HIGH IMPACT UVLED APPLICATIONS:

RECLAIMED WASTEWATER EFFLUENT IRRIGATION

BY

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ABSTRACT

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High Impact UVLED Applications: Reclaimed Wastewater Effluent Irrigation

Thesis directed by Professor Karl G. Linden

Concerns over water scarcity are turning global attention towards water reuse, the process of reclaiming wastewater for beneficial purposes like groundwater recharge, agriculture, and potable drinking water. UV disinfection is a common and effective treatment technology for drinking water, wastewater, and reclaimed water globally. Recent advancements in UV light emitting diodes (UVLEDs) have allowed for compact, electrically efficient, and customizable point-of-use UV disinfection options. This provides promising applications for UVLEDs to act as a disinfectant prior to and during water distribution.

UV inactivates cells through DNA damage, which some microorganisms can partially counteract or tolerate through repair mechanisms. The ability of microorganisms to repair and regrow after UVLED disinfection in distribution systems fed by recycled wastewater effluent is not well-studied. This research investigated factors that affect *P. aeruginosa* recovery following UVLED exposure and the significance for water reuse applications. More specifically, the effect of 265nm and 285nm UVLED exposure on *P. aeruginosa* recovery in both low and high nutrient waters was examined, and the contributions of repair and regrowth to total *P. aeruginosa* recovery were determined.

Enabling the effective reuse of wastewater effluents while control biofouling, or biofilm accumulation, is essential. Biofilms play a major role in disrupting the success of many water reclamation and reuse technologies. The higher concentration of nutrients, organic matter, and microorganisms in wastewater effluent can aggravate biofouling of water distribution systems, like drip irrigation lines. UVLED treatment of effluent could help to minimize biofilm formation during the distribution of wastewater effluent for reuse. This study applied a commercially available UVLED flow through device,

operating at 40 mJ/cm^2, to the inlet of a bioreactor fed by wastewater effluent. Biofouling was monitored through total coliform counts, crystal violet staining, and an ATP bioluminescence analysis. These results display the potential for biofouling mitigation by UVLEDs and highlight the need for further investigation into the effects of UV light as a pretreatment for wastewater effluent distribution during water reuse.

DEDICATION

This thesis is dedicated to my family. I would not be the researcher, engineer, or woman I am today without your unwavering love and support. Dad, thanks for the advice, tough love, and confidence in my ability to achieve. You are an incredible father and- as I move into adulthood, friend. Mom, you are the binding glue that holds our family together. I could not ask for a better role-model, support system, or cards opponent. I am so grateful for the influence you have had on my life and character. Elle, how lucky am I for a sister like you. You are an incredible young woman and have taught me empathy and compassion, which I am thankful for beyond words. I am so excited to witness your journey into adulthood, and for us to grow old as sisters.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Karl G. Linden, for his role in my academic and personal development throughout this research journey. The experience greatly improved my ability to conduct meaningful research and make impactful contributions to the academic community, all of which was taught through Dr. Linden's mentorship. This year brought a series of research disruptions through family health emergencies and a global pandemic, and I am so grateful for Dr. Linden's sincere and unwavering support throughout these events. I consider myself extremely fortunate to close this chapter with Dr. Linden as a mentor and friend.

I would also like to thank colleagues from the Technion Institute of Technology, Haifa including Dr. Eran Friedler, Dr. Yael Gilboa, and Inbar Shlomo. It was such a pleasure to work with you all, and your contributions to this research were invaluable. Although my trip to Israel was cancelled, I hope we can meet and celebrate this project sometime in the near future.

Dr. Kari Sholtes, thank you for your mentorship in the early days of this degree. You're an incredible researcher, teacher, and person. I am fortunate to have such an inspiring female role-model and am excited to follow your journey as a professor. I am also grateful for the larger community of researchers I was lucky to work alongside during this degree including Dr. Sydney Ulliman, Jasmine Gamboa, Eric Peterson, Bryan Liu, Dr. Ben Ma, Breanna Real, and Yarrow Linden. Dorothy Noble, thank you for being a fabulous lab manager. I will always cherish our hallway chats.

Finally, I would like to thank my friends who provided support on the bad days and celebrations on the good days. I could not have made it through this challenging experience without you all, and I will carry your love and support with me into the next chapter.

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BACKGROUND INFORMATION

WATER SCARCITY

In 2013, the Global Water Institute estimated 700 million people in 43 countries suffer from water scarcity (Hameeteman 2013). By 2050, the number of people living in water scarce areas is projected to increase to between 4.5 and 5.7 billion (Burek et al. 2016). The UN describes water scarcity as a physical shortage of water or a scarcity in access due to institutional failure to ensure a regular water supply or adequate infrastructure for water services (UN-Water 2018). In the eyes of most water-users, water scarcity is often interpreted as a lack of drinking water (Savenije 2000). However, roughly 70 percent of freshwater withdrawals are for irrigation as 40% of food is produced on irrigated lands (Wada et al. 2013; Wallace 2000). Therefore, reducing agricultural water demand through water conservation efforts, like agricultural water reuse, will be imperative for water scarcity alleviation.

WATER REUSE

Irrigation water demand has increased over the past 50 years despite advancements in agricultural-related technology (Jingsi Li et al. 2020). This is consistent with other literature that suggests reduction in water consumption by water-efficient technologies will be overwhelmed by population increase if water use per capita does not change (Bazilian et al. 2011). Thankfully, improvements in water treatment technologies have led to reduced per capita water consumption (National Research Council. 2012). Advances in the ability to treat previously discarded wastewater sources have instigated a new era in water management, focusing on reclaiming wastewater sources. These sources include the desalination of seawater and brackish groundwater, the recovery of groundwater impaired by previous anthropogenic activity, off-stream or underground storage of seasonal water surpluses, the recovery of rainwater and stormwater runoff, on-site greywater reuse, and the reuse of municipal wastewater effluent (National Research Council. 2012).

Reuse water, also known as reclaimed and recycled water, can originate from municipal wastewater that has been treated to meet specific water quality criteria with the intent of being used for beneficial purposes (Asano, Burton, and Leverenz 2007). Over the past several decades, reuse water from

municipal wastewater sources has created a new water supply and reduced demands on limited traditional water supplies like surface and groundwater (Asano, Burton, and Leverenz 2007; National Research Council. 2012). Reuse applications can be broken down into "potable" and "nonpotable" reuse. Potable reuse relates directly to drinking water while nonpotable reuse includes applications not related to drinking water, like industrial processes and reuse for landscape and agricultural irrigation. Irrigation with reuse water is highly impactful, as agriculture remains the largest source of water consumption globally (Asano, Burton, and Leverenz 2007).

REUSE FOR IRRIGATION

Using domestic wastewater for irrigation dates back to the Bronze Age (3200-1100 BC) and has been employed by a number of civilizations including those in China, Egypt, Mesopotamia, and Crete (Angelakis et al. 2018). The twentieth century brought significant technological and scientific advancements including the implementations of wastewater treatment plants (WWTPs). Moving into the latter part of the twentieth century, water reuse began gaining popularity because of both the demand for additional water sources and the development of water reclamation technologies that produce water of equal or greater quality than drinking water (Angelakis et al. 2018). More specifically, technologies like microfiltration, ultra filtration, reverse osmosis, ozone coupled with biological filtration, and UV advanced oxidation have been used to successfully remove contaminants of concern in reuse water (Asano, Burton, and Leverenz 2007). With the advancement of water treatment technologies, effluent quality improved making reuse water more suitable for modern agriculture. Today, the potential of reuse water for irrigation is well demonstrated; for instance, Israel uses more than 87 percent of treated wastewater effluents for agriculture (Marin et al. 2017). A large portion of this effluent receives treatment sufficient for use on crops without restriction. Other countries irrigating with reuse water include Australia, Japan, China, as well as states within the US such as Florida, Idaho, Colorado, and California (Sheikh 2016).

PROBLEMS- EMITTER CLOGGING

Like irrigation with reuse water, drip irrigation is a water efficient technology that dominates water delivery methods (Jovanovic et al. 2020). Advantages of drip irrigation include significant water saving, reduced energy consumption and pumping costs per unit area, efficient nutrient uptake as water is delivered directly to roots, suppression of weed growth and minimizing exposure of farmers and consumers to pathogenic microorganisms (Cararo et al. 2006; Jovanovic et al. 2020; Oliver, Hewa, and Pezzaniti 2014; Pei et al. 2014; Perry 2017; Puig-Bargués et al. 2005; Yan et al. 2009). Drip emitters have a narrow and winding internal structure making them susceptible to clogging. Clogging adversely effects emitter flow rate and uniform water application especially when irrigating with wastewater effluent (J. Li et al., 2009; Pei et al., 2014; Qian et al., 2017; Tarchitzky et al., 2013). Crops are susceptible to water stress once drip emitters are partially or fully clogged. The three main mechanisms for emitter clogging are 1) chemical, caused by precipitation of iron, manganese, phosphorus and calcium carbonate, 2) physical, due to sedimentation of organic and inorganic suspended solids and 3) biological, caused by biofilm formation within the emitters or bacterial growth on the outer side of the emitters. Research suggests the formation and growth of biofilms, also known as biofouling, during irrigation with reuse water is a key factor in emitter clogging (Duran-Ros et al. 2009; G. B. Li et al. 2012; 2012; Ravina et al. 1997).

SOLUTIONS

Strategies for biofouling control during irrigation with reuse water include, but are not limited to, filtration, flushing, and chlorination. Filtration is an important factor in preventing biofilm formation as it removes biofouling microorganisms and nutrients that facilitate their growth, and particles. Most manufacturers of drip irrigation systems recommend filtration; however, in the case of reuse waters, the main problem becomes clogging of the filter as opposed to clogging of the drip irrigation lines (Adin and Elimelech 1989; Ravina et al. 1997). Regardless, filtration is still an important step during irrigation with reuse water to prevent immediate clogging of emitters by large particles (Adin and Sacks 1991).

Flushing is the process of increasing the water velocity in an irrigation system so the flow hydraulic shear force rapidly sheds attached biofilms while also slowing down the clogging frequently caused by the shredded biofilm falling inside the emitter (Y. Li et al. 2015). Flushing studies have identified a method to effectively slow down emitter clogging in a reclaimed water drip irrigation system, and the longer period it was applied, the better controlling was observed (Q. Li et al. 2019; Y. Li et al. 2015). However, changing water velocity causes an immediate increase in bacteria numbers as the increase in shear stress resuspends biofilms (Lehtola et al. 2006). In one study examining the effects of flushing during irrigation with reclaimed water, lateral flushing failed to completely solve the emitter clogging problem and pairing flushing with additional measures was recommended (Y. Li et al. 2015).

Chlorination has been considered the most effective method of controlling biofouling during irrigation but it has many drawbacks (P. Song et al. 2017). Aside from being hazardous to humans, it has demonstrated adverse soil health effects when applied in high concentrations during short term use which is the common recommended treatment of irrigation lines (P. Song et al. 2019). When chlorine and chloramine react with organic matter, like that found in wastewater effluent, it forms hazardous disinfection by products (DBPs) (Doederer et al. 2014). Although the impact of DBPs on soil and crop health are not well-studied, their ability to persist in aquatic environments have been reported (Rostad 2002).

Ultraviolet disinfection (UV) is a common and effective treatment technology for drinking water, wastewater, and reclaimed water globally (Jacangelo et al. 1995; Meulemans 1987; Nguyen et al. 2019; Sommer et al. 2008; K. Song, Mohseni, and Taghipour 2016a). In 2011, there were more than 7000 municipal UV disinfection installations worldwide with a dramatic increase in the past decade (Westerling 2011). The absorption of UV by DNA or RNA in a microorganism causes a photochemical reaction forming a chemical dimer. The dimer prevents the formation of new DNA/RNA resulting in inactivation (inability to replicate) of affected microorganisms by ultraviolet light (Bolton and Linden 2003).

Microorganisms have developed UV repair and tolerance mechanisms, originally evolved to help cells adapt to increasing ultraviolet radiation at the earth's surface (Sinha and Häder 2002). The reactivation phenomenon was first observed by Kelner in 1951 and is well-documented for many bacteria of concern after water disinfection (Harris et al. 1987; Kelner 1951; Zimmer and Slawson 2002). Microbial population recovery after UV exposure can be attributed to damaged cell repair and viable cell growth. Repair mechanisms include dark and light repair. Photolyase, a DNA repair enzyme, absorbs energy through blue light wavelengths and reverses UV-induced DNA damage like pyrimidine dimers (Chiganças et al. 2000). Light repair is controlled by not exposing UV treated water to light; however, dark repair is much more difficult to control because it occurs under both light and dark conditions (Sinha and Häder 2002). As opposed to repair, regrowth is described as surviving cells producing more active cells and is governed by nutrient availability and the viability status of microorganisms (Kollu and Örmeci 2015; Sinha and Häder 2002). Because recovery is dependent on nutrient availability, the consequences of microbial recovery post-UV may be more severe when treating higher nutrient waters, like wastewater effluent.

