Simultaneous surface-adsorbed organic matter desorption and cell integrity maintenance by moderate prechlorination to enhance *Microcystis aeruginosa* removal in KMnO_4 –Fe(II) process

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ABSTRACT

The KMnO_4 –Fe(II) process was proved to have good application potential in *Microcystis aeruginosa* removal, although at relatively high doses. This study aims to improve the algae removal in KMnO_4 –Fe(II) process by moderate prechlorination, which can realize the desorption of surface-adsorbed organic matter (S-AOM) from algae cells without damaging cell integrity. S-AOM was proved to not only inhibit algae removal but also maintain cell integrity, using various dilution methods for algal suspension preparation. The dilution after filtration method mainly removed the dissolved organics in cultured *M. aeruginosa*, while the dilution after centrifugal cleaning method could also remove the S-AOM on algae cells. Compared with the S-AOM-removed algal suspension, the lower algae removal in KMnO_4 –Fe(II) process and the reduced proportion of damaged cells during prechlorination of algal suspension without S-AOM removed indicated the inhibitory role of S-AOM in algae removal and the protective function of S-AOM toward cell integrity, respectively. Moderate prechlorination of directly diluted *M. aeruginosa* could be realized at chlorine doses of below 0.5 mg/L, and the damaged cell ratios were below 4% after 5-min prechlorination. The ability of the KMnO_4 –Fe(II) process to remove algae was dramatically enhanced by the elevation of chlorine dose from 0 to 0.5 mg/L, as more S-AOM was desorbed during prechlorination. Additionally, algae cells were easily captured by flocs after moderate prechlorination, which benefited the floc aggregation for formation of tightly bounded algae flocs. Therefore, the desorption of S-AOM without damaging cell integrity is the key feature of moderate prechlorination, which can be applied in improving the algae removal of KMnO_4 –Fe(II) process.

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1. Introduction

The removal of algae cells in water sources is a continuing concern for drinking water treatment plants (DWTPs) worldwide. The traditional water treatment processes show limited efficiency in achieving satisfactory removal of algae cells, not only because of the high motility, low specific density, diverse morphology and negative surface charge characteristics of algae cells (Pieterse and Cloot, 1997), but also due to the existence of algal organic matter (AOM) (Pivokonsky et al., 2006). The surface-adsorbed organic

matter (S-AOM) was reported to stabilize the algae cells in water, which increases the difficulty of removing algae cells for DWTPs (Clasen et al., 2000; Takaara et al., 2010). S-AOM refers to all the organic matter adsorbed on algae cells, including both the extra-cellular organic matter (EOM) and natural organic matter (NOM). The thickness of S-AOM could be reached to more than 20 μm when the algae cells form colonies of different sizes with mucilage or sheaths under natural conditions (Reynolds, 2007; Wu et al., 2007; Fan et al., 2016).

Many strategies have been proposed to enhance algae removal based on the conventional treatment processes, such as the introduction of dissolved air flotation, ultrafiltration, hydrodynamic cavitation and electro-coagulation-flotation processes (Henderson et al., 2008; Gao et al., 2010; Yang and Chen, 2013; Li et al., 2014).

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However, these strategies are all hindered by the high investment required for facilities modification. The KMnO_4 –Fe(II) process was proposed in our former study to achieve enhanced algae removal without large-scale reconstruction (Ma et al., 2012a). The process was further optimized successively to achieve maximum algae removal and minimum residual Mn for the same Fe(II) dose, and the optimum molar ratio of KMnO_4 to Fe(II) [RKMnO_4 :Fe(II)] was found to be 1: 3 (Qi et al., 2016a). The *in-situ* formed Fe(III) in the KMnO_4 –Fe(II) process was proved to be more efficient for algae removal than pre-formed Fe(III), because of its large reactive surface area (Ma et al., 2012a). In addition, the *in-situ* MnO_2 formed from the reduction of KMnO_4 can also serve as nuclei to promote coagulation and contribute to floc growth and sedimentation (Chen and Yeh, 2005; Sun et al., 2009; Qi et al., 2016a). However, the algae removal efficiency is highly dependent on the doses of KMnO_4 and Fe(II) (Qi et al., 2016a), which need to be reduced.

