The Mechanism Insight Into the Inhibitory Effect of Artemisinin Sustained-release Inhibitors With Different Particle Sizes on *Microcystis aeruginosa*

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

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Highlights

1. Algicide with nanometer size were successfully prepared for algae 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 maybe used as a potential candidate for algal control.

Graphical abstract

Abstract

Environment-friendly algaecides based on allelopathy have been widely used to control harmful algal blooms. In this research, micro nano scale artemisinin sustained-release algal inhibitor was prepared, the optimal preparation conditions were explored and the inhibitory mechanism of artemisinin algaecides was perfected. The results showed that when the particle size of artemisinin sustained-release microspheres (ASMs) was 2/10000 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 algae 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), catalase (CAT) increased rapidly at first and then decreased, which indicated that ASGs and ASMs caused oxidative damage to *Microcystis aeruginosa* (*M. 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, 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 significant continuous inhibitory effect while the ASGs had 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

1.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* reproduction rate is the main reason for the outbreak of harmful blooms (Feng et al. 2013, Zhu 2012). The large-scale outbreak of *Microcystis 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, the need to control algae reproduction (Jancula & 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).

Artemisinin extracted from *Artemisia annua* L 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* L and confirmed that artemisinin could cause the physiological function disorder of algae cells through algae inhibitory test. (Wu et al. 2013) considered the water productivity around the production area of *Artemisia annua* might be restricted by the allelopathic effect of artemisinin, 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 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 algae inhibition, and its inhibitory mechanism needs to be explored. There is no doubt that allelochemicals affect the biological and biochemical characteristics of algae, and artemisinin is no exception. Most researchers have talked about 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) when 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 easy loss and is unable to reach the right concentration for algae inhibition. Therefore, embedding allelochemicals into sustained-release particles has become one of the most effective and safe means to inhibit the large-scale propagation of algae (Ni et al. 2018). At the same time, the sustained-release granules have the ability to sustained-term effective release of drugs, which can achieve the effect of long-term effect of algal inhibition and are widely used (Ni et al. 2015).

In a previous research, artemisinin sustained-release algae inhibiting 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. This optimization does not only improve the algae inhibitory effect, but also excludes the use of organic chemical reagents. It is a non-toxic, stable and mild operation method. The main objectives of this paper are to: (1) determine the optimal preparation conditions of artemisinin sustained-release microspheres (ASMs); (2) compare the algal inhibitory effect of two artemisinin sustained-release algae inhibitors; (3) perfect the inhibitory mechanism of artemisinin sustained-release algae inhibitors.

2. Materials and methods

2.1 Algal cultivation

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

2.2 Preparation of ASGs and ASMs

The drop preparation method was used to prepare ASGs, following the steps of (Ni et al. 2013), which has been described explicitly. The ASMs were produced with 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 inhibiting microspheres. The specific steps were 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, PH 4.7) solution to obtain a pregel, which was stirred for 30 min, crushed for 10 min, and then successively dripped the 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, PH 5.1) into the pregel, slowly stirring for 60 min, chitosan colloidal microspheres were formed which were then crushed for 1 min. After centrifugating at 12000 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 photo diode 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 C 18 column. The mobile phase was $0.02 \text{ mol L}^{-1} \text{ KH}_2\text{PO}_4$: acetonitrile (90:10 v/v) solution at a flow rate of 1 mL min^{-1} , and with a column temperature of 30°C .

To determine the optimal preparation of ASMs, the concentrations (in%, w/v) of materials mentioned above depended on an orthogonal test. The microspheres size were calculated by dynamic light scattering (DLS) analysis (Mastersizer 3000, UK). The encapsulation efficiency (%) and drug-loading rate (%) 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.

$$\text{Drug - loading rate (\%)} = M_1 / M_0 \times 100\% \quad (2)$$

where M_1 is the content of artemisinin in sustained release microspheres, and M_0 is the total mass of sustained release microspheres.

