CHEMICAL ENGINEERING TRANSACTIONS
VOL. 88, 2021
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Petar S. Varbanov, Yee Van Fan, Jiří J. Klemeš

ISBN 978-88-95608-86-0; **ISSN** 2283-9216
DOI: 10.3303/CET2188173

Quercetin Enhanced Cellulose Nanocrystals for the Removal of Harmful Algae *Phaeocystis Globosa*

Qing Liu[a], Mingda Che [a], Renliang Huang[b], Guojie Ding[c], Wei Qi[a], Zhimin He[a], Rongxin Su[a,b,*]

[a]State Key Laboratory of Chemical Engineering, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, P.R. China
[b]School of Marine Science and Technology, Tianjin University, Tianjin 300072, P.R. China
[c]Tianjin Rumi Novel Materials Co. Ltd, Tianjin 300350, P.R. China
surx@tju.edu.cn

The *Phaeocystis globosa* widely exists in the seawater, which becomes a great threat for the health of humans and animals in the ecological ecosystem. In this work, we reported a promising control strategy with quercetin(QT) enhanced cellulose nanocrystals (CNC). The quercetin enhanced cellulose nanocrystals (QT/CNC) was characterized through Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectrometer (FTIR) and X-ray diffractometer (XRD). The effects of concentrations of CNC and QT on controlled *Phaeocystis globosa* were investigated. The results showed that the removal rate of *Phaeocystis globosa* exhibited an optimal value (92.1 %) at 0.05 g L^{-1} CNC and 0.1 g L^{-1} QT. Furthermore, the chlorophyll-*a* content in the *Phaeocystis globosa* exhibited a remarkable decrease from 3.72 mg L^{-1} to 0.21 mg L^{-1} when QT/CNC was interacted. On the basis of these results, we concluded that *Phaeocystis globosa* could be removed by QT/CNC effectively. During the removal of *Phaeocystis globosa*, the pH of algal solution decreased from 9.45 to 7.36, indicating that QT/CNC could maintain the normal pH of water. In addition, the testing result of the QT/CNC zeta potentials was negative, which revealed that *Phaeocystis globosa* was removed through inhibition and flocculation. Accordingly, a possible removal mechanism of *Phaeocystis globose* by QT/CNC was presumed. This study suggested that QT/CNC might be a potential treatment materials on the removal of *Phaeocystis globosa*.

1. Introduction

Phaeocystis globosa is one of the harmful algal blooms (HABs), the outbreak of *Phaeocystis globosa* will bring huge losses to the local fishery resources. Even worse, Phaeocystis blooms threaten the safety of nuclear power stations because large colloidal colonies block the filters of nuclear power plants (Qiu et al., 2020), which has led to heightened scientific and regulatory attention. Therefore, it is essential to build efficient and feasible prevention mechanisms and removal methods (Heisler et al., 2008).

In recent years, many methods have been studied on controlling HABs, such as oxidation(Chen et al., 2017), photocatalysis (He et al., 2021), modified clay method (Lu et al., 2015) and so on. These methods can reduce the damage of HABs to a certain extent, but these applications are hindered due to almost all marine life can be oxidized by oxidants, the clay is not renewable and the photocatalytic method is expensive. Recently, cellulose nanocrystal (CNC) has attracted much attention because of its adsorption and flocculation for HABs. Cellulose nanocrystals (CNC) are mainly obtained from abundant natural cellulose fibers, which are biodegradable, renewable and environmentally friendly in nature. However, CNC can't flocculate HABs with strong mobility. In this respect, pyridine and 1-methylimidazol were used to modify CNC for the removal of HABs (Verfaillie et al., 2020). Nevertheless, the flocculation activity of CNC is still suppressed by the red tide algae. Quercetin (QT) is a kind of low-cost, non-toxic and biodegradable natural flavonoids. Previous work proved that QT could interfere with the physiology and inhibit the activity of *Microcystis aeruginosa* (Mecina et al., 2019). Therefore, we speculated that QT can be ued to promote the removal of *Phaeocystis globosa by* CNC. The

