UV Light Emitting Diodes (LED) for Degradation of Antibiotic Resistant Bacteria and Genes

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UV LIGHT EMITTING DIODES (LED) FOR DEGRADATION OF ANTIBIOTIC RESISTANT BACTERIA AND GENES

by

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ABSTRACT
UV LIGHT EMITTING DIODES (LED) FOR DEGRADATION OF ANTIBIOTIC RESISTANT BACTERIA AND GENES

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The presence of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) in the environment is a growing issue, which been exacerbated by the overuse and misuse of antibiotics in various healthcare and agricultural systems. One possible means of antibiotic resistance mitigation is through drinking water and wastewater treatment, specifically disinfection processes. Ultraviolet light emitting diodes (UV-LEDs) are an emerging disinfection technology that utilize LEDs that emit at varying peak wavelengths in the UV light spectrum. This study analyzed the effect of UV-LED on ARB and ARGs compared to conventional low-pressure UV (LP-UV) treatment and calculated electrical energy efficiencies for each system. Three peak polychromatic UV-LED wavelengths were utilized: 255 nm, 265 nm, and 285 nm; LP-UV emits a monochromatic wavelength at 254 nm. The ARB used here was TOP10 *E. coli* containing the pUC19 plasmid which carries resistance to ampicillin via the bla_{TEM} gene. Two forms of ARGs were used: intracellular (bla_{TEM} incorporated in the cell's DNA in intact cells) and extracellular (free floating bla_{TEM} plasmids). Increasing UV fluence increased log inactivation for both bacteria and genes. ARB tests indicated that damage to DNA (255 nm, 265 nm, LP-UV wavelengths) was more effective for inactivation than damage to cell proteins (285 nm wavelength) due to higher kinetic rate constants. Overall, the ARGs were less effectively damaged compared to bacteria. Furthermore, intracellular DNA was less effectively damaged than extracellular DNA, possibly because of the protective structures that encompass intracellular DNA. The UV-LEDs exerted larger energy demands compared to LP-UV treatment. This difference can be attributed to the low wall plug efficiencies for the LED wavelengths compared to LP-UV treatment. Energy efficiency was in the order of ARB < extracellular DNA < intracellular DNA, whereas the relative order was reversed considering first order rate constants for inactivation. Overall, UV-LEDs can successfully inactivate ARB and ARGs; however, for LEDs to become more competitive with LP-UV systems, energy efficiencies will have to improve greatly.

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DEDICATION

Nicole A. Heyniger

I would like to dedicate this thesis to my family; parents Susan and Nick Heyniger, and brother John Heyniger, whose support I am incredibly grateful for through my academic journey and time at Marquette. I would also like to dedicate this thesis to my incredible friends, who continually inspire me in all aspects of life. I would not be here without you all.

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1 INTRODUCTION

1.1 Motivation

The presence of potentially harmful antibiotic resistant microorganisms (and their related antibiotic resistance genes) in water is a major public health concern. Antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) pose a risk to health care systems, not only clinically but financially as well (Davies, 2010). Although naturally occurring, the overuse and misuse of antibiotics due to anthropogenic activity has created environmental reservoirs of resistance (Davies, 2010). These ARGs have been found in surface waters, groundwater, drinking water, wastewater, and biofilms (Stange et al., 2019).

Antibiotic resistance decreases the effectiveness of an antibiotic by developing the ability to survive and multiply in the presence of the antibiotic (*CDC,* n.d.). Deemed a critical human health challenge by a World Health Organization report in 2001, antibiotic resistance is a growing problem (Pruden et al., 2006). A 2019 report from the United States Centers for Disease Control and Prevention (CDC) stated that around 2.8 million infections are attributed to antibiotic resistance in the United States, resulting in more than 35,000 deaths (*2019 CDC Report*, n.d.). Considered an emerging contaminant, the need for research on solutions addressing antibiotic resistance is significant. Also, certain processes, industries, or microorganisms can select for resistance and contribute to its spread (Stange et al., 2019). ARB can then share their ARGs through gene transfer mechanisms, either vertically or horizontally. This can happen in cells that are alive, but also when they degrade or die because deoxyribonucleic acid (DNA) can lyse from the cell. In this case, free-floating DNA containing antibiotic resistance can be taken up by

new cells through transformation (Davies, 2010). Because the spread of antibiotic resistance is a growing issue, water and wastewater treatment plant processes are being studied for the mitigation of ARB and ARGs (Stange et al., 2019). Active development of advanced water treatment technologies is ongoing to address these concerns. One possible technology to mitigate the hazards of antibiotic resistance is ultraviolet (UV) treatment.

The use of UV light emitting diodes (UV-LEDs) is an emerging application in water treatment operations and is attracting significant attention. UV light interacts with DNA to cause base pair modification, thus inhibiting DNA replication. DNA absorbs UV light best at a wavelength around 260 nm, while proteins in the cell absorb UV best around 280 nm (Beck et al., 2017; Olson & Morrow, 2012). Compared to the conventional low-pressure UV (LP-UV) systems often used in water and wastewater treatment processes, UV-LED advantages include a fast start-up time, more options in configuration (and thus wavelengths), and the elimination of harmful mercury used in conventional UV processes (Nyangaresi et al., 2018). Moreover, UV-LEDs can be smaller, lighter, and less fragile than conventional LP-UV lamps (Beck et al., 2017). Since UV-LEDs have a Gaussian distribution of wavelengths, there is potential that a UV-LED system could act similarly to a medium pressure (MP) UV system, which can sometimes be more effective than LP-UV (Beck et al., 2017).

Disadvantages to UV-LED include low wall plug efficiency, which is expected to improve in the future (Beck et al., 2017; Chatterley & Linden, 2010). However, there is a lack of understanding of UV-LEDs that could lead to underutilization of the technology (Chen et al., 2017). This research seeks to advance understanding of UV-LED-based

destruction of an important class of emerging water contaminants: antibiotic resistant organisms.

1.2 Objectives

The goal of this research is to better understand how UV-LED systems affect the degradation of ARB and ARGs compared to conventional LP-UV treatment. Toward this aim, the efficiency of treating ARB and ARGs, both of which are emerging threats in drinking water, was evaluated using UV drinking water treatment. Specifically, the first objective was to evaluate the inactivation of ARB using UV-LEDs and compare this to inactivation using conventional LP-UV treatment, quantified as the inactivation of ARB, where the cell can no longer replicate. The second objective was to evaluate the inactivation of ARGs using UV-LEDs and compare this to inactivation using LP-UV treatment. This is defined as ARG reduction, which is quantified through quantitative polymerase chain reaction (qPCR), which captures DNA damage (where DNA is no longer quantifiable). The genes studied here were tested in two different configurations: intracellular (iDNA), or DNA that is located inside of the cell, and extracellular (eDNA), or DNA located outside of the cell. Using data from the first two objectives, the energy efficiency of inactivating ARB and ARGs from the water matrix using UV-LEDs in comparison to treatment using conventional LP-UV was calculated. As LEDs can be operated at different light wavelengths, which offers variable efficacy of disinfection, three different LED wavelengths were utilized for testing: 255 nm, 265 nm, and 285 nm. These wavelengths are predetermined output wavelengths from the manufacturer, however, are aimed to target DNA absorption (around 260 nm) and protein absorption (around 280 nm) (Beck et al., 2017; Olson & Morrow, 2012).

1.3 Thesis Structure

Chapter 2 provides a comprehensive literature review on the state of research involving UV-LED inactivation of ARB and ARGs. **Chapter 3** presents detailed methods of how experimental results were obtained. **Chapter 4** presents the project results and discussion. A summary of the research project and key conclusions gathered from the results is presented in **Chapter 5**.

2 LITERATURE REVIEW

2.1 Antibiotic Resistance and UV Light Treatment

Previous studies have found that UV disinfection can successfully inactivate ARB and ARGs (Childress et al., 2014; Guo et al., 2013; Rizzo et al., 2013; Yuan et al., 2015; Y. Zhang et al., 2015; Zheng et al., 2017). However, different UV fluences (these studies used UV fluences ranging from 5 to 4000 millijoule per centimeter squared (mJ/cm^2)), and differences in the microorganisms and genes studied affect the effectiveness of UV disinfection. Many studies highlight that ARB are more easily inactivated than their ARG counterparts (Pang et al., 2016; Stange et al., 2019; Z. Zhang et al., 2019). This could be due to bacterial inactivation resulting from a single critical gene disruption in the bacteria's genome, while ARG reduction is the damage to that particular gene itself. Differences between iDNA and eDNA have also been shown for gene mitigation using UV. For example, McKinney & Pruden (2012) discovered that the four ARB they evaluated (two gram-positive and two gram-negative) experienced 4-log inactivation at a UV fluence of 10-20 mJ/cm^2, while the iDNA and eDNA exhibited 3-4 log reduction at a UV fluence of 200-400 mJ/cm^2. Yoon et al. (2017) studied reduction of intracellular and extracellular genes with LP-UV treatment and found that intracellular genes showed lower rates of damage compared to extracellular genes.

