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Efficacy of Inactivation of Legionella pneumophila by Multiple-Wavelength UV LEDs

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Outline

- Introduction
  - ✓Background information and research motivation
  - ✓Research objectives
- Materials and Methods
- Results and Discussion
- Summary and Conclusion
- Further Study

Research Background

Legionellosis

- *Legionella pneumophila*, a waterborne pathogen, first caught the public's attention when an outbreak occurred in Philadelphia, PA in 1976.
  - 182 cases with 29 deaths
- There are an estimated 8,000 to 18,000 cases per year of Legionnaires' disease.
  - Estimated incident rate: 7.0 to 7.9 cases per 100,000 people
- One of the largest, most recent outbreaks occurred in Flint, MI.
  - 2014 outbreak: 45 cases, 7 deaths
  - 2015 outbreak: 46 cases, 7 deaths

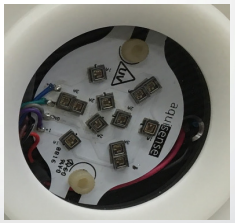
Research Background

Disinfection practices

- Chlorine is the most common way in the United States to treat pathogens in water.
  - Evaporates out at higher temperatures
  - Creates disinfection by-products
- Heat flushing is another commonly used treatment to inactivate *Legionella*.
  - Must be performed regularly
  - Can be costly and laborious to maintain
- Copper-silver ionization is another strategy used to combat microorganisms in water, especially in hospitals.
  - Difficult to maintain proper levels in a large distribution system
  - Potential health risks if too much is present in drinking water

Research Motivation – UV LEDs

- Ultraviolet (UV) light has been successfully used for treating a broad suite of pathogens.
  - No carcinogenic DBPs formation
- However, conventional mercury UV lamps have some practical limitations in water treatment applications.
  - Inefficiency of energy consumption
  - Potential mercury contamination
- An emerging UV LEDs (light emitting diodes) technology has enormous potential and could eliminate the aforementioned limitations.
  - Smaller, lighter, less fragile, and mercury-free
  - Provides the capability to be turned instantaneously on and off



Study Objectives

❖We investigated the efficacy of multiple-wavelength UV LEDs for inactivating *Legionella pneumophila* in water.

- ✓ Three major Opportunistic Premise Plumbing Pathogens (OPPPs)
  - *Legionella pneumophila*, *Pseudomonas aeruginosa*, & Nontuberculous mycobacteria

USEPA's contaminant candidate list (CCL) 4 microorganisms (2017)

Microbial Contaminant Name	Type	Diseases and Infections
Adenovirus	Virus	Respiratory illness and occasionally gastrointestinal illness.
Caliciviruses	Virus (includes Norovirus)	Mild self-limiting gastrointestinal illness.
Campylobacter jejuni	Bacteria	Mild self-limiting gastrointestinal illness.
Enterovirus	Viruses including polioviruses, coxsackieviruses and echoviruses	Mild respiratory illness.
Escherichia coli (0157)	Bacteria	Gastrointestinal illness and kidney failure.
Helicobacter pylori	Bacteria	Found in the environment capable of colonizing human gut that can cause ulcers and cancer.
Hepatitis A virus	Virus	Liver disease and jaundice.

Microbial Contaminant Name	Type	Diseases and Infections
Legionella pneumophila	Bacteria	Found in the environment including hot water systems causing lung diseases when inhaled.
Mycobacterium avium	Bacteria	Lung infection in those with underlying lung disease, and disseminated infection in the severely immuno compromised.
Naegleria fowleri	Protozoan	Parasite found in shallow, warm surface and ground water causing primary amebic meningoencephalitis.
Salmonella enterica	Bacteria	Mild self-limiting gastrointestinal illness.
Shigella sonnei	Bacteria	Mild self-limiting gastrointestinal illness and bloody diarrhea.

