Self-floating Capsule of Algicidal Bacteria *Bacillus* sp. HL and its Performance in the Dissolution of *Microcystis Aeruginosa*

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Research Article

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Abstract

Algicidal bacteria is known as efficient and environmentally friendly in treating *Microcystis aeruginosa* (*M. aeruginosa*). However, the practical application of algicidal bacteria in the natural water is limited by the interference of external factors and the low reuse capability. In this study, a biodegradation capsule for *M. aeruginosa* is prepared by biocompatible sodium alginate (SA) compositing with eco-friendly ethyl cellulose (EC). Bacterial strain HL was immobilized and the capsule was obtained under optimal usage concentrations of SA, Calcium chloride ($CaCl_2$) and EC at 2%, 3% and 3%. It has been observed that capsules immobilizing bacteria HL shows considerable advantages over traditional bio-treatment systems (free-living bacteria) and good reusability performance. A better dissolution rate of 77.67% ± 1.14% on the 7th day was obtained with the embedding of algicidal bacteria at 50 mL, which enhanced algae dissolution rate by 11.05% compared with free-living bacteria, and the dissolution rate for *M. aeruginosa* still reaches 68.57% ± 2.88% after three times repetitive use. Algicidal bacteria capsules were examined on the fluorescence and antioxidant system of *M. aeruginosa*. It was indicated that photosynthetic mechanisms of *M. aeruginosa* were destroyed, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) were all significantly induced as antioxidant response, and malondialdehyde (MDA) content increased. Overall, capsules prepared in this study can provide a desirable environment for algicidal bacteria HL and ensure algicidal bacteria to in-situ work well in the inhibition of algae.

Highlights

- A high-efficient biodegradation capsule for *Microcystis aeruginosa* is prepared.
- Algicidal bacteria HL is well retained in capsules.
- Capsules suppressed growth of *Microcystis aeruginosa*.
- Photosynthetic activities are inhibited.

Introduction

Water eutrophication aroused by the excessive discharge of nutrients and phosphorus has resulted in the overgrowth of harmful algal blooms (HABs) worldwide (Zhang et al. 2021a). *M. aeruginosa*, one kind of the cyanobacteria (Schmidt et al. 2020), not only influences negatively the water ecosystem (Lu et al. 2021b) but also produces toxic microcystins (MCs) (Zhang et al. 2020), which can pose toxic effect on liver, nervous and genital system (Zhang et al., 2020a). Up to now, many strategies have been applied to control *M. aeruginosa*, such as photocatalysis (Fan et al. 2021, Wang et al. 2020), hydrogen peroxide (Wang et al. 2019), copper sulfate (Anderson 2009), etc. Though these methods are fast and effective in the inhibition of *M. aeruginosa*, the potential threat to aquatic environment and the secondary pollution limit their large-scale application (Zhu et al. 2020). Currently, an enormous amount of effort has been devoted to developing a biological way to inhibiting the algae, different kinds of the bacteria, which are able to dissociate the algae, are found and applied to control *M. aeruginosa*. The reported algicidal bacteria species involved *Bacillus* sp. (Xuan et al. 2017), *Acinetobacter* (Yi et al. 2015), *Streptomyces* (Yu et al. 2019), etc. It was reported that *M. aeruginosa* could be inhibited by algicidal bacteria through direct feeding or secreting algicidal compounds and they can have a substantial effect on *M. aeruginosa* (Lu et al. 2021a).

The preparation of microbial inhibition reagents for the algae is usually conducted by gathering algicidal bacteria from water, soil and organisms, then enriching and culturing process. Thus,

most of current microbial algal inhibition reagents included algicidal bacteria and culture medium (Li et al. 2021).

Usually, microbial algal inhibition reagents are used by directly pouring in natural water, algicidal bacteria were then released and dispersed, which could inhibit HABs effectively. However, the environment and conditions for algicidal

bacteria, including temperature, water velocity and natural enemies, are complex. Especially, it is hard to ensure algicidal bacteria to be the predominant bacterial population in natural micro-ecosystem, and may disappear due to intense competition between them. Giving a desirable community environment for algicidal bacteria is thus essential to keep the activity and effect of microbial inhibition reagents.

Capsule immobilization of algicidal bacteria is a potential solution (Hu et al. 2020, Ma et al. 2021, Wang &Coyne 2020), which can provide favorable environment for algicidal bacteria and improve the algicidal effect. For example, *Shewanella* sp. IRI-160 was immobilized into several carriers (Agarose, alginate hydrogel, cellulosic sponge, and polyester foam) and used in inhabiting harmful dinoflagellates, a higher activity against the target species compared to free-living bacteria (Wang &Coyne 2020) was obtained. It is considered that a suitable immobilized carrier material is critical for keeping the activity of immobilized bacteria (Chen et al. 2013). Sodium alginate (SA), with good biocompatibility and adhesion property (Bennacef et al. 2021), is the most used in loading material because SA cross-links with divalent cations to form the hydrogel (Khalid et al. 2018, Rybak 2021, Yerramathi et al. 2021). It is widely used in medical pharmaceutical (Zhang et al. 2021b), food production (Yan et al. 2021) and microbial composite (Yamaguchi et al. 2019). It has been shown that the high-density of *M. aeruginosa* is upper water (Aparicio Medrano et al. 2013), and keeping microbial inhibition reagents enriching in upper water can take fully advantage of algicidal bacteria. It is assumed that SA was employed as the loaded material for greater biocompatibility and environmental safety to combine the gathered algicidal bacteria and form the capsule. Furthermore, ethyl cellulose (EC) was insoluble in water and exhibits good chemical, physical and mechanical properties (Lin et al. 2018). EC was chosen to coat on the surface of the capsule to keep the capsule floating on the water. It is supposed that algicidal bacteria enriching in the floating capsule structure is a feasible approach to provide a desirable microbial population.

In current study, algicidal bacteria HL, was merged by the protoplast fusion, exhibited a better performance in the inhibition of *M. aeruginosa*. The algicidal bacteria HL was mixed with SA to prepare the capsule, and EC was coated on the surface of the capsule to keep floating. Scanning electron microscope (SEM) was used to characterize the morphology of algicidal bacteria HL in the capsule. The algicidal efficiency of prepared capsules on algae cells and chlorophyll-a was evaluated and compared with free-living bacteria. The algae inhibition process of capsules was speculated by the changes in algal fluorescence and oxidative stress biomarkers in *M. aeruginosa*. This study can provide an effective approach for improving the efficiency of algicidal bacteria in the microbial treatment of HABs.

