



Reduction of methane emission from microbial fuel cells during sulfamethoxazole wastewater treatment

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ABSTRACT

Carbon emissions from wastewater treatment contribute to global warming and have received widespread attention. It is necessary to seek low-carbon wastewater treatment technologies. Microbial fuel cells (MFC) and osmotic microbial fuel cells (OsMFC) are low-carbon technologies that enable both wastewater treatment and energy recovery. In this study, MFC and OsMFC were used to treat sulfamethoxazole (SMX) wastewater, and direct carbon emissions during operation was calculated. The highest SMX removal rate can reach about 40%. Simultaneously, the CH₄ emission factor was significantly reduced to <6 g CO₂/kg of chemical oxygen demand. The accumulation of SMX-degrading bacteria competed with methanogens for carbon source utilization, leading to a significant decrease in the relative abundance of methanogens. It is hoped that this study can provide a sustainable approach to antibiotic wastewater treatment and promote the development of low-carbon wastewater treatment technologies.

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The large amount of greenhouse gases produced by human activities has received much attention [1]. It is necessary to reduce greenhouse gas emissions in various industries. It is estimated that the wastewater treatment industry is responsible for 1.3% of global greenhouse gas emissions [2]. Therefore, it is necessary to develop low-carbon wastewater treatment technology and decarbonize the wastewater treatment process.

Microbial fuel cells (MFC) can produce electricity while treating wastewater [3]. The osmotic microbial fuel cell (OsMFC) was invented by replacing the proton exchange membrane in MFC with a forward osmosis (FO) membrane [4]. In addition to wastewater treatment and energy recovery, the cathode draw solution in OsMFC can extract water from the feed solution and produce cleaner water [5,6]. MFC and OsMFC have been applied to treat different kinds of wastewater and have obtained better performance [7]. As green wastewater treatment technologies, the energy that they produce can offset indirect carbon emissions during wastewater treatment. Although MFC and OsMFC are still in the laboratory research stage, they will have broad application prospects in the future because they can realize wastewater treatment and electricity generation at the same time. However, the direct carbon emission of MFC and OsMFC in the wastewater treatment pro-

cess still needs attention, and it is necessary to further calculate the direct carbon emission and develop carbon reduction strategies. Anaerobic microorganisms in MFC and OsMFC anodes use organic matter in wastewater as a carbon source and organic matter can be degraded to CO₂. However, the presence of methanogens may lead to the generation of CH₄. Methane is one of the major gases contributing to global warming [8], and the production of methane reduces the environmental friendliness of this wastewater treatment technology. The presence of methanogens can also compete with electrogenic bacteria for electron utilization, resulting in lower electron utilization efficiency for electricity generation. There has been a study to add methanogenic chemical inhibitors to MFC, but this increases operating costs [9]. It is important to develop a pathway to reduce carbon emissions without additional chemicals, such as using pollutants in wastewater.

Sulfamethoxazole (SMX) is a widely used antibiotic and is stable in water environments [10]. Only a portion of SMX can be utilized by humans or animals, and the remainder is eliminated from the body to the environment [11,12]. In some household or community sewage, the concentration of SMX can even reach several milligrams per liter [13]. It is reported that the actual concentration of SMX in livestock wastewater is <1 mg/L [14]. To avoid harm to the environment or aquatic life, appropriate measures should be taken to treat SMX wastewater. When using MFC and OsMFC to treat wastewater containing 500 µg/L SMX, it was observed that

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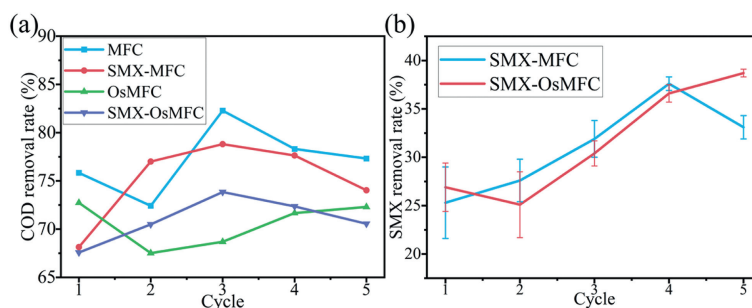


