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Research Paper

Exploration of the primary features of algal organic matter that impede the efficiency of harmful algal bloom control by modified clay*

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Abstract In recent studies, the most effective method for controlling harmful algal blooms (HABs) in field applications is the modified clay (MC) technology. While the MC technology has been proved highly effective for HABs control, emerging evidence highlights its limitations, particularly in the presence of algal organic matter (AOM) where the algal removal efficiency of MC can be significantly reduced. We compared the physicochemical properties of AOM derived from two dinoflagellate species, *Prorocentrum donghaiense* (also identified as *Prorocentrum shikokuense* Hada and *Prorocentrum obtusidens* Schiller) and *Amphidinium carterae*, and their effects on the MC flocculation of microalgae cells. The physicochemical properties of AOM in microalgal culture fluid can differ with species and growth phase. Various AOM samples had different effects on MC flocculation; for example, AOM derived from *P. donghaiense* in the exponential growth phase inhibited flocculation, while AOM derived from *A. carterae* in the exponential growth and stationary phases accelerated flocculation. The effect of AOM on MC flocculation was attributed to a combination of multiple mechanisms, with individual AOM properties displaying inconsistent effects under different sets of conditions. Notably, the ratio of the molecular weight to the electric charge (*M*/*E*) of the AOM can indicate how the AOM will affect the algae removal efficiency of MC: the larger the ratio is, the greater the promoting effect is likely to be, and vice versa.

Keyword: harmful algal bloom (HAB); algal organic matter (AOM); modified clay (MC); molecular weight; electrical property

1 INTRODUCTION

The frequency and size of harmful algal blooms (HABs) have increased in recent years owing to the intensification of human activities and the exacerbation of eutrophication in marine environments (Dai et al., 2023; Wang et al., 2023a). Thus, how to safely and efficiently prevent and control HABs is an urgent issue in marine environmental protection.

On the basis of the theory of clay surface modification, Yu et al. (2017) innovatively introduced polyaluminum chloride (PAC) as a surface modifier to increase the clay flocculation

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capacity, developing a modified clay (MC) material that demonstrates several-fold greater algal removal efficiency than conventional clays do, and successfully applied this MC in the field to control HABs. This MC is the first dedicated technical standard specialized for HAB management in China that also provides important technical support for the prevention and control of HABs in the United States, Chile, Malaysia, Peru, and other countries (Yu et al., 2017). During application, MC particles flocculate microalgal cells and form heterogeneous microalgal cell-MC flocs, which settle into the sediment to help the removal of microalgal biomass from the water body (Yu et al., 2017; Chu et al., 2024; Cao et al., 2025). Many environmental factors, including salinity, ionic strength, and dissolved organic matter, have been suggested to affect this process (Jiang et al., 2021; Wu et al., 2021; Zang et al., 2024), and algal organic matter (AOM) was recently identified as another potential influencing factor.

AOM is a biologically sourced form of organic matter that is derived mainly from algal metabolism that has a complex composition and variable properties (Pivokonsky et al., 2014; Villacorte et al., 2015; Rehman et al., 2017). AOM easily accumulates during blooms and leads to an increase in the dissolved organic carbon (DOC) concentration from a few milligrams per liter to tens of milligrams per liter within a short period (Oh et al., 2018; Xiang et al., 2023). Since the high concentrations of AOM in HAB-affected water represent a significant source of environmental pollution, it is almost impossible to meet purification standards during the blooming period (Nguyen et al., 2005; Dyble et al., 2008; Li et al., 2012; Pearson et al., 2016). Notably, AOM typically has a negative charge (Henderson et al., 2010), which impedes the flocculation efficiency of MC and further increases the difficulty of HAB control (Jiang et al., 2021; Wang et al., 2023b; Chu et al., 2024).

The impact of AOM on flocculation is related to its concentration (Wang et al., 2023b). In a specific concentration range, AOM can undergo electrical neutralization with the flocculant, promoting particle aggregation. However, at excessively high concentrations, its negative charge may compete with the flocculant and hinder effective collision between the flocculant and suspended particles, thereby lowering the flocculation effect (Henderson et al., 2010; Pivokonsky et al., 2012). Additionally, different types of AOM might exhibit different flocculation behaviors on the basis of their molecular weight (Safarikova et al., 2013), as components with higher molecular weights tend to be more involved in the flocculation process (Leloup et al., 2013; Pivokonsky et al., 2014). Furthermore, the AOM produced by different algae varies in terms of composition, concentration, and physicochemical properties (Huang et al., 2007; Pivokonsky et al., 2014), which may have different effects on flocculation.

Approximately 300 species of microalgae can cause marine HAB disasters (Lin et al., 2019). However, is the variation in the physicochemical properties of AOM produced by different microalgal species and growth phases a primary feature contributing to the inconsistent algal bloom treatment efficiency of MC? Additionally, which features of AOM are critical factors affecting the microalgae removal efficiency of MC? Answering these questions could provide theoretical guidance for the further optimization of MC technology, thereby providing methods for the efficient treatment of HABs. In this study, we selected typical dinoflagellates (*Prorocentrum donghaiense and Amphidinium carterae*) from a variety of HAB organisms with similar cell sizes and morphologies as the experimental organisms and collected their AOM to carry out the following experiments. The physicochemical properties of the AOM were determined, the interference patterns of different AOM types on MC flocculation with microalgal cells were summarized, and the critical factors related to AOM were explored.