Materials And Methods

2.1. Experimental algae and bacteria

M. aeruginosa (No. FACHB-905) was purchased from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The detailed cultivation steps are described in Text S1. *Bacillus* sp. HL, formed by the protoplast fusion of algicidal bacteria R1 and denitrifying phosphorous accumulating bacteria B8. The detailed cultivation steps and identity information are described in Figure S1 and Table S1-S2.

2.2. Preparation of algicidal bacteria capsule

Chemical grade SA (CAS 9005-38-3), chemical grade EC (CAS 9004-57-3), analytical grade $CaCl_2$ (CAS 10043-52-4) were purchased from Sinopharm Chemical Reagent Corporation (Shanghai, China). SA was dissolved in deionized water and stirred at 1500 revolutions per minute (rpm) in the constant-temperature magnetic agitator (HJ-6A, China) at 25 ℃ to prepare 2% (w/v) SA solution. For polymer coating, 3% (w/v) of EC solution with anhydrous ethanol. The curing agent was prepared by dissolving 3% (w/v) $CaCl_2$ solution in deionized water.

According to the growth curve of algicidal bacteria HL, HL was cultured in beef-protein medium for 12 h at the late logarithmic growth stage and cultures were diluted with beef-protein solid medium to OD_{600} of 1.5 before use. The supernatant was discarded after centrifugation of the diluted culture, and the bacterial suspension were obtained by the addition of deionized water. The prepared bacterial suspension was mixed with SA solution, and stirred by the magnetic mixer at 750 rpm. SA capsules were prepared by the dropping injection of the obtained mixing slurry. The mixed slurry was poured into the constant pressure hopper (The distance between the exit and the curing agent is set in 10 cm) to obtain spherical capsules. The spherical capsules were remained in the curing agent for 25 min to ensure gel reaction occur completely, and then filtered by strainer, washed with deionized water for three times and dried at 35 ℃.

The dried capsules were placed in the EC solution and stirred for 25 min, and filtered, were further washed by anhydrous ethanol and deionized water for three times. The prepared capsules were dried in the oven at 35 ℃ and finally stored in the refrigerator at 4 ℃.

2.3. Factors affecting performance of algicidal bacteria capsule

SA concentration was set in 0.5, 1, 1.5, 2, 2.5, 3, 3.5% (w/v). Both $CaCl_2$ and EC concentration were examined by 1, 2, 3, 4, 5% (w/v). 50 mL of the bacterial suspension was mixed with different concentrations solutions of SA, $CaCl_2$ and EC to prepare capsules with same method. The prepared capsules were introduced to examine the influences of SA, $CaCl_2$ and EC concentrations on and 7-day algae dissolution rate of *M. aeruginosa.* The concentrations of the above embedding bacterial suspensions were 50 mL and capsules prepared above were placed in *M. aeruginosa* to determine the floating performance and 7-day algae dissolution rate.

Floating performance was measured by counting in the algae solution using Eq. (1). 1 ± 0.001g capsules were placed in 0.1 mol·L^{-1} sodium citrate solution at pH 7.8 (Yang &Wang 2019) to crush the capsules, and releasing numbers of algicidal bacteria HL in the solution were counted by the coating plate dilution method using Eq. (2).

$$\text{Floating performance} = \frac{\text{Numbers of floating particles}}{\text{Total EquationNumbers of capsules}} \times 100\%$$

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$$\text{Embedding rate} = \frac{\text{Numbers of bacteria initially added -Numbers of bacteria in the supernatant}}{\text{Number of bacteria initially added}} \times 100\%$$

2

2.4. Characterization of algicidal bacteria capsule

The prepared capsules were treated with 2.5% glutaraldehyde solution for 3 h at room temperature and washed with deionized water. Then, the capsules were dipped in ethanol of gradient concentration (50%, 60%, 70%, 80%, 90%) for 10 min each. The morphological feature of the prepared capsules was observed by SEM (Nova Nano 450, USA).

2.5. Determination of algae growth

The growth of *M. aeruginosa* was determined by concentrations of cell density and chlorophyll-a. 500 mL of *M. aeruginosa* at logarithmic growth stage (7.5×10^5 cells·mL^{-1}) were selected for simulating natural water containing algae, and the magnetic stirrer was equipped to simulate the disturbance of the real water body. The concentration of *M. aeruginosa* was measured at OD_{680} every 24 h. The density of algae cells was measured by the optical density method and blood cell counting (Yuan et al. 2020). The cell density was calculated using the following established standard curve: $y=168.16x-1.11$, $R^2=0.99$, where y represents the cell density, 10^5 cells·mL^{-1}, and x represents the absorbance of *M.*

aeruginosa measured at 680 nm. The concentrations of cellular pigments, including chlorophyll-a, were determined according to a previous study (Siedlewicz et al. 2020), as shown in Eq. (3).

$$\text{Chlorophyll-a}(\text{mg}\cdot\text{m}^{-3}) = V_2 * \frac{\left[11.64 * \left(D_{663} - D_{750}\right) - 2.16 * \left(D_{645} - D_{750}\right) + 0.10 * \left(D_{630} - D_{750}\right)\right]}{V_1 * \delta} \quad (3)$$

where D_{630}, D_{645}, D_{663} and D_{750} represent the absorbance values of the extract at 630, 645, 663 and 750 nm, respectively; V_1 represents the volume of sample, namely algal fluid volume, mL; V_2 represents extract liquid product, namely the volume of 90% ethanol, 8 mL; δ represents light path of the colorimetric dish, 1 cm.

The formula for the inhibition rate of *M. aeruginosa* was calculated using the formula of $IR = (1 - N_0\text{-}N/N_0) \times 100\%$, where N represents the cell density of the experimental group, cells·mL^{-1}, N_0 represents the cell density of the control group, cells·mL^{-1}, and IR represents the inhibition ratio, %.

2.6. Determination of algal fluorescence

The algal fluorescence intensity was measured using flow cytometry (BD Accuri^{TM}C6 plus, USA), with an argon excitation laser (15 mW, 488 nm). Capsules were co-cultured in algal solution and BG11 medium was used as the control. On the 7th day, the experimental and control group were added with Propidium iodide (PI, Solarbio-C0080, China) solution and in complete darkness at room temperature for 30 min. PI is a fluorescent dye that binds only to DNA in dead or membrane-ruptured cells (Gumbo et al. 2014, Xiao et al. 2011). The fluorescence detection wavelength was set at 560–590 nm. PI fluorescence was collected by channel FL2 to detect cell membrane integrity, phycoerythrin (PC) fluorescence was collected by channel FL4, the fluorescence wavelength was greater than 630 nm, and chlorophyll-a fluorescence was collected by channel FL3. The obtained data were analyzed using flowjo_v10.

