Long-term operation of electroactive biofilms for enhanced ciprofloxacin removal capacity and anti-shock capabilities

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\textbf{ABSTRACT}

Few studies have focused on the feasibility of microbial fuel cells (MFCs) for removing quinolones antibiotics and their anti-shock capabilities. After 1.5 years of operation, the removal efficiency of 10 mg/L ciprofloxacin in MFCs increased to 99.00% in 88 h. These results are in accordance with the enhanced activity of biofilms and voltage output of MFCs. Additionally, the anti-shock capacities of the biofilms in MFCs were evaluated by treating ofloxacin and enrofloxacin and operating at different temperature and salinity. These MFCs can remove 87.31% and 40.81% of ofloxacin and enrofloxacin in 72 h, respectively. Even exposed to a low temperature of 10 °C or a salinity of 3%, the MFCs can achieve greater than 50% and nearly 80% of ciprofloxacin removal efficiency, respectively. The enrichment of \textit{Alcaligenes} and \textit{Chryseobacterium} contributed mostly to the removal of quinolones antibiotics. This study provides scientific evidences for treating wastewater containing quinolones antibiotics using MFCs.

\section{1. Introduction}

Currently, the widespread occurrence of antibiotic residues has resulted in their consideration as emerging contaminants, mainly resulting from their potential toxicity to ecological systems and persistent selecting pressure on antibiotic-resistant bacteria and antibiotic resistance genes (Baker et al., 2018). Quinolones antibiotics, mainly including ciprofloxacin, enrofloxacin and ofloxacin, are defined as "Highest Priority Critically Important Antimicrobials" by the World Health Organization and were among the most frequently prescribed antibiotics in hospitals in 2014 in Germany (BLV, 2016; Rusch et al., 2018; WHO, 2017). These synthetic antibacterial drugs are poorly...
metabolized by animals and human beings mainly because of their highly stable structure (Slana et al., 2014). Hence, their residues are often found to co-exist with concentrations ranging from μg/L to mg/L in many environments (Tran et al., 2018). These residues have caused the resistance of some bacteria to quinolone antibiotics (Ashfaq et al., 2016; Qamar et al., 2014). Therefore, frequent detection of antibiotic residues calls for an urgency to develop effective and economical treatments to remove these pollutants of emerging concern.

Bioelectrochemical systems are regarded as energy recovery alternatives for enhancing the removal of antibiotics (Yan et al., 2019). However, few researchers have focused on the removal capacities of quinolones antibiotics and the succession of the bacteria exposed to quinolones in bioelectrochemical systems, particularly for long-term operations. Additionally, previous studies have paid more attention to the degradation of one antibiotic, but it should be noted that co-existence of these emerging contaminants is ubiquitous, particularly in pharmaceutical wastewater which always contains several antibiotics contaminants belonging to the same class. Hence, the feasibility of bioelectrochemical systems for removing co-existence antibiotics contaminants needs further study.

Furthermore, the influencing factors in the environment are highly variable; however, few studies have paid attention to the abilities of biofilms in bioelectrochemical systems to withstand the sudden environmental shocks. For example, temperature in realistic surroundings fluctuates and plays an evident role during microbial catalytic processes (Zhou et al., 2016). However, many research studies have been conducted setting a stable temperature, resulting in a deficient evaluation of the effect of a sudden change in temperature on the antibiotic removal ratio. Additionally, wastewater always contains other carbon sources, and whether the substrate competition would disturb removal of target antibiotic pollutants also needs further study. Moreover, pharmaceutical wastewater, as another example, it always contains high-strength salinity (Ng et al., 2015; Shi et al., 2017). Whether bioelectrochemical systems maintain good biocatalytic ability when facing with the impact of different salinities remains unclear. Hence, it is essential to evaluate the anti-abilities of bioelectrochemical systems for removing antibiotics for their practical application in polluted environments.

In this study, microbial fuel cells (MFCs), as one type of typical bioelectrochemical systems, were chosen, and ciprofloxacin, one representative quinolones antibiotic, acted as a carbon source added to the anodic chamber of the MFCs. The main objectives of this study were to (1) characterize the removal dynamics of ciprofloxacin over 1.5 years in MFCs and test the feasibility of MFCs for removing enrofloxacin and ofloxacin; (2) evaluate the anti-shock capacities of electroactive biofilms in MFCs subject to variations in environmental factors, and (3) investigate the shift in microbial communities in understanding the microbial mechanism during long-term ciprofloxacin processing. This knowledge could provide a scientific guidance for treating with wastewater containing quinolones antibiotics using MFCs.