Materials & Methods

❖ *Legionella pneumophila* serogroup 1

- Philadelphia 02 (Lp02) & two environmental strains such as KMC (Ohio) and F7621 (Michigan)

- Log phase cells generated by incubation at 35°C for 48 hours in buffered yeast extract broth

❖ Microbial Stock Preparation

- Step 1: Growth of the culture (overnight culture, followed by 2-day incubation)

- Step 2: Washing the culture and making the stock

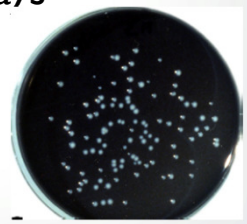
- Step 3: Sample preparation at 10^5 CFU/mL (10 mL per sample)

❖ Standard Culturable method

- Step 1: Spread plating on BCYE agar

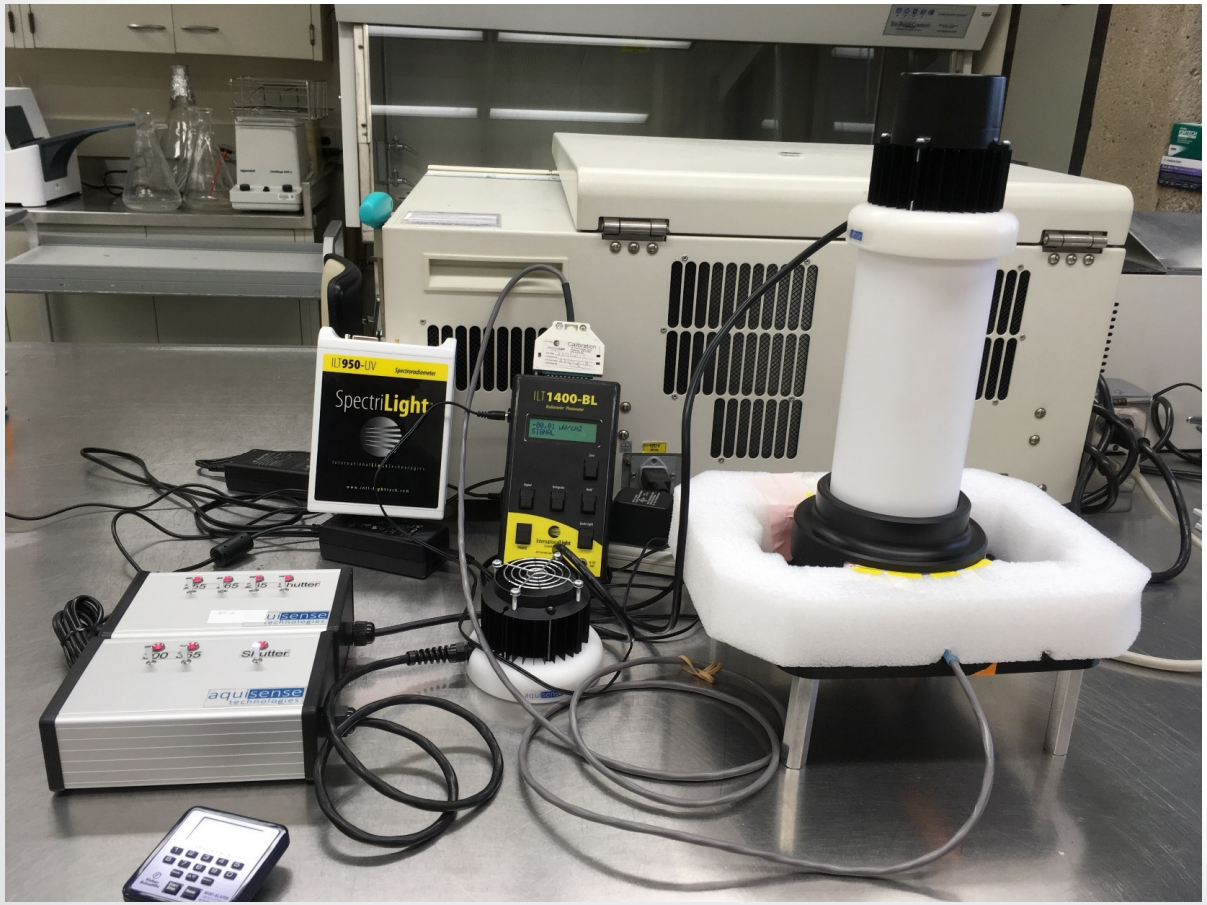
- Step 2: Incubate at 35°C for 4-5 days

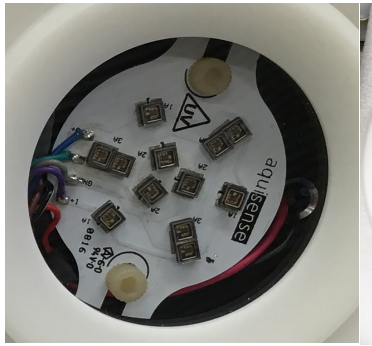
- Step 3: Counting colonies

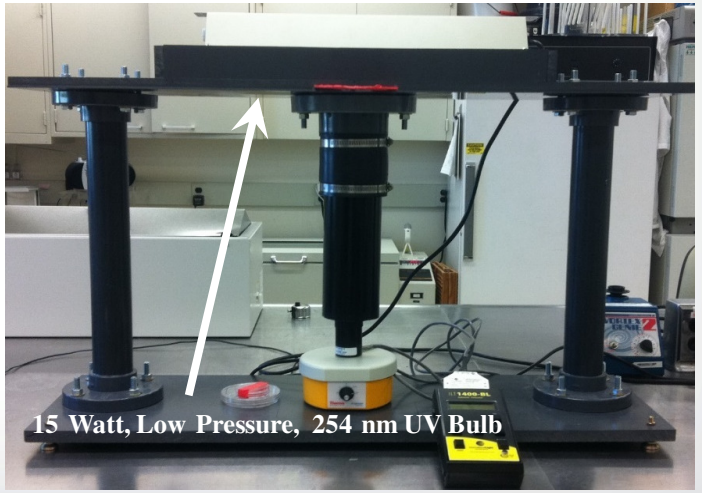


BCYE plate

UV Collimated Beam Apparatus



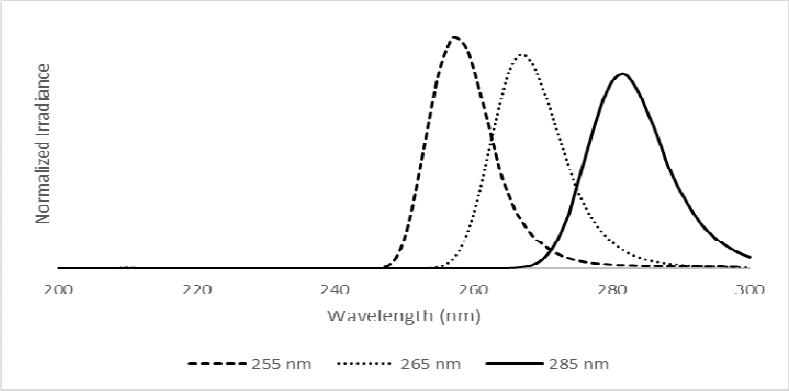




Emission Spectra

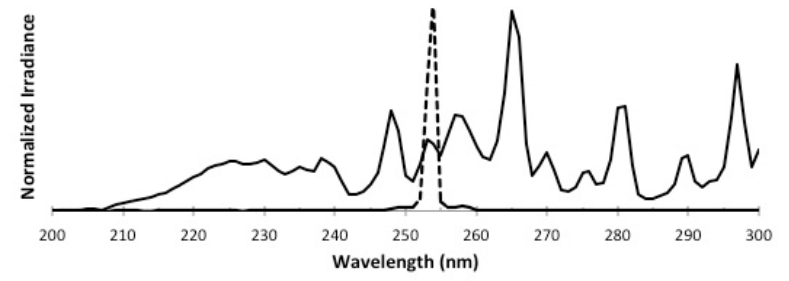
Emission spectra from LEDs and low-pressure (dashed) mercury vapor lamps

LEDs (255, 265, & 285 nm)



Peak wavelength emissions at 260.65 nm, 268.87 nm, and 282.98 nm with FWHM band widths of 10.5 nm, 11.7 nm, and 13.0 nm, respectively.

