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Removal of *Dolichospermum circinale*, *Microcystis aeruginosa,* and their metabolites using hydrogen peroxide and visible light

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ABSTRACT

Frequent cyanobacterial blooms in reservoirs used for human supply increase the risk of noxious secondary metabolites, endangering human health and ecological balance, and requiring constant monitoring by water companies. Although hydrogen peroxide (H_2O_2) has been widely reported as an effective agent for the control of cyanobacteria, being *Microcystis aeruginosa* one of the most studied species, very limited data is available on its effects over *Dolichospermum circinale*. Therefore, this study aimed to evaluate the impact of H_2O_2 on *D. circinale* and comparing it to the effects over the *M. aeruginosa*. The treatment was performed in cyanobacterial cultures with the application of 2 and 5 mg L^{-1} of H_2O_2 under visible light. To measure the impact of the treatment, intact cells were counted and cell re-growth monitored. Geosmin and microcystin, cell pigments, color, and organic matter in water were also analyzed during the treatment. The results showed that even the smallest H_2O_2 concentration (2 mg L^{-1}) was able to completely remove *D. circinale* cells. Although *M. aeruginosa* could only be completely removed using 5 mg L^{-1}, the few cells remaining after the application of 2 mg L^{-1} were not viable and did not re-grew after 15 days. Total microcystin concentration increased after *M. aeruginosa* was exposed to H_2O_2, suggesting that oxidative stress may increase the detection of this metabolite when the cells are lysed. While 2 mg L^{-1} was able to significantly decrease total geosmin, the addition of 5 mg L^{-1} did not improve removal. Chlorophyll-*a* was readily degraded after cell rupture but the same did not happen to phycocyanin, demonstrating its high resilience to this oxidant. Color and organic matter increased for the *M. aeruginosa* but decreased for the *D. circinale* suspension, probably because the higher concentration of the *M. aeruginosa* yielded more extracellular content to the water which was not able to be degraded by the amount of H_2O_2 applied.

1. Introduction

Cyanobacteria are a highly adaptive and diverse group of microorganisms that can exist in a wide range of habitats. Their blooms are increasingly becoming more intense and frequent in continental freshwater worldwide (Ho et al., 2010; Li et al., 2014) imposing a serious threat to water companies. These organisms are considered a problem primarily because they can produce toxic metabolites, which can negatively impact human health, aquatic biota, and local wildlife, or substances which are not harmful to health but can give a musty or earthy taste and odor (T&O) to water (Chang et al., 2018).

Microcystis is a genus of freshwater cyanobacteria which includes *Microcystis aeruginosa,* one of the most frequently studied organism and a source of great concern when present in reservoirs used for human or animal supply (Bittencourt-Oliveira et al., 2014; Fan et al., 2020) because they can produce toxic metabolites known as microcystins (MCs) (Wang et al., 2018; Zhou et al., 2018). The World Health Organization (WHO) has established a guideline value of 1.0 μ L^{-1} microcystin-LR equivalent (WHO, 2011) to ensure the safe use of drinking water. Exposure to microcystins is known to cause cellular damage and genotoxicity within the livers, kidney, heart, reproductive systems, and lungs of mammalians (Roegner et al., 2014). MC-LR was chosen as a reference because it is one of the most toxic and prevalent cyanotoxins found in freshwater (Meriluoto et al., 2017; Miao et al., 2010; Zong et al., 2013).

In addition to MCs, 2-methylisoborneol (MIB) and geosmin are a major problem in water distribution systems since they can cause unpleasant T&O in drinking water (Xie et al., 2015). Although these T&O

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compounds are not toxic, their unpleasant organoleptic characteristics contribute to a negative water quality perception and impel consumers to alternatives and riskier water sources (Doederer et al., 2019; Li et al., 2016). MIB and Geosmin can be produced by actinomycetes, proteobacteria, myxobacteria, and some fungi. However, cyanobacteria, especially the genera Dolichospermum and more specifically *Dolichospermum circinale*, are the major producers of those substances in freshwater (John et al., 2018; Kim et al., 2016; Li et al., 2016).

The removal of intact cyanobacterial cells from water is challenging because, in the process, cells can be damaged and the intracellular metabolites released. Cell lysis contributes to increasing the dissolved toxin and T&O compounds fraction, making it more difficult to remove them in a conventional water treatment plant (WTP) (Barrington et al., 2013).

Mathematical models can be important tools to estimate the concentration of these noxious cyanobacterial compounds when using easily measurable parameters, such as pigments, to alert for the presence of toxins in water and the application of complementary treatments (Aragão et al., 2020). Aragão et al. (2020) showed, for example, that the differences between calculated and measured MC concentrations were less than 5%, demonstrating that the models can be applied for cultured M. aeruginosa and, in the future, for environmental samples depending on further investigations.

Although dissolved MCs can be removed by environmental mechanisms, such as biodegradation, photolysis, and adsorption, it can take days or weeks (Gan et al., 2012). T&O compounds are biodegradable but extremely resilient to chemical oxidation, making it also hard to remove them in conventional WTP with short detention times. Furthermore, cell disruption may expose not only toxins and T&O compounds but other natural organic matter contributing to the formation of harmful disinfection by-products (Kim et al., 2016; Wert et al., 2014). Therefore, the water treatment plants should remove cyanobacteria cells without breaking them, so the intracellular content, such as toxins, T&O compounds, and other organic matter are not released into the water (Fan et al., 2013).

Despite the risks of damaging cyanobacteria, pre-oxidation, especially pre-chlorination, is commonly used to optimize phytoplankton coagulation and removal (Chen et al., 2009; Steynberg et al., 1996). Other options of pro-oxidants are ozone, potassium permanganate, and chlorine dioxide. However, besides being expensive, these technologies are limited by the potential formation of harmful by-products or staining water at the customer's tap (Chang et al., 2018; Li et al., 2014).

Advanced oxidation processes (AOP), which has a reaction that involves highly reactive hydroxyl radical, is an alternative method to control cyanobacteria in water (Chang et al., 2018). The advantage of this reaction is, besides the ability to disrupt cyanobacterial cells, it can destroy the toxins and T&O compounds produced (Chang et al., 2018). Hydrogen Peroxide (H_2O_2) is used in AOP and it is a naturally occurring reactive oxygen species produced by the photolysis of dissolved organic matter exposed to ultraviolet (UV) radiation and by phytoplankton metabolic processes (Burson et al., 2014). It is considered an environmentally benign algaecide because when associated with light, it quickly decomposes into water and oxygen, therefore not accumulating in the environment (Barrington et al., 2013; Chang et al., 2018).

