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Pre-ozonation for removal of algal organic matters (AOMs) and their disinfection by-products (DBPs) formation potential

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Abstract. As a result of algal bloom, algal organic matters (AOMs) are rapidly increased in surface water. AOMs can act as precursors for the formation of harmful disinfection by-products (DBPs), which are serious problems in water treatment and human health. The main aim of this study is to characterize the formation of DBPs from AOMs produced by three different algae such as *Oscillatoria sp*., *Anabaena sp*., and *Microcystis aeruginosa* under different algal growth phases. In an effort to examine formation of DBPs during chlorination, chloroform (TCM), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA) were determined under various CT (product of disinfectant concentration and contact time, mg·min/L) values. Generally, the amounts of DBPs tended to increase with increasing CT values at the most growth phases. However, there was a significant difference between the amounts of DBPs produced by the three algal species at different growth phases. This result is likely due to the chemical composition variability of AOM from different algae at different growth phases. In addition, the effect of pre-ozonation on coagulation for the removal of AOMs from three algal species was investigated. The pre-ozonation had a positive effect on the coagulation/flocculation of AOMs.
Keywords: algal organic matter(AOM); chlorination; disinfection by-products(DBPs); Pre-ozonation

1. Introduction

Algae blooms frequently occur in worldwide freshwater bodies due to anthropogenic activities, which have become the global environmental problem (Dong *et al*. 2021, Yao *et al*. 2019). Algae can interfere with the solid-liquid separation processes of coagulation, sedimentation and filtration in conventional water treatment processes, resulting in a series of problems (i.e., significant increase in coagulant dosing and a high turbidity in the effluent) (Coral *et al*. 2013, Fang *et al*., 2010 and Graham *et al*. 2010). On the other hand, algae bloom leads to the increase of algal organic matter (AOM), and the related problems occur in water treatment process (Janssen *et al*. 2019 and Pivokonsky *et al*. 2016). AOM is mainly comprised of proteinaceous substances, amino acids, and carbohydrate, which can be used as precursors of disinfection by-products (DBPs) (Lei *et al*. 2014, 2012, Wert and Rosario-Ortiz 2013, Zhu *et al*. 2015).

Chlorination of AOM can produce trihalomethanes (THMs) and haloacetic acids (HAAs) (Son *et al*. 2015). Generally, THMs and HAAs are major DBPs produced during chlorination of AOM (Krasner *et al*. 2006). THMs and HAAs which are reported to have adverse effects on human health, including carcinogenesis and mutagenesis (Fawell *et al*. 2003). Currently, many studies focused on the formation of DBPs (Fang *et al*. 2010, Hua *et al*. 2017, Liu *et al*. 2018, Park *et al*. 2006, Yao *et al*. 2022). Li *et al*. (2012) quantified the yields of DBPs during the disinfection of AOM produced by *Microcystis aeruginosa*. The formation potentials of chloroform, chloroacetic acid, and nitroso- dimethylamine were reported to be 32.44, 54.58 and 0.0189 μg/mg DOC, respectively. Goslan *et al*. (2017) investigated the DBP formation potentials from the AOMs of six different algal species (three cyanobacteria, one diatom, one green and an additional diatom). They reported that carbonaceous and nitrogenous DBPs were formed to 92.4 and 1.7 μg/mg DOC, respectively.

On the other hand, in order to remove algae and AOMs for drinking water safety various treatment technologies such as adsorption, coagulation, and chemical oxidation processes have been applied (Han *et al*. 2022). Among these treatments, ozone can be applied to treat the wastewater containing algae and their AOMs due to its efficient oxidizing and sterilizing properties. It is clear that pre-ozonation is an effective method for removal of algal cells (Hammes *et al*. 2007, Miao *et al*. 2009, Tang *et al*. 2020). However, a treatment process with only pre-ozonation was ineffective for removal of AOM. Chien *et al* (2018) used ozone to treat surface water with high cyanobacteria-derived organic matter and indicated that the removal efficiency of DOC was 19% with ozone treatment. Zhu *et al*. (2015) found that the effect of pre-ozonation on TCAA removal was not significant with increasing ozone doses. Therefore, a combined process consisting of pre-ozonation and adsorption/coagulation has been proposed. Bose and Reckhow (2007) treated DOC rich water using a combined application of pre-ozonation and

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alum coagulation. The result showed that the DOC removal rate increased after alum coagulation as the ozone dose increased. Schneider and Tobiason (2000) reported that pre-ozonation aided organic matter removal using polyaluminum chloride.

