This finding also suggests that this particular species and strain of *L. innocua* may not be environmentally robust compared with other proven test bacterial pathogens, moreover, it may not potentially reflect stress and adaptive responses of *L. monocytogenes*.

UV disinfection analysis typically expresses the inactivation rates of microorganisms in logarithmic units due to high microbial concentrations and variations in UV dose/output inactivation rates. However, for photoreactivation/dark repair experiments, expressing the data in units of percentage photoreactivation/dark repair or CFU/mL may also be warranted and perhaps necessary. Bacterial concentrations post UV treatment vary depending on the energy applied and so initial populations for photoreactivation analysis are often not uniform. Thus, logarithmic comparisons of data may be misleading and recent research has shown this to be the case (Menge *et al.,* 2018). It is therefore recommended in the case of photoreactivation analysis where bacterial concentrations vary according to the energy applied during the preceding UV treatment, interpreting the data as a % photoreactivation of initial bacterial population (post UV treatment) is preferred.

Minimal dark repair of *S. aureus* and *E. coli* was found post flow-through PUV disinfection (and also post LPUV disinfection). Potential reasons for the lack of dark repair exhibited by bacteria in this study may be due the length of exposure time applied during the experimental analysis. Jungfer *et. al.*, (2007) reported the activation of bacterial dark repair mechanisms (*rec*A mRNA protein) to be dependent on experimental incubation time which was found to be vary depending on the bacterial strain. For example, a dark incubation time of two hours induced *rec*A in drinking water bacteria *Caulobacter crescentus.* In contrast, *Enterococcus faecium* required an incubation period of six hours before dark repair

mechanisms were observed (Jungfer *et al.,* 2007). In this case, perhaps extending the exposure/incubation time of bacteria in the experimental dark repair conditions may have yielded higher bacterial dark repair rates. Alternatively, previous studies have also reported bacterial dark repair to occur within approximately 100 minutes of incubation post UV disinfection after which a bacterial decay period was observed (Sanz *et al.,* 2007; Salcedo e*t al.,* 2007). The dark repair analysis, carried out in conjunction with photoreactivation analysis, confirmed reactivation in darkness did occur but to a considerably lower degree than that in light repair conditions. Moreover, the authors concluded maximum dark repair of bacteria to occur sooner in comparison to maximum bacterial photoreactivation (Sanz *et al.,* 2007).

Populations of *S. aureus* exhibited greater photoreactivation rates in comparison to *E. coli* at the lowest PUV outputs of 486 mJ/cm^2 and 1026 mJ/cm^2. Photoreactivation has been observed previously for *E. coli* post static PUV treatment on gel mediums with a recovery rate of up to 2 $\log_{10}$ following a PUV dose 0.45 – 1 J/cm^2 (Kramer *et al.,* 2015). Studies investing PHR potential of *S. aureus* and *L. monocytogenes* post PUV treatment under static experimental conditions observed a photoreactivation response after PUV treatments of 1.5 J/cm^2 and 3.4 J/cm^2 respectively (Maclean *et al.,* 2008). The study found approximately 2% of *S. aureus* bacteria photoreactivated after a PUV exposure of 1.5 J/cm^2 (PHR exposure time not given). Similarly, results in this study found 1.65% of the *S. aureus* population to photoreactivate after 1.5 J/cm^2 PUV energy exposure. Furthermore, the authors also concluded that while the results reported a low degree of PHR for *S. aureus* at the PUV output energy applied, a less intense UV energy output would likely result in a

higher degree of photoreactivation which was the finding in this study (Maclean *et al.,* 2008).

A similar study investigated the ability of *E. coli*, *S. aureus* and *L. innocua* to synthesise new adenosine triphosphate (ATP) (a necessary molecule for cell regeneration) post PUV treatment (3 J/cm^2) in a static experimental set-up (Kramer *et al.,* 2017). The study found *S. aureus* to be to more susceptible to PUV treatment in comparison to *E. coli* which is in contrast to findings reported in this study and others in the literature whereby Gram-positive bacteria were found to be more resistant to PUV irradiation in comparison to Gram-negative bacteria (Anderson *et al.,* 2000; Farrell *et al.,* 2010). However, it was also concluded that *L. innocua* exhibited increased resistance to PUV treatment in comparison to *S. aureus* which is in agreement with corresponding analysis in the study. With regards to ATP synthesis, *S. aureus* was found to be the most affected out of the three strains post PUV treatment indicating it was the most sensitive in terms of cell regeneration. In the present research, *S. aureus* exibited a higher photoreactivation capacity in comparison to *E. coli* which does not fall in line with findings of Kramer *et al.,* (2017). However, while PHR and ATP synthesis are measures of certain capabalities of bacteria post UV treatment, the direct comparison of both factors may be improper owing to differences in molecular and microbiological techniques.

The results shown in Figure 6.9 indicate that the majority of photoreactivation occurs within the first hour for *S. aureus* and the first 90 minutes for *E. coli* thus avoiding bacterial exposure to light immediately after PUV disinfection is important when attempting to maintaining high inactivation rates for both *S. aureus* and *E. coli*. However, as is the case with LPUV disinfection, photoreactivation is avoidable when a high UV energy is applied

(Sanz *et al.*, 2007; Guo *et al.*, 2009). Nonetheless, this is a factor which should be considered if the PUV system were to be scaled up for water/wastewater treatment applications. Previous studies have suggested that a time-based comparison for photoreactivation experiments cannot be performed due to variations in lamp intensities i.e. the energy may vary from one lamp to another (Bohrerova & Linden, 2007). However, in the present research study a time-based comparison between *S. aureus* and *E. coli* was possible as the same experimental set-up and lamp was used for both bacteria and should be noted for future studies of this kind.

6.5 Conclusions

The aims of Chapter 6 were (i) to compare both UV systems for dairy pathogen inactivation in terms of UV output and (ii) to establish the potential of dairy pathogens to photoreactivate/dark repair post flow-through PUV and continuous-flow LPUV at various UV outputs. The findings are as follows;

- Continuous-flow LPUV treatment at 14 mJ/cm^2 yielded complete inactivation of *S. aureus*, *E. coli* and *L. innocua*. In addition, no photoreactivation or dark repair was found at this UV output.
- Bacterial inactivation via flow-through PUV disinfection suggests that inactivation is energy output dependent with *E. coli* being the most sensitive to PUV irradiation and *L. innocua* exhibiting the most PUV resistance.
- The experimental set-up for photoreactivation analysis was not conducive to *L. innocua* independent growth analysis thus PHR studies could not be completed on

these bacteria. This is a factor which should be noted when completing future PHR testing.

- Expressing photoreactivation data in units of percentage photoreactivation/dark repair or CFU/mL is advised as logarithmic data representation may be misleading.
- *S. aureus* exhibited approximately 10% higher photoreactivation in comparison to *E. coli* at the lowest UV outputs of 486 mJ/cm^2 and 1026 mJ/cm^2.
- Immediate exposure of bacteria to light post flow-through PUV treatment should be avoided to minimise photoreactivation of both *S. aureus* and *E. coli*. This finding is of particular relevance if scaling up of the system is considered for water/wastewater treatment.

Chapter 7
Methods of Pulsed UV Energy Determination & Energy Efficiency Comparison to LPUV Systems.

7.1 Introduction

The methods by which measurement and analyses of energy outputs/doses from PUV systems are conducted vary considerably within the literature with a number of methods being deployed. In this study three methods were deployed to analyse and measure (i) the output of the system (calculated), (ii) the dose received by samples (measured) and (iii) overall electrical energy consumption of the system (measured). As presented in Chapter 2, Section 2.3.7 this can lead to considerable variation in reported energy outputs and doses which can lead to challenges when comparing PUV technology to other UV systems and thus this study utilised different approaches for comparative purposes. In addition, the proportion of energy emitted from the UV and UV-C spectral range of the PUV system was also analysed. All energy measurements, both broadspectrum and UV/UV-C wavelengths were measured in air and not in water (Chapter 3, Section 3.3.6.2).

7.2 Calculated PUV Output

The broadspectrum and UV output of the PUV system was calculated using the specifications of the system (as per the Samtech Manual; Pulsed UV System PUV-01), the sample area (290 cm^2) and the sample distance from the lamp (10.75 cm) (see Chapter 3, Section 3.3.4 for further details). Table 7.1 illustrates the output energy of the system per pulse (1PPS setting; mW/cm^2) at each voltage setting for both the total broadspectrum output (E_A) across the full spectral range of 200 – 1100 nm and the output energy emitted below 300 nm ($E_{A\ <300\ nm}$). To calculate $E_{A\ <300\ nm}$, the ratio of energy emitted in the <300

nm range at varying sample distances was averaged and used to estimate the UV dose at 10.75 cm (Table 3.4). At 800 and 900 volts (the voltage settings predominantly used throughout the entire study), the energy output per pulse at < 300 nm was 8.1 mW/cm^2 and 10.3 mW/cm^2 respectively.

Table 7.1. Calculated total broadspectrum energy output per pulse and broadspectrum energy output <300nm per pulse of the PUV system.

Discharge voltage (V)	Joules per pulse (J)	Joules per unit time - (W)	Energy @ < 300 nm (W)	E_A (W/cm^2)	E_A (mW/cm^2)	$E_{A < 300\ nm}$ (mW/cm^2)
400	3.2	3.2	0.6	0.011	11.0	2.0
500	5.0	5.0	0.9	0.017	17.2	3.2
600	7.2	7.2	1.3	0.025	24.8	4.6
700	9.8	9.8	1.8	0.034	33.8	6.2
800	12.8	12.8	2.4	0.044	44.1	8.1
900	16.2	16.2	3.0	0.056	55.9	10.3
1000	20	20	3.7	0.069	69.0	12.7

7.3 Measured PUV Broadspectrum Dose

Analysis of the broadspectrum dose received by the sample from the PUV system was initially conducted with the use of a QE12LP-S-MB energy detector (1.2 mm square aperture) connected to a Maestro power and energy meter (Gentec-EO, Quebec, Canada). This particular detector had been used previously for measuring PUV dose (Levy *et al.*, 2012; Massier *et al.*, 2012). However, attempts to measure the pulse energy with this detector resulted in inconsistent readings with low repeatability and the data was found to

be unreliable. After discussion with Gentec regarding the issue, it was recommended to try a different thermopile power detector (Model: XLP12-3S-H2-IN, Gentec-EO, Quebec, Canada - see Chapter 3, Section 3.3.6.2 for further details). Gentec indicated the original detector used may not be suitable for this type of work. The second detector demonstrated high repeatability of results with low standard deviations (2% of average) when readings were averaged for any given PUV setting. High and low range settings available on the software allowed for increased accuracy – see Appendix C for further information. Figure 7.1 compares the calculated total broadspectrum output per pulse against the measured total broadspectrum dose per pulse. Results show that at each voltage system setting, an average of 27% ± 2% of the total broadspectrum energy emitted from the system was being absorbed by the detector as total broadspectrum dose.