Paper Received: 4 May 2021; Revised: 27 July 2021; Accepted: 1 October 2021
Please cite this article as: Liu Q., Che M., Huang R., Ding G., Qi W., He Z., Su R., 2021, Quercetin Enhanced Cellulose Nanocrystals for the Removal of Harmful Algae Phaeocystis Globosa, Chemical Engineering Transactions, 88, 1039-1044 DOI:10.3303/CET2188173

research objective of this paper is to remove *Phaeocystis globosa* using QT/CNC, which can achieve efficient treatment of *Phaeocystis globosa*, and its products will not pollute the marine ecological environment.
In this study, the QT enhanced CNC (QT/CNC) was fabricated and then used as a algicide for the removal of *Phaeocystis globosa*. The QT/CNC was characterized through Atomic Force Microscopy (AFM), Fourier-transform infrared (FTIR) and X-ray diffractometer (XRD). The fluorescence, chlorophyll-*a* (chl-a), turbidity and pH value were tested for evaluating the removal of *Phaeocystis globosa*. The effects of various reaction parameters, such as concentrations of CNC and QT on controlling *Phaeocystis globosa* were investigated. Furthermore, the zeta potentials of QT/CNC were determined and a possible removal mechanism of *Phaeocystis globosa* by QT/CNC was presumed.

2. Materials and methods

Quercetin enhanced cellulose nanocrystals (QT/CNC) are firstly prepared, and the algae removal experiments were then performed.

2.1 Reagents

Quercetin ($C_{15}H_{10}O_7$, AR),dimethyl sulfoxide (C_2H_6SO, AR) and sodium hydroxide (NaOH, AR) were supplied by Aladdin Reagent Company (Shanghai, China). Hydrochloric acid (HCl, AR), sulfuric acid (H_2SO_4, AR) were purchased from Jiangtian Chemical Technology (Tianjin, China). Medical absorbent cotton (Grade A) was purchased from Jiangxi Huazhong Textile Chemical Co., Ltd (Jiangxi, China). All chemicals were used without further purification. All water was purified by a water purification system (arium pro VF, Sartorius Germany).

2.2 The cultivation of *Phaeocystis globosa*

Phaeocystis globosa was bought from the Shanghai Guangyu Biotechnology Co., Ltd (Shanghai, China). The algae was cultured in f/2 culture medium at 20 ± 1 °C under a light intensity of approximately 65 μmol photons/m^2.s and a 12:12 light:dark cycle. The growth of the cultured algae was monitored by measuring the fluorescence using a fluorometer (Flurometer, AMIscience, USA), and the measurement method was calibrated by counting cells in a blood cell counting chamber. The algae removal experiments were performed using cultures in the mid-to-late exponential growth phase, with cell densities of 1.5×10^9 cells L^{-1}.

2.3 Preparation and modification of CNC

Cellulose nanocrystals (CNC) were prepared through sulfuric acid hydrolysis method (Wang et al., 2014). Take part of the CNC dispersion solution into the centrifuge tube and treat it in the ultrasonic cleaning machine for 5 min. At the same time, add the corresponding dose of QT solution, and then add a certain dose of artificial seawater. Different mass fraction of QT/CNC (25 wt%, 50 wt%, 75 wt%, 100 wt%, 200 wt%, 300 wt%, 400 wt%) were prepared.Then, the centrifuge tube was vibrated in the vortex shaking table for 5 min when the color of the mixture changed to light yellow, and there was no precipitation in the centrifuge tube after standing for a period of time, indicating that the preparation of QT/CNC was completed.