Several studies tested LP-UV and types of ampicillin-resistant *E. coli* or its related ARGs (as studied here). For example, Guo et al. (2012) demonstrated 3.5-log inactivation for ampicillin-resistant *E. coli* with a UV fluence of 5 mJ/cm^2. This result was similar to an earlier study by the same authors that reported a 3-log inactivation using a UV fluence of 5 mJ/cm^2 (Guo et al., 2009). Log inactivation values reported for

ampicillin-resistant *E. coli* ranged from 0.5-6.5 for a UV dose of around 5 mJ/cm^2 (Guo & Kong, 2019; Pang et al., 2016; Templeton et al., 2009; Yoon et al., 2017). Other studies showed a higher range of inactivation (up to 6-log inactivation) for a larger fluence range (up to 20 mJ/cm^2) (Quek & Hu, 2008; C. M. Zhang et al., 2017). One study found that it took a UV fluence up to 300 mJ/cm^2 to achieve a 4.7-log inactivation (Stange et al., 2019). Overall, ampicillin-resistant *E. coli* was shown to be effectively inactivated using LP-UV, although inactivation varied with UV fluence, type of bacteria, and testing conditions.

Chang et al. (2017) reported 1-log reduction for the bla_{TEM-1} plasmid with a UV fluence of 20-25 mJ/cm^2 using long amplicon qPCR (longer amplicons utilized during qPCR in attempt to cover entire gene sequences). The group also found 1-log inactivation with a much higher UV fluence (up to 400 mJ/cm^2) using short amplicon qPCR. They determined that longer amplicons should be used when trying to capture ARG transformation capability, not necessarily DNA damage, which is assessed in this study. Similar results reported by Destiani et al. (2018) indicate that a fluence of 159 and 21 mJ/cm^2 is needed for 1-log reduction for iDNA and eDNA bla_{TEM-1}, respectively. Pang et al. (2016) reported 1.2-log reduction of bla_{TEM-1} for a fluence of 80 mJ/cm^2, and that this ARG required a higher fluence for reduction than ampicillin-resistant *E. coli* also studied. Thus, there appears to be consensus that ARBs are more easily inactivated than their ARG counterparts.

2.2 UV-LEDs on Antibiotic Resistant Bacteria and Gene Mitigation

Many UV-LED studies have found that that UV-LED at a peak wavelength of around 265 nm achieves higher inactivation of microorganisms than other wavelengths

(Beck et al., 2017; Chatterley & Linden, 2010; Li et al., 2017; Nyangaresi et al., 2018; Oguma et al., 2013). For example, Beck et al. (2017) and Nyangaresi et al. (2018) observed up to 4.5-log inactivation with a UV fluence of 16 mJ/cm^2 for *E. coli* using a peak wavelength of 265 nm. However, Martino et al. (2021) reported the highest inactivation for *E. coli* at 255 nm, followed by 265 nm, then a significant drop in efficiency for the 285 nm LED. When compared to LP-UV treatment, two studies showed that the inactivation of *E. coli* was less efficient using UV-LED compared to LP-UV (Bowker et al., 2011; Rattanakul & Oguma, 2018).

Umar et al.'s (2019) review highlighted the need for more research on ARG reduction using UV-LED. Additionally, the role of UV-LED on iDNA and eDNA has not been widely studied (Zhang et al., 2019). To date, only two studies (to the author's knowledge) have evaluated extracellular ARG reduction using UV-LEDs. Krzeminski et al. (2020) studied treatment of tap water spiked with cell-free *E. coli* plasmids that contained resistance to ampicillin and kanamycin using UV-LED at 265 nm. They reported that the 265 nm wavelength damaged the cell-free plasmids, finding that 1-log reduction required a UV fluence of 73 mJ/cm^2 using a short amplicon analysis, and that a 23 mJ/cm^2 UV fluence was needed for the same log reduction using a long amplicon analysis (Krzeminski et al., 2020). Although both amplicon approaches can detect DNA damage, the short amplicon approach is more conservative. Umar et al. (2021) studied eDNA degradation using plasmids containing resistance to ampicillin and kanamycin as an eDNA-ARG model. The UV-LEDs used in the study operated at peak wavelengths of 265 nm or 285 nm, and results showed that both UV-LED wavelengths effectively damaged the ARGs, but 265 nm was more efficient (Umar et al., 2021).

2.3 Inactivation of Microorganisms and Genes as a Function of Electrical Energy Efficiency

Limited studies have considered the inactivation of microorganisms and genes as a function of electrical energy efficiency, assessed in this study as the electrical energy per order (EEO) measurement. Although UV-LED has the potential to replace traditional LP-UV systems, the research in this field is not yet sufficient to make logical conclusions (Nyangaresi et al., 2018). Studies on *E. coli* mainly show that LED wavelengths around 280-285 nm have higher electrical energy efficiency when compared to LED wavelengths around 255-265 nm for various bacteria and bacteriophages. Specifically, EEO values ranged from 0.137-0.6 kilowatt-hour per meter cubed (kWh/m^3) for wavelengths around 275-285 nm and 0.142-1.2 kWh/m^3 for wavelengths around 255-267 nm (Beck et al., 2017; Martino et al., 2021; Rattanakul & Oguma, 2018; Umar et al., 2019). However, LP-UV systems are still more efficient (values ranged from 0.006-0.0099 kwh/m^3), which can be attributed to higher wall-plug efficiencies (WPE) (Beck et al., 2017; Rattanakul & Oguma, 2018). There have been no studies on the electrical energy required for different levels of ARG inactivation using UV-LEDs (Umar et al., 2019).

2.4 Summary of Research Needs

As described in the review above, UV inactivation of ARB and ARGs is an active field of research. There is more literature on UV-LED inactivation of ARBs compared to ARGs, especially eDNA.

Current gaps in the literature, mainly regarding UV-LED reduction of ARGs (eDNA specifically) and assessing the electrical energy efficiency of ARGs using UV-

LED, highlight the need for more research. Rooted in the objectives described in Chapter 1, this thesis research aimed to generate knowledge on using UV-LEDs for antibiotic resistance mitigation, as well as assessments of energy efficiency for UV-LEDs compared to conventional LP-UV treatment. This work will specifically address the research gap of using UV-LED at variable wavelengths for ARG degradation. The specific objectives and hypotheses were:

Objective 1: Evaluate the inactivation of ARB using UV-LEDs and compare this to inactivation using conventional LP-UV.

Hypothesis: The UV-LED system more effectively inactivates ARB than the traditional LP-UV system because with multiple peak wavelengths, it can target damage to the bacterial cell's DNA and also cell's proteins (whereas LP-UV systems typically target DNA only and not proteins).

Objective 2: Evaluate the reduction of ARGs using UV-LEDs and compare this to reduction using conventional LP-UV.

Hypothesis: Based on previous literature, ARGs will be less effectively damaged using UV treatment compared to the bacteria themselves (Pang et al., 2016; Stange et al., 2019; Zhang et al., 2019).

3 METHODS

3.1 Bench Scale Experimental Approach for LED and Low-Pressure UV Setup

Bench-scale LED and LP-UV setups were utilized to test the effect of variable wavelength treatment on ARB and ARG inactivation.

3.1.1 Reactor Setup

Bench-scale UV testing was performed for all UV experiments. The LP-UV system was designed in-lab, based on protocols published by Bolton and Liden (2003) and Kuo et al. (2003), as described by Gerrity (2008). This system emits nearly monochromatic light at a wavelength of 254 nm. The UV-LED apparatus was the AquiSense Pearlbeam^{TM} (AquiSense Technologies, Erlanger, KY) a commercial system equipped with UV-LEDs. The system operates at 255 nm, 265 nm, or 285 nm peak wavelength options. Figure 1 depicts the two different UV systems (a shows the UV-LED system and b shows the LP-UV system).