2.7. Determination of oxidative stress biomarkers

Algae can enhance the antioxidant effect through the antioxidant enzyme system and improve the resistance of the organism to adversity (Li et al. 2020). The damage degree of algae cells at the logarithmic growth stage (7.5×10^5 cells·mL^{-1}) at 1th, 4th, 7th day with capsules containing different concentrations of bacterial suspension (50 mL, 100 mL and 150 mL) was analyzed. The control group was set up by capsules without the doping of algicidal bacteria HL. Enzyme activity of the capsules treated algae cells was determined by measuring contents of SOD, POD, CAT and MDA using the corresponding chemical assay kit (Nanjing Jiancheng Institute of Biological Engineering, China).

2.8. Reusability

The reusability test (three consecutive cycle) of capsules is conducted by placing capsules into *M. aeruginosa*, effect of capsules was accessed by determining the concentration of *M. aeruginosa. The* capsules were taken out every cycle and dried for next cycle experiment.

2.9. Statistical analysis

All data in this study were obtained by three replicates and expressed as mean ± standard deviation ($n = 3$). One-way analysis of variance (ANOVA), and differences were conducted through SPSS 22 (IBM, USA), with statistically significant when $P < 0.05$.

Results And Discussion

3.1. Optimization of factors affecting the performance of capsules

SA cross-linked with Ca^{2+} to form calcium alginate gelation, in which Na^{+} are replaced by Ca^{2+} (Eq. 4), and the egg-box model formed by combining two antiparallel chains of polyuronates with Ca^{2+} (Fig. 1). Usually, a large amount of free Ca^{2+} is still absorbed on the prepared SA capsules surface when the capsules are immersed into the EC solution, and the oxyethyl in EC is coated on the surface of SA capsules due to the electrostatic force. Moreover, part of hydroxyl groups in EC could interact with carboxyl groups in SA and form hydrogen bonds (Zhao et al. 2021). To ensure the floating performance and 7-day algae dissolution rate of capsules, the usage concentrations of SA, $CaCl_2$ and EC during the preparation process are need to be optimized.

$$2\mathrm{Na(Alginate)}+\mathrm{Ca}^{2+}\rightarrow\mathrm{Ca(Alginate)}_2+2\mathrm{Na}^{+}$$

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3.1.1. Effect of SA concentration

Figure 2a represents 7-day algae dissolution and floating performance of capsules with different usage concentrations of SA. When the SA concentration was increased to 2 g 100 mL^{-1}, 7-day algae dissolution and floating performance of capsules were significantly improved ($P < 0.05$). The highest 7-day algae dissolution activity of 73.84% ± 0.90% and floating performance of 86.77% ± 0.85% were observed with the introduction of SA at the concentration of 2 g 100 mL^{-1} ($P < 0.05$). With further increase in the SA usage concentration of 3.5%, 7-day algae dissolution activity decreased to 61.53% ± 1.26% and floating performance decreased to 53.43 ± 1.22%. 2 g 100 mL^{-1} was thus selected as the optimal usage concentration of SA for the preparation of capsules. It is reported that the gelation can be impacted by the usage concentration of SA (Guo et al. 2021). The higher concentration of SA (2.5–3.5 g 100 mL^{-1}) can enhance the viscosity of gel and the strength of capsules, which lowers pore size of capsules and improves immobilization efficiency. However, a denser capsule is also not favored to promoting the degradation efficiency of *M. aeruginosa*, because the diffusion and delivery of *M. aeruginosa* from outside of the capsules to inside is suppressed due to lower porosity. However, the lower usage concentrations of 0.5–1.5 g 100 mL^{-1} for SA would result in the fragile and loose capsules, which also negatively reduced the floatability of capsules.

3.1.2. Effect of $CaCl_2$ concentration

The type and usage concentration of the curing agent affect the stability and activity of immobilized algicidal bacteria (Sarma &Pakshirajan 2011). In this study, $CaCl_2$ was selected as the cross-link to promote the formation of capsule gel components. 7-day degradation efficiency of *M. aeruginosa* and floating performance of capsules with 1–5% $CaCl_2$ were evaluated and in Fig. 2b. It was observed that the maximum 7-day algae dissolution rate of 71.09% ± 0.79% and floating performance of 81.53% ± 0.76% ($P < 0.05$) with the use of 3 g 100 mL^{-1} of $CaCl_2$. The amount of Ca^{2+} was not enough to cause crosslinking reaction and combine with antiparallel chains at low concentrations of 1–2 g 100 mL^{-1}, which led to a poor embedding of algicidal bacteria (42.90% ± 2.64%, 50.68% ± 0.96%) and the fragile structure of capsules. Gelling process usually starts from outer towards inner core of capsules (Bennacef et al. 2021). The increase in the usage concentration of Ca^{2+} promotes cross-linking gelation rate and faster diffusion of Ca^{2+} in gels, which improves immobilization efficiency and the tightness of capsules. On the contrary, SA was fully cross-linked with Ca^{2+} when the

concentration increased to 4–5 g 100 mL^{-1}, which led to a reduction in porosity and the over-sealing internal structure of capsules.

3.1.3. Effect of EC concentration

1–5% of EC was coated on the surface of SA capsules, and the 7-day degradation efficiency of *M. aeruginosa* and floating performance of capsules were shown in Fig. 2c. It was observed that 68.33% ± 1.06% of *M. aeruginosa* was dissociated with the use of EC at the concentration of 1 g 100 mL^{-1}. As EC concentration increased to 2–3 g 100 mL^{-1}, algae dissolution rate was promoted to 70.39% ± 2.22% and 73.45% ± 0.24%, respectively. With further increase in concentration of EC to 5 g 100 mL^{-1}, a sharp decline to 28.58% ± 0.78% was observed. The floating rate increased with the increase in EC concentration, and floating performance was significantly affected when the usage concentration of EC was at 2–5 g 100 mL^{-1} ($P < 0.05$). 3 g 100 mL^{-1} of EC was considered as the suitable concentration for capsule coating. The coating concentration of EC is related with the mechanical strength and biological activity of capsules. Poor surface coating resulted in the sinking of the capsule and excessive coating of EC declined size of pores in capsules, which were not favored to promoting degradation efficiency of capsules for *M. aeruginosa*.

