Bioresource Technology 291 (2019) 121853

Contents lists available at ScienceDirect

Bioresource Technology

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

Phytoremediation of anaerobically digested swine wastewater contaminated by oxytetracycline via *Lemna aequinoctialis*: Nutrient removal, growth characteristics and degradation pathways

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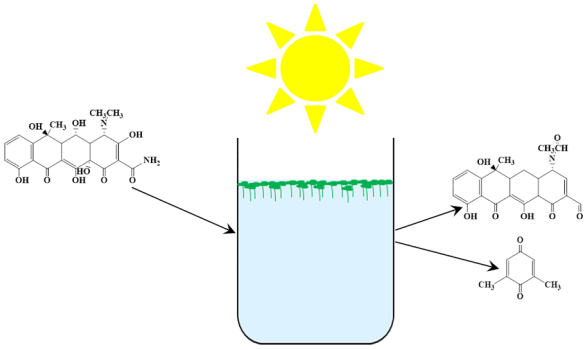
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Swine wastewater
Duckweed
Oxytetracycline
Phytoremediation
Antibiotic

ABSTRACT

The concentration of antibiotics in anaerobically digested swine wastewater (ADSW) usually gradually increases due to the addition of antibiotics in livestock feed. *Lemna aequinoctialis* was used to treatment synthetic ADSW contaminated by oxytetracycline (OTC) whose concentrations were 0.05, 0.25, 0.50 and 1.00 mg/L, and its influences on NH_3-N and TP remove were investigated. The fresh weight, photosynthetic pigment and protein content of duckweed were also investigated. Results have shown that nutrient removal and duckweed growth followed the "dose-response" relationships, and 0.05 mg/L OTC could significantly promote the synthesis of photosynthetic pigments and proteins in duckweed. Meanwhile, the protein content gradually decreased during investigation. More important, the degradation products and possible degradation pathways of OTC were diagrammatized via liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), and twelve intermediates were detected in the duckweed systems. This study can offer a novel view for phytoremediation of ADSW containing antibiotics by aquatic plants.

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https://doi.org/10.1016/j.biortech.2019.121853
Received 22 May 2019; Received in revised form 15 July 2019; Accepted 20 July 2019
Available online 22 July 2019
0960-8524/

1. Introduction

A large amount of anaerobically digested swine wastewater (ADSW) characterized by high concentration of ammonium and phosphorus could lead to pervasive water pollution if not managed appropriately (Luo et al., 2016). In order to promote growth of livestock as well as prevent the microbial infections, antibiotics and heavy metals are extensively used in livestock feed (Kümmerer, 2009). Unfortunately, most antibiotics cannot be absorbed by livestock rather than be excluded into the urine and feces (Lin et al., 2019a), which have given rise to high concentration of veterinary antibiotics in livestock wastewater. In addition, the concentrations of antibiotics could reach the level of ng/L or even μg/L in piggy wastewater because of the commonly used treatment methods cannot completely remove the antibiotics (Kümmerer, 2009; Chen et al., 2012). Therefore, the concentrations of antibiotics and ammonium and phosphorus are usually high in the anaerobically digested swine wastewater, which poses a serious challenge to the traditional wastewater treatment methods.

Oxytetracycline (OTC), a crucial broad spectrum antibiotic (Zhen et al., 2018), has been widely used in hoggery to control diseases evoked by various bacteria (Chen et al., 2012). OTC could be discharged into the swine wastewater due to it is difficult to be degraded via metabolisms, and resulted in severe antibiotic pollution in aquatic environments. OTC has been detected in groundwater, surface water, soils and sediments (Michael et al., 2013). It has been reported that OTC could not only affect the bioactivity of microorganisms but also perturb the function of some human organisms (Chi et al., 2010).

Wastewater phytoremediation is a widely used biotechnology on the basis of extraction, isolation or detoxification of contaminants via aquatic plants and microorganisms, and could be a cost-effective method to remove nutrients from ADSW (Cheng and Stomp, 2009; Al-Nozaily et al., 2000; Wen et al., 2016; Li et al., 2018; Zhou et al., 2018; Luo et al., 2016; ElMekawy et al., 2014, Dil et al., 2017). Phytoremediation has advantages over chemical and physical methods because aquatic plants could grow at a high rate without any secondary pollution. Additionally, aquatic plants could absorb nutrients including ammonia and phosphate from ADSW to produce proteins, starches and other substances, which could be utilized to generate biofuels and animal feeds (Pant and Adholeya, 2009, Cheng and Stomp, 2009).