Fig. 1. COD removal rate (a) and SMX removal rate (b) of different reactors. SMX-MFC represents the MFC used to treat SMX wastewater, and SMX-OsMFC represents the OsMFC used to treat SMX wastewater.

the amount of gas produced by the reactor anode was reduced and the smell changed. A study has shown that antibiotics can reduce greenhouse gas emissions from constructed wetland-microbial fuel cells [15]. This enlightens us on whether SMX in wastewater can be utilized to decrease direct carbon emissions from wastewater treatment.

In this study, MFC and OsMFC were used to treat SMX wastewater. The effects of 0.5 mg/L SMX in wastewater on the performance of MFC and OsMFC were analyzed, including wastewater treatment, electricity generation, water extraction, and electrochemical performance. Further analysis of differences in methane emissions and microbial composition revealed the mechanism of reducing MFC and OsMFC carbon emissions by 0.5 mg/L SMX. This study simultaneously achieved SMX wastewater treatment and methane emission reduction in MFC and OsMFC. It is hoped that this study can provide a reference for antibiotic wastewater treatment and low-carbon wastewater treatment technology.

The starting point of this study is to use SMX-containing wastewater to inhibit greenhouse gas emissions from MFC and OsMFC, rather than add SMX. In acclimated reactors, the presence of SMX did not significantly affect the wastewater treatment capacity of MFC and OsMFC. After >60 days of acclimation, further experiments were carried out. Chemical oxygen demand (COD) removal rates did not change significantly in the presence of SMX during the 5 monitoring cycles (Fig. 1a). In general, the COD removal rate can be maintained above 70% during the 25-day operation period. For wastewater containing SMX, both MFC and OsMFC had certain removal effects on SMX. The initial SMX removal rates were about 20%, and with the operation of the reactors, the SMX removal rates gradually increased and can reach up to about 40% (Fig. 1b). This indicates that with the extension of SMX exposure time, microorganisms gradually adapt to SMX and improve the ability of SMX degradation. The SMX removal rate can be further improved through optimization of the reactor and enrichment of relevant SMX degrading functional bacteria [16].

The potential changes of four groups of reactors over 5 cycles were measured. With the prolongation of operation time, the potential decreased gradually and the potential of the four reactors changed in the same trend (Fig. 2a). The decrease in potential may be attributed to the fouling of the proton exchange membranes and FO membranes, which reduces the proton or water fluxes [17]. In the same cycle, the potential differences among different reactors were not obvious. The initial exposure to SMX will cause changes in the microbial composition of the reactors, however, the microbial composition gradually stabilizes after a long time of acclimation. The differences in COD removal rates and electricity generation between the reactors can be ignored, which indicates that OsMFC can be well applied to the treatment of SMX wastewater. The output voltage of the four reactors remained stable, ranging from 0.53 V to 0.57 V over the 5 cycles. After 20 days of operation, the output voltage can still be stable at about 0.54 V.

Due to the introduction of the FO membrane, OsMFC can extract the water from the anode side to the cathode side [4]. The water production per cycle is shown in Fig. 2b. With the operation of two OsMFCs, the water production per cycle gradually decreased, which is due to the formation and development of membrane fouling. There was no significant difference in water production per cycle between the two OsMFC reactors, indicating that the introduction of SMX did not affect the water production process. The initial introduction of antibiotics will cause microorganisms to respond, including the secretion of extracellular polymeric substances based on self-protection mechanisms [18]. However, anodic carbon felts had all undergone SMX acclimation, and this response had disappeared. Therefore, there was no significant difference in membrane fouling and water flux.

