



Mechanistic insight into the inhibitory effect of artemisinin sustained-release inhibitors with different particle sizes on *Microcystis aeruginosa*

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Abstract

Environment-friendly algaecides based on allelopathy have been widely used to control harmful algal blooms. In this research, micro and nano scale artemisinin sustained-release algal inhibitor was prepared, the optimal preparation conditions were explored, and the inhibitory mechanism of artemisinin algaecides was turned perfect. The results showed that when the particle size of artemisinin sustained-release microspheres (ASMs) was 2/10,000 of artemisinin sustained-release granules (ASGs), the inhibitory effect was more remarkable. The optimal concentration of ASMs was 0.2 g L⁻¹, and the inhibitory effect reached 99% on the 10th day. The algal density and chlorophyll *a* both showed a downward trend, indicating that ASGs and ASMs could promote the degradation of chlorophyll *a*. The inhibition rate of ASGs was faster than that of ASMs on the 4th day, and the inhibitory effect of ASMs was more significant after the 5th day. The activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) increased rapidly at first and then decreased, which indicated that ASGs and ASMs caused oxidative damage to *Microcystis aeruginosa* and inhibited the activity of antioxidant enzymes. Furthermore, the content of the oxygen free radical (O²⁻) and malondialdehyde (MDA) continued to rise after the 5th day, and the protein, nucleic acid, and conductivity in the culture medium increased. These results showed that lipid peroxidation occurred in the algal cell membrane, and the permeability of the membrane increased. In summary, the ASMs had a significant sustained inhibitory effect while the ASGs had a better short-term effect. The main inhibitory mechanism of artemisinin algaecides is the irreversible damage of cell membrane.

Keywords Allelochemical · Particle sizes · Optimal manufacture · Inhibitory mechanism · Antioxidant enzyme activity · Cell membrane damage

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Highlights

1. Algicide with nanometer size was successfully prepared for algal inhibition.
2. Nano scale artemisinin algal inhibitor exhibited excellent inhibitory effect.
3. Destruction of algal cells was due to the irreversible damage of cell membrane.
4. Artemisinin microspheres may be used as a potential candidate for algal control.

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Introduction

Water eutrophication is a widespread phenomenon, and cyanobacteria bloom has become a threat to our water bodies necessitating a corresponding action (Zhao et al. 2010). The ever-increasing *Microcystis aeruginosa* (*M. aeruginosa*) reproduction rate is the main reason for the outbreak of harmful blooms (Feng et al. 2013; Zhu et al. 2012). The large-scale outbreak of *M. aeruginosa* leads to the decrease in dissolved oxygen in water, which breaks the stability and balance of the ecosystem (Greenfield et al. 2014; Harke et al. 2016). Therefore, it is imperative to control algae reproduction (Jancula and Marsalek 2011). Allelopathy has been considered as an important method to solve the algal succession and bloom. Many studies have shown that allelopathy can effectively inhibit the growth of cyanobacteria (Wu et al. 2010; Xiao et al. 2010). Phenolic acids have been

successfully extracted and purified from plants, making them the most widely investigated bioactive compounds. Allelopathic inhibition of algae has been proven by catechin (Laue et al. 2014), ginkgolic acids (Zhang et al. 2014), linoleic acid (Ni et al. 2018), and ferulic acid (Guo et al. 2013). Nonetheless, finding alternatives is necessary due to the potential negative effects of phenolic acids on non-target organisms and the complex transformation in the environment (Xie et al. 2014).

Artemisinin extracted from *Artemisia annua* has been proven to inhibit algae. Artemisinin can cause growth stagnation, injury, or death of the receptor organism through affecting cell proliferation, ultrastructure, protein synthesis, and biochemical reaction (Guo et al. 2019). Many researchers have begun to pay attention to the allelopathic effect of artemisinin on aquatic organisms. Ni et al. (2012) isolated and identified artemisinin from *Artemisia annua* and confirmed that artemisinin could cause physiological functional disorder of algal cells through algal inhibitory test. According to Wu et al. (2013), the allelopathic effect of artemisinin may hinder water productivity around the *Artemisia annua* production region, and the growth of *Chlorella pyrenoidosa* and *Scenedesmus obliquus* was significantly inhibited. Finaurini et al. (2012) found that the allelopathic effect of artemisinin on water hyacinth is reflected in its serious impact on the photosynthetic efficiency of water hyacinth. Therefore, the allelopathic effect of artemisinin on algae provides a new idea for algal inhibition, and its inhibitory mechanism needs to be explored. Most researchers discussed the influence of allelochemicals on photosynthetic system (Xing et al. 2018; Zhu et al. 2010), cell membrane (Shao et al. 2009), and enzyme activity (Lu et al. 2016; Zhang et al. 2010) while studying the inhibitory mechanism of allelochemicals. The inhibitory mechanism of artemisinin's consistency with the inhibitory mechanism of other allelochemicals still needs to be further explored.

