Pre-chlorination contact time and the removal and control of *Microcystis aeruginosa* in coagulation

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**Abstract.** The use of pre-oxidation is known to improve algae removal by coagulation and control the growth of algae. The contact time between oxidants and algae in pre-oxidation stage has been found as important parameter. This study investigated the effect of pre-chlorination contact time on the control and removal of cyanobacteria *Microcystis aeruginosa* by coagulation. The results showed that when the alum dose was sufficient, increasing contact time showed an improvement in algae removal by coagulation in case of high chlorine dose. The algae removal ratio at high chlorine dose, 3 mg L⁻¹ increased when contact time increased and it decreased after 30 minutes of contact time. In contrast, the result from chlorine dose, 2 mg L⁻¹ showed an unclear trend when contact time increased. Adding 2 mg L⁻¹ of pre-chlorination with 10 minutes of contact time was enough to control the regrowth of *M. aeruginosa*. In addition, dissolve organic carbon (DOC) and UV absorbance at 254 nm, which particularly indicates aromatic compounds, tended to increase when the contact time increased. The increased of DOC and UV 254 indicated the release of intracellular organic matter (IOM) from *M. aeruginosa*. High level of DOC, 0.68 mg L⁻¹ in this study showed negative effect on *M. aeruginosa* removal by coagulation and could not be removed by coagulation process.

1. Introduction
Eutrophication has caused algae-related problems in many water treatment facilities. The major algae-related problems in water treatment plant are unpleasant tastes, odors and filter clogging [1, 2]. Filter clogging by algae has been reported as a technical problem by many water treatment plants (WTPs). For example, Cheong Ju water treatment plant in South Korea faced the decrease of filter run time from 20 hours to below 5 hours by the occurrence of diatom, Synedra [3]. Moreover, the presence of filamentous diatom, *Melosira* sp., at Wahnbach Reservoir, Germany, resulted in reduction of the filter run time from 30 to 8 h, which was further reduced to 4 h as a result of a simultaneous influx of smaller cyanobacteria *C. naegelianaum* Unger [4]. The bloom of blue green algae, particularly *Anabaena* sp. and *Microcystis* sp. seriously impacted on filtration process in Morton Jaffray (MJ) water as evidenced by the increased backwashing frequency reported to be at every 4–8 h [5].

General methods to remove algae are physical processes such as filtration, membrane filtration, sonication and adsorption by activated carbon, biological processes such as activated sludge and chemical processes such coagulation and chlorination. Among these methods, the chemical treatment process has been considered to be cost-effective way because many chemical agents are inexpensive and they usually do not require plants to significantly change work-flow and structure [6, 7]. A number of studies have reported that pre-treatment using oxidants such as ozone, chlorine, potassium permanganate and potassium ferrate can improve algae removal by coagulation [4, 5, 8]. Oxidants has
the ability to enhance micro-flocculation and to reduce conventional coagulant dosages [9]. It was found that the change of algae cell surface or cell stability and the lysis of algae cell caused by pre-oxidation are factors leading to more effective coagulation [8]. In addition, cellular component released from algae caused by oxidation was found as coagulant aids that can promote coagulation [10, 11]. Contact time between oxidants and algae in pre-oxidation stage has been found as important parameter in algae removal by coagulation. Hoko and Makado (2011) [5] conducted experiments to optimize the removal of algae, mainly Anabaena sp. by using jar test and copper sulfate as an oxidant, found that removal efficiency increased with increasing contact time and the optimum contact time was around 30 minute. As pre-oxidation has been found to be associated with the change of algae cell architecture and the release of algogenic organic matter (AOM), which consists of intracellular organic matter (IOM) and extracellular organic matters (EOM), serving as coagulant aid [4, 10-12], this could be the reason why the above study found that the removal efficiency increased with increasing contact time [5]. According to the above mechanisms, the inappropiate contact time can severely damage cell or cause cell lysis releasing intracellular organic matter (IOM) and toxins in case of toxins producing algae such as cyanobacteria and then increase the levels of dissolved organic matter (DOM) [12, 13] which might be as precursor of disinfection by products. The large amount of intracellular organic matter (IOM) released by chlorination such as protein can show positive effect or negative effect depending on its concentration, composition and characteristics such as molecular weight (MW) distribution. The negative effect such as forming protein–coagulant complexes which inhibit algae removal and increase alum demand [12].

The effect of contact time of pre-chlorination, which is a common oxidant, on algae removal by coagulation is still not well study and it is practical to know the optimum contact time of pre-chlorination. This article describes the effect of pre-chlorination contact time and concentration on the removal and control of Microcystis aeruginosa (Kützing) Lemmermann. In addition, we also determined the effect of the contact time on dissolved organic carbon (DOC) concentration and UV absorbance at 254 nm, which specifically indicates aromatic compounds.