It is worth mentioning that the reaction between KMnO_4 and S-AOM has been reported to play an important role in enhancing algae removal for coagulation by destabilizing the algae cells (Xie et al., 2013; Qi et al., 2016a). The oxidation function of KMnO_4 to desorb the S-AOM on algae cells is weaker than for other oxidants, such as ozone and chlorine (Fan et al., 2013, 2014). This means that S-AOM desorption from algae cells can be enhanced by the introduction of more effective oxidants before the KMnO_4 –Fe(II) process. Chlorine, as the most widely used oxidant around the world, has been proposed for algae-laden raw water preoxidation (Shen et al., 2011). However, over-dosed chlorine pretreatment has been reported to damage cell integrity and induce intracellular organic matter (IOM) release (Daly et al., 2007; Lin et al., 2009; Ma et al., 2012b), which will not only inhibit coagulation but also increase disinfection by-products (DBPs) formation during disinfection (Garzon-Sanabria et al., 2013; Zhou et al., 2015; Zhu et al., 2015). Therefore, an ideal prechlorination step before the KMnO_4 –Fe(II)

process should be “moderate” to achieve not only the desorption of S-AOM but also the maintenance of cell integrity simultaneously.

In addition, lower-dosed oxidant has been reported to mainly react with the S-AOM without damaging the algae cell integrity (Xie et al., 2013; Qi et al., 2016a, 2016b). Thus, S-AOM can be conjectured to benefit the cell integrity maintenance during oxidation, and this effect can avoid the IOM release induced by cell damage to a certain extent. Therefore, the role of S-AOM needs to be assessed not only regarding its algae removal inhibitory effect, but also from the cell integrity protective aspect. However, no studies have been performed to directly prove the two roles of S-AOM, which is a fundamental guide for moderate prechlorination. Consequently, it is important to realize moderate prechlorination according to the two roles of S-AOM before applying prechlorination in the KMnO_4 –Fe(II) process.

Based on the above considerations, this study aims to: 1) investigate the inhibitory role of S-AOM in algae removal; 2) verify the protective function of S-AOM toward cell integrity; 3) clarify the moderate prechlorination process from both S-AOM desorption and cell integrity maintenance aspects; 4) evaluate the feasibility of applying moderate prechlorination in algae removal by the KMnO_4 –Fe(II) process.

2. Materials and methods

2.1. Materials and reagents

The algae species used in this study was *Microcystis aeruginosa* because of its prevalence in algae blooms and relevance to water quality and treatment challenges in waterworks (Kemp and John, 2006; Sano et al., 2011). *M. aeruginosa* (strain FACHB-905), as a cyanobacterium, previously described by Shen et al. (Shen and Song, 2007), was obtained from Wuhan Institute of Hydrobiology,

Fig. 1. Diagram of dilution method effects on the removal of dissolved organics and S-AOM from cultured *M. aeruginosa*.

Chinese Academy of Sciences, and cultured in BG-11 medium (Rippka et al., 1979). The detailed algae growth conditions are presented in Text S1. *M. aeruginosa* cultures were harvested at the exponential growth phase and diluted to the cell density of 1.0×10^6 cells/mL for all experiments. SYTOX green nucleic acid stain was purchased from Invitrogen, USA. Sodium hypochlorite (NaOCl, Sigma-Aldrich) stock solution, phosphate buffer solution (PBS; 10 mM, pH = 7.5) and ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) solution were prepared just before experiments with Milli-Q water. Potassium permanganate (KMnO_4) and sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) stock solutions of 0.5 g/L were prepared every week and stored in darkness. Source water was collected from the Qingcaosha reservoir located in Shanghai, China. The basic qualities of the source water were as follows: turbidity 3.8 NTU, pH 7.7, DOC 3.26 mg/L.