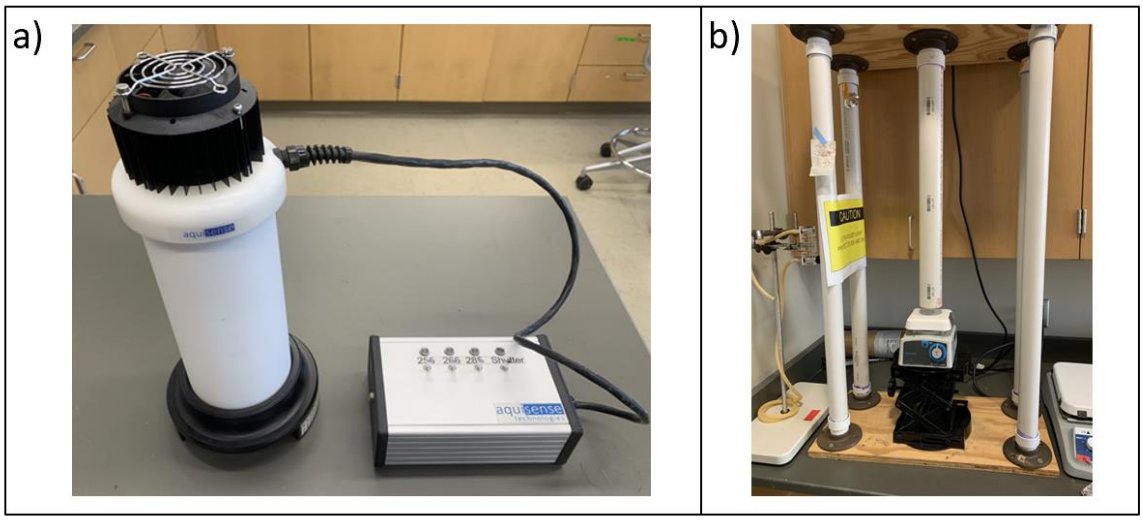

***Figure 1**: **UV systems used for the study**. a) AquiSense PearlBeam UV-LED system in-lab. b) Picture of the LP-UV system in-lab.*

The UV fluences (sometimes referred to as UV doses) were determined based on the time of exposure of the bacteria or gene samples and the irradiance of the UV wavelength, shown in Equation 1.

$$UV\ Fluence\ \left(\frac{mJ}{cm^2}\right) = Irradiance\ \left(\frac{mW}{cm^2}\right) * Time(seconds)$$ [Equation 1]

The irradiance of the beam for each wavelength was measured at the water surface and calibrated with a radiometer (ILT 1700 Research Radiometer with SED270 International Light, Newburyport, MA). The emission spectra of the beam was calibrated using the Ocean Optics Spectroscopy Wizards application. In order to calculate the actual average irradiance, correction factors were applied, as described by Bolton & Linden, (2003). The correction factors included the reflection factor (RF) = 0.975, divergence factor (DF) = 0.975, water factor (WF) ≈ 0.98, and petri factor (PF) ≈ 0.79 (specifically, 0.845, 0.862, 0.745, and 0.699 for 255 nm, 265 nm, 285 nm, and LP-UV, respectively). Since the LED emissions spectra is polychromatic, an Ocean HDX-UV-VIS spectrometer (Ocean Optics, Dunedin, FL) was used to calculate a sensor factor. The adjusted average irradiances incorporating the correction factors were calculated as 0.059, 0.439, 0.577, and 0.186 mW/cm^2 for the 255 nm, 265 nm, 285 nm, and LP-UV wavelengths, respectively.

3.1.2 Bacterial Cultivation and Inoculation

E. coli TOP10, a Gram-negative bacteria, hosting the plasmid backbone pUC19, was used as the model ARB in this study. The plasmid pUC19 (2686bps) is a commercially available *E. coli* cloning vector that contains ampicillin resistance

genes, which are the target ARGs in this study. Bacteria were initially grown by quadrant streaking a tryptic soy agar (TSA) plate containing 200 µg/mL ampicillin (AMP200) with a frozen stock culture of *E. coli* TOP10 with pUC19 (-80 °C). After 24 hours of incubation at 37 °C, the plate was deemed usable if isolated colonies were visible. The plate was then wrapped in parafilm and stored at 4 °C. The colonies were transferred every 2 weeks to a new TSA plate (TSA+AMP200) to ensure the bacteria remained viable (Bari & Yeasmin, 2021). Plate transfers were done until the bacteria were no longer viable, in which case the process (from frozen stock) would then be repeated.

3.1.3 Experimental Process

On each experiment day, bacteria were transferred from the TSA+AMP200 plate into BD Difco™ tryptic soy broth (TSB) containing AMP200. The bacteria were grown to log phase by incubating and shaking the bacteria at 37 °C while checking the optical density at a wavelength of 600 nm every 30 minutes – 1 hour until the target optical density of 0.4 was achieved (*The OD600 Basics* n.d.). A cell wash step was utilized to remove residual growth media from the *E. coli* to minimize organic competition. This process included three cycles of centrifugation at 2000 rotations per minute (rpm) for 10 minutes, pouring out the liquid, resuspending the bacterial pellet in buffered demand free (BDF) water (BDF, comprising 0.54 g of G-Biosciences® Na_2HPO_4 and 0.88 g of Sigma-Aldrich® ≥ 98% KH_2PO_4 in 1.0 L of Milli-Q water, pH adjusted to near neutral levels), and vortexing.

The water matrix used for both experimental samples and the subsequent serial dilutions was BDF water. Each test used 14 mL of BDF spiked with approximately

10^6 colony forming units (CFU) per 1 mL bacteria in a sterile, 60 x 15 mm quartz Petri dish. Before exposure, the LP-UV system warmed up for at least 15 minutes. During exposure, the Petri dish was centered under the collimated beam and stirred using a mini-magnetic stir bar. A range of fluences from 0.3 to 5.5 mJ/cm^2, and 1.2 to 740 mJ/cm^2, for ARB and ARG experiments, respectively, was tested under the different wavelength. Each test was conducted in triplicate. Following UV experimentation, 1 mL was transferred to a microfuge tube for serial dilutions. From the microfuge tube, serial dilutions were performed and plated using the spread plate technique on TSA + AMP200 plates. Plates were incubated at 37 °C for roughly 24 hours and CFUs were counted in the range of 30-300 CFU per plate.

3.2 ARG Analysis

3.2.1 DNA Extraction for iDNA analysis

Experiments were repeated for iDNA using the procedures above with the difference being that 10 mL were saved from each test in a centrifuge tube for iDNA analysis. The 10 mL sample was centrifuged at 10,000g for 30 minutes at 4 °C to concentrate DNA in the form of a pellet containing bacterial cells (resulting in a pellet containing only iDNA) (Corinaldesi et al., 2005). The supernatant was dumped, and the pellet was frozen at -20 °C until DNA extraction. DNA extraction was performed using the FastDNA Spin Kit (MP Biomedicals, Solon, OH). An initial cell lysis step was utilized using liquid nitrogen freeze thaw cycling (3x), followed by the manufacturer's protocol (Kimbell et al., 2021). The iDNA concentrations in the eluted DNA extracts were quantified and deemed suitable via A260/280 purity measurements with microspectrophotometry using a Nano-Drop (Nano-Drop™ Lite,

Thermo Scientific, Waltham, MA). DNA extracts were stored at -20 °C until further analysis.

3.2.2 eDNA

For eDNA experiments, the ARG bla_{TEM} plasmid was used as a representative extracellular ARG. The plasmid was obtained from pre-made qPCR standards, and stored at -20 °C. The bla_{TEM} qPCR standard was prepared as methods described by Kappell et al. (2015). A concentration of approximately 10^7 gene copies/μL of the plasmid was diluted (1:14,000) in molecular-grade water, mixed, and exposed under the variable UV wavelength treatment as described in Section 3.1.3. Following UV exposure, samples were stored at -20 °C until qPCR.

3.2.3 qPCR Analysis

Quantitative PCR was used to quantify gene copies in the samples. All qPCR assays were conducted on a Roche LightCycler® 96 (Roche Molecular Diagnostics, Pleasanton, CA). Each qPCR assay consisted of a total reaction volume of 20 μL, with 10 μL PowerUp™ SYBR® Green Master Mix (Master Mix), 2 μL of each forward and reverse primers (10 μM) (details shown in Table 1), 5 μL of DNA extract or sample, and 1 μL of molecular-grade water. Gene concentrations were analyzed in triplicate. Standard curves were created for the bla_{TEM} gene by conducting a ten-fold serial dilution of plasmid DNA yielding 10^0 to 10^7 copies per reaction. Standard curves and negative controls were conducted in triplicate and were included in each 96-well plate. The standard curve qPCR method was utilized to determine the quantity of the target gene in each experimental sample (Larionov et al., 2005). Negative controls were used in the qPCR analysis to ensure no contamination from

outside sources, and were composed of molecular grade water, qPCR primers, and Master Mix. By using this method, the qPCR output generates DNA concentration in gene copies / μL, which then can be used to calculate gene reduction.

Table 1: List of qPCR primers used for this research.

Target Gene	Antibiotic	Primers	Sequence	Amplicon size (bp)	Annealing Temperature °C
bla_{TEM}	Ampicillin	blaTEM-FX	GCKGCCAACTTACTTCTGACAACG	257	60
		blaTEM-RX	CTTTATCCGCCTCCATCCAGTCTA		

3.3 Data Analysis and Statistics

The Chick-Watson equation was utilized to model kinetics, microbial inactivation, and gene reduction, as shown in Equation 2.

$$\log\left(\frac{N}{N_o}\right) = -\Lambda_{CW}(It) \quad \text{[Equation 2]}$$

Where, N_o = initial microbe concentration via control plate counts (CFU/mL), N = microbe concentration following treatment (CFU/mL), Λ_{CW} = Chick-Watson coefficient of specific lethality (mJ/cm^2), I = UV irradiance (mW/cm^2), and t = time (seconds). The Chick-Watson coefficient of specific lethality was determined as the slope of the linear regression line on plots of UV fluence vs. log inactivation (mJ/cm^2), forced through the intercept (0,0).

