OHIO DEPARTMENT OF HIGHER EDUCATION HARMFUL ALGAL BLOOM RESEARCH INITIATIVE PROJECT SUMMARY FORM

PROJECT TITLE: Developing fast responding solutions for removing cyanobacteria, cyanotoxins, and nutrients with coagulation/flocculation/sedimentation by characterizing site-specific bloom-related environmental factors
INSTITUTION: University of Cincinnati and Ohio State University
DEPARTMENT OF HIGHER EDUCATION FUNDS: $316,524
MATCHING FUNDS: $316,829
PRINCIPAL INVESTIGATOR: Soryong R. Chae, Ph.D.
AFFILIATION: Department of Chemical and Environmental Engineering, University of Cincinnati
EFFORT: 1.24 Project Month
CO-PRINCIPAL INVESTIGATOR: Dionysios D. Dionysiou, Ph.D.
AFFILIATION: Department of Chemical and Environmental Engineering, University of Cincinnati
EFFORT: 1.24 Project Month
CO-PRINCIPAL INVESTIGATOR: Jiyoung Lee, Ph.D.
AFFILIATION: Division of Environmental Health Sciences, College of Public Health & Department of Food Science and Technology, Ohio State University
EFFORT: 2.22 Project Month
ASSOCIATE INVESTIGATOR: Arthur Helmicki, Ph.D.
AFFILIATION: Department of Electrical Engineering and Computer Science, University of Cincinnati
EFFORT: 0.26 Project Month

Note: The title, tasks, and budget were modified in response to the comments from the pre-proposal review panel.

OBJECTIVES

In cyanobacteria harmful algal blooms (cyanoHABs)-affected areas, drinking water treatment processes are optimized to ensure the absence of cyanotoxins in their finished water [1]. Coagulation, flocculation, and sedimentation (C/F/S) is considered the first barrier eliminating cyanobacterial cells and cyanotoxins entering drinking water treatment facilities [2]. However, there are significant knowledge gaps in understanding the effects of site-specific environmental factors in water chemistry and the population dynamics of cyanobacteria on their fate and transport through C/F/S. Such fundamental understanding is critical for the development of risk management tools of ecosystems, watersheds, and drinking water treatment facilities because cyanoHABs significantly impact fish and animal, habitat ecosystem services, and drinking water quality.

To enhance the removal of cyanobacteria, cyanotoxins, and nutrients from Ohio's lakes, reservoirs, and rivers, we aim to study the effects of site-specific bloom-related environmental factors and population dynamics of cyanoHABs on their fate and transport through C/F/S via three specific objectives. We will also develop a cyberinfrastructure-based user guidance protocol as a deliverable outcome for drinking water treatment facilities to optimize and enhance the performance of C/F/S (**Fig. 1**).

- **Objective 1.** Determination of temporal and spatial changes on population dynamics of cyanoHAB in Ohio's freshwater (Take 1 - Lee)
- **Objective 2.** Process optimization of C/F/S for the enhanced removal of cyanobacteria, cyanotoxins, and nutrients (Take 2 - Chae, Dionysiou, and Lee)
- **Objective 3.** Development of a cyberinfrastructure-based user guidance protocol of C/F/S for the fast response to cyanoHABs (Take 3 – Chae, Helmicki, Hunt, and Lee)

This project is aligned with the Ohio Environmental Protection Agency (OEPA) and Ohio Department of Health (ODH) priorities, which directly addresses 1) Strategies and tools to evaluate or mitigate cyanoHABs and protect (improve) drinking water source quality, 2) Identification, occurrence, and environmental drivers of emerging cyanobacteria and cyanotoxins in waters throughout Ohio, and 3) Cost-effective treatment technologies for drinking water treatment systems.

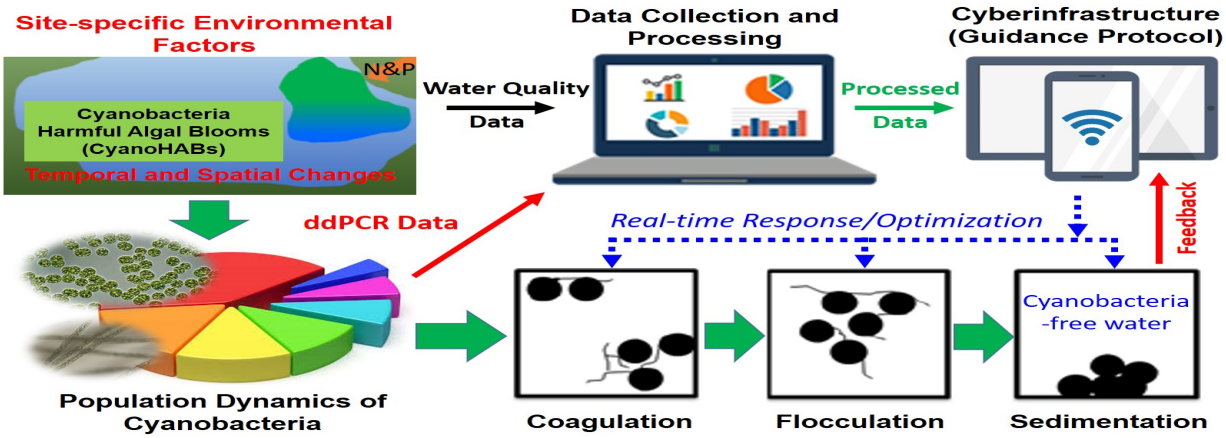

Fig. 1. The framework of data collection, processing, and cyber-infrastructures to develop fast responding solutions for removing cyanobacteria, cyanotoxins, and nutrients with C/F/S by characterizing site-specific cyanoHAB-related environmental factors.