LP UV



(adapted from Beck et al., 2017 Water Research)

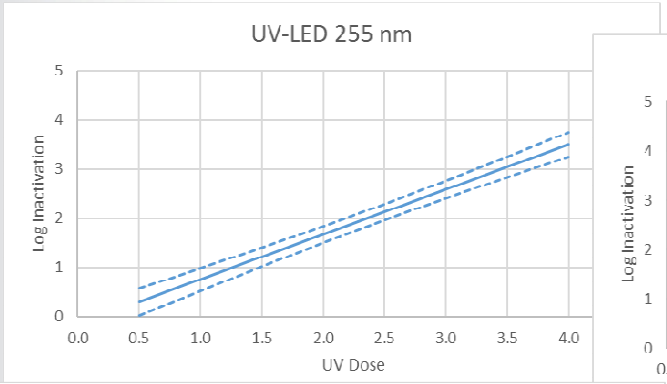
Materials & Methods

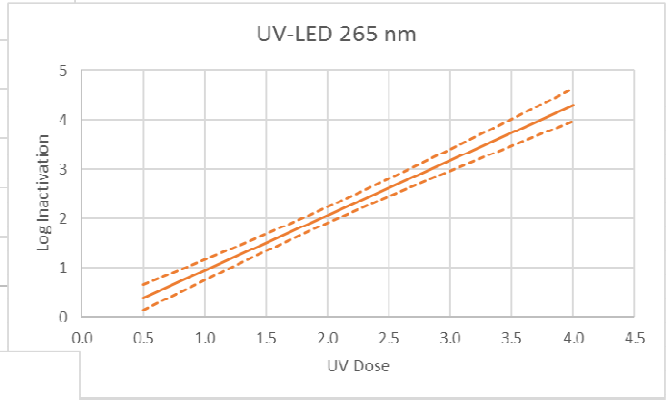
Statistical Analyses

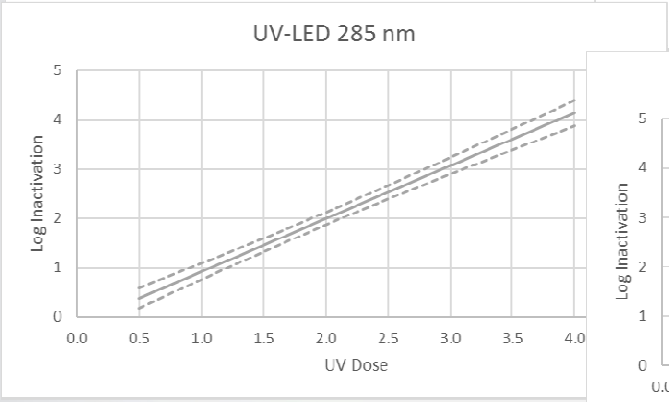
- For one experiment of a particular strain, three wavelengths of UV LEDs were tested (255 nm, 265 nm, and 285 nm) along with UV-LP (254 nm)
  - *Three experiments for each strain, for a total of nine experiments*
- Linear Regression was performed to generate the inactivation coefficient, as the strains appeared to follow the Bunsen-Roscoe Reciprocity Law.
- 95% Confidence Intervals were generated using the previously calculated slope.
- ANCOVA was used to analyze the difference between the inactivation coefficients between wavelengths of a single strain.
- A factorial design was used to determine if a difference existed between strains of a single wavelength.

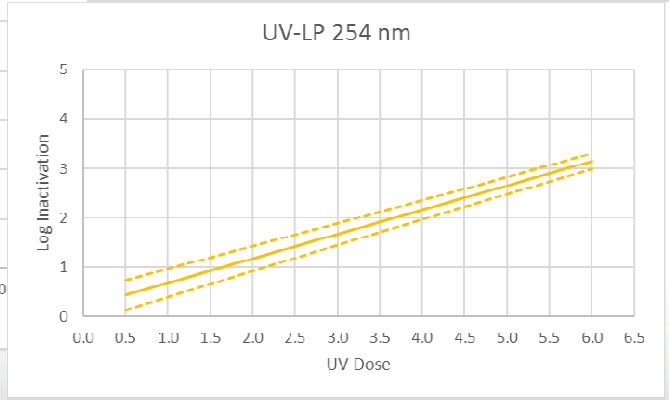
Results & Discussion

The inactivation rate of the LP02 strain varied for the different wavelengths.







