

1 **Photocatalytic removal of the cyanobacterium *Microcystis aeruginosa***
2 **PCC7813 and four microcystins by TiO₂ coated porous glass beads with**
3 **UV-LED irradiation**

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19 **Keywords:** Cyanobacteria, Photocatalysis, Cyanotoxins, Water Treatment,
20 Titanium Dioxide, UV-LED

21

22 **Highlights**

- 23 • Photocatalytic inhibition of *M. aeruginosa* PCC7813 (7.6×10^5 cells mL⁻¹ d⁻¹)
24 • 74% removal of four microcystins (intra- and extracellular)
25 • Porous glass beads made from recycled glass used as catalyst support
26 • UV irradiance supplied by low energy UV (365 nm) emitting LEDs

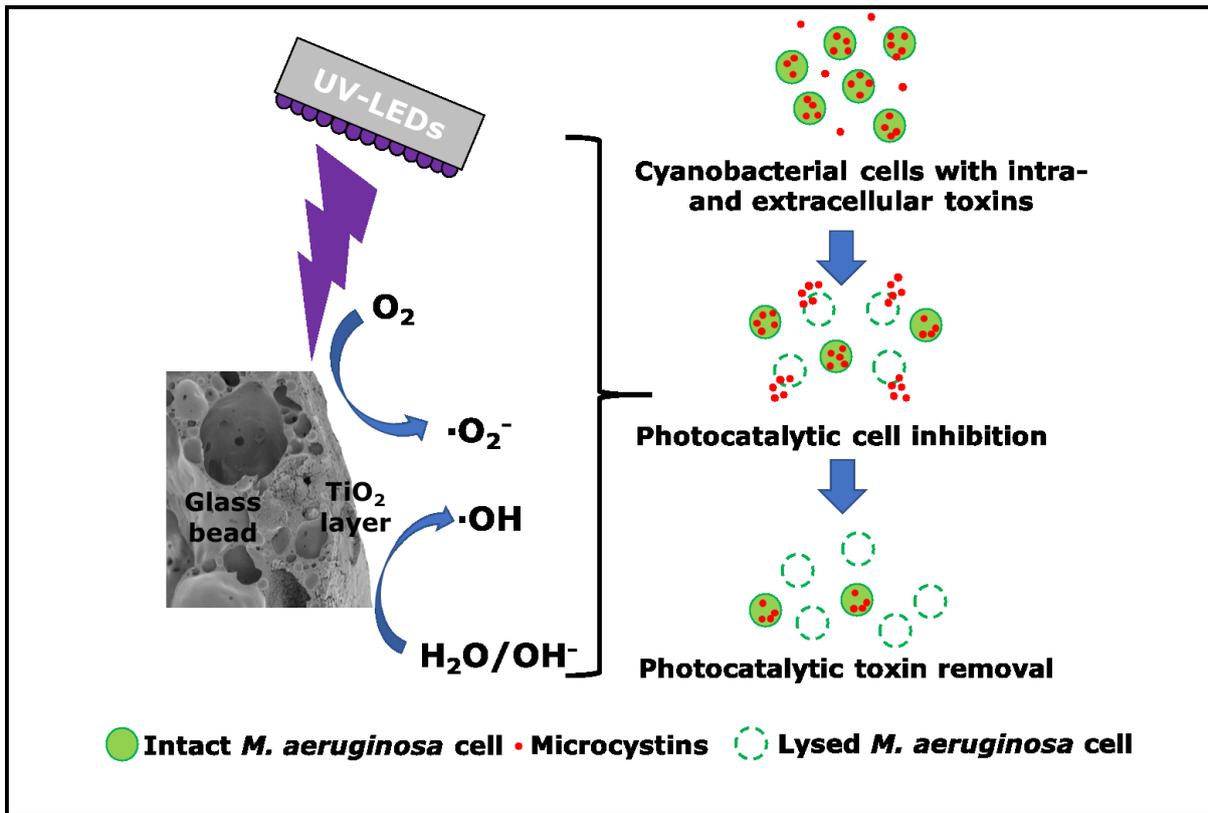
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31 **Graphical Abstract**



32

33 **Abstract**

34 Cyanobacteria and their toxic secondary metabolites are a challenge in water
 35 treatment due to increased biomass and dissolved metabolites in the raw water.
 36 Retrofitting existing water treatment infrastructure is prohibitively expensive or
 37 unfeasible, hence 'in-reservoir' treatment options are being explored. In the
 38 current study, a treatment system was able to photocatalytically inhibit the
 39 growth of *Microcystis aeruginosa* and remove released microcystins by
 40 photocatalysis using titanium dioxide coated, porous foamed glass beads and
 41 UV-LEDs (365 nm). A 35% reduction of *M. aeruginosa* PCC7813 cell density
 42 compared to control samples was achieved in seven days. As a function of cell
 43 removal, intracellular microcystins (microcystin-LR, -LY, -LW, and -LF) were
 44 removed by 49% from 0.69 to 0.35 $\mu\text{g mL}^{-1}$ in seven days. Microcystins that
 45 leaked into the surrounding water from compromised cells were completely

46 removed by photocatalysis. The findings of the current study demonstrate the
47 feasibility of an in-reservoir treatment unit applying low cost UV-LEDs and
48 porous foamed beads made from recycled glass coated with titanium dioxide as
49 a means to control cyanobacteria and their toxins before they can reach the
50 water treatment plant.

51

52 **1. Introduction**

53 Cyanobacteria are well known to form blooms in nutrient-rich waters, including
54 drinking water reservoirs. High cell densities challenge water treatment systems
55 by reducing the run time of filters leading to an increased demand of treatment
56 chemicals such as coagulants and disinfectants (De Julio *et al.*, 2010). This
57 problem is often further exacerbated by the release of toxic and/or noxious
58 metabolites produced by the cyanobacteria, further challenging water treatment
59 plant operators and decreasing water security (Chow *et al.*, 1999; Drikas *et al.*,
60 2001; Velzeboer *et al.*, 1995). The most commonly reported cyanobacterial toxic
61 metabolites are the microcystins. To date at least 247 microcystin congeners
62 have been described (Spoof and Catherine, 2017). The toxicity of microcystins
63 has been recognized as a global issue with the World Health Organisation setting
64 a recommended maximum allowable limit of $1 \mu\text{g L}^{-1}$ in drinking water (WHO,
65 2017).

