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EVALUATING THE EFFECTS OF LONG-TERM EXPOSURE TO NORFLOXACIN ON THE PERFORMANCE OF A MICROBIAL FUEL CELL

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ABSTRACT

Norfloxacin (NOR) is a widely used antibiotic, posing environmental risks due to its increasing presence in various media, including wastewater. This study investigates the long-term effects of NOR exposure on a double-chamber microbial fuel cell (MFC), focusing on pollutant removal efficiency and power generation. The MFCs were exposed to a wide range of NOR concentrations to simulate environmental and stressed conditions. Continuous monitoring, polarization curves, and internal resistance analysis were employed to evaluate the impact on power generation. Results indicate that while NOR exposure did not significantly affect chemical oxygen demand (COD) removal, it influenced nitrogen removal processes, stimulating nitrification but inhibiting denitrification. However, decreasing R_{ext} to 100 Ω seemed to mitigate the inhibitory effects of a high NOR concentration on the denitrification process. At 50 μg·L^{-1}, NOR enhanced electron transfer and power production in MFCs, although higher concentrations led to a slight decrease in power density. Additionally, electrochemical characterization revealed changes induced by NOR due to the lower maximum power output and the higher anodic internal resistance in MFC-NOR. Microbial community analysis identified potential electrogenic bacteria enriched in MFC-NOR.

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

1 INTRODUCTION

Norfloxacin (NOR) is a broad-spectrum antimicrobial active against Gram-positive and Gram-negative bacteria typically prescribed to treat urinary tract infections in humans and animals[1]. It is a representative of the fluoroquinolone class, which is the third most consumed antibiotic class in Brazil, only behind penicillins and macrolides[2]. As a consequence of its increasing consumption, NOR and its residues are frequently detected in environmental media (surface and groundwater, soil, plants, and animals) and wastewater streams[3,4]. NOR exposure can cause toxic effects in plants and aquatic organisms that might reduce their reproduction or even cause their death[5]. Likewise, NOR can contribute to an increase in antimicrobial resistance genes and bacteria among microbial communities, inducing irreversible adverse effects and threatening ecosystem function.

Bioelectrochemical systems, including microbial fuel cells (MFCs), have received increasing attention due to their capacity to harvest electrons from organic waste to produce value-added products from wastewater. Furthermore, MFCs can combine chemical and biological processes for simultaneous energy production and environmental remediation, e.g., removing conventional pollutants and micropollutants. The ability of MFCs to remove antibiotics from wastewater has already been demonstrated; however, this has typically been achieved with antibiotic concentrations higher than the environmentally relevant ones and under short operating times [6]. Therefore, this study aimed to understand the long-term effects of NOR exposure on the performance of a double-chamber MFC with biological anode and cathode. For this, we evaluated the pollutant removal efficiency and power generation of an MFC under a wide concentration range of NOR to cover the effects of both environmental and stressed conditions. Similarly, we evaluated the effects of external resistance on MFC performance.

2 MATERIAL & METHODS

The MFCs consisted of acrylic dual-chamber reactors with a total working volume of 200 mL bisected by a cationic exchange membrane (⌀ = 5 cm, CMI-7000S, Membranes International). Anode and cathode electrodes comprised six cartridges of stainless-steel woven (2.5 x 5.0 cm², 200 mesh, Telas Rocha Ltd, Brazil) filled with granular graphite (2.0 g, 300 μm, Nacional de Grafite Ltd, Brazil), arranged on a stainless-steel woven support (7 x 16 cm²), and attached to a stainless-steel rod for electrical connection. Intermittent aeration was provided on the cathode using an airstone and air pump to enable simultaneous nitrification-denitrification.

Both MFCs, namely MFC-CTL and MFC-NOR, were fed at the anode with synthetic wastewater constituted of 1200 mg COD·L^{-1}, 60 mg N-NH_4^+·L^{-1}, and other macro and micronutrients[7] by using a peristaltic pump. Meanwhile, the cathode was fed with the anode effluent through an external tube connecting both chambers. Only MFC-NOR was exposed to norfloxacin (analytical standard, Sigma-Aldrich, Germany) by mixing the antibiotic with the synthetic wastewater previously described to reach a concentration range from 50 – 1000 μg NOR·L^{-1}. Meanwhile, MFC-CTL was set as the control and was not exposed to NOR.

An anaerobic inoculum with a final concentration of 1.33 ± 0.10 g VSS·L^{-1} was seeded at the anode. This inoculum was obtained by mixing anaerobic pre-acclimated activated sludge from a municipal WWTP and enriched exoeletrogenic bacteria from an operating microbial desalination cell (MDC). Likewise, the cathode was inoculated with a mixture of enriched nitrifying consortium, enriched denitrifying consortium, and cathodic culture from the same MDC, with a final concentration of 1.31 ± 0.16 g VSS·L^{-1}.

