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Short Communication

Dynamics of antibiotic resistance genes in microbial fuel cell-coupled constructed wetlands treating antibiotic-polluted water

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HIGHLIGHTS

- Most ARGs in anode layer were higher than those in cathode and middle layers.
- ARGs in biofilm illustrated an obvious increase during treatment periods.
- No significant correlation between ARGs and 16S rRNA except for *sulI* and *tetW* genes.
- Significant correlations were observed among the most of ARGs copy numbers.

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ABSTRACT

Microbial fuel cell-coupled constructed wetlands (CW-MFCs) use electrochemical, biological, and ecological functions to treat wastewater. However, few studies have investigated the risks of antibiotic resistance genes (ARGs) when using such systems to remove antibiotics. Therefore, three CW-MFCs were designed to assess the dynamics of ARGs in filler biofilm and effluent over 5000 h of operation. The experimental results indicated that relatively high steady voltages of 605.8 mV, 613.7 mV, and 541.4 mV were obtained at total influent antibiotic concentrations of 400, 1,000, and 1600 μg L^{-1}, respectively. The 16S rRNA gene level in the cathode layer was higher than those in the anode and two middle layers, but the opposite trend was observed for the *sul* and *tet* genes. The relative abundance of the three tested *sul* genes were in the order *sulI* > *sulII* > *sulIII*, and those of the five *tet* genes were in the order *tetA* > *tetC* > *tetW* > *tetO* > *tetQ*. The levels of *sul* and *tet* genes in the media biofilm showed an increase over the treatment period. The effluent water had relatively low abundances of *sul* and *tet* genes compared with the filler biofilm. No increases were observed for most ARGs over the treatment period, and no significant correlations were observed between the ARGs and 16S rRNA gene copy numbers, except for *sulI* and *tetW* in the effluent. However, significant correlations were observed among most of the ARG copy numbers.

1. Introduction

Approximately 9,000, 5,000, and 6000 metric tons of antibiotics are consumed annually by the livestock industries in the United States, European Union, and China, respectively (Srinivasan and Sarmah, 2014). Moreover, the majority of veterinary antibiotics (VAs) used are excreted in feces and urine in their original form because they are metabolized slowly in the gut, resulting in large residue inputs into the environment (Huang et al., 2015b). Increasing VA use and disposal into the environment can promote antibiotic resistance gene (ARG) transfer between pathogenic and nonpathogenic bacteria, posing a high risk to the environment and human health (Huang et al., 2015a; Naquin et al., 2015). Higher relative abundance of ARGs have been observed in wastewater lagoons after biological treatment (Huang et al., 2015b). Hence, waste treatment technologies should be designed to both eliminate antibiotics and minimize ARGs (Zhang et al., 2016a).

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The microbial fuel cell-coupled constructed wetland (CW-MFC) system is a novel artificial ecosystem technology that embeds a MFC into a CW (Fang et al., 2015) and that is considered a cost-effective method for producing bioelectricity during wastewater treatment (Villaseñor et al., 2013; Doherty et al., 2015a). In CW-MFCs, a significant redox gradient develops vertically, and the anaerobic environment at the bottom and aerobic environment at the top can be regarded as an anode chamber and single air-cathode, respectively (Zhao et al., 2013; Doherty et al., 2015a). In the anode, organic matter can be used as a renewable resource to generate electrons and protons by electrochemically active bacteria (EAB) (Doherty et al., 2015a; Wang et al., 2016). This technique has attracted considerable interest for its comparatively low cost in terms of construction, operation, and maintenance compared to traditional MFCs (Higgins et al., 2011). CW-MFCs have been studied for the biodegradation of dyes (Fang et al., 2015), nitrate, and organics (Doherty et al., 2015b; Wang et al., 2016) in synthetic wastewater and municipal domestic wastewater. CW-MFCs possess a large effective area, driving the biodegradation of biorefractory and toxic compounds, which have recently raised concern (Fang et al., 2013; Cao et al., 2015; Zhang et al., 2016b). However, less attention has been paid to the potential of CW-MFCs to remove trace antibiotics.

Various bioelectrochemical methods have been used to enhance the removal rate of antibiotics via redox reactions. For example, penicillin can be degraded and produce electricity simultaneously in MFCs (Pham et al., 2008). Harnisch et al. (2013) studied the ability of a microbial bioelectrochemical system to remove sulfonamide from wastewater (Harnisch et al., 2013). Zhang et al. (2016a,b) observed that tetracycline (TC) and sulfamethoxazole (SMX) concentrations could be significantly reduced using CW-MFCs. Unfortunately, no studies on antibiotic removal have provided detailed information on antibiotic biodegradation along with the dynamic fate of ARGs.

In this study, TC and SMX, which are readily detected in environmental samples, were selected as target VAs (Rodriguez-Mozaz et al., 2015). Three *sul* genes (*sulI*, *sulII*, and *sulIII*) were selected as representative sulfonamide ARGs (Dan et al., 2013; Naquin et al., 2015; Wu et al., 2015), and five *tet* genes (*tetA*, *tetC*, *tetO*, *tetQ,* and *tetW*) were selected as representative TC ARGs (Barkovskii and Bridges, 2011; Huang et al., 2015b; Wu et al., 2015). The objectives of this study were to assess the development of ARGs in CW-MFCs and the dynamics of ARGs in effluent during the treatment process.