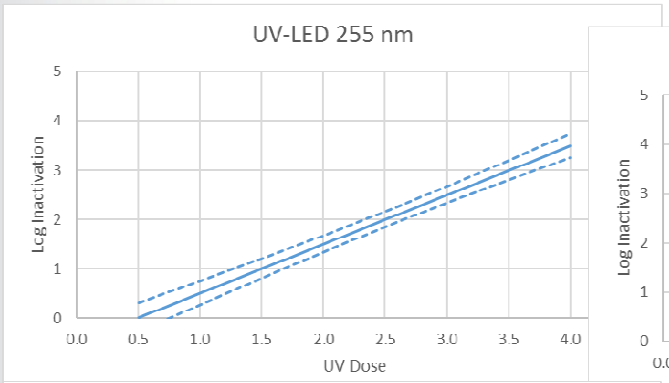


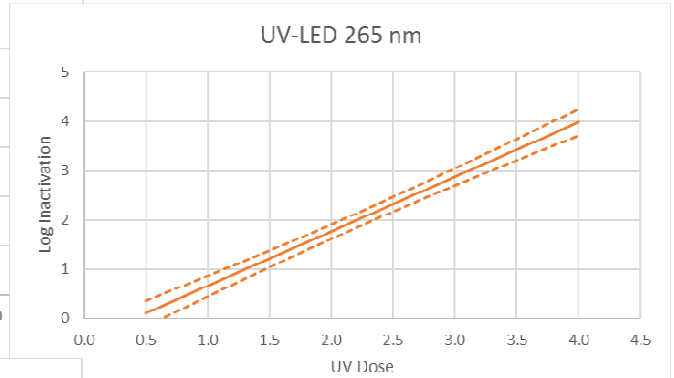
- Inactivation Coefficients:
  - 255 nm: 0.9135
  - 265 nm: 1.1165
  - 285 nm: 1.0727
  - 254 nm: 0.4934

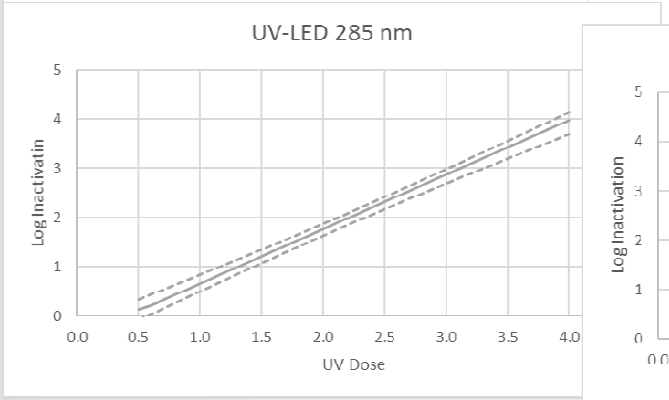
- All of the inactivation rates were significantly different from each other, except for the coefficients for 265 nm and 285 nm.

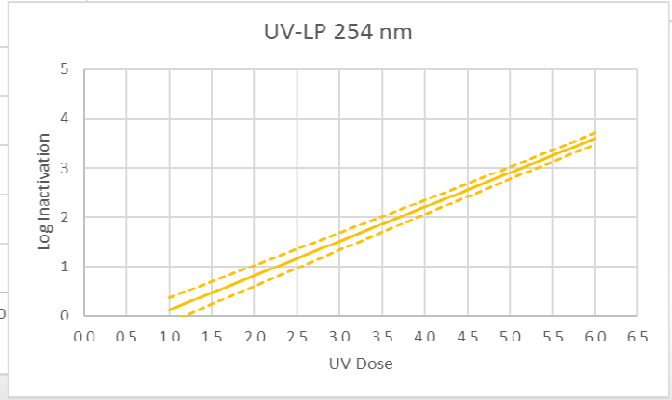
Results & Discussion

The inactivation rate of the KMC strain varied for the different wavelengths.