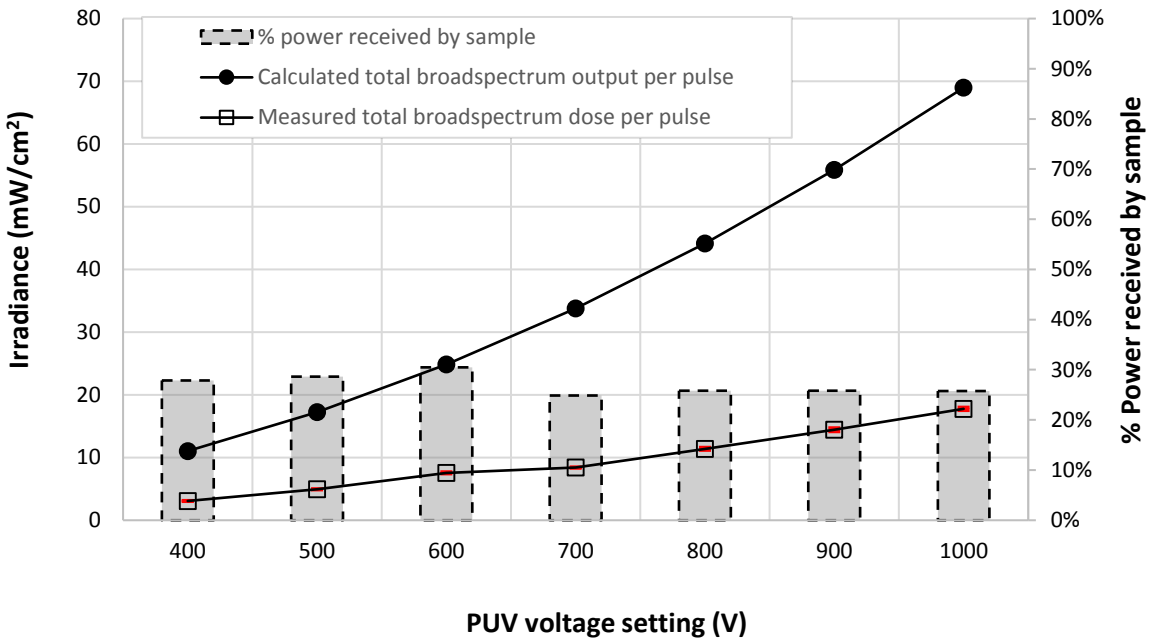


Figure 7.1. Comparison of the calculated broadspectrum energy output per pulse and the measured total broadspectrum dose per pulse of the PUV system with the % power received by the sample at each system voltage setting. Error bars are shown in red for the measured total broadspectrum dose, n = 9 for each voltage setting.

7.3.1 Impact of Lamp Distance on PUV Broadspectrum Dose Measurement

The PUV broadspectrum dose was measured at five lamp distances (from the sample/detector) of 10.75 cm (the height used for all experimental analysis), 15.75 cm, 20.75 cm, 25.75 cm and 30.75 cm. Figure 7.2 shows the impact of increasing the lamp distance from the sensor and the resulting broadspectrum irradiance measured at 800V and 900V (measurements were carried out at 800 V and 900 V only as these were the settings used during disinfection studies). The results indicated a relationship between the measured broadspectrum irradiance and the distance between the lamp and the sample.

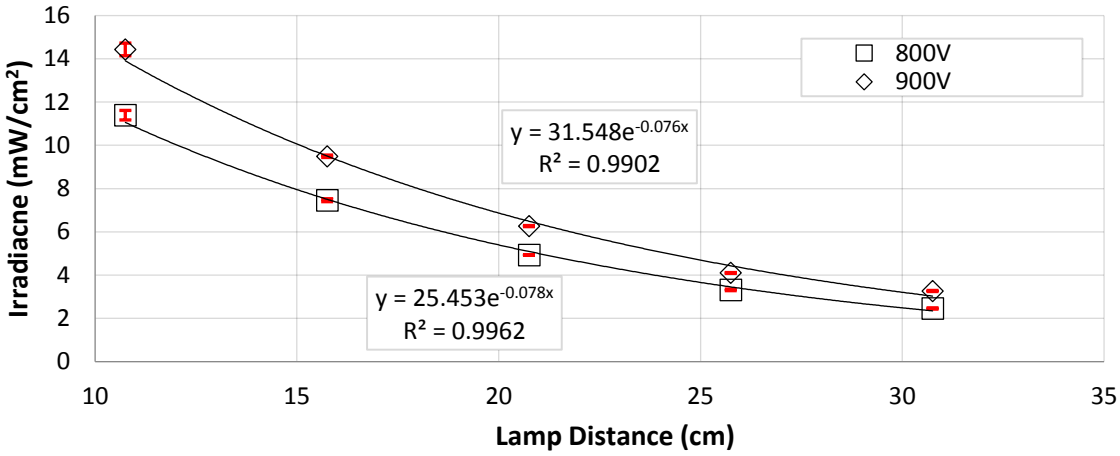


Figure 7.2. Measured total broadspectrum dose for the PUV system (800V and 900V settings; 1 PPS) at various distances from the lamp. Error bars are shown in red for the measured total broadspectrum dose, n = 9 for all points except 900V at 25 cm and 30 cm where n = 6.

7.3.2 UV Filters for PUV Broadspectrum Dose Measurement

Two longpass colour glass filters FGL 400 and FGL 280 were used in conjunction with the thermopile power detector to measure the portion of UV and UV-C energy imparted from the PUV lamp to the sample (see Chapter 3, Section 3.3.6.2 for further details). The FGL 280 filter filtered out wavelengths below 280 nm i.e. the UV-C range while the FGL 400 filter filtered out wavelengths below 400 nm i.e. the UV range.

The broadspectrum dose measured at various bandwidth regions of the PUV system at the 800V setting was given in the system manual and is outlined in Table 3.4. Figure 7.3 compares the total broadspectrum dose (the combined energy at all of the bandwidth regions at any given lamp distance) as per the Samtech Manual; Pulsed UV System PUV-01 with the measured total broadspectrum dose at 800V. The broadspectrum energy

measured with the Gentec sensor was higher than that given by the manufacturers. It can also be seen that the dose in the UV range as measured by the Gentec detector coincided with the broadspectrum dose given by the manufacturers. It was not possible to ascertain why this was the case.

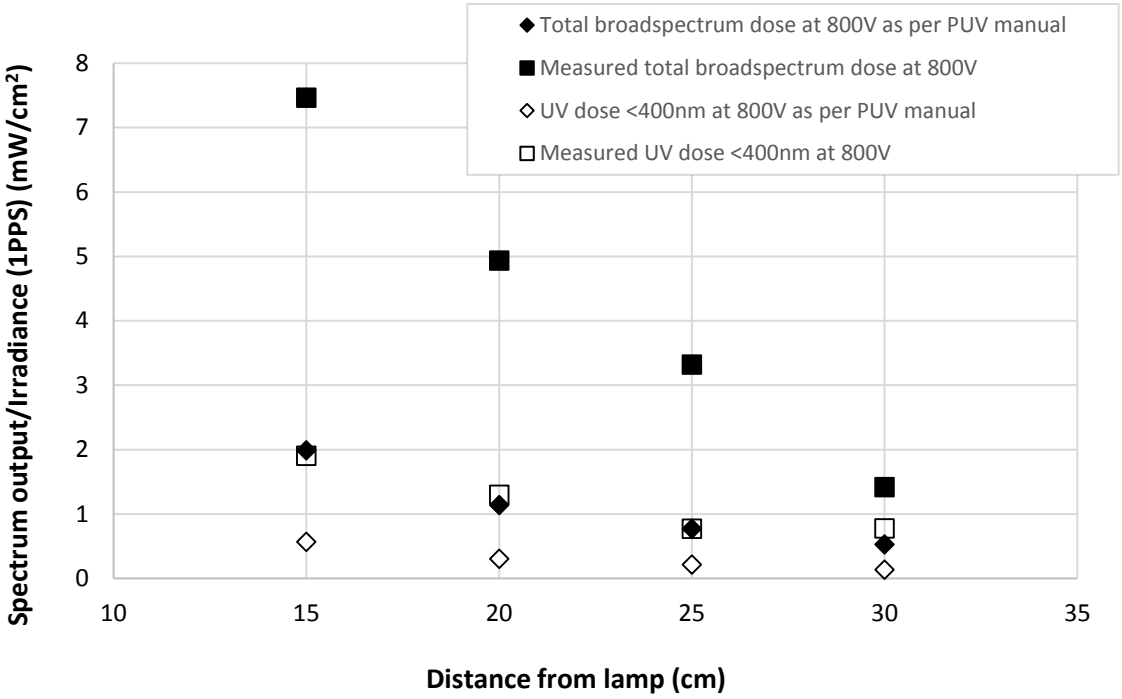


Figure 7.3. Comparison of the UV and total broadspectrum dose of the PUV system at 800V measured at various distances from the lamp as per Samtech Manual; Pulsed UV System PUV-01 and the measured total broadspectrum dose of the PUV system at 800V at various distances from the lamp.

Table 7.2 and Figure 7.4 detail the irradiance measurements with & without the filters at the range of distances detailed in Section 7.3.1 at 800 and 900 V.

Table 7.2. Irradiance measurements at various lamp distances for 800 V and 900 V system settings using colour glass UV and UV-C filters.

