Algal Research 50 (2020) 101962

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

# Effects of humic acids on biotoxicity of tetracycline to microalgae *Coelastrella* sp.

Mengying Tong[a,1], Xiang Li[a,b,1], Qian Luo[a,b,1], Chunping Yang[a,b,c,*], Wei Lou[c], Haiyang Liu[d], Cheng Du[b,**], Lijun Nie[b], Yuanyuan Zhong[b]

[a] College of Environmental Science and Engineering, Hunan University and Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha, Hunan 410082, China
[b] Guangdong Provincial Key Laboratory of Petrochemical Pollution Processes and Control, School of Environmental Science and Engineering, Guangdong University of Petrochemical Technology, Maoming, Guangdong 525000, China
[c] Hunan Provincial Environmental Protection Engineering Center for Organic Pollution Control of Urban Water and Wastewater, Changsha, Hunan 410001, China
[d] Datang Environment Industry Group Co., Ltd., Haidian, Beijing 100097, China

ARTICLE INFO

*Keywords:*
Tetracycline antibiotics
Humic acids
*Coelastrella* sp.
Biotoxicity
Hormesis

ABSTRACT

The release of antibiotics into aquatic environments would induce adverse effects on organisms, so the environmental impact and fate of antibiotics must be paid close attention. In this paper, the effects of tetracycline on the growth of microalgae *Coelastrella* sp. in the presence of humic acids were investigated at various tetracycline concentrations ranging from 0.5 to 10 mg/L in Blue-Green medium, and the underlying mechanisms were also discussed. Results revealed that the microalgae growth showed a hormesis dose-response phenomenon under tetracycline stress with stimulation at low levels (≤2 mg/L) while inhibition at higher levels (>2 mg/L). More importantly, tetracycline biotoxicity to *Coelastrella* sp. decreased significantly due to the increase in humic acids concentration, as evidenced by the increased biomass, chlorophyll-a and total proteins contents, as well as the reduced oxidative stress response in *Coelastrella* sp. cells. The strong complexation between tetracycline and humic acids was responsible for the reduced tetracycline biotoxicity. These findings are helpful for better understanding the environmental risk of antibiotics in eutrophic waters.

## 1. Introduction

Tetracycline, one of the most commonly used typical antibiotics, was widely used as therapeutic agent for animal infection treatment and as feed supplements to promote livestock growth and feed efficiencies [1–3]. However, antibiotics are often poorly metabolized in living organisms and a considerable proportion (30–90%) is released into surroundings by discharging animal manure and wastewater [4,5]. Application of animal manure in farmland has led to the widespread presence of antibiotics in multiple environmental media, including adjacent surface waters, sediments, soils and groundwater [1]. The concentration of antibiotics in contaminated freshwater range from ng/L to μg/L. In certain cases, their concentrations can reach up to 50 mg/L in locally contaminated sources in production facilities [6]. Antibiotics persist in the environment because of their low biodegradability and water solubility, and the residual fractions are receiving a lot of attention because they could stimulate the development of antibiotic resistant genes in bacterial populations [7]. In addition, their bioactive properties have also caused serious concern about biological responses triggered in non-target organisms [8]. A large number of studies have proven the potential toxicity of tetracycline to a variety of organisms, including duckweed [9], algae [10] and bacteria [11,12].

Microalgae, a key primary component in aquatic ecosystems, owing to its high biomass productivity, worldwide distribution, high growth rate and low arable footprint, can be used as a model organism to evaluate the potential toxicity of tetracycline in aqueous systems [13,14]. To date, studies of tetracycline have evaluated the effects on a variety of algal species and communities. Ye et al. [6] demonstrated that the toxicity to *M. aeruginosa* growth of the following antibiotics was tetracycline > chlortetracycline > oxytetracycline. Xu et al. [15] reported that high concentrations of tetracycline significantly enhanced the membrane permeability and changed the structure of algal cells.

* Correspondence to: Chunping Yang, College of Environmental Science and Engineering, Hunan University, Changsha, Hunan 410082, China.
** Corresponding author.
*E-mail addresses:* yangc@hnu.edu.cn (C. Yang), ducheng@gdupt.edu.cn (C. Du).
[1] These authors contribute equally to this paper.

https://doi.org/10.1016/j.algal.2020.101962
Received 18 December 2019; Received in revised form 26 May 2020; Accepted 26 May 2020
Available online 09 June 2020
2211-9264/ © 2020 Elsevier B.V. All rights reserved.

However, even if the toxicity mechanism of tetracycline alone has been discussed, it is still difficult to assess the toxicity of tetracycline in natural water due to its complexity. In addition, almost all reports on the toxicity of antibiotics have used deionized water. However, the toxicity mechanisms of antibiotics may vary under different conditions. The varying chemicals and factors (e.g. metal cations, organic ligands, and pH) can alter the reactivity, mobility and fractional distribution of tetracycline in solutions, thereby modulating the bioavailability of tetracycline to organisms [11,16].

Dissolved organic matter (DOM) is a heterogeneous mixture of autolysis or metabolism of cells in microorganisms and aquatic plants [17]. DOM is ubiquitous in aqueous environments, exists at a level of milligrams per liter, and is rich in organic functional groups (e.g., carboxyl, phenol, hydroxyl, and amide) with high active sites [18,19]. Therefore, DOM could efficiently interact with antibiotics through complexation, cation bridging, adsorption, hydrogen bonding and cation exchange [20]. Humic acids are important active components of DOM, which can be found in a variety of environments, including sediments, soil, groundwater and surface water [21,22]. It is well know that humic acids can catalyze the abiotic degradation of environmental pollutants in soil-water systems, for example, Salvestrini [23] indicated that low molecular weight humic acids-like compounds could catalyze diuron hydrolysis. Senesi and Testini [24] also emphasized the importance of the role of humus in the environmental fate of several herbicides. Aggregation of humic substances could result in the formation of both hydrophilic and hydrophobic components and can lead to complicated interactions with organic contaminants, which has been widely used in biotoxicity and bioavailability studies of aquatic organisms [25]. Zhang et al. [26] showed that in the presence of humic acids, the acute toxicity of graphene oxide to *S. obliquus* was mitigated by 28.6%. Tang et al. [27] revealed that $EC_{50}$ (concentration for 50% of maximal effect) of nano-ZnO to *Anabaena* sp. increased from 0.74 ± 0.01 to 1.15 ± 0.04 mg/L in the presence of 3.0 mg/L of humic acids. Furthermore, established models, including free ion activity model and biotic ligand model have been applied to predict the biotoxicity and bioavailability of heavy metals [28]. It has been demonstrated that DOM reduced the bioavailability and biotoxicity of Cu, Zn and Cd to algae through its role as a metal-binding ligand, which is consistent with free ion activity model [29,30]. The relationship between humic acids and bioavailability of metal ions has been reported for different algae species, but few similar studies have focused on antibiotics, and the underlying mechanisms have not been well examined. Besides, the interaction of DOM with antibiotics is critical for the ecotoxicity and transport of antibiotics in eutrophic water. Therefore, the effects of tetracycline to the microorganism in the presence of humic acids should be further studied to decrease the uncertainty of environmental risk assessment of tetracycline.

The purpose of this study was to assess the effects of different initial concentrations of tetracycline on its biotoxicity to the growth of microalgae *Coelastrella* sp. in the presence and absence of humic acids. The biotoxicity of tetracycline were characterized by the changes of biomass, chlorophyll-*a* (Chl-*a*) and total proteins contents of *Coelastrella* sp. cells. The oxidative stress responses were also examined to reflect the oxidative damage evoked by tetracycline. The mechanisms by which humic acids changes the biotoxicity of tetracycline to microalgae were also discussed. Our work could provide useful biotoxicity information for tetracycline while taking into account of its interaction with DOM, which is helpful to understand the environmental behavior of tetracycline in eutrophic water.

## 2. Materials and methods

### 2.1. Chemicals

Tetracycline hydrochloride (purity >99%) were supplied by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Humic acids sodium salt, selected as a DOM model owing to its solubility in water, was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methylalcohol, acetonitrile and formic acid were HPLC grade. The methylalcohol and acetonitrile were purchased from Tedia Company, Inc. (Fairfield, USA) and the formic acid was purchased from Tianjin Kermel Chemical Reagent Co., Ltd. (Tianjin, China). All other reagents were analytical grade, and ultrapure water was used in the whole experiment. Fresh stock solutions were prepared for each experiment and the prepared humic acids solutions were passed through 0.45 μm syringe filters to remove insoluble particles.

