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Bioelectrical power generation coupled with high-strength nitrogen removal using a photo-bioelectrochemical fuel cell under oxytetracycline stress

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ABSTRACT

Photo-bioelectrochemical fuel cell shows great potential as an environmental-friendly technology for converting solar energy and bioenergy into electricity with simultaneous wastewater treatment. The present work aims to assess the performance of an algal-bacterial biocathode photo-bioelectrochemical fuel cell (ABPBFC) operated with daily light/dark cycle for simultaneous bioelectrical power generation and high-strength nitrogen removal under oxytetracycline (OTC) stress by adding different concentrations of OTC into the biocathode. The results showed that the power generation of the ABPBFCs was significantly enhanced by the presence of OTC at all levels tested (5–50 mg/L) due to enhanced electron transfer from cathode to oxygen and nitrate mediated by degradation products of OTC, but the enhancement was not proportional to the rise in OTC concentration. The largest maximum power density of 54 mW/m^2 was achieved at 5 mg/L OTC during light period and 8.5 mW/m^2 was produced at 20 mg/L OTC during dark period, corresponding to a 1.8 and 7.5 fold increases compared to that of the ABPBFC without addition of OTC. The removal of nitrate was obviously accelerated by the addition of OTC with an initial OTC concentration lower than 20 mg/L. Increases in the concentration of OTC added to the biocathode did not result in continuous enhancement in power generation and nitrate removal due to the toxicity of OTC to biocathodic microbial community. Cathodic bioelectrochemical process enhanced photolysis of OTC, which was attributed to its contribution to basification of catholyte. The growth of some dominated genus related to biocathodic electron transfer, nitrogen removal and OTC degradation were stimulated at OTC concentrations less than 20 mg/L but inhibited at 50 mg/L, except for some OTC-resistant bacteria.

1. Introduction

The wastewaters containing both high-strength nitrogen and antibiotics, such as livestock wastewater and pharmaceutical wastewater, poses a serious threat to aqueous environment and ecological security assuming an improper handling due to the toxicity of nitrogenous compounds and widespread of antimicrobial resistance genes induced by antibiotics [1,2].

Bioelectrochemical fuel cell (BFC) as a promising technology for environmental friendly wastewater treatment with simultaneous bioelectrical energy recovery has aroused a great deal of attention in recent years [3]. Various BFCs have been developed during the last decade depending on the microbes used and reactor configuration. Among these, photo-bioelectrochemical cell (PBFC) shows greater application potential than traditional aerated cathode BFC due to significant improvement in sustainability by in-situ use of photosynthetic dissolved oxygen as cathodic electron acceptors while simultaneously enabling enhanced degradation of complex

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organic pollutants and conversion of inorganic pollutants in wastewater based on synergy of exoelectrogenic bacteria and photosynthetic microbes [4,5].

Previous studies have revealed that bioanode of the BFC can increase the removal rate of several antibiotics such as sulphamethoxazole [6], sulfamethoxazole [7] and oxytetracycline [8], which was attributed to enhanced microbial metabolism by using anode as electron acceptor. However, bioelectrical power generation was negatively affected by antibiotics degradation due to potential toxicity effects of antibiotics and its degradation intermediates on exoelectrogens and competitive electron consumption with anodic processes [7]. The biocathode has also been reported to accelerate reductive degradation of chlorinated and nitroaromatic antibiotic by using cathode as extracellular electron donor [9,10], but much less is known about how the biocathode biofilm community response to the antibiotic. Moreover, most of these studies were initially investigated for removal performance of antibiotics alone using the BFC and less attention has been given to the antibiotics degradation in cooperation with nitrogen removal by using the BFC which designed for treatment of wastewater containing both antibiotics and nitrogenous compounds.

The PBFC represent a unique configuration and operation mode which is totally different from that of traditional BFC. In the PBFC, algae or algal-bacterial consortium is usually used as biocathode catalyst [11]. Similar to the natural process that occurs in the surface water environments, algae consume carbon dioxide during daytime to produce oxygen and organic matter while consume oxygen and organic matter to release carbon dioxide at night. Thus, the algae activity under day/night cycle constructs alternate aerobic/anaerobic environment, resulting in periodic variation in availability of different terminal electron acceptors and consequent variation in metabolic activity of specific bacterial populations, which may provide heterogeneous niches to sustain diverse microbial communities and thereby providing multiple approaches for pollutants removal [12]. For instance, the photosynthetic dissolved oxygen released by alga can serve as not only a terminal electron acceptor for bioelectrical power generation but also oxygen sources for autotrophs or heterotrophs to degrade organic pollutants and conversion of inorganic pollutants in wastewater. Previous studies reported that simultaneous nitrification and denitrification can be achieved in an aerated biocathode by maintaining dissolved oxygen at specific level [13]. The main drawback of the biocathode is that extra energy was needed for aeration which makes the treatment is unsustainable, especially in long-term operation. Fortunately, this technical problem can be efficiently solved by using algal–bacterial biocathode PBFC due to in situ release of oxygen through photosynthesis of alga. Moreover, intermittent photosynthetic oxygen releases during day/night cycle could create alternate aerobic/anaerobic environment and enable occurrence of simultaneous or alternate nitrification and denitrification in the biocathode. However, how the algal–bacterial community in biocathode of PBFC may function together for synergistic antibiotic and nitrogen removal coupling with bioelectrical power generation has not been systematically explored. Moreover, little information was currently available on response of algal–bacterial biofilm communities to antibiotic pollutants under photo-biocathode microenvironment.

