Cyanophage Treatment Development for Mitigating Freshwater Harmful Algal Blooms Caused by Cyanobacteria

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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

Problem Harmful algal blooms (HABs) are an environmental problem of increasing concern because they directly affect public health and environmental quality. Viruses that exclusively infect cyanobacteria, cyanophages, are perhaps the most abundant, but also the most underutilized, biological resource on Earth. It is believed that cyanophages hold great promise and can be harnessed to mitigate cyanoHABs in a species-specific fashion, thanks to their host specificity.

Objective Develop a novel mitigation biotechnology by exploring the lytic life cycle of cyanophages.

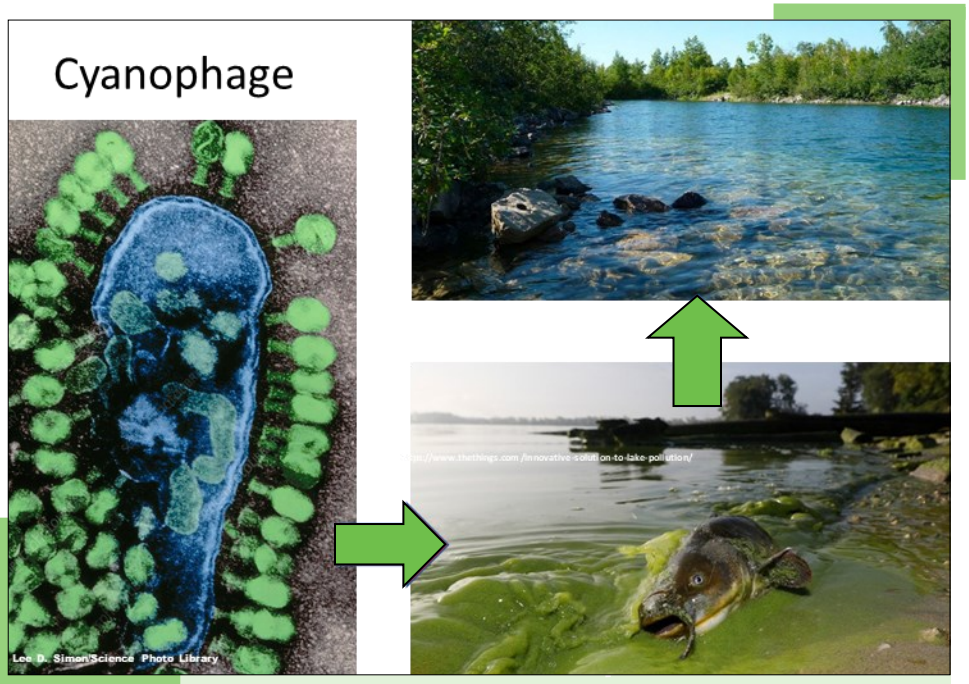


Figure 1: *A cyanophage-based biotechnology to be developed for restoring HAB-impacted lakes and reservoirs.*

Approach The first step was to isolate, identify, and characterize cyanophages from environmental samples collected from HABs-affected lakes. Methods were developed to infect cyanobacterial cultures with cyanophage, optimize biotic and abiotic conditions for inducing lytic life cycle, and quantify the disruption of cyanobacteria caused by cyanophage infection.

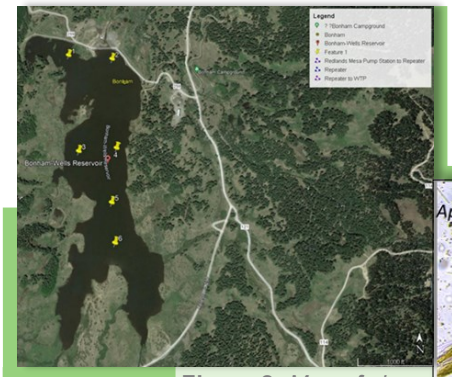
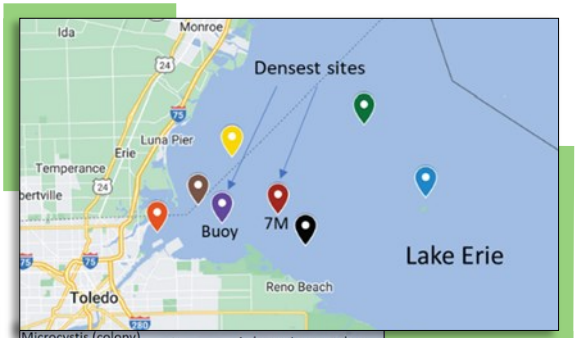