2.4 Removal experiment

The algae liquid in the middle and late exponential growth period was selected and placed in the colorimetric tube. A certain amount of QT/CNC dispersion was taken by a pipette and added to 50 ml of experimental algal liquid. The final concentration of CNC in algal liquid was 0.025, 0.05, 0.075 and 0.1 g L^{-1}. When the algal liquid was reversed and mixed well, it was still under normal culture conditions. In these hybrid systems, the effects of different mass ratios of CNC and QT on the removal efficiency of *Phaeocystis globosa* were studied. At the same time, the effect of different settling time on the removal of *Phaeocystis globosa* should be considered. 5 ml of the upper part of the mixed system was taken each time to measure the fluorescence, turbidity and chl-*a* value in vivo, and the pH value of the initial and residual algae solution was determined. Each experiment was performed in duplicate and the mean values were reported. The calculation formula of algae removal rate is as follows (Yu et al., 2004):

$$R_1 = \left(1 - \frac{F}{F_0}\right) \times 100\% \quad (1)$$

Where R_1 is the removal efficiency of fluorescence, F is the fluorescence value during the treatment, F_0 is the initial fluorescence value.

$$R_2 = \left(1 - \frac{C}{C_0}\right) \times 100\% \quad (2)$$

Where R_2 is the removal efficiency of chl-*a*, C is the chl-*a* concentration during the treatment, C_0 is the initial chl-*a* concentration.

$$R_3 = \left(1 - \frac{T}{T_0}\right) \times 100\% \quad (3)$$

Where R_3 is the removal efficiency of turbidity, T is the turbidity value during the treatment, T_0 is the initial turbidity value.

2.5 CNC characterization

The morphology of QT/CNC was observed using a atomic force microscopy (AFM, BRUKER AXS GMBH, Germany). X-ray diffraction (XRD) patterns of samples were obtained using an X-ray powder diffractometer (Bruker D8-Focus Advance) with Cu Kα radiation and acquired with the range of 5−60°. Fourier Transform Infrared Spectrometer (FTIR, Shimadzu IRAffinity-1S) analyses were carried out within a range of 400–4000 cm^{-1}. Zetasizer (Nano ZS, Malvern PANalytical, UK) were used to measure zeta potential. A turbidimeter (HACH2100N, HACH, Shanghai, China) were used to measure the turbidity and fluorometer (AMIscience, USA) were used to measure the fluorescence and chl-*a*.

3. Results and discussion

In this section, QT/CNC was characterized and its removal effect on *Phaeocystis globosa* was explored. Finally, the removal mechanism was analyzed.

3.1 Characterization of CNC and QT/CNC

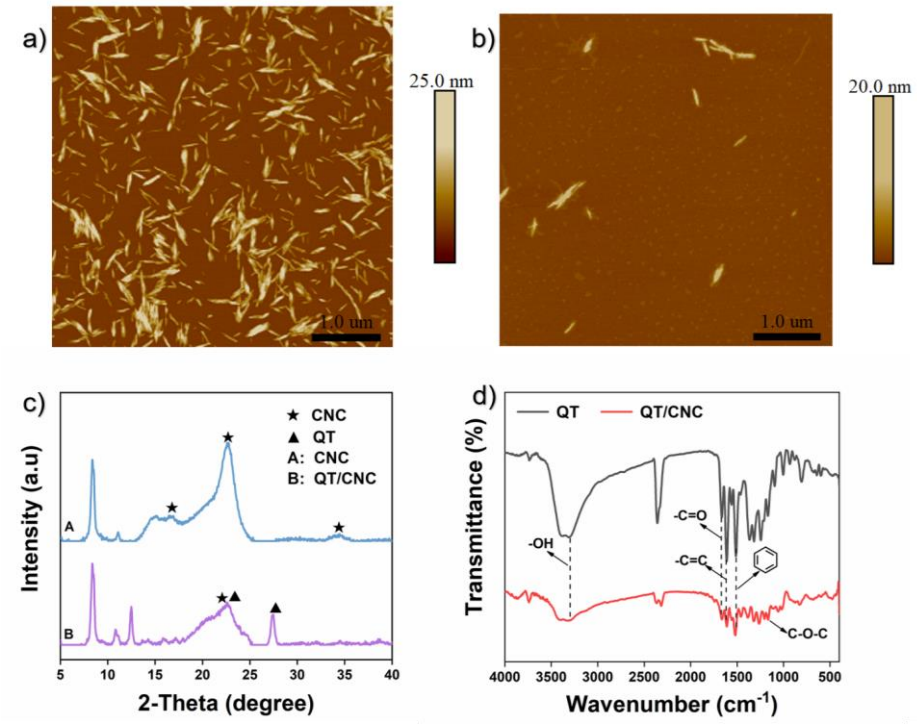

Figure 1: AFM height images of the (a) CNC and (b) QT/CNC; X-ray diffractograms of CNC and QT/CNC (c); FTIR spectra of QT and QT/CNC (d).

