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Rapid degradation of sulphamethoxazole and the further transformation of 3-amino-5-methylisoxazole in a microbial fuel cell

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ABSTRACT

Sulphamethoxazole (SMX) is extensively used in humans and livestock, but its appearance in natural water raises environmental concerns. This study demonstrated that SMX and its degradation product, 3-amino-5-methylisoxazole (3A5MI), could be effectively degraded in microbial fuel cell (MFC) reactors. Approximately 85% of 20 ppm SMX was degraded within 12 h, and this was a more rapid biodegradation rate than has been previously shown in the literature. In addition, 3A5MI, a toxic chemical that forms in the SMX degradation process, can be further mineralized. The degradation products of SMX were detected by mass spectrometry, and three speculated by-products were confirmed with chemical standards. It was observed that nitrogen atoms of SMX were progressively eliminated during the degradation process, which may relate with the degradation of SMX and 3A5MI. An antibacterial activity test showed that the biotoxicity of SMX towards *Shewanella oneidensis* MR-1 and *Escherichia coli* DH5α was greatly reduced after MFC treatment. Moreover, the ATP level of the MFC microbe was nearly threefold higher than that in open-circuit controls, which may be related to the rapid degradation of SMX in MFCs. This study can facilitate further investigations about the biodegradation of SMX.

1. Introduction

Few chemicals have profoundly improved human wellbeing as much as antibiotics. However, pharmaceutical factories, medical facilities, the breeding industry and patients discharge a large quantity of antibiotics. Because water-treatment facilities merely satisfy current standards, many emerging pollutants, such as pharmaceuticals and personal care products, are discharged into receiving waters without sufficient removal (Avisar et al., 2010). The long-term effects of antibiotics, including those bio-accumulated in food chains and boosting the growth of drug-resistant bacteria, severely threaten human health (Brown et al., 2006). On the other hand, the functional groups of antibiotics providing pathogen control may also hinder the biodegradation process, which makes the biodegradation of antibiotics a challenge.

Sulphamethoxazole (SMX) is a broad-spectrum antibiotic that is largely consumed in the breeding industry because it is cheap and effective. SMX is not easily biodegraded (Larcher and Yargeau, 2012; Onesios et al., 2009) and widely exists in the effluent of wastewater-treatment plants (Batt et al., 2006; Clara et al., 2005) and surface water (Wei et al., 2011). Numerous efforts have been devoted to study the removal of SMX by biological treatment. Müller et al. found that 50 ppm of SMX could be removed within 3 days if SMX was the sole carbon and nitrogen source in activated sludge reactor (Müller et al., 2013); Harnisch et al. reported that 6 ppb of SMX could be removed in microbial electrolysis cells within 7 days (Harnisch et al., 2013); Xu et al. reported that approximately 85% of 30 ppm SMX could be degraded in sediment system within 40 days, while the degradation of SMX was greatly affected by the ambient temperature but not readily related with its initial concentration (Xu et al., 2011). Moreover, certain microbes, such as *Rhodococcus rhodochrous* (Gauthier et al., 2010), *Rhodococcus equi* (Larcher and Yargeau, 2011), *Brevundimonas* sp. SMXB12 (Herzog et al., 2013), and *Pseudomonas psychrophila* HA-4 (Jiang

Abbreviations: 3A5MI, 3-amino-5-methylisoxazole; MFC, microbial fuel cell; ESI–Q-TOF/MS, electrospray ionization-quadrupole time-of-flight mass spectrometry; AC, abiotic control; HPLC, high-performance liquid chromatography; OC, open circuit control; SMX, sulphamethoxazole; UPLC, ultra-performance liquid chromatograph.

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et al., 2014), have been isolated for the degradation of SMX.

However, studies always refer to "removal" based on the SMX concentration change, rather than providing detailed information about the degradation mechanism. Additionally, certain degradation products of SMX may pose a toxic threat to animals (Gonzalez et al., 1995). For example, researchers found that 3-amino-5-methylisoxazole (3A5MI) was formed in both the chemical (Gómez-Ramos et al., 2011; Gao et al., 2014; Trovó et al., 2009) and microbial (Jiang et al., 2014; Ricken et al., 2013) degradation of SMX, but few studies have reported its further transformation. Because the degradation products of micro-pollutants may surpass their parent forms in either concentration or biotoxicity (Stadler et al., 2012), studies on the degradation mechanism and fate of degradation by-products would greatly assist in the evaluation of the environmental risk of SMX.

Electrodes have been used to eliminate recalcitrant chemicals via redox reactions, and researchers have determined that bio-electrochemical processes can enhance the removal rate of contaminants (Liang et al., 2013; Luo et al., 2009; Wang et al., 2012). Theoretically, the growth of anaerobe was restricted by the availability of electron acceptors, while the anode in microbial fuel cell could serve as an inexhaustible electron acceptor for microbes to enhance their degradation of organic contaminants under anaerobic conditions (Lovley and Nevin, 2011; Yan et al., 2012). Besides, researchers found that the microbe throughout the bulk of the electrode biofilm was metabolically active (Franks et al., 2010), and bio-electrochemical processes may improve the diffusion of molecules in biofilm (Renslow et al., 2013). These factors may make the microbial fuel cell a promising candidate for the removal of environmental contaminants. Since the explorations about utilizing microbial fuel cell to degrade recalcitrant chemicals (such as antibiotics) are still scarce, whether SMX could be removed in bio-electrochemical reactors aroused our interest.