In addition, it is worth mentioning that adding artemisinin directly into water has the disadvantages of being easily lost and is unable to reach the optimum concentration for algal inhibition. The encapsulation techniques provide a regulated medication delivery system by adsorbing active compounds into polymer skeleton materials and achieving long-term drug release. Ion crosslinking is widely used in the preparation of chitosan/sodium alginate particles. Chitosan, a natural hydrophilic basic polysaccharide, is an easy-to-form polycation electrolyte under acidic conditions and interacts with sodium alginate by electrostatic force to form tightly packed particles (Lee et al. 2011; Gokce et al. 2014). Compared with other methods, the encapsulation method is stable, mild, and non-toxic (without using organic chemical reagents), which reduces the additional toxicity of sustained-release carrier to a certain extent and safe. Meanwhile, research has shown that using two different

types of embedding materials with differing properties might improve the embedding rate and the materials' sustained-release impact (Li et al. 2020). As a result, alginate-chitosan microcapsule technology has emerged as one of the most effective and safe ways to prevent algae from spreading on a big scale (Ni et al. 2018). The sustained-release granules have the ability to release drugs for an extended period of time, resulting in a long-term effect of algal inhibition, and are widely used (Ni et al. 2015).

In a previous research, artemisinin sustained-release algal inhibition granules (ASGs) were successfully prepared by emulsification method, and their algal inhibitory effect and inhibitory mechanism were explored. In this research, we aim to prepare artemisinin sustained-release algal inhibition microspheres (ASMs) by ion crosslinking method combined with micro nanotechnology, reducing the particle size, swelling rate, and increasing the specific surface area of the sustained-release particles. The main objectives of this paper are (1) to determine the optimal preparation conditions of ASMs, (2) to compare the algal inhibitory effect of two artemisinin sustained-release algal inhibitors, and (3) to make the inhibitory mechanism of artemisinin sustained-release algal inhibitors perfect.

Materials and methods

Algal cultivation

M. aeruginosa FACHB-905 was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (China). It was cultured at Hohai University with sterilized BG-11 medium for 1 week and grew to logarithmic phase before the test. The algae were cultivated at 25°C under 40–60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (14 h light/10 h dark) (Ni et al. 2013) and had to be shaken several times a day.

Preparation of ASGs and ASMs

The drop preparation method was used to prepare ASGs, following the steps by Ni et al. (2013). The ASMs were produced with the ion crosslinking preparation method, combined with micro nanotechnology. Chitosan and sodium alginate were used as carrier materials to encapsulate artemisinin to prepare novel artemisinin sustained-release algal inhibition microspheres. The specific steps are shown in Fig. S1. In brief, calcium chloride (6 mg mL⁻¹, w/v) solution was slowly dripped (at a mixture speed of 700–1000 rpm) into sodium alginate (1 mg mL⁻¹, w/v) solution to obtain a pregel, which was stirred for 30 min, crushed for 10 min, then successively dripped artemisinin solution (10 mg mL⁻¹, w/v) and aqueous acetic acid (1%, v/v) solution containing chitosan (0.6 mg mL⁻¹, w/v) into the pregel, slowly stirring for 60 min, and chitosan

colloidal microspheres were formed which were then crushed for 1 min. After centrifugating at 12,000 rad/min for 30 min, rinsing several times with distilled water, and freeze-drying, the ASMs were then collected. This experiment was carried out with orthogonal test, and the optimal preparing conditions of ASMs were tested by high-performance liquid chromatography with a photodiode array (HPLC–PDA).

The artemisinin microspheres were washed three times with double-distilled water, grind-mixed with diatomite, and then dissolved in solvent to obtain artemisinin sustained-release solution. HPLC–PDA (Waters 2695 HPLC) was used to determine the artemisinin content of the sustained-release microspheres and to calculate the encapsulation efficiency based on the initial amount of ASMs. The HPLC–PDA method was performed on a Waters C18 column. The mobile phase was 0.02 mol L⁻¹ KH₂PO₄:acetonitrile (90:10 v/v) solution at a flow rate of 1 mL min⁻¹, with a column temperature of 30°C.

To determine the optimal preparation of ASMs, the concentrations (in %, w/v) of materials mentioned above were dependent on an orthogonal test. The size of microspheres was calculated by dynamic light scattering (DLS) analysis (Mastersizer 3000, UK). The encapsulation efficiency (%) of the artemisinin was calculated using the following equation:

$$\text{Encapsulation efficiency (\%)} = W_1/W_0 \times 100\% \quad (1)$$

where W_1 is the measured amount of encapsulated artemisinin in the sustained-release microspheres, and W_0 is the initial amount of ASMs.

