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# Allelopathic effect of the rice straw aqueous extract on the growth of *Microcystis aeruginosa*

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ABSTRACT

With the exploration and development of the practical technology for prevention and control of harmful algae blooms (HABs), cyanobacterium *Microcystis aeruginosa* (*M. aeruginosa*) blooms have been an urgent problem in the area of water resources protection. Allelopathic algicides are regarded as potential natural resources to constrain HABs for their exclusive and efficient characteristics. Previously, the allelopathic effect of rice has received great attention, but most of them are focused on herbicides to inhibit barnyard grass. In the present study, the effect of algal cells treatment with different concentrations of the rice straw aqueous extract were tested. A continuous culture system was established and the results suggested that the doses of rice straw aqueous extract in the range from 4.0 to 10.0 g $L^{-1}$ observably suppressed the growth of algae cells in a concentration-dependent way. After 9 days of treatment, the algae cells' metabolic activity decayed and the largest inhibition rate could be up to 98%, almost no algae growth was observed. In the case of eliminate the interference of nutrient elements, these results confirmed that rice straw aqueous extract may act as an algistatic agent.

## 1. Introduction

Freshwater eutrophication (water blooms) refers to lake, pond or fen responses to excess organic and mineral nutrients (especially nitrogen and phosphorus) that induce algae and cyanobacteria grow rapidly, cover the water surface flakily and deplete the oxygen supply (Zhu et al., 2014). This issue has caused serious aquatic environmental problems, such as smelly water, fisheries depletion, long-lasting cyanobacteria blooms, decreasing the aesthetic value of landscape water and blocking the water treatment systems, which attracted great concerns all over the world for decades (Lam et al., 1995). Cyanotoxins, a sort of metabolite from algae and cyanobacteria, are potential hazards for public health since these waters are usually supplied for production and daily life after a simple water treatment (Eriksson et al., 1990). Moreover, the tendency of carbon dioxide greenhouse effect is expected to stimulate the growth and proliferation of harmful algae blooms (HABs) (Zhu et al., 2014).

In China, HABs has become a growing serious problem on account of water eutrophication, which can cause the outbreak of HABs during the annual high temperature period within many fresh-water rivers, such as the Chaohu Lake and the Dongting Lake (Hong et al., 2009). *Microcystis aeruginosa* (*M. aeruginosa*), a kind of toxic cyanobacteria, is one of the commonest species in most of the eutrophicated waters in China. This species of algae could also be easily found in eutrophic lakes around the world. Therefore, with the purpose of reduce the harm of algal blooms, various studies had been done in this field, in which many known environmental factors of light intensity, temperature, nutrient, pH, trace elements and other effects on the influence of *M. aeruginosa* growth were involved (Hu et al., 2014; Jianping et al., 2008; Liu et al., 2016).

Many physical, chemical and biological technologies have been applied to prevent the outbreak of the eutrophication of water bodies, including ultrasonic treatment, modified biochar, chemical algicides and invasive aquatic plant (Broekman et al., 2010; Chen and Pan, 2012; Jančula and Maršálek, 2011). Although these methods are useful, they are associated with nonnegligible deficiency, including high costs, difficult to manage and secondary pollution. Additionally, some biological methods, such as algicidal bacteria and exotic algae-cating fishes,

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involve potential biological invasion risks and are lack of technical stability (McComas, 2003). Therefore, allelopathic effects of aquatic macrophytes are considered as environment-friendly and promising alternatives for controlling HABs has received increasing attention.

Numerous plants possessed of algistatic effects on harmful algae have been researched in aquatic ecosystem. However, almost of these reports were focus on the aquatic plants, including *Sagittaria* trifolia, *Myriophyllum verticillatum* and *Hydrilla verticillata* (Hilt et al., 2012; Mulderij et al., 2009; Zhang et al., 2011a). There were few researches on application of allelochemicals from dry plants and terrestrial plants in aquatic environment. The mechanism of algistatic effects from more common and effective allelopathic plants, especially some of the terrestrial plants, should be researched.

Many crops have been reported to be allelopathic towards other crops grown either simultaneously or subsequently (Tang et al., 2008). Barley, as a western diet, the straw part of it was confirmed to control blooms by Europe and America scientists (Wu et al., 2016), but the understanding on antialgal abilities of oriental terrestrial plant was insufficient. The allelopathic effect of rice has received great attention, but most of them are focused on herbicides to inhibit barnyard grass. Rice straw could inhibit the growth of other weeds by over-secretion of allelochemicals (Chung et al., 2001). Some rice varieties release biocidal allelochemicals which might affect major weeds, microbial and pathogenic diversity around rice plants, even soil characteristics.