Although many studies about DBPs from AOMs have been conducted using a single algal species, there was a lack of knowledge about formation characteristics of DBPs from various AOMs produced by three different algae species in the different growth phases such as exponential and stationary phases. Also, there were few studies examining effect of pre-ozonation based treatment method on removal of the different DBPs prepared under various experimental conditions such as chlorine contact times and concentrations. The objectives of this study are to investigate DBPs formation during chlorination of AOMs produced from three different algal species (*Oscillatoria sp*., *Anabaena sp*., and *Microcystis aeruginosa*) under different growth stages and to examine the effect of pre-ozonation on the formation of DBPs. Furthermore, application of combined methods (pre-ozonation + PAC/ $Al_2(SO_4)_3$) to evaluate the removal efficiency of AOMs was examined.

2. Materials and methods

2.1 Algal cultivation and AOM extraction

Three algal species (*Oscillatoria sp*., *Anabaena sp*., and *Microcystis aeruginosa*) were purchased from Korean Collection for Type Cultures (Daejeon, Korea), were cultured in batch cultivation using BG-11 medium to allow algal cells to grow into the stationary growth phase (Table 1).

The incubation bottles were illuminated with lamps at certain range of luminosity. Culture parameters (i.e., pH, temperature and light time) were strictly controlled during cultivation (Table 1). AOM solution was prepared in order to investigate characteristics of AOM produced from each algal species. Algae solutions were centrifuged in 7000 rpm speed for 5 min, and then supernatant was filtered using a 0.45-μm glass fiber membrane (Macherey-nagel, USA) (Chen *et al*. 2017). The concentration of AOM was measured using a total organic carbon (TOC) analyzer (TOC-V CSH, Japan), and algal cell numbers were counted by the method using a hemocytometer plate (Marienfeld Superior, Germany) and a light microscope (Olympus BX53M, Japan).

2.2 DBP analysis

In order to investigate DBPs formation from AOM during disinfection, chlorine reaction experiments were performed under various CT values (52, 59, and 68 mg • min/L). Three different CT values were selected for the chlorination experiments of AOMs to achieve a germicidal capacity of 99.9% (3-log). A stock solution of free available chlorine was prepared from sodium hypochlorite (NaClO) (free chlorine 6-14%, SAMCHUN Chemicals, Korea). Chlorination experiment was carried out in 500-mL brown glass bottles. Chlorine for 6-14% mass fraction of sodium hypochlorite solution was added in the bottles, and the free chloride content was calibrated with the *spectrophotometric N,N'-diethyl*-p-*phenylenediamine* (*DPD*) *method*. After the pH of sample was adjusted to 7.0 by either 0.1 mole/L NaOH or 0.1 mole/L HCl, the sample was stored in the brown glass bottle with Teflon lined cap and then placed in the dark at 20±1 °C (Fang *et al*. 2010). Meanwhile, to investigate the effect of chlorine contact time on DBP formation, the DBPs were measured using different reaction times (40min, 80min, and 130min) with the same dosage of chlorine (6.7 mg/L).

