Journal of Water Process Engineering 41 (2021) 102070

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Bacterial inactivation, photoreactivation and dark repair post flow-through pulsed UV disinfection

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ARTICLE INFO

Keywords:
Ultraviolet applications
Bacterial disinfection
Wastewater treatment
Cell injury
Pulsed UV
Disease mitigation

ABSTRACT

Pulsed UV (PUV) technology is accepted commercially for disinfection within the food packaging industry, but has yet to be deployed by the water/wastewater sector. This is partly due to a lack of robust, independently validated data for submerged or flow-through treatment applications. This study evaluated the efficacy of PUV for water disinfection under flow-through conditions. Bacterial pathogens of interest in the food and water/wastewater sector, namely *Escherichia coli*, *Staphylococcus aureus* and *Listeria innocua* (surrogate for *L. monocytogenes*) were used to investigate the potential for photoreactivation and/or dark repair post PUV flow-through disinfection. A continuous-flow low-pressure UV was also analysed under similar experimental conditions. Bacterial inactivation via flow-through PUV was dependant on energy output with *E. coli* exhibiting greatest sensitivity to PUV treatment (5.3 log $_{10}$ inactivation after treatment at 1539 mJ/cm ^{2} - output in UV range $<$ 300 nm); *L. innocua* exhibited the highest PUV resistance (3.0 log $_{10}$ inactivation after treatment at 1539 mJ/cm ^{2} – output in UV range $<$ 300 nm) under similar treatment conditions. Greater photoreactivation occurred at lower PUV outputs for both *S. aureus* and *E. coli* after flow-through PUV treatment. Thus exposure of treated bacteria to natural light, immediately post flow-through PUV treatment, should be avoided to minimise photoreactivation. The LPUV demonstrated inactivation of all bacteria below the limit of detection (1 CFU/mL) and inhibited the occurrence of photoreactivation. This study highlights the importance of considering bacterial repair potential and the need for further development of PUV technology for such applications.

1. Introduction

Ultraviolet (UV) disinfection is a well-established technology across a variety of different sectors including aquaculture, ballast water treatment, municipal wastewater treatment, drinking water treatment, agriculture, dairy and the beverage industry [1–3]. UV disinfection is typically seen as being user-friendly, free from toxic/hazardous chemicals, effective against chlorine-resistant microorganisms and exhibiting shorter contact times in comparison to chlorine treatment [4,5]. Low pressure UV (LPUV) and medium pressure UV (MPUV) are currently the de facto UV disinfection systems used for water/wastewater disinfection applications. Typically, UV light is generated within the lamps when a voltage is applied across a mercury gas mixture which results in the discharge of photons. The type of UV light produced is dependent upon the mercury vapour pressure; LPUV lamps produce monochromatic light at 253.7 nm under low vapour pressure while MPUV lamps produce a polychromatic light due to higher vapour pressures [6]. Pulsed UV (PUV) disinfection is a relatively new UV technology which differs to mercury vapour-based LPUV/MPUV light by utilising xenon gas to generate a high energy electron pulse, which typically lasts microseconds. PUV systems generally comprise three parts; the power supply, the pulse configuration system and the flash lamp [7]. An alternating current is stored in a capacitor where energy is discharged to create an intense pulse of light which spans across the polychromatic broad-spectrum of UV, visible and infrared light (200−1000 nm) [8]. The high peak power stored in the capacitor is a trademark of this system, which has been shown in some cases to offer shorter treatment times [9,10]. In addition, the power emitted from pulsed UV lamps is generally at least

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https://doi.org/10.1016/j.jwpe.2021.102070
Received 18 November 2020; Received in revised form 19 March 2021; Accepted 5 April 2021
Available online 19 April 2021

an order of magnitude higher than the power emitted from LPUV lamps; this is reflected in the respective energy outputs of both systems for microbial inactivation [11,12].

The application of PUV light as a microbial disinfection method within the food industry has been approved by the United States Food & Drugs Authority (FDA) under code 21CFR179.41 [7,13]. PUV disinfection is considered a favourable alternative to conventional thermal/-chemical disinfection processes owing to shorter treatment times 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 [14]. 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. Of those studies which have evaluated the disinfection potential of the system using a continuous 'flow-through' experimental set-up, authors reported log inactivation in terms of flow rates and 'number of passes' through the system; no UV doses were reported. Krishnamurthy et al. [15] investigated the PUV inactivation efficiency of *Staphylococcus aureus* via flow-through milk treatment. Results from this study reported bacterial inactivation rates of between 0.55 and 7.26 $\log_{10}$ CFU/mL which were dependent on the distance of the lamp from the sample, the flow rate and the 'number of passes' i.e. the amount of times the medium was recycled through the system. Complete inactivation of *S. aureus* (7.23$\log_{10}$) was achieved at a lamp distance of 8 cm and a flow rate of 20 mL/min for a 'one-pass' treatment; no UV doses were reported. Uslu et al. [16] evaluated the potential of flow-through PUV for the disinfection of *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) in wastewater. In this case, complete inactivation of *E. coli* (7.23 $\log_{10}$) and *B. subtilis* (7.13 $\log_{10}$) was achieved at a flow rate of 2 L/min for a 'one-pass' treatment in a synthetic wastewater mix. The variation in the sample media used creates difficulty in comparing both studies as UV absorption rates may vary depending on the medium [17]. Further detail on UV dose would also be necessary for comparison, this is an issue which limits wider comparisons within the literature in general. In addition, comparisons were not drawn between PUV flow-through treatment and LPUV disinfection.