Recently, a series of studies have been carried out on the allelopathic phenomenon of rice in Asian countries and regions rich in rice, and some encouraging results have been achieved (Carmichael and Boyer, 2016). As potential allelochemicals which could interact with surrounding environment, a large number of compounds , such as phenolic acids, aromatics, terpenes and flavonoids, have been identified in rice root exudates and rice straw aqueous extract (Huang et al., 2008). As these allelopathic interactions might be positive, they can be used as effective contributor for sustainable and eco-friendly agro-production system (Hong et al., 2009). A few rice varieties or rice straws left in the fields after harvesting and release allelochemicals into the fields which suppresses the growth of neighboring or successive crops/plant (Xiao et al., 2010).

Characterized by extensive fertilizer use, rice cultivation might lead to excess organic and mineral nutrients (especially nitrogen and phosphorus) within water and soil in paddy ecosystem (Everall and Lees, 1996). However, very few reports are available concerning the problem of eutrophication in rice fields. Barley straw was applied successfully in field trails (Spencer and Lembi, 2007). Therefore, we consider the successful model of barley straw to test the allelopathy of rice straw on algae. Previous researches have indicated the following four major mechanisms for plant inhibition of *M. aeruginosa* growth, including destruction of the internal structure of algae cells, adverse effects on photosynthesis of algae, the respiration of algae and enzymatic activities (Leu et al., 2002; Li and Hu, 2005; Nakai et al., 2000).

Physiological and biochemical parameters such as the algal biomass, content of chlorophyll-a (Chl-a), the concentration of the lipid peroxidation indicator malondialdehyde (MDA), protein contents, the specific antioxidant protective mechanisms (superoxide dismutase (SOD) and catalase (CAT)) and level of glutathione (GSH) are frequently utilized parameters to evaluate the *M. aeruginosa* physiological response to algae-inhibition chemicals. Under normal circumstances, cyanobacteria cells have activities of antioxidative enzymes to against the threat derived from reactive oxygen species (ROS) (Mallick and Mohn, 2000). Therefore *M. aeruginosa* can keep a dynamic equilibrium between ROS generation and removal. But overloaded free radicals, if not eliminated timely, might cause the oxidative stress and finally injury or death of *M. aeruginosa* cells. According to the literatures that algistatic chemicals from plants with the ability to kill algae could induce ROS production and then cause oxidative damage in *M. aeruginosa* cells (Hong et al., 2008; Wang et al., 2011; Zhang et al., 2011b). The specific objectives of the current research are: (1) provide laboratory data to prove the algistatic effect of rice straw on *M. aeruginosa*; (2) explore the potential mechanism of rice straw inhibiting algae growth, such as physiology and morphology; and (3) evaluate the feasibility of using widely grown terrestrial plants to treat HABs.

## 2. Material and methods

### 2.1. Strains and plant material preparation

An uncontaminated strain of *M. aeruginosa* FACHB-905 derived from Freshwater Algae Culture Collection of the Institute of Hydrobiology (Wuhan, China). Prior to initiation of the experiments, the *M. aeruginosa* was added into 1 L sterile flask containing 500 mL BG-11 medium, the pH of which was adjusted to 8.0 with 0.1 M NaOH and HCl in laboratory (Olvera-Ramírez et al., 2000). The rice straws were harvested from the experimental field under unified management of Dongting Lake plain (N23°10′42.14″ , E113°21′8.14″) which ensured that no pesticides, fertilizers and other artificial compounds were used in the rice growing. The rice straws were washed three times with deionized water to clear out superficial deposits, dried for 24 h at room temperature in October, then powdered and sifted by a 40 mesh sieve.

### 2.2. Chemical treatment

*M. aeruginosa* cells were cultured in the presence or absence of rice straw aqueous extract to evaluate its effects on the growth of living cells. To study the growth inhibition of *M. aeruginosa* by the rice straw aqueous extract, 10.0 g of straw powder was added into the prepared 1 L BG11 solution. These flasks were covered with cotton plugs to prevent contamination and evaporation and shaked for 7 days at normal ambient temperature. After 7 days, these cultures were filtered to clear away undissolved substances through filter paper, then filtrate was filtered through a millipore glass filter (0.22 μm pore size) to eliminate other microbe effects. Finally, these aqueous extract was gained and stored in a refrigerator at 4 °C. The equal volume of the *M. aeruginosa* in the logarithmic growing phase were added into five bottles containing BG11 nutrient solution under aseptic conditions. According to the standard of OECD (Guideline, 2001), the inoculation density of *M. aeruginosa* was set to be $5.8 \times 10^5$ cells $mL^{-1}$. After sterilization, the initial concentration gradients of rice straw aqueous extract for addition were designed as follows: 0, 2.0, 4.0, 8.0 and 10.0 g $L^{-1}$. The cultures without rice straw aqueous extract served as the control group. In our experiments, allelopathic inhibitory effect of rice straw aqueous extract on *M. aeruginosa* and its eco-physiological mechanism were investigated by continuous addition assays.

