Building Tomorrow's Society
Bâtir la Société de Demain

Fredericton, Canada
June 13 – June 16, 2018/ *Juin 13 – Juin 16, 2018*

ULTRAVIOLET LIGHT EMITTING DIODES (UV LEDS) FOR BIOFILM CONTROL

Stephanie Gora[1,2], Kyle Rauch[1], Carolina Ontiveros[1], Graham Gagnon[1]
[1]Dalhousie University, Halifax, Nova Scotia
[2]stephanie.gora@dal.ca

Abstract: Biofilm growth on treatment equipment and biofilm formation in drinking water distribution systems are major ongoing challenges in the drinking water industry. Control mechanisms include flushing, chemically intensive cleaning procedures, chlorination, and the removal of water matrix components that encourage the growth of biofilm-forming bacteria (e.g. natural organic matter). These procedures can be onerous and shorten the useful lifetime of the equipment and there is demand for novel disinfection technologies that can provide fast and effective disinfection without damaging equipment or infrastructure. Germicidal ultraviolet violet (UV) light is one potential option for these applications, and UV light emitting diodes (UV LEDs), which are smaller and more energy efficient than standard mercury UV lamps, are particularly promising.

In this study, *Pseudomonas aeruginosa* biofilms were grown on plastic and stainless steel coupons and exposed to UV LEDs emitting light at 265 nm for times ranging from 1 to 60 minutes. The biomass remaining on the coupons was then suspended in sterile phosphate buffered solution, which was then plated on tryptic soy agar to determine colony forming units and analyzed for adenosine triphosphate (ATP) concentration, a quick and effective measure of biological activity. Preliminary results indicate that 1.5-2 log inactivation can be achieved at a UV dose of 8.1 mJ/cm^2). This dose is higher than those reported for pure cultures of planktonic bacteria (Beck et al., 2017; Rattanakul and Oguma, 2018) in bench scale experiments but below that required to inactivate *Pseudomonas aeruginosa* in biofilms in real life medical applications (Bak et al., 2010).

The results of this study confirm that UV LED irradiation is a viable technology for surface disinfection. These findings have implications for the drinking water industry and for other industries that struggle to control biofilm growth. Future experiments will compare UV LED disinfection to standard biofilm mitigation strategies for water treatment equipment and water distribution infrastructure.

1 INTRODUCTION

UV emitting mercury lamps are commonly used to disinfect municipal water and wastewater in Canada. These lamps are large and power intensive, making them difficult to use in point of entry (POE) and point of use (POU) drinking water applications. UV LEDs, in contrast, are small, can be manufactured to emit nearly any wavelength of light, and are quickly becoming less expensive and more energy efficient (Ibrahim et al., 2014). Researchers in Canada, the United States, and Japan are spearheading research into the application of UV LEDs for drinking water disinfection. Beck et al. (2017) demonstrated that UV LEDs could be used to inactivate a wide range of common human pathogens and that the wavelength dependence and energy efficiency of UV LED disinfection varies from one organism to another. Researchers at the University

of Tokyo have demonstrated that significant levels of disinfection can be achieved in flow through UV LED units that may eventually be appropriate for POU drinking water treatment applications (Oguma et al., 2016, 2013).A standard method for the characterization of UV LED experiment apparatus has recently been published out of the University of British Columbia (Kheyrandish et al., 2017), and promises to increase the number of studies and improve the quality of UV LED research in the drinking water field.

Biofilms consist of microorganisms and extracellular polymeric substances attached to a surface. They are common in drinking water distribution systems and premise plumbing (Falkinham et al., 2015), and can provide a safe harbour for opportunistic human pathogens (Lau and Ashbolt, 2009). A recent study by Rattanakul and Oguma.(2018) showed that UV LEDs were effective for the inactivation of planktonic (free-floating) pure cultures of *Pseudomonas aeruginosa*, a common biofilm forming bacteria, and *Legionella pneumophilia*, a well known opportunistic pathogen. At a wavelength of 265 nm, a 2-log inactivation of *Pseudomonas aeruginosa* was achieved with a UV dose of 4 mJ/cm^2. Only 2 mJ/cm^2 of UV light was required to achieve this level of inactivation for *Legionella pneumophilia*.