The cyclic voltammetry (CV) and polarization curves of the four reactors were measured. According to the CV curves of the four reactors (Fig. 2c), the reactions in both MFC and OsMFC were irreversible. The area of the CV curve represents the capacitance and reflects the performance of the cell. The capacitance of the four reactors varied irregularly and did not increase or decrease with the presence of SMX. According to the polarization curve measurements (Fig. 2d), the maximum power density of the four reactors was not significantly correlated with the presence of SMX. Therefore, by analyzing CV and polarization curves, it can be determined that the electrochemical performance of MFC and OsMFC used to treat SMX wastewater did not deteriorate.

During the acclimation process, differences in gas production from the anodes of different reactors were observed. The gas volume of different reactors over 5 cycles was examined (Fig. 3a). When SMX was included in wastewater, the anode gas production of both MFC and OsMFC was greatly reduced. Further analysis of the gas composition reveals that the gas composition was mainly CO₂ and CH₄, and the concentration of N₂O was very low and negligible. The anode of MFC only undergoes anaerobic biological reactions, so N₂O cannot be produced in MFC. By calculating CO₂ emission factors, it is found that SMX had little effect on the CO₂ emissions of MFC and OsMFC. The CO₂ emission factors of the four reactors were about 1.05 g/kg COD (Fig. 3b).

The CH₄ emission factor showed an obvious difference. The MFC and OsMFC treated the wastewater containing SMX produced less CH₄ (Fig. 3c). The CH₄ emission factor of SMX-MFC was lower than 0.15 g/kg COD, and the CH₄ emission factor of SMX-OsMFC was about 0.175 g/kg COD. The CH₄ emission factors of MFC and OsMFC were higher than 0.2 g/kg COD. In contrast, the presence of SMX significantly reduced CH₄ emissions from both MFC and OsMFC, and their CH₄ emission factors were much lower than the default CH₄ emission factors given by the Intergovernmental Panel on Climate Change (IPCC) for anaerobic wastewater treatment processes [19].

According to the Fifth Assessment Report of IPCC, 1 kg of CH₄ has a global warming potential equivalent to 28 kg CO₂ [20]. The

