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Enhanced oxytetracycline removal coupling with increased power generation using a self-sustained photo-bioelectrochemical fuel cell

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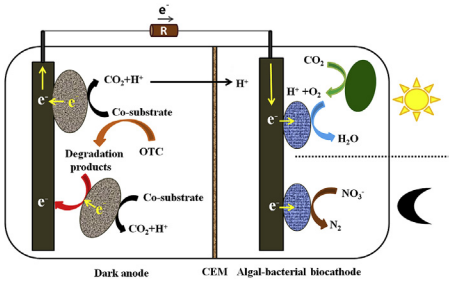
HIGHLIGHTS

- Simultaneous OTC removal and power generation in PBFC was firstly investigated.
- Bioelectrochemical enhanced co-metabolism responsible for OTC removal in bioanode.
- Power output of PBFC was significantly increased by feeding OTC to the bioanode.
- Degradation product of OTC mediated electron transfer from bacterial cell to anode.
- OTC-tolerant bacteria for degrading OTC and producing electricity were enriched.

GRAPHICAL ABSTRACT



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ABSTRACT

Photo-bioelectrochemical fuel cell (PBFC) represents a promising technology for enhancing removal of antibiotic pollutants while simultaneously sustainable transformation of organic wastes and solar energy into electricity. In this study, simultaneous antibiotic removal and bioelectricity generation were investigated in a PBFC with daily light/dark cycle using oxytetracycline (OTC) as a model compound of antibiotic. The specific OTC removal rate increased by 61% at an external resistance of 50 Ω compared to that in the open-circuit control, which was attributed to bioelectrochemically enhanced co-metabolic degradation in the presence of the bioanode. The OTC removal was obviously accelerated during illumination of cathode in contrast with a dark cathode due to the higher driving force for anodic bioelectrochemical reaction by using photosynthetic oxygen as cathodic electron acceptor during illumination than that using nitrate in dark. The bioelectrocatalytic activity of anodic biofilm was continuously enhanced even at an initial OTC concentration of up to 50 mg L^{-1}. The degradation products of OTC can function as mediators to facilitate the electron transfer from bacteria to the anode, resulting in 1.2, 1.76 and 1.8 fold increase in maximum power output when 10, 30 and 50 mg L^{-1} OTC was fed to the

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Power generation
Enhancement

bioanode, compared to the OTC-free bioanode, respectively. The OTC feeding selective enriched OTC-tolerant bacterial community capable of degrading complex organic compounds and producing electricity. The occurrence of ARGs during bioelectrochemical degradation of OTC was affected more greatly by the succession of the anodic bacterial community than the initial OTC concentration.

1. Introduction

Abuse of antibiotics in modern societies results in the release of large amounts of antibiotics into environment, raising worldwide concerns over the risk of promoting antibiotic resistance (Zhou et al., 2017; Binh et al., 2018; Li et al., 2018a). Tetracycline is the second most widely used antibiotics in the world (Bağda et al., 2014). As an important member of tetracycline antibiotic, Oxytetracycline (OTC) is most commonly used in human and veterinary medicine in developing countries due to its broad-spectrum antimicrobial activity and low cost. Based on recent statistics, the consumption of OTC is about 1360 tons, accounting for 20% of total usage of tetracyclines in China in 2013 (Zhang et al., 2015). It has also been reported to be at levels as high as 50 mg L^{-1} in the effluent from an OTC manufacturer in China (Li et al., 2008). Direct discharge or inadequate treatment of antibiotics-contained wastewater was mainly responsible for the continuous input of antibiotics to the environment and subsequent widespread dissemination of antibiotic resistance genes (ARGs), thus posing potential risks for ecological safety and public health (Zhang et al., 2015).

Various techniques have been explored to remove antibiotics from aqueous solution, including adsorption (Xiong et al., 2018), biological treatment (Copete-Pertuz et al., 2018), membrane separation (Cheng et al., 2018) and advanced oxidation (García-Galán et al., 2016). Among these techniques, biological processes, especially for anaerobic biological process, are more applicable for antibiotic-contained wastewater treatment, due to relatively high removal efficiency, less-energy consumption and environmental friendly (Ghattas et al., 2017). However, conventional anaerobic biological processes suffer from a major problem of slow-metabolism rates of anaerobic microorganisms which limit its application for antibiotic-contained wastewater treatment. Consequently, it is urgent to seek a high-efficient and sustainable alternative to conventional anaerobic biological processes for removing OTC from OTC-contained wastewater before being discharged into the environment.

Bioelectrochemical fuel cell (BFC) has recently gained increasing attention as a flourishing technology for wastewater treatment due to its innovative advantage of enhanced pollutants removal coupling with electrical energy recovery during wastewater treatment (Toczyłowskamamińska et al., 2018; Hemalatha et al., 2017). The anode of BFC can serve as an inexhaustible electron acceptor for enhanced degradation of recalcitrant organic pollutants in wastewater by using the pollutants as electron donor directly or accelerated co-metabolic degradation of the pollutants using biodegradable organic substrate as primary electron donor (Yu et al., 2016; Fan et al., 2017). Recent studies have shown that the BFC can stimulate and increase removal rate of antibiotic pollutants, such as sulfamethoxazole (Miran et al., 2018), chloramphenicol (Zhang et al., 2017) and oxytetracycline (Yan et al., 2018), due to enhanced microbial metabolism in the presence of anode. However, it is not clear whether the enhanced degradation of antibiotic by the bioanode is achieved by direct or co-metabolic bioelectrochemical process. Furthermore, less attention was given to the electrochemical response of the anodic electroactive biofilm to specific antibiotic and the power generation aspect of BFC. The underlying mechanisms of how the antibiotic and its degradation intermadiates take part in the anodic bioelectrochemical process are also poorly understood.