UV light applied during the application of H_2O_2 functions as a catalyst to produce hydroxyl ($^{\bullet}OH$) and hydroperoxyl ($^{\bullet}HO_2$) radicals which are the main reactive species responsible for the effective destruction of cyanobacterial cells and metabolites (Barrington et al., 2013). As UV radiation intensity is increased, the required dose of H_2O_2 may be reduced (Barrington et al., 2013). However, the most abundant kind of light spectrum in the sunlight is the visible light, therefore it is important to study a way to enhance the production of ($^{\bullet}OH$) from H_2O_2 under visible light (Chang et al., 2018). Previous studies have used low-intensity fluorescent light to simulate the effects of visible light on the activity of H_2O_2 on cyanobacteria and its metabolites (Barrington et al., 2013; Chen et al., 2016; Matthijs et al., 2012; Wang et al., 2019).

Several types of research used different conditions to observe the effects of H_2O_2 on the degradation of cyanobacteria, mainly *M. aeruginosa*, (Chen et al., 2016; Wang et al., 2019; Wen et al., 2017; Yang et al., 2018).On the other hand, researches that studied the effect of H_2O_2 under visible light on *D. circinale* cells and its regrowth potential, geosmin, organic matter, and pigment degradation have not been observed in the literature. Therefore, this work aimed to evaluate the effects of H_2O_2 on cultures of *M. aeruginosa* and *D. circinale* and their metabolites under visible light. To meet this goal we (1) investigated the effects of H_2O_2 on *M. aeruginosa* and *D. circinale* cell integrity and density after H_2O_2 treatment and their re-growth potential, (2) determined the fate of microcystins and geosmin, (3) investigated the impact on chlorophyll-*a*, pheophytin, phycocyanin, organic matter, and true color following the addition of H_2O_2.

2. Materials and methods

Axenic cultures of *M. aeruginosa* and *D. circinale* used in this experiment were isolated from reservoirs in the state of Ceará (Brazil) and are part of Selaqua/Federal University of Ceará collection. Both cultures were grown in ASM-1 medium (Gorham et al., 1964), a photoperiod of 12/12 h(L:D) at 22 μmol photons m^{-2} s^{-1} (Digital Lux Tester YF-1065), pH 8, 25 °C (±1 °C), and under continuous aeration. The *M. aeruginosa* used in this study mainly produced MC-LR and grew in single cells. *D. circinale* mostly produced Geosmin. Cell suspensions were prepared by diluting the cultures into ultrapure water to densities of 10^4 cells mL^{-1} for *D. circinale* and 10^5 cells. mL^{-1} for *M. aeruginosa*, concentrations normally found in reservoirs in the state of Ceará (COGERH, 2019).

The experiment was performed in continuous stirred-reactors (Total volume of 2 L), called from now on microcosms. The microcosms were filled with the cyanobacterial suspension (1.5 L) and dosed with H_2O_2 at the concentrations of 2 and 5 mg L^{-1}, under constant low levels of luminous flux (14 μmol photons m^{-2} s^{-1}) using fluorescent light (Sylvania 65 W) with no or undetectable amounts of UV radiation. These concentrations of H_2O_2 were used since the efficacy of low concentrations (<2 mg L^{-1}) on cyanobacterial suppression in lakes was already reported by Matthijs et al. (2012). After the proper dilution and before subdividing it into the microcosms, the culture suspension was analyzed in triplicate (Control BP) to identify its initial characteristics. Three microcosms were run without the application of H_2O_2 but using visible light (Control AP) to evaluate the effect of the new environment over the organisms. Finally, three microcosms were dosed with 2 mg L^{-1} and the other three, with 5 mg L^{-1} of H_2O_2, all with the same incidence of visible light. Samples were collected from these microcosms after the H_2O_2 degradation. Thus, the experiments were comprised of 12 samples in total for each species.

A Hydrogen peroxide solution, 30 wt. % in H_2O, (Merck, Germany), was dosed by adding the appropriate volumes to the microcosms containing the specific cyanobacterial suspension and kept under gentle and continuous agitation to avoid cell sedimentation. The concentration of H_2O_2 solution was verified using the iodometric method proposed by Skellon and Wills (1948). During the experiment, the concentration of H_2O_2 was monitored by Quantofix Peroxide Test Strips (Macherey-Nagel Company) until the H_2O_2 complete degradation.

The re-growth experiment was performed right after the H_2O_2 complete degradation. Aliquots of 50 mL of the treated samples were incubated into flasks containing 100 mL of ASM-1 medium (Gorham et al., 1964), using the same conditions adopted for growing the cultures explained previously. After 15 and 30 days of incubation, samples were removed from the flask, and cells were counted and measured.

Analysis of intra and extracellular microcystin, total geosmin, total chlorophyll *a*, total pheophytin, and total phycocyanin concentrations cyanobacterial cell density and size, true-color, and organic matter were performed. To determine cell density, aliquots of 1 mL were used. For the determination of chlorophyll-a (Chlo.a), phycocyanin (Phyc), intra

and extracellular microcystin, and geosmin, 100 mL was used for each analysis. For true color and organic matter determination, aliquots of 50 mL were used for each analysis.

Extraction and analysis of intra and extracellular MC-LR were performed by a modified method initially proposed by Lawton et al. (1994). For intracellular MC-LR, an aliquot of 100 mL was filtered through 110 mm GF/C discs (Whatman, Maidstone, Kent, UK). The GF/C discs with the cell-bound or intracellular microcystin were freeze-thawed and placed in glass beakers containing 10 mL of methanol (100 %) (J.T. Baker – Grade UV-HPLC) to extract the intracellular content for 1 h at room temperature (25 °C). The extract was filtered, evaporated, and the residue was resuspended in 1 mL of methanol (80 %) before analysis in high-performance liquid chromatography (HPLC). For the extracellular microcystin, the filtered samples (100 mL) were passed through C18 Cartridges (SupelClean LC-18 – SUPELCO 1 g and 3 mL) in a sample concentration system using a vacuum manifold (Varian Analytical Instruments, San Fernando, CA, USA) and after this, analyzed. Both analyses were performed using Agilent Model 1260 Infinity HPLC, with reverse-phase column (Waters Symmetry C18, 5 µm, 2.1 × 150 mm) at 40 °C and a DAD detector adjusted to wavelengths 238 and 222 nm. The mobile phases used consisted of 0.05 % TFA in water and 0.05 % TFA in acetonitrile with a flow rate of 0.5 mL min^{-1} and a total running time of 30 min. MC-LR, MC-LA, and MC-LY standards were provided by the Cyanosol Laboratory (Robert Gordon University, UK). The other reagents used were: Methanol, Acetonitrile (J. T. Baker - UV-HPLC Grade), and Trifluoroacetic Acid (TFA, Dynamic - UV-HPLC Grade).

Total geosmin quantification was performed by pre-concentrating the samples using the solid-phase microextraction technique (SPME) in the headspace, followed by analysis on a Thermo Scientific GC–MS (Gas chromatograph TRACE 1300 Series, splitless injection, injector at 220 °C and helium flow rate of 1 mL min^{-1} coupled to a Thermo Scientific Single Quadrupole, mass spectrometer at 150 °C and Electronic Impact Ionization) according to Graham and Hayes (1998). Calibration curves were prepared using standard Geosmin solution (Sigma Aldrich) diluted in Ethyl Acetate (Dynamic - UV-HPLC Grade).