### 2.3. Continuous culture system design

To explore the allelopathic effect of rice straw aqueous extract under different concentrations on *M. aeruginosa* cells growth, all cultures were prepared and classified into five treatment groups. Experimental cultivations were performed in 1 L flask containing 500 mL culture medium at 25 °C and 2000–2500 lx light intensity at the photoperiod of 12 L:12D h and relative humidity of 75%. There were two replicates for each treatment groups and control groups. All experiment groups were shaken by hand and their places were changed randomly three times a day. The *M. aeruginosa* biomass was determined every third day until tests reached the stationary phase. Under aseptic conditions, 50 mL different concentrations of extract were added into five groups every two days starting from the first day of the test.

### 2.4. Determination of physiological and biochemical parameters

10 mL *M. aeruginosa* cells suspension was harvested from all tests. The *M. aeruginosa* cell density was measured by a hemocytometer under a light microscope. The counted cell numbers were expressed as the cell

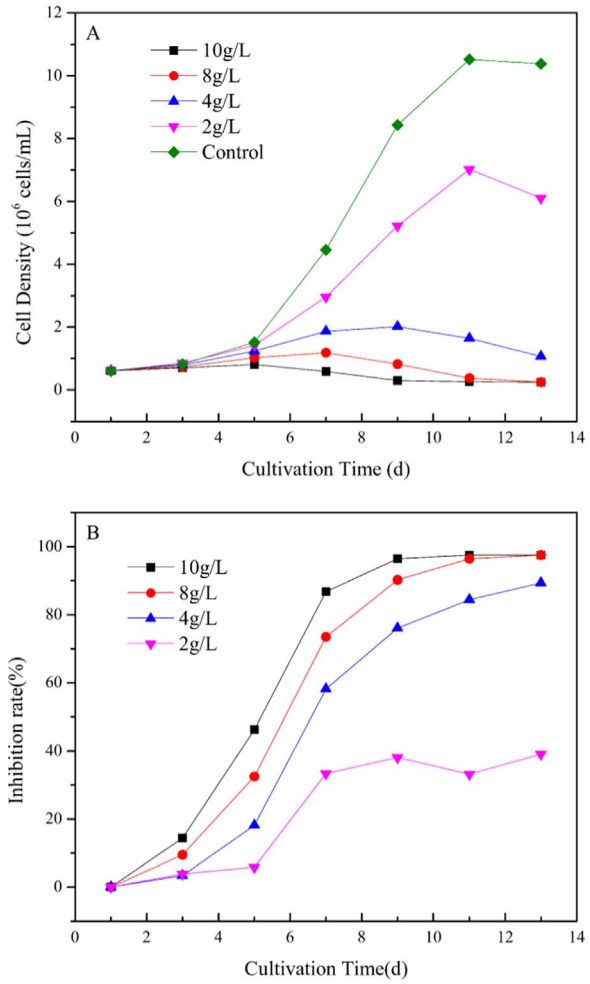


**Fig. 1.** Effects of different concentrations of rice straw aqueous extract on the growth of *M. aeruginosa*. (A)Growth curve, (B) inhibition curve.n = 3, $p < 0.05$.

density (cell $mL^{-1}$) in Fig. 1A, neglecting the physically destroyed cells.

The morphological and structural changes in the algal cells during the test were observed using a scanning electron microscope (SEM, JEOL JSM-6700 F, Tokyo, Japan).

Chl-a concentration was counted using spectropho-tometrically (Wang et al., 2010), and calculated on the basis of the method raised by Lichtenthaler and Wellburn (Lichtenthaler and Wellburn, 1983). *M. aeruginosa* cells were dealt with centrifugal treatment first and *M. aeruginosa* clusters were then isolated without illumination through 95% ethanol at 4 °C for 48 h. After isolation, all tests were treated with Centrifugal operation for 20 min at 2500 g and absorbance was identified in supernatants at 665 and 649 nm through a UV–Vis spectrophotometer. Blank solution was prepared by 95% ethanol. Chl-a concentration (mg/L) was measured by the following Eq. (1):

$$Chl{-}a = 13.95\ OD_{665} - 6.88\ OD_{649} \quad (1)$$

Algal cells were harvested by centrifugation at 4000 *g* for 25 min at 4 °C. The cell pellets were transferred into 10 mL centrifuge tubes. After removing the supernatant, 2 mL of phosphatebuffer (0.2 mol $L^{-1}$, pH 7.4) was added into the centrifugal tubes to re-suspend the *M. aeruginosa*. Then an ultrasonic cell pulverizer (SCIENTZ-IID, Ningbo Scientz Biotechnology Co., Ltd., China) at 300 W was used for a total time of 5 min (ultrasonic time: 2 s, rest time: 8 s) to get the cell disruption. All samples were placed in ice-water cubes to prevent the rise of temperature. These samples were treated with centrifugal operation at the speed of 4000 g for 15 min at 4 °C to obtain upper clear liquid, which was collected and used as a cell-free enzyme extract in the subsequent experiments.