In the past decade, ultraviolet light emitting diodes (UVLEDs) have become a dominant UV source due to their compactness, wavelength diversity, electrical efficiency, and robust options for design (Crook et al. 2015; Linden, Hull, and Speight 2019). In comparison, traditional mercury lamps are fragile and contain toxic mercury, which is difficult to dispose of and is hazardous to the environment. The development of UVLEDs has allowed novel integration of UV disinfection into devices like toothbrushes, coffee makers, and water coolers (Linden, Hull, and Speight 2019). LEDs are particularly well-suited for the intermittent flow applications of agricultural irrigation due to their small size, no-warm up time, and ability to turn on/off without negatively impacting the lifetime and performance of the device (Chen, Loeb, and Kim 2017; K. Song, Mohseni, and Taghipour 2016a).

GAPS IN KNOWLEDGE

UVLEDs have demonstrated an ability to treat wastewater effluent to US EPA guidelines for irrigation of processed food crops and non-food crops (Nguyen et al. 2019). Nguyen (2019) examined the ability of a flow through UVLED device to disinfect domestic wastewater for agricultural reuse and demonstrated promising results. To the best of the authors knowledge, there have been no studies examining the effects of UVLEDs on biofouling during the wastewater effluent distribution for reuse purposes; or more specifically, irrigation with reuse. The ability of common wastewater microorganisms to repair after UVLED exposure is not well developed. The few studies investigating microbial repair after UVLED irradiation found that *E.coli* can undergo light and dark repair (Li et al., 2017; Nyangaresi et al., 2018). Although *E.coli* is a relevant organism to water reuse, the repair ability of other biofilm-forming wastewater microorganisms in response to UVLEDs has not been investigated (Andersson et al. 2008a). To the authors knowledge, there is also no literature investigating the implications of microorganism repair after UVLED irradiation related to water reuse scenarios. The overall goal of this study was to examine a commercially available UVLED flow through device for recovery inhibition and biofouling control on drip irrigation line material during the distribution of wastewater effluent. Specific research objectives included understanding the factors that affect *P. aeruginosa* recovery following UVLED exposure and the significance for water reuse applications. The individual contributions of repair and regrowth to total *P. aeruginosa* recovery was also investigated. The effects of a UVLED device on irrigation material biofouling in a bioreactor fed by effluent was also tested through direct quantification assays (total coliforms) and indirect quantification assays (crystal violet, ATP).

**The following chapters is presented as a manuscript*

PSEUDOMONAS AERUGINOSA RECOVERY AFTER UVLED EXPOSURE AND IMPLICATIONS FOR WATER REUSE

ABSTRACT

Concern over water scarcity is turning global attention towards reclaiming wastewater to fulfill unmet water demands. Innovative water treatment solutions, like UVLEDs, are being applied in novel ways to treat wastewater effluent for water reuse. However, the ability of microorganisms to repair and regrow after UVLED disinfection in distribution systems fed by recycled wastewater effluent is not well-studied. Therefore, the objective of this research was to understand conditions that impact bacterial repair and regrowth following UVLED exposure and the significance for UVLED disinfection under reuse settings. Although UVLED repair investigations have been conducted with *E.coli*, this work examined *P.aeruginosa* repair mechanisms in response to UVLED exposure as *P. aeruginosa* is known to undergo significant photorepair and is a concerning organism for biofilm formation in reuse distribution systems. The effect of irradiation with 265nm and 285nm UVLEDs on *P. aeruginosa* repair and regrowth was studied in both low and high nutrient waters. As most water distributions systems are insulated from light (underground piping, closed storage tanks, drip irrigation systems), dark repair and regrowth mechanisms were of concern, whereas photorepair could occur in a holding pond during recharge or an agricultural reuse scenario. Interestingly, dark repair and regrowth were not observed in any of the treatment conditions. Water matrix did not have any statistically significant effect on *Pseudomonas* recovery. UV light emitted at 265nm inhibited percent photorepair at higher rates than 285nm, although 265nm had the highest absolute photorepair value ($10^{3.86}$ CFU/mL) after a 5 mJ/cm^2 exposure. A water reuse scenario, with 285nm LEDs and fluences up to 100 mJ/cm2, revealed that the maximum photorepair potential was reached within 45 minutes. For fluences 10 mJ/cm^2 and higher in wastewater effluent, the maximum *P. aeruginosa* percent photorepair was 0.81%.

INTRODUCTION

As population growth and climate change put pressure on limited water resources, water reuse has become a promising alternative for a variety of water-users. Examples of reuse applications include potable water supplies, groundwater storage, environmental restoration, and irrigation for agriculture (US EPA 2019a).

Ultraviolet disinfection is a common and effective treatment technology for drinking water, wastewater, and reclaimed water globally (Nguyen et al. 2019; Jacangelo et al. 1995; Meulemans 1987; Sommer et al. 2008; K. Song, Mohseni, and Taghipour 2016a). In the past decade, ultraviolet light emitting diodes (UVLEDs) have become a dominant UV source due to their compactness, wavelength diversity, electrical efficiency, and robust options for design (Crook et al. 2015; Linden, Hull, and Speight 2019). UVLEDs have also been proven to inactivate total coliforms in wastewater to meet water reuse guidelines for agriculture (Nguyen et al. 2019). These factors have contributed to novel applications of UVLEDs including integration in drinking water facets (Oguma and Watanabe 2020) and drip irrigation lines during reuse (Randall et al. 2020).

UV inactivates cells through DNA damage, which some microorganisms can partially counteract or tolerate through repair mechanisms. Initially, many of these repair and tolerance mechanisms evolved to help cells adapt to increasing ultraviolet radiation at the earth's surface (Sinha and Häder 2002). The reactivation phenomenon was first observed by Kelner in 1951, and is well-documented for most organisms of concern after water disinfection (Kelner 1951; Harris et al. 1987; Zimmer and Slawson 2002). Microbial population recovery after UV exposure can be attributed to either repair of damaged cells and/or growth of remaining viable cells. Repair mechanisms include dark and light repair. Photolyase, a DNA repair enzyme, absorbs energy through blue light wavelengths and removes UV-induced DNA damage like pyrimidine dimers (Chiganças et al. 2000). Light repair is controlled for by conducting experiments in the dark; however, dark repair is much more difficult to control for because it occurs under both light and dark conditions (Sinha and Häder 2002). As opposed to repair, regrowth is

described as surviving cells reproducing into more active cells and is governed by nutrient availability and the viability status of microorganisms (Kollu and Örmeci 2015; Sinha and Häder 2002). Because it is dependent on nutrient availability, the consequences of repair may be more severe when treating high nutrient waters, like wastewater effluent, than low nutrient waters, like drinking water.

Pseudomonas aeruginosa is an environmentally abundant organism with specific relevance to the water sector as both an opportunistic pathogen and biofouling organism (CDC 2019). Specific to reuse applications, *P. aeruginosa* has been found to persist in wastewater effluents (Peng et al. 2018b) and readily colonize biofilms in both low (Maes et al. 2019; Bédard, Prévost, and Déziel 2016) and high (Peng et al. 2018a) nutrient environments like wastewater (Andersson et al. 2008b). UVLED disinfection of *P.aeruginosa* as a planktonic cell and biofilm are well documented (Sholtes and Linden 2019; Lakretz, Ron, and Mamane 2010; Garvey et al. 2015). Despite the understanding that *Pseudomonas* is known to express DNA damage repair mechanisms via photolyase activation (Kim and Sundin 2001; Sidorenko, Jatsenko, and Kivisaar 2017), few studies have examined *P.aeruginosa* recovery following UV exposure (Ansa et al. 2017; Mun, Cho, and Yoon 2006).

The objective of this research was to understand the factors that affect *P. aeruginosa* recovery following UVLED exposure and the significance for water reuse applications. More specifically, we sought to understand the effect of 265nm and 285nm UVLED exposure on *P. aeruginosa* recovery in both low and high nutrient waters. The individual contributions of repair and regrowth to total *P. aeruginosa* recovery was examined. These results will help advance the general understanding of bacterial stress responses to UVLED irradiation as well as how these recovery mechanisms may affect the efficacy of UVLED treatment of filtered WW effluent meant for reuse.

METHODS

WASTEWATER EFFLUENT SAMPLE COLLECTION

Wastewater effluent was collected at the City of Boulder Water Resource Recovery Facility after biological nutrient removal and prior to UV disinfection. Water quality parameters (Table 1) were analyzed according to Standard Methods for the Examination of Water and Wastewater, Section 1060B (Bridgewater et al. 2017). The effluent was filtered through a 0.45 μm, 47mm, sterile polyvinylidene fluoride filter (Millipore, Germany) membrane filter and stored at 4°C. The effluent water was used within one week of sampling.

TABLE 1. AVERAGE WATER QUALITY PARAMETERS (N= 8) FOR THE BOULDER FACILITY SECONDARY WASTEWATER EFFLUENT SAMPLED PRIOR TO UV-DISINFECTION. STANDARD DEVIATIONS IN PARENTHESIS.

pH	7.48 (0.32)
T [°C]	21.3 (1.33)
DOC [mg-C/L]	6.83 (1.00)
UVT_{254nm}	72.7% (2.5%)

PREPARATION OF *PSEUDOMONAS AERUGINOSA* STOCKS

Pseudomonas aeruginosa (ATCC 15442) was streak-plated onto sterile Luria-Bertani broth (LB) (BD Difco™) agar plates, inverted, then incubated for 20 hours at 37°C. One isolated colony was selectively removed with a sterile inoculation loop. 25 mL sterile LB broth was inoculated with the isolated colony in a flask with side baffles to enhance aeration. The overnight culture was incubated 20 hours at 37°C with shaking (180 rpm) then centrifuged (5000 rpm, 10 minutes). The pellet was resuspended in sterile LB broth. Twenty percent (by volume) Glycerol (100% v/v, Microlytic) was added to produce a final concentration of $5.5x10^6$ CFU/mL and stocks were stored at -80°C.

Pseudomonas aeruginosa preparation for UV disinfection experiments

Log-phase cultures of *Pseudomonas aeruginosa* were grown from frozen stocks in 25mL LB broth for 14 hours at 37°C while agitated at 121 rpm in a baffled flask. Bacterial cultures were then pelletized by centrifugation (5000 rpm, 8 minutes). The supernatant was discarded, and the remaining pellet was re-suspended by vortexing in 25 mL phosphate buffered saline (PBS); this was repeated three times to "wash" the growth media from the cells. After the final wash, the pellet was resuspended in 10 mL PBS and the bacterial suspension was spiked into the test water (PBS or filtered wastewater (WW) effluent) to achieve a concentration of approximately $5x10^6$ CFU/mL.

BENCH-SCALE UV EXPERIMENTS

Bench-scale collimated beam tests were conducted according to Sholtes (2019) using a PearlBeam ultraviolet light emitting diode (UVLED) research device (AquiSense Technologies, Erlanger, Kentucky). The peak/weighted average wavelengths of the UVLED chips were 268nm/ 267.5nm and 283.6nm/284.6nm, respectively, measured used a Maya 2000 Pro spectrometer (OceanOptics, Dunedin, FL). The UV irradiance was measured before and after the exposures with a calibrated radiometer to ensure there was less than a 5% difference between final and initial irradiance (ILT2400, photodetector SED 240W-diffuser; International Light, Newburyport, Mass.). The calibrated plane of the detector was placed directly under the UVLED at the same height as the water surface of the irradiated samples. Because UVLEDs are a polychromatic light source, the radiometer was operated at a calibration factor corresponding to the weighted average wavelength of the UVLED.

REGROWTH

To understand the contribution of regrowth to the total recovery, regrowth was quantified according to Bohrerova (2015). To begin, *Pseudomonas aeruginosa* stocks were serially diluted to make a 10^6 CFU/mL solution. The solution was then irradiated with a 1000 mJ/cm^2 fluence from two low-pressure lamps (15 W Sankyo Denki, Japan) to inactivate cells beyond repair and create a high nutrient water matrix with nonviable *Pseudomonas* cells. The water matrix was then spiked with different concentrations of viable *Pseudomonas* cells (tens, hundreds, thousands CFU/mL) to mimic an irradiated water matrix with various levels of survivors.

DISINFECTION EXPERIMENTS

To control for unintended photorepair, the collimated beam experiments were conducted in dark conditions with a red-light source for visibility (700-1000nm). Although *Pseudomonas* is rapidly inactivated at 16 mJ/cm^2 (Sholtes and Linden 2019), exposures with fluences up to 100 mJ/cm^2 were performed to investigate whether repair is inhibited at higher fluences. Stirred suspensions of 18 mL (0.8 cm sample depth) were irradiated in 5.4 cm dishes at a 15 cm distance from the UVLED, corresponding to an average irradiance of 3.17e-2 mW/cm^2 for the 265nm LEDs and 7.13e-2 mW/cm^2 for the 285nm LEDs. Average UV fluences were determined similarly to previous reports (Bolton, Beck, and Linden 2003; Kheyrandish, Mohseni, and Taghipour 2018). The petri factor was >0.99 for all UVLED exposures. UV fluence is a product of average irradiance and time; therefore, pre-determined fluences were achieved by manipulating exposure time once average irradiance was determined. All experiments were performed in duplicate, and each sample had a replicate measurement. Samples were exposed randomly, 10-fold serially diluted and plated immediately.

