MENEZES, I., MAXWELL-MCQUEENEY, D., CAPELO-NETO, J., PESTANA, C.J., EDWARDS, C. and LAWTON, L.A. 2021. Oxidative stress in the cyanobacterium Microcystis aeruginosa PCC 7813: comparison of different analytical cell stress detection assays. *Chemosphere* [online], 269, article ID 128766. Available from: https://doi.org/10.1016/j.chemosphere.2020.128766

Oxidative stress in the cyanobacterium Microcystis aeruginosa PCC 7813: comparison of different analytical cell stress detection assays.

MENEZES, I., MAXWELL-MCQUEENEY, D., CAPELO-NETO, J., PESTANA, C.J., EDWARDS, C. and LAWTON, L.A.

2021

This document was downloaded from https://openair.rgu.ac.uk

Oxidative stress in the cyanobacterium *Microcystis aeruginosa* PCC 7813: comparison of different analytical cell stress detection assays

Indira Menezes[a,b*], Declan Maxwell-McQueeney[b], José Capelo-Neto[a], Carlos J. Pestana[b], Christine Edwards[b], Linda A. Lawton[b]

[a] Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Fortaleza, Brazil

E-mail: capelo@ufc.br

[b] School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen, United Kingdom

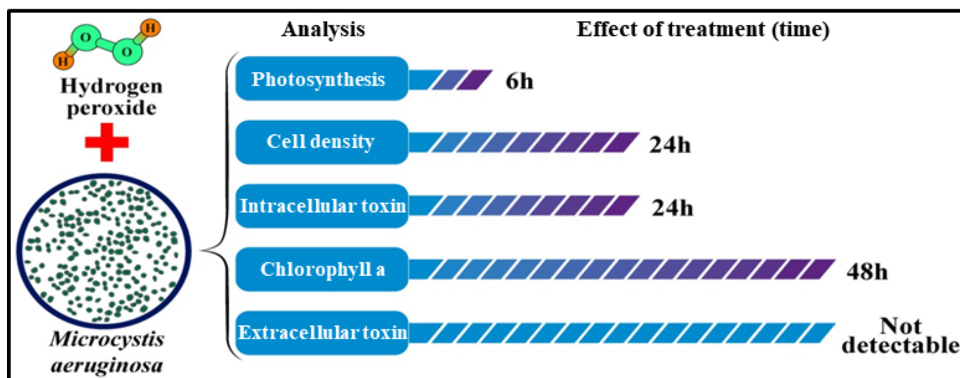
E-mail: l.lawton@rgu.ac.uk, c.edwards@rgu.ac.uk, c.pestana@rgu.ac.uk, d.maxwell4@rgu.ac.uk

*Corresponding author: i.de-menezes-castro@rgu.ac.uk

Highlights:

- Time lag observed between cell stress occurring and its detection by most methods
- Photosynthetic activity analysis was the fastest method for cell stress detection
- Advantages and drawbacks of five different cell stress detection assays elucidated

26 **Graphical Abstract**



27

28

29 **Abstract**

30 Cyanobacterial blooms are observed when high cell densities occur and are
31 often dangerous to human and animal health due to the presence of
32 cyanotoxins. Conventional drinking water treatment technology struggles to
33 efficiently remove cyanobacterial cells and their metabolites during blooms,
34 increasing costs and decreasing water quality. Although field applications of
35 hydrogen peroxide have been shown to successfully suppress cyanobacterial
36 growth, a rapid and accurate measure of the effect of oxidative stress on
37 cyanobacterial cells is required. In the current study, H_2O_2 (5 and 20 mg L^{-1})
38 was used to induce oxidative stress in *Microcystis aeruginosa* PCC 7813.
39 Cell density, quantum yield of photosystem II, minimal fluorescence and
40 microcystin (MC-LR, -LY, -LW, -LF) concentrations were compared when
41 evaluating *M. aeruginosa* cellular stress. Chlorophyll content (determined by
42 minimal fluorescence) decreased by 10% after 48 hours while cell density
43 was reduced by 97% after 24 hours in samples treated with 20 mg L^{-1} H_2O_2.
44 Photosystem II quantum yield (photosynthetic activity) indicated
45 cyanobacteria cell stress within 6 hours, which was considerably faster than

46 the other methods. Intracellular microcystins (MC-LR, -LY, -LW and -LF)
47 were reduced by at least 96% after 24 hours of H_2O_2 treatment. No increase
48 in extracellular microcystin concentration was detected, which suggests that
49 the intracellular microcystins released into the surrounding water were
50 completely removed by the hydrogen peroxide. Thus, photosynthetic
51 activity was deemed the most suitable and rapid method for oxidative cell
52 stress detection in cyanobacteria, however, an approach using combined
53 methods is recommended for efficient water treatment management.
54
55 **Keywords:** Cyanobacteria, fluorescence, microcystin, water treatment,
56 hydrogen peroxide.
57

58 1. Introduction

59 Climate change, and eutrophication contribute to cyanobacterial blooms in
60 freshwater reservoirs (Weenink et al., 2015). Cyanobacteria can be a threat
61 to drinking water quality since they are potential producers of a wide variety
62 of toxins. These toxins are a hazard to both human and animal health
63 (Falconer et al., 1983; Jochimsen et al., 1998; Pinho et al., 2015) and high
64 cell densities may complicate the potable water treatment process by
65 reducing filter run times and increasing the use of chemicals (e.g.,
66 coagulants and disinfectants), which raises the cost of water treatment (De
67 Julio et al., 2010).
68 Microcystins (MC) are one of the most commonly reported cyanotoxins
69 found in freshwater (Pinho et al., 2015). These toxins are cyclic
70 heptapeptides that share a common structure with two amino acid domains
71 which vary in positions 2 and 4 of the structure (Rinehart et al., 1994;

72 Harke et al., 2016). Currently, there are at least 247 identified microcystin
73 analogues (Spoof and Catherine, 2017). Microcystins are normally localized
74 inside the cells and are mostly released to the water after cell membrane
75 lysis and death (Tsai, 2015).
76 Conventional water treatment processes (coagulation, flocculation,
77 sedimentation and filtration) can be ineffective in removing high quantities
78 of cyanobacteria and their metabolites (Fan et al., 2013a; Zhou et al.,
79 2014). Chemical treatments using conventional oxidants may be used to
80 help remove cyanobacteria (e.g., ozone, chlorine, potassium permanganate
81 and chlorine dioxide) but can cause cell damage and the release of
82 cyanotoxins (Zamyadi et al., 2011; Chang et al., 2018). Due to the
83 inefficiency of conventional water treatment for the removal of
84 cyanobacteria and their toxins, it is necessary to evaluate complementary
85 technologies that can be applied in freshwater reservoirs, i.e., eliminating
86 cyanobacteria and their toxins prior to them entering the treatment plant.
87 Hydrogen peroxide (H_2O_2) has been applied as an algaecide to control
88 cyanobacterial blooms. H_2O_2 can generate reactive oxidative species (ROS)
89 that have high oxidative strength and are capable of compromising the cell
90 wall. Due to this, the detection of cell stress and/or damage is important
91 because it indicates the efficacy of the method and potential toxin release
92 (Chow et al., 1998).
93 Studies have suggested that H_2O_2 is an effective algaecide for
94 cyanobacterial treatment in-reservoir, however, it is necessary to compare
95 and verify the most suitable and rapid method to analyse the effects of H_2O_2
96 on cyanobacteria.

Cyanobacteria are particularly susceptible to H_2O_2 due to their physiology. One of the factors promoting the use of hydrogen peroxide is that it can be used directly in freshwater reservoirs as an algaecide to oxidize cyanobacterial cells and their toxins (Fan et al., 2013b; Fan et al., 2019) without producing oxidant residuals, as it decomposes into water and oxygen (Barroian and Feuillade, 1986).

Cell integrity and evaluation of cell numbers can be used to determine the effects of treatment technologies on cyanobacteria. Analytical methods to determine the effect of treatments on cyanobacteria, such as fluorescence detection of photosynthetic activity, can be used as a measure of cell stress providing an indirect measure of photoinhibition in photosynthesis (Campbell et al., 1998; Yang et al., 2013; Schuurmans et al., 2015; Weenink et al., 2015; Ogawa et al., 2017).

In this study, a range of methods for identifying cell stress in the cyanobacterium *Microcystis aeruginosa* PCC 7813 under the effect of different concentrations of H_2O_2 were compared to identify which analytical method provides the most accurate and rapid response. Further, although several studies have analyzed the effects of H_2O_2 on MC-LR (Qian et al., 2010; Matthijs et al., 2012; Papadimitriou et al., 2016; Kansole and Lin, 2017; Chang et al., 2018; Fan et al., 2019; Wang et al., 2015, 2018, 2019), here, for the first time, the degradation of intra- and extracellular concentration of four different microcystin analogues (MC-LR, MC-LF, MC-LY, and MC-LW) was evaluated under different concentrations of H_2O_2.