Algal inhibitory test

M. aeruginosa was introduced into the culture medium of 250-mL flasks, and $2 \sim 3 \times 10^6$ cells mL⁻¹ was set as the initial cell density to simulate the stationary phase of the algal growth. The concentration range of the ASMs was determined by pre-experiment, and then the algal inhibitory experiment was carried out. The blank group (without algal inhibitor) and the ASM groups with different concentrations (the dosages of ASMs were 0.1 g L⁻¹, 0.2 g L⁻¹, 0.4 g L⁻¹, 0.8 g L⁻¹, and 1 g L⁻¹, respectively) were set. The culture condition was the same as in the section “Algal cultivation.” During the 30-day culture phase, algal density was assessed on a regular basis using a conversion of optical density to reflect the growth of *M. aeruginosa*. By determining the algal inhibition rate of different concentrations of ASMs, the optimal effective dosage of sustained-release microspheres was determined.

Inhibitory mechanism studies

The dosage of ASG algal inhibitor was based on earlier research (Ni et al. 2013). Three experimental groups

were set up in this research: blank control group, ASG algal inhibitor experimental group, and ASM algal inhibitor experimental group. The three experimental groups were in triplicates. In this research, the initial algal density was 2×10^6 cells mL⁻¹, the optimal dosage of ASG algal inhibitor was 0.54 g L⁻¹, and the optimal dosage of ASMs was determined by the results of algal inhibitory test. The content of chlorophyll *a* and the activities of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD)) were measured every 5 days during the 30-day culture period. The degree of damage on cell membrane was characterized by the content of protein, nucleic acid, MDA (degree of lipid peroxidation), oxygen free radical (O²⁻), and the changes in conductivity.

Algal density and chlorophyll a content assays

The cell density of *M. aeruginosa* was determined by optical density method. The microscope counting method and the optical density at 680 nm were used to get a regression equation relating to algal density, that is

$$Y = 169.97X - 0.351 (R^2 = 0.9969) \quad (2)$$

where X is the absorbance value and $Y \times 10^5$ cells mL⁻¹ represents the density of algae.

The relative inhibition rate of algal inhibitor release on *M. aeruginosa* is as follows:

$$IR(\%) = (1 - N/N_0) \times 100\% \quad (3)$$

where N is the algal density of treatment group, and N_0 is the algal density of blank group.

The content of chlorophyll *a* was determined by ethanol extraction (Li et al. 2019). The absorbance of the extract and blank reference was measured at 665 nm and 750 nm, respectively. The calculation formula is as follows:

$$\text{Chl - a (mg/L)} = 11.93(A_{665} - A_{750}) \quad (4)$$

Cell membrane assays

The content of MDA was determined by thiobarbituric acid (TBA) spectrophotometry (Zhang et al. 2016a). The culture medium was treated with AP-TEMED system, and then the concentration of O²⁻ was determined by measuring its absorbance at 530 nm. The contents of protein and nucleic acid in the culture medium were mainly obtained by measuring the absorbance values of the supernatant at 280 nm and 260 nm, respectively (Zhang et al. 2016b). Conductivity is mainly measured by a conductivity meter.

Antioxidant enzyme activity assays

The total SOD activity was determined by NBT photoreduction method as described by Charles and Irwin (1971). The activity of CAT was determined by its ability to decompose H_2O_2 , and CAT activity was determined according to Aebi et al. (2010). POD can catalyze the reaction of peroxyphenol, and the activity detection method of POD referred to that of Liu et al. (2010).

Statistical analysis

All statistical analyses were performed using SPSS 22.0, and graphs were prepared in Origin 9.0. *p* values were derived from the one-way ANOVA test to determine differences between groups with normally distributed data. A *p* value < 0.05 was considered statistically significant.

Results and discussion

Optimal manufacture of ASGs and ASMs

The optimal preparation method of ASGs was based on our previous research (Ni et al. 2013), where the average particle size of ASGs was 5 μ m, with a maximum encapsulation efficiency of 52%. In this research, the optimal preparation concentrations (in %, w/v) of ASMs are as follows: chitosan (0.06), sodium alginate (0.1), calcium chloride (0.6), and artemisinin (1). Under the optimal preparation conditions, the microsphere size of ASMs is shown in Fig. 1. It is obvious that the microsphere size of ASMs is in the micro/nano level, which is far smaller than that of ASGs. According to the experimental data, the average particle size of ASMs was 1189.5 nm, and the encapsulation efficiency was 52.4%. It is well known that greatly reduced particle size is beneficial to reduce the swelling rate of the sustained-release algal inhibitor. Therefore, the novel preparation method used in this research is conducive to further improve the algal inhibitory effect.