METHODOLOGY

Task 1. Quantification of population dynamics of cyanoHABs in Ohio's freshwater with site-specific cyanoHAB-related environmental characteristics (Lee)

To determine the effects of temporal and spatial changes on the population dynamics of cyanoHABs (i.e., composition and abundance), duplicate surface water samples will be sampled from four different locations (i.e., Western Lake Erie, Great Lakes St. Mary, East Fork Lake, and Ohio River) in Ohio during cyanoHABs seasons.

Cyanobacteria quantification will be done with a QX200 droplet digital PCR (ddPCR) system (Bio-Rad) by targeting MC-producing gene (*mcy*E) gene of *Microcystis aeruginosa* and *Planktothrix sp. ana*C for anatoxin-*a* producing cyanobacteria and *sxt*A *for* saxitoxin-producing cyanobacteria [1].

For microbial community analysis, 16S rRNA sequencing will be conducted with the amplicons of V1-V3 regions of bacterial deoxyribonucleic acid (DNA) using Illumina MiSeq. Sequence cleaning, taxonomic classification, community analysis will be done by following our previous method [3]. Differential abundances in bacterial taxa and predictive pathways will be analyzed with QIIME 2 using DADA2 for amplicon sequence variant determination.

Analysis of cyanotoxins (i.e., MC, saxitoxin, anatoxin-a, and β-methylamino-L-alanine (BMAA)) from the source and C/F/S treated (from Task 2) water sample sets will be performed with ELISA methods [1] and subset of samples will be tested with LC-MS/MS.

Task 2. Optimization of C/F/S for the enhanced removal of cyanobacteria, cyanotoxins, and nutrients (Chae, Dionysiou, and Lee)

In Year 1, we will optimize a bench-scale C/F/S process using 2 L rectangular jars (PB-900, Phipps & Bird) to maximize the removal of cyanobacteria, cyanotoxins, and nutrients originated from Ohio's lakes, reservoirs, and rivers. We will determine the effects of operating conditions (i.e., chemical type and dosage, pH, and mixing speed/time) on (i) removal efficiency of turbidity, nutrients, total organic carbon (TOC), cyanobacteria and toxins, and (ii) cell damage and release of toxins from cells during C/F/S through batch experiments natural water samples in Ohio.

In Year 2, we will design and build ***a continuous flow C/F/S mobile system*** (treatment capacity = 5 - 50 L/day depending on feed flow rate) that consists of a feed pump, a feedwater storage tank (10 L), a C/F/S unit with chemical storage tanks (5 L) and injection pumps for coagulant/flocculant and pH control, and multiparameter sensors for pH, temperature, conductivity, dissolved oxygen, and turbidity (HANNA Instruments). A local area network of sensors will be installed on-site to measure and record all the pertinent readings.

We will determine the effects of temporal and spatial changes in water chemistry (e.g., nutrient loading and natural organic matter (NOM)) and the population dynamics of cyanobacteria on their fate and transport through C/F/S. Considering the site-specific difference in water quality and population dynamics of cyanobacteria, water samples will be *on-site* tested using the C/F/S mobile system under the optimum conditions obtained from the batch experiments at various lakes, reservoirs, and rivers in Ohio between May and October.

***Note:** *The selection of the test sites and timing will be determined through discussions with the OEPA and drinking water treatment facilities*.*

Task 3. Development of a cyberinfrastructure-based user guidance protocol of C/F/S for cyanoHABs (Chae, Helmicki, Hunt, and Lee)

In Year 2, we will develop a cyberinfrastructure-based fast-response user guidance protocol for the effective removal of cyanobacteria by C/F/S to prevent cyanotoxin accumulation and breakthrough in the following water treatment processes during cyanoHABs. The protocol will be composed of three main stages with six modules and two feedback loops. The processed data will be converted to Python (Python 3.4.2) and provided on the existing PI's website for public access.

The research project will be run daily by three graduate students (i.e., two at UC and one at OSU) and one undergraduate student. The faculty will have weekly research meetings with the students to guide their research and to assess research outcomes. The research teams at UC and OSU will have bi-weekly virtual meetings to discuss research outcomes and milestones. Quarterly and annual progress reports will be sent to the Ohio Department of Higher Education (ODHE).

Key research findings will be made publicly available in scientific journals and at national and international scientific conferences, such as the U.S. Symposium on Harmful Algae, International Conference on Harmful Algae, Conferences on Great Lakes Research, American Chemical Society, and American Geophysical Union. The proposed timeline of the project is in the following table.