While UV treatment is an effective disinfection method which produces no disinfection by-products (typically associated with chemical disinfection), a primary drawback includes that of microbial DNA damage repair post UV disinfection. UV-induced molecular lesions may be repaired or replaced by the microorganism either by (i) using enzymes that require light to repair DNA – i.e. photoreactivation (PHR) or (ii) employing enzymes that replace damaged DNA with undamaged nucleotides; excision or dark repair [18–20]. In the water/wastewater disinfection sector, PHR is of primary concern when incorporating UV systems into disinfection processes as microbial re-growth following exposure to sunlight can influence system effectiveness [21]. Literature investigating the photoreactivation potential of various pathogens in wastewater effluent post LPUV treatment has established the relationship between increasing UV dose and decreasing microbial photoreactivation rates [22–25]. However, in the case of bacterial PHR/dark repair post pulsed UV disinfection, study findings are mixed. Lee et al. [26] reported no PHR or dark repair activity of *E.coli* in distilled water mixed with humic acids after PUV treatment at 9 mJ/cm^2 (dose measured as the portion of UV energy within 200–400 nm range of the broadspectrum lamp), while MacLean et al. [27] confirmed photoreactivation of *S. aureus* after a PUV exposure (320–500 nm wavelength range) of 1500 mJ/cm^2 and also surmised a lower UV output/dose during treatment would most likely result in increased bacterial photoreactivation rates. Kramer et al. [28] investigated the PHR potential of *E. coli* and *L. innocua* after pulsed light exposure on a polysaccharide surface and reported photoreactivation for both test bacteria with relative recovery being PUV energy dependant. In all cases, pulsed UV disinfection analysis took place under static or 'batch' experimental set-up with fixed sample treatment. Indeed, the vast majority of PHR/dark repair studies in water typically involve prior UV disinfection carried out via batch analysis [21,24,29–32]. Limited UV disinfection studies have evaluated the potential of bacterial reactivation after flow-through UV disinfection for liquid treatments and to the best knowledge of the authors, all were performed with conventional UV systems.

In this study the efficiency of UV inactivation for *E. coli*, *S. aureus* and *L. innocua* (pathogens relevant in the food and water/wastewater sectors) was studied using LPUV and PUV systems in single-pass flow-through configuration. This also constitutes the first study to comprehensively compare bacterial photoreactivation and/or dark repair immediately following flow-through pulsed UV treatment. This study would be of general interest to the water/wastewater sector with a particular focus on the implementation of strategies of wastewater reuse.

2. Materials and methods

2.1. LPUV and PUV systems

The PUV system comprised a bench–scale pulsed power source (PUV-01, Samtech Ltd., Glasgow) which was used to power a low pressure (60 kPa) xenon-filled flashlamp (Heraeus Noblelight XAP type NL4006 series constructed from a clear UV transparent quartz tube) and produced a high intensity beam of polychromatic pulsed light (200−1100 nm). The lamp was placed 10.75 cm above a sterilised aluminium flow-through vessel (with a plan surface area of 290 cm^2, sample depth of 5.5 cm and hold-up volume of 750 mL) through which water was pumped with a peristaltic pump at specified flow rates (Fig. 1 (a)). The system was enclosed and a smooth aluminium cover was used on the inside of the lamp cover to reflect UV light onto the sample. The PUV system allowed for the input voltage and the pulse rate to be varied between 400 and 1000 V and for a pulse frequency of between 0.1 and 10 pulses per second (PPS). The energy output of the lamp (total energy and energy in specific wavelength ranges) was calculated by analysing lamp characteristics as supplied and verified by testing (by the manufacturer), the area of the vessel, the pulse frequency and the hydraulic residence time - HRT (Table 1) – see supplementary information for system details (Figure S1 and Table S1). The range of energy outputs chosen for this study were selected based on previous PUV system analysis outlined in Fitzhenry et al. [33] and Fitzhenry [34]. These outputs would approximate low, medium and high energy output capabilities from this lamp. Based on the characteristic curves for the PUV lamp, the expected energy outputs could be calculated to include only the wavelengths below 300 nm (i.e. comprises UV C and some of the UV B spectrum). This was done so provide context for in-situ measurements taken at $<$ 280 nm (described in Section 2.3).

The continuous-flow monochromatic LPUV system deployed in this study (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, an internal empty volume of 2.5 L and chamber dimensions of 95 cm length x6 cm diameter (Fig. 1 (b)). The lamp dimensions were 84.3 cm in length x1.6 cm diameter. The lamp irradiation was 30 mJ/cm^2 (at 1 s residence time). The UV energy output could be altered by varying the influent flow rate thereby altering the HRT and thus the exposure time. The flow rate used in the experiments was the maximum allowable (24.6 L/min) which corresponded to a HRT of 0.47 s. This equates to samples being exposed a UV output of 14 mJ/cm^2 (0.47 s x 30 mJ/cm^2 - this was the lowest exposure energy output achievable and resulted in almost full inactivation for all pathogens) (Table 1).

