Organisms and ecological systems are always affected by the transformation of marine ecological system. At the same time, large algae are very important to humans as biological resources, some species such as Hizikia fusiforme were more and more serious, and had become global environmental problems. China is the largest algae cultivation country in the world; some species such as Hizikia fusiforme are very famous, but coastal water pollution, especially heavy metal pollution, has damaging effects on large algae cultivation (Tan et al., 2002; Wang et al., 2009). However, little information exists concerning the physiological responses of algae cultivation (Tan et al., 2009). Lately, with the rapid economy development of coastal areas in China, the pollution conditions of inshore are more and more serious, and had become global environmental problems. China is the largest algae cultivation country in the world; some species such as Laminaria japonica and Porphyra are very famous, but coastal water pollution, especially heavy metal pollution, has damaging effects on large algae cultivation (Tan et al., 2002; Wang et al., 2009). However, little information exists concerning the physiological responses of large algae such as Hizikia fusiforme to heavy metal stress.

Hizikia fusiforme as a unique species in North-East Asia, recon as a sea vegetable. It is rich in trace elements, dietary fiber, polysaccharide sulfate and other bioactive substances (Pugh, 2011; Schepetkin et al., 2006). In recent years, with the evaluation and development of H. fusiforme food and medicines, the typical heavy metal contents determination and analysis in H. fusiforme was gradually attracted people’s attention. For the typical heavy metals such as arsenic, lead, cadmium, mercury, zinc and copper, the United Nations, the European Union, Japan and other countries have clear food testing method and evaluation standard (copper ≤ 50 mg/kg) (Besada et al., 2009; Dawczynski et al., 2007; Rodenas et al., 2009), but very few reports of such research appeared in China.

Heavy metals such as Cu$^{2+}$ and other elements exist in natural seawater at lower concentrations. However, the concentration of Cu$^{2+}$ could be increased by industrial activities and the sediments in coastal waters (Connan et al., 2011). China offshore pollution is mainly caused by rivers and the pollution can directly increase the soluble heavy metal and trace elements content. At the same time, it can also decrease the pH of the seawater. Some reports showed that the pH of the seawater dropped by 0.1 units since 1975, and the pH of the seawater will continue to drop of 0.2-0.4 units by the end of the century.

Introduction

Large algae are mainly distributed in coastal intertidal zones, which are also the primary producers and assure energy transformation of marine ecological system. At the same time, large algae are very important to humans as biological resources. Under the background of global environmental changes, marine organisms and ecological systems are always affected by the interactions of many environment factors (Brierley and Kingsford, 2009). Lately, with the rapid economy development of coastal areas in China, the pollution conditions of inshore were more and more serious, and had become global environmental problems. China is the largest algae cultivation country in the world; some species such as Laminaria japonica and Porphyra are very famous, but coastal water pollution, especially heavy metal pollution, has damaging effects on large algae cultivation (Tan et al., 2002; Wang et al., 2009). However, little information exists concerning the physiological responses of large algae such as Hizikia fusiforme to heavy metal stress.

Biological Adsorption and Accumulation Analysis of Hizikia fusiforme Response to Copper Stress Conditions

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Abstract

Coastal water pollution is an important environmental problem now days. Hizikia fusiforme is cultivated in coastal water, being considered as a healthy food. However, little information exists concerning on this species responses to copper stress conditions. Experiments were conducted to distinguish biological adsorption and biological accumulation of H. fusiforme in regard to copper stress; it was determined the long-term stress with lower concentrations of copper (0.25 mg/L and 0.50 mg/L) and short-term stress with higher concentrations of copper (1.5 mg/L and 3.0 mg/L) on H. fusiforme. Results suggested that H. fusiforme has different response to various copper stresses; lower concentration stress could significantly enhance the growth of H. fusiforme, while H. fusiforme growth was inhibited and mitigated injured by 0.25-0.50 mg/L copper stress. Under the highest stress, H. fusiforme was extremely harmed, the biomass loss was significant and dry weight/fresh weight was also significantly decreased. Results suggested that lower and higher concentrations of copper stress have different impacts on H. fusiforme; the biological adsorption amount is lower than that of biological accumulation amount under low copper stress conditions, but the biological adsorption amount is much higher under high concentration copper stress. A better understanding of H. fusiforme responses to heavy metal stress should bring more data about its physiological adaptation mechanism under such conditions.