Photo-bioelectrochemical fuel cell (PBFC), which uses microalgae and bacteria to assist cathodic reaction, represents a remarkable step toward development of a sustainable BFC (Xiao et al., 2012). In the PBFC, high concentration of dissolved oxygen is in situ produced through photosynthesis of algae and is subsequently reduced with the assistant of bacteria, so as to better drive the anodic bioelectrochemical reaction and thus achieve a sustainable transformation of organic wastes and solar energy into electrical energy. Nevertheless, the oxygen as cathodic electron acceptor was not supplied continuously because the photosynthetic oxygen production by algae is only carried out during daytime. Some compounds with high redox potential such as nitrate and sulfate, which are the most frequently detected components in wastewater, may serve as alternative electron acceptors to oxygen to drive anodic bioelectrochemical reaction at night (; Pous et al. (2014); Blázquez et al. (2017). Thus, the PBFC represents a unique operation mode which is totally different from that of conventional BFC. There has been no studies to-date that has reported the removal of OTC from aqueous solutions by PBFC with daily light/dark cycle.

This study therefore focused on the performance and mechanisms of simultaneous OTC removal and bioelectrical power generation by using a PBFC operated with daily light/dark cycle. The mechanisms of enhanced OTC degradation by the anodic bioelectrochemical process driven by the algal-bacterial biocathode were analyzed and the degradation pathway of OTC was proposed. Then, the effect of OTC degradation on power generation performance of the PBFC was investigated. Finally, the electrochemical activity and microbial community responses of the anodic biofilm to OTC were tested to reveal the relationship between OTC degradation and bioanode performance.

2. Materials and methods

2.1. Chemicals

OTC (90% purity) was purchased from Aladdin Industrial Corporation (Shanghai, China). All of the other chemicals were of analytical reagent grade and were obtained from commercial sources.

2.2. PBFC assembly

The PBFC consists of an anode chamber and cathode chamber, both of which had a total volume 256 mL (8 cm × 8 cm × 4 cm). They are separated by a cation exchange membrane (Zhejiang Qianqiu Group Co., Ltd. China). Carbon felt (5 cm × 6 cm × 0.2 cm) was used as the cathode and anode electrode. The anode and cathode was placed parallel to each other at 1 cm from the CEM with an external load of 500 Ω.

2.3. PBFC start-up and operation

The anode chamber were inoculated with anaerobic sludge from Liede wastewater treatment plant (Guangzhou, China) while the same sludge plus *Chlorella vulgaris* were used to inoculate cathode. The artificial wastewater served as an anode growth medium that contain glucose (500 mg COD/L), 50 mM phosphate buffer solution (PBS, pH = 7) and nutrients as described previously 18. The medium used in the cathode was identical to that of the anode, except for addition of 2 g/L $NaNO_3$, and replacement of glucose by $NaHCO_3$ (0.4 g/L). Different concentrations of OTC (10, 30 and 50 mg L^{-1}) were also added to the anode chamber to investigate the effect of initial concentrations of OTC on bioelectrochemical performance of anode. The PBFC cathode was operated under alternating 9 h light/15 h dark cycles to simulate natural day/night cycle. A light emitting diode (20 W, cover the entire visible light wavelength range from 380 to 780 nm) was used as light source for cathode illumination and placed at a distance of 5 cm from the cathode (2500 lux), while the anaerobic anode was wrapped in foil. All experiments were conducted at least in triplicate in a constant temperature room (30 ± 1 °C), and the average values were reported.

2.4. Analytical methods

2.4.1. OTC removal

The concentration of OTC was monitored by using a high-efficiency liquid chromatography (HPLC, Japan Shimatsu LC-16 series) equipped with a C18 column (4.6100 mm, 2.6 mm, Phenomenex, CA, USA). The degradation products of OTC were identified with an ultra-performance liquid chromatography (UPLC, Waters, USA) and electrospray ionization-quadrupole time-of-flight mass spectrometry (ESIeQ-TOF/MS, Bruker, Germany) detector system. OTC removal efficiency was calculated based on the concentration difference between influent and effluent. The degradation kinetics of OTC was assumed to follow apparent first-order reaction model as $C_t = C_0 e^{-kt}$, where C_t is OTC concentration (mg L^{-1}) at time t (h), C_0 is initial OTC concentration (mg L^{-1}) and k (h^{-1}) is rate constant.

2.4.2. Electrochemical measurement

The voltage across the external resistor was recorded every 7 min using a data acquisition device connected to a personal computer (Model 2700, Keithly Instruments, USA). The electrode potential was measured against saturated calomel electrode (SCE). When current output and OTC removal were stable, power and polarization test were conducted by varying the external resistance. For each resistor, a 30 min waiting time was used to obtain the stable cell voltage. The maximum power density and corresponding current density based on normalized anode surface area were calculated from power density curves.