The most commonly known DBPs such as TCM, DCAA and TCAA were measured in this study (Son *et al*., 2015). TCM was analyzed using Purge-Trap (Aquatec 100, Teledyne Tekmar, USA) and a gas chromatograph (GC) (7890A, Agilent, USA) with fame ionization detector (FID). The Supelco 24217-U capillary column (60 m × 0.32 mm I.D. with a film thickness of 1.8 μm) was used. The flow rate was 3 mL/min. The injector and detector temperatures

Table 1 Culture conditions of three different algae species Under

Axenic/ xenic	Luminosity (lux)	Light/dark cycle (h)	Cultivation temperature (℃)
xenic	3000-5000	12/12	27
xenic	3000-5000	12/12	27
xenic	3000-5000	12/12	27

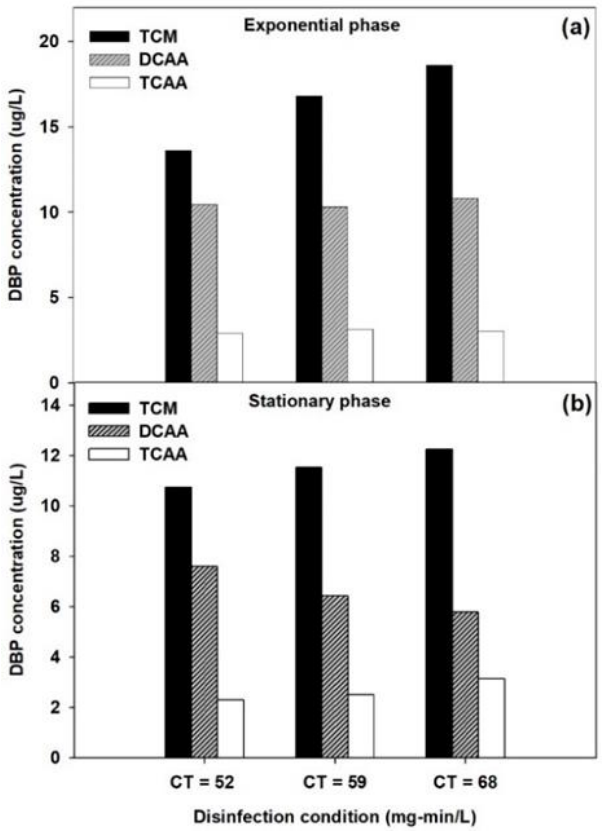

Fig. 1 Effect of CT on formation of DBPs at different growth phases of *Oscillatoria sp* : (a) DBPs during the exponential phase, (b) DBPs during the stationary phase

Table 2 Total amounts of DBPs from AOMs produced by *Oscillatoria sp* at different growth phases

Growth phase	CT value (mg • min/L)		
	CT = 52	CT = 59	CT = 68
Exponential phase (ug/L)	26.94	30.24	32.49
Stationary phase (ug/L)	20.61	20.48	21.16

Table 3 Total amounts of DBPs from AOMs produced by *Anabaena sp*.at different growth phases

Growth phase	CT value (mg • min/L)		
	CT = 52	CT = 59	CT = 68
Exponential phase (ug/L)	26.73	30.63	30.79
Stationary phase (ug/L)	26.72	32.70	41.99

Table 4 Total amounts of DBPs from AOMs produced by *Microcystis aeruginosa* at different growth phases

Growth phase	CT value (mg • min/L)		
	CT = 52	CT = 59	CT = 68
Exponential phase (ug/L)	27.88	27.10	25.49
Stationary phase (ug/L)	28.25	33.48	37.75