Specific task	Year 1	Year 2
1. Quantification of population dynamics of cyanobacteria and toxins in Ohio's freshwater	X	X
2.1 Optimization of C/F/S for the removal of cyanobacteria, toxins, and nutrients	X	
2.2 Fate and transport of cyanobacteria, cyanotoxins, and other constituents through C/F/S	X	
2.3 On-site testing of the C/F/S mobile system at various lakes, reservoirs, and rivers		X
3. Development of a cyberinfrastructure-based guidance protocol of C/F/S for cyanoHABs		X

RATIONALE

CyanoHABs represent a significant and expanding threat to human health, agriculture, and fishery resources throughout the United States and the world [1, 4-9]. Also, cyanoHABs have heavy economic impacts on local communities, including shellfish closures, wild or farmed fish mortalities, and scared consumers who avoid seafood. Indeed, toxic blooms reduce business during normally tourism-heavy weekends and holidays, resulting in heavy revenue losses [10, 11].

Local and state governments have placed a much emphasis on the improvement of water treatment facilities to avoid cyanobacteria and cyanotoxins in public water. The properly operated C/F/S process is efficient in removing microorganisms such as *Escherichia coli*, *Giardia, Cryptosporidium*, *Salmonella,* total and fecal coliform, as well as cyanobacteria[12-14]. However, the effects of site-specific environmental factors on water chemistry and the population dynamics of cyanobacteria on their fate and transport through C/F/S are not yet clearly understood.

The successful completion of this study will provide a risk management solution for cyanobacteria and its economic impacts and operating guidelines for (i) the enhanced removal of cyanobacteria and cyanotoxins by C/F/S and (ii) the prevention of cyanotoxin accumulation and breakthrough in the following water treatment processes such as sand filtration and activated carbon adsorption at drinking water treatment facilities in Ohio.

PROJECT NARRATIVE

A. Rationale

The global occurrence of toxic cyanobacteria in freshwater is increasing in both frequency and distribution [14]. The incidence of cyanobacteria harmful algal blooms (cyanoHABs) has been increasing in Ohio's freshwater in recent decades [10, 15-18], resulting in the accumulation of cyanobacteria in lakes, reservoirs, and storage ponds in Ohio [3, 19]. Some cyanobacteria produce cyanotoxins as their secondary metabolites that pose public health concerns [10]. Toxins produced by cyanobacteria have also been linked to mortality in aquatic macro-invertebrates, waterfowl, and other predators [20].

Among the various toxin-producing cyanobacteria genera, the most notable species of cyanoHABs is *Microcystis aeruginosa*, which is commonly found in the western basin of Lake Erie [15]. However, other cyanobacteria genera, including *Anabaena, Aphanizomenon, Cylindrospermophsis, Dolichospermum, Oscillatoria,* and *Planktothrix,* have been found in Ohio's freshwater [16, 21]. While the factors that stimulate or promote blooms are relatively well known, the factors that trigger toxin-production or the dominance of toxic versus non-toxic strains are considerably less understood [10].

The dominant species of cyanobacteria during HABs are determined by site-specific environmental factors, such as community characteristics, temporal changes in water chemistry, and nutrient abundance [21-23]. However, the effects of the site-specific environmental factors with temporal and spatial changes on the fate of toxin-producing cyanobacteria and cyanotoxins in source waters and the transport through C/F/S are not yet clearly understood.

In cyanoHABs-affected areas, drinking water treatment processes are optimized to ensure the absence of cyanotoxins in their finished water [1]. Coagulation, flocculation, and sedimentation (C/F/S) is considered the first barrier eliminating cyanobacterial cells and cyanotoxins entering public drinking water treatment facilities (serving over 80% of the American population) [2]. One critical concern regarding C/F/S is the potential for the disruption of cells and the subsequent release of toxins from cells and change their settling property. The disruption of cells during either physical (e.g., mixing and filtration) or chemical treatment (e.g., coagulation, flocculation, and disinfection), and the subsequent release of toxins, warrants significant concern for drinking water treatment operators [14].

These findings indicate that there are significant knowledge gaps in understanding the effects of site-specific environmental factors on water chemistry and the population dynamics of cyanobacteria on their fate and transport through C/F/S. Such fundamental understanding is critical for the development of risk management tools of ecosystems, watersheds, and drinking water treatment facilities in Ohio because cyanoHABs significantly impact fish and animal, habitat ecosystem services, and drinking water quality.

This project is aligned with the Ohio Environmental Protection Agency (OEPA) and Ohio Department of Health (ODH) priorities, which directly addresses 1) Strategies and tools to evaluate or mitigate cyanoHABs and protect (improve) drinking water source quality, 2) Identification, occurrence, and environmental drivers of emerging cyanobacteria and cyanotoxins in waters throughout Ohio, and 3) Cost-effective treatment technologies for drinking water treatment systems.

The successful completion of this study will provide (i) quantitative data related to temporal and spatial changes in water chemistry and nutrient abundance affecting the population dynamics of toxin-producing cyanobacteria through C/F/S using various advanced characterization techniques and (ii) an adaptable cyberinfrastructure-based fast-response user guidance protocol of C/F/S for risk management associated with cyanoHABs at drinking water treatment facilities.