2. Materials and methods

2.1. Chemicals and reagents

Ciprofloxacin, enrofloxacin, and ofloxacin (> 98%) were ordered from Dr. Ehrenstorfer (GmbH, Augsburg, Germany). Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Merck KGaA (Darmstadt, Germany). All of the other chemicals were bought from Sinopharm Group Co., Ltd. (Shanghai, China).

2.2. Reactor setup

Based on the method from the previous studies (Xiao et al., 2016; Yan et al., 2018), six groups of two-chamber MFCs were constructed using acrylic glass plates, and the working volume of each chamber was 140 mL (7.0 × 5.0 × 4.0 cm). The inoculation source for constructing the anodic biofilms of the MFCs was from pig manure (LeSen Farm, Xiamen, China). During the process of building biofilms, 20 g of pig manure was dissolved in one liter of phosphate buffer solution, and then 30 mL of the supernatant was added into each anodic chamber of MFCs. The artificial wastewater was served as an anolyte to feed mixed microbes from pig manure repeatedly. The catholyte contained 100 mmol/L K2[Fe(CN)6] and 50 mmol/L phosphate buffer solution (pH = 7.0). Both the anolyte and catholyte were replaced in batch mode. When the maximum voltage reached greater than 0.60 V after approximately 1 month, the MFCs were regarded as successfully developed. Thereafter, ciprofloxacin acted as a carbon source substituting for acetate in the anolyte to acclimate microbes in the anodic chamber of the MFCs until the end of the experiment. A dark incubator (LRH-500F, Keelrein instrument Co., Ltd., Shanghai, China) was used to prevent photodegradation of the ciprofloxacin and maintain the target temperature of the MFCs. A digital multimeter (Keithley Instruments, Inc., USA) was applied to record the output voltage of the MFCs. The autoclaved electrodes were used and set as abiotic controls.

2.3. Analytical methods

Ciprofloxacin of low concentrations (< 1 mg/L) were quantified using ultra-liquid chromatography with tandem mass spectrometry (LC–MS/MS; an ABI 3200 Q TRAP instrument, USA), and the test conditions detailed was provided in E-supplementary data.

Ciprofloxacin, enrofloxacin, and ofloxacin of high concentration were quantified using a Hitachi L-2000 series HPLC system (Hitachi, Japan) with an Agilent column (Zorbax Eclipse Plus C18, 250 × 4.6 mm i.d., 5 μm particle size). The detection wavelength for these three antibiotics was all at 276 nm through a diode array detector. The methods of the optimized isocratic elution for these three antibiotics was detailed in E-supplementary data.

The removal kinetics of the ciprofloxacin in the MFCs were fitted to the first-order kinetic model, whose equation is as follows:

\[ \ln C_t = -kt + \ln C_0 \]  

where \( C_t \) (mg/L) and \( C_0 \) (mg/L) note the concentration of the ciprofloxacin at time \( t \) and zero, respectively, and \( k \) (h\(^{-1}\)) is the removal rate constant.

The half-life (h) can be calculated as follows:

\[ t_{1/2} = \ln 2/k \]  

2.4. Evaluation of the anti-shock capacities of the biofilms in the MFCs

Firstly, to test the removal capacities for another two fluorquinolone antibiotics after long-term acclimation in the MFCs, enrofloxacin and ofloxacin were served as carbon sources substituting for ciprofloxacin in the anolyte and were added into the anodic chamber of the MFCs for three batches, respectively. The analyses of their concentrations change were conducted according to the effluent samples from the third circle.

Secondly, to measure whether other carbon sources would disturb the removal of the ciprofloxacin pollutants, glucose and acetate were chosen to investigate the effect of the type of carbon source on ciprofloxacin degradation in the system. During this test, the experimental temperature was controlled at 25 °C.

Thirdly, considering the temperature during summer in southern of China and during winter in the northern of China is approximately 40 °C and nearly 10 °C, respectively, the effect of experimental temperatures of 10, 20, 30, and 40 °C on ciprofloxacin removal in the MFCs was studied to test their anti-shock abilities within a temperature change.

Fourthly, to evaluate the anti-shock capacities of the biofilms in MFCs to different salinities, the removal efficiencies of the ciprofloxacin...
under salinities of 1, 3, 6 and 8% were tested. Salinities of 1, 3, 6 and 8% in the MFC influents were simulated by adding 10, 30, 60 and 80 g of NaCl into 1 L of wastewater, respectively. During this procedure, the temperature was set at 25 °C by the incubator.

