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Fate and mitigation of antibiotics and antibiotic resistance genes in microbial fuel cell and coupled systems

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Abstract

Microbial fuel cells (MFCs), known for their low energy consumption, high efficiency, and environmental friendliness, have been widely utilized for removing antibiotics from wastewater. Compared to conventional wastewater treatment methods, MFCs produce less sludge while exhibiting superior antibiotic removal capacity,

effectively reducing the spread of antibiotic resistance genes (ARGs). This study investigates 1) the mechanisms of ARGs generation and proliferation in MFCs; 2) the influencing factors on the fate and removal of antibiotics and ARGs; and 3) the fate and mitigation of ARGs in MFC and MFC-coupled systems. It is indicated that high removal efficiency of antibiotics and minimal amount of sludge production contribute the mitigation of ARGs in MFCs. Influencing factors, such as cathode potential, electrode materials, salinity, initial antibiotic concentration, and additional additives, can lead to the selection of tolerant microbial communities, thereby affecting the abundance of ARGs carried by various microbial hosts. integrating MFCs with other wastewater treatment systems can synergistically enhance their performance, thereby improving the overall removal efficiency of ARGs. Moreover, challenges and future directions for mitigating the spread of ARGs using MFCs are suggested.

Keywords: Microbial fuel cells, antibiotic resistance genes, mitigation, influencing factors, MFC coupled systems

1. Introduction

The emergence of antibiotics has made significant contributions to saving countless lives. Antibiotics have been widely applied in various industries, including the medical and agricultural sectors. However, the adverse effects of antibiotics were not taken seriously in the early days, leading to the current problem of antibiotic abuse (Hanna et al., 2023). The extensive usage of antibiotics has resulted in the emergence of antibiotic resistance genes (ARGs). The ARGs have been frequently detected in

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different water environments, posing potential risks to public health and ecosystems (Wang et al., 2020a). Amidst the COVID pandemic, the direct discharge of hospital wastewater into wastewater systems or water bodies creates additional stress on antibiotic-resistant bacterias (ARBs) and ARGs (Wang et al., 2022). In addition to human medicine, veterinary antibiotics pollution has also become a global environmental and health problem in recent decades (Gaballah et al., 2021; Xie et al., 2017; Zhang et al., 2015). Veterinary antibiotics have a significant impact on agricultural soil. The absolute abundance of ARGs in pig farm wastewater ranges from 10^{8} to 10^{10} copies/mL (Yang et al., 2019), while the relative abundance ranges from 10^{-3} to 10^{-1} copies/16S rRNA genes (Huang et al., 2022a). The direct application of manure and compost fertilization to land can result in the accumulation of tetracycline (TC), sulfonamides (SN), ciprofloxacin (CIP), and other substances in the soil, thus affecting the growth of plants and microorganisms (Fang et al., 2023).

Currently, the primary technology used in wastewater treatment plant is biological treatment process. However, in this process, microorganisms provide a conducive environment for the development and dissemination of ARGs. The types of ARGs may be increased in wastewater after the secondary treatment (Luo et al., 2024). Although tertiary treatments like chlorination or ultra-violet (UV) disinfection, and advanced oxidation processes (AOPs) (Qian et al., 2023a) are effective in degrading antibiotics and ARGs, they also have their limitations (Ma et al., 2024). For instance, UV disinfection has a brief contact time of 20 to 30 s, which might not be adequate for

complete removal of ARGs. Additionally, implementing UV disinfection involves a substantial investment in the large-scale wastewater treatment process (Luo et al., 2024). Fenton reaction, which uses hydroxyl radicals formed by ferrite salt and hydrogen peroxide to efficiently oxidize contaminants in water, requires demanding experimental conditions (e.g., temperature, pH) (Zhang et al., 2016b).

The photocatalytic oxidation method utilizes semiconductor materials, such as titanium dioxide, as catalysts to absorb energy and generate superoxide radicals and hydroxyl radicals (·OH). However, its treatment effectiveness and potential of practical applications are not optimistic (Gajdos et al., 2023).

