

## NWRI GRADUATE FELLOW SEMI-ANNUAL PROGRESS REPORT

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Project Title: Mechanisms and Sustainability of Wavelength-Tailored Ultraviolet Drinking Water Disinfection for Small Systems

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### Background and Introduction

*Overview:* This research is part of the EPA funded DeRISK (Design of Risk-reducing, Innovative-implementable Small-system Knowledge) research center investigating solutions for sustainable water technologies that are appropriate for small systems in the U.S. I investigate novel sources of ultraviolet (UV) light that output specific wavelengths, including light emitting diodes (LEDs) and excimer lamps, which have promise to be a sustainable solution for drinking water disinfection in small communities.

My research uses fundamental microbiological investigations to expand understanding of the mechanisms of UV disinfection beyond the understanding that nucleic acid (DNA and RNA) damage contributes to UV disinfection. Growing evidence indicates that protein damage is also important for UV disinfection<sup>1</sup>. This research will increase understanding of how different UV wavelengths damage the molecules that make up microorganisms, so that combinations of new and/or existing UV light sources can be optimized for more efficient drinking water disinfection.

LEDs and excimer lamps are new UV sources that can be designed to emit specific wavelengths. These new sources could be more sustainable than traditional UV lamps because they do not contain toxic mercury, have lower power requirements, are more compact, and are becoming more efficient as materials science advances. Because disinfection does not depend on the UV lamp type, these sources are as effective as traditional mercury UV lamps for inactivating bacteria, protozoa, and viruses for a given dose/wavelength combination<sup>2</sup>. Additionally, U.S. regulations of UV disinfection technology are governed by requirements for inactivation of viruses, which tend to be more resistant to UV disinfection<sup>2</sup>. Studies that inform current regulations used traditional low pressure (LP) mercury lamps that emit a single UV wavelength. Because viruses are resistant to this wavelength, the UV dose needed to achieve required 4-log viral inactivation (99.99% reduction) is high enough to sometimes be prohibitive for implementation in small systems. However, many microorganisms have varying sensitivity to different UV wavelengths<sup>2</sup>. For example, adenovirus is very resistant to single-wavelength (monochromatic at 254 nm) LP UV disinfection, but is more susceptible to multi-wavelength (polychromatic) medium pressure (MP) UV disinfection<sup>3</sup>.

*Hypotheses:* By optimizing wavelength selection, reactor design, and operation of mercury-free UV sources, equivalent or better disinfection performance can be achieved with less electricity, improving sustainability of UV disinfection. Additionally, this research can inform new regulations so that when optimized wavelengths are used, lower UV doses will be required, increasing the attainability (affordability and feasibility of implementation) of this technology for small systems.

*Objectives:* (1) Determine wavelength-specific protein damage, DNA damage, and inactivation for indicator organisms and pathogens using single, sequential, or simultaneous exposures of mercury-free (LED and excimer lamp) and/or traditional UV sources. (2) Validate disinfection performance at the bench and implement in a local small system the first commercially available LED flow-through reactor to study sustainability, robustness, and disinfection performance over time.

## Progress

*Experimental Design:* UV LEDs emitting various peak wavelengths from 255–285 nm are being tested to target the wavelengths corresponding to peak DNA absorbance and the secondary peak of protein absorbance. A benchtop UV LED system capable of emitting at these wavelengths is being supplied to our lab through an industry partnership. A KrCl excimer lamp emitting at 222 nm is used to target maximum protein absorbance, and is being supplied through another industry partnership. These wavelengths were selected to more efficiently target the advantages previously identified for polychromatic MP UV emissions<sup>4</sup>. The output spectra of these sources are shown in Figure 1.

We are testing these novel, non-mercury UV sources when illuminated individually, sequentially, or simultaneously to determine the optimum disinfection wavelength or combination(s) of wavelengths. These UV sources are also being tested in conjunction with a traditional monochromatic low pressure (LP) mercury UV lamp to determine if wavelength-specific sources targeting proteins can enhance LP disinfection, and decrease doses required to achieve regulatory compliance.