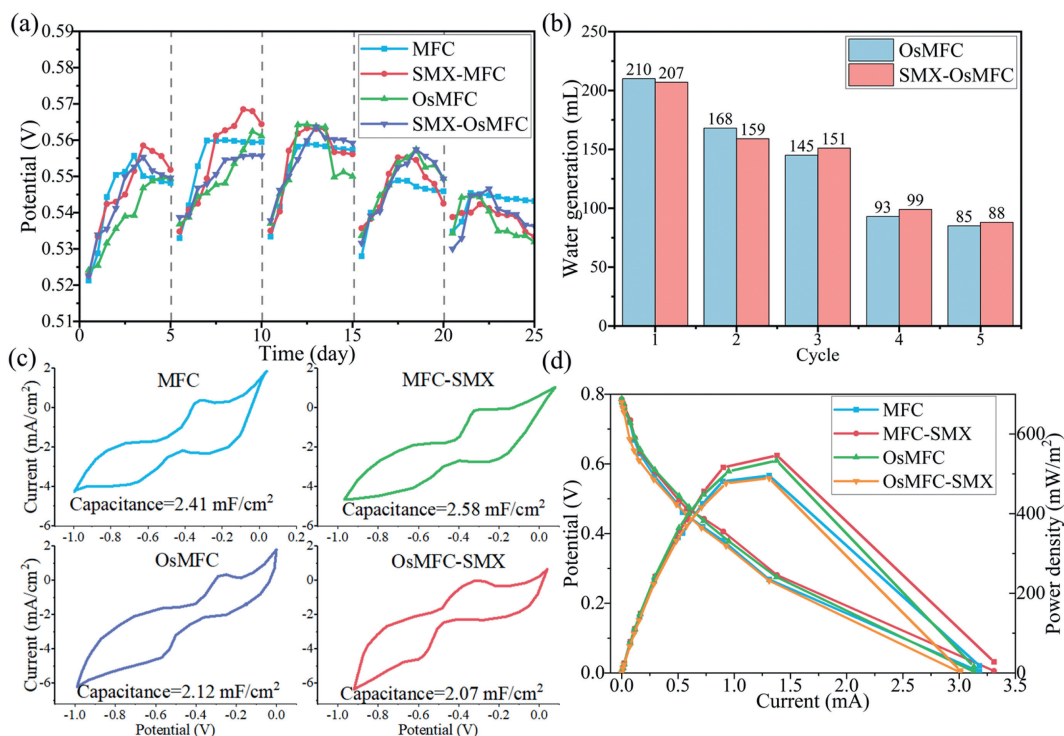


Fig. 2. Electricity generation (a), water extraction (b), cyclic voltammetry curve (c), and power density (d) of different reactors.

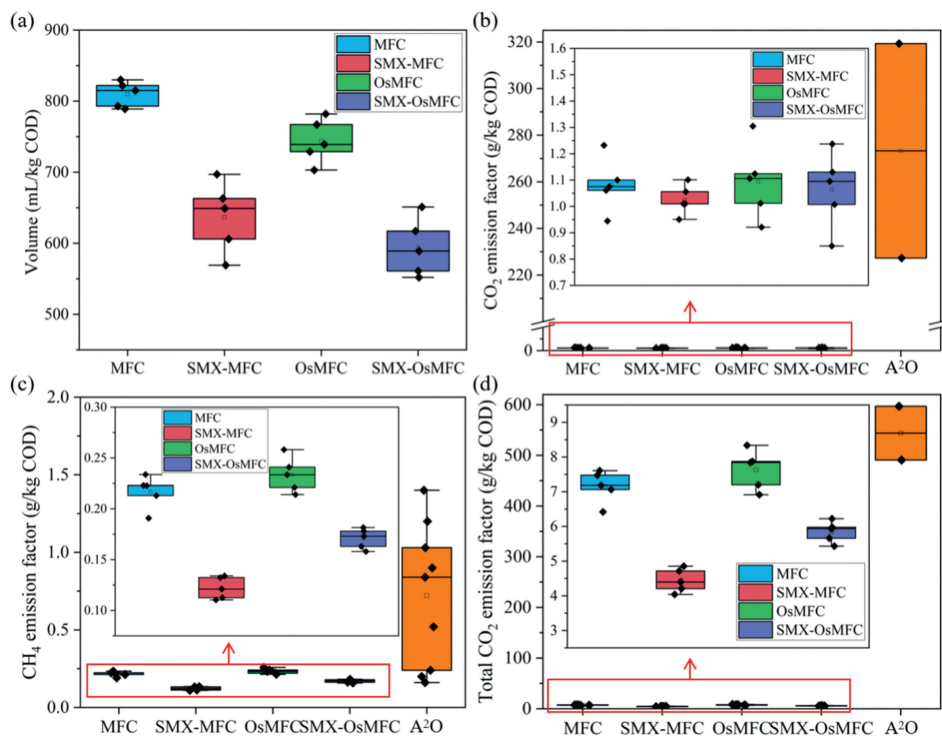


Fig. 3. Gas analysis of different reactors: Volume of generated gas (a), CO₂ emission factor (b), CH₄ emission factor (c), and total CO₂ emission factor (d).

total CO₂ emission factor can be calculated by converting CH₄ to equivalent CO₂ (Fig. 3d). After calculation, the total CO₂ emission factors of both MFC and OsMFC exceeded 7.0 g/kg COD. The total CO₂ emission factor of SMX-MFC was <5.0 g/kg COD. The total CO₂ emission factor of SMX-OsMFC was <6.0 g/kg COD. The greenhouse gas emissions of the anaerobic-anoxic-oxic (A²O) process in previ-

ous reports were much higher than those in this study [21]. Therefore, it can be concluded that using MFC and OsMFC to treat SMX wastewater can achieve simultaneous pollutant removal and carbon reduction.