The bioelectrochemical systems (BESs) are emerging technologies that utilize microorganisms to catalyze oxidation or reduction reactions at the anode or cathode. As one type of BESs, MFCs not only exhibits excellent degradation efficiency to antibiotics but also generates electrical energy through the enrichment of electrochemically active bacteria (EAB) (Al-Sahari et al., 2021). Compared to the processes of anaerobic digestion (AD) and cyclic activated sludge system (CAAS), the major merits of MFCs include capacity of energy recovery, low sludge production and effective degradation of contaminants (He et al., 2017). Thus, excellent studies are focusing on the application of MFCs in wastewater treatment, to accelerate its large-scale applications (Kumar et al., 2019; Logan, 2010). MFCs is considered as an efficient and feasible technology for the treatment of antibiotic wastewater (Lovley, 2008; Yu et al., 2020). A study conducted by Cheng et al. (2020) investigated the

efficiency of MFCs in the treatment of synthetic pig wastewater containing various SN. The results demonstrated that a removal rate of over 99% for sulfamethoxazole (SMX), 60% for sulfadiazine (SDZ) and sulfamethazine were achieved in the MFCs under certain conditions. The MFC systems performed higher removal efficiency of SN than that of conventional anaerobic reactors. Numerous studies have also confirmed the high performance of MFCs and its potential in reducing ARGs (Li et al., 2023b; Zhang et al., 2020b). In addition to the study of MFCs treatment of livestock breeding wastewater containing quinolones antibiotics, the number of ARGs decreased overall, among which aminoglycosider decreased by 62.7%，respectively, mobile genetic elements (MGEs) decreased by 57.3% (Chen et al., 2021a).

In MFC systems, many studies have determined that bio-electrochemical processes can enhance the removal rate of contaminant (Luo et al., 2009). The fate and removal of ARGs in MFCs could be influenced by various factors, such as cathodic potentials, anode electrode, antibiotics and their concentrations, dissolved oxygen and carbon source (Zhang et al., 2020a). For example, modifying the carbon source and controlling experimental conditions, can affect the structure and function of microbial communities and effectively control the richness and diversity of ARGs (Zhang et al., 2017). A lower sludge yield also inhibits the production and transmission of ARGs, thereby reducing the ecological risks associated with the evolution of multi-drug-resistant bacteria and ARGs (Chen et al., 2021b). Different concentrations of antibiotics induce different microbial regulatory mechanisms that affect the

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production and dissemination of ARGs in MFCs .Chen et al. (2023) investigated the effect of microbial enzyme changes in MFCs and found that under lower doses of SMX, microorganisms tend to up regulate catalase and *RpoS* regulon to induce *sul1*, *sul3* and 1 integron genes (*intI1*) (Hardwick et al., 2008). The microorganisms exposed to higher doses of SMX tend to up regulate superoxide dismutase and SOS response, a widespread regulatory network aimed at addressing DNA damage by repairing or bypassing lesions, to generate *sul2* and *sulA*. In addition, higher concentrations of antibiotics can induce more resistance mechanisms in microorganisms (Chen et al., 2023). Electrode potential is also one of the important factors. Yang et al. (2022) believed that although the higher current intensity is conducive to the removal of antibiotics, but will stimulate the oxidative stress of microorganisms and increase the pore size of cell membrane. High cathode potential also increases membrane permeability (Hibino et al., 1991), enabling the entry of antibiotics and subsequently promoting the growth of ARBs. This counterproductive consequence does not support the control of ARGs. Additionally, MFCs can also improve its performance through coupling with other technologies (Song et al., 2022), such as constructed wetlands (CWs), Fenton system, membrane bioreactors (MBRs) and photocatalysis. These alternative approaches have also demonstrated effectiveness in the removal of antibiotics and ARGs.

It has been proved that the significant potential of MFCs in removing antibiotics and controlling the dissemination of ARGs compared to conventional water treatment

technologies. This article provides a critical review of ARGs generation and transmission in MFCs, as well as the impact of various influencing factors on the fate of ARGs. These factors include potential voltage, electrode material, initial antibiotic concentration, different additives, temperature, and pH. Each of these factors plays a crucial role. They can directly cause changes in the relative abundance of ARGs or induce changes in ARGs indirectly by altering the microbial community and MFCs performance. Furthermore, the coupling of MFCs with other processes has been proven to enhance the reactor's performance in reducing ARGs in the effluent. By studying the mechanisms of these influencing factors and coupling techniques in MFCs for the removal of antibiotics and ARGs, we can deepen our understanding of ARGs and improve the removal efficiency of MFCs.