The samples used for cyanobacterial cell density were preserved with Lugol's Iodine. *M. aeruginosa* and *D. circinale* cell densities were measured using a Sedgewick-rafter chamber and an inverted optical microscope (Zeiss, Model Vert. A1) with a magnification of 40X adapted from APHA (2012). For the cell density count, each unicellular organism was considered as an individual. To determine the size of the cells, the scale overlay method (Hrycik et al., 2019; Sun, 2003) was used in which the microscope eyepiece contained a graduated scale and the sample slide contains another engraved scale.

Organic Matter is a complex mixture of organic materials such as humic acids, hydrophilic acids, proteins, lipids, amino acids, and hydrocarbons that may be found in various sources of natural waters (Pourzamani et al., 2015). True color results primarily from the presence of natural organic matter and, therefore, we used it as indicative of organic matter. True color and organic matter parameters were analyzed using the spectrophotometric method proposed by Albrektienė et al. (2012) and APHA (2012), respectively. Organic matter, measured by absorbance at a wavelength of 254 nm, was used as an alternative method for estimating the total organic carbon concentration in a water sample (Edzwald et al., 1985).

For the determination of chlorophyll-*a* and pheophytin, the spectrophotometric method (10200 H) proposed by APHA (2012) was used. Briefly, the method consisted of filtering (110 mm GF/C discs, Whatman, Maidstone, Kent, UK) the sample to retain the cyanobacterial cels, macerating the filter, extracting the pigments with acetone (90 %), filtering the extract, and analyzing it in a spectrophotometer (Genesys 10-S from Thermo-Scientific - USA) with wavelengths 664, 665, and 750 nm. Phycocyanin was analyzed using a spectrophotometric method adapted from Bennett and Bogorad (2011). Briefly, the method consisted of filtering the sample to retain the cyanobacterial cels, freeze-thawing the filters three times, extracting phycocyanin with sodium azide (0,02 g/mL), filtering the extract, and analyzing it in a spectrophotometer (Genesys 10-S from Thermo-Scientific - USA) with wavelengths 280, 615, and 652 nm.

Data normality and homogeneity of data variance was verified using the Shapiro-Wilk (P_{S-W}) and Levene test, respectively. For comparisons between the different conditions, the ANOVA test was used. Tukey test was performed for samples with normal distribution, while pairwise comparisons using the Wilcoxon signed-rank test were used for samples not normally distributed (Zar, 1996). All the statistical tests were performed using a significance level of 5 % ($\alpha = 5$ %) and the RStudio software (Team, R.C., 2018).

3. Results

To evaluate the full impact of the treatment, samples were collected after the complete degradation of H_2O_2, which happened after 24 and 48 h for the reactors dosed with 2 and 5 mg L^{-1}, respectively. The control AP (C_AP) was collected after 24 h. After the degradation of 2 mg L^{-1} (24 h), a decrease of 84 % in *M. aeruginosa* cell density was observed, and after the extinction of 5 mg L^{-1} (48 h), no intact *M. aeruginosa* cells were found (Fig. 1). After 15 days of the application of 2 mg L^{-1} (re-growth experiment), the number of cells did not show a significant difference ($p < 0.05$) when compared to the number just after the H_2O_2 degradation. After 30 days of the re-growth experiment, just cell fragments were present, indicating that the remaining cells were not viable, without the ability to reproduce.

M. aeruginosa cells showed a remarkable reduction in size from 8.658 ± 0.001 µm in the Controls BP and AP, to 6.197 ± 0.001 µm after the H_2O_2 degradation (2 mg L^{-1}), and then to 4.329 ± 0.002 µm after 15 days in the re-growth experiment, probably indicating the cell's response to the stress of the oxidative environment. A reduction of *M. aeruginosa* biovolume was also observed, from 44.49 ± 0.01 mm^3 L^{-1} in the Controls BP and AP to 2.64 ± 0.01 mm^3 L^{-1} after the H_2O_2 degradation (2 and 5 mg L^{-1}), and then to 0.63 ± 0.01 mm^3 L^{-1} after 15 days in the re-growth experiment, probably indicating a cell response to oxidative stress.

The *D. circinale* biovolume was also observed, 8.78 ± 0.01 mm^3 L^{-1} in the Controls BP and AP At the concentration used, *D. circinale* was noticeably more sensitive to H_2O_2 than *M. aeruginosa*, since, after the application of 2 and 5 mg L^{-1}, no cells were detected (Fig. 1) and,

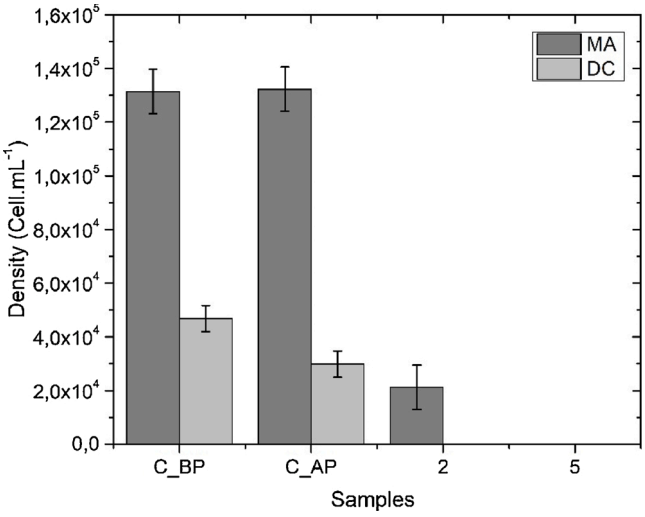

Fig. 1. Cellular densities of *M. aeruginosa* (MA) and *D. circinale* (DC) suspensions. Where: C_BP = C_BP = Control sample collected at time T = 0; C_AP = Control sample (with no H_2O_2) collected after 24 h from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 mg L^{-1} of H_2O_2 (24 h); 5 = Sample collected after the compete degradation of 5 mg L^{-1} of H_2O_2 (48 h).

therefore no biovolume measurement was possible. Comparing the Control BP to the Control AP showed that the experimental conditions did not cause significant variation ($p < 0.05$) of cell densities of either *M. aeruginosa* or *D. circinale*.

After the application of 2 and 5 mg L^{-1} H_2O_2, the concentrations of chlorophyll-a decreased significantly ($p < 0.05$) after 24 and 48 h for both species (Fig. 2A). The chlorophyll-a concentration did not show significant variation ($p < 0.05$) between the Control AP to the Control BP (Fig. 2A) also for both species, the same behavior observed for the cell densities. No pheophytin (Limit of Quantification <0.001 mg L^{-1}) was detected during the experiment.

Phycocyanin concentration varied between 0.6 and 0.9 mg L^{-1} for both H_2O_2 concentrations and both species (Fig. 2B), showing no significant difference ($p < 0.05$) except for the sample Control AP for culture *D. circinale* which presented a significant increase to approximately 2 mg L^{-1}. This value may have been generated from an error in the execution of the analysis and should, therefore, be disregarded.