2 MATERIAL AND METHOD

2.1 Microalgae cultivation

Two species of bloom-forming dinoflagellates, *P. donghaiense* and *A. carterae*, were obtained from the Key Laboratory of Marine Ecology and Environmental Sciences, Chinese Academy of Sciences and cultured in L1 media at 22±1 °C with a light intensity of approximately 65 μmol photons/(m^2·s) on a 12-h:12-h light:dark cycle. The growth of the two microalgae conformed to the S-curve of the logistic function, with the exponential growth phase lasting from 1 to 6 d, the stationary phase lasting from 6 to 12 d, and the decline phase lasting after 12 d. Microalgal density was determined via a combination of microscopic observations (Olympus CK33, Tokyo, Japan) and in vivo fluorescence measurements (Trilogy, Turner Design, USA).

2.2 Preparation of MC

Kaolin clay was obtained from Yankuang Beihai Kaolin Co., Ltd., China, and the modifier PAC was purchased from Tianjin Guangfu Fine Chemicals (Tianjin, China). Modified clay (PAC-MC) was prepared according to Yu et al. (2017), in which the surface of kaolin clay was modified with PAC (kaolin∶PAC=5∶1).

2.3 Preparation and characterization of the AOM

2.3.1 Preparation of the AOM

Culture media containing microalgae at different growth phases was subjected to vacuum extraction through a GF/F membrane (treated at 450 °C for 4 h) to eliminate algal cells and suspended particulate matter. The filtered medium, referred to here as the AOM solution, was added to saturated mercury dichloride and stored in a refrigerator at 4 °C. Different concentrations of AOM solution were prepared according to the methods of Wang et al. (2023b).

We also selected fucoidan as a representative AOM based on our previous work for further simulation studies (Chu et al., 2024). A total of 1.0 g of fucoidan (BMSG, China) was dissolved in 1 L of artificial seawater (ASW; details of the formulation are given in Supplementary Table S1). The solution was ultrasonicated at 900 W for 1 h and then filtered through a GF/F membrane. The prepared model organic compound solution was diluted with ASW to various molecular weights and electric charge, and investigated the impact of different molecular weight to electric charge (M/E) ratios on the MC flocculation efficiency.

2.3.2 Characterization of the AOM

(1) Concentration and molecular weight analysis

The AOM concentration was determined on the basis of the DOC content, which was quantified using a total organic carbon analyzer (Multi N/C 2100S, Analytik Jena, Jena, Germany). AOM components were separated by using Amicon Ultra-15 ultrafiltration centrifuge tubes with molecular weight cutoffs of 100, 50, 10, and 3 kDa according to the method described by Pivokonsky et al. (2014). The weight-average molecular weight of the AOM was determined using an 18-angle laser light scattering gel permeation chromatography system (Optilab T-rex) according to the method described by Wang et al. (2023b). Each treatment consisted of three replicates (n=3).

(2) Chemical composition analysis

In accordance with the methods of Wang et al. (2021), the organic composition of the AOM was analyzed via liquid chromatography with an organic carbon detector (LC-OCD; DOC-labor, Mold 9, Karlsruhe, Germany). LC separation was performed using a TSK HW-50S column (250 mm×20 mm, TSK HW 50S, Toso, Japan), and the AOM samples were diluted to a DOC concentration of less than 5.00 mg/L before analysis.

Fourier transform infrared (FT-IR) spectra was used to analyze the functional groups in various AOM samples. The solid samples were prepared using the KBr pellet method, and data were collected by performing 32 scans within the wavenumber range of 500–4 000 cm^{-1} with a resolution of 4 cm^{-1}. OMNIC 8.2 infrared analysis software was used to analyze the obtained spectra.

The zeta potential was used to represent the electrical characteristics of AOM (Henderson et al., 2008). The surface potentials of the high-molecular-weight components of the AOM were determined using a zeta potential analyzer (Nano ZS, Malvern, UK).

2.4 Determination of the impacts of AOM on the microalgae removal efficiency of MC

P. donghaiense and *A. carterae* were cultured as described in Section 2.1, and the microalgal cells were separated from the culture medium by gravity filtration. The cells were resuspended in the corresponding AOM solutions prepared as described in Section 2.3.1 to a final concentration of 1×10^5 cells/mL. Cells that were cultured in carbon-free ASW systems were used as controls. The MC stock solution was prepared according to the methods of Yu et al. (1994) and added to the abovementioned culture medium to a final concentration of 0.1 g/L.

Changes in cell density were determined using a fluorometer (Trilogy, Turner Design, USA) and quantified as relative fluorescence units (RFUs). The relationship between algal density and fluorescence intensity is shown in Supplementary Fig.S1. Both parameters exhibit linear distributions (R^2>0.99) across different algal species. Therefore, RFUs was used to represent microalgal density in this study. The removal efficiency in culture media with or without AOM was calculated according to the method described by Jiang et al. (2021).

2.5 Determination of the impacts of AOM on MC flocculation

By the method of Chu et al. (2024), 0.1-g/L MC

was added to a 100-mL quartz colorimetric cell with or without AOM. By adjusting the stirring speed of the external agitator (EUROSTAR, IKA, Gottingen, Germany), the particle flocculation was divided into three stages: aggregation, fragmentation, and regeneration (Jarvis et al., 2005; Jiang et al., 2021). A particle imaging velocimeter (PIV; FlowMaster, LaVision, Göttingen, Germany) was used to quantify the dynamic variations in floc particle size during flocculation. The zeta potentials of the MC particles in the AOM-treated group and the control group were measured at once using a Nano ZS zeta potential analyzer (Malvern, UK).