AFM images of CNC and QT/CNC were shown in Figure 1a and Figure 1b. The length of CNC and QT/CNC were 256.2 nm and 353.8 nm. The diameter of CNC and QT/CNC were 16.7 nm and 25.2 nm. The results showed that the morphology and shape of the modified CNC were basically the same as that of the CNC, and the effect of QT on the morphology and size of the CNC was negligible. X-ray diffractograms (XRD) patterns of CNC and QT/CNC were exhibited in Figure 1c. The diffraction peaks at 16.4°, 22.63° and 34.4° were assigned to the typical cellulose type I of CNC. The diffraction peak at 27.3° was attributed to the QT. The results indicated that QT/CNC had the structures of CNC and QT. The FTIR analysis results of QT and QT/CNC were shown in Figure 1d. The typical peaks for QT in absorption bands at 3314 cm^{-1} (-OH), 1667 cm^{-1} (-C=O), 1613 cm^{-1} (-C=C), 1513 cm^{-1}(benzene ring). In the spectrum of QT/CNC, the peaks at 1667 cm^{-1}, 1613 cm^{-1} and 1513 cm^{-1} moved to 1655 cm^{-1}, 1611 cm^{-1} and 1517 cm^{-1}. The new typical peaks are at 3330 cm^{-1} (-OH), 1167 cm^{-1}(C-O-

C). The results indicated that the interaction between CNC and QT appeared by hydrogen bond (Chen et al., 2017).

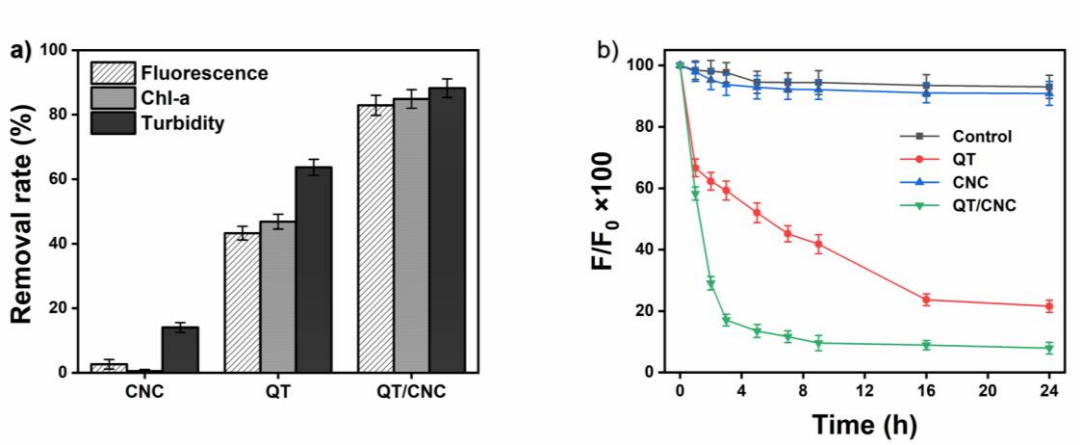

Figure 2: (a) Comparison of different pretreatment processes on algae removal. ([algal cell density] =1.5×10^9 cells L^{-1}, pH = 9.45, T = 25 ^{o}C, Time = 3h, [QT] = 0.1 g L^{-1}, [CNC] = 0.05 g L^{-1}, [QT/CNC] = 0.15 g L^{-1}) (b) Time curve of Phaeocystis globosa removal using CNC, QT and QT/CNC ([algal cell density] =1.5×10^9 cells L^{-1}, pH = 9.45, T = 25 ^{o}C, Time = 3h, [QT] = 0.1 g L^{-1}, [CNC] = 0.05 g L^{-1}, [QT/CNC] = 0.15 g L^{-1}).

