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Cometabolic degradation of chloramphenicol via a *meta*-cleavage pathway in a microbial fuel cell and its microbial community

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HIGHLIGHTS

- The MFC with acetate as electrons donor increased the removal rate of CAP.
- The CAP degradation was optimized using Box-Behnken model.
- Antibacterial activity of CAP was eliminated after treatment by MFC.
- The electrogenic bacteria enriched in MFC under the closed-circuit mode.

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ABSTRACT

The performance of a microbial fuel cell (MFC) in terms of degradation of chloramphenicol (CAP) was investigated. Approximately 84% of 50 mg/L CAP was degraded within 12 h in the MFC. A significant interaction of pH, temperature, and initial CAP concentration was found on removal of CAP, and a maximum degradation rate of 96.53% could theoretically be achieved at 31.48 °C, a pH of 7.12, and an initial CAP concentration of 106.37 mg/L. Moreover, CAP was further degraded through a ring-cleavage pathway. The antibacterial activity of CAP towards *Escherichia coli* ATCC 25922 and *Shewanella oneidensis* MR-1 was largely eliminated by MFC treatment. High-throughput sequencing analysis indicated that *Azonexus*, *Comamonas*, *Nitrososphaera*, *Chryseobacterium*, *Azoarcus*, *Rhodococcus*, and *Dysgonomonas* were the predominant genera in the MFC anode biofilm. In conclusion, the MFC shows potential for the treatment of antibiotic residue-containing wastewater due to its high rates of CAP removal and energy recovery.

1. Introduction

Since the discovery of the first natural antibiotic, penicillin, by Fleming, many natural antibiotics or their derivatives have been applied in medicine, animal feed, and agriculture (Andersson and Hughes, 2014). However, their widespread use has resulted in antibiotic residues becoming a serious problem. At present, medical facilities and pharmaceutical factories, particularly the animal-breeding industry, discharge large quantities of various antibiotics (Naquin et al., 2015), but few water-treatment plants have strictly implemented current standards, resulting in discharge of residual antibiotics into the environment (Wang et al., 2016). Residues of antibiotics in the environment are not only considered emerging contaminants but also pose a potential threat to human and animal health worldwide (Berendonk et al., 2015). The long-term effects of antibiotic residues include their bioaccumulation through the food chain and stimulation of the growth and spread of drug-resistant bacteria (Sarmah et al., 2006).

The broad-spectrum nitroaromatic antibiotic, chloramphenicol (CAP), was the first synthetic antibiotic introduced into clinical practice on a large scale (Henry et al., 1981). Due to its cost-effectiveness, it has been extensively used in the animal breeding industry globally since its discovery. Due to its serious toxicity (bone-marrow depression and aplastic anemia) in humans and animals (Martelli et al., 1991), CAP has been banned for use in food-producing animals in many developed countries; however, it is still extensively used in many developing countries because of its low cost. CAP is frequently present in the effluent of breeding farms

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and water-treatment plants (Fan et al., 2010; Resende et al., 2014). One or more functional groups is essential for the antibacterial activity of most antibiotics; the 1,3-propanediol formation and nitro group are the functional groups of CAP (Miller and Halpert, 1986). However, most studies have focused on the change in CAP concentration rather than the degradation pathway. Furthermore, acetylate and amine metabolites of CAP are produced by its microbial degradation (Yun et al., 2016), but whether they are further degraded is unclear. These aromatic metabolites pose a potential risk to human health (Kadlubar et al., 1992).

Various physicochemical methods have been used to investigate CAP degradation, such as photocatalytic reduction, zero-valent bimetallic nanoparticles, and the Fenton process (Sun et al., 2013). However, most of these methods consume energy and/or chemicals. Bioelectrochemical systems (BESs), including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) with biocatalyzed electrodes, can be used to degrade various pollutants (e.g., transforming nitroaromatics) into amino-aromatic compounds and to remove antibiotics such as sulfamethoxazole, ceftriaxone and ampicillin (Körbahti and Taşyürek, 2015; Liang et al., 2014; Wang et al., 2016; Wen et al., 2011). Biocatalytic electrochemical cells have also been used to degrade CAP using an external voltage and glucose as the electron donor (Sun et al., 2013). Unlike MECs, MFCs do not require an external input voltage and consume less energy. CAP degradation by MFCs with acetate as the sole electron donor has not been extensively investigated; therefore, CAP removal using MFC reactors requires further research.

This study investigated the removal of CAP by an MFC with acetate as the sole electron donor. A Box-Behnken experimental design (BBD) combined with response surface methodology (RSM) was applied to optimize the CAP degradation conditions. CAP metabolites were identified by mass spectrometry, their antibacterial activity was evaluated, and the degradation pathway was deduced. In addition, high-throughput sequencing was employed to analyze the microbial composition of MFC biofilm.