Among different aquatic plants, duckweed has attracted the special interest of researchers for the treatment of ADSW (Iatrou et al., 2017; Al-Nozaily et al., 2000; Zhou et al., 2019; Singh et al., 2018). Over other aquatic plants, duckweed has the following advantages: (I) easy to be obtained and removed from the environment; (II) high contents of proteins and starches; and (III) high biomass production (Cheng and Stomp, 2009; Mkandawire and Dudel, 2007). Chaiprapat et al. (2005) reported that *Spirodela punctata* 7776 could remove nearly all of the NH_3-N in 12 days and PO_4-P in 16 days in swine artificial medium (343.0 mg/L of initial NH_3-N and 135.0 mg/L of initial PO_4-P). Zhou et al. (2018) reported that *Lemna aequinoctialis* cultured in synthetic ADSW could remove more than 50% NH_3-N and 95% TP, respectively, and the fresh weight of *Lemna aequinoctialis* increased from 0.3 g to 1.8 g for 25 days.

Antibiotics in ADSW could inhibit the anaerobic digestion of swine waste solids and concentrated swine wastewater (Yang et al., 2019). Unfortunately, the effects of antibiotics on the nutrient removal and duckweed growth have not well been reported.

In this study, OTC was selected as the test antibiotic because of its high solubility in aqueous solution, strong toxicity, and extensive applications. The effects of various original concentrations of OTC on the nutrients removal from synthetic ADSW were evaluated. Meanwhile, the growth characteristics of duckweed including the changes in fresh weight, photosynthetic pigment and protein contents were also examined. In addition, the OTC's degradation pathways in the phytoremediation system were also studied. This work expected to provide some novel insights and data for phytoremediation of OTC-contaminated ADSW by duckweed and better understand the fate of OTC in this system.

2. Materials and methods

2.1. Chemicals

Oxytetracycline hydrochloride (OTC; purity 95–105%) was supplied by Hefei Bomei Biotechnology Incorporated Company (Hefei, China), which molecular formula was $C_{22}H_{24}N_2O_9{\cdot}HCl$. Acetonitrile, methyl alcohol and formic acid were HPLC grade and purchased from Tedia Company, Inc (Fairfield, USA) and Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China), respectively. The other reagents were analytical grade and gained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Duckweed cultivation conditions and synthetic ADSW preparation

The *Lemna aequinoctialis* applied in this work was taken from an artificial fresh water pond, Hunan, China. Pond water (NH_3-N = 30.15 ± 3.14 mg/L, COD = 165.62 ± 15 mg/L, TP = 5.08 ± 1.01 mg/L) was used to culture duckweed in 10 L polyethylene buckets and replaced every week. The buckets were placed in an illumination incubator at 25 °C under light/dark cycles of 14:10 h and the light intensity of 80 μmol m^{-2} s^{-1} for the further replication. The ADSW was man-made wastewater artificially synthesized using different chemical reagents. The pH of the synthetic ADSW was adjusted to 7.0 ± 0.5 by using 4.00 g/L NaOH. The regents used for synthetic ADSW preparation included NH_4Cl, KH_2PO_4, KNO_3, Na_2EDTA, $MgSO_4{\cdot}7H_2O$, $Na_2MoO_4{\cdot}2H_2O$, $FeSO_4{\cdot}7H_2O$, $MnCl_2{\cdot}4H_2O$, $ZnSO_4{\cdot}7H_2O$, $Ca(NO_3)_2{\cdot}4H_2O$, H_3BO_3, and $CuSO_4{\cdot}5H_2O$. Then the synthetic wastewater was sterilized in a steam sterilizer under the condition of 121 °C for 30 min ahead of the research.

2.3. Culture methods

The experiments were performed in 500 mL beaker in an illumination incubator. Robust duckweed was selected and washed with ultrapure water before the study. 400 mL synthetic ADSW and 0.30 g duckweed (fresh weight) was added into each beaker. The beakers were then placed in an illumination incubator for cultivation, and the conditions were: temperature 25 °C, photoperiod 14:10, irradiance 80 μmol m^{-2} s^{-1} offered via broad spectrum fluorescent tubes. The experiments were conducted in triplicates. The pH of synthetic ADSW was monitored every two days, and maintained at 7.0 ± 0.5 by 4.00 g/L NaOH.

Except the control group (0 mg/L), different volumes of 1.00 g/L OTC solution were added to synthetic ADSW to imitate different concentrations of OTC (0.05, 0.25, 0.50 and 1.00 mg/L), which were used as the original media to research the effects of OTC on the growth of *Lemna aequinoctialis* and remove nutrients from synthetic ADSW. For the sake of avoiding the lack of ammonium or phosphorus in synthetic ADSW, a 70-day preliminary experiment was conducted to ensure the effects of OTC on nutrients removal, and the appropriate exposure time was chosen to examine the effect of OTC on the *Lemna aequinoctialis* growth. Examine and replenish OTC every 2–3 days to guarantee a constant exposure concentration.