Energy efficiency of the system was evaluated using the EEO metric, shown in Equation 3 (Beck et al., 2017; Bolton et al., 2001; Martino et al., 2021).

$$EEO = \frac{A}{(3.6 * 10^6)(V)(\Lambda_{CW})(WPE)} \quad \text{[Equation 3]}$$

Where, EEO = electrical energy per order or the amount of energy needed to decrease the concentration of the contaminant by one order of magnitude (kW-hr/m^3), A = irradiated surface area (cm^2), V = sample volume (L), WPE = wall plug efficiency reported by the manufacturer (0.3% for 255 nm, 0.7% for 265 nm, 1.3% for 285 nm, and 32.7% for LP-UV). The factor 3.6 x 10^6 accounts for the conversion between hours and seconds, mW and kW, and m^3 to L.

All statistics were completed using GraphPad Prism 9 (GraphPad Software, San Diego, CA), with a significance level of $\alpha = 0.05$. One- or two-way ANOVA was used, along with Tukey's multiple comparison test to assess significant differences in the data.

4 RESULTS AND DISCUSSION

4.1 The impact of UV Wavelengths on ARB Inactivation

The impact of variable wavelength UV treatment on ARB inactivation was assessed for the ARB *E. coli* TOP10 with pUC19 plasmid. It was hypothesized that the ARB would be inactivated more effectively using UV-LED treatments compared to the traditional LP-UV system because depending on which peak wavelength is utilized, damage to the cell's DNA and also proteins can be targeted (whereas LP-UV systems typically target DNA only and not proteins due to it emitting monochromatic light at 254 nm). As per the methods described above, the ARB was added to water samples and exposed to UV light for varying times to ascertain how quickly the bacteria were inactivated using UV-LEDs compared to LP-UV.

4.1.1 Inactivation and Kinetics data for ARB

Greater bacteria inactivation was observed as fluence increased (Figure 2). Apart from 285 nm, up to 5.5-log inactivation was observed for UV fluences around 2-6 mJ/cm^2. Moreover, small standard deviations, defined as deviations close to the overall mean of the data set, are observed for the data points (ranging 0.001-1-log inactivation), meaning the experiments had high reproducibility. This, along with the R^2 values in Table 3 (above 0.96) also suggest that the data can be further used to accurately predict a certain level of bacterial inactivation with a given UV fluence. Every wavelength performed statistically different from the others, apart from the 255 nm vs. 265 nm comparison (Tukey's multiple comparison test, Table 2).

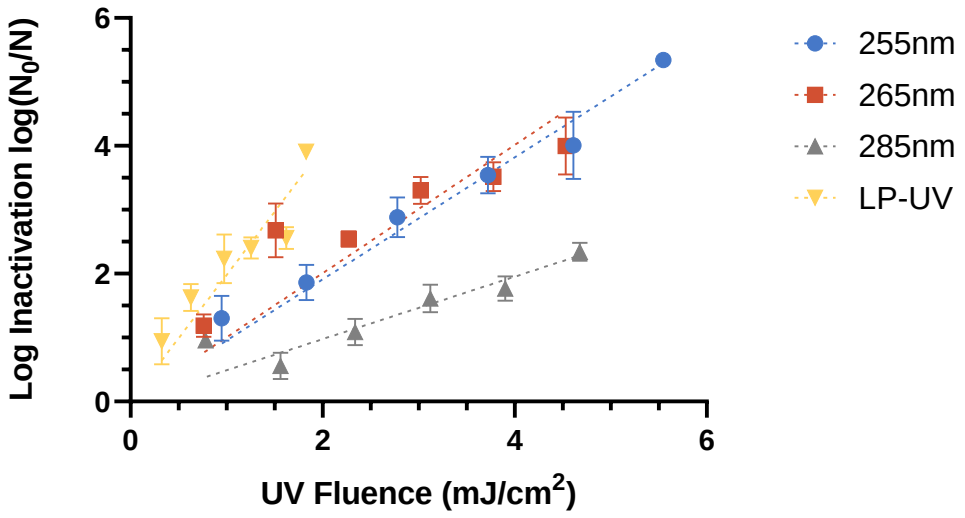

Figure 2: ARB inactivation for each wavelength of UV treatment. *Error bars show ± 1 standard deviation of triplicate experiments (points are means). The lines indicate linear regression models fit to each type of UV treatment system.*

Table 2: Tukey's multiple comparison test for the UV wavelengths during ARB inactivation.

Tukey's Multiple Comparison	Adjusted P value
255 nm vs. 265 nm	0.0731
255 nm vs. 285 nm	<0.0001
255 nm vs. LP-UV	<0.0001
265 nm vs. 285 nm	0.0236
265 nm vs. LP-UV	<0.0001
285 nm vs. LP-UV	<0.0001

Table 3 shows the modeled rates of inactivation (regression slopes using classic Chick-Watson disinfection kinetics), which indicate that LP-UV treatment was the most effective (highest slope), followed by UV-LED 265 nm and 255 nm (between which there was no statistical difference, p = 0.0731), and UV-LED at 285 nm. The finding that LP-UV was the most effective treatment compared to UV-LEDs is similar to results

reported by Rattanakul & Oguma (2018), and Bowker et al., (2011). With respect to the UV-LEDs, 265 nm outperformed the other LEDs, also reported by Chatterley & Linden (2010), Oguma et al. (2013), Beck et al. (2017), and Li et al. (2017). The drop in efficiency that the UV-LED system exhibited for the 285 nm wavelength is consistent with reports by Martino et al. (2021). This outcome could be due to the protein absorption of UV resulting in less damage than damage by DNA absorption of UV.

The outcome that LP-UV performed best deviates from the hypothesis that UV-LEDs could improve UV efficiency based on its ability to target DNA and protein damage. However, the results provide evidence suggesting that damage to the cellular DNA (expected to be greatest at UV wavelengths between 255 and 265 nm (Olson & Morrow, 2012)) is a more efficient means of mitigating ARB compared to damage to the cell's proteins (expected to be greatest at UV wavelengths around 280 nm (Beck et al., 2017)). Although each bacterial strain and test setup will differ, these findings demonstrate that the ARB tested here can be effectively inactivated in a short amount of time (Table 3), which could help guide the design of effective UV disinfection systems for ARB mitigation.

Table 3: Disinfection kinetics and inactivation efficiency for antibiotic resistant bacteria using UV-LED operated at peak wavelengths of 255 nm, 265 nm, or 285 nm in comparison to conventional LP-UV (254 nm).

UV System	**Kinetic rate constant (coefficient of specific lethality) (cm^2/mJ)**	**Model Fit (R^2)**	**Exposure time required to achieve target log inactivation (seconds)**		
			1 log	2 logs	3 logs
255 nm UV-LED	0.955	0.99	17	35	55
265 nm UV-LED	1.00	0.96	2.3	4.6	6.8
285 nm UV-LED	0.48	0.970	3.5	6.9	11
Low-pressure UV	1.98	0.970	2.7	5.4	8.6

In water treatment applications, the time required to disinfect bacteria can affect the size of the reactor or the retention time of the water, because a certain exposure time will be needed to achieve the desired UV fluence (Equation 1). Thus, it is useful to know the amount of time needed to disinfect bacteria to a level targeting minimal risk to human health. Using the kinetic rate constants and UV light irradiance for each of the four UV systems, the time required to inactivate 1, 2, or 3-logs of the ARB was calculated (Table 3). These results illustrate that the trend in efficiency based on the kinetic rate constants (LP-UV > 265 nm UV-LED ≈ 255 nm UV-LED > 285 nm UV-LED) was not the same as the trend in bacterial mitigation efficiency based on UV exposure time. Based on time to treat the bacteria, the 265 nm UV-LED system was most efficient, followed by the LP-UV system, 285 nm UV-LED, and lastly 255 nm UV-LED. This finding demonstrates the importance of not only designing UV treatment systems based on knowledge of traditional fluence-based kinetics of microbial inactivation, but also accounting for

energy efficiency (which varies amongst the systems tested here, as indicated by irradiation).

4.1.2 Energy Efficiency Calculations for ARB

The EEO metric indicates how effectively the ARB was inactivated using UV systems, where lower EEO values indicate greater energy efficiency. In Figure 3, the lowest EEO value was obtained for the LP-UV system, followed by the 265 nm UV-LED, 285 nm UV-LED, and 255 nm UV-LED. This result demonstrates that LEDs require more energy compared to the LP-UV system for the inactivation of ARB. The finding that the LP-UV system is more energy efficient compared to the UV-LEDs is consistent with other literature (Beck et al., 2017; Rattanakul & Oguma, 2018). The primary reason why LP-UV EEO values are so much lower is due to the WPE of the wavelengths. As noted in Section 3.3, the WPE values of the LEDs range from 0.3-1.3%, while the WPE value of the LP-UV system is 32.7%. The differences in these WPE and implications that arise with low LED-EEO values will be discussed more in Section 4.3.