B. Objectives

To enhance the removal of cyanobacteria, cyanotoxins, and nutrients from Ohio's lakes, reservoirs, and rivers, we aim to study the effects of site-specific bloom-related environmental factors and population dynamics of cyanoHABs on their fate and transport through C/F/S via laboratory and on-site experiments. We will also develop a cyberinfrastructure-based user guidance protocol as a deliverable outcome for drinking water treatment facilities to optimize and enhance the performance of C/F/S (**Fig. 1** in page 2). The proposed project aims to address those scientific questions through three specific objectives.

Objective 1. Determination of temporal and spatial changes on population dynamics of cyanoHAB in Ohio's freshwater (Take 1 - Lee)

In our previous study, we found that not only microcystin (MC)-producing cyanobacteria but also saxitoxin-producing cyanobacteria co-existed in a drinking water intake in Lake Erie (**Fig. 2**) [24]. For a fundamental understanding of cyanoHABs and their toxin ecology, microbial communities in those bloom-affected water sources should be examined. Recently, high-throughput sequencing and metagenomics techniques have been successfully applied to describe microbial communities in the water resources that are prone to cyanobacterial blooms in Lake Erie [25], inland lakes [3], and ponds. In our previous study, we observed that there was a dramatic shift to *Planktothrix* predominance in the microbial community during the bloom season (**Fig. 3**) [3]. ***The central hypothesis*** is that microbial community composition and toxin-producing cyanobacteria dynamics are unique in each lake or river because of site-specific abiotic factors (e.g., temperature, pH, and nutrients) and biotic factors, including interactions between cyanobacteria and other microbial species, affect bloom and toxin ecology.

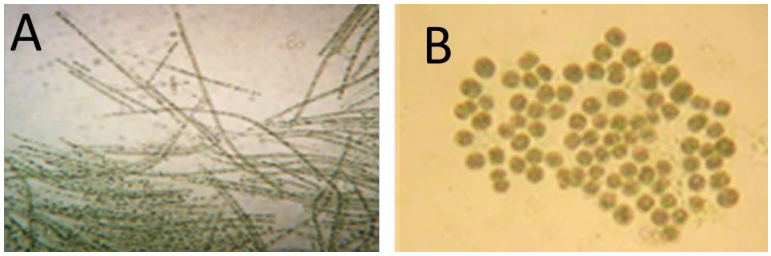

Fig. 2. *Aphanizomenon* spp. (potential saxitoxin producer; A) and *Microcystis aeruginosa* (MC producer; B) isolated from the same location in a drinking water intake in Lake Erie in 2013 (Lee, *unpublished data*).

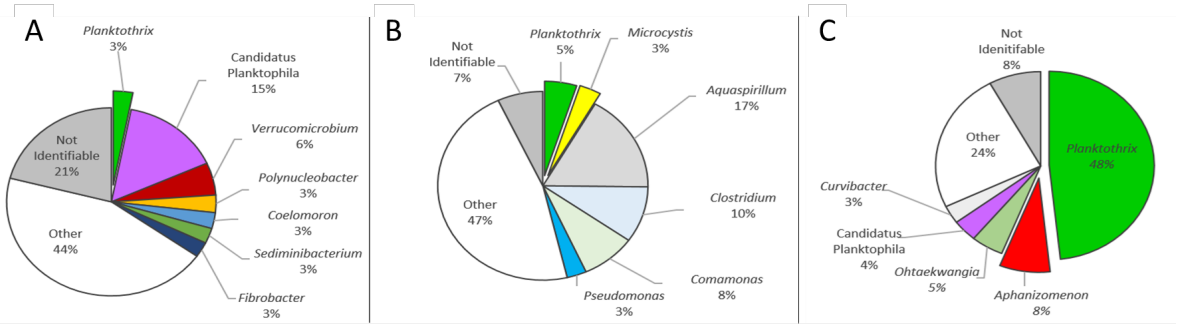

Fig. 3. Temporal dynamics of the microbial community in a eutrophic lake in central Ohio in June (A), early July (B), and late July 2015 (C). There was a dramatic microbial community shift to *Planktothrix* when the bloom peaked in late July [3].

Objective 2. Process optimization of C/F/S for the enhanced removal of cyanobacteria, cyanotoxins, and nutrients (Take 2 - Chae, Dionysiou, and Lee)

In our previous study funded by the Ohio Sea Grant, it was found that nutrient loading in a drinking water reservoir at GCWW increased by approximately two times from summer to fall in 2019 (**Fig. 4(a)**), resulting in a significant bloom increase measured by chlorophyll-*a* concentration in the reservoir (**Fig. 4(b)**). However, the removal efficiency of bloom (i.e., chlorophyll-*a*) by C/F/S in fall 2019 was higher than the one in summer 2019 under the same treatment conditions (*unpublished data*).

This finding indicates significant knowledge gaps in understanding the effects of temporal and spatial changes in water chemistry and the population dynamics of cyanoHABs on their removal through C/F/S. We ***hypothesize*** that temporal and spatial changes in water chemistry and population dynamics of cyanoHABs significantly affect the performance of C/F/S. The fundamental understanding of those changes will enhance the removal efficiencies of cyanobacteria, cyanotoxins, and nutrients by C/F/S through process optimization, including the selection of chemicals and the control of chemical dosage, pH, and mixing speed/time.