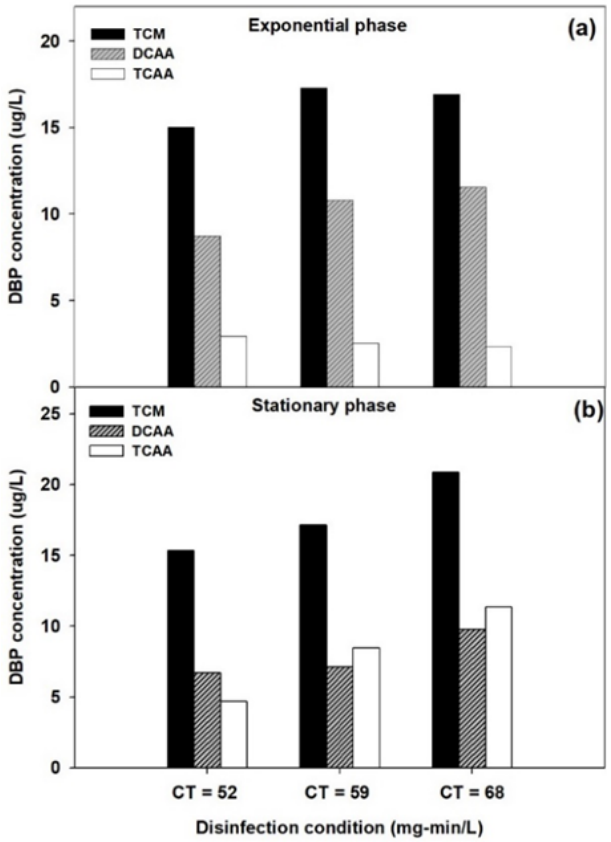

Fig. 2 Effect of CT on formation of DBPs at different growth phases of *Anabaena sp*.: (a) DBPs during the exponential phase, (b) DBPs during the stationary phase

were maintained at 220℃ and 250℃, respectively. The sample split ratio was 1: 40. Initial oven temperature was set at 35℃ for 4 min, and then increased to 180℃ at rate of 15℃/min and held constant for 3min. On the other hand, HAAs measurement was conducted by GC (7890B, Aglient, USA) using an electron capture detector (ECD), equipped with an Agilent 19091B-102 capillary column (25m×0.20mm I.D.×0.33μm). Initial oven temperature was set at 50℃ for 3 min, ramping to 150℃ at 10℃/min, and then ramping to 280℃ at 20℃/min and holding for 5 min. The flow rate was 0.42086mL/min, and the temperatures of injector and detector were held at 200℃ and 300℃, respectively.

2.3 Effect of pre-ozonation on absorption and coagulation

Ozone solution was prepared by an ozone generator (LAB-2B, Ozonia Korea Co., Ltd, Korea). The gas flow rate of pure oxygen wss 0.3 L/min and ozone was generated for 10 minutes to obtain an ozone concentration of 8.05 mg/L. The concentration of ozone in aqueous solutions was determined by the Indigo method (Bader and Hoigne 1981). The prepared ozone solution was mixed with the AOM solution to achieve the desired ozone concentration of 3 mg/L and AOM concentration of 5 mg/L, respectively. After the pre-ozonation, the coagulation (Jar test) was conducted with different PAC or $Al_2(SO_4)_3$.

3. Results and discussion

3.1 Effect of CT on formation of DBPs

The effect of CT on formation of DBPs from AOM produced by *Oscillatoria sp*. at two different growth phases under different CT values was summarized in Table 2. The total amount of DBPs in the exponential growth phase was greater than that in the stationary phase under all three conditions.

During the exponential growth phase, the total amount of DBPs formed for *Oscillatoria sp*. increased in the order of CT=52 mg·min/L < CT=59 mg·min/L < CT=68 mg·min (Fig. 1)

However, during the stationary phase, there was no significant difference between total amounts of DBPs under three different CT values. During the exponential growth phase, the amount of TCM increased with CT value, but in case of DCAA and TCAA no significant difference was found (Fig. 1a). On the other hand, during the stationary phase, the amount of DCAA decreased with CT value, but TCM and TCAA increased with CT value (Fig. 1b). For *Anabaena sp*., the effect of CT on formation of DBPs from AOM generated at different growth phases was shown in Fig 2. The total amount of DBPs in the exponential growth phase was less than that in the stationary phase under all three chlorine disinfection conditions (Table 3).

For both the exponential and stationary growth phases, the total amount of DBPs increased with CT value. During the exponential growth phase, the amount of TCM and DCAA increased with CT value, but no significant difference was found for TCAA (Fig. 2a).

On the other hand, during the stationary phase, all DBPs increased with CT value (Fig. 2b). Like *Anabaena sp*., total

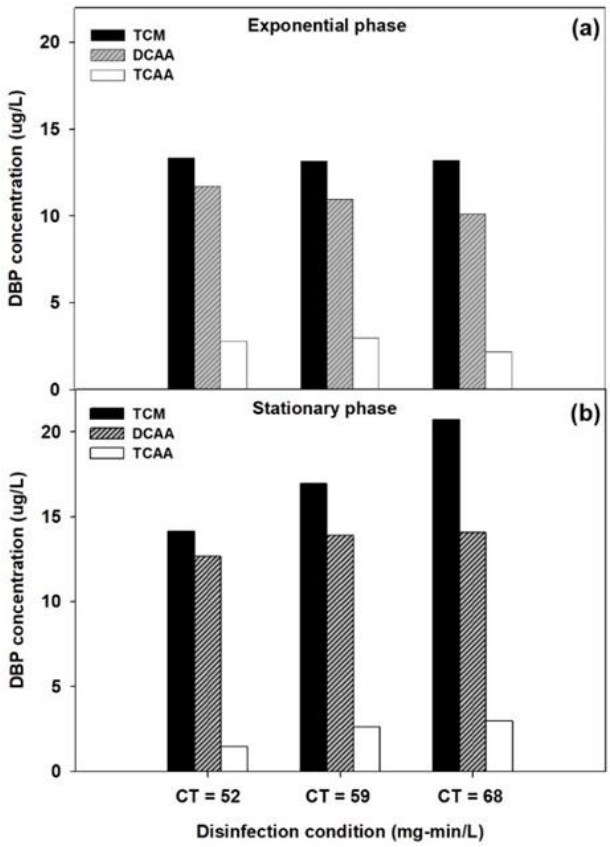

Fig. 3 Effect of CT on formation of DBPs at different growth phases of *Microcystis aeruginosa*: (a) DBPs during the exponential phase, (b) DBPs during the stationary phase

amounts of DBPs from AOM produced by *Microcystis aeruginosa* at two different growth phases increased with CT value (Table 4).

During the exponential growth phase, there was no significant difference between total amounts of DBPs under three different CT values (Fig. 3a). However, all three types of DBPs increased with CT value during the stationary phase (Fig. 3b).

For all three algae species, production of TCM was greater than the other DBPs (i.e., DCAA and TCAA) for both the exponential and stationary growth phases. In general, humic and fulvic acids are known the main precursors of TCM. Also, aromatic compounds with hydroxyl and amino groups (i.e., phenol and aniline) and with carbonyl and carboxyl groups have the potential to produce TCM (Reckhow *et al.* 1990). Therefore, the higher concentration of TCM than other DBPs may be due to the above-mentioned compounds present in the AOM (Fang *et al.* 2010). In addition, Yang *et al* (2011) demonstrated that TCM production was higher than other DBPs during the stationary phase of algae, which had good agreement with the results in this study. On the other hand, DCAA/TCAA ratios for *Microcystis aeruginosa* ranged from 3.69 to 5.32 (Fig. 3). This range was different from the values in the literature (0.48 to 1.75) (Huang *et al.* 2009, Qi *et al.* 2016, Son *et al.* 2015 and Goslan *et al.* 2017). This difference may be likely due to amino acid content associated with

