Ultrasound as a Source Water Reservoir Management Strategy To Control Cyanobacteria Blooms

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USACE Harmful Algal Bloom Research & Development Initiative

Delivering scalable freshwater HAB prevention, detection and management technologies through collaboration, partnership and cutting-edge science

INTRODUCTION: Ultrasound is a physical reservoir management strategy that may be an important bloom prevention and mitigation strategy with over 10,000 installations worldwide. From studies primarily in a lab setting at high power, ultrasound has been shown to: collapse gas vesicles in cells using buoyancy as an evaluation tool for gas vesicle collapse; break filamentous cyanobacteria; inhibit growth; reduce cell concentrations; inactivate cells; reduce photosynthetic activity; and increase sedimentation rates. Information on effects at low power used in reservoir management are limited. Compared to other physical strategies and use of algaecides, ultrasound has the advantage of low energy, no chemical addition, and the possibility that cell lysis and release of toxin does not occur.

However, at present, information on its effective use is mixed and rather limited. In particular, ultrasonic devices are installed to cover an entire reservoir and remain on for an entire season. Due to this mode of use, there are not paired control experiments to conclusively determine the mechanism by which ultrasound is working. Prior to this project, we investigated a mechanism cited by ultrasound vendors, collapse of gas vesicles, and did not find evidence that this mechanism was active.

PROJECT DESCRIPTION: This project aims to explore mechanisms that may be active in mitigating algal blooms to provide targeted guidance on deployment of these systems. We use smart deployment of ultrasound or combining ultrasound with an emerging algaecide, namely hydrogen peroxide (H_2O_2), which is anticipated to significantly improve its efficacy while decreasing algaecide use. Elucidating how ultrasound may affect cyanobacteria is important to resolve why it appears to be effective in some cases and not in other cases. This knowledge related to the use of ultrasound in reservoirs will allow for more informed decision making related to use of this emerging technology allowing for smart deployment.

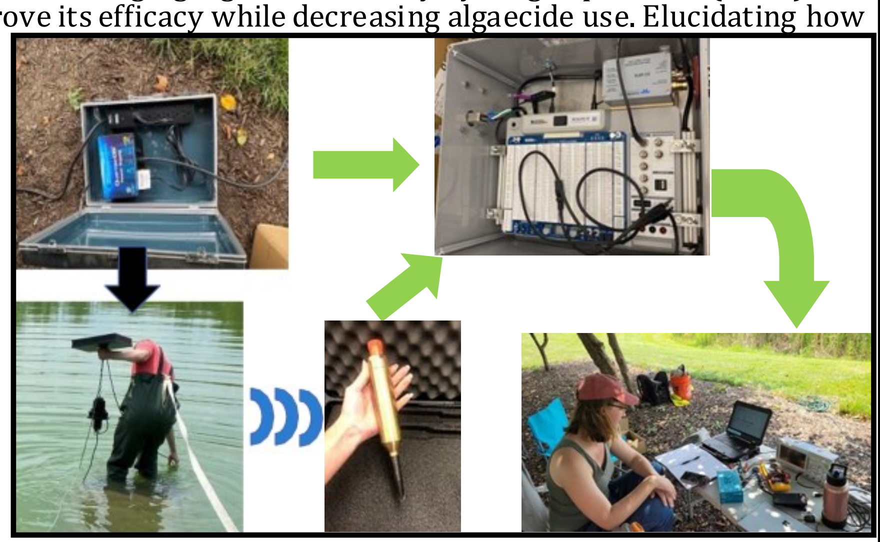

Figure 1: *Components for measuring ultrasound in lab and field deployments.*

Year 2 effort occurred in the laboratory and in the field. One aspect of lab work focused on whether sonoporation and/or endocytosis may be occurring. We modified methods using cell stains and flow cytometry to determine if cell permeance is higher in the presence of ultrasound. Additionally, in the laboratory, work has begun to explore the combined effect of ultrasound and hydrogen peroxide to determine if ultrasound can reduce the amount of algaecide required for treatment.

Moreover, in year 2 we measured ultrasound in the field in a reservoir and in a lab tank with a hydrophone, National Instruments ELVIS board and Lab View computer program (Figure 1). We have successfully monitored the sound field in a field deployed ultrasonic unit and collected water quality information with an EXO Sonde in two different reservoirs and on different days at different distances from the ultrasonic source.

Year 2 Results: *Container Studies.* To assess the potential for sonoporation to occur and be a mechanism affecting cyanobacteria exposed to field deployed ultrasound, we developed a method for testing this

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mechanism. Due to the natural fluorescence from phycocyanin and chlorophyll a, interfering with common stains used, we tested 5 stain combinations before finding suitable combination (SYTOX Green and DAPI). We confirmed that under ultrasound conditions in which human cells undergo sonoporation, Microcystis sp. also undergoes sonoporation. Sonoporation experiments over a 10 min time period revealed that sonoporation occurs rapidly with 70% of the cells stained with the impermeant stain in 5 min. Using a hydrophone, we confirmed that this condition is above the cavitation threshold, not consistent with field conditions. Next steps will be to perform experiments at conditions consistent with field ultrasound conditions to determine if sonoporation is occurring in the field deployed systems.

Additional container studies are being conducted in the presence of hydrogen peroxide, with and without ultrasound. We investigated the treatment of 10^5 and 10^6 Microcystis cells/mL with 2 and 4 mg/L hydrogen peroxide. At 10^5 cells/mL, degradation of hydrogen peroxide occurred over 24 hrs. Chlorophyll a increased in the no treatment and 2 mg/L hydrogen peroxide condition. Chlorophyll a remained stable in the 4 mg/L condition over the 3-day experiment. At the higher cell concentration of 10^6 cells/mL, hydrogen peroxide degraded over 24 hrs. Chlorophyll a decreased over 24 hrs in all conditions and then rebounded. Enumerations of the 10^6 cells/mL condition indicated an increase in cells in the control, decreases in the 2 mg/L and stable cell numbers in the 4 mg/L hydrogen peroxide condition. This work will be repeated prior to experiments in the presence of ultrasound.

In year 2, hydrophone measurements of ultrasound emanating from the field device was conducted in a small tank in the laboratory to determine if there was directionality of the sound from the device and to determine the stability of measurements with time. This work confirmed there is no directionality of the sound. We also determined that soaking the hydrophone and using an uninterruptible power supply improved the reproducibility of our measurements. In addition, the cycling of the ultrasound is such that we can reduce the time of our measurement capture while not biasing results, thereby improving the efficiency of our measurements.

Hydrophone and water quality measurements were conducted at two reservoirs at the City of Wilmington. The reservoir is generally well mixed over the depth we monitored based on temperature and water quality profiles with depth. In addition, no obvious water quality trends were observed at different distances from the ultrasonic unit. Hydrophone measurements indicated an exponential decrease in the ultrasonic signal with distance from the unit. Values measured are well below the cavitation threshold, suggesting a low likelihood for cell lysis and release of cyanotoxins from cyanobacteria present in the reservoirs. Work will continue in year 3 in reservoirs measuring field ultrasound and water quality. We have two new water utility partners that have recently installed ultrasonic units in reservoirs. We are developing a comprehensive field study in the reservoir nearest Columbus that has multiple similar reservoirs. Their reservoirs will allow us to monitor untreated and treated reservoirs for water quality to observe differences.

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