# Removal of Microcystis aeruginosa by UV-activated persulfate
## Performance and characteristics

Wang, Zongping; Chen, Yiqun; Xie, Pengchao; Shang, Ran; Ma, Jun

**DOI**
10.1016/j.cej.2016.04.125

**Publication date**
2016

**Document Version**
Accepted author manuscript

**Published in**
Chemical Engineering Journal

# Removal of *Microcystis aeruginosa* by UV-activated persulfate: Performance and characteristics

Zongping Wang[a,b], Yiqun Chen[a,b], Pengchao Xie[a,b,*], Ran Shang[c,**], and Jun Ma[d]

[a] *School of Environmental Science and Engineering, Huazhong University of Science and Technology (HUST), Wuhan 430074, China*

[b] *Key Laboratory of Water and Wastewater Treatment (HUST), MOHURD, Wuhan, 430074, China*

[c] *Department of Sanitary Engineering, Faculty of Civil Engineering and Geosciences, Delft University of Technology, P.O. Box 5048, 2600 GA Delft, The Netherlands*

[d] *State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China*

**Abstract**

Cyanobacteria blooms in source waters have become a worldwide issue for drinking water production. UV-activated persulfate (UV/PS) technology was firstly applied to remove cultivated *Microcystis aeruginosa* in bench scale. The presence of persulfate significantly enhanced both cytoclasis and algal organic matter mineralization compared with UV-C inactivation alone. Around 98.2% of algal cells were removed after UV/PS process treatment for 2 h at a dosage of PS being 1500 mg/L (approximately 6 mM). Both sulfate and hydroxyl radicals were proven to contribute

* Corresponding authors.
*E-mail address:* pengchao.x@gmail.com (P. Xie), r.shang@tudelft.nl (R. Shang).

to the removal of algae and the loss of cell integrity. The cultivated *Microcystis aeruginosa* in death growth phase were found to be more vulnerable to UV/PS treatment than those growing in log phase, thus a significant lower dosage of PS is needed to achieve the desired removal efficiency. This study suggested a novel application of UV/PS process in the removal of algae in source waters due to the high degradation efficiency of both algal cells and their derived organic matter.

*Keywords: Microcystis aeruginosa*; Ultraviolet; Persulfate; Cell integrity; Algal organic matter

## 1. Introduction

In recent decades, harmful algal blooms have frequently occurred in source waters such as lakes, rivers and reservoirs associated with eutrophication throughout the world [1-4]. *Cyanobacteria*, a prominent and ubiquitous issue, has attracted worldwide attention among the harmful algal blooms. Along with excessive algal cells, the algal organic matter (AOM) including extracellular organic matter (EOM) and intracellular organic matter (IOM) generated via metabolic excretion always cause serious water problems [5]. The AOM produced by some specific *Cyanobacteria* genera including *anabaena*, *microcystis*, *planktothrix*, etc. has been proven to comprise taste- and odor- substances, such as 2-methylisoborneol and geosmin [6], and a wide range of toxic intracellular metabolites being suggested to cause both acute and chronic effects on hepatocyte and central nervous system of aquatic organisms and biomagnify [7-10]. Moreover, algal cells and AOM have been reported to be important precursors of vast disinfection by-products (DBPs) including traditional trihalomethanes (THMs), haloacetic acids (HAAs) and emerging nitrogenous DBPs (N-DBPs) containing high genotoxicity and carcinogenicity [11-13].

However, the conventional drinking water treatment plant only shows limited removal efficiency on algal cells due to electrostatic repulsion, surface hydrophilicity and steric effects [14-16]. The residual cells after sedimentation could adhere to the filter material surface subsequently causing filter clogging, penetrate into the water supply pipe network, and finally impair the drinking water quality [17]. It should be

noted that the dissolved AOM generated via metabolic excretion are even more different than algal cells to be removed by using traditional coagulation-sedimentation-filtration process [18], which may adversely affect conventional water production via inhibition of coagulation [19, 20].

Activated persulfate (PS) oxidation has been studied as an alternative conventional advanced oxidation process (AOP) in water treatment [21-23]. The AOP using PS is mainly achieved by the formation of reactive sulfate radical ($SO_4^{\bullet-}$, $E^0$=2.65–3.1 V) through the decomposition of PS by heat, transit metals, light, microwave or ultrasound [24-26]. Similar to hydroxyl radical ($HO^{\bullet}$, $E^0$=1.8–2.7 V), electron-transfer is expected to be a vital reaction when $SO_4^{\bullet-}$ is used to degrade organic pollutants [27]. However, $SO_4^{\bullet-}$ is more selective in comparison with hydroxyl radical in general, thus might be more effective in the degradation of some organic pollutants in the presence of radical scavengers [27]. Particularly, PS activated by zerovalent iron was recently used for disinfection of ballast water and achieved a result that the species of marine phytoplankton could be inactivated by such a process without generating harmful byproducts [28]. However, to our knowledge, activated PS technology has not been utilized for algae removal in source waters.

Among the known PS-activation processes, ultraviolet (UV) radiation at 254 nm can activate the PS to generate $SO_4^{\bullet-}$ through Eq. 1 with a quantum yield of 0.7 mol E/s [29], and the other main chemical interactions subsequently take place were summarized in Eqs. 2 ~ 5 [30].

$$S_2O_8^{2-} \xrightarrow{UV} 2SO_4^{\bullet-} \qquad \phi = 0.7 \qquad (1)$$

$$SO_4^{\bullet-} + OH^- \longrightarrow SO_4^{2-} + HO^{\bullet} \qquad k = 6.5 \times 10^7\ M^{-1}s^{-1} \qquad (2)$$

$$SO_4^{\bullet-} + H_2O \longrightarrow HSO_4^{2-} + HO^{\bullet} \qquad k = 8.3\ M^{-1}s^{-1} \qquad (3)$$

$$OH^{\bullet-} + S_2O_8^{2-} \longrightarrow S_2O_8^{\bullet-} + OH^- \qquad k = 1.4 \times 10^7\ M^{-1}s^{-1} \qquad (4)$$

$$OH^{\bullet-} + SO_4^{\bullet-} \longrightarrow HSO_5^- \qquad k = 1 \times 10^{10}\ M^{-1}s^{-1} \qquad (5)$$

Furthermore, UV process has been applied to suppress algae growth in many cases accompanied by chlorophyll bleaching and inhibition of metabolic activity [31-35]. Therefore, the UV radiation can be regarded as one of the feasible techniques to activate the PS for drinking water treatment applications.

