

Relationship between flocculant dosage and efficiency: a case study on HAB mitigation using aluminum sulfate-modified clay*

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Received Sep. 5, 2025; accepted in principle Nov. 5, 2025; accepted for publication Dec. 4, 2025

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Abstract Flocculation is a common method for pollution treatment, and widely applied in various types of water pollution treatment due to its convenience and effectiveness. The dosage of flocculants is the key factor determining flocculation efficiency. There is a need to establish the relationship between the dosage of flocculant and the removal efficiency of pollutants (dosage-efficiency relationship), and the empirical method was deemed the most feasible way to address this problem. We successfully constructed the dosage-efficiency relationship among the dosage of modified clay, the cell density of red tide organisms, and algal removal efficiency, and assessed the empirical calculation equation of algal removal efficiency. The difference between the measured value and the theoretical value of the algal removal efficiency calculated by the equation was below 15%, the standard deviation was below 20%, and the empirical equation derivation method was proved suitable for various red tide organisms and various modified clays. By exploring the dosage-efficiency relationship between the dosage of aluminum sulfate-modified clay, the cell density of algae and algal removal efficiency, we provided data and theoretical support for the construction of a quantitative evaluation method for flocculation efficiency.

Keyword: flocculation; dosage-efficiency relationship; empirical model; modified clay; harmful algae bloom

1 INTRODUCTION

Flocculation is one of the most commonly used methods for water pollution control, and has been widely used in the pollution control of municipal sewage, industrial wastewater, and natural water in lakes and oceans, and can eliminate water pollutants such as microorganisms, organic matter, and inorganic particles effectively (Jarvis et al., 2005a; Verma et al., 2012; Milledge and Heaven, 2013; Lee et al., 2014; Gallardo-Rodríguez et al., 2019). Flocculation is a complex physicochemical process in which particles collide and combine, whose pollution control effect is affected by the type and dosage of

flocculants, the type and concentration of pollutants, and the environmental conditions of water bodies (Zhong et al., 2011; Cui et al., 2014; Potočár et al., 2020; Chen et al., 2021; Ghazali and Adachi, 2021; Metaxas et al., 2021). The complexity of influencing factors makes the flocculation effect difficult to quantify. As a result, when using the flocculation method to treat water pollution, the dosage of

* Supported by the Intergovernmental Innovation Cooperation Project of the Ministry of Science and Technology (No. 2022YFE0136400), the National Key Research and Development Program of China (No. 2022YFC3105204), and the National Natural Science Foundation of China (No. 42306230)

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flocculant used is often estimated by experience and experiments (Ahmad et al., 2008; Zahrim et al., 2011; Huang et al., 2016, 2020; Liu et al., 2016a, b), but will inevitably lead to waste of flocculant or unsatisfactory effects of the flocculation, which will bring greater losses, especially in the treatment of large-scale water pollution (Anderson, 2009; Yu et al., 2017). Red tides are a large-scale ecological phenomenon, which can cause great ecological and economic losses and impact areas reaching tens or even hundreds of square kilometers (Anderson, 1997; Anderson et al., 2012). At present, the modified clay method (MC) is one of the most promising red tide control methods, and has been widely used in the control of red tide in the coastal waters of China (Yu et al., 2017; Zhang et al., 2020; Song et al., 2021). This paper invokes the flocculation of modified clay on red tide organisms as a case study to explore the quantitative relationship between flocculant dosage and flocculation efficiency.

Flocculation is a complex process between particles, and the existing theoretical models of flocculation can only evaluate the strength of flocculation qualitatively (Jarvis et al., 2005a, b; Jiang, 2015). In the field of adsorption, the theoretical models such as Langmuir and Freundlich can be used to evaluate the adsorption capacity of materials to pollutants quantitatively (Proctor and Toro-Vazquez, 1996; Dynarowicz-Łatka et al., 2001), and then estimate the amount of adsorbent required based on the number of pollutants present (Schöene et al., 2017; Kalam et al., 2021). To study the change of a complex process such as flocculation, adopting empirical methods may make it easier to grasp the core of its operational rules. The Freundlich isotherm adsorption model was developed to create an empirical equation model, and then gradually study its theoretical basis. The empirical method makes it feasible to solve the problem of flocculant dosage estimation by studying the dosage-efficiency relationship between flocculant dosage and flocculation efficiency based on empirical methods.

This study is the first attempt to employ an empirical approach to construct a dose-effect relationship model between flocculant dosage and flocculation efficiency for establishing a quantitative evaluation methodology. Using modified clay as the research subject, with red-tide-inducing organism density (as pollutant) and modified clay concentration (as flocculant) serving as the main variables, experiments were conducted on two dominant algal

species commonly found in aquaculture waters, *Nannochloris* and *Heterosigma akashiwo*, to investigate algae removal. The dose-efficiency relationship between modified clay dosage and algal removal efficiency was successfully established. The development of this empirical evaluation method and the corresponding relationship not only enriches the flocculation theory and methodology but also provides more scientific and precise dosage guidance for the application of modified clay in aquaculture environments.

2 MATERIAL AND METHOD

2.1 Material

2.1.1 Modified clay

The clay material for preparing the MC in this study was kaolin purchased from Guangxi Yankuang Beihai Kaolin Co., Ltd. The zeta potential of kaolin in seawater was measured using a zeta potential meter (Zetasizer nano ZS, Malvern, UK), $\zeta_{\text{kaolin}} = -14.31 \pm 1.05$ mV. The particle size distribution of the original clay soil was measured by a laser particle size analyzer (Mastersizer A3000, Malvern, UK) as shown in Supplementary Fig.S1. Aluminum sulfate was purchased from Sinopharm Group (Shanghai, China). The clay required surface modification before use. For this study, aluminum sulfate-modified clay (denoted as MC II) was selected as the experimental material due to its prevalent application in aquaculture waters. It was prepared by thoroughly mixing kaolin with aluminum sulfate at the optimal empirical ratio of 5:1.879, which offers the best cost-performance balance (Liu et al., 2016a). The mixture was then added to natural seawater to form a homogeneous suspension in concentration of 25 g/L, which was used as the stock solution to ensure consistent dosage and minimize the weighing errors throughout the experiments.

2.1.2 Red tide organism

In the experiment, two species of algae were selected, and their cell densities during the mid-to-late exponential growth phase were used as the experimental conditions. *Nannochloris* is a common prey species in coastal aquaculture waters (Witt et al., 1981), with cells density of 10^6 – 10^7 cells/mL, the dominant algae in the water sample collected from shrimp culture waters in Dongying City, Shandong Province (Ding et al., 2021). The other one was *H. akashiwo*, a widespread harmful algal bloom species in global coastal areas, known for its

significant ecological hazards (Mardones et al., 2023). Its cell density ranged 1×10^5 – 2×10^5 cells/mL, from the algal collection of the Key Laboratory of Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences. The algae were cultured in L1 medium at 20 ± 1 °C, $65 \mu\text{mol photon}/(\text{m}^2 \cdot \text{s})$, and the light-to-dark ratio L:D=12 h:12 h (Guillard and Hargraves, 1993). The seawater was collected from Jiaozhou Bay, Qingdao and filtered through $0.7\text{-}\mu\text{m}$ GF/F membrane filters.