Therefore, the main goal of this study is to investigate the performance and mechanisms of simultaneous bioelectrical power generation and high-strength nitrogen removal in an algal-bacterial biocathode of PBFC (ABPBFC) under antibiotic stress. The ABPBFC was operated with daily light-dark cycle to comply with the metabolic pattern corresponding the natural growth of algae and the operation of the ABPBFC can be self-sustained by alternately using photosynthetic oxygen and nitrate as cathodic electron acceptors. Oxytetracycline (OTC), a widely used of veterinary antibiotic, was selected as a model antibiotic and added to the biocathode. The effect of different OTC levels on electrochemical and nitrogen removal performance of the biocathode and algal–bacterial biofilm community evolution were intensively studied to understand the potential bioelectrochemical and algal–bacterial interaction mechanisms under OTC stress. The pathways and main metabolites of OTC degradation were also investigated.

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. ABPBFC assembly

The ABPBFC 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 (CEM, 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 were placed parallel to each other at 1 cm from the CEM with an external load of 500 Ω.

2.3. ABPBFC 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 [12]. The medium used in the cathode was identical to that of the anode, except for addition of 1.2 g/L NH_4Cl, 2 g/L $NaNO_3$, and replacement of glucose by $NaHCO_3$ (0.4 g/L). Different concentrations of OTC (5, 10, 20, 50 mg/L) were also added to the cathode chamber to investigate the effect of initial concentrations of OTC on electrochemical and nitrogen removal performance of the biocathode. The ABPBFC cathode was operated under alternating 9 h light/15 h dark cycles to simulate natural day/night cycle. A light emitting diode (20W, 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) at time t (h), C_0 is initial OTC concentration (mg/L) 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, 0.241 V vs. standard hydrogen electrode). The maximum power density and corresponding current density based on normalized anode surface area were calculated from power density curves by varying the external resistance.

To investigate effect of OTC degradation on electrochemical activity of cathodic biofilm, cyclic voltammetry (CV) analysis was performed under three-electrode mode using an electrochemical workstation (Model 2273, Princeton Applied Research). The biocathode was the working electrode, and the counter electrode was the anode with a SCE. CV was also employed to detect the possible redox mediator in catholyte in a small electrochemical cell filled with spent catholyte 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 microbes prior to CV measurement.

2.4.3. Chemical analysis

The concentrations of ammonium (NH_3-N), nitrate (NO_3-N) and nitrite (NO_2-N) were analyzed according to Standard Method [14]. The DO in catholyte were continuously monitored online with an optic oxygen probe (Mettler–Toledo, Switzerland) connected to a personal computer.

2.4.4. Microbial analysis

Algal biomasses were measured by optical density measurements on a UV/vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at 680 nm. Optical density was then converted to dry weight of algae cells using a previously prepared calibration curve [15].

Cathode 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. Bioelectrical power generation

The effect of OTC concentration on bioelectrical power generation was evaluated in batch experiments with OTC concentrations ranged from 0 to 50 mg/L (Fig. 1). As shown in Fig. 1A, a continuous light/dark alternate current was generated and persisted for more than 168 h under daily light/dark cycle. There was a sharp increase in current when OTC was added into the biocathode due to improved cathode performance as indicated by a significant increase in cathode potential (Fig. 1B). The current generation seems to be not negatively affected by the OTC even at a high OTC concentration above 50 mg/L.

Furthermore, polarization data were obtained to characterize the bioelectrical power generation performance of the ABPBFC at different OTC concentrations (Fig. 2C–F). It can be seen that the addition of OTC into the bicathode significantly increased the power output and caused a low cathodic polarization during both the light and dark periods of the light-dark cycle (Fig. 2C and D). However, the improvements in power were not proportional to the rise in OTC concentrations. A largest maximum power density of 54 mW/m^2 was achieved at 5 mg/L OTC during light period while 8.5 mW/m^2 was produced at 20 mg/L OTC during dark period, corresponding to a 1.8 fold and 7.5 fold increase compared to that of the ABPBFC without addition of OTC (19 mW/m^2 during light period and 1 mW/m^2 during dark period), respectively. There was no remarkable improvement in power output with the addition of high concentration of OTC (50 mg/L), with only 86% increase during light period and 70% increase during dark period were observed, respectively.

Fig. 2E and F show that the increases in power were mainly attributed to the performance improvement of the cathode but not anode. The cathode potentials of the AMPBFC with addition of OTC were more positive than that of the OTC-free AMPBFC at the same current density, indicating that the performance of the biocathode was improved after addition of OTC. The effect of OTC on electrochemical performance of the biocathode followed the same trend as seen in power output. As is known, biocathode potential is controlled by the kinetics of electron transfer from the cathode to terminal electron acceptor mediated by microorganisms [16], so the performance improvement of the biocathode was probably due to an enhancement in electron transfer in the AMPBFC with addition of OTC. However, the microbial activity could be suppressed with addition of high concentration of OTC (50 mg/L), resulting in an insignificant improvement in biocathode performance. Previous studies revealed that an oxygen reducing biocathode hosted a distinctly different bacterial community composition from those in an autotrophic denitrifying biocathode [17,18]. Therefore, two different electron transfer processes which possibly mediated by two different groups of microorganisms should be considered simultaneously in the algal-bacterial biocathode since photosynthetic oxygen and nitrate/nitrite were alternately served as cathodic electron acceptors during the daily light/dark cycle. These results also indicated that the maximum concentration of OTC (50 mg/L) used in study was within the range of adaptation of microbial culture in the biocathode of the ABPBFC.