The result that the 265 nm LED had a lower EEO than the 285 nm LED does differ from other studies, where it was reported that 280-285 nm LEDs have a lower overall EEO (Beck et al., 2017; Martino et al., 2021; Umar et al., 2019). Also, the EEO values calculated in this study are lower in value than others. Here, UV-LED EEO is 0.12, 0.08, and 0.09 kWh/m^3 for 255 nm, 265nm, and 285 nm, respectively, where other studies reported EEO ranges of 0.4-1.2 kWh/m^3 for wavelengths around 255-267 nm, and 0.2-0.6 kWh/m^3 for wavelengths around 275-285 nm (Beck et al., 2017; Martino et al., 2021; Rattanakul & Oguma, 2018). The LP-UV EEO values for *E. coli* found by Beck et al. (2017) and Rattanakul & Oguma (2018) (0.006 and 0.0099 kWh/m^3, respectively) is

higher than the LP-UV value reported here (0.0009 kWh/m^3). This difference could be due to the differences in system design, peak wavelengths the system produces, and WPE (Umar et al., 2019). Also, different *E. coli* strains were utilized. Therefore, more research is needed to elaborate on this distinction

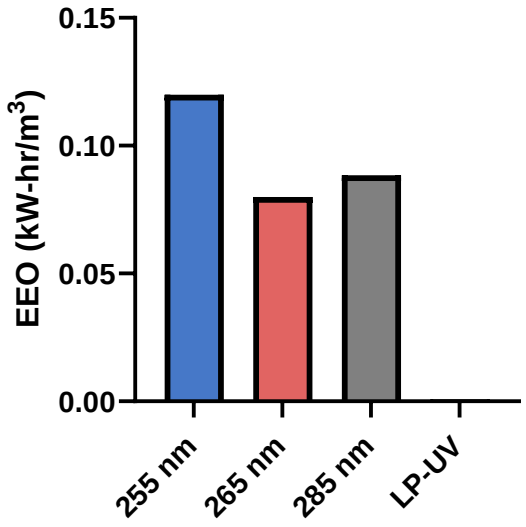

Figure 3: Electrical Energy per Order Inactivation of ARB using UV. *The LP-UV EEO was too low to be visible but was =0.0009 kW-hr/m^3. Bars are calculated from averaged rate constants.*

4.2 The Impact of UV Wavelengths on ARG Reduction

The impact of variable wavelength UV treatment on ARG reduction was assessed for iDNA and eDNA. It was hypothesized that genes would be less effectively damaged using UV treatment compared to the bacteria themselves, potentially because ARB inactivation can involve damage to cellular components, while ARG reduction involves damage to pyrimidine dimers (quantified through qPCR) (Pang et al., 2016; Stange et al., 2019; Zhang et al., 2019). To test this hypothesis, treatment of ARGs, both iDNA and eDNA, were tested using the UV-LED and LP-UV systems.

4.2.1 Intracellular DNA Tests

Intracellular DNA tests on UV fluences ranging from 0 to 700 mJ/cm^2 showed that an increase in UV fluence (x-axis) led to greater iDNA mitigation (log reduction, y-axis) (Figure 4). Compared to ARB, the poorer model fits for ARG reduction (as indicated by the lower R^2 values in Table 4) suggest that the genes were more difficult to accurately model compared to bacteria. In Figure 4, the 255 nm wavelength depicts a clear linear trend (R^2 = 0.99). However, the other wavelengths depict a less linear fit. Further research is needed to confirm the implications of the linearity/non-linearity of the data. Negative log reduction values are observed for some data points, especially at low fluences. This finding affirms the conclusion that iDNA are more difficult to model than ARB, and that overall, the reduction of iDNA is less effective than the inactivation of ARB.

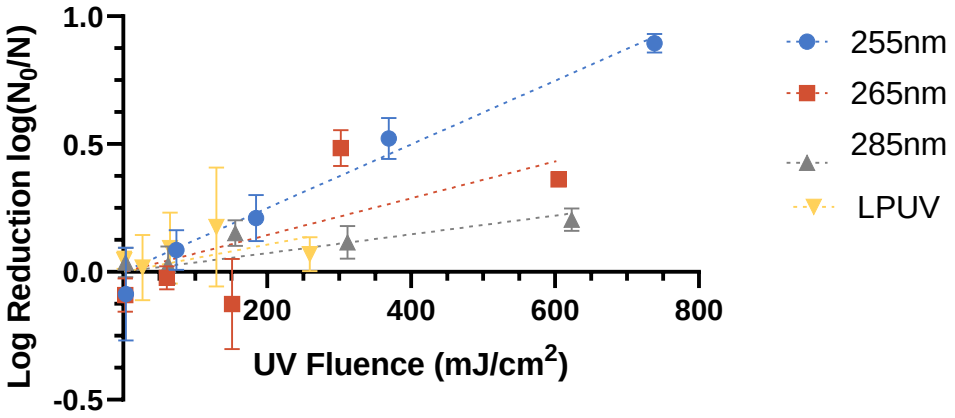

Figure 4: iDNA reduction for ARGs using variable wavelength UV treatments. *Each point represents the average of at least triplicate experiments and triplicate qPCR analyses. Error bars show ± 1 standard deviation for triplicate experiments (points represent means). The lines indicate linear regression models fit to each type of UV treatment system.*

Table 4 shows the modeled rates of reduction (regression slopes using classic Chick-Watson kinetics), which indicate that the UV-LED 255 nm wavelength treatment

was the most effective (highest slope), followed by UV-LED 265 nm, then LP-UV and UV-LED at 285 nm (not statistically different). This conclusion is different than the ARB findings, for which the LP-UV system was the most effective. The Tukey's multiple comparison test for each of the wavelengths showed that every wavelength was statistically different from each other ($p \leq 0.0006$, apart from the 285 nm vs. LP-UV comparison ($p = 0.838$)) (Table 5). This non-statistical difference, as well as LP-UV performing the worst at iDNA mitigation, could be due to the low reduction (≈0.05) at a high fluence of 250 mJ/cm^2 (Figure 4).

Table 4: Disinfection kinetics and reduction efficiency for antibiotic resistant genes (iDNA) using UV-LED operated at peak wavelengths of 255 nm, 265 nm, or 285 nm in comparison to conventional LP-UV (254 nm).

UV System	**Kinetic rate constant (coefficient of specific lethality) (cm^2/mJ)**	**Model Fit (R^2)**	**Exposure time required to achieve target log inactivation (seconds)**		
			1 log	2 logs	3 logs
255 nm UV-LED	0.0013	0.99	3.8	7.5	11.3
265 nm UV-LED	0.00072	0.64	0.86	1.8	2.6
285 nm UV-LED	0.00037	0.86	1.3	2.6	3.9
Low-pressure UV	0.00053	0.54	2.8	5.6	8.4

Overall, the lower kinetic rate constants compared to the ARB data in Table 3 demonstrate that ARG- present as iDNA were less effectively inactivated than ARB, thus confirming the hypothesis. This result is similar to other literature comparing iDNA and ARB (McKinney & Pruden, 2012; Yoon et al., 2017). These results suggest that

inactivation of ARB could stem from more than just DNA damage, such as damage to the cell wall or other cellular components. Also, reported by Stange et al. (2019), larger sections of DNA can remain unaffected by UV treatment, while some DNA strand breaks can result in total bacterial inactivation. This means that although bacterial inactivation could be observed under UV tests, resulting gene reduction might not happen to this extent. Further research using long amplicons during UV-LED treatment of iDNA is warranted to answer this question.

Like the ARB data, these results show that the trend in efficiency based on the kinetic rate constants (255 nm LED > 265 nm LED > LP-UV ≈ 285 nm LED) is different than the trend in ARG-iDNA mitigation efficiency based on treatment time (Table 4). Based on Table 4, the 265 nm UV-LED system was most efficient, followed by the 285 nm UV-LED, the LP-UV system, and lastly 255 nm UV-LED. This is different than the ARB time modeling data and can be explained by the differences in wavelength irradiance and the kinetic rate constants (Equation 1). Regarding the wavelength irradiance, 255 nm has the lowest (0.059 mW/cm^2), followed by LP-UV (0.186 mW/cm^2), 265 nm (0.439 mW/cm^2), then 285 nm (0.577 mW/cm^2). So, since the kinetic rate constants for iDNA differ from ARB, time modeling results could differ as well.

Table 5: Tukey's multiple comparison test for the UV wavelengths during iDNA inactivation.

Tukey's Multiple Comparison	Adjusted P value
255 nm vs. 265 nm	0.0006
255 nm vs. 285 nm	<0.0001
255 nm vs. LP-UV	<0.0001
265 nm vs. 285 nm	<0.0001
265 nm vs. LP-UV	<0.0001
285 nm vs. LP-UV	0.8377

4.2.2 Energy Efficiency Calculations for iDNA

The lowest EEO value for the iDNA data set was obtained for the LP-UV system, followed by the 265 nm UV-LED, 285 nm UV-LED, and 255 nm UV-LED (Figure 5). As discussed in Section 2.3, this (to the author's knowledge) is the first study assessing the energy required (expressed using the EEO metric) for iDNA reduction using UV-LEDs. Importantly, this trend in efficiency is the same as the ARB data in Figure 3. Thus, LEDs require more energy compared to the LP-UV system for the reduction of iDNA as well as ARB. However, higher overall EEO values are required for the reduction of iDNA compared to ARB, (2 magnitudes of difference). This result is to be expected, because the genes themselves were harder to inactivate than ARB; thus, more energy will be required to achieve similar log reduction values.

