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Research Paper

Different response pattern of cyanobacteria at development and maintenance stage to potassium permanganate oxidation

Xi Li [a,b,c], Jie Zeng [a,b,d], Xin Yu [e,*]

[a] Key Lab of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] CAS Key Laboratory of Urban Pollutant Conversion, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
[d] Department of Environmental Engineering, Graduate School of Engineering, Kyoto University, Kyoto University Katsura, Nishikyo, Kyoto 615-8540, Japan
[e] College of The Environment & Ecology, Xiamen University, Xiamen 361102, China

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ABSTRACT

Occurrence of successive cyanobacterial blooms in source waters can continuously impair drinking water quality. Previous studies have separately investigated potassium permanganate ($KMnO_4$) to treat high-viability cyanobacteria at just one stage of either development or maintenance. However, maintenance stage exhibited significantly higher cell-density and extracellular organic matters (EOMs) than development stage, which may result in a different $KMnO_4$ oxidation pattern. In this study, kinetics of oxidant decay, membrane integrity loss, and toxin degradation of high-viability cyanobacteria at both stages were compared. Results showed that cyanobacteria at maintenance stage became more resistant to $KMnO_4$ oxidation than that at development stage, since elevated cell-density and more proteins involved in EOMs resulted in lower oxidant exposure at this stage. Meanwhile, elevated cyanobacterial biomass became the main competitors to decrease toxin degradation efficiency at maintenance stage, leading to incapacity to degrade extracellular toxin to below safety guideline of 1 μg L^{-1}. Consequently, comparing with the best strategy for development stage (6 mg min L^{-1}, no membrane damage), a higher oxidant exposure (12 mg min L^{-1}) was recommended to treat cyanobacteria at maintenance stage even with slight membrane damage (19%), since it degraded extracellular toxin to below safety guideline and achieved the highest removal ratio of EOMs. Overall, this study demonstrated that stage of cyanobacteria can strongly affect $KMnO_4$ oxidation pattern, and it is necessary for water supplies to optimize $KMnO_4$ treatments depending on bloom stage.

1. Introduction

In recent years, harmful cyanobacterial blooms have occurred more frequently worldwide (Huisman et al., 2018). These blooms can directly affect the quality of drinking water through producing cyanotoxins (Merel et al., 2013; He et al., 2016). Among these cyanotoxins, microcystin-LR (MC-LR) has received the most attention from regulatory agencies with a guideline value of 1 μg L^{-1} for safe drinking water proposed by World Health Organization (WHO, 2014), since it has acute poisoning and chronic cancer promotion potentials to human beings. Additionally, the generation of algal organic matter (AOMs), including intracellular organic matter (IOMs) and extracellular organic matter (EOMs), can inhibit coagulation process in drinking water treatment plants (DWTPs) (He et al., 2016). AOMs are also precursors to form disinfection by-products (DBPs) after chlorination, deteriorating drinking water quality (Fang et al., 2010; Xie et al., 2013).

In DWTPs, pre-oxidants, including potassium permanganate ($KMnO_4$), chlorine, ozone, are commonly employed to promote the removal of cyanobacteria and associated metabolites (e.g., cyanotoxins, AOMs) by post-coagulation or filtration (Ma et al., 1997, 2012; Plummer and Edzwald, 2002; Chen and Yeh, 2005; Chen et al., 2009; Ma et al., 2012; Xie et al., 2013; Wang et al., 2013; Liu et al., 2017). After pre-chlorination or pre-ozonation, severe cell damage, intracellular metabolites release, and dramatical risk of DBPs formation were reported by many studies (Daly et al., 2007; Fang et al., 2010; Ma et al., 2012; Zamyadi et al., 2013; Xie et al., 2013; Fan et al., 2013b, 2014). In contrast, $KMnO_4$ did not induce the formation of DBPs and it had limited capacity to destroy membrane integrity of cyanobacteria, resulting in

* Corresponding author.
E-mail addresses: xli@iue.ac.cn (X. Li), jzeng19@hotmail.com (J. Zeng), xyu@xmu.edu.cn (X. Yu).

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little release of metabolites (Xie et al., 2013; Fan et al., 2013a, 2013b). To evaluate the application of $KMnO_4$ to treat cyanobacteria-laden source waters, amounts of $KMnO_4$ experiments have been conducted to determine its effects on membrane integrity and associated removal of cyanobacterial metabolites (Table S1). For examples, Fan et al. (2013b) observed a continuous decrease of extracellular toxin without cell lysis at $KMnO_4$ dosages of 1 and 3 mg L^{-1}. Xie et al. (2013) found that $KMnO_4$ of < 2 mg L^{-1} mainly stressed cells, leading to a section of EOMs adsorbed on cellular surfaces without the loss of membrane integrity. In summary, these studies have provided important references for the application of $KMnO_4$ as a pre-oxidant to treat cyanobacteria-laden source waters. However, these data of $KMnO_4$ experiments were always gained using just one stage of high-viability cyanobacteria (development or maintenance stage) (Table S1).

Recently, ecologists proposed a concept of "successive blooms" according to historical observations of cyanobacterial blooms in natural freshwaters (e.g., Taihu lake) (Tang et al., 2018; Wilhelm et al., 2020). A successive cyanobacterial bloom can last for several months in a year, including development, maintenance and decay stages (Tang et al., 2018; Wilhelm et al., 2020). Among the three stages, cyanobacteria at development and maintenance stages remained high-viability. However, cell-density at maintenance stage was much higher than that at development stage (Tang et al., 2018; Wilhelm et al., 2020; Li et al., 2020a). Meanwhile, toxin concentration increased at maintenance stage, posing a higher toxin risk than that at development stage (Rivasseau et al., 1998; Pietsch et al., 2002; Rueckert and Cary, 2009; Tang et al., 2018). Henderson et al. (2008), Leloup et al. (2013), and Pivokonsky et al. (2014) also observed a higher concentration of extracellular organic matters (EOMs) at maintenance stage than that at development stage. Li et al. (2020a) showed that elevated cell-density and EOMs at maintenance stage strongly affected chlorination process, including the decrease of oxidant exposure and the increase of toxin degradation rate. These results suggested that the change of cellular characteristic (e.g., cell-density, EOMs) at different stage may strongly affect $KMnO_4$ oxidation pattern to treat high-viability cyanobacteria. Consequently, it is strongly essential to compare $KMnO_4$ oxidation to treat high-viability cyanobacteria at development and maintenance stages.