Voltage (V) Distance (cm)	Filters used	Irradiance (mW/cm^2)	UV Irradiance (mW/cm^2)	% UV Irradiance	UV-C Irradiance (mW/cm^2)	% UV-C Irradiance
800 10.75	No filter	11.4±0.22	0.70	6%	0.27	2%
	< 280nm filter	11.1±0.12				
	< 400nm filter	10.7±0.31				
800 15.75	No filter	7.5±0.07	1.90	25%	1.23	17%
	< 280nm filter	6.2±0.11				
	< 400nm filter	5.6±0.08				
800 20.75	No filter	4.9±0.01	1.30	26%	0.98	20%
	< 280nm filter	4.0±0.04				
	< 400nm filter	3.6±0.12				
800 25.75	No filter	3.3±0.04	0.77	23%	0.40	12%
	< 280nm filter	2.9±0.17				
	< 400nm filter	2.6±0.05				
800 30.75	No filter	2.5±0.03	0.77	32%	0.34	14%
	< 280nm filter	2.1±0.05				
	<400nm filter	1.7±0.02				
900 10.75	No filter	14.4±0.29	1.12	8%	0.18	1%
	< 280nm filter	14.3±0.44				
	<400nm filter	13.3±0.25				
900 15.75	No filter	9.5±0.06	2.56	27%	1.49	16%
	< 280nm filter	8.0±0.12				
	<400nm filter	7.0±0.11				
900 20.75	No filter	6.3±0.03	1.74	28%	1.18	19%
	< 280nm filter	5.1±0.06				
	<400nm filter	4.5±0.07				
900 25.75	No filter	4.1±0.01	0.71	17%	0.53	13%
	< 280nm filter	3.6±0.10				
	<400nm filter	3.4±0.07				
900 30.75	No filter	3.3±0.02	1.09	33%	0.76	23%
	< 280nm filter	2.5±0.07				
	<400nm filter	2.2±0.02				

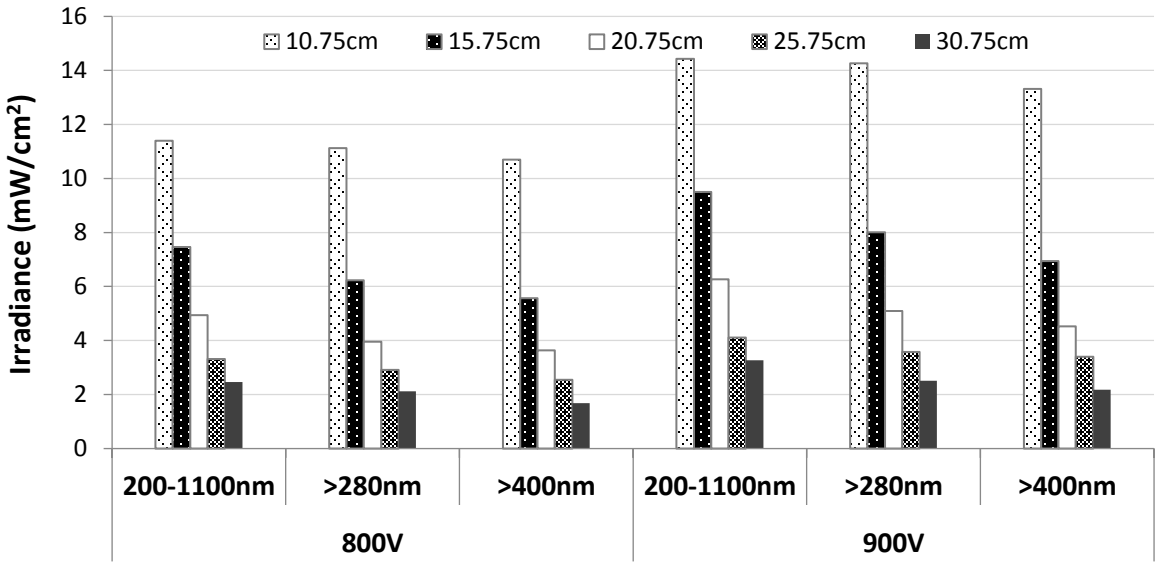
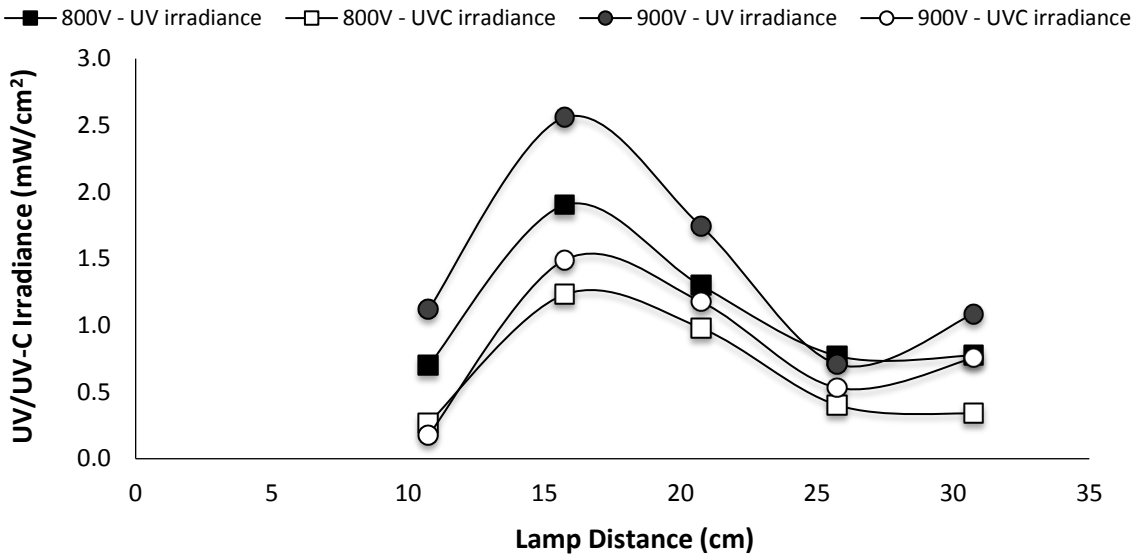


Figure 7.4. Irradiance measurements at various lamp distances for 800 V and 900 V system settings using colour glass UV and UV-C filters. Distance in the legend refers to the distance of the detector from the lamp.

The relationship between the measured UV and UV-C irradiance and the lamp distance was not found to be a linear one but rather a trend approximating a third order polynomial was observed. Figure 7.5 (a) shows the data as measured irradiance (mW/cm^2) while 7.5 (b) shows the data as a % of the total broadspectrum dose. Results show the proportion of UV and UV-C irradiance increased with increasing distance between the detector and lamp for distances between 10.75 cm and 15.75 cm. Thus, the proportion of UV and UV-C energy at each lamp height was found to vary with the highest proportion (and indeed highest absolute values) found at 15.75 cm lamp distance; thereafter irradiance in the UVC/UC spectrum was found to reduce before increasing again 25.75 cm and 30.75 cm.

(a)



(b)

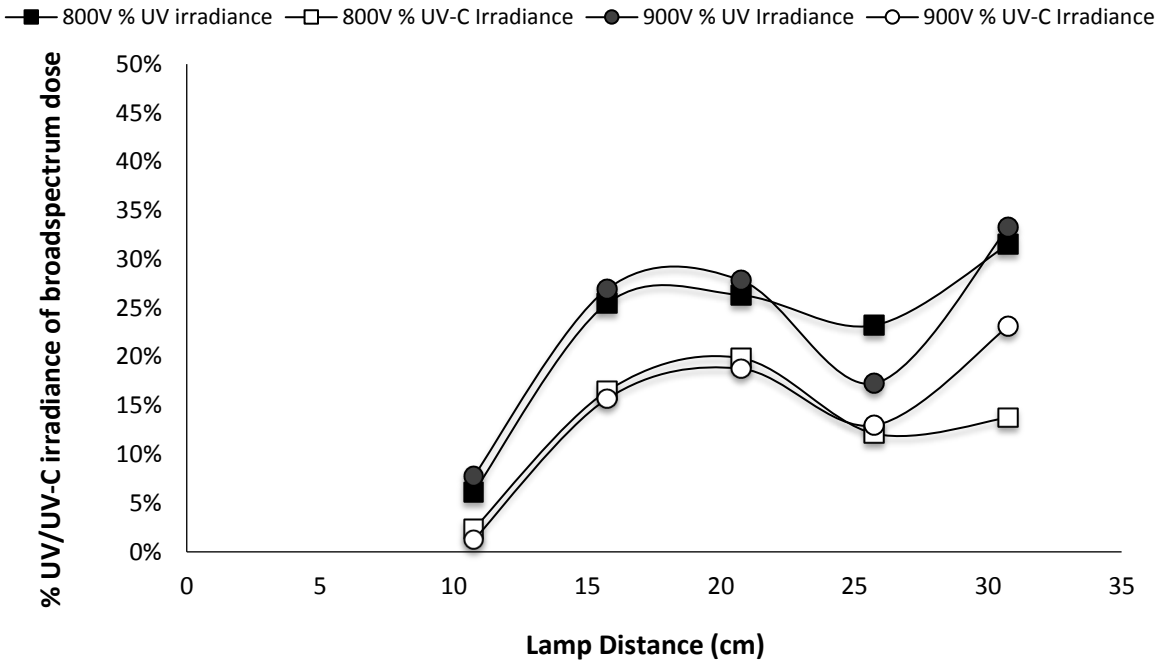


Figure 7.5. (a) Measured UV and UV-C irradiance at 800 V and 900 V system settings at various lamp distances and (b) % UV and UV-C energy of total broadspectrum dose at 800 V and 900 V at various lamp distances.

7.4 UV System Wall Plug Efficiency

The conversion efficiency of the UV systems from electrical energy to UV energy was analysed using a standard plug in energy monitor (see Section 3.3.6.3 for details). Table 7.3 details the energy monitor readings for a variety of settings for the PUV system and energy monitor readings for the fixed setting of the LPUV system. The theoretical UV power delivered from each system, derived from the system specifications is also included. A comparison between the total electrical energy consumption and the theoretical UV energy output (i.e. as per manufacturers specifications for LPUV and as calculated for the PUV; Section 3.4 & Section 7.2) of both systems showed the LPUV to be 77% efficient in converting electrical energy to UV energy while PUV efficiencies ranged between 17% and 35%. Losses due to heat were particularly noticeable with the PUV lamp however such losses were not measured in this study. The LPUV measurements may overestimate the electrical efficiency for the system and this is discussed in Section 7.5.3.