Inhibitory effect of ASMs on *M. aeruginosa*

The inhibitory effect on algae differs due to the dosages of algaecides (Ni et al. 2018). To investigate the effects of ASMs, algal density was determined and the results (Fig. 2) showed that the inhibitory effect of ASMs with higher concentration on *M. aeruginosa* was more remarkable and the inhibition time was longer than that with low concentration. As shown in Fig. 2, the blank control group showed an S-shaped growth curve (Li et al. 2010). At the dosage of 0.1 $g L^{-1}$, ASMs showed a transient inhibitory effect on *M. aeruginosa*. The inhibition rates were below 30% after the

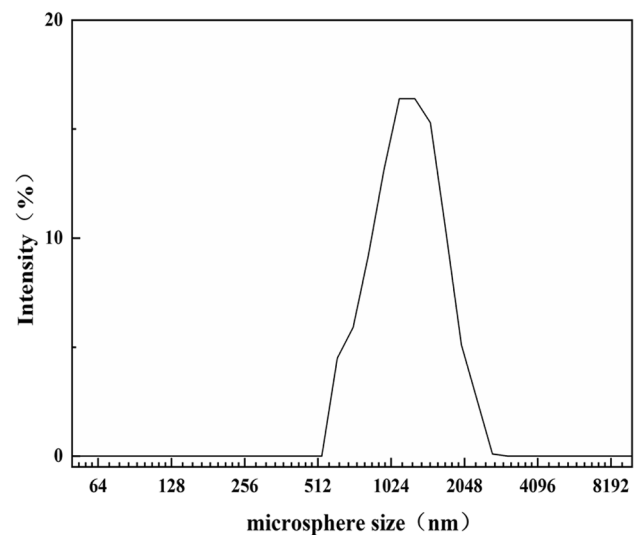


Fig. 1 Microsphere size distribution of ASMs under optimal preparation conditions

2nd day, and the inhibitory efficiency was only 10.20% at the end of the experiment (30 days). The growth of cyanobacteria was clearly suppressed with doses above 0.1 $g L^{-1}$. The dosages of 0.2 $g L^{-1}$ and 0.4 $g L^{-1}$ showed a very similar pattern. The growth of algae was in a constant inhibition state from the 4th to the 10th day and maintained high inhibitory efficiency (> 99%) after the 10th day. When the dosage was higher than 0.8 $g L^{-1}$, the inhibition rate was up to 99% on the 5th day. Considering the perspective of algal inhibitory test and economy, 0.2 $g L^{-1}$ of ASMs was selected as the optimal algal inhibitory concentration under laboratory conditions.

Effects of ASGs and ASMs on chlorophyll *a*

As an important index reflecting the photosynthetic capacity of algae, the content of chlorophyll *a* was affected by the growth of algal cells and photosynthesis (Maqbool et al. 2018). In this research, the effects of ASGs and ASMs on the density and chlorophyll *a* content of *M. aeruginosa* were investigated under the optimal algal concentration. The results (Fig. 3) were consistent with the results of many researchers (Espinoza-Avalos 2006), and the content of chlorophyll *a* in the blank control group was approximately positively correlated with the density of algae. At day 10 after being exposed to the two algal inhibitors, there were significant changes between the treatment groups and the blank control group ($p < 0.05$). In addition, chlorophyll *a* remained at a low level during the whole experiment. At the beginning of the experiment (the first 4 days), ASGs had more apparent inhibitory effect and faster inhibition rate. After day 5, the intensities of the

Fig. 2 Effects of different concentrations of ASMs on algal density and inhibition rate

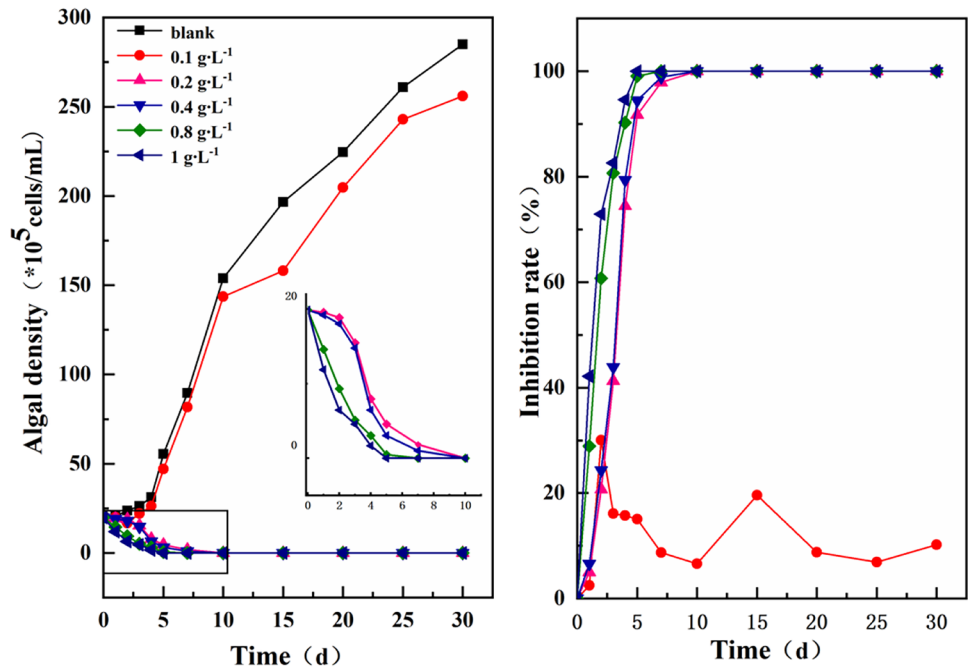
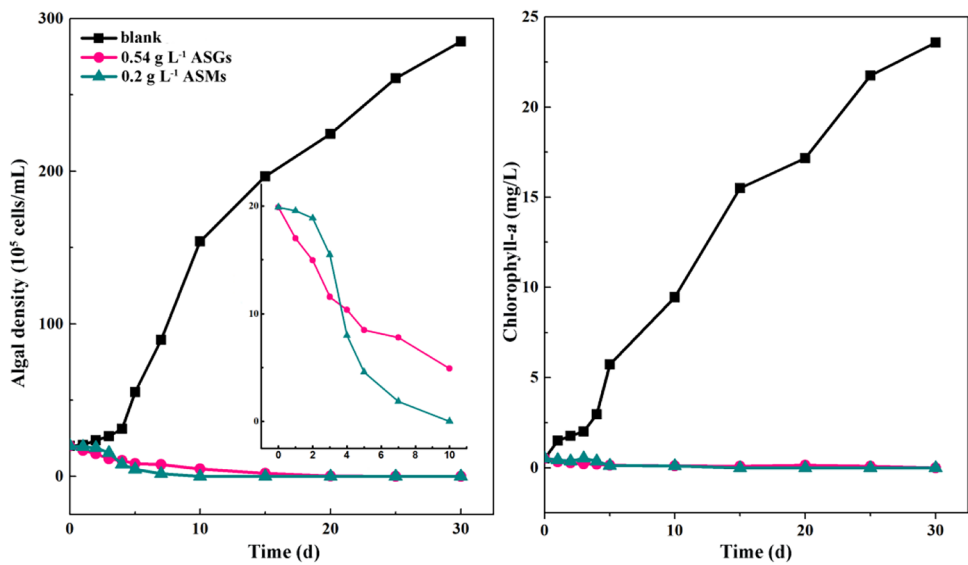