2.2. Experimental methods

Four dilution methods with source water were used for testing the inhibitory role of S-AOM in algae removal by the KMnO_4 –Fe(II) process, including direct dilution and dilution of the deposited algae cells after filtration (GF/F membrane, Whatman), centrifugation (4500 rpm, 10 min; supernatant removal), and centrifugal cleaning (4500 rpm, 10 min; three cycles of centrifugation and supernatant removal) respectively. The pH of this algal suspension was about 7.9. All jar tests were performed with 300 mL samples in 500 mL beakers and conducted on a programmable jar tester (MY3000-6, MeiYu, China). All samples were rapidly mixed with the added KMnO_4 at 250 rpm for 5 min before the addition of Fe(II). The subsequent process consisted of mixing at 200 rpm for 2 min and 40 rpm for 15 min, consecutively. Then, samples were quiescently settled for 30 min. After settling, samples were siphoned 2 cm below the water surface, and measured for residual algae by optical density at 680 nm (OD_{680}).

In order to test the protective function of S-AOM toward cell integrity, the cultured algae cells were suspended with PBS after filtration and centrifugal cleaning respectively. All samples were rapidly mixed at 250 rpm for 5 min after the addition of chlorine (0, 0.2, 0.4, 0.8 mg/L). $\text{Na}_2\text{S}_2\text{O}_3$ was then added to quench the prechlorination, and samples were siphoned for cell integrity detection.

The effects of prechlorination on cell integrity and S-AOM desorption were tested by the direct dilution of algae cells with source water. All the samples were rapidly mixed with the added chlorine (0–2 mg/L) at 250 rpm for 5 min. $\text{Na}_2\text{S}_2\text{O}_3$ was then added to quench the prechlorination. Samples were siphoned and divided in two subsamples: the first sample was evaluated for cell integrity detection. The remaining sample was immediately filtered through a 0.45 μm glass fiber filter for the concentration determination of DOC.

For testing the effects of prechlorination on algae removal by the KMnO_4 –Fe(II) process, samples were rapidly mixed with the added chlorine (0–2 mg/L) at 250 rpm for 5 min before the KMnO_4 –Fe(II) process. The KMnO_4 –Fe(II) process was conducted according to the former procedure described for jar tests. After settling, samples were siphoned and measured for residual algae by OD_{680} and algae flocs observation.

2.3. Analytical methods

2.3.1. Cell integrity detection

Cell integrity was determined for individual cells using a flow cytometer (FACSCalibur 4CLR, BD Biosciences, San Jose, USA) equipped with an argon ion laser emitting at a fixed wavelength of 488 nm for fluorescence measurement. SYTOX green nucleic acid stain was used to determine the cell integrity according to the

method of Daly (Daly et al., 2007). Full details of the cell integrity analysis are presented in Text S2.

2.3.2. OD_{680} of algal suspension

OD_{680} of the algal suspension was measured with a U-3100 spectrophotometer (Hitachi Co., Japan). The path length of the quartz cell was 10 mm. Milli-Q water served as the control.

2.3.3. Analysis of DOC

The DOC concentration in the dissolved phase of algal suspension was measured with a Shimadzu TOC-V_{CPH} analyzer.

2.3.4. Algae flocs observation

Scanning electron microscopy (SEM; SU-8020, Hitachi, Japan) was used to observe the morphologies of algae flocs formed during the KMnO_4 –Fe(II) process after prechlorination.

