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Pilot Study for UVA-LED Disinfection of *Escherichia coli* in Water for Space and Earth Applications

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Introduction

The purpose of the research is to investigate the efficiency of Ultraviolet A Light-Emitting Diodes (UVA-LEDs) for water disinfection. The study has significant applications to consistently provide potable water both in space and on Earth.[1] Ultraviolet light is an effective water purification method utilized since the 1950's. UVC lamps are commonly used in sterilization equipment and are highly effective, but contain mercury which is hazardous to human health. UVA-LEDs are advantageous because they require lower input energy and are mercury free, and furthermore can be installed in different places and angles due to their small size and resistance to vibrations.[2]

Abstract

To test the efficacy of UVA-LED disinfection, a solution of *Escherichia coli* was pumped through a modified drip flow reactor at a flow rate of 1 ml/min. The experiment was conducted in a controlled environment chamber to ensure that temperature did not cause disinfection. The reactor featured three wells with different treatments: UVA-LED irradiation, UVA-LEDs with TiO_2, and UVA-LEDs with nanosilver. Samples from each well were taken throughout a 340 hour period, inactivated, assayed, and analyzed for *E. coli* disinfection. Results of the duplicate experiments indicated longer exposure times are needed for UVA-LED disinfection of *E. coli* in water. Further research would consider a longer sampling period and different test conditions, such as increased contact area and various flow rates.

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[1] Mori, M., Hamamoto, A., Takahashi, A., Nakano, M., Wakikawa, N., Tachibana, S. ... Kinouchi, Y. (2007). Development of a new water sterilization device with a 365 nm UV-LED. *Medical and Biological Engineering and Computing, 45,* 1237-1241.

[2] Hamamoto, A., Mori, M., Takahashi, A., Nakano, M., Wakikawa, N., Akutagawa, M. ...Kinouchi, Y. (2007). New water disinfection system using UVA light-emitting diodes. *Journal of Applied Microbiology, 103,* 2291-2298.

Goals and Purpose of the Project

The goal of the project is to investigate a new method for disinfecting water in circumstances where energy requirements and size are the limiting factors. Ultraviolet light has been used for disinfection for nearly half a century. UV inactivates microorganisms by causing thymine dimerization of DNA or RNA, which prevents replication.[3] Sterilization equipment employs UV lamps with a 254 nm wavelength.[4] Shorter UVC wavelengths tend to be more effective for inactivating pathogens, but the optimal wavelength varies depending on the organism.[5] Although UV lamps are effective, they demand high energy inputs and contain mercury, which is hazardous to humans.[6] UVA-LEDs have longer wavelengths than commonly used disinfecting UV lamps that typically employ UVC wavelengths, but possess significant advantages. UVA-LEDs are low cost, require less energy, contain no mercury, and last much longer than UV lamps.[7] They can be positioned in many arrangements and at different angles given their small size.

The research utilized a modified drip flow reactor, where the bacteria solution flowed through wells containing UVA-LEDs at a rate of 1 ml/min. A circulating liquid sample is a realistic system, similar to current disinfection systems where water is pumped through a series of filters and treated before distribution.[8] The reactor featured three wells, where different treatments were investigated: UVA-LED irradiation, UVA-LEDs with TiO_2, and UVA-LEDs with nanosilver.

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[3] Maier, R.M., Pepper, I.L., & Gerba, C.P. (2000). *Environmental microbiology*. San Diego, CA: Academic Press. 553.
[4] Mori et al.
[5] Malley, James, & AWWA Foundation. (2004). *Inactivation of pathogens with innovative UV technologies.* American Water Works Association. 55.
[6] Hamamoto et al.
[7] Ibid.
[8] Pontius, F.W. (Ed.). (1990). *Water quality and treatment: A handbook of community water supplies* (4th ed.). New York, NY: McGraw-Hill. 177-182.

The research was conducted at the Space Life Sciences Laboratory (SLSL) at Kennedy Space Center, which specializes in biological research for exploration as well as processing experiments conducted on the International Space Station.[9] The Life Support and Habitation Systems project addresses the needs of humans in space, including food, air purification, and water purification. This pilot study is one of the water disinfection experiments being conducted at the SLSL.

My role in achieving the objectives of the assignment included a number of tasks. Before the sampling period, I assisted in culturing the bacteria, preparing the reactor, and sterilizing the necessary implements. I performed serial dilutions and plated the sample onto TSA plates, and counted the bacteria after the plates had been incubated. After the sampling period, I analyzed the data to draw conclusions. The experiment was conducted in duplicate.