2.2. Experimental setup

Table 1 summarises the experiments carried out with the LPUV and PUV systems in this study. In the case of the LPUV system, only one energy output was analysed (14 mJ/cm^2). For the flow-through PUV system, four different UV outputs were evaluated ranging from 486 mJ/

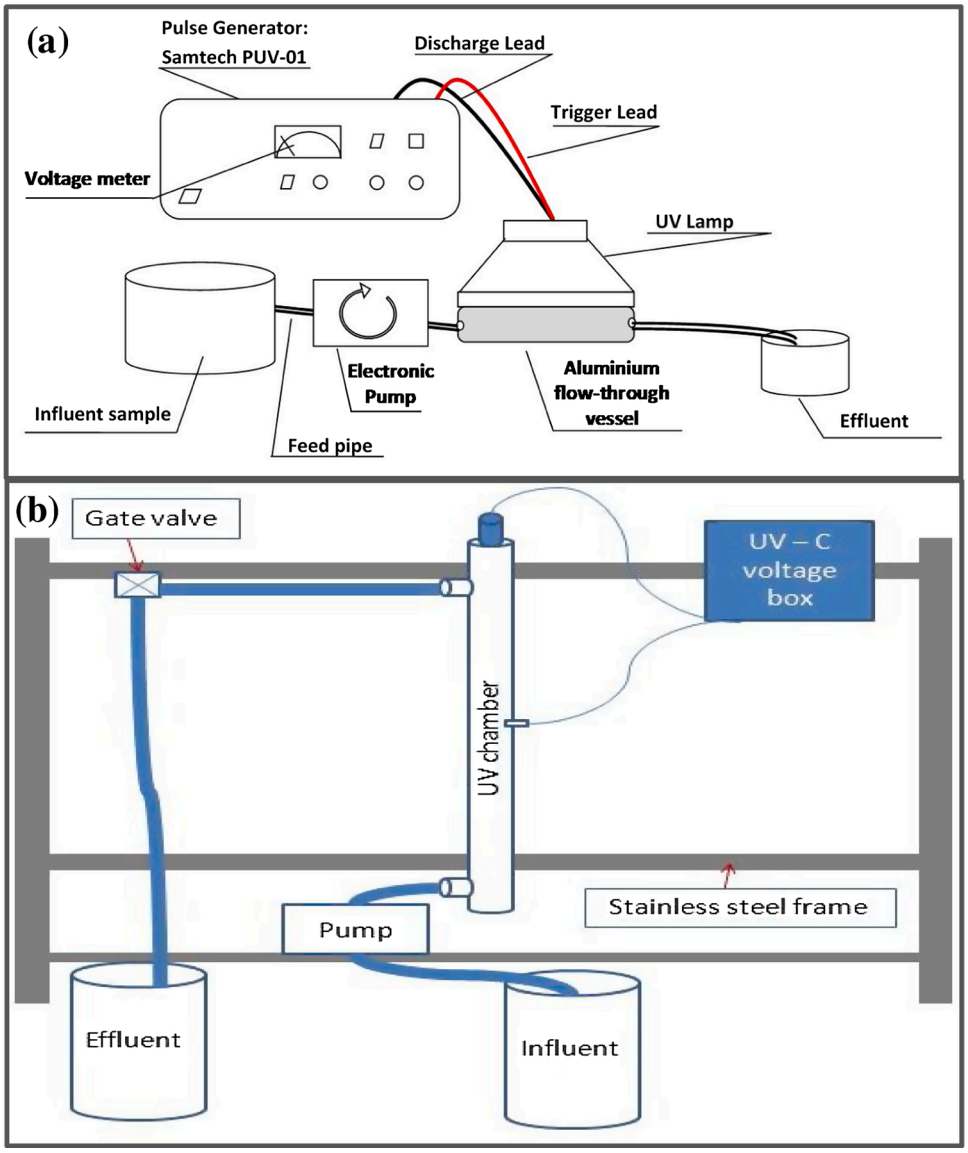

Fig. 1. Schematic of bench-top experimental set-up of (a) PUV system and (b) LPUV system.

cm^2 to 2052 mJ/cm^2.