Only a small number of studies have explored the use of UV light, UV LED or otherwise, for the inactivation of biofilm-bound bacteria on real surfaces. The studies that do exist suggest that mixed bacterial cultures and real clinical surfaces are more difficult to treat with UV light than biofilms grown from pure bacterial cultures under controlled lab conditions. A pair of studies conducted on medical catheters by Bak et al. illustrate this eloquently. In the first study (Bak et al., 2009), the researchers collected used catheters from a hospital, established the types of bacteria present in the biofilms attached to their inner surfaces, and then attempted to inactivate these bacterial communities with conventional UVC lamps. Species detected in the catheters included *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*. A dose of 1,500 mJ/cm^2 was required to achieve an overall 4 log kill of the bacteria present on the catheters. The researchers also noted that bacteria in thicker biofilms were more difficult to inactivate, likely due to the protective effects of EPS and light scattering cause by particulates in the biofilm matrix. In the second study (Bak et al., 2010), thin biofilms of *Pseudomonas aeruginosa* were grown in clean catheters in the laboratory and exposed to UVC LEDs. Approximately 8 mJ/cm^2 was required to achieve 4-log reduction of the bacteria present in these thin, single culture biofilms, which is just under 200 times less than the dose of UV light required to achieve this level of inactivation in multi-culture, mature biofilms from used catheters. The improved kill was attributed to the characteristics of the biofilms (thin, pure culture) as well as the increased efficiency achieved using UV LEDs, which can be applied nearly in-situ, compared to conventional UV lamps. These studies, though conducted in a medical setting rather than a civil engineering context, underscore the challenges of using UV light to inactivate biofilm-bound microorganisms, but also the promise that UV LEDs hold for biofilm mitigation.

2 METHODS

The collimated beam of light emitted by the UV LEDs at 265 nm was characterized using a methodology originally proposed by Bolton et al.(2003) and a spectrometer calibrated to measure in the UV range (Ocean Optics USB4000. The average intensity and petri factor of the light emitted by the LED apparatus were determined at five different distances from the edge of the collimator.

Pseudomonas aeruginosa biofilms were grown on polycarbonate or stainless steel coupons in a CDC biofilm growth reactor and exposed to UV LEDs emitting light at 265 nm. The biomass remaining on the coupons after treatment was suspended in sterile phosphate buffered solution, which was then plated on tryptic soy agar to determine colony forming units and analyzed for adenosine triphosphate (ATP) concentration, a quick and effective measure of biological activity, using LuminUltra's QGA method conducted on a bacterial sample cultured from the viable bacteria remaining on the coupon surface after UV exposure.

3 RESULTS

3.1 3.1 Characterization of UV LED Apparatus

The intensity of the beam across the illuminated surface at a distance of 50 mm from the collimator is depicted in Figure 1.