Table 7.3. Comparison of the wall plug efficiency of the PUV and LPUV systems using a standard energy monitor.

PUV System		Energy meter readings				UV power delivered (W)	Measured electrical power (W)	Calculated efficiency
System Voltage	PPS	Watts	Volts (V)	Amps (I)	V*I			
Off	0	0.0	234	0.00	0	-	-	-
0	0	19.3	233	0.12	28.7	-	-	-
400	0	19.4	234	0.12	28.8	-	-	-
600	0	19.4	234	0.12	29.0	-	-	-
800	0	19.2	234	0.12	29.0	-	-	-
1000	0	21.3	234	0.13	30.9	-	-	-
400	1	38.0	234	0.48	111.2	[1]3.2	18.6	17%
600	1	52.5	234	0.61	143.0	7.2	33.1	22%
800	1	67.5	234	0.68	158.2	12.8	48.3	27%
1000	1	91.5	234	0.73	171.1	20.0	70.2	28%
800	7	276.0	233	1.52	353.1	89.6	256.8	35%
LPUV system (40W)	-	52.0	233.6	0.22	51.4	[2]40.0	52.0	[3]77%

[1]UV power calculated as per Chapter 3, Section 3.3.6
[2]Based on manufacturer's specifications
[3]See section 7.5.3 for further details

7.4.1 Energy Efficiency per Treated Volume

The wall plug energy measurements were compared against the flow rates for both systems to determine the energy efficiency per litre treated. Table 7.4 compares the energy required per to treat 1 L of wastewater (or spiked water) via the PUV system at the lowest (62 s) and highest (120 s) HRTs used in this experiment and the energy required to treat 1 L via the LPUV system at the lowest (0.42 s) and highest (1.07 s) HRTs used in this study. The PUV system used approximately 44 times more energy per litre of wastewater treated in

comparison to the LPUV system and did not achieve the same inactivation rates for various pathogens. The measured broadspectrum dose per litre of wastewater treated via PUV in this study was of the same order of magnitude observed by Uslu *et. al.,* (2016) (243J/L at 3PPS, and 87 second HRT with relatively similar system settings) who investigated the disinfection of municipal wastewater via flow-through PUV. Thus the measurements in this study are comparable to those made in a previous study where broadspectrum dose was measured and details on residence time and system performance were available.

Table 7.4. Energy efficiency comparison of the flow-through PUV system and continuous-flow LPUV system per volume of water/wastewater treated.

UV System	System voltage - PUV only	HRT (s)	Energy consumption (J/L)	Measured broadspectrum dose at 800V & 1PPS (J/L)
PUV	800	62	5580	273
PUV	800	120	10800	529
LPUV	-	0.42	127	-
LPUV	-	1.07	292	-

7.5 Discussion

7.5.1 Methods of Reporting PUV System Energy

In many cases PUV system energy is not reported in studies with some studies advising caution particularly when reporting the energy received by the sample and in the cases where it is reported the methods by which PUV system energy are analysed vary (Gómez-López & Bolton, 2016; Gómez-López *et al.,* 2007). While a typical xenon gas PUV system comprises light emissions within the broadspectrum of UV, visible and infrared light, pulsed UV dose/output calculations and/or measurements in terms of the energy applied

from those wavelengths within the UV range (below 400 nm) should also be measured alongside 'total broadspectrum dose/output' which infers the total energy applied across the whole spectral range. This has been previously demonstrated in a number of studies with the aid of spectrometers/pyroelectric detectors and in some cases the UV dose from the PUV systems has fallen within the same order of magnitude as LPUV dose/outputs e.g. 1-100 mJ/cm^2 (Wang *et al.*, 2005; Lee *et al.*, 2009; Vimont *et al.*, 2015). In this study, broadspectrum energy measurements indicated that on average, at 800V and 900V, and a sample to lamp distance of 10.75 cm, 27% of the lamp energy impacted on the sample and of that, 6 - 8% fell within the UV wavelength range. Similar findings have been previously reported; Kramer & Muranyi (2013) also used an FGL 400 glass filter to determine the UV content emitted from a PUV system (Claranor, Avignon, France). At a distance of 10 cm, the measured broadspectrum dose (using a SOLO2 Power and Energy meter, Gentec – EO, Quebec, Canada) for voltage settings of 1000V and 3000V was reported to be 100 mJ/cm^2 and 1000 mJ/cm^2 respectively. Using the longpass filters, the UV content was measured at 6% for 100 mJ/cm^2 broadspectrum dose and 18% for 1000 mJ/cm^2 broadspectrum dose.

Vimont *et al.*, (2015) observed that 2% of the broadspectrum energy from a bench-top PUV system – the detector was located 8 cm from the lamp - (Sinteron 500, Xenon Corp., MA, USA) was measured between 235 – 307 nm (Vimont *et al.*, 2015). This finding is in agreement with results in this study which found the UV-C (200 – 280 nm) power to account for 1-2% of the total broadspectrum output of the lamp under the applied experimental conditions.

The calculated UV-C: broadspectrum output ratio ("UV-C" being < 300nm – Section 7.2) in this study indicated 18% energy allocation to the UV-C range which is significantly

higher than that of 1-2% as determined by the thermopile detector. The considerable difference between the energy emitted from PUV systems and the energy (particularly UV energy) impacting the sample demonstrates a level of uncertainty in PUV output measurements. The majority of the power emitted from PUV systems does not appear to lend itself to the microbial disinfection process (i.e. inactivation via UV-C disinfection) therefore future analysis should focus on system amendments to improve this issue.

7.5.2 Relationship between UV Irradiance and Lamp Distance

During the UV/UV-C irradiance measurement analysis it was expected that the relationship between increasing lamp distance and decreasing UV/UV-C irradiance would follow the inverse square law however this was not observed. UV irradiance measurements were higher at a lamp distance of 15.75 cm in comparison to 10.75 cm with a third order polynomial trend emerging as the distance increased. A study by Krishnmurthy *et al.*, (2007) reported similar findings. The authors investigated the inactivation of *S. aureus* via flow-through PUV disinfection in milk and compared bacterial inactivation at lamp distances of 5 cm, 8 cm and 11 cm (Krishnamurthy *et al.,* 2007). Results showed the inactivation of *S. aureus* to increase as the distance increased initially and then reduce due to less absorption of energy according to the authors (Figure 8.4). In contrast, a recent study investigating *L. monocytogenes* inactivation via static PUV treatment reported increasing microbial inactivation at lamp distances from 5 cm to 15 cm and voltage settings between 600V and 800V (Pollock *et al.*, 2017).

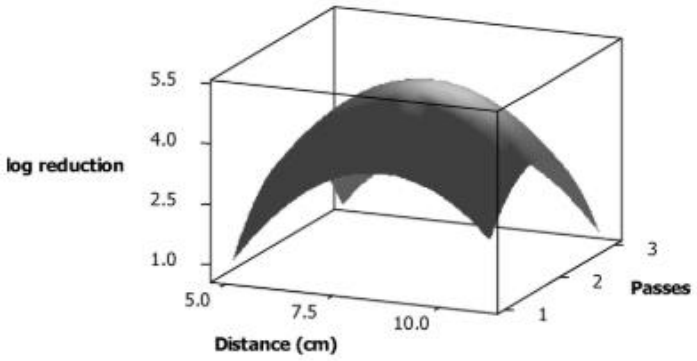


Figure 7.6. Inactivation of *S. aureus* via flow-through PUV disinfection at various lamp distances (Krishnamurthy *et al.*, 2007).

The spatial distribution and mapping of fluence at incremental distances inside a pulsed light treatment chamber has previously been studied (Hsu & Moraru, 2011). Findings confirmed a decrease in total broadspectrum fluence with increasing distance from the lamp (as expected) which is in agreement with findings in this study. The energy apportioned to the UV wavelengths (200 – 400 nm) at one lamp distance (distance not specified) was also determined using the integration of irradiance curves derived from spectrophotometric measurements of irradiance per wavelength for the entire broadspectrum output (100 – 1100 nm). Results showed UV fluence to account for approximately 40% of the total broadspectrum fluence, however the proportion of UV fluence at various lamp distances was not reported. Therefore, if PUV experimental analysis involves multiple lamp distances, or indeed a single distance, it may be necessary to measure both the broadspectrum energy and the UV energy to ensure the proportion of UV energy at any given height is correctly determined.

7.5.3 UV System Wall Plug Efficiency

Investigations into the energy efficiency of the PUV system in comparison to the LPUV system suggest the LPUV was more efficient at converting electrical energy to UV energy with PUV and LPUV efficiencies of 17 – 35% and 77% respectively. However, the energy efficiency of mercury based LPUV lamps typically reported in the literature is approximately 35% (IUVA, 2017; Schaefer *et al.*, 2007). The difference between results found in this study and those in the literature is likely due to a potential overestimation of the 'true UV power' emitted from the LPUV system. It should be noted that in studies which have measured the irradiance from standard LPUV lamps, less than half of the energy from the LPUV lamp was reported to be UV-C power e.g. a 250W LPUV lamp was shown to emit on average 109.3 ± 1.88 W of UV-C power (N = 10). When the UV-C power was compared to the measured wall plug power an average efficiency of 36.0 ± 0.4 was reported (IUVA, 2017). In the case of the present study, the predominant experimental focus was that of the flow-through PUV system and not the standard LPUV system thus irradiance analysis of the LPUV lamp was not carried out. Text to be added here pending correct data in Table 7.4.

The analysis of the energy efficiency of the PUV system used in this study was found to be in agreement with similar studies in the literature. Research investigating the conversion efficiency of electrical energy to broadspectrum energy reported conversion rates of 8 – 10% (Schaefer *et al.*, 2007) and 17% (Vasilyak, 2009). Interestingly, Schaefer *et al.*, (2007) also reported an increase in the % conversion efficiency as the pulse frequency of the system increased which is in agreement with the results found in the present study

(Schaefer *et al.*, 2007). The authors attributed the increased efficiency to a shorter pulse length at high PPS frequencies.