The *M. aeruginosa* culture showed a higher increase in dissolved organic material (organic matter 254 nm and true color) than *D. circinale* (Fig. 3), probably due to its higher concentrations of cells at the start of the experiment; 10^5 cells per mL^{-1} for *M. aeruginosa* as opposed to the 10^4 cells per mL^{-1} for *D. circinale.* A significant increase ($p < 0.05$) in dissolved organic material was observed in the *M. aeruginosa* culture from the Control BP to the other samples (Control AP, 2 and 5 mg L^{-1}) indicating that both, the environmental conditions and the H2O2 application, may have interfered in the release of intracellular content. This statement is only partially true for *D. circinale* where there was a significant increase ($p < 0.05$) in dissolved organic material from the Control BP to the Control AP but not to the treatment microcosms (2 and 5 mg L^{-1}). This indicates that the environmental conditions may have interfered in the release of intracellular content but the H2O2 application did not. Another possibility is that the amount of oxidant was sufficient to cause the cell lysis, the release, and the degradation of intracellular *D. circinale* content but not enough to degrade all the released intracellular content of *M. aeruginosa*.

Intracellular microcystin decreased while the extracellular fraction increased, both significantly ($p < 0.05$), from the Control BP to the treatment with 5 mg L^{-1} (Fig. 4A). This behavior was expected since the integrity of *M. aeruginosa* cells was impacted by the oxidant. What was not expected, though, was the increase in the total microcystin concentration from around 4–9 μg L^{-1}. The amount of H_2O_2 seemed not to be sufficient to degrade dissolved microcystin, similar to what happened to the dissolved organic material, even with 5 mg.L^{-1} being applied.

On the other hand, geosmin did not vary significantly ($p < 0.05$) from the Control BP to the Control AP, but decreased significantly ($p < 0.05$), from 264.82 ng L^{-1} in the Control AP to 72.02 ng L^{-1} in the microcosm with 5 mg L^{-1} of H_2O_2 (Fig. 4B). This concentration decrease, after the application of oxidant, was also observed for the dissolved organic material of the *D. circinale* suspension (Fig. 3).

4. Discussion

The main objective of this research was to evaluate the effects of H_2O_2 on cultures of *M. aeruginosa* and *D. circinale* and their metabolites under visible light. The application of H_2O_2 was effective in inducing cyanobacterial death and releasing intracellular content. The reduction of biovolume and cell numbers was evident for both strains studied.

In freshwater environments, *M. aeruginosa* cells size is reported to vary between 4 and 6 μm (Olenina et al., 2006), which differs from the initial cell size of our experiment (~8.66 μm). Cultured *M. aeruginosa* responds differently to different environmental conditions. According to Doers and Parker (1988), when exposed to different experimental conditions (1st condition: 30 °C, 30 μ E m^{-2} s^{-1} from white fluorescent lamps, and 2nd condition: 25 °C, 10 μ.E m^{-2} s^{-1} from white fluorescent lamps), *M. aeruginosa* presented morphological differences in terms of cell size, pigmentation, colony disintegration, mucilage sheath, the distance between cells, among others.

In addition to the reduction in cell size and biovolumes, the application of H_2O_2 reduced *M. aeruginosa* cell numbers. Zhou et al. (2018) observed *M. aeruginosa* lysis, reduction in growth, and a reduction of cell size from 2.1 to 1.7 μm on the 3rd day of the application of 24–51 mg L^{-1} H_2O_2. Barrington et al. (2013) also observed a selective reduction of cyanobacteria cells (up to 96 %) using 0.37 g L^{-1} of H_2O_2 in a lake containing high concentrations of cyanobacteria, mainly *M. aeruginosa*, *M. flos-aquae,* and *P. isothrix*. Matthijs et al. (2012) showed that the application of 2 mg.L^{-1} of H_2O_2 in a lake in the Netherlands (Lake Koetshuis), decreased the population of *P. agardhii* from 6.10^5 cells mL^{-1} to 1.10^4 cells mL^{-1}.

Yang et al. (2018) observed the effect of six different concentrations of H_2O_2 (0, 0.3, 0.9, 2.7, 8.1, and 24.3 mg L^{-1}) on 4 cyanobacteria (*Dolichospermum* sp., *Cylindrospermopsis* sp., *Planktothrix* sp., *Microcystis* sp.) After the treatment, *Dolichospermum sp.* was not detected even with low H_2O_2 concentrations (0.9 mg L^{-1}), showing to be significantly more sensitive than *Microcystis sp,* behavior also observed in our study. Lin et al. (2009) compared the cultures of *M. aeruginosa* and *D. circinale* after the addition of another oxidant, sodium hypochlorite (NaClO), and also

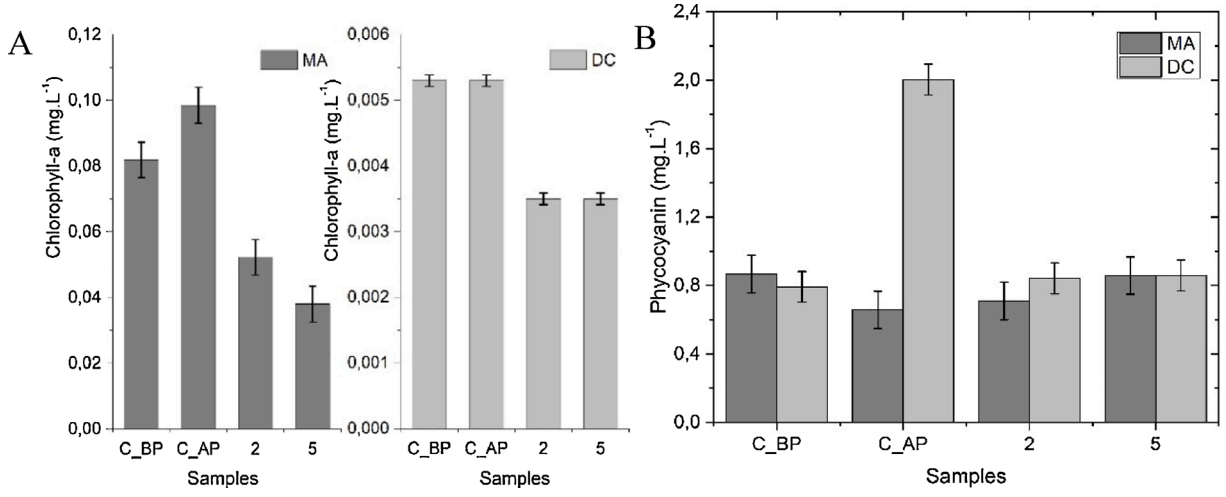

Fig. 2. Chlorophyll-a (A) and Phycocyanin (B) concentrations of *M. aeruginosa* (MA) and *D. circinale* (DC) suspensions. C_BP = Control sample collected at time T = 0; C_AP = Control sample (with no H_2O_2) collected after 24 h from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 mg L^{-1} of H_2O_2 (24 h); 5 = Sample collected after the compete degradation of 5 mg L^{-1} of H_2O_2 (48 h).