2.3. UV dose determination of PUV system

The 'UV dose' refers to the UV energy received by the sample (i.e. the UV exposure of the sample). The UV output is energy emitted from the lamp in the UV spectrum. Previous studies have shown the distance of the lamp from the sample to have an impact upon PUV fluence distribution and measurements [35]. Therefore, it was decided to measure the UV dose at the distances used in this study to allow for comparison to the calculated UV output. The UV and UV-C dose received by the sample from the PUV system was analysed using a thermopile power detector (Model: XLP12−3S-H2-IN, Gentec-EO, Quebec, Canada) and Integra software in addition to two longpass colour glass filters; FGL 400 (which filters out wavelengths above 400 nm to give the UV range (200−400 nm)) and FGL 280 (which filters out wavelengths above 280 nm to give the UV-C range (200–280 nm)) (Thorlabs GmbH, Dachau, Germany). The percentage transmission of both filters was as follows; for the 400 nm filter transmission at wavelengths greater than 400 nm was 89 % +/- 4% and for the 280 nm filter transmission for wavelengths greater than 280 nm was 91 % +/- 6% (Thorlabs GmbH, Dachau, Germany). The detector (12 mm aperture) was situated at the bottom of a metal cylinder 5 cm in depth through which the light was directed downwards towards the cylindrical detector surface. The detector was placed on the aluminium vessel, with the sensor being 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 broadspectrum energy readings in total after which an average value was obtained. Power was measured in irradiance units (mW/cm^2). The filters were then 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 were used to calculate the power emitted from the lamp within the UV and the UV-C range. The calculated UV output and equivalent measured UV and UV-C dose are listed in Table 2. At a distance of 10.75 cm there is a significant difference between the output of the lamp and the dose measured (as approximated by the inverse square law). Other factors contributing to the relatively low UV (and UV-C) dose relative to the lamp output is the broadspectrum nature of the output and heat generated within the PUV system. This has been previously noted in Fitzhenry et al., (2019) (33). It should be noted that the filters were removed for the disinfection studies and were only used to determine UV dose.

Table 1
Characteristics of the LPUV and PUV flow-through experimental runs performed with the three bacterial strains.

UV system	Voltage (V)	Pulse frequency (PPS)	HRT (s)	Flow rate (L/min)	UV output (mJ/cm^2)	Bacterial strains tested
LPUV	N/A	N/A	0.47	24.60	14.0[1]	*E. coli*, *S. aureus* and *L. innocua*
PUV	800	0.5	120	0.38	486.0[2]	*E. coli*, *S. aureus* and *L. innocua*
	900	1.0	100	0.45	1026.1[2]	*E. coli*, *S. aureus* and *L. innocua*
	900	2.0	75	0.60	1539.2[2]	*E. coli*, *S. aureus* and *L. innocua*
	900	2.0	100	0.45	2052.0[2]	*S. aureus* and *L. innocua*

N/A: Not Applicable.
[1] Output at 254 nm.
[2] Output at < 300 nm.

Table 2
Calculated broadspectrum UV output and equivalent measured UV and UV-C dose for PUV system.

Pulse frequency (PPS)	Residence time (s)	Voltage	Calculated UV Output (from lamp characteristics) $E_{A<300\ nm}$ (mJ/cm^2)	Average measured UV Dose (10.75 cm from lamp)* $E_{A<400\ nm}$ (mJ/cm^2)	Average measured UV-C Dose (10.75 cm from lamp)* $E_{A<280\ nm}$ (mJ/cm^2)
1	60	800	486	42	16
1	100	900	1026	112	18
2	75	900	1539	168	26
2	100	900	2052	269	35

E_A – energy per unit surface area·
* n = 9 for each of these measurements with a standard deviation of less than 2% of the average in each case·

2.4. Bacterial analysis via UV treatment

The bacterial strains used to investigate inactivation, photoreactivation and dark repair post LPUV and PUV disinfection were *Escherichia coli* ATCC 25922, *Staphylococcus aureus* DSM 1104 and *Listeria innocua* DSM 20649. These were chosen as they had been recently identified as pathogens of interest in wastewater streams from dairy processing facilities in Ireland [42]. The strain DSM 20649 was used as a non-pathogenic surrogate for *Listeria monocytogenes* [36]. All three freeze-dried cultures were reconstituted using tryptic soy agar (TSA, Sigma-aldrich, Wicklow, Ireland) and tryptic soy broth (TSB, Fisher Scientific, Dublin, Ireland) at 37 °C 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 PHR/dark repair analysis. 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 TSB (Fisher Scientific, Dublin, Ireland) for *L. innocua* and *S. aureus*. The broth(s) were cultured on a rotary shaker at 90 rpm for 24 h at 37 °C. For LPUV runs, batches of tap water (20 L) were spiked with 10 mL of broth strains to give a starting bacterial concentration of 6 $\log_{10} \pm 0.5$, while for PUV runs 2.5 L of distillate water was spiked with 1 mL of broth strains to give a starting bacterial concentration of 6 $\log_{10} \pm 0.5$). 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 [43] 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. The limit of detection was 1 CFU/mL, see Section 2.6 for log inactivation calculations.

2.5. Photoreactivation and dark repair analysis

Immediately following UV treatment, effluent samples were placed under light and dark conditions to study potential bacterial PHR/dark repair. For photoreactivation experiments, duplicate sample aliquots (40 mL) were placed into open petri dishes (diameter of 90 mm, surface area of 58 cm^2) at a distance of 9 cm from two compact fluorescent lamps (23 W power, luminous flux (Lm) 1450) which emitted light in the 300–700 nm spectral range (OSRAM model DPRO MITW 23 W/840 E27). Previous studies have reported the photolyase enzyme for bacterial PHR to respond significantly to 'blue light' in the 360–500 nm range [27]. 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 [43] using TBX agar (VWR, Dublin, Ireland) for *E. coli* and TSA (Sigma-aldrich, Wicklow, Ireland) for *S. aureus*.