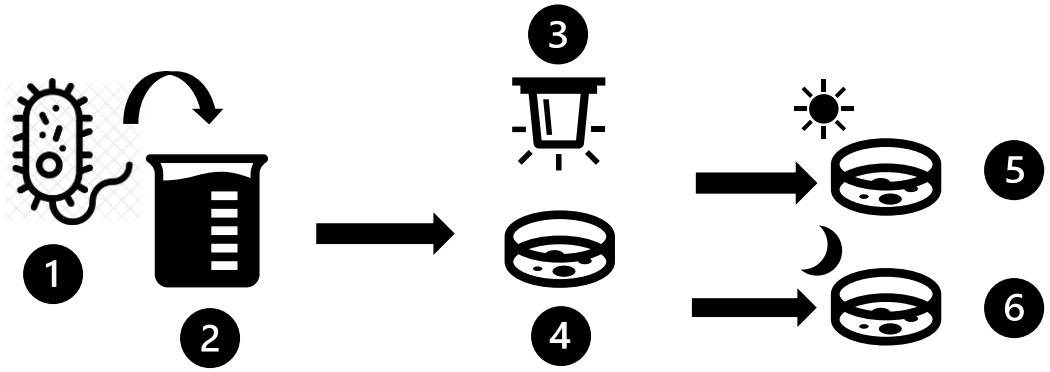


FIGURE 2.1: RECOVERY EXPERIMENT SCHEMATIC: *PSEUDOMONAS* CELLS (1) WERE ADDED TO THE TEST WATER (2) TO ACHIEVE A STARTING CONCENTARTION OF 5.5×10^6 CFU/ML, WHICH WAS THEN EXPOSED TO UV (3). THE CONCENTRATION OF THE IRRADIATED SAMPLES (4) WERE MONITORED OVER TIME IN LIGHT (5) AND DARK (6) CONDITIONS.

REPAIR

After UV exposures, the sample volume was split into separate 5.4 cm petri dishes as shown in Figure 2.1. One of the samples portions was kept in dark conditions, whereas the other was immediately placed under two full spectrum lamps (F17T8 VL Plus, 5500 K, Natural Lighting, Houston, TX) for light incubations. The distance between the lamps and samples was 10 cm with a light intensity of 191 mW/cm^2, as measured with a radiometer (ILT2400, SED 240; International Light, Newburyport, Mass.) calibrated to the average weighted wavelength of the lamps. The weighted average wavelength was determined by the emission spectra over wavelengths from 200-400nm measured using a Maya 2000 Pro spectrometer (OceanOptics, Dunedin, FL). The light repair fluence was calculated using the visible light spectra weighted by the action spectra of photolyase, a primary enzyme responsible for light repair in microorganisms (Bohrerova and Linden 2007). All samples were kept at room temperature (21°C) during the exposures. Non-UV-treated samples were placed in both repair conditions to control for growth or die-off.

ENUMERATION

Enumeration of *Pseudomonas* was conducted using a combination of spread plate and spot plate methods. For each dilution in spread plating, 1 mL of sample was applied to LB agar plates. For each dilution in spot plating, 200 uL of sample were added to a well in a 96-well plate. In total, 20 uL of sample was serially diluted into 180 uL of PBS, and 5 spots per dilution of 10 uL each, were added to LB plates with a multichannel pipette. After the sample absorbed into the agar, plates were inverted and incubated for 18 hours at 30°C. Samples were plated in triplicate. Colonies were counted, and bacterial concentrations expressed as colony-forming units (CFU)/mL.

DATA ANALYSIS/STATISTICS

Analysis of Variance (ANOVA) full factorial experiments were performed to examine the statistical significance of experimental effects. Model adequacy was verified by checking the three assumptions required for an ANOVA analysis: 1) equal variances, 2) residual normality, and 3) independent data. Results were presented as log concentration values ($\log10(N_x)$) and log reduction values (LRVs) ($\log10(N_0/N_x)$). To account for the impact of initial disinfection on repair, results were also expressed as percent photorepair as originally described in (Kelner 1951):

$$percent\ photorepair = \frac{N_p - N}{N_0 - N}\ x\ 100\%$$

where N_p *= cell number of the photo-reactivated sample (CFU/mL)*

N *= immediate survival after UV disinfection (CFU/mL)*

N_0 *= cell number in the control sample (CFU/mL)*

RESULTS AND DISCUSSION

REGROWTH

To understand the contribution of regrowth towards total recovery, the regrowth potential of *P. aeruginosa* was quantified over a six-hour period for various starting concentrations (Figure 2.2).

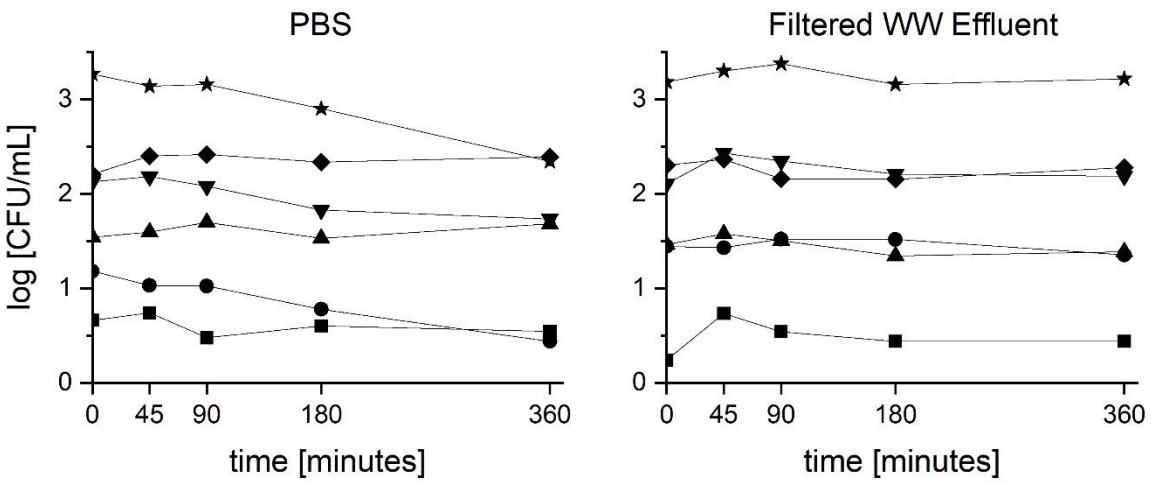


FIGURE 2.2: LOG CONCENTRATION VALUES (LRV) FOR DIFFERENT INITIAL CONCENTRATIONS OF *P. AERUGINOSA* IN A PBS (LEFT) AND WW (RIGHT) WATER MATRIX WITH 10^6CFU/ML OF IRRADIATED CELLS ACTING AS A NUTRIENT SOURCE.

In all samples, *P. aeruginosa* concentrations remained constant or experienced slight die-off despite the availability of nutrients in the form of lysed cell material. It also appears that water matrix (PBS, left; filtered WW effluent water, right) did not affect regrowth. This is consistent with Bohrerova (2015) who found no statistical difference in *E.coli* regrowth after a 48 hour holding time between wastewater effluent and drinking water. Guo (2011) observed similar results for *E. coli, B. subtilis,* and a fecal coliform strain in secondary and tertiary effluent over a 72-hour period at room temperature. Further studies examining nonirradiated cell growth of wastewater microorganisms (thermotolerant coliforms, fecal streptococci) found decreasing concentrations over time (Baron 1997). These regrowth results suggest that *P. aeruginosa* follows the same proposed trend in (Guo et al. 2011), that any increase in bacterial number after UV exposure results from the repair of injured bacteria as opposed to uninjured or repaired-cell growth.

BENCH-SCALE UV EXPERIMENTS

Results for the UV disinfection of *Pseudomonas* under both LEDs examined is presented in Figure 2.3. An ANOVA statistical analysis found that water matrix was statistically insignificant (p=0.057) compared to the effects of wavelength (p=0.003) and fluence (p=0.000). At a fluence of 5 mJ/cm^2, the 265nm UVLED outperformed the 285nm UVLED by roughly 2.5 LRV in both water matrices. At 10 mJ/cm^2, the 285nm UVLED achieved approximately equal $\log_{10}$ inactivation to the 265nm LED. The LRVs achieved in Figure 2.3 indicate that the LEDs produced first-order rate kinetics as reported in Sholtes (2019) and Rattanakul (2018), with the 265nm UVLED tailing off as the fluence approaches 10 mJ/cm^2.

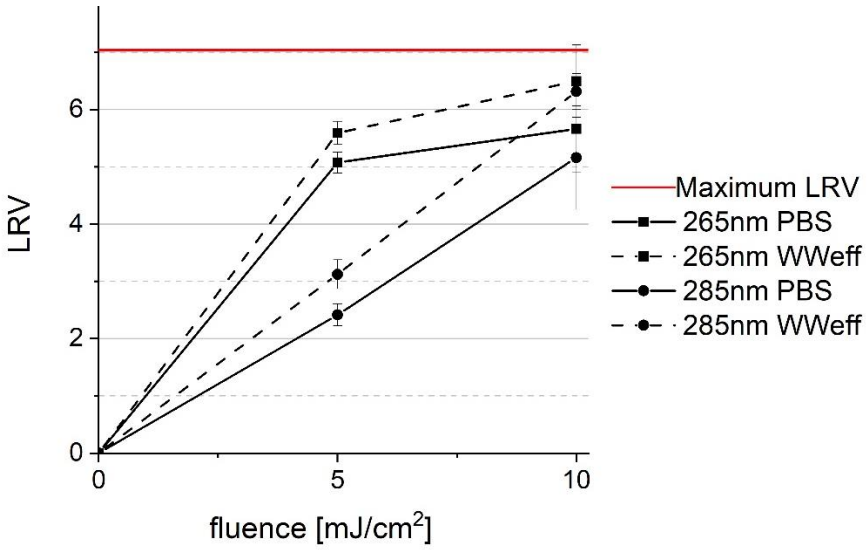


FIGURE 2.3: LOG REDUCTION VALUES (LRV) FOR (LOG10(N_0/N_x)) OF *P. AERUGINOSA* IN RESPONSE TO UV IRRADIATION FROM UVLEDS EMITTING AT 265NM, SQUARE MARKER, AND 285NM, CIRCLE MARKER, IN TWO DIFFERENT WATER MATRICES: 1) PHOSPHATE BUFFER SALINE (PBS) SOLUTION, SOLID LINE, AND 2) FILTERED WW EFFLUENT, DASHED LINE. THE RED LINE INDICATES THE MAXIMUM LRV, DETERMINED BY THE STARTING CONCENTRATION OF PSEUDOMONAS PRIOR TO UV.

PSEUDOMONAS AERUGINOSA LIGHT AND DARK REPAIR

Results from the repair investigation can be found in Figure 2.4. The recovery of *P. aeruginosa* was examined after exposure to fluence (5 or; 10 mJ/cm^2) by 265nm and 285nm UVLED sources in PBS

and filtered WW effluent after light and dark incubations. The control, either PBS or filtered WW effluent with 10^6 CFU/mL viable cells, was left to incubate at room temperature under light and dark conditions. Over the course of the full 6-hour experiment, the control samples experienced die off under all treatment combinations (indicated by negative log recovery values). This supports conclusions drawn from the data in Figure 2.2, that recovery mechanisms can be attributed to repair of injured *P. aeruginosa* cells as opposed to viable cell growth. Based upon the assumption that regrowth is not contributing to recovery under the observed conditions, recovery results will be referred to as repair when reported below.

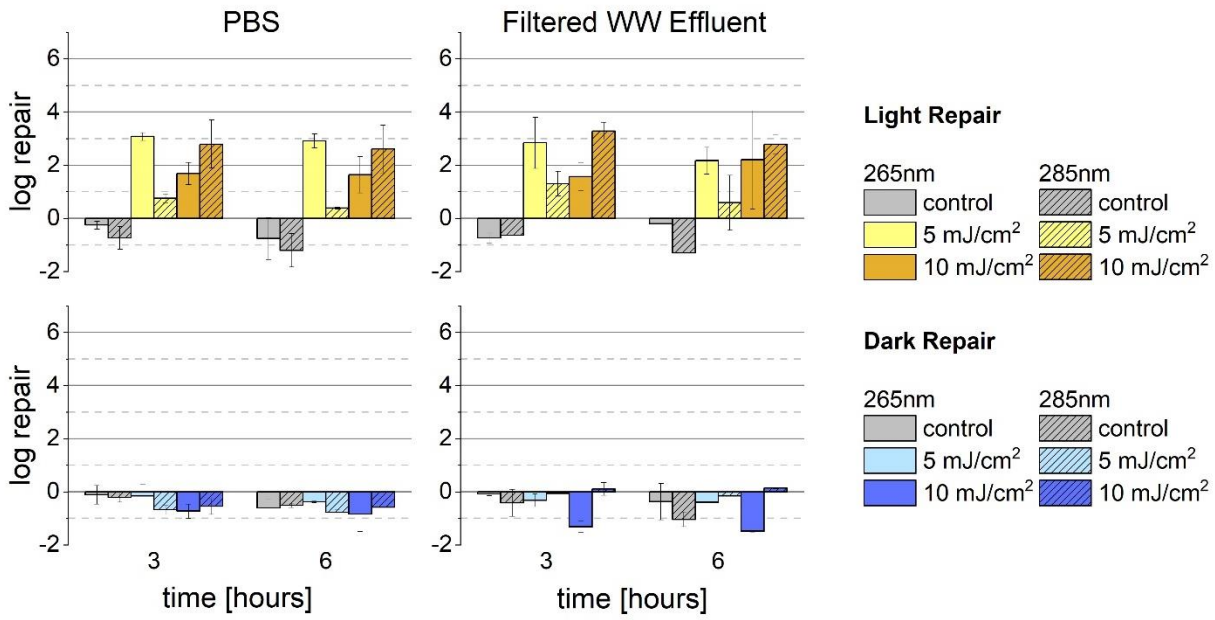


FIGURE 2.4: *P. AERUGINOSA* LOG REPAIR VALUES (LRV) FOR (LOG10(N_0/N_x)) AFTER EXPOSURE TO 5 AND 10 MJ/CM^2 FROM A 265NM AND 285NM UVLED IN TWO WATER MATRICES; PBS (LEFT) AND FILTERED WW EFFLUENT (RIGHT) UNDER LIGHT (TOP) AND DARK (BOTTOM) INCUBATION CONDITIONS.