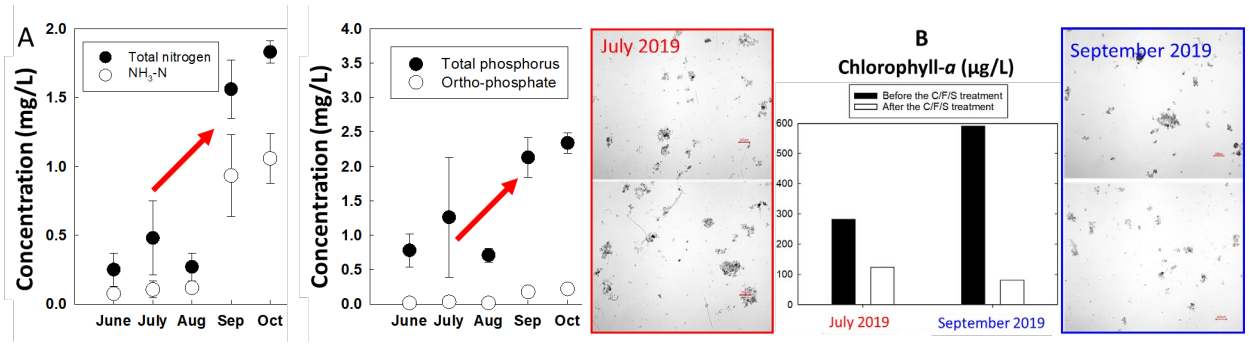

Fig. 4. The effect of temporal changes in nutrient loading (A) on the removal of chlorophyll-a through C/F/S (B). (Feed water turbidity: 245 NTU (July 2019) and 253 NTU (September 2019); Coagulation: 270 rpm (6 sec), alum dosage = 27.2 mg/L as Al_2O_3; Flocculation: 65 rpm (4 min)/40 rpm (4 min)/25 rpm (4 min); Sedimentation: 15 min; Scale bars on the microscopic images of water samples = 100 µm) (Chae, *unpublished data*).

Objective 3. Development of a cyberinfrastructure-based user guidance protocol of C/F/S for the fast response to cyanoHABs (Take 3 – Chae, Helmicki, Hunt, and Lee)

Including multidisciplinary aspects, such as physics, chemistry, microbiology, and process engineering, studies on water treatment heavily depend on experimental observations. The design and execution of effective and informative experiments in comparative studies on water treatment optimization are challenging due to their complexity and multi-disciplinarity.

We aim to develop a cyberinfrastructure-based user guidance protocol that underlines the crucial role of preliminary study and advanced data analysis in optimal designs and execution of water treatment experiments using C/F/S during cyanoHABs. By integrating a detailed experimental design, laboratory experiment execution, and advanced data analysis, more relevant conclusions and recommendations are likely to be delivered [26]. In fact, the experimentation can be considered as an iterative loop in which the feedback, including scientific understanding and statistical information, can be used to design more efficient subsequent virtual and real experiments.

C. Methodology

Our research team is composed of five senior researchers, three graduate students, and one undergraduate student in environmental engineering, environmental microbiology, statistics and data science, and computer science & information technology at the University of Cincinnati (UC) and the Ohio State University (OSU) aims to conduct three specific tasks. *All experiments will be performed in triplicate under the same conditions.*

Task 1. Quantification of population dynamics of cyanoHABs in Ohio's freshwater with site-specific cyanoHAB-related environmental characteristics (Lee)

To determine the effects of temporal and spatial changes on the population dynamics of cyanoHABs (i.e., composition and abundance), duplicate surface water samples will be sampled from four different locations in Ohio in three seasons (**Table 1**). Cyanobacteria quantification will be done with a QX200 droplet digital PCR (ddPCR) system (Bio-Rad) by targeting MC-producing gene (*mcy*E) gene of *Microcystis aeruginosa* and *Planktothrix sp. ana*C for anatoxin-*a* producing cyanobacteria and *sxt*A *for* saxitoxin-producing cyanobacteria [1].

For microbial community analysis, 16S rRNA sequencing will be conducted with the amplicons of V1-V3 regions of bacterial deoxyribonucleic acid (DNA) using Illumina MiSeq. Sequence cleaning, taxonomic classification, community analysis will be done by following our previous method [3]. Differential abundances in bacterial taxa and predictive pathways will be analyzed with QIIME 2 using DADA2 for amplicon sequence variant determination.

Analysis of cyanotoxins (i.e., MC, saxitoxin, anatoxin-a, and β-methylamino-L-alanine (BMAA)) from the source and C/F/S treated (from Task 2) water sample sets will be performed with ELISA methods [1] and subset of samples will be tested with liquid chromatography/mass spectrometry/mass spectrometry (LC-MS/MS). Functional gene pathways will be predicted from 16S rRNA genes using PICRUSt2.

For statistical analysis, ANCOM will be used to identify statistically significant taxa between sample groups, and LefSe will be used to determine differentially abundant taxa between groups after relative abundance normalization with $p < 0.05$ for both Kruskal-Wallis (between sample and control groups) and Wilcoxon tests. A principal coordinates analyses with Bray-Curtis dissimilarity matrices will be used for visualization of differences in bacterial community structure.