### 2.2. Microalgal cultivation conditions

The microalgae *Coelastrella* sp. used in the study was obtained from our laboratory [31]. After verification, microalgae *Coelastrella* sp. has been deposited in Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB), Wuhan, China, and the strain number of the microalgae is FACHB-2400.

The algae strains were pre-cultivated in Blue-Green (BG11) medium [32] in an illuminating incubator at 25 ± 1 °C, under a 14: 10 h light/dark cycle provided by cool white fluorescent lights at an intensity of 100 μmol/$m^2$/s. The cultures were regular shaken three times a day, and all operations were carried out under sterile conditions. These experimental conditions were also maintained in subsequent experiments.

### 2.3. Toxicity test

A 12-day batch experiment was conducted to study the toxicity of tetracycline on microalgae *Coelastrella* sp. with and without humic acids. After pre-cultivation, algae cells in exponential growth phase were collected by gentle centrifugation (3500 rpm, 10 min). The algae cells were then washed twice with BG-11 medium and resuspended in experimental medium to obtain an initial cell density of 0.03 g/L freeze-dried biomass. Except the control group (0 mg/L), test solutions were prepared by adding tetracycline (0.5, 1.0, 2.0, 5.0 and 10 mg/L) and/or humic acids (2 and 5 mg/L) to 400 mL of BG-11 medium in a 500 mL Erlenmeyer flask and allowed to equilibrate overnight. In this work, the tested tetracycline concentrations used were at the mg/L level (higher than realistic concentrations) to obtain toxicity data, which is necessary for the risk assessment of tetracycline antibiotics. In addition, in many published articles, the tested antibiotic concentrations used were at the mg/L level, which is helpful to compare the toxicity of different types of antibiotics [6,33]. Humic acids were spiked at two concentrations of 2 and 5 mg/L representing the lower range of river and oligotrophic lake concentrations [29]. The pH of the test solutions were adjusted to 7.0 with 1 mol/L HCl or NaOH before autoclaving.

### 2.4. Analytical methods

#### 2.4.1. Microalgae biomass

Microalgae biomass was employed as a growth indicator to detect tetracycline toxicity. During 12-day test period, for each treatment, 5.0 mL of water sample was taken from the erlenmeyer flask every two days and the optical density (OD) was analyzed using a ultraviolet-visible (UV–vis) spectrophotometer (UV-2550, Japan) at 680 nm wavelength. Considering the linear relationship between dry weight of algae cells and $OD_{680}$, the dry weight of *Coelastrella* sp. was calculated by Eq. (1) [34]:

$$\text{Dry weight (g/L)} = 0.3357 \times OD_{680},\ R^2 = 0.9962 \quad (1)$$

The yield inhibition rate μ was calculated by Eq. (2):

$$\mu = (Y_C - Y_T)/Y_C \times 100\% \quad (2)$$

where μ is the yield inhibition rate; $Y_C$ is the yield value of the control group; $Y_T$ is the yield value of treatment group.

*2.4.2. Measurement of chlorophyll a contents*

Chlorophyll *a* (Chl-*a*) contents were measured using a UV–vis spectrophotometer at 665 and 649 nm wavelength respectively after the algal cells were extracted with 95% ethanol in dark at 4 °C for 24 h. The control group was prepared by 95% ethanol. The Chl-*a* concentrations were calculated using the following equation [34]:

$$\text{Chl} - a\ (\text{mg/L}) = (13.95 \times \text{OD}_{665} - 6.88 \times \text{OD}_{649}) \div 5 \quad (3)$$

*2.4.3. Proteins content and oxidative stress response assays*

Microalgae cells of the cultured suspension were harvested by centrifugation (3500 rpm, 10 min) and washed twice with phosphate buffered solution (0.01 M, pH 7.4). The harvested microalgae were resuspended in 5 mL of phosphate buffered solution and homogeneously broken by ultrasonic treatment (XO-1000D, China) at 25% power for 5 min (ultrasonic time: 3 s; rest time: 8 s) under ice bath. Then, the broken cells were centrifuged again and the supernatant was used for biological analyses. The contents of total proteins and antioxidants, including superoxide dismutase (SOD), malondialdehyde (MDA) and reduced glutathione (GSH), were quantitatively measured by assay kits (Nanjing Jiancheng Bioengineering Institute, China).

*2.4.4. Analyses of tetracycline and humic acids concentration in solution*

During the 12-day exposure, both the concentrations of target antibiotic tetracycline and humic acids were regularly measured and replenished every 24 h to maintain stable exposure dose. In detail, the test solutions (5 mL) were collected and filtered using a syringe filter (0.45 μm). The concentration of tetracycline was analyzed using a HPLC (High Performance Liquid Chromatography) (1260 Infinity II, Agilent, USA) equipped with a UV detection and an Agilent ZORBAX SB-C18 (5um × 4.6 mm × 250 mm) reversed-phase column [35,36]. The operating conditions were as follows: the mobile phase consisted of acetonitrile and 0.01 M formic acid (20:80, v/v) with a retention time of 5 min, flow rate of 1 mL/min, detection wavelength of 360 nm, and an injection volume of 20 μL at 30 °C. The concentration of the humic acids in the solution was measured by a UV–vis spectrophotometer at 280 nm. The control of humic acids loss on the filters was systematically carried out and taken into account in the calculation when determining the amount of humic acids. After measuring the concentrations of tetracycline and humic acids in the test solution, different amounts of fresh storage solution (1 mg/L) of tetracycline and humic acids were added according to the test results.

*2.4.5. Complexation of tetracycline with humic acids*

The freely soluble and humic acids-bound tetracycline amounts in BG-11 medium were measured using solid-phase extraction method [11]. For the solid-phase extraction method, Ding et al. have proved that freely dissolved pharmaceutical in the mixture of DOM and pharmaceutical is fully adsorbed on the hydrophilic-lipophilic balance cartridge while DOM and DOM-associated pharmaceutical completely pass through the cartridge [37]. In detail, tetracycline (at 0.1, 0.5 and 1.0 mg/L) and humic acids (at 5.0, 25, and 50 mg/L) were mixed and equilibrated in BG-11 medium for 48 h. The supernatant (5.0 mL) of the mixture was passed through a hydrophilic-lipophilic balance cartridge, which retained the freely soluble tetracycline (absorbed by the cartridge) while allowing for the humic acids-tetracycline complex to elute through the cartridge. The retained tetracycline was eluted sequentially with 5.0 mL of methanol/water solution (1:1, v/v) containing 150 mg/L ethylenediaminetetraacetic acid, and 5.0 mL of methanol containing 1% (v/v) oxalic acid. Then, the eluant was combined and the tetracycline concentration was quantified using HPLC. The amounts of tetracycline complex with humic acids were determined as the difference between the tetracycline concentrations in the solution before and after contact with humic acids.