The hydraulic retention time of the aforementioned experiments under different conditions was all set to 3 days, for the MFCs could obtain nearly 80% removal efficiencies of ciprofloxacin over 3 days under most of experimental conditions. They were all performed in three batches and the changes of their corresponding concentrations were analyzed according to the effluent samples from the third circle.

2.5. Antibacterial activity assay

*Escherichia coli* K12 (E. coli K12) was selected to test the biotoxicity of the effluents from the MFCs after treating with ciprofloxacin, enrofloxacin, and ofloxacin. Three experimental groups were set, and the detailed experiments were provided in E-supplementary data.

2.6. Sample collection, 16S rRNA gene sequencing, and data analysis

The biofilm samples from the MFCs were collected during the following stages: (1) raw pig manure: inoculating source; (2) ciprofloxacin feeding stage: ciprofloxacin as substrate after the operation of 1.5 years. A description of the total DNA extraction; PCR amplification reaction, and its parameters; and 16S rRNA gene amplification, sequencing, and data processing was reported in detail in the previous study (Yan et al., 2018). The high-throughput sequencing was conducted at Shanghai Majorbio Bio-Pharm Technology Co., Ltd., (Shanghai, China).

2.7. Statistical analysis

The averages and standard deviations data were calculated using Excel 2010 (Microsoft, USA). Principal coordinate analysis (PCoA) based on Bray–Curtis distance, was organized using R language (version 3.1.0). Linear discriminant analysis (LDA) coupled with effect size algorithm (LEfSe) was used to search for the statistically significantly biomarkers of the bacterial communities between the samples of raw pig manure and MFCs (Segata et al., 2011). The fitted curves of the ciprofloxacin removal and the generation of other plots was obtained using Origin Pro 9.1 software (OriginLab, USA).

3. Results and discussions

3.1. Removal of ciprofloxacin during long-term operation

The changes in the ciprofloxacin concentration during a single cycle after operation for 1 month and 1.5 years are shown in Fig. 1A and B, respectively. Whenever the initial concentration was 0.65 mg/L or 10.00 mg/L, an obvious change in the ciprofloxacin concentration was 10.0 mg/L. All the reactors were operated in batch mode. MFC indicates microbial fuel cell with electroactive biofilms. The control represents microbial fuel cell with abiotic electrodes.

![Fig. 1. Change in ciprofloxacin concentration during one circle in the microbial fuel cells. (A) After operation for 1 month. (B) After operation for 1.5 years.](image)

Voltage output of the MFCs was considered as an important indicator of microbial activity and was recorded at different times (Fig. 2). When the substrate was switched from acetate to ciprofloxacin, the voltage significantly decreased from greater than 0.60 V to less than 0.10 V and was difficult to recover after several cycles (Fig. 2A), indicating that ciprofloxacin is severely toxic to many microorganisms and rigorously restrained the activity of electrochemically active bacteria. However, with the prolonged operation, the voltage outputs again recovered to approximately 0.60 V again (Fig. 2B), suggesting that microbial activities were recovered and bacteria in the MFCs could metabolize ciprofloxacin to grow and to yield electricity.

Additionally, the live/dead staining method was used to examine the microbial activities and formation of anodic biofilms. It was observed that during the initial 1 month, the anodic biofilms were not developed well under ciprofloxacin pressure (see E-supplementary data). In contrast, the microorganisms had attached well along the anodic electrodes after long-term operation, indicating that microbes had gradually adapted to the stress from the ciprofloxacin. Moreover, the microbes after 1.5 years of acclimation were obviously more active than those after 1 month, suggesting that the microbial activities of the functional bacteria were greatly improved (see E-supplementary data). This phenomenon agreed with the voltage output variation and
ciprofloxacin removal ratio change. The results of the scanning electron microscope also showed that with prolonged operation, microbes in the MFCs propagated in the electrodes and these functional bacteria were covered by a large amount of extracellular polymeric substances, resulted in a niche for micropollutant degradation and was conductive to the electron transportation (see E-supplementary data).