To investigate effect of OTC degradation on electrochemical activity of anode biofilm, cyclic voltammetry (CV) analysis was performed under three-electrode mode using an electrochemical workstation (CHI660E, Shanghai CH Instrument Company). The anode was the working electrode, and the counter electrode was the cathode with a saturated calomel reference electrode (SCE, 0.241 V vs. standard hydrogen electrode). CV was also employed to detect the possible redox mediator in anolyte in a small electrochemical cell filled with spent anolyte using three-electrode setup comprising glassy carbon electrode (3 mm diameter) as working electrode, platinum sheet (10 mm × 10 mm) as counter electrode, and saturated calomel electrode as reference electrode. The sample was filtered through 0.22 μm filter to remove suspended bacteria prior to CV measurements.

2.4.3. Microbial analysis

Anode biofilm samples with and without treatment of OTC were analyzed by high throughput sequencing while the diversity and abundance of ARGs were analyzed using real-time PCR. Both of them were described in detail in the Electronic Supplementary Material (ESM) accompanying this article.

3. Results and discussion

3.1. Bioelectrochemical degradation of OTC at the bioanode

In order to determine the contribution of anodic bioelectrochemical process to the degradation of OTC, comparisons in four groups under different operating modes with an initial OTC concentration of 30 mg L^{-1} were carried out (Fig. 1). Comparing with the abiotic group, the bioanode groups had shown more outstanding degradation performance of OTC throughout the entire operating process (Fig. 1a). The specific OTC removal rates of group with an external resistance of 50 Ω was 1.3 fold, 1.6 fold and 3.8 fold higher than the group with an external resistance of 500 Ω, the group under open circuit condition, and abiotic group, respectively. The enhanced removal of OTC under closed circuit was due to bioelectrical stimulation contrast with open-circuit control, which possibly has made PBFC more suitable niches for the OTC degradation by improving the metabolic rates of OTC-related microbes at the bioanode. While the superior effect shown by 50 Ω than 500 Ω maybe owing to the better electrochemical characteristics with a lower external resistance (Rismani-Yazdi et al., 2011). Besides, the decrease of OTC also took place in the abiotic anode which maybe resulted from the OTC adsorption onto carbon felt electrode and/or plastic surface of reactor parts (Liang et al., 2013). These results indicated that the biodegradation mainly contributed to the OTC removal, and the more effectively OTC degradation could be achieved by bioelectrical stimulation.

The mechanism of enhanced OTC removal by the bioanode was further investigated by varying the co-substrate concentration. And, an external resistor of 50 Ω was used to amplify the intensity of anodic bioelectrochemical reaction. As shown in Fig. 1b, the specific OTC removal rate reached 0.0105 h^{-1} in the presence of 500 mg COD L^{-1} of glucose which is 1.83 fold higher that obtained with 100 mg COD L^{-1} of glucose (0.0061 h^{-1}). It was obvious that the removal of OTC in the bioanode was a co-substrate-dependent biocatalytic process and the removal rate of OTC could be greatly increased by increasing concentration of co-substrate. This result was expected since the utilization of easily degradable carbon source, e.g. glucose as first substrate can provide enough energy for microbial biomass growth and/or counteract toxicity effects to maintained high metabolic activity which is required for persistent toxic organic compound biodegradation (Gadd, 2000). Thus, enhanced co-metabolic biodegradation of OTC by the accelerated transform of co-substrate in the presence of bioanode was demonstrated in current study.

The effect of initial OTC concentrations (10–50 mg L^{-1}) on OTC degradation by the bioanode of the PBFC was tested. As illustrated in Fig. 1C, the specific removal rate of OTC was decreased from 0.0113 to 0.0068 h^{-1} (by 40%) when the initial OTC concentration was increased from 10 to 30 mg L^{-1}. However, no further decrease in OTC removal rate was observed when the initial OTC concentration was further increased to 50 mg L^{-1}. These results indicated that the anodic electroactive bacteria capable of high tolerance to OTC. It was noted that the OTC removal was obviously accelerated during illumination of cathode in contrast with a dark cathode, especially in the case of the high initial OTC concentration. This was mainly due to the difference in dominant terminal electron acceptor at the biocathode during light and dark period. The high

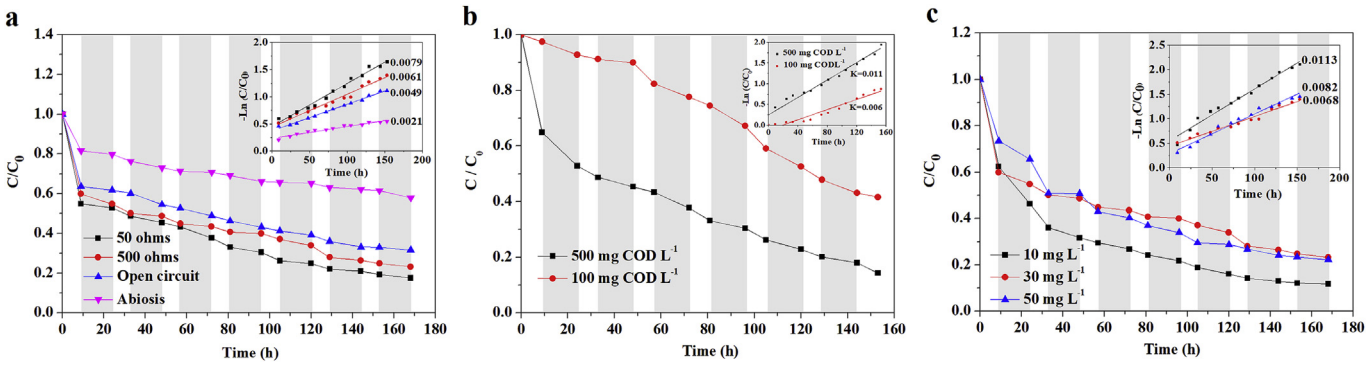

Fig. 1. OTC removal in the bioanode of PBFC under different operating modes (A), under different concentrations of glucose co-substrate (B) and under different initial OTC concentrations (C).

concentration of dissolved oxygen released by algae during illumination could maintain a higher cathodic potential as compared to the use of nitrate as cathodic electron acceptor in dark, and thus more effectively drive anodic bioelectrochemical reaction for OTC degradation.