Until the 7th operating day, we run the MFCs at open circuit voltage (OCV) and HTR of 2 d for each chamber. Then, we reduced the HTR to 1 d and defined an operating cycle of 12 h. We maintained the OCV operating mode until we assured the full functioning of the biological processes and closed the circuit with an external resistance (R_{ext}) of 1000 Ω. We investigated seven operating conditions (OC): from OC 1 to 5, we increased NOR concentration at MFC-NOR while maintaining a fixed R_{ext} of 1000 Ω. For OC 6 and 7, we reduced R_{ext} to 560 and 100 Ω, respectively, while keeping NOR at 1000 μg·L^{-1}(Table 1). All experiments were conducted at 27 ± 2°C.

We evaluated the performance of MFC-CTL and MFC-NOR regarding conventional pollutant removal and power generation. We assessed pollutant removal by quantifying ammonium ($N\text{-}NH_4^+$)[8], nitrite ($N\text{-}NO_2^-$)[9], nitrate ($N\text{-}NO_3^-$)[10], and COD[9] through colorimetric analysis at a spectrophotometer (DR 5000, HACH). We executed kinetics assays at the end of each OC; samples from the anode and cathode were collected periodically during one operating cycle (i.e., 12h), and the concentration of the pollutants was analyzed by ion chromatography (ICS 5000, Dionex), except for $N\text{-}NH_4^+$[8].

We developed an Arduino-based data acquisition system to monitor the power output generated by the MFCs. This system consisted of an Arduino board and a laptop connected in parallel to the external circuit of the MFCs to record the voltage (E_{MFC}) produced in each MFC. Then, we calculated the current (I) and power (P) by applying Ohm's law and divided I and P by the total working volume to get the respective densities. Electrochemical characterization comprised polarization and power curves, and internal resistance (R_{int}) analysis. Polarization and power curves were obtained by varying R_{ext} every two consecutive operation cycles (24 h) to ensure a stable voltage output[11]. R_{int} was assessed by 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 next-generation sequencing at the V3/V4 region of 16S rRNA[12].

3 RESULTS & DISCUSSION

We run the MFCs for 703 days. During this time, we did not observe significant changes in COD removal between MFC-CTL and MFC-NOR caused by NOR concentration or R_{ext}. Both reactors reached over 95% COD removal throughout the operation (Figure 1), with at least 90% of COD consumed in the first 6 hours. Moreover, the results of $N\text{-}NH_4^+$ removal from monitoring and kinetics suggest stimulation of nitrifying activity at the lower environmental concentration of NOR (50 μg·L^{-1}) (Table 1). On the other hand, total nitrogen (TN) removal seemed to be inhibited by NOR caused by the reduced efficiency of the denitrification step. These results are in agreement with other studies whose reported absence of inhibition of nitrifying bacteria but inhibition of the denitrifying ones [13,14].

We could not identify the effects of R_{ext} over nitrification on MFC-NOR once its efficiency was already near 100%. However, results from continuous monitoring suggest that after 50 days of operating with R_{ext}= 100 Ω, MFC-NOR recovered TN removal from 40 to 70% (Figure 1). This recovery was associated with an increased electron flow through the external circuit, which could promote denitrification via the electrotrophic pathway.

Table 1 Maximum ammonia removal rate and power density generated by MFC under different operating conditions.

Operating condition (OC)	Duration (d)	NOR (μg·L^{-1})	R_{ext} (Ω)	MFC-CTL		MFC-NOR	
				Maximum $N\text{-}NH_4^+$ removal rate (mg·L^{-1}·h^{-1})	Power output (mW·m^{-3})	Maximum $N\text{-}NH_4^+$ removal rate (mg·L^{-1}·h^{-1})	Power output (mW·m^{-3})
1	109 – 257	0	1000			5.88 ± 0.47	88 ± 81
2	258 – 294	50	1000			7.18 ± 0.12	175 ± 94
3	295 – 335	100	1000	5.23 ± 0.39	144 ± 69	4.03 ± 0.19	156 ± 56
4	336 – 369	500	1000			5.47 ± 0.28	163 ± 63
5	370 – 440	1000	1000			5.36 ± 0.43	156 ± 38
6	441 – 474	1000	560	5.07 ± 0.30	125 ± 31	4.89 ± 0.41	131 ± 44
7	475 – 703	1000	100	6.59 ± 0.45	13 ± 31	4.47 ± 0.41	25 ± 44