2.6 Statistical analysis

Pearson correlation analysis was employed to examine the AOM obtained during different growth periods affected the removal efficiency of MC, and SPSS 27.0 (IBM, USA) was used for analyzing significant differences in physicochemical properties among different groups of microalgae-derived AOM. Sequencing analysis was conducted using CANOCO for Windows 5.0 software (Microcomputer Power, Ithaca, NY, USA). On the basis of the results of the detrended correspondence analysis (DCA), redundancy analysis (RDA) was employed to investigate the relationships between the physicochemical properties of different AOM samples and the removal efficiency of MC. Origin 2023b software (OriginLab, Northampton, MA, USA) was used to generate the relevant graphs.

3 RESULT AND DISCUSSION

3.1 Physicochemical property of AOM derived from different microalgae

Despite the differences in cell density between the two microalgal species, their growth cycles and DOC change patterns were similar during cultivation (Fig.1). During natural cultivation, the AOM concentration in both microalgal systems remained within the range of 7–15 mg/L. At the same growth stage, the concentrations of AOM derived from *P. donghaiense* and *A. carterae* did not differ significantly (P>0.05), and the deviation rate remained within 10%. With prolonged culture duration, algal cells can secrete more AOM (Henderson et al., 2008). The AOM concentrations of the two microalgae during the stationary and decline phases were 1.5 and 2 times higher, respectively, than those during the exponential growth phase. However, the rate of increase in the

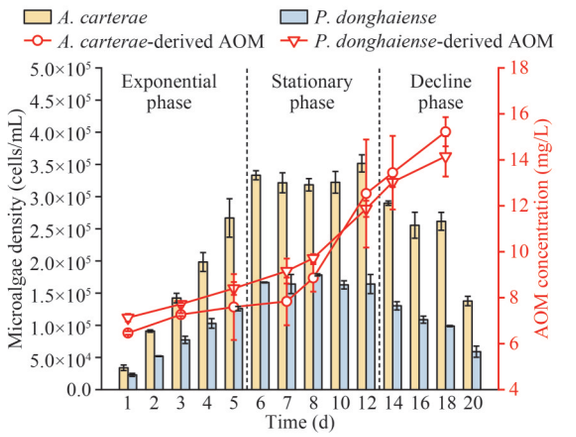

Fig.1 Concentrations of AOM derived from microalgae in different growth phases

AOM concentration was notably greater in the *A. carterae* culture during the middle and late stationary phases. The pronounced increase in AOM concentration during the decline phase is likely due to the disintegration of algal cells and subsequent release of endolytic organic matter (Zuo et al., 2021), resulting in the *A. carterae*-derived AOM concentration in the algal solution exceeding that of the *P. donghaiense*-derived AOM by the end of the culture period.

The chemical compositions of both types of microalgae-derived AOM were similar under the same cultivation conditions. Similar FT-IR absorption peaks were observed in the wavelength range of 500–4 000 cm^{-1} for both types of AOM (Fig.2a). Four characteristic peaks of different compounds typically found in AOM were observed in the spectra of AOM derived both species. The broad and intense absorption peaks in the range of 3 200–3 400 cm^{-1} primarily originated from O-H/N-H stretching vibrations (Zhou et al., 2014; Tang et al., 2017), the peak at 2 350 cm^{-1} was attributed to the sulfhydryl group in humic substances (Hadjoudja et al., 2010), the single peak at 1 640 cm^{-1} indicated C-O/C-N bonds in organic protein molecules (Lin et al., 2014), and the peaks at 1 070 and 983 cm^{-1} were associated with C-O/C-N bonds in polysaccharides (Tang et al., 2017). These findings suggest the presence of characteristic functional groups of polysaccharides, proteins, and humic substances in both types of microalgae-derived AOM, and no unique characteristic functional groups were identified. The results of the LC-OCD analysis revealed that hydrophilic constituents were the common primary component (Supplementary Table S2), accounting for approximately 85% and 91%

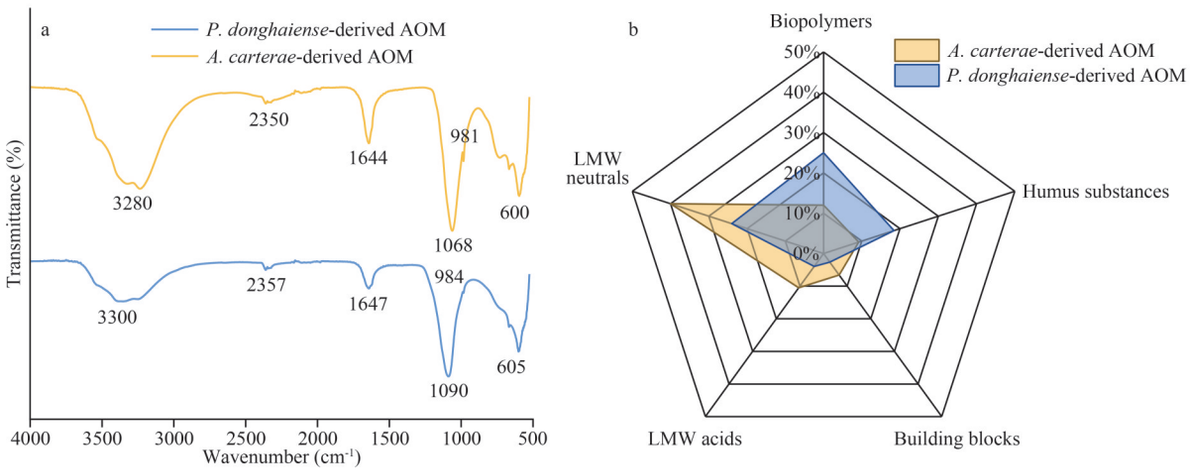

Fig.2 Components of AOM derived from different microalgae

a. Fourier transform infrared (FT-IR) spectra; b. relative abundances of the main components.

