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Research article

Removal of tetracycline from water by catalytic photodegradation combined with the microalga *Scenedesmus obliquus* and the responses of algal photosynthesis and transcription

Zhehua Chen [a,b], Dong Ou [b], Gan Gu [a,b], Shumei Gao [b], Xi Li [b], Changwei Hu [b,*], Xianrui Liang [a,**], Yuejin Zhang [b]

[a] College of Pharmacy, Zhejiang University of Technology, Hangzhou, 310014, China
[b] College of Biological, Chemical Science and Engineering, Jiaxing University, Jiaxing, 314001, PR China

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ABSTRACT

The antibiotic tetracycline (TC) and its degradation products (TDPs) in degradation solution present serious environmental problems, such as human health damage and ecological risk; thus further treatment is required before being released into the aquatic environment. Furthermore, their environmental impact on microalgae remains unclear. In this study, TC was degraded by photocatalysis using birnessite and UV irradiation, followed by biological purification using the microalga *Scenedesmus obliquus*. In addition, the photosynthetic activity and transcription of the microalgae were examined to evaluate the toxicity of TC and TDPs. The results show that photocatalytic degradation efficiency reached 92.7% after 30 min, and 11 intermediate products were detected. The microalgae achieved a high TC removal efficiency (99.7%) after 8 days. Exposure to the degraded TC solution (D) resulted in significantly lower ($p < 0.05$) biomass than the pure TC (T), and *S. obliquus* in the T treatment showed better resilience than the D treatment. Transcriptomic assays for different treatments revealed differential gene expression mainly involving the photosynthesis, ribosome, translation and peptide metabolic progresses. The up-regulation of photosynthesis-related genes and differential expression of chloroplast genes may be important for *S. obliquus* to acquire high photosynthetic efficiency and growth recovery when exposed to TC and TDPs. Our study provides a reference for TC removal using a combination of catalytic degradation and microalgal purification, and it is also helpful for understanding the environmental risk of TDPs in natural aquatic environments.

1. Introduction

Antibiotics are commonly used to prevent and treat infectious disease in humans and animals, as well as to promote growth in livestock industries (Kummerer, 2009). To maintain human health and reduce infection in aquaculture, tetracycline (TC) is one of the most commonly used antibiotics against many bacteria (Mathers et al., 2011). TC is not easily absorbed during animal metabolism, resulting in significant persistence in the aquatic environment (Dai et al., 2020). Furthermore, TC cannot be completely removed from conventional wastewater treatment plants (WWTPs) (Daghrir and Drogui, 2013); hence, it is widely detected in both the final effluent of WWTPs and the natural aquatic environment (Sarmah et al., 2006). In Yunfu City, China, 0.11–0.93 μg/L of TC was detected in WWTP effluent (Zhang et al., 2018). Additionally, high levels of TC (up to 334,000 μg/L) were detected in the effluent from pharmaceutical WWTPs in northern China (Hou et al., 2016). In some surface waters that receive effluent from WWTPs, detected TC concentrations varied from 3.3 ng/L to 2.0 mg/L (Li et al., 2021). The accumulation of TC in the water environment poses a potential threat to the health of the aquatic ecosystem. Babin et al. found that the EC_{50} values of TC for the enzymes ethoxyresorufin-O-deethylase and β-galactosidase were 167.63 mg/L and 84.59 mg/L, respectively, in two fish cells (RTG-2 and RTL-W1) (Babin et al., 2005). In addition, TC has been shown to have toxic effects on the photosynthesis and antioxidant enzyme activities of algae (Chang et al., 2015; Daghrir and Drogui, 2013; Jiang et al., 2010; Xu

* Corresponding authors.
** Corresponding authors.
E-mail addresses: changweihu@zjxu.edu.cn (C. Hu), liangxrvicky@zjut.edu.cn (X. Liang).

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et al., 2021).

The removal of TC from water is of great significance to minimize its adverse effects. Numerous techniques, including photocatalytic degradation, advanced oxidation, electrochemical methods, microalgal degradation, microbial degradation and activated sludge degradation, have been used for the removal of various antibiotics (Dai et al., 2019). Among these techniques, photocatalytic degradation is regarded as an efficient and eco-friendly way to remove various pollutants, including antibiotics (Dai et al., 2019), dyes (Yousefi et al., 2019, 2021b) and heavy metals (Majidnia and Fulazzaky, 2017; Fulazzaky et al., 2017). Photocatalytic degradation is widely used for TC removal and the final removal efficiency (RE) ranges from 27.2% to 99% under optimal degradation conditions (Dai et al., 2020; Soltani et al., 2019). Although photocatalytic degradation has been demonstrated as effective, it was found that a majority of the TC is transformed into intermediate products without complete mineralization, thereby simultaneously producing toxic TC degradation products (TDPs) (Jiao et al., 2008). Moreover, the maximum RE usually cannot be achieved in actual operation. Hence, because of their potential risks, it is better to treat the antibiotic and its residues, such as TC and its residual degradation products in the degradation solution, before releasing them into the aquatic environment.

Microalgal-based technologies are considered as an ecologically friendly and cost-effective reclamation strategy for antibiotics removal (Xiong et al., 2021). It has been demonstrated that microalgae have great potential to remove TC from water due to their high growth rate and wide distribution (Leng et al., 2020). For example, Pan et al. reported effective and rapid TC removal (up to 93.9% in 2 days) by *Chlorella pyrenoidosa*, with the microalgal RE depending closely on the initial TC concentration (Pan et al., 2021). Nevertheless, the potential for microalgae to remove TC residues in degradation solution has been paid very little attention. High concentrations of TC are toxic and could inhibit algal growth. After degradation, TC decreases to considerably low levels that have almost no inhibition effects on microalgae, making it feasible to use microalgae for the biotreatment of TC degradation solutions. In addition, studying the ability of microalgae to remove low concentrations of TC and its products helps to understand algal bioaccumulation and degradation of TC in WWTP runoff as well as in the natural aquatic environment.