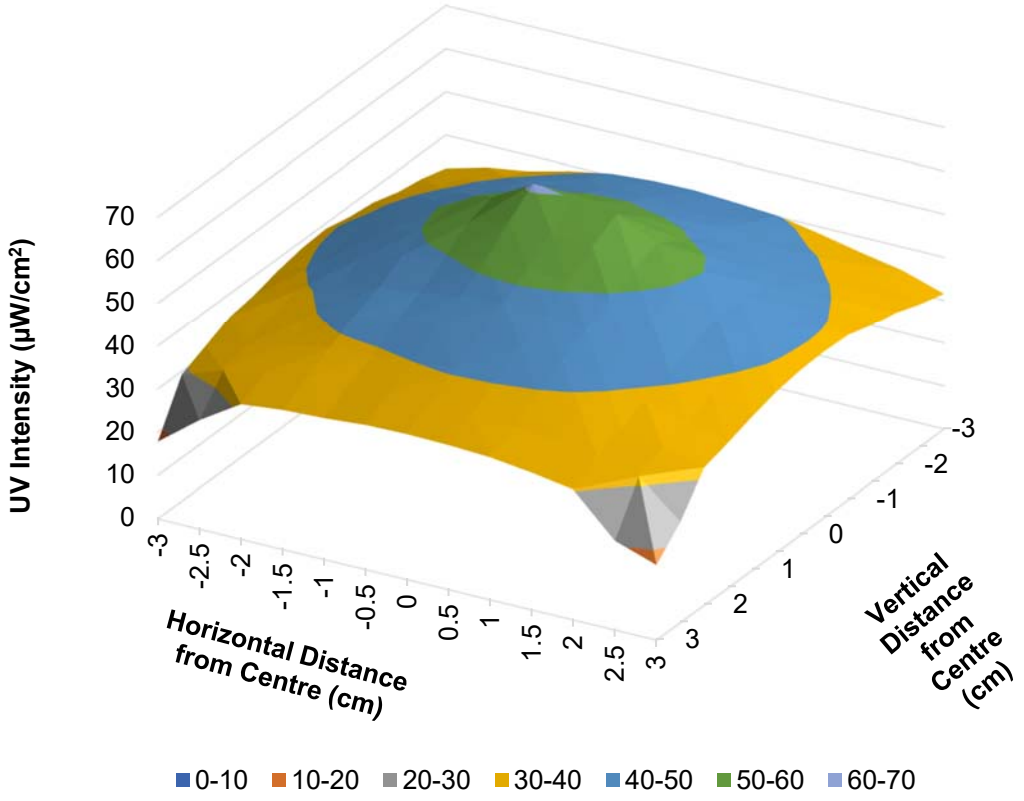

Figure 1: Intensity of UV light (265 nm) at different locations across the illuminated surface in the collimated beam apparatus at a distance of 50 mm from the collimator

The intensity distribution in Figure 1 shows that the intensity of the UV light beam varied substantially across the illuminated area (total area = 36 cm^2) and was higher in the centre than at the outer edges of the beam.

The average intensity and petri factor of the collimated beam issuing from the 265 nm LEDs in the collimated beam apparatus along with the time required to achieve a UV dose of 8 mJ/cm^2, the UV dose reported to provide 4-log reduction of *Pseudomonas aeruginosa* by previous researchers (Bak et al., 2010), at different distances from the collimator (Figure 2). Note that the average intensity and petri factor values in Figure 2 were calculated for the circular area required to illuminate the coupons (9 cm^2) rather than over the entire beam area.

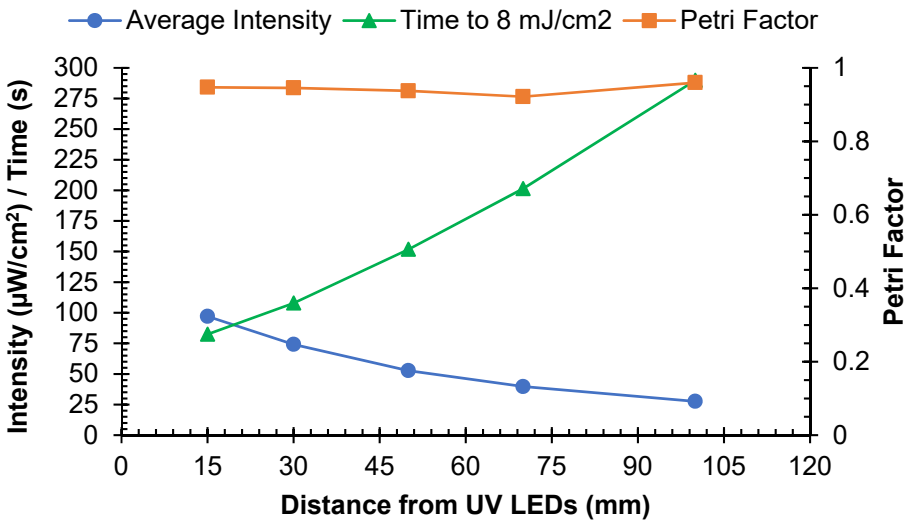

Figure 2: Intensity and uniformity (petri factor) of the light provided by the UV LED apparatus at different distances from the bottom of the collimator and the time required to achieve a UV dose of 8 mJ/cm^2 at each distance