3. Results and discussion

3.1. The inhibitory role of S-AOM in algae removal by the KMnO_4 –Fe(II) process

To investigate the role of S-AOM in algae removal and cell integrity, four dilution methods were used for algal suspension preparation, including direct dilution and diluting the deposited

Fig. 2. Effects of differences in dissolved organics and S-AOM induced by different dilution methods on algae removal by the KMnO_4 –Fe(II) process. Cell density of 1.0×10^6 cells/mL was obtained with source water. (a) KMnO_4 dose = 15 μM ; Fe(II) dose = 45 μM . (b) RKMnO_4 :Fe(II) = 1: 3. The results shown are mean data from triplicate experiments, and error bars indicate standard deviations based on triplicate analyses.

cells after filtration, centrifugation, and centrifugal cleaning respectively. The effects of dilution methods on the removal of dissolved organics and S-AOM from cultured algae are summarized in Fig. 1.

The directly diluted algal suspension contains both organics in the dissolved phase and S-AOM on algae cells. The filtration method can mainly remove the dissolved organics in cultured algae. Centrifugation has been widely used for separating the algae cells from the cultured suspension (Henderson et al., 2010; Takaara et al., 2010; Xie et al., 2013). The DOC concentration in the solution of cultured *M. aeruginosa* after centrifugal cleaning was 66.34 ± 3.19 mg/L, while only 39.61 ± 2.46 mg/L for the sample obtained after filtration. This result indicates that the dilution methods after centrifugation, especially after centrifugal cleaning, can remove not only the dissolved organics in cultured *M. aeruginosa* but also S-AOM on algae cells.

The effects of S-AOM on algae removal from source water are illustrated in Fig. 2a; the fixed doses of KMnO_4 and Fe(II) were 15 and 45 μM , respectively. The algae removal efficiency was 0.5% by the KMnO_4 – Fe(II) process for the directly diluted algal suspension, which contained not only dissolved organics but also S-AOM. A slight increase of 14% was obtained for the filtered algal suspension with the dissolved organics removed. Further enhancement of algae removal efficiency was achieved by the dilution method after centrifugation and centrifugal cleaning, with increases to 74% and 83.5% respectively. The highest algae removal efficiency was obtained when the most S-AOM was removed, in the centrifugal cleaning algal suspension. This result can directly prove that the S-AOM played a more serious inhibitory role in algae removal than the dissolved organics. AOM has been proved to be an inhibitory substance for coagulation (Bernhardt et al., 1989). S-AOM can stabilize the algae cells and enhance the difficulty of removing algae cells by coagulation (Clasen et al., 2000; Takaara et al., 2010). One of

the proposed mechanisms for the inhibitory function is the direct interaction of AOM with cations originating from the coagulant to form complexes, impeding the coagulation capability (Bernhardt et al., 1989), which can explain the role of S-AOM in stabilizing algae cells.

In order to further demonstrate the inhibitory role of S-AOM in algae removal from source water, the algae removal efficiency was tested with various doses of KMnO_4 and Fe(II) for the direct dilution and centrifugal cleaning dilution methods (Fig. 2b). The enhancement of algae removal after the centrifugal cleaning dilution method was remarkable, especially for low KMnO_4 doses of less than 20 μM . The KMnO_4 – Fe(II) process at RKMnO_4 : Fe(II) of 1: 3 was proved to have good performance in algae removal, and the removal efficiency increased with elevated doses of KMnO_4 and Fe(II) (Qi et al., 2016a). KMnO_4 was reported to mainly react with the dissolved organics and S-AOM on algae cells prior to Fe(II) addition (Xie et al., 2013; Qi et al., 2016a). The directly diluted algal suspension contains more S-AOM on algae cells, and needs a higher dose of KMnO_4 for oxidation. These results indicate that S-AOM can serve as a protective barrier stabilizing algae cells and inhibiting the algae removal.