2. Transfer mechanism of ARGs

2.1. Vertical gene transfer (VGT) and horizontal gene transfer (HGT)

Vertical gene transfer (VGT) refers to the transmission of ARGs from parent bacteria to offspring bacteria within the same microorganism. In contrast, horizontal gene transfer (HGT) is the primary mode of ARGs transfer between different bacterial species (Groussin et al., 2021), playing important role in the transmission of ARG. HGT can occur through several mechanisms, including conjugation, transformation, transduction, and vesiduction (Fig 1). Notably, mobile genetic elements (MGEs) such as plasmids, transposons, and integrons, serve as the primary carriers of HGT. They facilitate the widespread dissemination of ARGs through various means, independent

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of environmental changes (Huang et al., 2022b; Shao et al., 2018). Among these mechanisms, conjugation is considered the most stable and preferred method of transferring MGEs from donor cells to recipient cells through direct physical contact. Transformation, on the other hand, involves bacteria incorporating DNA fragments released by dead or damaged cells from their immediate surroundings to acquire new traits and functions. Transduction involves the packaging of DNA fragments containing ARGs by phages from donor cells and their integration into the chromosomes of recipient cells. Phages, being more resistant to degradation in the environment than naked DNA, can spread ARGs over longer distances and can remain dormant. Vesiduction is a process similar to transduction, where vesicles containing DNA are secreted from donor cells, transported to recipient cells, and enter the cytoplasm through vesiduction (Zhu et al., 2023). HRT can either deliver ARGs through direct contact on the cell surface or stimulate resistance expression by other bacteria through free MGEs. According to different resistance mechanisms.

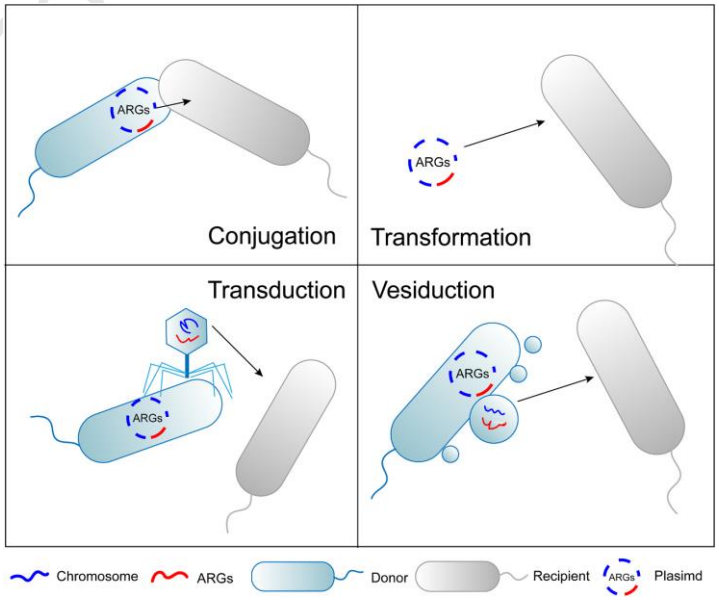

Fig 1. Four pathways of HGT, including conjugation, transformation, transduction and vesiduction (Zhu et al., 2023)

2.2. Resistance mechanisms generated by ARGs

Different resistance mechanisms categorize ARGs into efflux pumps, target protection (ribosome protection), enzyme modification (antibiotic inactivation), and other types of resistance genes (Kapoor et al., 2017; Ye et al., 2022). Efflux pumps play a role in limiting antibiotic expression by recognizing and extruding structurally diverse substrates outside bacterial cells (Alenazy, 2022). Modification of target sites previously targeted by antibiotics, according to Schaenzer et al. (2020), Resistance to glycopeptides and polymyxin antibiotics is manifested by enzyme activity that chemically alters the cell membrane elements required for antibiotic binding. Similarly, modifications of penicillin-binding proteins in some bacteria can also directly produce enzymes that degrade antibiotics to weaken the action of antibiotics (various types of β-lactamases degrade penicillin) (Thakur et al., 2021). Additionally, ARGs can work by stimulating gene mutations, altering bacterial metabolic pathways, membrane permeability, and protein synthesis.

When bacteria encounter the same antibiotic, multiple resistance genes can collaborate to limit the antibiotic's effectiveness. This multi-resistance mechanism allows bacteria to respond more effectively to the pressure of antibiotics, thereby increasing their ability to survive. For example, chloramphenicol (CAP) has three mechanisms of ARGs synergism: CAP acetyltransferases, encoded by cat gene,

catalyze the acetyltransfer reaction on CAP molecules; multidrug transporters or specific transporters, encoded by genes such as *cmlA*, *floR*, *fexA*, *pexA* and *fexB*, can export CAP from cells； rRNA methylase mediated by the cfr gene, adds methyl groups to rRNA molecules, and this methylation modification can regulate the formation and function of ribosomes, thereby affecting the protein synthesis process of cells (Schwarz et al., 2004). These genes are widely transmitted between different genera and species by MGEs.

