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Responses of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater

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ABSTRACT

Microalgae *Coelastrella* sp. could remove nutrients from anaerobically digested swine wastewater (ADSW) effectively, while its responses to the stress of Cu(II) were not well understood. In this paper, nutrients removal and growth of *Coelastrella* sp. were investigated at the presence of Cu(II) in ADSW. Results showed ammonium nitrogen concentration in ADSW decreased with culturing duration, while increased with an increased Cu(II) concentration. Total phosphorous concentration decreased with time, while did not drop in 4 days at Cu(II) concentration ≥1.0 mg/L. Microalgal growth was inhibited at all the Cu(II) concentrations, and ceased in about 6–8 days at Cu(II) concentration ≥1.0 mg/L. With an increased Cu(II) concentration, the contents of chlorophyll *a* and proteins decreased, those of malondialdehyde and superoxide dismutase, and ratios of octadecanoic acid (C18:0), hexadecanoic acid (C16:0) and octadecenoic acid (C18:1) to fatty acids in *Coelastrella* sp. increased, while octadecatrienoic acid (C18:3) gradually disappeared.

1. Introduction

Anaerobically digested swine wastewater (ADSW) is characterized by high levels of ammonium nitrogen (NH_3-N), chemical oxygen demand (COD) and total phosphorus (TP) (Luo et al., 2016). If the nutrients in ADSW were not managed properly, it could lead to severe environmental pollution including eutrophication of water bodies, soil pollution and air pollution (de et al., 2010).

Processes for treatment of nutrient and resource recovery from swine wastewater were investigated by a lot of researchers, including those on either biomass recovery (Cheng and Stomp, 2009; Colombo et al., 2017) or bioenergy recovery (Abubackar et al., 2012; Bajracharya et al., 2016; Fernández-Naveira et al., 2016; Pandey et al., 2016), and those on nutrient removal from and recycling of ADSW. These processes could also be grouped into physical adsorption (Huang et al., 2014; Guo et al., 2013), phytoremediation including wetland plants (Klomjek et al., 2016) and lemna (Gaur et al., 2017; Zhou et al., 2017; Cheng and Stomp, 2009), nutrient recycling (Peng et al., 2014), and microorganisms culture systems including using photosynthetic bacteria (Wen et al., 2016; Wang et al., 2000) and microalgae (Luo et al., 2016). Colombo et al. (2017) cultured *Spirulina* in cathodic compartments of photo-microbial fuel cells for wastewater treatment, and microalgae were considered to be appropriate for energy recovery as biodiesel due to high biomass productivity and ease to be cultured in liquid media (Ge and Champagne, 2015).

Microalgae culture systems showed potentials for cost-effective removal of nutrients from ADSW. Microalgae group could adapt to various water environment (Abou-Shanab et al., 2013), and many microalgae could store the triacylglycerol and starch which could produce biodiesel and ethanol. Meanwhile, microalgae-based systems could save energy for collecting organic matters and nutrients from swine wastewater (González et al., 2008; Abou-Shanab et al., 2013). So, microalgae culture systems were paid close attention for the treatment of agricultural wastewater (Ji et al., 2013; Luo et al., 2016).

Ji et al. (2013) cultivated three species (*Chlorella*, *Scenedesmus* and *Ourococcus multisporus*) in wastewater, which reached specific growth rate of 1.37, 1.14 and 1.00 day^{-1}, respectively. The removal efficiency of nitrogen and phosphorus exceeded 99% within 4 days in each

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medium, and *Chlorella* achieved a highest lipid productivity of around 0.164 g-lipid g-cell^{-1}day^{-1}, and 44% oleic acid was produced in the culture microalgae. Xu et al. (2015) cultivated *Scenedesmus obliquus* in piggery anaerobic digestate liquid, and the average removal efficiency of COD, TP and total nitrogen were 61.58%–75.29%, 70.09%–88.79% and 58.39%–74.63%, respectively, within 7 days. *Chlorella vulgaris* JSC-6 was cultured in swine wastewater, the removal efficiency of COD and NH_3-N reached to 60%–76% and 40%–91%, respectively, in 12 days, and the highest biomass of microalgae was 3.96 g/L, of which 58% was carbohydrate content (Wang et al., 2015). Luo et al. (2016) isolated microalgae strain *Coelastrella* sp. QY01 which removed 90% of NH_3-N and TP in anaerobically and aerobically treated swine wastewater, meanwhile as much as 22% lipids were reported in the cultured microalgae.