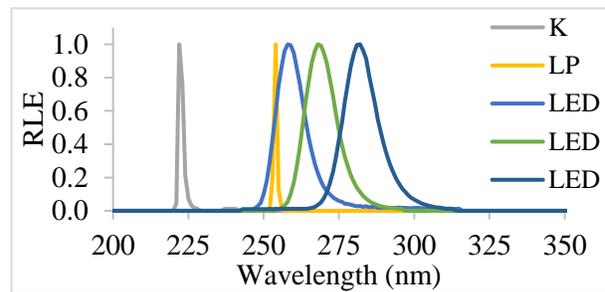


Figure 1 - Relative Lamp Emission (RLE) spectra for KrCl excimer lamp (K), low pressure lamp (LP), and light emitting diodes (LED)

Studies have shown that operating UV sources in pulsed mode provides the same level of disinfection at lower electrical requirements than continuous mode<sup>5-10</sup>. After optimizing wavelengths, we will test the UV source(s) in pulsed mode to explore further optimization of disinfection to achieve the same level of disinfection at lower electrical requirements. The ability to disinfect is being measured via cellular survival, viral infectivity assays, and molecular assays for protein and nucleic acid damage. The effectiveness of disinfection is being normalized to UV dose (fluence) and energy use, enabling comparisons between UV source combinations and modes of operation.

A flow-through LED reactor was designed using input from previous studies from our lab. We evaluated it at bench-scale to verify disinfection performance. The reactor is currently installed and being piloted at a local small system. Over the course of 6 months to 1 year, the UV transmittance (UVT) in the UV reactor influent is measured daily, and correlated with temperature, pH, and turbidity. Bi-weekly samples are collected in the treatment plant influent, slow sand filter effluent, and existing chlorine disinfection effluent for comparison to the pilot UV LED disinfection effluent, as shown in the water treatment plant schematic in Figure 2. Analytes for these bi-weekly samples include ATP, total coliform, *E. coli*, and TOC.

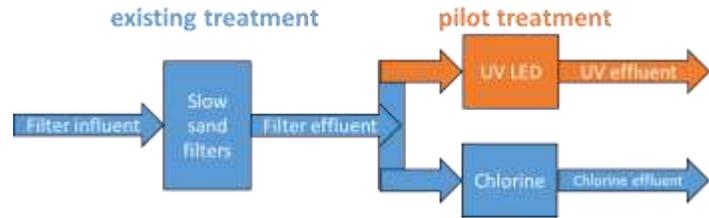


Figure 2 - Local small system drinking water treatment (existing and pilot)

Continued disinfection performance will be monitored using quarterly indicator virus challenge tests. Data on electrical and maintenance requirements are collected for life cycle sustainability analyses performed by partner DeRISK researchers. Additionally, biofilm development in pipes will be compared upstream and downstream of each disinfection process. DNA will be extracted and analyzed from biofilm that develops on 1-inch pipe sections to determine the effect of each disinfection process on microbial communities.

**Experimental Results:** Research for this project builds on work by a previous PhD student in the Linden Lab evaluating UV LEDs for their efficacy in inactivating the common water-borne pathogens, fecal indicators, and surrogates *E. coli*, MS2 coliphage, adenovirus 2, and *B. pumilus* spores<sup>11</sup>. Other work I have done in collaboration with the same student examining the contribution of wavelength-specific protein damage to the inactivation of adenovirus using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) is in preparation to submit for publication this year. Using the same adenovirus samples, I am using a novel enzyme-linked immunosorbent assay (ELISA) technique to investigate the wavelength specific contribution of DNA dimer formation to adenovirus inactivation. This will complement previous Linden Lab research that used qPCR to estimate adenovirus DNA damage after exposure to various UV wavelengths<sup>12</sup>.

The KrCl excimer lamp emitting UV light at a wavelength of 222 nm is being tested to determine how well it inactivates the common water-borne pathogen surrogate MS2 coliphage. The novel inactivation dose-responses for this UV source are being evaluated alone and when combined with LEDs and a LP UV lamp. UV exposures have been conducted so that the irradiation from each UV source was applied alone or sequentially. Experiments are underway to evaluate possible synergy from irradiating the sources simultaneously. We expect to determine the most effective lamp and lamp combinations and the molecular mechanisms by which that effectiveness is generated. Preliminary results indicate a disinfection advantage by incorporating the excimer lamp, and possible MS2 inactivation synergy from simultaneous exposure of the LP UV and excimer lamps.

Damage to MS2 proteins resulting from exposure to various wavelengths is being assessed by SDS-PAGE to determine contribution of protein damage to MS2 inactivation and compare to results published by our lab for adenovirus. We expect that protein damage will similarly contribute to MS2 inactivation because both viruses rely on protein integrity to attach to and infect their host cell. Knowing that the viral surrogate MS2 has similar molecular disinfection response to the target

pathogen adenovirus will contribute confidence to validations and test results for novel wavelength combinations using this viral surrogate.

The MS2 disinfection performance of the UV LED reactor was measured at various flowrates and UVTs at the bench in dechlorinated tap water, and in slow sand filter effluent water after installation at the local small system. The validation in the small system filter effluent aligned with modeled results from the bench testing, indicating no detrimental effects of the matrix water at the small system upon installation. Continued sampling is underway to monitor longitudinal performance.