In the present study, the performance of UV-activated PS (UV/PS) process on algae removal has been investigated using *Microcystis aeruginosa* (*M. aeruginosa*), a most abundant and common occurring cyanobacteria specie [36]. Additionally, variations in the characteristics of AOM during the UV/PS treatment were identified to further recognize the plausible by-products within the oxidation process.

## 2. Materials and methods

### *2.1. Materials*

The *M. aeruginosa* (No. FACHB-909) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, and laboratorial cultivated in a light growth incubator (Guohua Electric Co., Ltd., China). The *M. aeruginosa* was incubated under the specific growth conditions (25 ± 1 ℃ with a light-dark cycle of

12 h: 12 h) in BG-11 media [37]. The algae solutions in log phase were harvested and diluted using ultrapure water (18.5 MΩ cm) produced from a water purifier (PCDX-J, Pincheng Co. Ltd, China). Then a final cell density of $1 \times 10^6$ cell/mL was achieved for the experiments, which mimics to the practical density in harmful algal blooms [38].

All chemicals used in the experiments were of analytical reagent grade at least. Sodium persulfate ($Na_2S_2O_8$), sodium hydroxide, hydrochloric acid, methanol (MeOH) and tert-butyl-alcohol (TBA) were obtained from Sinopharm Chemical Reagent Co., Ltd., China. Powdered $Na_2S_2O_8$ was added to the prepared *M. aeruginosa* solutions to achieve designated concentrations of PS each time. Solution pH was subsequently adjusted to be 7.0 which is around the pH of natural water by using HCl and NaOH at a concentration of 0.1 M.

### *2.2. Experimental reactor and procedures*

Algae removal experiments were carried out in a cylindrical pyrex reactor (600 mL effective volume) with a low-pressure UV lamp (254 nm, 23W, GPH 436T5L/4, Philips Electronics Ltd., The Netherlands) (Fig. S1). An immersion well made of high purity quartz was placed inside the glass reactor. The UV lamp was fixed inside the immersion well. Cooling water was pumped through the thin annular zone of the immersion well to prevent overheating of the reaction solutions. In order to achieve a stabilized radiation intensity (measured to be 1.25 $mW/cm^2$ in average by the reported

116 method [39]), the lamp was always switched on for 15 min before being placed into
117 the reactor. A magnetic stirring apparatus at a speed of 200 rpm was used to
118 homogenize the solutions throughout the experiments. Samples were collected via the
119 sampling port at specific time intervals. To evaluate the reaction mechanisms in the
120 UV/PS system, MeOH and TBA were added as scavengers for hydroxyl and sulfate
121 radicals. Each batch of experiment was carried out in triplicate. As the formed $SO_4^{\bullet-}$
122 could be scavenged by high concentrations of $S_2O_8^{2-}$ and $Cl^-$ from BG-11 media and
123 HCl solution (section 3.2), the presence of anions including $NO_3^-$ and $HCO_3^-$ at
124 concentrations as usual in natural waters was expected to play little role in the
125 removal of algal cells (Fig. S2). Thus, the impacts of co-existing anions would not be
126 further discussed in the following sections.
127
128 ***2.3. Analytical methods***
129 The cell concentration of *M. aeruginosa* was measured using an UV-visible
130 spectrophotometer (U-3100, Hitachi, Japan) at a wavelength of 681 nm since the
131 optical density at 681 nm ($OD_{681}$) is linearly correlated with counted cell number by
132 microscope within the experimental range [40, 41]. Thus, the removal efficiency of
133 algal cells ($\rho$, %) can be calculated using Eq. 6.

134 $$\rho = \frac{(OD_{681_0} - OD_{681_t})}{OD_{681_0}} \times 100\% \qquad (6)$$

135 where $OD_{681_0}$ and $OD_{681_t}$ were the optical density values at 0 min and *t* min.

136 Chlorophyll-a (Chl-a) was extracted using acetone solution and then measured

using the spectrophotometer at wavelengths of 663 nm, 645 nm and 630 nm according to the reported method [42].

The algal cell integrity before and after treatment was monitored by a flow cytometer (Accuri C6, BD Biosciences, USA) equipped with an argon laser emitting (wavelength fixed at 488 nm) for fluorescence measurement. Algal cells were stained using SYTOX green nucleic acid stain (Invitrogen, Life Technologies, USA) [43]. Fluorescent filters and detectors were equipped to collect green fluorescence in channel FL1 (530 nm) and red fluorescence in channel FL3 (630 nm), which represents damaged and integrated cells, respectively, since SYTOX could penetrate damaged cells and stain the nucleic acid to emit green fluorescence.