2.2 Method

2.2.1 Algal removal experiment

Algae-rich water samples were added into 100-mL colorimetric tubes, and 0.001–0.2-g modified clay was added to the experimental group to produce the requisite concentrations of modified clay (0.01–2 g/L), while the control group received no modified clay. The colorimetric tube was left to stand, and the upper water sample was taken after 3 h. To ensure the accuracy and reproducibility of the experimental results, all tests were conducted in triplicate. For data fitting analysis, the average value of the algal removal rate was used as the input for the fitting calculation. The turbidity and fluorescence values of the water samples were measured using a scattering light turbidimeter (WZS-200, Anting, Shanghai) and in vivo fluorometer (Trilogy, Turner design, US), respectively. At the same time, 2-mL water samples were taken from each experimental group and treated with Lugol's for cell counting.

A microscope (CX33, Olympus, Japan) was used to count cells through a blood cell counting plate. According to the results of cell count and in vivo fluorescence value, the algal cell removal efficiency (E_f) was calculated using the following equation:

$$E_f(\%) = \left[1 - \frac{\text{cell density (in vivo fluorescence value) of experimental group}}{\text{cell density (in vivo fluorescence value) of control group}} \right] \times 100\%. \quad (1)$$

2.2.2 Measurement and calculation of particle concentration of modified clay

The MC was prepared in suspensions of 0.01, 0.02, 0.03, 0.05 g/L with deionized (DI) water, and added to $20 \times 50 \times 50 \text{ mm}^3$ quartz dishes. Particle image velocimetry (PIV) was used to record the flocculation process; the volume of the shooting

area was $1.2 \times 1.2 \times 2 \text{ mm}^3$, the shooting frequency was 5 Hz, and each recording time was 10 s. A total of 50 images were taken. Davis8.40 software was used to process the images, and the total number of particles in the 50 images calculated. Samples of each concentration were measured three times, and the relationship curve between modified clay concentration and particle concentration was drawn according to the measurement results (Fig.1). The relationship between the concentration of modified clay and the particle concentration was calculated as:

$$C_p = 4.80 \times 10^9 C_{MC} + 1.38 \times 10^6, \quad (2)$$

where C_p is the concentration of modified clay particles in water, inds./L; C_{MC} is the mass concentration of modified clay, g/L.

3 RESULT AND DISCUSSION

3.1 Change law of algal removal efficiency of MC

3.1.1 Effect of MC concentration and red tide biological density on algal removal efficiency of MC

As reported in previous studies on red tide control, cell density of red tide organisms and the dosage of MC are the key variables affecting the algal removal efficiency (Liu et al., 2016a; Qiu et al., 2020; Zhang et al., 2021). In this study, we focused on these two factors and adopted the method of variables controlling to explore the algae removal by MC. The variation rules of the efficiency, and the dosage-efficiency relationship among the dosage of MC, the density of red tide organisms, and the algae removal efficiency were established. When the MC concentration was kept at 0.5 g/L, as the algal cell density increased from 4.05×10^5 to

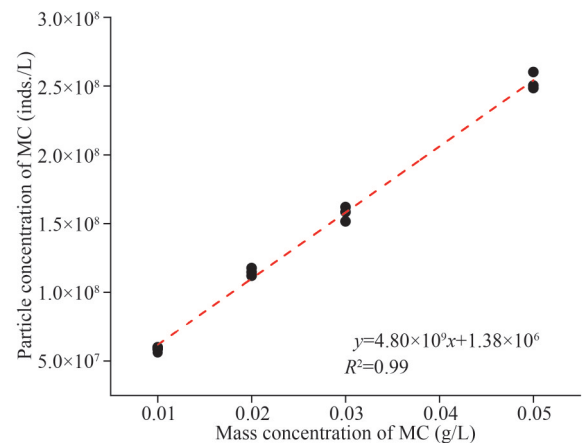


Fig.1 Relationship curve of concentration vs. particle concentration of modified clay

7.23×10^6 cells/mL, the algal removal efficiency of MCII showed a decreasing trend from 70% to below 50%. At same time, the removal number of algal cells by MCII increased significantly from 2.73×10^5 to 3.85×10^6 cells/mL (Fig.2a). Therefore, within the experimental gradient, the removal number of algal cells increased approximately linearly with increasing algal density. The algal cell density had a greater impact on the number of algae removed than the algal removal efficiency of the MC.

Using the amount of pollutant removed per unit dosage of flocculants or adsorbents is helpful for a more comprehensive assessment of their pollution control capacity (Madarász et al., 2014; Matter et al., 2019). We used the number of cells removed per modified clay particle (NRPC) to characterize the algal removal capacity of modified clay under different systems and were calculated as Eq.3. Within the experimental gradient, there was a positive correlation between NRPC and algal cell density ($R^2=0.99$), which indicated that when the density of MC particles was enough to possess sufficient flocculation ability, the higher the cell density of algae, the more algae removed per unit of MC particles.

$$\text{NRPC} = \frac{\text{cells removal amount}}{\text{modified clay particles density } (C_p)} \quad (3)$$

Under the condition of constant algal cell density, the removal efficiency and ratio of modified clay to algal cells removed increased with the increase of MC concentration. When the concentration of MC increased from 0.05 to 2 g/L, the removal efficiency increased from 14% to 83%, and the removal amount also increased, from 1.07×10^6 to 6.49×10^6 cells/mL. When the amount of modified clay was below 0.05 g/L, the removal efficiency of MC to algal cells was 0 or close to 0 (Fig.2c–d). The NRPC decreased with increased MC concentration, from 4.42 to 0.68 cells/ind., the power function equation was used to create Eq.4, and $R^2=0.85$. Within a certain range of algal density, as concentration of MC increased, the algal removal efficiency also increased, but the algal removal ability of single MC particles decreased.