Table 1. Water sampling plan from various lakes, reservoirs, and rivers in Ohio for Tasks 1 and 2.

Temporal effect	**Spatial effect**		
	Lake	Reservoir/Pond	River
Spring (May-Jun), Summer (July-Aug.), and Fall (Sep.-Oct.)	Western Lake Erie, Great Lakes St. Mary (GLSM)	East Fork Lake	Ohio River

Task 2. Optimization of C/F/S for the enhanced removal of cyanobacteria, cyanotoxins, and nutrients (Chae, Dionysiou, and Lee)

In Year 1, we will optimize a bench-scale C/F/S process using 2 L rectangular jars (PB-900, Phipps & Bird) to maximize the removal of cyanobacteria, cyanotoxins, and nutrients originated from Ohio's lakes, reservoirs, and rivers. As shown in **Table 2**, we will determine the effects of operating conditions (i.e., chemical type and dosage, pH, and mixing conditions) on (i) removal efficiency of turbidity, nutrients, total organic carbon (TOC), cyanobacteria and toxins, and (ii) cell damage and release of toxins from cells during C/F/S through batch experiments natural water samples in Ohio (see **Table 1**).

Table 2. Experimental conditions for the optimization of C/F/S during cyanoHABs.

Unit process	**Condition**
Coagulation	- Coagulant: $Al_2(SO_4)_3$, polyaluminum chloride, ferric and ferrous sulfate, $FeCl_3$ - Dosage: 10 ~ 100 mg/L; pH: 5 ~ 8; mixing speed: 150 ~ 350 rpm
Flocculation	- Flocculant: cationic (e.g., polydiallyl dimethyl ammonium chloride), anionic (e.g., sodium polyacrylate, and non-ionic (e.g., polyethylene oxide) - Dosage: 10 ~ 100 mg/L; pH: 5 ~ 8; mixing speed: 20 ~ 80 rpm
Sedimentation	- Gravity settling: 0 ~ 60 minutes

In Year 2, we will design and build ***a continuous flow C/F/S mobile system*** (treatment capacity = 5 - 50 L/day depending on feed flow rate) that consists of a feed pump, a feedwater storage tank (10 L), a C/F/S unit with chemical storage tanks (5 L) and injection pumps for coagulant/flocculant and pH control, and multiparameter sensors for pH, temperature, conductivity, dissolved oxygen, and turbidity (HANNA Instruments) **(Fig. 5)**.

A local area network of sensors will be installed on-site to measure and record all the pertinent readings. All sensors will be RS485 compatible, and dataloggers and peripheral equipment will be connected to a portable computing device to collect, process, and archive all incoming data. All data will be securely mounted within an enclosure.

We will determine the effects of temporal and spatial changes in water chemistry (e.g., nutrient loading and natural organic matter (NOM)) and the population dynamics of cyanobacteria on their fate and transport through C/F/S. Considering the site-specific difference in water quality and population dynamics of cyanobacteria, water samples will be *on-site* tested using the C/F/S mobile system under the optimum conditions obtained from the batch experiments at various lakes, reservoirs, and rivers in Ohio between May and October (see **Table 1**). ***Note:** *The selection of the test sites and timing will be determined through discussions with the OEPA and drinking water plant facilities*.*

We will determine the impact of C/F/S treatments on bacterial community composition, including cyanobacteria and their related microbial communities, their predicted function using the next-generation sequencing approach that was used in our previous studies [3, 7].

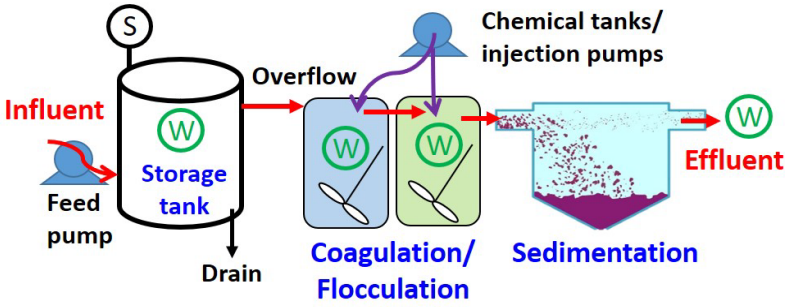

Fig. 5. Schematic diagram of a continuous flow C/F/S mobile system (S: multiparameter sensors, W: sampling points for water quality analysis, including cyanobacteria, cyanotoxins, nutrients, and NOM).

We will also track the fate of toxic cyanobacteria population abundance along with the C/F/S treatment processes, focusing on toxic cyanobacteria that pose public health risks, including MC-producing *Microcystis*, MC-producing *Planktothrix*, saxitoxin-producing cyanobacteria, and anatoxin-*a* producing cyanobacteria (The experimental methods are discussed in Task 1).

Changes in hydrodynamic diameter and zeta potential of colloidal matters in water through C/F/S will be measured using a particle size and zeta potential analyzer (NanoBrook Omni, Brookhaven Instruments Co.). Turbidity of water samples will be measured using a turbidimeter (TL2310, HACH, 0.01 – 1000 NTU). Temperature and pH of water samples will be measured using a bench-top pH meter (ThermoScientific). Ultraviolet (UV) absorbance at 254 nm (UV_{254}) and alkalinity of water samples will be measured using a UV/Vis spectrophotometer (DR6000, HACH).