Extracellular AOM was extracted from reaction solution by centrifuging the cell suspension at 8000 rmp for 15 min and subsequently filtering the supernatant through 0.45 μm cellulose acetate membranes [5]. The extracellular AOM was characterized on fluorescence spectroscopy, UV-Vis spectrum scan, and total organic carbon (TOC). A fluorescence spectrophotometer (F-4600, Hitachi, Japan) was used to measure the fluorescence excitation–emission matrix (EEM) spectroscopy of AOM. Excitation wavelengths (Ex) were scanned from 200 to 450 nm with 5 nm intervals and emission wavelengths (Em) from 280 to 550 nm with 2 nm intervals. The scanning speed was set at 1200 nm/min. Background signals were minimized by subtracting the signals of the blank (i.e. ultrapure water). The EEM data were analyzed by MATLAB 2010b (The MathWorks, Inc., USA). The UV-Vis spectrum scan of AOM ranging from 200

158 to 700 nm was measured using the U-3100 spectrophotometer. The concentration of
159 dissolved organic carbon (DOC) in the prepared sample was measured using a
160 TOC/TN analyzer (C/N 2100, Analytic Jena, Germany).

161 The persulfate concentration was measured by universal iodometric titration
162 method [44]. The procedure was conducted by mixing 2 mL samples and 10 mL KI
163 stock solution (10% mass fraction) in 40 mL ultrapure water. Then the resulting
164 solutions were equilibrated for 3 h in dark, and subsequently titrated by calibrated
165 $Na_2S_2O_3$ solution (0.1 M). A turbidimeter (Ruixin WGZ-2, China) was used to
166 measure the turbidity of algal solution before and after treatment. The *M. aeruginosa*
167 cells in suspension for morphology observation were firstly centrifuged at 6000 rpm
168 to collect the precipitate, and then dried by a vacuum freeze dryer (model FD-1A-50,
169 Shanghai Boyikang Instrument Co., Ltd., China). Thereafter, the dried algae samples
170 were sputter coated with gold by the sputter coater, and then photographed using a
171 scanning electron microscopy (SEM) (Sirion 200, FEI, USA) at 10 kV.

172

173 **3. Results and discussion**

174 ***3.1. Comparison of UV, PS and UV/PS processes on algae removal***

175 Fig. 1 shows the removal of *M. aeruginosa* based on the changes of $OD_{681}$ (Fig. 1a)
176 and Chl-a (Fig. 1b) by using three different oxidation processes, UV irradiation, PS
177 oxidation and combined UV/PS oxidation, at 25 ℃ with the initial reaction pH being
178 7.0. Negligible decrease of $OD_{681}$ and Chl-a was observed using PS oxidation alone at

a concentration of 1500 mg/L (about 6 mM) over a period of 120 min, indicating little to no oxidation of algal cells by PS directly. In contrast, UV irradiation could degrade algal cells to some extent with $OD_{681}$ and Chl-a being removed by 21.8% and 87.9%, respectively, after 120 min of radiation. The results corroborate that UV at 254 nm can effectively destroy the photosynthesis system of *M. aeruginosa*, but hardly cause large-scale cell cytolysis, which was also reported in other publications [32, 45]. UV/PS process showed the highest levels of removal efficiency for both $OD_{681}$ (98.2%) and Chl-a (99.8%) among the three oxidation processes.

As PS oxidation and UV irradiation had limited degradation efficiency on algal cells, it could be expected that the reactive radicals, i.e. $SO_4^{\bullet-}$ and $HO^{\bullet}$, produced in UV/PS system were responsible for the observed degradation of algal cells. The conclusion was further proved by the results shown in Fig. S3 that the presence of MeOH and TBA inhibited the removal of $OD_{681}$ significantly when using UV/PS process. MeOH is usually considered as an effective quencher for both the $SO_4^{\bullet-}$ and $HO^{\bullet}$ [46]. However, TBA is an effective quencher for $HO^{\bullet}$, but not for $SO_4^{\bullet-}$ [29]. Thus, they could be used to distinguish $SO_4^{\bullet-}$ and $HO^{\bullet}$ based on the difference of the degradation rate of target compounds. Fig. S3 shows that co-existence of either MeOH or TBA inhibited the removal of $OD_{681}$, while the inhibiting ability of MeOH is stronger than that of TBA. It proved that both $SO_4^{\bullet-}$ and $HO^{\bullet}$ contributed to the oxidation of algal cells in the experiment. It is known that activation of PS by UV only generate $SO_4^{\bullet-}$ (Eq. 1) without the production of $HO^{\bullet}$ [29]. However, $HO^{\bullet}$ was

proposed to be generated when $SO_4^{\bullet-}$ reacts with $OH^-$ at a rate constant of $6.5 \times 10^7$ $M^{-1} S^{-1}$ through Eq. 4 [30], or with water at a rate constant of 8.3 $M^{-1} S^{-1}$ through Eq. 3 [47]. Besides, the presence of chloride could convert $SO_4^{\bullet-}$ to $HO^{\bullet}$ through reactions shown in Eqs. 7 ~ 10 [48], especially when the reaction pH was higher than 5.0, which make sure the presence of $HO^{\bullet}$ in the experiment [49].

$$SO_4^{\bullet-} + Cl^- \rightleftharpoons SO_4^{2-} + Cl^{\bullet} \quad (7)$$

$$Cl^{\bullet} + OH^- \longrightarrow HO^{\bullet} + Cl^- \quad (8)$$

$$Cl^{\bullet} + H_2O \longrightarrow HOCl^{\bullet-} + H^+ \quad (9)$$

$$HOCl^{\bullet-} \rightleftharpoons HO^{\bullet} + Cl^- \quad (10)$$

### *3.2. Effect of initial PS dose on algae removal*

The effect of initial PS dose ranging from 0 mg/L to 1500 mg/L on the removal of *M. aeruginosa* by focusing on the changes of $OD_{681}$ and Chl-a was investigated (Fig. 2). The removal rates of $OD_{681}$ and Chl-a increased with the increase of initial PS dosage. When the dosage of PS increased from 0 mg/L to 1500 mg/L (about 6 mM), the removal rates of $OD_{681}$ and Chl-a increased from 21.8% and 87.9% to 98.2% and 99.8%, respectively, after treatment for 120 min. It should be noted that, the rapid decrease of Chl-a was observed in the initial 60 min treatment (including the only UV irradiation case), but not for the removal of $OD_{681}$. The result suggested that the photosynthetic system of *M. aeruginosa* was damaged immediately through synergetic irradiation and oxidation in UV/PS system, possibly due to the rapid destruction in gene expression of both *psbA* (for D1) and *cpc* (for phycocyanin) [50].