Figure 2: *Map of six sampling sites in Bonham Reservoir located in Colorado and a collected sample.*



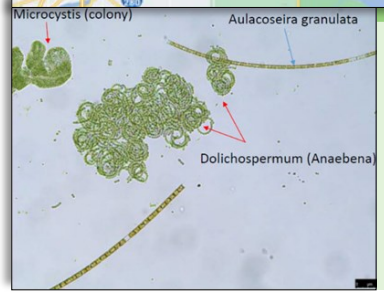


Figure 3: *Map of sampling sites at Lake Erie near Ohio and a collected sample.*

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Results

A wide range of multiplicity of infection (MOI) can be considered for cyanophage-based HAB control. When the cyanophage/cyanobacteria ratio is higher than 1:10,000, the HAB may be controlled.

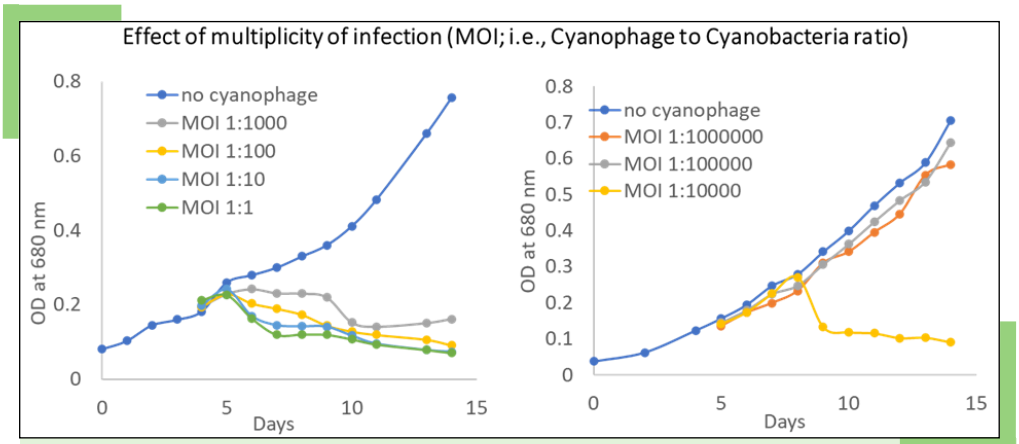

Figure 4: *A graph displaying multiplicity of infection.*

Major Milestones

Date	Milestone
FY21, Q1-Q2	Review article on cyanophage
FY21, Q3	Field collection of HAB samples
FY21, Q4	Field sample analysis (cyanobacterial community characterization)
FY22, Q1	Submission of cyanophage review manuscript, *Toxins* **2022**, *14*(6), 385; https://doi.org/10.3390/toxins14060385
FY22, Q2	Provide hands-on training for USBR lab personnel
FY22, Q3	Collect and characterize more field samples
FY22, Q3	Isolate and identify cyanophage from field samples
FY22, Q4	Develop protocols for long-term preservation of host cyanobacteria and cyanophage
FY23, Q1	Continue to isolate, identify, purify, and enrich host cyanobacteria and cyanophage from field samples
FY23, Q2	Continue to optimize protocols for long-term preservation of host cyanobacteria
FY23, Q3	Cyanophage genome sequencing and annotation
FY23, Q4	Lysogen isolation and cyanophage lytic cycle induction experiments using UV and/or $CuSO_4$
FY23, Q4	Technical report and conference presentation on physical/chemical induction of lytic cyanophage life cycle
Costs	**FY21: $37K** **FY22: $43K** **FY23:$100K** **TOTAL:$180K**

Partnership/Leveraging Opportunities

This work leverages multiple collaborations and other ERDC work units including: "Small regulatory ribonucleic acids for the control of harmful algal blooms", "Rapid, portable and multiplexed detection of freshwater harmful algal bloom-forming genera", "Development of a Near Real-Time Field Test Kit for the Rapid, Simultaneous Detection", and "Quantitation of High Priority Toxic Cyanobacteria".

Value to USACE Mission

This project should result in the development of a novel, cost-effective, species-specific and environmentally benign technology for HABs treatment in lakes and reservoirs. Such a technology would greatly benefit HAB management at USACE lakes and reservoirs.