2.4. Analytical methods

NH_3-N (HJ 535-2009) and TP (GB 11893-89) analysis was described by Zhou et al. (2018). The pH meter (PHS-3C) was used to monitor the pH of synthetic ADSW to evaluate the *Lemna aequinoctialis* growth. The nutrient level was monitor by destructive sampling via taking a whole beaker as a sample. The duckweed were washed three times by using ultrapure water after harvested, and then the fresh weight was

examined with the scale promptly after the water was sucked on the filter paper.

2.4.1. Determination the photosynthetic pigment and protein content of Lemna aequinoctialis

The fresh *Lemna aequinoctialis* (0.10 g) was uniformized in 4 mL of absolute ethanol containing a small amount of $CaCO_3$ after harvested. The homogenate was filtered and then the filtrate was made up to 10 mL used to judge the content of photosynthetic pigments in duckweed. The xanthophyll and chlorophyll *a* and *b* were measured at the absorbency 447, 665, and 649 nm, and then the content of them in duckweed was computed.

The fresh *Lemna aequinoctialis* (0.30 g) was mechanically homogenized under ice water bath conditions in 3 mL of normal saline after harvested. Then the homogenate was centrifuged for 10 min at 2500 rpm and the supernatant was analyzed using Total proteins quantitative assay kit (Coomassie brilliant blue method) to determine the content of proteins in duckweed.

2.4.2. Metabolites analysis

To investigate the degradation pathways of OTC after phytoremediation, the degradation products of OTC were analyzed via SPE-LC-MS/MS. 50 mL synthetic ADSW in which the OTC concentration was 1.00 mg/L was selected as the test sample. First, it would be percolated through 0.45 μm Millipore filter to remove impurities. Next, the degradation products of OTC in sample was extracted by SPE(solid-phase extraction). After that, the extracted sample was percolated again thorough a 0.22 μm Millipore filter and then it would be authenticated via a liquid chromatography-mass spectrometry-mass spectrometry (LC-MS/MS, Agilent 1290 series LC, 6460 Triple Quad LC/MS) equipped with a ZORBAX RRHD Eclipse Plus C18 column (2.1 × 50 mm, 1.8 μm). The elution was conducted by 0.1% (v/v) of formic acid aqueous solution (A) and a mixture of 60% acetonitrile and 40% methanol (B) under the flow rate was 0.20 mL min^{-1}. The flow phase gradients were described by Wang et al. (2018). The column temperature and the injection volume were 25 °C and 5 μL, respectively. OTC and its intermediates were measured in the positive ion mode using ESI, and the mass to charge ratio (*m/z*) of MS was scanned range from 50 to 500. The hydrolysis and photodegradation of OTC was also investigated by three-dimensional excitation-emission matrix fluorescence spectroscopy (3D-EEMs), which was described by Lin et al. (2019b) where the wavelengths of excitation (λEx) and emission (λEm) were 200–600 nm.

2.5. Statistical analysis

Origin 2017 (OriginLab Corporation, USA) was used for the construction of all figures in this work. The mean ± SE (standard error) of the three replicates used to describe the result, and the differences of data were computered by using SPSS 20 with one-way ANOVA (Chicago, USA).

3. Results and discussion

3.1. Nutrient removal

Applied duckweed to remove nutrients from ADSW has been studied by numerous researchers (Alaerts et al., 1996; Al-Nozaily et al., 2000; Xu and Shen, 2011; Cheng et al., 2002; Zhou et al., 2018). However, the influences of OTC on nutrient removal have not been implemented. The effects of OTC on nutrient removal by *Lemna aequinoctialis* within 70 days were showed in Fig. 1.

As could be observed from Fig. 1(a) that the NH_3-N concentrations after 70 days in the control group (0 mg/L OTC) was 23.57 mg/L, and the removal efficiency was 70.86%, which was slower than Cheng and Stomp (2009). Besides, the influence of OTC on the TP removal was

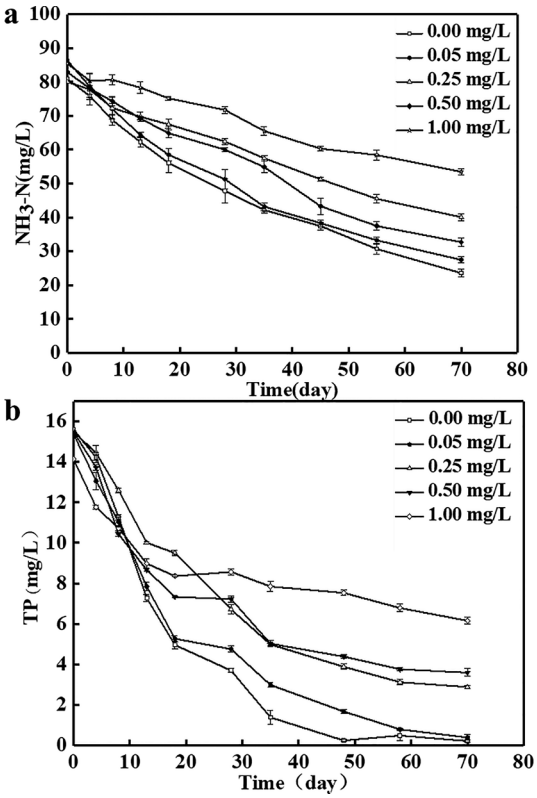


Fig. 1. Dynamic changes of nutrients level in synthetic ADSW at different concentrations of OTC in 70 days. (a) NH_3-N; (b) TP.