Fig. 3 Effects of ASGs and ASMs on algal density and chlorophyll *a* of *M. aeruginosa*



inhibitory effects of ASGs and ASMs were reversed. Both showed persistent and strong algal inhibitory efficiency after 10 days, with ASMs having a higher inhibitory intensity. At the end of the experiment (30 days), *M. aeruginosa* had no trace of recovery. The ASMs had a stronger long-term inhibitory effect, which could be due to the smaller particle size of ASMs compared to ASGs, combined with the multi-space structure on their surface, which considerably increased the effective contact area with *M. aeruginosa*. In addition, ASGs easily swell and sink in water, which reduces the contact time with *M. aeruginosa*. It is worth mentioning that in the previous research, the

apparent sudden release phenomenon of ASGs in the initial stage may be the real reason for the faster inhibition rate of ASGs. In general, both algal inhibitors were highly effective in inhibiting algae, although ASMs had a longer-lasting effect on algal inhibition and were more damaging to chlorophyll *a*.

Effects of ASGs and ASMs on cell membrane permeability

Cell membrane is an important structure of cells, and its important components are phospholipid bilayer and protein.

It can keep the cell microenvironment stable and has selective permeability function (Jonas et al. 2016). Generally, allelochemicals damage the integrity of cell membrane, reduce its selective permeability function, and make algal cells exude a lot of endolysates (Li et al. 2007). Therefore, we often use the change of cell membrane permeability to characterize the degree of membrane damage. Figure 4 shows the effects of the two algal inhibitors on the protein and nucleic acid content in the culture medium. The experiment was accompanied by natural apoptosis in algae, which was higher in the early stage than in the late stage, albeit being in a relatively low range (Zhou et al. 2009). The protein and nucleic acid concentration in the blank control group decreased at first, then increased, and then remained constant at a low level throughout. The two algal inhibitors induced a substantial rise in protein and nucleic acid content in *M. aeruginosa* ($p < 0.05$), and the protein and nucleic acid content in the ASG group was higher in the beginning of the experiment. It was reversed in the middle and later periods. Both of them appeared to be able to continuously release artemisinin in order to replenish the anti-algal active components in the algal inhibition system, but the damage effect of ASGs on cell membrane was faster, and the sustained inhibitory effect of ASMs was more impressive, which was consistent with their inhibition pattern. Protein deficiency in algae can damage or disrupt normal physiological metabolic processes, even resulting in algal death (Ni et al. 2013). As a result of the persistent stress of the two algal inhibitors, both algal cell membranes were severely damaged, and the decrease in membrane selective permeability led to the gradual leakage of protein and nucleic acid outside the cells. The degree of damage to algal cells increased over time as

artemisinin was released, and the damage caused by ASMs on the cell membrane was more effective and long-lasting.