The Chl-a concentration, SOD activity, CAT activity, GSH content and MDA level in *M. aeruginosa* cells were researched in this test. The SOD activity, CAT activity, GSH contents and MDA level were measured by assay kits purchased from Jiancheng Bioengineering Institute, Nanjing, China, according to the manufacturer's instructions (Agbakpe et al., 2014; Luo et al., 2011). The activities of all enzymes were investigated on a basis of protein content which was determined by a assay kit also purchased from Jiancheng Bioengineering Institute, Nanjing, China.

### 2.5. Statistical analysis

The allelopathic effect of rice straw aqueous extract on the biomass of *M. aeruginosa* was determined by inhibition over control which is defined by the following formula:

$$Inhibition\ (\%)\ over\ control = (1{-}N/N_0) \times 100 \quad (2)$$

where $N_0$ and N represent the cell numbers in the control and experimental cultures, respectively.

The data obtained in this study were presented with average value and respective standard deviation (SD). The statistical significance between all tests were analyzed using one-way analysis of variance (ANOVA), a Tukey multiple comparison test was then used to determine the difference (Wang et al., 2010). All the statistical analyses were performed using the Statistical Package for Social Science 19.0. The results shown in the figures represent the average of three independent replicate treatments. $p < 0.05$ was considered to indicate a significant difference.

## 3. Results and discussion

### 3.1. Effects of rice straw aqueous extract on M. aeruginosa growth

The growth period of *M. aeruginosa* could be divided into four stages: the lag, logarithmic, stationary, and aging phases. Growth variations of *M. aeruginosa* cell density exposed to different concentrations of the rice straw aqueous extract are presented in Fig. 1A. The rice straw aqueous extract exhibited a significantly algistatic effect on *M. aeruginosa* (Fig. 1B). In contrast to the control group, the algal biomass of *M. aeruginosa* at all four tested concentrations (2.0, 4.0, 8.0 and 10.0 g $L^{-1}$) were observably reduced during the whole experiment period and exhibited a concentration-dependent trend ($p < 0.05$).

The results indicated that from the first day to the third day, no marked differences were recorded among all groups. On the day 4–9, the percent inhibitions at all groups were gradually increased and were related to the concentrations of rice straw aqueous extract. Great algistatic effects ($p < 0.05$) were noted on day 9 with inhibitions of 37.27%, 75.31%, 89.87% and 96.57% exposed to 2.0, 4.0, 8.0 and 10 g $L^{-1}$, respectively. On day 9, the difference in algal biomass between the exposure concentrations of 4.0 g $L^{-1}$ and 8.0 g $L^{-1}$ were noteworthy ($p < 0.05$). From day 5–11, the algal cells were in the logarithmic growing phase, the algal biomass increased rapidly in the control sample but decreased gradually in the exposure concentrations of 4.0 g $L^{-1}$ and 8.0 g $L^{-1}$ samples. Moreover, The 10.0 g $L^{-1}$ group exhibited the lowest algal biomass among five groups during the 13 days period and the maximal inhibition of algal biomass was achieved on day 11. Compared with the control, the largest inhibition rate could be up to 98%. But the exposure concentrations of 2.0 g $L^{-1}$ were slowly increased the same with the control group.

Furthermore, by visual evaluation it was obvious that the biomass of *M. aeruginosa* increased as time extending. In the control groups, the greenness in culture medium was gradually deepening, while the culture medium was turned into transparent with yellow sediment after 13 days in all treatment groups. The results revealed that a dosage of 4.0–10.0 g $L^{-1}$ of rice straw aqueous extract could markdely constrain