2.6. Quantitative analysis

Bacterial inactivation via UV treatment using logarithmic scale and colony forming unit (CFU) /mL was determined using Eq. (1), where N_0 and N are the concentrations (CFU/mL) pre and post disinfection respectively.

$$\text{Inactivation(UV)} = log_{10}(N_0/N) \quad (1)$$

Bacterial photoreactivation and dark repair expressed as a percentage was determined with Eq. (2) adapted from methods used in Maclean et al. [27] and Shafaei et al. [25] where N_t is the concentration at time "t" after the start of the photoreactivation/dark repair experiment (light or dark). Note that at time zero N_t is equal to N.

$$\text{Repair}(\%) = \frac{N_t - N}{N_0 - N} \times 100 \quad (2)$$

3. Results & discussion

3.1. Bacterial inactivation via continuous-flow LPUV and flow-through PUV disinfection

Results for the LPUV system showed almost complete inactivation of all three bacterial strains at the UVC output of 14 mJ/cm^2 (Fig. 2). Log_{10} inactivations of 5.3 ± 0.3, 6.0 ± 0.2 and 5.9 ± 0.1 were observed from starting bacterial populations of 6.0 log_{10} ±0.5 for *S. aureus*, *E. coli* and *L. innocua* respectively at 14 mJ/cm^2. Therefore, given the LPUV system was operating at the shortest HRT (and thus the lowest UV output achievable) – no other HRTs were subsequently investigated. The inactivation of vegetative bacteria in water via LPUV is well established. 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 [4,37]. Thus, results in this study are in line with findings in the literature which illustrate vegetative cells are readily inactivated by LPUV disinfection at relatively low UV energies.

Research investigating bacterial inactivation in water/wastewater via flow-through PUV systems is limited. In this study, bacterial inactivation via flow-through PUV disinfection was linearly dependent on energy, with *E. coli* being the most sensitive and *L. innocua* exhibiting the most PUV resistance (Fig. 3).

The maximum inactivation observed for *E. coli* (5.3 ± 0.3 log_{10}) was achieved at a lamp UV output (< 300 nm) of 1539 mJ/cm^2 while a higher UV output (< 300 nm) 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. This finding is consistent with Farrell [38] who reported Gram-positive bacteria (*S. aureus* and *L. monocytogenes*) to be more resistant to PUV light in comparison to Gram-negative bacteria (*E. coli*) in a static experimental set-up. Uslu et al. [17] 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. In the aforementioned study, the UV dose/system output was not described rather the flow rate and broadspectrum energy output (J) per litre treated was given. Results showed complete inactivation of *E. coli* in synthetic wastewater at flow rates of 10 L/min (equivalent to a broadband energy of 39.7 J/L) for a two-pass treatment. A similar study [39] investigated the inactivation of *L. innocua* via flow-through PUV for water treatment. A PUV dose of 4,000 mJ/cm^2 (at a flow rate of 1 L/min) was required for 2 log_{10} *L. innocua* inactivation in water. Krishnamurthy et al. [16] 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. [17]. Log_{10} reductions were reported as a 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

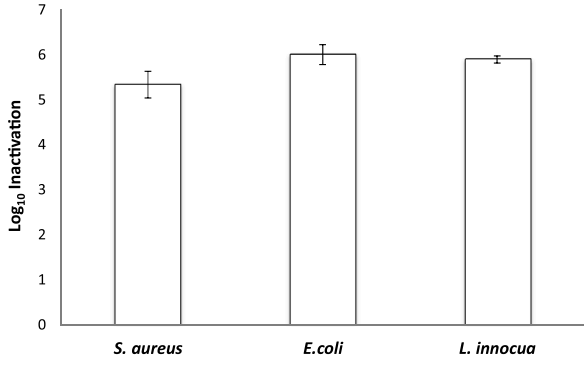

Fig. 2. Log_{10} inactivation of *S. aureus, E. coli* and *L. innocua* via continuous-flow LPUV disinfection at a UV output of 14 mJ/cm^2. Standard error bars shown (n = 3).

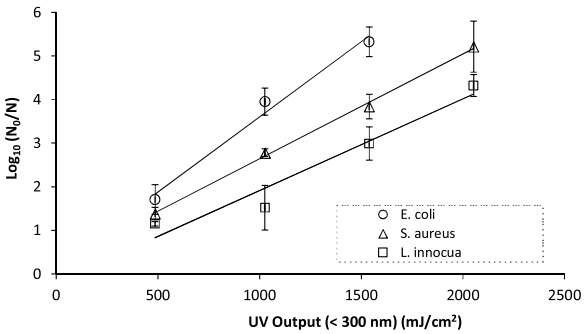

Fig. 3. Bacterial inactivation via flow-through PUV disinfection at various UV outputs. Data points are average values of three independent runs and error bars represent the standard deviation.

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 [16]. Finally, Fitzhenry et al. [33] recently reported a UV output (< 300 nm) of approximately 2,000 mJ/cm^2 was required to inactivate *Bacillus spp*. endospores by approx. 2 log_{10} in water via flow-through PUV disinfection.

These studies show that with flow-through pulsed UV technology (as investigated to date) the use of multi-pass is necessary for the inactivation of S. aureus and E. coli. In the case of L. innocua a single pass may be possible, however, to-date, clear information on the necessary UV dose has not been available. This study presents inactivation rates for various UV outputs for the PUV technology. This data is key as the overall capacity of the lamp in terms of UV energy output will be a key determining factor (alongside the desired pathogen removal rates) as to whether single pass or multi-pass should be deployed.