The complexation percentage μ of tetracycline bound to humic acids in BG-11 medium was calculated by Eq. (4):

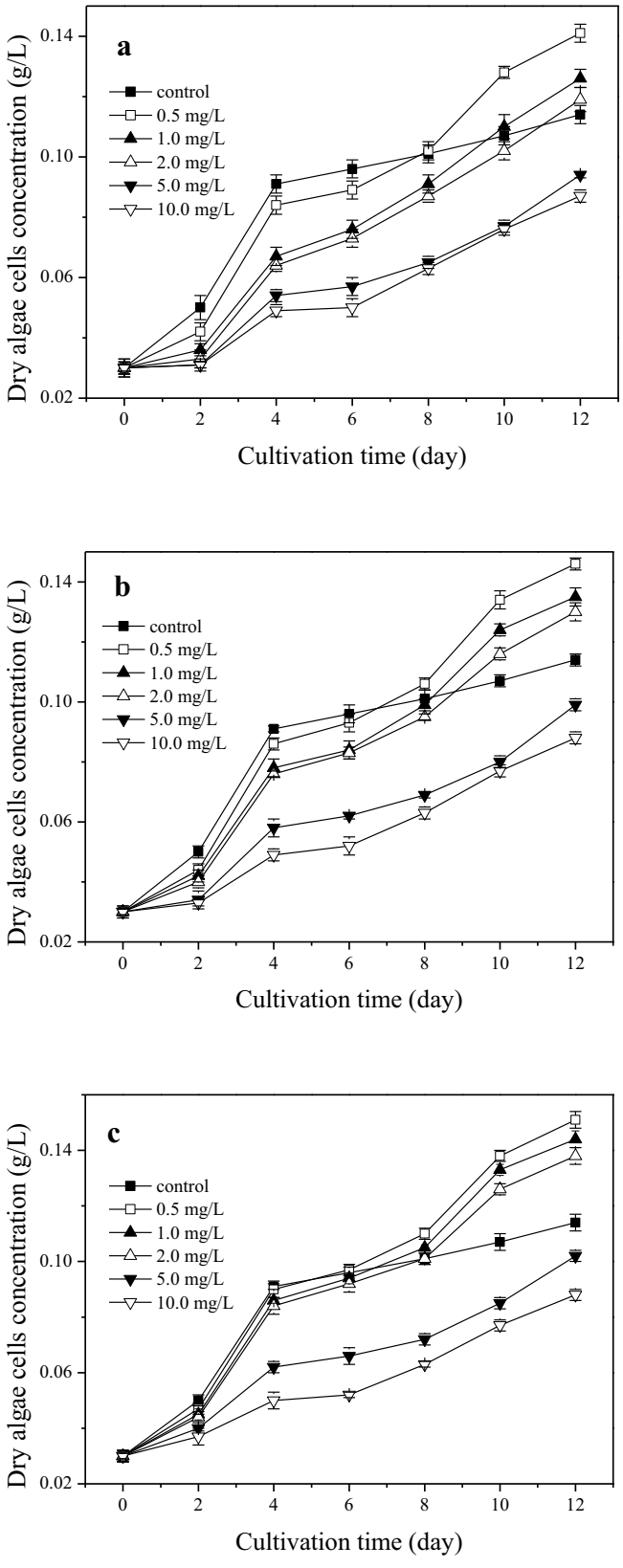


**Fig. 1.** Effects of humic acids on the growth of *Coelastrella* sp. under different tetracycline concentrations in BG-11 medium: (a) Control; (b) 2 mg/L humic acids; and (c) 5 mg/L humic acids. Data were expressed the average and standard deviation in biological duplicates ($n$ = 3).

$$\mu = (C_0 - C_1)/C_0 \times 100\% \quad (4)$$

where μ is the complexation percentage; $C_0$ is the tetracycline concentrations in the solution before contact with humic acids; $C_1$ is the tetracycline concentrations in the solution after contact with humic acids.

### 2.5. Statistical analyses

The data shown in this study were expressed as mean ± SD (standard deviation) of three replicates. All statistical analyses were performed with Origin 8.0 (OriginLab Co., USA) and SPSS 17.0 (SPSS, Chicago, IL, USA). One-way ANOVA (Chicago, USA) was conducted to test for a significant difference.

## 3. Results and discussion

### 3.1. Growth of Coelastrella sp. and biomass production

#### 3.1.1. Toxicity of tetracycline to microalgae in absence of humic acids

The growth curves of *Coelastrella* sp. at various initial tetracycline concentrations from 0 to 10.0 mg/L was presented in Fig. 1a. The growth of *Coelastrella* sp. was highly sensitive to the tested antibiotic throughout the treatment. Within the first 8 days, the biomass of *Coelastrella* sp. cultivated in all tetracycline-treated media was obviously lower than the control, which indicated that tetracycline had a significant toxicity to *Coelastrella* sp. in the early stage of cultivation. This result is similar with Yang et al. [38] where the growth of *M. aeruginosa* was highly inhibited when the tetracycline concentration is higher than 0.1 mg/L. Furthermore, the growth inhibition of *Coelastrella* sp. caused by tetracycline was essentially proportional to tetracycline concentration, as reported in previous literatures [6,15]. However, as the cultivation time increased, the biomass of the low-concentration of tetracycline-treated groups gradually exceeded the control. The cell density of the tetracycline-treated group at initial concentrations of 0.5, 1.0 and 2.0 mg/L exceeded the control group on day 8, 10 and 12, respectively. After 12-day cultivation, the dry weight of *Coelastrella* sp. in the control groups was relatively high, correspondingly increased from 0.03 to 0.114 g in 12 days. The final biomass of Coelastrella sp. was 0.141, 0.126, 0.119, 0.094 and 0.087 g under the tetracycline concentrations of 0.5, 1.0, 2.0 5.0 and 10 mg/L respectively. The algal biomass was correspondingly increased by 23.7%, 10.5%, 3.5%, −17.5% and −23.68%, respectively, compared with the control (Fig. 2). The final biomass of the low-concentration tetracycline-treated groups (< 2 mg/L) was significantly higher than the control, but a further increase in tetracycline concentration resulted in an obviously decrease in biomass. Similar patterns of stimulation at low levels and inhibition at higher levels were also revealed for tetracycline on *E. coli* [39].

This growth stimulatory effect under weak stress occurs in a variety of species and various exogenous chemicals [40]. Calabrese [40] called this phenomenon “hormesis” and suggested that it is probably owing to the over-corrected regulation of biosynthesis control mechanisms for low-level adverse attacks, leading to a greater growth than normal. Therefore, the effect of tetracycline on the growth of *Coelastrella* sp. should follow the hormesis dose-response relationship. In addition, in our experiment, the growth hormesis effect was not manifested in the beginning, but it gradually appeared after a period of cultivation under tetracycline stress. Furthermore, the hormesis effect related to the concentration of the target antibiotic, the higher the concentration, the later the hormesis appears. The hysteresis of the hormesis effect may be attributed to the fact that the target antibiotic concentrations set by our experiment are greater than the threshold of stimulus concentration, so it showed inhibition at the beginning. With the increase of time, owing to the adaptability of biological systems after long-term exposure to tetracycline, the microalgae may produce antibiotic resistance genes with enhanced tolerance, so the hormesis phenomenon gradually appeared.

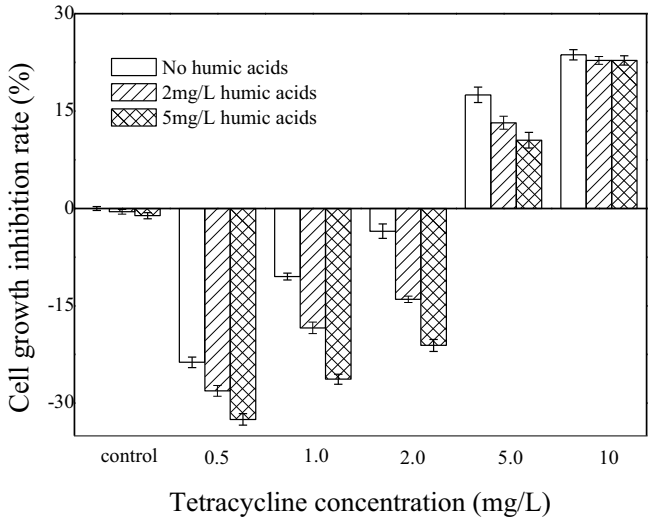


**Fig. 2.** Effects of humic acids on the growth inhibition rate of *Coelastrella* sp. under different tetracycline concentrations. Data were expressed the average and standard deviation in biological duplicates ($n$ = 3).

However, our results showed some differences compared with other literatures. Previous research have shown that antibiotics is highly toxic to algal growth, even at lower concentration [38]. In this study, the final growth performance of *Coelastrella* sp. was almost not adversely affected by tetracycline at concentrations below 2 mg/L, indicating that this algae species is highly tolerant to tetracycline. This may also be attributed to that previous studies have focused on the acute toxicity of antibiotics. Since the sensitivity of microorganisms to antibiotics may vary depending on the specific endpoint of test, therefore the long-term effects are also worth investigating. In general, the finding of the hormesis biphasic dose response of tetracycline to *Coelastrella* sp. has important implications regarding risk assessments of antibiotics in ecological systems.