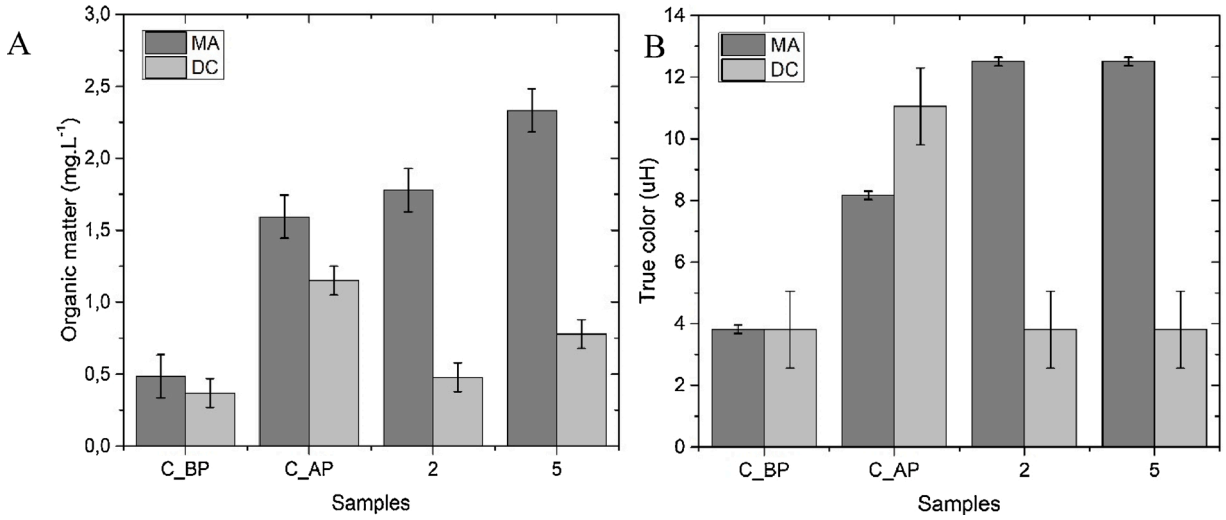

Fig. 3. Organic matter - 254 nm (A) and true color (B) of *M. aeruginosa* (MA) and *D. circinale* (DC) suspensions. C_BP = Control sample collected at time T = 0; C_AP = Control sample (with no H_2O_2) collected after 24 h from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 mg L^{-1} of H_2O_2 (24 h); 5 = Sample collected after the compete degradation of 5 mg L^{-1} of H_2O_2 (48 h).

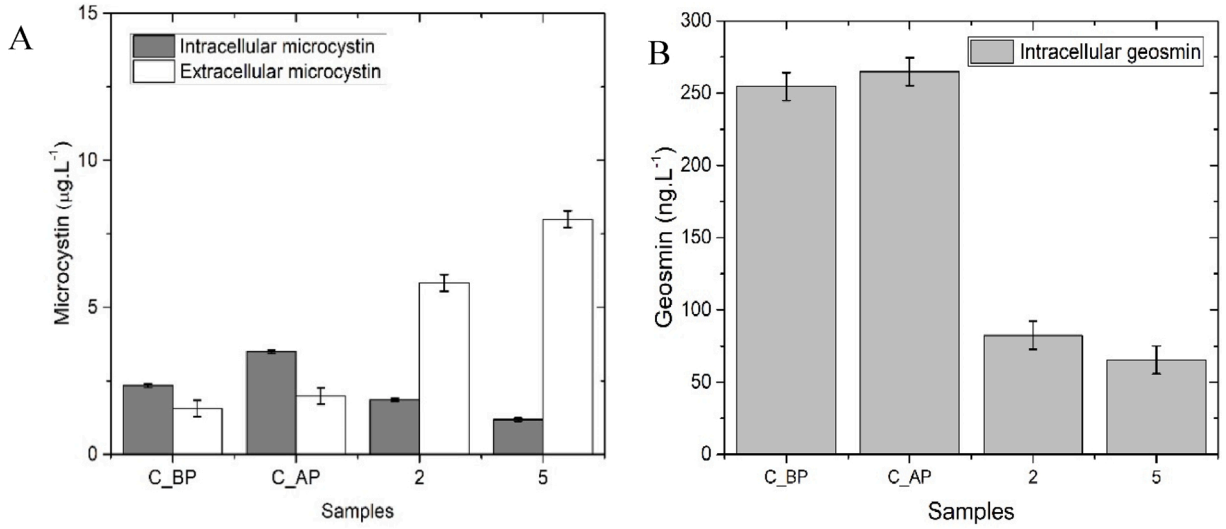

Fig. 4. Intra and extracellular Microcystin from *M. aeruginosa* (A) and Total Geosmin from *D. circinale* (B) suspensions. C_BP = Control sample collected at time T = 0; C_AP = Control sample (with no H_2O_2) collected after 24 h from the beginning of the experiment; 2 = Sample collected after the compete degradation of 2 mg L^{-1} of H_2O_2 (24 h); 5 = Sample collected after the compete degradation of 5 mg L^{-1} of H_2O_2 (48 h).

observed a higher rate of disruption of *D. circinale* cells, by a factor of 1.3–5.0 when compared to *M. aeruginosa* cells. According to Yang et al. (2018), *M. aeruginosa* cells are more resistant to changes in the external environment because they have greater fluctuating regulation, colonial morphology, and resistance to high radiation, giving the species greater competitive dominance. In addition, when microcystis cells occur in colonies, which is normally the case of strains encountered in natural waters, they are more resistant to H_2O_2 probably because the mucus formed in the colonies has antioxidant properties (Zhang and Benoit, 2019). This difference between cultivated and environmental strains should be considered when evaluating the concentration to be used in natural waters to controls cyanobacteria blooms.

The decrease in the chlorophyll-a concentrations after the application of H_2O_2, for both species, suggests that after the cell lysis chlorophyll-a is released and oxidized preferentially over the other organic material. Some authors verified the same effect after the application of H_2O_2. Liu et al. (2017) applied H_2O_2 (5 and 20 mg L^{-1}) to colonies of *Microcystis* sp. and observed a significant reduction of chlorophyll-a concentration after 12 h, from 60 µg L^{-1} to 39 µg L^{-1}. Yang et al. (2018) also found a significant reduction in chlorophyll-a (150 to 25 µg L^{-1}) in *M. aeruginosa* cultures due to the addition of 1, 3, 6.7, and 20 mg L^{-1} of H_2O_2. Barrington and Ghadouani (2008) studied the effects of H_2O_2 on the decay of chlorophytes and diatoms from environmental samples and observed that, under laboratory conditions, the application of 3 mg H_2O_2 per µgCl-a reduced *in vivo* photosynthetic activity of phytoplankton. According to this author, concentrations of chlorophyll-a were reduced to negligible values.