3.2. Ofloxacin and enrofloxacin removal

MFCs showed good removal capacity for ciprofloxacin, but the feasibility of the MFCs after long-term operation for treating other quinolone antibiotics should be paid to attention mainly because of the universality of the co-existence of antibiotics. Hence, the removal capacities of the ofloxacin and enrofloxacin belonging to the quinolone antibiotics in the MFCs were also tested and the results are shown in Fig. 3A. Within a hydraulic retention time of 3 days, MFCs could most efficiently remove ofloxacin, reaching up to 87.31% ± 0.63%. The enrofloxacin and ciprofloxacin removal efficiencies were approximately 40.81% ± 2.65% and 79.60% ± 1.81%, respectively. With the extension of hydraulic retention time, the removal efficiencies of these three antibiotics gradually increased, particularly those for ciprofloxacin. This result, that the MFCs could remove ofloxacin and ciprofloxacin without acclimation, might be associated with some functional bacteria in the MFCs. Previous studies have reported that some strains, such as Thermus sp. strain C419, possessed the capacity to biodegrade several fluoroquinolone antibiotics (Pan et al., 2018). Additionally, other literature has reported that the enzymes secreted by functional microbes that require primary biodegradation are class-specific rather than compound-specific (Müller et al., 2013). As three representative antibacterial drugs, ciprofloxacin, ofloxacin, and enrofloxacin all belong to the same class of antibiotics, and the chemical structures of enrofloxacin and ofloxacin are similar to that of ciprofloxacin. Hence, the bacterial communities exposed to ciprofloxacin for a long time would also possess biodegradation capacities for other structurally similar quinolone antibiotics. These results showed the considerable capability of MFCs for treating wastewater containing co-existent quinolone antibiotics.

Although the MFCs could efficiently remove ciprofloxacin, ofloxacin, and enrofloxacin, it should be tested that whether the effluents from the MFCs still remain toxic. Hence, the antimicrobial effects of the effluents from the MFCs after the treatment for these three quinolones antibiotics on E. coli K12 were investigated, respectively (Fig. 3B). In the group containing 10.0 mg/L ciprofloxacin, ofloxacin, and enrofloxacin, the optical densities of E. coli K12 were all constantly less than
0.01 all the time after inoculation. This indicated that 10.0 mg/L of ciprofloxacin, ofloxacin, and enrofloxacin are toxic such that they significantly inhibit the growth of *E. coli* K12. In contrast, it was observed that obvious growths occurred in the groups containing the effluents from the MFCs after treatment of ciprofloxacin and ofloxacin, whose growth curves were similar to those of *E. coli* K12 in the control group. These results suggested that after treatment by the MFCs, the antibacterial effects of ciprofloxacin and ofloxacin towards *E. coli* K12 were prominently eliminated in 5 days. Compared to the control group, the growth of *E. coli* K12 is not good in the MFC-ENR group although its maximum optical density of 600nm is nearly 0.60. This indicated that effluents from MFCs after treatment of enrofloxacin over 5 days still had a certain biotoxicity and a longer hydraulic retention time is needed to further improve the bio-availability of the effluents.

### 3.3. Anti-shock capabilities of the MFCs to environmental changes

Given the high variability of environmental factors and the future application of the MFCs in realistic polluted environments, other carbon sources, salinity and temperature were chosen as three environmental factors to evaluate the anti-shock abilities of the electroactive biofilms in the MFCs.

During long-term ciprofloxacin processing, the MFCs were operated under an oligotrophic environment, but the question as whether the presence of different types of carbon sources would disturb or favor ciprofloxacin degradation remains unclear. Fig. 4A shows that the removal efficiencies of ciprofloxacin under carbon sources of glucose and acetate. The roles of 1.0 g/L of glucose and acetate were similar and they favored the ciprofloxacin removal, obtaining a removal ratio of 99.89% ± 0.04% and 99.39% ± 0.11% at 3 days, respectively. This might be explained by the sufficient carbon sources that were easily utilized by the functional bacteria, which would provide sufficient energy for the quick increment of their biomass and metabolic activity to further promote ciprofloxacin removal.

Temperature as an environmental factor should be attracted sufficient attention, for this factor is highly fluctuant during 1 year, and even not stable during 1 day. Hence, it is crucial to evaluate the microbial catalytic capacity of bioelectrochemical systems as sudden temperature changes occur. Fig. 4B shows that the removal efficiencies of the MFCs for ciprofloxacin at 30 °C and 40 °C were similar, both reaching up to greater than 98.00%, which were higher than those exposed to 20 °C and 10 °C. It is suggested that a high temperature favors ciprofloxacin removal, while a sudden variation in low temperature results in an obvious decrease in removal efficiency. It is worth noting that the anodic biofilms exposed to a temperature of 10 °C could maintain a favorable removal ratio, up to 53.04% ± 4.83% at 3 days, showing the considerable anti-shock ability for a low temperature change. Previous literatures confirmed that biofilm technology is an effective upgrade for enhancing organic matter removal under low temperature (Zhou et al., 2018). Additionally, it may be associated with the relative expression of some key genes from the well-constructed anodic biofilms. Liang and his co-workers noted that it was concerned with maintaining the key genes, such as cytochrome c genes and hydrogenase genes (Liang et al., 2016). Hence, long-term operation contributed to the sound developments of the electroactive biofilms and aided in maintaining the considerable catalytic stability under low temperature.