2.3 Algal inhibitory test

M. aeruginosa in logarithmic growth phase was placed in a 250 ml conical flask, and the initial cell concentration was $2\sim 3 \times 10^6$ cells ml^{-1} . The concentration range of the ASMs was determined by pre-experiment, and then the algae inhibitory experiment was carried out. The blank group (without algae inhibitor) was set up, and the ASMs groups with different concentrations (the dosage 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). The culture condition was the same as described above in 2.1. The experimental period was 30 days, three parallel groups were set up, and the optical density was measured regularly. By determining the algal inhibition rate of different concentrations of ASMs, the optimal effective dosage of sustained-release microspheres was determined.

2.4 Inhibitory mechanism studies

The dosage of ASGs algal inhibitor referred to the previous research (Ni et al. 2013), and the dosage of ASMs algal inhibitor was determined by the results of algae inhibitory test. Three experimental groups were set up in this research: blank control group, ASGs algal inhibitor experimental group and ASMs algal agent experimental group. The three experimental groups were in triplicates, the test period was 30 days. In this research, the initial algal density was 2×10^6 cells ml^{-1} , the optimal dosage of ASGs algal inhibitor was 0.54 g L^{-1} , the optimal dosage of ASMs was determined by the results of algae inhibitory test. The content of chlorophyll-a and the activities of antioxidant enzymes (SOD, CAT and 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 (O^{2-}) and the changes in conductivity.

2.5 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:

$$Y = 169.97X - 0.351 (R^2=0.9969) \quad (3)$$

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

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

$$IR(\%) = (1 - N/N_0) \times 100\% \quad (4)$$

Where N is the algal density of treatment group, 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 were measured at 665 nm and 750 nm respectively. The calculation formula is as follows:

$$Chl - a(mg/L) = 11.93 (A_{665} - A_{750}) \quad (5)$$

2.6 Cell membrane assays

The content of MDA was determined by thiobarbituric acid (TBA) spectrophotometry (Zhang et al. 2016a). The culture solution was treated with AP-TEMED system and then the concentration of O^{2-} was determined by measuring its absorbance at 530 nm. The contents of protein and nucleic acid in the culture solution 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 conductivity meter.

2.7 Antioxidant enzymes activities assays

The total SOD activity was determined by NBT photoreduction method as described by (Charles et al. 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

192 POD referred to (Liu et al. 2010).

193 2.8 Statistical analysis

194 All assays were conducted in triplicate. The data in the paper used the average
195 value. All the data were analyzed by one-way ANOVA. SPSS and Origin were used
196 for statistical analysis, in which $p < 0.05$ represents significant difference and $P < 0.01$
197 represents extremely difference.

198 3. Results and discussion

199 3.1 Optimal manufacture of ASGs and ASMs

200 The optimal preparation method of ASGs referred to our team's earlier work in
201 2013 (Ni et al. 2013), the average particle size of ASGs was 5 mm and the maximum
202 embedding rate was 52% by the optimum preparation method. In this research, the
203 optimal preparation method of ASMs was obtained with the same orthogonal test, the
204 optimal preparation concentrations (in%, w/v) are as follows: chitosan (0.06), sodium
205 alginate (0.1), calcium chloride (0.6), artemisinin (1). Under the optimal preparation
206 conditions, the microsphere size of ASMs were shown in Fig.1. It is obvious that the
207 microsphere size of ASMs is in the micro nano level, which is far smaller than that of
208 ASGs. According to the experimental data, the average particle size of ASMs was
209 1189.5 nm, and the embedding rate was 52.4%. It is well known that greatly reduced
210 particle size is beneficial to reduce the swelling rate of the sustained-release algal
211 inhibitor. Therefore, the new preparation method used in this research is conducive to
212 further improve the algae inhibitory effect.

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

3.2 Inhibitory effect of ASMs on *M. aeruginosa*

M. aeruginosa in logarithmic growth stage was selected to carry out algal inhibitory test under the condition of adding different concentrations of ASMs. The results were shown in Fig.2, the results 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 curve growth, showing a normal growth cycle (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 all below 30% after the 2nd day, and the inhibitory efficiency was only 10.20% at the end of the experiment (30d). In addition, at the dosage of 0.2 g L^{-1} and 0.4 g L^{-1} showed similar inhibitory effect. The inhibitory effect increased significantly from the 4th day 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 as high as 99% on the 5th day. Considering the perspective of algae inhibitory test and economy, when the concentration of ASMs was 0.2 g L^{-1} , the algal inhibitory requirements of the laboratory had been met. As a result, the 0.2 g L^{-1} of ASMs was selected as the optimal algal inhibitory

concentration under laboratory conditions.