To our knowledge, no study has investigated these differences in oxidant exposure, membrane damage, toxin release/degradation and EOMs removal of high-viability cyanobacteria at development and maintenance stages by $KMnO_4$ oxidation. Hence, in this study, a bench-scale successive *Microcystis* bloom was simulated and *Microcystis* samples at development and maintenance stages were collected to conduct $KMnO_4$ experiments. Then, kinetics of oxidant decay, membrane integrity loss, and toxin degradation of high-viability *Microcystis* at development and maintenance stages were compared. Additionally, the removal of extracellular toxin and EOMs at both stages were discussed, aiming to optimize $KMnO_4$ to treat high-viability cyanobacteria at development and maintenance stages during a successive bloom.

2. Materials and methods

2.1. Materials and reagents

A toxic strain *Microcystis aeruginosa* FACHB-915 was used to conduct experiments, and associated details were described by previous study of Li et al. (2020a). Potassium permanganate ($KMnO_4$, analytical purity) crystals were dissolved in ultrapure water to prepare a stock solution of 1.0 g L^{-1}. Sodium thiosulfate crystals ($Na_2S_2O_3$, analytical purity) were dissolved in ultrapure water to prepare a stock solution of 4.0 g L^{-1}, and it was used to terminate oxidative reactions of $KMnO_4$ in *Microcystis* samples. SYTOX Green nucleic acid stain was obtained from Thermo Fisher Scientific (USA), and it was used for membrane integrity analysis by a flow cytometry. For cyanotoxins quantification, standard MC-LR (chromatographically grade) was obtained from Solarbio (China). Mobile phases (methanol and monopotassium) were also chromatographically grade and obtained from Xilong (China).

2.2. Preparing Microcystis samples at development and maintenance stages

To collect cyanobacteria at development and maintenance stages, a successive *Microcystis* bloom was simulated, as described by Li et al. (2020a). Pure-culture *Microcystis* of initial cell-density (about 1.0×10^7 cells mL^{-1}) was inoculated into a fresh BG-11 medium with a volume ratio of 1: 20 and it was cultivated for 80 d. During cultivation, both total cell-density and photosynthetic activity of *Microcystis* were measured at each 5–10 d. From 5–30 d, cell-density increased from 5.2×10^5 to 1.3×10^7 cells mL^{-1} (Fig. 1), suggesting that *Microcystis* growth was at development stage. Then, cell-density kept at high-level of about 1.3×10^7 cells mL^{-1} from 30 to 80 d (Fig. 1), implying that *Microcystis* growth had entered to maintenance stage. However, photosynthetic activity remained constant from development to maintenance stage ($P > 0.05$) (Fig. 1), suggesting that *Microcystis* at development and maintenance stages held the same high cell-viability (Li et al., 2020a, 2020b). Hence, high-viability *Microcystis* at 15 d (middle-development stage; total cell density: 6.0×10^6 cells mL^{-1}) and 50 d (middle-stationary stage; 1.2×10^7 cells mL^{-1}) were collected for subsequent experiments (Fig. 1; black arrows). These samples could well represent the average cellular characteristics (e.g., cell-density; EOMs; toxin fate) of *Microcystis* at development and maintenance stages during a successive bloom, respectively (Fig. 1).

2.3. $KMnO_4$ oxidation to treat high-viability Microcystis at development and maintenance stage

A guideline of chlorophyll-a threshold of 50 μg L^{-1} was set for severe blooms ($> 1 \times 10^5$ cells mL^{-1}) by Ohio Environmental Protection Agency (Kasich et al., 2014). Table S1 summarized almost all $KMnO_4$ experiments for *Microcystis* at development or maintenance stages by previous studies and the highest cell-density of about 2.0×10^6 cells mL^{-1} at maintenance stage was used for $KMnO_4$ experiments (Table S1). Here, *Microcystis* at maintenance stage has a much higher cell-density of 1.2×10^7 cells mL^{-1} than that used by previous studies. Thus, prior to experiments, *Microcystis* samples at development and maintenance stages were equally diluted to achieve a final cell-density of about 1.0×10^6 and 2.0×10^6 cells mL^{-1} with double distilled water (ddH_2O), respectively. The dilution of ddH_2O did not contain any organic or inorganic matters to affect $KMnO_4$ oxidation and it also could not cause membrane damage of *Microcystis*, as demonstrated by Li et al. (2020a). Furthermore, this study employed culture solutions as reaction

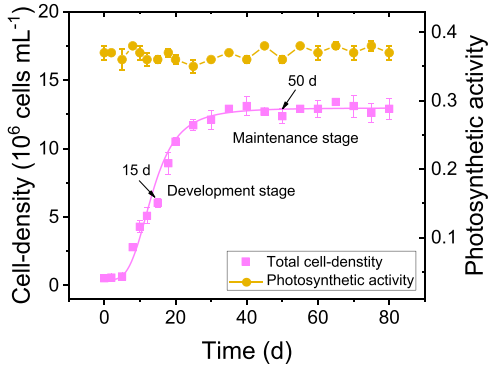

Fig. 1. *Microcystis* growth in BG-11 medium for 80 d to simulate a successive bloom in lab-scale, exhibiting classical development and maintenance stages via measuring total cell-density (Li et al., 2020a) and photosynthetic activity. Black arrows showed the timepoints at which these samples were collected for $KMnO_4$ experiments.

backgrounds, since it could show the effects of varied EOMs at development and maintenance stages on $KMnO_4$ oxidation.

All $KMnO_4$ experiments were conducted at 20 ± 2 ℃, since temperature could strongly affect cyanotoxin degradation efficiency (Kim et al., 2018). The pH values of source waters did not affect cyanotoxin oxidation by $KMnO_4$ treatments (Rodríguez et al., 2007a, 2007b), but pH values of cyanobacteria-laden source waters were always above 7.0 and previous studies have conducted $KMnO_4$ experiments at pH values of 7.0–8.0 (Table S1). We adjusted these samples at pH values of 7.5 ± 0.1 using 0.1 M sodium hydroxide or hydrochloric acid. In alkaline conditions, $KMnO_4$ oxidation was described by Eq. (1). Previous studies revealed that $KMnO_4$ to destroy cellular membrane was dosage dependent, among which dosage of > 2.0–3.0 mg L^{-1} could induce the loss of membrane integrity (Table S1). Hence, in this study, we used both low dosages of $KMnO_4$ (0.5, 1.0, 2.0 mg L^{-1}) and elevated dosage of 5.0 mg L^{-1} to treat *Microcystis* at development and maintenance stages, respectively.

$$MnO_4^- + 2H_2O + 3e^- \rightarrow MnO_2 + 4OH^- \quad E^0 = 0.60 \quad V \tag{1}$$

For $KMnO_4$ oxidation experiments, *Microcystis* at development and maintenance stages were treated with initial dosages of 0.5, 1.0, 2.0 and 5.0 mg L^{-1}, and mixed with a magnetic stirrer at a low speed (100 rpm min^{-1}), respectively. During $KMnO_4$ oxidation, samples were collected at 0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 h, and oxidant residual were measured. Meanwhile, samples of 1 mL were taken for membrane integrity analysis. Samples with a volume of 100 and 10 mL were immediately quenched with $Na_2S_2O_3$ and used for toxin and EOMs analysis, respectively. Control tests were also performed for $KMnO_4$ oxidation without adding *Microcystis* cells. These experiments were conducted in 5 L glass conical flasks.