3.1.2 Functional relationship between NRPC, algal density, and MC concentration

Results presented here showed that under the

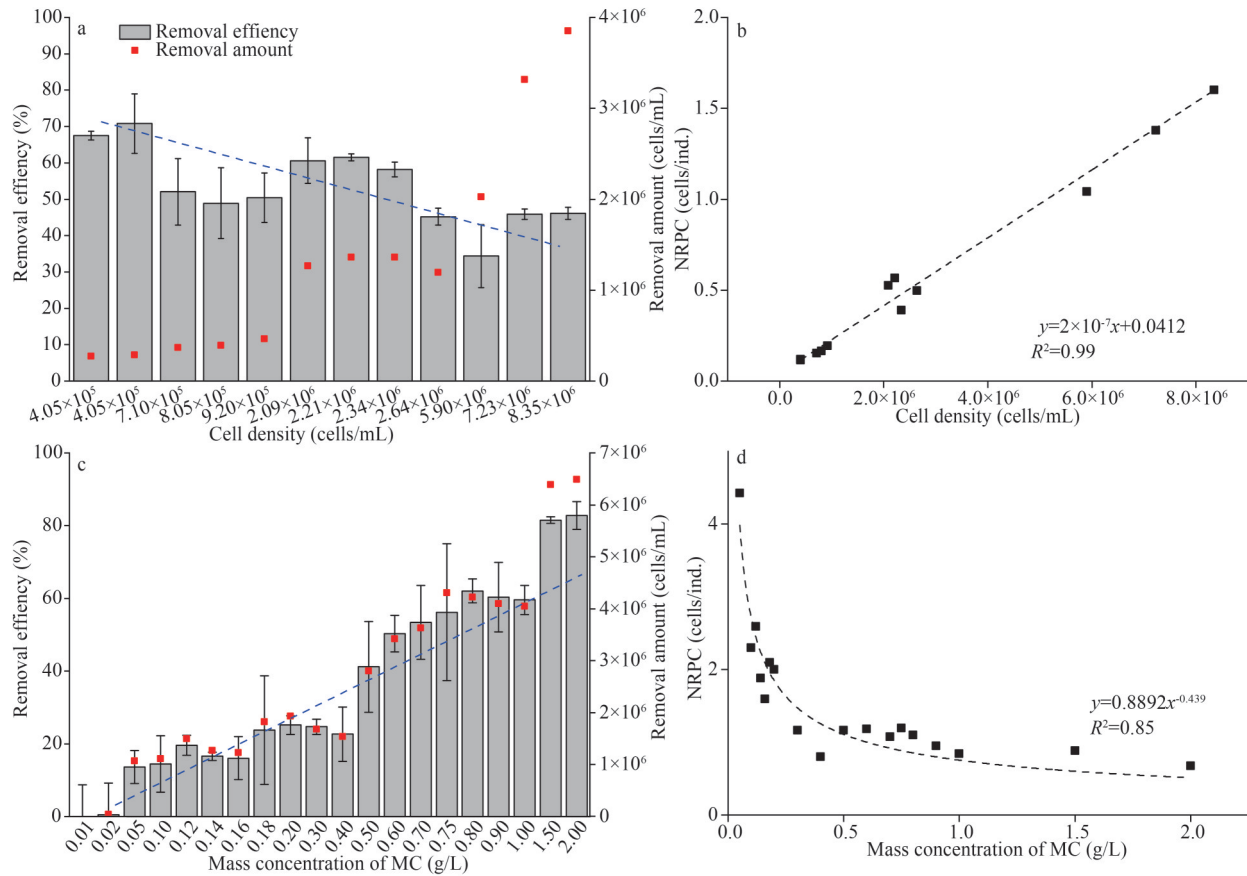


Fig.2 Effects of modified clay concentration and algal cell density on the removal efficiency of *Nannochloris*

conditions of 6.8×10^6 cells/mL (algal cells density) and 0.5 g/L (MC concentration), there was a good correlation between NRPC and the concentration of MC and the cells density of algae. To further explore the relationship between these three factors, relationship between the concentration of MC and NRPC at different algal densities was studied.

The relationship between the concentration of MC and NRPC was analyzed at algal densities of 2.06×10^6 , 3.44×10^6 , and 7.84×10^6 cells/mL (Fig.3a–c). Results show that there was a good power function relationship between the concentration of MC and NRPC under different algal densities ($\text{NRPC} = a \times C_{\text{MC}}^b$, $R^2 > 0.9$), and the parameters a and b in the relationship were different. With increases of cell density, parameter a gradually increased from 0.275 6 to 1.117 9, while parameter b fluctuated from -0.372 to -0.501. The change of parameter a indicates that under the same concentration of MC, the higher the algal cell density, the higher the NRPC, which is consistent with the result presented in Section 3.1.1, and further indicates that there is a linear relationship between cell density and NRPC. Therefore, the

change law of the algal removal ability of MC under different algal densities can be calculated by power function, which is helpful when dealing with the fact that the dosage of MC is difficult to estimate due to the great change of cell densities of red tides.

Furthermore, we analyzed the relationship between algae density and NRPC under different clay dosages and obtained the corresponding fitting equations (Fig.4). Under different concentrations of MC, there was a good linear relationship between algal cell density and NRPC ($\text{NRPC} = c \times C_{\text{algae}} + d$; $R^2 > 0.95$). With the increased dosage of MC, parameter c showed a decreasing trend and parameter d showed an increasing trend, indicating that the lower the dosage of MC, the greater the influence of cell density on NRPC.

Based on the fitting formulas (Eqs.4 & 5) we derived from Fig.2b & d, we used Eq.6 to derive the theoretical relationship between the concentration of MC and NRPC under different algal cell densities. The results are shown in Fig.3d, and the fitted curves of the theoretical equation are consistent with the fitted curves of the measured results. Although