3.2. Characterization of algicidal bacteria capsule

The optimal preparation conditions of algicidal bacteria capsules were set in SA, $CaCl_2$, EC usage concentrations of 2%, 3%, 3%, respectively. When bacterial suspension was 150 mL, 87.73% ± 0.21% of capsules is floated and 55.25% ± 2.78% of algicidal bacteria HL is embedded in capsules. Photos and SEM of algicidal bacteria capsules were depicted in Fig. 3. The appearance of prepared algicidal bacteria capsules was white and spherical. The micro surface of algicidal bacteria capsules was rough and porous (Fig. 3c-d). The porous gaps were favored to cell adhesion and the nutrient diffusion. The morphology of algicidal bacteria HL with rod shaped and a length of about 2 μm was observed. It is clearly showed aggregated rod-shaped bacterial cells were immobilized inside capsules.

3.3. Effects of algicidal bacteria capsule on the growth of M. aeruginosa

3.3.1 Algae cell

Figure 4a shows effects of algicidal bacteria capsules with different embedding concentrations of 50 mL, 100 mL, 150 mL compared with free-living bacteria on algae cells (7.5×10^5 cells·mL^{-1}) for seven days. During the experiment, capsules with all concentrations of algicidal bacteria HL can inhibit the growth of *M. aeruginosa* compared with free bacteria group. A better dissolution rate of 87.02% ± 0.67% than other concentrations at all stages ($P < 0.05$) at 7th day was obtained with the embedding of algicidal bacteria at 150 mL, which enhanced algae dissolution rate by 20.03% compared with free-living bacteria. It was found that 7-day algae dissolution rate though with lower embedding concentration of 50 mL still could reach 77.67% ± 1.14%, and algae dissolution rate was promoted by 11.05% comparing with free-living bacteria. These results indicated that capsules could provide a safe and stable environment for algicidal bacteria HL and algae dissolution rate is not impacted by environment factors.

3.3.2 Chlorophyll-a

Chlorophyll-a is an important indicator to reflect growth status of algae. Chlorophyll-a can bind with proteins through noncovalent bonds and deliver light to the central pigment in the reaction by inducing resonance (Zhang et al. 2013). Photosynthetic pigment destruction hinders photosynthesis of algae and inhibits the normal growth of algae. Figure 4

showed influences of different concentrations of algicidal bacteria (50 mL, 100 mL and 150 mL) on algae dissolution and chlorophyll-a. There was a dramatic increase in the concentration of chlorophyll-a in control group. The concentration of chlorophyll-a declined at 5th day as the introduction of different concentrations of algicidal bacteria. The content of 7-day Chlorophyll-a with the addition of 150 mL embedding algicidal bacteria was 764.01 ± 162.83 $mg{\cdot}m^{-3}$, and the best algae dissolution rate was 88.54% ± 1.23%, which was higher than free-living bacteria by 14.72%. The change in the concentration of chlorophyll-a was consistent with algae cell. The above results indicated that capsules were more effective in improving algae dissolution.

3.4. Effects of algicidal bacteria capsule on the algal fluorescence

Figure 5 shows the two-dimensional distribution point diagram of PI/chlorophyll-a fluorescence before and after the use of capsules with flow cytometry. In Fig. 5a-b, Q1 region is assigned to PI − / chlorophyll-a + cells, which indicates the cell membrane of *M. aeruginosa* is intact and in regular physiological state. Q2 region represents PI + / chlorophyll-a + cells, which suggests that though the appearance of algae cells may be same as regular cells, the cell membrane of *M. aeruginosa* is damaged. Q3 region represents PI + / chlorophyll-a − cells, is usually considered as death cells. Q4 region represents PI − / chlorophyll-a − cells, is usually assigned as double negative cells, which indicates most algal cells are dissociated. The dead algae cells refuse to dye PI because the DNA / RNA that can bind to PI in cells is destroyed. It is believed that no PI fluorescence is detected and chlorophyll-a fluorescence is strong in Q1 zone, and both PI and chlorophyll-a fluorescence are detected. PI fluorescence is strongly appeared in Q3 zone while chlorophyll-a fluorescence is absent. There is no PI fluorescence and chlorophyll-a fluorescence detected in Q4 zone. 91.0%, 0.25%, 0.39% and 8.39% of *M. aeruginosa* in the control group were live, cell debris, dead and selective permeabillity, which were displayed in the Q1, Q2, Q3 and Q4 zones. Most dots shift left remarkably, for example, 28.4% and 64.5% of cells shifted to Q2 and Q3 zones, which suggested that the live cells population of *M. aeruginosa* decreased with the application of capsules. Figure 5c-d and e-f presents chlorophyll-a and PC fluorescence of control and capsules respectively. Similar population shifting after treating with capsules (Hadjoudja et al. 2009). As the working of capsules, the fore peak of chlorophyll-a and PC fluorescence appeared and back peak decreased, suggesting that algae cells were destroyed and even scrapped.

3.5. Effects of algicidal bacteria capsule on the antioxidant system of M. aeruginosa

Antioxidant reaction is crucial for algae to resist interference from the external environment (Zhao et al. 2020). Effects of capsules on the antioxidant system of algae is helpful to reveal the process of algicidal bacteria capsules dissociating *M. aeruginosa*. Figure 6 shows influences of different embedding algicidal bacteri*a* HL concentrations (50 mL, 100 mL, 150 mL) on the antioxidant enzyme system of *M. aeruginosa* by accessing activities of SOD, POD, CAT and MDA on the 1th day, 4th day and 7th day.

Exogenous substance induces increase in various antioxidant active enzymes remove the harmful components and strengthen the defense function of algae. On the 1th and 4th of the experiment, SOD, POD and CAT show the similar tendency, which significantly increased compared with control group ($P < 0.05$, Fig. 6a-c), and the highest SOD, POD and CAT activity was observed at the 4th day with the treatment of 150 mL algicidal bacteria HL embedding capsules group. While the decreases in SOD, POD and CAT in the late period of the experiment, SOD, POD and CAT decreased to varying degrees, which also confirmed that the antioxidant enzyme system of algae had been greatly traumatized or even incapacitated with the usage of capsules. This result is similar to previous experiments (de la Rosa et al. 2020), in which SOD increases significantly initially by exogenous substances, while SOD content will decrease when it exceeds the threshold value. SOD is generally considered as the first barrier of the antioxidant system in algae cells and deal with the excess superoxide and convert it into H_2O_2 in order to avoiding the damage of reactive oxygen species (ROS) and

superoxide (Yu et al. 2021). However, excessive H_2O_2 produced by the reaction of SOD is highly toxic to algae cells, stimulating the algae cells to produce a large amount of POD and CAT to assist SOD defense and decompose H_2O_2 into H_2O and O_2. It can be found that capsules caused the huge damage to *M. aeruginosa* through the embedding of algicidal bacteria, and algae cells are faced with remarkable exogenous environmental pressure.