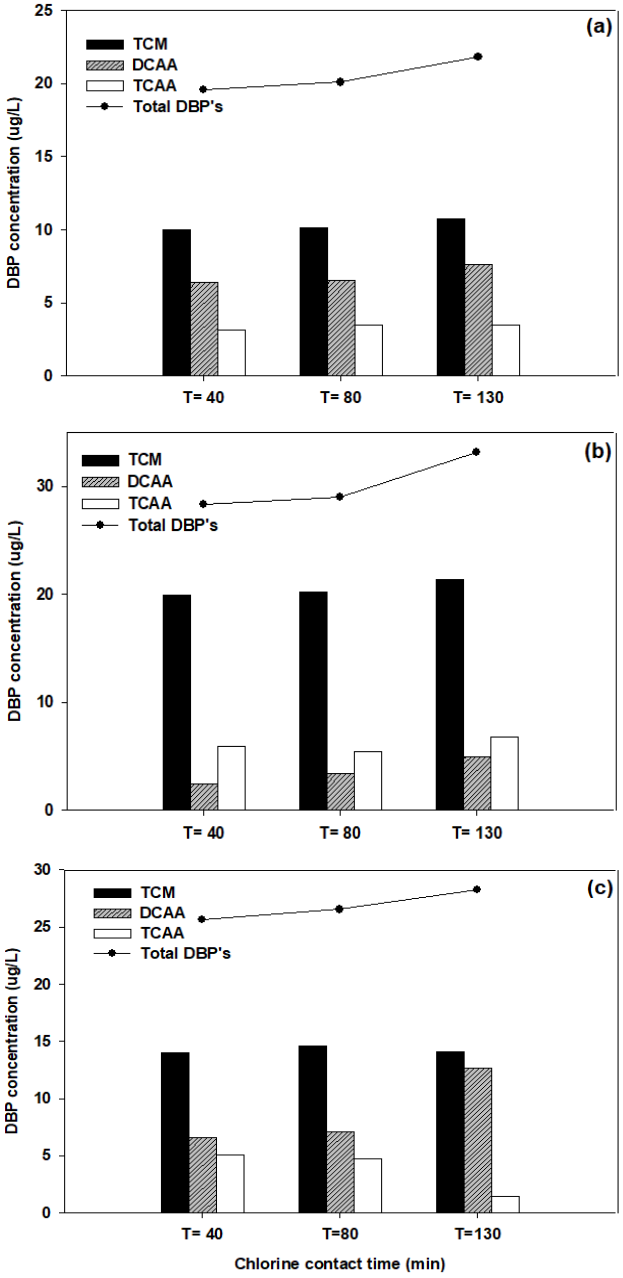

Fig. 4. The effect of contact time on formation of DBPs (chlorine dosage = 6.7 mg /L). a: *Oscillatoria sp.*; b: *Anabaena sp.*; c: *Microcystis aeruginosa*

HAAs production (Bond *et al.* 2009). In this study, the order of DCAA/TCAA ratio was *Microcystis aeruginosa* (4.73-5.32) > *Oscillatoria sp.* (1.85-3.32) > *Anabaena sp.* (0.84-1.42). This result implies that the amino acid content in the AOM of *Microcystis aeruginosa* is greater than the others.

The effect of chlorine contact time on DBP formation from AOM was shown in Fig. 4. For all the algae species, the total amount of DBPs formed by the AOM increased with the contact time. The results are in good agreement with literature values (Hua *et al.* 2008, Fang *et al.* 2010).

On the other hand, the contact time seems to have a greater impact on formation of HAAs than TCM. For *Oscillatoria sp.*, THM yields were 10.01 μg/L and 10.76