To fully understand the degradation of OTC by the PBFC, the samples collected from the bioanode at fixed time intervals were examined by LC-MS. As shown in Fig. 2a, total eight intermediate products of m/z 263.1, 279.3, 285.3, 301.2, 307.3, 383, 405.2, and 427, were detected in the bioanode at selected times of 12, 96 and 128 h. Fig. 2b shows the concentration changes of the major intermediates. All the intermediates in the anodic degradation processes were in a tendency of rising first and decreasing latter with the decreasing OTC (m/z 461), indicating that they were formed from the breakdown of the parent OTC and further degraded with the operation of the PBFC.

3.2. Effect of OTC degradation on bioelectrical power generation

The effect of OTC degradation on bioelectrical power generation of the PBFC was investigated by varing the initial OTC concentrations from 0 to 50 mg L^{-1}. As illustrated in Fig. 3a, continuous light/dark alternate current was generated and persisted for more than 168 h under daily light/dark cycle for all the tested OTC concentrations. It was demonstrated that the anodic exoelectrogenic bacteria could be acclimated for generating electricity with an OTC concentration of up to 50 mg L^{-1}. It is worth noting that there was a significant increase in current when OTC was fed into the bioanode and the current generation seemed not be distinctly impaired even when the OTC concentration was increased to 50 mg L^{-1}. Furthermore, polarization data were obtained to characterize the power generation performance of the PBFC at different OTC concentrations under light illumination of the cathode (Fig. 3c and d). It can be seen that the power generation performance of the PBFC was dramatically improved by feeding OTC to the bioanode. Maximum power density of 37, 53 and 54 mW/m^2 were achieved from the PBFC fed with 10, 30 and 50 mg L^{-1} OTC, corresponding to 1.2, 1.76 and 1.8 fold increase compared to the OTC-free PBFC (30 mW/m^2), respectively. There was no further remarkable improvement in power output with the addition 50 mg L^{-1} of OTC as compared to the 30 mg L^{-1} of OTC, indicating that the metabolic activity of the anodic electrogenic bacteria was nearly inhibited to some extent at this concentration. These results suggest that OTC-acclimatized anodic electroactive biofilm document a tolerance toward OTC toxicity within certain limits and the improvement in power production was deeply related to the OTC concentration. Previous study found that sub-minimal inhibitory concentrations of antibiotic tobramycin can induce electroactive biofilm formation because the tobramycin can serve as an efficient chemical signal to stimulate biofilm growth and allow the overexpression of cytochromes and pili to obtain more ATP (Zhou et al., 2017). However, the electrochemical performance of anodic electroactive biofilm was continuously enhanced until the OTC concentration was increased to 50 mg L^{-1} in this study, suggesting that some additional factors other than biological stimulation were responsible for the improved electrochemical performance of the bioanode in the presence of high concentration of OTC.

In order to further investigate the effect of OTC degradation on electrochemical activity of the anodic biofim and determine whether the OTC and its degradation intermediates could play as a redox mediator for accelerate electron transfer between bacteria and anode, the anodic biofim, pure OTC and sterilized spent anolyte were subjected to analysis by CV, respectively. As can be seen from Fig. 4a, the CV of OTC-fed anodic biofilm exhibited a new pair redox peaks and the peak current increased with the increases of OTC concentrations. This implied that a new redox specie had taken part in the bioelectrochemical reaction and the OTC-fed anodic biofilm exhibited higher catalytic activity toward substrate conversion to electricity compared to the OTC-free anodic biofilm.

Furthermore, CVs of sterilized spent anolyte from the OTC-fed and OTC-free bioanode were performed using a clean glassy carbon electrode to detect the possible redox species in the anolyte. As is shown in Fig. 4b, a distinct pair of redox peaks which were consistent with the peak position of CV of the OTC-fed anodic biofilm were only observed for the sterilized spent anolyte from the OTC-fed bioanode but absent at the sterilized spent anolyte from the OTC-free bioanode. The CVs of OTC in aqueous solution also exhibited a distinct pair of redox peaks but the peak position was totally different than that observed for sterilized spent anolyte from the OTC-fed bioanode. Thus, it can be concluded that the redox specie detected within the anodic biofilm is neither soluble electron shuttle secreted by the anodic bacteria nor OTC itself, but a degradation product of OTC.