#### 3.1.2. Toxicity of tetracycline to microalgae in presence of humic acids

The growth curves of *Coelastrella* sp. under tetracycline stress in the presence of 2 and 5 mg/L humic acids were shown in Fig. 1b and c, respectively. In the first 6 days, the tetracycline treated-group also showed an inhibitory effect compared to the control, which was the same as in Fig. 1a. Moreover, as the culture time increased, the growth of *Coelastrella* sp. also showed a hormesis dose response under tetracycline stress. Moreover, the phenomenon of tetracycline promoting *Coelastrella* sp. growth appeared earlier than that of the tetracycline-treated groups without humic acids. In the presence of 5 mg/L of humic acids, the biomass of microalgae treated by 0.5, 1.0 and 2.0 mg/L tetracycline exceeded the control group on day 6, 8 and 10, respectively. Two days earlier than the tetracycline-treated groups without humic acids, which indicated that humic acids reduced the available tetracycline concentration in the solution. This may be attributed to the fact that the complexation of humic acids and tetracycline reduced the available amount of tetracycline, causing the concentration of freely soluble tetracycline in the solution was decreased to approach the maximum response dose. In addition, it was also observed throughout the test that the biomass of *Coelastrella* sp. was significantly increased in the presence of 2 mg/L humic acids compared to the tetracycline-treated group without humic acids, which was more obvious in the presence of 5 mg/L humic acids. This suggested that humic acids might play a role in alleviating tetracycline toxicity. Fig. 2 shows that in the presence of 2 mg/L of humic acids, the biomass of *Coelastrella* sp. under the tetracycline concentration of 0.5, 1.0, 2.0 5.0 and 10 mg/L were increased by 4.4%, 7.9%, 10.5%, 4.3% and 0.88%, respectively,

compared with the tetracycline-treated group without humic acids. In addition, the microalgae biomass increased more obviously in the presence of 5 mg/L of humic acids, with a corresponding value of 8.8%, 15.8%, 17.6%, 7.0% and 0.88%, respectively. An increase in humic acids concentration from 2 to 5 mg/L showed an obvious decrease in growth inhibition rate.

The observed toxicity mitigation of tetracycline in the presence of humic acids is very consistent with previous studies on *E. coli*; the bioavailability of tetracycline to *E. coli* is diminished in the presence of humic acids, as evidenced by decreased expression of antibiotic resistance genes [11]. However, in this study there was almost no distinct difference of biomass between the treatments with and without humic acids when the initial concentration of tetracycline was 10 mg/L. This may be attributed to the high toxicity of tetracycline at high level, the mitigation effect of a small amount of humic acids is not sufficient to show an apparent increase in growth.

In addition, Fig. 2 revealed humic acids alone almost caused no effect in algae cell growth compared with the control, so the impact of humic acids at 2.0 and 5.0 mg/L alone to *Coelastrella* sp. is negligible, which is consistent with the conclusion reached by Tang et al. [27]. Freely soluble tetracycline (not complexion with humic acids) was considered to be the predominant fraction responsible for the evoked toxicity expression [11]. The reduced biotoxicity of tetracycline might due to the competitive complexion of humic acids with tetracycline. Thereby decreased the freely soluble tetracycline concentration in the medium and reduced the absorption of tetracycline by algal cells, consequently showed an obvious increase in microalgae biomass. Similar result was reported that DOM competed with cell surface functional groups of *Chlorella zofingiensis* for $Al^{3+}$ [41].

### 3.2. Effect of tetracycline on chlorophyll a and proteins contents in the presence of humic acids

To further assess the influence of tetracycline with a consideration of humic acids, the amounts of Chl-*a* and total proteins associated with *Coelastrella* sp. were evaluated with and without humic acids (Fig. 3).

#### 3.2.1. Chlorophyll a

Chl-*a* is usually used as an indication of photosynthesis capacity. It can be observed from the Fig. 3a that the Chl-*a* synthesis in *Coelastrella* sp. was promoted by tetracycline at low concentrations (≤2 mg/L), inhibited at high concentrations (>2 mg/L), which is consistent with the hormesis biphasic dose responses exhibited by the growth of the microalgae. At tetracycline concentration of 0.5, 1, 2 mg/L, the Chl-*a* content increased by 14%, 6.9%, 0.9%, respectively, indicating that low dose of tetracycline stimulated the synthesis of Chl-*a* in *Coelastrella* sp. cells. This may be attributed to the algae need to synthesize more photosynthetic pigments to cope with the low levels of tetracycline and alleviate the toxicity to algal cells. Similar stimulation effects in chlorophyll production have been reported by Guo et al. [42] in testing other antibiotics, including tylosin, lincomycin and trimethoprim. Although the reasons for the increase in chlorophyll content caused by low doses of exogenous toxic chemicals are unclear, it has been speculated that the electron transport between primary and secondary quinone receptors is activated by stressors, which in turn triggers photosystems I and II [3]. Hu et al. [9] also indicated that the stimulated secretion of biogenic amines in the body is the reason that low concentrations of tetracycline promote the synthesis of photosynthetic pigments in duckweed; the secretion of biogenic amines could alleviate environmental stress by maintain membrane stability and remove excess free radicals.

However, with increasing exposure concentrations of tetracycline, the amount of free radicals produced in algae is large, causing the function of antioxidant system to fail and the algae cells to be damaged. Ultimately, the higher concentrations of tetracycline resulted in a reduced Chl-*a* synthesis in *Coelastrella* sp. cells. The Chl-*a* content in

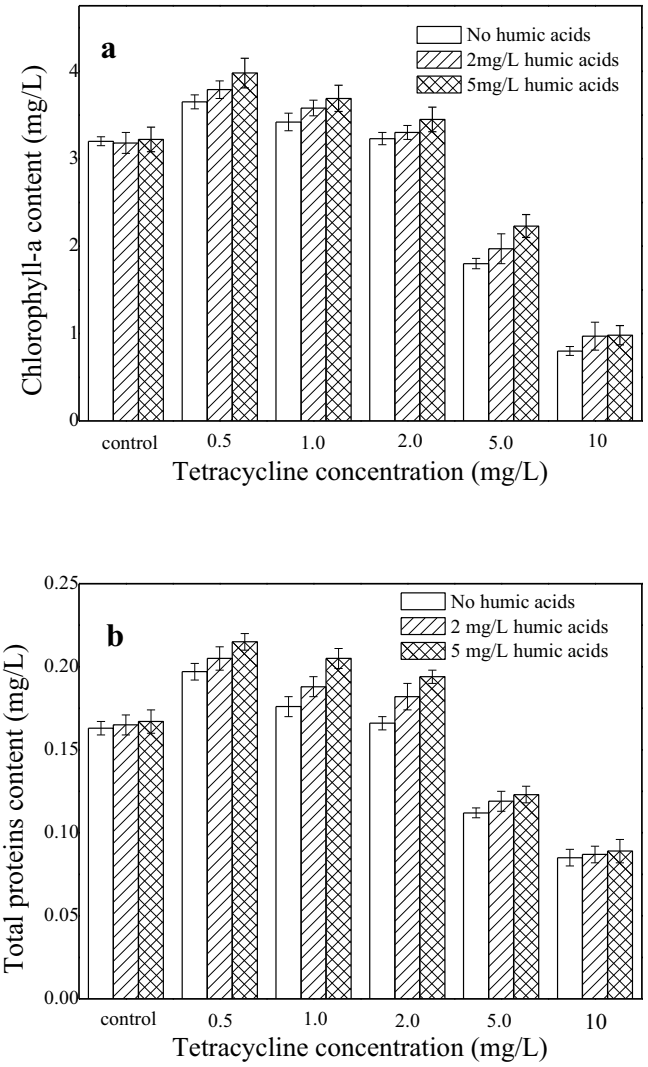


**Fig. 3.** Effects of humic acids on concentrations of (a) chlorophyll *a* and (b) total proteins in *Coelastrella* sp. under different tetracycline concentrations. Data were expressed the average and standard deviation in biological duplicates ($n = 3$).

