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Physicochemical Treatment of Cyanobacteria and Microcystin by Hydrodynamic Cavitation and Advanced Oxidation

Catherine C. Thomas, Afrachanna D. Butler, Victor F. Medina,
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February 2019

Environmental Laboratory

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Abstract

Cyanobacterial/harmful algal blooms (HABs) are a serious and growing threat to water resources. This project evaluated cavitation fields generated from four different nozzle configurations to determine their efficacy in HAB treatment performance and correlate oxygen radical production with HAB treatment. Oxygen radicals, particularly superoxides, have demonstrated their ability to transform organic contaminants, including microcystin toxins released from some cyanobacteria species. In this study, pure cultures of *Microcystis aeruginosa* and *Anabaena spp*. were subjected to two hours of cavitation treatments with each nozzle. It was found that the nano-micro bubble treatment nozzle was the only configuration that significantly decreased turbidity and chlorophyll α concentrations, in addition to notable reductions in microcystin toxin levels. Cavitation tests performed on environmental cyanobacteria samples using other treatment nozzles rendered no significant damage to algal cells. Results from electron paramagnetic resonance (EPR) spectra supports the treatment performance of the nano-micro bubble nozzle as hydroxyl (OH) and superoxide ($O_2^{\bullet-}$) radicals generated by the nozzle were detected in cavitated waters. Overall, the results from this study imply that hydrodynamic cavitation with the appropriate nozzle configuration can be used as an effective means of controlling certain species of cyanobacterial in HAB affected areas.

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Preface

The work was conducted at the U.S. Army Engineer Research and Development Center-Environmental Laboratory (ERDC-EL), Vicksburg, MS. Funding was provided by the Aquatic Plant Control Research Program (APCRP) under project number 450620.

This project was performed under the direct leadership of Dr. Catherine Thomas. Microcystin analysis was performed by Dr. Carina Jung of ERDC-EL Environmental Processes Branch. The report was reviewed by Mr. Roy Wade and Dr. Jose Mattei-Sosa of ERDC-EL.

This study was conducted under the direct supervision of Dr. W. Andy Martin, Chief, Environmental Engineering Branch, ERDC-EL and Mr. Warren P. Lorentz, Chief, Environmental Processes and Engineering Division, ERDC-EL, under the general supervision of Dr. Al Cofrancesco, Technical Director for Civil Works in the Environment, ERDC-EL. Dr. Jack Davis was Deputy Director, ERDC-EL and Dr. Ilker R. Adiguzel was Director, ERDC-EL.

COL Ivan P. Beckman was Commander of ERDC and Dr. David W. Pittman was the Director.

Acronyms and Abbreviations

APCRP	Aquatic Plant Control Research Program
CFU	Colony Forming Units
cm	Centimeters
DO	Dissolved Oxygen
DTPA	Diethylenetriamine-pentaacetic acid
EL	Environmental Laboratory
ELISA	Enzyme-Linked Immunosorbent Assay
EPR	Electron Paramagnetic Resonance
ESR	Electron Spin Resonance
ERDC	Engineer Research and Development Center
HAB	Harmful Algal Blooms
HPC	Heterotrophic Plate Counts
L	Liter
mm	Millimeters
NST	National Standard Thread
NTU	Nephelometric Turbidity Units
psi	Pounds per square inch
PVC	Polyvinylchloride

1 Introduction

1.1 Background

Harmful algal blooms (HABs) consisting of algae and cyanobacteria continue to be a serious issue facing federal and state agencies that manage waterways. These aquatic organisms can rapidly decrease dissolved oxygen, prevent light penetration, and gas exchange causing detrimental effects on the environment (Mezyk et al. 2013; Petrusevski et al. 1995; Steffensen 2008). To date, remediation of cyanobacteria has been particularly difficult, as chemical treatment methods that can control cyanobacterial growth have been found to affect the natural ecological processes of the environment, while physical methods often induce secondary pollution through the release of toxins from disrupted algal cells (Petrusevski et al. 1995; Sivakumar and Pandit 2002).

Due to the difficulty of removing cyanotoxins (such as microcystins) from water bodies, the treatment of HABs in contaminated waters is a sizeable challenge. In addition to algal removal, it is also necessary to treat the toxins generated by the algae. Disruption of the algae cells increases the release of toxins. The most current treatment technologies include the use of oxygen radicals such as ozone (O_3) and hydrogen peroxide (H_2O_2) to effectively decrease microcystin concentrations in aquatic environments (Barrington et al. 2013). However, there are concerns that strong oxidants like O_3 and H_2O_2 may adversely impact other organisms (Fan et al. 2013). To avoid employing harmful oxidants, other less harmful oxygen radicals generated by cavitation were investigated in this study. In previous work, superoxide anions (O_2 -) have demonstrated to degrade microcystins (Medina et al. 2016), while potentially causing less harm to other organisms due to their rapid dissociation in water (t1/2=10-9 sec).

Hydrodynamic cavitation is an emerging technology currently studied to control HABs (Gogate and Pandit 2001; Jancula et al. 2014; Li et al. 2015; Medina et al. 2016; Wu et al. 2012; Zhang et al. 2006). Harmful cyanobacteria are a growing epidemic as reports of HABs are occurring worldwide following perplexing trends that are only expected to intensify with climate change (Ho and Michalak 2015). In this study, oxygen radical generation without the use of a specialized, commercial reactor was investigated. Cavitation treatment has demonstrated to be a powerful

approach in the destruction of algae (Li et al. 2014). The physical mechanism of hydrodynamic cavitation alone can structurally damage the membranes of the cyanobacteria while radicals generated from the cavitation process oxidized the cyanotoxins released from the disrupted cell membranes. Since micro- and nano-bubbles produced in the cavitation field can generate strong turbulence during the collapsing of bubbles (Suslick et al. 2011; Wu et al. 2012), these mechanical forces can damaging the cyanobacteria's cell wall, rendering the injured algae, and its toxins, more sensitive to superoxidation. The resulting cavitation fields produce oxygen radicals without additional oxidizing agents.

1.2 Nano- and micro-bubble treatment of contaminants

Nano- and micro-bubbles are very small bubbles with respective diameters of <200 nm and 10–50 μm (Agarwal et al. 2011). According to the Young-Laplace equation (Equation 1), the rate at which the internal pressure of nano-micro-bubbles increase is inversely proportional to its size (Suslick et al. 2011).

$$P = Pl + 4\Omega/db \quad (1)$$

where:

P = gas pressure,
Pl = liquid pressure,
Ω = surface tension of the liquid,
db = bubble diameter.

High pressures up to 100 MPa are generated during the bubble collapse as the speed of this reaction is greater higher than the speed of sound in water (Suslick et al. 2011). Also, the temperature inside the collapsing bubble increases significantly (up to 10000 K) due to adiabatic compression (Agarwal et al. 2011). During the course of cavitation, micro-bubbles have been reported to maintain a negative charge under a wide range of pH values despite the influence of OH and H+ ions at the gas-water interface (Takahashi et al. 2009; Zhang and Hua 2000). An inherent difference in the two bubble types are that micro-bubbles gradually decrease in size and subsequently collapse after extended stagnation resulting in the dissolution of interior gases into the surrounding water, whereas nano-bubbles can remain in liquid media for long periods of time before collapsing (Agarwal et al. 2011). In contrast to nano- and micro-bubbles, larger, macro-bubbles

burst at the surface of water, releasing the inner gases (generally nitrogen and oxygen) into the atmosphere. During hydrodynamic cavitation, nano and micro sized bubble are produced. Li et al. (2014) investigated hydrodynamic cavitation treatment on *M. aeruginosa*. Findings from their study indicate that the extent of treatment was dependent on treatment time and pump pressure as considerable settling of the cyanobacteria was observed after ten minutes of treatment. Sedimentation and reduced chlorophyll α concentrations were attributed to damaged intracellular gas vesicles and photosystem apparatus, respectively. Other studies have reported cavitation treatment alone to have moderate treatment efficacy after testing (Jancula et al. 2014; Li et al. 2015). Wu et al. (2012) investigated hydrodynamic cavitation and ozone treatment on *M. aeruginosa* and found that their treatment alone only yielded 15 and 35% removal, respectively, after ten minutes of treatment.

1.3 Objectives

The objectives of this work were to evaluate the combined effect of hydrodynamic cavitation and advanced oxidation to:

1. Remove cyanobacteria and its associated toxins from water under various cavitation fields.
2. Determine the oxygen species generated from each cavitation field.

1.4 Approach

Taking into account the results reported from bench scale studies investigating hydrodynamic cavitation on cyanobacteria, it is thereby concluded that cavitation treatments can be optimized to increase treatment yields at significantly larger scales. In this study, varying types of water nozzles were used to generate cavitation fields in cyanobacteria contaminated water under constant pressures in 300 gal tanks.

1.5 Scope

Use of hydrodynamic cavitation to treat cyanobacteria. Algal contaminated water was treated using heavy-duty recirculation pumps equipped with varying types of bubble diffusers to create hydrodynamic cavitation which was conducted over different time intervals and analyzed for algal cell viability. Total organic carbon (biomass), turbidity, chlorophyll α, and microcystin analyses were used to determine efficiency of treatment.

2 Materials and Methods

2.1 Cyanobacteria cultures

Pure cultures of the toxin producing strain of *M. aeruginosa* (#LB 2385) and pure, non-toxin producing cultures of *Anabaena spp*. were obtained from the University of Texas at Austin. Environmental cyanobacteria samples were obtained from Barren River Lake (located in central KY). Pure cultures and environmental cyanobacteria samples were grown in half-strength Hoagland's Nutrient Media at 22° C under light intensity of 2100 lux. Both pure and environmental cyanobacteria samples were cultured in a stock tank until water turbidity measurements reached 0.1 Nephelometric Turbidity Units (NTU). Water, filtered via reverse osmosis, was used to fill each tank. Five gallons of the stock cyanobacteria culture were added to the experimental tanks. The total volume of each treatment tank was 250 gallons. Sixty milliliters of Shultz Plant Food Plus (10-15-10) was added to each tank. Cavitation treatments began after the cyanobacteria culture reached a turbidity measurement of 0.1 NTU.

2.2 Nozzle configurations

Three, national standard thread (NST) nozzles with a diameter of 25.4 millimeters (mm) were purchased from American Supply Company (Vicksburg, MS). The nozzles were selected based on extrusion molding characteristics that generate varying pressures and water stream structure. Nozzle 1 was a variable flow nozzle with surface molding that was positioned to generate a jet stream consisting of a wide core region, a discontinuous flow region, and a diffused flow region (Figure 1). Nozzle 2 was also a variable flow nozzle with closely spaced extrusion molding that was set to generate a jet stream with a wide core region and a discontinuous flow region. Nozzle 3 was a continuous flow nozzle that generated a forceful, narrow water stream (core region). Nozzles 1–3, are shown in Figure 2.

Figure 1. General structure of a high pressure water jet (photo adapted from www.wjta.org).