The variation of pHs over time under different initial PS doses was displayed in Fig. S4. Negligible change of pHs was observed under UV irradiation alone over a period of 120 min. However, solution pHs gradually reduced in UV/PS system during the oxidation, which was enhanced with the increase of PS doses. The results can be explained by the production of sulfate acid through $S_2O_8^{\bullet-}$ oxidation of water (Eqs. 11 and 12) [30, 51]:

$$SO_4^{\bullet-} + S_2O_8^{2-} \longrightarrow S_2O_8^{\bullet-} + SO_4^{2-} \quad (11)$$

$$S_2O_8^{\bullet-} + \frac{3}{2}H_2O \longrightarrow 2SO_4^{2-} + \frac{3}{2}O_2 + 3H^+ \quad (12)$$

Fig. S5 shows that the dissolved oxygen (DO) in the experimental solution significantly increased in the initial 30 min when using UV/PS process to treat algae, supporting the speculation on oxidation of water by $S_2O_8^{\bullet-}$. Then the gradually reduction of DO in the subsequent 90 min might due to the release into air, reacting with $HO^{\bullet}$ adducts to produce peroxyl transients [52], and being converted to oxygen-centered radicals which could be consumed by algae and its derived organic matter [53].

If the dosed persulfate was completely decomposed, formed sulfate at concentrations of 800 mg/L and 1200 mg/L would be achieved when the dosages of persulfate were 1000 mg/L and 1500 mg/L, respectively. A sulfate concentration that is higher than 600 mg/L in drinking water might cause taste alteration and diarrhea [54]. However, it should be noted that the concentrations of *M. aeruginosa* in most source waters were always lower than $10^6$ cells/mL. When the initial cell density was

242 reduced to 2.3 × $10^5$ cells/mL, the residual $OD_{681}$ and turbidity decreased by 57.9%
243 and 62.3%, respectively, after UV/PS process treatment for 120 min with an initial PS
244 dosage of 200 mg/L (Fig. S6).
245
246 ***3.3. Effect of growth phase on algae removal***
247 The growth of *M. aeruginosa* can be modeled with four different phases: lag phase,
248 log phase, stationary phase, and death phase. The removal of algae in their log phase
249 and death phase were investigated due to the large variation of cell population and
250 dissolved organic substances [55]. The effect of growth phase on the removal of *M.*
251 *aeruginosa* was studied under different PS doses (Fig. 3). The removal of $OD_{681}$ in
252 death phase kept high efficiencies (>92.8%) for all the initial concentrations of PS
253 ranging from 0 mg/L to 1000 mg/L over an oxidation period of 120 min. However, for
254 the cells in log phase, the removal of $OD_{681}$ only increased from 21.8% to 83.7% with
255 the initial dosage of PS increasing from 0 mg/L to 1500 mg/L after reaction for 120
256 min, which was much lower than the removal efficiencies for the algal cells in death
257 phase. Similarly, the removal efficiency of Chl-a ranging from 90.7% to 98.5% in
258 death phase by using UV/PS was also higher than that ranging from 87.9% to 97.6%
259 in log phase.
260 The easier removal of *M. aeruginosa* in death phase than that in log phase by using
261 UV/PS process was supposed to be connected to the disparity of biological activity,
262 EOM component, and morphologies of algal cells in different growth phases [56].

263 Besides, the autolysis of cells largely occurred in death phase [19], which might also
264 contribute to the high removal efficiency.
265
266 ***3.4. Mineralization of AOM***
267 Fig. 4 shows the residual TOC and extracellular DOC of *M. aeruginosa* suspension at
268 different PS doses. Negligible changes of TOC and extracellular DOC were observed
269 after single UV irradiation over a period of 120 min, indicating that limited
270 UV-induced mineralization of AOM occurred, which was in accordance with the
271 previous report [57]. On the other hand, the results reveal that the UV irradiation only
272 had limited damage on the structure of algal cell and did not cause large-scale
273 cytoclasis [58], since an increase in DOC concentration would be found due to the
274 release of IOM from damaged algal cells [25, 48].
275 With the dosage of PS increasing from 0 mg/L to 1500 mg/L (about 6 mM), the
276 TOC and extracellular DOC were decreased from 12.50 and 7.59 mg/L to 1.63 and
277 1.61 mg/L, respectively, over a reaction period of 120 min. The significant removal of
278 extracellular DOC (decreased by 79.6% at 1500 mg/L PS dosage) indicates that
279 UV/PS process can effectively mineralize AOM in the reaction solution. Moreover,
280 the result of higher reduced concentration of TOC than extracellular DOC in the
281 presence of PS can be explained by the fact that released dissolved IOM could make
282 up parts of mineralized DOC, reflecting that UV/PS oxidation can induce destruction
283 of algal cell structures. This speculation was supported by the result that residual TOC

(1.63 mg/L) and extracellular DOC (1.61 mg/L) were extremely approximate at an initial PS dosage of 1500 mg/L.

The UV-Vis spectra of *M. aeruginosa* solution before and after UV/PS treatment were also studied (insert graph in Fig. 4). The absorbance of solution ranging from 200 to 700 nm was significantly decreased after UV/PS treatment, which was in accordance with the changes of TOC and DOC. It is noteworthy that, the peak at 681 nm was disappeared after treatment, suggesting the effective removal of algal cells in UV/PS system.