CV tests of glassy carbon electrode in sterilized spent anolyte from OTC-fed bioanode (10 mg L^{-1} of OTC) have also been performed at different scan rates. As shown in Fig. 3C, the increases of current response of redox peaks on the glassy carbon electrode was directly proportional to the scan rates ranging from 40 to 350 mv s^{-1} and the oxidative to reductive peak current ratio is about one at given scan rates, indicating a reversible electron transfer process (Strycharz et al., 2011). In addition, both the oxidative and reductive peak current intensity were found to rise linearly with the square root of the scan rate (Fig. 3d), which implies a primarily diffusion-controlled electrochemical reaction (Nosheen et al., 2012). These

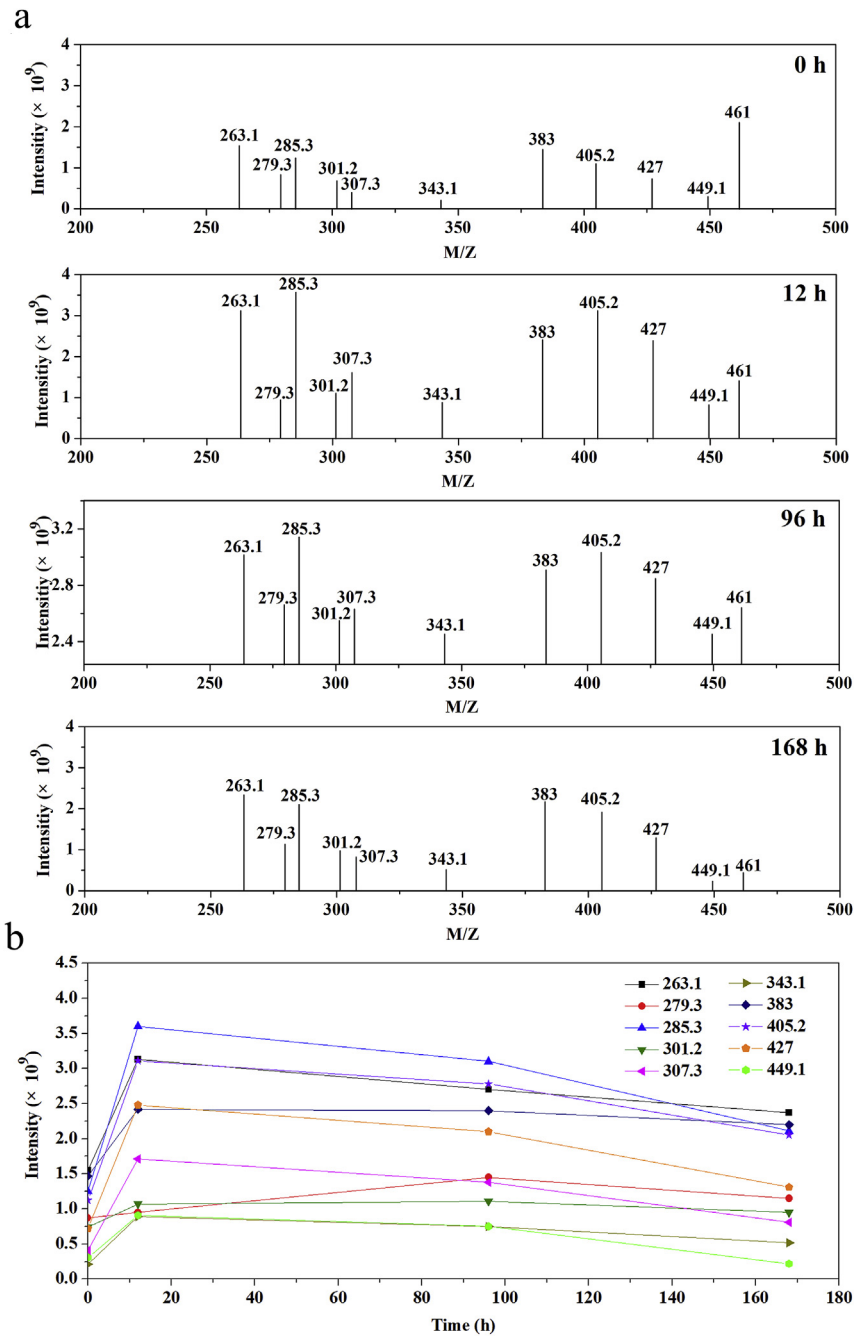

Fig. 2. LC-MS analysis of degradation intermediates of OTC in the bioanode at different times (A) and variation of the concentration of degradation intermediates of OTC as a function of operational time (B).

results indicate that the degradation product of OTC could serve as an efficient mediator for enhancing electron transfer in the bianode of the PBFC.

3.3. Response of anodic bacterial community to OTC degradation

Phylogeneticanalysis of 16S rRNA was performed to investigate the changes in anodic bacterial community structure and diversity in response to OTC degradation. (Fig. 5). At phylum level, a total 14 phylums were identified for all the samples and only 0.73–1.32% of the total sequences among the test samples were unclassified (Fig. 5a). There was no significant difference in the composition of the bacterial community between the OTC-fed and OTC-free bioanode. However, the abundance of *Bacteroidetes*, *Firmicutes*, *Synergistetes* and *Euyarchaeota* were enhanced, while those of *Proteobacteria* and *Chloroflexi* were decreased in the OTC-fed bioanode, as compared to the OTC-free bioanode. In addition, the extent of change in abundance of these dominant bacterial phylums was found to be proportional to the OTC concentration. In order to further investigate the effect of OTC degradation on dominant bacterial species and reveal their potential functions in the anodic bacterial community, phylogenetic analysis on genus level was conducted. As is shown in Fig. 4B, the bacterial community structures of OTC-fed bioanode were quite different from that of the OTC-free bioanode at genus level. The bacterial community of the OTC-free bioanode was largely dominated by *Pseudomonas*