of the AOM derived from *P. donghaiense* and *A. carterae*, respectively. Furthermore, both AOM samples contained five principal categories of organic matter, including biopolymers, humic substances, building blocks, low-molecular-weight acids (LMW acids), and low-molecular-weight neutrals (LMW neutrals) (Supplementary Fig.S2). Biopolymers in AOM are typically polysaccharides and proteins, such as cyanobacterial starch, algal polysaccharides, amylose/amylopectin from algae, cyanobacterial proteins, and peptidoglycan (Henderson et al., 2008; Qu et al., 2012; Pivokonsky et al., 2014). These findings indicate that the two types of microalgae-derived AOM are chemically similar.

However, there were differences in the contents of the different chemical components in the AOM from the two microalgae. The percentages of biopolymers, humic substances, building blocks, LMW acids, and LMW neutrals in *P. donghaiense*-derived AOM were 25%, 20%, 8%, 9%, and 24%, respectively, whereas these proportions in the *A. carterae*-derived AOM were 12%, 13%, 11%, 14%, and 40%, respectively (Fig.2b). *P. donghaiense*-derived AOM had higher concentrations of biopolymers and humic substances, constituting 45% of the total AOM, which was nearly twice that in the *A. carterae*-derived AOM. Conversely, LMW acids and LMW neutrals dominated the composition of *A. carterae*-derived AOM, constituting 65% of the total. These results indicate that although both types of microalgae-derived AOM have similar chemical compositions, the different proportions of the components may lead to different physicochemical properties.

AOM derived from different microalgae at the same growth phase presented different weight-average molecular weights (Table 1). During the experiment, the weight-average molecular weight increased and then decreased with prolonged cultivation, and the weight-average molecular weight of the *A. carterae*-derived AOM was greater than that of the *P. donghaiense*-derived AOM at the same growth stage. In addition, the overall molecular weight distributions exhibited a similar trend throughout the growth phases (Fig.3). During the exponential growth phase, the predominant molecular weight fractions of *P. donghaiense*-derived AOM were the >50-kDa (28%) and <3-kDa (38%) fractions, whereas *A. carterae*-derived AOM exhibited greater proportions of the >100-kDa (29%) and <3-kDa (42%) fractions, which is consistent with the findings reported by Yang et al. (2019). Only 13% of the *P. donghaiense*-derived AOM exhibited a molecular weight >100 kDa during the exponential growth phase, which was significantly lower than the proportion in the *A. carterae*-derived AOM (29%; $P<0.005$),

Table 1 Weight-average molecular weights of AOM derived from different microalgae

Microalgal species	Growth phase	Weight-average molecular weight (g/mol)
P. donghaiense	Exponential	24 838±315
	Stationary	30 911±159
	Decline	18 618±106
A. carterae	Exponential	44 067±148
	Stationary	47 866±120
	Decline	24 121±142

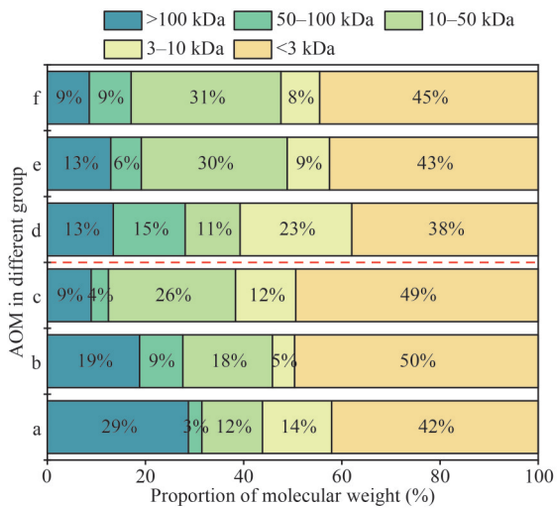

Fig.3 Molecular weight distributions of the AOM derived from different species in different growth phases

a. *A. carterae* in the exponential phase; b. *A. carterae* in the stationary phase; c. *A. carterae* in the decline phase; d. *P. donghaiense* in the exponential phase; e. *P. donghaiense* in the stationary phase; f. *P. donghaiense* in the decline phase.

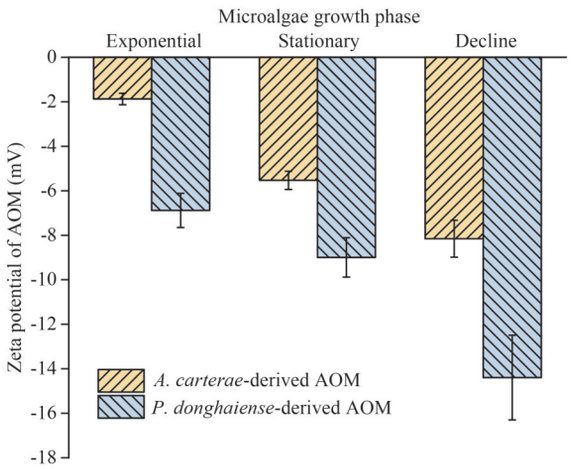

Fig.4 Zeta potentials of AOM derived from microalgae in different growth phases

indicating the greater capacity of *A. carterae* to secrete high-molecular-weight AOM. In contrast, more of the *P. donghaiense*-derived AOM was found in the 50–100-kDa fraction. With prolonged incubation duration, the macromolecular components were gradually degraded by bacteria and other microorganisms, and the proportion of >50-kDa components in the two types of microalgae-derived AOM gradually decreased, whereas the proportions of 10–50-kDa and <3-kDa components gradually increased.