2.4. Analytical methods

Microcystis samples of 10 mL were collected at each 5–10 d during cell growth for 80 d, and photosynthetic yield (φ_{PSII}) was measured using PHYTO-PAM phytoplankton analyzer (Walz, Germany) (Li et al., 2020a).

Prior to the analysis of $KMnO_4$ concentration, a standard curve of 0–10.0 mg L^{-1} was established by diluting the stock solution and establishing a linear relationship of absorbance at 525 nm (Stewart, 1973) (Fig. S1). During $KMnO_4$ oxidation, oxidant concentrationwas measured at 525 nm using a UV/VIS spectrophotometer (Thermo Scientific, UK) after removing cyanobacterial cells and manganese oxide via filtration with 0.22 μm nylon filters (Millipore, USA).

A visual flow cytometry (FCM) with SYTOX Green nucleic acid stain (Flowsight, Merck Millipore, USA) was employed to determine damaged cells, as described by Li et al. (2020a). The percentage of intact cells was estimated using flow cytometric analysis software (Flowsight, Merck Millipore, USA).

Microcystis aeruginosa FACHB-915 mainly produced MC-LR (Li et al., 2020a, 2020b). Mic*rocystis* samples of 50 mL were centrifuged by 6000 g for 5 min to collect cells for intracellular MC-LR measurements, and another volume of 50 mL were for total MC-LR measurements. Extracted MC-LR from each sample was concentrated by C18 solid-phase extraction (Nicholson et al., 1994), and the process exhibited a recovery ratio of 90–97% in this study (Li et al., 2020a). MC-LR concentration was measured using a high-performance liquid chromatography (HPLC) (Agilent 1200, USA) at a wavelength of 238 nm with a Bio-C18 column (Sepax, USA, 4.6 × 250 mm, 5 μm). The detailed protocols were described by Li et al. (2020a).

DOC and UV absorbance at 254 nm (UV_{254}) were used to analyze the amounts of EOMs and aromatic compounds (Matilainen et al., 2011). DOC concentration was measured by persulfate wet oxidation technique (Shimadzu TOC-V WP) after filtration for samples via a 0.45 μm glass fiber membrane (Millipore, USA) (Li et al., 2020a). UV_{254} was further measured using a UV3600 Spectrophotometer (Shimadzu, Japan). Protein concentration was quantified by Easy II Protein Quantitative Kit (TransGen Biotech, China).

2.5. Modeling

To compare oxidant decay of *Microcystis* at development and maintenance stages, a first-order kinetics was used to estimate rate constant of oxidant decay (k_{Mn}) by Eq. (2).

$$\ln\left(\frac{C_t}{C_0}\right) = -k_{Mn}t \tag{2}$$

where t = contact time; C_t = residual $KMnO_4$ after a given contact time; C_0 = initial concentration of $KMnO_4$ at t = 0 min; and k_{Mn} = rate constant of $KMnO_4$ decay.

To compare membrane integrity loss and total toxin degradation of *Microcystis* at development and maintenance stages, a second-order kinetics was used to estimate rate constant of membrane integrity loss (k_{loss}) and total toxin degradation (k_{total}) by Eq. (3).

$$\ln\left(\frac{N_t}{N_0}\right) = -kct \tag{3}$$

where ct = oxidant exposure; N_t = cell-density of intact cells or total toxin after a given oxidant exposure; N_0 = cell-density of intact cells or total toxin at ct = 0; and k = rate constant of membrane integrity loss or total toxin degradation.

2.6. Statistics analysis

$KMnO_4$ experiments had three replicates and the data were expressed as means ± standard deviation (SD). All parameters were compared across treatments with two-way ANOVA and the significance was set to $P < 0.05$. All statistical analyses were carried out using Origin 8.0.

3. Results

3.1. Kinetics of $KMnO_4$ decay

During $KMnO_4$ oxidation, oxidant decay was fast for both stages (Table S2). About 2.2 and 1.6 mg L^{-1} of oxidant was present after 8.0 h with initial $KMnO_4$ of 5.0 mg L^{-1} for *Microcystis* at development and maintenance stages, but there was no oxidant residual after a contact time of 1.0–6.0 h with low initial dosages of 0.5, 1.0, and 2.0 mg L^{-1} for both stages (Table S2).

First-order model fitted well to oxidant decay of high-viability *Microcystis* at both stages, since correlation coefficients (R^2) ranged from 0.95 to 0.99 (Fig. 2). The k_{Mn} of development and maintenance stages was in a range of about 0.07–2.30 and 0.11–3.31 h^{-1}, respectively (Fig. 2). With the same initial dosage of $KMnO_4$, k_{Mn} of maintenance stage was higher than that of development stage ($P < 0.05$) (Fig. 2).

3.2. Kinetics of membrane integrity loss

$KMnO_4$ of 0.5 mg L^{-1} did not cause membrane damage for both stages, but higher initial dosages of 1.0, 2.0 and 5.0 mg L^{-1} impaired membrane integrity with varied degrees (Fig. S2). Initial highest dosage of 5.0 mg L^{-1} induced completely membrane damage within 6.0 and 8.0 h for both stages (Fig. S2). Moreover, initial dosage of 1.0 mg L^{-1} resulted in 35% and 19% loss of membrane integrity for development and maintenance stages whereas higher percentages of 68% and 57% were disrupted with $KMnO_4$ of 2.0 mg L^{-1}, respectively ($P < 0.05$) (Fig. S2).