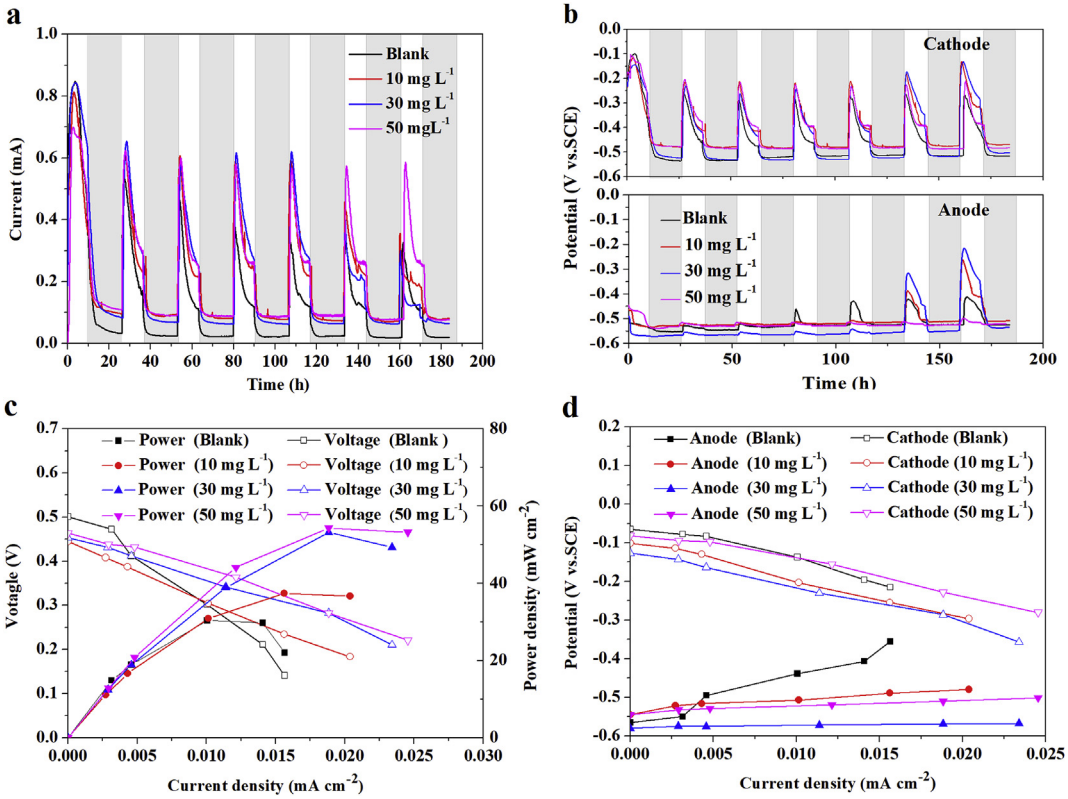

Fig. 3. Effect of OTC feeding to the bianode on Current generation (A), electrode potentials (B), power density (C) and electrode polarization (D) in the PBFC.

(53.16%), followed respectively by *Levilinea* (4.1%), *Ignavibacterium* (3.85%), *Trichococcus* (2.81%), *Petrimonas* (3.81%), *Clostridium sensu strict* (2.31%) and *Anaeroarcus* (1.03%). For the OTC-fed bioanode, *Pseudomonas, Ignavibacterium* and *Trichococcus* almost completely disappeared, coupled with a sharp increases in relative abundance of *Petrimonas, Anaeroarcus, Clostridium sensu stricto, Methanothrix*, *Cloacibacillus*, *Aquamicrobium*, *Parabacteroides, Sedimentibacter* and *Brooklawnia*. It is noteworthy that the increase in relative abundance of *Anaeroarcus*, *Aquamicrobium* and *Sedimentibacter* was largely inhibited while *Clostridium sensu strict*, *Methanothrix* and *Parabacteroides* were highly enriched at high OTC concentration (50 mg L^{-1}). In comparision, *Petrimonas* and *Cloacibacillus* did not show a strong response to an increase in OTC concentration. *Pseudomonas* is one of exoelectrogenic bacteria that can produce metabolites like pyocyanin and phenazine and use them as redox mediator for facilitating the extracellular electron transfer between bacterial cells and electrode in BFC (Qiao et al., 2011). The almost complete absence of *Pseudomonas* in the OTC-fed bioanode indicates that it was highly susceptible to OTC. The role of highly enriched bacteria belonging to *Anaeroarcus* genus in the bioanode of BFC has not been explored. This strain has been identified once from an upflow anaerobic sludge blanket reactor (UASB) for treating sugar refinery wastewater (Zhang et al., 2018). *Petrimonas*, *Clostridium* and *Cloacibacillus* are fermentation bacteria and possess the ability to degradate complex organic substrates (Li et al., 2014; Xie et al., 2014; Li et al., 2018c). *Cloacibacillus* has also been proved to be capable of producing electricity in BFC (Han et al., 2016). Members of the genus *Aquamicrobium* are widely distributed in the environment and are known for their abilities to degrade a wide variety of recalcitrant organic pollutants (Wang et al., 2017). *Parabacteroides* has been shown to be resistant to several kinds of antibiotic drug (Molina et al., 2014). *Sedimentibacter* was a genus of gram-positive bacteria which was frequently detected as a core member present in anaerobic batch reactors for degrading polycyclic aromatic compound (Gomes et al., 2014) and high-power generating biofilm in BFC (Lesnik and Liu, 2014).

