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HOW DOES LONG-TERM EXPOSURE TO CIPROFLOXACIN AFFECT THE PERFORMANCE OF A MICROBIAL FUEL CELL?

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ABSTRACT

Ciprofloxacin (CIP) is a synthetic antibiotic frequently detected in wastewater that poses potential risks to aquatic environments. The prolonged effects of CIP under environmental and stressed concentrations over the anodic and cathodic microbial communities of a microbial fuel cell (MFC) were investigated in this study. Two identical MFCs, namely MFC-CTL (control) and MFC-CIP were operated for 703 days. MFC-CIP was exposed to increasing concentrations of CIP to evaluate its performance regarding electricity generation and removal of COD and N. Except for COD removal, the performance indicators were reduced as we increased CIP concentration, mainly in stressed conditions. Also, reducing the R_{ext} from 1000 Ω to 560 and 100 Ω reduced CIP inhibitory effects on the biocathodes processes. However, electrochemical characterization suggested persistent damage to electricity generation due to increased anodic internal resistance and lower maximum power output in MFC-CIP at high CIP concentrations. Such findings demonstrate the resilience of bioprocesses for COD and N removal in dual-chamber MFCs when exposed to CIP-containing wastewater.

Keywords: Micropollutant. Bioelectrochemical systems. Waste-to-energy. Inhibition.

1 INTRODUCTION

The consumption of fluoroquinolones has increased considerably in recent years in several countries. In Brazil, fluoroquinolone consumption increased by 600% from 2000 to 2015, becoming the third most consumed antibiotic class, behind penicillins and macrolides[1]. Consequently, fluoroquinolones are detected in wastewater and several environmental matrices (surface and groundwater, soil, plants, and animals), with ciprofloxacin (CIP) being the most frequently detected compound in these samples [2,3]. Moreover, the World Health Organization considers CIP a critically important antibiotic[4], and its potential risks to aquatic environments are not yet well understood.

Microbial fuel cell (MFC) is a cutting-edge technology for harvesting electrons from organic waste oxidation to produce electricity. MFC has been successfully applied to remove complex compounds from wastewater, e.g., dyes, pesticides, and antibiotics[5]. However, the majority of the experiments are performed with micropollutant concentrations higher than the environmentally relevant ones [6]. Indeed, the effects of micropollutants' long-term exposition on MFC performance are still not fully understood. Therefore, this study aimed to investigate the effects of CIP long-term exposure on the performance of MFCs regarding pollutant removal efficiency and power generation. For this, we explored a wide concentration range of CIP to cover the effects of both environmental and stressed conditions. Likewise, we evaluated the effects of external resistance on MFC performance.

2 MATERIAL & METHODS

Two acrylic double-chamber MFCs with a working volume of 100 mL in each chamber were used for these experiments. Identical electrodes were used in both the anode and cathode chambers, consisting of six cartridges of stainless-steel woven (2.5 x 5.0 cm², 200 mesh, Telas Rocha Ltd, Brazil) filled with 2.0 g of granular graphite (mean size of 300 µm, Nacional de Grafite Ltd, Brazil). These electrodes were arranged on a stainless-steel woven support (7 x 16 cm²) and attached to a stainless-steel rod for electrical connection. A cationic exchange membrane (⌀ = 5 cm, CMI-7000S, Membranes International) was positioned between the anode and cathode chamber, and an airstone was installed at the cathode to facilitate oxygen supply.

The MFCs were designed to remove organic matter and nitrogen mainly at the anode and cathode, respectively. We used a peristaltic pump to feed the anode with synthetic wastewater, while the cathode was fed with the anode effluent through an external tube connecting both chambers. Synthetic wastewater composition was adapted from Lovley and Phillips[7] to provide 1.68 $g \cdot L^{-1}$ of sodium acetate and 0.24 $g \cdot L^{-1}$ of ammonium sulfate. Ciprofloxacin (HPLC grade, Sigma-Aldrich, Germany) was added to the synthetic wastewater in a concentration range from 50 – 1000 $\mu g \cdot L^{-1}$ for CIP exposure, which was performed in one of the MFCs (namely MFC-CIP), while the other reactor was set as the control (MFC-CTL).

The anode was inoculated with two bacterial sources (final concentration: 1.33 ± 0.10 g $VSS \cdot L^{-1}$): anaerobic pre-acclimated activated sludge from a municipal WWTP and an anodic consortium from an operating microbial desalination cell (MDC). Similarly, the cathode was inoculated with a mixture of three bacterial consortia (final concentration: 1.31 ± 0.16 g $VSS \cdot L^{-1}$): enriched nitrifying culture, enriched denitrifying culture, and cathodic consortium from the same MDC.

