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Profiling of co-metabolic degradation of tetracycline by the bio-cathode in microbial fuel cells†

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In this paper, a system of tetracycline (TEC) degradation by the bio-cathode in a microbial fuel cell (MFC) was constructed. Overall, the co-metabolic degradation performance of TEC was studied through single factor experiments and the ecological risk was evaluated using the *E. coli* growth inhibition rate and resistance genes. High throughput sequencing (HTS) was utilized to profile the biofilm community structure of the bio-cathode. Results showed that the degradation rate of TEC reached greater than 90% under optimal conditions, which was 10 mg L^{-1} initial TEC concentration, 0.2–0.7 g L^{-1} sodium acetate concentration and 12–18 L h^{-1} aeration. Furthermore, compared with the aerobic biodegradation of TEC, the degradation efficiency of the MFC bio-cathode for TEC was significantly increased by 50% and the eco-toxicity of TEC after 36 hour degradation was reduced by 60.9%, and TEC ARGs in effluent were cut down. HTS results showed that electrochemically active bacteria *Acetobacter* and TEC-resistant degradation bacteria *Hyphomicrobium*, *Clostridium* and *Rhodopseudomonas* were the main dominant bacteria in the cathode biofilm. Besides, based on 5 intermediates, degradation pathways involving deamidation, denitro dimethylation, dedimethylation and dehydroxylation of TEC were proposed. The degradation of TEC on the bio-cathode was mainly caused by microbial co-metabolism action. This study would enrich the study of MFC bio-cathodic degradation of antibiotics in water.

1. Introduction

In recent years, tetracycline (TEC) has been highly detected in water environments, with a detection range between μg and ng.[1–4] For example, in the Pearl River Basin of China, TEC is always detected and the highest concentration has been up to 385.5 ng L^{-1}.[5] Moreover, a great deal of studies have implied that TEC enriched in the aquatic environment can inhibit the growth of microbes, plants and aquatic organisms, reduce biodiversity, spread or diffuse the resistance genes, and even affect the survival and development of human beings,[6] which has attracted widespread attention. Therefore, it is imperative to reduce TEC in water.

Conventional water treatment processes cannot meet the requirements of removing TEC from municipal sewage, pharmaceutical wastewater and aquaculture wastewater.[7] Therefore, researchers have been committed to questing efficient and low-cost treatment methods to dispose wastewater containing TEC. For the past few years, many investigations have verified that microbial fuel cells (MFC) can degrade antibiotics and other refractory contaminants, among which some have explored the means of MFC to degrade TEC. Yan *et al.*[8] started MFC *via* inoculating pig manure supernatant and then replaced sodium acetate in the anolyte with TEC gradually. After a long-term operation for 330 d, the degradation rate of TEC with an initial concentration of 10 mg L^{-1} on MFC anode reached 99% within 72 h. Furthermore, compared with control groups containing microbes, the higher ATP concentration and continuous electrical stimulation in MFC process may be the reasons for the higher degradation efficiency of TEC. The results of high throughput sequencing (HTS) implied that the total resistance genes in the effluent of MFC were significantly reduced compared with that of the microbial control group.[8] Peng *et al.*[9] added glucose into the anode chamber of MFC for co-metabolism of TEC. Their results showed that MFC displayed good removal efficiency for TEC in a concentration range of 1–20 mg L^{-1}. When the concentration of TEC was 20 mg L^{-1}, the degradation rate of TEC could reach 87% within 80 h and the maximum power density was 660 mW m^{-3}. It was also proved that the toxicity of degradation products was weakened. Therefore, the anode of MFC has advantages of high efficiency and no harm to degrade TEC, but it still has disadvantages of long-running time and slow degradation rate.

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With the further investigations, it is found that degradation of antibiotics by anode of MFC has some problems, such as the low degradation efficiency, the slow microbial growth, the long start-up and running time, and so on. On the contrary, the bio-cathode of MFC usually don't need to add catalyst to improve the electron transfer efficiency to improve the degradation rate, indicating a higher degradation efficiency. Moreover, the cathode chamber is an aerobiotic environment, so the MFC start-up and running time can be accelerated. For example, Liang *et al.*[10] reported that the degradation rate of chloramphenicol (CAP) on bio-cathode was 96% within 24 h under an applied voltage of 0.5 V, which was much higher than that of non-bio-cathode (73%). They also found that the degradation process of CAP on bio-cathode would not generate highly toxic degradation intermediates. Sun *et al.*[11] also proved that the degradation rate of CAP on bio-cathode outclassed that on non-bio-cathode and was about 3.2 times. The electrochemical performance of bio-cathode was superior to non-bio-cathode as well. What is said above showed that degradation of antibiotics on bio-cathode possesses merits of high degradation efficiency/rate and non-toxic degradation products. Additionally, Chen *et al.*[12] also explored the degradation of oxytetracycline (OTC) by bio-cathode of MFC and confirmed the feasibility of bio-cathode in degrading TEC. Nonetheless, no further researches were conducted to investigate the degradation conditions, mechanism and efficiency of bio-cathode. In general, investigations involving the performance and mechanism of degrading TEC on bio-cathode are still scarce at present.