The environmental risk of TDPs derived from chemical degradation is also worth noting. However, limited research has focused on the toxicity of TDPs. Varying amounts of TDPs can be obtained depending on the different selected degradation methods. Up to 17 intermediate products, including anhydrotetracycline (ATC) and epitetracycline (ETC), were detected during the TC degradation process (Cao et al., 2019). The 96 h-EC_{50} value using the microalga *Chlorella vulgaris* indicated that the toxicity of ATC was higher than that of TC, whereas ETC had a lower toxicity (Xu et al., 2019). The combined toxicity of the degradation compounds was evaluated using the bacteria *Vibrio fischeri* (Jiao et al., 2008), *Salmonella typhimurium* (Ao et al., 2019) and *Escherichia coli* (Zhou et al., 2017), with the results revealing that the toxicity of the products had equivalent or even higher toxicity than TC. However, the combined toxicity of TDPs that remain in the degradation solution to microalgae, which are ubiquitous in the aquatic environment, has obtained less attention. In addition, the toxicological mechanisms of the combined TDPs to microalgae remain unknown.

The objectives of the present study were to 1) improve the RE of TC by combining two different removal methods, namely catalytic photodegradation and a microalgae-based method using the microalga *Scenedesmus obliquus*; 2) investigate changes in the photosynthesis and transcription of the microalgae; and 3) compare the photosynthesis and transcription characteristics of the microalgae employed to treat the degradation solution and pure TC, in order to demonstrate the effects and underlying mechanisms of the TDPs on microalgae. Our work contributes not only to the advanced purification of degraded TC solutions by microalgae, but also to understanding the effects of TC and TDPs at environmentally relevant concentrations on microalgae in the natural aquatic environment.

2. Materials and methods

2.1. Catalytic photodegradation of TC under UV irradiation

TC (CAS No. 60-54-8) was purchased from Shanghai Macklin Inc. (Shanghai, China). The catalyst birnessite was synthesized according to Lv et al. (2017). Briefly, 25 mL of $MnCl_2.4H_2O$ (0.4 M) was mixed with 25 mL NaOH (8 M) to form $Mn(OH)_2$ precipitate. Next, 50 mL $KMnO_4$ solution was added dropwise to oxidize the precipitate and to form birnessite.

Catalytic degradation was performed under different pH and dosages of catalyst under fixed irradiation intensity (200 μW/cm^2) of UV (365 nm, 250 W, XH-300UP, Xianghu Co. Ltd. Beijing, China). The effect of pH on the degradation efficiency was studied by adjusting the pH in the range of 5–10 in the presence of 0.67 g/L birnessite. The effect of catalyst dosage was studied by adding 0.1–1.0 g/L birnessite under the optimum pH. The reaction duration was 30 min. Finally, UV degradation was carried out under the optimum pH and birnessite dosage.

The degradation efficiency of TC (D, %) in water was calculated using the following Equation:

$$D = \frac{A_0 - A_e}{A_0} \times 100 \quad (1)$$

where A_0 and A_e are the initial and final concentrations (mg/L), respectively, of the TC solution, which was measured via liquid chromatography (LC, Agilent 1260 Infinity II, USA). The TDPs were detected using LC (Agilent 1100, USA) with mass spectrometry (MS, Thermos TSQ Quantum Ultra, USA) (LC-MS).

2.2. TC removal by the microalga Scenedesmus obliquus

The microalga *S. obliquus* (FACHB-417) was purchased from the Freshwater Algae Culture collection at the Institute of Hydrobiology, China. The *S. obliquus* was cultured in BG11 medium under a light intensity of 3000 Lux, light-dark cycle of 12 h:12 h and temperature of 25 ± 0.5 °C. The microalgae were subcultured by renewing the medium every 7 days.

The acute toxicity of TC to *S. obliquus* was previously reported by Xu et al. (2013). The calculated IC_{10} (10% inhibition concentration) was 1.6 mg/L, which was used to investigate the TC removal potential of *S. obliquus* in our study. After photocatalytic degradation of 50 mg/L TC solution for 30 min under the optimum conditions, the solution was filtered through a 0.22 μm membrane to remove the catalyst. The TC concentration was measured via LC (Agilent 1260 Infinity II, USA), and then the degraded TC solution was diluted to 1.6 mg/L with BG11 medium (hereafter referred to as D). In addition, 1.6 mg/L of pure TC solution was prepared using the stock solution and BG11 medium (hereafter referred to as T). The pure TC solution served as a base for comparing the effect of the TDPs remaining in the degradation solutions. *Scenedesmus obliquus* was transferred to the above two cultures to obtain an initial cell density of 2×10^5 cells/mL. A control group without TC (hereafter referred to as C) was simultaneously conducted. Under the same conditions, we used 2×10^6 cells/mL as the high cell density treatment to detect the RE of the microalgae at a high concentration. The microalgae were incubated for 8 days under the conditions described above.