MFCs were started up at open circuit voltage (OCV), HTR of 2 d for each chamber, and intermittent aeration at the cathode. On the 7th day, HTR was reduced to 1 d, and an operating cycle of 12 h was established. The OCV mode was kept for a longer period

to adjust the pH and aeration cycle, to guarantee the full functioning of the biological processes before closing the circuit with a 1000 Ω resistor (R_{ext}). Seven operating conditions (OC) were evaluated in this study: OC 1 to 5 assessed the effects of increasing CIP concentrations on MFC performance and OC 6 and 7 assessed the effects of reducing R_{ext} (560 and 100 Ω) while keeping high CIP concentration (1000 $\mu g \cdot L^{-1}$). The temperature was set at 27 ± 2°C.

MFCs' performance was evaluated in terms of pollutant removal and power generation. To assess pollutant removal, we conducted a colorimetric analysis of COD[8], ammonium ($N\text{-}NH_4^+$)[9], nitrite ($N\text{-}NO_2^-$)[8], and nitrate ($N\text{-}NO_3^-$)[10] using a spectrophotometer (DR 5000, HACH). Additionally, we performed kinetics assays at the end of each OC and the results were analyzed by ion chromatography (ICS 5000, Dionex), except for $N\text{-}NH_4^+$[9]. We assessed the power generation with a data acquisition system consisting of an Arduino board connected to a laptop to record the voltage (E_{MFC}) produced in each MFC every minute and applied Ohm's law to determine the current (I) and power (P) produced by the MFCs. The respective densities were calculated by dividing I and P by the total volume of the MFC, i.e., 200 mL. Polarization and power curves were carried out by varying R_{ext} every two consecutive operation cycles (24 h) to ensure a stable voltage response[11]. Moreover, we evaluated MFCs internal resistances (R_{int}) through electrochemical impedance spectroscopy (EIS) in two and three-electrode modes with a potentiostat (Palmsens4, Netherlands) and an Ag/AgCl electrode as the reference electrode. Finally, we analyzed microbial community diversity with high-throughput sequencing technology at the V3/V4 region of 16S rRNA[12].

3 RESULTS & DISCUSSION

MFCs operation lasted 703 days. Under the conditions investigated in this study, neither the increase of CIP, nor R_{ext} influenced COD removal. MFC-CTL and MFC-CIP achieved over 95% COD removal throughout the operation. On the other hand, the nitrification step of nitrogen removal was impaired by CIP at 500 and 1000 $\mu g \cdot L^{-1}$ but recovered its efficiency up to 100% with the decrease of R_{ext} at OC 7 (Table 1). These observations were confirmed by the kinetic assays performed at the end of each OC. They unveiled that the nitrification efficiency in OC 4 decreased by 23.15 ± 2.25% and 28.16 ± 1.10% compared to OC 1 (before CIP exposure) and MFC-CTL, respectively. Moreover, when we reduced R_{ext} to 100 Ω (OC 7) the nitrification efficiency recovered up to 91.75 ± 0.21%, indicating that the increase in electron flow helped reduce the inhibitory effects of CIP on cathodic bioprocesses.

Kinetics results also helped to understand the effects of CIP and R_{ext} over total nitrogen removal (TN). In all cases, TN efficiencies were lower than nitrification due to the low efficiency of denitrification. It could be partially explained by the high COD removal at the anode which decreased the COD/N ratio to 2, limiting nitrate conversion to N_2 via heterotrophic denitrification. Reducing R_{ext} improved TN removal in both MFCs because it favored the electron flow through the external circuit, which might have been used by bacteria to promote denitrification via the electrotrophic pathway.

Table 1 COD and ammonia removal and current density generated by MFC under different operating conditions.

Operating condition (OC)	CIP ($\mu g \cdot L^{-1}$)	R_{ext} (Ω)	MFC-CTL			MFC-CIP		
			COD removal (%)	$N\text{-}NH_4^+$ removal (%)	Current density ($mA \cdot m^{-3}$)	COD removal (%)	$N\text{-}NH_4^+$ removal (%)	Current density ($mA \cdot m^{-3}$)
0	0	OCV	86.2 ± 10.6	94.3 ± 5.5	-	86.7 ± 7.5	95.0 ± 3.9	-
1	0	1000				93.3 ± 3.0	94.7 ± 3.6	894 ± 412
2	50	1000				97.2 ± 1.4	97.5 ± 2.5	950 ± 156
3	100	1000	94.5 ± 2.5	95.3 ± 7.3	906 ± 268	95.0 ± 2.2	99.0 ± 0.7	694 ± 225
4	500	1000				95.7 ± 1.3	86.6 ± 4.8	713 ± 263
5	1000	1000				96.0 ± 0.9	45.6 ± 12.2	713 ± 244
6	1000	560	95.3 ± 1.2	98.6 ± 2.2	1163 ± 188	91.4 ± 4.7	83.2 ± 7.9	725 ± 181
7	1000	100	96.5 ± 2.1	95.9 ± 3.5	694 ± 712	96.5 ± 2.9	93.6 ± 8.7	1063 ± 706