Second-order model fitted well to membrane integrity loss during

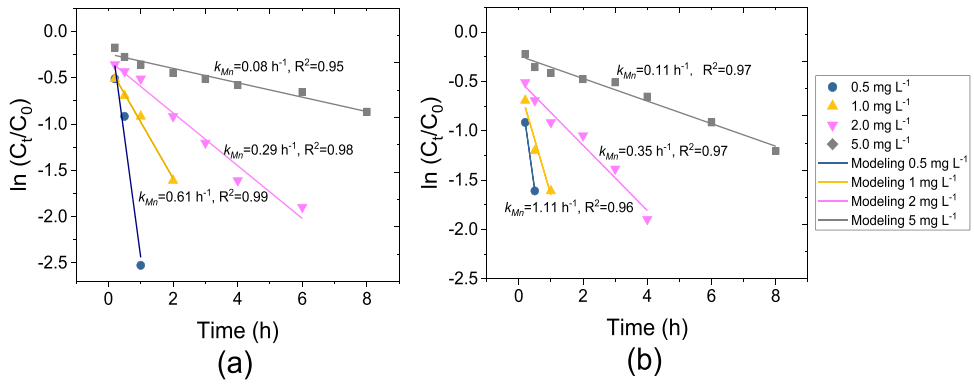

Fig. 2. Kinetics modeling of oxidant exposure and rate constants of oxidant decay (k_{Mn}) of *Microcystis* at development (a) and maintenance stage (b) treated with initial $KMnO_4$ of 0.5, 1.0, 2.0, and 5.0 mg L^{-1} after a contact time of 8.0 h.

$KMnO_4$ oxidation, since R^2 ranged from 0.96 to 0.99 (Fig. 3). With the initial $KMnO_4$ of 0.5 mg L^{-1}, k_{loss} was not estimated, since this treatment did not induce membrane damage (Fig. S2). The k_{loss} of development and maintenance stages ranged from about 11–30 and 8–27 M^{-1} s^{-1}(Fig. 3). With the same initial dosage of $KMnO_4$, k_{loss} of maintenance stage was lower than that of development stage ($P < 0.05$) (Fig. 3).

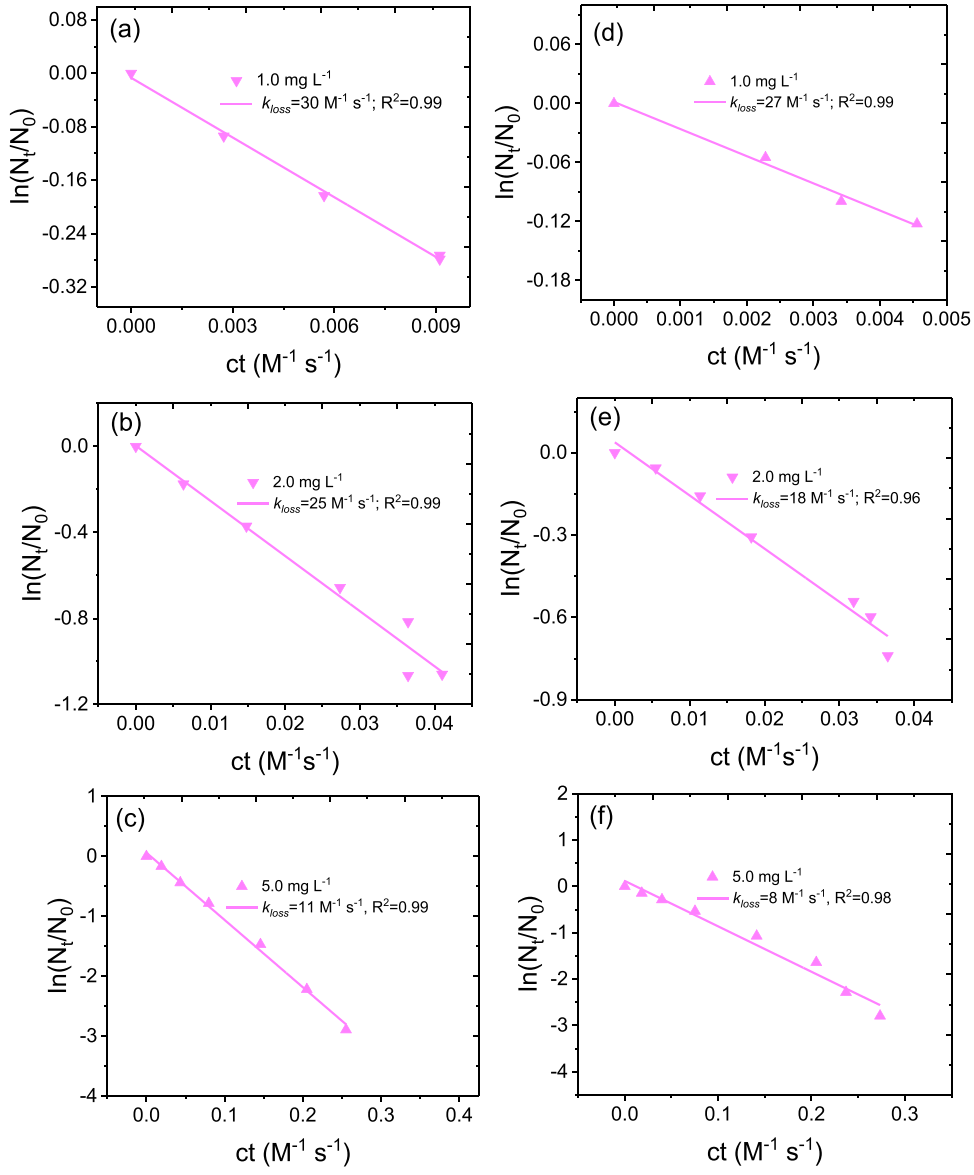

Fig. 3. Kinetics modeling of membrane damage and rate constants of membrane integrity loss (k_{loss}) of *Microcystis* at development (a-c) and maintenance stage (d-f) treated with various dosages of 1.0, 2.0, and 5.0 mg L^{-1}, respectively.

3.3. Kinetics of total toxin degradation

Total toxin was degraded with varied degrees via $KMnO_4$ oxidation, and their degradation percentage was depending on initial dosage and contact time (Fig. S3). Initial dosage of 5.0 mg L^{-1} could oxidize total toxin to below safety guideline (1 μg L^{-1}) after 6.0 and 8.0 h for both stages, but only 4–10% of total toxin was degraded after 1.0 h with $KMnO_4$ of 0.5 mg L^{-1} ($P < 0.05$) (Fig. S3).