The determination of phycocyanin is also important because it relates closely to the cyanobacteria biomass and, therefore, it's generally used to estimate the density of cyanobacteria (Yang et al., 2018). This pigment is a conjugated biliprotein and an accessory photosynthetic pigment. The chromospheres present in this pigment is the phycocyanobilin known to display a wide range of photochemical activity (Robertson et al., 1999). The oxidative stress after the application of H_2O_2 did not significantly affect the phycocyanobilins. Thus, phycocyanin has potential as an antioxidant agent due to its stability under oxidative

stress (Sonani et al., 2014). This argument reinforces the idea that peroxide did not affect phycocyanin concentration, as observed in our study.

The increase in organic matter 254 nm and true color may be related to the higher cell density of *M. aeruginosa* (10^5 cells mL^{-1}), as opposed to what happened to the *D. circinale* suspension (10^4 cells mL^{-1}). According to Jarusutthirak and Amy (2007), if there is a greater number of cells in the medium when H2O2 is added, there will be a greater level of organic matter to be released when the cells lyse. Additionally, the aromatic fraction present in some organic matter can not only consume hydroxyl radicals but also produce it. These new radicals react with other remaining particulate organic compounds, increasing the concentration of dissolved organic matter in the medium (Ho et al., 2002; Park et al., 2017). Thus, there is an optimum H2O2 dosage for a given system at which maximum removal of the target substance is achieved. Wang et al. (2000) showed that the degradation rate of humic acid during the UV/H2O2 process increased with the increase of hydrogen peroxide concentration until 0.01 % (2.94 mmol L^{-1}) and then decreased with further increase in H2O2 concentration.

An increase in extracellular microcystin and geosmin was expected because of the cell disruption caused by H_2O_2. Instead, a decrease of geosmin and an increase of total microcystin was observed. In the case of geosmin, some authors (Lürling et al., 2014; Rodríguez et al., 2007; Yang et al., 2018) have found that when enough H_2O_2 is applied, apart from breaking the cells, it can further degrade secondary metabolites. Other studies have shown that if H_2O_2 is applied in low concentrations, it can initially increase extracellular toxins and other metabolites and, after some time, these metabolites are significantly reduced after they are released to the surrounding waters. This may suggest that bacterial degradation can assist in the breakdown of dissolved toxins and other metabolites when treating waters using H_2O_2 in natural environments such as lakes and ponds (Miao et al., 2010; Papadimitriou et al., 2016). A possible explanation for the increase of total microcystin might be that this toxin was initially bound to internal proteins before the H_2O_2 addition, and therefore not visible to the HPLC method used. However, after cell death and internal content release, the toxin may have detached from the proteins and became visible to the analytical method (Schuurmans et al., 2018).

5. Conclusion

Hydrogen peroxide (H_2O_2) was used to control *M. aeruginosa* and *D. circinale* and their metabolites in the water. A low concentration of H_2O_2 (2 mg L^{-1}) was enough to reduce the concentrations of chlorophyll-a and geosmin, destroy cyanobacterial cells, or render them unviable, preventing their re-growth. Phycocyanin, however, was resilient to oxidation indicating that it may not be a good indicator of cyanobacterium density in natural waters when H_2O_2 is applied. This oxidant caused the release of intracellular content but was not enough to degrade it, increasing the dissolved organic material in the water, decreasing water quality, and increasing the potential formation of disinfection by-products during the conventional water treatment process. Total microcystin concentration doubled during the treatment, probably because it was detached from the proteins after cell lysis. Most of this microcystin was extracellular, representing an additional risk to conventional water treatment plants. Our findings suggest that, if the application of H_2O_2 *in situ* is to be considered for a reservoir used for drinking water purposes, it should be done at a distant point from the water treatment plant, allowing natural degradation processes of organic and toxic compounds, such as biological or photolysis degradation, to occur before the water reaches the water treatment plant intake.

CRediT authorship contribution statement

Marianna Correia Aragão: Methodology, Investigation, Data curation, Writing - original draft, Supervision. **Kelly Cristina dos Reis:** Validation, Formal analysis, Data curation. **Maria Aparecida Melo Rocha:** Methodology, Investigation, Resources. **Dayvson de Oliveira Guedes:** Investigation, Data curation. **Eduardo Costa dos Santos:** Formal analysis, Writing - review & editing. **Jose Capelo-Neto:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

We declare to Aquatic Toxicology that there is no conflict of interest. All authors mutually agree that they should be submitted to this journal.

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References

Albrektienė, R., Rimeika, M., Zalieckienė, E., Šaulys, V., Zagorskis, A., 2012. Determination of organic matter by UV absorption in the ground water. J. Environ. Eng. Landsc. Manag. 20, 163–167. https://doi.org/10.3846/16486897.2012.674039.

APHA, 2012. Standard Methods for Examination of Water and Wastwater, 22nd ed. American Public Health Association, Washington, DC.

Aragão, M.C., dos Reis, K.C., Souza, A.C., Rocha, M.A.M., Capelo Neto, J., 2020. Modeling total microcystin production by Microcystis aeruginosa using multiple regression. J. Water Supply Res. Technol. https://doi.org/10.2166/aqua.2020.128.

Barrington, D.J., Ghadouani, A., 2008. Application of hydrogen peroxide for the removal of toxic cyanobacteria and other phytoplankton from wastewater. Environ. Sci. Technol. 42, 8916–8921. https://doi.org/10.1021/es801717y.

Barrington, D.J., Ghadouani, A., Ivey, G.N., 2013. Cyanobacterial and microcystins dynamics following the application of hydrogen peroxide to waste stabilization ponds. Hydrol. Earth Syst. Sci. 17, 2097–2105. https://doi.org/10.5194/hess-17-2097-2013.

Bennett, A., Bogorad, L., 2011. Complementary chromatic adaptation in a filamentous blue-green alga complementary in a filamentous blue-green alga. J. Cell Biol. 58, 419–435.

Bittencourt-Oliveira, M., do, C., Piccin-Santos, V., Moura, A.N., Aragao-Tavares, N.K.C., Cordeiro-Araújo, M.K., 2014. Cyanobacteria, microcystins and cylindrospermopsin in public drinking supply reservoirs of Brazil. An. Acad. Bras. Cienc. 86, 297–310. https://doi.org/10.1590/0001-3765201302512.

Burson, A., Matthijs, H.C.P., de Bruijne, W., Talens, R., Hoogenboom, R., Gerssen, A., Visser, P.M., Stomp, M., Steur, K., van Scheppingen, Y., Huisman, J., 2014. Termination of a toxic Alexandrium bloom with hydrogen peroxide. Harmful Algae 31, 125–135. https://doi.org/10.1016/j.hal.2013.10.017.

Chang, C.-W., Huo, X., Lin, T.-F., 2018. Exposure of Microcystis aeruginosa to hydrogen peroxide and titanium dioxide under visible light conditions: modeling the impact of hydrogen peroxide and hydroxyl radical on cell rupture and microcystin degradation. Water Res. 141, 217–226. https://doi.org/10.1016/j.watres.2018.05.023.