2.3. Photosynthesis assay

An Aquapen system (Photon Systems Instruments, AP-C100, Brno, Czech Republic) was used to measure chlorophyll a fluorescence (OJIP) of *S. obliquus* according to Sun et al. (2020). The OJIP-test parameters

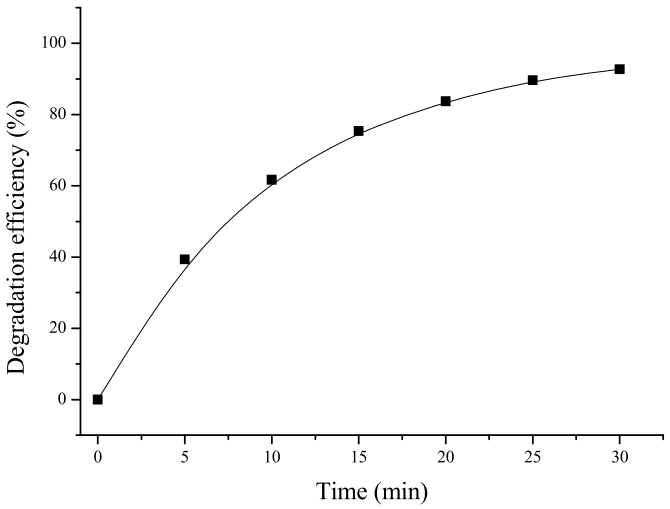

Fig. 1. The tetracycline (TC) degradation kinetics in BG11 medium by the catalyst under UV irradiation (50 mg/L initial TC concentration, pH 8, 0.6 g/L birnessite, and UV irradiation 200 μW/cm^2).

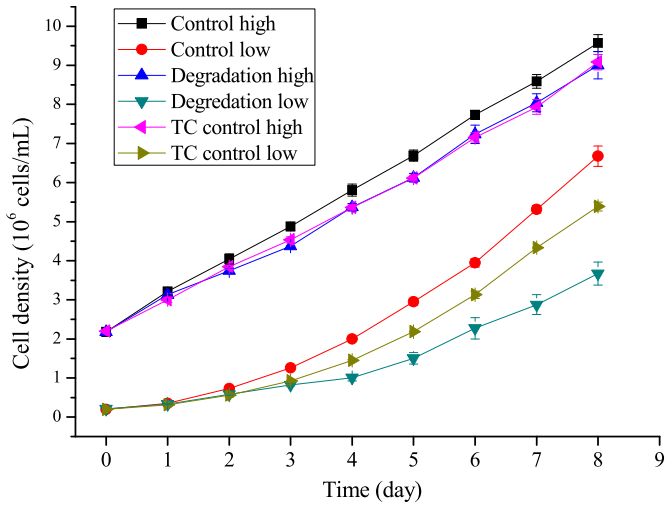

Fig. 2. Growth curves of the microalga *Scenedesmus obliquus* during the 8-d tetracycline (TC) removal period. High and low represent the initial cell densities of 2 × 10^6 and 2 × 10^5 cells/mL, respectively.

Table 1
Removal efficiency (RE) of tetracycline (TC) by the microalga *Scenedesmus obliquus* in the solutions of degraded tetracycline (D) and pure tetracycline (T) treatments. The data with the same superscript letter in each day indicates no significant difference ($p > 0.05$).

Treatment	Removal efficiency (RE, %)			
	1 d	2 d	4 d	8 d
D high	74.3 ± 0.6^b	73.5 ± 0.7^b	78.6 ± 0.2^a	99.7 ± 0.0^a
T high	78.0 ± 1.5^a	79.2 ± 0.4^a	78.6 ± 0.0^a	99.7 ± 0.0^a
D low	65.6 ± 0.8^c	68.0 ± 0.2^c	78.5 ± 0.1^a	99.7 ± 0.0^a
T low	46.3 ± 1.1^d	65.4 ± 3.5^c	78.4 ± 0.4^a	99.7 ± 0.0^a

extracted from the fast fluorescence transient, including PI_{ABS} (the performance index), ψ_o (the efficiency at which a trapped exciton can move an electron into the electron transport chain further than Q_A), φE_o (the quantum yield of electron transport) and φD_o (quantum yield (at t = 0) of energy dissipation), were selected to evaluate the effects and mechanisms of TC and TDPs according to previous literature (Chen et al., 2007, 2016; Strasser et al., 2000, 2004).

2.4. Transcriptomic analysis

Total RNA was extracted from triplicate C, T and D samples after 8 days of growth using Trizol Reagent (Invitrogen Life Technologies). A NanoDrop 2000 was used to determine the concentration and purity of the total RNA for the different samples. The total RNA samples were then submitted Shanghai Bioprofile Co. Ltd for preparation and construction of the mRNA library. Finally, the HiSeq X Ten System of the Illumina platform was used to complete transcriptomic sequencing.

2.5. Statistical analysis

The results of the cell density and photosynthetic parameters of *S. obliquus* are presented as the arithmetic mean with the corresponding standard deviation ($n = 3$). Statistical significance of differences observed among treatments was determined using one-way analysis of variance (ANOVA) and covariance (ANCOVA), followed by Tukey's pair-wise comparison at a significance level of $P < 0.05$.

3. Results and discussion

3.1. Photocatalytic degradation efficiency and products of TC

The optimum degradation conditions, specifically pH and catalyst dosage, were examined and the results are shown in Figs. S1 and S2. Based on these results, the degradation conditions of pH 8, 0.6 g/L birnessite and 200 μW/cm^2 UV irradiation were employed to investigate the degradation kinetics of TC in BG11 medium. The RE rapidly reached 83.7% within the first 20 min, and then gradually increased to 92.7% by 30 min, resulting in a high TC RE (Fig. 1).