The second-order model fitted well to total toxin degradation by $KMnO_4$ oxidation, as R^2 ranged from 0.80 to 0.99 (Fig. 4). Rate constants

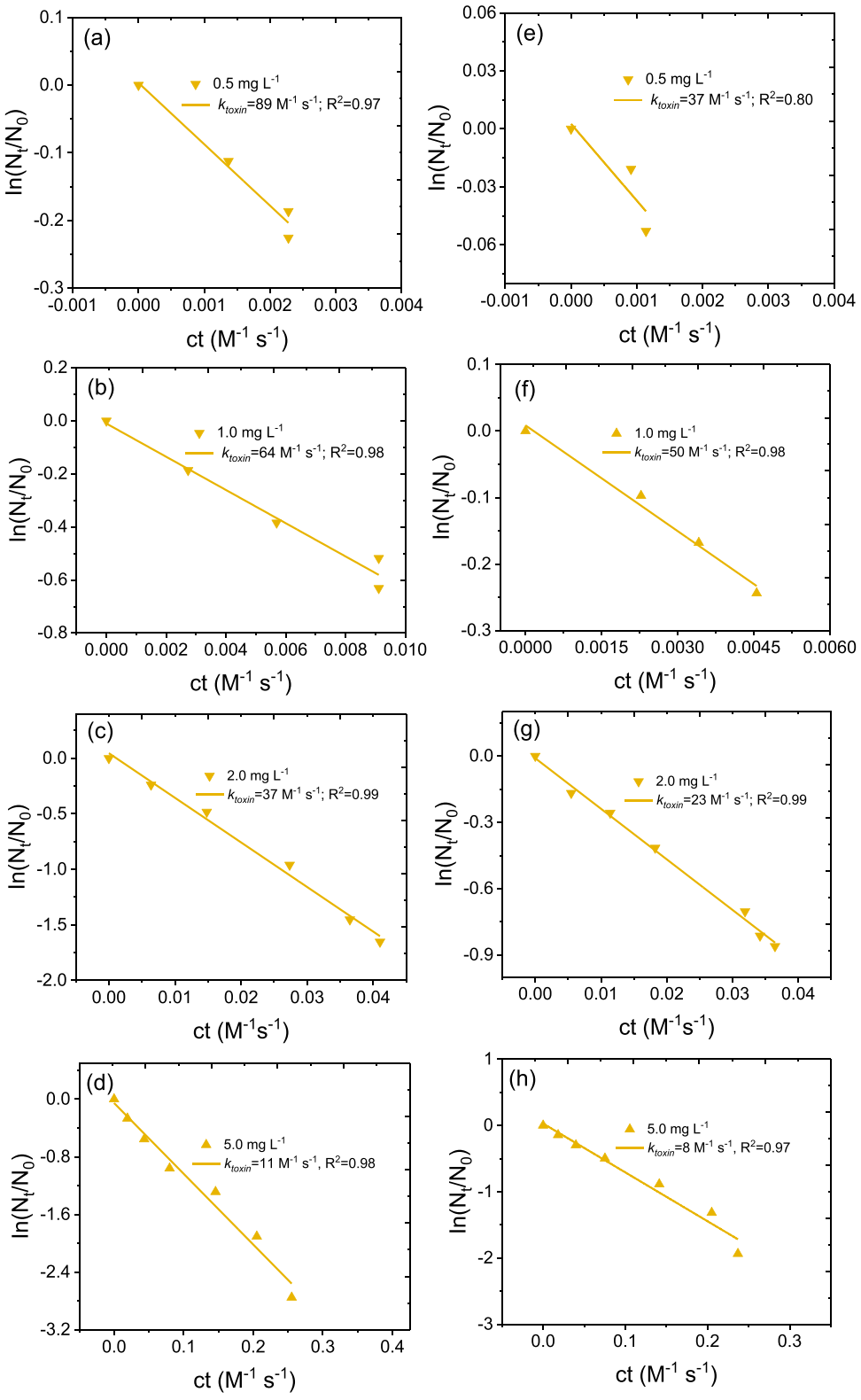

Fig. 4. Kinetics modeling of total toxin degradation and their rate constants (k_{toxin}) of *Microcystis* at development (a-d) and maintenance stage (e-h) treated with various dosages of 0.5, 1.0, 2.0, and 5.0 mg L^{-1}, respectively.

of total toxin degradation (k_{toxin}) of development and maintenance stages ranged from about 11–89 and 8–50 M^{-1} s^{-1} (Fig. 4). With the same initial dosage of $KMnO_4$, k_{toxin} of maintenance stage was lower than that of development stage ($P < 0.05$) (Fig. 4).

3.4. Extracellular toxin removal

Without membrane destruction, $KMnO_4$ of 0.5 mg L^{-1} continuously degraded extracellular toxin to below safety guideline (1 μg L^{-1}) for development stage while it could not be gained for maintenance stage (Fig. 5). A continuous decrease of extracellular toxin was observed with dosage of 1.0 mg L^{-1} and it was degraded to below the safety guideline within 1.0 h for both stages (Fig. 5). After dosing with higher dosages of 2.0 and 5.0 mg L^{-1}, extracellular toxin exhibited a pattern of increase-decrease and it could be oxidized to below the safety guideline after a longer contact time of 3.0–8.0 h for both stages (Fig. 5).

3.5. Extracellular organic matter (EOMs) removal

With $KMnO_4$ of 0.5 mg L^{-1}, extracellular DOC and UV_{254} of both stages exhibited a slight decrease of 4–6% and 3–10% after 1.0–2.0 h, respectively (Fig. 6). Despite the release of IOMs with membrane damage was induced by $KMnO_4$ of 1.0 mg L^{-1}, a higher degradation percentage of DOC (13–18%) and UV_{254} (15–18%) were observed for both stages (Fig. 6). With higher dosages of 2.0 and 5.0 mg L^{-1}, an increase-decrease pattern was observed for both stages (Fig. 6). The highest removal ratios of DOC (22%) and UV_{254} (39%) were gained for development stage with highest dosage of 5.0 mg L^{-1}, whereas a significant increase was observed for maintenance stage even after a contact time of 8.0 h (Fig. 6).

4. Discussion

4.1. Comparing oxidant exposure of high-viability Microcystis at development and maintenance stages

$KMnO_4$ decay was fast for both stages, in agreement with Fan et al. (2013a, 2013b) and Li et al. (2014), since $KMnO_4$ can react with cyanobacterial materials (e.g., mucilage) and EOMs (Xie et al., 2013; Li et al., 2014; Naceradska et al., 2017; Kim et al., 2018). With the same initial dosages of $KMnO_4$, rate constant of oxidant decay (k_{Mn}) became higher at maintenance stage than that at development stage, leading to its lower oxidant exposure (Fig. 2). This study also revealed that maintenance stage exhibited higher cell-density and EOMs than development stage. It suggested that lower oxidant exposure at maintenances stage was mainly due to elevated cell-density and EOMs.