Therefore, this study took TEC as the target pollutant and planned to construct a system to degrade TEC *via* bio-cathode of MFC. The influences involving initial concentration of TEC, type of carbon sources, concentration of carbon sources and aeration strength on the degradation of TEC *via* bio-cathode were investigated through single-factor experiments. From this, the suitable conditions for degradation of TEC on bio-cathode in MFC were obtained, and then, the pseudo-first order kinetics model was used to fit degradation kinetics. Moreover, the ecological risk of TEC degradation on bio-cathode in MFC was evaluated through analyzing the toxicity of degradation intermediates and the variations of antibiotics resistance genes (ARGs) in order to comprehensively assess the performance of TEC degradation by bio-cathode in MFC. Finally, the probable degradation pathway of TEC was speculated *via* authenticating degradation intermediates, while the mechanism of co-metabolic degradation for TEC on bio-cathode was elucidated *via* analyzing HTS of cathode biofilm communities. This study provided a theoretical basis for the decontamination of wastewater containing TEC by bio-cathode in MFC.

2. Materials and methods

2.1 Materials and reagents

The inoculated sludge was taken from the sludge concentration tank in the aerobic stage of a sewage treatment plant in Guangzhou, Guangdong province.

Tetracycline ($C_{22}H_{24}N_2O_8$) was purchased from Macklin Company. Sodium chloride (NaCl), ammonium chloride (NH_4Cl), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and glucose ($C_6H_{12}O_6$) were all purchased from Guangzhou Chemical Reagent Works.

2.2 Construction, inoculation and operation of MFC bio-cathode

The configuration of the two-chamber MFC reactor followed the previous literature published by our research group.[13] The two-chamber MFCs used were cubic in appearance with two cylinder reactors (2.2 cm in depth and 5 cm in diameter) in both sides, composed of carbon felt cathode and anode. The carbon felts used were cut into circles (3 cm in diameter) and cleaned by acetone before use. The main components of the anolyte were glucose (1000 mg L^{-1}), PBS buffer solution (50 mmol L^{-1}), vitamins and microelements (12.5 mL L^{-1}).[14] The catholyte mainly included sodium bicarbonate (500 mg L^{-1}), sodium acetate (500 mg L^{-1}), PBS buffer solution (50 mmol L^{-1}), vitamins and microelements (12.5 mL L^{-1}).

Due to the slow start-up speed of anaerobic anode, single-chamber MFC was used to make the anode operate in advance, and the anolyte was renewed every 7 d. After the output voltage stabilized, the anode was taken down and assembled into a new two-chamber MFC. Then, a concentration array including 5, 10, 15, and 20 mg L^{-1} TEC was added into the medium, with one concentration used for each cycle (3d), as described in previous reports.[15,16] The overall time for a successful operation was about half a month. The aeration rate in cathode chamber was about 12 L h^{-1}. The concentration of TEC was measured every 4 h. When the degradation rate of TEC (C = 20 mg L^{-1}) on bio-cathode reached more than 70%, the operation of this MFC reactor was considered to be successful.

2.3 Analysis and measurement methods

2.3.1 Determination of TEC and intermediates. The concentration of TEC was determined by high performance liquid chromatography (HPLC) and the detailed chromatographic conditions were as followed: chromatographic column was BEH-C18 (4.6 × 250 mm, 5 μm); mobile phases were acetonitrile and 0.01 mol L^{-1} oxalic acid (v/v = 31 : 69, v(acetonitrile) : v(oxalic acid)); detection wavelength was 355 nm; column temperature was 30 °C; flow rate was 1 mL min^{-1}; injection volume was 20 μL.

The degradation intermediates were detected by liquid chromatograph-mass spectrometer/mass spectrometer (LC-MS/MS). The samples were collected at the time of 12 h, 24 h and 36 h, and then mixed and centrifuged. Then, the supernatant was concentrated with nitrogen bubbling, with a concentration factor of 50 times. The concentrated solution was then filtered by 0.22 μm cellulose acetate membrane and injected into the LC-MS/MS instrument.