66 Retro-fitting water treatment plants with improved and advanced technology is
67 often prohibitively expensive and/or physically challenging, hence alternative
68 treatment technologies such as in-reservoir treatment need to be explored. The
69 application of algaecides in the reservoir is the simplest form of in-reservoir
70 treatment but studies have shown the negative effects of this practice, such as
71 toxicity to non-target organisms, development of bacterial resistances, increase

72 of potentially toxic/noxious dissolved metabolites and precursors of disinfection
73 by-products (Bishop *et al.*, 2017; García-Villada *et al.*, 2004; Greenfield *et al.*,
74 2014; Jančula and Maršálek, 2011).

75 In recent years, advanced oxidation processes, including titanium dioxide (TiO₂)
76 photocatalysis have been demonstrated to control cyanobacteria and their
77 secondary metabolites. Successful removal of cyanobacterial toxins by TiO₂
78 nanoparticulate photocatalysis have been reported by a number of studies
79 (Cornish *et al.*, 2000; Liu *et al.*, 2009; Pelaez *et al.*, 2011), especially for the
80 elimination of the commonly occurring group of cyanobacterial toxins, the
81 microcystins. One of the most critical technical challenges that has hampered
82 the application of photocatalysis in water treatment is the removal of the
83 nanoparticulate TiO₂ materials following treatment. The post treatment recovery
84 of TiO₂ is not only a technical challenge but also has ecotoxicological health
85 implications. It has been demonstrated that nanoparticulate TiO₂ can
86 bioaccumulate and damage biota (Heinlaan *et al.*, 2008; Wang *et al.*, 2007; Zhu
87 *et al.*, 2010). Further, the application of nanoparticulate TiO₂ represents a health
88 hazard to operators if inhaled (Grassian *et al.*, 2007). To avoid the problems of
89 free nanoparticulate TiO₂, immobilization of the photocatalyst onto a robust
90 carrier matrix is preferable. Matrices such as activated carbon, metal particles,
91 and glass have been explored, each with inherent advantages and disadvantages
92 (Kinley *et al.*, 2018; Liu *et al.*, 2007; Pestana *et al.*, 2015). Several design
93 parameters have to be considered when applying immobilized TiO₂ in a water
94 treatment context. For example, cyanobacteria occupy different positions in the
95 water column, depending on species and time of the day (Varuni *et al.*, 2017).
96 Thus, to ensure maximum efficiency of immobilized TiO₂, an even distribution
97 throughout the water column is desirable. Surface floating matrices will not

98 reach cyanobacteria deeper in the water column and likewise heavier matrices
99 that sink will miss cyanobacteria higher up in the water column. The use of
100 semi-bouyant foamed glass beads allows for even distribution in the water
101 column. Additionally, the use of low-cost (ca. USD 0.30 per LED), long life
102 (approximately 100,000 working hours), waterproof UV (365 nm) emitting LEDs
103 to activate TiO₂ would solve a further technological challenge in the application
104 of this *in-situ* treatment system, as in the past supplying cost-effective UV
105 irradiation of the required wavelength has been problematic. While in recent
106 years solar light-driven photocatalysis has been explored for the removal of
107 contaminants of emerging concern, including cyanobacteria and their toxins, the
108 application of this technology at scale suffers from drawbacks compared to the
109 use of for example UV-LEDs (Fagan et al., 2016). There are two major
110 drawbacks to this technology, one is the need to modify TiO₂ to shift its activity
111 into the visible light range, usually achieved doping with other materials such as
112 noble metals, carbon, or nitrogen (Wang et al., 2017, Fotiou et al., 2013). This
113 would increase the cost of the treatment as additional steps and materials are
114 required in the catalyst preparation. The other drawback of solar light-driven
115 catalysis is that sunlight hours vary across the globe and that it is only available
116 for a maximum of 12 h per day, thus rendering a purely solar light-driven
117 treatment system inactive overnight. Recently, we have shown the feasibility of
118 such a system for the photocatalytic removal of microcystin-LR (Gunaratne et
119 al., 2020). Applying a similar technology using TiO₂ coated porous glass beads
120 and UV-emitting LEDs, we now present a bench scale proof-of-principle in-
121 reservoir treatment system that aims to inhibit and eliminate cyanobacteria
122 while simultaneously removing toxins that are released and is energy efficient,
123 thus can be maintained in continuous use to limit cyanobacterial biomass and

124 dissolved metabolites entering water treatment plants. It is envisaged that the
125 pre-treatment system operates continuously avoiding the formation of intense
126 blooms and keeping the cyanobacterial biomass at a level that allows the
127 conventional water treatment process to completely remove any remaining
128 cyanobacteria, while at the same ensuring that no dissolved toxins enter the
129 plants that are ill equipped to remove dissolved contaminants, rather than a
130 point treatment used when cell numbers or toxin concentrations exceed national
131 threshold levels.

132

133 **2. Materials and Methods**

134 **2.1 Reagents**

135 All reagents for the preparation of artificial fresh water (AFW) and cyanobacterial
136 culture medium BG-11 were of reagent grade, obtained from Fisher Scientific
137 (UK), and used as received. Acetonitrile and methanol were of HPLC grade and
138 obtained from Fisher Scientific (UK). Ultrapure water (18.2 M Ω) was provided by
139 a PURELAB[®] system (ELGA Veolia, UK). Isoton II Diluent (Beckman Coulter,
140 USA) was used for cell enumeration and biovolume determination.

141

142 **2.2 Cyanobacterial cell culture**

143 *M. aeruginosa* PCC7813 was originally obtained from the Pasteur Culture
144 Collection (France) and cultured in sterilized BG-11 medium (Stanier *et al.*,
145 1971), at 22 \pm 1 °C with a 12h/12h light dark cycle at 20 μ mol photons m⁻² s⁻¹
146 under aseptic conditions. *M. aeruginosa* PCC7813 produces four main
147 microcystin analogues (MC-LR, MC-LY, MC-LW, and MC-LF) and does not contain
148 gas vesicles.

149

150 **2.3 Preparation of TiO₂ coated recycled porous glass beads**

151 Porous recycled foamed glass beads (1-4 mm diameter, Poraver, Germany) were
152 sieved to achieve > 2 mm, then washed with acetone, followed by deionised
153 water in a sonication bath (Scientific Laboratory Supplies Ltd., UK) and dried in
154 an oven at 80 °C for 18 h. After this pre-treatment, beads were coated with
155 titanium dioxide (P25, Rutile/Anatase: 85/15, 99.9 %, 20 nm particle size;
156 Degussa Evonik, Germany) according to a method by Mills *et al.* (2006) with
157 adaptations. In short, a slurry of P25 and water is prepared into which the pre-
158 treated glass beads are submerged. Coated beads are removed from the slurry
159 and allowed to dry, followed by calcination at 550 °C for 3h. Each coating
160 procedure deposits approximately 2% (w/w) of TiO₂ onto the beads. Coatings
161 are repeated until approximately 10% (w/w) of TiO₂ on the beads was achieved.
162 Characterization of the beads and the coating is recorded in the supplementary
163 material (S1 and figure S1).