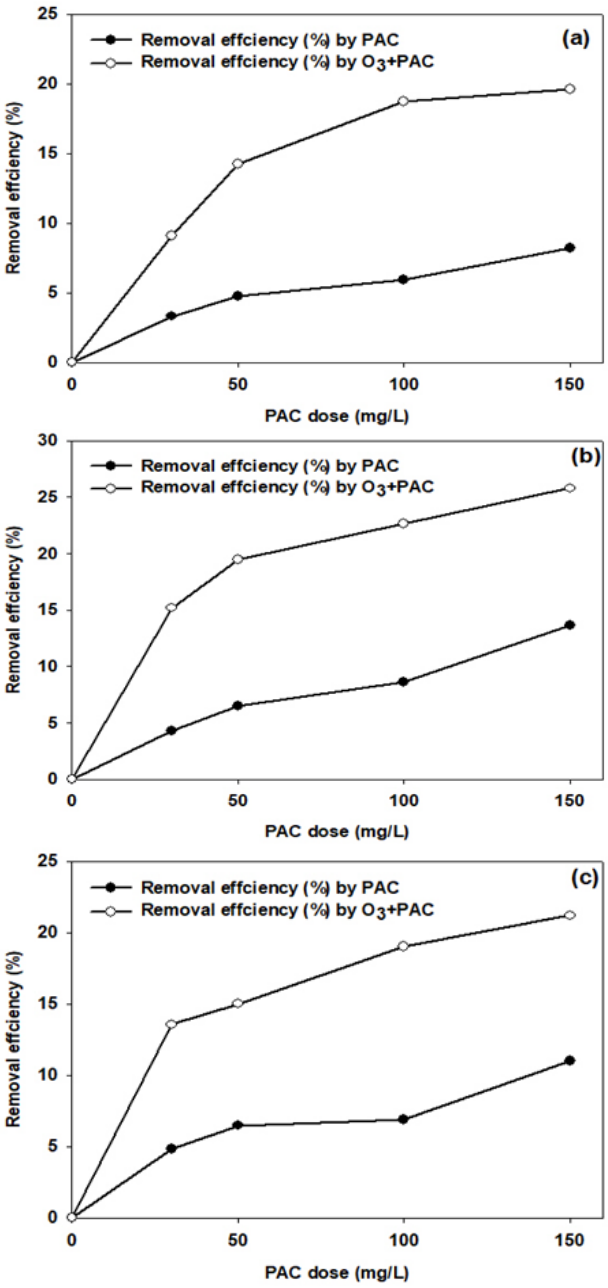

Fig. 5 C*omparison between* the PAC adsorption and the combined process of pre-ozonation and PAC on the *removal efficiency* of AOM at 0.6 mgO_3/mg C (a: *Oscillatoria sp*., b: *Anabaena sp*., c: *Microcystis aeruginosa*).

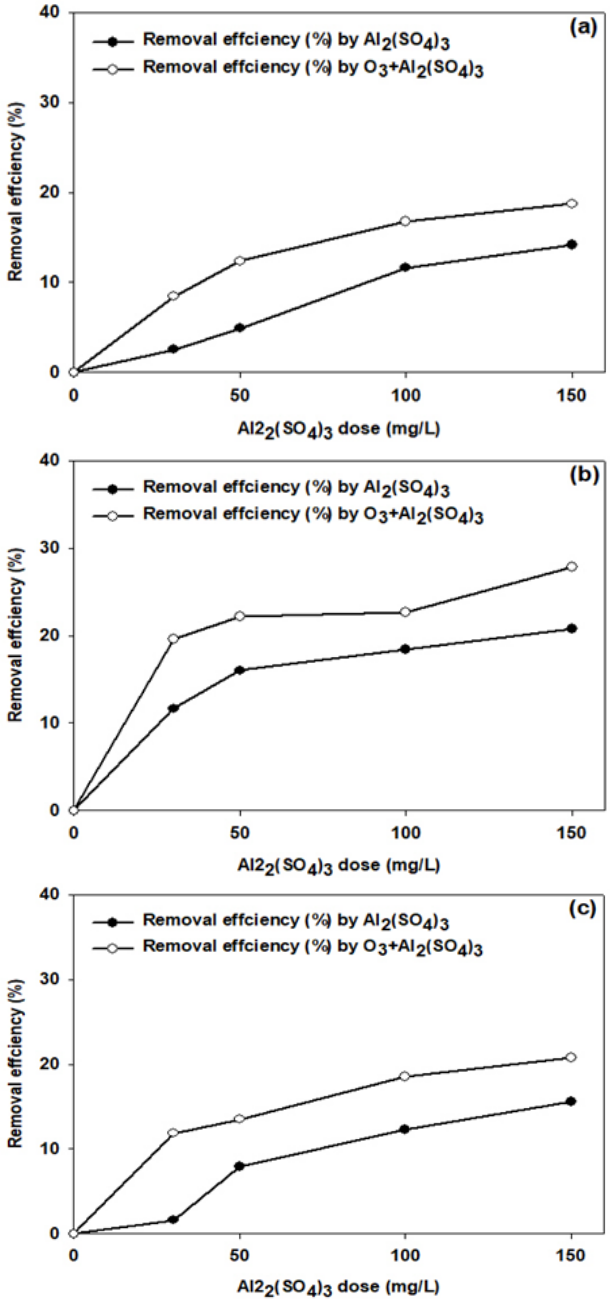

Fig. 6 C*omparison between* the $Al_2(SO_4)_3$ coagulation and the combined process of pre-ozonation and $Al_2(SO_4)_3$ on the *removal efficiency* of AOM at 0.6 mgO_3/mg C (a: *Oscillatoria sp*., b: *Anabaena sp*., c: *Microcystis aeruginosa*)