2. Materials and Methods

2.1 Cyanobacteria

123 The cyanobacterium *M. aeruginosa* PCC 7813 (Pasteur Culture Collection,
124 Paris) was cultured in BG-11 medium (Stanier et al., 1971) at 21±1 °C on a
125 12/12 h light/dark cycle illuminated by cool white fluorescent lights
126 (correlated color temperature 1400K to 5000K) with an average illumination
127 of 10.5 μmol photons m^{-2} s^{-1} without agitation. This particular strain of *M.*
128 *aeruginosa* produces four main microcystin analogues (MC-LR, MC-LY, MC-
129 LW, and MC-LF).
130

131 **2.2 *M. aeruginosa* PCC 7813 cell enumeration**

132 Cell counting by Multisizer for *M. aeruginosa* numbers and determination of
133 average cell diameter has been previously demonstrated (Wojtasiewicz and
134 Stoń-Egiert, 2016; Kim et al., 2020). A Multisizer 3 (Beckman Coulter, USA)
135 was used to enumerate *M. aeruginosa* PCC 7813 cell density, to evaluate
136 biovolume and average cell diameter. A 50 μm aperture was used, which
137 allows particle size detection from 1 to 30 μm. Samples were diluted 100 to
138 600-fold in Isoton carrier liquid (Beckman Coulter, USA), depending on the
139 sample density.
140

141 **2.3 Effect of H_2O_2 on cyanobacterium *M. aeruginosa* PCC 7813**

142 A 100 mL cell suspension of *M. aeruginosa* PCC 7813 (in 250 mL conical
143 flasks) with a final concentration of 5 x 10^6 cells mL^{-1} in BG-11 was
144 prepared and cultured for three days. Hydrogen peroxide (5 and 20 mg L^{-1})
145 was added to the conical flasks containing *M. aeruginosa* PCC 7813. Aliquots
146 of 3 mL were removed at known intervals (0, 6, 12, 24, 30, 36, 48, 60, 72,
147 84 and 96 h) over 4 days. Samples were incubated under the same
148 conditions as the strain was initially cultured. Treatments were performed in

149 triplicates. Aliquots were removed for analysis of H_2O_2 concentration (100
150 µL) and cell enumeration (900 µL), intra/extracellular microcystin
151 determination (1 mL) and photosynthetic activity measurements (1 mL).
152 The aliquots for toxin analysis were centrifuged for 10 minutes at 13000 x g
153 and the supernatant was transferred to a fresh microcentrifuge tube (1.5
154 mL) and stored at -20 °C, with the cell pellet also stored at -20 °C. The
155 aliquots for all other analyses were used immediately. A negative control
156 (no H_2O_2 addition) was also prepared in triplicate.
157

158 **2.4 Analysis**

159 **2.4.1 H_2O_2 analysis**

160 To determine the H_2O_2 concentration, a method by Drábková et al. (2007a)
161 with modifications by Fan et al. (2013b) was used. A phosphate buffer
162 solution was prepared with 0.5 M sodium phosphate dibasic (Na_2HPO_4)
163 solution and 0.5 M sodium phosphate monobasic (NaH_2PO_4) solution with a
164 final pH of 6 (all Sigma-Aldrich, UK). A solution with 0.1 g of N,N-diethyl-
165 1,4-phenylendiammoniumsulphate (Sigma-Aldrich, UK) in 10 mL of 0.1 N
166 sulfuric acid (H_2SO_4, Fisher, UK) was prepared (DPD solution). Further, a
167 horseradish peroxidase (HRP) (Sigma-Aldrich, UK) solution 1 mg L^{-1} in
168 ultrapure water was prepared.
169 For analysis, 900 µL of ultrapure water and 100 µL of phosphate buffer
170 solution were transferred into a 1 mL cuvette. Aliquots of the cell
171 suspension were centrifuged and 40 µL of the supernatant were added to
172 the cuvette, followed by 40 µL of DPD solution and 10 µL of HRP solution. A
173 blank was prepared by adding 900 µL of ultrapure water followed by 100 µL
174 of buffer solution, 40 µL of the supernatant from the control and 40 µL of

175 DPD solution into a cuvette. All the samples were measured using a UV/VIS
176 spectrophotometer (WPA Lightwave II, UK) at a wavelength of 551 nm.
177 H_2O_2 (30%, Fisher, UK) was used for the H_2O_2 degradation assay. Sodium
178 Thiosulfate ($Na_2S_2O_3.5H_2O$, Fisher, UK) was added to excess into the
179 supernatant after the H_2O_2 determination to quench the sample.
180

181 **2.4.2 High-performance liquid chromatography (HPLC) analysis of**
182 **extra- and intracellular microcystin concentrations**

183 The supernatant was removed and freeze-dried. Aliquots were resuspended
184 in methanol (1 mL), vortexed and centrifuged for 10 minutes at 13000 G.
185 Following this, 950 µL were transferred to a fresh microcentrifuge tube (1.5
186 mL) and dried in a Genevac (EZ-II evaporator, UK), resuspended in 80%
187 methanol (100 µL) and analyzed. To the cell pellet, 80% aqueous methanol
188 (250 µL) was added and sample tubes placed in a dispersive extractor for 5
189 minutes at 2500 rpm followed by centrifugation for 5 minutes at 13000 G
190 and if not analyzed immediately, samples were stored at -20 °C until
191 analysis.
192 The concentrations of four microcystin analogues (MC-LR, MC-LY, MC-LW,
193 and MC-LF) were quantified using a 2965 separation module and a 2996
194 photodiode array (PDA) detector (Waters, Elstree, UK). Separation of
195 analytes was achieved on a Symmetry C18 column (5 µm particle size, 2.1
196 mm IDx 150 mm long; Waters, Elstree, UK). The mobile phases used for
197 analysis were A: ultrapure water (18.2 MΩ) and B: acetonitrile each with
198 0.05% trifluoroacetic acid at a flow rate of 0.3 mL min^{-1}, an injection
199 volume of 35 µL and a column temperature of 40 ^{o}C. Initial condition was
200 set to 80% A and 20% B, increasing to 70% B over 25 minutes followed by

201 an organic wash and a return to the initial condition. All chromatograms
202 were analyzed at 238 nm and quantified using standards (as per Enzo Life
203 Sciences) for calibration between 0.05 and 25 µg mL^{-1} in the Empower
204 software (V3). The limit of quantification was 0.05 µg mL^{-1}.
205

206 **2.4.3 Determination of photosynthetic activity**

207 A Mini-PAM system (Walz, Germany) was used at room temperature to
208 determine the effect of H_2O_2 on the photosynthetic activity. This instrument
209 evaluates the photosynthetic activity by measuring the maximal values of
210 quantum yield of photosystem II (PSII) (F_V/F_M), where F_V is the difference
211 between the true maximal fluorescence (F_M) and the minimal fluorescence
212 (F_0).

213 F_0 is determined by emitting a low intensity measuring light for 20 seconds,
214 followed by a saturating pulse, which yields the maximal fluorescence (F_M).
215 After 40 seconds, actinic light is activated (actinic light intensity at specified
216 level 3), which allows the determination of the steady-state fluorescence
217 (F_S) (Ogawa et al., 2017). This is true for higher plants and green algae,
218 however, the photosynthetic complex in cyanobacteria functions differently
219 due to an effect called state transition. This means that there is a change in
220 energy allocation between the two photosystems (PSI and PSII) in the cells,
221 resulting in more energy in PSI (Schuurmans et al., 2015; Ogawa et al.,
222 2017). Due to this, it is necessary to add diuron (Sigma-Aldrich, UK), an
223 algaecide capable of inhibiting photosynthesis, under actinic light to detect
224 the true maximal fluorescence in cyanobacteria (F_M') by a saturating pulse
225 (Ogawa et al., 2017).

226 A sample of cells (400 µL) was added into a cuvette containing a small
227 stirrer bar for agitation. After measuring F_0 and F_M readings, Diuron (0.5 M)
228 was added to photoquench the sample and measure F_M' (Campbell et al.,
229 1998).
230

231 **2.5 Statistical analyses**

232 The values shown are the results of the mean of triplicates and all results
233 were analyzed using one-way ANOVA. A significance level of $p<0.05$ was
234 used to identify significant differences between the results.
235