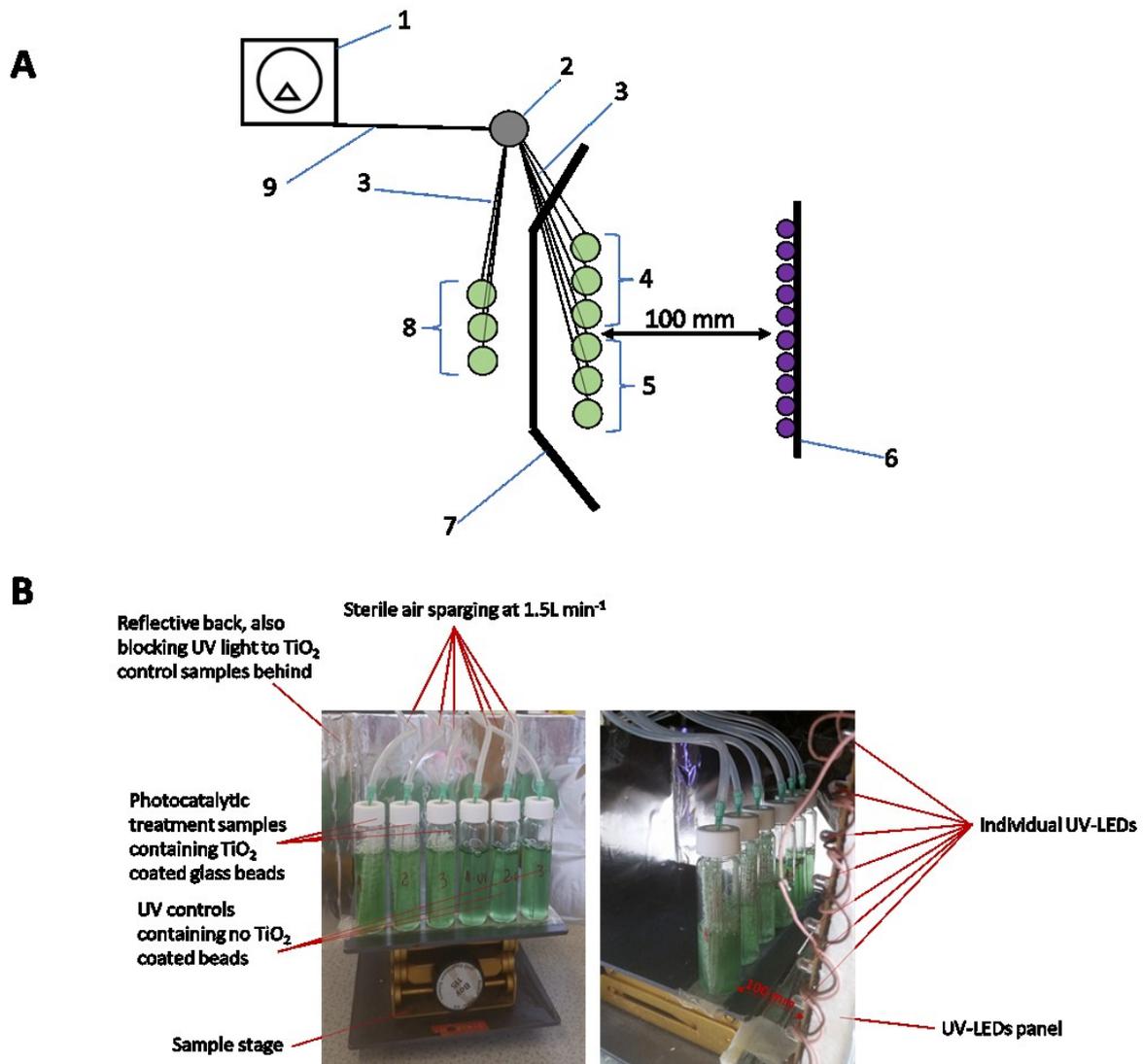
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165 **2.4 Photocatalytic removal of *M. aeruginosa* PCC7813 and microcystins**

166 Artificial fresh water (AFW) was used as an experimental matrix in the
167 photocatalysis investigation, and was prepared according to Akkanen and
168 Kukkonen (2003) by dissolving CaCl₂ (11.8 mg L⁻¹), MgSO₄ (4.9 mg L⁻¹), NaHCO₃
169 (2.6 mg L⁻¹) and KCl (0.2 mg L⁻¹) in ultrapure water. A three-week-old culture of
170 *M. aeruginosa* PCC7813 was diluted in AFW to achieve a final cell density of 15 x
171 10⁶ cells mL⁻¹. TiO₂ coated beads (700 mg, equivalent to 0.2% (w/v) TiO₂) were
172 placed in glass mesh pods (70 mm x 10 mm diameter) and placed into 40 mL
173 glass bottles (95 mm x 22 mm diameter) into which 30 mL of the cell
174 suspension was added. Three replicates containing the coated beads was
175 irradiated by a 550 cm² UV-LED panel with 90 individual UV-LEDs (AT

176 Technologies, UK) providing $2.8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (2.1 mW s^{-1}) at 365 nm
177 and at 100 mm distance (figure 1). Another three replicates, not containing
178 titanium dioxide coated beads was prepared at the same distance from the UV-
179 LEDs functioning as a UV control. While a third set of replicates with TiO_2 coated
180 beads was set up outside of the area of irradiation of the UV-LED panel to act as
181 a no-UV control. Typically, in photocatalysis the dark/no-UV control is performed
182 in complete darkness; however, cyanobacteria are photosynthetic organisms
183 that would not survive the duration of the experiment without light, hence this
184 third set of replicates was maintained in ambient light (no UV irradiation at 13
185 $\mu\text{mol s}^{-1} \text{m}^{-2}$ cool fluorescent irradiation). To maintain clarity 'TiO₂-control' will be
186 used throughout to identify samples that contain TiO_2 coated glass beads, but
187 are not exposed to UV irradiation. All samples were sparged at 1.5 L min^{-1} with
188 sterile ambient air. After taking a zero-time sample, each replicate was sampled
189 (1.1 mL) daily.