As expected, the average intensity of the beam increased as the distance between the spectrometer sensor and the UV LEDs decreased. The petri factor was high (> 0.9) indicating that the intensity of the light beam was nearly uniform across the 3 cm diameter illuminated area irrespective of the distance between the spectrometer sensor and the UV LEDs, which means that the three replicate coupons received a uniform dose of light at each height/intensity.

3.2 Inactivation of Pseudomonas Aeruginosa – Preliminary Results

The preliminary results of the inactivation experiments confirm that biofilm-bound bacteria can be inactivated by UV LEDs. As shown in Figure 3, 1.9 ± 0.4 log inactivation was achieved at a UV dose of 8.1 mJ/cm^2. This result is in line with recent results published by the Oguma research group at the University of Tokyo (Rattanakul and Oguma, 2018), which showed 2 log reduction of planktonic (free floating) *Pseudomonas aeruginosa* at approximately 4 mJ/cm^2 under otherwise similar treatment conditions. The slightly higher UV dose required to achieve inactivation in the current study is relative to the results published by Rattanakul and Oguma likely reflects the fact that the biofilm-bound bacteria in our study were protected from UV irradiation by the layers of EPS in the biofilm, unlike the planktonic bacteria used in their study, which had no such protection.

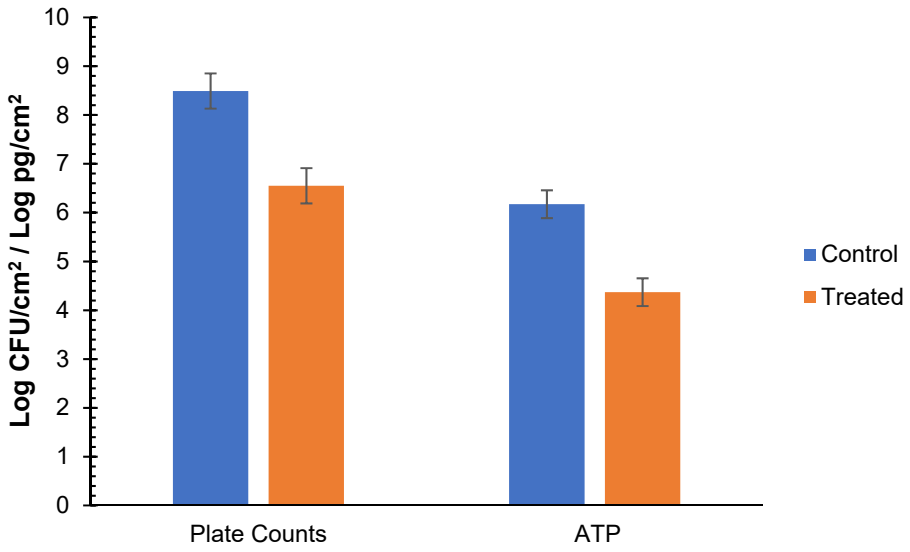

Figure 3: Log surface concentration of *Pseudomonas aeruginosa* and ATP in biofilms exposed to 8.1 mJ/cm^2 of UV light at a distance of 70 mm from the bottom of the collimator (n = 3, error bars represent the 95% confidence interval on the mean)

The UV dose required to achieve 2 log reduction of *Pseudomonas aeruginosa* in this study was similar to that applied by Bak et al. in their study with the same microorganism grown on the inner surfaces of catheters (Bak et al., 2010) but 200 times lower than that required to inactivate indigenous bacterial communities on real catheters (Bak et al., 2009). Although the Bak et al. studies were conducted in a medical setting, they do clearly show that indigenous bacterial communities in wild biofilms are likely to be more difficult to inactivate than bacteria in biofilms grown from pure cultures under controlled laboratory conditions. Given that the bacterial communities present on real drinking water infrastructure components will likely be complex, it is probable that higher UV doses will be required to achieve meaningful levels of bacterial inactivation in biofilms in real drinking water applications. The ATP results mirrored the plate count results, indicating that this parameter was a good measure of bacterial inactivation by UV LED irradiation.