3.2. OTC removal

As shown in Fig. 2A, more than 90% of OTC with an initial OTC concentration of 20 mg/L was removed within 29 h in the algal-bacterial biocathode of ABPBFC with an external resistance of 500 Ω. An OTC removal experiment in the ABPBFC under open circuit condition, resulted in an OTC removal of 86% within the same resting time period. Previous study showed that the photolysis of tetracycline antibiotics, such as such as OTC and tetracycline, were highly pH-dependent and strongly enhanced at high pH value [19,20]. The acceleration in removal of OTC in the ABPBFC operated in closed circuit mode as compared to open circuit mode can be linked to the pH increase in the biocathode since enhanced oxygen reduction and autotrophic denitrification of nitrate during daily light/dark cycle in the biocathode could resulted in accelerated basification of catholyte [21], as evidenced in Fig. S1. The abiotic cathode also showed a continuous decrease in OTC content over time, suggesting the possibility of photolysis of OTC under abiotic condition. The remaining OTC concentration (C/C_0) versus operating time was simulated as pseudo-first-order kinetics. Compared degradation rate constants of OTC between the biocathodic and abiotic treatments, OTC removal rate in the algal-bacterial biocathode was 2.2 and 4.1-fold higher than that in abiotic cathode under illumination and dark conditions, respectively. These results suggest that the biodegradation was the main contributor to OTC removal in the algal-bacterial biocathode instead of the photolysis.

Removal of OTC was also evaluated at initial OTC concentrations

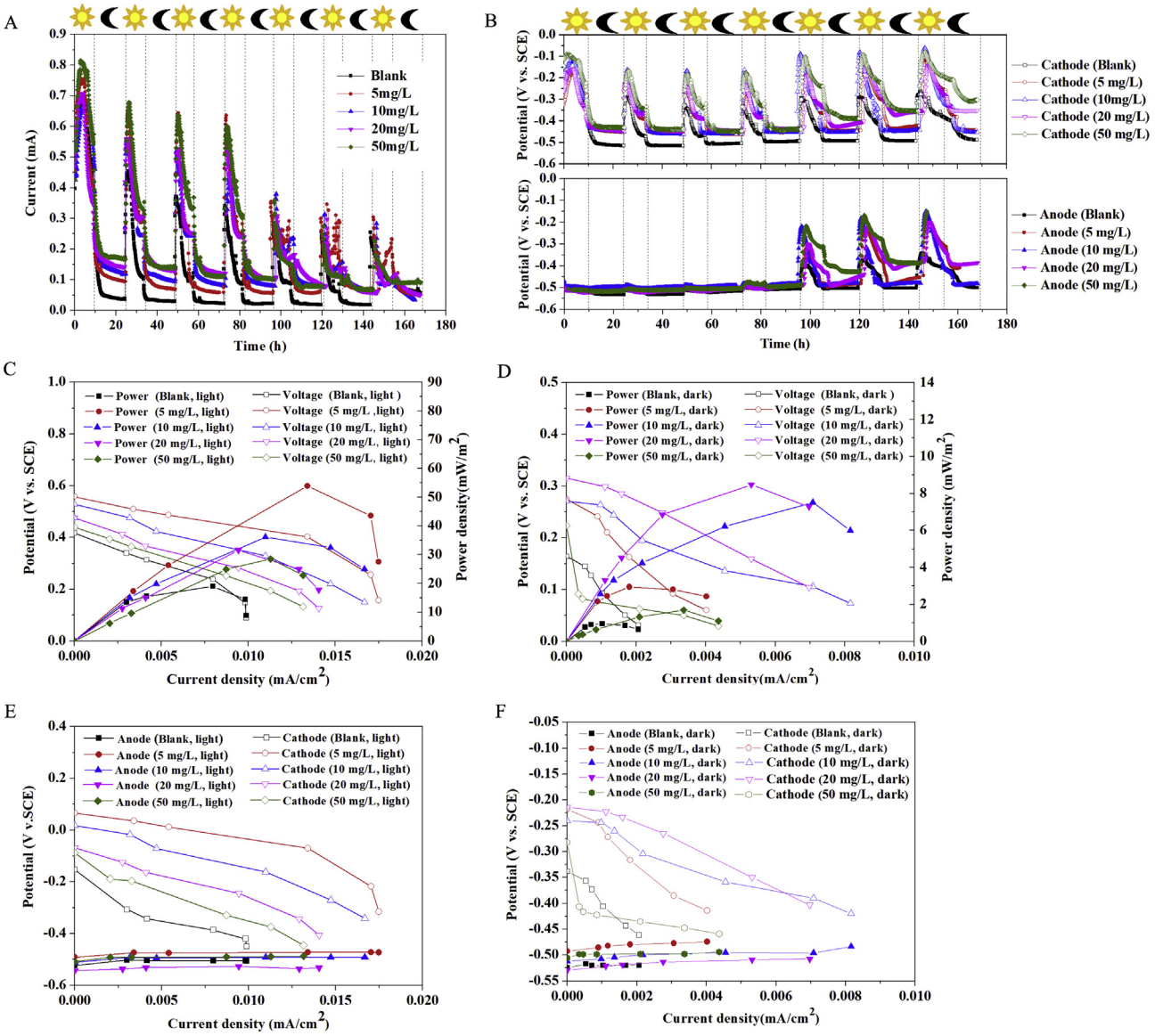

Fig. 1. Effect of the increases in OTC concentration on bioelectrical power generation in the ABPBFC: (A) current generation (500 Ω external resistance); (B) electrode potential (500 Ω external resistance); power output during the (C) light and (D) dark period; electrode polarization during the (E) light and (F) dark period.