7.6 Conclusions

- Comparisons of the calculated total broadspectrum output and measured broadspectrum dose highlighted that at each voltage system setting, an average of 27% ± 2% of the total broadspectrum energy emitted from the system was being absorbed by the detector as total broadspectrum dose.
- The measured PUV broadspectrum dose decreased exponentially with lamp distance for the 800 and 900 voltage system settings, which constituted the uppermost operational capabilities. This would infer that future studies should consider distances up to 10 cm from pulsed light sources.
- The proportion of measured PUV broadspectrum energy in the UV and UV-C range (as measured by the Gentec detector) was found to vary by lamp distance. The UV energy ranged from 6% to 32% of the total broadspectrum energy depending on lamp distance while the UV-C energy ranged from 2% to 20% depending on lamp distance. Furthermore, in contrast to the expected output in the UV range as provided by the manufacturers guidelines, the measured UV doses initially increased with lamp distance (up to 15 cm lamp distance before decreasing (between 15 cm and 25 cm distance) and increasing again (at 25 cm distance).
- The wall plug efficiency of the PUV system was lower in comparison to the LPUV system. In addition, the flow-through PUV system exhibited a lower energy

efficiency per volume of water/wastewater treated in comparison with the LPUV which indicated further optimization of the PUV system in a flow-through experimental set-up is required.

- Future studies should focus on improving the energy delivery from the PUV system to the target sample to ensure higher energy absorbance. In the case of water/wastewater treatment applications, submergence of the lamp should be considered to minimize distance and enhance the delivery of UV irradiation.
- The difference in energy measurements between the Gentec sensor and the measurements/predictions given the Samtech manual are notable and it is recommended that these differences are investigated further.

Chapter 8
Conclusions & Recommendations

8.1 Introduction

Water consumption rates within the Irish dairy industry are expected to increase due to industry expansion following the abolishment of milk quotas in April of 2015. Currently, water reuse practices within dairy plants remain low however with the potential introduction of stricter legislation (owing to industry expansion and stringent discharge standards), the industry appears open to strategies to minimise their water footprint. Pulsed UV (PUV) disinfection has recently been approved for application within the food industry for contact food and packaging treatments and differs from standard low pressure UV (LPUV) technology by emitting rapid, high energy pulses of broadspectrum light that has been shown to decrease treatment contact times and enhance microbial kill (Elmnasser *et al.,* 2007). This constitutes the first study that comprehensively tested and evaluated a modified PUV system under various operational configurations and settings as potential next-generation UV disinfection technology for water/wastewater treatment as a 'flow-through' approach. To date, very few studies have directly compared flow-through PUV to continuous-flow LPUV for bacterial inactivation efficiency.

Specifically, in this study, a modified flow-through PUV system was evaluated and compared to a standard continuous-flow LPUV system for the treatment of wastewater streams from dairy processing plants with a view of potential tertiary disinfection application in dairy WWTPs and/or dairy wastewater reuse at Irish dairy plants. The technologies were compared in terms of the inactivation efficiency of a variety of bacteria including pathogenic bacteria typically found in dairy wastewaters, faecal indicators and

bacterial endospores. Impacting factors on UV system efficiency such as wastewater parameters, culture media supplementation and bacterial photoreactivation potential were analysed to determine any additional benefits of employing modified flow-through PUV in water/wastewater treatment applications over a standard continuous LPUV system. Finally, a comparison of the methods of energy determination from the PUV system was performed to examine the proportion of absorbed broadspectrum and UV energy versus the energy output of the system.

8.2 Conclusions & Recommendations

The main conclusions and recommendations from this study are;

8.2.1 Dairy Plant Survey

1. A site survey at six representative Irish dairy plants indicated reuse of dairy wastewater had some potential: however, some facility managers alluded to reservations around ‘cow water’ reuse due to stigmas associated with inherent bacteria in the water. In addition, the site survey confirmed rainwater harvesting was not a priority at dairy plants at this time. Most facility managers agreed that future legislation of microbiological discharge standards in dairy WWTPs is a strong possibility and this would further drive the need for pathogen removal technology to be deployed.
2. Analyses of dairy wastewater samples from three contrasting Irish dairy plants highlighted the presence of total coliforms and *E. coli* in the majority of water samples tested that indicates faecal contamination. Out of the five target dairy pathogens investigated, *Salmonella spp.* went undetected in all the samples tested while *Listeria monocytogenes* was found to be most prevalent at Site 1. *L.*

monocytogenes is a significant pathogen for the food industry as it can grow at refrigeration temperatures and may cause serious human infections in the unborn foetus with up to 70% fatality rate where infective dose can be as low as 10 cells. *Staphylococcus aureus* was also found to be consistently present in samples collected at this site.

8.2.2 Application of Pulsed UV and Low Pressure UV Technology

1. This study showed that supplementing cultivation agar with $MnSO_4.H_2O$ aided the sporulation of members of the bacterial *Bacillus* genus, namely *B. pumilus* and *B. subtilis* and enhanced their resistance to both PUV and LPUV irradiation. This has implications for comparative evaluation of water-based disinfection whereby previous studies or comparison between studies and technologies have generally not accounted for this factor in determining performance.
2. The level of UV resistance of *B. pumilus*, *B. subtilis* and *B. cereus* increased significantly when relatively low concentrations (0.05 mM) of $MnSO_4.H_2O$ were added to cultivation agar. *B. pumilus* endospores appeared to be more readily affected by the addition of the supplement in terms of increased UV resistance, particularly in the case of LPUV disinfection. In general, *B. subtilis* endospores were inactivated to a higher degree by the PUV and LPUV systems in comparison *B. pumilus* endospores. Future research and validation studies should report $MnSO_4.H_2O$ supplementation and be cognisant of it when comparing between studies and technologies.
3. The continuous-flow LPUV system was found to achieve higher inactivation rates at lower system energy outputs when compared to the flow-through PUV system

with the LPUV system demonstrating $\log_{10}$ inactivations of 5.3, 6.0 and 5.9 for *S. aureus*, *E. coli* and *L. innocua* at UV dose or fluence of 14 mJ/cm^2. In contrast, *S. aureus*, *E. coli* and *L. innocua* inactivation via PUV disinfection was found to be energy dependant with a PUV output of 1539 mJ/cm^2 required for a 5.3 $\log_{10}$ inactivation of *E. coli* and an increased PUV output of 2052 mJ/cm^2 required for a 5.2 and 4.3 $\log_{10}$ inactivation of *S. aureus* and *L. innocua* respectively.

4. No photoreactivation of *E. coli* and *S. aureus* was observed post LPUV disinfection. This may be attributable to the relatively higher energy output of the system applied (14 mJ/cm^2). Typically, *E. coli* and *S. aureus* are inactivated at UV doses of < 10 mJ/cm^2 and studies have also shown PHR of *E.coli* to occur at low UV doses (5 mJ/cm^2) but not at high UV doses (15 mJ/cm^2) (Guo *et al.*, 2009; Malayeri *et al.*, 2016). Thus, in this study the LPUV system inhibited bacterial photoreactivation which further highlights its effectiveness and suitability for application in the water/wastewater treatment sectsector. The photoreactivation of *S. aureus* and *E. coli* was detected post PUV disinfection with *S. aureus* exhibiting approximately 10% higher photoreactivation in comparison to *E. coli* at the lowest PUV outputs of 486 mJ/cm^2 and 1026 mJ/cm^2. Results also showed the majority of photoreactivation post PUV disinfection occurred in the first 60 minutes for *S. aureus* and 90 minutes for *E. coli* thus if scaling up of the PUV flow-through system is considered for water/wastewater treatment, the immediate exposure of bacteria to light post PUV treatment should be avoided to minimise bacterial photoreactivation.

5. Comparative experimental studies highlight that conventional LPUV is superior to that PUV in its current configuration for microbial disinfection in flow-through treatment of dairy wastewater. While these findings support the use of conventional LPUV as an effective disinfection technology, it also emphasises that further modification of the PUV system must be considered to reflect its superior performance for contact food surface and air disinfection. It is also recommended that studies are performed to investigate the biocidal potential of the full broadspectrum pulse of PUV with the use of monochromators to analyse and compare disinfection potential of individual wavelength bands.

8.2.3 Impact of Suspended Solids and Organic Carbon on PUV and LPUV Performance

Turbidity can influence UV disinfection performance. Laboratory analysis regarding the use of bentonite and $CaCO_3$ (various particle sizes of both) to simulate inorganic TSS in water was shown to have no impact on the working efficiency of the flow-through PUV system in total coliform inactivation. In contrast, concentrations of organic TSS were shown to impact upon system efficiency of both the PUV and LPUV at TSS concentrations of >25-30 mg/L and >75 mg/L respectively. Similar analysis at site-scale level showed the inactivation of total coliforms via flow-through PUV to be variable. A relationship between TSS and COD concentrations and inactivation efficiencies was not observed. The inactivation of total coliforms via LPUV disinfection showed a more consistent trend with high inactivation rates of 4.6 ± 0.44 and 5.16 ± 0.09 at LPUV outputs of 14 mJ/cm^2 and 20 mJ/cm^2. However, TSS concentrations during site-scale studies did not surpass 30 mg/L for LPUV disinfection analysis thus the impact of high TSS concentrations was not fully

elucidated in this case. For PUV analysis, wastewater from a secondary treatment lab-scale IASBR yielded TSS concentrations from 5 mg/L to 70 mg/L. However, no clear trend could be observed between increasing TSS concentrations and decreasing PUV system efficiency as total coliform inactivations varied between 2 and 5 $\log_{10}$ throughout the range of TSS concentrations tested. For site-scale wastewater (the same wastewater as used above for LPUV system analysis), a slight trend was observed whereby increasing TSS concentrations appeared to decrease PUV inactivation at below 30 mg/L TSS concentration. It is therefore recommended in the case of deployment of flow-through PUV system for wastewater disinfection, effluent quality should be closely monitored to ensure optimal operation of the system.

8.2.4 Energy Analysis of PUV and LPUV Systems

8.2.4.1 Comparison of Methods of PUV Energy Determination and Influence of Lamp Distance.

Approximately 27% of the total broadspectrum energy emitted from the PUV system was measured by the detector at system voltage settings between 400 V and 1000 V at a lamp distance of 10.75 cm. A clear relationship between the measured broadspectrum dose and incremental lamp distances between 10.75 cm and 30.75 cm was found at the 800 and 900 V PUV system settings.

In the case of the PUV system, it is suggested that studies report full UV dose/output details including;

- Whether the reported UV dose/output accounts for the full broadspectrum or the UV portion alone.