Overall, the presence of OTC drives the anodic bacterial community shift toward selective enrichment of OTC-resistant bacteria that are responsible for both electricity generation and OTC degradation in the OTC-fed bioanode of the PBFC.

The presence of antibiotics may generate a selection pressure for ARGs, and some ARGs may persist even when the antibiotic pressure is gone (Johnsen et al., 2011; Jechalke et al., 2014). In order to investigate the occurrence of ARGs during the bioelectrochemical degradation of OTC, analysis of the relative abundance of the OTC-related ARGs was conducted. As shown in Table 1, total eleven OTC-related horizontal transfer genes which including nine tet genes, one transposase gene and one integron gene were detected in the OTC-fed bioanode. Compared to the OTC-free bioanode, the relative abundances of *tetA tetC, tetG, tetL, tetW, tetQ, tetX, Tn916/1545* and *intI 1* were increased by an approximate factor of 11.29, 69.82, 140.87, 4.45, 207.29, 56.26, 26.26, 1.24 and 30.24-folds, respectively, in the bioanode fed with 10 mg L^{-1} of OTC. The relative abundances of these ARGs were further increased by an approximate factor of 2.42, 1.76, 1.91, 7.33, 1.09, 1.09, 1.41, 4.34 and 3.64-folds, respectively, when a higher OTC concentration of 30 mg L^{-1} was applied. 50 mg L^{-1} of OTC feeding resulted in further increase in relative abundances of *tetL, tetW* and *tetQ*, but the relative abundances of *tetA tetC, tetG, Tn916/1545* and *intI 1* were decreased at this OTC concentration level. It has been reported that residual antibiotic concentration and antibiotic resistant bacteria were the main factors affecting the abundances of antibiotic ARGs (Johnsen et al., 2011; Pepper et al., 2018). Although the increment of the *tetM* and *tetO* seemed relatively insignificant, they were still show a

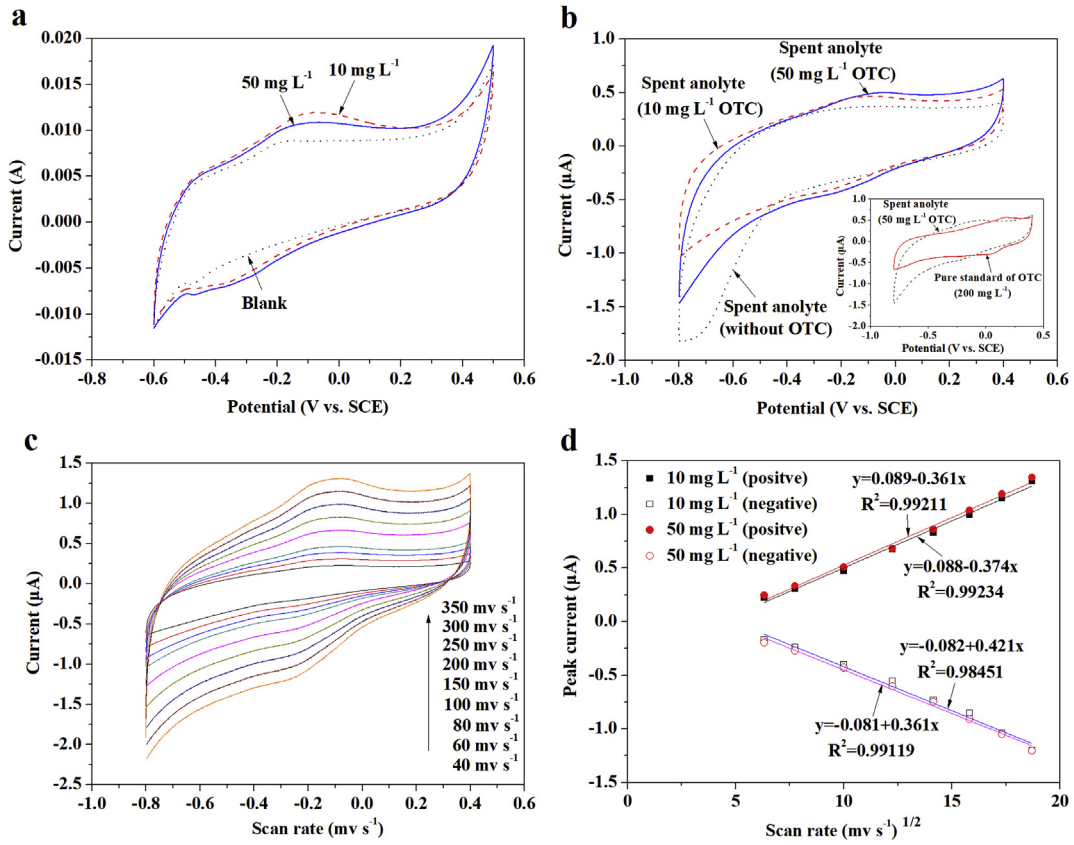

Fig. 4. Cyclic voltammograms of anodic biofilm in the OTC-fed bioanode (A), glassy carbon electrode in sterilized spent anolyte from OTC-free and OTC-fed bioanode (B) and glassy carbon electrode in sterilized spent anolyte from the bioanode fed with 10 mg L^{-1} OTC subjected to increasing scan rates (C), and redox peak heights showing linear dependence on the square root of scan rate (D).