2. Methodology

Microcystis aeruginosa (Kützing) Lemmermann, NIES-843 was purchased from The National Institute For Environmental Studies (NIES) and cultured in an incubator with following condition: 25 C, light intensity: 20-30 μmol photons/m²/sec and Light(L)/Dark(D) cycle: 10L:14 D. The density of algae was counted under an optical microscope and harvested during the exponential growth phase. The algae was centrifuged at rate 700 G 5 minutes to separate culture medium and algae. Then, only algae was transferred to synthetic medium. The final algae density subjected to the experiments was around 80×10⁶ cell L⁻¹.

Purified water was used as sample water. Turbidity, alkalinity and pH of sample were adjusted to average values during algae bloom in Chao phraya river, which were around 30-35 NTU, 100 mgL⁻¹ and 7.5, respectively. Alkalinity and turbidity were adjusted by adding sodium bicarbonate (NaHCO₃) and kaolinite clay, respectively. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used for pH adjustment. Chlorine solution for pre-chlorination was made by dissolving calcium hypochlorite (Ca(ClO)₂). Stirrer was used to perform pre-chlorination and coagulation experiment. Chlorine solution was added prior to coagulation process and mixed at 150 rpm with different contact time, including 0, 5, 10, 20, 30 and 60 minutes. The zero contact time in this study meant adding chlorine and alum together at the same time. After pre-chlorination, sample was collected immediately at the depth 5 cm below water surface for DOC and UV measurement. Then, alum solution was added and mixed at 200 rpm for 1 minute and 40 rpm for 15 minutes. After 10 minutes of sedimentation, water was sampled at the same depth, 5 cm below surface to measure DOC, UV, turbidity and algae density. The amount of algae was counted under an optical microscope. The dose of alum, 30 mg L⁻¹ was decided based on the pre-liminary result of coagulation experiment because it was found as an optimum dose for turbidity removal. To investigate the algae re-growth experiment, chlorine solution,
2 and 3 mg L\(^{-1}\) were added and mixed at 150 rpm with 10 and 30 minutes of contact time. After that, sample was collected and then centrifuged to remove chlorine solution. After removing chlorine solution and washing algae, algae was transferred to culture medium and cultured in incubator. The algae’s density was periodically checked.

pH was measured by electromatic method using HM-25R pH meter, DKK-TOA Corp., Tokyo, Japan. Turbidity was measured by nephelometric method using turbidity meter, 2100Q - HACH. DOC and UV254 were measured by UV spectrometer and TOC analyzers (A non-purgeable dissolved organic carbon method was employed) \[14\].

All analyses were conducted in triplicate. Removal ratio results were tested with oneway ANOVA to determine the significant effect of contact time.

3. Results and discussions

3.1. Effect of pre-chlorination dose and contact time on the removal of \(M.\ aeruginosa\) and turbidity

Pre-chlorination with chlorine 2 and 3 mg L\(^{-1}\) did not significantly improve \(M.\ aeruginosa\) removal by coagulation (p-value from ANOVA was higher than 0.05 in both concentration and at every contact time). However, Removal ratio of algae at chlorine 3 mg L\(^{-1}\) showed a significant change (p-value from ANOVA was 0.002) when contact time increased while removal ratio at chlorine 2 mg L\(^{-1}\) did not show a significant change with contact time (p-value from ANOVA was 0.821). Removal ratio at chlorine 3 mg L\(^{-1}\) increased with increasing contact time and reached the maximum 95.05% at contact time 30 minutes and then slightly decreased into 92.11% at 60 minutes of contact time (Figure 1). The increase and decrease of removal ratio in Figure 1 may result from the released intracellular organic matter (IOM) which has been found to show positive and negative effect on coagulation [12]. Black line in Figure 1 represents the removal ratio, which is 90.7 percent and has 3.51 of SD value, from coagulation with alum 30 mg L\(^{-1}\).

The result of turbidity after coagulation in pre-chlorination 3 mg L\(^{-1}\) experiment significantly changed with contact time (p-value from ANOVA was 0.002). It showed a reduction trend during 0 to 30 minutes of contact time and then sharply increased at 60 minutes of contact time (Figure 1). This turbidity result conformed to algae removal ratio of pre-chlorination 3 mg L\(^{-1}\) experiment shown in Figure 1. In case of pre-chlorination 2 mg L\(^{-1}\) experiment, turbidity did not significantly change when contact time increased (p-value was higher than 0.05).

3.2. Effect of pre-chlorination dose and contact time on the regrowth of \(M.\ aeruginosa\)

Figure 1. Relation of pre-chlorination contact time and algae removal ratio (Left) and turbidity (Light) after coagulation (at 30 mg L\(^{-1}\) of alum dose). The results are shown with average, standard deviations from triplicate experiments.
Adding pre-chlorination 2 mg L\(^{-1}\) with 10 minutes of contact time was sufficient to control the growth of M. aeruginosa. As shown in Figure 2, the density of algae remained stable after being reacted with chlorine 2 and 3 mg L\(^{-1}\), 10 and 30 minutes of contact time while the density of algae that did not expose to chlorine increased obviously. This means chlorine damaged the algae cell resulting to algae growth inhibition and the increase of DOC concentration.