Keywords: algae, biological adsorption, biological accumulation, copper stress, pollution

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(Doney et al., 2009). This result will also induce potential adverse impact on the solubility and uptake of heavy metals, and the growth and metabolic processes of the algae. In addition, Cu$^{2+}$ concentration in coastal water is always fluctuating and this also affects algae metabolism.

The adsorption of algae upon heavy metals can be divided into two categories: inactive algae adsorption and activated algae adsorption. Inactive algae adsorption rate and amount are affected by environmental temperature, pH, particle size and ion competition (Stirk et al., 2000). The adsorption processes of activity algae on heavy metals can divide into two steps: first step is called biological adsorption, and this step is very quickly and reversibly, the feature is that heavy metal ions adsorption is on the cell surface. The second step is very slow and is called biological accumulation. It is mainly similar with the heavy metal ions absorption into the cells.

At present, the researches on *Hizikia fusiforme* responding to heavy metal stress were always focus on photosynthesis, growth and development, enzyme activity analysis and evaluation, and no information exists concerning the adsorption and accumulation mechanism of this species to heavy metal (Alahverdi et al., 2012; Zhu et al., 2011).

The aim of this paper was to distinguish biological adsorption and biological accumulation of *Hizikia fusiforme* to copper stress and to determine the biomass and Cu$^{2+}$ concentration changes to long-term of lower concentration copper stress and short-term of higher concentration copper stress.

### Materials and methods

#### Plant material and copper stress treatments

*Hizikia fusiforme* adopted in this experiment was cultivated by Lufeng breeding institute in Zhejiang Province of China. Uniform samples (morphology and plant height) were screened, impurity were removed, and then clamped the seedlings by 15 cm of 100 polyethylene ropes; each rope contained 7 seedlings before treatments. When *H. fusiforme* seedlings were cultured for 24 h in natural seawater, each group was used for copper stress: (1) lower concentration stress conditions (0.25 mg/L and 0.5 mg/L) for 7 d, and biomass of the samples was measured in 1 d, 3 d, 5 d and 7 d during stress periods; (2) higher concentration stress conditions (1.5 mg/L and 3.0 mg/L) for 24 h, and then biomass of the samples was measured. Four replications were used in each stress condition. Seawater was replaced every 24 h, and the environment factors during experiment were presented in Table 1.

### Measurement of *H. fusiforme* biomass changes to copper stress

The samples were washed with tap water followed by distilled water and then put into the gauze bag (15 × 20 cm) which was soaked in distilled water, then centrifuged at 1,400 r/min for 2 min. Fresh weight (G initial total mass) was measured after removing excess water from the algae surface (Hai-Zao et al., 2009; Zhu et al., 2011).

\[
Y_{\text{net increase}} = \frac{G_{\text{initial total mass after stress treatment}} - G_{\text{initial total mass}}}{G_{\text{initial total mass after stress treatment}}} \times 100\%
\]

**Table 1. The environment factors of copper adsorption**

| Factors         | Light intensity (μmol/m²/s) | Photoperiod (Day/night) | Temperature (°C) | Salinity (%) | Cu$^{2+}$ concentration in nature seawater (mg/L) | CO$_2$ (ppm) | pH     |
|-----------------|-----------------------------|-------------------------|------------------|-------------|-----------------------------------------------|-------------|--------|
| Value           | 151 ~ 155                  | 12:12 (6:00 ~ 18:00)    | 23               | 35          | <0.009                                        | 450         | 7.8    |

Data analysis

The data were analyzed by one-way ANOVAS using SPSS 13.0 (SPSS Inc, Chicago, IL, USA). The means and standard errors (SE) were reported.

