# Performance of microbial fuel cell for treating swine wastewater containing sulfonamide antibiotics

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**Abstract**

The proper treatment of swine wastewater with relatively high concentrations of antibiotics is very important to protect environmental safety and human health. Microbial fuel cell (MFC) technology shows much promise for removing pollutants and producing electricity simultaneously. A double-chamber MFC was investigated in this study. Synthetic swine wastewater with the addition of sulfonamides was used as the fuels in the anode chamber. Results indicated that COD could be effectively removed (>95%) and virtually not affect by the presence of sulfonamides in the MFC. A stable voltage output was also observed. The removal efficiencies of sulfamethoxazole (SMX), sulfadiazine (SDZ), and sulfamethazine (SMZ) in the MFC were in the 99.46% to 99.53%, 13.39% to 66.91% and 32.84% to 67.21% ranges, respectively. These totals were higher than those reported for a traditional anaerobic reactor. Hence, MFC revealed strong resistance to antibiotic toxicity and high potential to treat swine wastewater with antibiotics.

**Keywords:** Swine wastewater, microbial fuel cell, sulfonamide antibiotics, biodegradation

## 1. Introduction

The occurrence and accumulation of antibiotics in the environment has led to critical attention worldwide regarding their harmful effects on the ecosystem and contribution to antibiotic resistance (Singh et al., 2019). Such resistance can reduce or eliminate the effectiveness of antibiotics in the treatment of infectious diseases, since multidrug-resistant bacteria that can strongly resist various antibiotics could lead to untreatable diseases and endanger people's health (Ma et al., 2018). The discovery of multidrug-resistant bacteria in the environment was recently reported (Lee et al., 2018). According to one report by the United States Centres for Disease Control and Prevention (US CDC), at least 23,000 people died due to infection by antibiotic resistant-bacteria every year in the United States (O'Neill, 2014). The European Centre for Disease Prevention and Control (ECDC) also reported that more than 25,000 deaths were caused by antibiotic resistance in Europe each year (O'Neill, 2014). Antibiotics which make a major contribution to the development of antibiotic resistance, exert positive effects on the accumulation and spread of ARGs in the environment (Cheng et al., 2019).

Swine wastewater is one of the major antibiotics' sources in the environment, owing to large amounts of antibiotics used as drugs and feed additives in swine industries. Sulfonamides (SMs) constitute one of the oldest and widely employed antibiotics for swine farms considering their economic and relative efficacy in some common bacterial diseases (Broll et al., 2004). According to Cheng et al. (2018), the concentration of SMs in swine wastewater worldwide is up to 324.4 μg/L. As the global population increases and the demand for pig products also increases, the consumption of SMs will continue to rise in the future. Therefore, a variety of treatment technologies, including biological, physicochemical and bioelectrochemical systems, have been conducted by researchers to remove antibiotics from wastewaters (Homem & Santos, 2011). Of these technologies, the microbial fuel cell (MFC) is the subject of increasing attention due to its advantages of effective removal of organic matter, moderately expensive operating requirements, low sludge production, and ability to produce power (Lovley, 2008). The effectiveness of MFC for enhancing the removal of refractory organic pollutants such as pesticides, toluene, phenol, indole, and azo dye from wastewater has been proved in other analyses (Huang et al., 2011).

Although all SMs have the same mechanism of action, there are significant differences in activity and antibacterial spectrum due to the various physicochemical characteristics of SMs. Therefore, sulfonamide combinations are usually used as feed ingredients in swine production instead of individual sulfonamides, resulting in the residual of sulfonamide combinations in swine wastewater. Unfortunately, most current research only pays attention to the removal of individual sulfornamides in the MFC, while few studies have focused on the removal and degradation kinetic of the simultaneous sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ) from swine wastewater in the MFC (Wang et al., 2018; Wu et al., 2020). Moreover, combined antibiotics may inhibit more seriously the performance of bioreactors than individual ones (Cheng et al., 2018). Hence, this study aimed to explore the effect of different concentrations of sulfonamide combinations on the electricity generation and organic matter removal in a double-chamber MFC. The removal efficiency and degradation kinetics of sulfonamide combinations (SMX, SMZ and SDZ) in the MFC were also analyzed in the present study.