190



191

192 **Figure 1:** A) Schematic diagram of the UV-LED photocatalytic experimental design (top-
 193 down view). 1- air pump, 2- air distribution hub to achieve equal air pressure across all
 194 samples, 3- silicone tubing of equal length, 4- TiO₂/UV treatment samples in triplicate, 5-
 195 UV control samples in triplicate, 6- UV-LED panel with 90 UV-LEDs (365 nm, 67.5 mW
 196 total output) in 9 rows of 10 LEDs; output at 100 mm 2.6 mW s⁻¹, 7- reflective surface;
 197 also blocking UV irradiation from LED panel to TiO₂-controls (8), 8- TiO₂-control samples
 198 in triplicate, 9- silicone tubing. B) Photographic representation of the reactor and the
 199 TiO₂/UV and UV control samples.

200

201

202 2.5 Sample analysis

203 2.5.1 Cell enumeration and sample pre-treatment

204 For cell enumeration, cell volume determination, and determination of the
 205 average cell diameter of *M. aeruginosa* PCC7813, 0.1 mL of each sample was
 206 diluted in 20 mL of Isoton II diluent and analysed by a Multisizer (Beckman

207 Coulter, USA). For this a 50 μm aperture was used, allowing the determination
208 of particles sized between 1 and 30 μm , particles ranging in size from 2.8 to 6.9
209 μm were considered intact cells based on published data of cell size ranges for
210 *M. aeruginosa* (Harke *et al.*, 2016; Komárek and Komárková, 2002). This cut-off
211 had to be introduced to ensure that cell fragments smaller than 2.8 μm are not
212 considered cells which would artificially increase the cell densities. For
213 microcystin analysis, the remaining 1 mL of each sample was centrifuged (13000
214 G) in microcentrifuge tube (1.5 mL) for 10 min to separate cells and medium.
215 The supernatant was evaporated to dryness on an EZ-II Evaporator (Genevac,
216 United Kingdom). The cell pellet was stored at -20 °C until further processing.
217 Prior to analysis, aqueous methanol (80 %) was added to the cell pellets which
218 were subsequently placed in a dispersive extractor for 5 minutes at 2500 rpm
219 and then centrifuged (13000 G). The supernatant was analysed to determine
220 intracellular toxin. The dried extracellular component was also resuspended in
221 aqueous methanol (80%, 150 μL), vortexed and centrifuged (13000 G). The
222 intra- and extracellular microcystins were analysed by HPLC.

223

224 **2.5.2 High performance liquid chromatography analysis of microcystins**

225 Chromatographic separation of microcystin analogues was carried out using a
226 2965 separation module with a Symmetry C18 column (2.1 x 150 mm, 5 μm
227 particle size) and a 2996 photodiode array (PDA) detector. Mobile phases were
228 ultrapure water (18.2 M Ω) and acetonitrile both with 0.05% trifluoroacetic acid.
229 Separation was achieved with a linear gradient from 35 to 70% organic phase
230 over 25 min followed by an organic solvent wash (100%) and re-establishment
231 of starting conditions. Column temperature was 40 °C. Scanning range for the

232 PDA was 200 to 400 nm, with microcystins integrated at 238 nm. The limit of
233 quantification of this method was 5 ng mL⁻¹.

234

235 **2.5.3 Statistical analysis**

236 All values shown are mean of triplicate treatments with error of one standard
237 deviation. For statistical significance testing results were analyzed using one-way
238 ANOVA. The significance level was set to $p > 0.05$ to identify significant
239 differences between results.

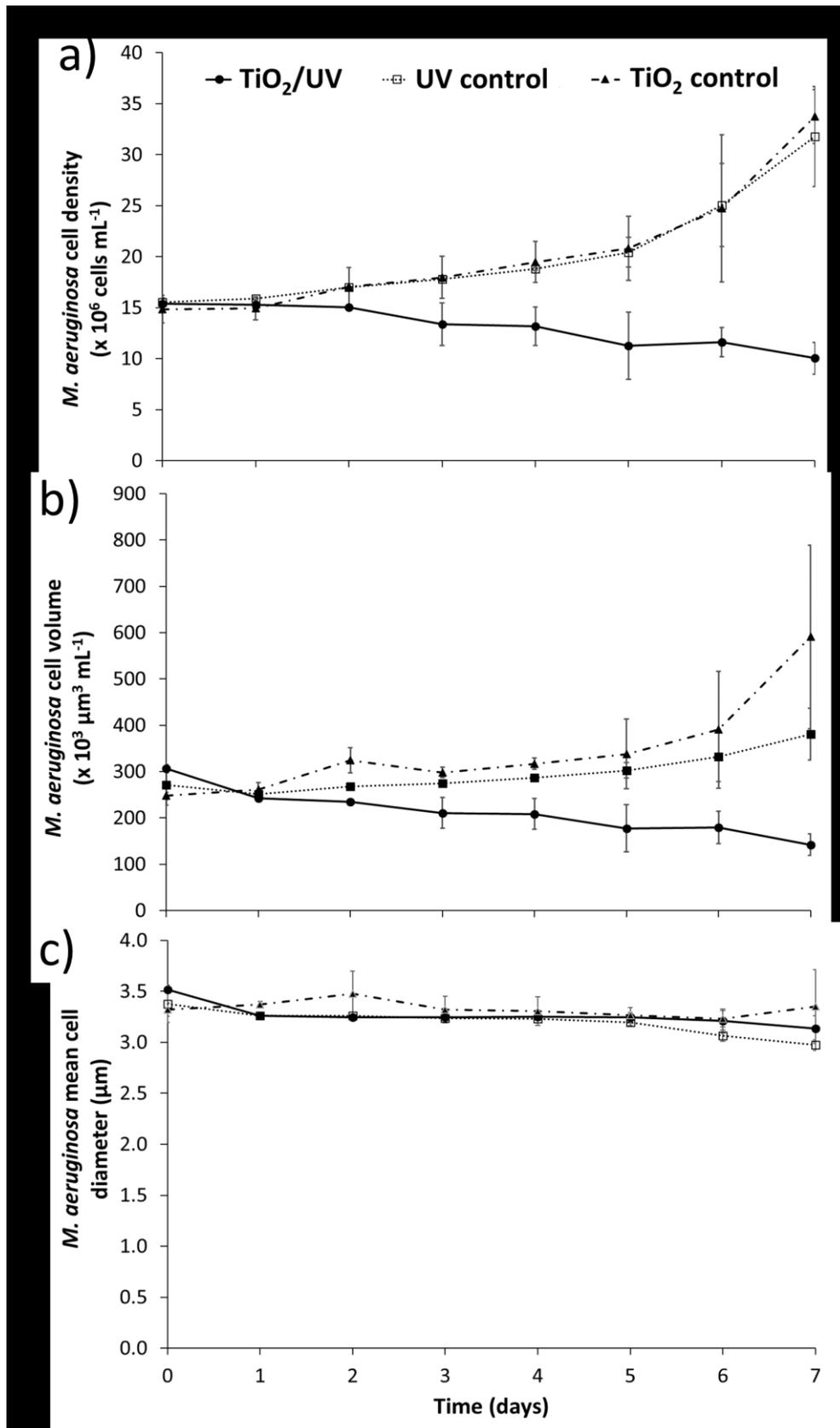
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241 **3. Results and Discussion**