3.2. The protective function of S-AOM toward algae cell integrity during prechlorination

Fig. 3 shows the effects of S-AOM on algae cell integrity, and the ratios of damaged algae cells were quantified by a flow cytometer. The algal suspension was prepared in PBS in order to exclude the influence of organics in the water body, and the maintenance of cell integrity in PBS has been proved by previous research (Wert et al., 2013; Qi et al., 2016a). The fluorescence were collected in channel FL1 (530 nm) and FL3 (630 nm) respectively to identify the algae cell integrity. The red dots in the upper area for each sample refer to

Fig. 3. Effects of S-AOM differences induced by different dilution methods on the damage of algae cell integrity during prechlorination. Cell density of 1.0×10^6 cells/mL was obtained with PBS. Prechlorination time: 5 min. FL1: 530 nm, FL3: 630 nm.

those algae cells which have lost cell integrity. It can be observed that the ratios of damaged cells increased with the elevation of chlorine dose for both of the dilution methods. In addition, the damaged cell ratios for the dilution after centrifugal cleaning method were all higher than the ratios after filtration, with the same chlorine dosage for preoxidation. When the chlorine dose was 0.4 mg/L, the ratio of damaged cells rose from 4.1% for the filtered algal suspension to 62.4% for the centrifugal cleaning suspension. The chlorine dose should be increased to 0.8 mg/L to obtain a similar damaged cells ratio, i.e., 62.9%, for the filtered algal suspension.

Chlorine has been reported to be capable of damaging algae cells, and the damage level is highly dependent on the oxidant dose (Wert et al., 2013; Fan et al., 2014). Chlorine at low dose, i.e., 0.2 mg/L, mainly reacted with the S-AOM rather than damaging the algae cells during the 5-min preoxidation for the filtered algal suspension, as indicated by the low ratios of damaged cells in Fig. 3a. The algae cells diluted after centrifugal cleaning were more easily damaged by prechlorination compared with the cells after filtration. These results indicate that S-AOM might play a protective role for algae cells during the prechlorination period. Similar results have been reported that oxidant at lower doses mainly reacts with the S-AOM without damaging the algae cell integrity (Xie et al.,

2013; Qi et al., 2016a, 2016b).

3.3. Effects of prechlorination on algae cell integrity and S-AOM desorption

Fig. 4 shows the effect of prechlorination on the cell integrity of directly diluted *M. aeruginosa* in source water, with chlorine doses from 0 to 2 mg/L. The ratios of damaged algae cells increased with the elevation of chlorine dose. Chlorine at doses below 0.5 mg/L showed little effect on cell integrity, and the ratios of damaged cells were below 4% after 5-min preoxidation. However, the ratios of damaged algae cells increased from 3.3% to 18.2% with the elevation of chlorine dose from 0.5 mg/L to 0.6 mg/L. When the chlorine dose was elevated to 2 mg/L, almost all the algae cells were damaged after prechlorination.

Chlorine at low doses, i.e., below 0.5 mg/L, mainly reacted with the dissolved organics and S-AOM rather than directly damaging the algae cells during the 5-min preoxidation, as indicated by the low ratios of damaged cells. This result is in agreement with the previous findings that chlorine is capable of damaging algae cells, and the damage extent is mainly influenced by the oxidant dose (Wert et al., 2013; Fan et al., 2014). The protective function of S-AOM on cell integrity has also been proved as shown in Fig. 3,

Fig. 4. Effects of chlorine dose on algae cell integrity during prechlorination. Cell density of 1.0×10^6 cells/mL was obtained by directly diluting with source water. Chlorine doses: (a) 0 mg/L; (b) 0.1 mg/L; (c) 0.2 mg/L; (d) 0.3 mg/L; (e) 0.4 mg/L; (f) 0.5 mg/L; (g) 0.6 mg/L; (h) 0.7 mg/L; (i) 0.8 mg/L; (j) 0.9 mg/L; (k) 1 mg/L; (l) 2 mg/L. Prechlorination time: 5 min. FL1: 530 nm, FL3: 630 nm.