Notably, EOMs composition at both stages was quite complex, and which substances could determine oxidant exposure was unknown. Kim et al. (2018) and Laszakovits et al. (2020) reported that $KMnO_4$ had a high reactivity with aromatic molecules. This study found that development stage held higher concentration of aromatic compounds (UV_{254}) than maintenance stage, possibly leading to lower oxidant exposure. However, this hypothesis was contrary with the result in Fig. 2, indicating that aromatic compounds could not be the main factor to determine oxidant exposure at both stages during $KMnO_4$ treatments.

Other studies revealed that alkenes preferred to be oxidized by $KMnO_4$, as it showed a higher rate constant (10^{-1}-10^{2} M^{-1} s^{-1}) than aromatics (10^{-4}-10^{-3} M^{-1} s^{-1}) (Waldemer and Tratnyek, 2006; Guan et al., 2010; Blotevogel et al., 2018). However, Laszakovits et al. (2020) found that $KMnO_4$ did not oxidize alkene groups in cyanobacterial-derived dissolved organic matters (DOMs), since alkenes were likely to be positioned in hydrophobic pockets, hindering $KMnO_4$ oxidation. This study conducted $KMnO_4$ oxidation for both stages with cyanobacterial-derived DOMs, suggesting that the influence of alkenes on $KMnO_4$ oxidation could be negligible.

Additionally, Laszakovits et al. (2020) noted that $KMnO_4$ reacted preferentially with nitrogen-containing organic compounds that were abundant in algal-derived DOMs. Among AOMs, protein/peptide-like compounds were rich in nitrogen. Henderson et al. (2008) and Pivokonsky et al. (2014) found that there was a rise in peptide/protein content at maintenance stage than that at development stage due to the release or active secretion of IOMs (Fig. S4). This study also certified that there were more abundant proteins in EOMs at maintenance stage than that at development stage ($P < 0.05$) (Fig. 7). Thus, comparing with development stage, the lower oxidant exposure at maintenance stage could be ascribed to more nitrogen-containing proteins involved in EOMs.

4.2. Comparing membrane integrity loss of high-viability Microcystis at development and maintenance stages

With the same initial dosages of $KMnO_4$, rate constants of membrane integrity loss (k_{loss}) was lower for maintenance stage than that for development stage (Fig. 3). It seemed that *Microcystis* at maintenance stage became more resistant to $KMnO_4$ oxidation than that at development stage, in disagreement with chlorination by Li et al. (2020a), in which they have demonstrated that *Microcystis* at maintenance stage was less resistant to chlorine oxidation due to its poor cellular surfaces. Comparing with $KMnO_4$, chlorine was a stronger oxidant to destroy cellular membrane of *Microcystis* and it could induce completely membrane damage even with low dosage of 1 mg L^{-1}, while *Microcystis* was less sensitive to $KMnO_4$ oxidation (Fan et al., 2013a).

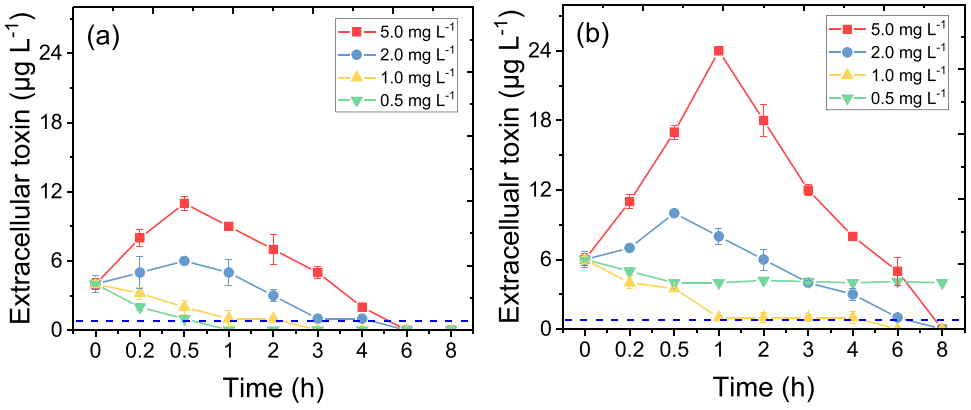

Fig. 5. Extracellular toxin of *Microcystis* at development stage (a) and maintenance stage (b) treated with initial $KMnO_4$ of 0.5, 1.0, 2.0, and 5.0 mg L^{-1} within a contact time of 0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 h. Blue dotted line: the safety guideline of 1 μg L^{-1} for drinking water proposed by WHO (2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

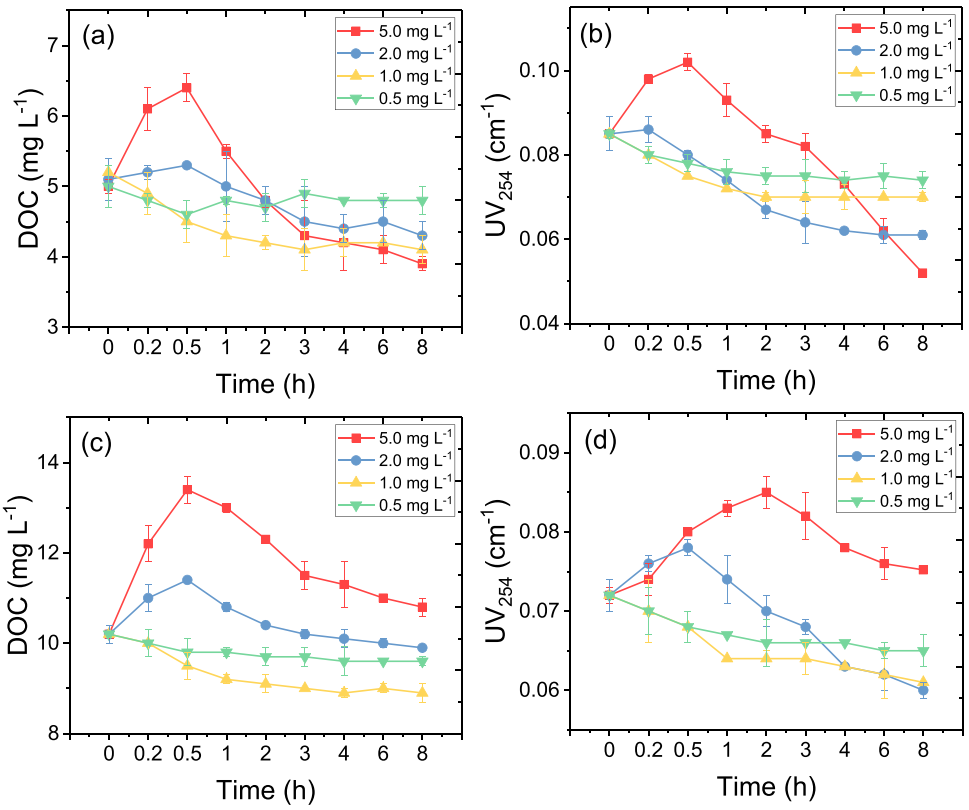

Fig. 6. DOC and UV_{254} of *Microcystis* at development stage (a-b) and maintenance stage (c-d) treated with initial $KMnO_4$ of 0.5, 1.0, 2.0, and 5.0 mg L^{-1} within a contact time of 0, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, and 8.0 h.