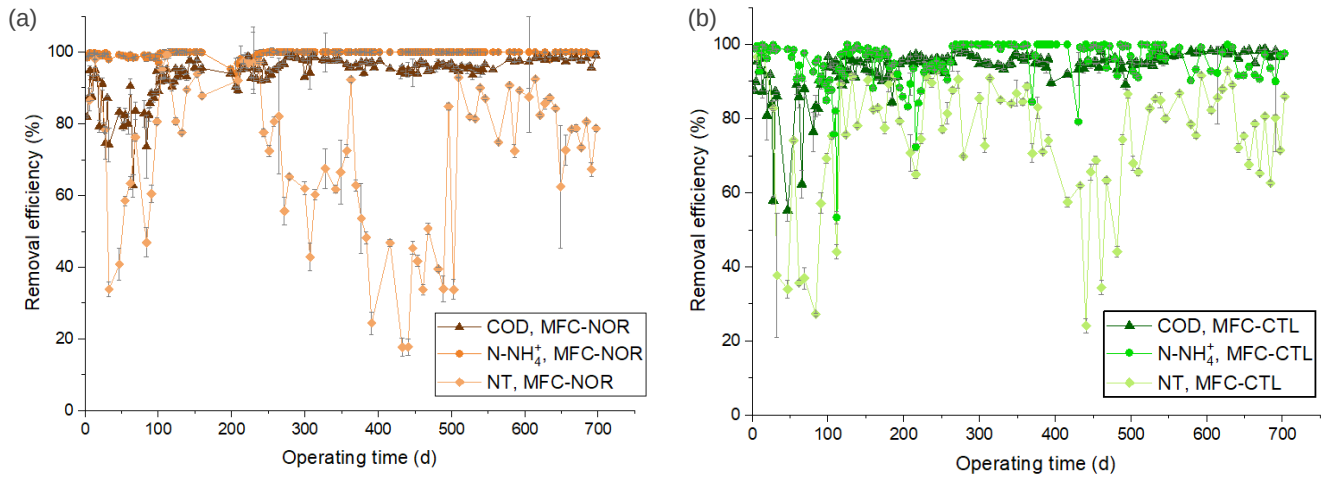

Figure 1 Removal efficiencies of COD, $N\text{-}NH_4^+$, and TN of (a) MFC-NOR and (b) MFC-CTL.

The effects of NOR exposure over power generation in MFCs were assessed through continuous voltage monitoring, polarization and power curves, and internal resistance (R_{int}). Intermittent aeration at the cathode increased the voltage output during the aerobic period while decreasing voltage during the anoxic phase, leading to a high standard deviation in electrochemical measurements. Results from power production monitoring (Table 1) suggest NOR enhanced the electron transfer in MFC-NOR, mainly at 50 $\mu g \cdot L^{-1}$ (OC2). At this condition, the power production increased approximately 2-fold compared to the OC1 of the same reactor. However, the continuous increase of NOR slightly decreased the power density in MFC-NOR, but the power production was still higher than in MFC-CTL (Table 1). Even so, results from electrochemical characterization performed at 1000 $\mu g \cdot L^{-1}$ of NOR suggest that NOR impaired electricity production to some extent. The maximum power output (MPO) obtained from power curve analysis was 14% smaller in MFC-NOR than in MFC-CTL, while anodic R_{int} was 46% higher in MFC-NOR. These findings reinforce the need for a detailed system characterization to identify possible changes induced by micropollutants in MFCs and establish optimal operating conditions.

Finally, next-generation sequencing results showed that *Methanosaeta concilii* was the main microorganism in both reactors, and its relative abundance reduced from 73.5% in MFC-CTL to 62% in MFC-NOR. On the other hand, bacteria from the Proteobacteria phylum (e.g., *Comomonas* spp., *Devosia* spp., *Paracoccus* spp., and *Rhodopseudomonas* spp.) were enriched at the anode of MFC-NOR compared with MFC-CTL. These bacteria have already been identified with at least one of the mechanisms for extracellular electron transfer and might have played a role in power production in MFC-NOR.

4 CONCLUSION

In conclusion, our findings demonstrated that while long-term exposure to norfloxacin did not affect COD removal, it influenced nitrogen removal processes, stimulating nitrification but inhibiting denitrification. 50 $\mu g \cdot L^{-1}$ of NOR enhanced electron transfer and power production in MFCs, although the exposure to increasing NOR concentrations led to a slight decrease in power density. The decrease of R_{ext} seemed to mitigate the inhibitory effects of a high NOR concentration on the denitrification process. Moreover, electrochemical characterization highlighted changes induced by NOR in the MFC-NOR not observed during the continuous operation. Under the experimental conditions explored in this work, NOR long-term exposure induced shifts in the microbial community, with potential electrogenic bacteria enriched in MFC-NOR. These results underscore the complex interactions between micropollutants and MFC performance, highlighting the need for further research on effective strategies for sustainable wastewater treatment and energy production.

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