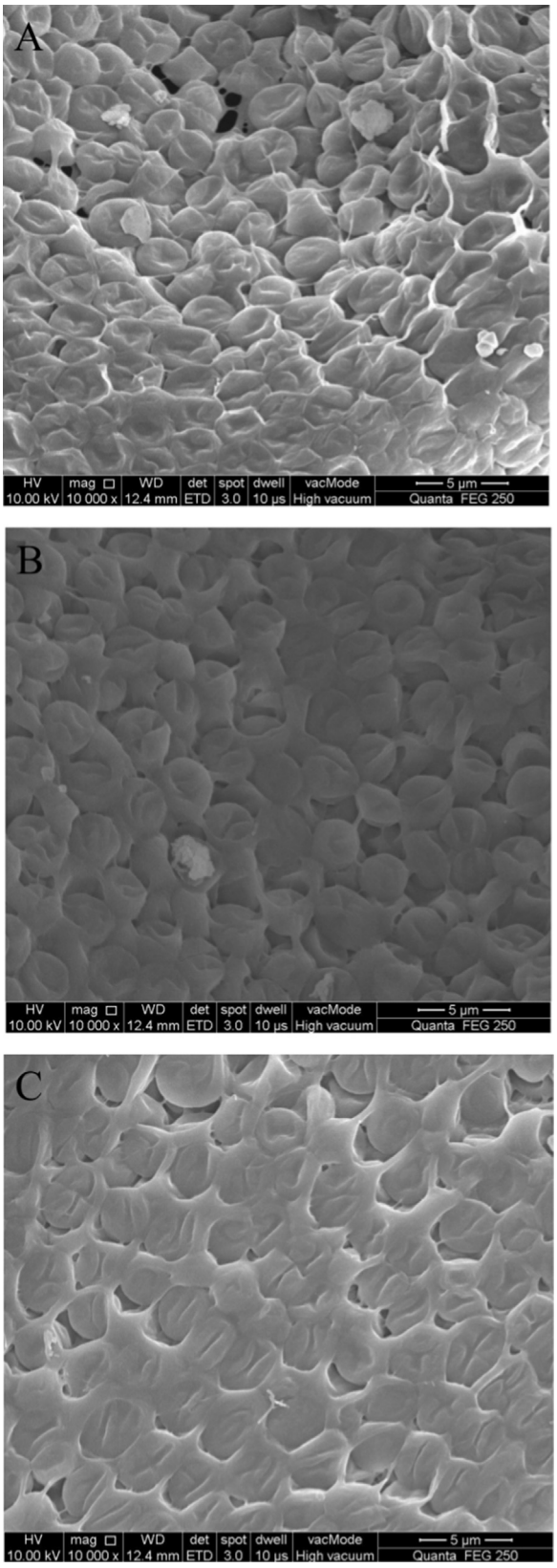


**Fig. 2.** SEM images: (A) control sample; (B) after the exposure to rice straw aqueous extract for 7 days; (C) after the exposure to rice straw aqueous extract for 11 days. n = 3, $p < 0.05$.

the growth of *M. aeruginosa*. When the dosage of the rice straw aqueous extract was 10.0 g $L^{-1}$, almost no algae growth was observed. It's manifested by Ma that there might exsit phenolic acids and oxygenic terpenoids in rice straw , which might be the main two factors suppressed the growth of *M. aeruginosa.* (Ma et al., 2014)

### 3.2. Morphological changes in M. aeruginosa

Over the past few years, scanning electron microscopy (SEM) has been regarded as an essential instrument for cell damage evaluation. (Ma et al., 2012; Sun et al., 2012). To understand the effect of rice straw aqueous extract on the surface morphology of the *M. aeruginosa* cells, the morphological changes were recorded before and after the addition of rice straw aqueous extract in the 10.0 g $L^{-1}$ group. The cells of *M. aeruginosa* were initially found to be round and plump with a smooth exterior, similar to the normal cells (Fig. 2A). However, after the addition of rice straw aqueous extract, the surface morphologies of algal cells changed. As shown in Fig. 2B , after reaction for 7 days, the size of the algal cells did not change significantly but they were distorted from their normal spherical shape and appeared flattened, and the surfaces became less smooth. After longer contact time, a distinct alteration of morphology was observed in Fig. 2C. Some of the cells cracked, the cell membrane of *M. aeruginosa* cells had been lysed, and the inclusion leaked out. This experimental phenomenon indicated that rice straw aqueous extract had a slight potential effect to accelerate *M. aeruginosa* cell lysis, thereby inhibiting its growth but not an immediate damage.

### 3.3. Chlorophyll a content of M. aeruginosa

Chl-a content is an indispensable index in Photosynthetic rate determination and plays an essential part in energy capture and transfer during photosynthesis (Zhang et al., 2013). Chl-a is the most primary photosynthetic pigment in *M. aeruginosa*, and Chl-a content can also be considered as anmonitoring factor of *M. aeruginosa* potential photosynthesis capacity, which turns out the water bodies primary productivity (Ni et al., 2015; Wang et al., 2014). Previous study showed that once the synthesis of Chl-a had been inhibited, the reproduction of the cell is inhibited as well (Carmichael and Boyer, 2016). The impacts on the growth of *M. aeruginosa* could be revealed by the decreased Chl-a content (Ni et al., 2012).