The intermediate products were identified by LC-MS. As shown in Fig. S3, eleven byproducts (namely P-114, P-130, P-374, P-415, P-417, P-421, P-435, P-443, P-445, P-477 and P-493) were detected. It was reported that various intermediate products can be found depending on the degradation method and conditions used. Cao et al. (2019) reported that 17 intermediate products, including all products mentioned above, were found when TC was degraded by peroxymonosulfate (PMS) activated with zero-valent iron. Lv et al. (2017) detected 6 products of TC (P-402, P-360, P-348, P-302, P-261 and P-217) in the presence of birnessite under 30 min microwave treatment. Jiang et al. (2021) reported that the products during the TC degradation process were mainly P-459, P-445 and P-134 in a UV-heterogeneous Fenton-like system with a $BiFeO_3$ (BFO) catalyst. The differences in the products indicate that different degradation mechanisms are involved in the individual methods and conditions.

Photocatalytic technology exhibits great potential in the field of wastewater treatment because it is a low cost, environmental friendly and sustainable treatment process (Chong et al., 2011; Mahdi et al., 2022). Various catalysts, such as Co/Co_3O_4, $Ni/Ni(OH)_2$ and $CdHgI_4/HgI_2$ nanocomposites, have been synthesized and show excellent catalytic efficiency in pollutant removal (Yousefi et al., 2016; Yousefi et al., 2017; ; Yousefi et al., 2021a). Although TC is difficult to degrade due to its broad spectrum antibacterial characteristic and stable naphthalene structure (Daghrir and Drogui, 2013), various catalysts and methods have been used to degrade TC in solutions, with satisfactory REs obtained (Dai et al., 2020; Saadati et al., 2016). The maximum RE of TC by birnessite was reportedly 99% under microwave irradiation in a highly acidic medium at 400 W for 30 min (Liu et al., 2014). However, most treatment processes show low mineralization yields, indicating that the majority of contaminants are transformed into by-products (Daghrir and Drogui, 2013). Thus, TC and TDPs with relatively low concentrations in the effluents may still be harmful to aquatic organisms. Hence, further treatment is required to reduce TC and TDPs in the degradation solutions.

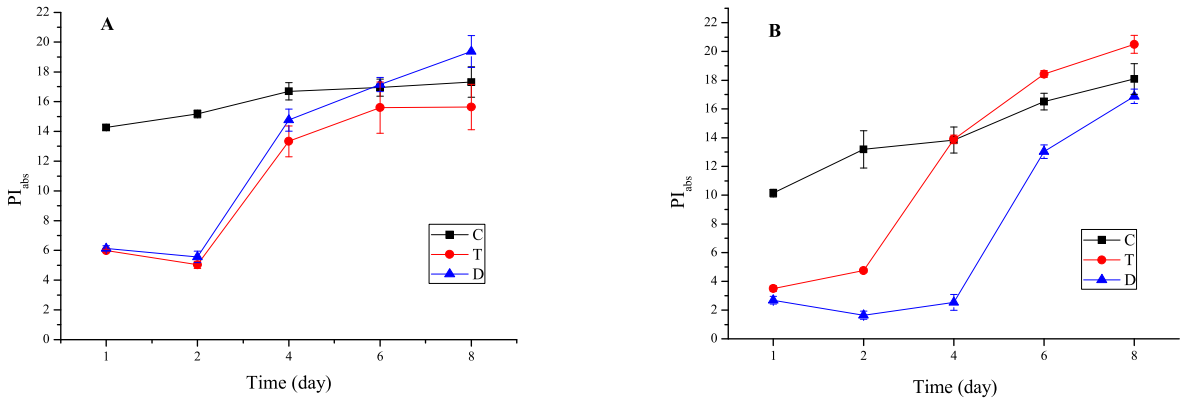

Fig. 3. Variation of the photosynthetic parameter PI_{abs} of the microalga *Scenedesmus obliquus* during the 8-d period. A) high initial cell density; B) low initial cell density.

Table 2
The values of the three selected photosynthetic indices of *S. obliquus* during 8-d exposure.