MDA is often recognized as an indicator of the degree of membrane oxidative damage. During the experiment, MDA content in all treatment groups were significantly higher than in the control group (Fig. 6d, $P < 0.05$), which indicated that oxidative stress reactions occurred in *M. aeruginosa* after the treatment of capsules. The significant increase for MDA in the 4th and 7th days indicated that the degree of lipid peroxidation of algae was deeper. The disruptive membrane system stability of *M. aeruginosa* is one of the main mechanisms of damage to algae cells, which indicated that free radical formation and removal of dynamic balance is disrupted, the excess free radicals play a destructive role in algae cell macromolecules, decreasing their unsaturation, membrane fluidity and increasing membrane permeability (Liu et al. 2020).

3.6. Reusability

The reusability of capsules is also important during practical applications. Therefore, the stability of algicidal bacteria capsules (50 mL) were evaluated by three repetitive recycle experiments and results were represented in Fig. 7. The algicidal bacteria capsules after the experiment were recovered and immersed in deionized water to remove residual *M. aeruginosa*. The dissolution efficiency of *M. aeruginosa* by algicidal bacteria capsules declined with the increase in the use time, which can be attributed to the pore block and structure collapse of capsules (Daâssi et al. 2014). Even so, three repeated experimental results demonstrated that the dissolution efficiency of *M. aeruginosa* by algicidal bacteria capsules was still higher than 68%, which is just reduced by 10% than first time. It is considered that the prepared capsules are stable in the dissolution efficiency of *M. aeruginosa*, which suggests that the capsules can be recovered and be used repetitively. The prepared capsules in this study can be used in the control algae in summer by throwing into the lake or rivers at June and July. The capsules in the lakes or rivers cannot be salvaged in the next two or three months. Most of algicidal bacteria can remain and survive due to desirable environment and popular community in the capsules, and play significant role in the dissolution of algae for ages. Thus, algae blooms in summer can be effectively inhibited by throwing capsules in the target waters, and capsules can be salvaged over the summer and reused next year.

Conclusions

In this study, the capsule was prepared by sodium alginate mixing with algicidal bacteria well and ethyl cellulose to protect bacteria from leaking and from environmental change. Experiments results demonstrated good retention of *Bacillus* sp. HL in SA-EC capsules. With the optimal concentrations of SA, $CaCl_2$, EC (w/v) and bacterial suspension at 2%, 3%, 3% and 150 mL, the floating and embedding rate of capsules were 87.73% ± 0.21% and 55.25% ± 2.78%, respectively. In the simulated treatment of *M. aeruginosa*, capsules were significantly more effective than free-living bacteria in dissolution of both algal cells and chlorophyll-a. Moreover, though the capsules are reused repetitively for 3 times, more than 68% of *M. aeruginosa* biodegradation rate are still obtained. The results of algal fluorescence indicated that the photosynthetic mechanisms of *M. aeruginosa* were significantly inhibited by algicidal bacteria capsules. The synchronous increase in content of SOD, POD and CAT indicated that capsules could cause oxidative stress for *M. aeruginosa*, and significant increases in MDA content, suggesting ROS were not completely removed in a short time and lipid peroxidation damage occurred. Overall, this study revealed that capsules immobilized algicidal bacteria HL may be an environmentally friendly and reusable approach for the control of *M. aeruginosa*.

Declarations

Data availability

The datasets used and/or analysed during the current study may be made available from the corresponding author on reasonable request.

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Contributions

Jinjie Huang: Writing and editing, Data curation. **Wenyi Zhang:** Investigation, Methodology, Funding acquisition. **Mingchen Xu:** Conceptualization, Formal analysis. **Linqiang Mao:** Writing and editing, Visualization, Funding acquisition.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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Figures