242 **3.1 Photocatalytic removal of *M. aeruginosa* PCC7813**

243 The removal of *M. aeruginosa* PCC7813 in a photocatalytic reactor with TiO₂
244 coated porous glass beads and UV-LED irradiation was initially investigated. Over
245 the course of seven days treatment the cell concentration of *M. aeruginosa*
246 PCC7813 increased significantly in both controls, UV with no catalyst and no UV
247 irradiation ($p < 0.05$ each), achieving 32 and 34 x 10⁶ cells mL⁻¹ respectively,
248 representing a per cent increase of 213 and 226%. There was no statistical
249 difference between the UV- and the TiO₂-controls ($p > 0.05$). No effect of the UV
250 only control would be expected as the UV light emitted by the UV-LED is
251 comparatively low in energy at 2.6 mW s⁻¹ and the emitted wavelength is
252 insufficient to be germicidal (germicidal wavelength <254 nm; Ou *et al.*, 2012).
253 On the other hand, in the treatment samples the initial cell concentration (15 x
254 10⁶ cells mL⁻¹) was significantly reduced to 10 x 10⁶ cells mL⁻¹ (35%,
255 $p = 0.00004$) when compared to the TiO₂-control (figure 2). The biovolume of the
256 *M. aeruginosa* PCC7813 culture also decreased over the course of the
257 experiment (66% of the TiO₂- control), which corresponds to and corroborates

258 the observed decrease in cell density. There was no statistical difference
259 between the two controls with respect to the cell volume ($p>0.05$). The diameter
260 of the intact cells (2.8-6.5 μm) did not significantly change ($p>0.05$) from either
261 the initial cell size at time zero or after seven days treatment when compared to
262 either control (UV with catalyst and no UV irradiation). This indicates that the
263 treatment fragmented the cells into particles smaller than 2.8 μm rather than
264 affect the cell diameter since the mean cell diameter did not change. Cell
265 fragmentation during photocatalytic treatment was also observed by Wang *et al.*
266 (2017) where *M. aeruginosa* (strain 913 from Wuhan Institute of Hydrobiology)
267 cells were treated with floating, expanded perlite particles that were coated with
268 F-Ce doped TiO_2 .
269
270



271

272 **Figure 2:** a) Removal of *M. aeruginosa* PCC7813 cells by photocatalysis using TiO₂
 273 coated porous glass beads over a seven-day period under 2.8 μmol photons m⁻² s⁻¹ at
 274 365 nm (2.6 mW s⁻¹) at 100 mm distance, as well as the effect of the treatment on *M.*
 275 *aeruginosa* PCC7813 b) cell volume, and c) mean cell diameter. (n=3, Error=1SD)

276 From 48 h onwards, a decline in cell density was observed for the treatment with
277 TiO₂/UV (figure 2a). Other studies have reported the inhibition of *M. aeruginosa*
278 growth by TiO₂ photocatalysis in one hour (Liao *et al.*, 2009; Pinho *et al.*, 2015),
279 however, there are marked differences in the application of the TiO₂
280 photocatalysis in terms of light source, *M. aeruginosa* strain, and presentation of
281 TiO₂. The UV-LED panel employed in the current investigation had a total output
282 of 67.5 mW (with each individual LED having an output of 750 μW, and the
283 panel having a total of 90 LEDs) providing a very low energy input into the
284 system. By comparison Pinho *et al.* (2015), who investigated the removal of *M.*
285 *aeruginosa* LEGE 91094 (IZANCY-A2) with particulate TiO₂, used simulated solar
286 irradiation at a UV equivalent of 44 W m⁻², and Liao *et al.* (2009), who
287 investigated the effect of silver-doped TiO₂ particulates on an unspecified *M.*
288 *aeruginosa* strain, used a UV-C lamp with 4 W output at 253.7 nm. The UV-LEDs
289 (67.5 mW) deployed in the current investigation use almost sixty times less
290 energy than the 4 W lamp used in the other study. An additional advantage of
291 employing LEDs is their longer life span in comparison to light bulbs, ca. 100,000
292 h compared to ca. 8,000 to 25,000 h for other UV irradiation sources (Heering,
293 2004). Furthermore, while rapid cell death is recorded when nanoparticulate TiO₂
294 is used, the removal of catalyst has been a barrier to deployment of this
295 technology. A particular advantage of the current system is the use of
296 immobilized TiO₂. While the reactive surface area is markedly reduced compared
297 to particulate catalyst systems, immobilized catalyst offers a much more facile
298 post-treatment separation of catalyst and water compared to (nano)particulate
299 TiO₂. In addition, most of these other studies which investigated the inhibition of
300 *M. aeruginosa* by TiO₂ photocatalysis, used modified TiO₂ composite materials.
301 Liao and co-workers (2009) used Ag-doped TiO₂ and Wang *et al.* (2017) used F-

302 Ce-doped TiO₂ further increasing the photocatalytic activity compared to TiO₂
303 alone. The doping of TiO₂ offers the advantage of shifting reactivity into the
304 visible spectrum, however, this has to be weighed against the cost of the doping
305 material and the complexity of preparation. Additionally, the intended application
306 has to be considered. The current design is aimed at continuous operation within
307 a reservoir to ease the burden on the water treatment process within a
308 treatment plant. Thus, materials used need to be plentiful, economically
309 affordable, and easy to obtain, which is not the case when doping with, for
310 example, noble metals.