AOM derived from different microalgae at the same growth phase presented different surface charges (Fig.4). The particle surface potentials of *P. donghaiense* and *A. carterae*-derived AOM at the exponential, stationary, and decline stages were -6.9, -9.0, and -14.4 mV and -1.87, -5.53, and -8.15 mV, respectively. The AOM was consistently negatively charged, with the magnitude increasing with prolonged culture time. This observation aligns with the findings reported by Henderson et al. (2008). The surface charge of AOM during different growth phases may be influenced by the AOM concentration; as the concentration of AOM increases, so does its surface charge (Wang et al., 2023b). However, at the same growth phase and concentration, the magnitudes of the surface charges of the different microalgae-derived AOM significantly differed (P<0.05), with the *P. donghaiense*-derived AOM being more negatively charged than the *A. carterae*-derived AOM. Polysaccharides and humic substances typically exhibit a highly negative surface charge (Pivokonsky et al., 2015; Liu et al., 2024). Therefore, the high proportion of polysaccharides and humic substances in *P. donghaiense*-derived AOM likely contributed to its more negative surface charge. In contrast, *A. carterae*-derived AOM contains lower proportions of these substances, resulting in a less negative surface charge. The lower zeta potentials of the AOM at pH values between 1 and 4 can be attributed to the ionization of carboxyl functional groups in charged polysaccharides and proteins, as demonstrated by Henderson et al. (2008). Moreover, the proportions of biopolymers and humic substances may also play crucial roles in the variations in the electrical characteristics of AOM during different growth phases. In the decline phase, microalgal cell apoptosis, cell wall lysis (polysaccharides), and the release of intracellular materials (proteins) contribute to an increase in the percentage of biopolymers, thereby enhancing the negative surface charge of AOM.

3.2 Effect of AOM on the particle characteristics and flocculation behavior of MC

The surface electrical properties of MC particles critically influence microalgae removal through flocculation. Different types of microalgae-derived AOM altered the surface electrical properties of the MC particles, and this effect increased with prolonged cultivation time (Fig.5). However, the extent of the influence of AOM on surface electrical properties differed between AOM types, as *P. donghaiense*-derived AOM exerted a more

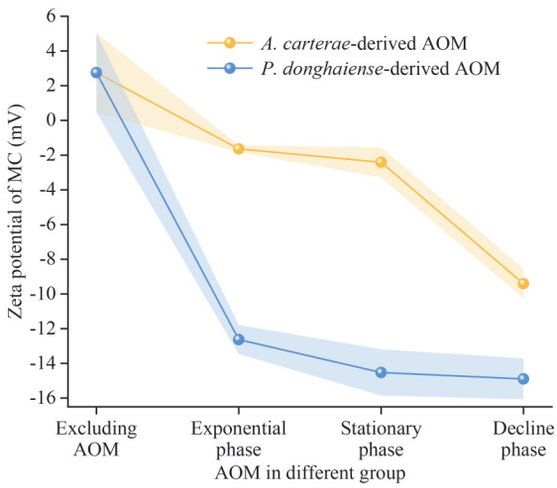

Fig.5 Changes in MC zeta potential in the presence of AOM

significant effect on the surface potential of MC particles than *A. carterae*-derived AOM did (P< 0.005). *P. donghaiense*-derived AOM induced a significant decrease in the surface potential of MC particles from +2.75 to -12.63 mV during the exponential growth phase (P<0.005) and remained at -14.7±0.26 mV throughout the other growth phases. In comparison, *A. carterae*-derived AOM had a smaller effect on the surface electrical characteristics of MC, resulting in MC surface potentials of -1.64 and -2.41 mV during the exponential growth and stationary phases, respectively, before decreasing further to -9.40 mV during the decline phase. According to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, a smaller absolute value of zeta potential indicates a weaker repulsive force among particles, resulting in reduced stability of a dispersed system and increased susceptibility to coagulation and flocculation. Conversely, a larger absolute value of zeta potential implies a stronger repulsive force between particles, leading to enhanced stability of a dispersed system and a decreased likelihood of flocculation (Derjaguin et al., 1987). Therefore, the different effects of microalgae-derived AOM on the MC surface potential may lead to different flocculation phenomena.

Algae removal involves electric neutralization or adsorption bridging between MC particles and algal cells, resulting in the formation of algal cell-MC heterogeneous flocs. These flocs are subsequently removed from the water for water purification (Yu et al., 2017; Cao et al., 2025). Therefore, microalgae-derived AOM affect MC removal efficiency primarily through their influence on particle flocculation. The effects of the AOM from different microalgae on MC flocculation behavior differed (Fig.6). Specifically, *A. carterae*-derived AOM promoted MC flocculation, whereas *P. donghaiense*-derived AOM inhibited flocculation.