*Coelastrella* sp. cells considerably decreased 43.8% at 5.0 mg/L tetracycline, and sharply dropped 75.0% at 10.0 mg/L tetracycline, which suggested that the photosynthetic system in the algae cells was damaged. Li et al. [10] also reported similar result that the production of Chl-*a*, Chl-*b* and carotenoids significantly declined with the increasing tetracycline concentrations. The decrease in photosynthetic pigment content may be resulted from changes in chloroplast ultrastructure and membrane lipid peroxidation [9]. Li et al. [10] also reported that toxic chemicals can inhibit the activities of protochlorophyllide reductase, which plays a key role in chlorophyll synthesis. Fig. 3a also revealed the favorable role of humic acids in promoting Chl-*a* synthesis at all tetracycline concentrations. For example, the levels of Chl-*a* in algal cells treated with 5 mg/L tetracycline increased by 9.4% and 23.9% in the presence of 2 and 5 mg/L humic acids, respectively, indicating that humic acids relieved the damage of tetracycline to algal photosynthetic system. In the presence of humic acids, the increased Chl-*a* synthesis at low tetracycline levels (≤2 mg/L) indicated the enhanced hormesis effects. This is also the result of humic acids reducing the concentration of available tetracycline in the solution to approach the maximum response dose. The mitigation effect in Chl-*a* production by humic acids have also been reported in other studies when the algae cultures was treated with graphene oxide [26], $Zn^{2+}$ [27] and As(III) [43].

### 3.2.2. Proteins

Tetracycline is believed to interfere with the protein synthesis machinery and the effects of the tetracycline were further evaluated by isolation and quantification of the total proteins in *Coelastrella* sp. cells, as shown in Fig. 3b. It can be observed that the concentrations of tetracycline have a great impact on the production of proteins in *Coelastrella* sp. cells. Similar with the Chl-*a* synthesis, the effects of tetracycline on proteins production also presented the hormesis dose-response relationships, with a clear decline observed at higher concentrations and an increase at lower concentrations of tetracycline. Exposure to low doses of tetracycline (0.5–2 mg/L) caused a significant increase in total proteins of 1.8–20.9%. Furthermore, at tetracycline concentration of 0.5 mg/L, the proteins content in *Coelastrella* sp. was higher than any other groups, which showed that 0.5 mg/L was the closest tetracycline concentration to the maximum response dose. The reasons for promoting the formation of total proteins may be that stress caused by xenobiotic can evoke metabolic pathways of proteins in plants to against the physiological stress caused by biotic or abiotic factors [9]. As the tetracycline stress increased, the total proteins content is lower than the control, the total proteins contents under the tetracycline concentrations of 5.0 and 10.0 mg/L were 68.7% and 52.1% of the control, respectively. This may be due to the fact that reactive oxygen species (ROS) evoked by toxic chemicals can react quickly with proteins, causing nonreversible peroxidation damage and consequently lead to cell necrosis or apoptosis [44–46]. Antibiotics have also been reported to combine the 16S part of the 30S ribosomal subunit and avoid the aminoacyl tRNA from attaching to the A-site of the mRNA-ribosome complex, thus inhibiting protein synthesis and cell growth [47].

Fig. 3 also revealed the favorable role of humic acids in promoting total proteins synthesis at all tetracycline concentrations. The alleviation effects of humic acids on protein synthesis is similar to the Chl-*a* as a concentration-related promotion. For example, the levels of total proteins in algal cells treated with 5 mg/L tetracycline increased by 6.3% and 9.8% in the presence of 2 and 5 mg/L humic acids, respectively.

### 3.3. Oxidative stress

Antioxidant response is an important defense mechanism for plants scavenging ROS, which can mitigate the peroxidation damage induced by stress of xenobiotics [48]. In order to examine the mechanisms of tetracycline toxicity in *Coelastrella* sp. and the effect of humic acids on oxidative damage induced by tetracycline, we conducted toxicity tests with *Coelastrella* sp. cells. The cellular antioxidase activities including superoxide dismutase (SOD), malondialdehyde (MDA) and non-enzymatic antioxidant reduced glutathione (GSH) were considered as the sensitive biomarkers for a variety of environmental stress, which were measured and presented in Fig. 4.

SOD was generally considered to be the first line of defense against ROS, which could prevent the accumulation of superoxide in microalgae cells [10]. It could be observed from Fig. 4a that the SOD contents in *Coelastrella* sp. cells increased significantly under tetracycline stress and were positively correlated with the concentration of target antibiotic. The activity values from the tetracycline concentrations of 0.5 to 10 mg/L were 2.2, 3.5, 4.5, 10.6 and 20.1 times of the control, respectively. When the tetracycline concentration was higher than 5 mg/L, significant increase of SOD activities were observed. The increased activities of SOD indicated an enhanced ability of *Coelastrella* sp. to remove the $O_2\cdot$ and $H_2O_2$ radicals, suggesting that tetracycline triggered excessive ROS and damaged the oxidative system. Similarly, Liu et al. [49] reported that the activities of SOD increased with the increase of ROS triggered by spiramycin and amoxicillin in *Microcystis aeruginosa*. Accordingly, oxidative damage could be a common cause of the toxicity of tetracycline to *Coelastrella* sp. cells.

As shown in Fig. 4b, the MDA activities of *Coelastrella* sp.

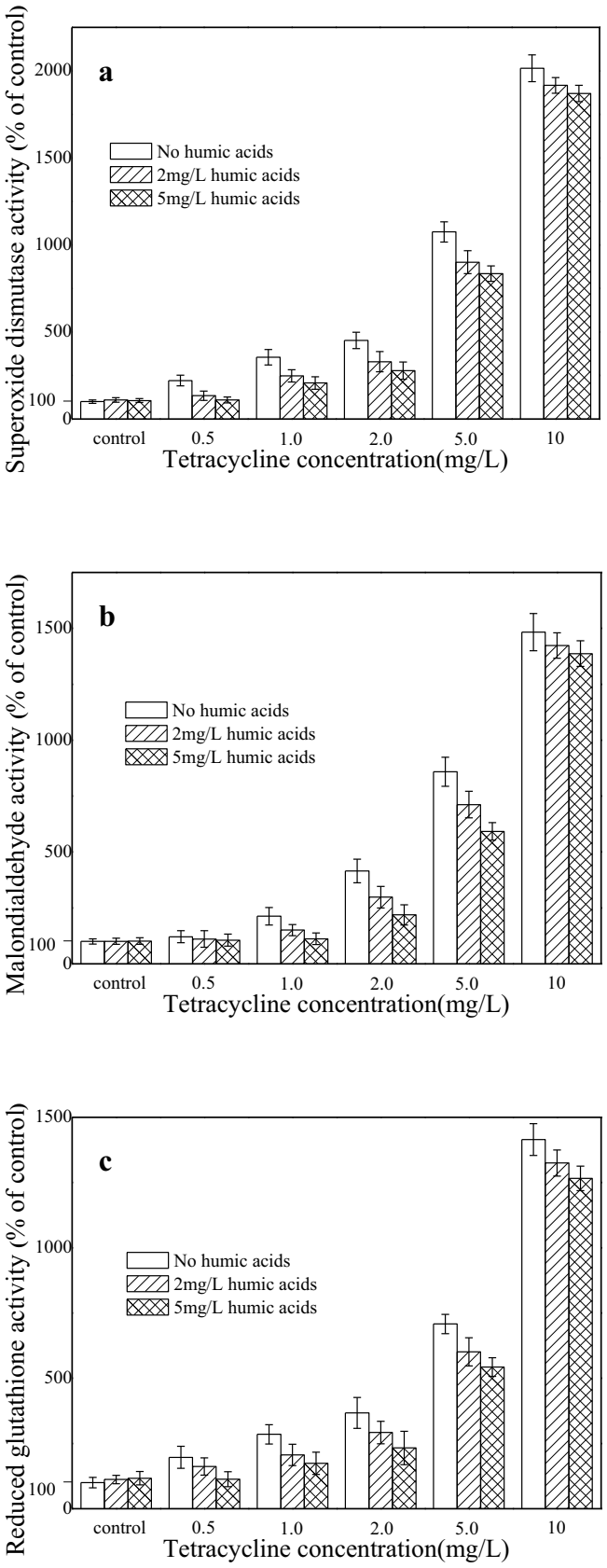


**Fig. 4.** Effects of humic acids on oxidative stress response of *Coelastrella* sp. to tetracycline: (a) superoxide dismutase activity; (b) malondialdehyde activity; and (c) reduced glutathione. Data were expressed the average and standard deviation in biological duplicates ($n = 3$).

significantly increased in response to the increasing tetracycline stress and the contents were 1.2, 2.1, 4.2, 8.6 and 14.8 times of the control, respectively. This indicated that tetracycline not only produced oxidative stress on *Coelastrella* sp., but also generated lipid peroxidation to the algal cell membrane. However, at 0.5 mg/L tetracycline, almost no increase in MDA content was observed. This may be because the stimulation of antioxidants could effectively eliminate ROS, thereby preventing the algal cells from oxidative damage under weak tetracycline stress. However, with the increase of tetracycline concentration, these antioxidant responses in algal cells were not sufficient to prevent the damage due to ROS, and then the cells were damaged and even apoptosized. GSH is important non-enzymatic antioxidant and plays important roles in scavenging ROS and biotransformation of xenobiotics in plants [50]. In the study, GSH activities were observed to be stimulated by tetracycline in *Coelastrella* sp. cells and the responses were 1.97–14.15 times of the control (Fig. 4c).