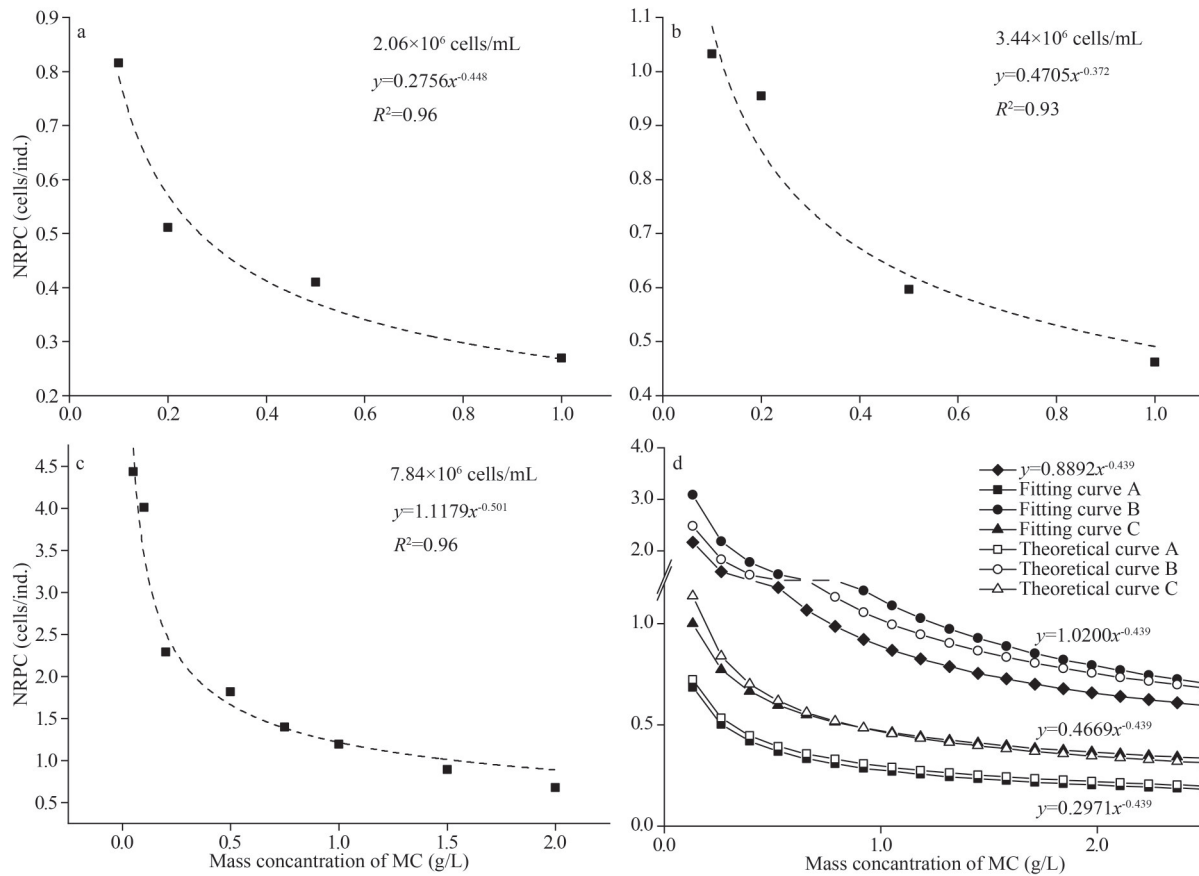


Fig.3 Theoretical and fitted power function between MC concentration and NRPC under different cell density

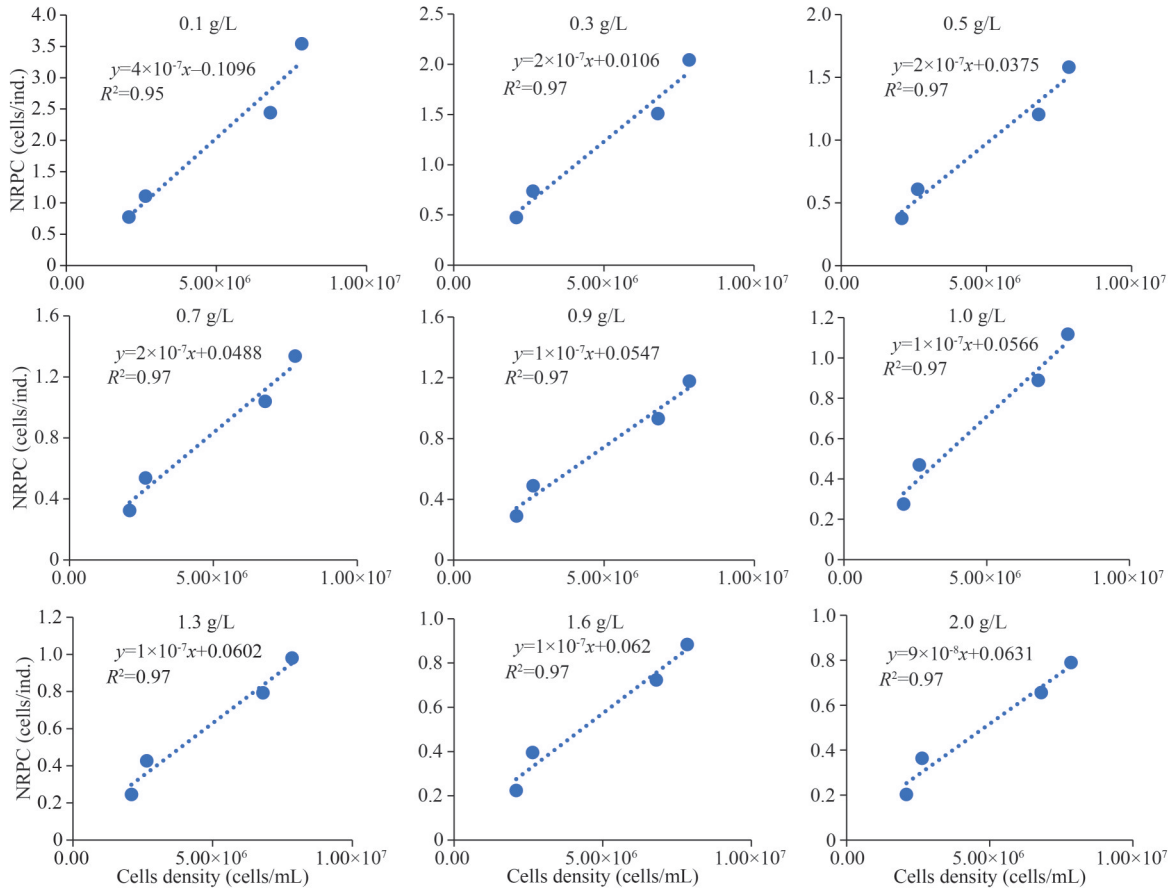


Fig.4 Linear function between cells density and NRPC under different concentration of MC

only parameter a changed, the value of parameter b is -0.439, which is slightly different from the parameters measured, the two parameters calculated by this method are close to the parameters measured, i.e., the theoretical curve is consistent with the measured curve. Therefore, this method can be used to calculate NRPC values under different algal cell densities and MC dosage.

$$y = 0.8892 \times x^{-0.439}, \quad (4)$$

$$y = 2 \times 10^{-7}x + 0.0412. \quad (5)$$

Therefore, under different algal cell densities and different concentrations of MC, NRPC has a good linear relationship with algal cell density, and a good power-function relationship with the concentration of MC. Thus, the functional relationship among algal cell density, MC concentration, and NRPC can be described by the following equations:

$$\frac{\text{NRPC}}{0.8892 \times C_{\text{MC}}^{-0.439}} = \frac{2 \times 10^{-7} \times C_{\text{algae}} + 0.0412}{2 \times 10^{-7} \times 6.8 \times 10^6 + 0.0412}, \quad (6)$$

where, C_{MC} represents the mass concentration of MC (g/L); C_{algae} represents algal cells density (cells/mL).