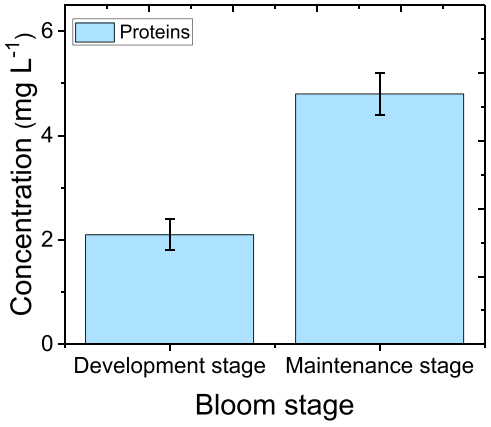

Fig. 7. Protein concentrations of *Microcystis* at development and maintenance stages before $KMnO_4$ oxidation.

Furthermore, a range of studies proposed that the percentage of membrane integrity loss was highly depending on oxidant exposure (ct) (Fan et al., 2013a, 2013b; Xie et al., 2013; Wert et al., 2014; Liu et al., 2017). In this study, *Microcystis* at maintenance stage exhibited higher cell-density and lower oxidant exposure (Fig. 2; S2), indicating that the actual $KMnO_4$ exposure in each *Microcystis* cell at this stage was much lower than that at development stage. Consequently, despite poor cellular surfaces of *Microcystis* at maintenance stage was observed (Li et al., 2020a), it exhibited higher resistance to $KMnO_4$ oxidation than development stage mainly benefited from its lower oxidant exposure after dosing with the same initial dosages of $KMnO_4$.

4.3. Comparing total toxin degradation efficiency of high-viability Microcystis at development and maintenance stages

With the same initial dosage of $KMnO_4$, rate constants of total toxin degradation (k_{toxin}) became lower at maintenance stage than that at development stage (Fig. 4), in disagreement with chlorination reported by Li et al. (2020a), which showed that toxin degradation efficiency at maintenances stage was higher than that at development stage. It may be ascribed to different mechanism to treat cyanobacteria. For chlorination to treat *Microcystis* at both stages, Li et al. (2020a) speculated the possible formation of reactive chlorinated-intermediates to accelerate toxin degradation at maintenance stage and effects of elevated cell-density and EOMs were negligible. In contrast, elevated cyanobacterial biomass and EOMs were possible strong competitors to decrease k_{toxin} at maintenance stage during $KMnO_4$ oxidation.

Until now, there was an argument about the competitive inhibition of toxin degradation by cyanobacterial cells and EOMs. Li et al. (2014) proposed that effects of coexisting EOMs on toxin oxidation via $KMnO_4$ was negligible, since there was no significant difference of k_{toxin} in EOMs solutions and ddH_2O. However, Laszakovits and MacKay (2019) found that the competition of toxin degradation depended on the amounts of dissolved organic matters (as referred EOMs in this study), and Jeong et al. (2017) also found aromatic molecules (e.g., humic or fulvic acid) could strongly inhibit the removal of cyanotoxin (MC-LR). In this study, *Microcystis* at maintenance stage held fewer aromatic compounds, but it showed lower k_{toxin} than that at development stage. Besides, after dosing with the same ratio of [oxidant: DOC] for both stages, k_{toxin} of maintenance stage remained lower than that of development stage (Table S3). These results suggested that the amounts of EOMs and aromatic molecules did not determine the difference of k_{toxin} between the two stages.

$KMnO_4$ could react with cyanobacterial cells, including surface mucilage, cellular membrane and intracellular chlorophyll-a etc., suggesting that cyanobacterial cells are potential competitors to toxin

degradation by $KMnO_4$ oxidation (Fan et al., 2013a; Xie et al., 2013; Li et al., 2014). Nevertheless, Laszakovits and MacKay (2019) proposed that cyanobacterial cells were not efficient scavengers of $KMnO_4$ to affect toxin degradation in cyanobacteria-impacted waters via measuring chlorophyll-a. Actually, when $KMnO_4$ was applied to treat cyanobacteria, oxidant must firstly disrupt surface mucilage, cell wall and cellular membrane, and then, it further destroyed intracellular chlorophyll-a after membrane destruction (Li et al., 2014). It suggested that the parameter of chlorophyll-a was sensitive to determine $KMnO_4$ reaction with cyanobacterial cells, possibly leading to the underestimation of inhibitory effect by cyanobacterial cells. In this study, *Microcystis* at maintenance stage held higher cell-density than that at development stage (Fig. 1), indicating elevated cyanobacterial biomass could be the major competitor to decrease k_{toxin} for maintenance stage during $KMnO_4$ oxidation.

4.4. A comparison of extracellular toxin and extracellular organic matters (EOMs) removal at development and maintenance stages

$KMnO_4$ of 0.5–5.0 mg L^{-1} showed a great capacity to degrade toxin for both stages, in agreement with many previous studies (Rodríguez et al., 2007a, 2007b; Fan et al., 2013a; Li et al., 2014; Kim et al., 2018; Laszakovits and MacKay, 2019). With initial high dosages of 2.0 and 5.0 mg L^{-1}, extracellular toxin increased attributed to the release of intracellular toxin after severe membrane destruction (> 57%). After a sufficient contact time of 6.0–8.0 h, extracellular toxin decreased to below safety guideline of 1 μg L^{-1} for both stages (Fig. 5). This result suggested that it was quite important for water supplies to provide sufficient contact time to completely remove extracellular toxin for both stages after severe membrane damage. $KMnO_4$ of 1.0 mg L^{-1} induced slight membrane damage but it degraded extracellular toxin to below safety guideline within 1.0 h for both stages (Fig. 5). It demonstrated that slight membrane damage did not affect the removal of extracellular toxin for both stages by $KMnO_4$ oxidation. Without membrane destruction, $KMnO_4$ of 0.5 mg L^{-1} degraded extracellular toxin to below detection limits within 0.5 h for development stage, but residual extracellular toxin was above 1 μg L^{-1} for maintenance stage mainly due to the decrease of k_{toxin}. These results suggested that a higher risk of extracellular toxin residual occurred at maintenance stage than that at development stage after dosing with low initial dosage of $KMnO_4$ (0.5 mg L^{-1}).