Cupric salts were one of most frequently used additive in many animal feeds (Xiong et al., 2010; Wu et al., 2017). Absorption of Cu(II) by farm animals was low, and most Cu(II) in feeds was excreted (Li et al., 2007). Usually, only about 10%–20% of Cu(II) added in feeds was absorbed by pigs (Li et al., 2007), so a high Cu(II) concentration in swine wastewater could be resulted in. As a vital biological trace element, copper (as Cu(II)) at high concentrations could be toxic to most microorganisms (Nakajima et al., 1979). Cu(II) in swine wastewater could inhibit microalgal growth and consequently reduce the removal efficiency of NH_3-N and TP in ADSW. Therefore, Cu(II) discharge from livestock farming including in swine wastewater has been paid close attention (Xiong et al., 2010).

The responses of microalgae *Coelastrella* to stress of cupric ions could be expressed by nutrient removal and biomass growth at various conditions as well as some important biochemical indicators. Contents of proteins, chlorophyll *a* (chl *a*), malondialdehyde (MDA), superoxide dismutase (SOD) and fatty acid methyl esters (FAME) were considered to be such indicators (Kagalou et al., 2002; Sabatini et al., 2009; Somerville et al., 1995). Content of chl *a* was applied to estimate total biomass and photosynthetic rate frequently, and also was used to assess short-term inorganic chemical toxicity to microalgae (Perez et al., 2006). SOD could remove reactive oxygen species (ROS) which was produced during visible and ultraviolet illumination in microalgae (Janknegt et al., 2007). Due to its special physiological activity, SOD is responsible for biological removal of free radicals in microalgae cells. When microalgae grew under Cu(II) stress, ROS played a primary role in Cu(II) toxicity to microalgae (Stefanie et al., 2008). ROS damaged cells not only through the biological membrane of polyunsaturated fatty acid peroxide, but also through the production of the decomposition of hydrogen peroxide, and ROS could attack polyunsaturated fatty acids of biofilms and generate lip peroxide, thereby produced MDA (Bandyopadhyay et al., 1999). Therefore, the content of MDA in *Coelastrella* sp. could reflect the degree of lipid peroxidation in the cell and indirectly reflect the degree of cell damage, and composition of FAME in microalgae were used to indicate the biodiesel quality. Unfortunately, little information on the performances of microalgae for nutrient removal and biomass growth as well as mechanisms and the mechanisms as indicated by the biochemical indicators at the presence of cupric ions in treatment of ADSW are available.

This work is expected to help fill this gap in which the effects of various Cu(II) concentrations on the growth of *Coelastrella* sp. and the removal efficiency of NH_3-N and TP will be examined. The contents of MDA and SOD, proteins, chl *a*, and FAME in *Coelastrella* sp. at various Cu(II) concentrations will also be studied to better understand the mechanisms.

2. Materials and methods

2.1. Anaerobically digested swine wastewater

Anaerobically digested swine wastewater (ADSW) was obtained from a local pig farm in Hunan, China (28°09′23″N, 112°53′34″E). ADSW was centrifugalized for 5 min at 10,000 rpm, and then the supernatant of ADSW was stored at 4 °C. The concentrations of NH_3-N, TP and Cu(II) in the ADSW were 1317 ± 10, 20.2 ± 0.5 and 7.3 ± 0.5 mg/L, respectively.

Autoclaved supernatant of ADSW was diluted with H_2O (distilled water) to 10%, and batch experiments were performed in 1 L conical flasks containing 800 mL of autoclaved supernatant of 10% ADSW.

2.2. Microalgae strains

Microalgae *Coelastrella* sp. were collected from a pond near a pig farm (26°36′95″ N, 112°05′08″ E) in Hunan, China on September 2014, and were isolated and cultured according to the methods described by Luo et al. (2016). Microalgae cells were grown in sterile distilled water with BG-11 medium (Luo et al., 2016). *Coelastrella* sp. strains were inoculated in 1 L conical flasks containing 800 mL of sterilized basal medium which were kept in an illuminating incubator at 25 ± 1 °C and a light intensity of 80 ± 10 μmol m^{-2} s^{-1} of fluorescent lights, and the daily light/dark cycle (L: D) was 14:10 h. The flasks were agitated three times each day, and all the operations were carried out under sterile condition. 0.05 mol/L Tris(hydroxymethyl)aminomethane (Tris-HCl) was used to wash microalgae cells and the pH of Tris-HCl was adjusted by phosphate buffer saline (PBS) at 7.4.