3.3 Effect of Intensity on Inactivation

Establishing the effect of intensity on the inactivation of biofilm-bound *Pseudomonas aeruginosa* was one of the goals of the current project. A previous study completed Sommer et al. (1998) examined the effect UV intensity had on inactivation of *E. coli*, MS2, ψX174, B40-8, and *B. subtillis.* Their study concluded that an additional 1 log reduction in *E. coli* was achieved with the high intensity (0.2 mW/cm^2) versus the low intensity (0.002 mW/cm^2), and no difference in the inactivation was found for the other microorganisms studied.

3.3.1 Plate Counts

As in earlier experiments, polycarbonate coupons coated with *Pseudomonas aeruginosa* biofilms were exposed to the 265 nm light in the UV LEDs apparatus. The intensity of the light hitting the biofilm-covered coupons was varied by placing the coupons at different distances from the collimator as summarized in Table 1. The exposure time was varied to the ensure that all samples were exposed to the same UV dose (8.1 mJ/cm^2).

Table 1: Relationship between sample placement and light intensity at 265 nm in UV LED apparatus

Distance from Collimator (mm)	Intensity (mW/cm^2)	Exposure Time (min)
15	0.097	1.4
30	0.074	1.8
50	0.053	2.6
70	0.040	3.4

A one-way ANOVA was used to determine whether there were any significant differences between the biofilms exposed to different intensities of UV LED light. All of the treatment levels were significantly different from the control at a 95% confidence level. There was less obvious variability between the treatments, and the only statistically significant difference was between 0.0527 mW/cm^2 and 0.0970 mW/cm^2. When the samples prepared at 0.0397 mW/cm^2 (height = 70 mm) were removed from the analysis, however, there was a linear relationship between concentration and intensity (Figure 4).

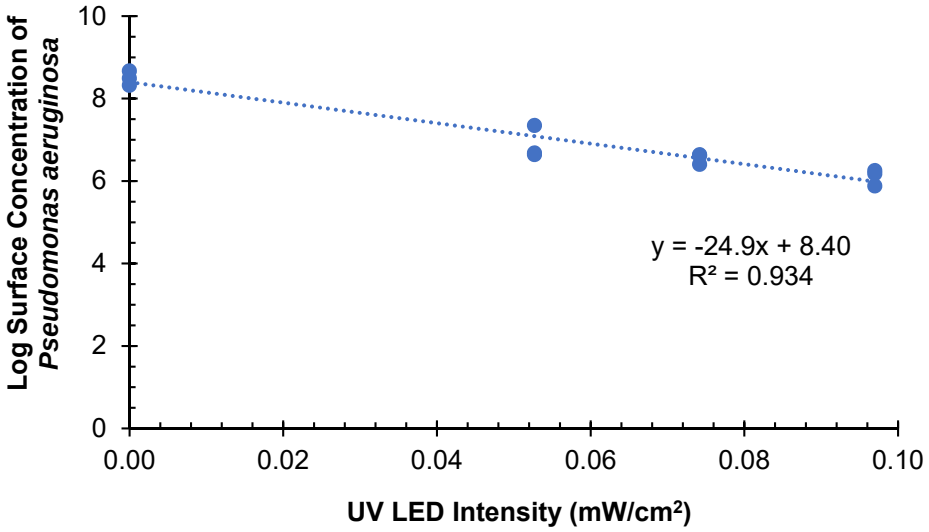

Figure 4: Relationship between intensity and log surface concentration of *Pseudomonas aeruginosa* after exposure to 8.1 mJ/cm^2 of 265 nm UV light at different intensities (n = 3)

3.3.2 ATP Results

As with the plate count results, the ATP content of the biofilms recovered from the control and treated coupons was analyzed with a one-way ANOVA at the 95% confidence level. Although the biofilms recovered from all of the treated coupons contained significantly less ATP than those recovered from the control biofilms, there were no significant differences between biofilm coated coupons exposed to different intensities of UV light.