Chen, J.-J., Yeh, H.-H., Tseng, I.-C., 2009. Effect of ozone and permanganate on algae coagulation removal – pilot and bench scale tests. Chemosphere 74, 840–846. https://doi.org/10.1016/j.chemosphere.2008.10.009.

Chen, C., Yang, Z., Kong, F., Zhang, M., Yu, Y., Shi, X., 2016. Growth, physiochemical and antioxidant responses of overwintering benthic cyanobacteria to hydrogen peroxide. Environ. Pollut. 219, 649–655. https://doi.org/10.1016/j.envpol.2016.06.043.

COGERH, 2019. Qualidade das águas dos açudes monitorados pela COGERH – Campanha de agosto/2019 [WWW Document].

Doederer, K., Gale, D., Keller, J., 2019. Effective removal of MIB and geosmin using MBBR for drinking water treatment. Water Res. 149, 440–447. https://doi.org/10.1016/j.watres.2018.11.034.

Doers, M.P., Parker, D.L., 1988. Properties of microcystis aeruginosa and m. Flos-aquae (cyanophyta) in culture: taxonomic implications. J. Phycol. 24, 502–508.

Edzwald, J.K., Becker, W.C., Wattier, K.L., 1985. Surrogate parameters for monitoring organic matter and THM precursors. J. Am. Water Works Assoc. 77, 122–132. https://doi.org/10.1002/j.1551-8833.1985.tb05521.x.

Fan, J., Daly, R., Hobson, P., Ho, L., Brookes, J., 2013. Impact of potassium permanganate on cyanobacterial cell integrity and toxin release and degradation. Chemosphere 92, 529–534. https://doi.org/10.1016/j.chemosphere.2013.03.022.

Fan, G., Zhou, J., Zheng, X., Luo, J., Hong, L., Qu, F., 2020. Fast photocatalytic inactivation of Microcystis aeruginosa by metal-organic frameworks under visible light. Chemosphere 239, 124721. https://doi.org/10.1016/j.chemosphere.2019.124721.

Gan, N., Xiao, W., Zhu, L., Wu, Z., Liu, J., Hu, C., Song, L., 2012. The role of microcystins in maintaining colonies of bloom-forming Microcystis spp. Environ. Microbiol. 14, 730–742.

Gorham, P.R., McLachlan, J., Hammer, U.T., Kim, W.K., 1964. Isolation and culture of toxic strains of Anabaena flos-aquae (Lyngb.) de Bréb. SIL Proceed. 1922-2010 15, 796–804. https://doi.org/10.1080/03680770.1962.11895606.

Graham, D., Hayes, K.P., 1998. Application of solid phase microextraction for the analysis of off-flavours in water. In: Proceedings of the Australian Water Association WaterTECH Conference. Brisbane, Australia.

Ho, L., Newcombe, G., Croué, J.-P., 2002. Influence of the character of NOM on the ozonation of MIB and geosmin. Water Res. 36, 511–518. https://doi.org/10.1016/S0043-1354(01)00253-6.

Ho, L., Kayal, N., Trolio, R., Newcombe, G., 2010. Determining the fate of Microcystis aeruginosa cells and microcystin toxins following chloramination. Water Sci. Technol. 62, 442–450. https://doi.org/10.2166/wst.2010.448.

Hrycik, A.R., Shambaugh, A., Stockwell, J.D., 2019. Comparison of FlowCAM and microscope biovolume measurements for a diverse freshwater phytoplankton community. J. Plankton Res. https://doi.org/10.1093/plankt/fbz056.

Jarusutthirak, C., Amy, G., 2007. Understanding soluble microbial products (SMP) as a component of effluent organic matter (EfOM). Water Res. 41, 2787–2793. https://doi.org/10.1016/j.watres.2007.03.005.

John, N., Koehler, A.V., Ansell, B.R.E., Baker, L., Crosbie, N.D., Jex, A.R., 2018. An improved method for PCR-based detection and routine monitoring of geosmin-producing cyanobacterial blooms. Water Res. 136, 34–40. https://doi.org/10.1016/j.watres.2018.02.041.

Kim, T.-K., Moon, B.-R., Kim, T., Kim, M.-K., Zoh, K.-D., 2016. Degradation mechanisms of geosmin and 2-MIB during UV photolysis and UV/chlorine reactions. Chemosphere 162, 157–164. https://doi.org/10.1016/j.chemosphere.2016.07.079.

Lawton, L.A., Edwards, C., Codd, G.A., 1994. Extraction and high-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. Analyst 119, 1525. https://doi.org/10.1039/an9941901525.

Li, L., Shao, C., Lin, T.-F., Shen, J., Yu, S., Shang, R., Yin, D., Zhang, K., Gao, N., 2014. Kinetics of cell inactivation, toxin release, and degradation during permanganation of Microcystis aeruginosa. Environ. Sci. Technol. 48, 2885–2892. https://doi.org/10.1021/es405014g.

Li, X., Dreher, T.W., Li, R., 2016. An overview of diversity, occurrence, genetics and toxin production of bloom-forming Dolichospermum (Anabaena) species. Harmful Algae 54, 54–68. https://doi.org/10.1016/j.hal.2015.10.015.

Lin, T.-F., Chang, D.-W., Lien, S.-K., Tseng, Y.-S., Chiu, Y.-T., Wang, Y.-S., 2009. Effect of chlorination on the cell integrity of two noxious cyanobacteria and their releases of odorants. J. Water Supply Res. Technol. 58, 539–551. https://doi.org/10.2166/aqua.2009.117.

Liu, M., Shi, X., Chen, C., Yu, L., Sun, C., 2017. Responses of Microcystis colonies of different sizes to hydrogen peroxide stress. Toxins (Basel) 9, 306. https://doi.org/10.3390/toxins9100306.

Lürling, M., Meng, D., Faassen, E., 2014. Effects of hydrogen peroxide and ultrasound on biomass reduction and toxin release in the cyanobacterium, Microcystis aeruginosa. Toxins (Basel) 6, 3260–3280. https://doi.org/10.3390/toxins6123260.

Matthijs, H.C.P., Visser, P.M., Reeze, B., Meeuse, J., Slot, P.C., Wijn, G., Talens, R., Huisman, J., 2012. Selective suppression of harmful cyanobacteria in an entire lake with hydrogen peroxide. Water Res. 46, 1460–1472. https://doi.org/10.1016/j.watres.2011.11.016.

Meriluoto, J., Spoof, L., Codd, G.A., 2017. Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, 1st ed. Wiley.

Miao, H.F., Qin, F., Tao, G.J., Tao, W.Y., Ruan, W.Q., 2010. Detoxification and degradation of microcystin-LR and -RR by ozonation. Chemosphere 79, 355–361. https://doi.org/10.1016/j.chemosphere.2010.02.024.

Olenina, I., Hajdu, S., Edler, L., Andersson, A., Wasmund, N., Busch, S., Göbel, J., Gromisz, S., Huseby, S., Huttunen, M., Jaanus, A., Kokkonen, P., Ledaine, I., Niemkiewicz, E., 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea. HELCOM Balt.Sea Environ. Proc. No 106, 144. https://doi.org/10.15468/rgugm4.