2.3. Culturing methods

Coelastrella sp. used in this study was inoculated to the 10% ADSW medium, maintaining a biomass concentration at around OD_{680} 0.10. Then $CuSO_4$ was added to the diluted ADSW at various concentrations of Cu(II) including 0.0, 0.10, 0.50, 1.0, 2.0 and 3.0 mg/L, the light and temperature condition were same as above and microalgae were cultured for 16 days. Microalgae could adsorb Cu(II) in aqueous solutions (Zeraatkar et al., 2016), so Cu(II) concentration was measured every 6 h during culture experiments to maintaining a stable cupric concentration.

2.4. Analytical methods

2.4.1. Nutrients and cupric

The Standard Methods for Water and Wastewater Monitoring and Analysis (SEPAC, 2002) was applied to measure the concentration of NH_3-N (GB 7479-87) and TP (GB 11893-89). And cupric concentration in aqueous solutions was measured using atomic absorption spectroscopy (AAS, PEAA700, America).

2.4.2. Microalgae biomass

On consideration of a linear relationship between dry cell weight and OD_{680} (APHA, 1998), *Coelastrella* sp. dry weight was measured and calculated by using Eq. (1):

$$DW(g\ L^{-1}) = 0.3357 \times OD_{680}, \quad R^2 = 0.9962 \tag{1}$$

The specific growth rate μ in the exponential phase of algal growth was calculated by Eq. (2) (Luo et al., 2016):

$$\mu = (\ln N_1 - \ln N_0) \div (t_1 - t_0) \tag{2}$$

Where N_1 and N_0 are the dry cell weight at time t_1 and t_0, respectively.

Biomass productivity (P) was calculated using Eq. (3) (Issarapayup et al., 2009):

$$P(mg\ L^{-1}\ day^{-1}) = (W_1 - W_0) \div (t_1 - t_0) \tag{3}$$

Where W_1 and W_0 are dry biomass (mg/L) at time t_1 and t_0, respectively.

2.4.3. Chlorophyll a concentration

Chlorophyll *a* concentration was measured by spectrophotometer (Wang et al., 2010), and calculated using the method described by Li

et al. (2016). Microalgae samples of 25 mL were centrifuged for 25 min at 4000 rpm, liquid supernatant were poured out, and then distilled water was added before centrifuged again at the same speed for 10 min. The microalgae residues were then extracted in dark with 5 mL of 95% ethanol at 4 °C for 24 h. After the extraction, samples were centrifuged for 5 min at 2500 rpm, and the absorbance of the liquid supernatants were measured at 665 and 649 nm, respectively. Blank solution was prepared by 95% ethanol. Chlorophyll *a* concentration was calculated using Eq. (4):

$$\text{Chl}-a\,(\text{mg/L}) = (\text{OD665} \times 13.95 - \text{OD649} \times 6.88) \div 5 \quad (4)$$

2.4.4. FAME

Fatty acid composition was analyzed according to the methods described by Abou-shanab et al. (2013) and Luo et al. (2016). The procedures included preparation of fatty acid methyl esters (FAME) and Gas Chromatography and Mass Spectrometry (GC–MS) analysis. Dried samples of 0.10 g were poured into screw-top glass tubes and mixed with 10 mL of solution composed of chloroform, methanol and concentrated sulfuric acid whose volume ratio was 5:4.25:0.75. Tubes sealed tightly with caps to avoid leakage were put into 90 °C water for 1 h before cooled to room temperature. Then 2.5 mL of distilled water was added into the tubes and shook for 30 s. After liquid stratification, the lower phase was transferred to a 5 mL glass bottle and dried with anhydrous Na_2SO_4. After taken out solids of Na_2SO_4, the liquid phase of samples in the bottles were analyzed by GC–MS (QP2010, Shimadzu). The composition of the samples was identified using the NIST Mass Spectral Database and quantified by the area normalization method.