Figure 5 shows the change in electrical conductivity under the action of the two artemisinin sustained-release algal inhibitors. In general, the electrical conductivity of the blank control group did not fluctuate significantly, but the ASM group increased significantly after the 7th day ($p < 0.05$). On the 2nd day, the conductivity of the treatment group culture medium was higher than that of the blank control group under the stress of the two sustained-release algal inhibitors. After the 7th day, the conductivity of the culture medium under the stress of ASMs was gradually higher than that of ASGs. The results showed that under

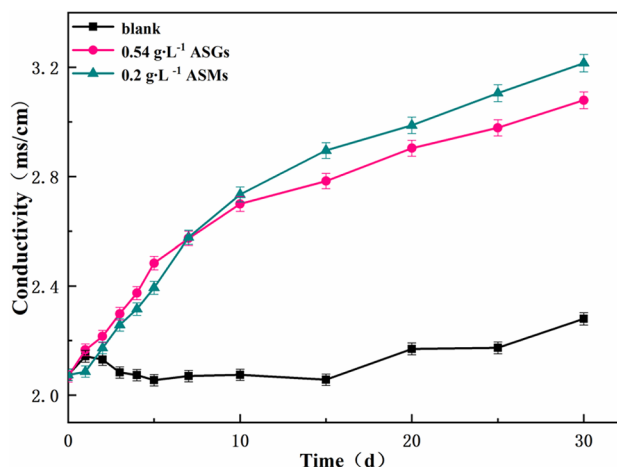
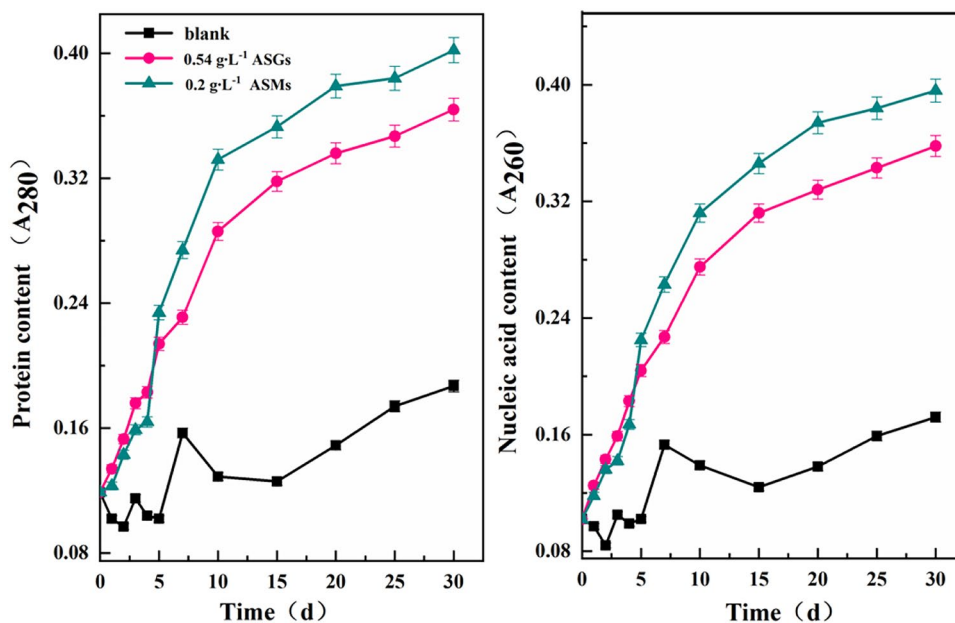


Fig. 5 Effects of ASGs and ASMs on the conductivity of the culture medium

Fig. 4 Effects of ASGs and ASMs on the protein and nucleic acid content of the culture medium



the sustained stress of the two artemisinin sustained-release algal inhibitors, the degree of damage on cell membrane was gradually aggravated, and its permeability was also gradually increased, which led to the leakage of electrolytes including K^+ , PO_4^{2-} , and other ions in the algal cell to the external environment. These phenomena indicated that artemisinin active substance could cause irreversible damage to cell membrane (Peng et al. 2016).