Samples in dark conditions experienced a similar declining concentration to the control samples, indicating there was no *Pseudomonas* dark repair mechanism expressed under the treatment conditions. Previous studies have observed dark repair after exposure to a 40 mJ/cm^2 fluence, which was attributed to the protein RecA (Jungfer, Schwartz, and Obst 2007). However, this study supports a growing body of

research which shows statistically insignificant dark repair after LP and MP UV for microorganisms that commonly inhabit wastewater including *E. coli, Salmonella*, heterotrophic bacteria, somatic coliphages, total coliforms, and fecal coliforms (Bohrerova, Rosenblum, and Linden 2015; Kollu and Örmeci 2015; Kashimada et al. 1996; Baron 1997). The nondetectable dark repair has been demonstrated in both wastewater and buffered water matrices for up to 48 hours (Kollu and Örmeci 2015; Kashimada et al. 1996; Bohrerova, Rosenblum, and Linden 2015). Recent UVLED recovery investigations, however, indicate minimal percent dark repair (<0.02%) in *E.coli* following LEDs emitting around 267nm (Nyangaresi et al. 2018). This is promising for reuse and point-of-use UVLED applications, as the rate of repair is low and most devices are currently manufactured with LED chips closer to 280nm.

A full factorial ANOVA analysis revealed that wavelength ($p=0.000$) and fluence ($p=0.000$) had significant effects of percent photorepair of *Pseudomonas*, whereas water matrix ($p=0.16$) and repair time ($p=0.96$) were not significant. Although the 265nm UVLEDs had the highest absolute log repair value (2.92), they produced consistently lower percent photorepair values (Table 2). The 265nm UVLEDs produced percent photorepair values from 0.00%-1.96%, whereas the 285nm UVLEDs produced values between 0.48% and 19.81%. This discrepancy between absolute repair and percent repair highlights the importance of normalized repair calculations that account for the initial disinfection experienced by microorganisms.

TABLE 2: *P. AERUGINOSA* PERCENT PHOTOREPAIR (%) AFTER EXPOSURE TO 265NM AND 285NM LED FLUENCES OF 5 AND 10 FOLLOWED BY 3 AND 6 HOUR LIGHT INCUBATIONS IN BOTH PBS AND FILTERED WW EFFLUENT (STANDARD DEVIATION IN PARENTHESIS).

Water matrix		PBS				Filtered WW effluent			
LED wavelength [nm]		265nm		285nm		265nm		285nm	
Fluence [mJ/cm^2]		**5**	**10**	**5**	**10**	**5**	**10**	**5**	**10**
Incubation time [hrs]	**3**	1.96% (0.59%)	0.02% (0.00%)	8.53% (0.39%)	2.04% (0.46%)	0.84% (1.35%)	0.00% (0.00%)	6.67% (5.36%)	0.48% (0.49%)
	6	2.40% (4.69%)	0.03% (0.05%)	8.51% (1.14%)	6.91% (0.63%)	0.06% (0.05%)	0.03% (0.04%)	19.81% (11.41%)	0.81% (1.02%)

The least inhibitive conditions for repair were 5mJ/cm^2 delivered by the 285nm UVLEDs. The most inhibitive conditions for repair were 10 mJ/cm^2 delivered by the 265nm UVLEDs. A fluence of 5 mJ/cm^2 produced higher repair than 10 mJ/cm^2 in both absolute and percent recovery results. Studies investigating the effects of fluence on photorepair have seen mixed results, with some reporting that higher fluences produce lower repair (Baron 1997; Peccia and Hernandez 2001; Bohrerova and Linden 2006), no change in repair (Bohrerova, Rosenblum, and Linden 2015), or higher repair (Jungfer, Schwartz, and Obst 2007). This illustrates the complex relationship between recovery mechanisms and microorganism, UV wavelength, incubation time, and water matrix. Guo (2011) showed similar results to this study, with a LP 5 mJ/cm^2 fluence causing the highest level of bacterial photorepair.

The 265nm UVLEDs produced lower levels of *Pseudomonas* percent photorepair than the 285nm UVLEDs. This conflicts with previous UVLED repair studies, which found 265nm LEDs to produce statistically higher levels of photorepair in *E.coli* (G.-Q. Li et al. 2017; Nyangaresi et al. 2018). In those studies, *E.coli* experienced a 30%-33% photorepair after an initial 3-log disinfection from 267nm UVLEDs. Compared to our study, *P. aeruginosa* experienced 2.40% photorepair under the same conditions. This suggests that *Pseudomonas* incurs more irreversible cell damage to ~265nm UV

irradiation than *E.coli*, or that *E.coli* has more efficient repair mechanisms following 265nm UV. Following 280nm UVLED exposure, *E.coli* experienced a 16% photoreactivation compared to a 8.53% achieved by *Pseudomonas* in this study (G.-Q. Li et al. 2017). This highlights the need to investigate repair mechanisms post-UVLED exposure in a larger community of wastewater microorganisms, as two different microorganisms can exhibit different repair trends under the same conditions. Exposure wavelength is a critical aspect of recovery mechanisms as different wavelengths can target different components of the cell. Although 285nm was expected to inhibit enzymatic function by damaging the photolyase enzyme and interfering with the photorepair process (as had been observed in *E.coli*), this was not observed for *P.aeruginosa* (G.-Q. Li et al. 2017; Nyangaresi et al. 2018).

Unexpectedly, filtered WW effluent does not provide a more favorable environment for *P. aeruginosa* recovery after UVLED exposure when compared to a buffer solution. Bohrerova (2015) saw higher rates of *E.coli* photorepair in wastewater effluent treated for reuse water following a 40 mJ/cm^2 MP and LP UV exposure but no significant difference between waters when examining regrowth. In this study, *P. aeruginosa* regrowth was similarly unaffected by water matrix. Water matrix has been suggested to affect repair mechanisms but not regrowth because water matrix may have a significant effect for cells under stress (Bohrerova, Rosenblum, and Linden 2015). It is possible that *P. aeruginosa* did not exhibit differences in repair between water sources because the applied fluence was too low to trigger the proposed stress mechanisms. Conversely, Kollu (2015) observed lower regrowth in wastewater. This trend was attributed to the presence of inhibiting or toxic compounds in wastewater matrices. Another explanation for lower recovery in filtered WW effluent (despite the higher levels of nutrients in the secondary wastewater effluent) is that upstream treatment processes, like biological nutrient removal, leave only recalcitrant or non-biodegradable compounds which would not affect microbial recovery mechanisms.

Repair Under Reuse Conditions

In water reuse settings, fluences up to 100 mJ/cm^2 are typical. Because bacterial repair has been shown to be affected by the level of UV fluence applied, recovery was examined at higher fluences (20, 40, 80, 100 mJ/cm^2) delivered by 285nm UVLEDs in filtered wastewater effluent (Figure 2.5). While 255nm and 265nm UVLEDs are more germicidal, 285nm UVLEDs were choosen for the reuse study because it is the most electrically efficacious wavelength and, therefore, the design choice for commerically available UVLED devices (Sholtes and Linden 2019).

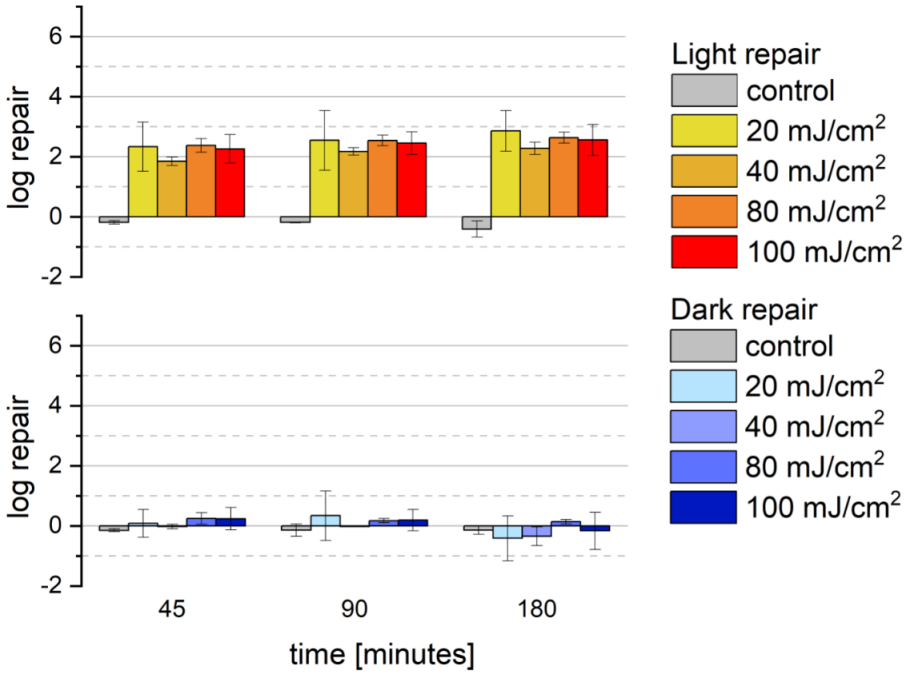


Figure 2.5: *P. aeruginosa* log recovery values (LRV) for ($\log_{10}(N_0/N_x)$) after exposure to 285nm UVLED irradiation with fluences ranging from 20 to 100 mJ/cm^2 in filtered WW effluent under light (top) and dark (bottom) incubation conditions.

Similar to Figure 2.4, the control and dark repair samples experienced die off during the three hour incubation in filtered WW effluent. This confirms prior conclusions, that the observed *Pseudomonas* recovery in these experiments can be attributed to photorepair as opposed to dark repair or natural growth mechanisms.

Figure 2.4 reveals *P. areuginosa* log photorepair values between 1.85 and 2.86 with no effect of fluence or incubation time. These results are consistent with Mun (2006) that found 2.6 log *P. aeruginosa* repair by 30 minutes, which held steady until 180 minutes after both LP and MP irradiation. It has been proposed that due to the small number of photolyase enzymes in a microbial cell (~20) and the maximum rate of dimer repair possible per enzyme, complete repair to pre-UV concentrations is not possible (Guo et al. 2011). This implies that a maximum amount of photorepair exists, limited by irreversible UV cell damage. For *P.areuginosa*, it appears that this value may be between 2.5 and 3. It should be noted that rates of photorepair have been reported to differ significantly within strains and species of microorganisms. The conditions of photorepair also play a significant role, highlighting the importance of standardized methods and detailed descriptions of repair conditions for proper interpretation of results (Bohrerova and Linden 2006).

Table 3 demonstrates the *Pseudomonas* percent repair values after exposure to 285nm UVLEDs under light conditions. A full factorial ANOVA analysis revealed that fluences of 20, 40, 80, and 100 mJ/cm^2 produced statistically similar percent repair values. This is consistent with other studies observing the same photorepair plateau with fluences equal or greater to $20mJ/cm^2$ in both E.coli and fecal coliforms (Guo et al. 2011). Previous literature suggests that there is an optimal time for repair mechanisms, after which no further repair is observed (Baron 1997; Kelner 1949; Lindenauer and Darby 1994). Ansa (2017) observed maximum photorepair for *P. aeruginosa* following pulsed-LPUV after 30 minutes. This agrees with the data presented in Figure 2.5, showing maximum photorepair values reached by 45 minutes. When the log photorepair values were transformed to percent photorepair (Table 3); however, an ANOVA analysis found that percent photorepair increased slightly with incubation time ($p=0.012$). The results presented in Table 2 also revealed no statistical difference between percent photorepair at 3 and 6 hours for fluences up to 10 mJ/cm^2, indicating that *P. aeruginosa* reaches the highest log photorepair and percent photorepair under 45 minutes and 3 hours, respectively.

TABLE 3: *P. AERUGINOSA* PERCENT PHOTOREPAIR (%) AFTER EXPOSURE TO 285NM UVLED FLUENCES OF 20, 40, 80, 100 MJ/CM^2 FOLLOWED BY 45, 90, AND 180-MINUTE LIGHT INCUBATIONS (STANDARD DEVIATION IN PARENTHESIS).