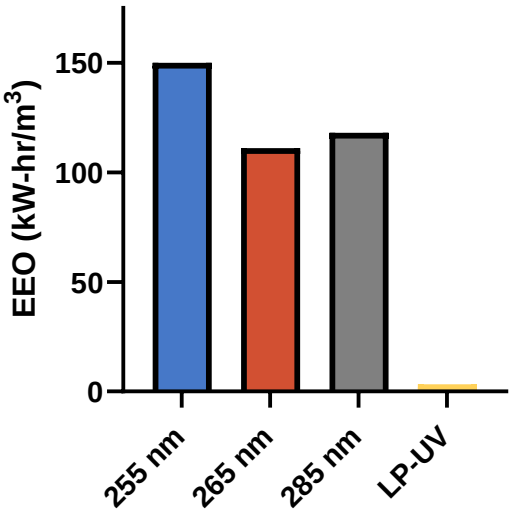

Figure 5: Electrical energy per order (EEO) for iDNA treatment. *Bars are calculated from averaged rate constants.*

4.2.3 Extracellular DNA Tests

Extracellular DNA tests were performed using the same UV doses as the iDNA tests, ranging from 0 to 700 mJ/cm^2. Increasing UV fluence (x-axis) led to greater ARG eDNA reduction (log inactivation, y-axis) (Figure 6). For the range of fluences tested, 0-2.5-logs of inactivation were observed. This result is lower than other studies, that reported up to 0.4-logs inactivation with UV fluences between 20 and 400 mJ/cm^2 (Chang et al., 2017; Destiani et al., 2018; McKinney & Pruden, 2012). Of note, Krzeminski et al. (2020), studied eARGs inactivation using UV-LED at 265 nm, and reported that 1-log reduction required a fluence of 73 mJ/cm^2 (assessed using short amplicons), which is a better efficiency than this study. Differences in reduction efficiency could be attributed to using different genes or test setups.

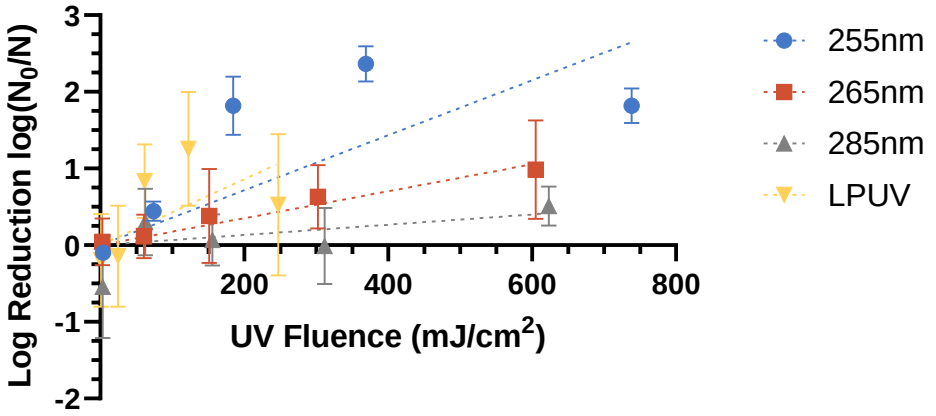

Figure 6: eDNA reduction for each wavelength UV treatment. *Each point represents the average of at least triplicate experiments and triplicate qPCR analyses. Error bars show ± 1 standard deviation. The lines indicate linear regression models fit to each type of UV treatment system.*

Comparison of the magnitude of the slopes of the linear regression lines shown in Figure 6 allowed for comparative evaluation of the efficacy of each of the four different types of UV systems. Table 6 shows the modeled rates of reduction (regression slopes using classic Chick-Watson kinetics), which indicate that the LP-UV wavelength treatment was the most effective (highest slope), followed by UV-LED 255 nm, then UV-LED 265 nm and UV-LED at 285 nm. These results differ slightly from the ARB and iDNA data. However, wide variability in reduction (as indicated by large error bars) contributed to there being no statistical difference amongst eDNA treatment using the different UV systems, with the exception of 285 nm and LP-UV ($p = 0.0246$) (Table 7). However, the high initial log reduction values for LP-UV could be the reason it is statistically different than the 285 nm LED. This result makes sense since eDNA does not contain proteins, and 285 nm LED mainly targets proteins; thus, it would be expected to have the lowest log reduction compared to a wavelength that targets DNA (i.e., LP-UV).

Table 6: Disinfection kinetics and reduction efficiency for antibiotic resistant genes (eDNA) using UV-LED operated at peak wavelengths of 255 nm, 265 nm, or 285 nm in comparison to conventional LP-UV (254 nm).

UV System	Kinetic rate constant (coefficient of specific lethality) (cm^2/mJ)	Model Fit (R^2)	Exposure time required to achieve target log inactivation (seconds)		
			1 log	2 logs	3 logs
255 nm UV-LED	0.0036	0.75	1.3	2.6	3.9
265 nm UV-LED	0.0018	0.90	0.35	0.72	1.1
285 nm UV-LED	0.00067	0.35	0.72	1.4	2.2
Low-pressure UV	0.0043	0.58	0.33	0.68	1.0

Like the ARB and iDNA data, Table 6 shows that the trend in efficiency based on the fluence-based kinetic rate constants (LP-UV ≈ 255 nm LED ≈ 265 nm LED ≈ 285 nm LED) was different than the trend in ARG mitigation efficiency based on treatment time. Based on treatment time, the LP-UV system was most efficient, followed by the 265 nm UV-LED, the 285 nm UV-LED, and lastly 255 nm UV-LED. These results show that UV treatment of eDNA is not as efficient as UV treatment of ARB, for the same reasons discussed in the iDNA section. However, for the same UV fluence range, UV treatment of eDNA was more efficient than iDNA. This could be due to the protective role of cellular structures encompassing iDNA, whereas for the "free-floating" eDNA, the DNA bear the full brunt of the UV treatment. A similar conclusion was reached by Yoon et al. (2017), where RNA screening or proteins in the cell can protect the iDNA from damage.

Table 7: Tukey's multiple comparison test for the UV wavelengths during eDNA inactivation.

Tukey's Multiple Comparison	Adjusted P value
255 nm vs. 265 nm	0.8185
255 nm vs. 285 nm	0.5198
255 nm vs. LP-UV	0.3758
265 nm vs. 285 nm	0.9565
265 nm vs. LP-UV	0.0782
285 nm vs. LP-UV	0.0246

4.2.4 Energy Efficiency Calculations for eDNA

The energy efficiency of eDNA treatment in the different UV systems was evaluated using the EEO metric, where the lowest EEO value was obtained for the LP-UV system, followed by the 265 nm UV-LED, 255 nm UV-LED, and 285 nm UV-LED (Figure 8). Similar to the ARB and iDNA EEO data, this result demonstrates that LEDs require more energy over the LP-UV system for the reduction of eDNA. The 285 nm LED had the highest EEO out of the LEDs, possibly due to the very low kinetic rate constant of 285 nm (Table 6) compared to the other wavelengths, which is not the case for iDNA. This is an interesting result, compared to alternate reports in some of the literature, where EEO values for 275-285 nm were lower compared to 255-265 nm LEDs (Beck et al., 2017; Martino et al., 2021; Nyangaresi et al., 2018; Oguma et al., 2013; Rattanakul & Oguma, 2018). However, these studies did not assess eDNA. Overall, EEO values for eDNA are higher than ARB, but lower than iDNA. This result relates back to the differences in kinetic rate constants of eDNA and iDNA. The kinetic reduction of eDNA is more efficient than iDNA but less efficient than ARB. This is logical because

the harder bacteria or genes are to damage, the more energy will be needed for reduction, and thus the kinetic rate constants will be lower as well.

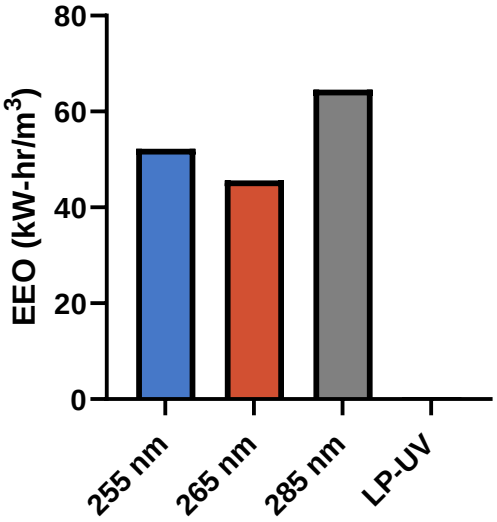

Figure 7: Electrical energy per order (EEO) for the eDNA experiments. *The LP-UV EEO was too low to be visible but was = 0.4* kW-hr/m^3. *Bars are calculated from averaged rate constants.*

4.3 EEO Comparison Summary

Calculating the EEO aimed to establish how efficiently the ARB and ARG were inactivated using the UV systems. Table 8 summarizes the EEO values shown in Figures 3, 5, and 7. The lower EEO values for LP-UV compared to LEDs is consistent with other literature (Beck et al., 2017; Rattanakul & Oguma, 2018). The iDNA-ARG had the highest EEO values, followed by eDNA-ARG, then ARB. Overall, higher EEO values were observed using UV-LED compared to the LP-UV system. EEO values for the ARB and ARGs related to their respective kinetic inactivation efficiencies, attributed to microorganism vulnerability, as described by Nyangaresi et al. (2018), which would

change for different wavelengths. Additionally, EEO is impacted by the energy efficiency of each UV system.