## 2. Materials and methods

### 2.1 MFC construction and inoculation

A double-chamber MFC was employed in this study, and the anode and cathode chamber has the same effective volume of 0.35 L. A cylindrical graphite felt (3 cm in diameter and 6 cm thickness) and a carbon-fiber brush (3 cm diameter and 3 cm length) served as the anode and cathode of MFC, respectively. Two chambers were separated by a cation exchange membrane (CEM) (CMI7000, Membranes International Inc., USA) and connected by a copper wire via a resistor of 1000 Ω. The anode chamber was inoculated by anaerobic sludge collected from a pilot scale anaerobic digester and fed with synthetic swine wastewater (3000 mg/L COD, 223 mg/L $NH_4Cl$, 66 mg/L of $KH_2PO_4$, 54 mg/L $MgSO_4 \cdot 7H_2O$ and 4 mg/L $CaCl_2 \cdot 2H_2O$). The synthetic swine wastewater was adjusted to pH 7.5 ± 0.1 and purged with $N_2$ gas for 15 minutes prior to feeding to the anode chamber. Meanwhile, distilled water was used to fill in the cathode chamber. The cathode chamber was continuously purging air to maintain DO concentration at around 6 mg/L.

### 2.2 Experimental design and operation

In order to investigate the removal efficiency of SMs in the MFC and compare the different outcomes from the open-circuit mode, reactors in this study were conducted in closed-circuit mode (MFC) and open-circuit mode (OC) simultaneously. The OC disconnects the anode and cathode chamber, which was regarded as the conventional anaerobic reactor. All reactors were conducted in batch running mode at room temperature (around 25℃). Synthetic swine wastewater was pumped into the anode chamber by a peristaltic pump and then self-circulated at a flow rate of 20 mL/min, which was replayed after each running circle (120 h). After the reactor achieved stable COD removal and voltage generation, three target SMs (SMX, SMZ and SDZ) (≥ 99.0%, supplied by Sigma-Aldrich, Australia) were dded into the synthetic swine wastewater simultaneously and pumped into the anode chamber. The initial concentration for SMX, SMZ and SDZ were 100 μg/L in the first operating circle. They were then increased to 200 and 300 μg/L in the sequential second and third operating circles. Duplicate samples were collected from the MFC and OC, respectively, at different operating times and filtered by a syringe filter (0.2μm) prior to testing. Their mean and standard deviation were calculated.

**2.3 Analytical methods**

The concentrations of SMX, SMZ and SDZ in the collected sample were analyzed by using a triple quadrupole mass spectrometer LCMS-8060 (Shimadzu). The separation of antibiotics was achieved by a Phenomenex C18 column (Luna, 3.0 × 100 mm, 3 μm) with the mobile phase of water and acetonitrile (0.1% (V/V) formic acid) under a flow rate of 0.4 mL/min. The detailed method has been reported in our previous study (Cheng et al., 2020). The COD analysis was performed by the Standard Methods (Federation and American, 2005). During the experimental period, the voltage generation of MFC was recorded through a universal digital meter (VC86E, Shenzhen City Station Win Technology Co. Ltd., Shenzhen, China).

**3. Results and discussion**

**3.1 Impacts of SMs on power generation of MFC**

The voltage generation under different initial concentrations of SMs is presented in Fig. 1. The stable voltage output was achieved before SMs were added into the MFC with the average value of 551.1 mV, which indicates the enrichment of exoelectrogenic bacteria on the anode surface and the successful start-up of the MFC. From Fig. 1, the average voltage was 555.1 mV and 536.4 mV after the injection of 100 μg/L and 200 μg/L of SMs into the MFC in successive operating cycles. Stable voltage production was observed during the operating period, which reflected that microorganisms in the anode chamber have a strong tolerance to SMs. A slight increase in voltage production (average of 583.6 mV) was observed by further increasing the initial concentration of SMs to 300 μg/L. Wu et al.'s (2020) research also discovered that the presence of SMX in the anode of MFC: firstly, enhanced the abundance of exoelectrogens; and secondly, increased the power density by 18%. The study by Wen et al. (2011) revealed that adding ceftriaxone into the MFC had positive effects on the production of electricity. It is clear that the electricity production in the MFC system occurred through the oxidation of organic matter through the biocatalysis of microorganisms. During this process, the produced electrons were transferred from the cell to the anode electrode and then flowed to the cathode through an external circuit to produce electricity. Therefore, the electricity generation in the MFC was determined by the activity of exoelectrogenic bacteria and the transfer of electrons between bacterial cells and the electrode. Based on this kind of mechanism for producing electricity, it is suggested that the addition of SMs to 300 μg/L in the MFC might improve the activity of exoelectrogenic bacteria and/or the ability of electrons to transfer from microbe cell to the anode.