ranging from 10 to 50 mg/L (Fig. 2B). The OTC was almost completely removed within 48 h when the initial OTC concentration was lower than 20 mg/L, while the time required for completely removal of OTC was prolonged to 86 h at the initial OTC concentration of 50 mg/L. The specific removal rates of OTC in the algal-bacterial biocathode of the ABPBFC were 0.066, 0.054 and 0.033 h^{-1} at the initial OTC concentrations of 10, 20 and 50 mg/L, respectively. These results indicated that the microbial activity was not significantly affected by OTC in concentration up to 50 mg/L.

The degradation products of the OTC were analyzed by LC-MS (Fig. 2C–F). The peak at *m/z* 461.1, which corresponding OTC, was almost completely disappeared within 12 h while peaks at *m/z* 173.1 (species A) and *m/z* 235.1 (species B) increased dramatically. These observations can be explained by the decomposition of OTC to form species A and B, which was consistent with the results in Fig. 2B. Further degradation of species A and increased formation of species B were verified based on comparison between the two species for mass spectral peak intensities at 12 h and at the end of the cycle.

Total seven OTC-related ARGs including one transposase gene and one integron gene regarded as horizontal transfer genes were detected in the biocathode with OTC addition and their relative abundance has a positive correlation with the OTC concentration (Fig. 2F). In particular, the relative abundance of *tet (c)* and *tet (L)* increased by 49% and 27% in the biocathode with 5 mg/L OTC addition as compared to the OTC-free biocathode. Addition of 10 mg/L OTC resulted in further increases in relative abundance of *tet (c)* and *tet (L)*, almost 104% and 40.1%, respectively. In contrast, the increases in relative abundance of *tet (A)*, *tet (G)*, *tet (O)*, *Tn916/154* and *intI1* were relatively insignificant. It was reported that transposons were identified commonly carry a tetracycline ARGs such as encoding an efflux pump for tetracycline, and its abundance levels are positively associated with OTC concentration [22]. With respect to integrons, tetC has been reported to be positively correlated with *intI1*, indicating that *intI1* played an important role in the propagation of *tetC* in this study [23]. Overall results indicated that the accumulation of OTC-related ARGs is strongly associated with the initial concentration of OTC.