showed in Fig. 1(b). It was showed that the concentrations of TP reduction rapidly in 18 days and then decreased slowly. There was a phosphorus removal of 98.65% in 70 days in the control group, which suggested that the *Lemna aequinoctialis* has a great removal efficiency on phosphorus. This result was consistent with the result of Zhou et al. (2018). However, the removal rate of TP decreased rapidly when the concentration of OTC increased from 0.05 to 1.00 mg/L, which were 97.46%, 81.39%, 76.81% and 56.40%, respectively. In addition, compared with the control group, the NH_3-N removal efficiency decreased to 68.16%, 49.93%, 60.57% and 37.42%, respectively when the concentration of OTC was 0.05, 0.25, 0.5 and 1.00 mg/L. It was indicated that the removal rate of NH_3-N was decreased with the increase of OTC concentrations. Based on the results, the nutrient removal could be inhibited significantly with the increase of OTC concentrations.

The influence of antibiotics on the removal of nutrients from synthetic ADSW via duckweed was multifaceted. Baciak et al. (2016) have indicated that it would stimulate the secretion of various biogenic amines (BAs) in the body to alleviate the damage when duckweed exposed to tetracycline (TC). BAs played an important role in anti-stress defense mechanisms, which has been pointed out in numerous publications (Gill and Tuteja, 2010; Alcázar et al., 2010). As a low molecular organic base, BAs generally exists in floristic cells (Kuznetsov et al., 2006) and are responsible for such mechanisms as remove free radicals or maintaining membrane stability (Bouchereau et al., 2000). The effect of OTC on the removal of nutrients via *Lemna aequinoctialis* from synthetic ADSW should follow the “dose-response” relationships. When the concentration of OTC was lower, the BAs could remove excess free radicals and maintain membrane stability, thereby alleviated stress. However, with the increase of concentration of OTC, the free radicals produced in the duckweed were quantity, which caused the antioxidant system failed its function and the duckweed cells were damaged. Finally, the higher concentrations of OTC could result in a decreased in the removal efficiency of nutrients. Meanwhile, the higher concentrations of OTC could also produce oppressive toxicity on

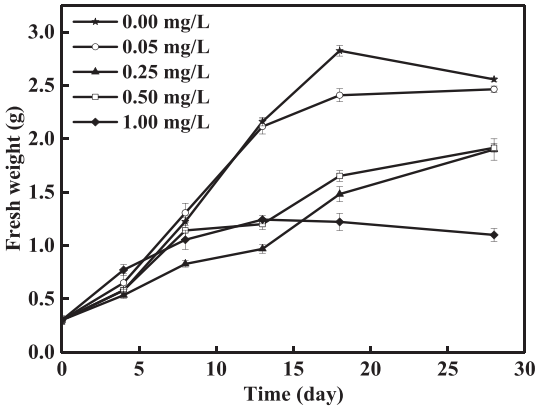


Fig. 2. Mass curve for *Lemna aequinoctialis* under different initial concentrations of OTC in 28 days.

rhizosphere microorganisms including the nitrifying and denitrifying bacteria (Zhen et al., 2018), and thereby inhibit the nutrient removal from synthetic ADSW. Therefore, OTC is toxic for duckweed to remove nutrients from synthetic ADSW, and the effects followed the "dose-response" relationships.

3.2. Biomass production

The effect of different original OTC concentrations on the *Lemna aequinoctialis* growth was studied and showed in Fig. 2. It could be seen from Fig. 2 that there was no obvious lag phase compared with the report by Cheng et al. (2002), which meant that duckweed might grew well in the early stage of the culture. It was probably because the culture medium used in the further replication in this study contains more microbial communities than the medium which was used to incubate the *Spirodela punctala* (Cheng et al., 2002), or because of the species differences in duckweed employed in both experiments.