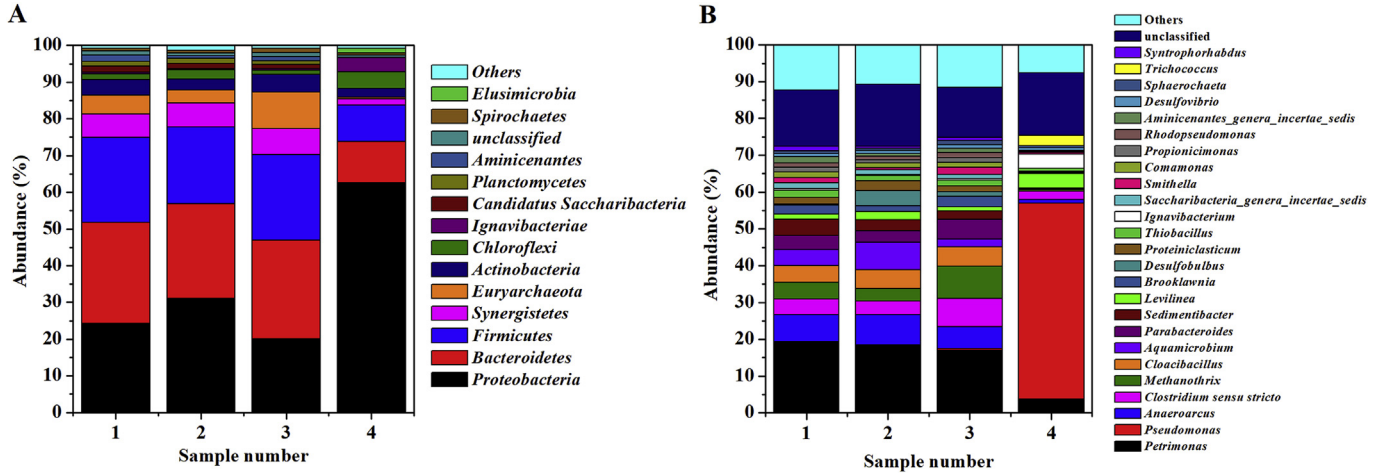

Fig. 5. Pyrosequencing results of DNA from the microbial community of the bioanode with and without feeding OTC at the phylum (A) and genus (B) levels.

clear-cut correlation with OTC initial concentration. The variation of the abundances of OTC-related ARGs with the initial concentration of OTC could be largely dependent on the change in anodic bacterial community structure under OTC selection pressures since similar variation trend in abundance of ARGs and dominant bacterial populations in the bioanode was observed. It could be also illustrated by the fact that some ARGs have the same or different host bacteria and their abundances are positive correlation with their host bacteria (Qian et al., 2016). The *Tn916/154* was classified a transposon gene which had been reported to play a critical determinants of ARGs, especially in gram-positive bacteria. It had been identified commonly carry a tetracycline ARGs such as encoding an efflux pump for tetracycline (Zhu et al., 2013). With respect to *intI1*, it has been demonstrated to be enriched in conventional biological treatment process (Yuan et al., 2016), and was found to be positively correlated with *tetC*. Thus the abundance of *tetC* was more likely to be constrained by *intI1* in this study (Qian et al., 2016). Previous study demonstrated that both the

Table 1
Relative abundance of each OTC-related ARGs type in the bioanodes fed with different concentrations of OTC to the OTC-free bioanode revealed by high throughput sequencing and real-time quantitative PCR analysis.

ARGs type	Relative abundance		
	10 mg L^{-1} OTC	30 mg L^{-1} OTC	50 mg L^{-1} OTC
tetA	11.28816	27.34481	15.87558
tetC	69.81935	123.4369	57.20109
tetG	140.8699	269.0342	137.8075
tetL	4.447552	32.57988	101.9397
tetM	0.365897	0.389242	0.571233
tetW	207.292	226.0696	320.7912
tetO	0.515366	1.185488	2.071851
tetQ	56.26081	61.52163	92.89672
tetX	26.26328	36.97727	33.15738
Tn916/1545	1.23796	5.374662	2.33145
intI 1	30.24364	110.0527	22.35457

abundances of antibiotic resistance bacteria and ARGs were enriched with increasing current in the bioanode of the BFC due to stimulated growth of antibiotic resistance bacteria and upregulation of the ARG expression (Li et al., 2018b). The observed results revealed that the abundance of OTC-related ARGs was strong positive associated with the succession of anodic bacterial community structure which was indirectly affected by the initial OTC concentrations and the anodic bioelectrochemical process. The large enrichment of OTC-resistant bacteria was mainly responsible for the occurrence and accumulation of OTC-related ARGs in the OTC-fed bioanode since the bacteria are the main carries of ARGs.

4. Conclusions

Simultaneous enhanced OTC degradation and increased power output were achieved using a self-sustained PBFC with an algal-bacterial biocathode. The accelerated transform of co-substrate in the presence of the bioanode was mainly responsible for the enhanced OTC degradation. The electrochemical activity of the anodic biofilm was moderately enhanced by the presence of OTC, and the degradation intermediates of OTC can function as mediators to improve the electron transfer in the anodic bioelectrochemical process. The OTC feeding selective enriched OTC-tolerant bacterial community capable of degrading complex organic compounds and producing electricity. The occurrence of ARGs during bioelectrochemical degradation of OTC was affected more greatly by the succession of the anodic bacterial community than the initial OTC concentration. The PBFC demonstrates great potential for enhanced treatment of OTC-contained wastewater with additional electrical energy recovery.

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Appendix A. Supplementary data

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