When SMX was present in wastewater, greenhouse gas emissions from the wastewater treatment processes of MFC and OsMFC

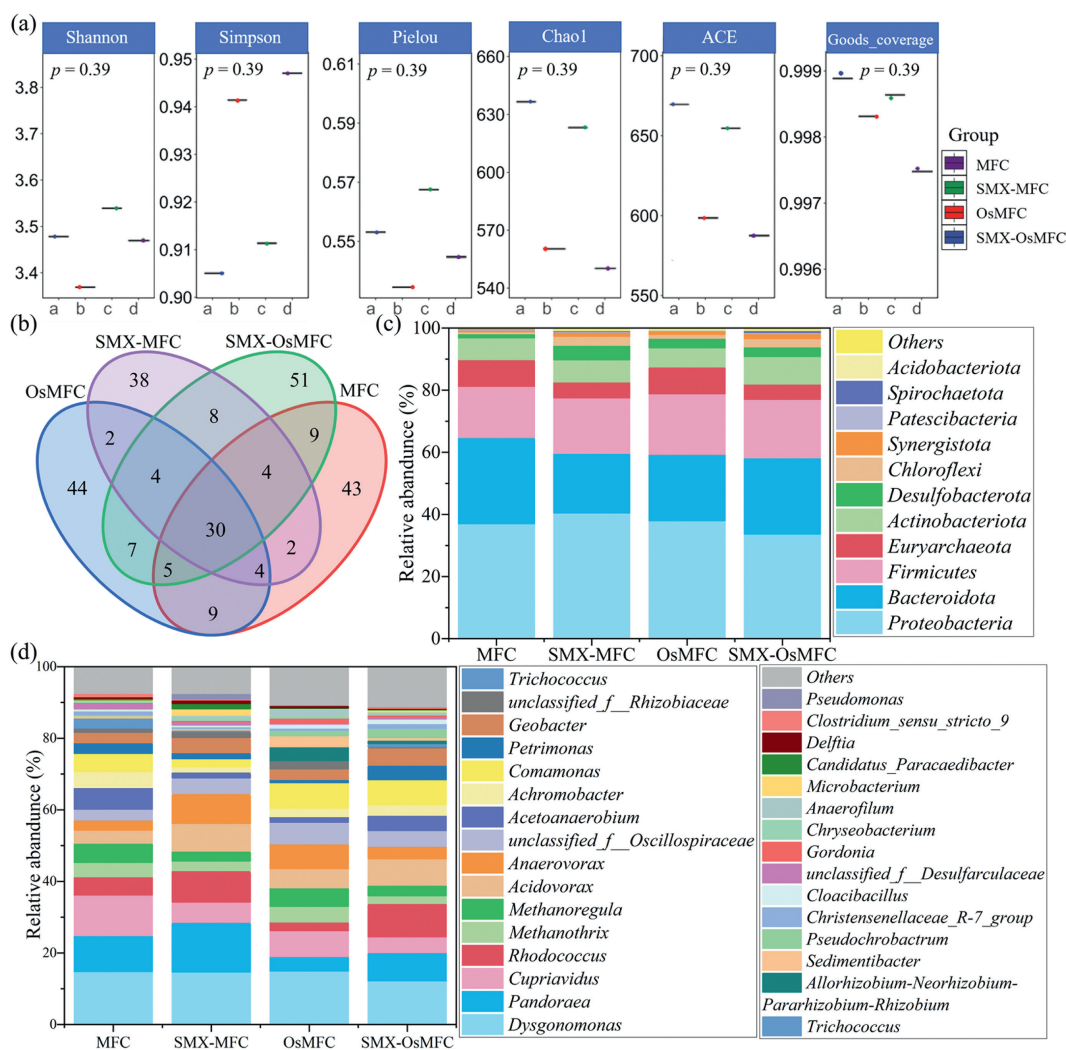


Fig. 4. Alpha-diversity indexes (a), Venn diagram (b), phylum-level relative abundance (c), and genus-level relative abundance (d) of different reactors.