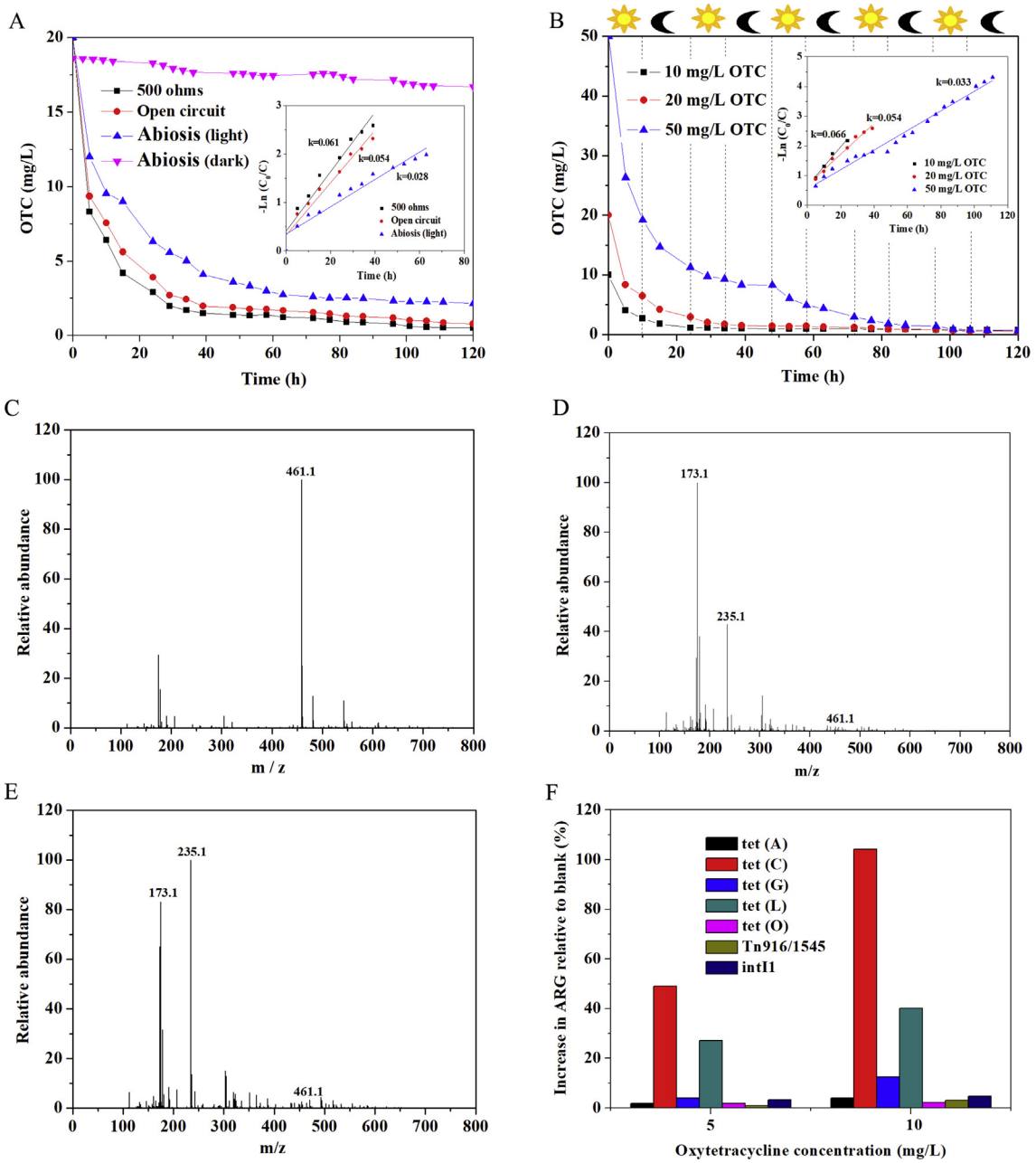

Fig. 2. OTC degradation and occurrence of ARGs in the algal-bacterial biocathode: (A) OTC degradation under different condition; (B) OTC degradation at different initial OTC concentrations daily light/dark cycle; (C) LC-MS spectra of OTC and degradation products of OTC at (D) 12 h and (D) at the end of cycle. (D).

3.3. Nitrogen removal and cathodic bioelectrochemical process

The nitrogen removal performance of OTC-treated biocathode with the addition of different concentration of OTC was investigated and compared with the OTC-free biocathode. As is shown in Fig. 3A, the removal rates of NH_4^+-N were decreased in the OTC-treated biocathdoes at all levels of OTC tested (5–50 mg/L) during the initial 36 h of batch cycle when compared with the OTC-free biocahtode and after that was almost similar to that in the OTC-free biocahtode. This may be partially due to the temporary inhibition of alga and/or nitrifying bacteria activities in the presence of OTC but revival of their activity when OTC was degraded, which is consistent with the results of OTC degradation shown in Fig. 2B that the OTC was almost degraded completely within 36 h at initial OTC concentrations of 10 and 20 mg/L and degraded by 82% at an initial OTC concentration of 50 mg/L. The results indicated that the concentration of OTC used in this study did not significantly affect the NH_4^+-N removal in the biocathode. Similarly, Prado et al. [24] did not observe significant differences in nitrification of ammonia before and after addition of a high concentration of tetracycline (40 mg/L) in a semi-industrial membrane reactor. Additionally, Shi et al. [25] did not detect any negative effect of 10 mg/L of tetracycline on nitrification performance in a conventional granular system.

The removal of NO_3^--N was obviously accelerated by addition of OTC with an OTC concentration lower than 20 mg/L during initial 96 h and after that the acceleration in NO_3^--N removal was significantly reduced. However, the removal of NO_3^--N was not enhanced

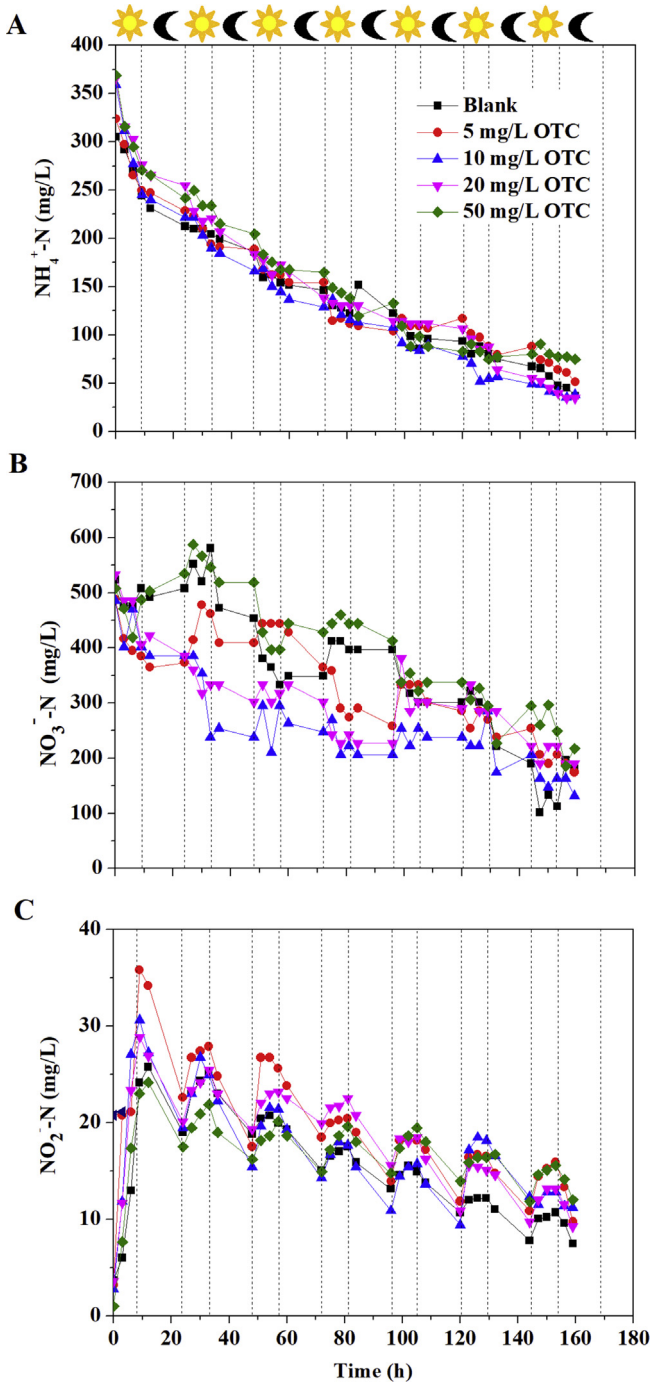

Fig. 3. Effect of the increases in OTC concentration on nitrogen removal in the algal-bacterial biocathode during daily light/dark cycle: (A) NH_4^+-N; (B) NO_3^--N; (C) NO_2^--N.