3.2. Bacterial photoreactivation & dark repair post UV treatment

Experimental results for the potential photoreactivation of the three pathogens following LPUV disinfection at 14 mJ/cm^2 indicated that bacterial concentrations after light and dark repair analysis were negligible (see Supporting Information, Figure S2). Previous research [24] has 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 h before photoreactivation was observed. While photoreactivation of *E. coli* was reported after LPUV treatment at 5 mJ/cm^2, the same study [24] reported a lack of photoreactivation when the bacteria were exposed to a higher UV dose of 15 mJ/cm^2. Sanz et al. [23] reported a similar conclusion when investigating the photorepair potential of total coliforms post LPUV treatment in a wastewater treatment facility. Their 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. 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. Unfortunately, limitations regarding decreasing the system energy output of the LPUV system did not allow for analysis of bacterial PHR/dark repair post lower LPUV outputs. Nonetheless, considering the high inactivation efficiencies found for the three experimental bacterial strains and the lack of photoreactivation/dark repair at the minimum UV output possible (14 mJ/cm^2), and at a relatively high flow rate (24.6 L/min equating to 0.47 s HRT), the results further highlight the suitability of the LPUV system as an effective technology to inactivate S. aureus, E. coli and L. innocua

The particular strain of *L. innocua* (DSM 20649) selected for this study appeared to be impacted by the photoreactivation experimental conditions. Previous studies have discussed various light sources used in PHR experiments including 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 [27], therefore these factors were taken into consideration when deciding the optimum conditions for photoreactivation experiments. Fig. 4a shows the results of the photoreactivation and dark repair experiments for *L. innocua* post flow-through PUV treatments at PUV outputs (< 300 nm) of 486, 1026 and 2052 mJ/cm^2. While minimal dark repair was observed for *L. innocua*, populations of *L. innocua* survivors post PUV irradiation were observed to decrease in numbers during the period of photoreactivation conditions. Upon further investigation, it was confirmed that *L. innocua* was inactivated by the compound fluorescent lamp with no prior UV exposure (Fig. 4b). Thus, it was decided to eliminate it from the photoreactivation analysis. 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). The results confirmed that the photoreactivation experimental conditions were not impacting upon cell viability as was the case with *L. innocua* (Fig. 4(b)). A previous study reported photoreactivation of the same strain of *L. innocua* as was used in this study [28]. Kramer et al. [28] 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. These results showed photoreactivation rates of between 10^2 and 10^6 CFU/mL after 24 h of lamp exposure on tryptic soy agar at 37 °C 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 lower spectrum output of the lamp and the difference in experimental conditions (illumination on agar and not water as was the case in this study) in the Kramer et al. [28] study were more conducive to *L. innocua* photoreactivation analysis i.e. agar medium designed to enable bacterial growth as opposed to water medium. 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 360–380 nm. 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 experimental design and the particular bulbs used for PUV treatments may be noteworthy for future studies.

Photoreactivation was observed for both *E. coli* and *S. aureus* post flow-through PUV treatment at various energy outputs over time (Fig. 5 (a) and 5 (b)). Results for *S. aureus* at the lowest output of 486 mJ/cm^2 showed a sharp increase in PHR between 10 and 60 min after which inactivation were relatively stable. However, this was not constant and the % PHR increased slightly again after this time point. Increases in the