Inset Fig. 1

**3.2 Impacts of SMs on COD removal in the MFC**

Referring to practical application, the MFC's performance in removing organic matter from swine wastewater and the effect of antibiotics on their removal were also critical. Hence, the removal efficiency of COD in the MFC and OC under different SMs concentrations was monitored and the results are illustrated in Fig. 2. As observed from Fig. 2 (a), the degradation rate of COD declined by increasing the initial concentration of SMs, which reflected the fact that the larger concentrations of antibiotics showed more inhibition to the anaerobic microbes. This finding is consistent with previous reports (Cheng et al., 2018). High COD removal efficiencies were achieved in both MFC (98.85%) and OC (94.21%) before the addition of SMs into the reactor (Fig. 2 (b)). By adding 100, 200 and 300 μg/L of SMs into the reactor, the overall removal efficiency of COD in the MFC remained quite stable (95.28% - 98.66%) while its removal in OC fell significantly to 58.72%, 51.65% and 18.82%, respectively. The high and stable degradation efficiency of organic matter in MFC systems was also found by adding other types of antibiotics in the MFC (Wang et al., 2018; Wen et al., 2011; Zhou et al., 2018). This finding indicated that the MFC could eliminate the toxicity of SMs to microorganisms, which has great potential for treating wastewater containing antibiotics. The high and stable removal efficiency of COD in the MFC with the addition of SMs was consistent with the electricity production, which was also stable.

Inset Fig. 2

**3.3 Degradation of SMs in the MFC**

The concentration change and removal efficiency of SMX, SMZ and SDZ in the MFC and OC under the initial concentrations of 100, 200 and 300 μg/L are presented in Fig. 3. It is observed that the MFC revealed higher and faster removal of all the SMs than those in OC under all the initial concentrations. Xue et al. (2019) and Song et al. (2018) documented the rapid removal of SMX and SDZ in the MFC and their low residual concentrations in the MFC's effluent in comparison with the effluent originating from OC. One possible explanation for this is that the stimulation of electron transfer could enhance the growth of microorganisms and the microbial metabolisms in the MFC anode (Cao et al., 2015; Zhang et al., 2017). Based on the previous study, the removal of SMs in anaerobic reactors is attributed to the biodegradation of microorganisms (Cheng et al., 2020). The degradation of SMX, SDZ and SMZ in the MFC followed the first-order kinetic reaction model and the parameters are summarized in Table 1.

Inset Fig. 3 and Table 1

High removal efficiency of SMX can be achieved in the MFC (>99%) at all tested concentrations, and its degradation rate was faster than the degradation of SDZ and SMZ, with a much higher degradation rate constant (K) and less time for the degradation of 50% SMs (DT50) (Table 1). The MFC's effective performance for removing SMX was also reported by Xue et al. (2019) and Wu et al. (2020), and these authors indicated that SMX could be completely degraded into less harmful byproducts without affecting the performance of MFC and its removal was less affected by the initial concentration. Comparatively, the removal efficiency of SDZ and SMZ was in the 13.39% to 66.91% and 32.84% to 67.21% ranges at the studied concentrations. The research by Harnisch et al. (2013) also reported the complete removal of SMX and only partial removal of SDZ in the MFC. This phenomenon demonstrated that the degradation of SMs by microorganisms in the MFC has substance-specific properties. With the increase in the initial concentration of SMs from 100 to 200 μg/L, the efficiencies in removing SDZ and SMZ in the MFC fell from 66.91% to 13.39% and 67.21% to 32.84%, and the DT50 increased from 129.81 to 693.1h and 78.68 to 228.77 h, respectively. This phenomenon might be due to the reduced bioactivity of the degrading microorganisms in the anode compartment. Conversely, the SDZ removal efficiency recovered to 40.1% by further increasing the addition concentration to 300 μg/L, and a slight increase for SMZ (38.25%) was also observed. This outcome suggested that microorganisms in the MFC might have gradually adapted to the presence of SMs to some extent and became more active after a period of acclimation. Wang et al. (2016) also stated that the ability of microbes to degrade recalcitrant chemicals could be enhanced by a long acclimation period. This result agrees with the improved voltage generation at 300 μg/L of SMs. Compared to the removal of individual SDZ in a MFC as reported previously, its removal efficiency in this study was quite low (Wang et al., 2018). Probably due to sulfonamide combinations are more toxic to their degrading microorganisms than the individual antibiotic (Cheng et al., 2018).