3.2 Construction and verification of the empirical equation for algal removal efficiency of MC

3.2.1 Construction method of algal removal efficiency equation

NRPC represents the amount of algal removal per MC particle, and the algal removal amount of all MC can be obtained by multiplying it by the particle concentration of MC (C_p), and the algal removal efficiency of MC can be obtained by dividing it by the algal cell density. Thus, the algal removal efficiency calculation Eq.7 is obtained. In the results above, it is shown that NRPC has a definable functional relationship with the concentration of MC (C_{MC}) and algal cell density (C_{algae}), which can be expressed as Eqs.8 & 9, respectively. Based on these functional relationships, Eqs.8 & 9 can be combined to obtain the amounts of NRPC under different algal densities and dosages of MC. The relationship between NRPC, C_{MC} , and C_{algae} was described as Eq.10, and then Eq.11 was obtained by substituting Eq.10 into Eq.7 to calculate the algal removal efficiency of MC with C_{MC} and C_{algae} as variables.

Based on Eq.11, the algal removal efficiency can

be calculated when using a specific amount of MC to treat red tide with known cells density. Conversely, the dosage of MC needed to achieve the target algal removal efficiency for red tide with known cells density can also be calculated. The parameters a – d in the Eqs.8–12 were determined according to the method in Section 3.1 and C_p as in Eq.2. After all parameters and expressions are substituted into the equation, only C_{MC} and C_{algae} are variables in the Eqs.8–12. Based on the data in Section 3.1, Eq.12 for the removal efficiency of *Nannochloris* by MC was constructed. The value range of C_{MC} was 0.01–2 g/L, and the value range of C_{algae} was 4×10^5 – 8×10^6 cells/mL. The value of C_{MC} covers the requirements of conventional application scenarios, and the value of C_{algae} covers the algal density range of *Nannochloris* red tide.

$$E_f = \frac{NRPC \times C_p}{C_{algae}}, \quad (7)$$

$$NRPC_0 = a \times C_{MC}^b, \quad (8)$$

$$NRPC = c \times C_{algae} + d, \quad (9)$$

$$\begin{aligned} \frac{NRPC}{NRPC_0} &= \frac{c \times C_{algae} + d}{c \times C_0 + d} \xrightarrow{\text{substitute } NRPC_0} NPPC \\ &= \frac{c \times C_{algae} + d}{c \times C_0 + d} \times a \times C_{MC}^b, \end{aligned} \quad (10)$$

$$\begin{aligned} E_f &= \frac{NRPC \times C_p}{C_{algae}} \xrightarrow{\text{substitute } NRPC} E_f \\ &= \frac{\frac{c \times C_{algae} + d}{c \times C_0 + d} \times a \times C_{MC}^b \times C_p}{C_{algae}}, \end{aligned} \quad (11)$$

$$E_f = \left[\frac{2 \times 10^{-7} \times C_{algae} + 0.0412}{2 \times 10^{-7} \times C_0 + 0.0412} \times 0.8892 \times C_{MC}^{-0.439} \times (4.80 \times 10^6 \times C_{MC} + 1378.1) \right] / C_{algae}, \quad (12)$$

where, $NRPC_0$ is defined as the number of algal cells removed per unit mass of MC particles at a specific algal density, while $NRPC$ refers to this value under any condition (cells/ind.); C_0 represents the background algal density of $NRPC_0$, which was 6.8×10^6 cells/mL in this section; C_{MC} represents the mass concentration of MC (g/L); C_{algae} represents algal cells density (cells/mL).

3.2.2 Verification of removal efficiency equation of *Nannochloris*

To verify the applicability of Eq.12, we verified the Eq.12 based on 19 groups of experiments performed under different algal densities or MC concentrations (in different batches of experiments calculated using empirical equations). The algal cell density range of the verification experiment was 0.41×10^6 – 7.23×10^6 cells/mL, and the MC concentration range was 0.01–1 g/L. The algal cell density and MC concentration were substituted into the equation to calculate the theoretical values of $NRPC$ and removal efficiency, and the difference and standard deviation between them and the measured results were calculated.

The experimental data in Fig.5a & b (with further detailed data presented in Supplementary Table S1) show that the theoretical model yields predictions generally consistent with the measured values for

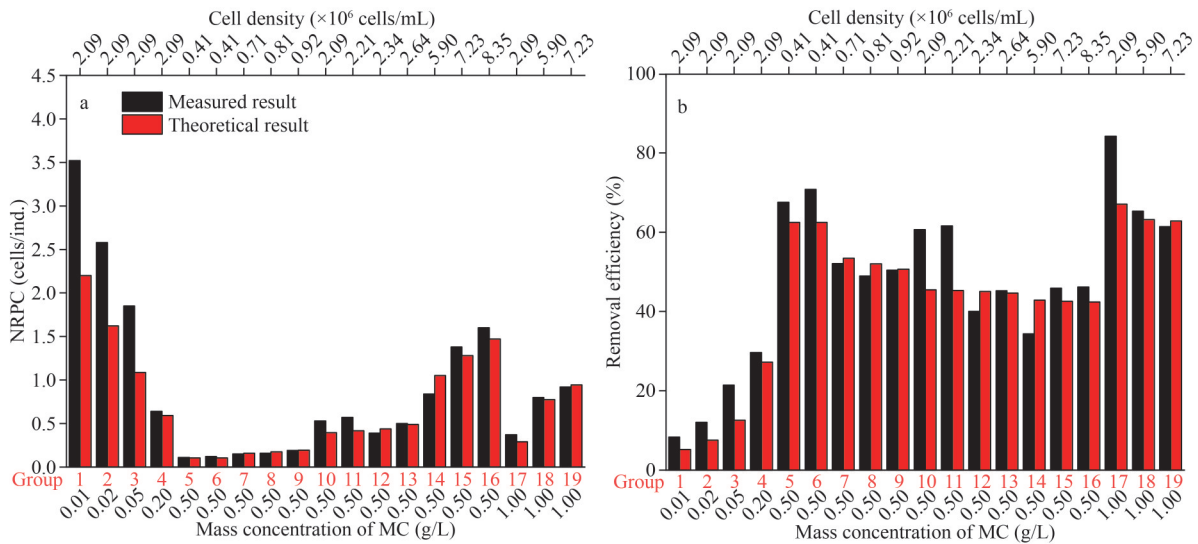


Fig.5 Comparison of theoretical and measured results of NRPC (a) and removal efficiency (b)

both NRPC and removal efficiency, with only specific groups exhibiting notable deviations. For NRPC, the most distinct deviation was observed in Groups 1–3 at low MC concentrations, where the theoretical values were substantially lower than the measured values with a maximum discrepancy of 1.32, while the consistency improved markedly with the increase of MC concentration: the discrepancy between theoretical and measured values was ≤ 0.05 in over 50% of the experimental groups (10 groups), < 0.20 in more than 70% of the groups (15 groups), and the standard deviation between the two values was also < 0.20 in over 80% of the groups (16 groups). For removal efficiency, the theoretical values were in good agreement with the measured results overall, with only Groups 11, 12, and 17 showing significant deviations (theoretical values notably lower than measured values, maximum discrepancy of 17.11%); the discrepancy was $< 10\%$ in more than 70% of the experimental groups (15 groups), $< 20\%$ across all groups, and the standard deviation between theoretical and measured values was $< 10\%$ in more than 80% of the groups (16 groups). Despite the minor predictive inaccuracy of the model within specific concentration ranges, the algae removal efficiency is required to be maintained at no less than 70% in practical application scenarios, and thus such partial inaccuracy has no adverse impact on the practical use of the proposed formula.