Time [mins]	Fluence [mJ/cm^2]			
	20	40	80	100
45	0.08% (0.10%)	0.07% (0.04%)	0.09% (0.08%)	0.08% (0.06%)
90	0.18% (0.23%)	0.15% (0.10%	0.15% (0.13%)	0.11% (0.06%)
180	0.50% (0.62%)	0.28% (0.26%)	0.35% (0.39%)	0.30% (0.33%)

All treatment combinations in Table 3 produced *P. areuginosa* percent photorepair values under 0.5%. Compared to a similar study investigating recovery for common wastewater microorganisms after LPUV, it appears that *P.aeruginosa* is capable of slightly higher rates of photorepair after exposure to highly inactivating fluences (>20mJ/cm^2) from LEDs. After a LPUV fluence of 20 mJ/cm^2 and light incuabtion time of 4 hours, Guo (2011) reported *E.coli* and fecal coliform percent recovery to be 1.21e-3 % and 2.08e-3 %, respectively. Comparatively, *Pseudomonas* achieved a 0.50 % recovery in three hours after a 20 mJ/cm^2 fluence delivered by 285nm LEDs. Following 285nm UVLED exposure; however, *P. aeruginosa* experiences lower rates of photorepair than *E.coli* (G.-Q. Li et al. 2017). These results suggest that 1) LPUV is more efficent at inhibiting *P.aeruginosa* repair than 285nm UVLEDs and 2) *Pseudomona*s may have a less robust photorepair ability at 285nm than other common wastewater micororganisms like *E.coli*. Further studies examining recovery mechanisms of these common wastewater microorganisms after UVC exposure would help clarify this distinction.

CONCLUSIONS

Recovery mechanisms of *P. aeruginosa* were examined after UVLED exposure in PBS and a filtered WW effluent. Results indicate that:

1. No difference in *P. aeruginosa.* repair or regrowth was observed between PBS and filtered WW effluent water matrices;
2. Dark repair does not contribute to *P. aeruginosa* recovery;
3. 265nm UVLEDs inhibited percent *P. aeruginosa* photorepair at higher rates than UV285nm LEDs;
4. Fluences above 20 mJ/cm^2 produce statistically similar P. aeruginosa photorepair; and,
5. The maximum *P. aeruginosa* photorepair is 2.86 logs in filtered WW effluent.

These results provide interesting implications for UVLED disinfection during water reuse. Without the ability to undergo dark repair or regrowth, *Pseudomonas* recovery is expected to have minimal effects during reuse where most water infrastructure is insulated from light (underground piping, closed storage containers, light impermeable tubing, etc..), but repair could occur during open storage of wastewter such as in a recharge basin and agricultural reuse. Further studies examining UVLED recovery in other wastewater microorganisms will be a critical next step in this body of research. As UVLEDs are becoming more common in disinfection applications, this study provides novel observations for recovery in microorganisms disinfected under UVLED irradiation.

FLOW THROUGH UVLED DEVICE CHALLENGE TESTING

RATIONALE

Before implementing the UVLED device for biofouling mitigation studies, a series of challenge tests were performed to establish a relationship between operating flow rate and fluence for varying water qualities. Qβ bacteriophage was chosen as the challenge organism because of its ability to survive high fluence levels, providing countable concentrations for the low-flow rate/ high-fluences testing conditions (Malayeri et al. 2016). The log reduction value (LRV) of Qβ bacteriophage were determined in three water matrices with varying UV transmittances (UVT_{254nm}) (93%, 80%, and 70%).

METHODS

MICROBIAL STOCKS

QB BACTERIOPHAGE

Qβ bacteriophage stock (ATCC 23631-B1) was propagated by GAP EnviroMicrobial Services Ltd. (London, Ontario, Canada). An *Escherichia coli* K-12 (ATCC 23631) host culture was made in tryptic soy broth (TSB) with shaking at 35ºC. Once the *E. coli* reached log-phase growth, 1mL of $1x10^{10}$ cfu/mL Qβ stock was added. After an overnight incubation with shaking, the stock was centrifuged to remove cellular debris, leaving the phage in the supernatant. A concentration of approximately 1×10^{10} cfu/mL was achieved. Stocks were stored at 40°C.

E.COLI FAMP

E.coli Famp (ATCC #700891) was used as the Qβ bacteriophage stock host for challenge testing. The Famp was separately streak-plated onto sterile nutrient broth (NB) (BD Difco™) agar plates and incubated for 20 hours at 37°C. One isolated colony was selectively removed with a sterile inoculation loop and suspended in 25 mL sterile NB for overnight incubation for 20 hours at 37°C with shaking (180 rpm). The overnight culture was then centrifuged at 5000 rpm for 10 minutes. The supernatant was removed, and the pellet was resuspended in sterile NB. Twenty percent (by volume) Glycerol (100% v/v,

Microlytic) was added to produce a final concentration of $9x10^8$ CFU/mL *E. coli* Famp. Stocks were stored at -80°C.

BENCH-SCALE UV DISINFECTION EXPERIMENTS

TEST WATER

Three water matrices with varying UV transmittances (UVT_{254nm}) (93%, 80%, and 70%) were used for challenge testing. Qβ bacteriophage stock was serially diluted into dechlorinated tap water to achieve a test water concentration of approximately $5x10^6$ PFU/mL, resulting in a 93-95% UVT at 254nm. UV transmittance was adjusted for the 80% and 70% UVT_{254nm} waters using a mix of SuperHume (UAS of America, Lake Panasoffkee, FL, USA) and vanillin according to NSF/ANSI 55 standards. Dechlorination of test water was verified with a Hach DPD Free Chlorine colorimetric test.

UV DISINFECTION

Bench-scale collimated beam tests with ultraviolet light emitting diodes (AquiSense Technologies, Erlanger, Kentucky) and low pressure (LP) lamps (15 W Sankyo Denki, Japan) were conducted to create reduction equivalent fluence (REF) equations for use with the flow-through UVLED devices. The UVLED collimated beam experiments followed Sholtes (2019), whereas the LP experiments followed Bolton (2003). The peak and weighted average wavelength of the UVLED over a 200-300nm spectrum were 281.5nm and 283.5nm, respectively, measured using a Maya 2000 Pro spectrometer (OceanOptics, Dunedin, FL). Incident irradiance (mW/cm^2) was measured with a radiometer (IL2400, SED240, International Light, Newburyport, Massachusetts) at the calibration factors corresponding to the weighted average wavelength (283.5nm) for the polychromatic UVLED chips and 254nm for the monochromatic LP lamps. The calibrated plane of the detector was placed directly under the UVLED at the same height as the water surface of the irradiated samples. Stirred suspensions of 10 mL (0.55cm sample depth) were irradiated in 5.4 cm dishes at a 15 cm distance from the UVLED and 41cm from the LP lamps. The UV irradiance was measured before and after the exposures with a calibrated radiometer to ensure there was no decay in lamp output over the exposure periods and less than a 5% difference between final and initial irradiance (US EPA, Office of Water 2006). The petri factor was >0.99 for all

UVLED exposures. UV fluence is a product of average irradiance and time; therefore, pre-determined fluences were achieved by manipulating exposure time once average irradiance was determined. All experiments were performed in duplicate, and each duplicate sample had a replicate measurement. Samples were exposed in random order over the target fluence levels.

Flow-Through Experiments

The UVLED flow through units were set up in accordance to package instructions. Greater than five reactor and effluent tube volumes of laboratory grade deionized (DI) water were pumped through the entire system as a rinse. A sample of this DI rinse water was taken to test for contamination from previous testing in the system line prior to each experiment.

The test water was contained in a glass bottle, connected to integrated tubing, and a peristaltic pump for additional flow control. An untreated sample was taken from the glass bottle after sufficient mixing to quantify the starting concentration. To start experimentation, the peristaltic water pump (MasterFlex L/S 7518-62) was turned on. Once water was flowing through the UVLED system, a sample was taken before the UVLED device was turned on to test for disinfection due to non-UV factors. Upon changing the system flow rate, five times the reactor and effluent tube volume were allowed to flush the system before further experimentation. Samples from influent and effluent were taken in duplicate.

Once the relationship between fluence and LRV was established, the reduction equivalent fluences (REF_{280nm}) for various flow rates were back calculated based on the Qβ LRVs. A REF at 254 was used to Similar to Nguyen (2019), a REF at 280nm was chosen to calculate the flow rates (as opposed to 254nm). Using a REF_{280nm} more accurately represents the wavelength-specific UV-induced stress experienced by microorganisms in the 280nm UVLED flow through device.

Enumeration

US EPA 1602 method and Standard Methods 9224 B.3 were followed with minor adjustments including the exclusion of percent recovery steps and a streptomycin/ampicillin antibiotic (“Method 1602: Male-Specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure” 2001;

Bridgewater et al. 2017). The bacterial host was *E. coli* Famp (ATCC #700891). Enumeration of Qβ was conducted using a combination of spread plate and spot plate methods and each sample was plated in triplicate. For each dilution in spread plating, 1 mL of sample was applied to soft agar plates. For each dilution in spot plating, 20 µL of sample was serially diluted into 180 µL of PBS and five spots, 10 µL each, were added to the soft-agar plates with a multichannel pipette. After the sample absorbed into the agar, plates were inverted and incubated for 20 hours at 37°C. Plaques were counted and concentrations expressed as plaque-forming units (PFU)/mL. For each experiment, the PBS dilution water, dechlorinated tap water, and pre-wash purified water were plated to test for contamination. Microbial reduction at each dose was expressed as $\log_{10}(N_0/N_x)$. N_0 represents the initial concentration in PFU/mL in the control sample without UV exposure. N_x represents the concentration in PFU/mL after exposure to the various experimental flow rates.

RESULTS AND DISCUSSION

QB DOSE RESPONSE

The F-specific RNA bacteriophage Qβ was chosen as the biological indicator for flow through UVLED device challenge testing because it shows first-order rate kinetics at high UV fluences (>40mJ/cm^2) and can capture the high inactivation rates produced under slow flow rates. It is a commonly used for flow through UV device challenge testing (Oguma, Rattanakul, and Bolton 2016; Oguma, Kita, and Takizawa 2016; Jenny et al. 2014) and is the NSF/ANSI 55 challenge organism for UV device challenge testing ("NSF/ANSI 55" 2019). A LP 254nm exposure was performed to ensure quality control of the microbial stocks and comparable results to other QB LP literature values. In addition to the LP 254nm exposures, 280nm UVLEDs were chosen to illuminate the response of microorganisms inactivated in the UVLED flow through device also manufactured with 280nm LED chips.

The doses response of Qβ in three varying UVT_{254nm} waters is presented in Figure 3.1. As expected, the response is the same between UVTs for both UV devices as the fluence calculations account for the UV transmittance of the water sample (Bolton and Linden 2003). LP 254nm produces higher first-

order rate kinetics in Qβ with an inactivation rate constant of 0.0728 cm^2/kJ, similar to other Qβ LRVs following LP 254nm exposure (Jenny et al. 2014; Aoyagi et al. 2011; Beck et al. 2015; Rattanakul et al. 2014; Oguma et al. 2013). The 280nm UVLED produced a lower inactivation rate constant of 0.0410 cm^2/kJ, also in line with previous studies (Aoyagi et al. 2011; Oguma, Rattanakul, and Bolton 2016).

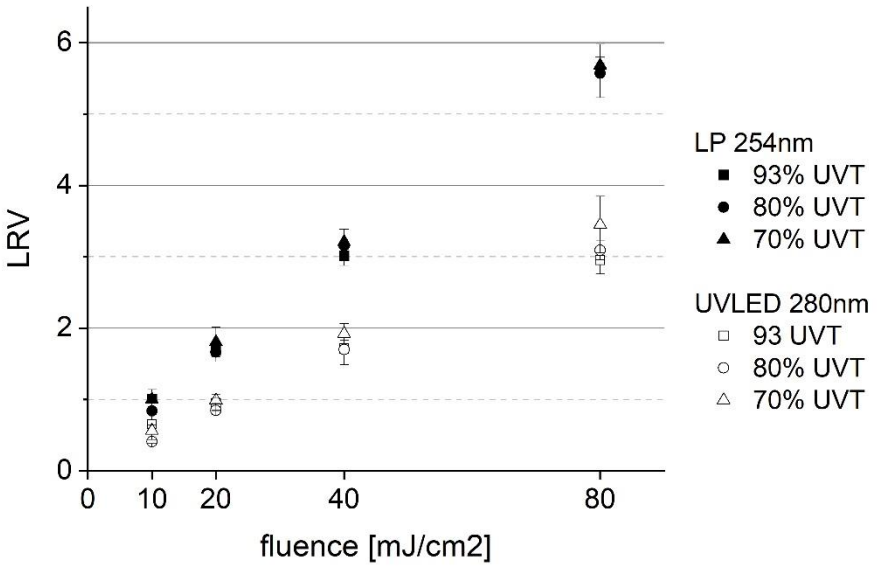


FIGURE 3.1: QB BACTERIOPHAGE LOG REDUCTION VALUES (LRV) (LOG10(N_0/N_x)) OVER THREE UVT_{254nm} WATERS IN RESPONSE TO UV IRRADIATION FROM BOTH POLYCHROMATIC UVLEDS EMITTING AT A PEAK OF 280NM AND A MONOCHROMATIC 254NM LP LAMP.

FLOW-THROUGH RESULTS

The flow through results (Figure 3.2) display a tailing behavior in flow rates over 1 L/min. Tailing has also been observed in other UVLED flow through reactor studies indicating higher inactivation efficiencies at higher flow rates (Oguma et al. 2013). Declining disinfection performances with increasing flow rate is expected as a higher flow rate corresponds to a shorter residence time, and shorter LED exposure, within the reactor (Barstow, Dotson, and Linden 2014). A previous study examining flow through UVLEDs for domestic wastewater disinfection for agricultural reuse found that the REF_{280nm} values were high enough (>50 mJ/cm^2) to meet water reuse guidelines for agriculture of both processed-food crops and non-food crops (US EPA 2012; Nguyen et al. 2019). The results from this study

illustrate that similarly high REF280s at low (<0.05 L/min) flow rates can be achieved; however, the REF decreases as expected at higher flow rates especially for low UVT waters.