- Whether it is calculated or measured.
- The number of pulses applied.
- The pulse rate.
- The energy per pulse.

In this way, clear comparisons can be drawn between PUV inactivation studies. Moreover, in the case of flow-through PUV inactivation studies details such as the HRT and vessel area should also be clearly stated.

8.2.4.2 Determination of Proportion of UV/UV-C Energy Emission from PUV System

The use of FGL 400 and FGL 280 UV filters during the thermopile power detector analysis showed the relationship between the proportion of UV/UV-C energy measured at incremental lamp distances and the increasing lamp distance did not follow the inverse square law as had been expected i.e. more proportional UV/UV-C energy was measured at a lamp distance of 15.75 cm in comparison to 10.75 cm. It is recommended that future studies investigate if/how PUV lamp distance might impact upon the amount of UV energy reaching the sample as similar studies on this topic were limited.

8.2.4.3 Wall plug Efficiency of PUV and LPUV Systems

1. A comparison of the wall plug efficiency for both systems confirmed the LPUV system was more efficient at converting electrical energy to UV energy in comparison to the PUV system. Efficiencies of between 17 – 35% were reported for the PUV system depending on the voltage and pulse frequency applied whereas the LPUV system illustrated a conversion efficiency of 77% at the fixed power output of 40 W. However, it should be noted that the energy data used to calculate

the conversion efficiency was the theoretical (calculated) energy output of the system and not the measured energy output which may influence the % CE as has been shown in previous studies.

2. UV system energy efficiency comparison in terms of volume of water treated showed the LPUV system to require less ‘wall plug’ energy per litre of wastewater treated in comparison to the flow-through PUV system. It is recommended that if flow-through PUV were to be considered for upscaling, optimization of the system in terms of enhanced energy delivery should be investigated to decrease overall energy consumption at the wall.

8.2.5 Summary

This study evaluated and compared the effectiveness of a conventional LPUV system to that of a potentially more efficient novel UV treatment system for the disinfection of dairy water pathogens in a flow-through set-up. The rationale for supporting this concept relates to previously published studies illustrating the PUV to demonstrate superior disinfection performance to LPUV for contact surface treatments of foods and packaging. The findings conclude that for the pulsed UV to be a viable technology for wastewater treatment application, significant enhancements would be necessary as it did not offer advantages over low pressure UV disinfection in this study. This study also demonstrated the effectiveness of existing LPUV as a technology suitable for dairy wastewater disinfection and reuse.

8.3 Future Research

Key areas of further research are highlighted below.

1. The potential for the recycling of condensate water at Irish dairy plants should be analysed in more detail. Condensate water is produced in large quantities at some dairy plants and results in this study indicate it to be contaminated to a lesser degree in comparison to other wastewater streams. Moreover, findings in the present study illustrated condensate water to be the most common source for water reuse at Irish dairy plants. Therefore, future studies should investigate this waste stream further in terms of both its chemical and microbiological profile to determine suitability for water reuse at dairy plants on a larger scale.
2. Future studies should focus on optimising the energy delivery of the pulsed UV light source in flow-through applications. For example, the submergence of the xenon lamp into the water sample (as is the case with closed LPUV reactors) would minimise the distance between the water column and PUV rays thus enabling optimum inactivation efficiency. Furthermore, the optimisation of the UV portion of the broadspectrum system output would enhance the microbial disinfection process. Focusing on this aspect would aid system efficiency and perhaps decrease HRTs/contact time necessary for flow-through PUV. In addition, analysis of the efficacy of the xenon lamp itself over time is also recommended. Such information would assist with cost/benefit analysis of PUV systems in terms of lamp life and frequency of lamp replacement etc.
3. Both physical and chemical methods of PUV energy measurement should be evaluated in future studies with a view of forming standard guidelines which should be used when reporting the energy from PUV systems. It is imperative that consistent terminology and measurement methods are used and applied to allow for clearer comparisons between studies and individual PUV systems internationally.

4. Future studies should investigate cell and molecular mechanisms by which microorganisms are inactivated during flow-through PUV treatments as the majority of the literature focuses on microbial PUV damage on solid surfaces or in air as opposed to water/liquid media. Moreover, there is no single study that comprehensively investigates concurrent and sequential stresses occurring in PUV-treated microorganisms based upon fluence that highlights onset of irreversible damage (cell death) in flow-through treatments so as to inform and optimise future system modifications, which needs to be addressed.
5. Future studies focused on the cost-benefit analysis of water/wastewater reuse at dairy plants should include an environmental assessment of water/wastewater reuse to determine the potential impact on reducing water abstraction and wastewater discharges. In addition, the incentives that would drive industry to adopt such practices should be identified. Notwithstanding the fact that the significant bacterial pathogens such as *Listeria monocytogenes* were isolated from Irish dairy wastewater samples during this study that merits mitigation.
6. The impact of adding agar supplement $MnSO_4.H_2O$ to bacterial culture media should be investigated further. Specifically, analysis should focus on whether the addition of manganese sulphate monohydrate might offer protection to endospores from other external stresses e.g. heat or alternative radiation treatments. It should be established whether this phenomenon is limited to UV treatment only or has implications further afield.
7. The photoreactivation capacity of microorganisms post LPUV disinfection should be analysed in more detail with regard to UV doses currently applied in the

water/wastewater industry. Molecular analysis should be included to confirm complete microbial inactivation. While microbiological methods are widely used and accepted, they cannot detect dormant phases exhibited by some bacteria e.g. ‘viable but non-culturable’ states which allow the microorganism to reactivate at a later stage. The addition of molecular analysis allows for confirmation of microbial metabolic inactivation.

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Appendix A

SAMTECH
SWITCHING ACCELERATOR AND MODULATOR TECHNOLOGY

PULSED UV SYSTEM PUV-01
OPERATING INSTRUCTIONS

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Contents

Samtech has no liability for any consequences arising from the misuse of the High Voltage Pulsed UV Generator and/or this Operating Manual.

1. Health and Safely Guidelines

Warning - High Voltage

High Voltage Operating Guidelines

This Pulsed UV System contains high voltages that are **potentially lethal**. The Pulsed UV System must only be operated by qualified personnel who have :-

1. Read and understood the contents of this manual.

2. Undergone appropriate safety training by an experienced high voltage engineer.

3. Understood necessary precautions and undertaken necessary safety procedures outlined in local safety guidelines pertaining to the operation of high voltage equipment.

Suitable care and judgement must always be exercised when using this Pulsed UV system. The following is a list of precautionary measures that must be adhered to when using this UV System.

1. Before turning on the UV System, ensure that all covers are in place and fastened securely.
2. Correct grounding of the input ac power is essential and must be undertaken to minimise the risk of electrical shock, and to comply with local safety requirements.
3. Use extreme caution when connecting the input ac power and only use the voltage levels indicated in this manual.
4. Make sure the correct BNC output corresponds to the correct Flashlamp cable, i.e. **Discharge Output = Black cable**, **Trigger Output = Red Cable**

5. Always make sure all appropriate connections are made before switching the UV system on.
6. Never handle the high voltage output connection or lamp when the UV System is operating.
7. The high voltage connection and lamp should be placed inside a suitable screened enclosure that can be locked with a key, to prevent accidental access during operation of the UV system. The operator of the UV System should hold the key when the UV System is turned on.
8. Always replace fuses with the same type and Volt/Amp rating.
9. Never attempt to use the UV System for anything other than the purpose it was designed for.
10. Do not remove the high voltage and safety warning labels from the UV System.
11. Always wear appropriate safety equipment when operating the UV System i.e. UV protective eye glasses, any skin protective equipment.

2. Introduction

The **SAMTECH pulsed UV system PUV-01** is a portable, bench-top pulsed UV light source. Its low-pressure (450 torr) xenon filled flash lamp can produce high intensity UV illumination, making it widely useable in microbial disinfection and related fields.

Specifications:

- Intense UV output covering UVC, UVB and UVA regions.
- Continuously adjustable discharge voltage from 400 to 1000V.
- Continuously adjustable energy per pulse from 3.2 to 20 Joule.
- Manual trigger and automatic trigger.
- Adjustable flash frequency from 0.1 to 10 pulses per second, depending on discharge voltage.

3. System Components:

Front and Rear Panels of the System

Figure 1. Front and rear panels

- **Driver Unit** - unit consisting trigger and discharge outputs, frequency control, trigger control and discharge voltage control
- **Flash lamp** - xenon filled flash lamp mounted in the flash lamp unit
- **Trigger cable** - cable connecting the trigger output of the driver unit with the trigger electrode of the flash lamp
- **TRIGGER -** HV BNC connector for the trigger output
- **DISCHARGE** - HV BNC connector for the discharge output
- **Fan** - fan of the driver unit.
- **Flash lamp unit** - holds the flash lamp and its reflector
- **Discharge cable** - cable connecting the discharge output of the driver unit with the lamp anode and cathode
- **Mains IEC connector** - for mains cord connection.
- **Mains cord** - supplied with the product for mains power connection.
- **Charging Voltage** meter - showing the voltage of the charge on the discharge capacitor (V).
- **Auto, Man/Ext** Trigger - switch for choosing the mode of triggering - automatic trigger (left) or manual trigger (right).
- **Single Shot** –switch for manual triggering.
- **Power** - switch for mains power on or off.
- **Enable** - switch the HV charging system on.
- **Voltage Control** dial - adjusts the charging voltage from 0 to 1000 V.
- **Frequency** control **0.1-1 pps** - adjustment of the flash frequency from 0.1 to 1Hz.
- **Frequency** control **1-10 pps** – adjustment of the flash frequency from 1 to 10 Hz.

WARNING: DO NOT BLOCK THE VENTILATION HOLES IN THE TOP OF THE UNIT.

4. Connecting Up The System

- Two output cables are connected to the flash lamp unit - the trigger cable (red) and the discharge cable (black). Connect the trigger cable to the connector marked TRIGGER and the discharge cable to DISCHARGE. Both BNC connectors are on the back panel of the driver unit.