Indices	Time (day)	High initial cell density (2×10^6 cells/mL)			Low initial cell density (2×10^5 cells/mL)		
		C	T	D	C	T	D
Ψ_O	1	0.79 ± 0.00^a	0.74 ± 0.00^b	0.74 ± 0.00^b	0.76 ± 0.00^a	0.70 ± 0.00^b	0.67 ± 0.00^c
	2	0.82 ± 0.00^a	0.75 ± 0.00^b	0.76 ± 0.00^b	0.79 ± 0.00^a	0.73 ± 0.00^b	0.65 ± 0.01^c
	4	0.83 ± 0.00^a	0.82 ± 0.00^a	0.82 ± 0.00^a	0.80 ± 0.00^a	0.79 ± 0.00^b	0.69 ± 0.01^c
	6	0.83 ± 0.00^a	0.83 ± 0.01^a	0.83 ± 0.00^a	0.82 ± 0.00^a	0.82 ± 0.00^a	0.80 ± 0.00^a
	8	0.83 ± 0.00^a	0.83 ± 0.01^a	0.83 ± 0.01^a	0.83 ± 0.00^a	0.83 ± 0.00^a	0.82 ± 0.00^a
Φ_{Eo}	1	0.61 ± 0.00^a	0.52 ± 0.00^b	0.52 ± 0.00^b	0.57 ± 0.00^a	0.46 ± 0.00^b	0.43 ± 0.00^c
	2	0.63 ± 0.00^a	0.50 ± 0.00^b	0.52 ± 0.01^b	0.60 ± 0.00^a	0.50 ± 0.00^b	0.39 ± 0.02^c
	4	0.63 ± 0.00^a	0.61 ± 0.01^a	0.62 ± 0.00^a	0.61 ± 0.00^a	0.61 ± 0.00^a	0.43 ± 0.02^b
	6	0.64 ± 0.00^a	0.62 ± 0.01^a	0.63 ± 0.00^a	0.63 ± 0.00^a	0.64 ± 0.00^a	0.60 ± 0.00^b
	8	0.64 ± 0.01^a	0.62 ± 0.01^a	0.64 ± 0.01^a	0.64 ± 0.00^a	0.65 ± 0.00^a	0.63 ± 0.00^a
Φ_{Do}	1	0.23 ± 0.00^a	0.30 ± 0.00^b	0.29 ± 0.00^b	0.24 ± 0.00^a	0.34 ± 0.00^b	0.35 ± 0.00^b
	2	0.23 ± 0.00^a	0.33 ± 0.00^b	0.32 ± 0.01^b	0.24 ± 0.00^a	0.32 ± 0.00^b	0.41 ± 0.01^c
	4	0.23 ± 0.00^a	0.25 ± 0.01^a	0.24 ± 0.00^a	0.23 ± 0.00^a	0.24 ± 0.00^b	0.37 ± 0.02^c
	6	0.23 ± 0.00^a	0.24 ± 0.01^a	0.23 ± 0.00^a	0.23 ± 0.00^a	0.22 ± 0.00^a	0.24 ± 0.00^a
	8	0.24 ± 0.00^a	0.25 ± 0.01^a	0.23 ± 0.00^a	0.23 ± 0.00^a	0.22 ± 0.00^a	0.23 ± 0.00^a

Notes: C, control; T, pure tetracycline solution; D, tetracycline degradation solution. The data with the same superscript letter in each day and each index indicates no significant difference ($p > 0.05$).

3.2. Algal growth during biological degradation

Two initial cell densities (2×10^6 and 2×10^5 cells/mL) of *S. obliquus* were used to purify the D and T solutions. Growth of the microalgae is shown in Fig. 2. *Scenedesmus obliquus* with the higher cell density grew fast and did not experience an obvious lag phase. The microalgae maintained exponential growth during the 8-d culture. Differences among the three treatments (C, D and T) were observed from day 3, and lasted the following 5 days. Compared with the control, exposure to 1.6 mg/L TC resulted in low *S. obliquus* biomass, which was reflected by the average cell densities. D exposure led to a similar cell density that was close to the T treatment. The microalgae with the low initial cell density experienced an evident lag phase during the first 3 days, and then produced less biomass than that of the high initial cell density at 8 d. Both the D and T treatments significantly inhibited algal growth from day 3. The differences in algal growth between D and T appeared from day 4. The T treatment caused significantly lower ($p < 0.05$) biomass than the control. However, the D treatment inhibited algal growth more severely than the T treatment, suggesting that the TDPs and the residual TC in the D solution may have had more adverse effects on *S. obliquus*.

Microalgae are more sensitive to TC than crustaceans and fish (Daghrir and Drogui, 2013). For example, it was observed that the EC_{50} values of TC were 0.09 and 2.2 mg/L for *Microcystis aeruginosa* and *Selenastrum capricornutum*, respectively (Halling-Sørensen, 2000). TC hydrochloride concentrations above 2 mg/L significantly inhibited the photosynthesis and growth of two microalgae (*Dictyosphaerium pulchellum* and *Micractinium pusillum*) on agar plates (Bashir and Cho, 2016). However, Tong et al. (2020) found that growth of the microalgae *Coelastrella* sp. Showed a dose-dependent response to TC with stimulation at low levels (≤2 mg/L) and an inhibitory effect only observed at higher levels (>2 mg/L). Our results show that 1.6 mg/L of pure TC inhibited the growth of *S. obliquus* at the low cell density, which is the cell density recommended by OECD for toxicity tests (OECD, 2006). However, the biomass of the microalgae used for bioremediation is usually much higher than 2×10^5 cells/mL. Therefore, 1.6 mg/L of TC has no significant inhibitory effect on microalgal growth at relatively high cell densities.

When compared with the parent compound, few toxicity studies have investigated the TDPs. Zhou et al. (2017) examined the toxicity of TDPs produced by *E. coli* and did not find intermediate products of higher toxicity. Halling-Sørensen et al. (2002) investigated the individual toxicity of 13 TC products on aerobic sludge bacteria, and the results showed that most of the products (10 of 13) had lower toxicity than TC. Furthermore, the authors speculated that these compounds possess a different mode of action than the parent compound. However, the mechanisms of TC and its products on bacteria remain unknown.

