THE UNIVERSITY OF ALABAMA IN HUNTSVILLE | COLLEGE OF ENGINEERING

Honors Capstone Research (HCR)
Summer Program 2024

Influence of Pyocyanin Pigment on the Effectiveness of Non Thermal Argon Gas Plasma Sterilization

Caroline Waugh, College of Engineering, MAE
Mentor: Tatyana Sysoeva, College of Science, Microbiology

Introduction

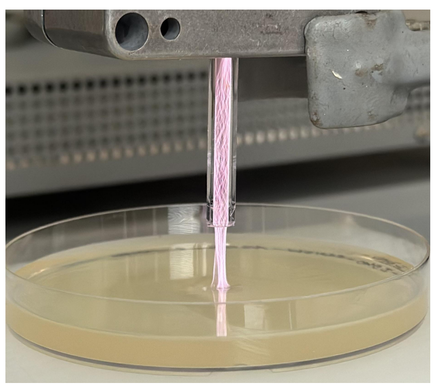

Figure 1: Non Thermal Argon Gas Plasma Plume

Non Thermal Plasma (NTP), or Cold Atmospheric Plasma (CAP), is a type of plasma formed by passing a gas through a high voltage, partially ionizing it. The properties of NTP make it ideal for bacterial sterilization applications.

Pyocyanin is a type of blue/green redox-active pigment found in several species of bacteria, including *Pseudomonas aeruginosa*, and there is evidence[1] that the presence and abundance of this pigment affects the survival rates of the bacteria after exposure to NTP.

A series of experiments were performed using multiple strains of *P. aeruginosa* with varying Pyocyanin production levels with the goal of gaining a better understanding of the effects Pyocyanin pigment production has on the effectiveness of NTP sterilization.

Methods

- PA01, PA14, and PA14 Pyocyanin over producing strains of *P. aeruginosa* cells were inoculated in LB broth for ~6-6.5 hours before being spread onto LB Agar plates and exposed to a 6 kV Argon gas NTP plume (Figure 1), for 3 minutes and incubated overnight (Figure 2).
- The UV visible absorption spectrum of each of the strains prior to plasma exposure was measured using a spectrophotometer (Figure 3)
- GIMP was used to analyze the sterilization effectiveness on the cells in terms of “kill area” (Figure 2).
- A Student’s T-Test was used to determine the statistical significance of the results

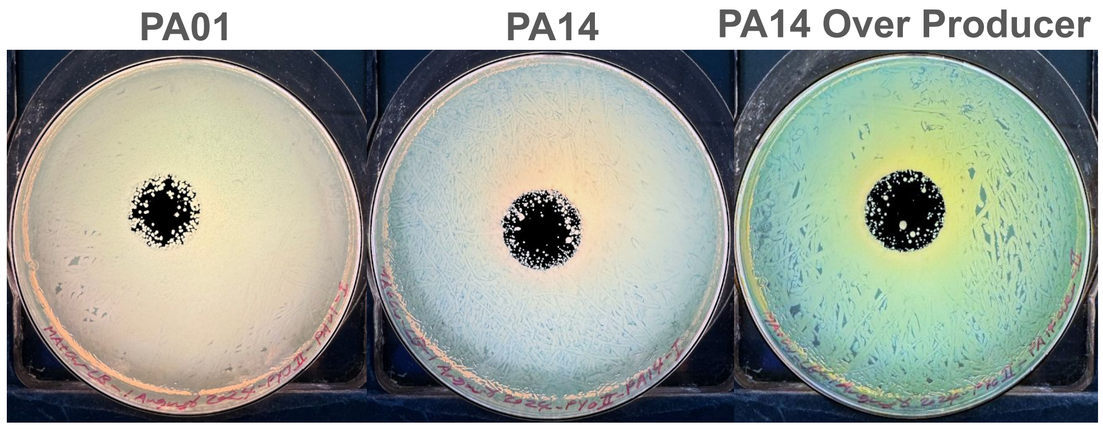

Figure 2: GIMP Analysis of Actual Kill Area from Run 2 (PYO II)

