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Effects of voltage on sulfadiazine degradation and the response of *sul* genes and microbial communities in biofilm-electrode reactors

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ABSTRACT

Few studies have been performed on both the potential and the risks of biofilm-electrode reactors (BERs) with regard to the removal of antibiotics. This study used 33 BERs to investigate the removal rate and degradation pathway of sulfadiazine (SDZ). Furthermore, the effects of additional electrons on *sul* genes and microbial community composition were examined. The study found that rapid elimination rates of 20 mg/L SDZ were observed during the first 3 h with different DC voltage rates. Even high concentrations (160 mg/L) could be rapidly removed after 24 h of system operation. Pyrimidin-2ylsulfamic acid and aniline were noted to be principal products, and an SDZ degradation mechanism was proposed. The study identified 41 species of microorganism; based on bacterial community divergence caused by voltage, and six samples were grouped into four clusters. The relative abundances of *sul* genes from biofilm were in the following order: *sulII* > *sulIII* > *sulI* > *sulA*. The *sulI*, *sulII*, and *sulA* genes were enhanced with electrical stimulation in the cathode layer. It is noteworthy that *sul* genes were not detected in the effluent after 24 h of operation.

1. Introduction

Sulfonamide antibiotics are widely used to treat infections. They are also used for growth promotion in industrial livestock farming as well as in aquaculture, resulting in large residue inputs into the environment (Cheng et al., 2015; Engelhardt et al., 2015). Sulfonamide antibiotics have frequently been detected in water resources and soil, indicating their persistence and insufficient degradation in natural environments (Deng et al., 2016; Zhang et al., 2017). The benefits of global antibiotic use have come at the cost of bioaccumulation in the environment and the promotion of growing antibiotic-resistant bacteria (ARB) (Yamashita et al., 2017) and antibiotic resistance genes (ARGs), which confer a significant risk to both the environment and human health (Bergeron et al., 2015). Sulfadiazine (SDZ) as a “high priority” sulfonamide and it is frequently discovered in natural environments (Deng et al., 2016). It is essential to comprehend the environmental fate of SDZ and its biodegradation, which has been reported to have an significant role in sulfonamide removal in rivers, soil and biological waste treatment systems (Li and Zhang, 2010).

Investigations of biological treatments have been devoted to the elimination of SDZ. Li et al. (2010) reported that the removal efficiencies of SDZ via biodegradation were calculated as 23.8% with a hydraulic retention time (HRT) of 17 h in the activated sludge process. Tappe et al. (2013) found that the SDZ molecule was pervasively mineralized and the phenyl moiety was partly assimilated by *Microbacterium lacus*. The biodegradation of antibiotics presents a challenge, as functional groups in antibiotics are resistant to biodegradation by virtue of their lethality to microbial communities (Srinivasan and Sarmah, 2014). Moreover, compared with chemical oxidation methods, biodegradation is advantageous due to its low operational costs, robustness, and wide applicability, but it is a time-consuming process, as SDZ is not readily biodegraded (Li and Zhang, 2010). Therefore, effective, inexpensive processes must be developed for the removal of antibiotics. Bioelectrochemical systems have been applied to enhance the removal performance of sulfonamide antibiotics via redox reactions. Harnisch et al. (2013) reported that sulfonamides can be principally removed from wastewater using microbial bioelectrochemical systems. In addition, Wang et al. (2015) found that sulfonamides (e.g., 200 mg/L) could be rapidly degraded using microbial fuel cells. Furthermore, Zhang et al. (2016a) revealed that ~90% of 200 μg/L sulfonamide antibiotic was degraded in a microbial bioelectrochemical system with an HRT of 17 h.

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Biofilm-electrode reactors (BERs) are electrochemical and anaerobic biological reactors that enhance the biological degradation of pollutants (Sakakibara and Kuroda., 1993). Unfortunately, traditional BERs consume substantial amounts of electrical energy to produce carbon dioxide and hydrogen gas, and have relatively low efficiencies (Feleke et al., 1998). However, it is still early in the development of BERs for the treatment of landfill leachate, nitrate, and organic pollutants (Nageswara Rao et al., 2009). Furthermore, previous studies found that cells throughout the biofilm are metabolically active (Franks et al., 2010), and the effective diffusion coefficients in biofilm respiration are improved in bio-electrochemical processes (Renslow et al., 2013). These findings suggest that BERs are a promising tool for the degradation of recalcitrant chemicals.

Based on the previously demonstrated persistence of anodic microbial biofilms in bioelectrochemical systems against antibiotics at high concentrations, it became clear that these biofilms were toxic to planktonic cells (Harnisch et al., 2013). Additionally, some of the degradation products of sulfonamides may be toxic (Wang et al., 2016). Investigating the degradation mechanism and by-products would support evaluations of the environmental risks and fate of SDZ. In addition, the effects of electrical stimulation on ARGs during anaerobic processes of BERs, which serve as a candidate for energy-efficient antibiotic removal, are of great interest. Therefore, the purposes of this experiment were to (1) explore the removal and degradation pathway of SDZ (2) and examine the effects of electrons on the development of ARGs and the microbial community composition in a BER.

2. Materials and methods

2.1. BER configuration

The BER consisted of a glass column (6 cm in height and 3.5 cm in diameter) as an electrolytic tank. Graphite as the anode (5 cm in length, 0.3 cm in diameter) was installed at the axial center. Active carbon fiber (5 cm in height, 5.5 cm in inner diameter, 1.2 mm thickness) and a stainless steel ring (5 cm in height, 5.5 cm in inner diameter, and 1 mm in thickness; Nanjing Zhongdong Chemical Glass Instrument Co., Ltd., Nanjing, China) with conductive adhesive (Nanjing Zhongdong Chemical Glass Instrument Co., Ltd., Nanjing, China) were used around the anode as the cathode. The effective working volume of the reactor was 0.55 L.