further when the OTC concentration was increased to 50 mg/L (Fig. 3B). Previous studies showed that autotrophic denitrification by using the cathode as electron donor played a major role in NO_3^--N removal in biocathode of BFC [26]. The increases in NO_3^--N rates in the presence of OTC in this study could due to enhanced electrons transfer among cathode, microorganisms and nitrate mediated by degradation products of OTC. The reduction of acceleration in NO_3^--N removal at the end of the cycle was probably due to the exhaustion of the electron donor (glucose) in the anode, and thus could not provide enough electrons for cathodic reduction of nitrate, which is consistent with the change in anode and cathode potential shown in Fig. 1B. In contrast, OTC additions did not resulted in a significant acceleration in NO_2^--N removal at all levels of OTC tested which might be due to the fact that reduction of nitrite requires a more negative potential than nitrate reduction [27]. In the algal–bacterial biocathode used in this study, the photosynthetic dissolved oxygen and nitrate were served alternately as cathodic electron acceptors during daily light/dark cycle, and the effect of OTC on the bioelectrochemical and nitrogen removal performance of the biocathode could be attributed mainly to its action on algal–bacterial activity.

CVs were obtained to reveal the biocatalytic activities of the biocathodic biofilms with and without addition of OTC (Fig. 4A). Compared to the OTC-free biocathodic biofilm, the OTC-treated biocathodic biofilm exhibited larger current responses in potentials ranging between −0.6 and 0.5 V and showed higher distinct redox peaks, indicating higher electrochemical activity of biofilms. However, the increase in redox peak current was not proportional to the rise in OTC concentration since 2.5-fold increases in OTC concentration (from 20 to 50 mg/L) did not result in further significant increases in redox peak current, indicating that the biocatalytic activities of the biocathodic biofilms could be inhibited at high OTC concentration. This could partly explain the trend that is observed in bioelectrical power generation and autotrophic denitrification of nitrate by using cathode as sole electron donor at different OTC concentrations.

To examine whether the degradation products of the OTC could play as a redox mediator for enhancing electrons transfer between cathode and terminal electron acceptor (oxygen and nitrate) in the algal–bacterial biocathode, CV tests were performed using five samples with a bare glassy carbon electrode, including fresh cathodic medium, spent medium obtained from an OTC-free biocathode and spent cathodic mediums obtained from biocathodes treated with 10, 20 and 50 mg/L of OTC. As is shown in Fig. 4B, the spent cathodic mediums obtained from OTC–treated biocathodes showed a couple of distinguishable redox peaks with the oxidation peak potential located at −0.12 V (vs.SCE) and the reduction peak potential located at −0.24 V (vs.SCE) and the redox peak current increased with increasing concentration of OTC, this could be the evidence of the mediator contained in the spent cathodic mediums obtained from OTC–treated biocathodes. In contrast, there was no peak was found using the fresh cathodic medium and the spent medium obtained from an OTC-free biocathode, excluding the possibility that the mediator was excreted by the cathodic microorganisms or the components of the cathodic medium. Thus, it is concluded that the mediator contained in the spent cathodic mediums obtained from OTC–treated biocathodes was the degradation products of the OTC. For the redox reactions in the biocathode, the NAD^+/NADH redox couple of cathodophilic bacteria, which has the lowest redox potential of −0.32 V vs. NHE (−0.56 V vs. SCE), seems to set the limit of redox mediators' application [28]. As is shown in Fig. 4B, the intercept of the line connecting the oxidation and reduction peak with the x-axis indicated the standard redox potential of the degradation product of OTC, at around −0.22 V vs. SCE (0.02 V vs. NHE), which is more positive than the potential of NAD^+/NADH redox couple but negative than the redox potential of O_2/H_2O (1.23 V vs. NHE) and NO_3^-/NO_2^- (0.367 V vs. NHE) [27], indicating it can act as redox mediator for facilitating electrons transfer from bacterial cell to oxygen and nitrate. Similarly, our previous study has also shown that the degradation products of azo dye can also be served as redox mediator for enhancing electron transfer in the BFC [29]. These results demonstrate the contribution of OTC addition to the performance improvement of the biocathode for simultaneous bioelectrical power generation and NO_3^--N removal, as consistent with the former hypothesis (Figs. 1 and 3).