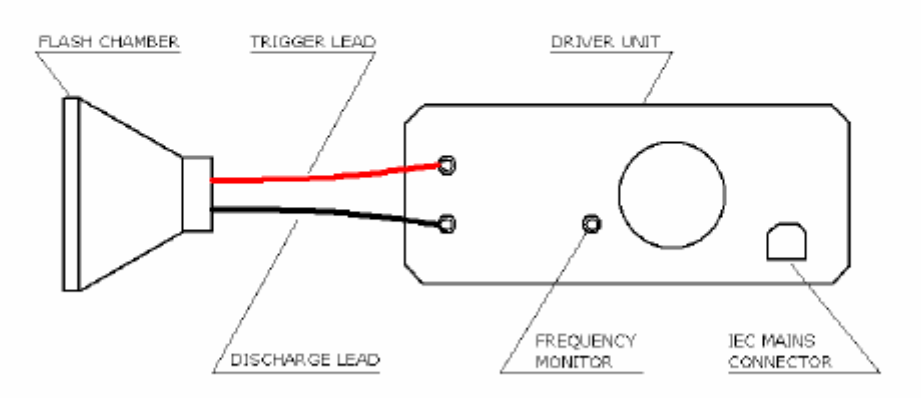


Figure 2. Connecting the PUV-01

5. System functional check procedure

- Make sure the charge/ discharge **Voltage Control** dial is on zero position and the triggering is on the **Man**ual/ **Ext**ernal position.
- Turn the **Frequency** control dial (**0.1-1 pps**) fully clockwise and the dial (**1 - 10 pps**) fully anticlockwise. The flash frequency is 1Hz with these positions.
- Turn on the **Power** switch.
- Turn on the **Voltage** switch.
- Adjust the **Voltage Control** to 500V.
- Press the **Single Shot** button. The lamp will flash correspondingly.
- Test the automatic trigger by switching to **Auto.** The flash lamp will flash at 1Hz.
- Turn the **1-10 pps Frequency** control dial fully clockwise slowly. The flash frequency will increase correspondingly from 1Hz to 10Hz.
- Turn the **1-10 pps Frequency** control dial fully anti-clockwise. The flash frequency returns to 1Hz.
- Turn the **0.1-1 pps Frequency** control dial fully anti-clockwise slowly. The flash frequency will decrease from 1Hz to 0.1Hz correspondingly.
- Turn the **0.1-1 pps Frequency** control dial fully clockwise. The flash frequency returns to 1Hz.
- Switch the automatic trigger mode to **Man**ual/ **Ext**ernal trigger. The lamp will stop flashing.
- Return the charge/ discharge **Voltage Control** to zero.
- Push the single shot button to discharge residual energy.
- Turn off the Charging **Voltage** switch.
- Turn off the mains **Power** switch.

Note: When using the manual trigger the frequency control dials should be set for 1Hz.

Note: The adjustment of the flash frequency is not linearly proportional to the position of the frequency control dials.

6. Operating the system

In addition to the general operating procedures, topics concerning the operation of the system are listed in this section.

General operating procedures:

- Choose either AUTO trigger or MAN/EXT trigger mode.
- To preset the frequency in automatic mode, connect a frequency meter to the FREQUENCY MONITOR BNC connector and adjust the frequency dials until the preset value is achieved. Return the mode switch to MAN/EXT.
- Turn on the charging voltage ENABLE switch and set the desired charging voltage
- For manual triggering press the MAN/EXT button to fire the flash lamp
- For automatic triggering flip the mode switch to AUTO. Return the mode switch to MAN/EXT to stop the triggering.
- When finished using the unit turn off the ENABLE switch and switch off the mains POWER.

Note: After switching off the unit the discharge capacitor may still have a high voltage charge, which will register on the Charging Voltage meter. This charge will decay through a bleeder resistor over a period of about 15 minutes.

Discharge voltage and energy per pulse

Table 1 lists the discharge voltage and the corresponding energy per pulse input into the flash lamp in the applications with this system. For the values not listed in this table, Formula 1 can be used to calculate the corresponding energy per pulse.

Voltage V	400	500	600	700	800	900	1000
Energy J	3.2	5.0	7.2	9.8	12.8	16.2	20

Table 1. Discharge voltage and energy per pulse

Frequency and discharge voltage:

The range of the flash frequency is controllable from 0.1 to 10 Hz depending on the discharge voltage. The rated power of the flash lamp is 90 W, which determines the maximum frequency that can be operated at the selected discharge voltage, as shown in Table 2.

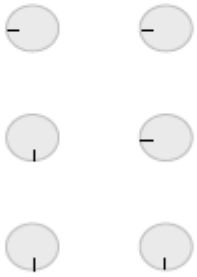
V. (V)	400	500	600	670	800	900	1000
Max. Freq. (Hz)	10	10	10	10	7	5.6	5

Table 2. Maximum operating frequency and discharge voltage

In the automatic trigger mode, the system can control the lamp power within the rated value, even when the frequency is set higher than the listed values in Table 4. The system will keep the flash lamp at the rated power by reducing the discharge voltage automatically. e.g. when the system is preset at 1000V, and the frequency is set to 10 Hz, the system will only charge to 670V rather than the preset 1000V.

Frequency Control

The frequency control in the automatic trigger mode is performed by adjusting the two control dials. The positions and the corresponding frequency are shown below. The initial positions can be adjusted by turning two dials fully anticlockwise, which corresponds to 0.1 Hz. Turning the left dial (0.1-1Hz) fully clockwise will increase the frequency to 1 Hz. If the left dial is kept the same position and the right one (1-10 Hz) is turned fully clockwise, the frequency will be increased from 1 Hz to 10 Hz. The desired frequency between 0.1 to 10 Hz can be obtained between the initial and final positions.



Initial positions f = 0.1Hz

Middle positions f = 1 Hz

Final positions f = 10 Hz

The maximum frequency at different voltages should be adhered to, and it should be noted that the frequency does not change linearly with dialling.

Voltage Monitor

The charging voltage can be adjusted and shown on the front panel of the driver unit. The meter will always point to the preset voltage except during the fast flash operations when the pointed value is lower than the preset voltage due to the slow movement of the pointer. The actual discharge voltage will keep the preset value for all the operations despite the low voltage display.

7. System Protection

Measures are taken to protect the equipment from being damaged in abnormal operating conditions, which include:

- A thermal fuse is installed in the driver unit to shut down the high voltage section if the unit becomes too hot. The thermal fuse incorporates hysteresis, so the system will remain shut down until the internal temperature drops significantly below the trip temperature. Note that if the mains switch is left on, the system will start up as soon as the thermal fuse resets.
- A 5A fuse is used in the IEC main socket on the back panel of the driver unit and a 5A fuse is used in the mains cord

8. Technical Description

To use the pulsed UV system effectively, you need to understand the following basic concepts.

- Pulsed operation.
- Discharge voltage, energy and UV irradiance or dose.
- Spectrum emitted by the flash lamp.

Pulsed operation

The pulsed UV system is developed by combining the pulse power and flash lamp technology. It differs from the traditional continuous UV in that a much higher intensity of UV illumination can be produced within a short time. Figure 3 below shows the block diagram of the pulsed UV system.

The pulsed operation of this system enables the release of electrical energy stored in the capacitor into the flash lamp within a short time and produces the high current and high peak power required for emitting the intense UV flash.

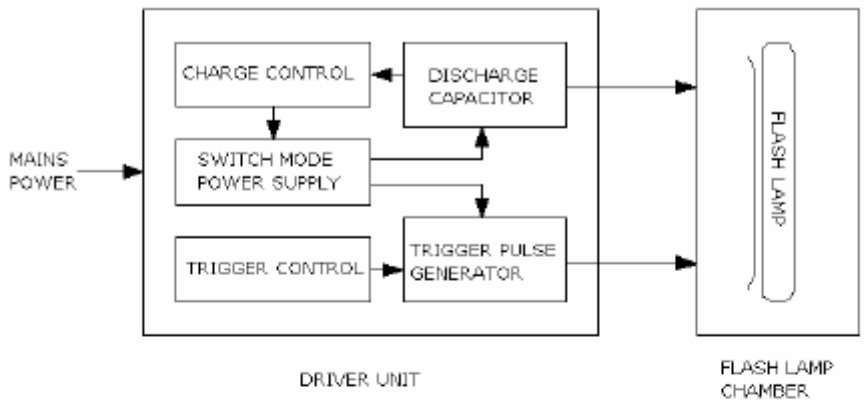


Figure 3. Block diagram of the pulsed UV system.

When the system operates, the flash lamp is kept at the preset D.C discharge voltage across its anode and cathode by the charge/ discharge **Voltage control** circuit, until a trigger voltage is generated by the **trigger circuit**. The high pulse trigger voltage will ignite the flash lamp and release the stored energy into the flash lamp to produce the pulsed UV illumination. The waveforms related to the pulse operation are shown in Figure 4.

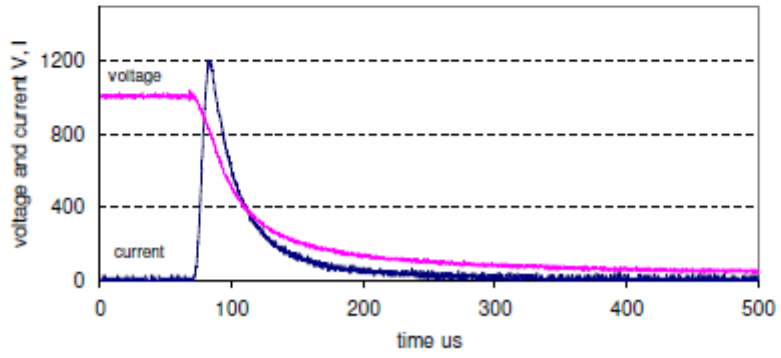


Figure 4. Waveforms of the lamp current and discharge voltage

The pulse operating parameters of the flash lamp are listed in Table 3, including the peak current, peak power, peak admittance and current rise/fall time at different discharge voltage from 500 to 1000V.

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

Table 3. **Discharge parameters of the flash lamp**

Discharge voltage, energy, UV irradiance and dose

The UV irradiance or the dose at a distance from the flash lamp is variable with the discharge voltage. The energy input into the flash lamp per pulse is also dependent on the discharge voltage. The relation between the discharge voltage and the energy per pulse is shown in Formula 1:

$$E = 0.5\ CV^2 \qquad C = 40\ \mu F \qquad (1)$$

With the discharge voltage varying from 400V to 1000V, the energy per pulse will increase from 3.2 joule to 20 joule. As the dose (mJ/cm^2) is the product of irradiance (mW/cm^2) and the integration time (s), the required dose can be realized by adjusting the discharge voltage or by applying a different number of pulses.