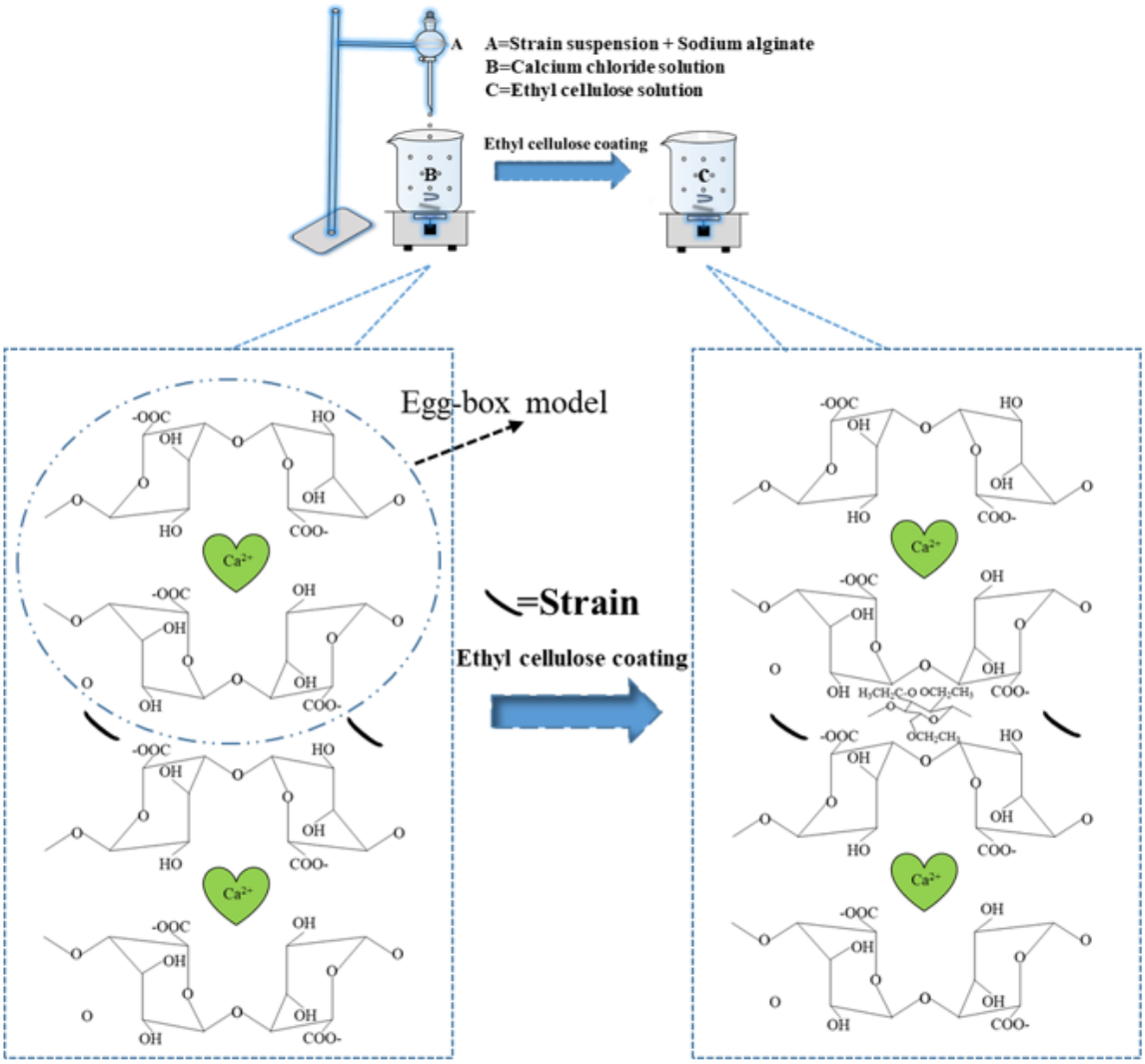

Figure 1

Preparation procedure and mechanism of algicidal bacteria capsule

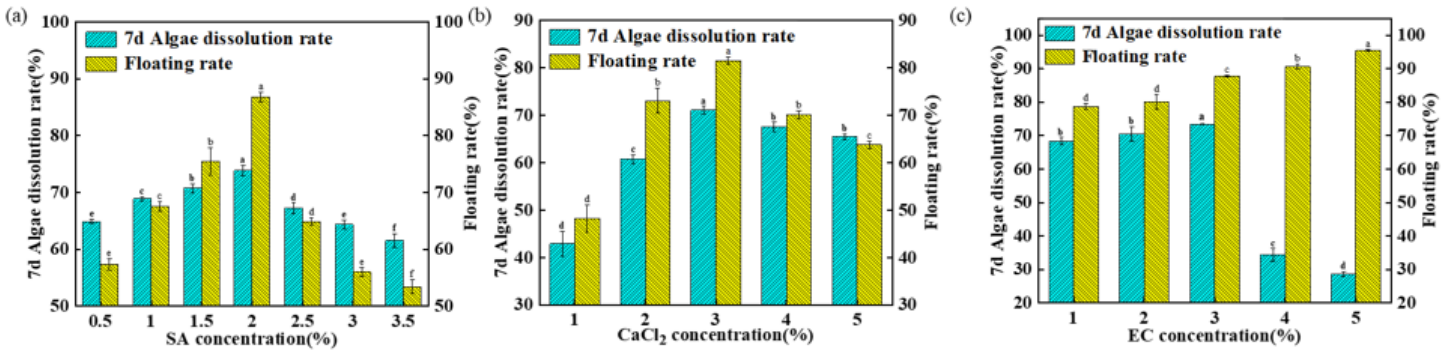

Figure 2

Effects of (a) SA, (b) CaCl2, (c) EC usage concentrations on the 7th algae dissolution rate and floating performance of capsules

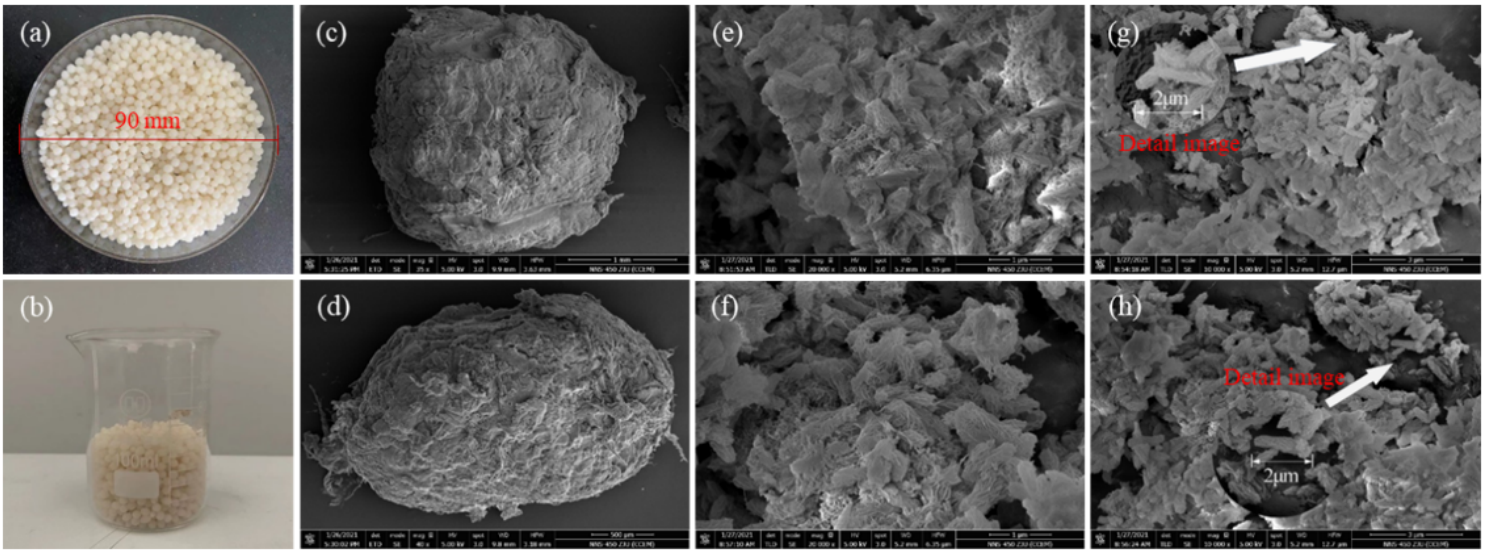

Figure 3

(a-b) Photographs of capsules, (c-d) surface of capsules, (e-h) algicidal bacteria HL inside capsules.