### Results

#### The biomass changes of *H. fusiforme* under copper stress conditions

The biomass growth rate of the control group (Cu$^{2+}$ < 0.009 mg/L) sample increased with increasing culturing time during 7 d, and the biomass growth rate reached 9.49% at 7 d. A similar trend of biomass growth rate to that of the control group was observed also under 0.25 mg/L stress group, but the value at 1 d was less than that of the control group, and the values were much higher than for control group at the other 3 culturing days, the biomass growth rate reached 11.73% at 7 d. The biomass growth rate of the 0.5 mg/L Cu$^{2+}$ stress group presented a negative growth trend, the value was sharply decreased at 5 d and the biomass was lost at the extreme at 7 d, when it reached 40.83%. The biomass growth rate of the 1.5 mg/L stress group and 3 mg/L stress group were both decreased since 1 d of stress treatment, more under the 3 mg/L stress group, the value of that being 17.56%. The biomass growth rate decreased with the increasing copper concentration at 1 d in all five groups (Fig 1).
The C and T values for the control group were the highest at 1 d: 13.41% and 7.26%, respectively. The minimum T value appeared at 3 d. However, C value appeared at 5 d. The values reached 10.07% and 7.18% at the end of the culturing days. The changing trends of C and T values were different and the changes showed that the biomass loss was significant after treatments with HCl and EDTA0Na (Fig. 2A).

The C value and T value of the 0.25 mg/L copper stress group were the highest at 1 d: 12.71% and 6.97%, respectively. The minimum T value appeared at 3 d (5.51%), and then increased slowly at 5 d and 7 d. The minimum C value appeared at 5 d (10.44%) and then increased. The values reached 10.64% and 6.94% at the end of the culturing period. A similar trend was found with the control group during the culturing days, and 0.25 mg/L of Cu$^{2+}$ had a significant effect on C value (Fig. 2B).

The change trend of C value and T value in the 0.5 mg/L stress group was similar with that of the 0.25 mg/L stress group. The T value decreased from 6.89% to 5.76% at 3 d, and the C value decreased from 12.21% (max) to 11.21% (min) at 5 d. The values reached 11.72% and 6.86% at the end of the culturing period, respectively. The changes showed that the biomass loss was significant after being treated with HCl and EDTA-Na (Fig. 2C).

The C values were 10.96% and 10.33%, while the T values were 6.50% and 6.04% in the 1.5 mg/L stress group and 3 mg/L stress group after 24 h copper stress. The C values decreased with increasing copper concentration at 1 d stress in all five groups (Fig. 2D).

**The Cu$^{2+}$ content before treatment (C) and after chemical treatment (T)**

Copper adsorption content of C and T values of the control group decreased from 1 to 5 d, and then increased from 5 d to 7 d. All of the T values were higher than C values during the culturing days. The max C and T values were both reached at 1 d, which were 10.4 mg/kg and 11.6 mg/kg. The min C and T values were reached at 5 d, and were 4.8 mg/kg and 7.6 mg/kg.
The results showed that the biological adsorption and accumulation amount of the Cu$^{2+}$ significantly decreased with the increasing culturing time (Fig. 3A).

The C and T values for the first stress group were significantly higher than that of the control group at 1 d: 250.0 mg/kg and 49.6 mg/kg, respectively. Both C and T values significantly increased from 1 d to 3 d, and then decreased from 3 d to 5 d. The maximum values were 79.2 mg/kg and 96.4 mg/kg, reached at the end of the culturing days. The changing trends of C value and T value were similar in this group, however, the T values were higher than C values at the same culturing day (Fig. 3B).

The changing trends of C value and T value were different in the 0.5 mg/L stress group. The C value and T value were 224.0 mg/kg and 73.8 mg/kg at 1 d, and were significantly higher than that of the control group and 0.25 mg/L stress group. The C values showed irregular changes, but the T value increased gradually during the whole culturing time and reached the maximum value of 230.0 mg/kg at 7 d (Fig. 3C).