In the *P. donghaiense*-derived AOM system, across the various growth phases, the rate of MC floc formation was relatively slow, and the particle size stabilized at 156±8 μm. This represents a significant reduction of approximately 250 μm compared with the floc size in the ASW system without AOM (P<0.005). Furthermore, there were no significant changes in particle size following fragmentation and regeneration, indicating that MC flocculation was substantially inhibited in the presence of *P. donghaiense*-derived AOM. In contrast, in the *A. carterae*-derived AOM system, the variations in the MC flocculation behavior were associated with the microalgae growth phase. During the exponential and stationary phases, the flocs exhibited accelerated growth and effective regeneration following fragmentation. The particle size of the stabilized flocs reached approximately 553±32 μm, which is approximately 150 μm greater than the particle size in the ASW system without AOM. These findings suggest that *A. carterae*-derived AOM enhances MC flocculation. However, during the decline phase, *A. carterae*-derived AOM exhibited an inhibitory effect on flocculation that was similar to that observed with AOM from *P. donghaiense*.

The weak negative surface charge of *A. carterae*-derived AOM during the exponential and stationary

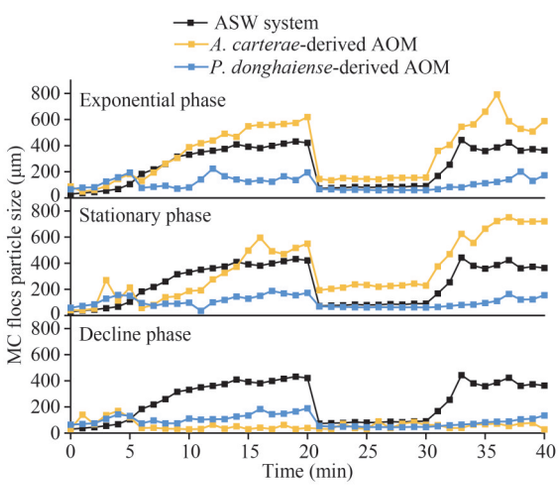

Fig.6 MC flocculation behavior in the presence of different types of microalgae-derived AOM

phases maintained the zeta potential of the MC system at approximately -2 mV. A study conducted by Xiao et al. (2019) revealed that flocculation is more likely to occur when the zeta potential of the system approaches zero. When the absolute value of the zeta potential in the system is low, the interparticle forces are weak, facilitating particle aggregation and the formation of large flocs. *P. donghaiense*-derived AOM and *A. carterae*-derived AOM in the decline phase were characterized by a high negative surface charge, which led to a reduction in the zeta potential of the MC system to less than -10 mV. This decrease enhanced the electrostatic forces among particles while lowering the collision efficiency, resulting in reduced MC flocculation efficiency and the increased retention of small particles in the water.

The flocculation behavior of MC can directly affect its microalgae removal efficiency. Similar to the effects of the AOM derived from the two microalgal species on MC flocculation, *P. donghaiense*-derived AOM significantly reduced microalgae removal efficiency (P<0.005), whereas *A. carterae*-derived AOM increased microalgae removal efficiency (P< 0.005). During the exponential growth phase, the removal of microalgal cells by MC in the presence of *P. donghaiense*-derived AOM (with an AOM concentration of 8.4 mg/L) was only 10%, and the microalgae removal efficiency of MC consistently remained low across the various growth phases (Fig.7). Wang et al. (2023b) reported that *P. donghaiense*-derived AOM decreased the removal efficiency of MC by approximately 50% at a concentration of 8 mg/L, whereas it increased the removal efficiency at concentrations ranging from 1 to 3 mg/L. The impact of *A. carterae*-derived AOM on the efficiency of microalgae removal by MC was found to be dependent on the growth phase of the microalgae. Before entering the decline phase (at an AOM concentration of 8–10 mg/L), the algae removal efficiency of MC consistently remained at approximately 70%. However, at the beginning of the decline phase, the AOM concentration increased to 15.67 mg/L, and the algae removal efficiency of MC decreased to 50%.

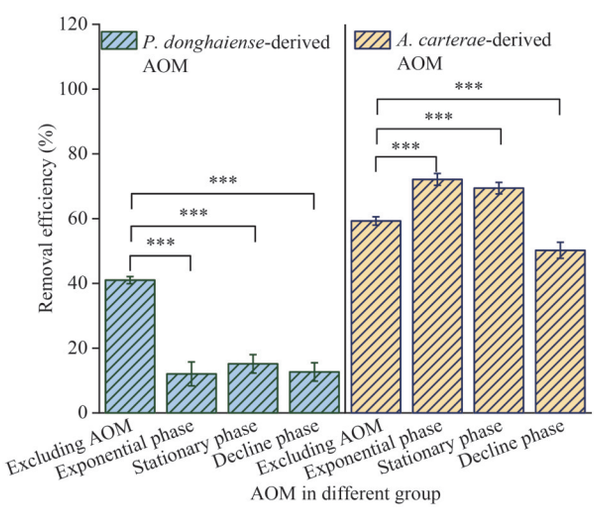

Fig.7 Microalgae removal efficiency of MC in the presence of different types of microalgae-derived AOM

***: significant difference at P<0.005.

The floc settling rate is typically accelerated by gravity, with larger floc particles settling faster. The presence of *A. carterae*-derived AOM obtained during both the exponential and stationary phases promoted the formation of larger flocs, which facilitated the settling and removal of the MC-microalgal cell flocs from the water and enhanced the algae removal efficiency. Conversely, *P. donghaiense*-derived AOM and decline phase *A. carterae*-derived AOM increased the electrostatic repulsion between the MC particles and algal cells, resulting in the formation of flocs with a small particle size and failure to achieve the desired microalgae removal effect. A comparison of the effects of the same concentration of *P. donghaiense* and *A. carterae*-derived AOM on the microalgae removal efficiency of MC revealed a 30% reduction in removal efficiency with the *P. donghaiense*-derived AOM but a 12% increase in removal efficiency with the *A. carterae*-derived AOM. These findings suggest that the effects of AOM from different microalgae on MC flocculation and microalgae removal are influenced by both the AOM concentration and physicochemical properties.