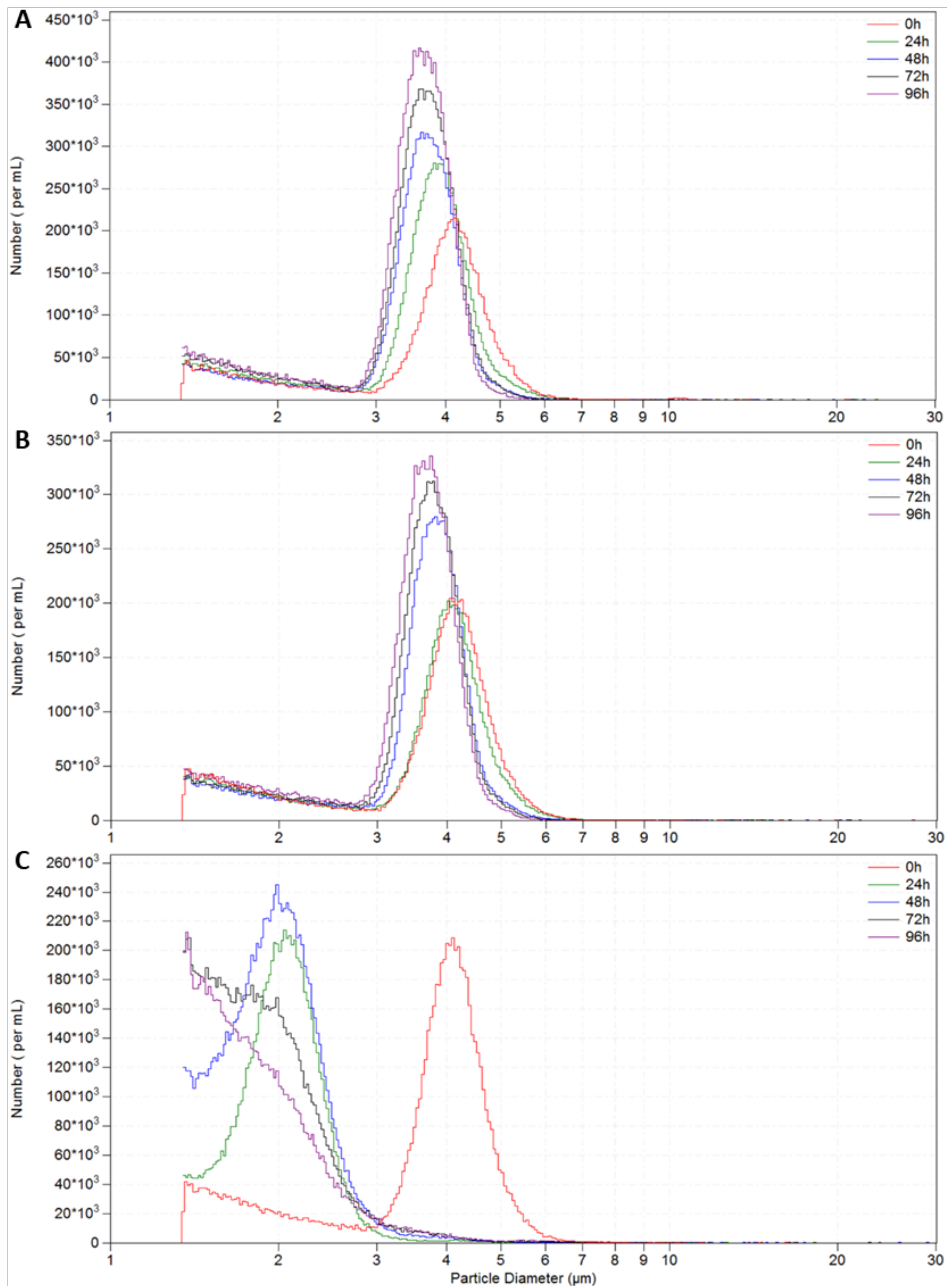
236 **3. Results and Discussion**

237 **3.1. Cell enumeration and characterization**

238 When analyzing *M. aeruginosa* PCC 7813 cell density, the distribution was
239 observed for the particle diameter range of 2.8 to 6.9 µm (Figure 1A and
240 1B) for all the samples at time T_0 which were considered cells. Particles in
241 the range of 1.3 to 2.7 µm were considered cell fragments. The decision to
242 consider particles in the range of 2.8 and 6.9 µm cells was based on the
243 size distribution of the initial sample (Figure 1) and published data. For
244 example, Komárek et al. (2002) report average cell sizes from 4 to 6 µm for
245 *M. aeruginosa* and Harke et al. (2016) report cell sizes from 1 to 9 µm for
246 the genus *Microcystis*. A decrease in the average particle diameter and an
247 increase in the distribution maxima caused by a rise in cell density were
248 observed in the control (Figure 1A) and the 5 mg L^{-1} (Figure 1B) samples
249 from 0 to 96 hours. The particle diameter decrease is likely due to
250 cyanobacterial reproduction by binary fission, which leads to a decrease in

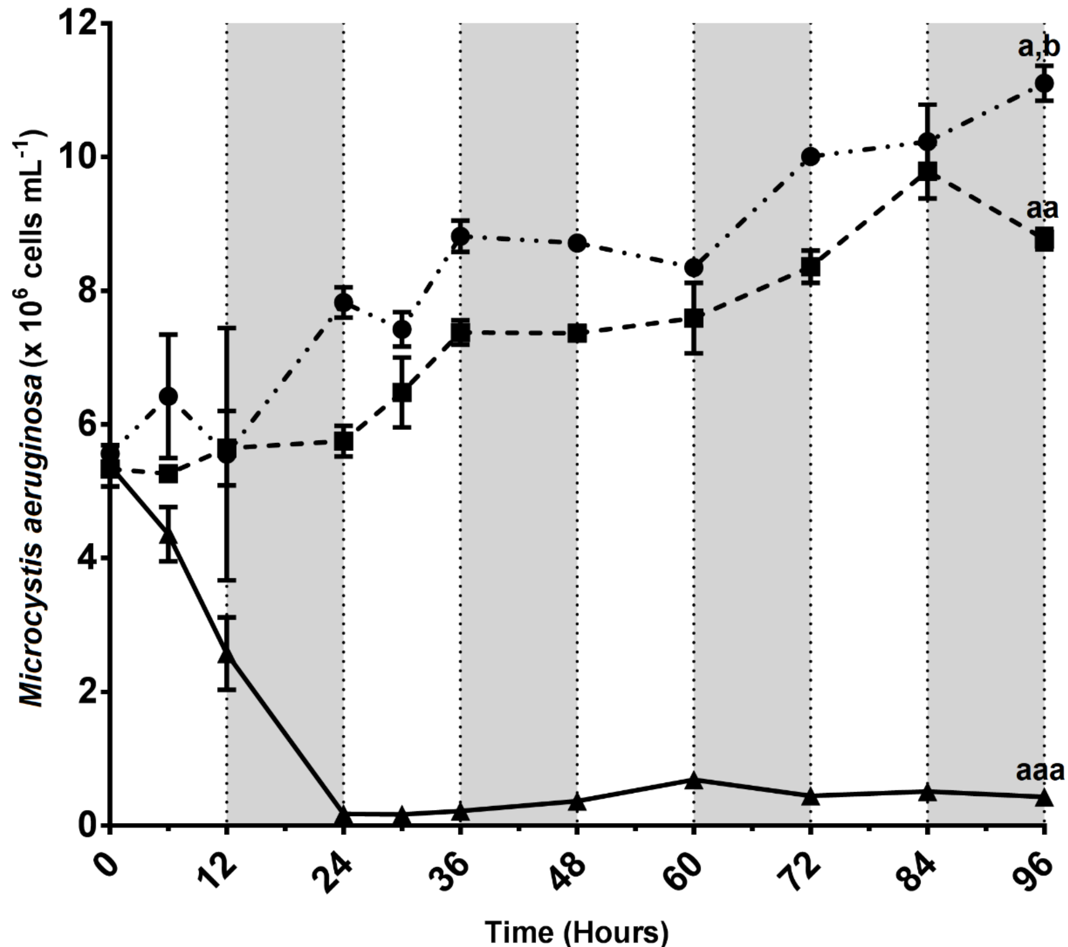
251 individual cell diameter due to cell division (Cassier-Chauvat and Chauvat,
252 2014).
253 The 20 mg L^{-1} of H_2O_2 caused extensive cell damage to *M. aeruginosa* PCC
254 7813, increasing the number of fragments (particles in the range of 1.3 to
255 2.8 μm) (Figure 1C). The effect of 20 mg L^{-1} H_2O_2 was immediate with cells
256 rapidly fragmenting by 97%. Over the next 24 hours the cell density
257 increased slightly again from about 45 hours onwards (Figure 2), which
258 could represent post-treatment recovery. Although cells did not show
259 immediate removal in the 5 mg L^{-1} H_2O_2 samples, there is evidence of
260 growth inhibition with cell density significantly lower ($p=1 \times 10^{-4}$) than that
261 of the control (Figure 2).

262
263



264 **Figure 1:** *Microcystis aeruginosa* PCC 7813 cell density (A) control, (B) 5 and (C)
265 20 mg L^{-1} hydrogen peroxide over 96 hours under cool white fluorescent lights of
266 10.5 µmol photons m^{-2} s^{-1}.

267



268

269 **Figure 2:** *Microcystis aeruginosa* cell density for particle size range 2.8 to 6.9 µm
270 after exposure to 0 (●), 5 (■) and 20 (▲) mg L^{-1} H_2O_2 over 96 hours under cool
271 white fluorescent lights of 10.5 µmol photons m^{-2} s^{-1}. Grey sections on the graph
272 denote dark time. a, aa, aaa = significantly different from time T_0; b = significantly
273 different from 96 hours 5 mg L^{-1}. (n = 3, error bars = σ_{-1}).

274

275 Particles in the range from 1.3 to 2.8 µm were classified as cell debris
276 (Figure 3), which included damaged cells and cell fragments. No significant
277 change (p=9 x 10^{-2}) was observed between 0 mg L^{-1} and 5 mg L^{-1} H_2O_2.
278 However, the amount of cell debris in the 20 mg L^{-1} sample significantly
279 increased (p=9.2 x 10^{-6}) over 60 hours of exposure as cells continued to
280 fragment. There was an increase of cell fragment density over the first 60
281 hours which then decreased as the cell debris continued to break down into
282 smaller fragments to the point where fragments had decreased in size to