The effects of CIP exposure over power generation in MFCs were assessed through continuous voltage monitoring, polarization and power curves, and internal resistance (R_{int}). The high standard deviation in electrochemical parameters resulted from the intermittent aeration at the cathode, which increased voltage output during the aerobic period followed by a decrease in voltage during the anoxic phase. All results indicated that CIP impaired the activity of exoelectrogen bacteria. Before CIP exposure, at OC 1, MFC-CTL and MFC-CIP generated a similar amount of current density (Table 1). However, the exposure to 100 $\mu g \cdot L^{-1}$ CIP deteriorated the current density of MFC-CIP by 22% compared to the performance before the exposure. Those findings are supported by polarization and power curves results (Figure 1), which indicated a decrease in the maximum power output (MPO) of 10% for MFC-CIP compared to MFC-CTL. Finally, EIS results showed that the anodic R_{int} of the MFC-CIP was 43.6% higher than the control. Although current density monitoring indicated that reducing R_{ext} helped to recover the electrochemical performance under stressing CIP concentrations (1000 $\mu g \cdot L^{-1}$), the polarization curve and EIS data obtained at the end of the experiments (OC7) suggest a persistent damage of exoelectrogenic bacteria activity and electricity generation.

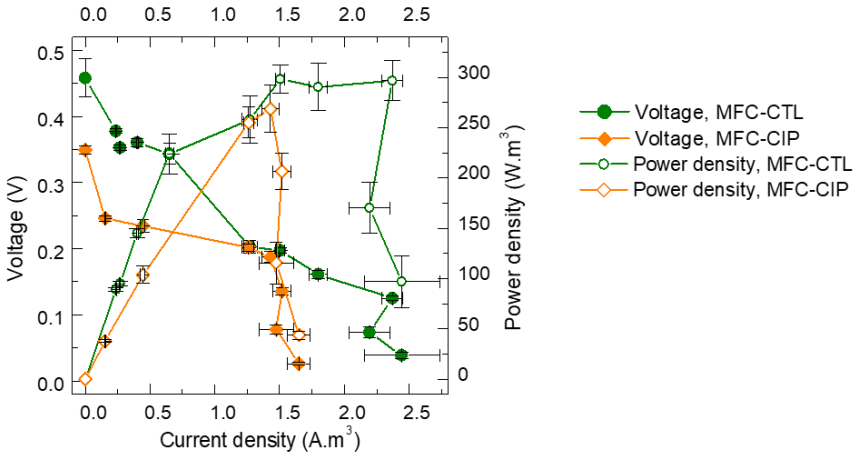

Figure 1 Polarization and power curves of MFC-CTL and MFC-CIP.

The composition of the microbial community by genetic sequencing showed that, in the long-term operation, CIP exposure did not change the main groups responsible for the removal of conventional pollutants, i.e., *Methanosaeta concilii* in the anode and *Nitrosomonas europaea* and Candidatus *Nitrospira defluvii* on the cathode). Even so, the relative abundance of these microorganisms decreased in MFC-CIP, corroborating with the previous discussions of performance parameters and electrochemical characterization.

4 CONCLUSION

This work demonstrated the resilience of bioprocess for COD and N removal incorporated into a double-chamber MFC when exposed to synthetic wastewater contaminated with CIP. The nitrification process at the cathode of MFC-CIP was compromised after exposure to 500 $\mu g \cdot L^{-1}$ of CIP, while just 100 $\mu g \cdot L^{-1}$ inhibited exoelectrogenic activity. The decrease of R_{ext} seemed to mitigate the inhibitory effects of a high CIP concentration on the process parameters performance; however, intrinsic inhibition to the microbial community has remained as shown by R_{int} and MPO. Under the experimental conditions explored in this work, CIP long-term exposure reduced the relative abundance of the principal microorganisms responsible for COD and N removal (*M. concilii* at the anode and *N. europaea* and *N. defluvii* at the cathode).

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