In the presence of humic acids, the relative SOD, MDA and GSH levels reduced significantly in proportion to the concentration, which reflected that humic acids could alleviate the severe oxidative damage of tetracycline to *Coelastrella* sp. cells. Similar results were also reported that humic acids reduced the oxidative damage in algae cells caused by nano-$TiO_2$ [51] and graphene oxide [26]. It has been also reported that humic acids could serve as an antioxidant by reacting with ROS, which would further decrease the toxicity of tetracycline on algal cells [52].

### 3.4. Complexation of tetracycline with humic acids

To further assess whether the humic acids-tetracycline complexation resulted in a decrease in the concentration of freely soluble tetracycline in the medium, the humic acids-bound and freely soluble tetracycline fractions in BG-11 medium were quantitatively analyzed. As shown in Fig. 5, the free soluble tetracycline concentration was markedly reduced in the presence of humic acids. At tetracycline of 0.1 mg/L, the proportion of free tetracycline decreased from 82.5% to 54.5% of the total when humic acids increased from 5 to 50 mg/L. This indicated that under low concentration of tetracycline, a considerable fraction of tetracycline could complex with the humic acids in solution, which manifested a relatively stronger affinity capability. Ding et al. [37] reported that at pH 8.0, the presence of 5 mg/L humic acids could complex about 25.9% tetracycline (50 μg/L) in water, and the complex fraction increased up to 93.2% as the humic acids concentration increased to 200 mg/L. Since free-dissolved tetracycline was considered as the dominant component responsible for its toxicity; the complexation of tetracycline with humic acids could diminish the content of freely soluble tetracycline in medium, thus lowering its bioavailability and biotoxicity. The relatively strong interaction between tetracycline and humic acids could potentially affect the uptake of tetracycline by cells, thereby regulating the selective pressure exerted on natural algal communities in eutrophic water.

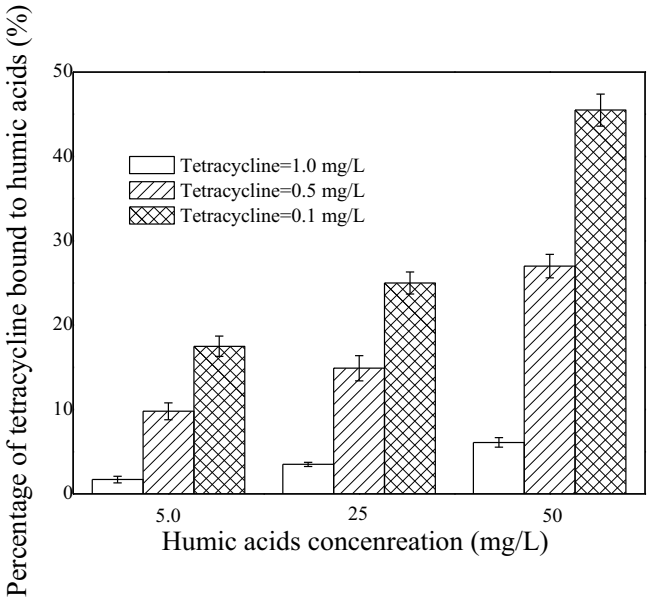


**Fig. 5.** Percentage of tetracycline bound to humic acids in BG-11 medium (pH = 7.0). The values represent mean ± SD ($n$ = 3).

Several techniques have been successfully used to characterize the binding of humic acids with organic contaminants, including fluorescence quenching, solid phase micro extraction, dialysis equilibrium [37]. H-bonding, cation bridging and ion exchange have been considered as the main mechanisms for the tetracycline and humic acids complexation [20]. In addition, the adsorption of tetracycline onto humic acids has been found to obey the Freundlich and the adsorption-desorption hysteresis was observed which may further impede the release of absorbed tetracycline and deduce its bioavailability [20]. However, as tetracycline increased to 1 mg/L, proportion of the complexed tetracycline only increased from 1.7% to 2.1% of total after humic acids increased from 5 to 50 mg/L. The proportion of complexed tetracycline decreased with the increasing total tetracycline concentrations. The observed tetracycline toxicity reduction in the presence of humic acids was lower than or equal to expected. It is speculated that the complexation between humic acids and tetracycline may not be the only mechanism controlling the bioavailability of tetracycline to *Coelastrella* sp. cells. It is also reported that the humic acids could be adsorbed on cells surface and acts as a barrier to the entry of tetracycline into the bioreceptor, thereby diminishing the cells surface site available to toxic substances and reducing tetracycline uptake by algae [53]. In addition, humic acids adsorbed on the algae cells surface can also adsorb tetracycline, which could further prevent the approaching of tetracycline to cells.

## 4. Conclusions

The growth of *Coelastrella* sp. presented a biphasic hormesis dose-response phenomenon under tetracycline stress. In addition, humic acids decreased the biotoxicity of tetracycline to *Coelastrella* sp., as evidenced by the mitigation in the tetracycline-induced reduction of biomass, Chl-*a*, total proteins and oxidative stress response. The formation of humic acids-tetracycline complex is responsible for reducing uptake of tetracycline by the microalgae. Extensive findings of the study have important significance on the environmental toxicity and risk evaluation strategies to avoid overestimate the risk associated with antibiotics.

## CRediT authorship contribution statement

**Mengying Tong**: Methodology, Writing - original draft. **Xiang Li**: Investigation. **Qian Luo**: Investigation. **Chunping Yang**: Conceptualization, Methodology. **Wei Lou**: Methodology. **Haiyang Liu**: Formal analysis, Data curation. **Cheng Du**: Methodology. **Lijun Nie**: Formal analysis, Data curation. **Yuanyuan Zhong**: Writing - review & editing.

## Declaration of competing interest

The authors declare there no conflict of interest that could perceive to influence the results of the research.

## Acknowledgments

Support from the National Natural Science Foundation of China (Grant No.: 51978178, 51478172 and 51521006), the Department of

Science and Technology of Guangdong Province of China (Contract No.: 2019A1515012044), Maoming Municipal Science and Technology Bureau of Guangong Province of China (2018S0011), the International S&T Cooperation Program of China (Contract No.: 2015DFG92750), and the Department of Science and Technology of Hunan Province of China (Contract No.: 2017JJ2029 and 2017SK2362) is highly appreciated.

## Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

## Authors' agreement to authorship and submission

All persons designated as author agree to submit the manuscript for peer review.

## References

[1] A.K. Sarmah, M.T. Meyer, A.B. Boxall, A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment, Chemosphere 65 (2006) 725–759, https://doi.org/10.1016/j.chemosphere.2006.03.026.

[2] Y. Lin, X. Wu, Y. Han, C.P. Yang, Y. Ma, C. Du, Q. Teng, H.Y. Liu, Y.Y. Zhong, Spatial separation of photogenerated carriers and enhanced photocatalytic performance on $Ag_3PO_4$ catalysts via coupling with PPy and MWCNTs, Appl. Catal. B Environ. 258 (2019) 117969, https://doi.org/10.1016/j.apcatb.2019.117969.