3. Fate and mitigation of ARGs in MFC systems

3.1 Fate and mitigation of ARGs related to the fate of antibiotics in MFC systems

The use of antibiotics directly contributes to the development of ARBs and ARGs. Commonly used antibiotics target curtails cellular functions, such as inhibiting bacterial growth and interfering with the physiological and biochemical metabolism of pathogenic microorganisms. For example, β-lactam antibiotics inhibit the synthesis of bacterial cell walls (Hemez et al., 2022; Zabiszak et al., 2023) , polymyxin antibiotics alter the permeability of bacterial cell walls, aminoglycosides, tetracycline, and macrolides interfere with protein synthesis in bacterial cells (Akkaya et al., 2019; Glinka et al., 2020), quinolones inhibit nucleic acid synthesis, and SN hinder folic acid synthesis (Wang et al., 2015). Bacteria have developed various resistance mechanisms, including internal production and external acquisition, to resist the action of these antibiotics. Although the current water treatment process reduces antibiotic load, its effectiveness is insufficient, leading to the production and accumulation of ARGs in

water. ARGs are transferred through the sharing of genetic information via VGT and HGT.

The MFC systems holds significant potential for the efficient removal of antibiotics and ARGs due to its advantages of high efficiency and low energy consumption. It has been extensively researched and applied in the treatment of antibiotic wastewater. In an MFC, antibiotics serve as electron donors, entering the anode chamber where microorganisms break them into inorganic substances, electrons, and protons. The protons move through the proton exchange membrane, separating the anode and cathode chambers, while the electrons flow through the external circuit into the cathode. Typically, oxygen acts as the electron acceptor at the cathode, combining with hydrogen ions to form water.

Currently, wastewater treatment plants (WWTPs) achieve an antibiotic removal rate of approximately 43.5~88%, with the removal rate of ARGs displaying considerable variability. Moreover, the accumulation of microorganisms in sludge can exacerbate ARGs accumulation (Wang et al., 2024; Wang et al., 2021). In contrast, most MFCs and their coupled systems maintain a removal rate ranging from 80% to 99% (Zhang et al., 2020a), and the efficient removal of antibiotics by MFCs can help alleviate the selection pressure of ARBs, leading to a reduction of ARGs, as shown in table 1. This is particularly important as the increased concentration and variety of antibiotics gene mutation and recombination rates, leading to increased ARGs pollution, MFCs capable of degrading antibiotics within a tolerable range have shown excellent

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performance in antibiotic removal. For example, research by Guo et al. (2016) compared the effects of CAP at different initial concentrations on the results, and found that although CAP removal rate decreased from 90% to 61% with the increase of the initial concentration during the 48-hour experimental period, the removal rate was not strongly inhibited at high concentrations. Moreover, the absolute removal of CAP per hour increased with higher concentrations of CAP, indicating that the decrease in removal efficiency with increasing CAP concentration was mainly attributed to the CAP loading effect.

Further experimental evidence from Wu et al. (2020) demonstrated that an appropriate concentration of SMX can enhance the activity and performance of electroactive biofilms. The maximum power density of an MFC with 20 mg/L SMX and l g/L sodium acetate is 18% higher than that of an MFC with l g/L sodium acetate. Similarly, Xue et al. (2019b) observed that the MFC's electrical production performance reached its peak when the initial concentration of SMX was 10 mg/L. At low concentrations, sodium acetate facilitated co-metabolism with antibiotics. Once both entered the anode, electroproducing bacteria first hydrolyzed easily degradable sodium acetate to generate electrons. The released electrons are used to split the S-N bond for SMX under anaerobic conditions (Muller et al., 2013). Consequently, this process significantly weakened the inhibitory effect of SMX on the anode biofilm, and the degradation products of SMX continued to serve as a carbon source after sodium acetate consumption. However, it is important to note that even slight electrical

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stimulation can affect the physical properties of bacteria, such as transmembrane permeability and membrane potential, potentially resulting in uncontrolled ARGs and ARBs within the MFC (Zhang et al., 2020a).Therefore, while MFCs show promise in antibiotic removal, careful consideration of potential unintended consequences is necessary.

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Table 1 Removal of antibiotics and ARGs in different MFCs

Antibiotics	Reactor structure	Antibiotic Removal efficiency (%)	ARGs reduction	Bacterial Phylum or Family hosts	Bacterial Genus hosts	References
			sul1, *sul2*, *tetG* and $bla_{TEM,}$	● Actinobacteria, Firmicutes		(Yu et al., 2020)

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				and Proteobacteria	
chloramphenicol	dual-chamber	small multidrug resistance protein, b-lactamase, major facilitator superfamily efflux pump membrane fusion protein	●	Proteobacteria, Firmicutes, Bacteroidetes and Euryarchaeota	(Liang et al., 2019)