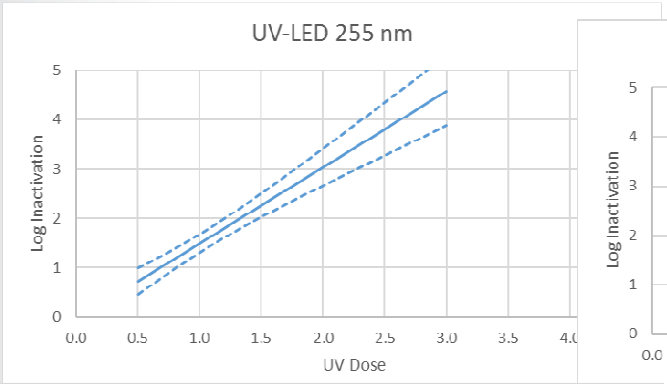


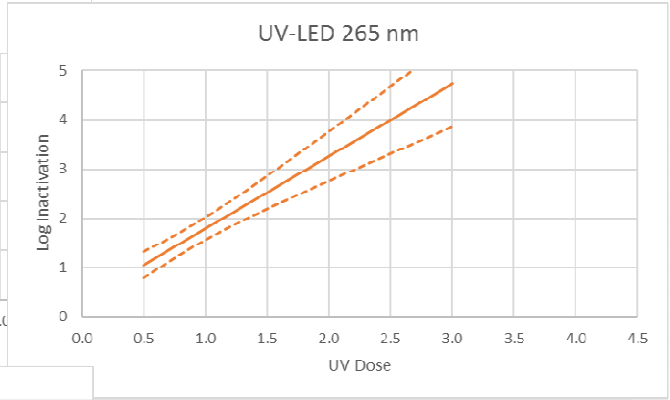


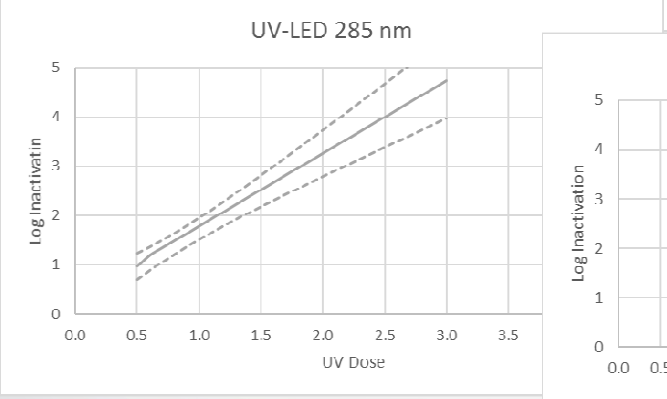
- Inactivation Coefficients:
  - 255 nm: 0.9945
  - 265 nm: 1.1063
  - 285 nm: 1.0788
  - 254 nm: 0.6936
- All of the inactivation rates were significantly different from each other, except for the coefficients for 265 nm and 285 nm.

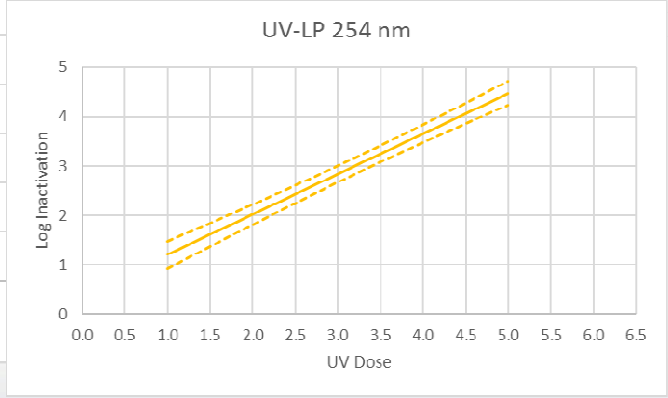
Results & Discussion

The inactivation rate of the F7621 strain varied for the different wavelengths.