[3] Y. Xiong, D. Hozic, A.L. Goncalves, M. Simões, P.-Y. Hong, Increasing tetracycline concentrations on the performance and communities of mixed microalgae-bacteria photo-bioreactors, Algal Res. 29 (2018) 249–256, https://doi.org/10.1016/j.algal.2017.11.033.

[4] L. Bai, Z. Zhao, C. Wang, C. Wang, X. Liu, H. Jiang, Multi-spectroscopic investigation on the complexation of tetracycline with dissolved organic matter derived from algae and macrophyte, Chemosphere 187 (2017) 421–429, https://doi.org/10.1016/j.chemosphere.2017.08.112.

[5] F.B. Liu, W.T. Zhang, W.J. Chen, J. Wang, Q.F. Yang, W.X. Zhu, J.L. Wang, One-pot synthesis of $NiFe_2O_4$ integrated with EDTA-derived carbon dots for enhanced removal of tetracycline, Chem. Eng. J. 310 (2017) 187–196, https://doi.org/10.1016/j.cej.2016.10.116.

[6] J. Ye, Y. Du, L. Wang, J. Qian, J. Chen, Q. Wu, X. Hu, Toxin release of cyanobacterium *Microcystis aeruginosa* after exposure to typical tetracycline antibiotic contaminants, Toxins 9 (2017), https://doi.org/10.3390/toxins9020053.

[7] D. Chen, L.B. Chu, J.L. Wang, Z.L. Yang, Q. Yang, Y.P. Shen, Degradation of antibiotic cephalosporin C in aqueous solution and elimination of antimicrobial activity by gamma irradiation, Chem. Eng. J. 374 (2019) 1102–1108, https://doi.org/10.1016/j.cej.2019.06.021.

[8] T.A. Brimner, G.J. Boland, A review of the non-target effects of fungi used to biologically control plant diseases, Agric. Ecosyst. Environ. 100 (2003) 3–16, https://doi.org/10.1016/S0167-8809(03)00200-7.

[9] H. Hu, Q. Zhou, X. Li, W. Lou, C. Du, Q. Teng, D.M. Zhang, H.Y. Liu, Y.Y. Zhong, C.P. Yang, Phytoremediation of anaerobically digested swine wastewater contaminated by oxytetracycline via *Lemna aequinoctialis*: nutrient removal, growth characteristics and degradation pathways, Bioresour. Technol. 291 (2019) 121853, , https://doi.org/10.1016/j.biortech.2019.121853.

[10] J. Li, X. Zheng, K. Liu, S. Sun, X. Li, Effect of tetracycline on the growth and nutrient removal capacity of *Chlamydomonas reinhardtii* in simulated effluent from wastewater treatment plants, Bioresour. Technol. 218 (2016) 1163–1169, https://doi.org/10.1016/j.biortech.2016.07.080.

[11] Z. Chen, Y. Zhang, Y. Gao, S.A. Boyd, D. Zhu, H. Li, Influence of dissolved organic matter on tetracycline bioavailability to an antibiotic-resistant bacterium, Environ. Sci. Technol. 49 (2015) 10903–10910, https://doi.org/10.1021/acs.est.5b02158.

[12] R. Xu, Z.H. Yang, Y. Zheng, Q.P. Wang, Y. Bai, J.B. Liu, Y.R. Zhang, W.P. Xiong, Y. Lu, C.Z. Fan, Metagenomic analysis reveals the effects of long-term antibiotic pressure on sludge anaerobic digestion and antimicrobial resistance risk, Bioresour. Technol. 282 (2019) 179–188, https://doi.org/10.1016/j.biortech.2019.02.120.

[13] X.W. Yang, R. Zhang, J. Fu, S. Geng, J.J. Cheng, Y. Sun, Pyrolysis kinetic and product analysis of different microalgal biomass by distributed activation energy model and pyrolysis-gas chromatography-mass spectrometry, Bioresour. Technol. 163 (2014) 335–342, https://doi.org/10.1016/j.biortech.2014.04.040.

[14] B.O. Abo, E.A. Odey, M. Bakayoko, L. Kalakodio, Microalgae to biofuels production: a review on cultivation, application and renewable energy, Rev. Environ. Health 34 (2019) 91–99, https://doi.org/10.1515/reveh-2018-0052.

[15] D. Xu, Y. Xiao, H. Pan, Y. Mei, Toxic effects of tetracycline and its degradation products on freshwater green algae, Ecotoxicol. Environ. Saf. 174 (2019) 43–47, https://doi.org/10.1016/j.ecoenv.2019.02.063.

[16] V. Sharma, D. Pant, Structural basis for expanding the application of bioligand in metal bioremediation: a review, Bioresour. Technol. 252 (2018) 188–197, https://doi.org/10.1016/j.biortech.2017.12.070.

[17] Z.M. Wei, X. Zhang, Y.Q. Wei, X. Wen, J.H. Shi, J.Q. Wu, Y. Zhao, B.D. Xi, Fractions and biodegradability of dissolved organic matter derived from different composts, Bioresour. Technol. 161 (2014) 179–185, https://doi.org/10.1016/j.biortech.2014.03.032.

[18] Q. Zhou, X. Li, Y. Lin, C.P. Yang, W.C. Tang, S.H. Wu, D.H. Li, W. Lou, Effects of copper ions on removal of nutrients from swine wastewater and on release of dissolved organic matter in duckweed systems, Water Res. 158 (2019) 171–181, https://doi.org/10.1016/j.watres.2019.04.036.

[19] Y.X. Zhang, P.Y. Zhang, J.B. Guo, W.F. Ma, L.P. Xiao, Spectroscopic analysis and biodegradation potential study of dissolved organic matters in sewage sludge treated with high-pressure homogenization, Bioresour. Technol. 135 (2013) 616–621, https://doi.org/10.1016/j.biortech.2012.09.034.

[20] C. Gu, K.G. Karthikeyan, S.D. Sibley, J.A. Pedersen, Complexation of the antibiotic tetracycline with humic acid, Chemosphere 66 (2007) 1494–1501, https://doi.org/10.1016/j.chemosphere.2006.08.028.

[21] L. Zhao, F. Luo, J.M. Wasikiewicz, H. Mitomo, N. Nagasawa, T. Yagi, M. Tamada, F. Yoshii, Adsorption of humic acid from aqueous solution onto irradiation-cross-linked carboxymethylchitosan, Bioresour. Technol. 99 (2008) 1911–1917, https://doi.org/10.1016/j.biortech.2007.03.030.

[22] Z. Wu, P.Y. Zhang, G.M. Zeng, M. Zhang, J.H. Jiang, Humic acid removal from water with polyaluminum coagulants: effect of sulfate on aluminum polymerization, J. Environ. Eng. 138 (2012) 293–298, https://doi.org/10.1061/(Asce)Ee.1943-7870.0000412.

[23] S. Salvestrini, Diuron herbicide degradation catalyzed by low molecular weight humic acid-like compounds, Environ. Chem. Lett. (2013) 11, https://doi.org/10.1007/s10311-013-0415-5 .

[24] N. Senesi, C. Testini, Theoretical aspects and experimental evidence of the capacity of humic substances to bind herbicides by charge-transfer mechanisms (electron donor-acceptor processes), Chemosphere (1984) 13 doi:461-468.10.1016/0045-6535(84)90104-8.

[25] C.F. Lin, T.Y. Lin, O.J. Hao, Effects of humic substance characteristics on UF performance, Water Res. 34 (2000) 1097–1106, https://doi.org/10.1016/S0043-1354(99)00273-0.

[26] Y. Zhang, T. Meng, L. Shi, X. Guo, X. Si, R. Yang, X. Quan, The effects of humic acid on the toxicity of graphene oxide to *Scenedesmus obliquus* and *Daphnia magna*, Sci. Total Environ. 649 (2019) 163–171, https://doi.org/10.1016/j.scitotenv.2018.08.280.

[27] Y. Tang, S. Li, Y. Lu, Q. Li, S. Yu, The influence of humic acid on the toxicity of nano-ZnO and $Zn^{2+}$ to the *Anabaena* sp, Environ. Toxicol. 30 (2015) 895–903, https://doi.org/10.1002/tox.21964.