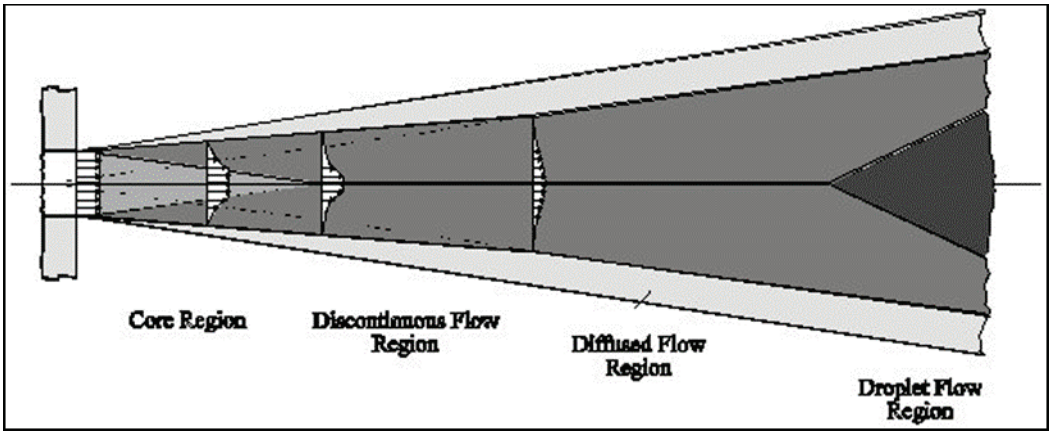


Figure 2. Nozzle configurations 1 (left), 2 (right), and 3 (center) employed in cavitation treatments.

Additionally, a 12.7 mm nano-micro bubble nozzle (BT-50) was purchased from River Forest Corporation (Escondido, CA). This nozzle was designated as Nozzle 4 (Figure 3). A gas flow (compressed air) line was connected to the nozzle in order to generate nano-micro sized cavitation bubbles in water. Optimum gas pressure requirement for the nozzle was 5% of water flow pressure. Air pressure was set at 1.2 pounds per square inch (psi), and water flow pressure was calculated as 25.3 psi.

Figure 3. Nozzle configuration 4 employed in cavitation treatment.

2.3 Treatment tank assembly

Five, 1,200 liter (L) tanks were used in growing cyanobacteria cultures under controlled greenhouse conditions. Three tanks were plumbed together with 25.4 mm polyvinylchloride (PVC) pipe to utilize one water pump in an effort to maintain pressure consistencies between treatments (Figure 4). One tank was used as a stock, and the other as a control (Figure 5). Treatment nozzles #1–4 were affixed to 0.91 Meter (m) sections of hose that consisted of quick connect locks that could attach to the primary hose (from water pump) to eliminate the task of changing nozzles between treatments (Figure 6). Flow rates for each nozzle configuration used for cyanobacterial treatment are provided in Table 1.

Figure 4. Water treatment assembly for treatment tanks.

Figure 5. Stock (left) and control (right) tanks for cyanobacteria.

Figure 6. Treatment nozzles attached to interchangeable 3 ft hose sections (A and B respectively).

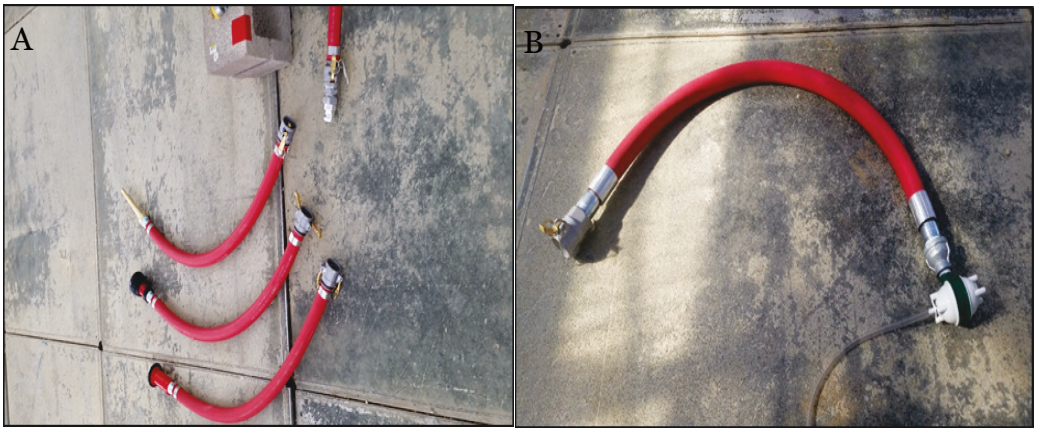


Table 1. Flow rates for each nozzle configuration used for cyanobacterial treatment.

Treatment Nozzle #	Nozzle Configuration	Flow Rate (L/min)
1	VF; Moderately Spaced Extrusion Molding	25.5
2	VF; Closely Spaced Extrusion Molding	61.5
3	CF; Narrow Stream	40.1
4	Nano-Microbubble Nozzle	22.3

2.4 Oxygen radical production tests

Superoxide [O^{2}-] and hydroxyl [OH] radical production were investigated using a 5,5-dimethyl-1-pyrroline-n-oxide (DMPO) radical trap to stabilize radical adducts for analysis. Radical trap buffer solutions were prepared with 20 microliter (µL) of 1 molar (M) DMPO, and 70 µL of a 100 millimolar (mM) phosphate buffer solution containing 25 micromolar µM diethylenetriamine-pentaacetic acid (DTPA). In clean water, cavitation treatments were conducted with each test nozzle. Nozzles 1–3 were positioned at water surface, and nozzle 4 was positioned beneath water surface. Treatments were conducted for 30 minutes before sample collection. The 110 µL samples were collected and dispensed into radical trap buffer solution. Samples were analyzed via electron paramagnetic resonance (EPR) immediately after collection. All samples were collected in triplicate.

2.5 Above and submerged water cavitation treatment of Barren River Lake cyanobacteria

In above water cavitation treatments, nozzles were positioned six inches above the water surface (Figure 7). Each tank was treated for 40 minutes.

At 5, 10, 20, and 40 minutes, 1L samples were collected at 15 inches from the treatment impact area. Afterwards, samples were collected at 48, 96, and 162 hours.

Submerged water cavitation treatment were conducted in the same manner, with the exception of the position of test nozzles #1–3 which were placed three inches beneath the water surface (Figure 8).

Figure 7. Above water cavitation treatment with nozzles 1–3.

Figure 8. Submerged water cavitation treatment with nozzles 1–3.

2.6 Cavitation treatment of *M. aeruginosa* and *Anabaena spp.*

M. aeruginosa was tested with treatment nozzles #1–4. Nozzle #4 was tested separately on pure cyanobacteria cultures. This nozzle was incorporated into the treatment process due to the advanced oxidation potential of the micro-nano bubble nozzle configuration. Nozzle #4 was also down-selected for treatment of the pure culture of *Anabaena spp*. For each individual test, the treatment nozzle (1, 2, or 3) was partially submerged at water surface through the duration of the test (Figure 9). Each tank was treated for 120 min. Samples were collected 7.62 – 12.7

centimeters (cm) below the water surface in 1 L, sanitized Nalgene bottles at 0, 0.5, 1.0, 1.5, and 2.0 hrs. Cavitation tests with treatment nozzle 4 was conducted with nozzle positioned 40.64 – 45.72 cm beneath the water surface (Figure 10). Water samples were collected in aforementioned manner at 0, 0.5, 1.0, 1.5, and 2.0 hrs. All collected samples were stored at 4° C for 24 hours before analysis.

Figure 9. Cavitation treatment of *M. aeruginosa* with partially submerged treatment nozzles 1–3 (A, B, and C, respectively).

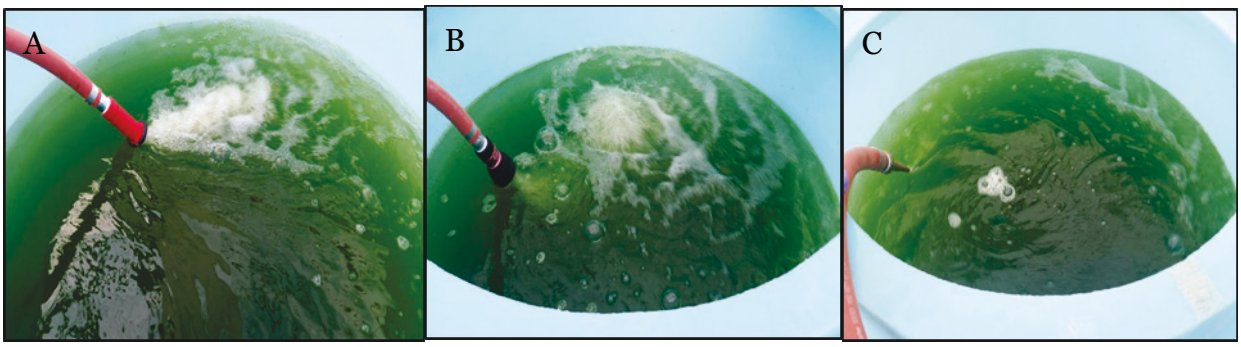
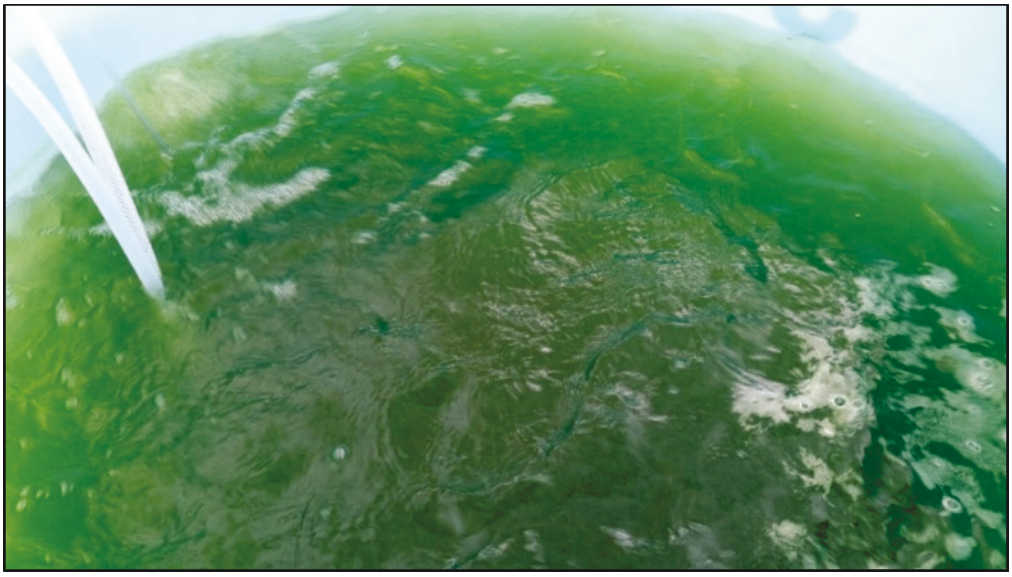


Figure 10. Cavitation treatment of *M. aeruginosa* with treatment nozzle 4.