Moreover, OTC had a great impact on the increase of *Lemna aequinoctialis* biomass. The fresh weight of *Lemna aequinoctialis* in the control group was the highest, correspondingly up to 2.56 g during 28 days, its maximum biomass increased by 28.18% compared with the previous study (Chaiprapat et al., 2005). The mass of *Lemna aequinoctialis* was 2.46 g, 1.90 g, 1.92 g and 1.10 g under the OTC concentration of 0.05–1.00 mg/L, which was decreased by 3.91%, 25.78%, 25.00% and 57.03%, respectively compared with the control group (Fig. 2). Similar with the nutrients removal, the effect of OTC on biomass production might also result by the "dose-response" relationships. The mechanism of the toxic effect of OTC on duckweed growth could be attributed to the following reasons. First of all, high concentrations of OTC could cause the deficiency of the roots of *Lemna aequinoctialis*, which given rise to absorb the essential nutrients for *Lemna aequinoctialis* growth from synthetic ADSW suffocated and the duckweed growth to be blocked. Secondly, rhizosphere microorganisms have a significant role in facilitating the plant growth via producing the plant hormones such as abscisic acid, auxins, ethylene, gibberellins and cytokinins (Cheng et al., 2016; Hayat et al., 2010; He et al., 2017). While antibiotics have intolerable toxicity to rhizosphere microorganisms (Yang et al., 2018a; Yang et al., 2010; Wu et al., 2017; Zhen et al., 2018), which resulted in insufficient secretion of hormones and inhibited the growth of *Lemna aequinoctialis*.

3.3. Effects on photosynthetic pigments and proteins

3.3.1. Effect on photosynthetic pigments

Photosynthesis is the basis of plant material transformation and energy conversion, and the basis of photosynthesis is chlorophyll. In addition, xanthophyll, a kind of photosynthetic pigments, was an

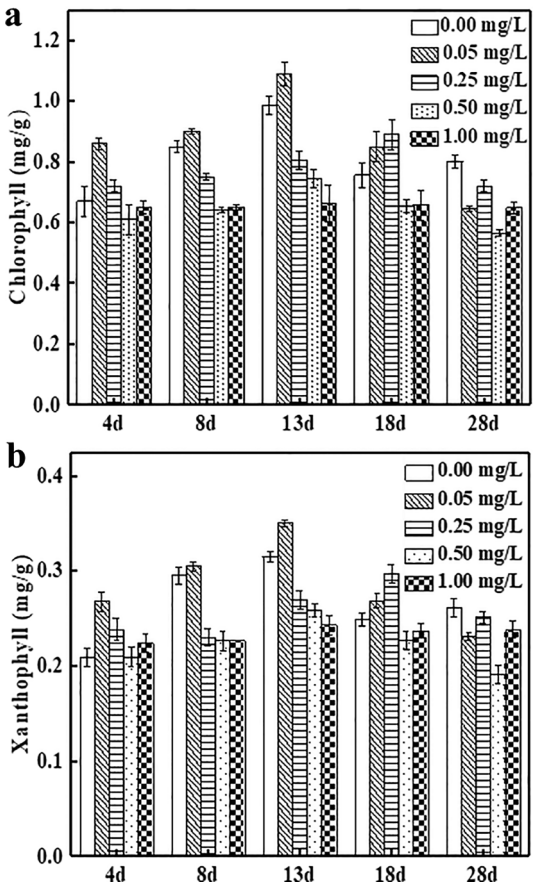


Fig. 3. Photosynthetic pigments content in *Lemna aequinoctialis* under different concentrations of OTC in 28 days. (a) chlorophyll; (b) xanthophyll.

excellent antioxidant, which can resist the damage evoked by free radicals to plant cells and organs (Boon et al., 2010). Consequently, the effects of OTC on chlorophyll and xanthophyll in *Lemna aequinoctialis* were presented in Fig. 3. It could be noticed from the Fig. 3 that the chlorophyll and xanthophyll production in *Lemna aequinoctialis* was significantly ($P < 0.05$) enhanced when the concentration of OTC was 0.05 mg/L in the synthetic ADSW, which was consistent with the results reported by Di Baccio et al. (2017). For instance, on the fourth day, compared with the control group, the content of chlorophyll and xanthophyll were increased by 28.36% and 28.57%, respectively. Nevertheless, the synthesis of chlorophyll and xanthophyll in *Lemna aequinoctialis* were inhibited, and thereby it would result in restricted growth of duckweed (Fig. 2), when the concentrations of OTC was higher (> 0.25 mg/L). Low concentrations of tetracycline antibiotics could promote the formation of photosynthetic pigments in duckweed due to the large amount of biogenic amines were accumulated in duckweed (Baciak et al., 2016). The polyamines (PA) metabolism regulates the photosynthetic apparatus, and the involvement of PA in the function of photosynthetic apparatus involves the protection of photosystem II (PSII) and Light Capture Complex II (LHC II), which contributed to the increase of chlorophyll and xanthophyll in duckweed, consistent with the results showed in Fig. 3.