Pharmaceutical wastewaters containing high levels of salinity are a troublesome problem for traditional treatment processes, given the adverse effect on biological processes (Larsson et al., 2007). Fig. 4C shows the change in the removal efficiencies of ciprofloxacin under different salinities. With salinities of 1% and 3%, the high removal ciprofloxacin efficiencies were maintained, particularly at the salinity of 1% (reaching up to 94.82% ± 0.14%); Feng et al. (2015) demonstrated that electrical stimulation could enhance bacterial resistance to salinity (Feng et al., 2015). Additionally, this favorable anti-salinity ability of electroactive biofilms may be associated with withstanding capacities of extracellular polymeric substances and enhanced solution conductivity. Some researchers have reported that a certain level of salinity increases the conductivity of wastewater and further attributed this to the redox reaction of pollutants (Guo et al., 2018). However, inhibition to microbial activity of functional bacteria occurred and it was exacerbated with the increment in salinity, causing an apparently

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**Fig. 4. Effects of environmental factors on ciprofloxacin removal.** (A) Effects of carbon source on ciprofloxacin removal. Glucose 1 represents 1.0 g/L of glucose. The scale of break in the Y axis is from 2 to 4; (B) Effects of temperature on ciprofloxacin removal. The scale of break in the Y axis is from 10 to 40; (C) Effects of salinity on ciprofloxacin removal. The scale of break in the Y axis is from 2 to 4.
decreased ciprofloxacin removal. At a salinity of 6% and 8%, the removal efficiency of ciprofloxacin decreased to 21.82% ± 0.14% and 6.46% ± 0.56%, respectively. These results indicated that the anti-salinity ability of well-constructed electroactive biofilms is considerable, but this capacity remain limited when biofilms were exposed to a salinity greater than 6%, mainly because of the decline in microbial activity.

3.4. Succession of microbial community structure

After operation of 1.5 years, the succession of microbial communities played a prominent role in enhancing the removal capacities for ciprofloxacin and the anti-shock abilities of biofilms in the MFCs. Therefore, high-throughput sequencing method was explored to determine their variation. The number of sequences of each sample varied from 41,410 to 62,691 and a total of 306,377 high-quality sequences were recovered from all six samples. The good coverage indices indicated that this sequencing analysis was credible and covered most microbes in all of the samples. The indices of community richness (Chao 1 and Ace) and community diversity (Shannon and Simpson) suggested that the structure of the microbial community in the sample of raw pig manure was more complex than that in the MFCs after long-term ciprofloxacin. This is understandable given the microbes from raw pig manure originated from the gut of pigs, which were fed by many different complex substrates. With the subsequent inoculation into the MFCs, ciprofloxacin as a substrate predominantly selected the overall bacterial communities, causing the dominance of some specific functional bacteria (see Table 1).

Principal coordinates analysis based on Bray–Curtis distance was performed to examine the β-diversity of the two groups. The two axes of PC1 and PC2 explained 98.11 and 1.68%, respectively, which obviously separated the microbial species into the two sections and showed a clear distinction between raw pig manure and the MFCs (Fig. 5). These results showed that the role of ciprofloxacin as substrate coupled with the effect of operation time together resulted in a profound selection on the bacterial communities.

3.5. Potential functional dominant microbes

To find specialized and functional communities, the LEiSe tool was used to determine the statistically significant differences between the samples of raw pig manure and the MFCs. Groups represented by corresponding colors are shown in cladograms and the inner to outer circles corresponded to the phylum to genus levels (Fig. 6A). At a strict LDA threshold of 4.0, 65 statistical biomarkers were found and their corresponding taxon names are shown in Fig. 6B.

At the phylum level, in the sample of raw pig manure, Firmicutes (73.16% ± 4.27%) and Spirochaetae (6.24% ± 0.89%) as statistical biomarkers were enriched (Fig. 6A). However, with the prolonged of operation, the relative abundance of Proteobacteria, accounting for 53.82% ± 1.16%, significantly increased (see E-supplementary data).