Table 1
Effects of chlorine doses on DOC in algal suspension after prechlorination.^a

Chlorine doses (mg/L)	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1	2
DOC (mg/L)	4.27	4.35	4.41	4.52	4.63	4.74	4.95	5.03	5.16	5.36	5.51	5.62

^a Cell density of 1.0×10^6 cells/mL was obtained by directly diluting with source water. The results shown are mean data from triplicate experiments.

Fig. 5. Effects of chlorine dose on algae removal by the KMnO_4 –Fe(II) process. Cell density of 1.0×10^6 cells/mL was obtained by directly diluting with source water. $\text{RKMnO}_4\text{:Fe(II)} = 1:3$. The results shown are mean data from triplicate experiments, and error bars indicate standard deviations based on triplicate analyses.

which can explain the low ratios of damaged cells. However, an elevated chlorine dose, i.e., above 0.6 mg/L, could induce high ratios of cell damage due to the loss of protective S-AOM.

The variation of DOC values after the prechlorination of directly diluted *M. aeruginosa* is shown in Table 1. The elevation of chlorine dose from 0 to 2 mg/L increased the DOC value from 4.27 to 5.62 mg/L. The DOC value increased, i.e., 0.47 mg/L, after the 5-min preoxidation by 0.5 mg/L chlorine, even though the ratio of damaged cells induced was below 4%. Combined with the low damaged cell integrity result, it can be inferred that the DOC increase after lower dose chlorine (i.e., below 0.5 mg/L) preoxidation is mainly caused by the desorbed S-AOM rather than released IOM. In addition, the further increased DOC values at higher chlorine doses, i.e., above 0.6 mg/L, were induced by the released IOM from damaged cells, which has been reported to adversely influence waterworks to a large degree (Garzon-Sanabria et al., 2013; Feng

et al., 2014). Therefore, the algae cell lysis problem can be prevented with moderate prechlorination, which can avoid not only the demand of extra chlorine to oxidize released toxins (Daly et al., 2007) but also the formation of DBPs at problematic level (Qi et al., 2016b). Considering that the effect of prechlorination on algae cells is strongly dependent on cell density and water matrix, the influence of chlorine dose in algae cell integrity and S-AOM desorption should be further verified in the actual water bodies to achieve the appropriate chlorine range.

3.4. Effects of prechlorination on algae removal by KMnO_4 –Fe(II) process

To illustrate the effects of prechlorination on the removal of directly diluted *M. aeruginosa* in source water by the KMnO_4 –Fe(II) process, chlorine at doses from 0 to 2 mg/L was added, and the KMnO_4 doses were 24, 27, 30, and 40 μM, respectively (Fig. 5). The removal of algae by KMnO_4 –Fe(II) process increased with increasing chlorine dose up to 0.5 mg/L but decreased at higher concentrations at all the fixed KMnO_4 doses. When the KMnO_4 dose was 30 μM, the algae removal was increased from 71% to 96% with the elevation of chlorine dose from 0 to 0.5 mg/L. However, further elevation of chlorine dose from 0.6 to 2 mg/L decreased the algae removal efficiency. The lowest algae removal was obtained with 2 mg/L chlorine pretreatment, which was even lower than the case without prechlorination. In addition, the algae removal efficiency increased with the elevation of KMnO_4 and Fe(II) doses when the chlorine dose was fixed. Without chlorine preoxidation, the highest algae removal efficiency, i.e., 90%, was obtained at the KMnO_4 dose of 40 μM. Similar algae removal of 89% could be achieved after being preoxidized by 0.3 mg/L chlorine for the KMnO_4 dose of 30 μM.