The changes of chloroplast ultrastructure and peroxidation of membrane lipids may result in the decrease of photosynthetic pigment content (Xu et al., 2010). The structure-activity analysis by Aristilde et al. (2010) indicated that fluoroquinolone antibiotics can mediate the role as quinone site inhibitors in photosystem II (PSII), and inhibit the PSII role in photosynthetic electron transport. Therefore, the photosynthetic pigment content of *Lemna aequinoctialis* in the control group was higher than that in the other groups when the concentrations of

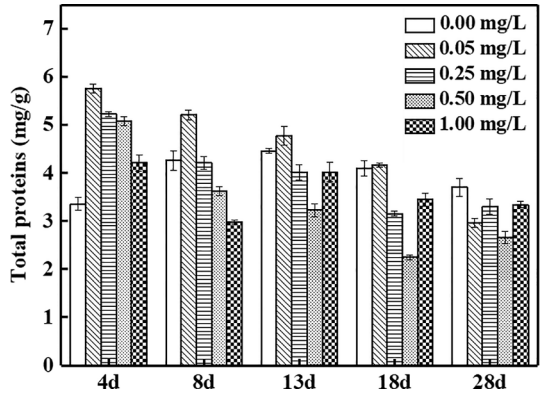


Fig. 4. Proteins content in *Lemna aequinoctialis* under different concentrations of OTC in 28 days.

OTC in synthetic ADSW was higher (> 0.25 mg/L). At the latest stage of study (Day 28), the content of chlorophyll and xanthophyll of *Lemna aequinoctialis* in OTC-contaminated synthetic ADSW was lower than that in the control group. This was consistent with the reduction of chlorophyll in duckweed triggered by TC revealed by Baciak et al. (2016). With the extension of research, the inhibition impact of OTC on PSII was violent, which resulted in the photosynthetic pigment content of *Lemna aequinoctialis* in other groups was lower than that in the control group.

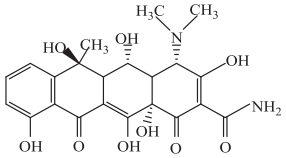
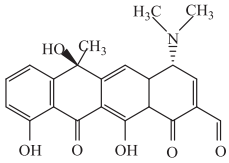
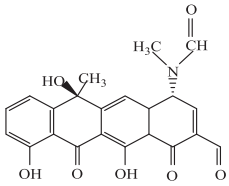
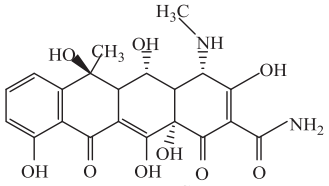
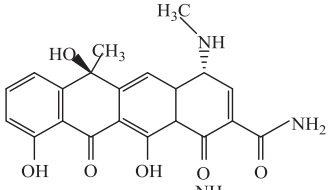
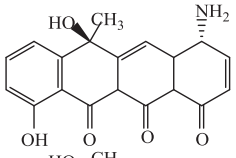
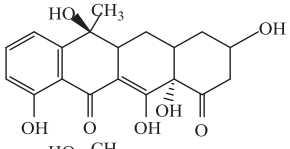
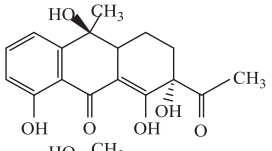
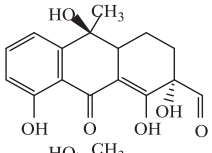
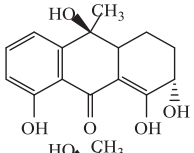
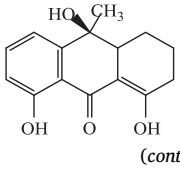
3.3.2. Effect on proteins

Duckweed could accumulate proteins by removing nutrients when grew in wastewater, which was the main process for nutrient removal (Gaur and Suthar, 2017). The stress evoked by xenobiotic could trigger the metabolic pathways of proteins in plants, which could be as a part of a defence mechanism against the physiological stress engendered by biological or abiotic factors (Taiz and Zeiger, 2010). In this work, the protein content in *Lemna aequinoctialis* under various concentrations of OTC (0.05–1.00 mg/L) contaminated synthetic ADSW were showed in Fig. 4. It could be noticed that OTC has a great influence on the synthesis of proteins in *Lemna aequinoctialis*. At the beginning of the culture, in order to resist the chemical stress caused by OTC, duckweed could furnish amino acids for protein synthesis to promote more suitable for duckweed growth (Cooke et al., 1980). Therefore, it was observed that the protein content of *Lemna aequinoctialis* increased rapidly, which was significantly ($P < 0.05$) outnumber that in the control group (Fig. 4). Zhou et al. (2018) have reported that the rapid decreased of NH_3-N in the early stage of culture might also be the reason for the rapid protein generation in duckweed because the nitrogen is the essential element for the synthesis of proteins. Moreover, the protein content in *Lemna aequinoctialis* was the highest compared with the other groups when the concentration of OTC was 0.05 mg/L, which meant that 0.05 mg/L was the optimal OTC concentration for protein synthesis in *Lemna aequinoctialis*. The possible reason might be because low concentrations of OTC (0.05 mg/L) could stimulate the activity of enzymes related to protein synthesis and promoted the protein synthesis in duckweed. The protein content gradually decreased with the extension of time, which might be due to the low synthesis and degradation of enzymes in duckweed (Singh et al., 2018). It was caused by oxidative modifications of peptide cleavage, reactions of peptides with lipid and carbohydrate oxidation products, etc. amino acid side chains, (Valavanidis et al., 2006). Similar results have been reported by Singh et al. (2018).