## 4. Conclusion

Large quantities of organic matter in swine wastewater could be removed in the MFC and was slightly affected by the presence of SMs. Moreover, stable voltage was generated continuously in the MFC by feeding it with synthetic swine wastewater. The addition of SMs might increase the electricity production through the improved activity of exoelectrogenic bacteria after a period of domestication and/or the enhanced ability of electrons transfer from microbe cell to the anode. The simultaneous removal of SMX, SDZ and SMZ in the MFC was higher than those in the conventional anaerobic reactor.

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**Figure Captions**

Fig. 1 The voltage generation under different initial concentrations of SMs in MFC

Fig. 2 The removal efficiency of COD in MFC and OC under different SMs concentrations

Fig. 3 The concentration change and removal efficiency of SMs in MFC and OC at different initial concentrations.

**Table Captions**

Table 1. Fitting Results of SMX, SDZ and SMZ degradation in MFC using the first-order kinetic model

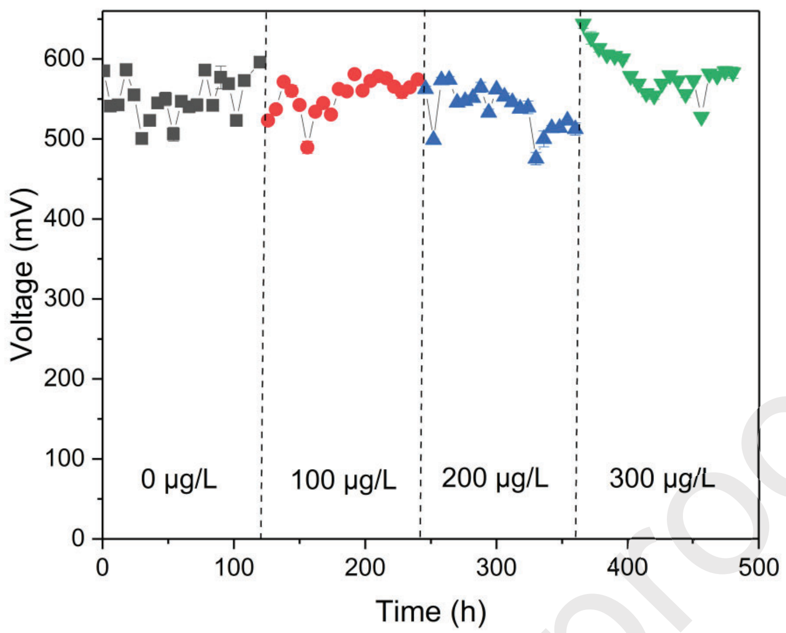


Fig. 1 The voltage generation under different initial concentrations of SMs in MFC

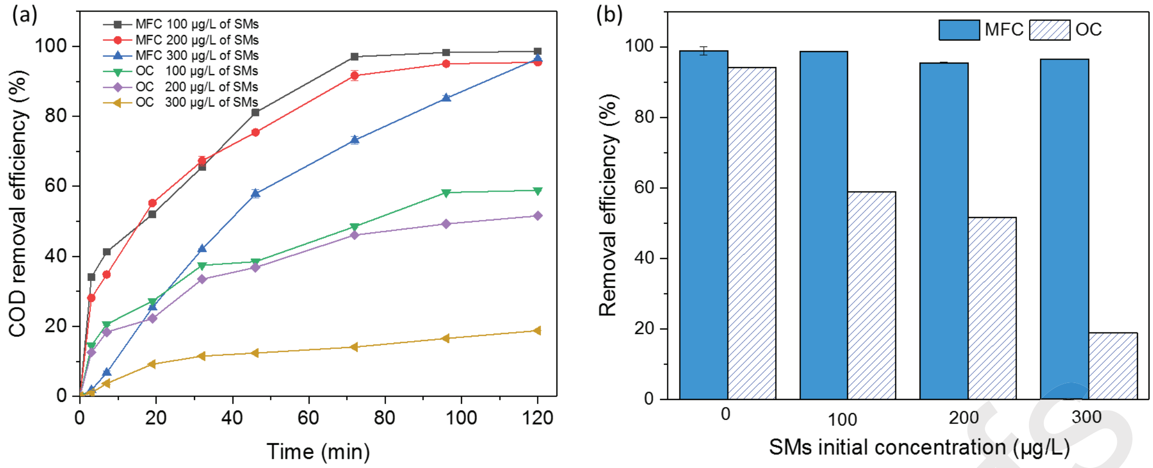


Fig. 2 The removal efficiency of COD in MFC and OC under different SMs concentrations