The increase of algae removal with elevation of chlorine dose from 0 to 0.5 mg/L can be explained by the low ratios of damaged cells found in Fig. 4, which indicated that chlorine at low doses mainly reacted with the organic matter, such as dissolved organics and S-AOM on the cells. The S-AOM proved to serve as a protective barrier can stabilize the algae cells, and decrease the algae removal efficiency (Fig. 2). The desorption of S-AOM from algae cells can benefit the algae removal (Clasen et al., 2000; Xie et al., 2013; Qi

Fig. 6. SEM micrographs of algae flocs formed after the KMnO_4 –Fe(II) process (a) without and (b) with prechlorination. Cell density of 1.0×10^6 cells/mL was obtained by directly diluting with source water. Chlorine dose (if any) = 0.5 mg/L. $\text{RKMnO}_4\text{:Fe(II)} = 1:3$; KMnO_4 dose = 24 μM.

et al., 2016a), and elevation of chlorine doses from 0 to 0.5 mg/L can lead to a more pronounced desorption effect. This result is consistent with a previous study showing that the low level of AOM in a water body induced by lower-dose chlorine oxidation mainly benefits coagulation (Bernhardt et al., 1985). In addition, the decrease in algae removal efficiency with further elevation of chlorine dose from 0.6 to 2 mg/L can be explained by the high level of IOM released from the large ratios of damaged cells found in Fig. 4, which has been reported to adversely influence algae removal (Garzon-Sanabria et al., 2013). A high level of IOM can decrease algae removal efficiency and increase the required coagulant dose by forming IOM-coagulant complexes (Pivokonsky et al., 2006).

Fig. 6 shows the SEM results for algae flocs formed after the KMnO_4 –Fe(II) process with and without prechlorination. As we can see from Fig. 6a, the algae cells were loosely bound by flocs formed in the KMnO_4 –Fe(II) process, and needed more surrounding flocs in order to be aggregated. With 0.5 mg/L chlorine preoxidation, the algae cells were easily captured by the flocs formed in the KMnO_4 –Fe(II) process, which benefited the floc aggregation for formation of tightly bounded algae flocs (Fig. 6b). This result can directly prove the positive function of moderate prechlorination in improving coagulation and aggregation. With moderate prechlorination, the S-AOM on the cells can be desorbed well without damaging the cell integrity, which finally benefits the algae removal by the KMnO_4 –Fe(II) process. A previous study (Xie et al., 2013) also reported that the improvement of algae removal by coagulation–sedimentation after preoxidation was likely due to the destruction of S-AOM on the cells. In addition, the algae cells still maintained their morphology after prechlorination compared with the original algae cells in Fig. S1. This result is consistent with the previous findings that chlorine can inactivate the algae cells and reduce the algae colloid stability without changing the cell morphology (Ma and Liu, 2002; Lin et al., 2009; Ma et al., 2012c).

4. Conclusions

This study directly proved that S-AOM could not only inhibit the algae removal by the KMnO_4 –Fe(II) process, but also protect the cell integrity from prechlorination damage. Moderate prechlorination can effectively eliminate the inhibitory role of S-AOM without damaging large ratios of cells, and finally promote the algae removal by the KMnO_4 –Fe(II) process. Chlorine doses of below 0.5 mg/L could achieve moderate prechlorination of directly diluted *M. aeruginosa*, and the ratios of damaged cells were below 4% after 5-min prechlorination. With moderate prechlorination, the problems of toxins release and DBPs formation at problematic level can be avoided. The algae removal of the KMnO_4 –Fe(II) process could be dramatically enhanced by the elevation of chlorine doses from 0 to 0.5 mg/L as more S-AOM was desorbed from algae cells during the 5-min preoxidation. In addition, moderate prechlorination could benefit the floc aggregation for formation of tightly bounded algae flocs, since the S-AOM-desorbed algae cells were easily captured by the flocs formed in the KMnO_4 –Fe(II) process. Thus, the implementation of moderate prechlorination may be practically applicable to enhance the algae removal in the KMnO_4 –Fe(II) process. It should be noted that all the findings were obtained with *M. aeruginosa*, and further verification is needed to extend the findings to other algal species.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2016.09.042>.

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