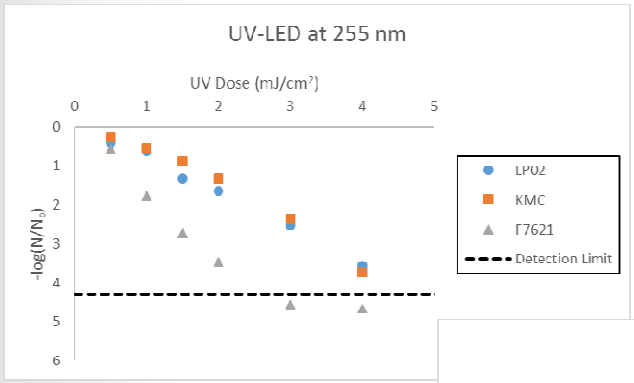


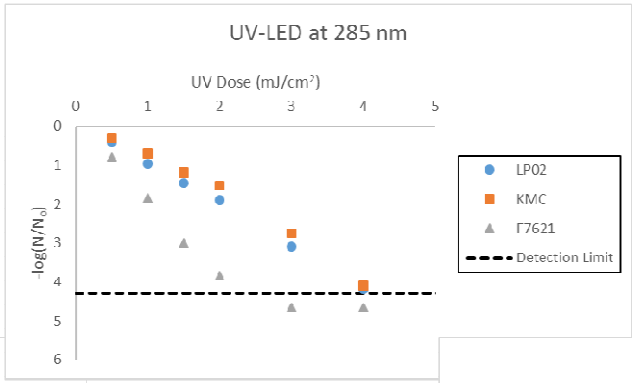


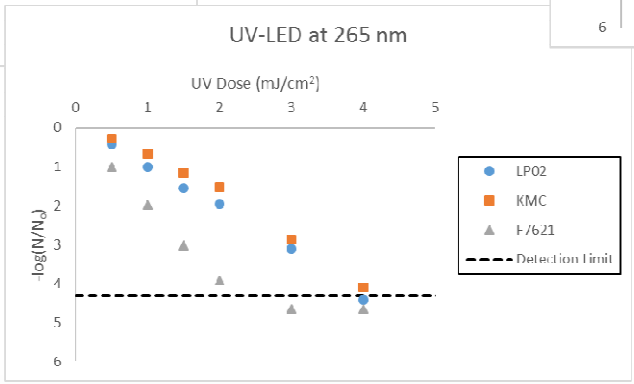
- Inactivation Coefficients:
  - 255 nm: 1.5440
  - 265 nm: 1.4736
  - 285 nm: 1.5262
  - 254 nm: 0.8196
- LP UV at 254 nm had the least inactivation rate.