Fig 2 Effects of the different concentration of ASMs on algae density and inhibition rate.

3.3 Effect 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 algae 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), the content of chlorophyll-a in blank control group was approximately positively correlated with the density of algae. In addition, the two kinds of algal inhibitors had strong inhibitory effect on the growth of *M. aeruginosa*, and chlorophyll-a remained at a low level during the whole experiment. At the beginning of the experiment (the first 4 days), ASGs had more obvious inhibitory effect and faster inhibition rate. After the 5th day of the experiment, the algal inhibitory effect and inhibition rate of ASMs shown an obvious reverse phenomenon. After 10 days, both of them showed persistent and high algal inhibitory efficiency while the inhibitory intensity of ASMs was higher. At the end of the experiment (30

days), *M. aeruginosa* had no trace of recovery. The reason is that the ASMs had more significant inhibitory effect, which may be because the particle size of ASMs which was far smaller than that of ASGs, coupled with the multi-space structure on its surface, greatly increasing 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 obvious sudden release phenomenon of ASGs in the initial stage may be the real reason for the faster inhibition rate and further effect of ASMs in the initial stage. In general, both algal inhibitors had high efficiency of algae inhibition, but ASMs had a more significant effect on algae inhibition and more destructive to chlorophyll-a.

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

3.4 Effect of ASGs and ASMs on cell membrane permeability

Cell membrane is an important structure of cells. 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 through allelopathy, reduce its selective permeability function, and make algae 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. Fig.4 shows the effect of the two algal inhibitors on the protein

and nucleic acid content of *M. aeruginosa*. There are the death of some the algae in the experimental process, although in a relatively low range, the proportion is relatively high in the early stage and relatively low in the later stage (Zhou et al. 2009). The content of protein and nucleic acid in the blank control group first decreased and then increased. It was apparent that the contents of protein and nucleic acid in algal solution increased significantly after adding the two kinds of algal inhibitors, and the contents of protein and nucleic acid in ASGs group were higher in the early stage of the experiment. In the middle and later period, it was the opposite. Obviously, the damage effect of ASGs on cell membrane was more rapid and obvious in the early stage, and the ASMs were more dominant in the middle and later stage, which was consistent with the inhibition law of the two algal inhibitors. Therefore, the above results showed that both algal cell membranes were seriously damaged under the continuous stress of these two algal inhibitors, and the decrease of membrane selective permeability resulted in the gradual overflow of protein and nucleic acid from the algae cells to the outside of the cells; during the whole experimental period, the degree damage of algal cells increased with the passage of time, and the damage of ASMs on the cell membrane was more effective and lasting.

Fig 4 Effects of ASGs and ASMs on protein content and nucleic acid content of *M. aeruginosa*.

Fig 5 Effects of ASGs and ASMs on conductivity of *M. aeruginosa*.

Fig 5 shows the change in electrical conductivity under the action of the two kinds of artemisinin sustained-release algal inhibitors. In general, the electrical conductivity of the blank control group did not fluctuate significantly, but increased after adding the two kinds of sustained-release algal inhibitors. On the 2nd day, the conductivity of algal solution was higher than that of blank control group under the action of the two kinds of sustained-release algae inhibitors. After the 7th day, the conductivity of algal liquid under the action of ASMs was gradually higher than that of ASGs. The results showed that under the continuous action of the two kinds of 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 algae cell to the external environment. These phenomena indicated that artemisinin active substance could cause irreversible damage to cell membrane (Peng et al. 2016), and the damage of ASMs was stronger.