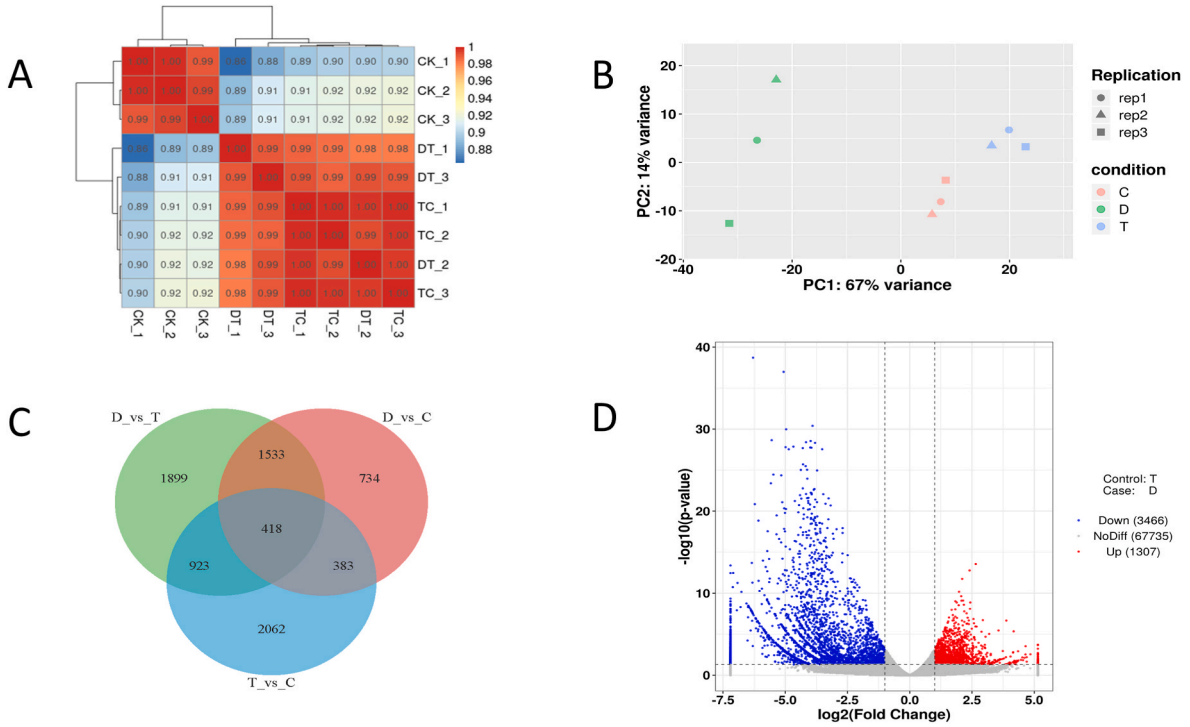

Fig. 4. Transcriptomic profiles of *Scenedesmus obliquus* in the three different treatments (C, T and D). (A) Correlation analysis of gene expression patterns in the three different treatments; (B) Principal Components Analysis based on the expression quantity in the three different treatments; (C) Venn diagram based on differentially expressed genes (DEGs) in the three groups; (D) Volcano plot based on the DEGs in the D vs T group.

Table 3
Statistical analysis of expression differences.

Control	Treat	Up-regulation	Down-regulation	DEG Number
C	D	943	2125	3068
C	T	2157	1629	3786
T	D	1307	3466	4773

Notes: C, control; T, pure tetracycline solution; D, tetracycline degradation solution.

3.3. Removal efficiency of tetracycline in degraded and pure tetracycline solutions by the microalgae

The TC concentrations in the solutions of the D and T treatments were monitored using LC-MS, and the REs were calculated (Table 1). When the high initial *S. obliquus* biomass was selected, high REs of more than 74% were achieved after a 1-d exposure, and the REs basically remained steady during the first four days. By 8 d, 99.7% of TC was removed in both the D and T treatments. Conversely, significantly lower REs ($p < 0.05$) were observed for the low biomass on day 1. The microalgae in the T treatment exhibited a significantly lower RE ($p < 0.05$) than that of the D treatment. The RE increased to approximately 78% during the following three days, reaching 99.7% by 8 d. The culture media of the microalgae was also examined for the TC products after 8 d, but no TDPs were detected.

Microalgae have been intensively used for the removal of antibiotics in recent years (Leng et al., 2020). The reported REs vary greatly and previous work has usually focused on pure antibiotics without TDPs. Norvill et al. (2017) reported that TC removal by algae with activated sludge in a high rate algal pond exceeded 99% at a hydraulic retention time of 7 days. The RE of TC in our study is consistent with this previous study. Different to the high cell density, the low initial *S. obliquus* density experienced an evident lag phase during the first 4 days, which may also be influenced by TC and responsible for the low REs. However, the REs of the microalgae of both cell densities exceeded 78% without differences at 4 d, and reached 99.7% by 8 d, indicating the great potential of *S. obliquus* in TC removal. In addition, the TDPs accompanying TC seemed to have no influence on the RE of the microalgae. A previous study used the bacteria *Pseudomonas aeruginosa* for biodegradation of oxytetracycline intermediates and the results showed that nearly 70% of the intermediates could be removed at a cell concentration of 1.5 g/L (Subramani et al., 2019).

3.4. Photosynthetic responses of the microalgae to tetracycline during biological degradation

The photosynthetic parameter PI_{abs} is defined as the performance index for the energy conservation of photons absorbed by photosystem II (PSII) to the reduction of intersystem electron acceptors (Strasser et al., 2004). It is regarded as a sensitive indicator to reflect the photosynthetic efficiency and is frequently used to evaluate the toxicity of contaminants to plants and microalgae (Oukarroum et al., 2007; Sun et al., 2020). For the high initial cell density, the PI_{abs} value of *S. obliquus* in the control group (C) slowly increased from 14.2 on day 1–17.3 on day 8 (Fig. 3A). However, both the D and T treatments started with a very low PE, which was indicated by a PI_{abs} value close to 6, suggesting the stresses form TC and the TDPs in the solutions. After 4 days of growth, the PEs of the two treatments rapidly increased, reaching that of the control on 8 d. No significant differences ($p > 0.05$) between the three treatments were observed after 8 days of growth. The low initial cell density led to much lower PI_{abs} values at the beginning of the culture (Fig. 3B). During the 8-d culture period, the PI_{abs} value of *S. obliquus* in the C group increased from 10.1 to 18.1. Similarly, both the D and T treatments showed remarkably low PEs on day 1, and then the PI_{abs} values increased over the following seven days. However, the PE of *S. obliquus* in the D treatment was significantly lower ($p < 0.05$) than that of T on day 2, which lasted until day 4, and it then promptly recovered to close to that of C in the following period. On day 8, the PE of T was significantly higher ($p < 0.05$) than that of C, while D was the opposite.