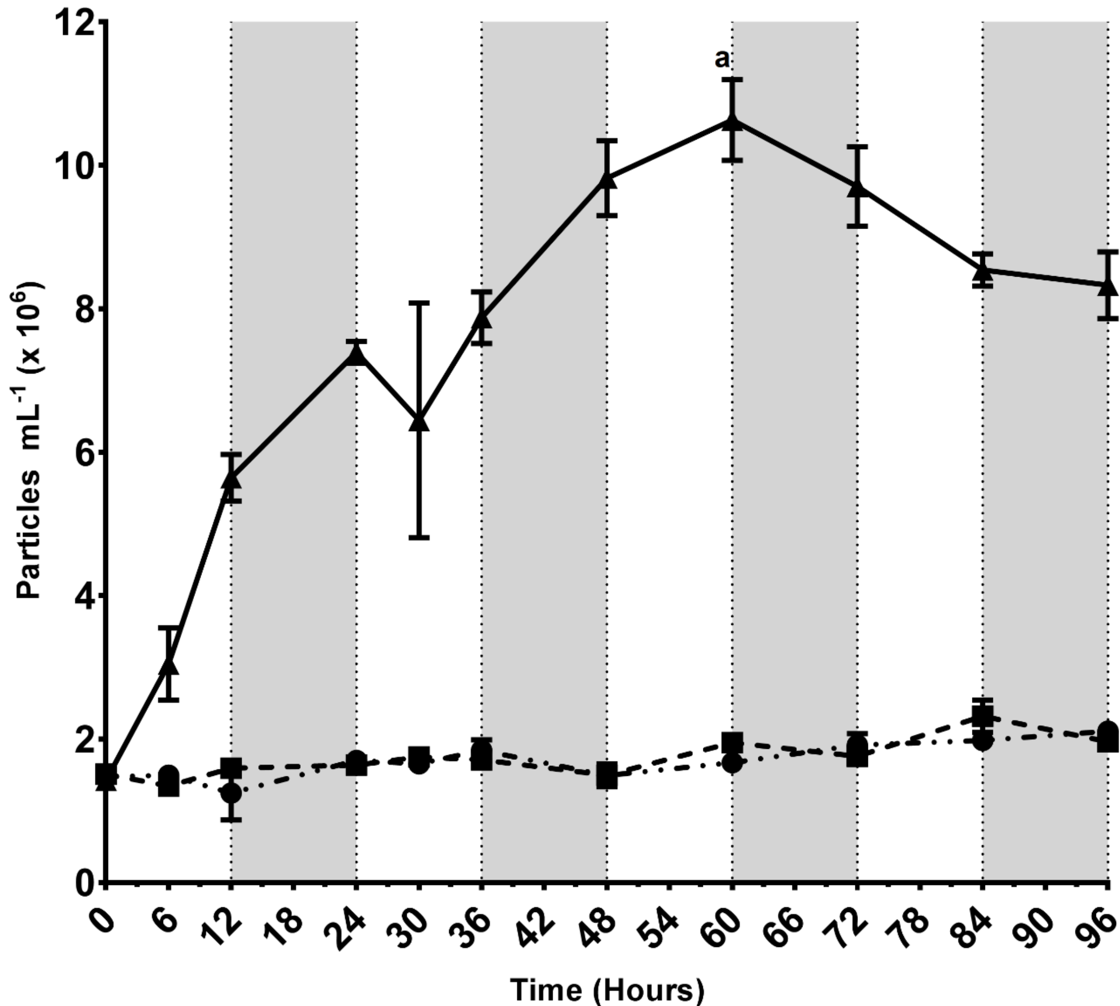
283 below 1.3 μm and were undetectable (Figure 1C). A similar observation was
284 made by Fan et al. (2013b) where the effects of hydrogen peroxide doses
285 (10.2, 51 and 102 mg L^{-1}) on *M. aeruginosa* (strain 338) membrane
286 integrity were evaluated over 7 days. There were no significant differences
287 in cyanobacterial cell density after 7 days of exposure to H_2O_2, although it
288 was possible to observe a decrease in cell density in the present study when
289 using 20 mg L^{-1}. However, despite the differences in cell density results
290 between the two studies, in Fan et al. (2013b), the cells decreased in size
291 and fragmented under all the H_2O_2 concentrations which suggests a similar
292 response as indicated by the cell debris observed in the current study.
293 The cyanobacterial degradation processes by H_2O_2 occur by the production
294 of ROS that attack and destroy the cyanobacterial cell membrane. After
295 that, ROS enter the cell resulting in photoinhibition while the cyanobacterial
296 intracellular material is released into the extracellular matrix. Finally, ROS
297 facilitate the oxidation of pigments (e.g., chlorophyll *a*) (Wang et al., 2017).
298 Comparing the current study with the study of Fan et al. (2013b), there
299 were differences in the study design that could explain the results observed:
300 the growth light intensity and the light intensity used during the current
301 experiment were almost five times lower compared to the Fan et al.
302 (2013b) study. Further, the growth media used in the current study (BG-
303 11) had a higher iron content than the one used (ASM-1) in the study by
304 Fan et al. (2013b). This higher iron content may lead to photo-fenton
305 reactions, intensifying cell disruption in the present study. The toxicity of
306 H_2O_2 on cyanobacteria depends on several factors such as light intensity,
307 pre-adaptation to growth at a higher light intensity and the generation of
308 hydroxyl radicals by the photo-fenton reaction of H_2O_2 with Fe^{2+} ions

309 present in the medium (Drábková et al., 2007a; Chen et al., 2016). Further,

310 a different strain of *M. aeruginosa* was used in the current investigation

311 which might cause further differences in the results.

312



313

314 **Figure 3**: *Microcystis aeruginosa* PCC 7813 particle density for particle size range
315 1.3 to 2.8 µm after exposure to 0 (●), 5 (■) and 20 (▲) mg L^{-1} H_2O_2 over 96 hours
316 under cool white fluorescent lights of 10.5 µmol photons m^{-2} s^{-1}. Grey sections on
317 the graph denote dark time. a = significantly different from time T_0. (*n = 3,* error
318 bars = σ_{-1}).

319

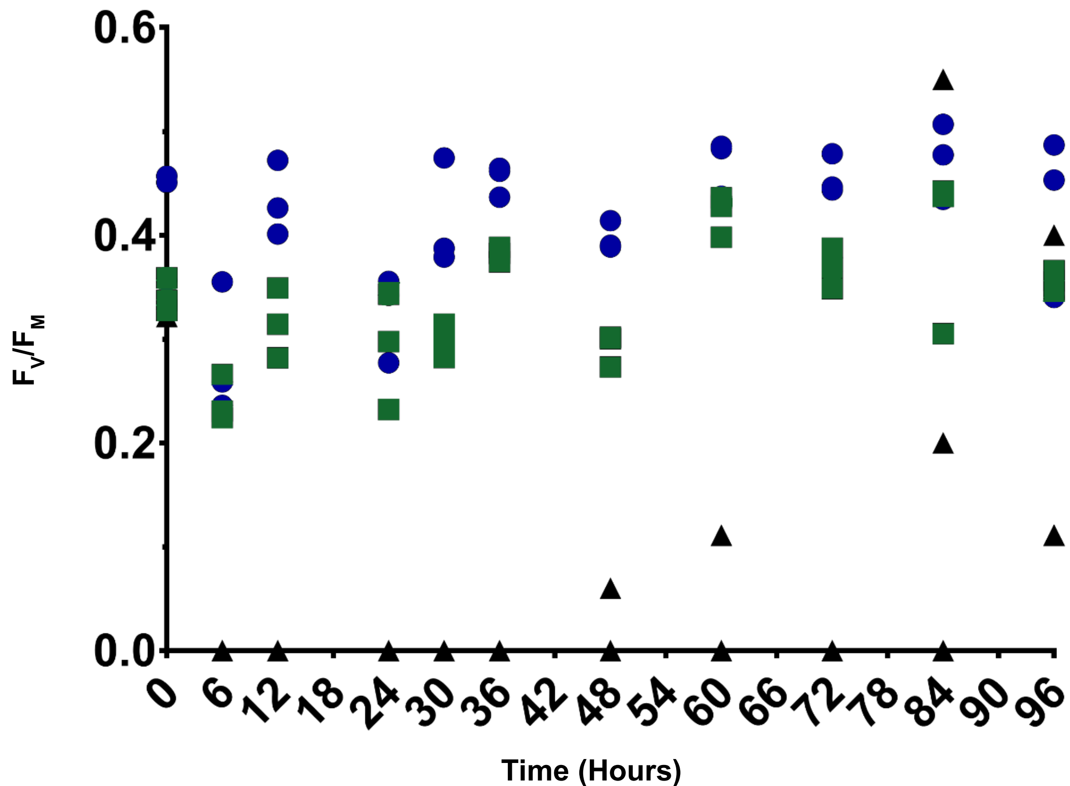
320 **3.2 *M. aeruginosa* photosynthetic activity assay**

321 A factor that can represent the level of stress in a cyanobacterial cell is

322 photosynthetic activity as expressed as the F_V/F_M ratio (Yang et al., 2013).

323 Photosynthesis is the primary production in cyanobacteria/algae. Energy in

324 the form of light is captured and drives the synthesis of sugar while
325 consuming carbon dioxide and generating oxygen. The addition of H_2O_2 can
326 generate the production of intracellular ROS that are mainly created in
327 cyanobacteria when the absorption of light energy by chlorophyll *a* is higher
328 than the amount of energy that can be used by the photosynthetic
329 apparatus of the cell. These ROS cause damage in cyanobacteria by
330 blocking the electron transport of PSII thus decreasing the photosynthetic
331 activity in a process known as photoinhibition (Lupínková and Komenda,
332 2004; Wang et al., 2019). Photoinhibition in cyanobacteria causes a
333 decrease in F_V/F_M and, when F_V/F_M is close to or zero, the cells are so
334 damaged or stressed that photosynthetic activity is absent. F_V/F_M was not
335 significantly different for the control and the 5 mg L^{-1} samples of the
336 experiment (for all samples $p>0.05$; Figure 4). F_V/F_M decreased in the 20
337 mg L^{-1} samples and the photosynthetic activity was inhibited from 6 to 48
338 hours indicating that the photosynthetic system of *M. aeruginosa* cells was
339 inhibited (Figure 4). It must be noted that the observed increase in F_V/F_M
340 after 48 hours is likely to be an artifact as one of the triplicate samples
341 started showing signs of recovery.
342 The immediate decrease of F_V/F_M was detected following 6 hours of
343 exposure to 20 mg L^{-1} H_2O_2. It is possible that F_V/F_M was affected before 6
344 hours of treatment but was not detected earlier because the first sampling
345 was only performed after six hours of exposure to H_2O_2.
346



347

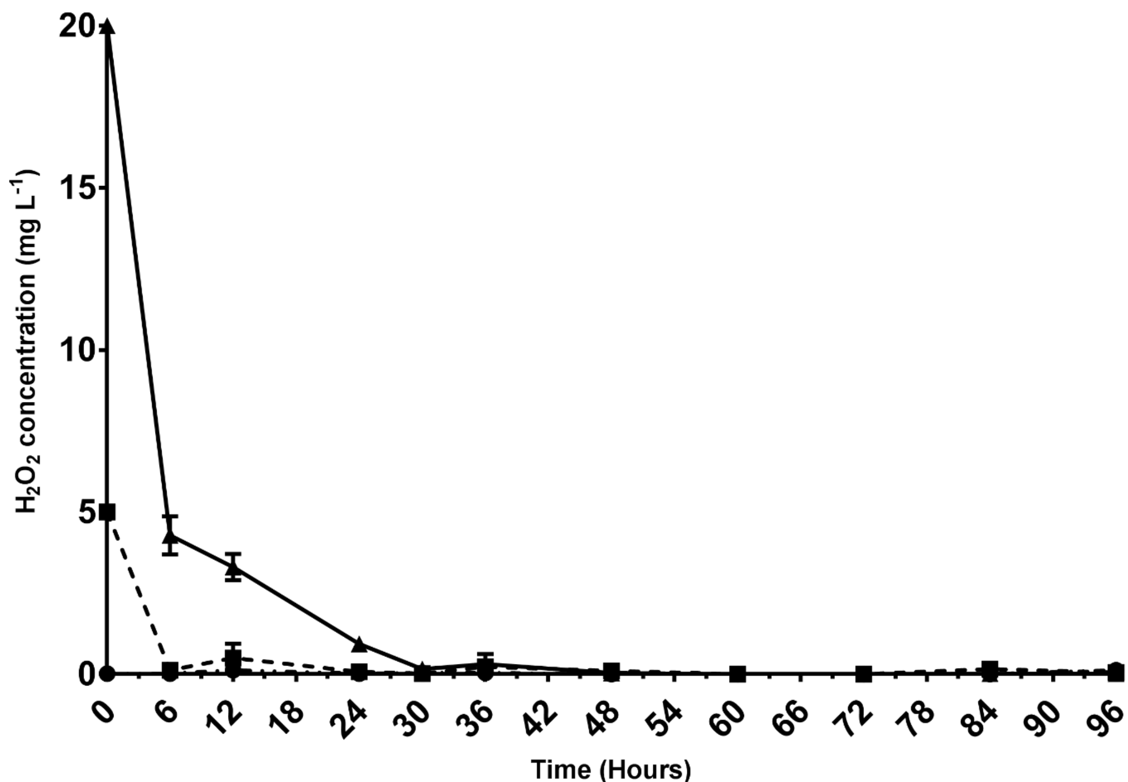
348 **Figure 4**: *Microcystis aeruginosa* PCC 7813 maximal quantum yield results after
349 being treated with 0 (●), 5 (■) and 20 (▲) mg L^{-1} H_2O_2 over 96 hours under cool
350 white fluorescent lights of 10.5 µmol photons m^{-2} s^{-1}. Each point represents the
351 individual replicates.

352

353 The difference between replicates in the 20 mg L^{-1} dose from 60 hours
354 onwards can be explained by the fact that the differences after the
355 photoinhibition were very small (Figure 4), therefore even small variance
356 can cause large error. The F_0 varied between 7 and 15 and the F_M varied
357 between 7 and 24. Further, one of the samples showed signs of recovery
358 with a F_V/F_M increasing from 0.06 to 0.44 between 48 and 60 hours.
359 The H_2O_2 concentration was analyzed over the 96 hours of treatment. After
360 30 hours, the H_2O_2 was completely consumed in the 5 and 20 mg L^{-1}
361 samples (Figure 5). The fast decrease of the H_2O_2 concentration in the 20
362 mg L^{-1} samples could have made recovery possible for the *M. aeruginosa*

363 PCC 7813 cells, which, in turn, would have prevented longer-term

364 suppression.



365

366 **Figure 5**: Decrease in hydrogen peroxide concentration of different dosages 0 (●),
367 5 (■) and 20 (▲) mg L^{-1} over 96 hours in the presence of *Microcystis aeruginosa*
368 PCC 7813 under cool white fluorescent lights of 10.5 µmol photons m^{-2} s^{-1} (*n = 3,*
369 error bars = σ_{-1}).

370

371 Several investigations described the behavior of *M. aeruginosa*

372 photosynthetic yield when treated with different H_2O_2 concentrations. In a

373 study by Wang et al. (2019), *M. aeruginosa* FACHB-905 was treated with

374 different concentrations of H_2O_2 (0, 2, 5, 8 and 10 mg L^{-1}) for 2 hours. A

375 decrease in the F_V/F_M was observed for every dosage used in the study by

376 Wang et al. (2019), even for lower dosages than the H_2O_2 concentration

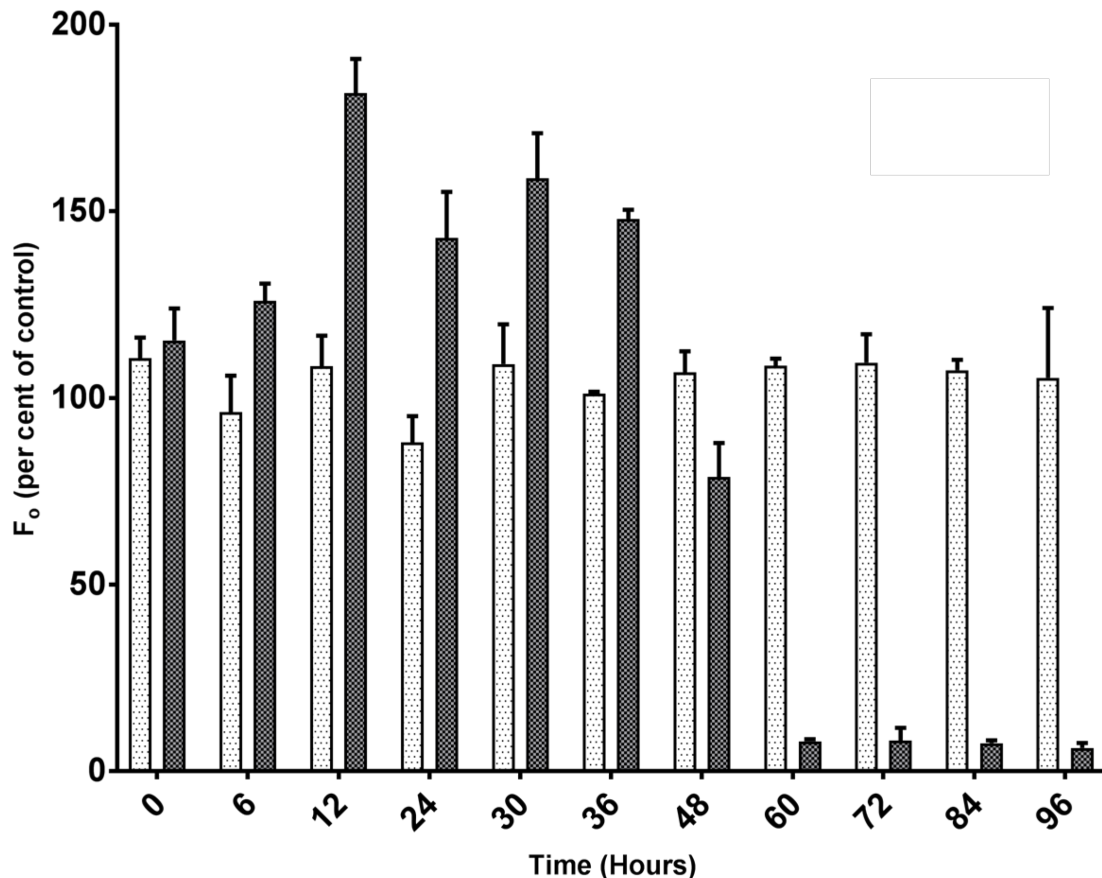
377 used in the present investigation (20 mg L^{-1}). This decrease could be

378 because the photosystem II of the cells was not completely quenched, so

379 part of the energy was allocated to photosystem I. This reallocation of light

380 energy from one photosystem to another in cyanobacteria is called state
381 transition (Ogawa et al., 2017). To suppress state transition, diuron was
382 added in the current study, allowing a determination of the true maximal
383 fluorescence F_M value in cyanobacteria. Further, in the Wang et al., (2019)
384 study the cells were centrifuged before analysis, which could have further
385 affected the cell stress response.
386 Wang et al. (2018) demonstrated the effects of H_2O_2 at different dosages
387 (0, 2, 5 and 10 mg L^{-1}) on *M. aeruginosa* FACHB-905 cells using the same
388 initial cell density analyzed in the present study. In the Wang et al. (2018)
389 study, a significant decrease in the F_V/F_M ratio after 72 hours was observed
390 even when using lower dosages of H_2O_2 (5 mg L^{-1}) compared to the present
391 study (20 mg L^{-1}). Again, this could have happened due to the reallocation
392 of energy to PSI, decreasing F_V/F_M value as no addition of state transition-
393 suppressing chemicals was reported. The higher illumination and
394 temperature used by Wang et al. (2018), 40 µmol m^{-2} s^{-1} at 25 °C
395 compared to 10.5 µmol m^{-2} s^{-1} and 21°C, could be another factor that
396 influenced the results.
397 Other studies evaluated different cyanobacterial species. Weenink et al.
398 (2015) studied the effects of different concentrations of H_2O_2 (2.5, 5, 10, 20
399 and 50 mg L^{-1}) treating *Planktothrix*-dominated lake samples containing
400 three phytoplankton groups (cyanobacteria, green algae, and diatoms). In
401 the concentrated sample, the fluorescence value increased after 4 days of
402 treatment in all H_2O_2 concentrations, showing similar signs of recovery from
403 the ones found in the current study. In another large-scale study using
404 different cyanobacterial species, Matthijs et al. (2012) verified a similar
405 decrease in the photosynthetic viability when analyzing *Planktothrix*

406 *agardhii*-dominated lake samples under the effects of lower dosages of H_2O_2
407 (0, 0.5, 1, 2 and 4 mg L^{-1}).
408 In the present study, the F_0 was used as an indirect indicator of chlorophyll
409 *a* concentration which plays a central role in cyanobacterial photosynthesis
410 (Qian et al., 2010). After 6 hours, the impact on photosynthetic activity
411 (decrease in F_V/F_M) was already obvious in the 20 mg L^{-1} samples (Figure 4)
412 but a decrease in F_0 (i.e., chlorophyll *a*) was only noticed after 48 hours of
413 H_2O_2 exposure (Figure 6).
414 Increase in the fluorescence in 20 mg L^{-1} samples when compared to the
415 control between 6 and 36 hours (Figure 6) was also observed by Chen et al.
416 (2016). When analyzing the effects of 0, 1, 5, and 20 mg L^{-1} on lake
417 samples dominated by *Microcystis* sp. and *Anabaena* sp. over 72 hours,
418 they also found a chlorophyll *a* increase between 12 and 24 hours for 5 and
419 20 mg L^{-1} H_2O_2 samples. An increase in F_0 values is connected to cell
420 damage after stress (Drábková et al., 2007b) so it is possible that the
421 increase in F_0 is a fast chemical response due to an increase in measurable
422 chlorophyll *a* as a cell protection mechanism or a physical change in the
423 cells which allow more chlorophyll *a* to be detected.



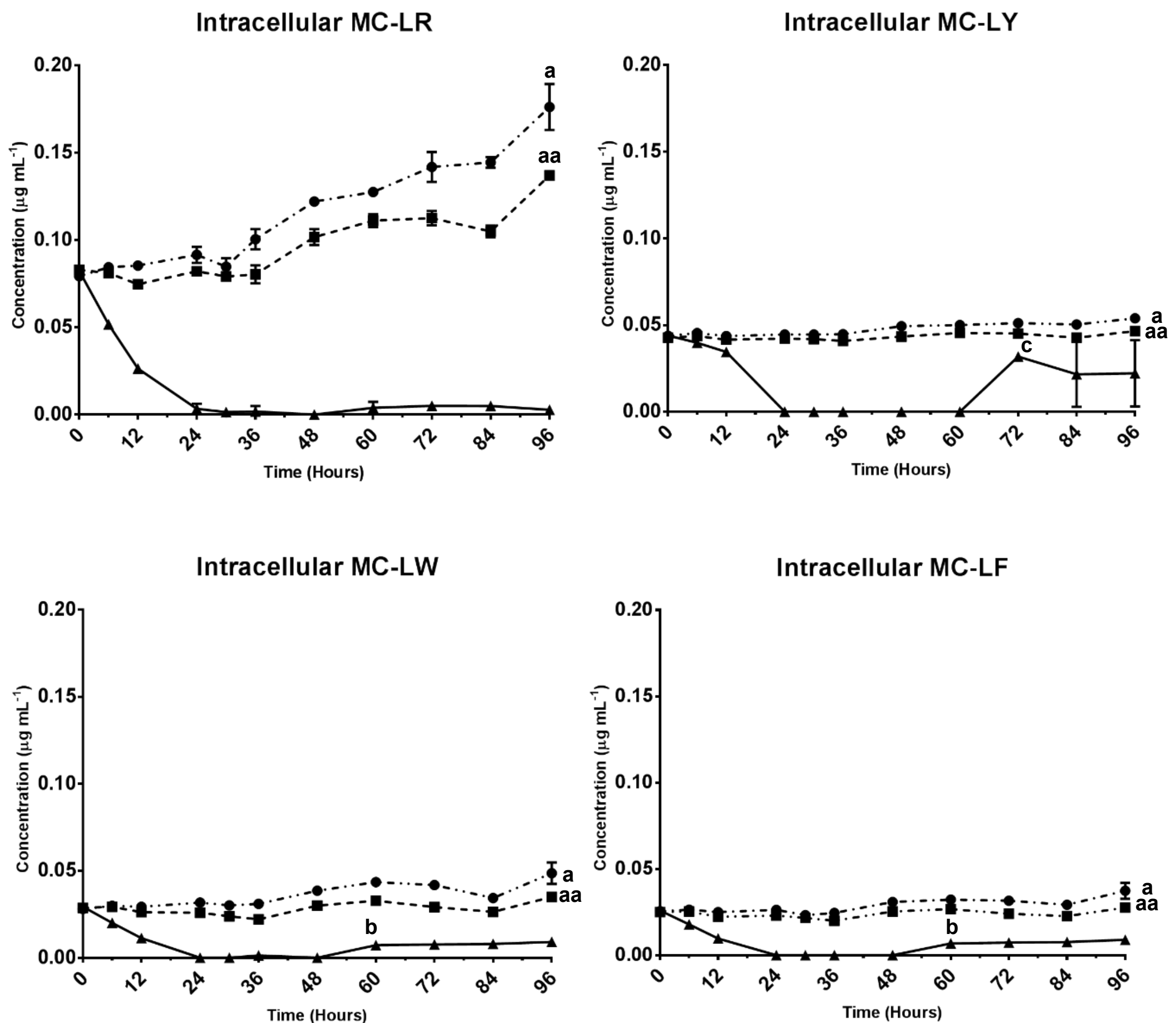
424
425 **Figure 6**: *Microcystis aeruginosa* PCC 7813 minimal fluorescence (indicative of
426 chlorophyll *a*) results (percentage of the control) after being treated for 96
427 hours with different H_2O_2 dosages ( ) 5 and ( ) 20 mg L^{-1} under cool white
428 fluorescent lights of 10.5 µmol photons m^{-2} s^{-1} with different dosages of H_2O_2 (*n* =
429 *3,* error bars = σ_{-1}).

430

431 3.3 Effect of H_2O_2 on intracellular and extracellular microcystins

432 The removal efficiency of microcystins by H_2O_2 depended on the dosage of
433 H_2O_2 (Figure 7). All four intracellular microcystin analogues were completely
434 degraded after 24 hours of treatment when using 20 mg L^{-1} H_2O_2, i.e., as
435 cells were lysed and microcystins released, toxin was rapidly degraded.
436 However, a significant increase of intracellular MC-LW and -LF after 60
437 hours, and in MC-LY after 72 hours (for all the samples tested $p<0.05$) was
438 observed. There was an increase ($p<0.05$) of intracellular MC-LR, -LY, -LW
439 and -LF after 96 hours of exposure when using 0 and 5 mg L^{-1} of H_2O_2 which

440 corresponds to the increase in *M. aeruginosa* PCC 7813 cell density (Figure
441 2) for the same dosages due to cell growth. The increase for MC-LR was
442 more marked than for the other microcystin analogues produced by *M.*
443 *aeruginosa* PCC 7813 which can be explained by the fact that MC-LR is the
444 main microcystin produced by this organisms and has been observed
445 previously (Pestana et al., 2020).



446
447 **Figure 7**: Removal of intracellular MC-LR, -LY, -LW and -LF after exposure to 0 (●),
448 5 (■) and 20 (▲) mg L^{-1} hydrogen peroxide over 96 hours under cool white
449 fluorescent lights of 10.5 µmol photons m^{-2} s^{-1}. a, aa = significantly different from
450 T_0; b = significantly different from 48 hours; c = significantly different from 60
451 hours. (*n = 3,* error bars = σ_{-1}).

452

453 Microcystins are normally contained within the cells, and are released into
454 the surrounding water along with other intracellular content when cell
455 integrity is compromised. H_2O_2 is capable of lysing cells and releasing the
456 microcystins. After the liberation of intracellular microcystins in all the 20
457 mg L^{-1} samples (Figure 7), no extracellular microcystin was detected. This
458 suggests that the intracellular microcystins that were released into the
459 surrounding water were rapidly removed by H_2O_2.
460 A few studies have investigated the removal of MC-LR by H_2O_2, but this is
461 the first time that the effect of H_2O_2 on these four microcystin analogues,
462 both intra- and extracellular, has been evaluated (MC-LR, -LY, -LW and -
463 LF). Papadimitriou et al. (2016) showed that both intra- and extracellular
464 MC-LR decreased over 4 hours in a study with naturally occurring MC-LR
465 and 4 mg L^{-1} H_2O_2. The study by Papadimitriou et al. (2016) observed that
466 the use of H_2O_2 caused cyanobacterial cell lysis followed by a release of
467 intracellular microcystin which became extracellular microcystin and then
468 was removed by H_2O_2. The successful removal of MC-LR even at lower H_2O_2
469 dosages was most likely due to a lower initial concentration of dissolved
470 toxins used by Papadimitriou et al. (2016), intracellular and extracellular
471 microcystins were below 2.5 μg L^{-1} and 0.5 μg L^{-1}, respectively.
472 Kansole and Lin (2017) showed similar results to the present study when
473 evaluating cyanobacterium *M. aeruginosa* PCC 7820 with a similar initial cell
474 density (2 x 10^6 cells mL^{-1}) and under the effects of several H_2O_2 dosages
475 (0, 1, 2, 3, 5, 10 and 20 mg L^{-1}). The highest dosage of H_2O_2 used was able
476 to reduce both MC-LR and *M. aeruginosa* PCC 7820 cells by 40% and 95%,
477 respectively. It should be noted that *M. aeruginosa* PCC 7813 is almost
478 identical to *M. aeruginosa* PCC 7820 (isolated from the same original

479 bloom), producing the same microcystins, with the exception that the PCC
480 7813 strain does not contain gas vesicles. As would be expected that the
481 absence of gas vesicles would not affect cell inhibition by H_2O_2.
482

483 **3.4 Comparison of cell stress detection methods**

484 While there are more sophisticated methods that can be used for cell stress
485 determination, such as flow cytometer with nucleioid acid dyes or cellular
486 ROS determination, the methods used in the current study to analyse
487 cyanobacterial cell stress were selected considering operation and
488 availability in a water treatment company laboratory. Each method
489 presented advantages and disadvantages for the determination of H_2O_2
490 induced oxidative stress in *M. aeruginosa* PCC 7813. It is possible to notice
491 an early stress response from the cells when analyzing the inhibition of
492 photosynthetic activity in cyanobacterial cells compared to other methods
493 (Table 1), such as cell density analysis, chlorophyll *a* and toxin
494 determination. While F_0 (the indicator of chlorophyll *a*) decreased after 48
495 hours (Figure 6) and cell density indicated complete removal after 24 hours
496 (Figure 2), F_V/F_M provided a more accurate and rapid response
497 demonstrating cyanobacteria cell stress detectable at 6 hours after
498 exposure to H_2O_2 (Figure 4). Chlorophyll *a* concentration is a poor indicator
499 of cellular stress, as it is initially intracellular and its concentration is only
500 affected a considerable time after cell lysis and intracellular matter leakage
501 into the surrounding water. Detection of chlorophyll *a* only indicates that
502 this pigment is present in the samples. It is not a measure of cell viability,
503 as demonstrated by the fact that a decrease in F_0 was detected only after
504 48 hours but decline cell density was clearly seen after 24 hours.

505 Similar limitations are true for microcystins as an indicator cell lysis and
506 death. After the degradation of the cell membrane, intracellular microcystin
507 is released into the surrounding water which was observable after 24 hours,
508 thus, while a reliable proxy measurement for cell integrity, the effects on
509 the intracellular microcystins are less rapid than measurement of the
510 photosynthetic activity. Since it is not possible to detect any differences in
511 extracellular microcystin concentration over 96 hours, this method is not
512 suitable to identify cyanobacterial cellular stress responses during oxidation.
513 The measurement of extracellular levels of microcystin as an indicator of
514 cell lysis is confounded by the on-going destruction of the toxins by the
515 oxidant. As the microcystins increase in concentration in the surrounding
516 media due to leakage from cells they will rapidly be chemically degraded by
517 the H_2O_2. Hence, the detected concentration is a result of the total excreted
518 microcystin minus the toxin which has been oxidized.
519 A further consideration when selecting a suitable cell stress detection
520 method is the applicability of the required equipment. The time from
521 sampling to results with the Mini-PAM fluorescence detection system is less
522 than 10 minutes and it can be used as a portable instrument. Furthermore,
523 the Mini-PAM can be operated on a boat during in-reservoir treatment,
524 compared to laboratory analysis taking at least one hour for sample
525 preparation and HPLC analysis of microcystins. While results by a particle
526 counter can be acquired in a similar time frame to the Mini-PAM, the
527 Multisizer can only be used in laboratory, however, both methods using the
528 Mini-PAM (indication of photosynthesis and chlorophyll *a*) are non-specific,
529 which means that in a natural water sample these would also measure the

530 photosynthesis and chlorophyll *a* from other organisms, such as diatoms

531 and green algae.

532

533 Table 1: Comparison of different analytical methods for cell stress determination

Method	Response time	Advantages	Disadvantages
F_v/F_M photosynthetic activity (photosynthesis)	6 hours	• Fastest response of cell stress • Applicable for *in-situ* and laboratory • Rapid analysis (< 10 minutes)	• Requires diuron which is hazardous • Non-specific (no differentiation between phytoplankton species)
Cell density (by particle counter)	24 hours	• Detects changes in particle distribution • Rapid analysis (< 10 minutes)	• Laboratory analysis • Does not differentiate viable from non-viable cells • Less suitable for filamentous organisms
Minimal fluorescence F_0 (chlorophyl *a*)	48 hours	• Applicable for *in- situ* and laboratory • Rapid analysis (< 10 minutes)	• Slowest detection of cellular stress • Non-specific (no differentiation between phytoplankton species) • Detection of chlorophyll, but not viability
Intracellular toxin	24 hours	• Good proxy for cell integrity	• Laboratory analysis • Requires long time for sample preparation and analysis (> 1 hour)
Extracellular toxin	Not detectable	• Can indicate cell lysis if toxins are not oxidized	• Laboratory analysis • Requires long time for sample preparation and analysis (> 3 hours) • Unsuitable cell stress detector due to inability to measure dissolved toxins after oxidation

534

535 ## 4. Conclusions

536 Many studies evaluate the effects of H_2O_2 on cyanobacteria, but a reliable
537 and rapid detection method for cell stress caused by oxidative processes is
538 needed to allow inter-study comparison. Several approaches are suitable as
539 a measure of cell stress in cyanobacteria with the present study was able to
540 compare different cell stress assessment methods which could be available
541 to most water treatment laboratories.
542 The current study clearly demonstrates efficacy of hydrogen peroxide in
543 reducing cyanobacterial cell numbers, viability and microcystin contents.
544 From the five methods investigated, the Mini-PAM fluorometer which
545 measured photosynthetic activity (F_V/F_M) provided the most rapid analysis
546 (< 10 minutes) and presented the fastest response time (i.e., was the
547 method which detected stress first), and is therefore the most suitable
548 method for cyanobacterial cell stress detection. To have a complete
549 understanding of algaecide treatment it is desirable to combine
550 photosynthesis, cell number and intracellular toxin detection methods.
551 Therefore, an approach using combined methods is advisable for successful
552 water management and to determine the efficacy of cyanobacterial removal
553 methods.
554

555 ## 5. Acknowledgements

556 The authors would like to acknowledge the Engineering and Physical
557 Sciences Research Council (EPSRC) [EP/P029280/1], the Coordination for
558 the Improvement of Higher Education Personnel - CAPES [PROEX 20/2016
559 and PrInt 88887.311806/2018-00], the Brazilian National Research Council
560 – CNPq [403116/2016-3 and 304164/2017-8] and the Ceará Research

561 Support Foundation - FUNCAP [PNE-0112-00042.01.00/16] for financial
562 support. In addition, the authors would like to thank Len Montgomery for
563 proof-reading the manuscript.

564

565 **6. References**

566 Barroin, G., Feuillade, M., 1986. Hydrogen peroxide as a potential algicide
567 for *Oscillatoria Rubescens* D.C. Water Res. 20, 619–623.
568 Campbell, D., Hurry, V., Clarke, A.K., Gustafsson, P., Oquist, G., 1998.
569 Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and
570 acclimation. Microbiol. Mol. Biol. Rev. 62, 667–83.
571 Cassier-Chauvat, C., Chauvat, F., 2014. Cell division in cyanobacteria. cell
572 Biol. cyanobacteria.
573 Chang, C.W., Huo, X., Lin, T.F., 2018. Exposure of *Microcystis aeruginosa* to
574 hydrogen peroxide and titanium dioxide under visible light conditions:
575 Modeling the impact of hydrogen peroxide and hydroxyl radical on cell
576 rupture and microcystin degradation. Water Res. 141, 217–226.
577 https://doi.org/10.1016/j.watres.2018.05.023
578 Chen, C., Yang, Z., Kong, F., Zhang, M., Yu, Y., Shi, X., 2016. Growth,
579 physiochemical and antioxidant responses of overwintering benthic
580 cyanobacteria to hydrogen peroxide. Environ. Pollut. 219, 649–655.
581 https://doi.org/10.1016/j.envpol.2016.06.043
582 Chow, C.W.K., House, J., Velzeboer, R.M.A., Drikas, M., Burch, M.D.,
583 Steffensen, D.A., 1998. The effect of ferric chloride flocculation on
584 cyanobacterial cells. Water Res. 32, 808–814.
585 https://doi.org/10.1016/S0043-1354(97)00276-5
586 De Julio, M., Fioravante, D.A., De Julio, T.S., Oroski, F.I., Graham, N.J.D.,

587 2010. A methodology for optimising the removal of cyanobacteria cells
588 from a brazilian eutrophic water. Brazilian J. Chem. Eng. 27, 113–126.
589 https://doi.org/10.1590/S0104-66322010000100010
590 Drábková, Michaela, Admiraal, W., Maršálek, B., 2007a. Combined exposure
591 to hydrogen peroxide and light-selective effects on cyanobacteria,
592 green algae, and diatoms. Environ. Sci. Technol. 41, 309–314.
593 https://doi.org/10.1021/es060746i
594 Drábková, M., Matthijs, H.C.P., Admiraal, W., Maršálek, B., 2007b. Selective
595 effects of H_2O_2 on cyanobacterial photosynthesis. Photosynthetica 45,
596 363–369. https://doi.org/10.1007/s11099-007-0062-9
597 Falconer, I.R., Beresford, A.M., Runnegar, M.T.C., 1983. Evidence of liver
598 damage by toxin from a bloom of the blue-green alga, *Microcystis*
599 *aeruginosa*. Med. J. Aust. 1, 511–514. https://doi.org/10.5694/j.1326-
600 5377.1983.tb136192.x
601 Fan, F., Shi, X., Zhang, M., Liu, C., Chen, K., 2019. Comparison of algal
602 harvest and hydrogen peroxide treatment in mitigating cyanobacterial
603 blooms via an in situ mesocosm experiment. Sci. Total Environ. 694,
604 133721. https://doi.org/10.1016/j.scitotenv.2019.133721
605 Fan, J., Daly, R., Hobson, P., Ho, L., Brookes, J., 2013a. Impact of
606 potassium permanganate on cyanobacterial cell integrity and toxin
607 release and degradation. Chemosphere 92, 529–534.
608 https://doi.org/10.1016/j.chemosphere.2013.03.022
609 Fan, J., Ho, L., Hobson, P., Brookes, J., 2013b. Evaluating the effectiveness
610 of copper sulphate, chlorine, potassium permanganate, hydrogen
611 peroxide and ozone on cyanobacterial cell integrity. Water Res. 47,
612 5153–5164. https://doi.org/10.1016/j.watres.2013.05.057