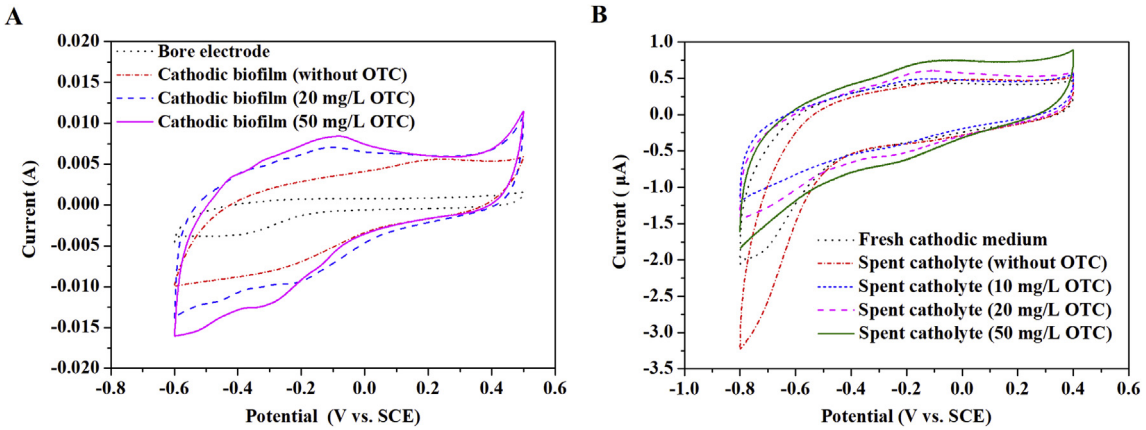

Fig. 4. (A) CV curves of the OTC-free biocathode and the OTC-treated biocathodes with different initial concentration of OTC, with a scanning rate of 5 mV/s; (B) CV curves of a glassy carbon electrode in the spent catholyte obtained from the OTC-free biocathode and OTC-treated biocathodes with different initial concentration of OTC, with a scanning rate of 100 mV/s.

3.4. Algal activity and microbial community analysis

In order to examine whether OTC addition has a negative affection on algal activity in the biocathode, the photosynthetic oxygen release and algal growth were monitored in the presence of different concentrations of OTC. As shown in Fig. 5A, the photosynthetic oxygen production was not negatively affected by the addition of 10 mg/L of OTC since similar or slight increase in photosynthetic oxygen level was observed in the OTC-treated biocathode with an OTC concentration of 10 mg/L when compared with the OTC-free biocathode. In contrast, photosynthetic oxygen level was generally lower in the OTC-treated biocathode with higher OTC concentrations than that in the OTC-free biocathode, especially at an OTC concentration of 50 mg/L. It was noted that the decrease in photosynthetic oxygen level was more prominent during the initial 57 h of the operation cycle and after that the photosynthetic oxygen differences between the OTC-treated biocathode and the OTC-free biocathode were gradually decreased. Algal growth in the presence of different concentrations of OTC showed a similar trend as that observed for the photosynthetic oxygen production (Fig. 5B), indicating that the photosynthetic oxygen concentration was closely related to algal growth. However, the photosynthetic oxygen consumption was a complicated process since the oxygen can serve as not only a terminal electron acceptor for bioelectrical power generation but also oxygen sources for nitrification of NH_4^+-N and biodegradation of OTC. The results could partly explain the bioelectrical power generation and NH_4^+-N removal performance of the biocathode during light period in the presence of different concentration of OTC (Figs. 1B and 3A). However, the positive effect of the enhancement in electron transfer from the cathode to terminal electron acceptors mediated by the degradation products of OTC could exceed the negative effect of toxicity of OTC to algal-bacterial consortium, as evidenced by increased power output in the OTC-treated biocathode with different concentrations of OTC in comparison to the OTC-free biocathode.

Phylogenetic analysis on genus level reveals bacterial community changes under addition of different concentrations of OTC (Fig. 6). *Comamonas, Aquamicrobium, Nitrosomonas, Truepera, Moheibacter, Segetibacter, Gemmatimonas* and *Brucella* are common dominated genus in the five samples but showed different relative abundance among the five samples. The abundance of *Comamonas* was increased from 13.6% in the OTC-free biocathode to 14.0%, 21.2% and 25.47% in the OTC-treated biocathode with OTC concentrations of 5, 10 and 20 mg/L, respectively while it was decreased to 8.1% in the OTC-treated biocathode with an OTC concentration of 50 mg/L. *Comamonas* was reported to degrade various complex organic compounds [30,31] and possess

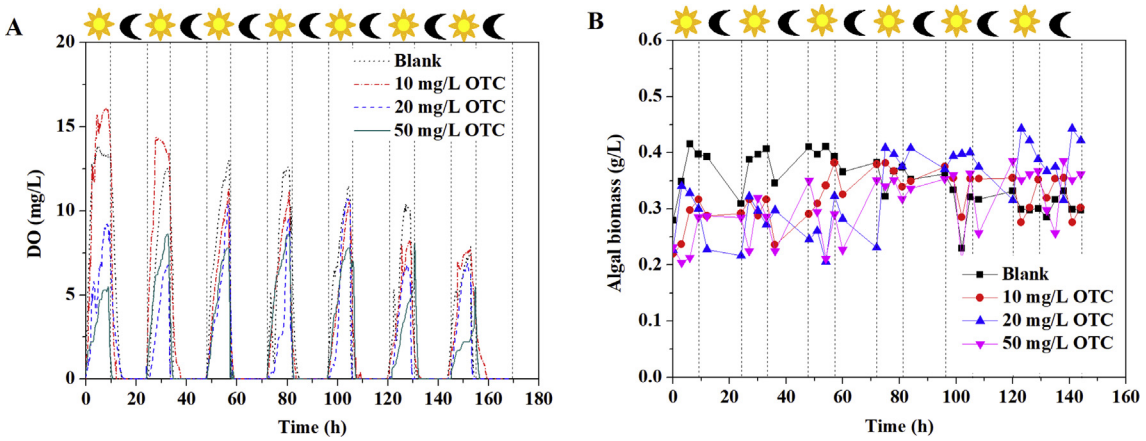

Fig. 5. (A) Time-variation of DO and (B) algal biomass in the OTC-free biocathode and the OTC-treated biocathodes with different initial concentrations of OTC during light/dark cycle.