3.2 Effects of different substances and reaction time on removal efficiency of *Phaeocystis globosa*

Figure 2a shows the impacts of three processes including CNC, QT and QT/CNC on the removal of *Phaeocystis globosa*. An approximate removal performance was observed compared with the control group by using a flocculation process of CNC, which only achieved limited removal rates of 2.6 %, 0.49 % and 14.06 % for fluorescence, chl-*a* and turbidity, respectively. The removal rates almost unchanged in 24h, indicating that little algae could be removed by CNC. When the algae was removed by the QT, the removal rates of fluorescence, chl-*a* and turbidity were 43.28 %, 46.84 % and 63.68 %. The remaining amount of algae cells was 21.57 % after 24 h. Among all the chosen removal processes, the combined process of QT/CNC treatment achieved the highest removal rate for algae as that fluorescence, chl-*a* and turbidity were 82.92 %, 84.89 %, and 88.21 %. After 24 h, the removal rate was nearly stable (Figure 2b). The results demonstrated that treatment by QT/CNC could not only could remove *Phaeocystis globosa*, but also improve the flocculation efficiency.The effect of the concentration and proportion of QT and CNC combination in QT/CNC on algae cell removal rate can be further explored.

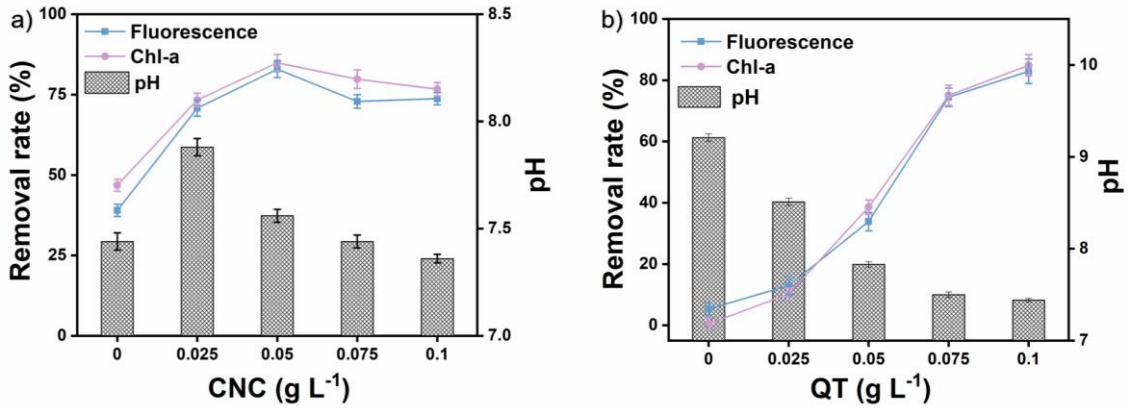

Figure 3: (a) Effect of CNC doses on algae removal. ([algal cell density] =1.5×10^9 cells L^{-1}, pH = 9.45, T = 25 ^{o}C, Time = 3h, [QT] = 0.1 g L^{-1}) (b) Effect of QT doses on algae removal. ([algal cell density] =1.5×10^9 cells L^{-1}, pH = 9.45, T = 25 ^{o}C, Time = 3h, [CNC] = 0.05 g L^{-1})

3.3 Optimization process of different parameters

3.3.1 Effects of CNC dose

Figure 3a showed the effect of CNC dosages ranging from 0.025 to 0.1 g L^{-1} on algae removal under the premise of keeping the QT concentration at 0.1 g L^{-1}. The removal rate of *Phaeocystis globosa* was enhanced with the increase of initial CNC dosage rom 0.025 to 0.05 g L^{-1}, while decreased with the initial CNC dosage further increasing to 0.075 g L^{-1}. An optimum CNC dose for this experimental condition was advised to be 0.05 g L^{-1}, at which the removal rates for fluorescence and chl-*a* reached up to 82.92% and 84.89%. As aforementioned, CNC could flocculate and precipitate the algae cells. The removal rate of algae cells was expected to be heightened with increasing CNC dosage. However, overdose of CNC might reduce the inhibition of QT on algae cells and impair settlement efficiency.