2. Materials and methods

2.1. Reactor configurations

Three CW-MFCs were constructed in polyacrylic plastic chambers (diameter: 20 cm, height: 55 cm) containing four layers, from the bottom to the top: the bottom anode layer, two middle layers, and one cathode layer (Fig. 1). The bottom and middle layers (thickness of each: 20 cm) were both filled with sand and soil (sand: soil volume ratio, 40:1; diameter: 2–3 mm). The anode (thickness: 8 cm) and cathode (thickness: 6 cm) layers were both filled with granular activated carbon (GAC)/titanium mesh (diameter: 2–5 mm, specific area: 500–900 $m^2\ g^{-1}$). 8 *Oenanthe javanicas* were planted in the cathode layer. The sampling outlets were set up to collect samples vertically along the reactor at the same interval from the anode layer, first middle layer, second middle layer, and cathode layer. The external circuit was connected with an external resistance of 1000 Ω (Zhang et al., 2016b).

2.2. Inoculation and operation of CW-MFCs

Anaerobic sludge (mixed liquor-suspended solids: 60 g L^{-1}) collected from the South City Municipal Wastewater Treatment Plant (Nanjing, China) was used as the inoculum source. The sludge was pretreated by mixing with GAC (GAC/sludge volume ratio: 3:1) and introduced into the cathode and anode layers of the CW-MFCs. Synthetic domestic wastewater was composed of glucose (0.225 g L^{-1}), NaCl (0.15 g L^{-1}), NH_4Cl (0.020 g L^{-1}), KH_2PO_4 (0.004 g L^{-1}), and 0.20 mL of concentrated trace elements solution (which contained per liter: 15 g $CaCl_2$, 15 g $MgSO_4{\cdot}7H_2O$, 2.2 g $MnSO_4{\cdot}H_2O$, 2 g $ZnSO_4{\cdot}7H_2O$, 1 g $FeSO_4$, 0.24 g $CoCl_2{\cdot}6H_2O$, 10 mg $FeCl_3{\cdot}6H_2O$, 5 mg $ZnCl_2$, 2 mg $NiCl_2{\cdot}6H_2O$, 1 mg $CuCl_2{\cdot}2H_2O$, and 0.4 mg $Na_2MoO_4{\cdot}2H_2O$) (Cao et al., 2015; Zhang et al., 2016b).

The synthetic domestic wastewater was fed continuously into the CW-MFCs through the bottom inlet using peristaltic pumps after inoculation. The hydraulic retention time was set to 2.5 d. The SMX and TC concentrations in the influent were 200, 500, and 800 μg L^{-1} respectively. The stable operation was obtained after 4 weeks of operation. The CW-MFCs were covered with black adhesive tape at room temperature (28 ± 2 °C) continuously for 5000 h.

2.3. Quantification of ARGs

Samples were collected from the anode layer, middle layers, cathode layer, and effluent in triplicate during five sampling periods, in April, May, June, July, and August 2015. Microbial genomic DNA was extracted using a Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) following the manufacturer's instructions. Genomic DNA concentration and quality were assessed with spectrophotometry (UV-9100; Lab Tech, Ltd., Beijing, China) and gel electrophoresis, respectively. The primer sequences (Table S1) and protocols (Table S2) employed for the quantitative PCR (qPCR) of the various ARGs were based on previous publications (Huang et al., 2015b; Rodriguez-Mozaz et al., 2015; Wu et al., 2015). Plasmids carrying the ARGs were generated (Sangon Biotech, Shanghai, China) to create standard curves (Table S3). Eight ARGs (*sulI*, *sulII*, *sullII, tetA, tetC, tetO, tetQ,* and *tetW*) were quantified using a CFX Connect Real-Time PCR System (Bio-Rad, Shanghai, China), with four repetitions for each qPCR reaction.

2.4. Instrumentation and statistical analysis

Effluent was filtered through 0.45-μm fiber filters and extracted using Oasis HLB extraction cartridges (6 mL; Waters, USA). SMX and TC were measured using a liquid chromatography-mass spectrometry system (LCQAD-60000; Thermo Fisher Scientific, Waltham, MA, USA) (Huang et al., 2015b; Wu et al., 2015). Voltage (V) data were monitored with a data acquisition module (DAM-3057; Art Technology Co., Ltd., China). The averages and standard deviations of all data were calculated using Microsoft Excel 2007. The statistical analyses were performed using SPSS ver. 21 (IBM, Armonk, NY, USA), and all figures were plotted with Microsoft Excel 2007, SigmaPlot ver. 11.0 (Systat Software, Inc., San Jose, CA, USA), and Adobe Photoshop CS6 (Adobe Systems Inc., San Jose, CA, USA).

3. Results and discussion

3.1. Effects of antibiotics on electricity production

Voltage data were collected to analyze the effects of antibiotics on the electricity production of the CW-MFCs (Fig. 2). After the acclimation period, the average voltages of the systems were 605.8 mV, 613.7 mV, and 541.4 mV at the SMX and TC concentrations were 200, 500, and 800 μg L^{-1} respectively. During this