This result demonstrates that LEDs require more energy, and thus could have higher associated costs (Umar et al., 2019). The difference in EEO values can be attributed to the WPE values, where LP-UV bulbs are currently the most efficient at converting input energy into UV output, while lower wavelength UV-LEDs are currently less efficient. Current WPE for other UV-LED systems range from 1-3%, while LP-UV systems range from 30-40% (Umar et al., 2019). However, if the WPE of germicidal UV-LEDs improve, they will have a much more competitive advantage against LP-UV systems.

Table 8: Energy efficiency of the UV systems for treating antibiotic resistant bacteria and genes (characterized using the "electrical energy per order metric").

	Electrical energy per order (kWh/m^3)		
UV-System	**Antibiotic resistant bacteria**	**Antibiotic resistance genes – iDNA**	**Antibiotic resistance genes – eDNA**
255 nm UV-LED	0.120	150	52.2
265 nm UV-LED	0.0799	111	45.6
285 nm UV-LED	0.0884	118	64.6
LP-UV	0.000867	3.22	0.40

5 CONCLUSION

The overarching goal of this research was to assess ARB and ARG mitigation using an emerging water treatment technology, UV-LED (at wavelengths of 255 nm, 265 nm, or 285 nm), compared to conventional LP- UV treatment. Specifically, the objectives aimed to address the research gaps on ARG studies using UV-LEDs, as well as EEO calculations for ARGs under these conditions. The objectives were investigated using bench-scale UV systems to expose a synthetic water matrix containing TOP 10 *E. coli* with the pUC19 plasmid (ARB, and iDNA through DNA extraction), or bla_{TEM} plasmids (model eARG). Plating methods and gene analysis were utilized to develop inactivation/reduction curves using Chick-Watson disinfection kinetics. Treatment time was modeled, and EEO values were calculated and compared for each wavelength and system. The key findings for this research were:

1. Overall, an increase in UV fluence increased inactivation/reduction, for every system tested, as indicated by the positive kinetic rate constants in Figures 2, 4, and 6. ARB exhibited the highest rate constants, and thus had the most efficient inactivation compared to iDNA and eDNA.
2. A drop in efficiency is noted for the 285 nm wavelength for ARB studies. This trend is also shown in literature (Martino et al. (2021)) and could be attributed to UV wavelengths that target DNA (255 nm – 265 nm) being more effective than UV wavelengths that target proteins (275-285 nm) at inactivating bacteria.
3. ARGs were less effectively damaged than ARB (higher UV fluences were needed for less log inactivation). Furthermore, iDNA exhibited more resistance to UV

than eDNA. Results from these studies suggest that ARB damage could be attributed to more than solely DNA damage, such as damage to other cellular components (i.e., cell wall – inactivation through necrosis from lipid damage). Or, a single point of DNA damage could result in total bacterial inactivation. Because the eDNA was damaged easier than iDNA, this suggests that the protective structures that encompass iDNA might better protect it from UV damage. This result is consistent with another study (Yoon et al., 2017). Finally, the ARGs were overall more difficult to accurately model than the ARB.

4. For each ARB/ARG system and UV wavelength, efficiency based on inactivation/reduction kinetics was different than efficiency based on treatment time modeling. Even though both ways of modeling incorporate the kinetic rate constant values and irradiance of the wavelength through Chick-Watson disinfection, the differences highlight the need to design a system with both models in mind.
5. The EEO was highest for UV-LEDs compared to LP-UV, for both ARB/ARG. This difference is credited to the low WPE values of the LEDs (1-3%) compared to LP-UV (30-40%). Although LED WPEs are expected to improve in the future, high EEO values for the UV-LEDs make them non-competitive against current LP-UV technology. The trend in EEO requirements matched how well the ARB and ARGs were inactivated via kinetic rate constant values (iDNA > eDNA > ARB). Future research assessing more wavelengths, ARB/ARG types, and different system operation would offer a better understanding of how UV-LEDs compare to LP-UV systems.

BIBLIOGRAPHY

2019 Antibiotic Resistance Threats Report | CDC. (n.d.). Retrieved April 1, 2022, from https://www.cdc.gov/drugresistance/biggest-threats.html

Bari, M. L., & Yeasmin, S. (2021). Microbes Culture Methods. *Reference Module in Biomedical Sciences*. https://doi.org/10.1016/B978-0-12-818731-9.00128-2

Beck, S. E., Ryu, H., Boczek, L. A., Cashdollar, J. L., Jeanis, K. M., Rosenblum, J. S., Lawal, O. R., & Linden, K. G. (2017). Evaluating UV-C LED disinfection performance and investigating potential dual-wavelength synergy. *Water Research*, *109*, 207–216. https://doi.org/10.1016/j.watres.2016.11.024

Bolton, J. R., Bircher, K. G., Tumas, W., & Tolman, C. A. (2001). Figures-of-merit for the technical development and application of advanced oxidation technologies for both electric- and solar-driven systems. *Pure and Applied Chemistry*, *73*(4), 627–637. https://doi.org/10.1351/PAC200173040627/MACHINEREADABLECITATION/RIS

Bolton, J. R., & Linden, K. G. (2003). Standardization of methods for fluence (UV Dose) determination in bench-scale UV experiments. *Journal of Environmental Engineering*, *129*(3), 209–215. https://doi.org/10.1061/(ASCE)0733-9372(2003)129:3(209)

Bowker, C., Sain, A., Shatalov, M., & Ducoste, J. (2011). Microbial UV fluence-response assessment using a novel UV-LED collimated beam system. *Water Research*, *45*(5), 2011–2019. https://doi.org/10.1016/J.WATRES.2010.12.005

Chang, P. H., Juhrend, B., Olson, T. M., Marrs, C. F., & Wigginton, K. R. (2017). Degradation of Extracellular Antibiotic Resistance Genes with UV254 Treatment. *Environmental Science and Technology*, *51*(11), 6185–6192. https://doi.org/10.1021/acs.est.7b01120

Chatterley, C., & Linden, K. (2010). Demonstration and evaluation of germicidal UV-LEDs for point-of-use water disinfection. *Journal of Water and Health*, *8*(3), 479–486. https://doi.org/10.2166/WH.2010.124

Chen, J., Loeb, S., & Kim, J.-H. (2017). LED revolution: fundamentals and prospects for UV disinfection applications. *Environmental Science: Water Research & Technology*, *3*(2), 188–202. https://doi.org/10.1039/C6EW00241B

Childress, H., Sullivan, B., Kaur, J., & Karthikeyan, R. (2014). *Effects of ultraviolet light disinfection on tetracycline-resistant bacteria in wastewater effluents*. https://doi.org/10.2166/wh.2013.257

Corinaldesi, C., Danovaro, R., & Dell'Anno, A. (2005). Simultaneous Recovery of Extracellular and Intracellular DNA Suitable for Molecular Studies from Marine Sediments. *Applied and Environmental Microbiology*, *71*(1), 46. https://doi.org/10.1128/AEM.71.1.46-50.2005

Davies, J. (2010). Origins and evolution of antibiotic resistance. In *Microbiology and Molecular Biology Reviews* (Vol. 74, Issue 3, pp. 417–433). American Society for Microbiology (ASM). https://doi.org/10.1128/mmbr.00016-10

Destiani, R., Templeton, M. R., & Kowalski, W. (2018). Relative Ultraviolet Sensitivity of Selected Antibiotic Resistance Genes in Waterborne Bacteria. *Environmental Engineering Science*, *35*(7), 770–774. https://doi.org/10.1089/ees.2017.0179

Gerrity, D., Ryu, H., Crittenden, J., & Abbaszadegan, M. (2008). Photocatalytic inactivation of viruses using titanium dioxide nanoparticles and low-pressure UV light. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering*, *43*(11), 1261–1270. https://doi.org/10.1080/10934520802177813

Guo, M., Hu, H., Bolton, J. R., & El-Din, M. G. (2009). Comparison of low- and medium-pressure ultraviolet lamps: Photoreactivation of Escherichia coli and total coliforms in secondary effluents of municipal wastewater treatment plants. *Water Research*, *43*(3), 815–821. https://doi.org/10.1016/J.WATRES.2008.11.028

Guo, M., Huang, J., Hu, H., Liu, W., & Yang, J. (2012). UV inactivation and characteristics after photoreactivation of Escherichia coli with plasmid: Health safety concern about UV disinfection. *Water Research*, *46*(13), 4031–4036. https://doi.org/10.1016/J.WATRES.2012.05.005

Guo, M. T., & Kong, C. (2019). Antibiotic resistant bacteria survived from UV disinfection: Safety concerns on genes dissemination. *Chemosphere*, *224*, 827–832. https://doi.org/10.1016/j.chemosphere.2019.03.004

