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Biodegradation of oxytetracycline and electricity generation in microbial fuel cell with *in situ* dual graphene modified bioelectrode

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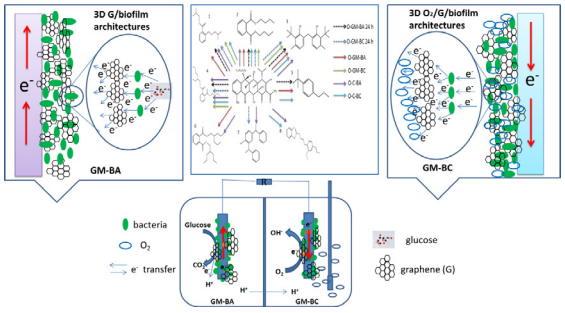
GRAPHICAL ABSTRACT



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ABSTRACT

A three-step method to prepare dual graphene modified bioelectrode (D-GM-BE) in microbial fuel cell (MFC) in previous studies. This study explored the biodegradation of oxytetracycline (OTC) and electricity generation in O-D-GM-BE MFC. The OTC removal efficiency of graphene modified biocathode and bioanode (O-GM-BC, O-GM-BA) was 95.0% and 91.8% in eight days. The maximum power density generated by O-D-GM-BE MFC was 86.6 ± 5.1 mW m^{-2}, which was 2.1 times of that in OTC control bioelectrode (O-C-BE) MFC. The Rct of O-GM-BA and O-GM-BC were decreased significantly by 78.3% and 76.3%. OTC was biodegraded to monocyclic benzene compounds by bacteria. O-GM-BA was affected strongly by OTC, and *Salmonella* and *Trabulsiella* were accounted for 83.0%, while typical exoelectrogens (*Geobacter*) were still enriched after the maturity of biofilm. In O-GM-BC, bacteria related with OTC biodegradation (*Comamonas*, *Ensifer*, *Sphingopyxis*, *Pseudomonas*, *Dechloromonas*, etc.) were enriched, which contributed to the high removal efficiency of OTC.

1. Introduction

Oxytetracycline (OTC) is a significant member of tetracyclines (TCs), which are crucial classes of broad-spectrum antibiotics and have been widely used to prevent disease and promote growth in livestock (Liu et al., 2016a). The abuse of OTC/TCs in animals would lead to accumulation of antibiotics in meat, milk or chicken eggs, and the accumulation is most likely to have serious impacts on human health (Kim

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et al., 2014). So far, OTC has been detected broadly in aquatic environment, soil system, and sediments etc. on a global scale (Liu et al., 2016b).

Due to the antibiotic nature, hydrophilic property and stable naphthacene ring structure, studies have shown that traditional water treatment processes are not effective to remove OTC from water (Zhao et al., 2013). However, biodegradation of OTC is also proved to be feasible in recent studies (Chang et al., 2014; Coban et al., 2016). Migliore et al. (2012) conducted a test of OTC degradation in liquid medium by the use of ligninolytic fungus *Pleurotus ostreatus*, the data demonstrated that *Pleurotus ostreatus* was not only able to survive and grow in the presence of OTC, but also nearly degraded OTC in few days. *Cerrena* laccase was proved to be most effective to degrade OTC over 80% within the first 12 h (Yang et al., 2017). Chang and Ren (2015) found that aerobic conditions were feasible to degrade OTC and the degradation efficiency was related with particle size, enzyme extract, etc. Anaerobic digestion (AD) was regarded not only to degrade OTC, but also produce valuable biogas (methane) (Akyol et al., 2016), and the relationship between OTC concentration and its removal efficiency in AD process was described with exponential (40–100 mg/kg) (Yin et al., 2016). Five strains isolated from Chilean fjord sediments showed activities on OTC degradation and were identified as *Trichoderma harzianum*, *Trichoderma deliquescens*, *Penicillium crustosum*, *Rhodotorula mucilaginosa*, and *Talaromyces atroroseus*, which decreased OTC concentration by 92%, 85%, 83%, 73% and 72%, respectively, after 21 days of treatment (Ahumada-Rudolph et al., 2016). All these studies indicated that OTC could be biodegraded by microorganism in aerobic or anaerobic conditions and biodegradation had great potential to degrade OTC. Biodegradation is not only environmentally friendly but also generating valuable by-products in anaerobic conditions. The studies of antibiotics biodegradation were the current hot spots and focal points, and the enrichment of degrading bacteria was the key to the biodegradation of antibiotics.

Microbial fuel cell (MFC) could convert biochemical energy into electrical energy and be used for environmental remediation meanwhile (Logan et al., 2015; Wang et al., 2017). Hassan et al. (2016) built *Bacillus subtilis*-catalysed MFC for bioelectricity generation and degradation of 2,4-dichlorophenol, and found that *Bacillus subtilis*-catalysed MFC was effective to obtain the maximum power density of 9.5 mW m^{-2} and the degradation efficiency of 60%. Cao et al. (2015) investigated hexachlorobenzene degradation pathway by soil MFC, which obtained maximum power density of 77.5 mW m^{-2} and proved that hexachlorobenzene could be degraded through the reductive dechlorination pathway in anaerobic condition. In view of MFC was effective to degrade refractory organics in wastewater, and acquire renewable energy at the same time, MFC could also be deemed as a new approach to degrade OTC and generate electricity. The improvement of bioelectrode was the development direction, and it was the key to the enrichment of degrading bacteria.