3.3 Possible effect mechanism of AOM on microalgae removal by MC

In this study, it was experimentally confirmed that AOM derived from different microalgae have significantly different effects on microalgae removal through MC flocculation (Figs.6 & 7; P<0.005). To determine the factors influencing these differences, we performed RDA to investigate the correlations among AOM physicochemical properties, such as weight-average molecular weight, concentration, and electric charge (explanatory variables) and microalgae removal efficiency and stable MC floc particle size (response variables); these results are presented in Fig.8. For *A. carterae*-derived AOM,

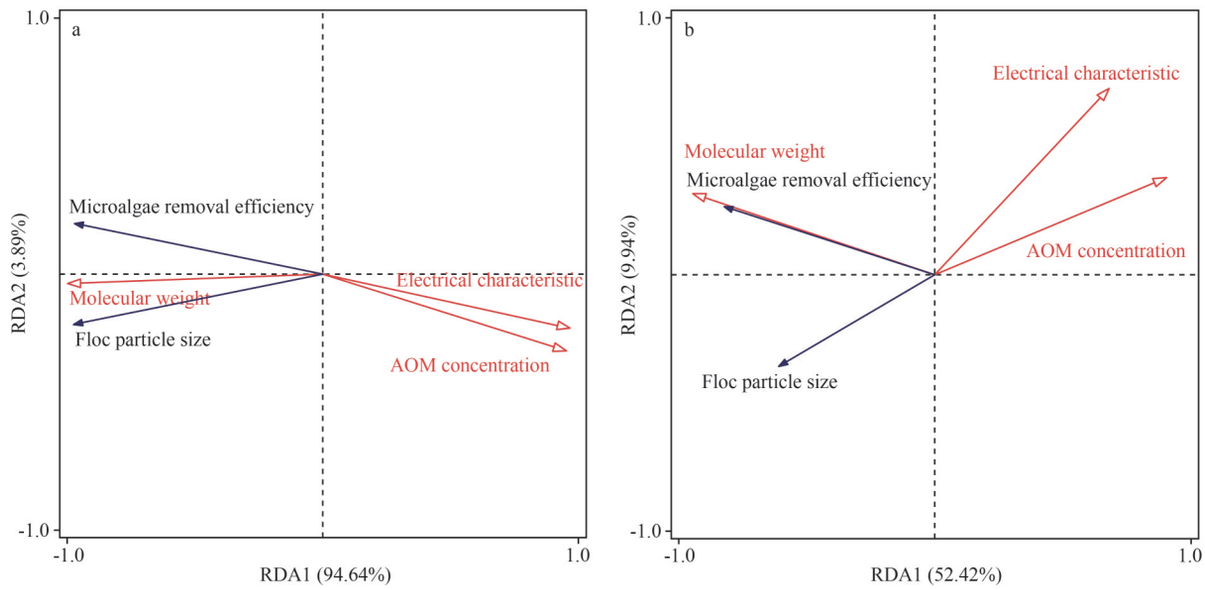

Fig.8 Relationships between microalgae removal through MC flocculation and the main characteristics of the AOM

a. *A. carterae*-derived AOM; b. *P. donghaiense*-derived AOM.

the angle between the weight-average molecular weight and microalgae removal efficiency was less than 90 degrees, indicating a significant positive correlation. Specifically, as the molecular weight of AOM increases, the microalgae removal efficiency of MC also increases. Conversely, the angles between the AOM concentration, electrical properties and microalgae removal efficiency were greater than 90 degrees, suggesting significant negative correlations (Fig.8a). With increasing AOM concentration and negative surface charge, the effect of *A. carterae*-derived AOM on the microalgae removal efficiency of MC shifted from promotion to inhibition. *P. donghaiense*-derived AOM exhibited a similar association (Fig.8b). *P. donghaiense*-derived AOM also promotes microalgae removal through MC flocculation when present at lower concentrations with a less negative surface charge than those investigated in this study (Wang et al., 2023b). The effects of the two microalgae-derived AOM samples on microalgae removal through MC flocculation exhibited similar patterns in this study with respect to the main physicochemical factors. Specifically, AOM from both microalgae inhibited microalgae removal through MC flocculation when the AOM had a low molecular weight, strong negative surface charge, and high concentration. Conversely, the two AOM types promoted microalgae removal through MC flocculation when they had a high molecular weight, weak negative surface charge, and low concentration. However, at the same AOM concentration, the electrical properties and molecular weights of the AOM derived from the two microalgae were significantly different (P<0.05). These variations led to different flocculation behaviors, ultimately resulting in distinct algae removal efficiencies during MC flocculation (Supplementary Fig.S3). To thoroughly investigate the key AOM characteristics influencing microalgae removal through MC flocculation, this study focused on how the electrical properties and molecular weight affect MC flocculation and microalgae removal at a constant AOM concentration.