2.2. System operation

Anaerobic sludge was used to inoculate 33 reactors (the sludge was composed of mixed liquor suspended solids, 60 g/L; Municipal Wastewater Treatment Plant of Nanjing, China). Continuous voltages (LP3003D; Shenzen Lodestar S.T. Co., Ltd., Shenzen, China) of 0 (no voltage), 0.3 (anode: 0.067 V, cathode: 0.242 V), 0.6 (anode: 0.369 V, cathode: −0.255 V), 0.9 (anode: 0.649 V, cathode: −0.268 V), 1.2 (anode: 0.936 V, cathode: −0.276 V), and 1.5 V (anode: 1.248 V, cathode: −0.285 V) were applied separately to evaluate the effects of electricity on the BER with regard to the elimination of SDZ (20 mg/L) (LEICI 323 reference electrode; Shanghai Instrument Scientific Instrument Co., Ltd.). The current density ranged from 0 to 6.213 A/m^3. Furthermore, SDZ concentrations of 10, 20, 40, 80, and 160 mg/L under 0.9 V were tested to evaluate the removal efficiency of SDZ using BERs. The current density ranged from 1.243 to 5.812 A/m^3 in the systems (32 ± 1 °C). The mineral solutions were changed biweekly. The SDZ removal performance of the system was measured after four weeks of operation. The nutrient solution contained glucose (0.125 g/L), KH_2PO_4 (0.005 g/L), NaCl (0.1 g/L), NH_4Cl (0.025 g/L) and 0.15 mL of a trace essential element solution containing (per liter) $CaCl_2$ 15 g, $MgSO_4{\cdot}7H_2O$ 15 g, $FeSO_4$ 1 g, $MnSO_4{\cdot}H_2O$ 2.2 g, $CoCl_2{\cdot}6H_2O$ 0.24 g, $ZnSO_4{\cdot}7H_2O$ 2 g, $FeCl_3{\cdot}6H_2O$ 10 mg, $CuCl_2{\cdot}2H_2O$ 1 mg, $NiCl_2{\cdot}6H_2O$ 2 mg, $ZnCl_2$ 5 mg, and $Na_2MoO_4{\cdot}2H_2O$ 0.4 mg.

2.3. SDZ analysis

Water samples were filtered through 0.22-mm fiber filters before testing. An Agilent 6400 Series Triple Quad LC/MS System was used to analyze SDZ at 269 nm (Agilent Technologies, USA). Separation of SDZ was performed with a Phenomenex C18 column (250 mm × 4.6 mm; Thermo). The mobile phase was composed of 0.1% formic acid solution (85%) and acetonitrile (15%) (Wu et al., 2015). SDZ concentrations were determined from external calibration curves and the determination coefficients (R^2) exceeded 0.996.

The degradation products of SDZ were determined by mass spectrometry (Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap; Thermo Fisher Scientific, CA, USA) and an UltiMate™ 3000 RSLCnano (Thermo Fisher Scientific, CA, USA) in both positive and negative electrospray ionization modes (Wang et al., 2016). Chromatography was performed with a Hypersil GOLD C18 column (Acquity UPLC BEH C18; 100 mm × 2.1 mm, 3 μm) with a mobile phase of acetonitrile (15%) and 0.1% formic acid solution (85%) at a rate of 0.3 mL/min (Wu et al., 2015). The capillary temperature was 350 °C, the capillary voltage was 3.8 (±) kV, and the collision energy was 8 eV. The collision energy was set to 50 eV for high-energy collisional dissociation fragmentation, and the sheath gas pressure was set at 35 arb.

2.4. Microbial community analysis

First, 5 g active carbon fiber of each cathode layer was collected and smashed under sterile conditions four weeks of operation, and DNA extraction was performed using the Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). The DNA concentration was tested with a UV-9100 microspectrophotometer (Lab Tech Ltd., Beijing, China), and sample integrity was tested by agarose gel electrophoresis. Each qualified DNA segment was used to construct a library. Bioinformatics analysis will soon be carried out with the Illumina platform (BGI Co., Ltd., Shenzhen, China) (Bokulich et al., 2013).

2.5. ARG analysis

Cathode layer samples (5 g) were collected in 0 and 1.5 V BERs (20 mg/L) after four weeks and 24 h of operation, respectively. DNA was extracted using a DNA isolation kit (MoBio, Carlsbad, CA, USA). *Sul* resistance genes (*sulI, sulII, sulIII*, and *sulA*) and the 16 S rRNA gene were quantified using a CFX Connect Real-Time PCR System (Wcgene Biotechnology, Shanghai) (Zhang et al., 2016a). The qPCR conditions, primer sequences targeting these genes, and the PCR protocol were based on previously reported methods (Rodriguez-Mozaz et al., 2015; Wu et al., 2015). Each reaction contained 1.2 μL of primers (forward and reverse primers, 0.6 μL of 10 mM each; Bio-Rad), 0.8 μL of DNA templates, 12 μL of SYBR Green qPCR Mix (Bio-Rad), and 11 μL of ddH_2O (Bio-Rad) (Zhang et al., 2016b). Each PCR reaction was carried out in triplicate. Regression coefficients (R^2) were higher than 0.990 for standard curves.

2.6. Measurement and statistical analysis

Taxonomic ranks were assigned to OTU using the Ribosomal Database Project (RDP). To display the differences in OTU composition from the different samples, a two-dimensional graph was constructed from a principal component analysis (PCA) to summarize the main factors responsible for this difference. Heat maps were generated using the R software package (v3.1.1). All data were analyzed with Microsoft Excel 2010, and other figures were plotted in SigmaPlot ver. 11.0.