2. Materials and methods

2.1. Chemicals and reagents

CAP (>98% purity) was purchased from Solarbio (Beijing, China). High-performance liquid chromatography (HPLC)-grade methanol was purchased from Sigma-Aldrich (St. Louis, MO, USA). The ultrapure water used in this experiment was generated by a MilliQ system (Bedford, MA, USA). All of the other chemicals were of analytical reagent grade and were obtained from commercial sources.

2.2. Reactor setup

Dual-chamber MFC reactors separated by cationic exchange membranes (CMI-7000s, Membrane International Inc., Ringwood, NJ, USA) were assembled. Each chamber of the MFC reactor had a working volume of 120 mL. Active-carbon felt (3 cm diameter, 3 cm length) was used for the cathode and anode. The two electrodes were connected via a titanium wire (1 mm in diameter) with an external load of 1000 Ω. The MFC reactor output voltages were recorded using a digital multimeter (Keithley 2700, Cleveland, OH, USA).

2.3. Reactor operation

Before starting the MFC reactors, the microbial consortium used as the anode inoculum was pre-enriched using activated anaerobic sludge obtained from a wastewater treatment plant (Mianyang, China). The anaerobic sludge was fed with medium containing acetate in 50 mM phosphate buffer solution (PBS; 2.45 g/L $NaH_2PO_4{\cdot}H_2O$, 4.57 g/L Na_2HPO_4, 0.13 g/L KCl, 0.31 g/L NH_4Cl) supplemented with 5 mL/L vitamins and 12.5 mL/L mineral solutions in the presence of 50 mg/L CAP (Lovley and Phillips, 1988; Zhang et al., 2015).

To start the MFC reactors, the anode chambers were inoculated with the anaerobic-acclimated sludge. Then CAP was injected into the anodes of the MFC reactors (final concentration, 50 mg/L). The reactors were randomly divided into three groups and operated in closed-circuit mode, open-circuit (OC) mode, and abiotic control (anodes were autoclaved). The OC test without a circuit load was regarded as the traditional anaerobic digestion test. The medium was purged with N_2 gas (99.9%). After 6 months, CAP was rapidly degraded in the anode chamber and the anolyte was changed weekly thereafter. The catholyte was potassium ferricyanide solution (50 mM $K_3[Fe(CN)_6]$ in 50 mM PBS) and was replaced after each cycle. For sampling, the anolyte was withdrawn from the anode chamber and filtered through 0.22 μm porosity polytetrafluoroethylene (PTFE) membranes prior to chemical analysis. Experiments were performed at room temperature (28 ± 2 °C) and the anode chambers were covered with silver paper to exclude light.

2.4. Analytic method

The CAP concentration was determined using an Agilent 1260 HPLC system (Agilent, Santa Clara, CA, USA) with UV detection at 275 nm. CAP separation was achieved using an Agilent C18 column (4.6 × 250 mm, 5 μm) with a methanol/water mobile phase at a flow rate of 0.6 mL/min. CAP removal was calculated using the following equation:

$$P = (A - B)/A \times 100\% \quad (1)$$

where P is the CAP removal percentage, A is the initial concentration of CAP in the anolyte, and B is the CAP concentration in samples. Analysis of CAP degradation products was performed by electrospray ionization-quadrupole time-of-flight mass spectrometry (ESI-Q-TOF-MS; Bruker, Germany). Positive and negative ESI modes with a capillary voltage of 4.5 kV were used to detect samples. The nebulizer was set at a 0.8 bar and the flow of dry gas was set at 6.0 mL/min (dry heater at 180 °C). High-purity nitrogen (N_2) (99.999%) was used as the collision gas.

2.5. Optimization of the degrading conditions

Based on the results of a single-factor experiment, the temperature, pH, and initial CAP concentration were selected as three independent variables. The dependent variable was the CAP degradation rate over 3 days in the MFC reactors. A three-factor and three-level BBD of RSM was applied to evaluate the combined effects of three independent variables on the CAP degradation rate using the Design Expert (DX) 8.0.5 software (Stat-Ease Inc., Minneapolis, MN, USA). The design matrix and levels of each variable are shown in Table 1. Seventeen experimental sets were conducted to assess the interactive effects of the three independent variables,

Table 1
Range and levels of independent variables and code values in BBD.

Independent variables	Symbols	Range and levels		
		−1	0	1
pH	X1	6.0	7.0	8.0
Temperature (°C)	X2	20	30	40
CAP concentration (mg/L)	X3	100	150	200