were significantly reduced. CH_4 is difficult to dissolve in water, and CO_2 has low solubility. Therefore, even if some greenhouse gases are dissolved, MFC and OsMFC exhibit ideal carbon emissions compared to existing wastewater treatment technologies. While the reactor performance was not significantly affected, the SMX in wastewater was removed and the microbial response to SMX was utilized to reduce carbon emissions from MFC and OsMFC at the same time. In this way, wastewater treatment and utilization can be maximized.

The alpha diversity of microorganisms in the four reactors was evaluated (Fig. 4a). Compared to MFC and OsMFC, the biodiversity of SMX-MFC and SMX-OsMFC was significantly reduced. This may be attributed to the antibacterial action of SMX, which eliminates certain microorganisms. A similar study has also found a decrease in alpha diversity after the addition of antibiotics [18]. Venn analysis of operational taxonomic units was performed after clustering (Fig. 4b). Each of the four reactors contained the same species while also cultivating unique species. Community composition at the phylum level (Fig. 4c) reveals that the dominant phylum included Proteobacteria, Bacteroidota, Firmicutes, Euryarchaeota, Actinobacteriota, and Desulfobacterota. Many bacteria in Proteobacteria and Firmicutes are electroactive and involved in the transformation of SMX [22]. Euryarchaeota exhibits the highest relative abundance in the archaeal community, which contains many methanogens [23].

The electricity production is directly related to electricity-producing bacteria, and the methane production is related to the methane-producing functional bacteria. After a long period of acclimation, the SMX removal ability of MFC and OsMFC improved due to the enrichment of SMX-degrading functional bacteria. Long-term exposure to SMX increased the relative abundance of bacteria that can degrade SMX or hardly degradable substances in SMX-MFC and SMX-OsMFC (Fig. 4d), including *Pandoraea*, *Rhodococcus*, *Achromobacter*, and *Pseudomonas* [22,24]. Electricity generation functional bacteria mainly included *Comamonas*, *Dysgonomonas*, *Cupriavidus*, *Geobacter*, *Acidovorax* and *Cloacibacillus* [25–27]. Overall, there was no significant change in the abundance of electrogenic functional bacteria (Fig. 4d).

In MFC and OsMFC where SMX was present, the relative abundance of *Methanotherix* and *Methanoregula* was significantly reduced. *Methanotherix* uses acetic acid as a substrate, and *Methanoregula* uses hydrogen as a substrate [15]. The degradation of SMX is mostly completed by co-metabolism, and the increase in SMX-degradation related bacteria intensified the competition with methanogens for carbon source utilization. A study showed that methanogens are inhibited only when the SMX concentration exceeds 15 mg/L [28]. Therefore, the decreased CH_4 emission factor and the relative abundance of methanogens may be attributed to competition between electrogenic bacteria and SMX-degrading bacteria and methanogens.