From Fig. 3, it demonstrated that the Chl-a content was markedly affected with rice straw aqueous extract concentration enrichment ($p < 0.05$). The Chl-a content showed a "S" type growth curve during the whole experiment period in the control group. Different from the control group, all experimental groups exhibited Chl-a content reduction significantly in the last 5 days. Especially for the treatment with 10.0 g $L^{-1}$ of extract, the Chl-a content was reducing from day 5 to end.

On day 1, the Chl-a content were almost exactly the same at all

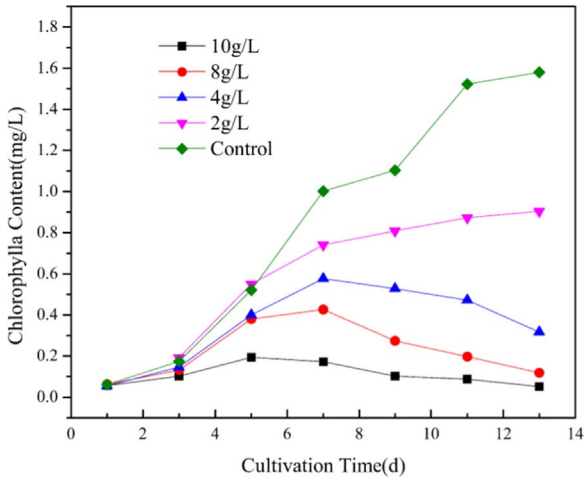


**Fig. 3.** Chlorophyll a concentration of *M. aeruginosa* cells in control group and treatment groups with different concentrations of rice straw aqueous extract.

treatments. On day 3, the concentration differences in Chl-a content at 2.0, 4.0 and 8.0 g $L^{-1}$ were not obvious compared with the control, but at 10.0 g $L^{-1}$, the Chl-a content was markedly different ($p < 0.05$). However, on day 7 at 2.0 g $L^{-1}$, Chl-a content was slightly lower than the control group and at 4.0, 8.0 and 10.0 g $L^{-1}$, Chl-a content were much lower. When the concentration of rice straw aqueous extract was 10.0 g $L^{-1}$, the content of Chl-a declined to 9.3% on day 9 ($p < 0.05$). The most remarkable reductions were noted on day 9, highly consistent with the algal biomass results.

The results showed that Chl-a as one of primary photosynthetic pigment in cells was damaged by rice straw aqueous extract, the situation of which accorded with previous studies that photosynthesis activity in several types of phytoplankton was suppressed by allelochemicals because of photosystem dysfunction (Zhu et al., 2010; Yan et al., 2011; Ni et al., 2012). The decrease of Chl-a in the present tests could be demonstrated by the inhibition of photosynthetic by the rice straw aqueous extract and further led to malfunctions of normal physiological metabolism in *M. aeruginosa* cells. The results of photosynthesis potential showed that the rice straw aqueous extract on the physiological activity of algae cells was inhibitive and not fatal. Similar to the Ni research (Ni et al., 2012), the result might indicate one type of algae suppressive mechanisms. It was suggested that the application of field control algae should be considered continuously or repeatedly.

### 3.4. MDA content of *M. aeruginosa*

To research whether the oxidative stress caused oxidative harm to *M. aeruginosa*, membrane lipid peroxidation was evaluated. The decomposition product, MDA, has been employed continually as a biomarker to estimate oxidative damage in cells (Shao et al., 2011). As is shown in Fig. 4A, the content of MDA increased markedly with exposure concentration enrichment and time extension. After 5 days treatment, the result that MDA content increase significantly were only noted in *M. aeruginosa* cells exposed to 8.0 and 10.0 g $L^{-1}$ ($p < 0.05$). With treatment time extension to day 9, MDA content in the *M. aeruginosa* cells in all groups treated with rice straw aqueous extract increased significantly (Fig. 4A). The maximal MDA value was 14.25 μmoL $10^{-7}$ cells at the concentration of 10 g $L^{-1}$, which was more than twice as much as that of the control group. Compared with control group, all the treatment groups exposed to rice straw aqueous extract were markedly decreased as time prolonged ($p < 0.05$). However, the MDA contents in the control groups kept relatively stable.

It's well known that algae cell membrane are composed of unsaturated phospholipids, which are susceptible to reactive oxygen species (ROS) (Wang et al., 2016). When exposed to rice straw aqueous extract at day 5, MDA content in the algal cells increased significantly (Fig. 4A). Unquestionably, abnormal lipid peroxidation in *M. aeruginosa* was induced by rice straw aqueous extract, which indicated that free oxygen radicals induced by rice straw aqueous extract could result in severe oxidative stress to *M. aeruginosa* and caused damage to cytomembrane polyunsaturated acid.

