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Effect of electrical stimulation on the fate of sulfamethoxazole and tetracycline with their corresponding resistance genes in three-dimensional biofilm-electrode reactors

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HIGHLIGHTS

- Satisfactory removal of sulfamethoxazole and tetracycline were acquired in 3D-BER.
- Antibiotic resistance genes in a GAC cathode were higher than those in a GAC anode.
- A low current density could not increase the development of antibiotic resistance genes.
- Microbial communities were significantly affected by current stimulation.

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ABSTRACT

Three-dimensional biofilm-electrode reactors (3D-BERs), which possess a large effective area to drive the reductive degradation of contaminants, have recently attracted attention for wastewater treatment. There have been few studies of the potential and risks of the application of this system on the removal of antibiotics. Here four 3D-BERs were designed to initially assess the potential for electrical stimulation to remove sulfamethoxazole (SMX), tetracycline (TC) and chemical oxygen demand, and to study the fate of the corresponding antibiotic resistance genes. The results indicated that the 3D-BER could significantly reduce antibiotic concentrations in wastewater, achieving removal rates of 88.9–93.5% and 89.3–95.6% for SMX and TC, respectively. The concentrations of target genes (*sulI, sulII, sulIII, tetA, tetC, tetO, tetQ*, and *tetW*) in a granular-activated carbon (GAC) cathode were higher than those in a GAC anode in the 3D-BR (reactor with biological sludge and no voltage) and 3D-BER. An obvious increasing trend in the relative abundances of all target genes was observed in the GAC. A low current density could not increase the development of *sul* and *tet* genes in the 3D-BER. The total resistance was in the following order: 3D-BER > 3D-BR > 3D-ER (reactor with 0.8 V and without biological sludge). In addition, the dehydrogenase activity of the microorganisms in the 3D-BER was significantly higher than in the 3D-BR ($p < 0.05$). High-throughput sequencing revealed that the microbial communities and relative abundance at the phyla level were affected by current stimulation.

1. Introduction

Biofilm-electrode reactors (BERs) (Sakakibara and Kuroda., 1993), as well as electrochemical and biological reactors, involve the direct immobilization of autotrophic denitrifying microorganisms on the surface of the cathode and the production of hydrogen gas as an electron donor by the electrolysis of water (Feleke et al., 1998). However, traditional BERs consume significant amounts of electrical energy to produce CO_2 and H_2, and have a relatively low efficiency. To enhance BER performance, it is possible to enlarge the effective electrochemical activity of the three-dimensional (3D) electrode by providing a large effective area (Hao et al., 2013). A 3D-BER has been investigated for the treatment of groundwater (Zhou et al., 2007), landfill leachate (Nageswara Rao et al., 2009), groundwater containing nitrate and organic

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pollutants (Zhou et al., 2009), Acid Orange 7 wastewater (Zhao et al., 2010), and municipal wastewater (Hao et al., 2013).

Antibiotics are among the most commonly used and successful group of pharmaceuticals applied in human and animal medicine (Bouki et al., 2013). China is the global leader in antibiotic production. Approximately 210,000 t of antibiotics are produced annually, of which 85% is utilized in animal agriculture and medicine (Luo et al., 2011). However, the majority of the applied antibiotics are excreted through feces and urine in an unchanged form because they are weakly absorbed and do not completely metabolize in the gut of humans and animals (Huang et al., 2015). Excreted antibiotics eventually enter river environments through a variety of pathways, including point discharges from wastewater treatment plants (WWTPs), animal feeding operations (AFOs), and fish hatcheries or nonpoint sources, such as overland flow from fields where manure or biosolids have been applied (Storteboom et al., 2010). The overuse and misuse of antibiotics has led to the occurrence and spread of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs), which raises additional concerns and poses a health risk to humans. In addition, antibiotics promote ARG transfer between nonpathogenic and pathogenic bacteria by vertical gene transfer (VGT) and horizontal gene transfer (HGT) (Huang et al., 2015). Hence, treatment processes that are effective and inexpensive for the removal of antibiotics, ARBs, and ARGs are required.

However, novel 3D-BER technology is still in its early developmental period, and the potential advantages and risks of a 3D-BER for antibiotic removal are still a cause of concern (Zhou et al., 2009; Hao et al., 2013). In this study, a 3D-BER was used to treat artificial wastewater containing sulfamethoxazole (SMX) and tetracycline (TC) for eight months. In addition, three *sul* genes (*sulI, sulII,* and *sulIII*) and five *tet* genes (*tetA, tetC, tetO, tetQ,* and *tetW*) were chosen as representatives of the antibiotic resistance genes due to their frequent reporting in livestock manure and wastewater treatment lagoons (Liu et al., 2014; Wu et al., 2015). The main objectives of the study were: (1) to analyze the effect of electrical stimulation in the 3D-BER on the removal efficiencies of chemical oxygen demand (COD), SMX, and TC from synthetic domestic sewage, (2) to assess the influence of electrical stimulation on the development of ARGs during the treatment process; and (3) to study the potential impact of low current density on electrochemical characteristics, dehydrogenase activity (DHA), and the microbial community.