3.3. Effect of pre-chlorination dose and contact time on DOC and UV 254

DOC concentration before coagulation of chlorine 3 mg L\(^{-1}\) was higher than those 2 mg L\(^{-1}\) at every contact time (Figure 3). DOC concentration before coagulation of chlorine 3 mg L\(^{-1}\) increased gradually during 5 to 20 minutes of contact time and then largely increased at 30 minutes of contact time. At 60 minutes of contact time, DOC concentration remained same as contact time 30 minutes. In case of the lower chlorine dose, 2 mg L\(^{-1}\), DOC concentration did not show important change within 30 minutes of contact time, but it showed sharp increase at 60 minutes. The increase of DOC concentration indicated the release of AOM, especially IOM as pre-chlorination has been found to damage algae cell wall and release IOM of algae [12, 13]. Absorption of UV at 254 has been used as organic compounds monitoring parameter, especially aromatic compounds throughout water treatment plants. In this study, UV 254 showed large increase in case of chlorine 3 mg L\(^{-1}\), particularly at 30 minutes of contact time and then decreased at 60 minutes of contact time (Figure 3). The decrease of UV 254 at 60 minutes might be resulted from the degradation of high MW organics to lower MW organics by chlorine [12, 13]. In addition, UV 254 value of chlorine 2 mg L\(^{-1}\) showed no large different when contact time increases. Since UV 254 specially indicates aromatic compounds, so not only contact time but the dose of chlorine also affects characteristics of organic matter.

![Figure 2](image)

**Figure 2.** The growth of algae after exposing to chlorine. The results are shown with average and standard deviations from triplicate experiments.
Figure 3. Relation of pre-chlorination contact time and DOC concentration (Left) and UV 254 (Right) before coagulation (at 30 mg L$^{-1}$ of alum dose). The results are shown with average and standard deviations from triplicate experiments.

According to the result of DOC concentration, the trend of DOC and UV were similar, especially at 30 minutes of contact time of chlorine 3 mg L$^{-1}$ which shows obviously increased in both DOC and UV 254.

The intracellular organic matter (IOM) has been found to affect coagulation process and may influence coagulation performance for removing algae in this study as shown in Figure 4. The removal ratio and DOC increased when contact time increased until 30 minutes of contact time. After that, removal ratio showed decreasing while DOC remained stable. The increase and decrease trend of algae removal ratio could be influenced by DOC concentration as it has been found that the low level of DOM benefited coagulation while high level of DOM inhibited the aggregation of algae cells and increased the alum dose [12, 15]. In addition, the low ratio of coagulant/DOC was found to decrease coagulation performance by causing charge stabilization [16]. Based on the above result, the high level of DOC in this study showed negative effect on algae removal.

The results of DOC concentration after coagulation in Figure 5 shows that low level of DOC, produced in chlorine 2 mg L$^{-1}$ experiment, was removed by coagulation process to the same level of blank experiment. In contrast, high level of DOC in chlorine 3 mg L$^{-1}$ experiment remained high after coagulation process (Figure 5). This means 30 mg L$^{-1}$ of alum dose in this experiment was not sufficient to remove high level of released organic matter.

Figure 4. Relation between pre-chlorination contact time, DOC concentration (before coagulation) and algae removal (at 30 mg L$^{-1}$ of alum dose). DOC value of 0 contact time is value of water containing algae.
Figure 5. Relation of pre-chlorination contact time and DOC concentration (before and after coagulation at 30 mg L\(^{-1}\) of alum dose). The results are shown with average and standard deviations from triplicate experiments. (There is no result of before coagulation at zero contact time because that contact time is adding alum and chlorine at the same time).

4. Conclusion
Adding 2 and 3 mg L\(^{-1}\) of pre-chlorination did not show a significant improvement in \(M.\ aeruginosa\) removal by coagulation. However, pre-chlorination contact time showed a significant effect on \(M.\ aeruginosa\) removal ratio at chlorine 3 mg L\(^{-1}\). The success in algae growth control and the increase of DOC concentration supported that chlorine damaged algae cell and released AOM, especially IOM. According to results from DOC, and UV 254, pre-chlorination contact time in this study affected removal ratio of \(M. aeruginosa\) by increasing organic level, especially 3 mg L\(^{-1}\) of chlorine at 20–60 minutes of contact time. The increase of organic concentration increased and decreased the removal ratio as shown in Figure 6. In addition, 30 mg L\(^{-1}\) of alum was sufficient for removing algae but not sufficient to remove organic compounds produced at high chlorine dose. Moreover, due to the increase of disinfection by product precursor found in this study, the pre-chlorination contact time should less than 30 minutes when high chlorine dose is used.

5. References
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