Guo, M. T., Yuan, Q. Bin, & Yang, J. (2013). Microbial selectivity of UV treatment on antibiotic-resistant heterotrophic bacteria in secondary effluents of a municipal wastewater treatment plant. *Water Research*, *47*(16), 6388–6394. https://doi.org/10.1016/j.watres.2013.08.012

How Antibiotic Resistance Happens | Antibiotic/Antimicrobial Resistance | CDC. (n.d.). Retrieved August 31, 2021, from https://www.cdc.gov/drugresistance/about/how-resistance-happens.html

Kappell, A. D., De Nies, M. S., Ahuja, N. H., Ledeboer, N. A., Newton, R. J., & Hristova, K. R. (2015). Detection of multi-drug resistant Escherichia coli in the urban waterways of Milwaukee, WI. *Frontiers in Microbiology*, *6*(APR), 336. https://doi.org/10.3389/FMICB.2015.00336/ABSTRACT

Kimbell, L. K., Lamartina, E. Lou, Kappell, A. D., Huo, J., Wang, Y., Newton, R. J., & McNamara, P. J. (2021). Cast iron drinking water pipe biofilms support diverse microbial communities containing antibiotic resistance genes, metal resistance genes, and class 1 integrons. *Environmental Science: Water Research & Technology*, *7*(3), 584–598. https://doi.org/10.1039/D0EW01059F

Krzeminski, P., Feys, E., Anglès d'Auriac, M., Wennberg, A. C., Umar, M., Schwermer, C. U., & Uhl, W. (2020). Combined membrane filtration and 265 nm UV irradiation for effective removal of cell free antibiotic resistance genes from feed water and concentrate. *Journal of Membrane Science*, *598*, 117676. https://doi.org/10.1016/J.MEMSCI.2019.117676

Kuo, J., Chen, C., & Nellor, M. (2003). Standardized Collimated Beam Testing Protocol for Water/Wastewater Ultraviolet Disinfection. *Journal of Environmental Engineering*, *129*(8), 774–779. https://doi.org/10.1061/(ASCE)0733-9372(2003)129:8(774)

Larionov, A., Krause, A., & Miller, W. R. (2005). A standard curve based method for relative real time PCR data processing. *BMC Bioinformatics*, *6*(1), 1–16. https://doi.org/10.1186/1471-2105-6-62/FIGURES/12

Li, G. Q., Wang, W. L., Huo, Z. Y., Lu, Y., & Hu, H. Y. (2017). Comparison of UV-LED and low pressure UV for water disinfection: Photoreactivation and dark repair of Escherichia coli. *Water Research*, *126*, 134–143. https://doi.org/10.1016/j.watres.2017.09.030

Martino, V., Ochsner, K., Peters, P., Zitomer, D. H., & Mayer, B. K. (2021). Virus and Bacteria Inactivation Using Ultraviolet Light-Emitting Diodes. *Https://Home.Liebertpub.Com/Ees*, *38*(6), 458–468. https://doi.org/10.1089/EES.2020.0092

McKinney, C. W., & Pruden, A. (2012). Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater. *Environmental Science and Technology*, *46*(24), 13393–13400. https://doi.org/10.1021/es303652q

Nyangaresi, P. O., Qin, Y., Chen, G., Zhang, B., Lu, Y., & Shen, L. (2018). Effects of single and combined UV-LEDs on inactivation and subsequent reactivation of E. coli in water disinfection. *Water Research*, *147*, 331–341. https://doi.org/10.1016/j.watres.2018.10.014

Oguma, K., Kita, R., Sakai, H., Murakami, M., & Takizawa, S. (2013). Application of UV light emitting diodes to batch and flow-through water disinfection systems. *Desalination*, *328*, 24–30. https://doi.org/10.1016/j.desal.2013.08.014

Olson, N. D., & Morrow, J. B. (2012). DNA extract characterization process for microbial detection methods development and validation. *BMC Research Notes*, *5*, 668. https://doi.org/10.1186/1756-0500-5-668

Pang, Y., Huang, J., Xi, J., Hu, H., & Zhu, Y. (2016). Effect of ultraviolet irradiation and chlorination on ampicillin-resistant Escherichia coli and its ampicillin resistance gene. *Frontiers of Environmental Science and Engineering*, *10*(3), 522–530. https://doi.org/10.1007/s11783-015-0779-9

Pruden, A., Pei, R., Storteboom, H., & Carlson, K. H. (2006). Antibiotic resistance genes as emerging contaminants: Studies in northern Colorado. *Environmental Science and Technology*, *40*(23), 7445–7450. https://doi.org/10.1021/es060413l

Quek, P. H., & Hu, J. (2008). Indicators for photoreactivation and dark repair studies following ultraviolet disinfection. *Journal of Industrial Microbiology and Biotechnology*, *35*(6), 533–533. https://doi.org/10.1007/S10295-008-0314-0

Rattanakul, S., & Oguma, K. (2018). Inactivation kinetics and efficiencies of UV-LEDs against Pseudomonas aeruginosa, Legionella pneumophila, and surrogate microorganisms. *Water Research*, *130*, 31–37. https://doi.org/10.1016/J.WATRES.2017.11.047

Rizzo, L., Fiorentino, A., & Anselmo, A. (2013). Advanced treatment of urban wastewater by UV radiation: Effect on antibiotics and antibiotic-resistant E. coli strains. *Chemosphere*, *92*(2), 171–176. https://doi.org/10.1016/J.CHEMOSPHERE.2013.03.021

Stange, C., Sidhu, J. P. S., Toze, S., & Tiehm, A. (2019). Comparative removal of antibiotic resistance genes during chlorination, ozonation, and UV treatment. *International Journal of Hygiene and Environmental Health*, *222*(3), 541–548. https://doi.org/10.1016/j.ijheh.2019.02.002

Templeton, M. R., Oddy, F., Leung, W. K., & Rogers, M. (2009). Chlorine and UV disinfection of ampicillinresistant and trimethoprim-resistant Escherichia coli. *Canadian Journal of Civil Engineering*. https://doi.org/10.1139/L09-040

The OD600 Basics | Best OD600 Tool To Generate Microbial Growth Curves. (n.d.). Retrieved March 4, 2022, from https://www.implen.de/od600-diluphotometer/od600/

Umar, M., Anglès D'auriac, M., & Wennberg, A. C. (2021). Application of UV-LEDs for antibiotic resistance genes inactivation – Efficiency monitoring with qPCR and transformation. *Journal of Environmental Chemical Engineering*, *9*(3), 105260. https://doi.org/10.1016/J.JECE.2021.105260

Umar, M., Roddick, F., & Fan, L. (2019). Moving from the traditional paradigm of pathogen inactivation to controlling antibiotic resistance in water - Role of ultraviolet irradiation. *Science of the Total Environment*, *662*, 923–939. https://doi.org/10.1016/j.scitotenv.2019.01.289

Yoon, Y., Chung, H. J., Wen Di, D. Y., Dodd, M. C., Hur, H. G., & Lee, Y. (2017). Inactivation efficiency of plasmid-encoded antibiotic resistance genes during water treatment with chlorine, UV, and UV/H2O2. *Water Research*, *123*, 783–793. https://doi.org/10.1016/j.watres.2017.06.056

Yuan, Q.-B., Guo, M.-T., & Yang, J. (2015). *Fate of Antibiotic Resistant Bacteria and Genes during Wastewater Chlorination: Implication for Antibiotic Resistance Control*. https://doi.org/10.1371/journal.pone.0119403

Zhang, C. M., Xu, L. M., Wang, X. C., Zhuang, K., & Liu, Q. Q. (2017). Effects of ultraviolet disinfection on antibiotic-resistant Escherichia coli from wastewater: inactivation, antibiotic resistance profiles and antibiotic resistance genes. *Journal of Applied Microbiology*, *123*(1), 295–306. https://doi.org/10.1111/JAM.13480

Zhang, Y., Zhuang, Y., Geng, J., Ren, H., Zhang, Y., Ding, L., & Xu, K. (2015). Inactivation of antibiotic resistance genes in municipal wastewater effluent by chlorination and sequential UV/chlorination disinfection. *Science of the Total Environment*, *512–513*, 125–132. https://doi.org/10.1016/j.scitotenv.2015.01.028

Zhang, Z., Li, B., Li, N., Sardar, M. F., Song, T., Zhu, C., Lv, X., & Li, H. (2019). Effects of UV disinfection on phenotypes and genotypes of antibiotic-resistant bacteria in secondary effluent from a municipal wastewater treatment plant. *Water Research*, *157*, 546–554. https://doi.org/10.1016/j.watres.2019.03.079

Zheng, J., Su, C., Zhou, J., Xu, L., Qian, Y., & Chen, H. (2017). Effects and mechanisms of ultraviolet, chlorination, and ozone disinfection on antibiotic resistance genes in secondary effluents of municipal wastewater treatment plants. *Chemical Engineering Journal*, *317*, 309–316. https://doi.org/10.1016/j.cej.2017.02.076