3.4. The possible degradation pathways of OTC

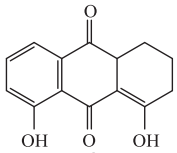
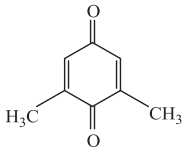
Possible degradation mechanisms of antibiotics in phytoremediation include hydrolysis in the dark, photodegradation during

Table 1
Possible metabolites of OTC in synthetic ADSW.

Products	RT time (min)	*m/z*	Possible structure
OTC	8.737	461	
P1	9.773	396.9	
P2	28.490	409	
P3	10.507	448	
P4	13.425	399	
P5	9.557	340	
P6	26.954	362	
P7	25.944	318	
P8	25.089	274	
P9	0.950	305	
P10	0.950	261	

(*continued on next page*)

Table 1 (*continued*)

Products	RT time (min)	*m/z*	Possible structure
P11	15.592	246	
P12	13.425	135	

illumination and biodegradation through plant uptake (Di Baccio et al., 2017; Gatidou et al., 2017). Rhizosphere microorganisms degradation might also be the mechanisms for OTC degradation, but this process might be negligible due to the toxic effect of antibiotics on rhizosphere microorganisms (Singh et al., 2018). Xenobiotics could be uptake by plants through a diffusion process (Iatrou et al., 2017), and then different extracellular and intracellular enzymes, including peroxidases and cytochrome oxidases, catalyze the oxidation of xenobiotics and convert them into compounds with low toxicity or bioavailability (Gatidou et al., 2017; Larue et al., 2010). Di Baccio et al. (2017) reported that ibuprofen could be degraded by *Lemna gibba* L. and obtained 11 degradation products; degradation of five benzotriazoles through *Lemna minor* has also reported by Gatidou et al. (2017).

Thus, twelve degradation intermediates of OTC were detected by LC-MS/MS and the possible metabolites were showed in Table 1. To further understand the biodegradation process of OTC by *Lemna aequinoctialis* systems, the possible degradation pathways of OTC was proposed according to the previous reports. Pathway I is that the OTC was degraded by *Lemna aequinoctialis* under the action of intracellular enzymes lost three ·OH and one amino group to produce the intermediate product P1, and then the intermediate P2 was obtained via P1 through oxidatively losing one methyl group on $-N(CH_3)_2$ and formed a carbonyl group. Pathway 2 mainly included the detachment of N-methyl and amino group as well as the ring-opening action of the aromatic ring. First, OTC lost one methyl group and three hydroxyl groups by oxidation to obtain intermediates P3 and P4, respectively. Since the $-N(CH_3)H$ and the amide group of intermediate P4 are easily oxidized, these functional groups are further detached to form the intermediate P5. The intermediate could also be produced by oxidation of the intermediate P2 to lose two carbonyl groups and one methyl group which is one of the final products by phytoremediation. As the primary intermediate P3 has acylamino and methyl amino group that is easy to be lost, then with the detachment of a hydroxyl group, dehydroxylation, ring opening, deethylation, and addition reaction of the benzene ring, formed intermediate P6 and P7, respectively. Intermediate P7 has two possible degradation pathways. One is to lose a methyl group and a carbonyl group through acetylation to obtain the intermediate P8, Yang et al. (2018b) have reported this intermediate in the previous study. Another is to remove a methyl group and get the intermediate P9. The intermediate P10 could obtain via intermediate P8 through removing one hydroxyl group, and also yielded by P9 via removing a carbonyl group and a hydroxyl group. P10 lost a methyl group after being oxidized, and the hydroxyl group was substituted by a ketone group, then undergoes ring-opening of the benzene ring and cleavage of the functional group to form P11 and final product P12. Various intermediates and final products of OTC were returned to the environment with the root exudate (Toyama et al., 2009) and decomposition of *Lemna aequinoctialis*. The above description was explained the fate of OTC in phytoremediation of ADSW by *Lemna aequinoctialis*.