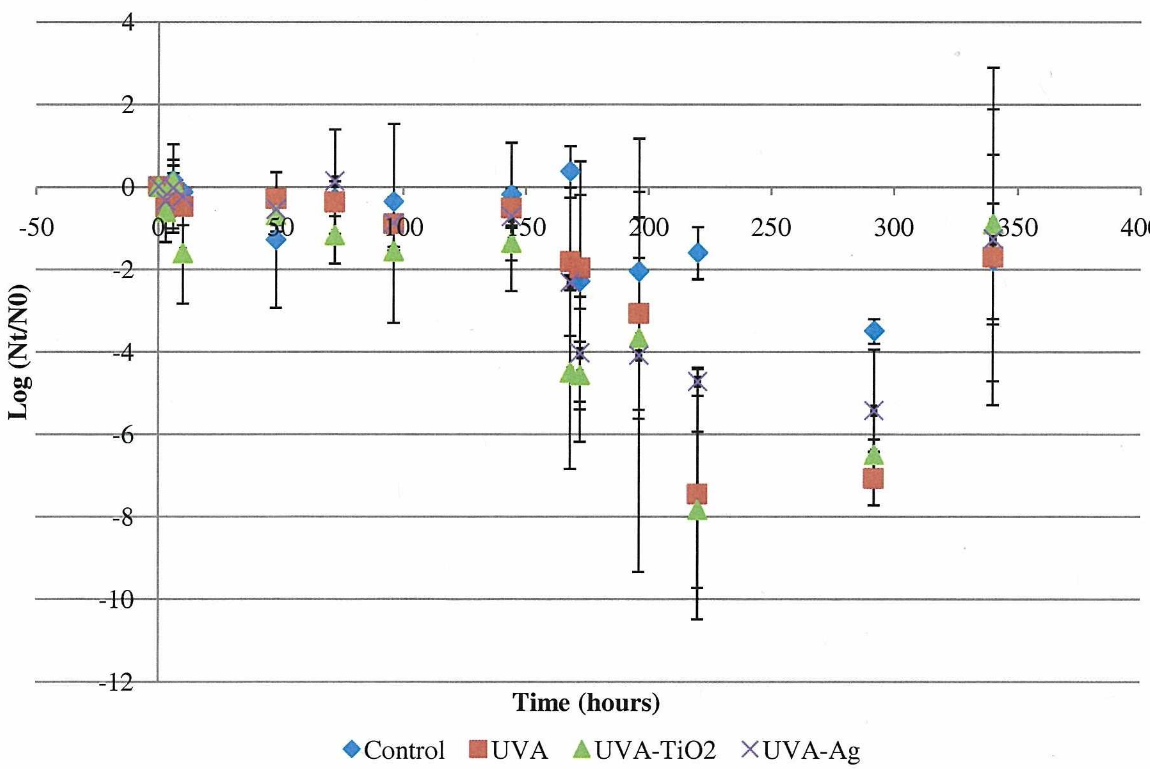

Figure 1. Disinfection of *Escherichia coli* by UVA-LEDs in a modified drip flow reactor system.

[9] Heise, J. (Ed.). (2006). *Space Life Sciences Lab.* Retrieved from http://slsl.lssc.nasa.gov/

The duplicate data in Figure 1 show that all treatments demonstrated >5 log reduction at t = 292, with control having a 3.4 log reduction. At t = 340, all treatments had less reduction than control. At t = 220, UVA-LEDs with TiO_2 had 7.8 log reduction and UVA-LEDs had 7.4 log reduction. However, at t = 292, UVA-LEDs had 7.0 log reduction and UVA-LEDs with TiO_2 had 6.4 log reduction. Further testing with a longer sampling period should be conducted to investigate whether UVA-LEDs can inactivate *E. coli* in water at the conditions tested with longer exposure time, and to discern whether the change in the relative reduction capacities of UVA-LEDs and UVA-LEDs with TiO_2 is due to bacterial reactivation.

ANOVA	**P-Value**
Control, UVA, UVA-TiO2, UVA-Ag	0.284
Control, UVA, UVA-TiO2	0.166
Control, UVA, UVA-Ag	0.389
Control, UVA-TiO2, UVA-Ag	0.125
UVA, UVA-TiO2, UVA-Ag	0.668
UVA, UVA-TiO2	0.519
UVA, UVA-Ag	0.880
UVA-TiO2, UVA-Ag	0.393

Table 1. Analysis of variance and P-values of the different treatments investigated.

Given a P-value of > 0.05, the null hypothesis that all treatments are equal cannot be rejected. Our conclusion for this pilot study from the ANOVA of the data is that there is no statistically significant difference among the treatments and the control. Therefore, UVA-LEDs alone or in combination with TiO_2 or nanosilver do not inactivate *E. coli* under the conditions tested during a period of 340 hours. Existing research demonstrates that UVA-LEDs can inactivate *E. coli* DH5α in well plates with a static sample and a contact area of 0.15 cm^3, conditions dramatically different from the ones investigated in this study.[10] Future research should investigate longer exposure times, different flow rates, and increased contact area.

[10] Mori et al.

Impact of the MUST Internship on My Career Goals

For my project I worked primarily one-on-one with my mentor, which was extremely helpful since I have limited technical experience after my first year of undergraduate study. I had the chance to ask plenty of questions and establish a rapport with an experienced researcher. Several opportunities allowed me to learn about the research being conducted in other labs and work with other researchers and interns. I found it interesting to work with people from different generations, which does not happen often in my academic career. Coming into a situation where previous relationships have been formed and individuals have different levels of experience was eye-opening, since in college my classmates have similar technical experience.

I was motivated to learn as much as possible and honed my networking skills while gaining technical experience. My mentor taught me how to perform serial dilutions and plating, statistically analyze data, and gave me feedback to improve my research and writing skills. Furthermore, I was able to take a firsthand look at NASA facilities and learn more about the Space Shuttle and International Space Station programs. In my spare time, I learned about Scanning Electron Microscopy and assisted in the Applied Chemistry Lab, allowing me to develop an interest in materials science.

Assisting with a project that has such broad applicability was fascinating, and as a professional I hope to make a positive, tangible impact. I would like to explore genetics and materials science in the future as I begin to narrow my area of expertise as a biomedical engineer. As an economics minor, the business aspects of NASA to which I was exposed at meetings deepened my interest in the field. It is undeniable that the NASA employees I have met enjoy the work they are doing, and my brief tour of duty has been so rewarding that I would eagerly consider spending a portion of my professional career at NASA.