2.3.2 Analysis of ARGs and microbial communities. Samples collected from the inoculated activated sludge, TC degraded in MFC bio-cathode after operating 60 d and TC

degraded by aerobic sludge for 60 day operation were prepared respectively. DNA was extracted using kit and amplified by PCR. The amplified samples were sent to Beijing BioMaker Company for determination.

In order to investigate the effects of TEC on the succession of microbial communities in bio-cathode, HTS analysis was adopted. The samples of original activated sludge, sludge containing TEC and operated for 60 d, sludge containing no TEC and operated for 60 d, and finally, the samples of open circuit MFC containing TEC and operated for 60 d were collected respectively. After extracting the DNA with the kit, the DNA was amplified by PCR and then sent to BioMaker Company (Beijing) for sequencing.

3. Results and discussion

3.1 TEC degradation performances and kinetics on MFC bio-cathode

By carrying out the singlet-factor degradation experiment, it was helpful to explore the impacts of main factors on the biological degradation of TEC in bio-cathode system, as well as inquiry into the degradation kinetics according to the pseudo-first order kinetic fitting. The results are shown in Fig. 1.

3.1.1 Initial concentration of TEC. In order to explore the effect of the initial concentration on the degradation efficiency of TEC by bio-cathode in MFC, the initial concentration was set as 2, 5, 10, 20 and 30 mg L^{-1} to conduct the experiments. As shown in Fig. 1(a), the degradation rates of TEC at the initial concentration of 2, 5, 10, 20 and 30 mg L^{-1} were 68.58%, 81.43%, 89.99%, 73.23% and 50.23%, respectively. By fitting, the degradation kinetics accorded with the pseudo-first order kinetics model. The corresponding degradation rate constants in Fig. S1(a)† were 0.039, 0.061, 0.070, 0.042 and 0.023 h^{-1}, respectively. The effect of the initial concentration was clear. It can be seen that the degradation rate of TEC increased as the initial concentration increased when the initial concentration was as low as that between 2 and 10 mg L^{-1}. When the initial concentration of TEC was 10 mg L^{-1}, the degradation rate of TEC reached a maximum. On the contrary, when the initial concentration of TEC was higher than 10 mg L^{-1}, the degradation rate of TEC would decrease as the concentration increased. This phenomenon suggested that excess TEC can inhibit the degradation performance of bio-cathode in MFC, possibly due to the strong inhibitory effect on the life activities of microbes on the bio-cathode under exorbitant high concentration of TEC. Previous studies have verified that when the concentration of some antibiotics was too high, the degradation ability of MFC would be weakened accordingly, such as SMX[17] and CAP.[18]

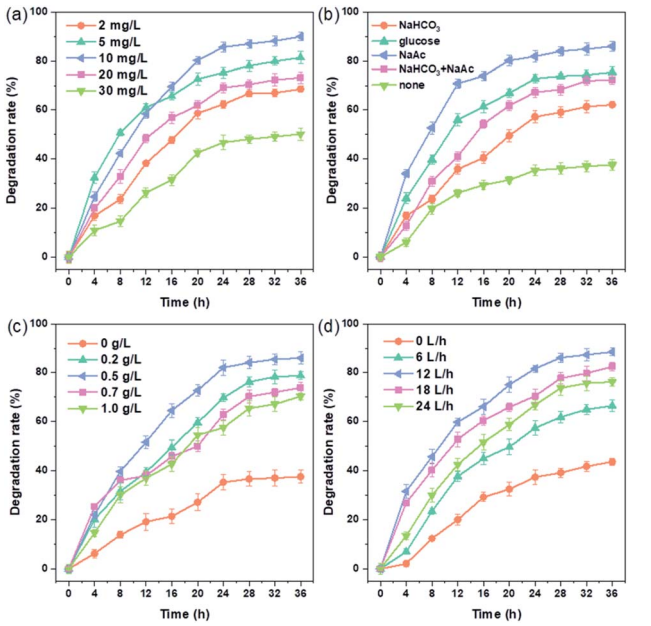

Fig. 1 Influence factors on TEC degradation: (a) initial concentration of TEC, (b) type of carbon sources, (c) concentration of carbon sources (sodium acetate), (d) aeration intensity.