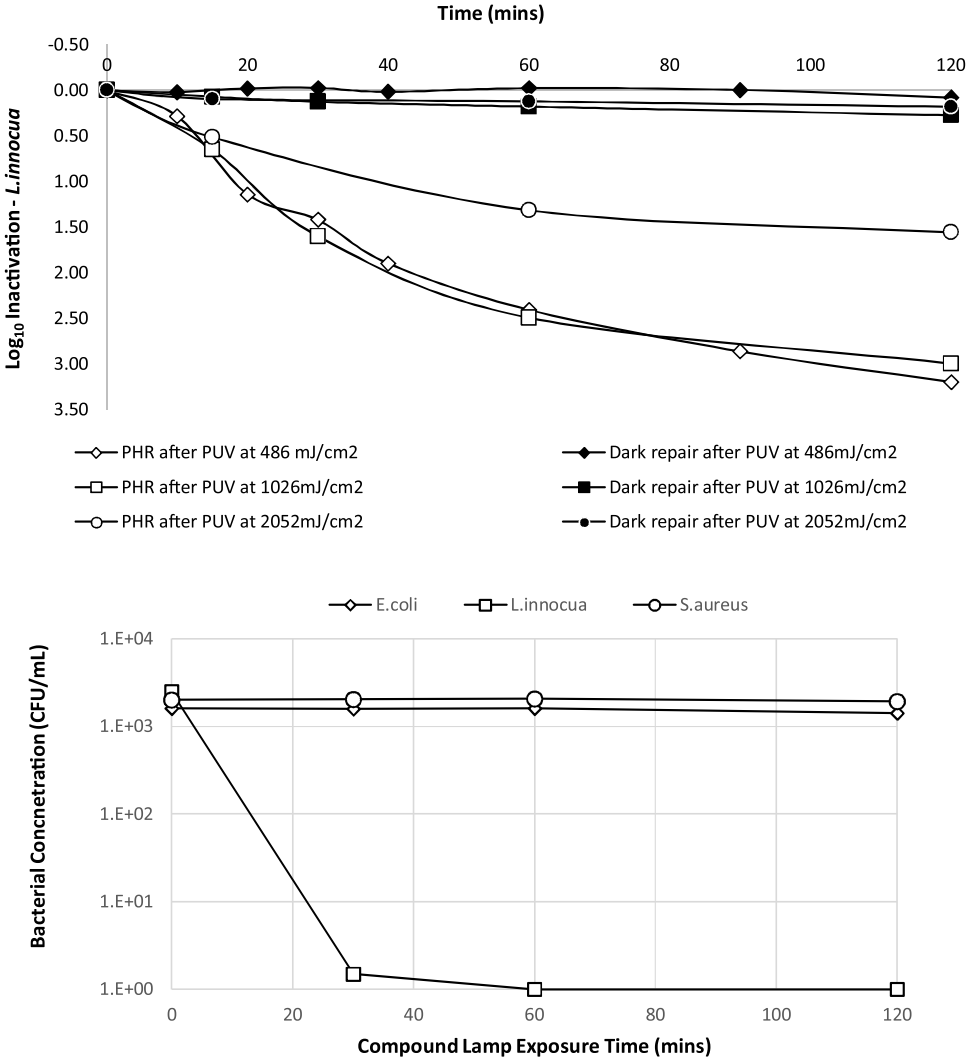

Fig. 4. (a). photoreactivation and dark repair post PUV disinfection at PUV outputs (< 300 nm) of 486, 1026, 2052 mJ/cm^2 and 4(b) inactivation of *L. innocua* under photoreactivation (PHR) lamp only, without any prior PUV treatment.

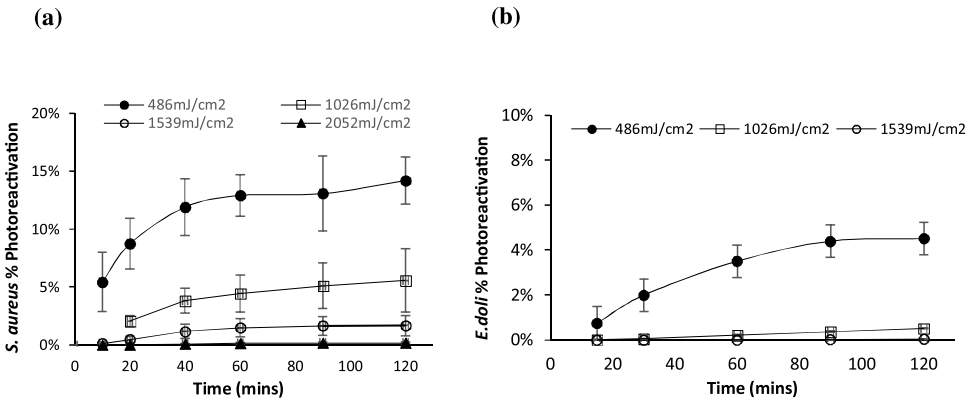

Fig. 5. Percentage photoreactivation over time of (a) *S. aureus* and (b) *E. coli* at various UV outputs (< 300 nm) following flow-through PUV treatment.

percentage photoreactivation of *S. aureus* were less pronounced at higher UV system outputs with negligible PHR occurring at any time point after PUV treatment at 2052 mJ/cm^2 (Fig. 5 (a)). Analysis of *E. coli* photoreactivation over time shows a slightly different trend to that of *S. aureus* post PUV treatment (Fig. 5 (b)). At 486 mJ/cm^2, the PHR of *E. coli* steadily increased between 15 and 90 min before a tailing effect was observed. At 1026 mJ/cm^2 the percentage of photoreactivation *E. coli* was relatively low, while at 1539 mJ/cm^2 it was found to be negligible. Therefore, it was decided to not investigate photoreactivation of *E. coli* at higher UV system outputs. Previous studies [21] have suggested that a time-based comparison for photoreactivation experiments cannot be performed due to variations in lamp intensities i.e. the energy outputs of lamps can vary even where they are the same model. 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. Photoreactivation has been observed previously [28] for *E. coli* post static PUV treatment on gel mediums with a recovery rate of up to 2 $\log_{10}$ following a PUV dose of 450–1000 mJ/cm^2. Maclean et al. [27] investigated the PHR potential of *S. aureus* and *L. monocytogenes* post PUV treatment under static experimental conditions and observed a photoreactivation response after PUV treatments of 1500 mJ/cm^2 and 3400 mJ/cm^2 (within 320–500 nm range) respectively. The study found approximately 2% of *S. aureus* bacteria photoreactivated after a PUV exposure of 1500 mJ/cm^2 (PHR exposure time not given). Similarly, results in the present study found 1.7 % of the *S. aureus* population photoreactivated after 1500 mJ/cm^2 PUV energy exposure at 90 min. However, in this case, a flow-through experimental set-up was applied. While Maclean et al. [27] did report a low degree of PHR for *S. aureus* at the PUV output energy applied, it was surmised that a less intense UV energy output would likely result in a higher degree of photoreactivation which was the finding in this study. The majority of photoreactivation occurred within the first hour for *S. aureus* and the first 90 min for *E. coli* thus avoiding bacterial exposure to light immediately after flow-through PUV disinfection may be important when attempting to maintain high inactivation rates for both *S. aureus* and *E. coli*. However, it should also be noted that this study did not investigate delayed exposure to light i.e. PHR after some dark incubation, thus caution may need to be exercised in this case also. Nonetheless, as is the case with LPUV disinfection, bacterial photoreactivation is reported to be avoidable when a high UV energy is applied [23,24].