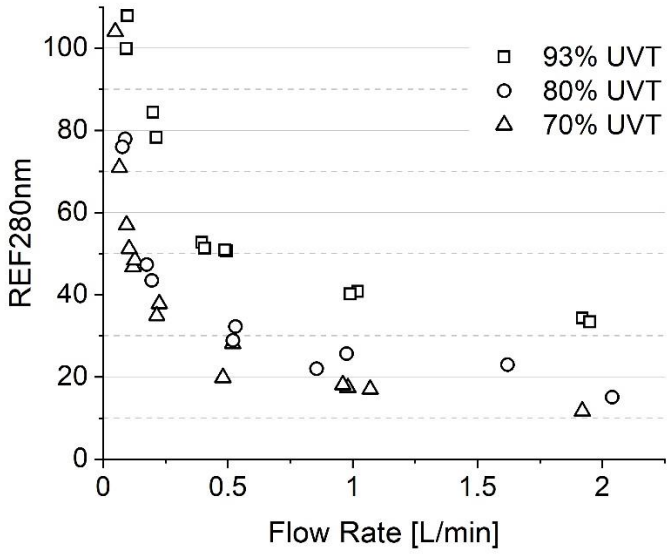


FIGURE 3.2: QB REDUCTION EQUIVALENT FLUENCE VALUES (REF_{280nm}) FOR THE FLOW THROUGH UVLED DEVICE TESTED WITH THREE CHALLENGE WATERS. THE 93% UVT_{254nm} WATER WAS UNALTERED WITH UV ABSORBING COMPOUNDS, WHILE THE 80 AND 70% UVT_{254nm} WATERS WERE ADJUSTED WITH AQUAHUME.

Higher reduction equivalent fluences were reached for the 93% UVT_{254nm} water, with lower values in the 80 and 70% UVT_{254nm} waters. The lower germicidal performance for 80 and 70% UVT_{254nm} waters was anticipated as low UVT indicates a smaller percent of light is transmitted through the water sample. At a flowrate of ~2 L/min, the REF_{280nm} values were 34, 15, and 12 mJ/cm^2 for the 93, 80, and 70% UVT_{254nm} waters, respectively. The 22 mJ/cm^2 REF_{280nm} difference between the 93% and 70% UVT_{254nm} waters is challenging for applications of UVLEDs for wastewater effluent reuse if higher fluence levels are required. Most effluents fall between 70-80% UVT_{254nm} while most UVLED flow through devices are developed for drinking-water applications (93% UVT_{254nm} and higher). This highlights an interesting juxtaposition between the intended use of commercially available UVLEDs versus the available and growing applications for the devices.

The primary objective of this study was to test the UVLED device's ability to disinfect planktonic microorganisms in waters with UV transmittances ranging from 70-93%. A target REF for water reuse at 40 mJ/cm^2, corresponding to a 0.16 L/min flow rate in a UVT_{254nm} 72.7+/-2.5% water, was investigated in the biofilm studies described in the next chapter.

CONCLUSIONS

Challenge testing was conducted for a UVLED flow through device using three test waters of varying UVT_{254nm} (93, 80, 70%). The study concluded that the UVLED device examined in this study performed similarly to other UV flow through devices discussed in literature, with tailing at higher flow rates and decreased performance for low UVT waters (Oguma et al. 2013; Barstow, Dotson, and Linden 2014). When operating at low flow rates, the device produced fluences (>40 mJ/cm^2) able to cause high levels of inactivation of *E.coli* and total coliforms, corresponding to US EPA guidelines for pretreated water during agricultural water reuse of food crops (nondetectable fecal coliforms), and processed/non-food crops (<200 fecal coliforms/100mL) (US EPA 2012). However, this fluence level was not achievable at higher flow rates, as a 1.9 L/min flow rate produced only an 11 mJ/cm^2 REF_{280nm} in the 70% UVT_{254nm} test water.

**The following chapter is presented as a manuscript*

EVALUATION OF DECENTRALIZED UVLEDS FOR BIOFOULING CONTROL DURING DISTRIBUTION OF REUSE WATER

ABSTRACT

While wastewater reuse in agriculture is practiced worldwide, water conservation measures with drip irrigation can lead to excessive clogging when emitters are fed by wastewater effluent. UVLEDs can treat wastewater effluent to US EPA reuse guidelines but, the impacts of UVLED treatment on microbial biofilm formation in the drip irrigation system during the distribution of wastewater effluent for agricultural reuse is unknown. A commercially available UVLED flow through device, operating at 40 mJ/cm^2, was examined for control of biofilm on irrigation pipe material fed by wastewater effluent. Biofouling development was monitored through total coliform counts, a crystal violet staining assay, and ATP analysis. A UV fluence of 40 mJ/cm^2 at 280nm retarded biofilm formation in wastewater effluent; however, complete biofilm prevention was not achieved despite the high inactivation of planktonic cells. The total coliform counts indicated a stable biofilm cell concentration was reached; however, the crystal violet assay showed biofilm biomass accumulation with time. This adds an important discussion surrounding the contribution of viable cells, as represented by total coliforms, and extracellular polymeric substance (EPS) to total biofilm biomass. This study also highlighted the lack of literature surrounding the relationship between ATP responses and complex UV-stress responses of microbial communities as opposed to pure cell cultures. Future studies should examine the effects of higher reduction equivalent fluences (>40 mJ/cm^2) on biofouling during reuse scenarios and the relationship between ATP production in mixed cell communities post-UV to advance the understanding of microbial community response to UV-induced damage.

INTRODUCTION

Water reuse is a promising water management strategy to alleviate water scarcity currently affecting every continent (UN-Water 2018). Water sources for potential reuse include stormwater, industry process water, and municipal wastewater (US EPA 2019a). Uses for reuse water include environmental restoration, groundwater recharge, and agricultural irrigation. Wastewater reuse for agriculture is particularly promising; for instance, Israel recycles 87% of its wastewater for irrigation of both food and non-food crops (Marin et al. 2017). However, irrigating with wastewater effluent comes with challenges including biofouling of drip lines and other infrastructure meant to transport water.

Biofouling, or biofilm accumulation, is the unwanted deposition of microorganisms and sticky extracellular polymeric substances (EPS) on surfaces (Hans-Curt Flemming 2002). Biofilms are established in five stages; 1) attachment of planktonic cells to a surface, 2) attached bacteria divide and EPS is excreted, 3) the EPS cell mixture expands, 4) the biofilm matures, and 5) the mature biofilm releases planktonic bacteria back into the environment (Hans-Curt Flemming, Neu, and Wozniak 2007). Biofilms play a major role in many water reclamation and reuse technologies like membrane reactors and filtration systems used in specific reuse systems (Bishop 2007). The higher concentration of nutrients, organic matter, and microorganisms in wastewater effluent can aggravate biofouling of water distribution systems, like drip irrigation lines. Strategies for biofouling control during distribution of effluent include filtration, flushing, and chlorination. Filtration is an important factor in preventing biofilm formation as it removes biofouling microorganisms and some nutrients that facilitate their growth. Most manufacturers of drip irrigation systems recommend filtration; however, in the case of effluent, filter clogging is a big concern in addition to the clogging of the drip irrigation lines (Ravina et al. 1997). Flushing studies have found that running water at high velocities can effectively slow down emitter clogging in a reclaimed water drip irrigation system, and the longer period it was applied, the better controlling was observed (Q. Li et al. 2019; Y. Li et al. 2015). However, changing water velocity causes an immediate increase in bacteria numbers as the increase in shear stress resuspends biofilms (Lehtola et al. 2006). Chlorination has been considered the most effective method of controlling biofouling during irrigation but has many

drawbacks (P. Song et al. 2017). Chlorine has recently demonstrated adverse soil health effects when applied in high concentrations during short term use which is the common recommendation for treatment of irrigation lines (P. Song et al. 2019).

UV disinfection is a common and effective treatment technology for drinking water, wastewater, and reclaimed water globally (K. Song, Mohseni, and Taghipour 2016b; Nguyen et al. 2019). Recent advancements in UV light emitting diodes (UVLEDs) have allowed for compact, electrically efficient, and customizable point-of-use UV disinfection options. This includes integration of UVLEDs into devices like toothbrush holders, coffee makers, and water coolers (Linden, Hull, and Speight 2019). UVLEDs have demonstrated an ability to treat wastewater effluent to US EPA guidelines for irrigation of processed food crops and non-food crops (Nguyen et al. 2019). However, the impact of UVLED disinfection on biofouling during irrigation with wastewater effluent has not been studied.

The overall goal of this study was to examine a commercially available UVLED flow through device for biofouling control on drip irrigation line material during the distribution of wastewater effluent. The effects of the UVLED device on biofilm growth in a bioreactor fed by effluent was also tested through direct quantification assays (total coliforms) and indirect quantification assays (crystal violet, ATP).

METHODS

WASTEWATER EFFLUENT SAMPLE COLLECTION

Wastewater effluent was collected at the City of Boulder Water Resource Recovery Facility after biological nutrient removal and prior to UV disinfection. Water was analyzed according to Standard Methods for the Examination of Water and Wastewater, Section 1060B (Bridgewater et al. 2017). Parameters tested include pH, oxidation-reduction potential, temperature, phosphate, nitrate, UV transmittance, and total coliforms.

BIOREACTOR SET-UP

Native wastewater effluent biofilms were grown on high density polycarbonate coupons in a Centers for Disease Control and Prevention (CDC) biofilm reactor (model CBR 90-1, Biosurface Technologies Corp., Bozeman, MT). High density polycarbonate coupons were chosen to mimic the adhesion surface found in drip irrigation lines. The bioreactors were operated for five days under semi-batch conditions with effluent replenished after day 1 and day 3. Internal stirring speed of the bioreactor, 60 rpm, was chosen to mimic the retention time of a standard drip irrigation line and shear stress experienced through a drip emitter. Flow rate was based on the REF_{280nm} equations of the UVLED device, used to achieve the target fluence level, and the operating limits of the inlet tubing (MasterFlex L/S 16). The experimental design is illustrated in Figure 4.1.

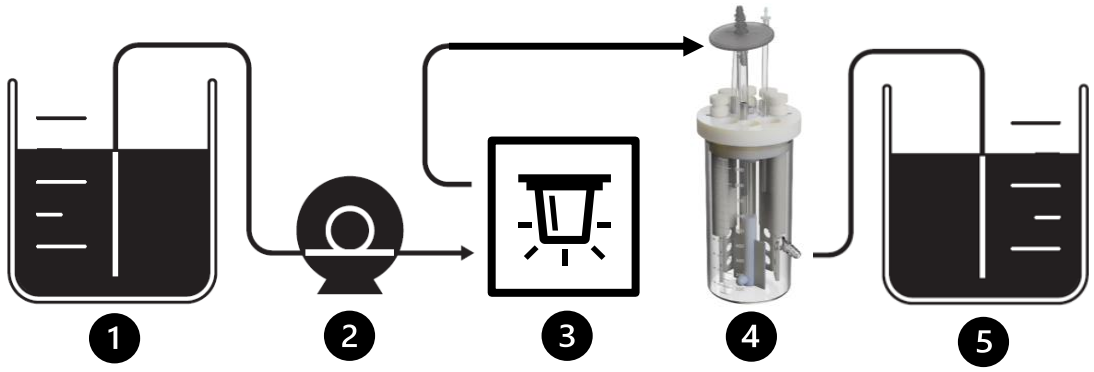


FIGURE 4.1: FLOW THROUGH SCHEMATIC FOR THE FIVE-DAY BIOFILM STUDIES. REUSE WATER (1) IS PUMPED BY A PERISTALTIC PUMP (2) THROUGH THE UVLED FLOW THROUGH DEVICE (3) INTO THE CDC BIOREACTOR (4) WHICH DRAINS INTO THE WASTE CONTAINER (5).

BIOFILM ENUMERATION

TOTAL COLIFORMS BY PLATE COUNT

Total coliforms were enumerated through membrane filtration on m-ENDO agar LES (NutriSelect™ Plus). Each fouled coupon was rinsed in 30 mL PBS for 20 seconds to remove planktonic cells then immersed in a 10 mL PBS solution. A vortex-sonication series was used to disaggregate biofilm

off the coupons. The UltraSonic cleaner (Branson 8210, 50/60 Hz) was degassed for 5 minutes prior to testing. Samples were then sonicated for 30 seconds, vortexed for 30 seconds, with each step being repeated three times. Serial dilutions of 10mL were passed through 0.45 μm membrane filters and the filters were placed on the surface of m-ENDO agar plates, filter side up. Plates were incubated at 35 +/- 0.5ºC for 24 +/- 2.0 hours. Coliforms were identified by red colonies with a golden-green metallic sheen.

ATP BIOLUMINESCENCE

ATP bioluminescence is an indirect quantification method using ATP (adenosine triphosphate) as a proxy marker which infers biofilm viability and biomass. ATP is a nucleoside triphosphate which acts as the primary energy source in all organisms, making it a strong indication of biofilm viability and biomass (Christina Wilson et al. 2017). Coupons were rinsed in 30 mL PBS to remove extracellular ATP then immersed in 2 mL PBS. Coupons were then subjected to 30 s vortexing followed by five minutes of sonication. Samples were then left to incubate at 37ºC for four hours to diminish any ATP increase due to stress induced by UV irradiation (Rauch et al. 2019). Intracellular ATP was quantified using LuminUltra's standard Quench-Gone Aqueous (QGA) Test (LuminUltra Technologies Ltd., New Brunswick, Canada).