TOC will be analyzed according to the U.S. EPA Method 415.3, Rev.1.2 [27] using Teledyne Tekmar TOC Fusion. Size distribution of DOC in water samples will be analyzed using liquid chromatography – organic carbon detection (LC-OCD) (Model 8, DOC-Labor GmbH, Germany) [28]. LC-OCD can separate five size fractions: biopolymers (> 20,000 Da), humics (~ 1000 Da), building blocks (300 ~ 500 Da), low material weight (LMW) acids (< 350 Da), and LMW neutrals [29]. Excitation and emission matrix (EEM) analysis of NOM will be conducted using a UV-Vis/fluorescence spectroscopy (Aqualog, Horiba Instruments Inc.) as described in the previous study by Chen et al. (2003) [30].

Task 3. Development of a cyberinfrastructure-based user guidance protocol of C/F/S for cyanoHABs (PIs Chae, Helmicki, Hunt, and Lee)

In Year 2, we will develop a cyberinfrastructure-based fast-response user guidance protocol for the effective removal of cyanobacteria by C/F/S to prevent cyanotoxin accumulation and breakthrough in the following water treatment processes during cyanoHABs. The protocol will be composed of three main stages with six modules and two feedback loops (**Fig. 6**).

Stage 1. Experimental Planning: Parameters (see **Table 2**) for laboratory batch experiments will be tested for the optimization of C/F/S based on the systematic analysis of water chemistry and population dynamics of cyanobacteria. Controllable and uncontrollable factors (such as the variation of influent constituents and the fluctuation of the feed pump) will be identified. By considering them and their interactions as potential design factors, factorial experiments will be created [31]. Costs of chemicals and operation of C/F/S will be surveyed and evaluated through various batch tests.

Stage 2. Experiment Execution: Selected experimental parameters at Stage 1 will be tested using natural water samples in Ohio (see **Table 1**) and a bench-scale jar tester to optimize C/F/S. In Year 2, water samples will be tested *in-situ* using the C/F/S mobile system under the optimal conditions from the batch experiments with consideration of the site-specific difference in water quality and population dynamics of cyanobacteria at the selected sites in Ohio.

Particularly, in the case of water treatment experiments, it is a challenge to develop a standardized method for experimental works that can be validated in different laboratories due to their undefined and complex characteristics. Therefore, quality assurance (QA)/quality control (QC) activities will be

conducted in the routine tasks of an experiment (i.e., collecting samples, performing analyses, checking running systems, processing, and reporting experimental results). More importantly, experimenters must define a document-control procedure as a mean for data defensibility for each treatment and each analytical test.

Stage 3. Advanced Data Analysis: After data collection, instead of conventional visually comparing the values of the results, results will be analyzed by applying statistical data analysis in R (R 4.0.0) using the *nlme* package with the *lme* function [32] to avoid subjective conclusions. All data collected from experiments will be analyzed for the process optimization using the response surface methodology (RSM) [33, 34] and initially be stored as a Microsoft Excel document on a local computer. The processed data will be converted to Python (Python 3.4.2) and provided on the existing PI's website for public access (https://mystudy4livingwater.org/protocol). After analyzing the results from the cyberinfrastructure, engineers/operators will draw practical conclusions about treatment systems and recommend subsequent actions to decision-makers.

Feedback Loops: Throughout the guidance protocol, it is crucial to keep an iterative process for discovering the underlying mechanisms of the systems or processes. The first loop (*Stage 3* → *Stage 1*) indicates the conventional progress of the experimental works in which the researchers tend to change the investigation area of design factors, add new or removal parameters. The second loop (*Stage 3* → *Stage 2*) aims to adjust system inputs or change to a different comparison approach by replacing it with other response variables.

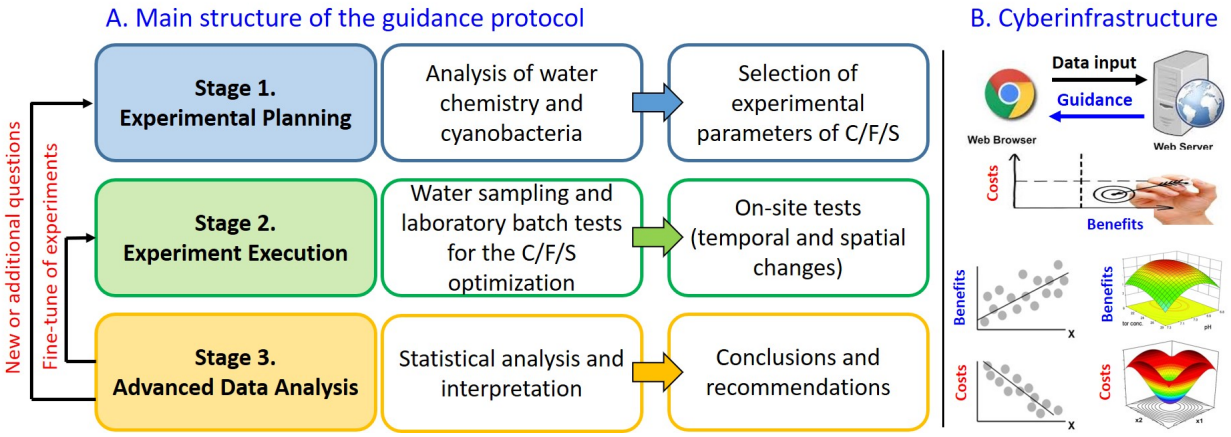

Fig. 6. Main structure of the user guidance protocol (A) and cyberinfrastructure (B) for fast responding C/F/S optimization during cyanoHABs.