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chloramphenicol	dual-chamber	96.0		● Proteobacteria		(Liang et al., 2013)
cefazolin sodium	Single-chamber	1.2~6.8 mg/L/h		● Proteobacteria, Bacteroidetes and Spirochaetae		(Zhang et al., 2018a)
Sulfamethoxazole	dual-chamber	96.1	*sul1*, *sul2*, *sul3*, *sulA*, *intI1*, and *intI2*	● Geobacteraceae	● Alcaligenes, Pseudomonas and Achromobac	(Xue et al., 2019c)

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					ter	
Sulfamethoxazole	MFC-CW-BER	99	*sul1*, *sul2*, and *sul3*	●	Euryarchaeota, Proteobacteria, Chloroflexi, Bacteroidetes, Parcubacteria and Firmicute	(Li et al., 2018b)

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				s		
chloramphenicol	dual-chamber	92.5	*cmlA*, *floR*, *tetC*, *sul1*, and *intI1*		• Pseudomonas, Byssovorax and Dechloromonas	(Guo et al., 2018)
chloramphenicol	dual-chamber	99		• Proteobacteria, Bacteroidetes, Firmicute	• Geobacter, Rhodopseudomo-nas, Desulfovibrio,	(Yun et al., 2016)

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			s and Synergistetes	Pseudomonas, Dysgonomonas, Petrimonas and Cloacibacillus		
chloramphenicol	dual-chamber	80	● Proteobacteria, Firmicutes and	● Burkholderia-Paraburkholderia, Alcaligenes,		(Wang et al., 2017)

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				Bacteroidetes	Geobacter, Stenotrophomonas and Enterobacter	
oxytetracycline	dual-chamber	99	*Tp614*, *sul2*, *intI*	● Firmicutes and Proteobacteria	● Eubacterium spp	(Yan et al., 2018)
Sulfamethoxazole	dual-chamber	83.3		● Proteobacteria, Firmicutes and	● Thauera	(Miran et al., 2018)

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				Bacteroid etes	
tetracycline and sulfamethoxazole	CW-MFC	99	*tetA*，*tetC*，*tetO*，*tetQ*，*tetW*，*sul1* and *sul2*		(Zhang et al., 2016a)

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3.2 Fate and mitigation of ARGs related to the sludge produced in MFC systems

Residual sludge from WWTP contains a high concentration of ARBs and ARGs from human and livestock wastewater (Calero-Caceres et al., 2014). The content of antibiotics in the sludge is significantly higher than that found in wastewater discharge (Munir et al., 2011). In addition, the ARGs content and density of bacteria in sludge were higher than that of free phages, particularly for *sul1* (8 $\log_{10}$ gene copies (GC)/g, in bacterial DNA; 4.4 $\log_{10}$ GC/g, in phage DNA). The presence of nitrogen, phosphorus, heavy metals, organic compounds and microorganisms in the sludge promote HGT within the biofilm, potentially leading to an increase in ARGs abundance during the biological treatment process (Zhu et al., 2021).

The bacteria in MFCs convert chemical energy produced through cellular metabolic processes into electricity by breaking down and utilizing the energy stored in the chemical bonds of substrates (e.g., various biomass and waste compounds, pure or mixed inoculations) (Baniasadi et al., 2021; Pant et al., 2010). Since a significant portion of the energy is converted into electricity, the available energy for biomass growth is reduced, leading to decreased sludge output (Logan et al., 2006). This reduction in sludge production results in lower levels of ARBs and MGEs, effectively inhibiting the transmission of ARGs by limiting the presence of receptors and vectors for gene transfer.

Moreover, compared to AD and CAAS processes, MFCs produce less residual activated sludge. Operating costs associated with treating sludge in sewage treatment

plants typically constitute more than 50% of the total operating investment (Xiao et al., 2011). Therefore, reducing sludge production is a key strategy to effectively decrease the operational expenses of sewage treatment plants (Ting et al., 2007). Yu et al. (2020) integrated BESs with conventional anaerobic composting techniques to expedite the degradation of residual sludge, offering insights for the future advancement of urban wastewater treatment systems (Wang et al., 2021).

Various traditional treatment methods have been employed for the removal of antibiotics and ARGs in wastewater treatment, including the activated sludge process, disinfection techniques (such as UV, chlorine, and UV/chlorine) and adsorption. However, the activated sludge method for treating sludge can potentially lead to secondary pollution and increase the operational costs of water treatment plants (Xue et al., 2019a). Disinfection methods may induce bacterial dormancy and trigger delayed bacterial responses, although the suppression of HGT cannot be sustained over an extended period (Ye et al., 2022). Adsorption processes have shown effectiveness in removing TC and fluoroquinolones. On the other hand, the elimination of SNs, macrolides, trimethoprim, lincomycin, and CAP in the aqueous phase of wastewater treatment plants is primarily attributed to degradation mechanisms (Zhou et al., 2013). Most antibiotics exhibit a positive correlation with ARGs (*bla* (*blaCTXM*, *blaTEM)*, *sul* (*sul1*, *sul2*), *tet* (*tetO*, *tetQ*, *tetW*) and *ermB* genes) commonly found in WWTP. Therefore, MFCs present a promising solution for efficiently degrading antibiotics and reducing sludge production, offering significant potential in controlling the

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transmission of ARGs.