Proteobacteria as dominant microbes has been found in many bioelectrochemical systems for treating antibiotics and it also plays an important role in the bioreactors exposed to ofloxacin, norfloxacin, and ciprofloxacin (Amorim et al., 2014; Zhang et al., 2017b). Additionally, the relative abundance of Bacteroidetes and Actinobacteria increased from 15.55% ± 2.81% to 34.26% ± 1.26% and from 0.38% ± 0.05% and 3.92% ± 0.16%, respectively, and they were identified as another two statistical biomarkers in the MFCs group (see E-supplementary data). Other studies have also found a similar result, that Bacteroidetes, as mainly dominated microbes, occur in the ciprofloxacin-degrading microbial community and Actinobacteria is notably more abundant during diclofenac and carbamazepine biodegradation (Liao et al., 2016; Thelusmond et al., 2018; Zhang et al., 2018).

At the genus level, Terrisporobacter and Clostridium_sensu_stricta_1 as the main dominant genera were found in the sample of raw pig manure. Nevertheless, 10 groups of microbes were found to be significantly enriched in the MFCs, namely Alcaligenes, Chryseobacterium, Myroides, Stenotrophomonas, Eubacterium, Ochrobactrum, Acinetobacter, Gordonia, Pseudomonas, and Dysgonomonas (Fig. 6). Their relative abundances accounted for 27.19, 20.71, 8.95, 5.10, 4.92, 4.70, 3.98, 3.79, 3.31, and 2.66%, respectively (see E-supplementary data). As the most abundant genus, some strains belonging to Alcaligenes exhibited excellent properties in the biodegradation of wastewater containing sulfonamide antibiotics (Yang et al., 2016). Additionally, the gene maiaI in Alcaligenes faealais JQ135 encoding a maleic acid cis-trans isomerase was also found to be a key gene in the metabolism of pyridine derivatives (Qiu et al., 2018).

Moreover, previous studies have also noted that bacteria within the genus Alcaligenes could secrete some enzymes, such as HpaM, to catalyze the decarboxylative hydroxylation of the target pollutants (Qiu et al., 2018; Zhang et al., 2017a). These conclusions indicate that Alcaligenes plays an important role in biodegradation of complex contaminants. Another dominant genus Chryseobacterium was also found in other studies about biodegradation of enrofloxacin (Alexandrino et al., 2017). It has been also reported that Chryseobacterium acts a predominant role in the degradation of complex pollutants in the MFCs (Zhao and Kong, 2018). Previous study has proven that some strains of Myroides spp. could degrade aromatic hydrocarbon pollutants, such as 3,4-dichloroaniline (Li et al., 2012). It has also been found in other studies that Stenotrophomonas is the dominant genus for biodegrading ciprofloxacin and other emerging contaminants, indicating that the genus Stenotrophomonas may possess an ability to degrade ciprofloxacin (Lee et al., 2014; Yang et al., 2017). In addition Eubacterium and Pseudomonas as the main functional genera occurred in

![Fig. 5. Principal coordinate analysis based on the Bray-Curtis distance as shown in the overall distribution pattern of bacterial taxa in the microbial communities. RPM indicates the sample of raw pig manure; MFC represents the biofilm samples from the microbial fuel cells fed with ciprofloxacin.](image-url)
the MFCs for biodegrading oxytetracycline and biocathodes for treating chloramphenicol, respectively (Guo et al., 2017; Yan et al., 2018). Additionally, some species of these two genera belong to electrochemically active bacteria and are responsible for power generation in MFCs (Jiang et al., 2016; Yong et al., 2015). From these results, it has been demonstrated that after long-term operation, the MFCs could effectively select potential functional dominant microbes and the enrichment of these bacteria was tightly associated with the efficient removal of ciprofloxacin antibiotics.

4. Conclusion

This study showed the good feasibility of the MFCs in removing quinolones antibiotics. After long-term operation, MFCs favor anti-shock capacities to cope with variations in environmental factors. Particularly, when exposed to a sudden change to a low temperature of 10°C and a salinity of 3%, MFCs maintained a greater than 50% and nearly 80% ciprofloxacin removal efficiency, respectively. With high-throughput sequencing, it demonstrated that the enrichment of Alcaligenes and Chryseobacterium as well as other genera contributed to the removal of quinolones antibiotics. This research provides a scientific guidance for treating with wastewater containing quinolones antibiotics using MFCs.

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Appendix A. Supplementary data

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