2.4.5. Protein content, MDA and SOD

Microalgae cells in 500 mL of cultured suspension were harvested by centrifuging at 4000 rpm for 10 min and washing with 5 mL of Tris-HCl. The harvested cells were put on ice, and broken using ultrasonication (XO-1000D, China) at 200 W for 8 min (3 s on and 8 s off). The broken cells were centrifuged at 4000 rpm and 4 °C for 10 min, and the supernatants were stored at 4 °C before biochemical analysis. Content of proteins, malondialdehyde (MDA) and superoxide dismutase (SOD) were measured by using assay kits purchased from Nanjing Jiancheng Bioengineering Institute, China.

3. Results and discussion

3.1. Nutrient removal

Luo et al. (2016) have cultured *Coelastrella* sp. for nutrient remove in ADSW, that study concerned with nutrient removal by microalgae in different concentrations of ADSW, while the influence of Cu(II) on nutrient remove was not taken into account. In this study, microalgae were cultured in ADSW media to examine the effect of different initial concentrations of copper on nutrient removal. The dynamic variations of NH_3-N and TP concentration at different initial Cu(II) concentrations for 16 days are described in Fig. 1.

Before the ammonia was totally used up in ADSW, the nitrate would not be consumed (Luo et al., 2016). In this study, nitrogen removal analysis was by measuring the amount of ammonium. It can be seen from Fig. 1(a) that NH_3-N concentration decreased with culturing time for all the concentrations of Cu(II), and NH_3-N removal efficiency decreased with an increased concentration of Cu(II) except for 2.0 mg/L of Cu(II). In the control test without Cu(II) addition, about 80.0% of NH_3-N was removed on day 16. When the concentration of Cu(II) was increased to 0.10, 0.50, 1.0, 2.0 and 3.0 mg/L, the removal efficiency decreased to 61.6%, 58.8%, 45.1%, 50.7% and 38.6%, respectively.

Fig. 1(b) showed that TP concentrations decreased for all the concentrations of Cu(II) on day 4, and subsequently slight increases of TP concentration were observed when Cu(II) concentration was higher than 1.0 mg/L. On day 16, the removal of TP were 79.0%, 70.5%, 84.9%, 18.2%, 28.2% and 12.6% at different concentrations of Cu(II) of 0.0, 0.10, 0.50, 1.0, 2.0 and 3.0 mg/L Cu(II), respectively. TP removal efficiency was maximized at 84.9% under 0.50 mg/L of Cu(II). But the maximized removal efficiency of NH_3-N and TP in the control group were lower than that previously reported (Luo et al., 2016), the main reason was that the microelements in ADSW were different.

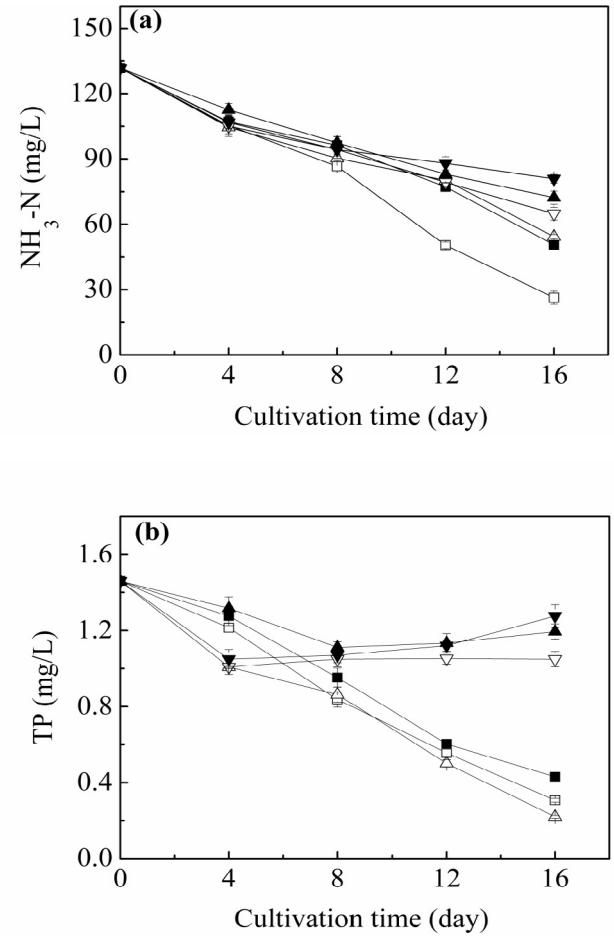

Fig. 1. Dynamic changes of contents of NH_3-N and TP at various concentrations of Cu(II) during culturing of *Coelastrella* sp. □: control; ■: 0.10 mg/L; △: 0.50 mg/L; ▲: 1.0 mg/L; ▽: 2.0 mg/L; ▼: 3.0 mg/L.