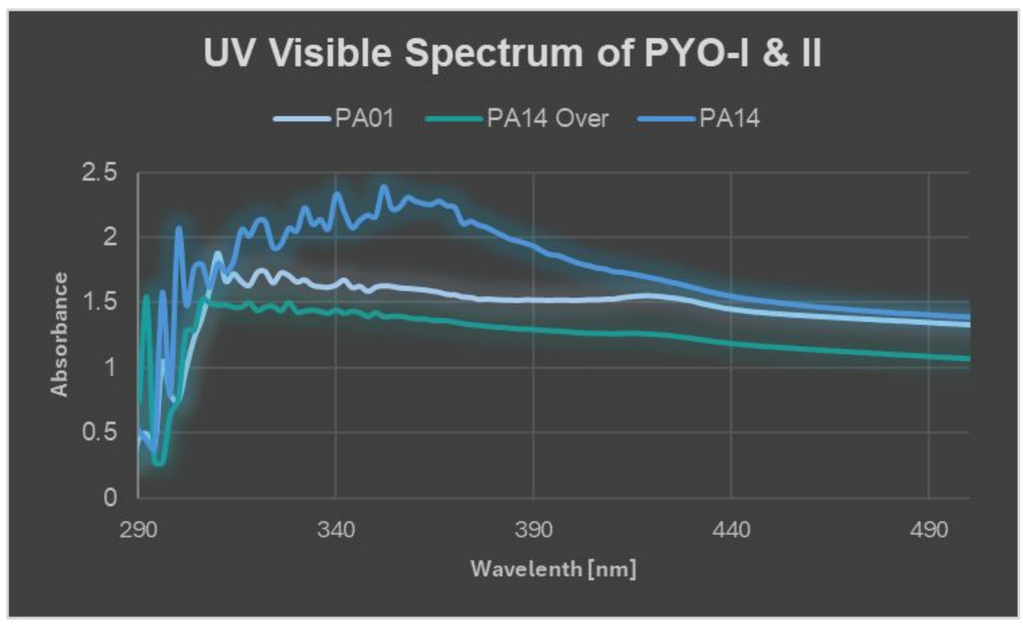

Figure 3: UV Visible Absorption Spectrum Graph

Results

- The results varied significantly between separate runs of the experiment, although the aggregate data revealed a trend of increased NTP sensitivity with increased Pyocyanin levels.
- Incubation and inoculation time differences between runs resulted in differing optical densities (ODs) and colony sizes, and may have contributed to the trends seen.
- Only absorbance values in the UV range were able to be properly detected with the methods used. Scattering effects from the liquid medium the cells were suspended in most likely obstructed any visible wavelength measurements of the pigment

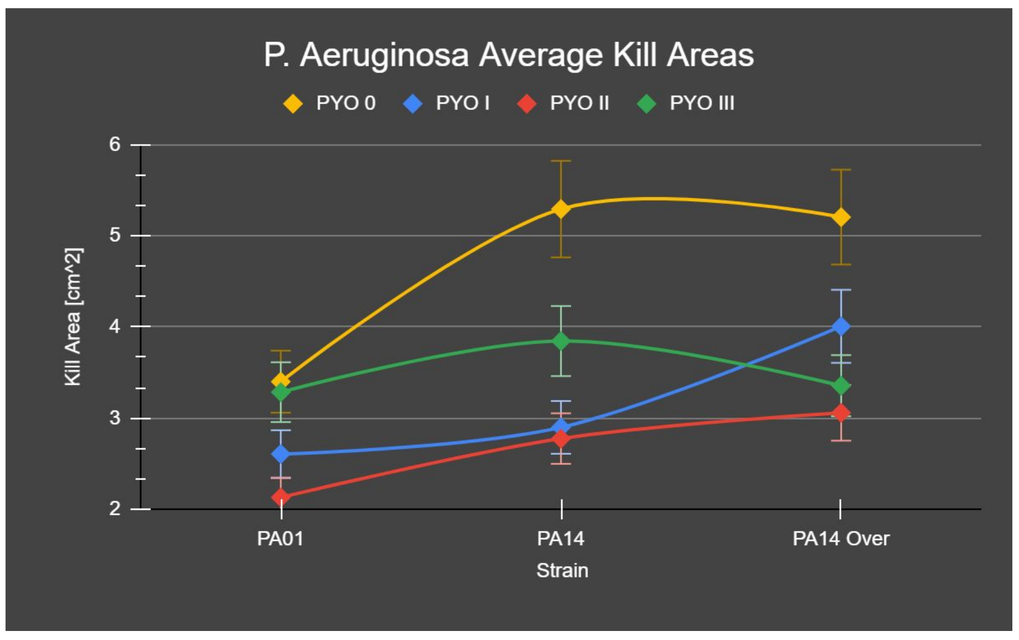

Figure 4: Average Actual Kill Area Graph

Conclusions

- Initial testing showed a trend, though not necessarily statistically significant, of increased vulnerability with higher Pyocyanin production, which is the opposite of what was expected based on existing literature.
- Further experimentation with more precise procedure methods is needed to validate these results and ensure Pyocyanin levels are the actual cause of the differences

Acknowledgements

Funding for this project was provided by the UAH Honors College.
This project was done in collaboration with Marina Ajinov under the additional mentorship and support of Dr. Azita Amiri, Amy LeBleu-DeBartola, Dr. Gabe Xu, and Dr. John Mayo

References

1. Zhou, H., Yang, Y., Shang, W., Rao, Y., Chen, J., Peng, H., Huang, J., Hu, Z., Zhang, R., & Rao, X. (2022). Pyocyanin biosynthesis protects Pseudomonas aeruginosa from nonthermal plasma inactivation. Microbial biotechnology, 15(6), 1910–1921. https://doi.org/10.1111/1751-7915.14032

HONORS COLLEGE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE