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Article

Inhibition of CO_2 Fixation as a Potential Target for the Control of Freshwater Cyanobacterial Harmful Algal Blooms

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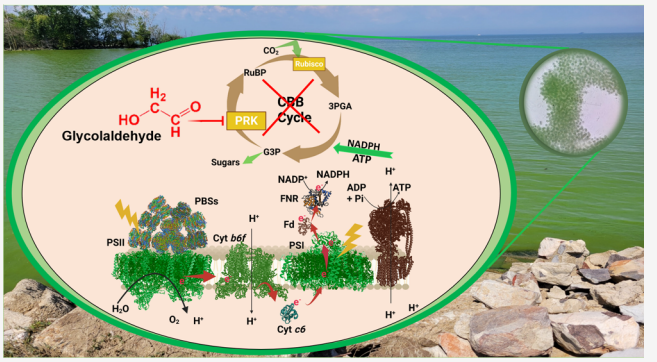
ABSTRACT: Global warming and climate change have created various problems, one of which is the increase in harmful algal blooms (HABs) in freshwater systems. Annually, numerous freshwater bodies experience cyanobacterial HABs, which pose significant ecological and public health risks. In this study, we investigated a novel strategy for controlling cyanobacteria by inhibiting CO_2 fixation using glycolaldehyde (GLA) as a nonoxidative algicide. After treatment of *Microcystis aeruginosa* (*M. aeruginosa*), a model cyanobacterium, with various doses of GLA, changes in cell number and biomass were observed, along with alterations in the photochemical efficiency of Photosystem II and electron transfer processes in *M. aeruginosa*, as measured through chlorophyll *a* fluorescence transients. Treatment with GLA concentrations of 2.5 mM and above completely suppressed the growth and photosynthesis of the *M. aeruginosa* cells. However, the use of 0.5 mM GLA led to a hormetic effect in *M. aeruginosa*. When GLA was tested on cyanobacteria-laden water samples collected from a lake, primarily consisting of colonial *M. aeruginosa* cells, the obtained results indicated that GLA is equally effective against them. These results suggest that GLA could potentially control both *M. aeruginosa* and other phytoplankton. Our findings led us to consider target-specific approaches for the control and mitigation of cyanobacterial HABs.



KEYWORDS: *algicides, cyanobacterial harmful algal blooms, hormesis, Microcystis aeruginosa, OJIP fluorescence transient, photosynthetic CO_2 fixation*

INTRODUCTION

Climate changes have led to an imbalance in the Earth's biosphere, resulting in an increase in the number of cyanobacterial blooms in freshwater and marine water bodies. Cyanobacterial blooms in freshwater bodies are particularly of great concern, as they are commonly used for potable water supply. A well-known example is Lake Erie, which has been suffering from cyanobacterial harmful algal blooms (cyanoHABs) for several decades, affecting the water supply for over 12 million people across USA and Canada.[1,2] CyanoHABs produce and release cyanotoxins, which can inflict damages on the vital organs of those who ingest them.[3] Additionally, cyanotoxins negatively impact fish populations by disrupting proteins associated with the cytoskeletal structure, stress response, and DNA repair mechanisms.[4] Therefore, cyanoHABs in freshwater bodies not only pose significant ecological and public health threats, but they have a profound negative impact on the local economy.[5,6]

Recently, various studies have been conducted around the world to better mitigate and control cyanoHABs.[7] Copper (Cu) and hydrogen peroxide (H_2O_2)-based algicides have been used to mitigate cyanoHABs. Peroxide-based algicides are known to have fewer environmental side effects due to their shorter lifespan, which sometimes coincides with their limited treatment efficacy.[8] They are also ineffective against green algae,[8] which can also cause problems in potable water production.[9,10] On the other hand, Cu-based algicides control both cyanobacteria and green algae, but their continuous use is known to cause spontaneous mutation in cyanobacteria, leading to resistance to the treatment.[11,12] Furthermore, the continuous use of Cu-based algicides results in the accumulation of Cu in the sediments, which can have long-term toxic effects on the environment.[13] Additionally, both Cu- and peroxide-based algicides damage nontarget organisms, which play a crucial role in the ecosystem.[14] Another major disadvantage of commercial algicides is that they can cause rapid cell lysis of cyanobacteria and algae, releasing intracellular

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algal organic matter and cyanotoxins, which, in turn, requires significant effort for their removal during potable water production.[15] Due to the emerging resistance to the use of commercial algicides and their adverse effects, there is a pressing need to explore and expand the repertoire of other algicides that are highly selective against cyanobacteria and green algae while exhibiting minimum toxicity toward other living organisms. In cyanobacteria, organelles are not compartmentalized, and, thus, there is no chloroplast *per se*, in contrast to green algae and higher plants. Cyanobacteria have all the photosynthetic proteins inside their cells.[16] Compared with green algae, various metabolic processes in cyanobacteria are much more affected by chemical inhibitors and stress-inducing agents. Targeting the crucial metabolic pathways with chemical compounds that have high specificity and an ecofriendly nature is expected to help control the growth of both cyanobacteria and green algae.

In this study, glycolaldehyde (GLA) was tested as an inhibitor of the Calvin−Benson−Bassham (CBB) cycle to obstruct the growth of *M. aeruginosa*, a well-known cyanoHAB contributor. GLA is a small nonsugar chemical compound that also exists in the metabolites of green algae and higher plants;[17] it has been previously used to investigate CO_2 fixation in photosynthetic organisms and has been reported to bind to the active site of phosphoribulokinase (PRK), an enzyme of the CBB cycle.[18,19] While GLA had been tested to study active CO_2 and HCO_3^- transport in the cyanobacterium *Synechococcus* UTEX 625, there have been no studies examining the algicidal effect of GLA on cyanoHABs.[19] In our study, we examined the effect of GLA on photosynthesis in both a pure laboratory culture and cyanobacteria-laden water samples obtained from Lake Erie during a cyanoHAB season. Cell density, photosynthetic pigments, and a set of parameters from chlorophyll (Chl) *a* fluorescence transients were measured to determine the inhibition of cell growth and changes in the photochemical efficiency of PSII, different energy fluxes, and electron transfer events.

MATERIALS AND METHODS

Glycolaldehyde. Analytical grade GLA (99.9% purity) was obtained from Sigma-Aldrich (Mo, USA) and used to assess its efficacy in controlling the growth of both pure *M. aeruginosa* and mixed species of cyanobacteria. GLA exists in cloudwater, where it is made from isoprene and ethane; further, it is oxidized into other small organic compounds. Concentrations of 1−5 μM of GLA have been recorded in cloudwater at various times and locations.[20] The lifetime of GLA in the atmosphere is 1.0 day for reaction with hydroxyl radical and more than 2.5 days for photolysis. The side effect of GLA is that it can inhibit the plant growth when present in a higher amount.[21] GLA is not listed on the EPA Toxic Substance Control Act Inventory (https://www.epa.gov/tsca-inventory).

Cell Growth Inhibition Tests. The *M. aeruginosa* strain (UTEX LB 2385) was purchased from the UTEX Culture Collection of Algae (University of Texas at Austin, USA), and grown in a sterile BG-11 medium inside a temperature-controlled incubator at 25 °C, under 30 μmol photons m^{-2} s^{-1} of continuous cool-fluorescent white light (light/dark cycle, 12 h/12 h). *M. aeruginosa* was harvested and diluted to an optical density (OD) of 0.033 at 680 nm, equivalent to a cell density of approximately 1.5×10^6 cells mL^{-1} in 1× BG-11 medium. Aliquots of 250 mL of the diluted cell suspensions were used for inhibition tests by adding GLA at concentrations of 0.5, 1.0, 2.5, and 5.0 mM. A solution without GLA served as a negative control. All tests were conducted in triplicate. The inhibitory action of GLA against mixed species of cyanobacteria was also evaluated using cyanobacteria-laden water samples (containing colonial *M. aeruginosa* and other bacterioplankton) collected from Lake Erie during the summer of 2022. Aliquots of 250 mL of samples with Chl *a* fluorescence level of 80 relative fluorescence units (RFU) were added to 1.5 L flasks. The optimum GLA dose and testing procedure established from our tests on pure *M. aeruginosa* cells were applied to this mixed culture. 16S rRNA gene-targeted next-generation amplicon sequencing and downstream analysis were also conducted to determine the GLA impacts on the prokaryotic and eukaryotic community composition. For further details, see the Supporting Information (Text S1).

Determination of Light Harvesting Pigments and Changes in Photochemical Efficiency of PSII. The light-harvesting pigments, Chl *a* and phycocyanin, were measured using a fluorometer (Trilogy, Turner Designs, CA, USA) from time zero before algicide application, up to 96 h after the treatment. Changes in the photochemical efficiency of PSII in the pure *M. aeruginosa* cells and in lake water samples containing mixed species of cyanobacteria were evaluated using a set of several Chl *a* fluorescence parameters obtained from the OJIP fluorescence transients, especially the ratio between the maximal variable (F_V) to maximum (F_M) fluorescence emitted by PSII, $F_V/F_M = (F_M - F_O)/F_M$, a well-known parameter that has been shown to be a proxy for the maximum quantum yield (QY) of PSII photochemistry.[22] For these measurements, a fluorometer (FluorPen FP 110, Photon System Instruments, Czech Republic) was used. The samples were dark adapted for 15 min before the measurement to relax the system. A saturating light pulse of 3,000 μmol photons $m^{-2}s^{-1}$ was used to excite the photosystems, producing light emission in the form of Chl *a* fluorescence, mostly from PSII; its value increases from the minimum fluorescence (F_O) until it reaches a peak, in less than 1 s, which is the maximum fluorescence (F_M).[23]

Measurement of Chlorophyll Fluorescence Transients. To monitor the effect of GLA on the physiological condition of the photosynthetic apparatus, specifically changes in the efficiency of PSII photochemistry and subsequent electron transfer, we measured the Chl *a* fluorescence induction, during 1 s illumination of dark-adapted cells with saturating light, known as the OJIP transient.[24−27] During this period, the fluorescence increased from its minimum value F_O to the maximum value F_M. From the measured OJIP curves, various parameters, including the apparent antenna size of active PSII, quantum efficiencies, probabilities, and energy fluxes, of various processes were calculated using the JIP-test.[25,28] These parameters characterize not only the effective absorption cross section of PSII, excitation energy trapping, and plastoquinone reduction by PSII but also the electron transport toward the final acceptors of PSI (see Figure S1).[24−27] A summary of the fluorescence parameters used here is available in Table S1.

***M. aeruginosa* Phosphoribulokinase Homology Modeling and Molecular Docking.** The structure of phosphoribulokinase (PRK) of *M. aeruginosa* was constructed using AlphaFold Protein Structure Database to gain an understanding of how GLA interacts with the active site of PRK.[29,30] The nucleotide sequence of PRK of *M. aeruginosa* was downloaded from Uniprot, and its structure was constructed

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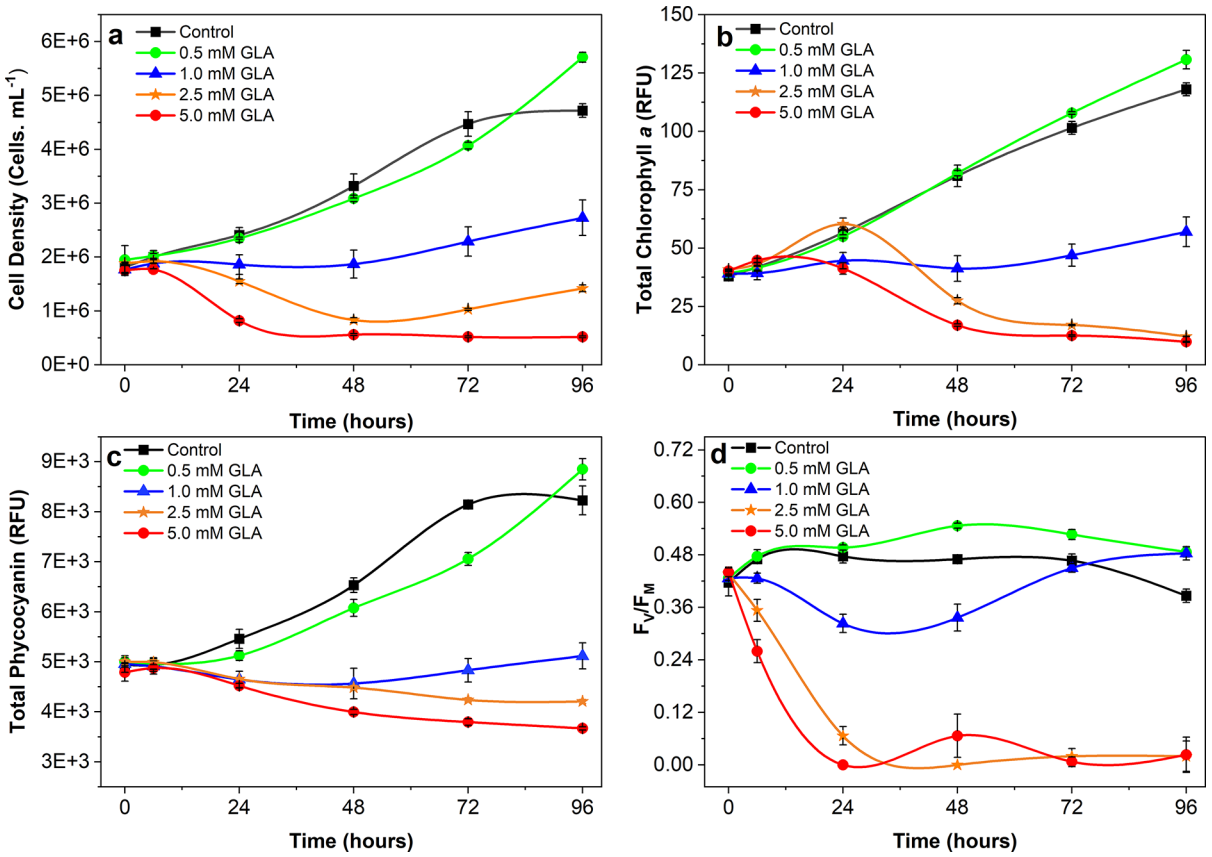

Figure 1. Effect of the various concentrations of GLA on *M. aeruginosa*: (a) cell density, (b) total Chl *a* content, (c) total phycocyanin, and (d) F_V/F_M.

using the template of *Synechococcus* sp. strain PCC 6301 PRK (PDB ID 6HZK) on AlphaFold Protein Structure Database.[31] The GLA molecule was docked with the active site of the constructed PRK of *M. aeruginosa* to assess the molecular interactions.

RESULTS AND DISCUSSION

Inhibitory Effect of Glycolaldehyde on *M. aeruginosa*.

Cell growth. The growth of *M. aeruginosa* cells treated with different concentrations of GLA was measured for 96 h (Figure 1a and Figure S2). All the samples show similar growth trends for the initial 6 h, but significant differences were observed thereafter. *M. aeruginosa* cells treated with 0.5 mM GLA initially had slow growth, but after 72 h, unobstructed growth was observed, even slightly higher than that of the control sample. *M. aeruginosa* cells treated with 1.0 mM GLA showed very slow growth compared to the control and displayed a yellowish color after 96 h of treatment (Figure S2). However, cells treated with 2.5 and 5.0 mM GLA showed no growth and did not survive during the test period. The growth of *M. aeruginosa* cells was also observed when hydrogen peroxide[32] or copper-based[33] algicide was used in low doses. Cyanobacteria treated with a low dose of algicide initially exhibited slow growth, but over time, these cells grew even higher than the untreated cells.[33] Besides *Microcystis* sp., other cyanobacteria, such as *Anabaena*, *Planktothrix*, and *Cylindrospermopsis*, when treated with low dose of hydrogen peroxide, also showed higher growth compared to the control samples.[8] This heightened growth of cyanobacteria in response to low-dose exposure to an inhibitor (0.5 mM GLA in this study) is referred to as hormesis, a phenomenon where organisms exhibit a positive response to low doses of a stressor.[34] The results presented here indicate that GLA works in a dose-dependent manner, displaying both algistatic (at 1.0 mM GLA) and algicidal properties (at 2.5 and 5.0 mM GLA).

Light Harvesting Pigment Content. The inhibitory effect of GLA on light harvesting pigments, such as phycocyanin and Chl *a*, was examined. The amount of Chl *a* and phycocyanin in the control sample increased continuously after 6 h of incubation during the exponential growth stage, indicating a healthy growth of the *M. aeruginosa* cells (Figure 1b,c). In the presence of 0.5 mM GLA, *M. aeruginosa* cells initially showed a growth pattern slower than that of the control sample. However, after a 24 h period, the Chl *a* amount in the treated cells surpassed that of the control. The phycocyanin level also increased when its exponential growth time was close to 96 h (Figure 1c). In contrast, *M. aeruginosa* cells exposed to 1.0 mM GLA did not show any increase in Chl *a* and phycocyanin until 72 h; however, after that time, a slight increase was observed, consistent with the trend in cell growth. As compared to the initial levels, *M. aeruginosa* cells treated with 2.5 and 5.0 mM GLA showed, after 24 h, a significant decrease in both Chl *a* and phycocyanin levels. The use of a $CuSO_4$-based algicide in the early exponential growth phase of *M. aeruginosa* was also shown to result in a dose-dependent decrease of Chl *a* and phycocyanin content.[35] For example, Cu-based commercial algicides, such as Algimycin and Cutrine (0.7 and 1.0 mg Cu/L), have been shown to lower the level of Chl *a* by 70 and 80%, and that of phycocyanin by 83 to 96%, after 3 days of treatment,[36] which is similar to the effective concentration of

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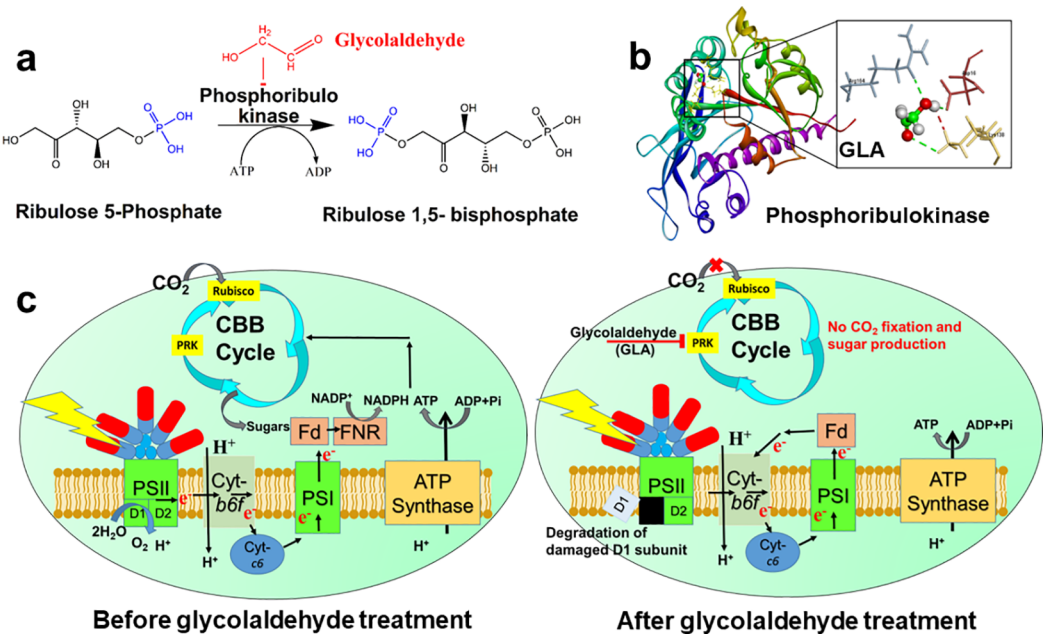

Figure 2. Schematic diagram of photosynthesis in *M. aeruginosa* cells and its inhibition by GLA targeting the CBB cycle. (a) PRK-based phosphorylation of ribulose 5-phosphate to ribulose 1,5-bisphophate using ATP, and its inhibition by GLA in *M. aeruginosa*, (b) molecular interaction between GLA and PRK, and (c) photosynthesis before and after the inhibition of the PRK by GLA: the normal degradation of the D1 subunit of PSII (left) and the blockage of *de novo* biosynthesis of D1 after CO_2 fixation inhibition (right).

GLA (i.e., 2.5 mM) used in this study. Recently, several other photosynthesis inhibitors, such as acetylacetone and 2-hydroxychalcone have been shown to decrease the growth of *M. aeruginosa* cells, besides the commercial algicides.[26,37,38] These inhibitors have been shown to block the electron transfer steps of photosynthesis, resulting in the depletion of adenosine triphosphate (ATP) and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) needed for the CBB cycle. For example, the application of acetylacetone to *M. aeruginosa* cells has been shown to inhibit the overall photosynthesis process, ultimately leading to complete degradation of the phycocyanin content after 96 h of treatment.[37] We note that a similar level of decrease in the level of phycocyanin was observed in *M. aeruginosa* cells treated with 5 mM GLA (Figure 1c). However, acetylacetone has been suggested to target the iron−sulfur cluster in Photosystem I, which also exists in the mitochondria of all living organisms.[37] Therefore, treatment with acetylacetone may potentially impact other organisms in freshwater systems.

Inhibitory Effects of Glycolaldehyde on Photosynthesis. *Photochemical efficiency of PSII.* To assess the functionality of the photosynthesis process in*M. aeruginosa*cells under GLA treatment, the photochemical efficiency of PSII (as inferred from F_V/F_M) was measured for each GLA concentration (Figure 1d). Without GLA treatment, the F_V/F_M increased from 0.42 to 0.48 with time, until 72 h during the log phase of growth, but then, it declined slowly to a value of 0.39 after 96 h treatment. For *M. aeruginosa* cells treated with 0.5 mM GLA, the F_V/F_M was around 0.4 in the first 24 h and then increased to 0.55 at 48 h after treatment, surpassing that of the control sample. Afterward, the F_V/F_M slowly declined until 0.48 from 48 to 96 h after treatment. This shows that the recovery of the photosynthesis process in *M. aeruginosa* allows their repair and growth. In the samples treated with 1.0 mM GLA, the F_V/F_M continuously declined until 48 h, followed by a rebound between 72 and 96 h, which is in agreement with the results of total Chl *a* and phycocyanin measurements (Figure 1b,c). With 2.5 and 5 mM GLA, the F_V/F_M in the treated samples declined with time and became zero after 24 h of treatment, showing that there was a blockage of the entire photosynthesis process.

The changes described above in the photochemical efficiency of PSII demonstrate the efficacy of GLA in inhibiting the photosynthesis of *M. aeruginosa* cells. Chen et al. (2021) have shown that using a high H_2O_2 dose (i.e., 20 mg/L—two times higher than the maximum allowed application dose by US EPA) results in lowering the F_V/F_M value to zero after 24 h of application.[39] However, after 4 days, *M. aeruginosa* cells fully regrew, attaining an F_V/F_M value similar to the control sample.[39] In contrast to the results of Chen et al. (2021),[39] our study showed that GLA completely stopped the growth of *M. aeruginosa* cells at concentrations of 2.5 mM and above. This suggests that the inhibition of CO_2 fixation not only affects the growth of *M. aeruginosa* cells but also disrupts the normal repair processes in the cells, particularly that of PSII through the production of new D1 subunits after treatment.[18] Treatment with H_2O_2 (1−4 mg/L) has been shown to cause a dramatic decrease in the photosynthesis activity of *M. aeruginosa* cells (>80% in 4 h vs 24 h for GLA at 2.5 mM).[40] The reason for this decrease is that H_2O_2 directly damages the cell membrane and the photosynthetic proteins, while GLA first enters the cells, binds to the target enzyme, and primarily blocks the photosynthetic carbon assimilation process (see the following section).

Inhibition of Phosphoribulokinase. Figure 2 shows a schematic diagram of the photosynthesis process with and without GLA inhibition. During photosynthesis, the oxygen-evolving complex of PSII utilizes water molecules to generate electrons, protons, and molecular oxygen (Text S2 for a detailed explanation for photosynthetic light reactions). When GLA is applied, it binds to the active site of the PRK of the CBB cycle (Figure 2a) and inhibits the normal ATP-dependent

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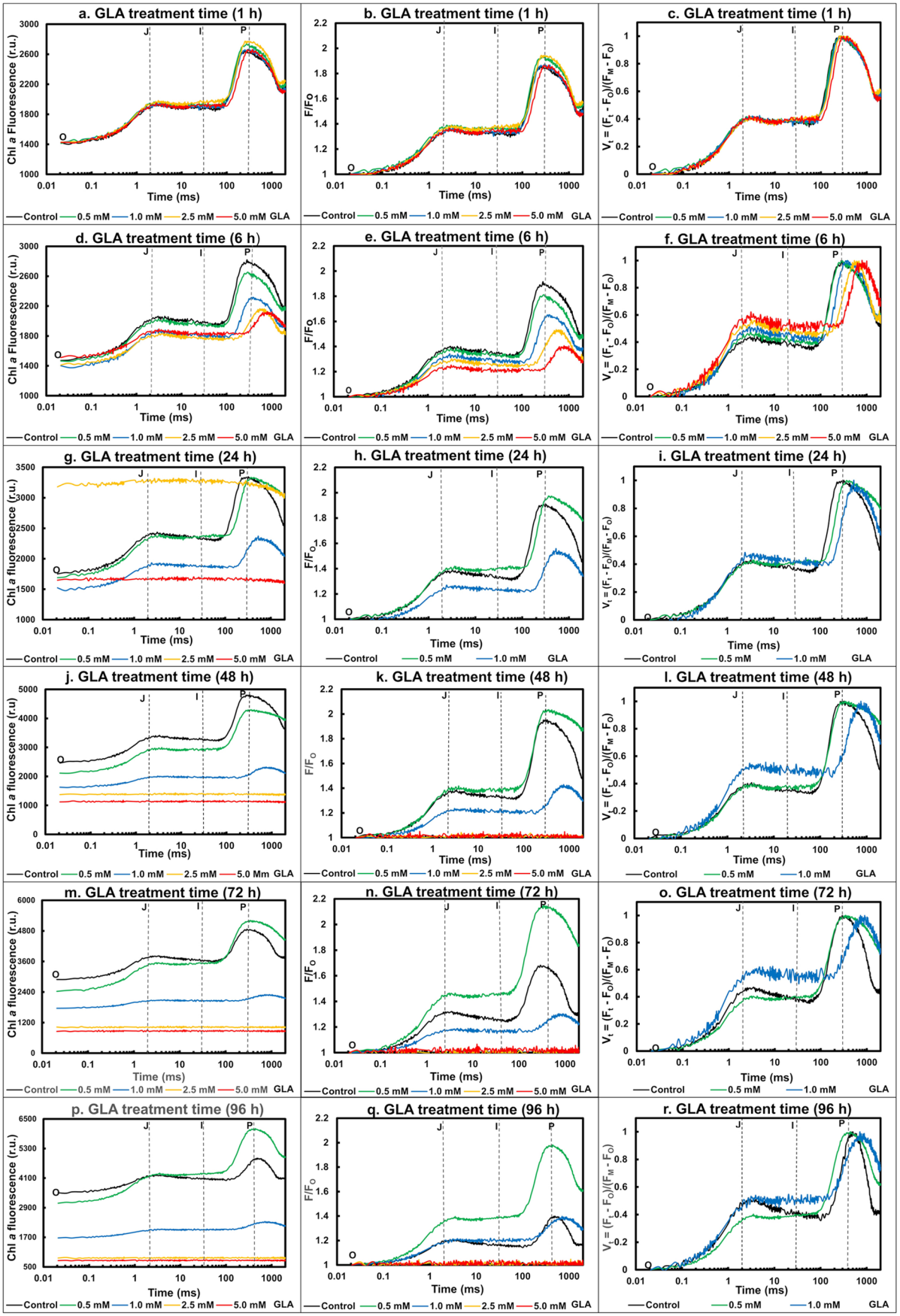

Figure 3. OJIP fluorescence transients measured at 1 to 96 h after treatment with 0.5, 1, 2.5, and 5 mM GLA. The first column shows the measured OJIP curves, the middle column the OJIP curves normalized to F_O (i.e., F/F_O), and the third column for the OJIP curves normalized to F_O and F_M (i.e., V_t), where $V_t = (F_t - F_O)/(F_M - F_O)$.

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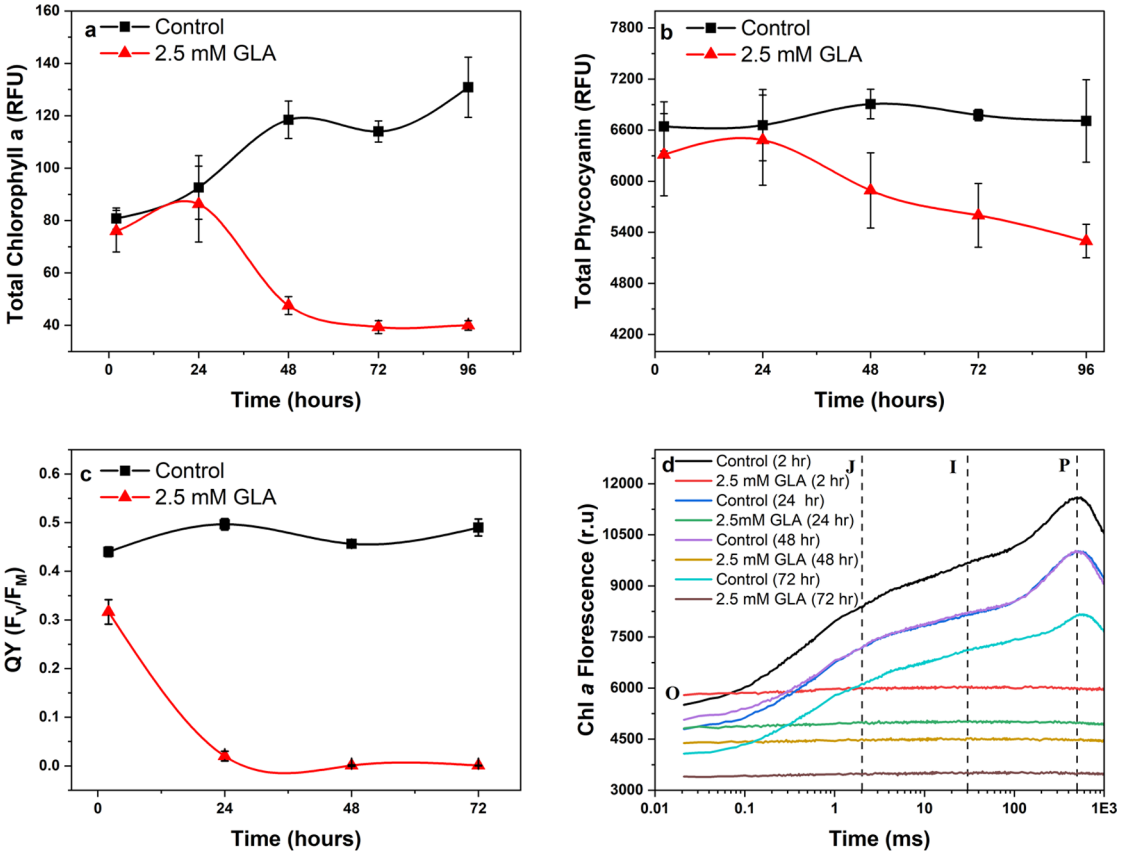

Figure 4. Response of the mixed phytoplankton culture from Lake Erie treated with 2.5 mM GLA: (a) total chlorophyll *a*, (b) total phycocyanin, (c) F_V/F_M, and (d) Chl *a* fluorescence transients (the OJIP curves) of mixed *M. aeruginosa* samples at different time intervals after GLA application. Each transient is an average of three replicates.

conversion of ribulose 5-phosphate (RuP) into ribulose 1,5-bisphosphate (RuBP),[41] which is the substrate of 1,5-bisphosphate carboxylase/oxygenase (RuBisCo).[42]Figure 2b illustrates the molecular docking of GLA with the active site of PRK. The molecular docking results show that GLA interacts with Asp16, Lys138, and Arg164 of the active site of PRK. Due to the unavailability of the RuBP for the ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCo), CO_2 cannot be fixed, which ultimately leads to the inhibition of *de novo* biosynthesis of the D1 subunit of PSII and a decrease in the entire light-dependent reactions of photosynthesis, including the linear and cyclic electron transfer processes (Figure 2c).[18,19]

Chlorophyll a Fluorescence Transients. The Chl *a* fluorescence induction curves were measured after one h of GLA application. The fast (less than 1 s) Chl *a* fluorescence transient (the OJIP curve) provides valuable information not only about the PSII but also about the electron transfer across both photosystems, offering insights into how and where the GLA affects these processes (Figure S3 and Text S3).[26]Figure 3 shows the fluorescence transient curves measured on *M. aeruginosa* cells at different times after GLA treatment. The plots in the middle column are curves normalized with respect to the initial fluorescence (F_O, i.e., F/F_O), while the plots in the third column are the relative variable fluorescence curves (V_t), normalized to both F_O and F_M (i.e., $V_t = (F_t - F_O)/(F_M - F_O)$). One hour after the treatment, both the control and all the GLA-treated samples exhibited relatively similar OJIP transients, indicating that the energy trapping and electron transfer process were normal, and *M. aeruginosa* cells were in a normal functional state (Figure 3a–c). The transients recorded after 6 h of GLA treatment showed clear changes, due to the inhibition of the photosynthesis process, varying in magnitude depending upon the GLA concentration (Figure 3d–f). Although all the GLA-treated samples had normal-looking OJIP transients, their amplitudes relative to the control decreased gradually with increasing GLA concentration. On the other hand, the V_J in the relative variable fluorescence curves (Figure 3f) increased with GLA concentration, indicating a slowdown in the electron transport from Q_A^- to Q_B.[25] Additionally, as shown in Figure 3c, the relative variable fluorescence reached the peak ($V_P = 1$) almost at the same time in the control and the *M. aeruginosa* cells treated after 1 h with GLA (i.e., t_{Fmax} = ~300 ms). However, after 6 h treatment, the t_{Fmax} gradually increased at higher GLA concentrations (see Figure 3f). This suggests a decrease in the number of active PSII reaction centers in these samples.[25] When compared with the OJIP curve of the *M. aeruginosa* control, which reaches a maximum fluorescence of approximately 5,000 in arbitrary units after 96 h, the *M. aeruginosa* cells treated with 0.5 mM GLA show an increase in Chl *a* fluorescence that reaches a maximum higher than 6,000 at 96 h (Figure 3p); this confirms the hormetic effect of 0.5 mM GLA on the *M. aeruginosa* cells that we observed from the *M. aeruginosa* growth curves and photosynthetic pigment concentration measurements (Figure 1a–c). However, between 24 and 96 h of treatment, the samples treated with 2.5 and 5.0 mM GLA showed no variable fluorescence, and the

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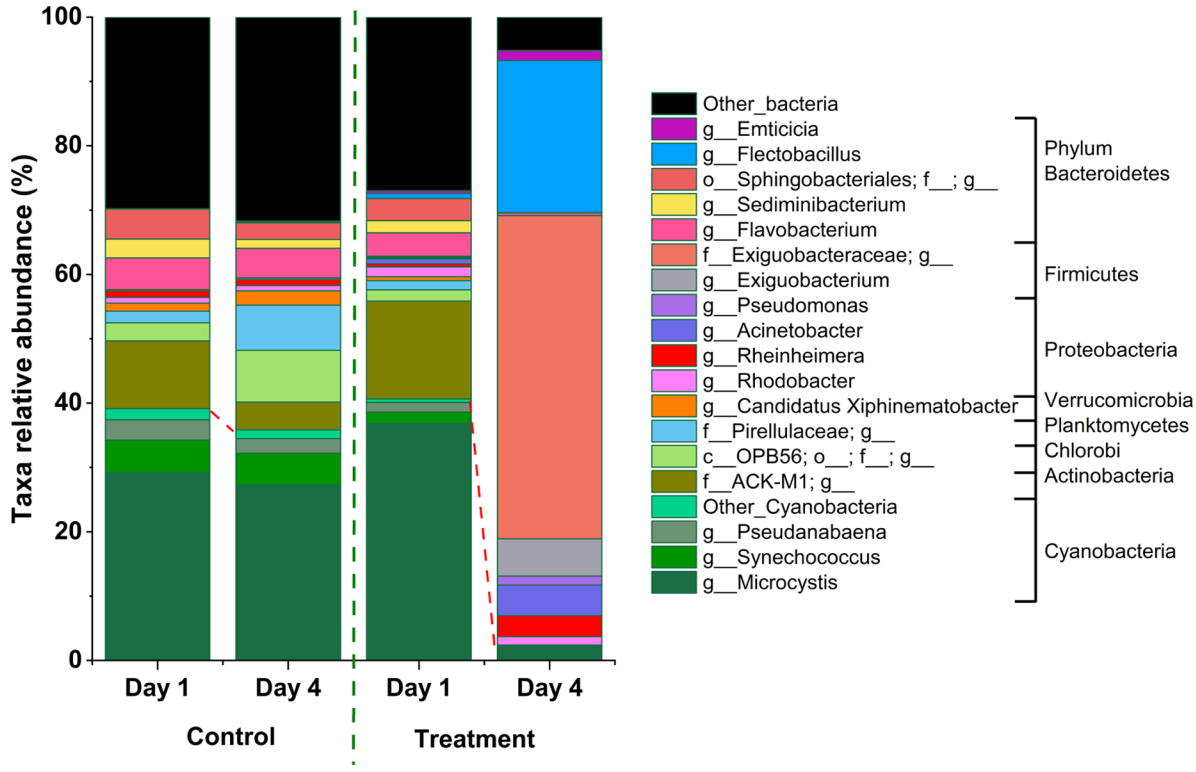

Figure 5. Changes in bacterioplankton communities in the control and 2.5 mM GLA-treated groups: relative abundance of cyanobacteria and other heterotrophic bacteria at the genus and phylum level. If they could not be classified at the genus level, taxonomic levels above the genus level, such as order and family, were labeled as the nearest lower taxonomic level. The acronyms "o__", "f__", and "g__" represent order, family, and genus, respectively.

fluorescence intensity remained almost constant (Figure 3g,j,m,p), which indicates no electron transport in these samples.

We speculate that it is likely due to the destruction of the D1 subunit in PSII (i.e., no biosynthesis) induced by the GLA treatment (Figure 2). On the other hand, the decrease of the Chl *a* fluorescence in these two samples after 24 h is probably due to the "destruction" of *M. aeruginosa* cells.[18] Similar observations have been reported for other types of inhibitors used in similar studies. For example, Chl *a* fluorescence transients of*M. aeruginosa* cells treated with paraquat (methyl viologen) also became flat (complete inhibition of photosynthesis).[43] When *M. aeruginosa* cells were treated with 10 mg/L 2-hydroxychalcone, the cells and the photosynthesis process were also seriously damaged after 48 h of treatment.[38] We also used the OJIP transients of *M. aeruginosa*cells treated with GLA to calculate several other Chl *a* fluorescence parameters than F_V/F_M (see Figure S4, Tables S1 and S2, and Text S4 for related discussion).[25,28] Between these parameters, we found that the so-called performance index (PIabs), which is mostly used in the assessment of photosynthetic tolerance to various abiotic stress factors,[44] gave very similar results with those obtained with the parameter F_V/F_M presented earlier (see Figure 1d).

GLA Effect on Mixed Species of Cyanobacteria. The inhibitory action of GLA was also tested on cyanobacteria-laden water samples (primarily *M. aeruginosa*) collected from Lake Erie during the summer of 2022.

GLA Effect on Biomass and the PSII Photochemical Efficiency of Phytoplankton. Based on our results obtained on pure *M. aeruginosa* cells, 2.5 mM GLA was selected for tests with mixed *M. aeruginosa* cultures. The total Chl *a* level in the control and GLA-treated sample was similar until 24 h. However, a 40% reduction in Chl *a* content was observed in the GLA-treated sample (48 h) and a 50% drop was observed after 72 and 96 h (Figure 4a). Initially, the total amounts of phycocyanin in the control and 2.5 mM GLA-treated samples was similar. However, after 24 h, a continuous decline in phycocyanin levels was observed for the remainder of the experimental period (Figure 4b). Compared to our findings with the pure *M. aeruginosa* culture, where both the Chl *a* and phycocyanin levels decrease within 24 h after GLA treatment (Figure 1b,c), the mixed *M. aeruginosa* cultures showed no significant decrease in the first 24 h. However, this decrease became larger after 48 to 72 h. We note that the mixed culture of *M. aeruginosa* cells is quite different, both morphologically and physiologically, compared to the unicellular *M. aeruginosa* culture (Figures S5 and S6). It is known that the effectiveness of various commercial algicides and chemical inhibitors against *M. aeruginosa*-dominant cyanoHABs depends upon the size of colonial aggregates, nutrient level, and bloom period in the season.[45,46]

The photochemical efficiency of PSII (represented by the values of F_V/F_M) measured for the mixed culture treated with 2.5 mM GLA showed a noticeable decrease after 2 h of GLA application (Figure 4c). This decrease was time dependent, and on the second day, the F_V/F_M decreased to zero, indicating a complete shutoff of photosynthesis. This result also implies that the GLA was efficiently transported in the colonial aggregates of cyanobacterial cells and thus inhibited the photosynthesis process, just as it had for the pure culture (Figure 2). In both the laboratory and the mixed cultures, the

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ACS EST Water XXXX, XXX, XXX−XXX

F_V/F_M dropped to less than 0.1 when treated with 2.5 mM GLA, in contrast to the value of ~0.45 observed in the controls (see Figures 4c and 1d). This demonstrates that GLA has the potential to be a potent inhibitor of CO_2 fixation in cyanobacteria and algae, providing an alternative to current available commercial algicides for water treatment, which have various disadvantages.

OJIP Transients. Chlorophyll *a* fluorescence transients, the OJIP curves, for the 2.5 mM GLA-treated mixed culture and the control sample, are shown in Figure 4d. In the control sample, the O−J, J−I, and I−P phases are clearly distinguished from each other, displaying a much higher J−I phase than in the pure *M. aeruginosa* culture, as shown earlier (Figure 3), which is probably due to the presence of some green algae in the sample.[47] For the control, both F_O and F_M values were similar at 24 and 48 h after the first measurement but lower than in the initial fluorescence transients. However, they become even lower at 72 h after GLA treatment. This was also reflected in the performance index (PI_{abs}) values, which decreased from its maximum of 0.49 at 48 h to 0.36 at 72 h post-treatment (see Table S3). Compared to the control sample, the OJIP curves of GLA-treated mixed culture were flat ($F_V = F_M - F_O \approx 0$) at 2, 24, 48, and 72 h post-treatment. This is in agreement with the inhibition of the CBB cycle for CO_2 fixation and disruption of the photosynthetic system, demonstrating the effectiveness of GLA against the mixed cyanobacteria and other phytoplankton present in lake water.[18] Changes in the Chl *a* fluorescence curves (Figure 4d) have revealed that the effect of 2.5 mM GLA was also pronounced in the mixed *M. aeruginosa* culture, as in pure cultures (Figure 3), showing no variable fluorescence after 2 h. The raw Chl *a* fluorescence of GLA-treated mixed culture (Figure 4d) and that of the pure *M. aeruginosa* cells treated with 2.5 and 5 mM GLA at 24 to 96 h (Figure 3g,j,m,p) show similar flat lines, implying the disintegration of the photosynthetic apparatus of mixed cyanobacteria and other phytoplankton after CO_2 fixation is inhibited. High doses (13−39 mg/L) of H_2O_2 application on *M. aeruginosa* cells mostly damage the photosynthetic apparatus and can result in inhibition of linear electron transfer, as inferred from fast O−J phase formation with accumulation of $Q_A^-Q_B$ or $Q_A^-Q_B^-$ or the complete loss of the I−P phase (due to damage of PSI).[48,49] Our observations on the OJIP curves with GLA treatment differ significantly from earlier studies on *M. aeruginosa* inhibition with hydrogen peroxide, which is not target specific and can damage various parts of cyanobacteria and other microorganisms.

Impact of GLA on Mixed Species of Cyanobacteria. 16S rRNA gene sequencing was performed to investigate the influence of GLA treatment on the bacterioplankton communities. Until day 1, the overall bacterial diversity was comparable between the control and GLA treatment groups. Furthermore, *Microcystis* and other cyanobacterial species (including *Synechococcus* and *Pseudanabaena*) maintained their dominance up to 39% of their relative abundances (Figure 5). However, on day 4 (96 h) after the GLA treatment, the relative abundance of *Microcystis* and other cyanobacteria decreased to around 2%, suggesting the algicidal effect of GLA on cyanoHABs. Along with these cyanobacteria, the genus *Flavobacterium* and unclassified *Pirellulaceae* decreased after GLA treatment (Figure 5). The relative abundances of the genus *Sediminibacterium* and unclassified *ACK-M1* also decreased after the GLA treatment, but they also decreased in the control group, indicating that the experimental conditions may not be favorable for their growth. Meanwhile, in the GLA-treated group, the relative abundance of non-photosynthetic bacteria in the phylum Bacteroidetes (e.g., the genera *Flectobacillus* and *Emticicia*) and in the phylum Firmicutes (e.g., the family *Exiguobacteraceae*) increased to 25 and 56%, respectively, by the end of the experiment period (Figure 5).

Previous studies have reported that during algal blooms, Bacteroidetes species demonstrated their ability to degrade polysaccharides and thrive in environments abundant with dissolved organic matter.[50−52] *Flectobacillus* and *Emticicia* in the phylum Bacteroidetes could benefit from the release of AOM following algal death. Since they are also microcystin degraders, the released intracellular toxins after the algicide treatment could provide an additional advantage for their growth.[44] *Exiguobacterium* possesses algicidal activity against a wide range of algal groups;[53] thus, it is expected to help suppress cyanoHABs along with the GLA treatment (Figure 5). *Pseudomonas* and *Rhodobacter* are metabolically versatile to degrade organic matters[54,55] and also known to produce cobalamin (vitamin B12)[56−58] that is an essential growth factor for various aquatic organisms.[59,60] *Acinetobacter* is known as human pathogenic bacteria;[61] thus, further studies are warranted to determine whether *Acinetobacter* and other bacteria observed after GLA treatment have beneficial or adverse impacts on the aquatic environment. Furthermore, quantifying the absolute quantities of these bacteria, through techniques such as quantitative real-time PCR, could enhance the assessment of GLA treatment effects on changes in bacterioplankton in ponds, reservoirs, and lakes, paving the way for future applications. Metagenomic and metatranscriptomic analyses would also be required in the future to verify the functional roles of microbes that changed after GLA treatment.

CONCLUSIONS

The complete inhibition of cyanoHABs requires a multifaceted effort to safeguard the freshwater system from various toxin-releasing cyanobacteria. Public water systems face a continuous challenge of uncontrolled growth of cyanobacteria, as they are becoming increasingly resistant to commercial algicides.[11,12,15] In this study, we demonstrate that GLA effectively blocks the photosynthesis process in *M. aeruginosa* cells, in both pure and mixed cultures, within hours, resulting in a subsequent decrease in their biomass. The OJIP fluorescence transient data provide a detailed photosynthetic description and metabolic fitness of *M. aeruginosa* cells upon exposure to various doses of GLA. GLA, a broad-spectrum inhibitor, may control both cyanobacteria and other phytoplankton, potentially offering more efficient water treatment. The biotransformation of GLA into other biochemical compound that can easily be utilized as a carbon source by nonphotosynthetic and microcystin-degrading bacteria in water reservoirs may help in its residual removal on time. Thus, the unique potential of GLA as an inhibitor of cyanobacteria and as a possible carbon source for beneficial nonphotosynthetic bacteria, can be fully explored in future studies. Selective inhibitors like GLA for the control of cyanobacteria and algae offer opportunities to develop novel inhibitors for the photosynthetic enzymes with suitable selectivity and specific toxicity toward phytoplankton. Large-scale mesocosm based studies in open environment for the control of cyanoHABs and the release of cyanotoxins will

https://doi.org/10.1021/acsestwater.4c00191
ACS EST Water XXXX, XXX, XXX−XXX

further reveal the importance of GLA to be considered as a commercial algicide for water treatment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.4c00191.

Additional materials and methods, information about figures and tables and photographs of experimental setup; (Text S1) genomic analysis of the mixed culture of cyanoHABs; (Text S2) photosynthetic light reactions; (Text S3) OJIP curves; (Text S4) JIP test parameters; Tables S1−S3 and Figures S1−S6 (PDF)

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CRediT: **Syed Lal Badshah** conceptualization, data curation, formal analysis, investigation, methodology, visualization, writing-original draft, writing-review & editing; **Alexandrina Stirbet** data curation, formal analysis, investigation, methodology, writing-review & editing; **Mashuk Siddiquee** data curation, investigation, methodology, writing-review & editing; * **Govindjee** data curation, formal analysis, investigation, writing-review & editing; **Dae-Wook Kang** data curation, formal analysis, funding acquisition, investigation, methodology, writing-review & editing; **Thomas Bridgeman** funding acquisition, resources; **Youngwoo Seo** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, writing-original draft, writing-review & editing.

Notes

The authors declare no competing financial interest.

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