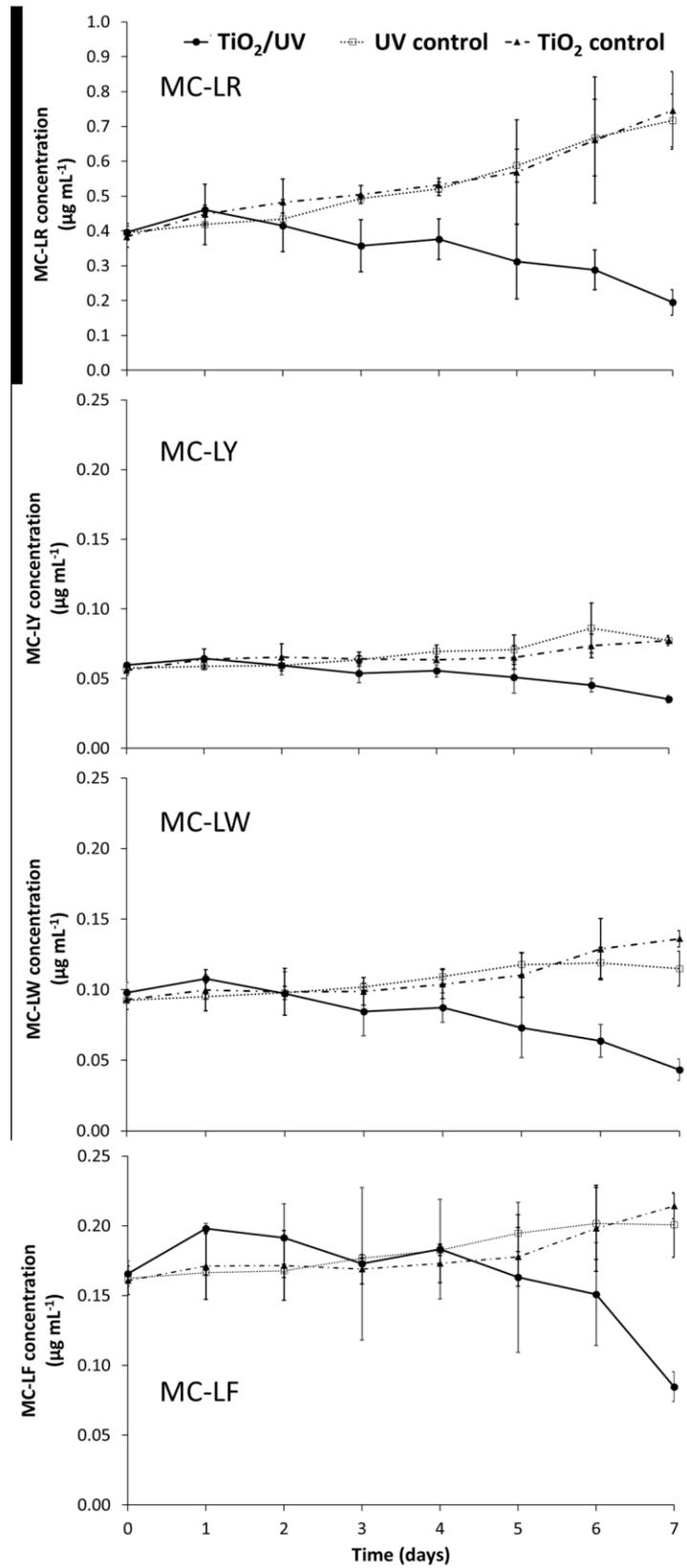
311 The UV irradiation (365 nm) alone had no observable effect on the cell number,
312 cell volume, or cell diameter (figure 2), which was what might have been
313 expected since antimicrobial UV treatments tend to employ irradiance in the UV-
314 C spectrum of a wavelength of 260 nm and below (Wolfe, 1990). This was
315 demonstrated in the Liao *et al.* (2009) study where approximately 12%
316 difference in the chlorophyll *a* content between an untreated and the UV(C)
317 controls was observed.

318

319 **3.2 Photocatalytic removal of four microcystin congeners**

320 The strain of *M. aeruginosa* PCC7813 used in the current investigation produces
321 four main microcystin congeners (MC-LR, -LY, -LW, and -LF). During the
322 photocatalysis of *M. aeruginosa* PCC7813 both the intracellular (figure 3) and
323 extracellular (figure 4) microcystin concentrations were monitored. As
324 microcystins are usually encountered in the intracellular space until cell integrity
325 is compromised and the intracellular toxins leak into the surrounding water,
326 monitoring the intracellular concentration during photocatalysis can be used as a
327 proxy measurement of cell integrity. The distribution of the four congeners at

328 the start of the experiment was MC-LR 58%, MC-LY 9%, MC-LW 14%, and MC-
329 LF 19% of the total intracellular microcystin concentration. A significant
330 ($p=0.0009$ to 0.045) decrease of intracellular toxin concentration was observed
331 for all four microcystin congeners over the course of seven days (figure 3)
332 during photocatalytic treatment. Combined intracellular microcystin content
333 decreased by 49% from 0.69 to $0.35 \mu\text{g mL}^{-1}$. Individually the concentrations for
334 MC-LR, -LY, -LW, and -LF decreased by 53, 34, 60, and 54% respectively from
335 the initial concentration present in the cells. The profile of different intracellular
336 microcystin variants at the end of the seven-day experiment remained largely
337 unchanged, with MC-LR remaining the main congener produced (54%), followed
338 by MC-LF (23%) and MC-LY and MC-LW (11% each). There was no statistical
339 difference *viz* the intracellular toxin concentration in either of the two controls
340 ($p>0.05$). Compared to the TiO_2 and UV with no catalyst controls the
341 concentration of the total intracellular microcystin in the treated samples was
342 reduced by 67% with individual concentrations for MC-LR, -LY, -LW, and -LF
343 decreased by 74, 50, 68, and 71% respectively. It is predicted that decrease in
344 cell density and toxins concentration would continue and be maintained at a low
345 level if this treatment system is used *in-situ* in a reservoir.