Abou-Shanab et al. (2013) examined six microalgae species including *Ourococcus multisporus*, *Chlamydomonas*, *Scenedesmus*, *Nitzschia*, *Chlorella* and *Micractinium* in the coupling of nutrient removal and biodiesel production, and the highest removal efficiencies of nitrogen (initial concentration: 53 mg/L) and phosphorus (initial concentration: 7.1 mg/L) were 62% and 28% by *Chlamydomonas*. Xu et al. (2015) cultivated Scenedesmus obliquus for nutrient removal in piggery anaerobic digestate liquid, the removal efficiencies of TN and TP were 58.39–74.63% and 70.09–88.79%, respectively. However, all of the studies were concerned about nutrient removal by microalgae which grew in swine wastewater without cupric ions. *Coelastrella* sp. could adapt cupric ions and high concentration of nitrogen and it was good at NH_3-N and TP removal.

3.2. Microalgal growth

Biomass production of *Coelastrella* sp. cultured in ADSW media at the various concentrations of Cu(II) was presented in Fig. 2. Within the first 4 days, biomass increased for all the Cu(II) concentrations, which indicates that inhibition from cupric ions was not obvious for microalgal growth during this period. Then, algae biomass gradually stopped increasing and eventually decreased when Cu(II) concentration was no

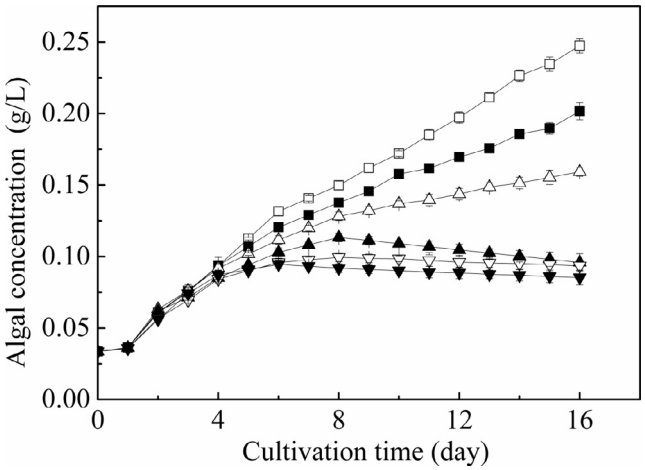

Fig. 2. Dynamic changes of *Coelastrella* sp. concentration at various concentrations of Cu (II) in ADSW. □: control; ■: 0.10 mg/L; △: 0.50 mg/L; ▲: 1.0 mg/L; ▽: 2.0 mg/L; ▼: 3.0 mg/L.

less than 1.0 mg/L. This result was similar as those by Bilgrami et al. (1997) in which copper highly inhibited the phytoplankton species at a copper concentration higher than 0.1 mg/L.

From Fig. 2, it can also be seen that microalgae mass decreased with an increased concentration of Cu(II) on day 16. And the specific growth rate and biomass productivity for the microalgae decreased correspondingly. These results suggest that Cu(II) decreased *Coelastrella* sp. production, which led to decreased removal efficiency for NH_3-N and TP.

Figs. 1 and 2 showed that there was a high correlation between the microalgal growth rate and removal efficiency of NH_3-N and TP. A higher growth rate of the microalgae led to higher removal efficiencies of NH_3-N and TP.

3.3. Physiological and biochemical properties

3.3.1. Chlorophyll *a* and protein contents

The effect of Cu(II) on the contents of chl *a* (Chlorophyll *a*) and proteins in the microalgae on day 16 were evaluated (Fig. 3). Fig. 3(a) shows that chl *a* concentration in *Coelastrella* sp decreased considerably at 0.50 mg/L of Cu(II), and dropped sharply at 1.0 mg/L of Cu(II). It was fond that concentration of chl *a* was decrease with an increased concentration of Cu(II), and when Cu(II) concentration was higher than certain level, chl *a* concentration decreased sharply. This is close to the study that was reported by Kagalou et al. (2002), who reported the effect of different concentrations of Cu(II) on chl *a* in microalgae, and the concentration of chl *a* decreased rapidly in the first 2 days when cultured in 1 mg/L Cu(II).