### 3.5. Effects of rice straw aqueous extract on *M. aeruginosa* enzymatic antioxidants activities (SOD and CAT)

Alexova et al. (2011) raised that *M. aeruginosa* with cellular defending capacity through the enzymic system, including SOD, CAT and other enzymes, which could exhibit antioxidation effect by eliminating ROS and OFR. SOD plays an indispensable role in antioxidant defense system in *M. aeruginosa* cells, which is capable of scavenging superoxide radicals via converting them into either ordinary molecular oxygen or hydrogen peroxide (Guo et al., 2014). Analogous to SOD, CAT is also an imperative enzyme with ability for catalytic decomposition of hydrogen peroxide in resistance to oxidation damage caused by ROS (Cakmak and Marschner, 1992; Vranová et al., 2002).

As shown in Fig. 5A, SOD activity of *M. aeruginosa* was stimulated

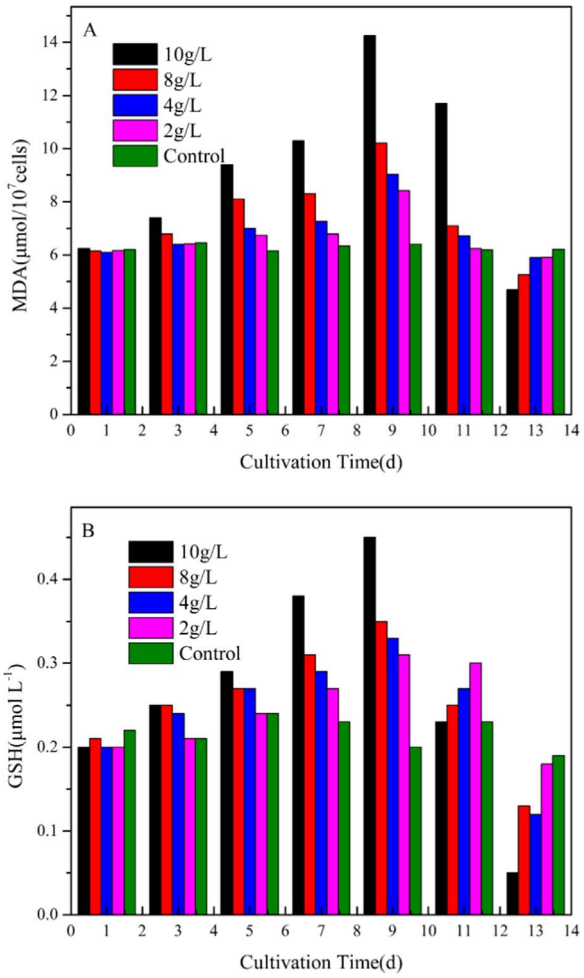


**Fig. 4.** (A) Effects of rice straw aqueous extract on MDA content in *M. aeruginosa*. (B) Effects of rice straw aqueous extract on the activities of GSH in *M. aeruginosa*. n = 3, $p < 0.05$.

markedly by the rice straw extract. This demonstrated that SOD was induced by rice straw aqueous extract in the early stage to resist the stress, which is identical with previous research (Hong et al., 2009), SOD deceased with the passage of time. It indicated that the rice straw extract could abate or impair the antioxidant defense system and therefore exhibited the strong algal inhibiting ability.

As shown in Fig. 5, compared with the first day, SOD and CAT activities increased significantly ($p < 0.05$) on day 5 in all treatment groups with the superoxide radical enrichment in *M. aeruginosa* in the initial stage of study. Normally, antioxidases in *M. aeruginosa* were adequate for eliminating ROS which protected cells from oxidative damage. In this research, However, ROS increased dramatically and oxidative stress was induced as SOD concentration was insufficient to scavenge all ROS when under 4.0, 8.0 and 10.0 g $L^{-1}$, triggering the defense system collapse. Analogous results could be obtained from other pressures, such as heavy metal on *M. aeruginosa* (Chen et al., 2015), copper oxide nanoparticles on *Chlamydomonas reinhardtii* (Melegari et al., 2013) and streptomycin on *Chlorella vulgaris* and *M. aeruginosa* (Qian et al., 2012). Despite existing fluctuations, the activities of SOD and CAT of 0, 2.0, 4.0, 8.0 g $L^{-1}$ groups seemed to restore the initial level in the last stage of the study. While in 10.0 g $L^{-1}$ groups, a new balance between the activities of SOD and CAT was achieve data much lower level ($p < 0.05$). The activities of SOD and CAT in *M. aeruginosa* cells initially increased as a stress response to the oxidative pressure induced by rice straw aqueous extract, while then decreased with time extending.