Therefore, the removal efficiency of *Nannochloris* by modified clay can be effectively estimated by using the empirical equations, as long as two of the three parameters are known, the value of the third term can be calculated, but the applicability and boundary conditions of the equation need to be further explored.

3.2.3 Verification of removal efficiency equation for *Heterosigma akashiwo*

To further investigate the application scope of the algal removal efficiency equation, we used *H. akashiwo*, a common red tide organism in offshore China, as the experimental organism to explore the variation rule of the removal efficiency of *H. akashiwo* under different algal cell densities and MC concentrations, and the removal efficiency equation was constructed.

Similar to *Nannochloris*, the removal efficiency of *H. akashiwo* increased with increasing MC concentration and decreasing algal cell density, but the trend of NRPC was slightly different. With the increase of cells density, NRPC showed a trend of

increasing at first and then tending to be flat. With the increase of MC concentration, NRPC showed a trend of first increasing and then decreasing (Fig.6a–b), indicating that the change of NRPC was not monotonic, there was a limit value, taking *H. akashiwo* as an example, the limit value of NRPC was around 0.25 cells/mL. However, as far as the current experimental results were concerned, in the conventional density of algal cells and concentration of MC, most of the NRPC showed monotonic changes, and the limit values of NRPC were in the range of low algal removal efficiency. The discussion here mainly focuses on the monotonic function relationship between algal cell density and MC concentration and NRPC, which was sufficient to estimate the algal removal efficiency of the MC accurately.

After excluding the data with algal removal efficiency lower than 20%, NRPC changed with the MC concentration and algal cells density as a power function and a linear function respectively ($R^2 > 0.85$), and the corresponding function equation were obtained (Fig.6c). According to the method described in Section 3.2.1, Eq.13 for the removal efficiency of *H. akashiwo* was constructed. In this study, 20 sets of experimental data were used to verify Eq.13. The algal cell density range of the verification experiment was 8.15×10^3 – 1.55×10^5 cells/mL, and the MC concentration range was 0.01–0.5 g/L. The theoretical value of NRPC obtained was relatively close to the measured value, and the difference was not higher than 0.03 (Supplementary Table S2). Further, the theoretical value of the removal efficiency was also relatively close to the measured value. In 60% of the experimental groups (12 groups), the difference between the theoretical and measured values of removal efficiency did not exceed 15%, and the standard deviation of the theoretical and measured values of 85% of the experimental groups (17 groups) did not exceed 10% (Fig.6d; Supplementary Table S3).

The construction method of the empirical equation for the algal removal efficiency of MC was suitable for *H. akashiwo*, and the constructed equation can be used to estimate the algal removal efficiency.

$$E_t = \left[\frac{6.6 \times 10^{-7} \times C_{\text{algae}} + 0.00334}{6.6 \times 10^{-7} \times 1.08 \times 10^5 + 0.00334} \times 0.0237 \times C_{\text{MC}}^{-0.567} \times (4.80 \times 10^6 \times C_{\text{MC}} + 1378.1) \right] / C_{\text{algae}}, \quad (13)$$

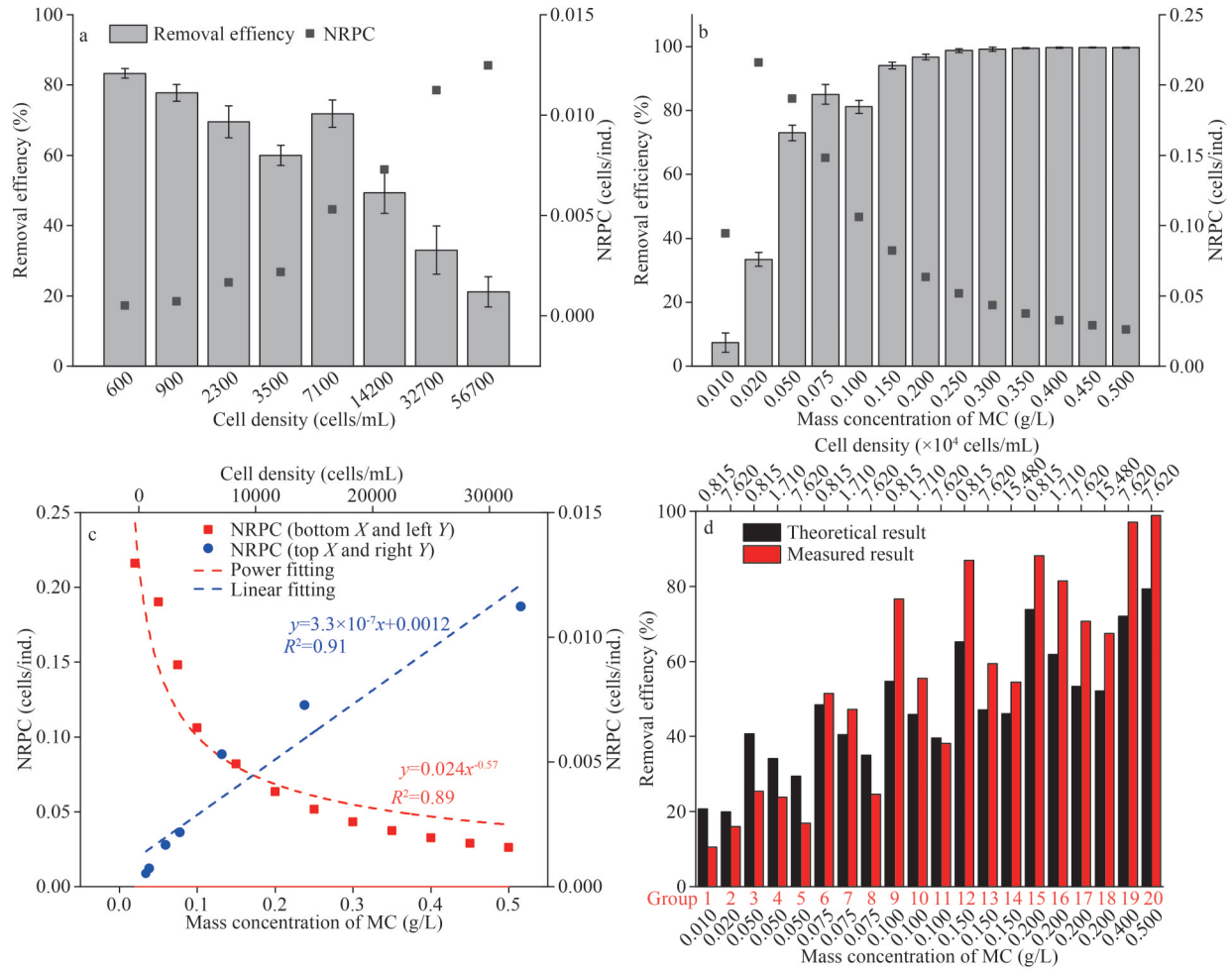


Fig.6 Effects of modified clay concentration and algal cell density on the removal efficiency of *Heterosigma akashiwo*