### *3.5. Evolution in fluorescence EEM spectra of extracellular AOM*

The effects of UV/PS process treatment on fluorescence EEM spectra of extracellular AOM are shown in Fig. 5 and Table S1. There were four fluorescence peaks at Ex/Em wavelengths of 270/442 nm (peak A), 350/432 nm (peak C), 230/330 nm (peak $T_2$), and 280/330 nm (peak $T_1$) in the control sample (Fig. 5a), which represented fulvic-like, humic-like and protein-like substances, and dissolved microbial metabolites, respectively [59, 60]. The strong signal at peak $T_1$ suggested high concentration of protein-like substances contained in EOM, which was similar to other reports [61, 62].

However, after UV/PS process treatment for 5 min, peaks A and C were observed to be significantly enhanced, accompanied with the disappearance of peaks $T_1$ and $T_2$ in contrast (Fig. 5b). This can be explained that UV/PS process was supposed to cause

cell secretion and decomposition which was expected to produce fulvic-like and humic-like substances [63, 64].

When the treatment time was over 30 min, all the four peaks were disappeared (Figs. 5c and 5d), whose possible reasons were proposed as below: 1) UV/PS process could further oxidize and even mineralize the formed humic-like and fulvic-like substances (Fig. 4); and 2) the large-scale cell cytoclasis and metabolites release mainly occurred in the initial reaction period under UV/PS oxidation, which was similar to the ozonation of *cyanobacteria* [65].

Additionally, comparative experiments of single UV irradiation, PS alone and UV/PS process treatment on the changes of the fluorescence EEM spectra of extracellular AOM were conducted (Fig. S7). After treatment for 30 min, UV irradiation caused significant decrease of peak $T_1$, but slight enhancement of peak C. While the treatment of algae by PS alone had negligible impact on the fluorescence EEM spectra of the extracellular AOM. In comparison with UV irradiation or PS oxidation alone, UV/PS treatment caused the most significant decline of all the peaks on the fluorescence EEM spectra. Thus, it is reasonable to conclude that the formed reactive species, such as hydroxyl and sulfate radicals, in UV/PS process contribute to the degradation of organic matter.

### *3.6. Cell integrity and cellular morphology*

Fig. 6 shows the impacts of PS oxidation, UV irradiation, and UV/PS treatment on

326 cell integrity by using a flow cytometer to measure changes in cell permeability and
327 chlorophyll auto-fluorescence. P1 and P2 regions represent damaged and integrated
328 cell population, respectively. In Fig. 6a, the proportion of live cells was 96.1%,
329 reflecting that the vast majority of *M. aeruginosa* cells without any treatment were
330 intact. Compared with the control sample, the damaged cells shown in P1 region was
331 found to be only slightly increased by 1.8% after 2 days reaction with PS alone (Fig.
332 6b), indicating that individual PS oxidation played little to no roles in destruction of
333 *M. aeruginosa* cells . In contrast, UV was observed to be more effective in decreasing
334 the live algal cells as shown in regional P2 of Fig. 6c that the amount of live algal
335 cells was reduced. However, the amount of algal cells shown in regional P1 was also
336 decreased. Since the green fluorescence in channel FL1 (Regional P1) was from the
337 stained nucleic acid by SYTOX [43], the results can be explained by the fact that UV
338 irradiation could damage nucleic acid [66, 67]. Among the four treatment processes,
339 UV/PS process had the strongest ability to damage algal cells (Fig. 6d). After
340 treatment for 30 min, the integrated cell population was significantly reduced,
341 indicating that the reactive substances in UV/PS system, such as $SO_4^{\bullet-}$ and $HO^{\bullet}$, can
342 strongly damage the cell integrity. No signals were found in regional P1 suggested
343 that UV/PS could further oxidize nucleic acid effectively due to the synergetic effect
344 of UV irradiation and the generated reactive radicals.

345 The surface morphologies of *M. aeruginosa* cells before and after UV/PS treatment
346 were observed using the SEM. Compared to the algal cells without any treatments

(Fig. 7a), the surface morphologies of cells were deformed and the cellular structure was completely lost after the UV/PS treatment (Fig. 7b). Some apparent materials were found to be released from the cells, which were probable to be amino acids and carbohydrates [28].

### *3.7. Persulfate decomposition*

Fig. 8a shows the persulfate decay under different initial PS doses ranging from 500 to 1500 mg/L. In each case, residual persulfate was less than 2% over a reaction period of 120 min. The decomposition of persulfate followed the first-order kinetics model (Fig. 8b), suggesting that the presence of algal cells and AOM had little impact on the self-decomposition of persulfate, which was supported by the insignificant oxidation potential of PS on algal cells and AOM compared to UV/PS process (Figs. 1 and S7). The added PS was almost completely decomposed ($\geq$ 99.9%) during the reaction when the initial concentration of PS was less than 1000 mg/L. Even at initial dosages of 1250 mg/L and 1500 mg/L, the average residual PS concentrations were only 2.14 mg/L (about 0.2%) and 23.6 mg/L (about 1.6%) after treatment for 120 min, respectively. Thus, it is concluded that application of UV/PS process in the removal of algae in natural water had little potential to cause undesired risk from the residual PS.

## 4. Conclusions

UV/PS process is demonstrated to be an effective technology for the removal of *M.*

*aeruginosa* in laboratory. The formed reactive species including $SO_4^{\bullet-}$ and $HO^{\bullet}$ were proven to be the most important reasons for the removal of algal cells. Increasing PS dosage could improve the removal of algal cells and the mineralization of AOM. With the addition of 1500 mg/L (about 6 mM) PS, removal rates up to 98.2% and 99.8% of algal cells and Chl-a, respectively, were achieved in UV/PS system over a reaction period of 120 min. *M. aeruginosa* in death phase was easier to be removed by using UV/PS treatment than that in log phase. Although the UV/PS process significantly damaged the structures of algal cell and caused the release of IOM, these derived organic compounds along with the dissolved EOM could be further mineralized in the subsequent reaction period. The fast decomposition of persulfate might insure the safety of using UV/PS to remove algal cells to some extent.