Cupric ion's damage to chl *a* was a complex process. Cu(II) affected both light and dark reactions of photosynthesis (Krupa et al., 1995), and disturbed the synthesis of the D1 protein which assembles chl *a* molecules in microalgae cells (Patsikka et al., 1998). In addition, Cu(II) substituted for magnesium ion (Mg^{2+}) and consequently directly affected chlorophyll photosynthesis, and inhibited enzymes and various sites of photosystem II (PSII), enhanced photoinhibition and oxidative stress, thus disturbed the uptake of essential microelements (Kupper et al., 2002). Therefore when excessive Cu(II) existed in the ADSW, the Cu(II) affected synthesis of chlorophyll *a* and disturbed the uptake of nitrogen and phosphorus in *Coelastrella* sp. cells, thereby decreased the TP and NH_3-N removal ability.

From Fig. 3 (b) it can be seen that content of proteins in microalgae *Coelastrella* sp. was highly sensitive to Cu(II) concentration. At Cu(II) concentration of 0.0, 0.10, 0.50, 1.0, 2.0 and 3.0 mg/L, the ratios of proteins in *Coelastrella* sp. cells to dry cell mass (protein: dry cell mass) were 0.702, 0.297, 0.259, 0.297, 0.214 and 0.194, respectively. Copper

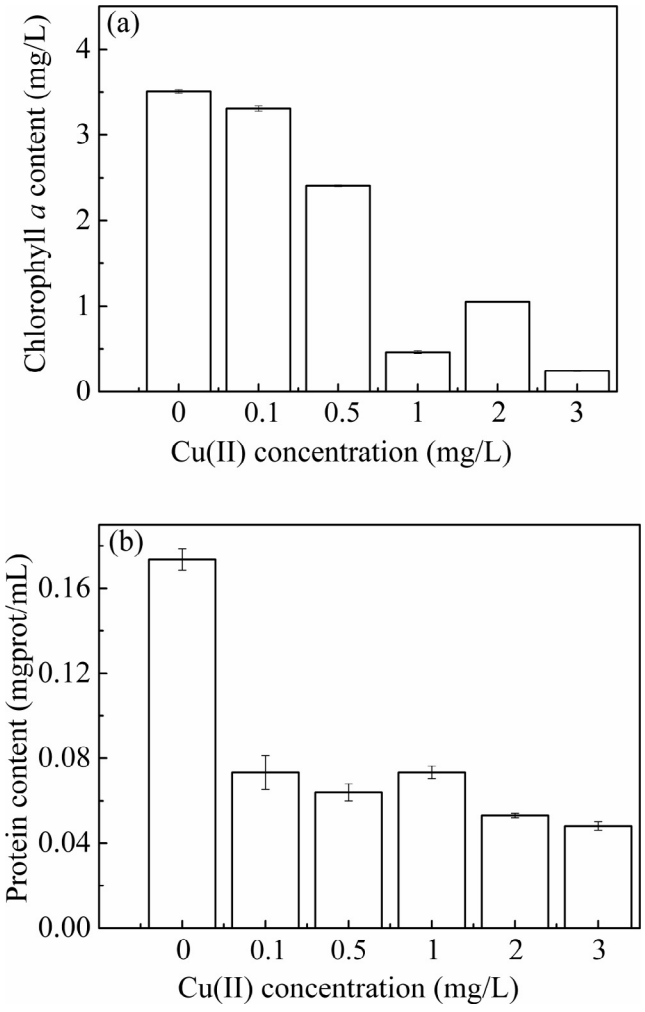

Fig. 3. Contents of chlorophyll *a* and proteins in Coelastrella sp. under various concentrations of Cu(II) on day 16.

could accumulate in microalgae, and induce toxicity and oxidative damage by producing reactive oxygen species (ROS) such as O_2, H_2O_2 and OH (Iseri et al., 2011). ROS could react rapidly with proteins, which led to irreversible peroxidation damage to proteins and oxidative stress and consequent cell death via apoptosis or necrosis (Vera-Estrella et al., 1994; Palanikumar et al., 2013).