To further explore the influences of TC and TDPs on *S. obliquus*

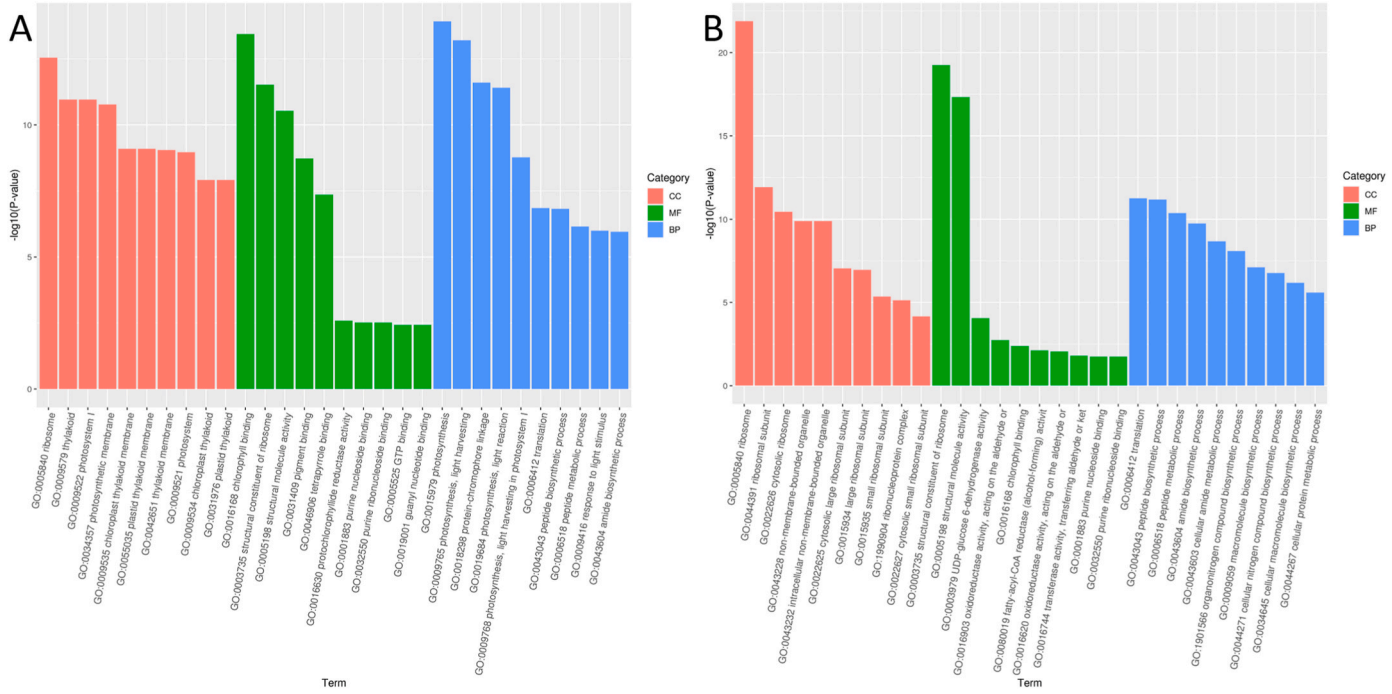

Fig. 5. GO enrichment analysis of differentially expressed genes (DEGs). (A) T vs C; (B) D vs T. (C, control; T, pure tetracycline solution; D, tetracycline degradation solution).

photosynthesis, the photosynthetic indices ψ_o, φE_o and φD_o were monitored on days 1, 2, 4, 6 and 8, and the results are shown in Table 2. When the high cell density was used, the ψ_o and φE_o values in both D and T were significantly inhibited ($p < 0.05$) during the first 2 days, while φD_o was enhanced. The results illustrate low efficiencies of light absorption and electron transport, and a high energy dissipation in the D and T treatments. However, these effects disappeared after day 4. Moreover, no significant differences ($p > 0.05$) between D and T were observed. For the low initial cell density, similar effects on ψ_o, φE_o and φD_o were detected during the first 4 days. The inhibitory effects of D were significantly higher than that of T. This suggests that the TDPs in the D treatment had additional adverse influences on the microalgae. TC has been demonstrated to inhibit the photosynthetic activity of the microalgae *D. pulchellum* and *M. pusillum*, which was evidenced by the quantum yield of PSII (F_v/F_m), but the inhibitory mechanisms were not discussed in this work (Bashir and Cho, 2016). Our study indicates that light absorption, electron transport and energy dissipation of the microalgae were affected by TC. However, the microalgae showed resistance to these effects and recovered photosynthetic activity to a normal level after the 8-d exposure.