4. Influencing factors for the removal of ARGs in MFCs

4.1. Cathode potential

In the process of antibiotics degradation by MFCs the variation of biocathode voltage can provide different degrees of electrical stimulation to the electroactive biofilm, thereby affecting the composition of the microbial community. Different numbers of electron donors can also affect the degradation rate of antibiotics (Commault et al., 2013). It has been observed that higher external resistance in MFCs results in increased output voltage, a decrease in current intensity, and a more negative anode potential.

Guo et al. (2017) established three MFCs with gradient cathode potential (-1.25V, -1V, -0.5V) to remove CAP and found that the CAP removal efficiency tended to increase when applying more negative cathode potential. The reason is that more negative cathode potential can provide enough electrons for CAP reduction. The change of microbial community structure at -1.25V was also one of the main reasons. With the cathode potential decreases, the abundance of Proteobacteria gradually decreased from 82.1% to 58.3%. The decrease of hosts directly leads to the decrease of CAP resistance genes abundance. The application of a strong electric field can stimulate the expansion of cell membrane pore size and improve membrane permeability (Hibino et al., 1991). The CAP can enter the cell and then cause the death of bacteria that do not carry CAP resistance genes, leading to the decrease of the total

biomass and selectively increase of the ARBs abundance (Li et al., 2013). At a lower negative potential (-0.5 v), further dechlorination of $AMCL_2$ (reduced amine product of CAP) was prevented and more CAP was retained, resulting in a high ARBs abundance.

Kong et al. (2017) also obtained similar results. The author found that the reduction rate constant *k* and reduction efficiency at 1h of nitrofurazone were 1.202 ± 0.124 mg/h and 70.60% ± 4.21% when the applied voltage was 0.8V, which reduced to 0.677 ± 0.069 mg/h and 42.25 ± 1.35% while the applied voltage was 0.2 V. The same trend was observed with different carbon sources. Further investigation found that lower cathode potential resulted in shorter retention of nitrofurazone on the three main intermediates, during the degradation process, and almost all of them were removed at 48h. Yuan et al. (2016) also found that the high current intensity (9.6-20.2 mA) in MFCs was not conducive to control ARGs, and this might related to the process of a large proportion of extracellular electrons flowing to the electrode in the MFC systems. Moreover, high current intensity may lead to oxidative stress in ARBs and result in the release of ARGs. The higher the externally applied voltage, the higher the energy consumption of the whole system, so it is more appropriate the potential to further improve the control of ARGs and power output while ensuring the antibiotic removal efficiency.

4.2. Electrode material

Electrode materials are the basis of MFCs. EAB and antibiotic-degrading bacteria adhere to the electrode surface through various forces and molecular reactions to form

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biofilms, degrade antibiotics and carry out electron transfer simultaneously (Champigneux et al., 2018). Currently, most MFCs utilize metal or carbon-based materials as electrode materials (carbon felt, carbon cloth and carbon brush, etc.). The choice of cathode material affects the degradation pathway and removal efficiency of antibiotics by influencing the cathode potential and circuit current. Wu et al. (2017) compared the removal of CAP by using carbon rod (CR), copper foam (Cu), and nickel foam (NF) as the biological cathode of BES, considering the characteristic advantage of metal foam with highly open porous walls and large specific surface area. The author found that the Cu cathode showed the best performance in CAP (32 mg/L) degradation at an applied voltage of 0.3V. By contrast, the CAP could be removed completely in 24h, 36h and more than 120h by using Cu, CR and NF cathode under the same conditions, respectively.

In addition, different electrode materials also affect the formation of final products. At high potential, the electrical stimulation could cause the reaction skip some intermediates, leading directly to the breaking of the bond in he terminal carbon containing two chlorine atoms. This ultimately resulted in the conversion to CO_2 and H_2O on the copper electrode through free radical reactions, ring opening reactions and mineralization reactions. The final product under the action of CR was nitrobenzene. The final product under NF was 4-nitrobenzyl alcohol. Copper foam exhibited a higher reduction potential compared to the other two materials, and the use of metal foam expanded the range of electrode materials. A deeper understanding of the relationship

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between electrodes, microorganisms, and ARGs will facilitate the quicker discovery of suitable electrode materials and modification methods.