3.4 Effect of Coupon Material

Experiments are ongoing to examine the impact of coupon material (polycarbonate vs. stainless steel) on biofilm development and UV LED treatment efficacy.

4 CONCLUSIONS

The UV dose required to achieve 2 log inactivation of *Pseudomonas aeruginosa* in this study was in line with previous studies on pure cultures, confirming that the methods employed in this study were congruent with previous research in the field. The UV dose required in the current study was, however, lower than the UV doses required to achieve this level of inactivation in a study that employed mixed bacterial cultures on real clinical surfaces, and it is expected that similarly higher UV doses will be required in real drinking water applications as well.

The effects, if any, of UV intensity on *Pseudomonas aeruginosa* inactivation were difficult to elucidate because of the small range of intensities explored in this study. This limited range was imposed by the configuration of the apparatus used in this study, specifically the length of the collimator. Nonetheless, the plate counts from this part of the study suggest a positive relationship between intensity and inactivation and justify further investigation into this phenomenon.

Acknowledgements

This work was financially supported by NSERC (Engage Grant) and BlueLight Analytics. The authors would like to acknowledge the contributions of Chris Felix, Leeza Hack, and Bronwyn Woolfrey of BlueLight Analytics and Amina Stoddart at the Centre for Water Resources Studies at Dalhousie University.

References

Bak, J., Ladefoged, S.D., Tvede, M., Begovic, T., Gregersen, A., 2010. Disinfection of Pseudomonas aeruginosa biofilm contaminated tube lumens with ultraviolet C light emitting diodes. Biofouling 26, 31–38. doi:10.1080/08927010903191353

Bak, J., Ladefoged, S.D., Tvede, M., Begovic, T., Gregersen, A., 2009. Dose requirements for UVC disinfection of catheter biofilms. Biofouling 25, 289–296. doi:10.1080/08927010802716623

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 Res. 109, 207–216. doi:10.1016/j.watres.2016.11.024

Bolton, J.R., Linden, K.G., Asce, M., 2003. Standardization of Methods for Fluence: UV Dose Determination in Bench-Scale UV Experiments. J. Environ. Eng. 129, 209–215.

Falkinham, J., Pruden, A., Edwards, M., 2015. Opportunistic Premise Plumbing Pathogens: Increasingly Important Pathogens in Drinking Water. Pathogens 4, 373–386. doi:10.3390/pathogens4020373

Ibrahim, M.A.S., Macadam, J., Autin, O., Jefferson, B., 2014. Evaluating the impact of LED bulb development on the economic viability of ultraviolet technology for disinfection. Environ. Technol. (United Kingdom) 35, 400–406. doi:10.1080/09593330.2013.829858

Kheyrandish, A., Mohseni, M., Taghipour, F., 2017. Development of a method for the characterization and operation of UV-LED for water treatment. Water Res. 122, 570–579. doi:10.1016/j.watres.2017.06.015

Lau, H.Y., Ashbolt, N.J., 2009. The role of biofilms and protozoa in legionella pathogenesis: Implications for drinking water. J. Appl. Microbiol. 107, 368–378. doi:10.1111/j.1365-2672.2009.04208.x

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. doi:10.1016/j.desal.2013.08.014

Oguma, K., Kita, R., Takizawa, S., 2016. Effects of Arrangement of UV Light-Emitting Diodes on the Inactivation Efficiency of Microorganisms in Water. Photochem. Photobiol. 92, 314–317. doi:10.1111/php.12571

Rattanakul, S., Oguma, K., 2018. Inactivation kinetics and efficiencies of UV-LEDs against Pseudomonas aeruginosa, Legionella pneumophila, and surrogate microorganisms. Water Res. 130, 31–37. doi:10.1016/j.watres.2017.11.047