3.5. Transcriptomic responses of the microalgae to tetracycline

A total of 140, 058, 092 transcripts were detected in the *S. obliquus* samples (Table S1). The maximum and mean transcript lengths were 13,643 and 1163.56 bp, respectively. In this study, the correlation coefficient among the three replicates of each *S. obliquus* sample (C, T and D treatments) was 0.99–1.00, indicating a high correlation among biological replicates (Fig. 4A). The correlation coefficient between C and T was 0.88–0.92, while that between T and D was 0.99–1.00. The results show that the gene expression pattern of D was more similar to T than to C. According to the expression quantity, principal components analysis (PCA) showed that the clusters for each type of sample were clearly distinguished (Fig. 4B).

DESeq was used for differential analysis of gene expression for the three treatments, and the results are shown in Table 3 and Fig. 4C. In the D vs C group, a total of 3068 differentially expressed genes (DEGs) were identified (945 up- and 2125 down-regulated). In the T vs C group, 3786 DEGs were identified (2157 up- and 1629 down-regulated). Fig. 4D illustrates that 1307 up-regulated DEGs, 3466 down-regulated DEGs and 67,735 non-significantly DEGs were detected in the D vs T group. This suggests that in the pure TC and degraded TC solution, although the gene expression pattern of *S. obliquus* in T and D treatments was similar (Fig. 4A), some differences in expressed genes still existed.

For further analysis of expression differences in the S. *obliquus* of the T and D treatments, differential gene enrichment analysis was conducted. GO enrichment analysis was used to estimate the effect of TC and TDPs on the growth and photosynthetic performance of S. *obliquus* for the categories of BP (biological process), CC (cell components) and MF (molecular function) (Fig. 5 and Table S2). In the T vs C group (Fig. 5A), the GO items were associated with photosynthetic progress (GO: 0009522 photosystem I; GO: 0009521 photosystem; GO: 0034,357 photosynthetic membrane; GO: 0016,168 chlorophyll binding; GO: 0031,409 pigment binding), with all GO enriched items up-regulated in the T treatment relative to the C treatment, which indicates that the enriched GO items detected in the T vs C group played a vital role in the TC-associated inhibition of *S. obliquus* growth and photosynthetic performance. For the D vs T group (Fig. 5 B), the GO items associated with ribosome, translation and peptide metabolic progresses (GO: 0005840 ribosome; GO: 0003735 structural constituent of ribosome; GO: 0044,391 ribosomal subunit; GO: 0005198 structural molecule activity; GO: 0006412 translation; GO: 0043,043 peptide biosynthetic process; GO: 0006518 peptide metabolic process) were down-regulated in the D treatment relative to the T treatment. Functional pathway analysis based on the KEGG database confirmed that TC and TDPs affected the RNA mechanism and photosynthesis of *S. obliquus*. Detailed information on the functional pathways (top 20 pathways) is provided in the supporting information (Fig. S4).

In our study, after the 8-d exposure to pure TC, the DEGs involved photosynthesis and chloroplast synthesis were up-regulated in *S. obliquus*, which may be responsible for the recovery of the PE. Microalgae and plants can adapt to external stresses and show stress responses for cell protection by up-regulating multiple genes. For example, up-regulation of photosynthesis-related and ribosomal genes were found

in two cyanobacterial species (*Microcystis aeruginosa* and *Synechocystis* sp.) that were exposed to a ternary antibiotic mixture (TC, ciprofloxacin and sulfamethoxazole) (Xu et al., 2022). Ma et al. (2016) found that the expression of photosynthesis- and chloroplast-related DEGs were up-regulated in a high drought-tolerant rice cultivar (IAC1246).

The ribosome is one of the major targets within bacterial cells for antibiotics (Nguyen et al., 2014), and bacteria can become resistant to TCs via the ribosomal protection mechanism (Connell et al., 2003; Taylor and Chau, 1996). This mechanism has not been previously found in microalgae. In the present study, the ribosomal genes were not significantly influenced by pure TC, but they were comparatively down-regulated in the D treatment (Table S2), which have led to low productivity of *S. obliquus* in the D treatment at day 8. It is worth noting that the expression of photosynthesis- and chloroplast-related DEGs were also up-regulated in the D treatment compared with the C treatment. Thus, maintenance of high photosynthetic efficiency may be crucial for *S. obliquus* when exposed to TC together with TDPs.

4. Conclusions

In the present study, TC was photocatalytically degraded by birnessite and UV irradiation, and then the TC residue and TDPs that remained in the solution were further purified by the microalga *S. obliquus*. The results show that the combined catalytic degradation and algal purification method exhibited an encouraging RE (99.7%) and could be used to remove TC in wastewater. Furthermore, dilution of the degradation solution could help to reduce the stress of TC on the microalgae, allowing for a high growth rate and photosynthetic efficiency, which are beneficial for the RE of the microalgae.

Transcriptomics was performed to investigate the response of the microalgae to TC and TDPs. The results reveal that differential gene expression of the three treatments (C, T and D) mainly involved photosynthesis, ribosome, translation and peptide metabolic progresses. The up-regulation of photosynthesis- and chloroplast-related DEGs may be crucial for *S. obliquus* to obtain a high PE and growth recovery when exposed to TC and TDPs. Our study provides a reference for research into the algal response to TC and TDPs in both wastewater and the natural aquatic environment.

Author contribution statement

Zhehua Chen: Writing – original draft, Methodology, Validation. **Dong Ou**: Conceptualization, Validation. **Gan Gu**: Conceptualization, Methodology. **Shumei Gao**: Methodology, Validation. **Xi Li**: Conceptualization, Validation. **Changwei Hu**: Project administration, Funding acquisition, Supervision, Writing – reviewing & editing. **Xianrui Liang**: Writing – reviewing & editing. **Yuejin Zhang**: Reviewing & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

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