2.7 Chemical analysis

Samples collected from treatment tanks were analyzed for pH, temperature, and dissolved oxygen (DO) using an Oakton multiparameter meter. Chlorophyll α analyses were performed after 90% acetone extraction using a Hach DR 2800 VIS spectrophotometer. Turbidity measurements were also determined using the Hach spectrophotometer. Heterotrophic plate counts (HPC) were determined using HPC Pour Plate

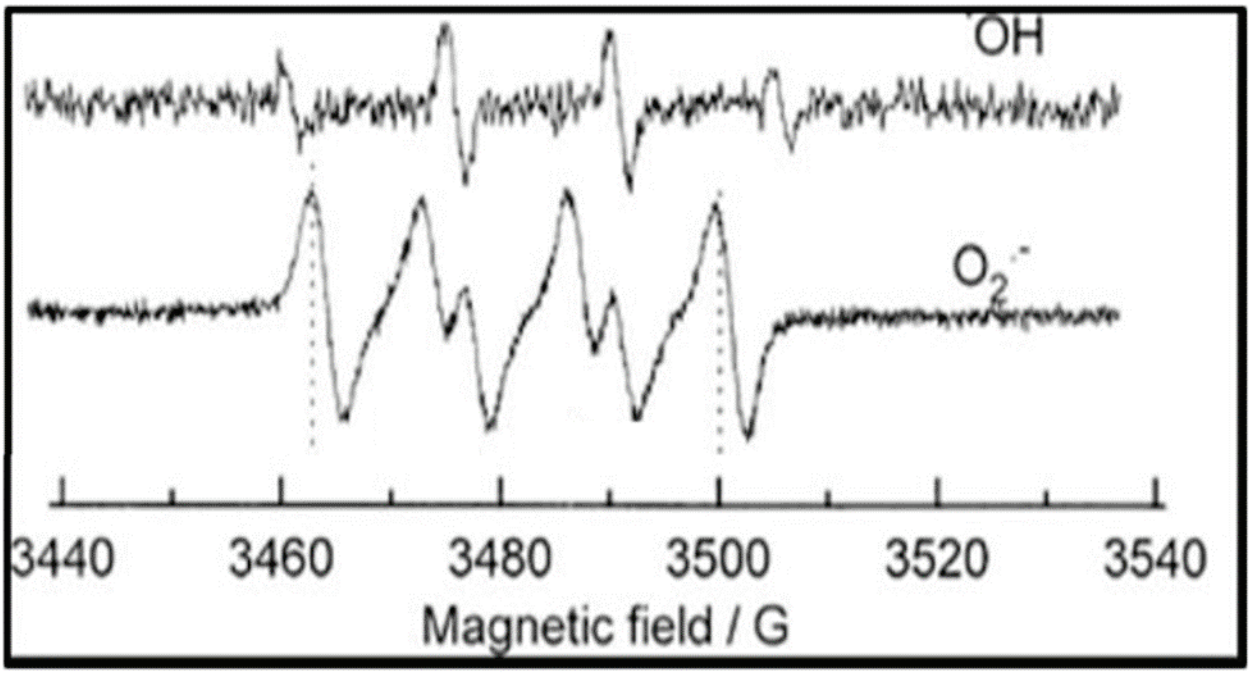
Method 8241 (Standard Methods for the Examination of Water and Wastewater). Microcystin concentrations were determined by Abraxis enzyme-linked immunosorbent assay (ELISA) for cyanotoxins. For EPR analysis of O_2- and OH radicals in clean water samples, an Active Spectrum Micro-Electron Spin Resonance (ESR) analyzer (lower detection limit: 0.1 μM) was used.

3 Results and Discussion

3.1 Oxygen radical production tests

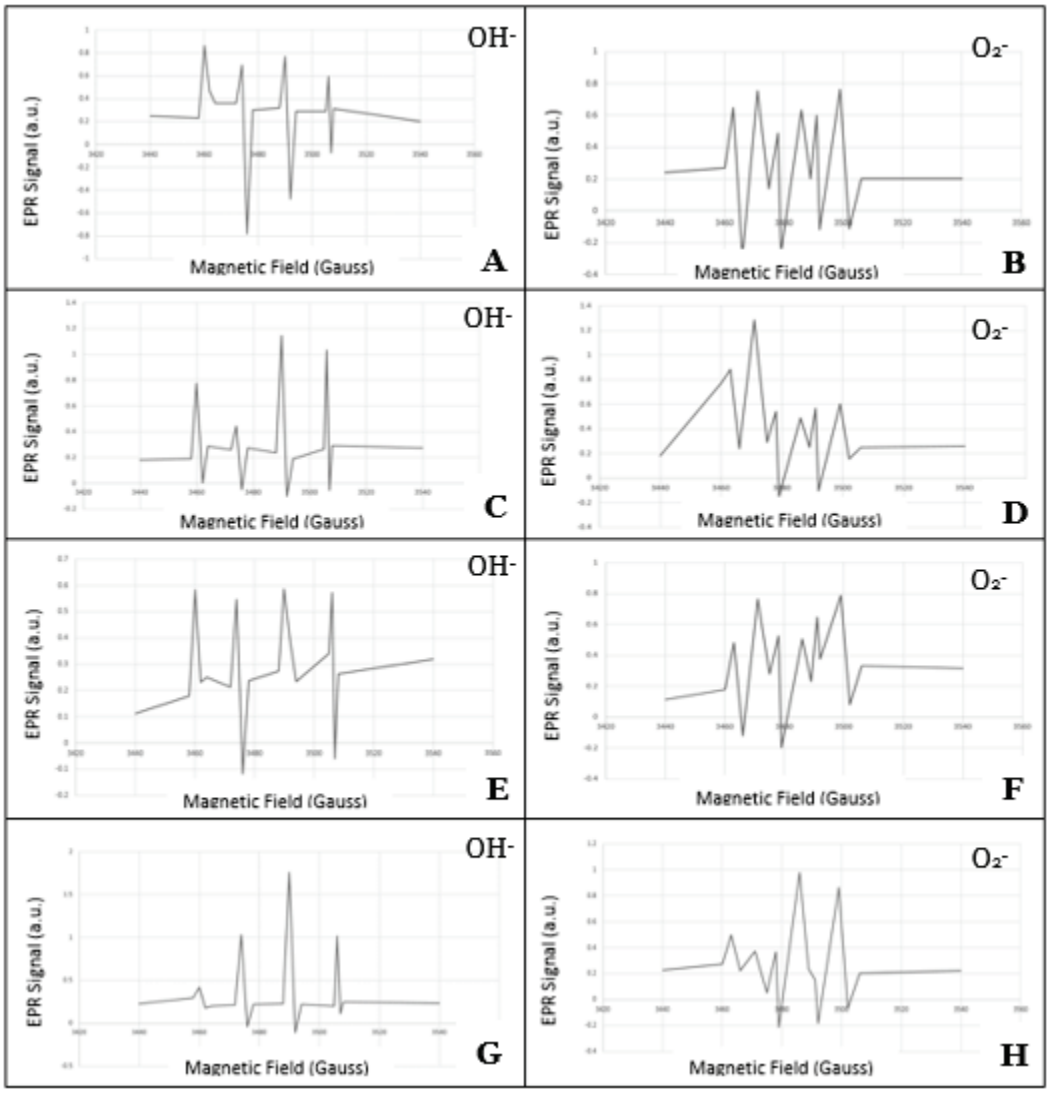
The EPR spin trapping technique was employed to detect free radical production during the hydrodynamic cavitation treatment process. Reference spectra for the magnetic field fingerprint region of OH and O_2- are shown in Figure 11. All EPR spectra reflect reported data points which were generated from the averaging of magnetic field (G) values and manual plotting of raw data.

Figure 11. Reference spectra for superoxide and hydroxyl radicals (adapted from Guo et al. 2003).



Samples collected from cavitated waters induced by treatment nozzles #1 and #3 yielded no OH or O_2- radicals as indicated by EPR spectra (Figure 12). No correlation was observed for either of the oxygen radicals of interest. EPR spectra for water samples collected during treatment with nozzle #2 indicated a slight correlation with reference spectra of OH radicals (Figure 12c). It is possible that OH radicals were present in low concentrations, or either detected during the rapid decay of the DMPO/OH adduct. No correlation between EPR sample and reference spectra was observed for O_2- radicals produced from nozzle #2. These results are in part due to the nozzle configurations that produced primarily macro-bubbles into the water.

Figure 12. EPR Spectra for hydroxyl and superoxide radical generated from nozzle# 1–4. EPR signal for OH (left) and O_2^- (right) for nozzle #1 (A and B), nozzle #2 (C and D), and nozzle #3 (E and F) show little to no correlation with reference spectra indicating the presence of OH and O_2^- radicals. EPR spectra for nozzle #4 showed direct correlation with reference spectra of OH, and a moderate correlation with that of O_2^- (G and H).



In cavitation samples collected during the treatment with nozzle #4, EPR spectra indicated that hydroxyl radicals were present in samples as the averaged data points generated peaks in the OH magnetic fingerprint region similar to the computer generated spectra in Figure 12g. EPR spectra for O_2- radicals also indicate that these radicals were present, possibly at low concentrations, as peaks correlate to those observed in the fingerprint region for O_2- (Figure 12h). This outcome was anticipated as nozzle #4 was a micro-bubble nozzle specifically designed to generate negatively charged micro- and nano-bubbles. The mode of action is to

aspirate water droplets and air by way of gas-water circulation (Agarwal et al. 2011).

3.2 Above and submerged water cavitation treatment of Barren River Lake cyanobacteria

Environmental samples collected from Barren River Lake, KY were subjected to cavitation treatments using nozzles #1–3 positioned above the water surface. The trends observed in particulate biomass concentrations after treatment with nozzles #2 and #3 indicate that these nozzles inhibited algal growth up to 168 hours. Rapid proliferation of the algae began after 48 hours of cavitation treatment with nozzle #1. Algal cell concentration in the water column appeared to be reduced immediately following treatment, but regenerated quickly after 96 hours. This outcome was anticipated as nozzle #1 configuration generated the least amount of pressure of the three test nozzles. Additionally, it was expected that cavitation generated in the water would not exceed 1–2 ft. below the water surface. Configurations for treatments nozzles #2 and #3 generate a relatively greater amount of pressure that can contact algae beyond 2 ft of the water surface. Algal biomass data correlates directly with turbidity measurements for each treatment. Although the shear stress on the algae induced by water circulation was able to break up large cyanobacteria aggregates, the overall effect of the cavitation treatment on the cyanobacteria was minimal.

Chlorophyll α measurements correlate directly with turbidity data indicating that algal cells were least affected by nozzle configuration #1, as chlorophyll α concentrations spiked at 168 hours after treatment. These results imply that the photosynthetic activity of the algae were not affected. Chlorophyll α concentrations slightly increased at 48 hours compared to the observed trend in turbidity. This increase in chlorophyll α observed after treatment with nozzles #1 and #2 could be due to chlorophyll released from physically damaged or lysed cells. Because chlorophyll is not very stable and is quickly de-graded by light, these results could suggest a slow, continuous release of the compound into the water. Relative to treatments with the other nozzle configurations, samples collected after cavitation treatment with nozzle #3 show the greatest effect in treating and controlling the cyanobacteria up to 168 hours. This indicates that pressure is directly proportioned to algal biomass reduction as it relates to the occurrence of shear stress only. Turbidity and chlorophyll α values for samples collected at each sampling interval after

cavitation treatment are shown in Figures 13 and 14. Due to spatial constraints during above and submerged water preliminary treatments, cavitation tests were not replicated.

Figure 13. Turbidity measurements of cyanobacteria samples collected at time 0, 0.7, 48, 96, and 168 hours.

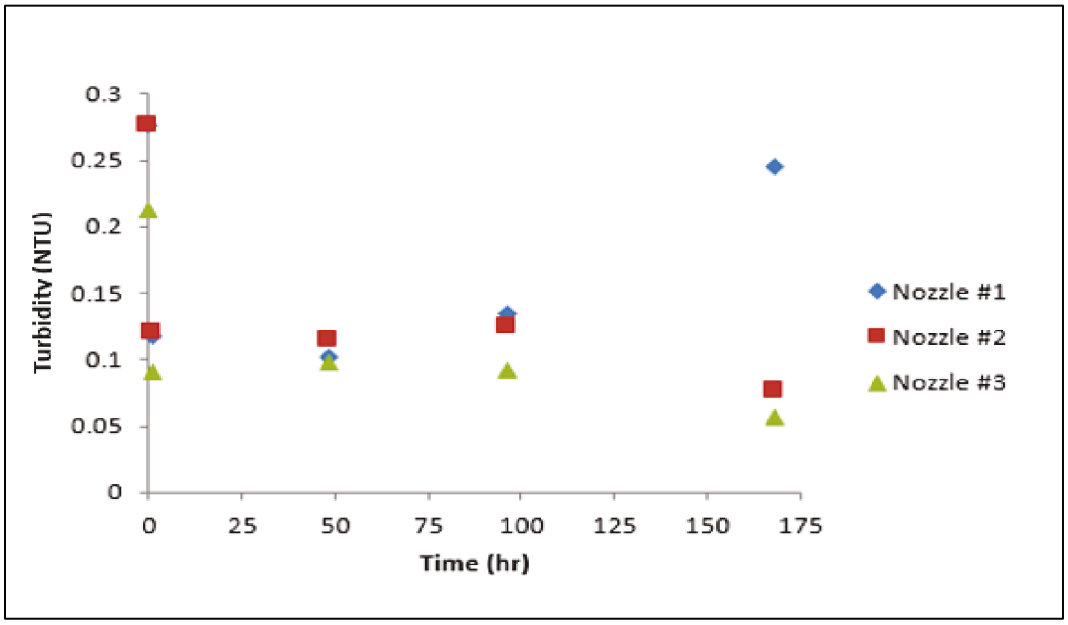
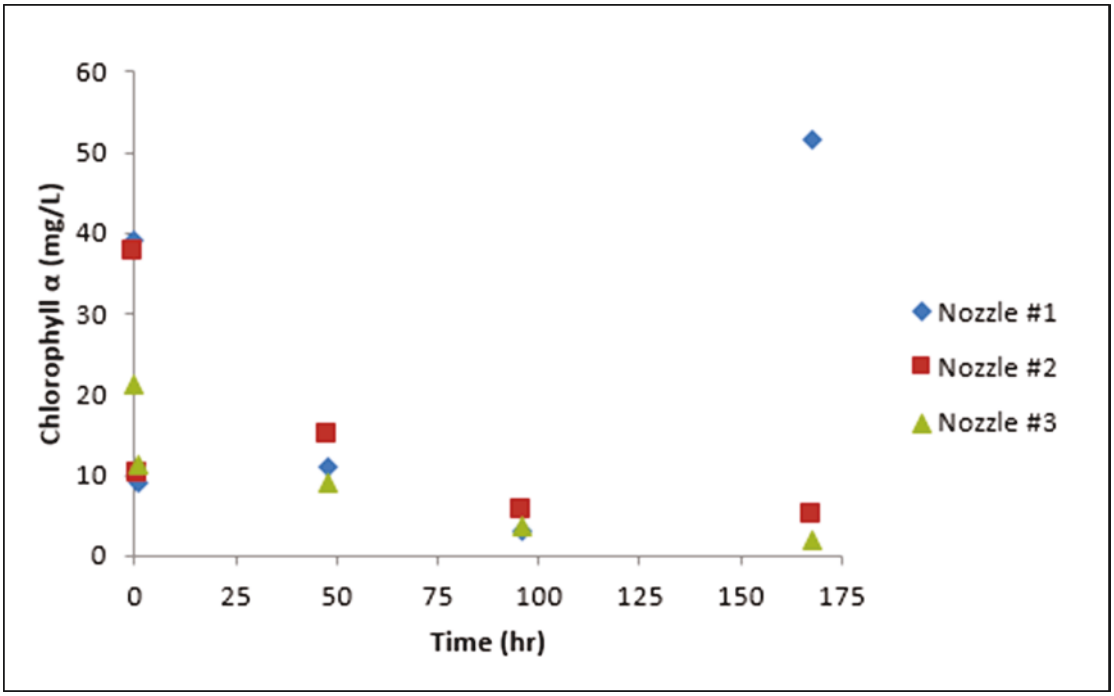


Figure 14. Chlorophyll α concentration for cyanobacteria samples collected after 0, 0.7, 48, 96, and 168 hours of treatment.



In submerged water treatments, algal biomass was moderately reduced in samples collected after cavitation treatment with nozzle #1, but greatly reduced in samples treated with nozzles #2 and #3 up to 96 hours. While this trend is encouraging, the rapid regeneration rate of the cyanobacteria observed at 168 hours in tanks treated with nozzles #2 and #3 is a highly unfavorable outcome. The algal biomass concentration increased by an average of 58% above the initial biomass concentrations. These data imply that the greater water pressures are effective for only up to 96 hours after treatment, while the treatment with the least pressure yielded only subtle changes in algal biomass. In addition to the increased biomass reductions, the submerged nozzles were able to create turbulence at greater depths than if the nozzles were positioned above the water surface.

It is likely that the only mode of treatment occurring in the tanks from each test nozzle is by physical stresses from turbulence, as no air is incorporated with the nozzle output to generate the air bubbles in the liquid medium capable of chemically affecting the algal cells. Treatment using nozzle #1 impeded rapid proliferation of the algae, reducing algal biomasses. Trends observed in turbidity and chlorophyll α data (Figures 15 and 16) also follow that of the algal biomass concentrations. Because shear stress is not effective enough to completely inhibit algal growth, nozzles #1–3 are not recommended for cyanobacterial treatment.

Figure 15. Turbidity measurements of cyanobacteria samples collected at time 0, 0.7, 48, 96, and 168 hours.

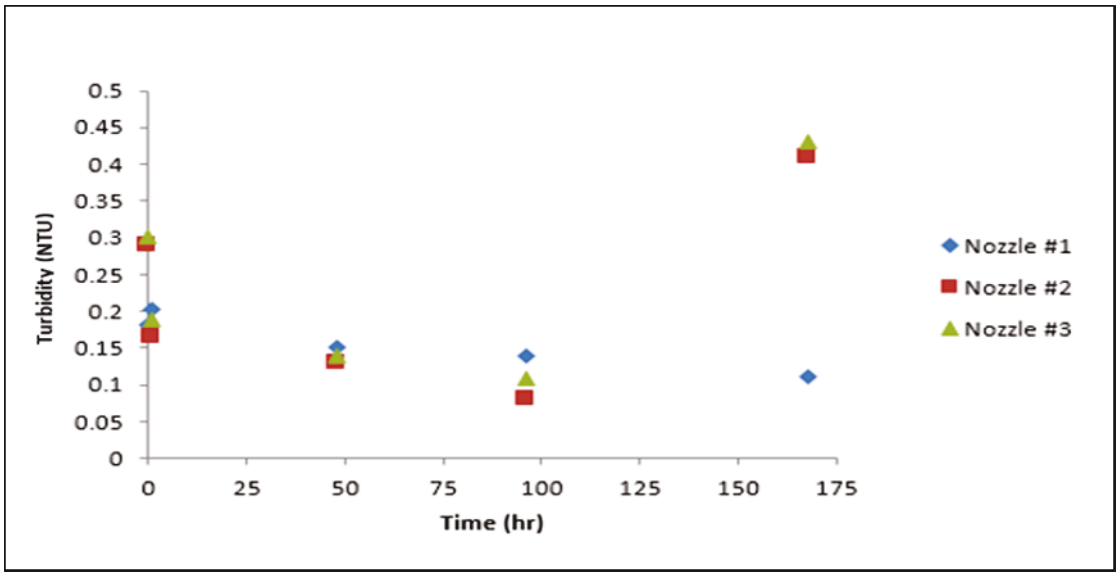
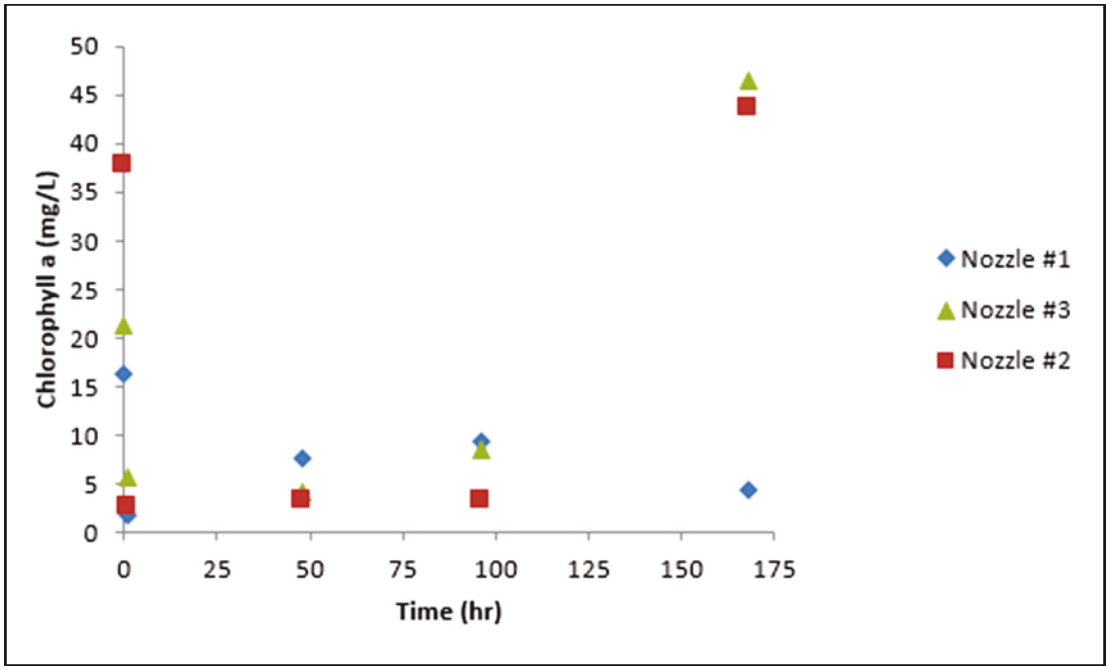


Figure 16. Chlorophyll α concentration for cyanobacteria samples collected after 0, 0.7, 48, 96, and 168 hours of treatment.



3.3 Cavitation treatment of *M. aeruginosa* and *Anabaena spp.*

3.3.1 *M. aeruginosa* treatment

Cavitation treatment with nozzles #1–3 were not effective in damaging cell structures and inhibiting growth of *M. aeruginosa*. Results from turbidity and chlorophyll α analysis showed no significant decreases in viable cell density in samples collected between 0.5–2 hours. Turbidity and chlorophyll α were moderately decreased upon initial circulation of the water between 0 and 0.5 hours. In tanks treated with nozzles #1–3, the algal cell aggregates subjected to cavitation between time 0 and 0.5 hours are likely undergoing shear stress which occurs during water recirculation. This type of stress can lead to cell damage, however, this effect is usually mild. As reported in several studies, shear stress only causes a modest amount of damage to algal photosynthetic activity (Jancula et al. 2014; Roelke et al. 2013).

Samples collected from cavitated waters produced by nozzle #4 showed notable reductions in turbidity and chlorophyll α values over the two-hour treatment period. For each sample, particulate mass and turbidity measurements decreased moderately after one-hour of treatment with nozzle #4, whereas chlorophyll α measurements continued to decrease. The presence of cell debris yielded a relatively constant particulate mass

over 1.5 to 2 hours. This may be in part due to the sedimentation of algal cells (and debris) that often occurs after cavitation treatment in which cell vacuoles are ruptured. Turbidity measurements continued to decrease slightly after 1.5 hours relative to particulate mass values primarily due to the attenuation of free chlorophyll α in the liquid medium. The continued reduction in chlorophyll α is a direct result of the decomposed free chlorophyll and the possible damage sustained to the photosystems of the affected algal cells.

Table 2 shows the average turbidity and chlorophyll α measurements in samples after cavitation treatment with nozzles #1–4 at each sampling interval. Table 2 shows the algal cell colony forming units (CFU) from Heterotopic Plate Counts (initial and final) after 48 hours of incubation. It is important to note that initial concentrations of the cyanobacteria varied greatly between tanks, particularly in turbidity and chlorophyll α concentrations. Therefore, plate counts were performed to more accurately quantify algal removal of each treatment. Overall, algal cell colonies determined from heterotrophic plate counts support the general trend observed for turbidity and chlorophyll α in samples treated with nozzles #1–4 (Table 3). Figures 17–20 show turbidity and chlorophyll reductions in *Microcystis aeruginosa* samples collected at each time interval during the two-hour treatment period with each nozzle.

Table 2. Average particulate mass, turbidity and chlorophyll α measurements after cavitation treatment with nozzles #1–4.

	Particulate Mass (g/L)		Turbidity (NTU)		Chlorophyll α (µg/L)	
Nozzle Configuration	Initial	Final	Initial	Final	Initial	Final
Nozzle #1	5.41	3.04	0.26	0.07	13.8	4.8
Nozzle #2	3.61	3.25	0.46	0.08	22.4	5.2
Nozzle #3	7.86	4.13	1.10	0.08	35.1	5.8
Nozzle #4	3.42	1.55	0.15	0.02	9.6	0.5

Table 3. Algal cell colony forming units (CFU) from heterotopic plate counts (initial and final) after 48 hours of incubation.

	CFUs (10^8 cells/mL)		
Nozzle Configuration	Initial	Final	Percent Reduction
Nozzle #1	187	103	44%
Nozzle #2	258	126	51%
Nozzle #3	217	104	52%
Nozzle #4	121	39	68%

Figure 17. Turbidiy and chlorophyll α concentrations of *M. aeruginosa* collected at 0, 0.5, 1.0, 1.5, and 2.0 hours after treatment with nozzle #1. Turbidity and chlorophyll α concentrations stabilized after 0.5 and 1.0 hours of treatment, respectively.

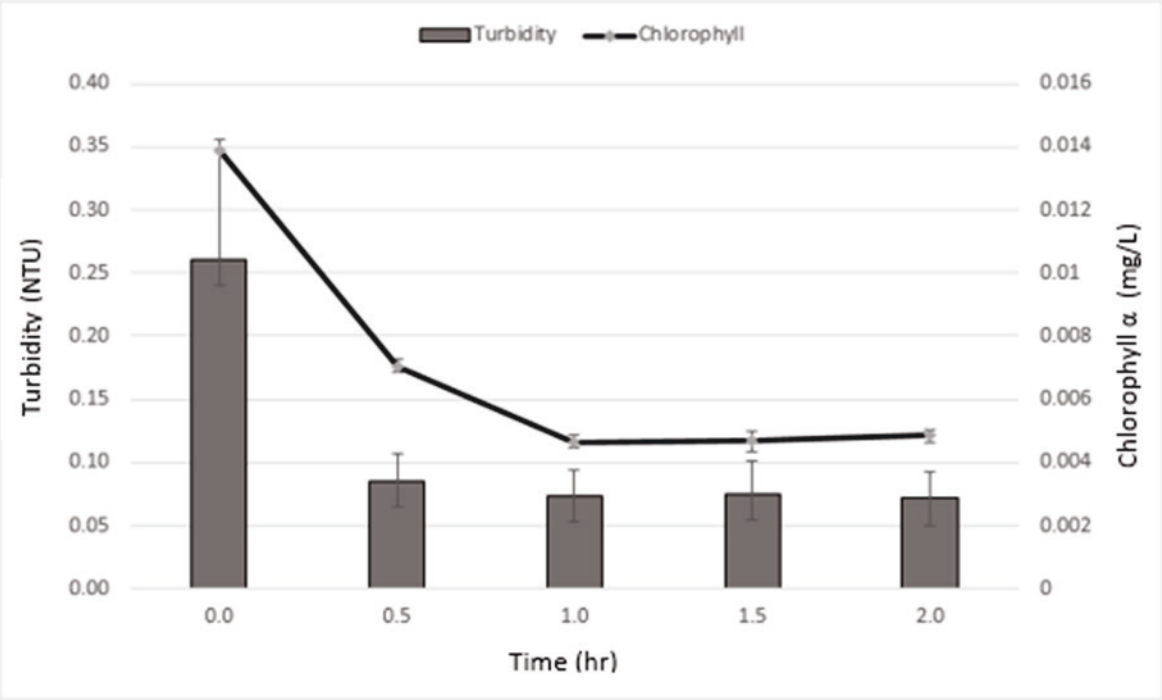


Figure 18. Turbidiy and chlorophyll α concentrations of *M. aeruginosa* collected at 0, 0.5, 1.0, 1.5, and 2.0 hours after treatment with nozzle #2. Both turbidity and chlorophyll α concentrations stabilized after 0.5 hours of treatment.

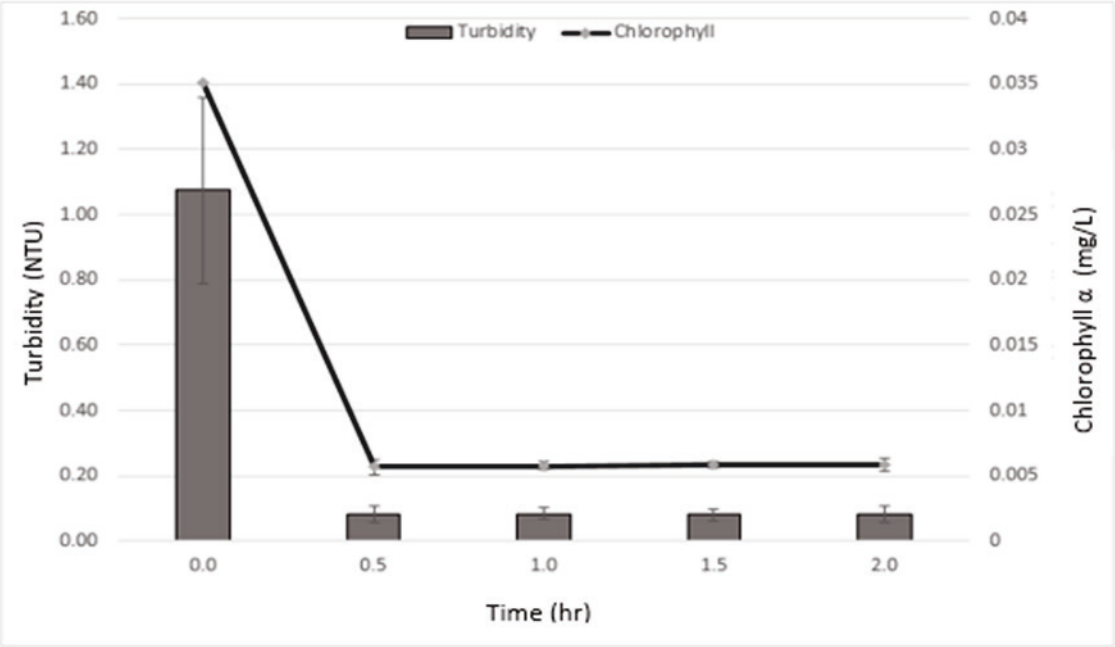


Figure 19. Turbidiy and chlorophyll α concentrations of *M. aeruginosa* collected at 0, 0.5, 1.0, 1.5, and 2.0 hours after treatment with nozzle #3. Both turbidity and chlorophyll α concentrations stabilized after 0.5 hours of treatment.

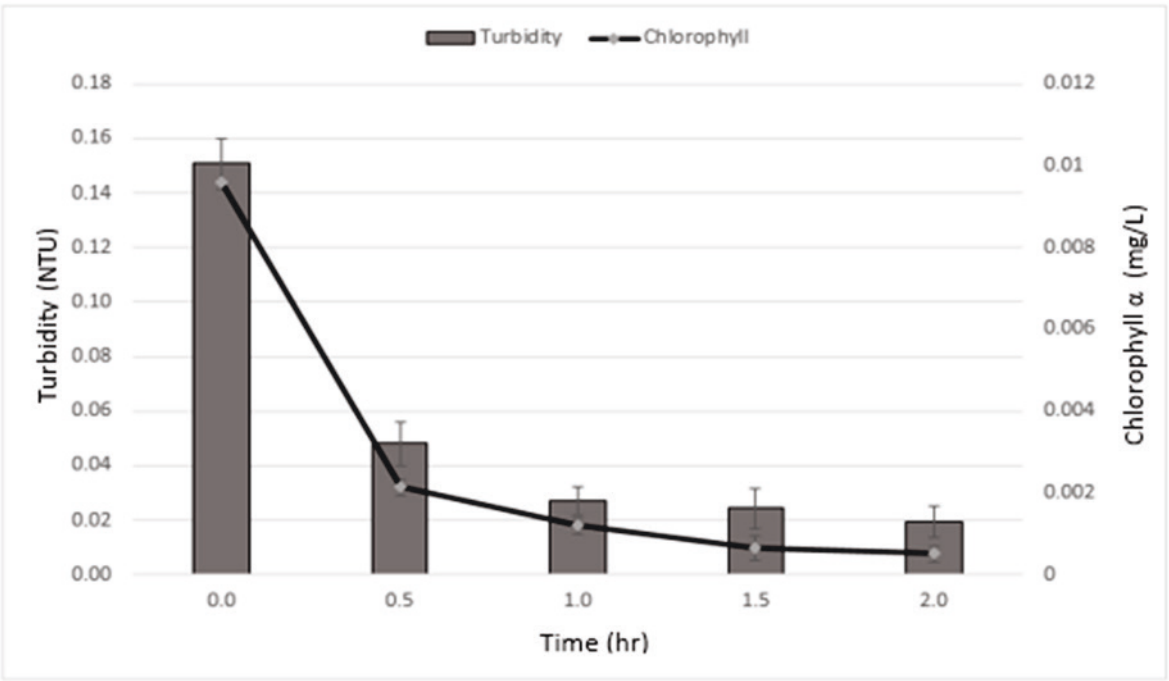
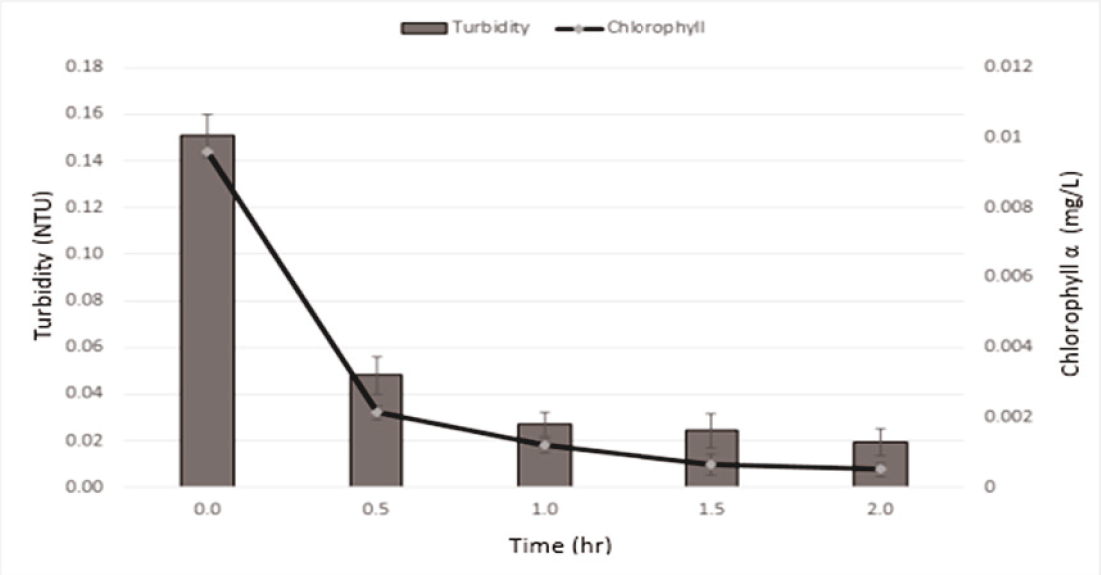
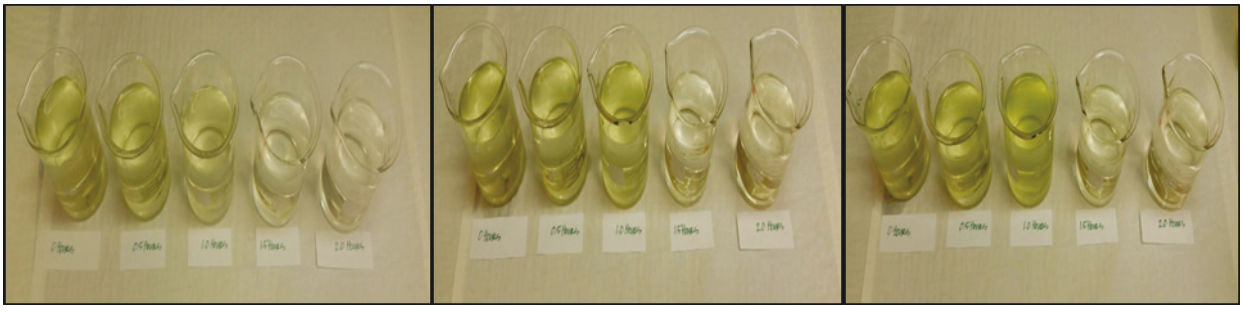


Figure 20. Turbidiy and chlorophyll α concentrations of *M. aeruginosa* collected at 0, 0.5, 1.0, 1.5, and 2.0 hours after treatment with nozzle #4. Both turbidity and chlorophyll α concentrations continued to decrease up to 2 hours of treatment.



Figures 21 shows the increased clarification in water samples over the two-hour cavitation period after treatment with nozzle #4. The percent reduction in viable algal cells was similar after repeated testing with each nozzle, indicating good reproducibility in treatment. Comparing treatments with nozzles #1–3 to that of nozzle #4, chlorophyll α concentrations decreased an average of 65, 79, and 83% over two hours after treatments with nozzles #1-3, respectively, and an average of 95% with nozzle #4.

Figure 21. *M. aeruginosa* collected at each sampling interval after treatment with nozzle # 4. Samples shown above (a, b, and c) represents the treatment replicates of nozzle #4.



When correlating EPR data with the outcome of algal treatment it is likely that the micro-bubble generation could have treated the microcystin toxin and affected the algal cells. Similar outcomes have been reported in studies investigating hydrodynamic cavitation on the gas vacuolated alga species *M. aeruginosa*. These studies found that the cell density and photosynthetic

activity were reduced by nearly 90 % when subjected to cavitation (Li et al. 2015). Arrojo and Benito (2008) reports that the inhibitory effect of hydrodynamic cavitation on algal growth potential is thought to be similar to that of ultra-sound, which induces the collapse of the gas vesicles within the algae and inflicts immediate damage on photosynthetic activity. Other researchers have also suggested that the subjection of algae to cavitation could trigger a secondary collapse of gas vacuoles inside the cells which may initiate a secondary production of free radicals within the cell that damages the photosynthetic apparatus. This phenomenon further explains the ineffectiveness of cavitation on vacuole-negative algae as a mild effect as only shear stress is inflicted on the alga (i.e. Chlorella spp.). In this study, the dual phase treatment attributed to the observed outcome in which the shear stress physically damaged the cells, and the cavitation microbubbles collapsed cell vacuoles and likely oxidized toxins released by the alga. Table 4 shows average percent reduction in microcystin concentrations measured in initial, intermediate, and final samples collected after cavitation treatments with each test nozzle.

Table 4. Microcystin concentrations (µg/L) in samples treated with nozzles #1–4.

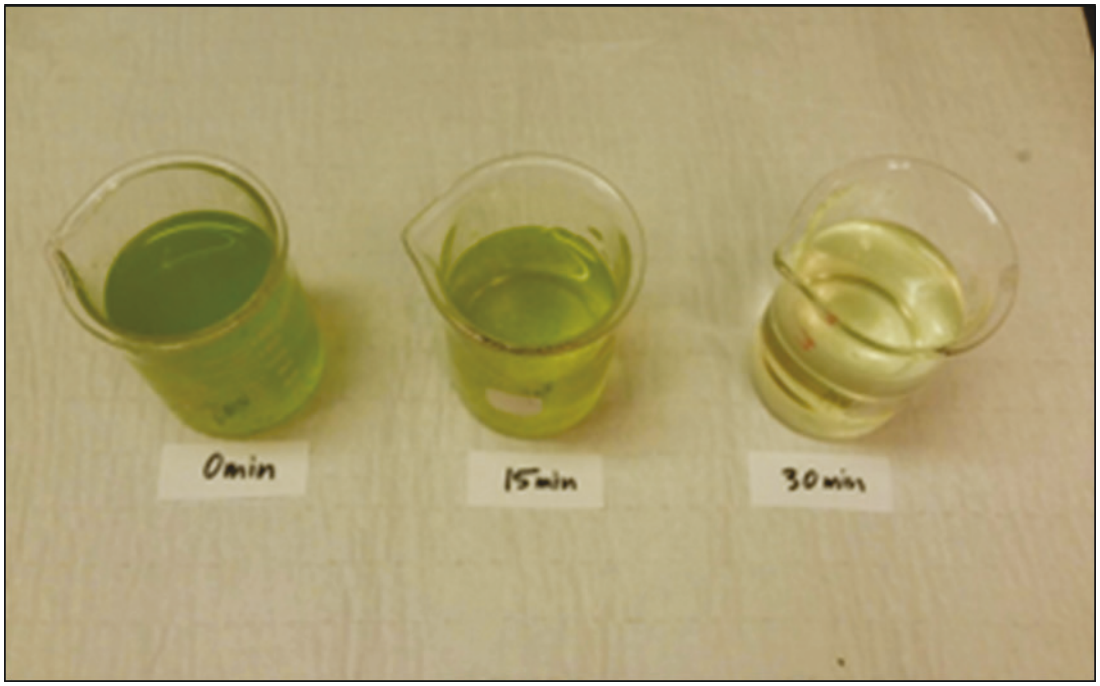
	Microcystin Concentration (ug/L)		
Nozzle Configuration	Initial	Final	Percent Reduction
Nozzle #1	0.322	0.214	33.54%
Nozzle #2	0.434	0.341	21.42%
Nozzle #3	0.636	0.531	16.51%
Nozzle #4	0.535	0.281	47.48%

Final microcystin concentrations decreased an average of 33, 22, and 17 percent in cavitated waters induced by treatment nozzles # 1–3, respectively. Initial microcystin concentrations in the tank treated with nozzle #3 were greatest, this correlates with initial turbidity and chlorophyll α values. Results from this test showed that the greatest reduction of microcystin concentrations occurred after cavitation treatments with nozzle #4, with an averaged reduction at 48%. Effects induced by nozzle #4 could have been due to the pyrolytic decomposition that takes place within the collapsing bubbles and the OH radicals, and shock waves generated at the gas-liquid interface. However, noticeable variability is observed in final microcystin concentrations among samples collected from cavitated waters generated by nozzle #1–3. Although the decreases are modest, there is still a replicable trend observed in the

microbubble treated samples in which destruction of the microcystin toxin in observed. This outcome is still consistent with other studies that have tested the microcystin toxin against hydrodynamic cavitation treatments.

In the 1,200 L tanks, water clarification was visually observed after two hours of treatments with the microbubble nozzle. In an effort to determine the rate at which the algae was effectively treated, a smaller scale cavitation test was performed in a 18.9 L bucket to compare treatment times with that observed in the large tanks. It is likely that the algae is treated immediately after being aspirated from the micro bubble nozzle. Results showed significant clarity of the samples after thirty minutes of treatment (Figure 22).

Figure 22. *M. aeruginosa* (15 L) treated with micro bubble nozzle for thirty minutes.



3.3.2 *Anabaena spp.* treatment

Anabaena spp. treated with nozzle #4 yielded considerable reductions in chlorophyll α concentrations after two hours of treatment. It was anticipated that the vacuolated, filamentous cyanobacteria would be more susceptible to the physical phase of the cavitation treatment due to its relatively larger size compared to *M. aeruginosa*. Treatment efficacy for both species were comparable. However, *M. aeruginosa* was slightly more susceptible to the cavitation treatments. Overall, chlorophyll α concentrations were reduced by an average of 86%, and turbidity

decreased by an average of 75%. Figure 23 shows turbidity and chlorophyll α concentrations over the two hour treatment period. Particulate mass concentrations are shown in Table 5.

Figure 23. Turbidity and chlorophyll α concentrations of *Anabaena spp.* samples treated with nozzle #4.

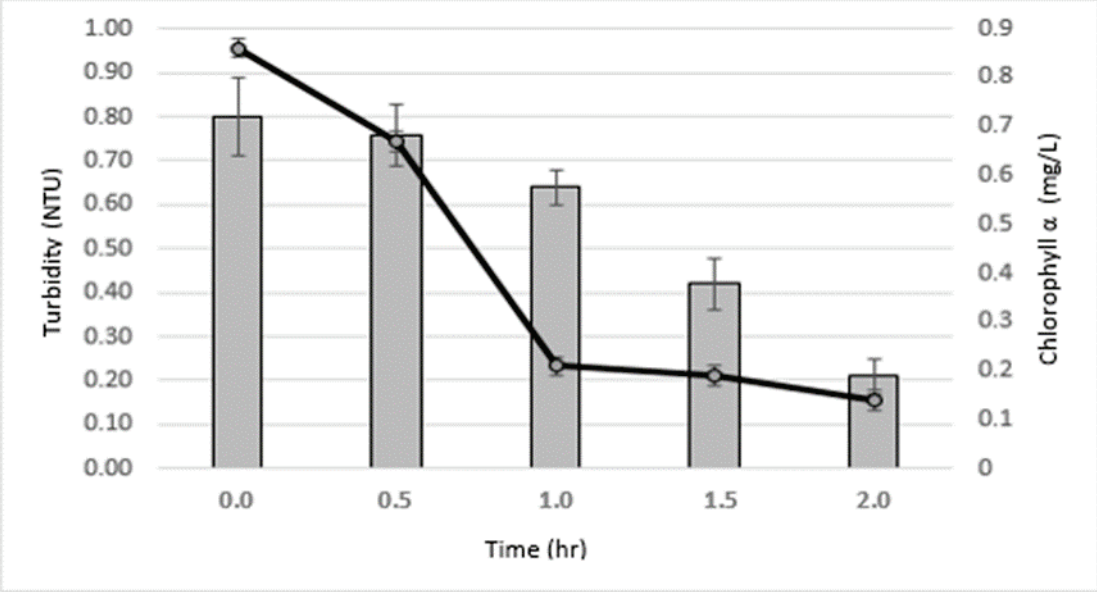


Table 5. Particulate mass concentration (g/100 mL) of *Anabaena spp.* in triplicate samples collected over the two hour testing period with nozzle #4.

Time (hr)	Rep 1	Rep 2	Rep 3	AVG	SD
0.0	0.9820	0.5960	0.6613	0.7460	0.2070
0.5	0.3314	0.0679	0.0843	0.1610	0.1480
1.0	0.3018	0.0808	0.1229	0.1690	0.1170
1.5	0.3098	0.0973	0.0107	0.1390	0.1540
2.0	0.0963	0.0950	0.1915	0.1280	0.0550

4 Conclusions and Recommendations

This study demonstrates that hydrodynamic cavitation is useful in the destruction of *M. aeruginosa* and the reduction of microcystin toxins in water. The results outlined in this report are consistent with other hydrodynamic cavitation studies with algae and clearly indicate the effect of shear stress on algae including the impact of cavitation on vacuolated algae. Algal sedimentation, turbidity, particulate mass, and chlorophyll α values were all consistent with results reported in literature. A notable exception of this study is that the hydrodynamic cavitation process was induced by a small, inexpensive micro-bubble nozzle and water circulation system. It is understood that the efficacy of hydrodynamic cavitation on algae depends on inlet pressure, algae concentrations, and treatment times. However, this work can be optimize with the addition of micro-nano bubble treatment nozzles, and greater throughput volumes. It has also been determined that hydrodynamic cavitation is more effective in removing vacuolated algae than vacuole-negative algae. According to Zhang et al. (2006), the reasons for this treatment variability are the different cell structures, presence of cellulose in cell wall, and the absence of gas vacuoles.

The following conclusions can be drawn from this work:

- *M. aeruginosa* suffers damage due to shear stress.
- Exposure to free radicals can further damages cellular mechanisms including the rupture of gas vacuoles, and the potential disruption of photosynthetic activity.

Moreover, these results imply that hydrodynamic cavitation has the potential to be used as an effective means of controlling cyanobacterial blooms in targeted areas, while potentially leaving more beneficial algae which tend to lack gas vacuoles less affected.

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Appendix A: Background Measurements

Table A1. Parameter measurements for cyanobacteria (environmental sample) from Barren River Lake, KY.

Sample ID		pH	DO (%)	Biomass (mg/L)
Time (hrs)	Control	6.8	15.3	148.3
0.0	Treatment 1	6.7	15.4	120.0
	Treatment 2	6.8	15.7	105.0
	Treatment 3	6.5	15.5	80.0
0.67	Treatment 1	6.0	16.3	28.3
	Treatment 2	6.8	16.5	20.0
	Treatment 3	6.1	16.6	25.0
48	Treatment 1	6.1	15.7	23.3
	Treatment 2	5.6	15.5	26.7
	Treatment 3	6.1	15.7	26.7
96	Treatment 1	6.0	15.4	61.7
	Treatment 2	5.8	15.6	45.0
	Treatment 3	5.2	15.3	21.7
168	Treatment 1	6.5	15.2	131.7
	Treatment 2	6.8	15.3	1.7
	Treatment 3	6.7	15.1	3.3

Table A2. Parameter measurements for *M. aeruginosa*.

	pH			Temperature (°C)			Dissolved Oxygen (mg/L)			Particulate Mass (g/100 mL)		
Treatment Nozzle 1	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*
Time 0.0 hrs	5.91	6.04	5.88	23	23	23	5.1	4.7	5.3	0.4362	0.6231	0.5622
Time 0.5 hrs	4.58	5.76	5.71	23	23	23	6.4	6.5	5.5	0.3028	0.3417	0.3457
Time 1.0 hrs	4.51	5.51	5.35	23	23	23	6.2	6.4	5.8	0.3248	0.3218	0.3018
Time 1.5 hrs	4.53	5.02	4.79	23	23	23	6.6	6.6	6.0	0.2994	0.3393	0.3097
Time 2.0 hrs	4.61	4.87	4.69	23	23	23	6.3	6.4	6.2	0.2952	0.3278	0.3611
	pH			Temperature (C)			Dissolved Oxygen (mg/L)			Particulate Mass (g/100 mL)		
Treatment Nozzle 2	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*
Time 0.0 hrs	6.02	6.13	6.01	23	23	23	5.8	5.9	6.0	0.4055	0.2953	0.3985
Time 0.5 hrs	5.03	4.47	4.91	23	23	23	6.1	6.2	6.1	0.3749	0.3422	0.3664
Time 1.0 hrs	4.82	4.51	5.03	23	23	23	6.2	6.1	6.1	0.3261	0.3574	0.3504
Time 1.5 hrs	4.51	4.49	4.72	23	23	23	6.2	6.2	6.3	0.3339	0.3425	0.3324
Time 2.0 hrs	4.38	4.52	4.51	23	23	23	6.4	6.3	6.2	0.2908	0.3371	0.3477
	pH			Temperature (C)			Dissolved Oxygen (mg/L)			Particulate Mass (g/100 mL)		
Treatment Nozzle 3	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*
Time 0.0 hrs	6.24	6.31	6.09	23	23	23	6.1	6.0	5.9	0.4414	0.9874	0.9301
Time 0.5 hrs	5.51	5.41	5.72	23	23	23	6.2	6.2	6.1	0.3608	0.4876	0.4012
Time 1.0 hrs	5.03	4.98	5.01	23	23	23	6.2	6.1	6.3	0.3765	0.4555	0.3904
Time 1.5 hrs	4.76	4.76	4.67	23	23	23	6.4	6.3	6.3	0.3701	0.4508	0.3274
Time 2.0 hrs	4.52	4.26	4.51	23	23	23	6.3	6.2	6.3	0.3586	0.4922	0.3877
	pH			Temperature (C)			Dissolved Oxygen (mg/L)			Particulate Mass (g/100 mL)		
Treatment Nozzle 4	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*	*Rep 1*	*Rep 2*	*Rep 3*
Time 0.0 hrs	4.61	4.52	4.51	23	23	23	5.8	6.1	6.0	0.3052	0.3168	0.3986
Time 0.5 hrs	4.48	4.42	4.47	23	23	23	6.4	6.3	6.2	0.1313	0.3580	0.2234
Time 1.0 hrs	4.53	4.45	4.43	23	23	23	6.4	6.5	6.4	0.2069	0.2182	0.0966
Time 1.5 hrs	4.51	4.51	4.41	23	23	23	6.7	6.8	6.5	0.2061	0.2022	0.1112
Time 2.0 hrs	4.62	4.49	4.43	23	23	23	6.5	6.6	6.5	0.1676	0.1950	0.1036

Figure A1. Barren River Lake cyanobacteria treated with nozzle #1 at 0 (left) and 40 minutes (right) after submerged water test.

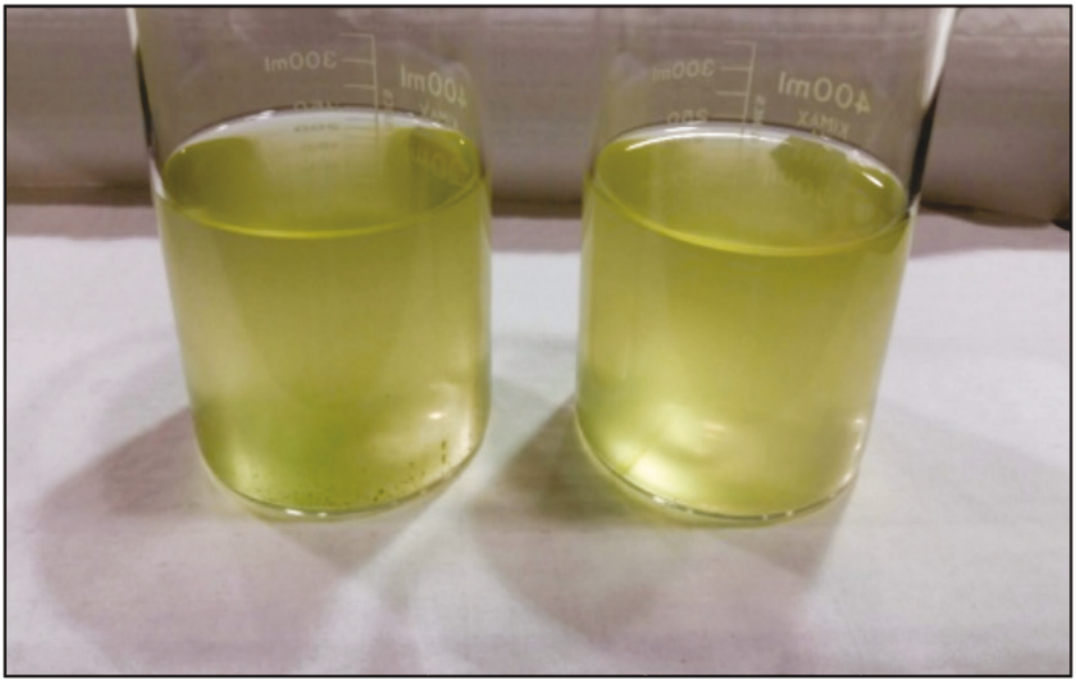


Figure A2. Barren River Lake cyanobacteria treated with nozzle #2 at 0 (left) and 40 minutes (right) after submerged water test.

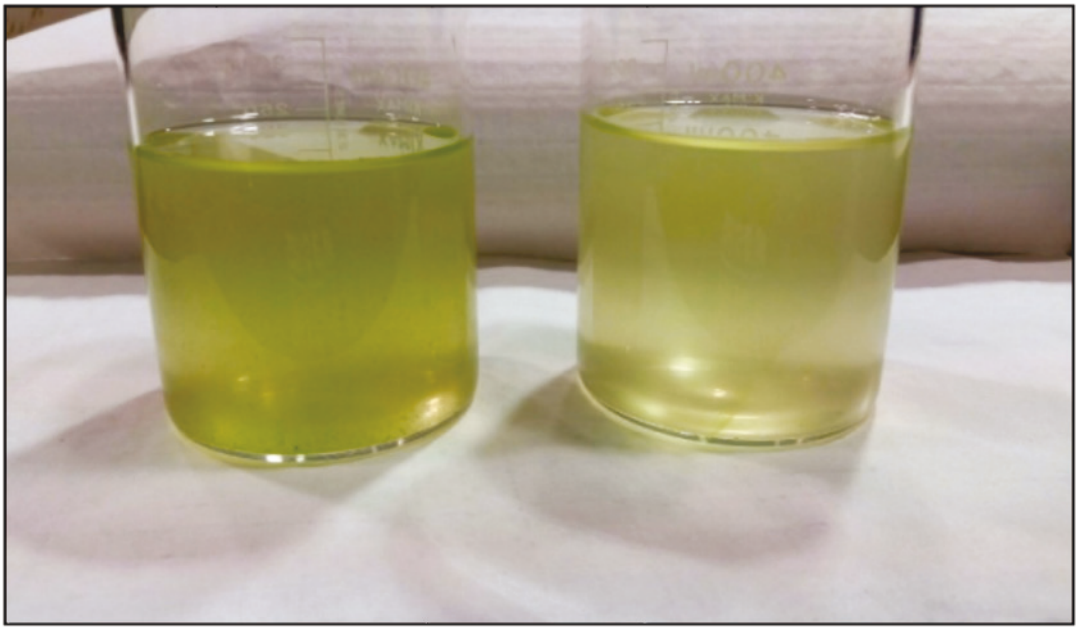


Figure A3. Barren River Lake cyanobacteria treated with nozzle #3 at 0 (left) and 40 minutes (right) after submerged water test.

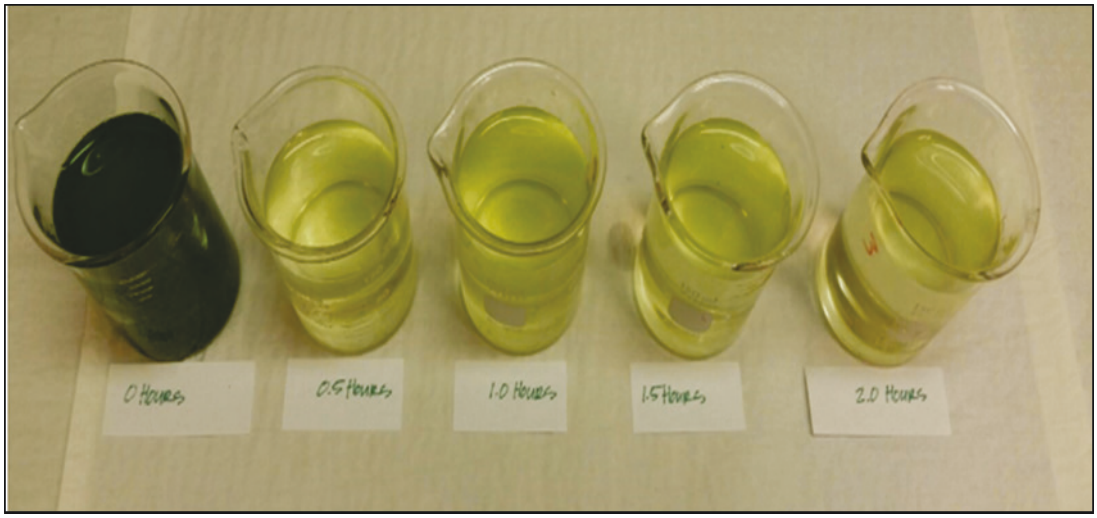


Figure A4. *M. aeruginosa* cyanobacteria treated with nozzle #2 at 0, 0.5, 1, 1.5, and 2 hours.

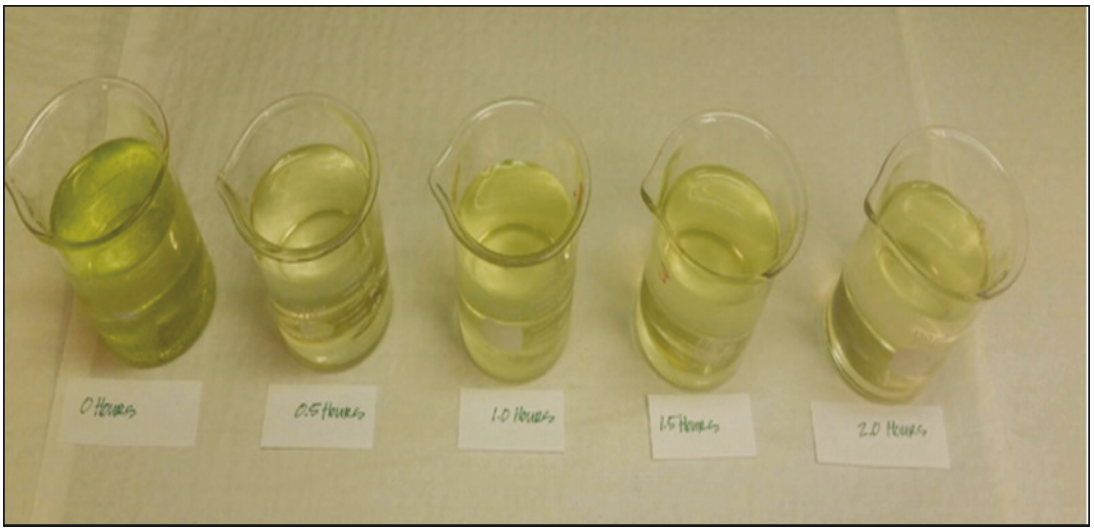
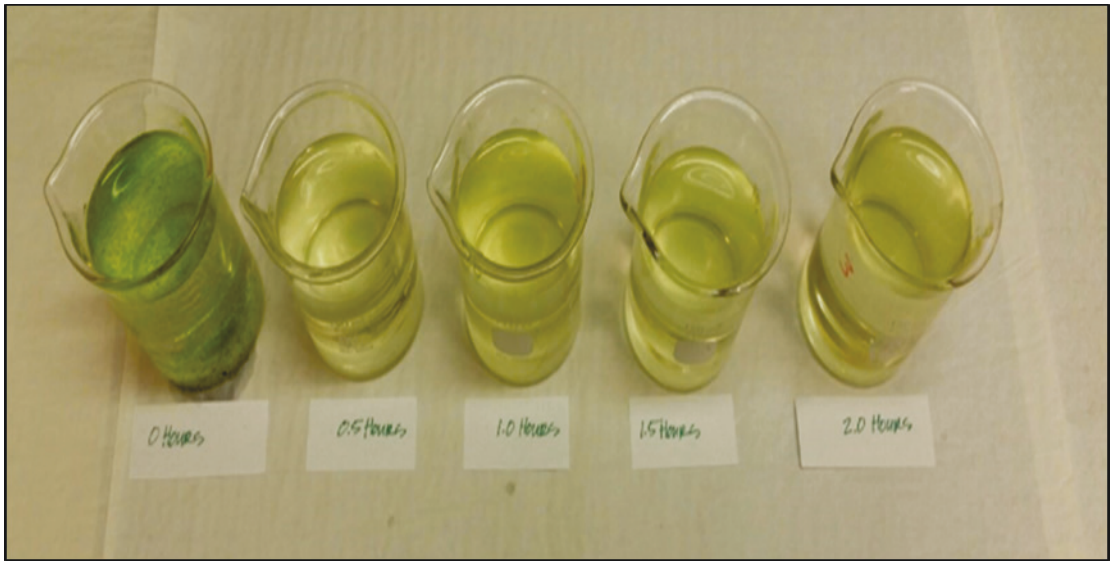


Figure A5. *M. aeruginosa* cyanobacteria treated with nozzle #3 at 0, 0.5, 1, 1.5, and 2 hours.



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14. ABSTRACT

Cyanobacterial/harmful algal blooms (HABs) are a serious and growing threat to water resources. This project evaluated cavitation fields generated from four different nozzle configurations to determine their efficacy in HAB treatment performance and correlate oxygen radical production with HAB treatment. Oxygen radicals, particularly superoxides, have demonstrated their ability to transform organic contaminants, including microcystin toxins released from some cyanobacteria species. In this study, pure cultures of *Microcystis aeruginosa* and *Anabaena spp.* were subjected to two hours of cavitation treatments with each nozzle. It was found that the nano-micro bubble treatment nozzle was the only configuration that significantly decreased turbidity and chlorophyll α concentrations, in addition to notable reductions in microcystin toxin levels. Cavitation tests performed on environmental cyanobacteria samples using other treatment nozzles rendered no significant damage to algal cells. Results from electron paramagnetic resonance (EPR) spectra supports the treatment performance of the nano-micro bubble nozzle as hydroxyl (OH) and superoxide ($O_2^{\bullet}$-) radicals generated by the nozzle were detected in cavitated waters. Overall, the results from this study imply that hydrodynamic cavitation with the appropriate nozzle configuration can be used as an effective means of controlling certain species of cyanobacterial in HAB affected areas.

15. SUBJECT TERMS		
	Environmental protection	Algal blooms
	Water quality	Cyanobacterial blooms
	Cavitation	Cyanobacteria

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