Spectrum emitted by the flash lamp

The emitted spectrum from the flash lamp is shown in Figure 5. The recorded spectrum at 600, 800 and 1000V exhibit the similar profile, which has a rich output in the UVC region from 220nm to 280nm. Three peak outputs are emitted at 229nm, 247nm and 260nm separately.

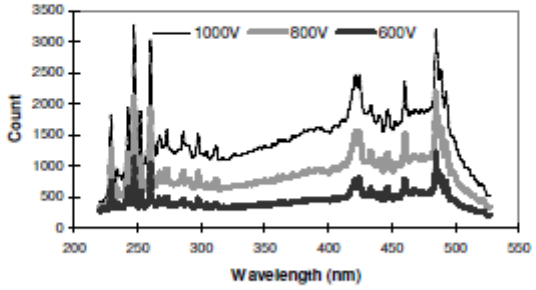


Figure 5. The emitted spectrum of the system at different discharge voltages

By examining the optical output at different bandwidth, it was seen that visible light and IR are also emitted from this light source, as shown in Table 4. The system operates at 800 V.

The dose measurement of less than 300nm revealed that the distance distribution in this wavelength region follows the inverse square law when the distance is further than 15cm from the flash lamp. By varying the distance, the UV dose received by the tested sample can be changed.

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

Table 4. Spectrum output at different bandwidth region with the distance from the flash lamp, $\mu J/cm^2$

9. WARRANTY

This product is warranted to be free of manufacturing defects for one year.

Should any manufacturing defects arise, please contact Samtech Ltd, or their representatives, to arrange a return number (RN) from the company. The customer, quoting the RN, should return the product to Samtech Ltd. Should a manufacturing defect be identified, this will be rectified and the product returned to the customer.

This warranty does not cover misuse, accidental damage or any fault that has arisen through incorrect operation. Also excluded from this warranty is degradation in product performance due to normal operation (aging of the flashlamps etc).

Appendix B
Wastewater Characterisation Analysis

Table B.1 outlines all of the data collected for heterotrophic bacteria, faecal indicator and pathogenic bacterial analysis in all dairy wastewater samples.

Table B1. Complete data set of all experimental analysis of water&wastewater streams at three Irish dairy plants.

Site	Day	Sample Type	HPC - abundance (CFU/100mL)		Total coliforms (MPN/100mL)	E. coli (MPN/100mL)	Salmonella detection (100mLs)	Listeria monocytogenes detection & enumeration (cfu/100mL)	Campylobacter spp detection (100mL)	S. aureus (cfu/100mL)	B. cereus (cfu/100mL)
			37°C	22°C							
1	1	Process water pre-treatment	Inconclusive	8.35E+05	1.87E+02	3.10E+00	*ND	Detected	ND	4.40E+03	4.48E+03
		WWTP influent	Inconclusive	7.30E+09	4.61E+06	1.85E+04	ND	Detected	ND	4.32E+03	5.04E+03
		WWTP effluent	Inconclusive	2.65E+08	4.28E+05	8.66E+02	ND	Detected	ND	4.08E+03	5.26E+03
	2	Process water	2.85E+05	6.20E+04	3.26E+02	3.00E+00	*N/A	<1 cfu/mL	N/A	1.63E+03	1.04E+03
		WWTP influent	3.75E+09	4.80E+09	1.50E+06	1.15E+04	N/A	<1 cfu/mL	N/A	1.63E+03	9.60E+02
		WWTP effluent	1.41E+09	4.20E+08	2.42E+05	1.73E+03	N/A	<1 cfu/mL	N/A	1.85E+03	1.07E+03
	3	Process water	5.00E+03	4.00E+03	6.49E+02	4.22E+01	ND	8.40E+03	ND	<1	9.80E+02
		WWTP influent	5.70E+09	4.60E+09	3.89E+05	4.48E+03	ND	7.90E+03	ND	<1	9.40E+02
		WWTP effluent	7.00E+07	9.10E+07	3.45E+04	1.07E+03	ND	6.20E+03	ND	<1	9.23E+02
2	1	WWTP influent	8.10E+07	7.80E+07	8.66E+04	1.46E+01	ND	Detected	ND	1.46E+03	1.99E+03
		WWTP effluent	2.02E+07	3.20E+07	5.17E+06	2.75E+01	ND	Detected	Detected	1.25E+03	1.67E+03
		Condensate	0.00E+00	1.40E+04	0.00E+00	0.00E+00	ND	Detected	ND	1.10E+03	1.84E+03
		Cooling water	5.30E+06	4.20E+06	1.02E+04	5.48E+02	ND	Detected	Detected	1.16E+03	1.96E+03
	2	WWTP influent	6.30E+08	6.80E+08	4.11E+06	1.11E+04	ND	3.60E+02	ND	<1	1.05E+03
		WWTP effluent	5.50E+05	2.50E+05	5.56E+03	1.83E+01	ND	6.40E+02	ND	<1	1.06E+03
		Condensate	0.00E+00	0.00E+00	0.00E+00	0.00E+00	ND	<1	ND	<1	1.05E+03
		Cooling water	7.60E+06	8.40E+06	1.31E+04	2.42E+03	ND	1.10E+02	ND	<1	9.60E+02

3	1	Cheese process effluent	2.03E+09	4.20E+09	2.42E+08	5.83E+01	ND	ND	ND	<1	1.08E+03
		Mixed process effluent excl. whey	2.00E+08	1.40E+08	1.55E+05	2.42E+03	ND	ND	ND	<1	1.06E+03
		Whey process effluent	3.32E+08	2.85E+08	7.80E+03	5.37E+03	ND	ND	ND	<1	1.05E+03
		Condensate	3.40E+06	3.30E+05	0.00E+00	0.00E+00	ND	ND	ND	<1	9.67E+02
		WWTP effluent	7.00E+05	2.80E+06	6.30E+04	2.28E+02	ND	ND	ND	<1	1.02E+03
	2	Cheese process effluent	2.41E+09	3.00E+09	4.48E+07	3.10E+04	ND	ND	ND	<1	1.04E+03
		Mixed process effluent excl. whey	2.00E+08	4.80E+08	9.32E+05	1.78E+02	ND	Detected	ND	<1	1.01E+03
		Whey process effluent	1.07E+07	9.10E+07	4.10E+02	3.10E+02	ND	ND	ND	<1	1.06E+03
		Condensate	3.36E+07	2.92E+07	1.05E+03	0.00E+00	ND	ND	ND	<1	9.84E+02
		WWTP effluent	7.40E+06	9.70E+06	6.13E+04	2.61E+02	ND	ND	ND	<1	1.06E+03

*ND – Not detected *N/A – test not performed

Appendix C
Thermopile Power Detector - Operational Settings Analysis

The thermopile power detector and Integra software (Model: XLP12-3S-H2-IN, Gentec-EO, Quebec, Canada) was used to measure the PUV broadspectrum dose, UV irradiance and UV-C irradiance from the PUV system. A variety of measurement ranges were available to use on the software depending the strength of the applied energy in question (Table C.1).

Table C.1. Power/irradiance settings available on the thermopile power detector and Integra software (Gentec-EO, Quebec, Canada).

Power/irradiance measurement ranges available	
300	µW
1	mW
3	mW
10	mW
30	**mW**
100	mW
300	mW
1	W
3	**W**

Energy measurements from the PUV system could be read at any desired measurement range providing the reading was above/close to the range chosen. For example, at an energy

measurement of 13.33 mW/cm^2, a setting of 30 mW or above could be used but not 10 mW or below as the reading was above the threshold setting of the detector. An 'Out' message would be given by the software in this case. Figure C.1 illustrates the irradiance measurements for a range of PUV system settings where the values were measured at both low range (either 10 mW or 30 mW depending on the output energy of the system) or high range (3 W). The results show a difference of approximately 2 mW/cm^2 for the same energy output between the low and high range settings at each of the lamp distances. In the case of the energy measured at a lamp distance of 30 cm, no value was given for the high range setting as it is assumed that the sensitivity was too low to read the low energy value at this height. In comparison, the energy at 30 cm could be read when a low range setting was used as it appeared more sensitive to the energy at this height to give a reading. For this reason, it was decided to use the low range settings for all of the PUV measurement data to ensure increased accuracy among energy readings.

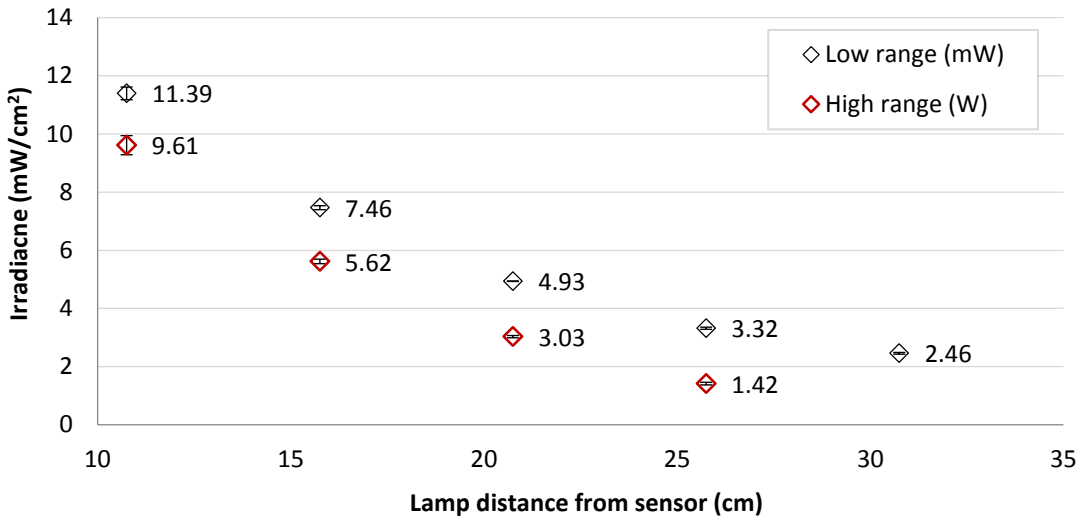


Figure C.1. Comparison of low and high range settings for irradiance measurements at various distances from the PUV.