3.3 Applicability and potential theoretical basis of the empirical equation of algal removal efficiency

3.3.1 Application range of the empirical equation of algal removal efficiency

In this study, we performed regression analysis on 120 sets of data using the nonlinear fitting function in Origin 2021 software, and evaluated the accuracy of the fitting results through the value of R^2 . The main variables in these experiments were MC type, red tide species, and modifier dosage (Supplementary Tables S3–S4). Among them, the concentration of MC in 111 groups showed a power function relationship with NRPC (Eq.9), $R^2=99$ groups were >0.8 , $R^2=92$ group was >0.9 , and $R^2=80$ group was >0.95 . In the 9 groups that did not show a power function relationship, there were 1–4 data points with removal efficiency $<20\%$, which made NRPC change non-monotonically or irregularly with the increase of concentration of MC. In the 11 groups whose $R^2 < 0.8$, there was also a data point with

removal efficiency $<20\%$, which makes NRPC show non-monotonically change law.

In general, under the influence of three factors: MC type, red tide species, and modifier dosage, the NRPC of MC was described as a power function relationship with the increase of MC concentration, indicating that Eq.11 proposed in this study is suitable for application to a variety of MC to treat different red tide organisms. Equation 11 is currently constructed based on removal efficiency greater than 20%, and thus applicable to the prediction of removal efficiency greater than 20%. In addition, we aimed to explore the quantitative variation laws of algal removal efficiency of MC, and the selected six modified clays mainly rely on flocculation to control red tide. As a result, the empirical equation method proposed would also be applicable to such MC.

3.3.2 The potential theoretical basis of empirical equation

The results presented here demonstrate interesting quantitative relationships in the flocculation process

which can be used to calculate the flocculation efficiency. An understanding of the mechanism behind these relationships will help to improve understanding of the flocculation process.

It is well known that MC mainly relies on flocculation to control red tide (Liu et al., 2016b; Yu et al., 2017; Qiu et al., 2020), but MC particles will not only flocculate with algal cells, but also collide and combine with other MC particles to cause self-flocculation (Jiang et al., 2021). The NRPC decreased as the concentration of MC increased or the cells density of algae decreased, which may be affected by the self-flocculation of MC particles. For example, when the concentration of MC was low (0.05 g/L), there were few MC particles in the water (Fig.1), and the degree of self-flocculation was low. Thus, it mainly collided with algal cells, and a single MC particle can remove more algae cells, so NRPC was as high as 4.43 cells/ind. With high concentration of MC (2 g/L), the NRPC dropped to 0.68 cells/ind., indicating the degree of self-flocculation of the clay was high. A high NRPC indicates that the MC mainly flocculates with algal cells, while a low NRPC indicates that the MC mainly self-flocculates. The self-flocculation of MC may be the main reason for the regular change of its algal removal efficiency and effective utilization rate. Therefore, we will use the particle collision theoretical model to discuss the potential theoretical mechanism for the algae removal efficiency empirical equation of MC.

In this study, the algal removal and flocculation process of MC was studied with the help of the models of traditional flocculation theory. Smoluchowski (1917) proposed a particle collision rate function and a particle collision frequency function (Eq.14) based on six assumptions. (1) The fluid is a laminar shear motion. (2) The particles are monodispersed. (3) The flocs will not break up after they are formed. (4) All particles are solid spheres before and after the collision. (5) The collision occurs only between two particles. (6) All the particles will combine after the collision, that is, the collision efficiency $\alpha=1$. In this study, the flocculation efficiency of MC particles and algal cells was quite different from the self-flocculation efficiency of MC particles, so the assumption (6) is no longer applicable. In addition, we mainly compared the collision efficiency between the various objects in the system at the initial moment of the MC flocculation process of red tide biological process. Therefore, the flocculation process is simplified and it was considered that only one binding occurred between particles (no further

binding occurs after particle binding). This adds a new assumption to replace the previous assumption (6): the binding between particles occurs only once. Finally, the collision frequency function β in Eq.15 and the particle collision rate equation dn/dt in Eq.16 were obtained. The flocculation efficiency f is equal to the product of the particle collision efficiency and the collision rate, and the inter-particle flocculation efficiency equation (Eq.17) was obtained accordingly. As the particle concentration increases, the flocculation efficiency increases. For the self-flocculation process of MC, the increase of MC concentration will have an exponential effect on the self-flocculation efficiency f_2 (proportional to n_c^2); for the algae removal process of MC, refer to algae flocculation efficiency f_1 , increasing the concentration of MC has a linear effect (proportional to n_c). Therefore, the increased concentration of MC has a greater influence on self-flocculation.

$$\frac{dn_k}{dt} = \frac{1}{2} \sum_{i+j=k} \beta(i,j) n_i n_j - \sum_{i=1}^{\infty} \beta(i,k) n_i n_k, \quad (14)$$

$$\beta = \frac{1}{6} \times \frac{du}{dy} \times (d_i + d_j)^3, \quad (15)$$

$$\frac{dn}{dt} = \frac{1}{2} \beta(i,j) n_i n_j, \quad (16)$$

$$f = \alpha \times \frac{dn}{dt} = \frac{1}{2} \alpha \beta(i,j) n_i n_j, \quad (17)$$

$$f_1 = \frac{1}{2} \alpha_1 \beta(c, a) n_c n_a, \quad (18)$$

$$f_2 = \frac{1}{2} \alpha_2 \beta(c, c) n_c^2, \quad (19)$$

where, i , j , and k represent the particle size gradation; n_i , n_j , and n_k represent the concentration of particles i , j , and k (inds./mL); dn/dt and β represent collision and collision frequency, respectively; du/dy represents the velocity gradient of the fluid (/s); d_i and d_j represent the particle diameters of particles i and j (cm); α denotes the collision efficiency and β denotes the collision rate between particles; α_1 and $\beta(c, a)$ represent the collision efficiency and collision rate between modified clay particles and algal cells, respectively, while α_2 and $\beta(c, c)$ represent the collision efficiency and collision rate among modified clay particles, respectively; n_c and n_a represent the number concentration of MC and algal cells (inds./mL, cells/mL) respectively; t is the collision time.