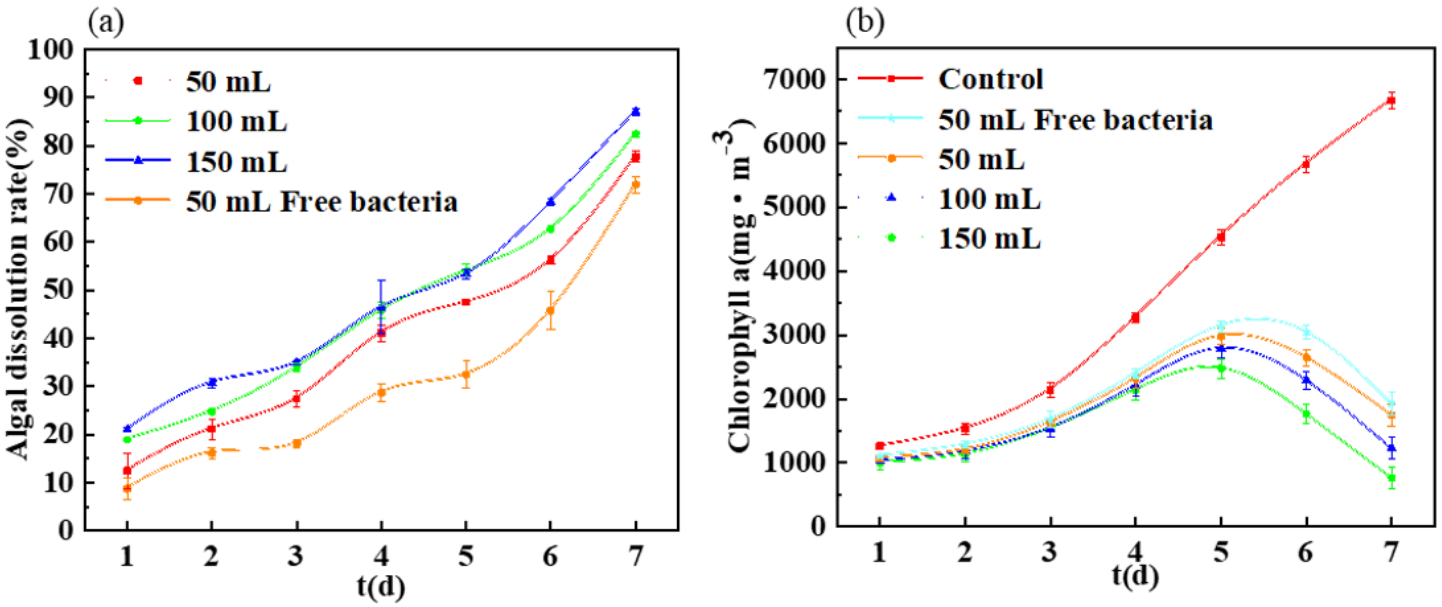

Figure 4

(a) Algae dissolution rate and (b) chlorophyll-a with the addition of 50 mL, 100 mL, 150 mL algicidal bacteria HL

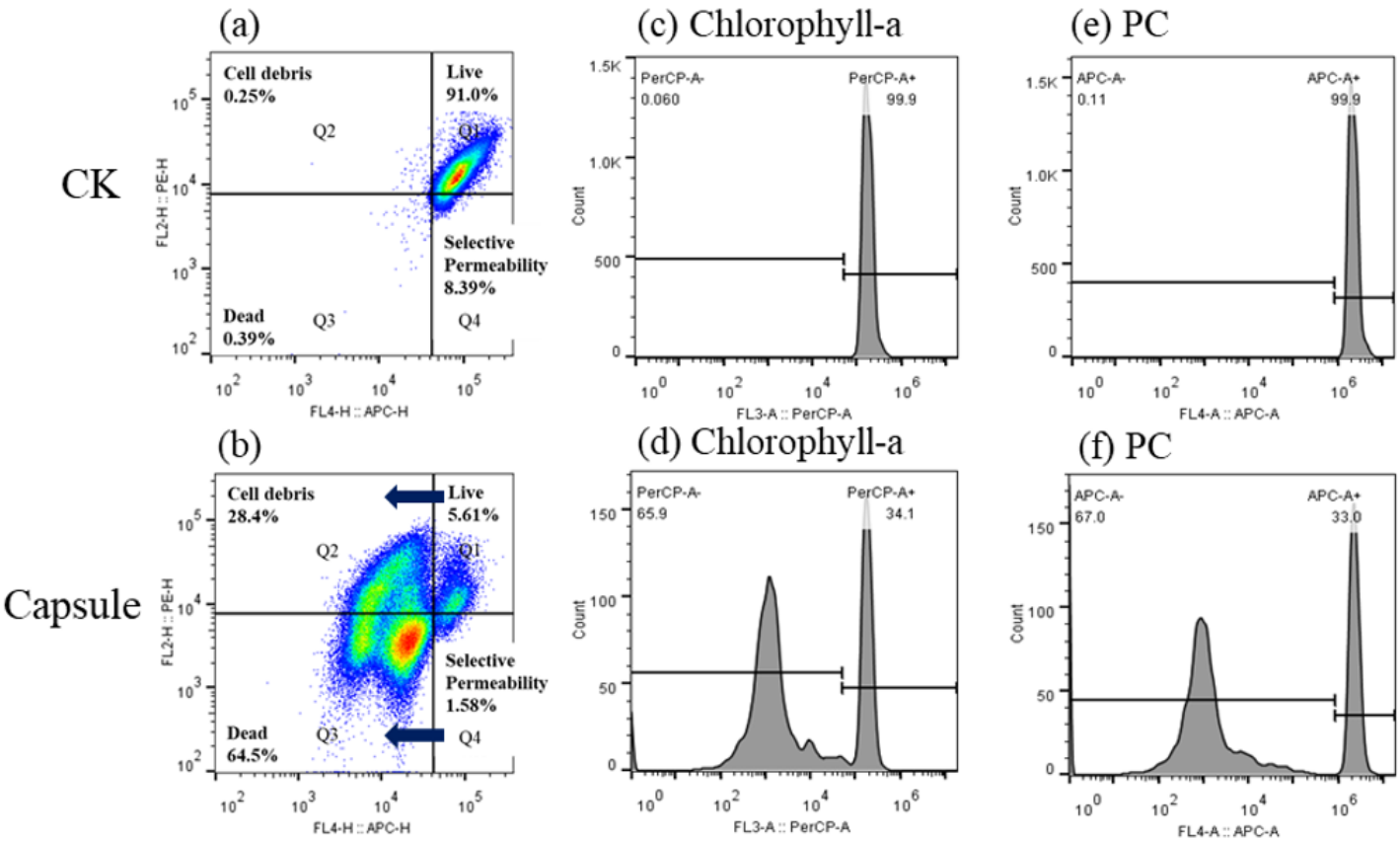

Figure 5

Flow cytometry of M. aeruginosa after treatment with capsule. Histograms of FL3, FL4, (c-d) chlorophyll-a fluorescence, (e-f) PC fluorescence