The C value and T value increased with increasing copper concentration at 1 d in all of the five stress groups. The C values were higher than T values, especially for higher concentration gradients of copper stress (1.5 mg/kg and 3 mg/kg), except for 0.25 mg/L of copper stress group, which had C value lower than T value (Fig. 3D).

**Discussions**

In natural conditions, the environment of algae growth has higher or lower conditions than the optimal growth factors, such as the periodical changes of light intensity, temperature and other environmental factors. Long and short term stress conditions together with stress intensity may also have different impacts on algae growth. Several reports have indicated that only the changes of long term growth factor could induce photosynthesis, as well as other key metabolic process changes within plants (Hai-Zao, 2009; Ruan, 2001; Zou et al, 2005). Results obtained from the current study clearly demonstrated the different responses of H. fusiforme to long-term of lower concentration copper stress (0.25 mg/L and 0.50 mg/L) and short-term of higher concentration copper stress (1.5 mg/L and 3.0 mg/L). In addition, artificial culture conditions in the experiment also enhanced the interference factor and provided data about suitable environmental conditions for H. fusiforme growth and development.

H. fusiforme lives in alternation of wetting and drying conditions due to the environments of large algae growth. At this time, H. fusiforme produced and secreted algin in order to adapt to the changing environment. Under drying conditions, H. fusiforme slowed the water loss and kept cell activity, while slowed water absorption under wetting conditions (Zou et al, 2005). In addition, algin is an important biological active component of large algae, which account for 48.28-63.73% of the dry weight of H. fusiforme (Wang, 2009).

In the current research, biological accumulation of Cu$^{2+}$ must finish the process of biological adsorption by algin in H. fusiforme. While for other large algae such as Sargassum and Padina, the main factor affecting the adsorption of heavy metals is the cell wall polysaccharides, and the formation of functional groups such as S=O and N–H in the cell wall (Andradea et al., 2010; Plazaa et al., 2011; Romeraa et al., 2007; Shenga et al., 2004). In this study, the samples treated with 0.01 mol/L HCl and EDTA-Na could effectively retain the copper in tissue cells. This design can distinguish biological adsorption and biological accumulation of H. fusiforme to copper stress.
Biological adsorption and accumulation of *H. fusiforme* to 0.25 mg/L copper stress was significantly higher than that of the control group, and this was mainly dependent on the stress time. The phenomenon reflects that *H. fusiforme* has strong resistance to 0.25 mg/L copper stress, and copper can be used as a necessary trace element for *H. fusiforme* growth under lower stress concentration (Yrula et al., 2000). At the same time, the phenomenon also shows the good adsorption capacity on copper ion adhered on the surface of *H. fusiforme* and the rapid transfer ability of mannuronic acid on copper ion. However, under higher copper concentration and short time stress conditions, biological adsorption and accumulation of *H. fusiforme* increased. The values of the adsorption were 211.2 times and 159.6 times of the control group under 1.5 mg/L and 3 mg/L, indicating that physical adsorption of copper may be closely related to the algin outside of the epidermis, which needs further research.

Conclusions

In summary, this study clearly showed that *Hizikia fusiforme* had different responses to various copper stresses, whereas lower concentration could significantly enhance its growth. Under high copper concentration (>0.5 mg/L) stress, *H. fusiforme* was extremely harmed, the biomass loss was significant and dry weight/fresh weight significantly decreased. Results suggested that the stress induced by lower and higher copper concentrations have different impacts on *H. fusiforme* and the biological adsorption is lower than that of biological accumulation under low copper stress conditions, while the biological adsorption is much higher under high concentration copper stress. A better understanding of *H. fusiforme* responses to heavy metal stress should bring more clarification upon its physiological adaptation mechanism and this data might facilitate the effective utilization of this species in seawater.

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