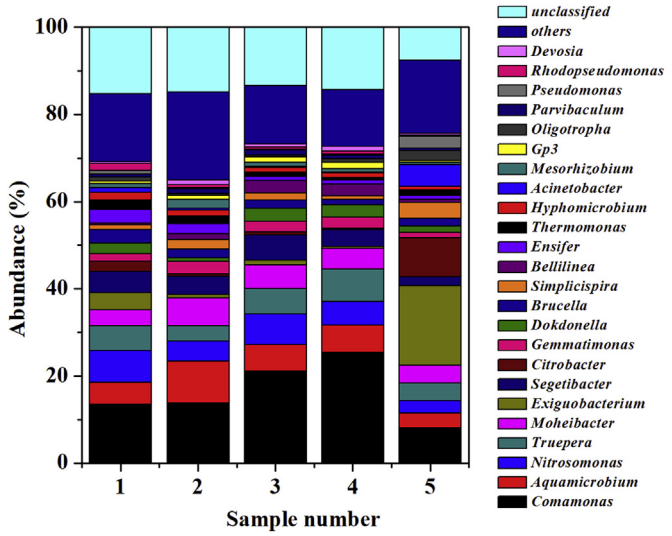

Fig. 6. Comparison of microbial communities in the OTC-free biocathode and the OTC-treated biocathodes with different initial concentrations of OTC at (A) phyla level and (B) genus level. 1: OTC-free; 2: 5 mg/L OTC; 3: 10 mg/L OTC; 4: 20 mg/L OTC; 5: 50 mg/L OTC.

nitrification and denitrification abilities (Zhang et al., 2012; Chen and Ni, 2011). *Comamonas* has also demonstrated its advantage for the extracellular electrons transfer to oxygen reduction and autotrophic dechlorination reaction in biocathode [32]. Similarly, *Aquamicrobium* were accounted for 9.5%, 6.1% and 6.2% in the OTC-treated biocathode with OTC concentrations of 5, 10 and 20 mg/L, respectively, which were much higher than that in the OTC-free biocathode (4.96%), but were accounted for only 3.5% in the OTC-treated biocathode with an OTC concentration of 50 mg/L. *Aquamicrobium* sp. is often highly versatile in its ability to degrade heterocyclic compounds [33]. The presence of *Comamonas* and *Aquamicrobium* could largely contribute to the nitrogen removal and OTC degradation in the biocathode. In contrast, the abundance of *Nitrosomonas* in all the OTC-treated biocathodes was lower than that in the OTC-treated biocathode, indicating that *Nitrosomonas* has a low tolerance to OTC. *Nitrosomonas* belongs to the typical ammonia-oxidizing bacteria and was frequently detected in biological nitrogen removal system [34]. *Truepera* showed obvious tendency to be higher abundance in the OTC-treated biocathodes with OTC concentrations of less than 20 mg/L, but showed a relatively lower abundance in the OTC-treated biocathodes with an OTC concentration 50 mg/L, as compared to the OTC-free biocathode. The genera *Truepera* containing several well-characterized thermophilic radiation-tolerant species could also take part in nitrogen removal since it may harbour nitrogenases (Ivanova et al., 2011). *Exiguobacterium* (18.26%), *Citrobacter* (8.86%), *Acinetobacter* (5%) and *Simplicispira* (3.65%) were only largely enriched in the OTC-treated biocathodes with an OTC concentration of 50 mg/L, indicating selective enrichment of them under high OTC stress. *Exiguobacterium* has been reported to degrade 4-chloroindole [35] and azo dye [36], while *Citrobacter* is capable of degradation of antibiotic [37] and aromatic compounds [38]. The results clearly show that OTC exerts a selection pressure to the biocathode microbial community. Most of the members of microbial community were not affected or were even enhanced at an OTC concentration less than 20 mg/L, but was totally inhibited at high OTC concentration of 50 mg/L, excepting for certain bacteria that have high resistance against OTC. Overall, the flourishing of these microorganisms expounded the formation of functional microbial groups for the simultaneous bioelectrical power generation, OTC degradation and high-strength nitrogen removal in the algal–bacterial biocathode of the ABPBFC.

4. Conclusion

Bioelectrical power generation and NO_3^--N removal in the PBFC were enhanced by the addition of low concentration of OTC due to the fact that the degradation products of OTC can serve as redox mediator for enhancing electron transfer from cathode to oxygen and nitrate during daily light/dark cycle, but the acceleration was not proportional to the rise in OTC concentration due to the toxicity of OTC to biocathodic microbial community. The NH_4^+-N removal rate was negatively affected by the OTC addition, probably due to its toxicity to alga and ammonia-oxidizing bacteria, but can be recovered to the level similar to that of the OTC-free biocathode when OTC was largely degraded. The cathodic bioelectrochemical process resulted in basification of catholyte which contributed to enhanced photolysis of OTC. Most of species related to cathodic electron transfer, nitrogen removal and OTC degradation were stimulated in the presence of OTC at concentrations of less than 20 mg/L but inhibited at 50 mg/L.

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Appendix A. Supplementary data

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