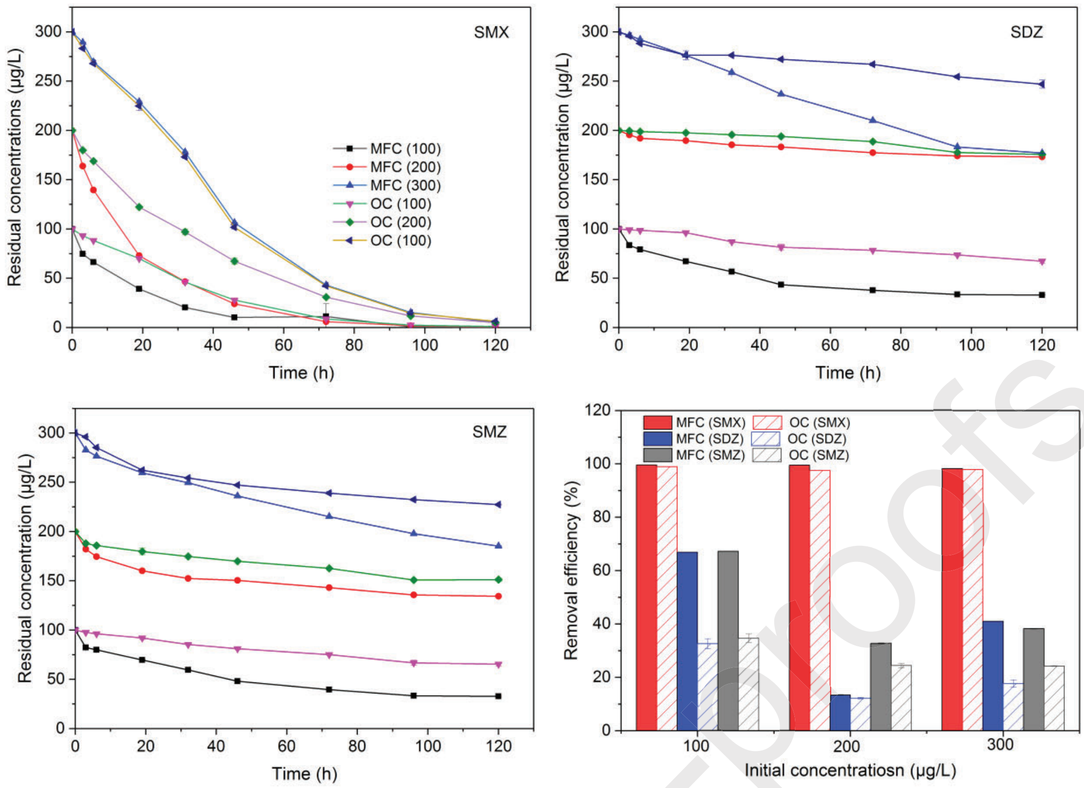


Fig. 3 The concentration change and removal efficiency of SMs in MFC and OC at different initial concentrations.

Table 1. Fitting Results of SMX, SDZ and SMZ degradation in MFC using the first-order kinetic model

| Kinetic formula | Antibiotic | Initial concentration (μg/L) | K | $R^2$ | DT50 (h) |
|---|---|---|---|---|---|
| $\frac{dC}{dt} = -k_1 \cdot C$ $\leftrightarrow C_t = C_0 \cdot e^{-k_1 \cdot t}$ (Chen et al., 2017) | SMX | 100 | 0.045 | 0.999 | 15.79 |
| | | 200 | 0.045 | 0.996 | 15.44 |
| | | 300 | 0.033 | 0.953 | 20.88 |
| | SDZ | 100 | 0.0053 | 0.864 | 129.81 |
| | | 200 | 0.001 | 0.906 | 693.15 |
| | | 300 | 0.0049 | 0.993 | 142.04 |
| | SMZ | 100 | 0.00889 | 0.975 | 78.68 |
| | | 200 | 0.00309 | 0.970 | 228.76 |
| | | 300 | 0.00399 | 0.992 | 177.28 |

$C_0$ is initial concentration of SMs; $C_t$ is SMs concentration at time t; k is the degradation rate constant. DT50 is time for the degradation of 50% SMs (DT50=ln 2/k) (Chen et al., 2017).

**Author Contribution Statement**

Dongle Cheng: investigation, writing - original draft, methodology, formal analysis, data curation
Huu Hao Ngo: supervision, investigation, project administration, conceptualization, review & editing
Wenshan Guo: supervision, investigation, review & editing
Dujong Lee: methodology, formal analysis, resources, review