## Conclusions

*Implications:* LEDs were demonstrated to successfully inactivate pathogens, indicators, and surrogates. This provides proof of concept that these emerging sources can be an effective drinking water disinfection technology. Synergy was not observed for simultaneous irradiation of LEDs for inactivation or nucleic acid damage, but comparatively less energy was required to inactivate *E. coli* using LEDs. This provides evidence for the practicality of LEDs as an emerging disinfection technology. The extent of protein damage is being investigated for adenovirus and MS2. This is important for the design of a reactor using tailored wavelengths that will be equally or more effective at lower doses than provided by LP UV. Mechanistic understanding of how surrogate and pathogenic microorganisms are inactivated can contribute to more informed regulatory decision-making, especially when mechanisms for the surrogate match the target pathogen. Longitudinal evaluation of the flow-through UV LED system will provide data necessary for practical operation, design improvements, and scale-up, allowing faster adoption in the future.

*Novel Aspects:* Synergies between wavelength-specific excimer lamps, LEDs, and traditional LP lamps have not previously been investigated to determine how molecular protein and nucleic acid damage contribute to increased UV disinfection efficiency (especially at low wavelengths). Use of a simple viral surrogate, MS2 coliphage, to assess protein damage is a novel technique for evaluation of performance of these UV sources. This is the first longitudinal pilot investigation of a flow-through UV LED disinfection reactor at a drinking water treatment plant.

## Next Steps

Future bench-scale research plans include simultaneous UV exposures, combining LEDs tested with Excimer lamp and/or LP UV, to optimize molecular damage and cellular inactivation. The best wavelength combination(s) will be tested in pulsed mode to determine if further electrical optimization is possible. As for adenovirus, MS2 will be exposed to wavelengths across the germicidal spectrum to determine the spectral protein damage response. It is expected that the protein damage spectrum for MS2 will resemble that for adenovirus, just as their inactivation action spectra are similar. Work with the excimer lamp also demonstrates promise for design and evaluation of a reactor combining it with either LEDs or LP UV to obtain enhanced disinfection from low wavelengths. The spring runoff season is just beginning at the local water treatment plant, which may prove challenging for the UV LED reactor due to spikes in turbidity.

## References

- (1) Eischeid, A. C.; Linden, K. G. *Appl. Environ. Microbiol.* **2011**, *77* (3), 1145–1147.
- (2) WRF. *WRF 4376: Guidance for Implementing Action Spectra Correction with Medium Pressure UV Disinfection*; 2015.
- (3) Eischeid, A. C.; Thurston, J. A.; Linden, K. G. *Crit. Rev. Environ. Sci. Technol.* **2011**, *41* (15), 1375–1396.
- (4) Linden, K. G.; Thurston, J.; Schaefer, R.; Malley, J. P. *Appl. Environ. Microbiol.* **2007**, *73* (23), 7571–7574.
- (5) Azar Daryany, M. K.; Massudi, R.; Hosseini, M. *Curr. Microbiol.* **2008**, *56* (5), 423–428.
- (6) Willert, C.; Stasicki, B.; Klinner, J.; Moessner, S. *Meas. Sci. Technol.* **2010**, *21* (7), 75402.
- (7) Massier, S.; Bouffartigues, E.; Rincé, A.; Maillot, O.; Feuilloley, M. G. J.; Orange, N.; Chevalier, S. *J. Appl. Microbiol.* **2013**, *114* (1), 186–195.
- (8) Tran, T.; Racz, L.; Grimaila, M. R.; Miller, M.; Harper, W. F. *Water Sci. Technol.* **2014**, *70* (9).
- (9) Li, J.; Hirota, K.; Yumoto, H.; Matsuo, T.; Miyake, Y.; Ichikawa, T. *J. Appl. Microbiol.* **2010**, *109* (6), 2183–2190.
- (10) Wengraitis, S.; McCubbin, P.; Wade, M. M.; Biggs, T. D.; Hall, S.; Williams, L. I.; Zulich, A. W. *Photochem. Photobiol.* **2013**, *89* (1), 127–131.
- (11) Beck, S. E.; Ryu, H.; Boczek, L. A.; Cashdollar, J. L.; Jeanis, K. M.; Rosenblum, J. S.; Lawal, O. R.; Linden, K. G. *Water Res.* **2017**, *109*, 207–216.
- (12) Beck, S. E.; Rodriguez, R. A.; Linden, K. G.; Hargy, T. M.; Larason, T. C.; Wright, H. B. *Environ. Sci. Technol.* **2014**, *48* (1), 591–598.