The addition of inorganic substances also improves the performance of the reactor at a richer level and limits the delivery of ARGs. Metal-organic framework (MOFs), as a new carbon-based material composed of organic and inorganic parts, has attracted wide attention because of its unique skeleton, large specific surface area, flexible pore structure and low cost (Tian et al., 2017). Li et al. (2021) used self-synthesizing Ni-MOF-74 to replace Pt/C as the cathode catalyst in MFCs, which not only has a good degradation effect on SMX, the relative abundance of ARGs was also decreased (the relative abundance of *sul1* decreased by approximately 62%, *sul2* decreased by approximately 73%).

4.3. Initial antibiotic concentration

The concentration of initial antibiotic influences the selective pressure generated by ARGs. Antibiotics can also facilitate the formation and spread of ARGs in sludge, thereby exerting pressure on both ARBs and ARGs. In the MFC set by Yang et al. (2022), the relative abundance of ARGs increased significantly with the increase of SMX concentration. This trend was more obvious when the concentration of SMX increased to 2 mg/L and 10 mg/L. Such results indicated that there is a direct relationship between ARGs production and antibiotic concentration, and a positive relationship was observed because the contamination load of MFCs increases and SMX removal efficiency decreased with increasing initial concentration. However, different

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concentrations of SMX have different microbial regulatory mechanisms. A comparative study demonstrated that microorganisms tend to up regulate catalase and *RpoS* regulon to induce *sul1*, *sul3* and *intI1* at the SMX concentration of 10 mg/L and 20 mg/L, and the microorganisms tend to up regulate superoxide dismutase and SOS response to generate *sul2* and *sulA* when the SMX concentration increased to 30 mg/L and 40 mg/L (Chen et al., 2023).

Xue et al. (2019b) conducted experiments on the degradation of SMX at four concentrations in MFCs and found that the MFC had the best electrical generation performance when the amount of SMX was 10 mg/L, and the degradation product 3-amino-5-methyl-isoxazole could be completely degraded to less harmful alcohol and CH_4 in the MFC. Two *sul* genes (*sul1*, *sul2*) and *intI1* were detected in the effluent and biofilm with high removal efficiencies (>99%), and the reduction of integron demonstrated the inhibition of HGT, which would kill the potential ARBs bacteria in the incubator.

In general, low concentrations of antibiotics (<0.5 mg/L) have minimal impact on microorganisms, but high concentrations of antibiotics can be toxic to microorganisms, triggering the SOS response. Meanwhile, high concentrations of antibiotics can show inhibitory effects on the current intensity and the diversity of microbial communities. The changes in bioelectrochemical characteristics caused by the addition of different concentrations of antibiotics may stimulate the metabolic activity of microorganisms and alter the efficiency of substance exchange, thereby regulating the relative

abundance of ARGs (Li et al., 2018c; Maslowska et al., 2019; Sultan et al., 2018).

4.4. Carbon source

Adding organic compounds as substrates can improve the performance of MFCs in the treatment of antibiotic wastewater. Generally, high concentrations of antibiotics have an inhibitory effect on microbial metabolism. Even though antibiotics can also provide energy as a carbon source, the introduction of non-toxic nutrients can stimulate the activity of enzymes in microorganisms, thereby improving the degradation rate of antibiotics. Balancing microbial competition for carbon sources is known as co-metabolism (Xiong et al., 2020). Organic substrates (e.g., glucose and sodium acetate) or other nutrient substrates (e.g., nitrogen and phosphorus sources) are often used as cometabolic additions. Adding different carbon sources can help us better understand the fate of ARBs and ARGs in MFCs.

MFCs utilize microbial metabolism to convert organic matter in wastewater into electricity, with the microbial community playing a crucial role in determining the production capacity. When a single antibiotic serves as an anode electron donor, it restricts the growth of microorganisms. However, the addition of suitable organic matter can enhance microbial uptake of carbon and energy, thereby improving microbial activity. This leads to improved degradation of antibiotics, inhibition of ARGs, increased number of anode electron donors, and enhanced energy conversion rate. Therefore, the choice of substrate in MFC is critical as it greatly influences their power generation performance (Liu et al., 2009). Acetate, an end product of the

metabolism of various complex carbon sources, is commonly used to stimulate the growth of electroactive bacteria (Bond et al., 2002), it significantly enhances the degradation rate of antibiotics. However, due to its relatively high sludge yield, more complex carbon sources, such as glucose, are often chosen for fuel purposes.Wen et al. (2011) found that introducing a mixture of 1 g /L glucose and 50 mg/L penicillin into a single-compartment MFC powered by an air cathode resulted in the maximum power output being 6 times higher than the combined power output of 1 g /L glucose and 50 mg/L penicillin individually. In another study by Sun et al. (2013), using sludge fermentation liquid or glucose as carbon source of biological cathode to promote the degradation of CAP, and found that the highest degradation rate was 3.2-3.6 times that of non-biological cathode.