**Acknowledgments**

This work was supported by the National Natural Science Foundation of China (Grant Nos. 51578259). We also thank the Analytical and Testing Center of Huazhong University of Science and Technology for related analysis.

**Appendix A. Supplementary data**

Supplementary data (Figs. S1 to S7 and Tab. S1) associated with this article can be found, in the online version.

## References

[1] B.E. Lapointe, L.W. Herren, D.D. Debortoli, M.A. Vogel, Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon, Harmful Algae 43 (2015) 82-102.

[2] J.D. Isaacs, W.K. Strangman, A.E. Barbera, M.A. Mallin, M.R. McIver, J.L.C. Wright, Microcystins and two new micropeptin cyanopeptides produced by unprecedented Microcystis aeruginosa blooms in North Carolina's Cape Fear River, Harmful Algae 31 (2014) 82-86.

[3] M.A. Burford, S.A. Johnson, A.J. Cook, T.V. Packer, B.M. Taylor, E.R. Townsley, Correlations between watershed and reservoir characteristics, and algal blooms in subtropical reservoirs, Water Research 41 (2007) 4105-4114.

[4] V.H. Smith, Eutrophication of freshwater and coastal marine ecosystems: a global problem, Environmental Science and Pollution Research 10 (2003) 126.

[5] R.K. Henderson, A. Baker, S.A. Parsons, B. Jefferson, Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms, Water Research 42 (2008) 3435-3445.

[6] J.O. Jo, S.D. Kim, H.J. Lee, Y.S. Mok, Decomposition of taste-and-odor compounds produced by cyanobacteria algae using atmospheric pressure plasma created inside a porous hydrophobic ceramic tube, Chemical Engineering Journal 247 (2014) 291-301.

[7] J.P. Berry, P.D. Gibbs, M.C. Schmale, M.L. Saker, Toxicity of cylindrospermopsin,

410 and other apparent metabolites from Cylindrospermopsis raciborskii and
411 Aphanizomenon ovalisporum, to the zebrafish (Danio rerio) embryo, Toxicon :
412 official journal of the International Society on Toxinology 53 (2009) 289-299.
413 [8] R. Bhattacharya, K. Sugendran, R.S. Dangi, P.V.L. Rao, Toxicity evaluation of
414 freshwater cyanobacterium Microcystis aeruginosa PCC 7806: II Nephrotoxicity in
415 rats, Biomedical and Environmental Sciences 10 (1997) 93-101.
416 [9] C. MacKintosh, K.A. Beattie, S. Klumpp, P. Cohen, G.A. Codd, Cyanobacterial
417 microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A
418 from both mammals and higher plants, FEBS letters 264 (1990) 187-192.
419 [10] M.J. Bakke, T.E. Horsberg, Effects of algal-produced neurotoxins on metabolic
420 activity in telencephalon, optic tectum and cerebellum of Atlantic salmon (Salmo
421 salar), Aquatic toxicology 85 (2007) 96-103.
422 [11] J. Fang, J. Ma, X. Yang, C. Shang, Formation of carbonaceous and nitrogenous
423 disinfection by-products from the chlorination of Microcystis aeruginosa, Water
424 Research 44 (2010) 1934-1940.
425 [12] Y.S. Lui, J.W. Qiu, Y.L. Zhang, M.H. Wong, Y. Liang, Algal-derived organic
426 matter as precursors of disinfection by-products and mutagens upon chlorination,
427 Water Research 45 (2011) 1454-1462.
428 [13] X. Yang, W. Guo, Q. Shen, Formation of disinfection byproducts from
429 chlor(am)ination of algal organic matter, Journal of hazardous materials 197 (2011)
430 378-388.

[14] J. Edzwald, Algae, bubbles, coagulants, and dissolved air flotation, Water Science & Technology 27 (1993) 67-81.

[15] C.W. Chow, M. Drikas, J. House, M.D. Burch, R.M. Velzeboer, The impact of conventional water treatment processes on cells of the cyanobacterium Microcystis aeruginosa, Water Research 33 (1999) 3253-3262.

[16] M.R. Teixeira, M.J. Rosa, Comparing dissolved air flotation and conventional sedimentation to remove cyanobacterial cells of Microcystis aeruginosa, Separation and Purification Technology 52 (2006) 84-94.

[17] Q. Shen, J. Zhu, L. Cheng, J. Zhang, Z. Zhang, X. Xu, Enhanced algae removal by drinking water treatment of chlorination coupled with coagulation, Desalination 271 (2011) 236-240.

[18] D.L. Widrig, K.A. Gray, K.S. McAuliffe, Removal of algal-derived organic material by preozonation and coagulation: Monitoring changes in organic quality by pyrolysis-GC-MS, Water Research 30 (1996) 2621- 2632.

[19] M. Pivokonsky, O. Kloucek, L. Pivokonska, Evaluation of the production, composition and aluminum and iron complexation of algogenic organic matter, Water Research 40 (2006) 3045-3052.

[20] M. Ma, R. Liu, H. Liu, J. Qu, W. Jefferson, Effects and mechanisms of pre-chlorination on Microcystis aeruginosa removal by alum coagulation: Significance of the released intracellular organic matter, Separation and Purification Technology 86 (2012) 19-25.

[21] H. Hori, A. Yamamoto, E. Hayakawa, S. Taniyasu, N. Yamashita, S. Kutsuna, H. kiatagawa, R. Arakawa, Efficient decomposition of environmentally persistent perfluorocarboxylic acids by use of persulfate as a photochemical oxidant, Environmental Science & Technology 39 (2005) 2383-2388.