In addition, the hydrolysis and photodegradation processes of OTC

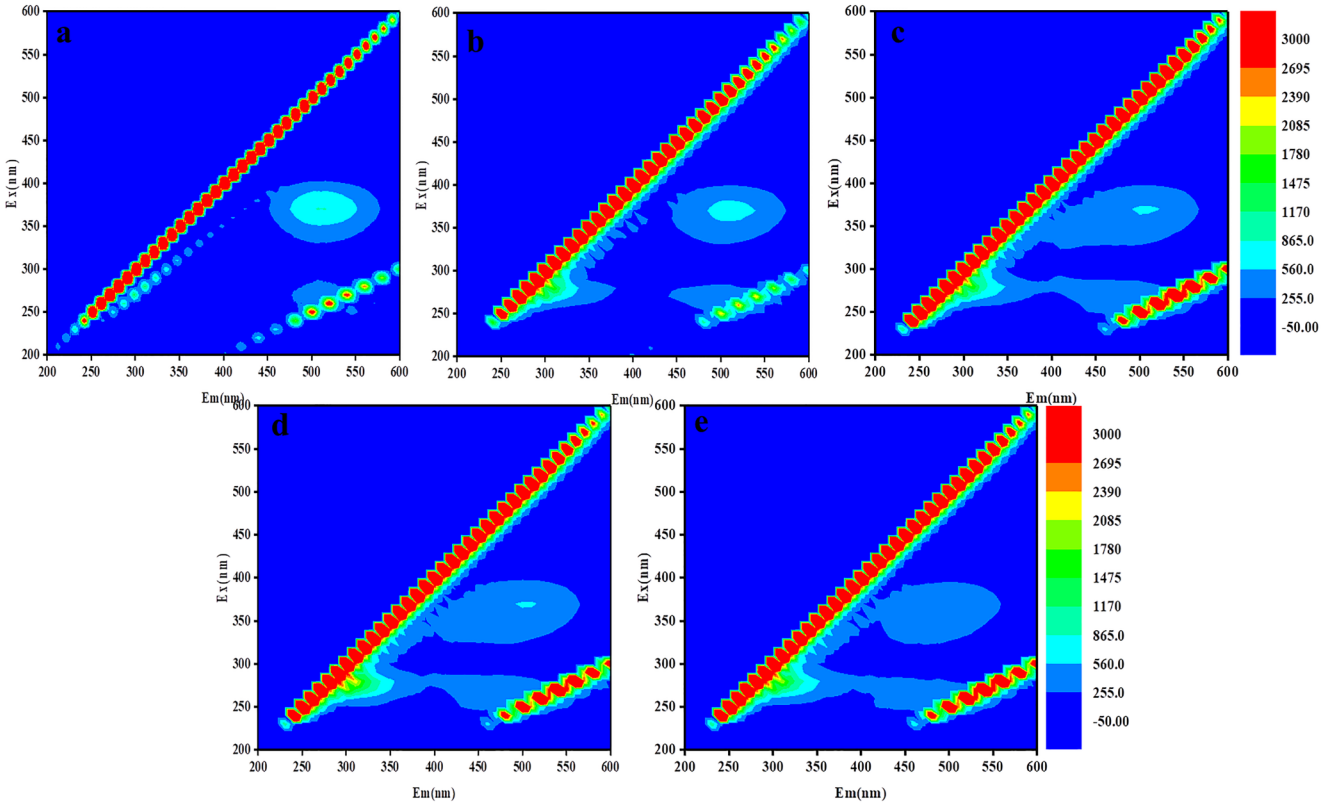


Fig. 5. Three-dimensional excitation-emission matrix fluorescence spectra (3D-EEMs) of the OTC in synthetic ADSW. (a) obtained before the cultivation; (b)–(e) collected at day 3, day 5, day 7 and day 9, respectively.

in synthetic ADSW were also studied using 3D-EEMs, and the results were showed in Fig. 5. It could be seen from Fig. 5(a) that when OTC has just been added to synthetic ADSW, a fluorescence peak appeared in the area with the wavelength at λEx/λEm = (370–380 nm)/(500–510 nm), which was humic acid-like organic matters (Chen et al., 2003). With the extension of culture time, the peak intensity gradually decreased (Fig. 5(b)–(e)), which indicated that OTC was gradually degraded via photodegradation and hydrolysis in synthetic ADSW, which might be related to the decomposition of condensed aromatic parts and fragmentation of macromolecules into smaller fragments (Yang et al., 2018b). The peak intensity weakened to disappear after nine days, which might be attributed to the degradation of a portion of the intermediate and the evolution to CO_2 and H_2O.

4. Conclusions

OTC-contaminated synthetic ADSW was treatment using *Lemna aequinoctialis* systems. The removal efficiency of NH_3-N and TP obviously inhabited with the increase of OTC concentration during the study. Similar result for duckweed growth was also revealed. The photosynthetic pigment and protein content were significantly promoted when the concentration of OTC was 0.05 mg/L. The protein content in *Lemna aequinoctialis* gradually reduced with the prolongation of time due to the toxic effect of OTC on duckweed. Twelve intermediates and possible degradation pathways were proposed based on results of LC-MS/MS, and 3D-EEMs illustrated that the peak intensity reduced to invisibility after nine days.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant Nos.: 51478172 and 51521006), the Department of Science and Technology of Guangdong Province of China (Contract No.: 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 Nos.: 2017JJ2029 and 2017SK2362).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.121853.

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