3.1.2 Type of carbon sources. The growth of microbes on MFC bio-cathode is closely related to the carbon sources, and generally speaking, carbon sources with simple structure are easier to be used to improve the system performance.[19] However, the response of the different types of carbon sources on bio-system is not consistent. Therefore, experiments about TEC degradation under different carbon sources was carried out and the results are shown in Fig. 1(b). In the absence of any other carbon source, the degradation rate of TEC by bio-cathode was only 37.55%, indicating that TEC itself was difficult to be used as the only carbon source for microbial metabolism. In other words, the TEC degradation in MFC bio-cathode was a process of co-metabolism. When the carbon sources were sodium acetate, glucose, sodium bicarbonate and sodium acetate/sodium bicarbonate complex, the degradation rates of TEC by bio-cathode were 85.99%, 75.43%, 62.23% and 72.33%, while the corresponding degradation kinetic constants in Fig. S1(b)† were 0.062, 0.054, 0.040 and 0.031 h^{-1}, respectively. Generally, it can be obviously found that the organic carbon sources (sodium acetate and glucose) are more conducive to the degradation of TEC on bio-cathode than the inorganic carbon source (sodium bicarbonate). When sodium acetate was used as carbon source, the degradation rate of TEC was the largest, which was about two times the inorganic carbon source (sodium bicarbonate) and more than five times no carbon source. This result was similar to the conclusions obtained by Shao *et al.*,[20] in regard to the exploration of degradation characteristics for tetracycline by one aerobic tetracycline degrading bacteria SQY5, indicating that sodium acetate may well be an appropriate carbon source for microbes to co-metabolize tetracycline. It is worth mentioning that one reason for sodium bicarbonate to promote the degradation of TEC is its role of the inorganic carbon source for microbial growth and metabolism.

3.1.3 Concentration of carbon sources. The concentration of carbon sources is another vital factor that affected the growth and metabolism of microbes in bio-cathode. According to the facts obtained previously, sodium acetate was chosen to be the research object in this part. As shown in Fig. 1(c), when the concentration of carbon source was 0.5 g L^{-1}, the degradation

rate of TEC reached the highest (86.02%), followed by the concentration of 0.2 g L^{-1} (78.85%). When the concentration of carbon source came up to 1.0 g L^{-1}, the degradation rate of TEC showed a downward trend compared with 0.5 g L^{-1} instead. As can be seen in Fig. S1(c),† the corresponding kinetic constants of the concentration gradient were 0.016, 0.048, 0.064, 0.039 and 0.036 h^{-1} from bottom to top was 0, 0.2, 0.5, 0.7 and 1.0 g L^{-1}, respectively. Furthermore, when the external carbon source was insufficient, the cathode chamber was in an oligotrophic environment, so that the growth and metabolism of microbes were inhibited and the degradation rate of TEC was low accordingly. However, when the concentration of carbon source increased to 0.7 g L^{-1}, the degradation rate of TEC was suppressed. It was clear that when the concentration of carbon source was up to 1.0 g L^{-1}, the inhibition effect on TEC degradation was more significant. Therefore, the concentration of sodium acetate was set to be 0.2–0.7 g L^{-1} as the optimal condition and regulatory strategy for the degradation of TEC by bio-cathode in MFC.

3.1.4 Aeration strength. The cathode chamber in MFC with bio-cathode is an aerobic system, so the degradation efficiency is relevant to the intensity of aeration in cathode chamber. As shown in Fig. 1(d), corresponding to the aeration intensity of 0, 6, 12, 18 and 24 L h^{-1}, the degradation rates of TEC were 43.67%, 66.45%, 88.53%, 82.52% and 76.29%, respectively. In Fig. S1(d),† the kinetic constants were 0.019, 0.034, 0.068, 0.051 and 0.046 h^{-1} corresponded to the aeration intensity from less to more. When the intensity of aeration was 12 L h^{-1}, the degradation rate of TEC was the highest. It is well known that the changes of aeration can affect the concentration of dissolved oxygen in cathode chamber of MFC. When the aeration was stopped, the concentration of dissolved oxygen in cathode chamber would drop rapidly and the metabolic activities of plentiful aerobic microbes were weakened, leading to a decline in the degradation rate of TEC. These aerobic microbes were originally enriched in the bio-cathode by inoculating activated sludge. Based on the facts above, the degradation performance of bio-cathode in MFC for TEC was not ideal under lower intensity of aeration on the negative side of 12 L h^{-1}. When the intensity of aeration arose to 18 L h^{-1}, the degradation rate of TEC decreased to a degree because of the strong hydraulic flow in the cathode chamber of MFC caused by high aeration intensity, which was not conducive for the stability of biofilm system onto the cathode. Therefore, the intensity of aeration was set to be 12–18 L h^{-1} as the optimal aeration strength for subsequent researches.