CRYSTAL VIOLET ASSAY

Gram staining is a common and optimized indirect biofilm quantification method (Christina Wilson et al. 2017). Crystal violet is a basic trianiline dye that permeates cell membranes in both gram positive and negatives cells. The dye leaves cells violet in color, and after a de-colorization step with an ethanol solution, the biofilm can be quantified via spectroscopy. The crystal violet (CV) assay was conducted according to Charlton (2008). A calibration curve was established between CV concentration and optical density, and a linear relationship was found. After treatment, coupons were immersed in a 2 mL 0.3% CV solution for 90 minutes followed by a PBS rinse and a subsequent DI rinse. Coupons were allowed to air dry, then transferred to a 2 mL 95% ethanol solution. Further dye solubilization was achieved by a 30 second vortex followed by 5 minutes of sonication. In total, 125 μL of sample were added to a 96-well plate and absorbance was measured at 540nm. Five replicate measurements were taken

per sample. Background absorbance was determined with clean HDPE coupons taken through the same stain and rinse steps.

The amount of biofilm remaining on coupons was expressed as percent reduction (PR) where C_{av} is the average control absorbance, B_{av} is the average background absorbance, and T_{coupon} is the treatment coupon absorbance at 540nm.

$$PR = \frac{(C_{av} - B_{av}) - (T_{coupon} - B_{avg})}{(C_{av} - B_{av})}$$

DATA ANALYSIS/STATISTICS

Analysis of Variance (ANOVA) full factorial experiments were performed to examine the statistical significance of experimental effects. Model adequacy was verified by checking the three assumptions required for an ANOVA analysis: 1) equal variances, 2) residual normality, and 3) independent data.

RESULTS AND DISCUSSION

DIRECT QUANTIFICATION METHODS

Direct methods for biofilm quantification are those that rely on a direct observations for quantification of parameters like number of cells or total biofilm volume (Christina Wilson et al. 2017). Inferring biofilm quantity through viable cell numbers should be supported with other assays as many factors affect a biofilms carrying capacity of cells. Carrying capacity is defined as the maximum potential population size a given landscape is capable of supporting and is a common attribute used to describe population dynamics (Stilling 2003). Biofilm cell carrying capacity is dependent on; but not limited to, carbon and oxygen depletion, shear stress, temperature, and iron availability (Madigan et al. 1949). These factors also regulate the rate at which biofilms shed planktonic cells.

TOTAL COLIFORMS

The UV treated and control bioreactors had $10^{2.22}$ and $10^{3.51}$ TC/coupon on day one, comparatively (Figure 4.2). The 1.29 log difference, corresponding to a 3000 TC/coupon difference, indicates the UV treated bioreactor had less biofouling initially. Referencing the dose response of Qβ in Figure 3.1, a

280nm fluence of 40 mJ/cm^2 produced a similar Qβ log reduction value (1.64 LRV) as the coupons on day one (1.29 LRV). This indicates that initially, biofilm formation is inhibited at similar levels to the inactivation of planktonic cells achieved by the UVLED device. After day 1, the control and UV treated coupons both approach concentrations of 10^4 TC/coupon as an apparent coupon carrying capacity was reached. An important distinction not investigated in this study is whether the viable planktonic cells are growing within the bioreactor, then adhering to the coupons and forming biofilms, or if the planktonic cells immediately form biofilms then release more planktonic cells as biofilm maturity is reached.

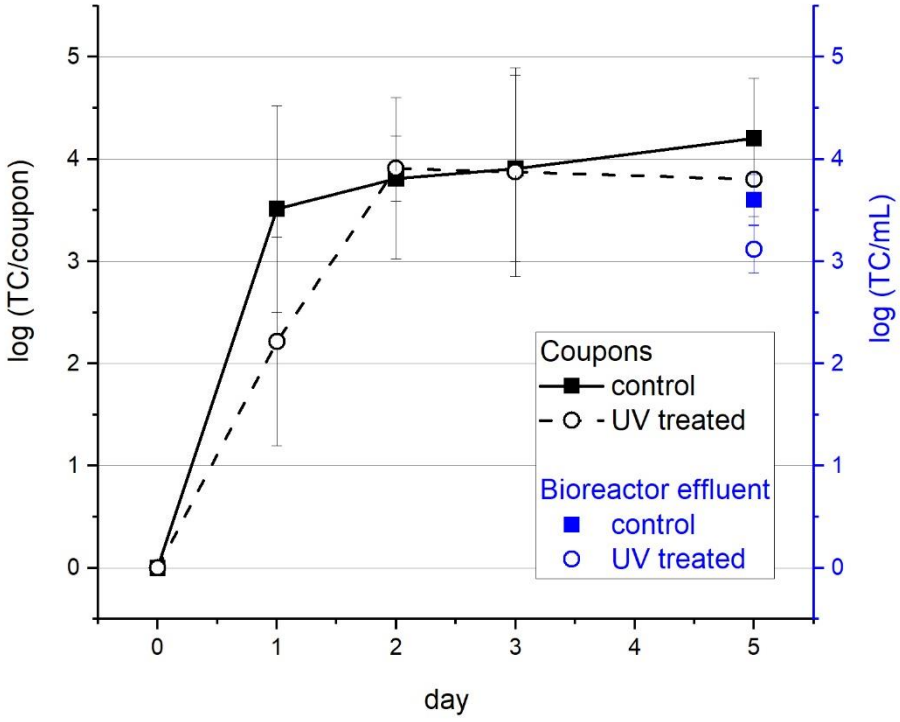


FIGURE 4.2: TOTAL COLIFORM CONCENTRATIONS EXPRESSED AS LOG TC PER COUPON (LEFT AXIS) AND LOG TC PER ML OF BIOREACTOR EFFLUENT (RIGHT AXIS) OVER A FIVE-DAY PERIOD. VERTICAL ERROR BARS REPRESENT STANDARD DEVIATION.

To understand how the UVLED device may change microbial conditions within the bioreactor, bioreactor effluent coliform concentrations were measured on day 5. A full factorial ANOVA analysis revealed that the day 5 bioreactor effluent coliform values were not statistically different (p=0.65). This supports the previous conclusion that any viable cells surviving UV disinfection could potentially

colonize the bioreactor. Interestingly, the cell carrying capacity of the coupons (~10^4 CFU/coupon) is similar to the planktonic cell concentration of the wastewater effluent within the bioreactors (~$10^{3.25}$ CFU/mL). The data suggests that either the planktonic cell concentration environment is influencing the carrying capacity of the biofilms, or the opposite, the biofilm may be influencing the planktonic cell concentration in the bioreactor. If biofilm maturity is reached rapidly, planktonic cell dispersion from the mature biofilm may influence the planktonic cell concentration in the bioreactor effluent.

INDIRECT QUANTIFICATION METHODS

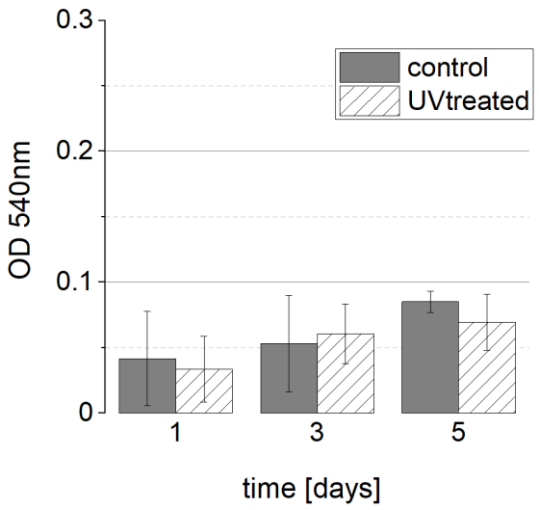
Biofilm growth can be inferred through proxy markers which infer biofilm quantity. There is a general assumption that the proxy substance being measured is directly related to cell concentration within the cell (Azeredo et al. 2017). However, these biofilm quantification techniques are often dependent on metabolic function and biomolecule production which can be dependent on many factors that may not affect cell concentration. This study followed the recommendation provided in Wilson (2017) to pair indirect quantification methods with a direct quantification method.

CRYSTAL VIOLET STAINING

Crystal violet staining was first described by Christensen (1985) and is now one of the most optimized microbiological methods for identification and visualization of bacteria (Christina Wilson et al. 2017). A crystal violet assay was performed days 1, 3, and 5 of the experiment. Although gram positive and gram-negative cells can be differentiated via microscopy after crystal violet staining, this was not examined. The main disadvantage of crystal violet assays is the inability to distinguish between dead and living cells (Christina Wilson et al. 2017); however, a prerinse step was performed prior to crystal violet staining to attempt to "wash" planktonic cells from the coupon biofilm.

The crystal violet adhered to the coupons, represented by optical density, appears to increase slightly over time with no statistical difference between the control and UV treated samples (Figure 4.3). A full factorial ANOVA analysis confirmed that biofilms did accumulate with time ($p=0.033$) and there was no significant difference between the optical density of coupons from the control versus UV treated

bioreactors. The results disagree with the findings in Figure 4.2, which found no biofouling increase after day 2. A possible explanation is that crystal violet may be staining dead or lysed cell materials contained in the EPS, which would not be removed during the wash step (Hans-Curt Flemming and Wingender 2010). In this scenario, the dead/lysed cell material would contribute more crystal violet to the optical density readings suggesting higher biofouling than a viable cell assay, like total coliforms. This hypothesis is supported by McSwain (2005) who observed significant effects by cell lysis and contamination by dead biomass in EPS leading to different and opposing conclusions in biofilm quantification assays. Future studies may benefit from an EPS-specific quantification assay, such as a ruthenium red dye specific to carbohydrates or scanning electron microscopy (SEM) which detects the presence of EPS (Azeredo et al. 2017; Figueroa and Silverstein 1989). This would allow independent observations of the contribution of viable cells and EPS to the total biofilm biomass, provided by the CV assay. Regardless, both total coliform and crystal violet assays indicate that a reduction equivalent fluence of 40 mJ/cm^2 retarded, but did not fully prevent, biofouling in CDC bioreactors fed by wastewater effluent.



day	1	3	5
$OD_{540nm,\ control}$	0.0417 (0.0361)	0.0533 (0.0368)	0.0852 (0.0081)
$OD_{540nm,\ UVtreated}$	0.0338 (0.0251)	0.0607 (0.0227)	0.0695 (0.0213)

FIGURE 4.3: OPTICAL DENSITY (OD_{540NM}) PRESENTED GRAPHICALLY AND NUMERICALLY AT 540NM FOR THE CRYSTAL VIOLET SOLUTIONS DERIVED FROM THE DECOLORIZATION OF CRYSTAL VIOLET STAINED CONTROL AND UV TREATED COUPONS. STANDARD DEVIATIONS ARE REPRESENTED BY THE VERTICAL ERROR BARS AND PARENTHESIS.

ATP BIOLUMINESCENCE

Due to the high cost of ATP analyses relative to the other assays, the ATP analysis was performed on day 5 only. ATP was sampled from the bioreactor coupons as well as bioreactor effluent. UV prevents cells from replicating but does not inhibit ATP production in the cell. To observe differences between UV and non-UV treated samples, the coupon and bioreactor effluent samples were incubated for four hours post-sampling at 37°C to allow for replication of active cells (Rauch et al. 2019). Allowing cells to undergo replication cycles post-UV provides resolution between untreated and non-UV treated samples that would not be detectable immediately following UV disinfection.

On day five, the UV treated bioreactor appears to have slightly higher coupon ATP (54.8 pg ATP/mm^2) than the control coupons (42.9 pg ATP/mm^2) (Figure 4.4). A similar trend is observed for the bioreactor effluents, the UV treated effluent has higher ATP (1678 pg ATP/mL) than the control effluent (1014 pg ATP/mL). Previous studies have reported higher ATP values in *E.coli* following UV (Villaverde, Guerrero, and Barbe 1986). However, these results disagree with the total coliform (Figure 4.2) and crystal violet (Figure 4.3) results, which did not see statistically different results between bioreactors by day five.

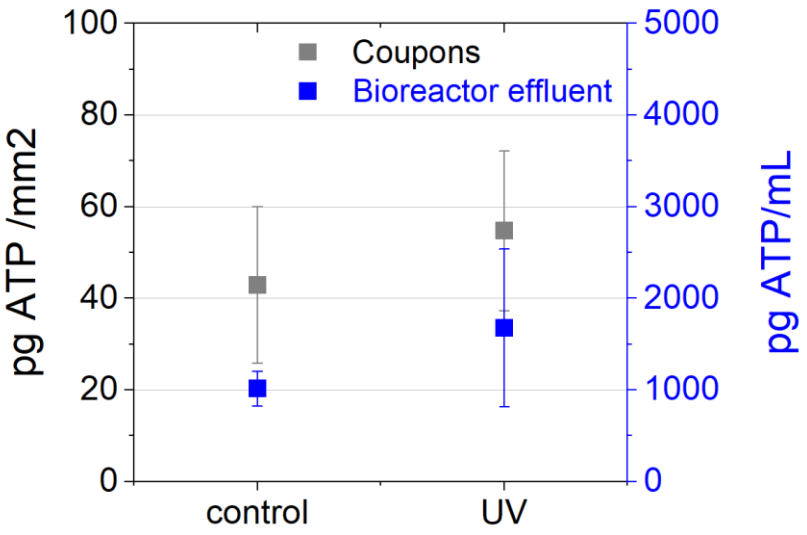


FIGURE 4.4: DAY FIVE ADENOSINE TRIPHOSPHATE (ATP) VALUES FOR THE COUPONS [PG ATP/MM^2] AND EFFLUENT [PG ATP/ML] OF THE CONTROL AND UV TREATED BIOREACTORS.