Oxidative damage

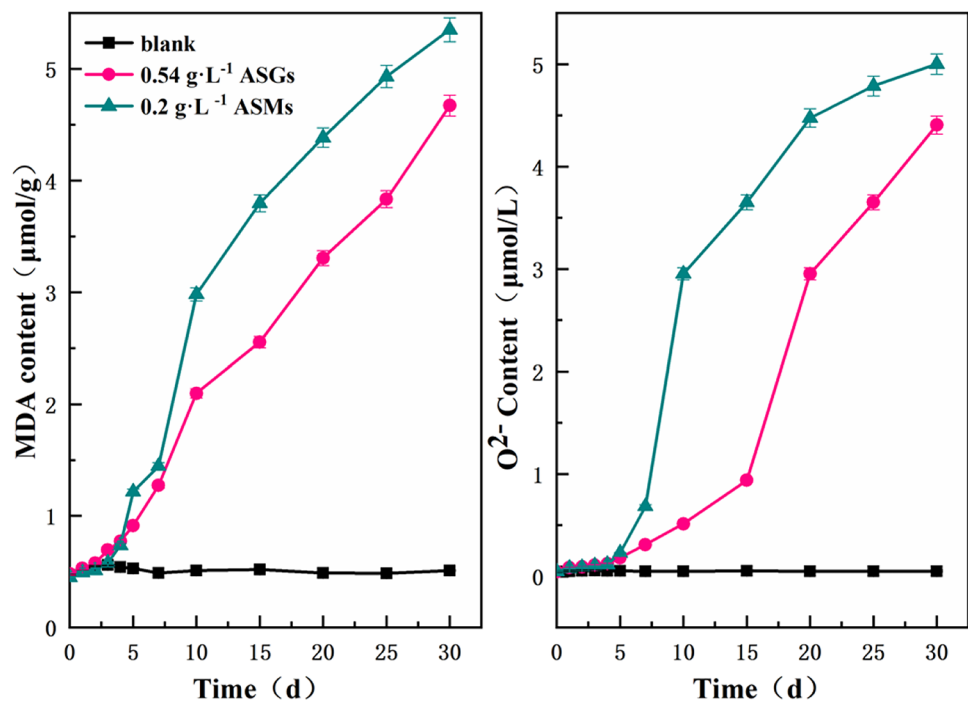
Under the condition of environmental stress, the production and elimination of $O_2^{\cdot-}$ in the algal cell system will be out of balance. A large number of $O_2^{\cdot-}$ will destroy the biological macromolecules in the algal cells and cause membrane lipid peroxidation (Huang et al. 2013a; Saed-Moucheshi et al. 2014). MDA is the product of membrane lipid peroxidation. Membrane lipid peroxidation can destroy the structure of cell membrane and make it abnormal (Huang et al. 2013b). The higher the content of MDA, the higher the degree of lipid peroxidation and the more serious the damage of cell membrane (Ali et al. 2006). As shown in Fig. 6, the content of $O_2^{\cdot-}$ and MDA in the blank control group was nearly invariable, which indicated that *M. aeruginosa* did not suffer membrane lipid peroxidation and grew in a normal state. In addition, the change in trend for $O_2^{\cdot-}$ and MDA content in the two artemisinin algal inhibitor groups was basically the same, showing different degrees of upward trend, which indicated that the algae experienced different degrees of lipid peroxidation after adding the two sustained-release

algal inhibitors, and the algal cells were damaged in different degrees. Specifically, the increase rate of $O_2^{\cdot-}$ and MDA content in the ASG group was higher than that in the ASM group 4 days prior to the experiment, but it was reversed after the fifth day. On the 10th day of the experiment, the content of MDA and $O_2^{\cdot-}$ in the ASM group increased significantly, while the significant increase appeared in the ASG group on the 15th day ($p < 0.05$). This phenomenon was consistent with the algal inhibitory effect and the abovementioned pattern of intracellular solute extravasation. These phenomena indicated that the algal inhibitory effect of ASGs was better than that of ASMs in a short time while ASMs had better sustained algal inhibitory effect. To some extent, the results indicated that the smaller the particle size of the algal inhibitor, the more favorable the contact between the algal inhibitor and the algal cell, and the algal inhibitory effect was more remarkable.

Inhibition of antioxidant enzyme activities

Antioxidant system is the protective system of organism against harsh environment. The key antioxidant enzymes are SOD, POD, and CAT (Gulzar and Siddiqui 2015). Antioxidant enzymes usually eliminate excessive reactive oxygen species (ROS) to ensure the homeostasis of internal oxidation metabolism. SOD is the key protective enzyme of the body, which can reflect the degree of damage and the ability to resist the adverse environment (Blackhall et al. 2004). CAT and POD play an important role in the antioxidant system of algal cells, which can promote the decomposition

Fig. 6 Effects of ASGs and ASMs on the MDA and $O_2^{\cdot-}$ content of *M. aeruginosa*



of H_2O_2 and stabilize the ROS in the organism at a normal level, in order to avoid damage to cells (Tang et al. 2010).

The activities of SOD, CAT, and POD were measured to evaluate the effect of the two artemisinin sustained-release algal inhibitor on antioxidant enzymes (Fig. 7). The activities of SOD, POD, and CAT in the two artemisinin sustained-release algal inhibitor groups showed a trend of rapid increase and then decrease, showing a dynamic change process from induction to inhibition. The SOD activity of treatment groups decreased gradually after reaching the maximum, and the ASG group and ASM group reached maximum on the 5th day and 10th day, respectively, and then declined sharply ($p < 0.01$) on the 25th day. The SOD activity of treatment groups was lower than that of the blank control group on the 15th day. Furthermore, the decrease in SOD activity in the ASM group was much greater than that in the ASG group. The activities of POD and CAT reached the maximum on the 5th day, then decreased sharply after

15 days ($p < 0.01$) until beyond the detection range. Excess ROS was created as a result of the continual release of artemisinin, preventing algal cells from developing normally and even destroying the antioxidant system. The activity of antioxidant enzymes decreased and eventually inactivated as intracellular components (nucleic acid, proteins, ions, etc.) were lost (Hejl and Koster 2004).