To our knowledge, there was an argument about the removal of EOMs via $KMnO_4$ oxidation mainly ascribed to its selective oxidation of AOMs. Wang et al. (2013) speculated that $KMnO_4$ can oxidize low molecular weight (MW) organic matters (< 10 kDa) to CO_2. Kim et al. (2018) and Laszakovits et al. (2020) reported that $KMnO_4$ can effectively degrade aromatic molecules. Naceradska et al. (2017) suggested that newly formed MnO_2 can further remove organic matters via physical adsorption. However, some other studies found that $KMnO_4$ shows a weak reactivity with high-MW AOMs (e.g., polysaccharide) (Wang et al., 2013; Jeong et al., 2017; Kim et al., 2018). This study observed that $KMnO_4$ of 0.5–5.0 mg L^{-1} effectively degraded EOMs, and it was possible to be ascribed to oxidation/adsorption with low-MW AOMs and aromatic compounds. Strikingly, an increase of DOC and UV_{254} occurred for maintenance stage with initial dosages of 5.0 mg L^{-1} even after a long contact time of 8.0 h. It could be due to a continuous release of IOMs with complete membrane destruction, or more high-molecular organic matters involved in AOMs at maintenance stage, that were difficult to be oxidized to CO_2 by $KMnO_4$ treatments (Wang et al., 2013; Pivokonsky et al., 2014) (Fig. S2; S4; 6). Overall, these results demonstrated that $KMnO_4$ decreased EOMs with varied degrees for both stages, but the risk of elevated EOMs was striking for maintenance stage after complete membrane destruction.

4.5. Evaluating the appropriateness of $KMnO_4$ as a pre-oxidant to treat high-viability cyanobacteria at both stages

Although high dosages of $KMnO_4$ (5.0 mg L^{-1}) showed a strong capacity to degrade extracellular toxin and EOMs with complete membrane destruction at both stages, it could not be ignored that oxidant residual (1.6–2.2 mg L^{-1}) would result in pink color and an increasing risk of DOC and UV_{254} occurred for maintenance stage. Moreover, $KMnO_4$ of 2.0 mg L^{-1} induced severe membrane damage (57–68%), and thus, a sufficient contact time of at least 3.0–8.0 h was quite important to degrade extracellular toxin to below safety guideline and decrease EOMs. However, generally, a short contact time of < 1.0 h was used for pre-oxidation process in DWTPs. Hence, high dosages of $KMnO_4$ (2.0 and 5.0 mg L^{-1}) as a pre-oxidant to treat cyanobacteria at both stages should be carefully assessed in practice.

Many previous studies concluded that maintaining membrane integrity was the best strategy for $KMnO_4$ oxidation to treat cyanobacteria-laden source waters (Fan et al., 2013a; Xie et al., 2013; Liu et al., 2017; Naceradska et al., 2017). For development stage, $KMnO_4$ of 0.5 mg L^{-1} degraded extracellular toxin to below safety guideline and continuously decreased EOMs without membrane destruction. It demonstrated that this traditional strategy was quite applicable for cyanobacteria at this stage. For maintenance stage, this treatment could not degrade extracellular toxin to below the safety guideline mainly attributed to the decrease of k_{toxin}. However, higher initial dosage of 1.0 mg L^{-1} degraded extracellular toxin to below 1 μg L^{-1} and the highest degradation percentages of DOC and UV_{254} were gained. Meanwhile, the percentage of membrane damage (19%) at maintenance stage was much lower than that at development stage (35%) due to its higher resistance to $KMnO_4$ oxidation. Therefore, despite slight membrane damage was caused by initial $KMnO_4$ of 1 mg L^{-1} (higher ct of 12 mg min L^{-1}), it was recommended as a better strategy to treat cyanobacteria at maintenance stage than initial dosage of 0.5 mg L^{-1}.

4.6. Practical implications

This study has demonstrated that $KMnO_4$ oxidation pattern was strongly affected by cyanobacterial biomass and EOMs concentration/composition at maintenance stage, including the decrease of oxidant exposure, membrane damage and toxin degradation rate. These results would contribute to understand the influences of bloom stage on pre-$KMnO_4$ process to treat high-viability cyanobacteria in DWTPs. Considering bloom stage as an important factor to optimize $KMnO_4$ treatments, this study proposed that a different strategy should be adopted to treat high-viability cyanobacteria at development (6 mg min L^{-1}, without membrane destruction) and maintenance stages (12 mg min L^{-1}, with slight membrane destruction), respectively. In general, to better optimize pre-$KMnO_4$ process to treat cyanobacteria-laden source waters, it was quite important for water supplies to real-time monitor cyanobacterial biomass and EOMs concentration/composition during a successive bloom.

5. Conclusions

This study demonstrated that stage of high-viability cyanobacteria could strongly affect $KMnO_4$ oxidation pattern, and different strategy of $KMnO_4$ treatments should be adopted depending on the stage of cyanobacterial bloom. The main findings were below:

(i) Cyanobacteria at maintenance stage held lower oxidant exposure than that at development stage mainly attributed to its elevated cell-density and more nitrogen-containing proteins involved in EOMs.
(ii) With the same initial dosages of oxidant, cyanobacteria at maintenance stage became more resistant to $KMnO_4$ oxidation

than that at development stage benefited from its lower oxidant exposure.

(iii) Elevated EOMs at maintenance stage did not cause the decrease of toxin degradation, but cyanobacterial biomass became the main competitor to decrease toxin degradation efficiency at this stage.

(iv) Without membrane destruction, initial low dosages of $KMnO_4$ (0.5 mg L^{-1}) resulted in higher risk of residual extracellular toxin at maintenance stage than that at development stage mainly due to the decrease of toxin degradation efficiency.

(v) With complete membrane destruction, the risk of elevated EOMs was striking for maintenance stage while the highest removal ratio of EOMs was observed for development stage after a sufficient contact time of 8.0 h.

CRediT authorship contribution statement

Xi Li: Experimental design, Writing/revising manuscript, Investigation, Data curation, Conceptualization. **Jie Zeng:** Investigation, Revising manuscript, Polishing language. **Xin Yu:** Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.126492.

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