3.3.2 Effects of QT dose

The impact of QT dose on the removal of *Phaeocystis globosa* under the premise of keeping the CNC concentration at 0.05 g L^{-1} was tested (Figure 3b). The removal rates of algae and chl-*a* were enhanced with the increase of QT dose within 0.1 g L^{-1}. An optimum QT dose of 0.1 g L^{-1} was proposed, at which time the highest removal rates of no less than 82 % for chl-*a* and fluorescence were achieved. Moreover, the removal of Chl-a was observed to maintain at high efficiencies (75.1 % – 84.9 %) ranging from 0.075 to 0.1 g L^{-1}, which was proposed to be attributed to the effective destroy of photosynthetic system of *Phaeocystis globosa* by QT (Huang et al., 2015).

3.4 The mechanism on the removal of *Phaeocystis globosa*

To better reveal the mechanisms on the removal of *Phaeocystis globosa* by QT/CNC, the zeta potentials of CNC, QT and QT/CNC were observed by Zetasizer (Figure 4). The zeta potentials were negative, which were consistent with the charge of algae cells. there is no charge neutralization between QT/CNC and algae cells, so the surface charge is not the main reason affecting the removal effect.To further confirm the role of CNC and QT in algae removal system, the effect of QT/CNC on the pH of algal solution was analyzed. The pH value of algal liquid decreased with the increase of CNC and QT concentration, and the maximum range was from 9.45 to 7.36. The rapid growth of HABs will lead to the increase of pH value, and properly reducing the pH value of water body is conducive to inhibit the growth of algae cells.

Based on the foregoing results, a possible mechanism of the removal of *Phaeocystis globosa* by QT/CNC was illustrated in the schematic diagram (Figure 4). After the addition of QT/CNC into the *Phaeocystis globosa* water, QT/CNC was responsible for the destruction of alage. QT was a potent pro-oxidative agent which could induce higher reactive oxygen species(ROS) production in differents cells. As a result, excessive production and intracellular concentrations of ROS, destruction of intracellular structures and possess decreased activity, which made it more liable to be destabilized and aggregated (Torres et al., 2008). Therefore, algal cell could be effectively adsorbed by QT/CNC. At the same time, most of Na^+ was adsorbed on the surface of deprotonated anionic sulfate ester groups under alkaline conditions(Qi et al., 2019). Consequently, electrostatic shielding occured, which could result in the aggregation and sedimentation of the CNC, which benefits the removal of *Phaeocystis globosa*.

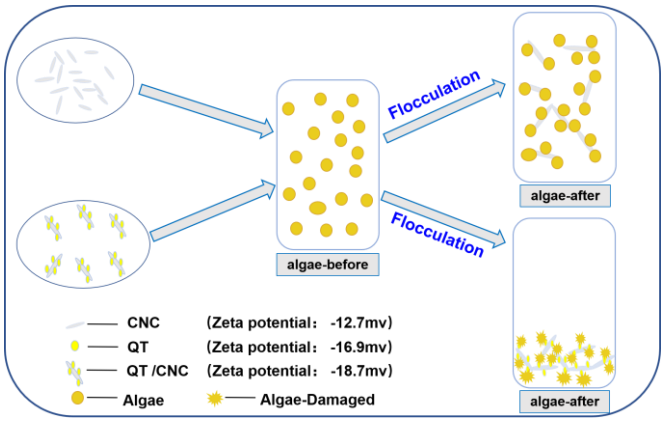

Figure 4: Schematic diagram for the Modification of QT/CNC and the removal of Phaeocystis globosa

4. Conclusions

This study indicates that the process of QT/CNC process flocculation is competent in removing *Phaeocystis globosa* cells, with simple operation, high removal rate in shorter running time, eco-friendly and no secondary pollution. Under optimal conditions, the removal rate of fluorescence, chl-*a* and turbidity were 82.92%, 84.89%, and 88.21% in 3 hours. The results demonstrated that the destruction of *Phaeocystis globosa* occured on the cell surface due to QT/CNC could induce higher ROS production in algae cells. Additionally, algal cell could be effectively adsorbed by QT/CNC, which could destabilize algae cells. The controlling of QT/CNC depends on the trade-off between the destruction of intracellular structures by generated higher ROS and the aggregation by the behavior of CNC. On the basis of the highly efficient removal for the *Phaeocystis globosa*, the QT/CNC might have hopeful prospects in the HABs control in the near future. The effects of QT/CNC on marine organisms are required to be carried out in future.