SOD can convert O^{2-} into H_2O_2 and O_2 specifically to avoid cell injury (Ni et al. 2018). CAT and POD can remove excessive hydrogen peroxide in algal cells, so the change in trend for the three antioxidant enzymes was consistent, until the antioxidant enzymes were inactivated. To sum up, under the action of algal inhibitory substances, ROS increased, and the contents of SOD, CAT, and POD increased. With the constant release of artemisinin, antioxidant enzymes were gradually inactivated, resulting in the damage of algal cells, thus inhibiting the growth of algae (Li et al. 2013).

Conclusions

In summary, the inhibitory effects of the two artemisinin sustained-release inhibitors were both significant. The results showed that the optimal concentration of ASMs was 0.2 g L^{-1} , and the inhibition rate was up to 99% on the 10th day. Under the optimal conditions, the algal inhibitory effect of ASMs with small particle size was more effective than that of ASGs on the whole, and the ASMs had significant sustained inhibitory effect while ASGs had better short-term effect.

The inhibitory mechanism of ASGs was consistent with that of ASMs. They inhibited the growth of *M. aeruginosa* by destroying the photosynthetic system, causing oxidative damage, inhibiting antioxidant enzyme activity, and destroying cell membrane. The decrease in chlorophyll *a* content indicated that both algal inhibitors could inhibit the photosynthesis of algal cells. Under the stress of algal inhibitor, the algal cells were stimulated to generate a large amount of O^{2-} , which resulted in lipid peroxidation of cell membrane and irreversible damage to the permeability of algal cell membrane. At the same time, the production of a large amount of O^{2-} can induce the increase of SOD, POD, and CAT activities. With the prolongation of inhibition time, the generated excess ROS prevented the algal cells from growing normally and ultimately led to the inactivation of antioxidant enzymes. The accumulation of MDA showed that the cell membrane was damaged to a certain extent, which led to the leakage of substances in the cell, resulting in the increase of protein, nucleic acid, and electrical conductivity in the culture medium. ASMs had a remarkable anti-algal effect, and the preparation method was environmentally friendly, and economical to produce, which provided an unlimited possibility for the application of ASMs in controlling red tide in natural lakes in the future.

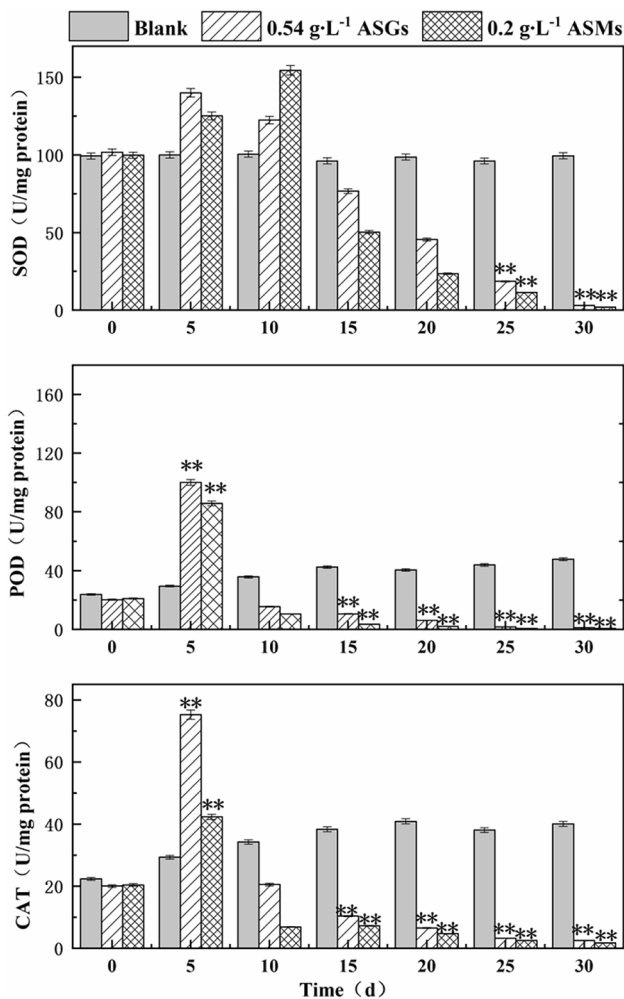


Fig. 7 Effects of ASGs and ASMs on activities of SOD, CAT, and POD of *M. aeruginosa* (* $p < 0.05$, ** $p < 0.01$)

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11356-022-21534-x>.

Author contribution Zhiyun Jiang: writing of the original draft, formal analysis, and data curation. Lixiao Ni: funding acquisition, conceptualization, and project administration. Xianglan Li: software and methodology. Chu Xu: investigation. Xuqing Chen: conceptualization and resources. Shiyin Li: writing, with review and editing.

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Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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