Therefore, the main purpose of this study was to investigate the removal of SMX in microbial fuel cells. The removal dynamics of SMX were analysed and the degradation pathway of SMX was proposed. Then, the antibacterial effects of SMX degradation by-products were examined. Finally, the microbial activity of suspended microbes was tested to reveal the relationship between SMX degradation and microbe activity. These results may provide a systematic elucidation of the biodegradation of SMX in MFC.

2. Materials and methods

2.1. Chemicals and reagents

Sulphamethoxazole (>99%) was purchased from Sigma–Aldrich (St. Louis, USA). The molecule 3-amino-5-methylisoxazole (3A5MI, >97%) was purchased from Tokyo Chemical Industry (Tokyo, Japan). Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Aladdin Industrial Corporation (Shanghai, China). All other chemicals used were of analytical grade and purchased from Sinopharm Group Co. Ltd. (Shanghai, China). SMX was dissolved in methanol at a concentration of 25 mg/mL (SMX stock solution), and 3A5MI was dissolved in methanol at a concentration of 50 mg/mL (3A5MI stock solution). Both stock solutions were stored in a cabinet without light. Pure water (18 mΩ) was prepared using a Milli-Q system (Millipore, Germany). The effluent sample was filtered through a 0.22-μm-pore-size filter (Millipore, Germany) before test.

2.2. Reactor setup and operation

Two-chamber MFC reactors were assembled by bolting acrylic glasses into a hollow cube, and each chamber had a 115-mL working volume. The anode and cathode chamber were separated by a cation-exchange membrane (Zhejiang Qianqiu Group Co., Ltd., China). Carbon felt (Haoshi Carbon Fiber Co., Ltd., China, 4 × 4 cm^2) was used as an electrode and connected with titanium wire (1 mm in diameter, Shanghai GuiTai Titanium Group Co., Ltd., China), with an external load of 1000 Ω. The output voltages of the bio-electrochemical reactors were recorded with a digital multimeter (Keithley Instruments, Inc., USA).

The anode chamber of MFCs was inoculated with 20 mL of sludge collected from a thermostatic anaerobic digester (Hayi coal gasification wastewater treatment plant, Harbin, China) and fed with artificial wastewater (1 g/L sodium acetate, 20 mM phosphate buffered solution, pH 7.0 and 2 mL mineral solution (Lovley and Phillips, 1988)). The reactors were kept in an incubator with the temperature maintained at 35 ± 2 °C, and the solution was changed in batch mode. No stirring or oxygen was provided during the operating process, and the dissolved oxygen in the anolyte was less than 0.5 mg/L. One month later, three reactors were randomly selected and operated under open-circuit (OC) mode, and another three reactor electrodes were autoclaved and set as an abiotic control (AC), as illustrated in Fig. 1. Then, SMX with 20 mM PBS and a mineral solution was injected into the anode chamber of the reactors (the final concentration of SMX was 20 ppm), and the anolyte was changed biweekly. Six months later, we observed that SMX could be rapidly removed from the anode chamber, so the anolyte was changed weekly. A potassium ferricyanide solution (100 mM $K_3[Fe(CN)_6]$ and 20 mM PBS) was utilized as the catholyte and replaced biweekly.

2.3. Analytical methods

The SMX and 3A5MI concentrations were detected using Hitachi L-2000 series high-performance liquid chromatography (HPLC), with UV detection at 203 nm for SMX and 200 nm for 3A5MI. SMX separation was achieved by a Phenomenex C18 column (Luna, 4.6 × 250 mm, 5 μm) with mobile phases of water (70%) and acetonitrile (30%) at a flow rate of 1.0 mL/min. 3A5MI separation was achieved by an Agilent column (Zorbax Eclipse Plus C18, 4.6 × 250 mm, 5 μm) with a mobile phase of water (100%) at a flow rate of 1.0 mL/min. Effluent samples were filtered through a membrane of 0.22μm pore size before testing.

Analysis of the SMX degradation products was performed using ultra-performance liquid chromatography (UPLC, Waters, USA) and electrospray ionization-quadrupole time-of-flight mass spectrometry (ESI–Q-TOF/MS, Bruker, Germany). A Waters C18 column (Acquity UPLC BEH C18, 2.1 × 100 mm, 1.7 μm) was used with a flow rate of 0.2 mL/min (30% acetonitrile and 70% water). Both negative and positive modes of electrospray ionization (ESI) were used to detect samples, with a capillary voltage of 2.8 kV and a collision energy of 8 eV. The nebulizer was set at 1.6 bar, and the flow of dry gas was set at 8.0 L/min (Dry Temp: 200 °C). High-purity nitrogen (99.9996%) was used as the collision gas.

2.4. Antibacterial activity measurement

Shewanella oneidensis strain MR-1 and *Escherichia coli* DH5α were cultured in the reactor effluent samples (sterile). One-hundred -ninety milligrams of SMX per litre was added into the MFC reactors and maintained for one week. Then, the reactor effluent (approximately 120 mL) was centrifuged (7500 rpm, 20 min) three times, and the suspension was filtered through a Millipore filter (0.22μm pore size).

Three test groups were used: the control group (containing 20 mL Luria–Bertani medium and 80 mL 20 mM PBS, pH 7.0), the reactor effluent group (containing 20 mL Luria–Bertani medium and 80 mL reactor effluent) and the SMX group (containing 20 mL