3.5 Oxidative damage

Under the condition of environmental stress, the production and elimination of O_2^- in the algal cell system will be out of balance. A large number of O_2^- 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 damage of cell membrane (Ali et al. 2006). As shown in Fig 6, the contents of O^{2-} and MDA in the blank control group were almost unchanged, which indicated that the algae did not experience membrane lipid peroxidation and grew normally. In addition, the change in trend for O^{2-} and MDA content in the two kinds of artemisinin algal inhibitor groups was basically the same, showing different degrees of upward trend, which indicated that the algae cells experienced different degrees of lipid peroxidation after adding the two kinds of sustained-release algal inhibitors, and the algal cells were damaged in different degrees. Specifically, the increase rate of O^{2-} and MDA content in the ASGs group was higher than that of ASMs group four days prior to the experiment, but it was the opposite after the fifth day. This phenomenon was consistent with the algal inhibitory effect and the above-mentioned law of intracellular solute extravasation. These phenomena all indicated that the algal inhibitory effect of ASGs was better than that of ASMs in a short time while ASMs had better persistent algal inhibitory effect. In addition, it is worth mentioning that, at the point in time when the content of O^{2-} and MDA in the ASMs group increased significantly, than that of the ASGs group, and the content of O^{2-} and MDA in the ASMs group was higher than that of the ASGs group. To some extent, this phenomenon 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 significant.

Fig 6 Effects of ASGs and ASMs on MDA and O₂⁻ content of *M. aeruginosa*.

3.6 Inhibition of antioxidant enzymes activities

Antioxidant system is the protective system of organism against harsh environment. The key antioxidant enzymes are superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) (Gulzar & 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 of H₂O₂ and stabilize the ROS in the organism at a normal level, so as to avoid damage to cells (Tang et al. 2010).

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

The activities of SOD, CAT and POD were measured to evaluate the effect of the two kinds of artemisinin sustained-release algal inhibitor on antioxidant enzymes (Fig.7). The activities of SOD, POD and CAT in the two kinds of 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 the two kinds of artemisinin sustained-release algal inhibitor groups decreased significantly after reaching the maximum value, and the SOD activity was lower than that of the blank control group on the 15th day. The difference was that the SOD activity of ASGs group reached the maximum value on the 5th day, while the ASMs group reached the maximum value on the 10th day. In addition, the decrease of

SOD activity in ASMs group was much greater than in ASGs group. With the stress going on, the content of reactive oxygen species in algal cells increased to exceed the capacity of its enzyme activity system, and the activity of SOD began to decrease until it was inactivated (Hejl &Koster 2004). The activities of POD and CAT reached the maximum on the 5th day, then decreased sharply ($P<0.01$) until beyond the detection range.

SOD can convert $O_2^{\cdot-}$ into H_2O_2 and O_2 specifically to avoid cell injury (Ni et al. 2018). This can also explain why the rate of $O_2^{\cdot-}$ production is low in the initial stage. CAT and POD can remove excessive hydrogen peroxide in algae 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 time of inhibition, antioxidant enzymes were gradually inactivated, resulting in damage of algal cells, thus inhibiting the growth of algae (Li et al. 2013).

4. Conclusions

In summary, the algal inhibitory effect of the two kinds of artemisinin sustained-release algal inhibitors were both significant. The results showed that the optimal concentration of ASMs was 0.2 g L^{-1} , and the inhibition rate was as high as 99% on the 10th day. Under the optimal conditions, the algal inhibitory effect of ASMs with small particle size was better than that of ASGs on the whole, and the ASMs had significant continuous 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 reducing chlorophyll-a content, causing oxidative damage, inhibiting antioxidant enzyme activity and destroying cell membrane. The decrease in chlorophyll-a content in the process of inhibition indicated that both algal inhibitors could inhibit the photosynthesis of algal cells. Under the stress of algal inhibitor, the algal cells were stimulated to produce a large amount of $O_2^{\cdot-}$, 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^{2-} can induce the increase of SOD, POD and CAT activities. With the prolongation of inhibition time, ROS produced exceeded its own resistance ability, and finally 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. The results of this research when compared with the previous research, showed that ASMs had more significant anti algae effect, and the preparation method was more environmentally friendly, and the economic cost was greatly reduced, which provided unlimited possibility for the application of ASMs in controlling red tide in natural lakes in the future.

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Declarations

● Ethics approval and consent to participate

Not applicable

● Consent for publication

Not applicable

● Availability of data and material

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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The authors declare that they have no competing interests.

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