Validation and Further Optimization *of the user guidance protocol should be conducted at various full-scale facilities as a further study. This information can then be used for the design and development of the technology with the aim of adjusting it for greater and broader use in the water industry.*

The application of advanced data analytics, including, but not limited to, artificial intelligence (AI), deep learning, and machine learning can be considered to mine for further understanding within the data of the correlative and causal effects in water treatment and processing and leading to more refined decision-making capabilities for the cyberinfrastructure fast response protocol.

D. Timeline/Project schedule

The research project will be run by three graduate students (i.e., two at UC and one at OSU) and one undergraduate student under the supervision of Drs. Chae, Dionysiou, Helmicki, and Hunt at UC, and Dr. Lee at OSU. The research teams at UC and OSU will have weekly internal meetings with the students to guide their research and to assess research outcomes. The research teams will have bi-weekly virtual meetings to discuss research outcomes and milestones (**Table 3**). Annual review and evaluation will be performed through research team workshops. Then, quarterly and annual progress reports will be sent to the Ohio Department of Higher Education.

Key research findings will be made publicly available in scientific journals and at national and international scientific conferences, such as the U.S. Symposium on Harmful Algae, International Conference on Harmful Algae, Conferences on Great Lakes Research, American Chemical Society, and American Geophysical Union.

Table 3. The proposed timeline of specific tasks in this project.

Specific task	Year 1	Year 2
1. Quantification of population dynamics of cyanobacteria and toxins in Ohio's freshwater	X	X
2.1 Optimization of C/F/S for the removal of cyanobacteria, toxins, and nutrients	X	
2.2 Fate and transport of cyanobacteria, cyanotoxins, and other constituents through C/F/S	X	
2.3 On-site testing of the C/F/S mobile system at various lakes, reservoirs, and rivers		X
3. Development of a cyberinfrastructure-based guidance protocol of C/F/S for cyanoHABs		X

E. Related work

The properly operated C/F/S process is efficient in removing microorganisms such as *Escherichia coli*, *Giardia, Cryptosporidium*, *Salmonella* as well as cyanobacteria[12-14]. The presence of cyanobacteria in drinking water sources necessitates a fundamental understanding of not only how the processes of C/F/S remove cyanobacteria but also how these processes disrupt cells during treatment because hydraulic and chemical stresses during treatment may cause damage to cells and trichomes, leading to the release of cyanotoxins [35]. However, the effects of temporal and spatial changes in water chemistry and the population dynamics of cyanobacteria on the removal of cyanobacteria and cyanotoxins by C/F/S are not yet clearly understood.

Our groups at UC and OSU have carried out extensive research on the fate and transport of cyanobacteria and cyanotoxins in natural water resource and drinking water treatment processes supported by the ODHE, Ohio Sea Grant, and US EPA [1, 3, 7, 9, 10, 24]. In addition, we have studied the physical, chemical, biological, and photocatalytic removal/decomposition of contaminants of emerging concern in water sources [36-45].

F. How the research supports Ohio industry, commerce, and business

CyanoHABs represent a significant and expanding threat to human health, agriculture, and fishery resources throughout the United States and the world [1, 4-9]. Also, cyanoHABs have heavy economic impacts on local communities, including shellfish closures, wild or farmed fish mortalities, and scared consumers who avoid seafood. Indeed, toxic blooms reduce business during normally tourism-heavy weekends and holidays, resulting in heavy revenue losses [10, 11]. Local and state governments have placed a much emphasis on the improvement of water treatment facilities to avoid cyanobacteria and cyanotoxins in public water.

The successful completion of this study will provide a risk management solution for cyanobacteria and its economic impacts and operating guidelines for (i) the enhanced removal of cyanobacteria and cyanotoxins by C/F/S and (ii) the prevention of cyanotoxin accumulation and breakthrough in the following water treatment processes such as sand filtration and activated carbon adsorption at public drinking water treatment facilities in Ohio.

G. Project partners

The research groups at UC and OSU have ongoing collaboration on the monitoring of cyanobacteria and cyanotoxins at various surface water sources in Ohio, as well as with drinking water treatment facilities.

Research collaboration activities with Andrew P. McClure (The City of Toledo Water Treatment Plant), Todd E. Hone (Celina Water Treatment Plant), Mary Anne Evans (U.S. Geological Survey), and Bruce Whitteberry (Greater Cincinnati Water Works) will include sharing water samples, water quality data, and visits by students to research facilities of the collaborators, and mentorship of undergraduate and graduate students (*see the Letters of Collaboration*).

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