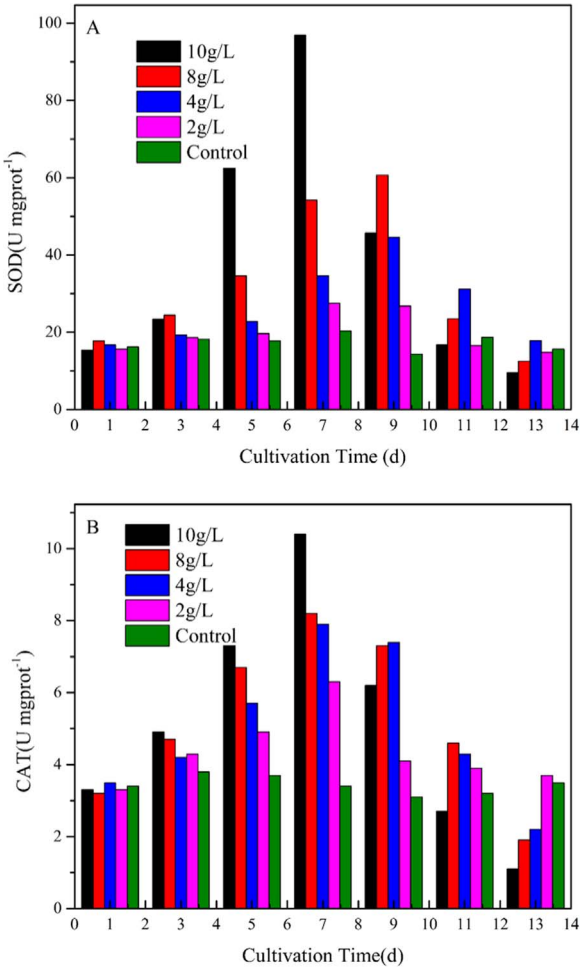


**Fig. 5.** Effects of rice straw aqueous extract on the activities of enzymatic antioxidants in *M. aeruginosa*. (A) SOD activity fluctuation with culture time. (B) CAT activity fluctuation with culture time. n = 3, $p < 0.05$.

Changes in antioxidative responses of algal cells suggested that rice straw aqueous extract could release allelochemicals to inhibit activity of antioxidant enzyme system in algal cells, thus, the algal could not scavenge intracellular free radicals, leading to that highly reactive and toxic free radicals could react with algal cells, terminating the *M. aeruginosa* growth, and the *M. aeruginosa* cells then died.

### *3.6. Effects of rice straw aqueous extract on M. aeruginosa non-enzymatic antioxidant activity (GSH)*

GSH is a significant non-enzymatic antioxidant involved in $H_2O_2$ detoxification in the ascorbate–glutathione cycle (AGC) and proteins protection from denaturation. (Chen et al., 2015). The effects of the rice straw aqueous extract on GSH content were also quantified. From Fig. 4B, we could note the evident difference in treatment groups in contrast with the control group. GSH content showed no prominent difference in all tests on day 3 but then became stimulated observably in the treatment groups and reached its peak on day 9 ($p < 0.05$), at a 2.25-fold level relative to the control groups. There was a sharp decrease after culture on day 11 in treatment groups. In the last stage of the experiment, GSH levels in 10.0 g $L^{-1}$ were 0.05 μmol $L^{-1}$, 26.3% of the value found in control group on day 13 (Fig. 4B). The GSH content had been slightly reduced periodically in control group.

The determination of glutathione contents were executed to investigate whether the non-enzymatic antioxidants participate in the cellular defense system against the oxidative stress from rice straw aqueous extract in *M. aeruginosa*. The GSH pools play an important in the cellular machinery of AGC. The increases in concentration and the regeneration rates of GSH at day 9 hinted that AGC were activated by rice straw aqueous extract. Based on the experimental data, the GSH content increase in our study not only indicated that the production of $H_2O_2$ was elevated, but also manifested that GSH were involved in antioxidant response to the oxidative stress when exposed to rice straw aqueous extract in the early stage.

## 4. Conclusions

Through the analysis of these physiological indexes, the inhibition mechanism of rice straw aqueous extract on *M. aeruginosa* growth was explored. The results demonstrated that the decrease of algal density was directly related to the activity of antioxidant system of algal cells. Apparently, allelochemicals secreted from rice straw aqueous extract can restrain antioxidant enzyme system activity in algal cells, which leads to uncleared highly reactive and toxic intracellular free radicals reacting with algal cells, suspending algal growth and causing cellular death. Therefore, this approach to suppress HABs could decrease the use of harmful chemical herbicides and provide an alternative sustainable technology of terrestrial plants management.

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