[28] C.M. Zhao, P.G.C. Campbell, K.J. Wilkinson, When are metal complexes bioavailable? Environ. Chem. 13 (2016) 425–433, https://doi.org/10.1071/En15205.

[29] B. Koukal, C. Gueguen, M. Pardos, J. Dominik, Influence of humic substances on the toxic effects of cadmium and zinc to the green alga *Pseudokirchneriella subcapitata*, Chemosphere 53 (2003) 953–961, https://doi.org/10.1016/s0045-6535(03)00720-3.

[30] W. Shi, X. Fang, X. Wu, G. Zhang, W. Que, F. Li, Alteration of bioaccumulation mechanisms of cu by microalgae in the presence of natural fulvic acids, Chemosphere 211 (2018) 717–725, https://doi.org/10.1016/j.chemosphere.2018.07.200.

[31] X. Li, C. Yang, G. Zeng, S. Wu, Y. Zhong, Nutrient removal from swine wastewater with growing microalgae at various zinc concentrations, Algal Res. 46 (2020) 101804, , https://doi.org/10.1016/j.algal.2020.101804.

[32] X. Li, W.L. Yang, H. He, S. Wu, Q. Zhou, C. Yang, G. Zeng, L. Luo, W. Lou, Responses of microalgae *Coelastrella* sp. to stress of cupric ions in treatment of anaerobically digested swine wastewater, Bioresour. Technol. 251 (2018) 274–279, https://doi.org/10.1016/j.biortech.2017.12.058.

[33] B.K.M. Imran, C. Man-Gi, The effect of kanamycin and tetracycline on growth and photosynthetic activity of two chlorophyte algae, Biomed. Res. Int. 2016 (2016) 1–8, https://doi.org/10.1155/2016/5656304.

[34] L. Luo, H. He, C. Yang, S. Wen, G. Zeng, M. Wu, Z. Zhou, W. Lou, Nutrient removal and lipid production by *Coelastrella* sp. in anaerobically and aerobically treated swine wastewater, Bioresour. Technol. 216 (2016) 135–141, https://doi.org/10.1016/j.biortech.2016.05.059.

[35] S.H. Wu, H.J. He, X. Li, C.P. Yang, G.M. Zeng, B. Wu, S.Y. He, L. Lu, Insights into atrazine degradation by persulfate activation using composite of nanoscale zero-valent iron and graphene: performances and mechanisms, Chem. Eng. J. 341 (2018) 126–136, https://doi.org/10.1016/j.cej.2018.01.136.

[36] Y. Lin, S.H. Wu, X. Li, X. Wu, C.P. Yang, G.M. Zeng, Y.R. Peng, Q. Zhou, L. Lu, Microstructure and performance of Z-scheme photocatalyst of silver phosphate modified by MWCNTs and Cr-doped $SrTiO_3$ for malachite green degradation, Appl. Catal. B Environ. 227 (2018) 557–570, https://doi.org/10.1016/j.apcatb.2018.01.054.

[37] Y.J. Ding, B.J. Teppen, S.A. Boyd, H. Li, Measurement of associations of pharmaceuticals with dissolved humic substances using solid phase extraction, Chemosphere 91 (2013) 314–319, https://doi.org/10.1016/j.chemosphere.2012.11.039.

[38] W. Yang, Z. Tang, F. Zhou, W. Zhang, L. Song, Toxicity studies of tetracycline on *Microcystis aeruginosa* and *Selenastrum capricornutum*, Environ. Toxicol. Pharmacol. 35 (2013) 320–324, https://doi.org/10.1016/j.etap.2013.01.006.

[39] L. Migliore, A. Rotini, M.C. Thaller, Low doses of tetracycline trigger the *E. coli* growth: a case of hormetic response, Dose-Response 11 (2013) 550–557, https://doi.org/10.2203/dose-response.13-002.Migliore.

[40] E.J. Calabrese, Hormesis: why it is important to toxicology and toxicologists, Environ. Toxicol. Chem. 27 (2008) 1451–1474, https://doi.org/10.1897/07-541.1.

[41] X.Z. Zhang, P. Amendola, J.C. Hewson, M. Sommerfeld, Q. Hu, Influence of growth phase on harvesting of *Chlorella zofingiensis* by dissolved air flotation, Bioresour. Technol. 116 (2012) 477–484, https://doi.org/10.1016/j.biortech.2012.04.002.

[42] J. Guo, K. Selby, A.B. Boxall, Effects of antibiotics on the growth and physiology of chlorophytes, cyanobacteria, and a diatom, Arch. Environ. Contam. Toxicol. 71 (2016) 589–602, https://doi.org/10.1007/s00244-016-0305-5.
[43] J.Y. Zhang, Y.Y. Ni, T.D. Ding, C.L. Zhang, The role of humic acid in the toxicity of arsenite to the diatom *Navicula* sp, Environ. Sci. Pollut. Res. 21 (2014) 4366–4375, https://doi.org/10.1007/s11356-013-2413-3.
[44] Q. Zhou, Y. Lin, X. Li, C. Yang, Z. Han, G. Zeng, L. Lu, S. He, Effect of zinc ions on nutrient removal and growth of *Lemna aequinoctialis* from anaerobically digested swine wastewater, Bioresour. Technol. 249 (2018) 457–463, https://doi.org/10.1016/j.biortech.2017.10.044.
[45] Y.J. Chen, H.J. He, H.Y. Liu, H.R. Li, G.M. Zeng, X. Xia, C.P. Yang, Effect of salinity on removal performance and activated sludge characteristics in sequencing batch reactors, Bioresour. Technol. 249 (2018) 890–899, https://doi.org/10.1016/j.biortech.2017.10.092.
[46] H.J. He, Y.J. Chen, X. Li, Y. Cheng, C.P. Yang, G.M. Zeng, Influence of salinity on microorganisms in activated sludge processes: a review, Int. Biodeterior. Biodegrad. 119 (2017) 520–527, https://doi.org/10.1016/j.ibiod.2016.10.007.
[47] J.B. Murray, S.O. Meroueh, R.J.M. Russell, G. Lentzen, J. Haddad, S. Mobashery, Interactions of designer antibiotics and the bacterial ribosomal aminoacyl-tRNA site, Chem. Biol. 13 (2006) 129–138, https://doi.org/10.1016/j.chembiol.2005.11.004.
[48] Z.H. Wei, L.Q. Bai, Z.X. Deng, J.J. Zhong, Enhanced production of validamycin A by $H_2O_2$-induced reactive oxygen species in fermentation of *Streptomyces hygroscopicus 5008*, Bioresour. Technol. 102 (2011) 1783–1787, https://doi.org/10.1016/j.biortech.2010.08.114.
[49] Y. Liu, Y. Guan, B. Gao, Q. Yue, Antioxidant responses and degradation of two antibiotic contaminants in *Microcystis aeruginosa*, Ecotoxicol. Environ. Saf. 86 (2012) 23–30, https://doi.org/10.1016/j.ecoenv.2012.09.004.
[50] G.B. Liang, X.Y. Liao, G.C. Du, J. Chen, A new strategy to enhance glutathione production by multiple $H_2O_2$-induced oxidative stresses in *Candida utilis*, Bioresour. Technol. 100 (2009) 350–355, https://doi.org/10.1016/j.biortech.2008.06.012.
[51] D.H. Lin, J. Ji, Z.F. Long, K. Yang, F.C. Wu, The influence of dissolved and surface-bound humic acid on the toxicity of $TiO_2$ nanoparticles to *Chlorella* sp, Water Res. 46 (2012) 4477–4487, https://doi.org/10.1016/j.watres.2012.05.035.
[52] J. Fabrega, S.R. Fawcett, J.C. Renshaw, J.R. Lead, Silver nanoparticle impact on bacterial growth: effect of pH, concentration, and organic matter, Environ. Sci. Technol. 43 (2009) 7285–7290, https://doi.org/10.1021/es803259g.
[53] P. Sanchez-Marin, R. Beiras, Adsorption of different types of dissolved organic matter to marine phytoplankton and implications for phytoplankton growth and Pb bioavailability, J. Plankton Res. 33 (2011) 1396–1409, https://doi.org/10.1093/plankt/fbr039.