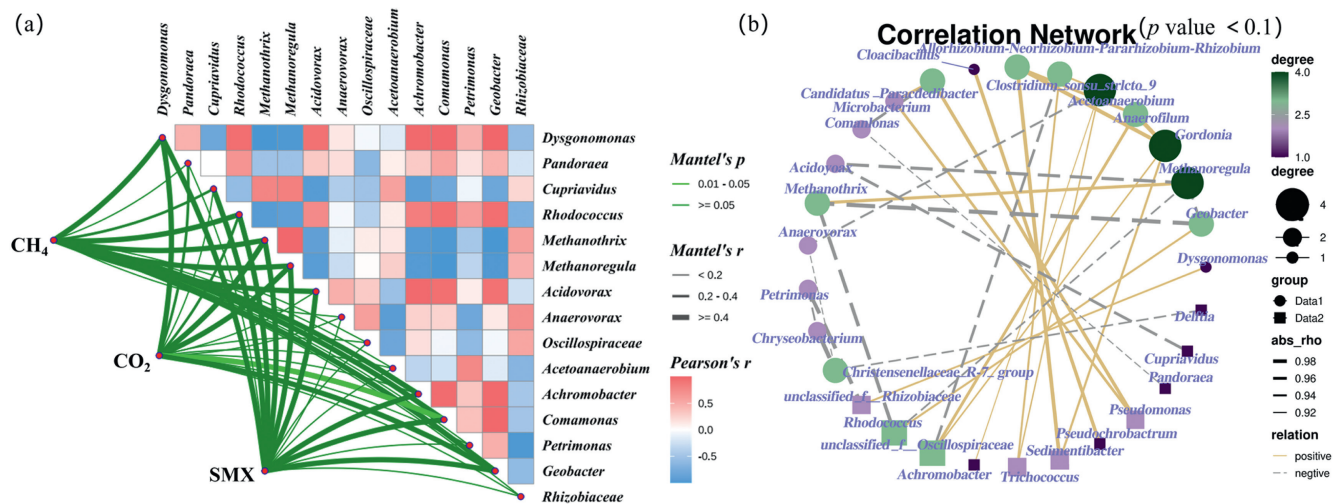


Fig. 5. Microbial mantel test (a) and microbial correlation network analysis (b).

Through the partial Mantel test, correlations among SMX, CO₂ production, CH₄ production, and differences in microbial community composition were analyzed (Fig. 5a). A strong correlation was observed between SMX and the three functional bacteria. In addition, there was a significant correlation between the three functional bacteria and greenhouse gas emissions. According to the correlation analysis between bacteria genera (Fig. 5a), there was a negative correlation between methanogens (*Methanotrix* and *Methanoregula*) and SMX-degrading bacteria (*Pandoraea*, *Achromobacter*, and *Rhodococcus*). In addition, methanogens were negatively correlated with electrogenic bacteria (*Comamonas*, *Dysgonomonas*, *Acidovorax* and *Geobacter*). According to the microbial correlation network (Fig. 5b), *Methanoregula* was significantly negatively correlated with *Geobacter*, *Acidovorax* and *Rhodococcus*. *Methanotrix* was significant negative correlation with *Geobacter* and *Rhodococcus*. These findings suggest that both SMX-degrading bacteria and electrogenic bacteria compete with methanogens. This explains why the total CO₂ emission factors of MFC and OsMFC are significantly lower than those of the A²O process, and the total CO₂ emission factors of MFC and OsMFC that were exposed to SMX were further reduced.

At present, the problem of carbon emissions in the wastewater treatment process is urgent. This study presents a more low-carbon SMX wastewater treatment technology and provides a new reference for the application of MFC and OsMFC. However, MFC and OsMFC still face challenges of operating costs and reactor scale-up. It is believed that the gradual solution of these problems will promote the practical application of MFC technology.

The study explored the simultaneous removal of SMX and the reduction of carbon emissions in MFC and OsMFCs. The effects of SMX contained in wastewater on greenhouse gas emissions during wastewater treatment were investigated, and it was found that 0.5 mg/L SMX significantly reduced methane production. In the process of treating SMX wastewater, SMX can reduce greenhouse gas emissions without negatively impacting the wastewater treatment, electricity generation, and electrochemical performance of MFC and OsMFC. The removal rate of COD can be kept above 70%, and the output potential was stable above 0.53 V. The study also provides insights into microbial community changes and their relationship with carbon emissions. The addition of SMX resulted in the enrichment of SMX-degrading bacteria and a decrease in methanogenic bacteria.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Shilong Li: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Liang Duan:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization. **Qisheng Gao:** Visualization, Validation, Formal analysis. **Hengliang Zhang:** Writing – review & editing, Project administration, Methodology, Investigation, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2025.110997.

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