2. Materials and methods

2.1. Experimental design and configuration of 3D-BERs

Experiments were conducted indoors in four 3D-BERs, as shown schematically in Fig. 1. The 3D-BER was assembled using a polyacrylic plastic column (internal diameter: 140 mm; height: 350 mm). The reactor was composed of four layers (from upper to lower): (1) a 20-mm active carbon fiber layer/titanium mesh anode, (2) a 200-mm layer filled with a granular-activated carbon (GAC, diameter: 3–5 mm; specific area: 500–900 m^2 g^{-1}), (3) a 20-mm active carbon fiber layer/titanium mesh cathode, and (4) a 60-mm layer with gravel (diameter: 3–4 mm). The volume of the whole container was 10.1 L, with an effective working volume (liquid volume) of 3.3 L.

2.2. Inoculation, system start-up and experiments

Anaerobic sludge (mixed liquor suspended solids (MLSS): 60 g L^{-1}) was obtained from the South City Municipal Wastewater Treatment Plant of Nanjing, China. The sludge was pretreated by mixing with GAC (sludge volume ratio of 2.5:1) and introduced into a 3D-BR and 3D-BER for microbial inoculation. Inactivated sludge (autoclaving: 121 °C, 15 min) was mixed with GAC (sludge volume ratio 2.5:1) and introduced into a 3D-R and 3D-ER. The nutrient solution contained glucose (0.125 g L^{-1}), NH_4Cl (0.025 g L^{-1}), KH_2PO_4 (0.005 g L^{-1}), NaCl (0.1 g L^{-1}), and 0.15 mL of a trace essential element solution (Cao et al., 2015).

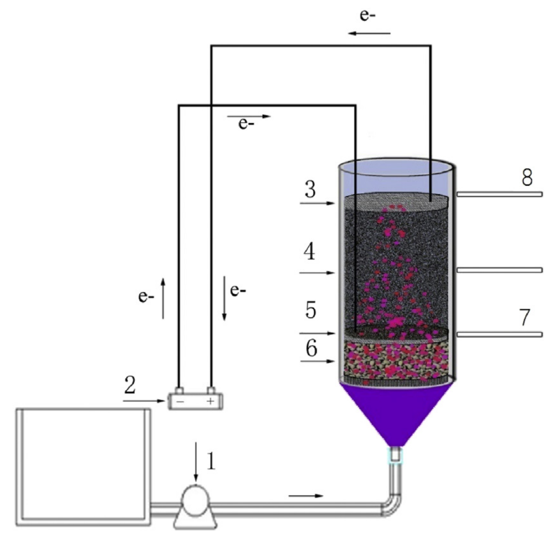

Fig. 1. Schematic diagram of the 3D-BER: (1 peristaltic pump; 2 DC regulated power supply; 3 active carbon fiber/Ti mesh anode; 4 granular activated carbon; 5 active carbon fiber/Ti mesh cathode; 6 gravel; 7 cathode effluent; 8 anode effluent.).

The nutrient solution was fed continuously into the 3D-BERs using peristaltic pumps (BT100-1L, Baoding Longer Precision Pump Co., Ltd., Baoding, China). The concentrations of TC and SMZ in the influents were 200 μg L^{-1} for all 3D-BERs. The hydraulic retention time was 40 h. All experiments were conducted in the dark at 28 ± 2 °C. The experiment was conducted for eight months from April 2015 to November 2015. The 3D-ER and 3D-BER were operated under a continuous voltage of 0.8 V provided by a DC power supply (LP3003D; Shenzen Lodestar S.T Co., Ltd., Shenzen, China) (Liu et al., 2015). The reactors were defined as follows: 3D-R, reactor without biological sludge and no voltage; 3D-ER, reactor without biological sludge, under a voltage of 0.8 V; 3D-BR, reactor with biological sludge and no voltage; 3D-BER, biofilm-electrode reactor under a voltage of 0.8 V. The current density ranged from 31.5 to 46.3 mA m^{-2}.

2.3. Quantification of antibiotics and COD removal

Effluent samples at the anode and cathode were collected in triplicate during three sampling periods in June, September, and November of 2015. The antibiotics in the effluents were detected using an LC-MS system (LCQAD-60000, Thermo Fisher Scientific, Waltham, MA, USA). Water samples were filtered through 0.45-μm fiber filters and extracted using Oasis HLB (6 mL, Waters, Milford, MA, USA) extraction cartridges. Antibiotics analysis in the samples was based on a published method (Huang et al., 2015; Wu et al., 2015). The antibiotics concentrations were determined from external calibration curves; the determination coefficients (R^2) exceeded 0.996 (Table SM-1). The COD in the effluents were determined in May, June, July, August, and November of 2015, based on guidelines provided by the American Public Health Association (APHA, method 5220).

2.4. DNA extraction and ARG analysis

GAC samples from the anode and cathode were collected in