The maximum pathogen photoreactivation and dark repair after 120 min following flow-through PUV treatment is shown in Fig. 6. As indicated in Fig. 5, *S. aureus* exhibited higher PHR rates in comparison to *E. coli*; this trend appeared consistent for dark repair analysis of *S. aureus* at 486 mJ/cm^2 albeit the percentage of reactivated bacteria was low. For example, in the case of *S. aureus*, 0.75 % of the surviving bacterial population (post PUV treatment at 486 mJ/cm^2) repaired under dark

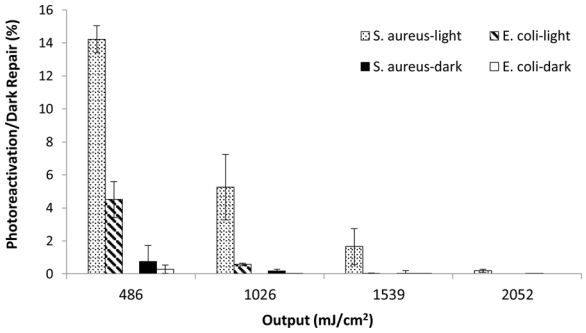

Fig. 6. Photoreactivation (light) and dark repair (dark) after 120 min exposure for *S. aureus* and *E. coli* post flow-through PUV treatment at various UV outputs.

experimental conditions in comparison to 0.29 % of *E.coli* at the same UV output. Aside from slight bacterial reactivation at the lowest PUV output, dark repair for both strains was found to be minimal after PUV flow-through treatments of 1026 mJ/cm^2 and above. Potential reasons for the lack of dark repair exhibited by bacteria in this study may be due the length of dark exposure time applied during the experimental analysis. Jungfer et al. [40] reported the activation of bacterial dark repair mechanisms (*rec*A mRNA protein) to be dependent on experimental incubation time which was found to 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 [40]. However, previous studies [23,41] have also reported bacterial dark repair to occur within approximately 100 min of incubation post UV disinfection after which a bacterial decay period was observed. Nebot Sanz et al. [23] confirmed dark repair analysis, carried out in conjunction with photoreactivation analysis, did occur but to a lower degree that in light repair conditions. Moreover, the authors [23] concluded maximum dark repair of bacteria occurred sooner in comparison to maximum bacterial photoreactivation. In this study, stable photoreactivation rates were achieved during the maximum experimental exposure time of 120 min, and for this reason dark repair experimental analysis was also carried out for the same duration. Some of the differences in findings between studies may be attributed to the variation in Gram positive and negative composition in terms of cell membrane(s) and amount of cell wall material present (peptidoglycan) (38).

It is possible that low UV outputs also puts less biocidal stress on the treated bacterial cells with potentially greater ability to repair as less physical, genomic and metabolic damage – lower doses may not cause irreversible damage.

4. Conclusions

This constitutes the first study to demonstrate that bacterial (S. aureus and E. coli) photoreactivation is possible post flow-through PUV disinfection and that the level of bacterial photoreactivation is dependent upon the output energy applied during prior UV disinfection treatment. Furthermore, it was shown that dark repair is less significant when compared to light repair. The results agree with similar studies in the literature involving static and continuous-flow UV disinfection studies whereby the UV dose/output applied during the UV disinfection phase influences the degree to which bacteria can repair. Should flow-through PUV systems be considered for full-scale operation as a disinfection system for water reuse/wastewater treatment, sufficient energy should be applied to avoid bacterial reactivation. The immediate exposure of bacteria to light post flow-through PUV treatment should also be avoided to minimise photoreactivation of both S. aureus and E. coli. The continuous-flow LPUV system used in this study successfully inactivated all three bacteria to a higher degree than the PUV system deployed (with significantly lower energy consumption). The LPUV was also successful in the inhibition of E. coli and S. aureus photoreactivation post LPUV treatment. The study confirmed the suitability of existing LPUV as a technology in achieving adequate inactivation percentages with limited potential for repair in both light and dark conditions. However, further work would be required in the development of PUV systems to enable them be efficiently and cost-effectively deployed in larger scale water/wastewater treatment scenarios. As with all disinfection technology the required doses for adequate removal of targeted pathogens will depend on the nature of the wastewater being treated.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The authors would like to acknowledge the Department of Agriculture, Food and the Marine of Ireland (Ref: 13-F-507) for funding this research. Dr. Val del Rio was supported by the Spanish Government (CTM2014-55397-JIN project co-funded by FEDER) and Xunta de Galicia postdoctoral fellowship.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jwpe.2021.102070.

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