3.2 Mechanism of TEC degradation on MFC bio-cathode

3.2.1 Analysis of TEC degradation process. As shown in Fig. 2, the degradation rates of hydrolysis, adsorption, electrochemical degradation, biodegradation and MFC bio-cathode degradation were 9.95%, 13.06%, 19.49%, 55.79% and 88.86%, respectively. The corresponding kinetic constants were 0.0025, 0.0788, 0.0076, 0.0169 and 0.0320 h^{-1}, respectively. It can be seen that hydrolysis and adsorption were not the prime reasons for TEC degradation in this system. Additionally, the

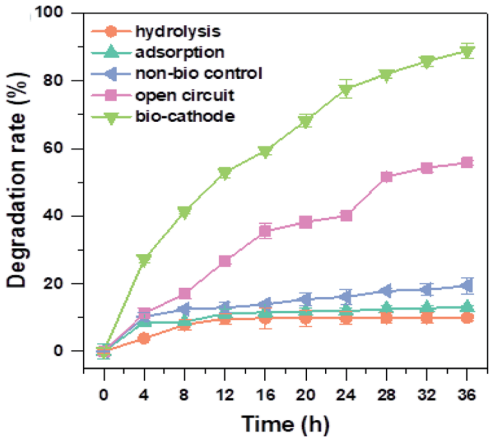

Fig. 2 TEC degradation processes in different units (degradation condition: C_{TEC} = 10 mg L^{-1}, NaAC as carbon source, $C_{carbon\ source}$ = 0.5 g L^{-1}, aeration strength = 12 L h^{-1}).

electrochemical oxidative degradation of TEC depended on the amount of hydroxyl free radicals produced by the cathode, so that the electrochemical oxidative degradation of TEC was not significant due to the less generation of hydroxyl free radicals on the MFC bio-cathode.[21–23] Most noteworthy, the degradation rate of TEC by open circuited MFC cathode within 36 h reached 55.79%, indicating that biodegradation played a key role in degrading TEC on MFC bio-cathode, which was also proved by the numerous TEC-degrading bacteria on the bio-cathode.

The degradation efficiency of TEC in MFC bio-cathode system was 88.86%, which was about 50% higher than that in bio-degradation system, and the reaction rate was about twice as high as that in bio-degradation system. The reason might be that the weakly electric stimulation of MFC can promote the growth and metabolism of microbes, thus improved the degradation efficiency and rate of TEC. However, further verifications were needed from the perspective of microbial communities.

3.2.2 Microbial community evolution of bio-cathode biofilm. By HTS technology, the relative abundance of bacterial communities at phylum and genus levels was obtained, as shown in Fig. 3. Among all the microbiological samples, A1 was the bio-cathode acclimated with TEC, A2 was the bio-cathode without TEC, A3 was the unacclimated sludge, and A4 was the aerobic microbes acclimated with TEC.

Compared with the TEC-free sample A2 and open circuited cathode sample A4, the MFC bio-cathode sample A1 produced different microbial communities, indicating that different external conditions had a great influence on the evolution of microbial communities. At gate level in Fig. 3(a), *Proteobacteria* had the highest content in all the four systems, which were 60.58% (A1), 86.08% (A2), 40.43% (A3) and 64.21% (A4), respectively. Other dominant bacteria were *Firmicutes*, *Bacteroides* and *Actinobacteria*. By comparison with the unacclimated sludge, the content of *Proteobacteria* in A1, A2 and A4 raised by 20.15%, 45.65% and 23.78%, respectively. Especially, in the bio-cathode sample without TEC (A2), *Proteobacteria* was the