3.3.2. Physiological stress

MDA and SOD contents were measured to evaluate the physiological stress of Cu(II) concentration on the microalgae (Fig. 4). As showed in Fig. 4, the MDA content of the microalgae cultured with 0.0 mg/L Cu(II) was the lowest, correspondingly up to 0.705 ± 0.021 nmol/mgprot. While *Coelastrella* sp. was cultured in 0.10 mg/L, MDA concentration increased dramatically to 2.0 ± 0.1 nmol/mgprot. MDA concentration was 3.3 ± 0.1, 5.3 ± 0.1, 6.5 ± 0.4 and 5.7 ± 0.4 nmol/mgprot at corresponding Cu(II) concentration of 0.50, 1.0, 2.0 and 3.0 mg/L, Sabatini et al. (2009) found the same results working with microalgae. Lipid peroxidation could indirectly reflect the influence of ROS generated by oxidative stress, the increased of MDA content could result from cell damage (Bandyopadhyay et al., 1999). These results suggest that cupric ions induced peroxidation of *Coelastrella* sp. cells, so high contents of MDA in *Coelastrella* sp. were produced at high Cu(II) concentrations.

Cu-Zn SOD which could remove oxygen free radicals and hydrogen peroxide, and SOD level in organisms could be an indicator of aging and death (Cao et al., 2009). From Fig. 4 it can be seen that the lowest SOD

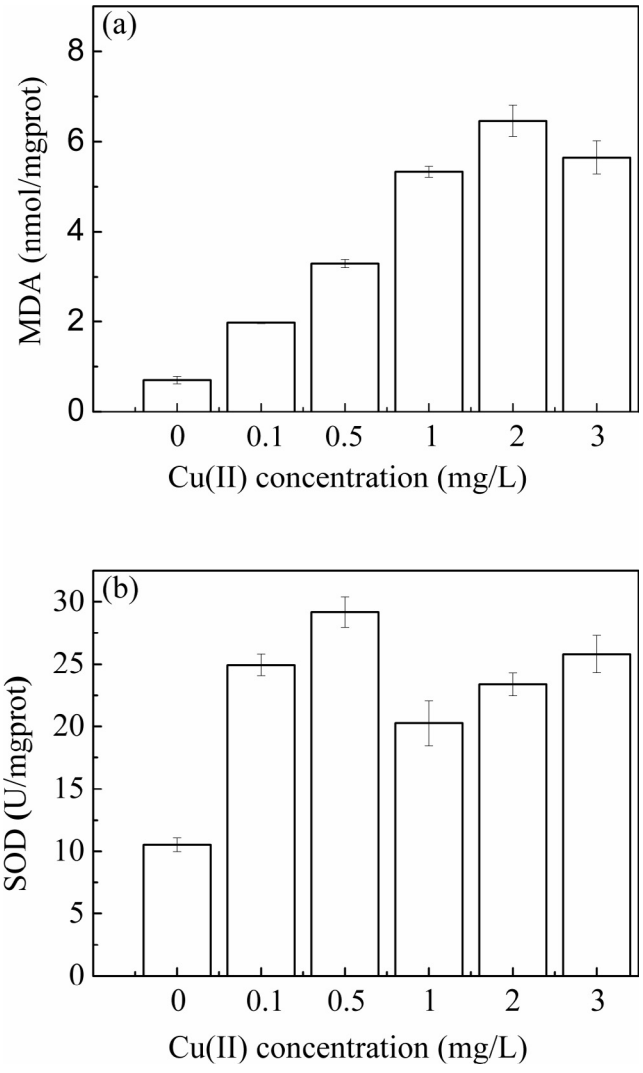

Fig. 4. MDA and SOD contents in proteins of *Coelastrella* sp. under various concentrations of Cu(II) on day 16.

content of protein was 10.52 ± 0.54 U/mgprot for the control test, and the highest SOD content was 29.2 ± 1.2 U/mgprot at 0.5 mg/L Cu(II). The SOD content at 0.10, 1.0, 2.0 and 3.0 mg/L of Cu(II) was 24.9 ± 0.9, 20.3 ± 1.8, 23.4 ± 0.9, 25.8 ± 1.5 U/mgprot, respectively. Therefore, SOD content increased with an increased Cu(II) concentration ranging either from 0.0 to 0.5 mg/L or from 1.0 to 3.0 mg/L, while decreased when Cu(II) concentration was higher than 1.0 mg/L of Cu(II).