346

347 **Figure 3:** Removal of the four main intracellular microcystin analogues (MC-LR, MC-LY,
 348 MC-LW, MC-LF) produced by *M. aeruginosa* PCC7813 during a seven-day photocatalytic
 349 treatment with TiO₂ coated porous foamed recycled glass beads and UV-LED provided UV
 350 irradiation at 2.8 µmol photons m⁻² s⁻¹ at 365 nm (2.6 mW s⁻¹) at a distance of 100 mm.
 351 (*n*=3, Error=1SD)

352 For most of the congeners the amount of toxin per cell decreased (table 1),
 353 which is indicative that some of the cells detected by the particle counter were
 354 damaged, but had not yet completely fragmented. Zilliges and co-workers
 355 (2011) have observed that intracellular microcystins concentrations decrease as
 356 a response to oxidative stress. In their study Zilliges *et al.* (2011) were able to
 357 observe intracellular microcystins bind to intracellular proteins in the presence of
 358 hydrogen peroxide (0.34 mg L⁻¹). Hydrogen peroxide is a strong oxidizing agent
 359 and under UV irradiation hydrogen peroxide can lead to the creation of hydroxyl
 360 radicals, an even stronger oxidizing agent. Thus, the oxidative stress response of
 361 *M. aeruginosa* exposed to hydrogen peroxide may be comparable to the stress
 362 response to TiO₂ photocatalysis (where hydroxyl and superoxide radicals are
 363 created), indicating that the decrease in intracellular microcystin concentrations
 364 could also be caused by microcystins binding to intracellular proteins although
 365 this would require further investigation.

366

367 **Table 1:** Reduction of intracellular microcystin congener concentration in *M. aeruginosa*
 368 PCC7813 after seven days of treatment in a photocatalytic reactor under UV-LED
 369 irradiation (at 2.8 μmol photons m⁻² s⁻¹ at 365 nm (2.6 mW s⁻¹)) at 100 mm distance in
 370 the presence of TiO₂ coated porous glass beads. (*n*=3, Error=1SD).

MC congener	Time 0 (fg cell ⁻¹)	Time 7d (fg cell ⁻¹)	Per cent reduction
MC-LR	25.8 ± 2	19.4 ± 2	25*
MC-LY	3.8 ± 0.1	3.5 ± 0.4	8
MC-LW	6.4 ± 0.4	4.3 ± 0.6	32*
MC-LF	10.8 ± 0.7	8.5 ± 0.8	21*

371 *difference significant (p>0.05)

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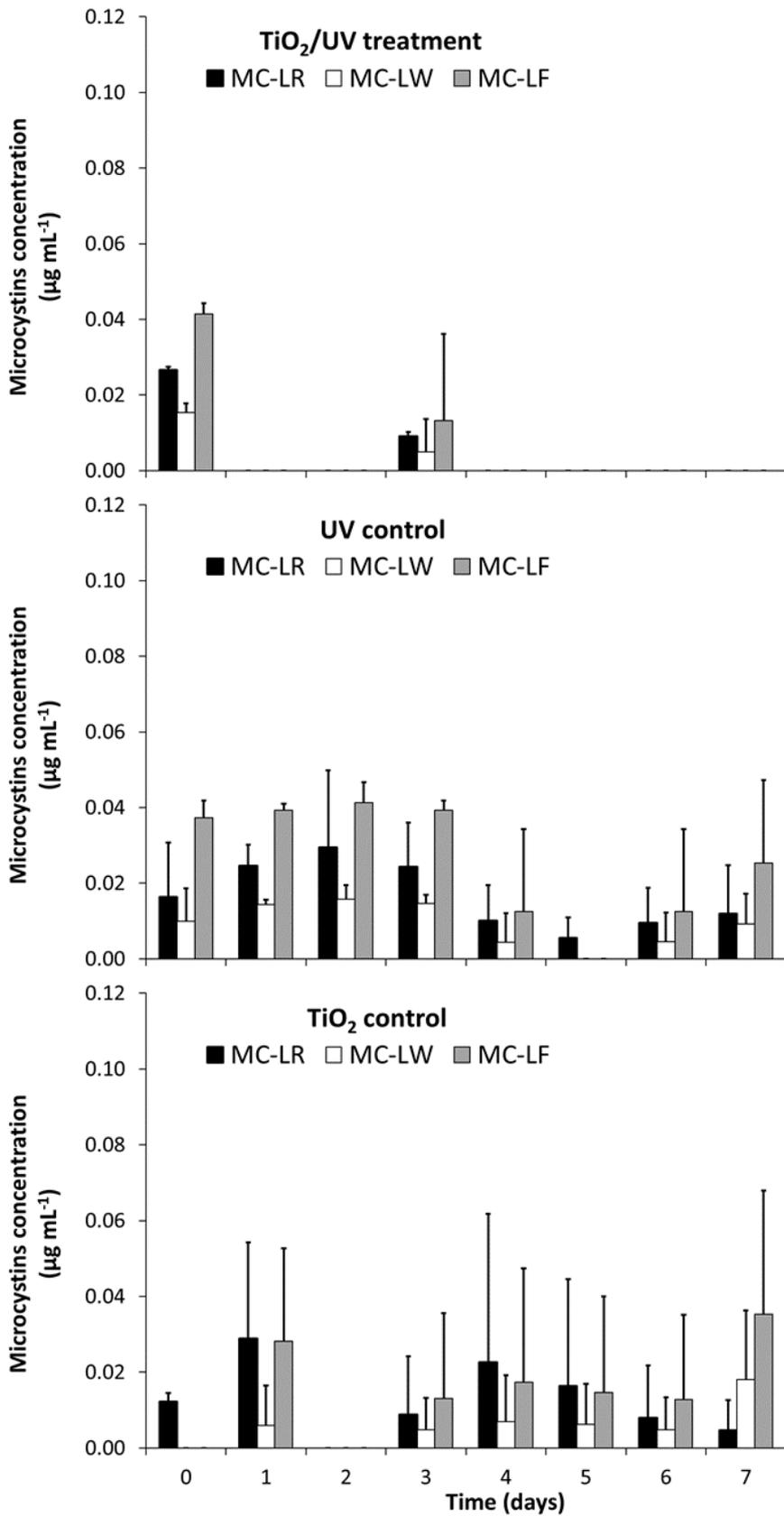
374 When the cell integrity of microcystin-producing cyanobacteria is compromised
 375 by oxidative processes, the intracellular organic material leaks into the
 376 surrounding water, including any microcystins (Daly *et al.*, 2007). Therefore, it is
 377 important that water treatment systems either avoid compromising cell integrity
 378 or, failing that, the system should also be able to remove microcystins that are

379 released into the water. Failing to remove the dissolved organic matter,
380 including microcystins, exacerbates the challenges faced by water treatment
381 processes, as conventional water treatment is more suited to the removal of
382 particulate and colloidal than dissolved components (Chow *et al.*, 1999; Li *et al.*,
383 2012). In the current investigation, extracellular concentrations of the four main
384 microcystin congeners produced by *M. aeruginosa* PCC7813 were also monitored
385 (figure 4).