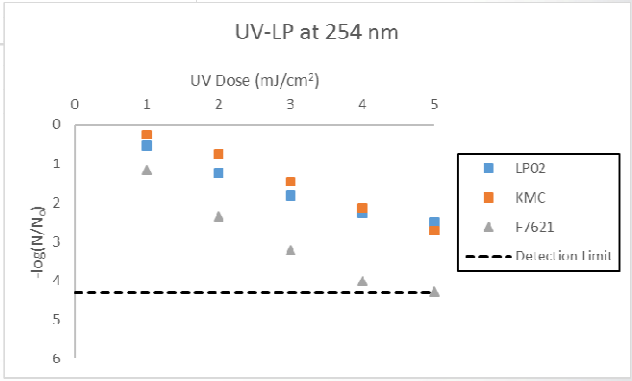
Results & Discussion

Comparison of the Three Strains





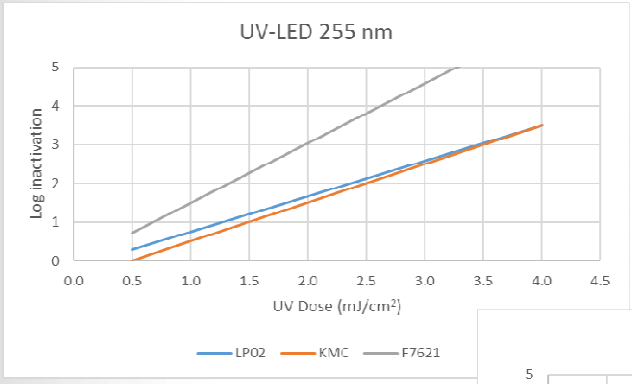


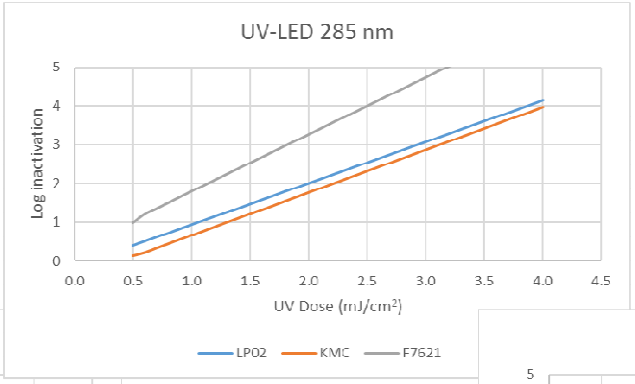


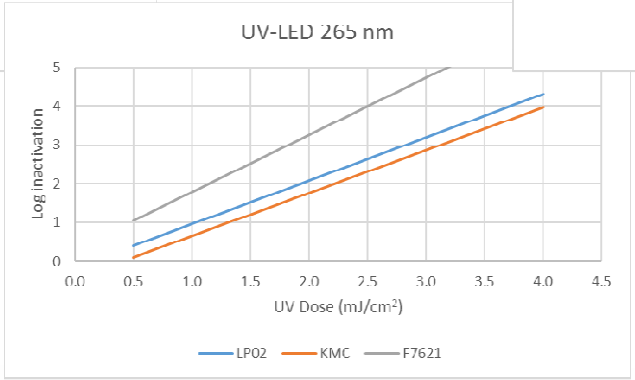
Overall Inactivation Efficacy: LP02 = KMC < F7621

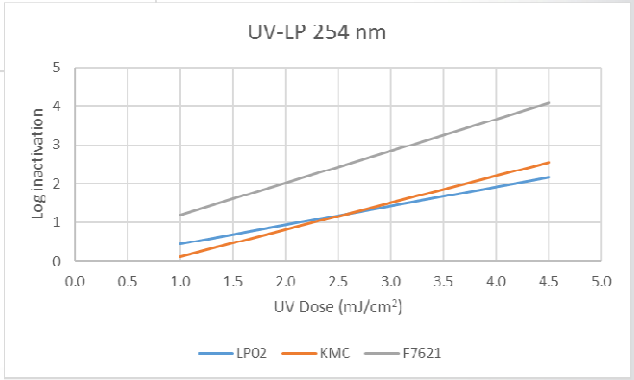
Results & Discussion

Comparison of the Three Strains









Overall Inactivation Efficacy: LP02 = KMC < F7621

Results & Discussion

Comparison of Inactivation Coefficients

	Strain LP02 (Lab)	Strain KMC (OH Environmental)	Strain F7621 (MI Environmental)
UV-LED 255 nm	0.9135	0.9945	1.5440
UV-LED 265 nm	1.1165	1.1063	1.4736
UV-LED 285 nm	1.0727	1.0788	1.5262
UV-LP 254 nm	0.4934	0.6936	0.8196

Summary

- **LP02 and KMC strains**
  - ✓ UV LEDs were more effective than conventional mercury vapor lamps for inactivating these strains.
  - ✓ The 265 nm and 285 nm wavelengths had the greatest inactivation coefficients.
- **Michigan Environmental Strain F7621 Study**
  - ✓ UV LEDs resulted in greater inactivation when compared to the mercury lamps for this strain.
  - ✓ No significant difference of inactivation coefficients among three wavelengths of LEDs, whereas LP UV had the least inactivation coefficient.
- **Comparison of the Strains**
  - ✓ While the LP02 and KMC strains had statistically equivalent inactivation coefficients, the F7621 strain had greater inactivation coefficients for each wavelength, compared to the other two strains.

Conclusions

- UV LEDs showed the capability to effectively inactivate all three strains of *L. pneumophila* serogroup 1 tested. For the LPO2 strain,
  - LEDs: approximately 4 mJ/cm^2 for achieving 4-log inactivation
  - LP UV at 254 nm: approximately 8 mJ/cm^2 for achieving 4-log inactivation
- When choosing a wavelength to inactivate *L. pneumophila* serogroup 1, 265 nm and 285 nm performed the best across all strains.
- All wavelengths of the UV LED outperformed conventional UV LP.

Further **Study**

- ✓ CCL microbes & other Premise Plumbing Pathogens - *Pseudomonas & Mycobacterium*
- ✓ Synergistic effect of UV LEDs coup ed with the Cu-Ag ionization – beneficial to hospital water systems
- ✓ Improving the efficacy of a germicidal UV system
- ✓ Development of a POU devices and its performance evaluation

EPA Disclaimer

- ***The U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for external publication. Any opinions expressed in this paper are those of the authors and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.***

Questions ?

Thanks For Your Attention!

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