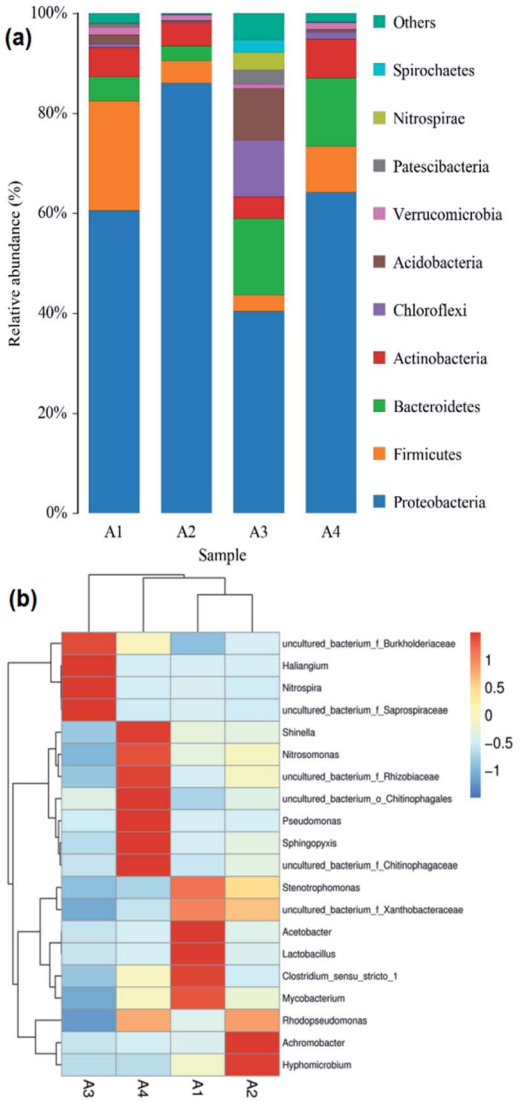

Fig. 3 Relative abundance of bacterial communities at phylum (a) and genus (b) levels.

dominant group plenarily, because *Proteobacteria* is a common dominant bacteria phylum in wastewater treatment systems and it contains a large number of electric-producing bacteria.[24] It is worth mentioning that in sample A1 with TEC, the relative abundance of *Firmicutes* was 21.93%, which was 18.68% higher than the original sample (3.25%), while the relative abundance of *Firmicutes* in another sample A4 containing TEC also increased by 5.98%. However, in sample A2 without TEC, the relative abundance of *Firmicutes* did not change significantly. It implied that *Firmicutes* had a strong resistance to TEC, which might be the vital bacteria in cathode biofilm to degrade TEC, and the result was consistent with the research results of Wang *et al.*[25]

The heat map of bacterial community distribution at genus level for different biological samples was shown in Fig. 3(b). Under the stress of TEC and electron transport, the abundance and species of dominant microflorae in samples A1, A2 and A4 evolved greatly, compared with that in sludge sample A3. However, A1 and A2 had relatively similar bacterial community, might because that both of them were microbial electrochemical systems, leading to the enrichment of a large number of electrochemically active bacteria on the cathode. In detail, the dominant microbe on cathode biofilm included *Rhodopseudomonas*, *Hyphomicrobium*, *Acetobacter*, *Clostridium*, *etc.* There into, *Rhodopseudomonas* was enriched in all the three samples A1, A2 and A4. Wu *et al.*[26] proved that *Rhodopseudomonas* has a good degradation performance for TEC. Besides, *Hyphomicrobium* was a common facultative anaerobe in bioelectrochemical systems with the function of promoting hydrolysis and reducing refractory organic contaminants.[27] The existence of *Hyphomicrobium* in the bio-cathode was likely to promote the degradation of TEC. *Acetobacter* was also an electrochemically active bacterium. When the carbon source was sodium acetate, *Acetobacter* can easily accomplish electron transfer process in MFC.[28] It could be inferred that *Acetobacter* is primarily a medium for electron transport to the bio-cathode, thus provided continuous electrical stimulation for microbes. In addition, *Clostridium* was a TEC-resistant bacterial genus commonly found in animal intestines.[29] In sample A4, the TEC degrading bacterium *Rhodopseudomonas* was the dominant specie in the process of aerobic microbial degradation and the relative abundance was 18.5%, which was much higher than other microbes. However, the relative abundance of electrochemically active bacterium *Acetobacter* in A4 was much lower than in sample A1, indicating that aerobic microbial degradation system could enrich TEC degrading bacteria, but could not enrich electrochemically active bacteria.[30]

In conclusion, in the process of degrading TEC by MFC bio-cathode, a large number of electroactive bacteria and TEC degrading bacteria were enriched on the bio-cathode. As a result, TEC could be rapidly degraded under the interaction of electrochemically active bacteria and TEC degrading bacteria. Therefore, the efficient performance of MFC bio-cathode to degrade TEC was further proved from the perspective of microbial communities.