μg/L for 40 min and 130min contact times, respectively, while the HAAs yields were 9.56 μg/L and 11.99 μg/L under the same conditions, respectively. This trend is consistent with the result of the previous study (Kanan and Karanfil 2011). In addition, HAAs from AOM produced by *Oscillatoria sp*. and *Microcystis aeruginosa* contained higher amounts of DCAA than TCAA, however, HAAs from AOM produced by *Anabaena sp*. contained higher amounts of TCAA than DCAA. The results are likely due to chemical properties of precursor functional groups. The transient β-diketone groups (R-CO-CH_2-CO-R') undergo hydrolysis and acidification to form the common precursor of DCAA and TCAA. When the R-group is a hydroxyl group (-OH), DCAA is the final product. However, when the R-group is an oxidizable functional group, further chlorination promotes the formation of TCAA (Hong *et al*., 2009).

3.2 Effect of pre-ozonation on AOM adsorption and coagulation

Fig. 5 shows the removal efficiencies of AOM by PAC

alone and the combination of pre-ozonation and PAC at the concentration of ozone of 3 mg/L and pH 7. In case of PAC process alone, removal efficiency of AOM increased as PAC was added for all three algae species. Additionally, when pre-ozonation was combined with PAC absorption, the removal efficiency of AOM was significantly higher than PAC treatment alone. Ozonation was found to be able to convert high molecular weight compounds to low molecular weight ones (Paralkar and Edzwald 1996, Bose and Reckhow 2007 and Wei *et al*. 2016). The removal efficiency of the combined process with AOM produced by *Anabaena sp*. was higher than both *Oscillatoria sp*. and *Microcystis aeruginosa*. This result may be attributed to a greater amounts of low molecular weight organic compounds in the presence of AOM produced by *Anabaena sp*. than the others. In addition. the ozonation alone was not an effective method for removal of AOM without a subsequent processes (Lin *et al*. 2021). Thus, the ozonation effect of improving removal efficiency of AOMs by adsorption and coagulation was investigated in this study.

Fig. 6 shows the removal efficiency of a combined process of pre-ozonation and coagulation with alum ($Al_2(SO_4)_3$) for three algal AOMs. For coagulation process alone, removal efficiency of AOM increased as $Al_2(SO_4)_3$ was added for all three algae species. Driscoll and Schecher (1990) found that negatively charged aromatic compounds with conjugated double bonds were more easily removed by electro-neutralization with positively charged aluminum salt. In addition, when pre-ozonation was combined with $Al_2(SO_4)_3$ coagulation, the removal efficiency was higher than that of the coagulation alone. Schneider and Tobiason (2000) hypothesized that pre-ozonation could reduce the surface charge of particulates, leading to better coagulation by charge neutralization. The results in this study were in good agreement with the literature (Masoomi *et al*. 2019 and Novotná *et al*. 2020).

4. Conclusions

In this study the formation of DBPs during chlorination of AOMs form three different algal species under various experimental conditions was investigated. The total amounts of DBPs tended to increase with increasing CT values for both exponential and stationary growth phases. After chlorination with AOM in the most experimental conditions, the amount of TCM was higher than DCAA and TCAA. This result implies that DBPs formation may be attributed to the characteristics of AOMs which are mainly due to the hydrophobic organic matter content (i.e., humus-like substances). The effect of the combined methods (pre-ozonation + PAC/ $Al_2(SO_4)_3$) on the removal of AOM was examined. The pre-ozonation changed the structure and functional group charge of AOM, which directly improved the efficiency of absorption and coagulation of AOM.

Acknowledgments

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