Rauch (2019) developed an ATP biomass recovery method in *E.coli* and wastewater communities following UV exposure. Adoption of the method has been successfully reproduced in studies examining the effects of UV on pure cell cultures ATP (Gora et al. 2019). Despite development with both pure cultures and microbial communities, the method has yet to be verified in non-pure cell cultures. Miller (2020) found that a 2-hour incubation was enough to diminish background noise for ATP measurements at a pilot-scale direct potable reuse facility, and higher sensitivity was achieved from ATP than flow cytometry following UV-AOP. Hammes (2010) was able to calculate an average ATP-per-cell value

($1.75x\ 10^{-10}$ nmol/cell) from microbial samples taken from a variety of aquatic environments including drinking water, groundwater, river water, and wastewater effluent. Although the study standardized ATP production per cell, the authors also recognized the high ATP heterogeneity of microbial communities of different samples and variations in ATP extraction and analysis procedures when different natural water microbial communities are analyzed (Hammes et al. 2010; Schneider and Gourse 2004; Eydal and Pedersen 2007; Charles Wilson, Stevenson, and Chrzanowski 1981). Rauch (2019) also recognized that variations in microbial communities (like slow-growing organisms) may complicate the method. The ATP results presented in this study suggest that the four-hour 37°C incubation was not sufficient at suppressing heightened ATP production in microbial communities post-UV. Future studies should examine the relationship between UVLED irradiation and natural microbial community ATP, as opposed to pure cell cultures, over time frames beyond four hours.

A different explanation for the ATP results day 5 is the high level of variability characteristic to biofilms and their quantification. Many coliform-specific biofilm studies have noted this stochastic behavior including a study examining coliform retention and biofouling within irrigation pipes (Shelton et al. 2013) and a study investigation drinking water systems plagued by coliform regrowth (Camper, Jones, and Hayes 1996). Through an 18-month survey of 31 water systems in North America, LeChevallier (1996) concluded that the occurrence of coliforms is dependent upon complex interactions between chemical, physical, operational, and engineering parameters. This reinforces that recommendation made in Wilson (2017), to draw upon multiple biofilm assessment methods that include both direct and indirect techniques to improve understanding and knowledge surrounding biofilms.

CONCLUSIONS

A UVLED flow through device was assessed for its ability to prevent biofouling in a CDC bioreactor fed by wastewater effluent operating at a 40 mJ/cm^2 REF_{280nm}. The study concluded the following:

1. The 40 mJ/cm^2 pretreatment delivered by 280nm UVLEDs retarded biofouling in a bioreactor fed by wastewater effluent. By day five, both bioreactors approached 10^4CFU/coupon as well as $10^{3.75}$ coliforms per mL of bioreactor effluent. Future studies should examine higher reduction equivalent fluences or more extensive pretreatments to enhance the biofouling retardation observed at 40 mJ/cm^2. Understanding the interaction between planktonic cells and sessile cells (i.e., whether the planktonic cell concentration is influencing biofouling; or if a rapidly-formed mature biofilm are dispersing planktonic cells) would help advance the conclusions drawn in this study.
2. The ATP results suggest there was higher biofouling on day five in the UV treated bioreactor, despite a four-hour incubation to allow microorganisms to undergo replication cycles and eliminate ATP spikes as cell attempt to repair UV damage. This can be attributed to either 1) the high variability known to exist within biofilm behavior and resulting quantification or 2) insufficient incubation conditions following UV. Natural microbial communities in wastewater samples can vary dramatically between regions and may exhibit varying physiological UV-stress responses depending on community. Future studies examining the relationship between ATP production in mixed cell cultures or microbial communities post-UV should be conducted to strengthen the hypothesis made by Rauch (2019), and to develop a greater understanding of microbial response to UV-induced stress.

UVLEDS FOR WATER REUSE IN LOW-INCOME SETTINGS

WATER SCARCITY

Economic growth and increased demand for food driven by population growth have raised concerns about water scarcity and freshwater resource overexploitation worldwide (Gheewala et al. 2018). According to the UN, global water use has been increasing at twice the rate of population growth, limiting many regions ability to sustainably deliver water (UN-Water 2018). By 2030, 700 million people worldwide could be displaced by intense water scarcity (UN-Water 2018). Integrated water resource management (IWRM) is a process that promotes coordinated development and management of water and land resources to maximize economic and social benefits without compromising vital ecosystems and the environment (UN Department of Economic and Social Affairs 2014). IWRM provides a framework to manage water demand and balance water needs for all users, including those in low-income countries.

IWRM IN INTERNATIONAL DEVELOPMENT

Many government and non-government organizations (NGOs) have started to adopt IWRM practices to meet the needs of clients without comprising vital ecosystems. This is especially relevant in the global engineering sector as marginalized groups are also more likely to suffer from water scarcity (M. Li et al. 2020). In 2006, an IWRM task force was created under UN Water and in 2008 the task force completed its mandate when it presented the 'Status Report on Integrated Water Resources Management and Water Efficiency Plans' at the sixteenth session of the Commission on Sustainable Development (UN Department of Economic and Social Affairs 2014). IWRM was also a critical aspect of the Millennium Development Goals (MDGs), eight international development goals established after the 2000 Millennium Summit of the UN (UN 2015). In July of 2014, the UN General Assembly Open Working Group (OWG) wrote a document containing 17 goals that set the ground for the new Sustainable Development Goals (SDGs) and the global development agenda spanning from 2015-2030. SDG 6 is described as “ensure the availability and sustainable management of water and sanitation for all” with a specific target to implement integrated water resources management at all levels, including through transboundary cooperation as appropriate. Under this goal, the UN created an IWRM Action Program that

provides practical support for countries to enhance the availability and sustainability of water resources ("SDG 6 Synthesis Report on Water and Sanitation Archives" 2018).

In practice, many development organizations have implemented their own IWRM policies. Water for People, a global nonprofit focusing on clean water and sanitation across nine countries, has included IWRM practices in their service approach. This includes an adoption of four IWRM principles: 1) water is a finite and vulnerable resource, 2) water development and management should be based on participatory approaches, 3) women play a central role in water management, and 4) water is an economic good with efficient and equitable use (Water for People 2019). World Vision, a humanitarian aid, development, and advocacy organization, has also integrated IWRM practices into their service strategy. World Vision has utilized the approach to implement multiple-use water systems providing water for domestic use, and agriculture, livestock, and livelihoods. More specifically, "through the IWRM approach, our programmes enable equitable access to water while also considering the impact of pollution in relation with human activities. WASH committees, the basic entities of local WASH governance, consult with the community on needs and the status of the current water supply" (World Vision n.d.).

IWRM FOR AGRICULTURAL WATER REUSE

Adoption of IWRM practices related to agriculture and water reuse have become more common in the past decade (IRC Wash n.d.; Asano, Burton, and Leverenz 2007; Angelakis et al. 2018). Reuse water, also known as reclaimed water and recycled water, is defined as wastewater that has been treated to meet specific water quality criteria with the intent of being used for beneficial purposes (Asano, Burton, and Leverenz 2007). Reuse water has created a new water supply and reduced demands on limited traditional water supplies like surface and groundwater (Asano, Burton, and Leverenz 2007; National Research Council. 2012). This may be especially important with the improvement of water infrastructure in low-income regions. Improved ability to extract and transport water puts strain on existing water resources. IWRM encourages creative water management schemes that look beyond the traditional water supply to meet the needs of agriculture water. This includes a framework for

incorporating water reuse and reclamation into water supply planning to achieve short term water needs goals and long term water supply reliability (Esposito et al. 2005). Because rural low-income regions are highly dependent on agriculture for socio-economic development (M. Li et al. 2020), agricultural water reuse may be particularly impactful.

AGRICULTURAL REUSE IN LOW-INCOME REGIONS

Water reuse is the process of reclaiming water for beneficial purposes like groundwater replenishment, ecological restoration, and agriculture (US EPA 2019b). Many water scarce regions have already adopted water reuse practices including Namibia, Morocco and West Asia (commonly known as the Middle East (Ajam n.d.). The first reuse treatment facility ever developed sits in Windheok Namibia where today, the water reuse treatment facility produces 21000 m^3 of drinking water per day for 350,000 inhabitants. This has also led to a 6 % urban growth of the Windhoek city, attributed to the economic growth fostered by increased water availability ("Namibia: Windhoek Has Been Producing Drinking Water from Its Wastewater for 50 Years" n.d.). Despite the success in Namibia, the funding and engineering resources poured into this project are highly unrealistic for other low-income regions. For example, IRC Wash has reported that only 7% of the population in low-income regions are served by wastewater collection and treatment facilities. Most cities are unsewered and the sewers that do exist discharge untreated sewage into the nearest drainage channel or water course (IRC Wash n.d.). Reuse cannot begin until either improvements in centralized treatment are made or technologies for decentralized treatment, at the point of use, are improved. Developing decentralized wastewater treatment options for agricultural reuse provides promising results.

DECENTRALIZED WASTEWATER TREATMENT

In small communities located in agricultural regions, there is significant potential for reusing wastewater for agricultural irrigation through decentralized or point of use (POU) treatment (Nelson 2005). The important characteristic that distinguishes this type of wastewater treatment from larger centralized treatment is the proximity between the treated water and the potential reuse sites (Nelson 2005). This is especially relevant to low-income settings where water infrastructure for wastewater

collection and distribution from a centralized treatment facility is often lacking or inadequate (IRC Wash n.d.). By treating wastewater in smaller quantities and localized regions, the treatment can be coordinated for specific uses and many barriers to stakeholder engagement are removed.

Common decentralized wastewater treatment technologies include sand filtration, ceramic filtration, and solar disinfection (SODIS) (Barstow, Dotson, and Linden 2014). Recently, UV disinfection has become more popular for decentralized wastewater treatment due to the development of UV light emitting diodes (UVLEDs). In low-income settings, UVLEDs are especially more suitable than traditional mercury lamps due to their low cost, high durability, lower voltage requirements, and lack of toxic mercury (Nguyen et al. 2019). However, there are many challenges to wastewater disinfection with UV technology including the high amounts of suspended solid and turbidity particles, organic matter, and low transmittance characteristics of wastewater (Carré et al. 2018; Mamane 2008). Treatment technologies, like slow sand filters, may alleviate these barriers for decentralized treatment during agricultural reuse. Slow sand filters have demonstrated high efficiency in conventional wastewater treatment for a variety of chemical, physical, and biological pollutants (Jianan Li, Zhou, and Campos 2018; Haig et al. 2014; Paredes et al. 2016). In 2019, a UVLED system fed with wastewater from a slow sand filter, disinfected domestic wastewater in accordance with US water reuse guidelines for irrigation of both processed food and non-food crops (Nguyen et al. 2019; US EPA 2012). This novel study demonstrated the ability of UVLEDs to meet US and international reuse guidelines for decentralized treatment of wastewater for agricultural reuse.

LOOKING FORWARD

Despite these promising results, there are technical obstacles of UVLED wastewater disinfection that have not been demonstrated at field-scale. For example, powering the UV devices may be challenging in regions with an undeveloped electrical grid. The lower power requirements of UVLEDs make solar options feasible but this is yet to be validated at field-scale. Additionally, maintenance requirements related to UV disinfection, like fouling and device lifetime, have not been examined for decentralized

wastewater treatment beyond bench-scale studies. Arik (2004) proposed that LEDs will not be as affected by fouling as traditional mercury lamps due to differences in heat management. Furthermore, Hull (2019) investigated the ability of UVLEDs operating at a reduction equivalent fluence of 40 mJ/cm^2 to treat municipal scale drinking water. Through lab tests and the field studies lasting one year, the device was tested in challenging conditions (i.e., swings in turbidity and temperature) without maintenance like device cleaning. The reactor demonstrated disinfection efficacy and resilience equivalent to the chlorination system (Hull, Herold, and Linden 2019). The researchers also called for future work that considers scale-up to meet flow demands of municipal systems and modifications for lower UVT water such as wastewater and reclaimed water. Accordingly, a separate study examining UVLEDs performance while treating wastewater effluent found organic fouling in the device after 2 days of operation (Nguyen et al. 2019). This is consistent with the results found in this study, that a reduction equivalent fluence of 40 mJ/cm^2 delivered by UVLEDs did not prevent biofouling in distribution systems transporting wastewater effluent. Therefore, to fully demonstrate the efficacy of UVLEDs for agricultural reuse in low-income settings, more robust treatments (like slow-sand filtration) or higher fluences must be investigated. The cost, power, and maintenance requirements of these systems must also be examined in context-specific studies. Once these technical investigations are completed, the social appropriateness of UVLED technology and long-term implications must be assessed in the project-specific environment prior to implementation.

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