[22] A. Tsitonaki, B.F. Smets, P.L. Bjerg, Effects of heat-activated persulfate oxidation on soil microorganisms, Water Research 42 (2008) 1013-1022.

[23] H. Liu, T.A. Bruton, F.M. Doyle, D.L. Sedlak, In Situ Chemical Oxidation of Contaminated Groundwater by Persulfate: Decomposition by Fe(III)- and Mn(IV)-Containing Oxides and Aquifer Materials, Environmental Science & Technology 48 (2014) 10330-10336.

[24] S. Su, W. Guo, C. Yi, Y. Leng, Z. Ma, Degradation of amoxicillin in aqueous solution using sulphate radicals under ultrasound irradiation, Ultrason Sonochem 19 (2012) 469-474.

[25] Y. Ji, Y. Fan, K. Liu, D. Kong, J. Lu, Thermo activated persulfate oxidation of antibiotic sulfamethoxazole and structurally related compounds, Water Research 87 (2015) 1-9.

[26] T.K. Lau, W. Chu, N.J. Graham, The aqueous degradation of butylated hydroxyanisole by UV/$S_2O_8^{2-}$: study of reaction mechanisms via dimerization and mineralization, Environmental Science & Technology 41 (2007) 613-619.

[27] P. Neta, V. Madhavan, H. Zemel, R.W. Fessenden, Rate constants and mechanism of reaction of sulfate radical anion with aromatic compounds, Journal of the American

Chemical Society 99 (1977) 163-164.

[28] S. Ahn, T.D. Peterson, J. Righter, D.M. Miles, P.G. Tratnyek, Disinfection of ballast water with iron activated persulfate, Environmental Science & Technology 47 (2013) 11717-11725.

[29] G. Mark, M.N. Schuchmann, H.P. Schuchmann, C. von Sonntag, The photolysis of potassium peroxodisulphate in aqueous solution in the presence of tert-butanol: a simple actinometer for 254 nm radiation, Journal of Photochemistry and Photobiology A: Chemistry 55 (1990) 157-168.

[30] P. Neta, R.E. Huie, A.B. Ross, Rate constants for reactions of inorganic radicals in aqueous solution, Journal of Physical and Chemical Reference Data 17 (1988) 1027-1284.

[31] Y. Tao, X. Zhang, D.W. Au, X. Mao, K. Yuan, The effects of sub-lethal UV-C irradiation on growth and cell integrity of cyanobacteria and green algae, Chemosphere 78 (2010) 541-547.

[32] H. Ou, N. Gao, Y. Deng, H. Wang, H. Zhang, Inactivation and degradation of Microcystis aeruginosa by UV-C irradiation, Chemosphere 85 (2011) 1192-1198.

[33] M.G. Antoniou, A. Armah, D.D. Dionysiou, Degradation of microcystin-LR using sulfate radicals generated through photolysis, thermolysis and e− transfer mechanisms, Applied Catalysis B: Environmental 96 (2010) 290-298.

[34] N.S. Shah, X. He, H.M. Khan, J.A. Khan, K.E. O'Shea, D.L. Boccelli, D.D. Dionysiou, Efficient removal of endosulfan from aqueous solution by UV-C/peroxides:

494 a comparative study, Journal of hazardous materials 263 (2013) 584-592.
495 [35] V.K. Sharma, T.M. Triantis, M.G. Antoniou, X. He, M. Pelaez, C. Han, W. Song,
496 K.E. O'Shea, A. Armah, T. Kaloudis, Destruction of microcystins by conventional and
497 advanced oxidation processes: a review, Separation and Purification Technology 91
498 (2012) 3-17.
499 [36] P. Xie, J. Ma, J. Fang, Y. Guan, S. Yue, X. Li, L. Chen, Comparison of
500 Permanganate Preoxidation and Preozonation on Algae Containing Water: Cell
501 Integrity, Characteristics, and Chlorinated Disinfection Byproduct Formation,
502 Environmental Science & Technology 47 (2013) 14051-14061.
503 [37] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic
504 assignments, strain histories and properties of pure cultures of cyanobacteria, Journal
505 of General microbiology 111 (1979) 1-61.
506 [38] P. Li, Y. Song, S. Yu, Removal of Microcystis aeruginosa using hydrodynamic
507 cavitation: Performance and mechanisms, Water Research 62 (2014) 241-248.
508 [39] R.O. Rahn, Potassium iodide as a chemical actinometer for 254 nm radiation: use
509 of lodate as an electron scavenger, Photochemistry and Photobiology 66 (1997)
510 450-455.
511 [40] W. Liang, J. Qu, L. Chen, H. Liu, P. Lei, Inactivation of Microcystis aeruginosa
512 by continuous electrochemical cycling process in tube using Ti/RuO2 electrodes,
513 Environmental Science & Technology 39 (2005) 4633-4639.
514 [41] G. Zhang, P. Zhang, B. Wang, H. Liu, Ultrasonic frequency effects on the

