NWRI GRADUATE FELLOW SEMI-ANNUAL PROGRESS REPORT

DATE:	December 1, 2017
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 treatment 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, for drinking water disinfection. 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 causes UV disinfection. Growing evidence indicates that protein damage also causes UV disinfection[1]. 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, as shown in Figure 1. 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 disinfect bacteria, protozoa, and viruses just as well as traditional mercury UV lamps for a given dose and wavelength[2].

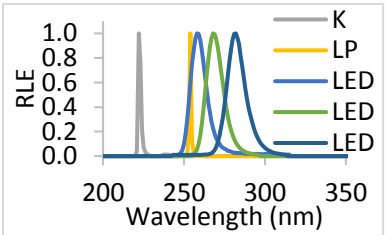

Figure 1 - Relative Lamp Emission (RLE) spectra for the KrCl excimer lamp (K), low pressure mercury lamp (LP), and light emitting diodes (LEDs) used in this study.

U.S. EPA guidance for UV disinfection of drinking water are governed by virus inactivation using traditional low pressure (LP) mercury lamps that emit a single UV wavelength[3]. 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 cost-prohibitive for small systems. However, microorganisms vary in their sensitivity to different UV wavelengths[2]. For example, adenovirus is

resistant to single-wavelength (monochromatic at 254 nm) LP UV disinfection, but is more susceptible to multi-wavelength (polychromatic) medium pressure (MP) UV disinfection[4]. This varying wavelength sensitivity offers an opportunity to optimize UV disinfection.

Hypotheses: By optimizing wavelength selection, reactor design, and operation of mercury-free UV sources, we can achieve equivalent or better disinfection performance with less electricity, thereby improving the sustainability of the UV disinfection process. 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: A benchtop UV LED system emitting various peak wavelengths from 255 – 285 nm shown in Figure 1 is being supplied to our lab through an industry partnership. A KrCl excimer lamp emitting at 222 nm is being supplied through another industry partnership. We are testing these wavelengths to more efficiently target absorbance spectra of DNA and proteins, to simulate the disinfection advantages previously identified for polychromatic MP UV emissions[5]. 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 LP lamp to determine if wavelength-specific sources targeting DNA and proteins can enhance LP disinfection, and lower the combined dose to achieve 4-log virus disinfection required for regulatory compliance. After optimizing wavelengths, we will test the UV source(s) in pulsed mode[6–11] to explore further optimization 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 and it is being piloted at a local small system. Over the course of 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.

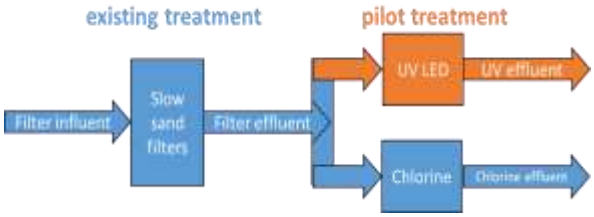

Figure 2 - Local small system drinking water treatment (existing and pilot).

Analytes for these bi-weekly samples include ATP, total coliform, *E. coli*, and TOC. 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 by extracting, quantifying, and sequencing DNA.

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 *E. coli* bacteria, MS2 coliphage (nonpathogenic surrogate virus), adenovirus 2 (pathogenic virus), and *B. pumilus* bacterial spores[12]. Other work I have done in collaboration with this student examined the contribution of wavelength-specific protein damage to adenovirus inactivation using SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), and was recently accepted for publication[13]. I am currently investigating wavelength-specific damage to MS2 proteins using SDS-PAGE to compare to adenovirus results. Preliminary results indicate that protein damage similarly contributes to MS2 inactivation, likely because both viruses rely on protein integrity to attach to and infect their host cell. I am also using a novel enzyme-linked immunosorbent assay (ELISA) 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[14]. These mechanistic studies demonstrating 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 KrCl excimer lamp is being tested to determine how well it inactivates MS2. 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, or in pulsed mode. 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.

The MS2 disinfection performance of the flow-through UV LED reactor was measured at various flowrates and UVTs measured at 285 nm (the wavelength emitted by the LEDs) at the bench in dechlorinated tap water, as shown in Figure 3. MS2 disinfection was also measured in slow sand filter

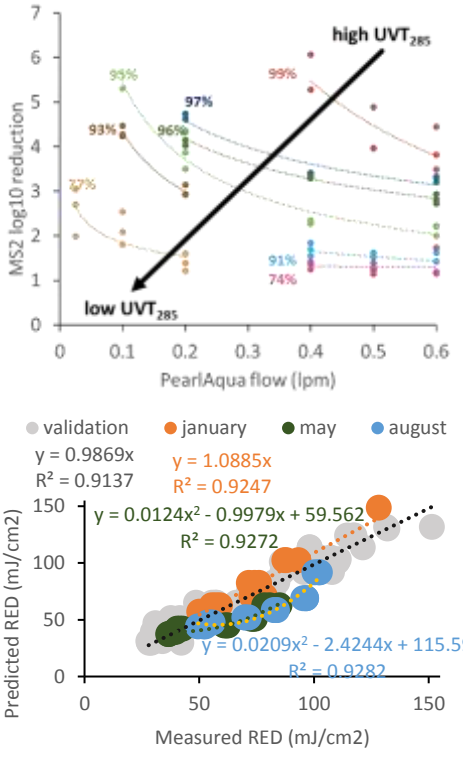

Figure 3 – MS2 disinfection performance of the UVLED reactor at the bench (first panel) and in the field (second panel). Reduction equivalent dose (RED) is the collimated beam UV Dose that achieved equivalent log_{10} reduction of MS2 in the flow through reactor. Predicted RED was modeled using bench data in the first panel by a combined variable approach[15] for comparison to measured field data in the second panel.

effluent water after installation at the local small system in January. Experimental results of MS2 disinfection in January aligned with model projections based on the previous bench testing, indicating that the disinfection performance of the LED reactor could be predicted using bench-scale data when installed in the field and treating a different water matrix. Ongoing testing in May and August demonstrated greater disinfection than predicted by the bench-testing model.

Conclusions

Implications: LEDs were demonstrated to successfully inactivate pathogens, indicators, and surrogates. This provides evidence for the practicality of LEDs as an emerging disinfection technology. The contribution of UV wavelength-specific protein damage and DNA dimer formation to inactivation is being investigated for adenovirus and MS2. This is important for the design and validation 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 of inactivation are the same for both the surrogate and 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, which will combine LEDs tested with Excimer lamp and/or LP UV to optimize molecular damage and inactivation efficacy. Although our bench UV LED system cannot feasibly operate in pulsed mode, the excimer lamp will be investigated to determine if further electrical optimization by pulsing is possible. Investigations of wavelength-specific protein damage and dimer formation for MS2 and adenovirus are ongoing. Work with the excimer lamp demonstrates promise for design and evaluation of a reactor combining it with either LEDs or LP UV to obtain enhanced disinfection from low wavelengths. When the year-long pilot study of the flow-through reactor ends, pipe biofilm microbial communities will be extracted and sequenced if sufficient volume accumulated.

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