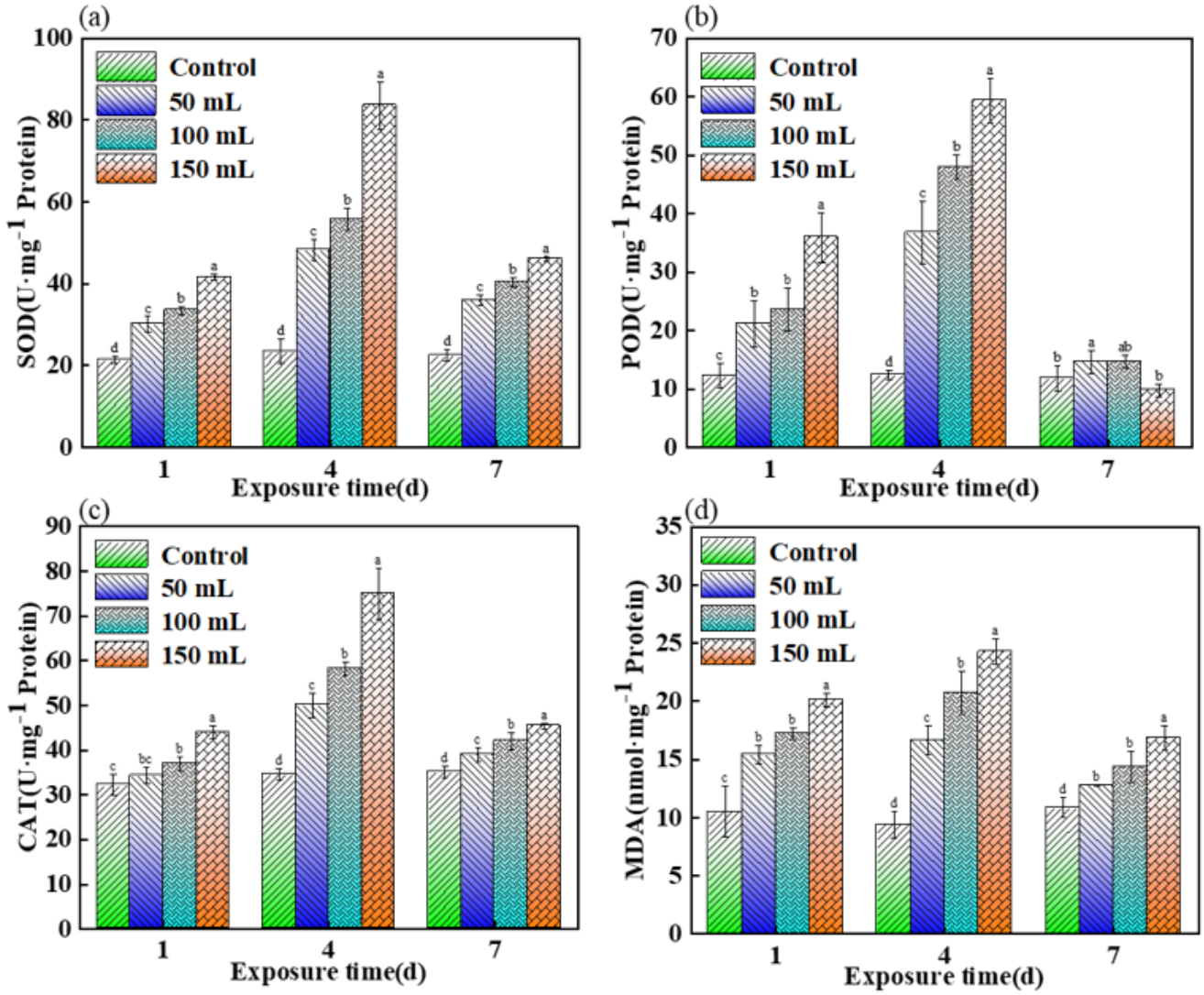

Figure 6

Effects of different embedding algicidal bacteria HL concentrations (50 mL, 100 mL, 150 mL) in capsules on (a) SOD, (b) POD, (c) CAT and (d) MDA of M. aeruginosa

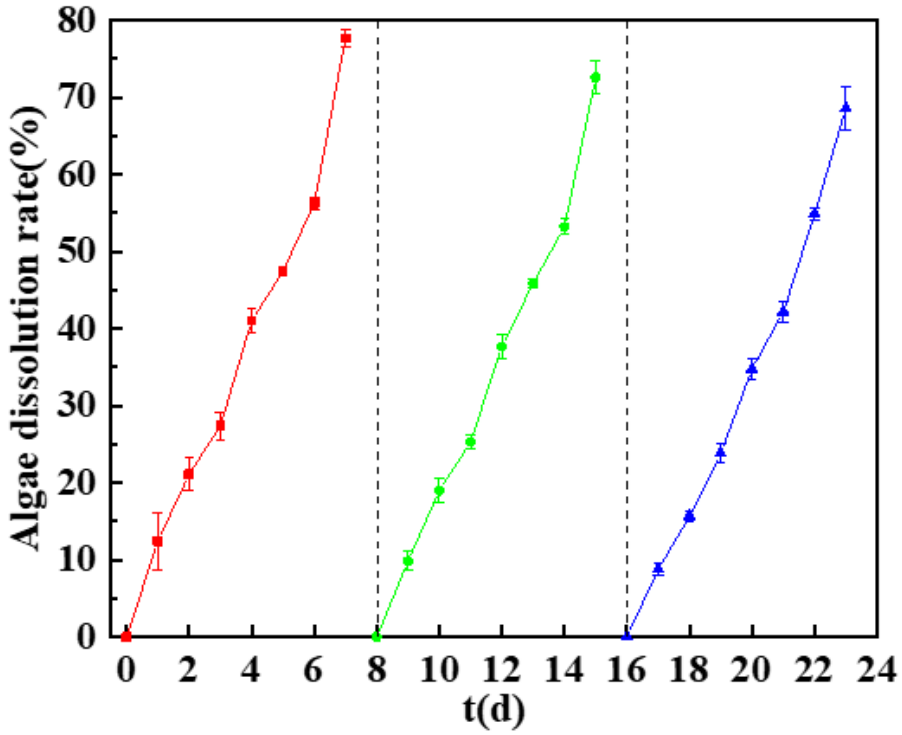

Figure 7

Algae dissolution rates of capsules during three repetitive times

Supplementary Files

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