In previous study, a three-step method to prepare *in situ* dual graphene modified bioelectrode (D-GM-BE) were proposed by *in situ* microbial-induced reduction of graphene oxide (GO) and polarity reversion in MFC, which demonstrated excellent electrochemical properties and contributed to enhance the extracellular electron transfer (EET) process and oxygen reduction reaction (ORR) in GM-BE (Chen et al., 2017b). Based on these, this study expanded the application of D-GM-BE MFC, and the degradation conditions of OTC were in line with the running conditions of MFC, therefore, OTC was used as typical organic pollution to test the degradation ability of D-GM-BE MFC. This study would provide possible references to degrade OTC and electricity generation synchronously by *in situ* D-GM-BE MFC.

In this study, *in situ* D-GM-BE MFC was built firstly, then a certain concentration of OTC was added into GM-BE to explore the degradation efficiency and mechanisms. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were operated to expound the electrochemical activity of GM-BE with OTC, and the power density and polarization curves were conducted to illuminate the electrochemical performance of MFC; confocal scanning laser microscopy (CSLM) was employed to interpret the viability of microbial biofilm in GM-BE; high performance liquid chromatography (HPLC) was carried out to detect the concentration of OTC; gas chromatograph mass spectrometer (GC–MS) was used to detect the degradation products; next generation sequencing technology was used to investigate the bacterial community composition. This study would not only provide a novel approach to degrade OTC in aerobic and anaerobic conditions, but also generate electricity at same time. This study would provide technical support and theoretical basis for the degradation antibiotics.

2. Materials and methods

2.1. Start-up and running of D-GM-BE MFC

A double-chamber MFC was equipped with carbon felt (5 cm × 6 cm) as basic electrode (anode and cathode), and separated by cation exchange membrane (CEM) as previous studies (Chen et al., 2017a; Li et al., 2014). Active sludge was obtained from Liede Sewage Treatment Plant in Guangzhou City. GO solution was synthesized via improved Hummers method (Zhang et al., 2018), and GO solution (1 mg L^{-1}) was added into anode chamber together with carbon source, Phosphate buffer solution (PBS, pH 7.0), trace mineral and vitamin solution. *In situ* D-GM-BE MFC was prepared through a three-step method: graphene modified bioanode (GM-BA) was initially prepared by microbial-induced reduction of GO in anode; then graphene modified biocathode (GM-BC) was completed on the basis of polarity reversion of GM-BA; GM-BA was formed once again in anode as the first step (Chen et al., 2017c). The GM-BA chamber was slowly mixed by a diminutive magnetic stirrer to minimize mass transfer limitations and dissolved oxygen (DO) in GM-BC was maintained at 6–7 mg L^{-1} with an aeration device. All reactors were placed in a constant-temperature room (30 ± 1 °C), all tests were manipulated at least in duplicate and the average value was reported for all data.

2.2. Electrochemical tests

The voltage was recorded each 10 min by a data acquisition system (Model 2700, Keithly Instruments, USA). The external resistor was set the range of 5000–50 Ω as the voltage output approached steady state to acquire the power density and polarization curves. The electrochemical characteristics of GM-BE were measured by CV curves and EIS by an electrochemical work station (Model 2273, Princeton Applied Research, USA). All the electrochemical measurements were conducted *in situ* in a conventional three-electrode cell mode by setting working electrode, counter electrode and reference electrode (saturated calomel electrode, SCE). Prior to each measure, the MFC was operated under open circuit condition for more than 2 h until open circuit potential was stable. CV curves was conducted by applying a potential ramp to the working electrode, at a scan rate of 3 mV s^{-1} over the range from -0.8 V to 0.2 V. EIS test was operated in a frequency range of 100 kHz to 10 mHz with an AC signal of 10 mV amplitude, and the experimental data were accommodated according to predetermined equivalent electrical circuit (Chen et al., 2015).

2.3. Detection of OTC and products during D-GM-BE MFC

OTC (CAS No. 79-57-2; > 95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The concentration of OTC was detected by HPLC (e2695, Waters, Mil- ford, MA, USA) equipped with a C_{18} reverse phase column (150 mm × 4.6 mm, 5 μm) with acetonitrile (0.005 mol L^{-1})/methanoic acid solution (20/80, v/v %) as the mobile phase at a flow rate of 1 mL min^{-1}. The detection wavelength was 360 nm, with a column temperature of 35 °C and a sample injection volume of 20 μL. The degraded products were detected and identified by GC–MS (GCMS-