In the presence of the two types of microalgae-derived AOM, the stable floc particle size generated upon MC flocculation was significantly positively correlated with the microalgae removal efficiency of MC (P<0.005), and the larger the floc particle size was, the higher the microalgae removal efficiency of MC was (Fig.8). Therefore, the microalgae removal ability of MC in the presence of AOM can be inferred from the floc particle size. Although the electrical properties and molecular weight are crucial indicators of the impact of AOM on MC flocculation, their influences exhibit different trends. To comprehensively analyze the influence of these factors, the ratio of molecular weight to electric charge (M/E) was employed as a composite index to further investigate the mechanism by which AOM affects MC flocculation. We investigated the changes in floc particle size as the M/E changed in model organic matter (Fig.9). The results revealed a linear relationship between the M/E of the model

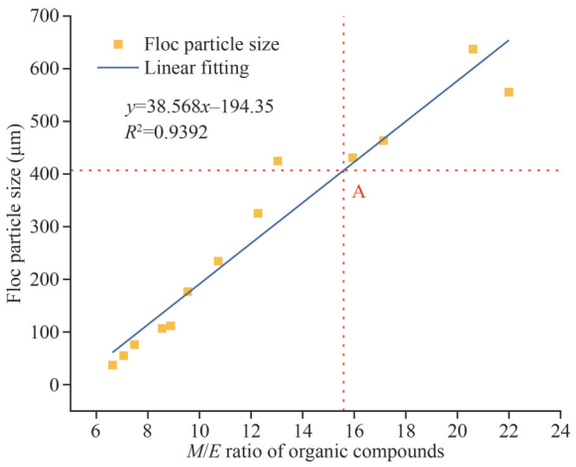

Fig.9 Relationship between the *M*/*E* ratio of the organic compounds and floc particle size

Point A represents the floc particle size when the *M*/*E* ratio of the organic compounds is 15.4.

organic matter and the floc particle size (R^2=0.939 2). When *M*/*E* was low, the floc particle size was small, which made aggregation of the particles in the system difficult. As the *M*/*E* of the model organic matter increased, the floc particle size gradually increased, facilitating the formation of larger flocs that could be more easily removed from the water body. In the ASW system without AOM, the floc particles formed by MC flocculation had a size of approximately 407 μm. When the *M*/*E* of the model organic matter was 15.4, MC generated flocs with particle sizes comparable to those observed in the absence of AOM (as shown in Fig.9, point A). Thus, it is reasonable to infer that at this *M*/*E*, the influence of organic matter in the water body is minimal, and the flocculation behavior of MC closely resembles that in the absence of AOM. Therefore, in the experimental system, when the *M*/*E* of the AOM exceeds 15.4, the AOM aids in aggregation, enabling the MC to form flocs with larger particle sizes. Conversely, when the *M*/*E* of the AOM is less than 15.4, MC flocculation is inhibited, resulting in flocs with smaller particle sizes.

The *M*/*E* values of the *A*. *carterae* and *P. donghaiense*-derived AOM at different growth phases were evaluated on the basis of the trend of influence of the model organic matter on MC flocculation; these results are presented in Supplementary Table S3. The measured *M*/*E* values of AOM and their effects on MC flocculation align with the general trends observed with the model organic matter, as depicted in Fig.9. In the exponential and stationary growth phases, the *M*/*E* values of *A*. *carterae*-derived AOM were 27.12 and 21.63, respectively, both of which are higher than 15.4; thus, these systems may facilitate MC flocculation by promoting the formation of "bridges" between microalgal cells and MC particles, generating larger flocs that can settle and separate from the water body, thus increasing the efficiency of microalgae removal (Pivokonsky et al., 2006; Henderson et al., 2008; Gonzalez-Torres et al., 2014). In contrast, the *M*/*E* values of the *P. donghaiense*-derived AOM in each growth stage (exponential, stationary, and decline) and *A*. *carterae*-derived AOM in the decline phase were all less than 15.4, with values of 1.97, 2.14, 1.26, and 2.58, respectively. The adsorption of AOM onto the surface of the MC particles altered the zeta potential of the system, thereby decreasing the number of effective collisions between MC and microalgal cells. This process leads to the formation of a stable particle suspension in the water body and makes the particles resistant to removal by flocculation (Xiao et al., 2019). These findings indicate that the different flocculation behaviors of MC in the presence of various types of AOM are influenced primarily by the *M*/*E* of the AOM. When the *M*/*E* is high, AOM acts as a flocculation aid to increase the efficacy of microalgae removal by MC; conversely, when the *M*/*E* is low, AOM inhibits MC flocculation, thereby reducing the efficiency of microalgae removal.

4 CONCLUSION

In this study, we investigated the physicochemical properties of different types of microalgae-derived AOM and their interference with microalgae removal through MC flocculation as well as the underlying mechanisms. The results reveal that the concentrations of *P. donghaiense* and *A*. *carterae*-derived AOM were similar during incubation, but their electrical properties and molecular weights significantly differed (P<0.05). We found that the molecular weight to electric charge ratio (*M*/*E*) of AOM strongly influences the efficiency of microalgae removal by MC. A high *M*/*E* of AOM benefits the formation of bridges between MC particles and microalgal cells, which promotes the generation of large flocs and improves the microalgae removal efficiency. However, a low *M*/*E* of AOM decreases the zeta potential of MC, which increases interparticle electrostatic repulsion and decreases the flocculation ability of MC.

Although this study has derived some inspirational laws based on the laboratory conditions, their guidance for the practical application still needs to be investigated in the complex water body.

5 DATA AVAILABILITY STATEMENT

Data are available on request to authors.

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Electronic supplementary material

Supplementary material (Supplementary Tables S1–S3 and Figs.S1–S3) is available in the online version of this article at https://doi.org/10.1007/s00343-025-5102-4.