Based on the self-flocculation efficiency (f_1) and algal removal efficiency (f_2) of modified clay, the proportion of collision binding frequencies between modified clay and algal cells in the collision binding frequencies of the whole system could be analyzed, and the dominant process in the algal removal system can be identified. Assuming that there are only two types of particles in the experimental system at the initial moment, MC particles and algal cells, there are only three collision processes in the system, namely algae-algae, algae-MC, and MC-MC. The algae are difficult to combine with each other after collision, so the collision process of algae and algae is neglected. It can be seen that the number of algae-MC collisions ($f_1 \times t$) divided by the sum of MC-MC collisions, and algae-MC collisions ($f_1 \times t + f_2 \times t$) can obtain the proportion of algae removal flocculation process in the total flocculation process (η) (Eq.20). As the definition, η should have a positive correlation with NRPC.

$$\begin{aligned} \eta &= \frac{f_1 \times t}{f_1 \times t + f_2 \times t} = \frac{\frac{1}{2} \alpha_1 \beta(c, a) n_c n_a}{\frac{1}{2} \alpha_1 \beta(c, a) n_c n_a + \frac{1}{2} \alpha_2 \beta(c, c) n_c^2} \\ &= \frac{\alpha_1 \beta(c, a)}{\alpha_1 \beta(c, a) + \alpha_2 \beta(c, c) \frac{n_c}{n_a}}. \end{aligned} \quad (20)$$

Both α_1 and α_2 are collision efficiencies, which are mainly related to the particle properties of the MC and algal cells, and are regarded as fixed values here. $\beta(c, a)$ and $\beta(c, c)$ are related to du/dy and particle size; in the same experimental system, du/dy

is regarded as a constant value and can be approximated in the equation. The particle size of cells is also constant, so $\beta(c, a)$ and $\beta(c, c)$ can also be considered constants. Therefore, η in Eq.20 is only affected by two variables, n_c and n_a . When the algal cell density n_a is constant, the higher the MC concentration n_c , the lower the proportion η of the algae removal and flocculation process in the total flocculation process, the unit The algae removal capacity (NRPC) of the MC was lower, which was consistent with the previous findings (Sections 3.1 and 3.2).

According to the theoretical model Eq.20, there is a proportional relationship between the ratio of algal cell density to MC concentration (initial diatomite particle ratio, n_a/n_c) and η . Thus, according to the model, there is a positive correlation between the initial n_a/n_c and NRPC, under the premise of that the higher the η , the higher the NRPC. However, the horizontal axes in Fig.2b and d were converted into the initial n_a/n_c as shown in Fig.7. It is clear that the initial n_a/n_c was positively correlated with NRPC, which indicated that the conclusions obtained from the experimental results and the results obtained from the theoretical analysis were consistent. In fact, the initial n_a/n_c reflects the relative dosage of MC, and the lower the initial n_a/n_c , the higher the relative dosage of MC. The change of this parameter directly affects the self-flocculation efficiency of MC and the flocculation efficiency on algal cells, resulting in quantifiable and regular changes in algae removal efficiency. The empirical equation

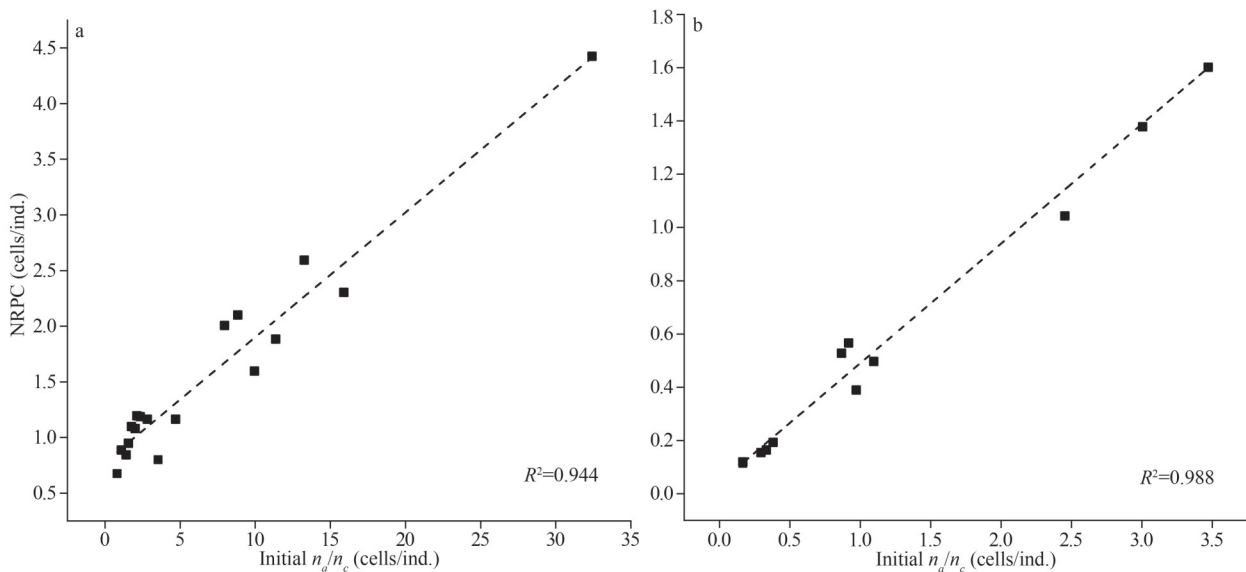


Fig.7 Correlation between initial n_a/n_c and NRPC

method of MC removal efficiency proposed in this study quantifies the relationship of MC concentration vs. algal cell density and algae removal efficiency. The accurate estimation of the algae removal efficiency of the MC provides an important reference for the estimated dosage of the MC in practical applications.

4 CONCLUSION

In this study, by systematically investigating the effects of modified clay concentration and red tide organism density on algae removal efficiency, we clarified the dose-effect relationships between these two factors and flocculation-based algae removal efficiency, and on this basis, constructed the corresponding mathematical models. For *Nannochloris* and *Heterosigma akashiwo*, empirical calculation formulas for removal efficiency based on key parameters were further established. Validation results indicated that the deviations between the theoretical values calculated by the formulas and the measured values were mostly less than 15%, with all standard deviations not exceeding 20%, which demonstrates the high predictive accuracy and reliability of the models. The construction method of the empirical formulas exhibits excellent applicability and scalability, and can be extended to other systems for red tide organism control using modified clay, thereby providing a theoretical basis and technical support for the precision dosing of modified clay and efficiency evaluation in red tide mitigation.

5 DATA AVAILABILITY STATEMENT

The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files.

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Electronic supplementary material

Supplementary material (Supplementary Tables S1–S4 and Fig.S1) is available in the online version of this article at <https://doi.org/10.1007/s00343-026-5303-5>.