3.2.3 Analysis of degradation intermediates and pathways. The degradation intermediates of TEC by MFC bio-cathode were identified by LC-MS technology, and the molecular fragments detected were 444 *m*/*z*, 430 *m*/*z*, 427 *m*/*z*, 417 *m*/*z*, 404 *m*/*z* and 384 *m*/*z*. These substances were represented by A444, B430, C427, D417, E404 and F384 respectively. The main degradation intermediates of TEC were inferred by comparing with literatures in Table S1.†

The overall degradation pathways of TEC degraded on MFC bio-cathode was shown in Fig. S3.† The substance with the molecular fragment of 444 *m*/*z* was judged as TEC parent. Among the degradation intermediates of TEC by MFC bio-cathode, the substances represented by the fragments of 430 *m*/*z* and 427 *m*/*z* could be inferred as the demethylation and deamination products of TEC respectively, based on the structural formula of TEC molecular. The molecular fragment of 417 *m*/*z* might be the further demethylation product of B430, while the molecular E404 was the further demethylation product of D417. Besides, F384 was the deamination product by removing

two methyl groups of C427. It could be seen from these intermediates that the degradation process of TEC by MFC bio-cathode mainly includes demethylation, deamination, dedimethylation and other processes. In the process of TEC degradation, the active sites of amides and dimethyl groups on TEC were gradually destroyed and the antibacterial activity of tetracycline decreased, which explained why the eco-toxicity of effluent from MFC bio-cathode was eliminated to some extent.

3.2.4 Degradation mechanism analysis. Based on the analysis of microbial communities on the bio-cathode and deduction of degradation pathways, the fundamental mechanism of TEC degradation on MFC bio-cathode was proposed. As seen in Fig. 4, the mechanism mainly included two following points: (I) under the action of electroactive microbes such as *Acetobacter* and *Rhodopseudomonas* that enriched on MFC bio-cathode, the electrons on the bio-cathode received from the anode will accelerate the metabolic activities of TEC-degrading bacteria to secrete more enzymes for biochemical reaction. Thus, the methyl, hydroxyl, dimethyl and amide groups on TEC fall off and the TEC parent was degraded. (II) The TEC-degrading bacteria such as *Hyphomicrobium*, *Clostridium*, *Stenotrophomonas* and *Mycobacterium* are able to co-metabolize TEC using sodium acetate as the effective carbon source.

3.3 Ecological risk assessment of TEC degradation on MFC bio-cathode

3.3.1 Toxicity assessment of TEC intermediates. The potential ecological risk of TEC and its intermediates during the degradation process of bio-cathode in MFC was assessed by the growth inhibition rate of *E. coli* in different degradation stages. The results were shown in Fig. 5a. As the reaction goes on, it can be seen that the growth inhibition rate of *E. coli* showed an overall downward trend. The growth inhibition rate of TEC (C = 10 mg L^{-1}) on *E. coli* was 23.0%, while the growth inhibition rate of the sample in 12 hour degradation stage was nearly no difference. Further, the growth inhibition rate of the sample that collected after 24 hour degradation began to drop to 12.3% and gradually fell as the operation time extended. After operating the MFC for 36 h, the growth inhibition rate of degraded sample on *E. coli* was very weak, declining to 9.0%. Compared with the TEC parent substance, the growth inhibition rate of degradation intermediates was reduced by 60.9% after 36 hour degradation by bio-cathode in MFC. This might be due to the destruction of the active functional groups such as dimethyl group and amide group during the degradation process of TEC by bio-cathode in MFC, leading to the reduced antibacterial activity of TEC in the effluent. The facts above obviously proved that bio-cathode in MFC can effectively reduce the potential ecological risk of TEC to microbes, which inured to the benefit of the microbial system in wastewater treatment process.

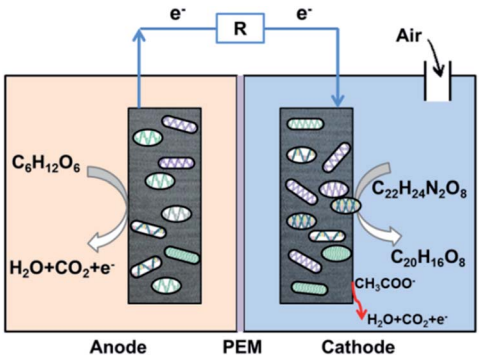

Fig. 4 Mechanism of TEC degradation on MFC bio-cathode.