613 Harke, M.J., Steffen, M.M., Gobler, C.J., Otten, T.G., Wilhelm, S.W., Wood,
614 S.A., Paerl, H.W., 2016. A review of the global ecology, genomics, and
615 biogeography of the toxic cyanobacterium, *Microcystis* spp. Harmful
616 Algae 54, 4–20. https://doi.org/10.1016/j.hal.2015.12.007
617 Jochimsen, E.., Carmichael, W.W., An, J., Cardo, D.M., Cookson, S.T.;
618 Holmes, C.E.M.; Antunes, B.C.; Melo Filho, D.A.; Lyra, T.M.; Barreto,
619 V.S.T; Azevedo, S.M.F.O. & Jarvis, W.., 1998. Liver failure and death
620 after exposure to microcystins. N. Engl. J. Med. 338, 873–878.
621 https://doi.org/10.1080/13504509.2013.856048 M4 - Citavi
622 Kansole, M.M.R., Lin, T.F., 2017. Impacts of hydrogen peroxide and copper
623 sulfate on the control of *Microcystis aeruginosa* and MC-LR and the
624 inhibition of MC-LR degrading bacterium *Bacillus* sp. Water
625 (Switzerland) 9, 1–18. https://doi.org/10.3390/w9040255
626 Kim, M., Moon, J., Lee, Y., 2020. Biomass and Bioenergy Loading effects of
627 low doses of magnesium aminoclay on microalgal *Microcystis* sp . KW
628 growth , macromolecule productions , and cell harvesting. Biomass and
629 Bioenergy 139, 105619.
630 https://doi.org/10.1016/j.biombioe.2020.105619
631 Komárek, J., Komárková-Legnerová, J., Sant'anna, C.L., Azevedo, M.T. de
632 P., Senna, P.A.C., 2002. Review of the European *Microcystis-*
633 *morphospecies* (Cyanoprokaryotes) from nature Two common
634 *Microcystis* species (Chroococcales, Cyanobacteria) from tropical
635 America , including *M. panniformis* sp . nov . Cryptogam. Algol. 23,
636 159–177.
637 Lupínková, L., Komenda, J., 2004. Oxidative Modifications of the
638 Photosystem II D1 Protein by Reactive Oxygen Species: From Isolated

Protein to Cyanobacterial Cells. Photochem. Photobiol. 79, 152. https://doi.org/10.1562/0031-8655(2004)079<0152:omotpi>2.0.co;2

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

Ogawa, T., Misumi, M., Sonoike, K., 2017. Estimation of photosynthesis in cyanobacteria by pulse-amplitude modulation chlorophyll fluorescence: problems and solutions. Photosynth. Res. 133, 63–73. https://doi.org/10.1007/s11120-017-0367-x

Papadimitriou, T., Kormas, K., Dionysiou, D.D., Laspidou, C., 2016. Using H_2O_2 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

Pestana, C.J., Portela Noronha, J., Hui, J., Edwards, C., Gunaratne, H.Q.N., Irvine, J.T.S., Robertson, P.K.J., Capelo-Neto, J., Lawton, L.A., 2020. Photocatalytic removal of the cyanobacterium *Microcystis aeruginosa* PCC7813 and four microcystins by TiO_2 coated porous glass beads with UV-LED irradiation. Sci. Total Environ. 745, 141154. https://doi.org/10.1016/j.scitotenv.2020.141154

Pinho, L.X., Azevedo, J., Miranda, S.M., Ângelo, J., Mendes, A., Vilar, V.J.P., Vasconcelos, V., Boaventura, R.A.R., 2015. Oxidation of microcystin-LR and cylindrospermopsin by heterogeneous photocatalysis using a tubular photoreactor packed with different TiO_2 coated supports. Chem. Eng. J. 266, 100–111. https://doi.org/10.1016/j.cej.2014.12.023

Qian, H., Yu, S., Sun, Z., Xie, X., Liu, W., Fu, Z., 2010. Effects of copper

665 sulfate, hydrogen peroxide and N-phenyl-2-naphthylamine on oxidative
666 stress and the expression of genes involved photosynthesis and
667 microcystin disposition in *Microcystis aeruginosa*. Aquat. Toxicol. 99,
668 405–412. https://doi.org/10.1016/j.aquatox.2010.05.018
669 Schuurmans, R.M., Van Alphen, P., Schuurmans, J.M., Matthijs, H.C.P.,
670 Hellingwerf, K.J., 2015. Comparison of the photosynthetic yield of
671 cyanobacteria and green algae: Different methods give different
672 answers. PLoS One 10, 1–17.
673 https://doi.org/10.1371/journal.pone.0139061
674 Spoof, L., Catherine, A., 2017. Cyanobacteria samples: preservation,
675 abundance and biovolume measurements, in: Meriluoto, J., Spoof, L.,
676 A. Codd, G. (Eds.), Handbook of Cyanobacterial Monitoring and
677 Cyanotoxin Analysis. John Wiley & Sons, Chichester, UK, pp. 526–537.
678 https://doi.org/10.1002/9781119068761
679 Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971.
680 Purification and properties of unicellular blue-green algae (order
681 Chroococcales). Bacteriol. Rev. 35, 171–205.
682 https://doi.org/10.1128/mmbr.35.2.171-205.1971
683 Tsai, K., 2015. Ecotoxicology and Environmental Safety Effects of two
684 copper compounds on *Microcystis aeruginosa* cell density, membrane
685 integrity, and microcystin release. Ecotoxicol. Environ. Saf. 120, 428–
686 435. https://doi.org/10.1016/j.ecoenv.2015.06.024
687 Wang, B., Song, Q., Long, J., Song, G., Mi, W., Bi, Y., 2019. Optimization
688 method for *Microcystis* bloom mitigation by hydrogen peroxide and its
689 stimulative effects on growth of chlorophytes. Chemosphere 228, 503–
690 512. https://doi.org/10.1016/j.chemosphere.2019.04.138

691 Wang, B., Wang, X., Hu, Y., Chang, M., Bi, Y., Hu, Z., 2015. The combined
692 effects of UV-C radiation and H_2O_2 on *Microcystis aeruginosa*, a bloom-
693 forming cyanobacterium. Chemosphere 141, 34–43.
694 https://doi.org/10.1016/j.chemosphere.2015.06.020
695 Wang, J., Chen, Z., Chen, H., Wen, Y., 2018. Effect of hydrogen peroxide on
696 *Microcystic aeruginosa*: Role of cytochromes P450. Sci. Total Environ.
697 626, 211–218. https://doi.org/10.1016/j.scitotenv.2018.01.067
698 Wang, Xin, Wang, Xuejiang, Zhao, J., Song, J., Wang, J., Ma, R., Ma, J.,
699 2017. Solar light-driven photocatalytic destruction of cyanobacteria by
700 F-Ce-TiO_2/expanded perlite floating composites. Chem. Eng. J. 320,
701 253–263. https://doi.org/10.1016/j.cej.2017.03.062
702 Weenink, E.F.J., Luimstra, V.M., Schuurmans, J.M., van Herk, M.J., Visser,
703 P.M., Matthijs, H.C.P., 2015. Combatting cyanobacteria with hydrogen
704 peroxide: A laboratory study on the consequences for phytoplankton
705 community and diversity. Front. Microbiol. 6, 1–15.
706 https://doi.org/10.3389/fmicb.2015.00714
707 Wojtasiewicz, B., Stoń-Egiert, J., 2016. Bio-optical characterization of
708 selected cyanobacteria strains present in marine and freshwater
709 ecosystems. J. Appl. Phycol. 28, 2299–2314.
710 https://doi.org/10.1007/s10811-015-0774-3
711 Yang, W., Tang, Z., Zhou, F., Zhang, W., Song, L., 2013. Toxicity studies of
712 tetracycline on *Microcystis aeruginosa* and *Selenastrum capricornutum*.
713 Environ. Toxicol. Pharmacol. 35, 320–324.
714 https://doi.org/10.1016/j.etap.2013.01.006
715 Zamyadi, A., Macleod, S.L., Fan, Y., Mcquaid, N., Dorner, S., 2011. Toxic
716 cyanobacterial breakthrough and accumulation in a drinking water

717 plant : A monitoring and treatment challenge 6.
718 https://doi.org/10.1016/j.watres.2011.11.012
719 Zhou, S., Shao, Y., Gao, N., Zhu, S., Li, L., Deng, J., Zhu, M., 2014.
720 Removal of Microcystis aeruginosa by potassium ferrate (VI): Impacts
721 on cells integrity, intracellular organic matter release and disinfection
722 by-products formation. Chem. Eng. J. 251, 304–309.
723 https://doi.org/10.1016/j.cej.2014.04.081
724