515 removal of Microcystis aeruginosa, Ultrasonics Sonochemistry 13 (2006) 446-450.
516 [42] O. Holm-Hansen, B. Riemann, Chlorophyll a determination: improvements in
517 methodology, Oikos (1978) 438-447.
518 [43] R.I. Daly, L. Ho, J.D. Brookes, Effect of chlorination on Microcystis aeruginosa
519 cell integrity and subsequent microcystin release and degradation, Environmental
520 Science & Technology 41 (2007) 4447-4453.
521 [44] I. Kolthoff, E. Carr, Volumetric determination of persulfate in presence of organic
522 substances, Analytical Chemistry 25 (1953) 298-301.
523 [45] H. Ou, N. Gao, Y. Deng, J. Qiao, H. Wang, Immediate and long-term impacts of
524 UV-C irradiation on photosynthetic capacity, survival and microcystin-LR release risk
525 of Microcystis aeruginosa, Water Research 46 (2012) 1241-1250.
526 [46] P. Xie, J. Ma, W. Liu, J. Zou, S. Yue, X. Li, M.R. Wiesner, J. Fang, Removal of
527 2-MIB and geosmin using UV/persulfate: Contributions of hydroxyl and sulfate
528 radicals, Water research 69 (2015) 223-233.
529 [47] X.-Y. Yu, Z.-C. Bao, J.R. Barker, Free Radical Reactions Involving Cl, Cl2-, and
530 SO4-in the 248 nm Photolysis of Aqueous Solutions Containing S2O82-and Cl, The
531 Journal of Physical Chemistry A 108 (2004) 295-308.
532 [48] G.P. Anipsitakis, D.D. Dionysiou, M.A. Gonzalez, Cobalt-mediated activation of
533 peroxymonosulfate and sulfate radical attack on phenolic compounds. Implications of
534 chloride ions, Environmental science & technology 40 (2006) 1000-1007.
535 [49] H.V. Lutze, N. Kerlin, T.C. Schmidt, Sulfate radical-based water treatment in

presence of chloride: Formation of chlorate, inter-conversion of sulfate radicals into hydroxyl radicals and influence of bicarbonate, Water research 72 (2015) 349-360.

[50] Y. Tao, X. Mao, J. Hu, H.O. Mok, L. Wang, D.W. Au, J. Zhu, X. Zhang, Mechanisms of photosynthetic inactivation on growth suppression of Microcystis aeruginosa under UV-C stress, Chemosphere 93 (2013) 637-644.

[51] H. Liu, T.A. Bruton, W. Li, J. Van Buren, C. Prasse, F.M. Doyle, D.L. Sedlak, Oxidation of Benzene by Persulfate in the Presence of Fe (III)-and Mn (IV)-Containing Oxides: Stoichiometric Efficiency and Transformation Products, Environmental science & technology (2015).

[52] R. Szabó, C. Megyeri, E. Illés, K. Gajda-Schrantz, P. Mazellier, A. Dombi, Phototransformation of ibuprofen and ketoprofen in aqueous solutions, Chemosphere 84 (2011) 1658-1663.

[53] B. Lee, M. Lee, Decomposition of 2, 4, 6-trinitrotoluene (TNT) by gamma irradiation, Environmental science & technology 39 (2005) 9278-9285.

[54] D. Guimaraes, V.A. Leao, Batch and fixed-bed assessment of sulphate removal by the weak base ion exchange resin Amberlyst A21, Journal of hazardous materials 280 (2014) 209-215.

[55] M. Melkonian, Release of Dissolved Organic Substances by the Green Alga Fritschiella tuberosaIyeng. (Chaetophorineae) During Different Growth Phases, Zeitschrift für Pflanzenphysiologie 94 (1979) 125-133.

[56] J. Huang, N. Graham, M.R. Templeton, Y. Zhang, C. Collins, M. Nieuwenhuijsen,

557 A comparison of the role of two blue-green algae in THM and HAA formation, Water
558 Research 43 (2009) 3009-3018.
559 [57] H. Xu, H. Jiang, UV-induced photochemical heterogeneity of dissolved and
560 attached organic matter associated with cyanobacterial blooms in a eutrophic
561 freshwater lake, Water research 47 (2013) 6506-6515.
562 [58] H. Sakai, H. Katayama, K. Oguma, S. Ohgaki, Kinetics of Microcystis
563 aeruginosa Growth and Intracellular Microcystins Release after UV Irradiation,
564 Environmental Science & Technology 43 (2009) 896-901.
565 [59] N. Hudson, A. Baker, D. Reynolds, Fluorescence analysis of dissolved organic
566 matter in natural, waste and polluted waters - A review, River Research and
567 Applications 23 (2007) 631-649.
568 [60] P.G. Coble, Characterization of marine and terrestrial DOM in seawater using
569 excitation. Emission matrix spectroscopy, Marine Chemistry 51 (1996) 325-346.
570 [61] W. Huang, H. Chu, B. Dong, J. Liu, Evaluation of different algogenic organic
571 matters on the fouling of microfiltration membranes, Desalination 344 (2014)
572 329-338.
573 [62] H. Ou, N. Gao, Y. Deng, J. Qiao, K. Zhang, T. Li, L. Dong, Mechanistic studies
574 of Microcystic aeruginosa inactivation and degradation by UV-C irradiation and
575 chlorination with poly-synchronous analyses, Desalination 272 (2011) 107-119.
576 [63] E.J. Rochelle-Newall, T.R. Fisher, Production of chromophoric dissolved organic
577 matter fluorescence in marine and estuarine environments: an investigation into the

578 role of phytoplankton, Marine Chemistry 77 (2002) 7-21.
579 [64] L. Li, C. Shao, T.-F. Lin, J. Shen, S. Yu, R. Shang, D. Yin, K. Zhang, N. Gao,
580 Kinetics of cell inactivation, toxin release, and degradation during permanganation of
581 Microcystis aeruginosa, Environmental science & technology 48 (2014) 2885-2892.
582 [65] H. Miao, W. Tao, The mechanisms of ozonation on cyanobacteria and its toxins
583 removal, Separation and Purification Technology 66 (2009) 187-193.
584 [66] R.P. Sinha, D.-P. Häder, UV-induced DNA damage and repair: a review,
585 Photochemical & Photobiological Sciences 1 (2002) 225-236.
586 [67] J. Cadet, C. Anselmino, T. Douki, L. Voituriez, New trends in photobiology:
587 Photochemistry of nucleic acids in cells, Journal of Photochemistry and Photobiology
588 B: Biology 15 (1992) 277-298.