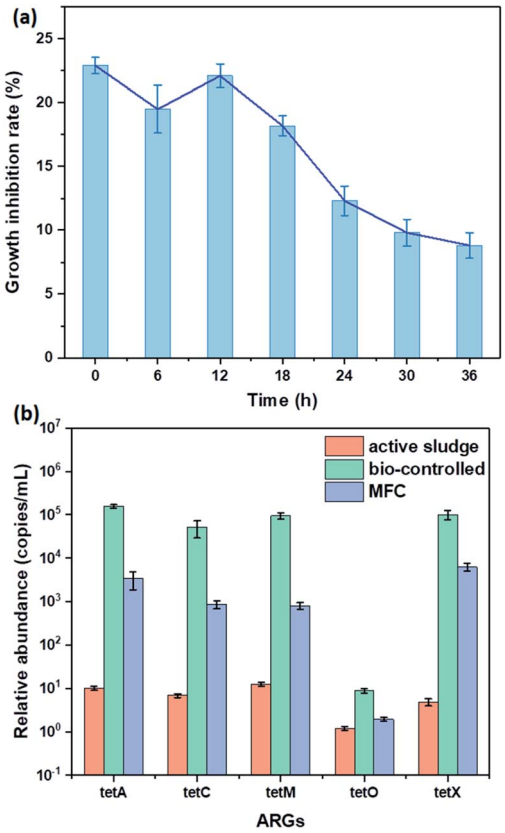

Fig. 5 (a) Toxicity changes of TEC in the degradation process. (b) Variation of ARGs during degradation of TEC by MFC bio-cathode.

3.3.2 Variation of ARGs. In order to evaluate the possible ecological risks caused by the accumulation of ARGs in the degradation process of TEC by bio-cathode in MFC, the quantitative analysis was carried out, involving five types of TEC ARGs (*tet*A, *tet*C, *tet*M, *tet*O, *tet*X) in three samples collected from primordial activated sludge, MFC bio-cathode and degraded antibiotics by aerobic microbes. The determination results were shown in Fig. 5b. In the three samples, the concentration of *tet*O all remained low, indicating that there was no enrichment of *tet*O in all samples. In the primordial sludge sample without adding TEC, the concentrations of five TEC ARGs were all within the range of 10^1 copies per μL, representing the very low abundance of TEC-resistant bacteria in the sludge. However, the concentrations of *tet*A, *tet*C, *tet*M and *tet*X in aerobic microbes were 1.6×10^6, 5.1×10^5, 9.4×10^5 and 1.0×10^6 copies per μL respectively, while the concentrations of *tet*A, *tet*C, *tet*M and *tet*X in MFC bio-cathode were 3.4 ×

10^3, 8.8×10^2, 8.1×10^2, 6.2×10^3 copies per μL respectively. So that, under prolonged stress from TEC, the bio-cathode in MFC would significantly enrich drug-resistant bacteria. It was clear that the former was much higher than the latter, indicating that degradation of TEC by bio-cathode in MFC was more effective to reduce the ARGs in effluent than biodegradation by aerobic microbes. Yan *et al.*[8] reported that bio-anode in MFC was more effective to reduce ARGs in effluent by using bio-anode for anaerobic degradation of TEC, which was consistent with the conclusion in this study.

4. Conclusions

To sum up, the method of gradient domestication was utilized to successfully construct and operate the MFC containing bio-cathode, as well as to realize the high-efficiency degradation of TEC. The optimal degradation conditions were determined as followed: 10 mg L^{-1} of initial concentration of TEC, sodium acetate as the carbon source, 0.2–0.7 g L^{-1} of concentration of carbon source, 12–18 L h^{-1} of aeration strength, and the highest degradation rate of TEC reached more than 90% after 36 hour degradation. The degradation efficiency was significantly enhanced compared with aerobic biodegradation or MFC bio-anodic degradation of TEC. The growth inhibition rate of *E. coli* indicated that the final degraded sample showed less toxicity compared with TEC parent, reducing by 60.9%, leading to the very low ecological toxicity of the effluent. Moreover, the results of ARGs determination showed that the concentrations of *tet*A, *tet*C, *tet*M and *tet*X were all enriched in MFC bio-cathode system except for *tet*O, but the magnitude of ascent was much lower than in the aerobic biodegradation system, implying that MFC bio-cathode degradation was beneficial to cutting down ARGs in the effluent. The analysis of HTS showed that dominant microflorae on bio-cathode were electrochemically active bacteria such as *Acetobacter*, *Rhodopseudomonas* and TEC-resistant degradation bacteria such as *Hyphomicrobium*, *Clostridium*, *Stenotrophomonas* and *Mycobacterium*. Five kinds of intermediates were determined. Meanwhile, two degradation pathways involving demethylation, deamination, dehydroxyl and dedimethylation of TEC were speculated. The degradation of TEC by MFC bio-cathode was owing to the action of microbes and the improvement of microbial metabolic activity under electrical stimulation, and that the degradation of TEC was improved by leaps and bounds consequently.

Author contributions

Conceptualization, Luxaing Wang; methodology, Luxaing Wang and Dongmin Liang; software, Luxaing Wang; investigation, Luxaing Wang, Dongmin Liang and Yunqi Shi; data curation, Dongmin Liang and Yunqi Shi; writing – original draft preparation, Luxaing Wang. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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