Acknowledgments

This study was supported by the Natural Science Foundation of China (No. 21776212) and Tianjin S&T Commission (20YFZCSN00650).

References

Chen H., Yao Y., 2017, Phytoglycogen improves the water solubility and Caco-2 monolayer permeation of quercetin, Food Chemistry, 221, 248-57.

Chen Y. Q., Xie P. C., Wang Z. P., Shang R., Wang S. L., 2017, UV/persulfate preoxidation to improve coagulation efficiency of *Microcystis aeruginosa*, Journal of Hazardous Materials, 322, 508-15.

He X. H., Wu Pian., Wang S. L., Wang A. Z., Wang C. L., Ding P., 2021, Inactivation of harmful algae using photocatalysts: Mechanisms and performance, Journal of Cleaner Production, 289,125755.

Heisler, J., Glibert P. M., Burkholder J. M., Anderson D. M., Cochlan W., Dennison W. C., Dortch Q., Gobler C. J., Heil C. A., Humphries E., Lewitus A., Magnien R., Marshall H. G., Sellner K., Stockwell D. A., Stoecker D. K., Suddleson M., 2008, Eutrophication and harmful algal blooms: A scientific consensus, Harmful Algae, 8, 3-13.

Huang H. M., Xiao X., Ghadouani A., Wu J. P., Nie Z. Y., Peng C., Xu X. H., Shi J. Y., 2015, Effects of natural flavonoids on photosynthetic activity and cell integrity in *Microcystis aeruginosa*, Toxins, 7, 66-80.

Lu G. Y., Song X. X., Yu Z. M., Cao X. H., Yuan Y. Q., 2015, Effects of modified clay flocculation on major nutrients and diatom aggregation during Skeletonema costatum blooms in the laboratory, Chinese Journal of Oceanology and Limnology, 33, 1007-19.

Mecina, G. F., Chia M. A., Cordeiro M. K., Bittencourt M. D., Varela R. M., Torres A., Molinillo J. M. G., Macias F. A., Silva R. M. G., 2019, Effect of flavonoids isolated from Tridax procumbens on the growth and toxin production of *Microcystis aeruginos*, Aquatic Toxicology, 211, 81-91.

Qi W. H., Yu J. H., Zhang Z. S., Xu H, N., 2019, Effect of pH on the aggregation behavior of cellulose nanocrystals in aqueous medium, Materials Research Express, 6, 125078.

Qiu L. X., Yu Z. M., Cao X. H., Ji H. N., Song X. X., 2020, The mechanism of a new type of modified clay controlling *Phaeocystis globosa* growth, Journal of Oceanology and Limnology, 38, 1270-82.

Torres M. A., Barros M. P., Campos S. C. G., Pinto E., Rajamani S., Sayre R. T., Colepicolo P., 2008, Biochemical biomarkers in algae and marine pollution: A review, Ecotoxicology and Environmental Safety, 71, 1-15.

Verfaillie A., Blockx J., Praveenkumar R., Thielemans W., Muylaert K., 2020, Harvesting of marine microalgae using cationic cellulose nanocrystals, Carbohydrate Polymers, 240, 116165.

Wang Q. Q., Zhao X. B., Zhu J. Y., 2014, Kinetics of strong acid hydrolysis of a bleached kraft pulp for producing Cellulose Nanocrystals (CNCs), Industrial & Engineering Chemistry Research, 53, 11007-14.

Yu Z. M., Sengco M. R., Anderson D. M., 2004, Flocculation and removal of the brown tide organism, Aureococcus anophagefferens (Chrysophyceae), using clays, Journal of Applied Phycology, 16, 101-10.