386

387

388



389

390 **Figure 4:** Extracellular microcystins (MC-LR, MC-LW, MC-LF) produced by *M. aeruginosa*
 391 PCC7813 during a seven-day photocatalytic treatment with TiO₂ coated porous glass
 392 beads and UV-LED provided UV irradiation at 2.8 µmol photons m⁻² s⁻¹ at 365 nm (2.6
 393 mW s⁻¹) at 100 mm distance. (n=3, Error=1SD)

394 At the start of the experiment, relatively low concentrations ($0.02\text{-}0.04\ \mu\text{g mL}^{-1}$)
395 of extracellular MC-LR, MC-LW, and MC-LF were detected, while no extracellular
396 MC-LY was detected. Over the course of seven-day photocatalytic treatment, the
397 extracellular microcystin concentrations remained low, not exceeding $0.05\ \mu\text{g}$
398 mL^{-1} in the treated samples, and were completely undetectable after day four of
399 the UV/TiO₂ treatment. As the intracellular microcystins concentrations decrease
400 due to loss of structural integrity of the cyanobacterial cells, extracellular toxin
401 concentrations should increase, however this was not observed in the
402 photocatalytically treated samples. Instead the intracellular microcystins were
403 photocatalytically decomposed once they were released into the water. The
404 efficacy of photocatalytic removal of dissolved microcystins has been
405 demonstrated previously (Gunaratne *et al.*, 2020; Lawton *et al.*, 2003; Liu *et al.*,
406 2009; Pestana *et al.*, 2015). The decreased microcystin concentrations in the
407 TiO₂-control compared to the UV only control can be explained with adsorption of
408 the microcystin congeners onto the surface of the TiO₂ layer on the glass beads,
409 as previously observed (Pestana *et al.*, 2015). The sum of the intracellular and
410 extracellular microcystin concentrations of the TiO₂-control represents the total
411 microcystin. Comparing this to the total microcystins of the photocatalytically
412 treated samples allows the determination of the individual removal of the
413 different microcystin congeners (table 2).

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420 **Table 2:** Reduction of total microcystins (intra- and extracellular) produced by *M.*
 421 *aeruginosa* PCC7813 after seven days of treatment in a photocatalytic reactor under UV-
 422 LED irradiation ($2.8 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 365 nm (2.6 mW s^{-1})) at 100 mm distance
 423 in the presence of TiO_2 coated porous glass beads. ($n=3$, Error=1SD)
 424

MC congener	Mean total microcystins TiO_2 control ($\mu\text{g mL}^{-1}$)	Mean total microcystins photocatalytic treatment ($\mu\text{g mL}^{-1}$)	Mean Δ total microcystins time 7d ($\mu\text{g mL}^{-1}$)	Mean per cent reduction total microcystins
MC-LR	0.79 ± 0.04	0.19 ± 0.04	0.6 ± 0.07	76 ± 6
MC-LY	0.08 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	55 ± 3
MC-LW	0.15 ± 0.02	0.04 ± 0.01	0.12 ± 0.01	72 ± 2
MC-LF	0.25 ± 0.03	0.08 ± 0.01	0.17 ± 0.03	66 ± 5
Combined	1.28	0.35	0.93	73

425
 426 In the control samples (UV with no catalyst and TiO_2 with no UV) there were no
 427 cell-disrupting processes occurring which would lead to the liberation of
 428 microcystins. Thus, as expected, the extracellular microcystins concentrations
 429 (MC-LR, -LW, -LF) remained relatively consistent in both control samples over
 430 the course of seven days. Lack of cellular disruption is evidenced by the increase
 431 in cell numbers over the course of the seven days (figure 2) and intracellular
 432 microcystin concentrations (figure 3) in the TiO_2 -control. The doubling rate of *M.*
 433 *aeruginosa* PCC7813 in the TiO_2 -control is approximately seven days (from
 434 1.5×10^6 at time 0 to 3.4×10^6 cells mL^{-1} at time 7 d). Wilson and co-workers
 435 (2006) report the average doubling time for environmental isolates of *M.*
 436 *aeruginosa* cultured in BG-11 medium as 2.8 days. In the current study *M.*
 437 *aeruginosa* PCC7813 was placed in artificial fresh water which contained none of
 438 the main nutrients required for growth, which can explain the slower doubling
 439 rate. Another factor that will affect the growth rate of *M. aeruginosa* is the initial
 440 inoculation cell density. In the current study initial cell density was 15×10^6 cells
 441 mL^{-1} in 30 mL, which represents a very high inoculation cell density. In a
 442 laboratory study Dunn and Manoylov (2016) have demonstrated that *M.*
 443 *aeruginosa* UTEX2385 does not grow as rapidly with a higher (7×10^5 cells mL^{-1})

444 initial inoculation cell density compared to a lower (1×10^5 cells mL^{-1}) one in Bold's
445 medium under laboratory conditions. No extracellular MC-LY was detected over
446 the entire experimental period in neither the treatment samples or controls. This
447 can be explained by the low intracellular concentrations of MC-LY ($0.06 \mu\text{g mL}^{-1}$)
448 present.

449

450 **4. Conclusions**

451 In the current study we have demonstrated that a simple photocatalytic system
452 of recycled, TiO_2 coated, porous, foamed glass beads with low level UV
453 irradiation supplied by UV emitting LEDs can successfully inhibit cyanobacterial
454 growth and eliminate released microcystins. The design of the treatment system
455 is readily scalable. The housing of the beads can be increased in size to contain
456 more TiO_2 -coated beads and the application of waterproof UV-LEDs in long strips
457 attached to the side of the bead housing would facilitate the required UV
458 irradiation. These LEDs may be powered by integrated floating solar panels that
459 would provide a self-contained and sustainable treatment system.

460 The proposed treatment system:

- 461 • is energy efficient due to the use of UV emitting LEDs requiring a lower
462 energy in-put compared to conventional bulb light sources (mW power
463 input compared to W)
- 464 • could be powered *in situ* by photovoltaic cells to further increase the
465 energy efficiency
- 466 • does not exacerbate the treatment challenge of, especially dissolved,
467 cyanobacterial secondary metabolite and intracellular organic material by
468 photocatalytically removing intracellular toxins

- 469 • represents a “green” treatment option through the use of recycled
470 materials, catalyst, and low-energy LEDs (which could be further
471 enhanced by the application of photovoltaic cells).

472

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479

480 **6. References**

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