SOD content increased when *Coelastrella* sp. was exposed to the ADSW with Cu(II), because the SOD must adapted the levels of MDA in microalgae cells. As showed in Fig. 4 SOD content increased from 0.10 mg/L Cu(II) was twice as the control, and MDA content was similar with the result. However, with the increase of MDA content in high Cu (II) concentration, these responses in cells were not enough to prevent the damaged by ROS, the cells were damaged even dead, and then the production of enzymes decreased. Therefore Cu(II) inhibited SOD produced at Cu(II) concentration no less than 0.50 mg/L. These results agreed with Sabatini et al. (2009).

3.3.3. FAME composition

FAME compositions in the microalgae which were applied to assess the potential value of microalgae as biodiesel and the fatty acid components of C16 and C18 were favorable for biodiesel production (Miao et al., 2009). FAME was measured at the various concentrations of Cu (II) after the microalgae were cultured in ADSW for 16 days (see Fig. 5). Fig. 5 showed the main fatty acids in *Coelastrella* sp. were hexadecanoic acid (C16:0), octadecatrienoic acid (C18:3) and octadecadienoic acid (18:2) when the Cu(II) concentration in ADSW was no higher than 1.0 mg/L. When the *Coelastrella* sp. was cultured in 2.0 and 3.0 mg/L of Cu(II), the octadecatrienoic acid (C18:3) disappeared, and hexadecanoic acid (C16:0) increased to 43.3%–57.8%, and octadecenoic acid (C18:1) increased to 34.4%–41.2%. With the increase of concentration of Cu(II) in ADSW media, octadecanoic acid (C18:0) in the microalgae was also increase. Cu(II) could increased the saturation of C18 and C16. This was close to the instauration of fatty acids that was reported by Yang et al. (2015). When there insisted high compositions of saturated fatty acids (SFA) in microalgae cells, the kinematic viscosity, pour point and melting point of biodiesel might increase (Luo et al., 2016). The increased ratios of saturated fatty acids could decrease the resistance to cold for microalgae cells. The high content of SFA could decrease the stability of chloroplasts and mitochondria at the cold condition, therefore affect the photosynthesis and growth of microalgae (Somerville et al., 1995). All the results indicated that when the microalgae was used to participate treatment of swine wastewater or heavy-metal-polluted wastewater for collect biodiesel, it was better to control the Cu(II) concentration for preventing the saturation of fatty acids.

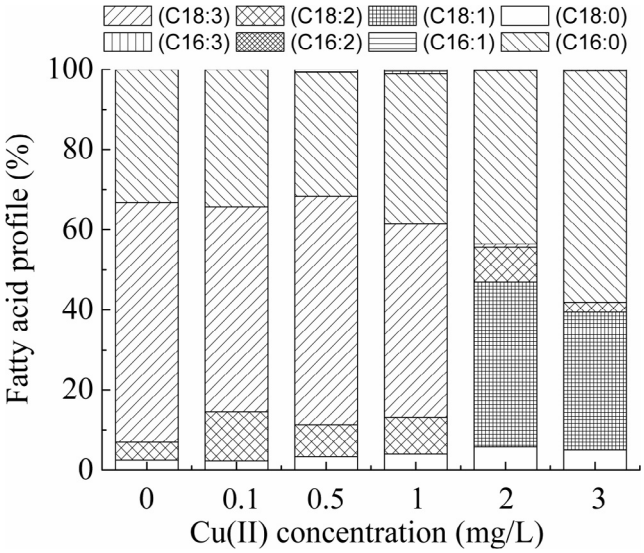

Fig. 5. FAME compositions in *Coelastrella* sp. cultured in ADSW at different concentrations of Cu(II) on day 16.

4. Conclusions

Cu(II) could inhibit NH_3-N and TP removal by *Coelastrella* sp. cultured in ADSW, and reduce the microalgal growth. The inhibition of Cu (II) to the microalgal photosynthesis was resulted from lipid peroxidation, protein denaturation and fatty acid saturation. A higher Cu(II) concentration led to a lower nutrient removal. When Cu(II) was higher than 1.0 mg/L, microalgae mass stopped increasing, meanwhile NH_3-N and TP removal dropped and stabilized, respectively. At 0.5 mg/L of Cu (II), TP removal efficiency maximized 84.9%, NH_3-N removal efficiency was 58.8%, and the biomass productivity and the tolerance to Cu(II) poison were optimal.

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