Papadimitriou, T., Kormas, K., Dionysiou, D.D., Laspidou, C., 2016. Using H2O2 treatments for the degradation of cyanobacteria and microcystins in a shallow hypertrophic reservoir. Environ. Sci. Pollut. Res. 23, 21523–21535. https://doi.org/10.1007/s11356-016-7418-2.

Park, J.-A., Nam, H.-L., Choi, J.-W., Ha, J., Lee, S.-H., 2017. Oxidation of geosmin and 2-methylisoborneol by the photo-Fenton process: kinetics, degradation intermediates, and the removal of microcystin-LR and trihalomethane from Nak-Dong River water, South Korea. Chem. Eng. J. 313, 345–354. https://doi.org/10.1016/j.cej.2016.12.086.

Pourzamani, H., Majd, A.M.S., Attar, H.M., Bina, B., 2015. Natural organic matter degradation using combined process of ultrasonic and hydrogen peroxide treatment. Anuário do Inst. Geociências - UFRJ 38, 63. https://doi.org/10.11137/2015_1_63_72.

Robertson, P.K.J., Lawton, L.A., Cornish, B.J.P.A., 1999. The involvement of phycocyanin pigment in the photodecomposition of the cyanobacterial toxin, Microcystin-LR. J. Porphyr. Phthalocyanines 03, 544–551. https://doi.org/10.1002/(SICI)1099-1409(199908/10)3:6/7<544::AID-JPP173>3.0.CO;2-7.

Rodríguez, E., Majado, M.E., Meriluoto, J., Acero, J.L., 2007. Oxidation of microcystins by permanganate: reaction kinetics and implications for water treatment. Water Res. 41, 102–110. https://doi.org/10.1016/j.watres.2006.10.004.

Roegner, A.F., Brena, B., González-Sapienza, G., Puschner, B., 2014. Microcystins in potable surface waters: toxic effects and removal strategies. J. Appl. Toxicol. 34, 441–457. https://doi.org/10.1002/jat.2920.

Schuurmans, J.M., Brinkmann, B.W., Makower, A.K., Dittmann, E., Huisman, J., Matthijs, H.C.P., 2018. Microcystin interferes with defense against high oxidative stress in harmful cyanobacteria. Harmful Algae 78, 47–55. https://doi.org/10.1016/j.hal.2018.07.008.

Skellon, J.H., Wills, E.D., 1948. Iodimetric methods of estimating peroxidic oxygen. Analyst 73, 78. https://doi.org/10.1039/an9487300078.

Sonani, R.R., Singh, N.K., Kumar, J., Thakar, D., Madamwar, D., 2014. Concurrent purification and antioxidant activity of phycobiliproteins from Lyngbya sp. A09DM: an antioxidant and anti-aging potential of phycoerythrin in Caenorhabditis elegans. Process Biochem. 49, 1757–1766. https://doi.org/10.1016/j.procbio.2014.06.022.

Steynberg, M.C., Pieterse, A.J.H., Geldenhuys, J.C., 1996. Improved coagulation and filtration of algae as a result of morphological and behavioural changes due to pre-oxidation. J. Water Supply Res. Technol. - AQUA 45, 292–298.

Sun, J., 2003. Geometric models for calculating cell biovolume and surface area for phytoplankton. J. Plankton Res. 25, 1331–1346. https://doi.org/10.1093/plankt/fbg096.

Team, R.C, 2018. R: a Language and Environment for Statistical Computing.

Wang, G.-S., Hsieh, S.-T., Hong, C.-S., 2000. Destruction of humic acid in water by UV light–catalyzed oxidation with hydrogen peroxide. Water Res. 34, 3882–3887. https://doi.org/10.1016/S0043-1354(00)00120-2.

Wang, J., Chen, Z., Chen, H., Wen, Y., 2018. Effect of hydrogen peroxide on Microcystic aeruginosa: role of cytochromes P450. Sci. Total Environ. 626, 211–218. https://doi.org/10.1016/j.scitotenv.2018.01.067.

Wang, B., Song, Q., Long, J., Song, G., Mi, W., Bi, Y., 2019. Optimization method for Microcystis bloom mitigation by hydrogen peroxide and its stimulative effects on growth of chlorophytes. Chemosphere 228, 503–512. https://doi.org/10.1016/j.chemosphere.2019.04.138.

Wen, G., Zhu, H., Wei, Y., Huang, T., Ma, J., 2017. Formation of assimilable organic carbon during the oxidation of water containing Microcystis aeruginosa by ozone and an advanced oxidation process using ozone/hydrogen peroxide. Chem. Eng. J. 307, 364–371. https://doi.org/10.1016/j.cej.2016.08.073.

Wert, E.C., Korak, J.A., Trenholm, R.A., Rosario-Ortiz, F.L., 2014. Effect of oxidant exposure on the release of intracellular microcystin, MIB, and geosmin from three cyanobacteria species. Water Res. 52, 251–259. https://doi.org/10.1016/j.watres.2013.11.001.

WHO, 2011. Guidelines for Drinking Water Quality, 4th ed. World Health Organization, Geneva.

Xie, P., Ma, J., Liu, W., Zou, J., Yue, S., Li, X., Wiesner, M.R., Fang, J., 2015. Removal of 2-MIB and geosmin using UV/persulfate: contributions of hydroxyl and sulfate radicals. Water Res. 69, 223–233. https://doi.org/10.1016/j.watres.2014.11.029.

Yang, Z., Buley, R.P., Fernandez-Figueroa, E.G., Barros, M.U.G., Rajendran, S., Wilson, A. E., 2018. Hydrogen peroxide treatment promotes chlorophytes over toxic cyanobacteria in a hyper-eutrophic aquaculture pond. Environ. Pollut. 240, 590–598. https://doi.org/10.1016/j.envpol.2018.05.012.

Zar, J.H., 1996. Biostatistical Analysis. Prentice-Hall International Inc., Upper Saddle River, New Jersey.

Zhang, S., Benoit, G., 2019. Comparative physiological tolerance of unicellular and colonial Microcystis aeruginosa to extract from Acorus calamus rhizome. Aquat. Toxicol. 215, 105271 https://doi.org/10.1016/j.aquatox.2019.105271.

Zhou, T., Zheng, J., Cao, H., Wang, X., Lou, K., Zhang, X., Tao, Y., 2018. Growth suppression and apoptosis-like cell death in Microcystis aeruginosa by H2O2: a new insight into extracellular and intracellular damage pathways. Chemosphere 211, 1098–1108. https://doi.org/10.1016/j.chemosphere.2018.08.042.

Zong, W., Sun, F., Sun, X., 2013. Oxidation by-products formation of microcystin-LR exposed to UV/H2O2: Toward the generative mechanism and biological toxicity. Water Res. 47, 3211–3219. https://doi.org/10.1016/j.watres.2013.03.037.