Chen et al. (2018) established a tetrachloroethylene (PCE)-dechlorinating biocathode systems using glucose, NaAc and $NaHCO_3$ as carbon sources, and found that the structure and composition of the cathode biofilm were significantly affected by the addition of different carbon sources to the constructed biofilm. In the biocathode supplemented with glucose, more electricogenic or dechlorinated bacteria, such as lactococcus, sulfospira and anaerobes, were enriched. The relative abundance of ARGs were also increased correspondingly, which depended on the relative concentration changes of different bacteria and ARGs under the three carbon sources. It can be inferred that *pceA* and *tceA* were more possibly involved in the dechlorination of PCE/TCE to cis-1,2-DCE. As an extracellular electron transfer related gene, *rdhA1* was

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involved in the further dechlorination of cis-1,2-DCE and *omcX*, was also positively correlated with the concentration of electrogenic bacteria. This suggests that carbon source plays an important role in predicting the functional mechanism of ARGs.

4.5. Additives added to MFCs

Electron donor materials such as Cu can also be used in redox reactions, Cu^{2+} and Zn^{2+} are often used with antibiotics in farms or fisheries. Zhu et al. (2022) found that the removal rate of enrofloxacin combined with 10 mg/L Cu^{2+} and Zn^{2+} was 67.6% ± 7.1%, much higher than the MFC without Cu^{2+} and Zn^{2+} (only 33.8 ± 9.1%). The coexistence of Zn^{2+} and antibiotics can improve the power generation performance of MFC, while Cu^{2+} can enhance the ability of MFC to degrade antibiotics. However, when heavy metals coexist with antibiotics, can further exacerbate the spread of ARGs through co-selection. Due to their stable chemical properties and resistance to degradation, some bacteria may die due to the toxicity of heavy metals, while others may develop tolerance to heavy metals and stably inherit this trait. The resistance genes to heavy metals disrupt bacterial growth metabolism, activate bacterial metal protection stress responses and growth states, and may also lead to bacterial resistance to antibiotics (Baker-Austin et al., 2006).

Biochar (BC) is also often used as a catalyst in MFCs due to its strong properties (large specific surface area, rich surface functional groups, high catalytic activity) (Chen et al., 2022; Fan et al., 2022; Zhang et al., 2024). The catalytic performance of BC can also be optimized by a variety of modifications (Qian et al., 2023b).

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Benefiting from these characteristics of BC, the transformation of ARGs can be effectively inhibited (Fang et al., 2022). The previous studies have demonstrated that eDNA can be significantly adsorbed by BC, mainly through electrostatic interaction, cation bridging between eDNA and negatively charged functional groups of BC, and π-π interaction between eDNA and the aromatic surface of BC (Fang et al., 2021). Similarly, some additives that target ARGs removal and transfer can also be used in MFCs. For instance, resistance mechanism inhibitors inhibit the HGT of ARGs, and resistance is usually exerted by specialized efflux pumps or antibiotic degrading enzymes. Adding an antibiotic and a resistance mechanism compound at the same time can enhance the effect of antibiotics while reducing the selective advantage of resistance genes (Drawz et al., 2010).

4.6. Salinity in wastewater

It is necessary to study the fate of ARBs and ARGs in the anode chamber of MFCs under different salinity, considering the high salinity in pharmaceutical wastewater produced in antibiotic production process. N. Guo et al. (2018) used NaCl to control the effect of four salinities (0%, 0.5%, 2% and 6%) on CAP degradation in MFCs, and found that the abundance of *intI1* at 0.5% salinity was significantly higher than that in the control. Most of the ARGs showed no significant correlation with *intI1* at different salinities, except that the relative abundance of *sul1* can encode to inhibit SN antibiotics. For example, the typical ARGs encoding efflux pumps, *cmlA* and *floR*, increased at low salinity but were slightly inhibited at high salinity. The relative abundance of *tetC*

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increased significantly with the increase of salinity, mainly due to the wider host range of *tetC*.

However, increasing the salinity of the anode and cathode chamber can improve the conductivity and proton transfer ability of the solution by reducing the antibiotic selection pressure (Guo et al., 2021). High salinity would disperse the selective pressure on CAP and reduce the growth of ARGs under residual CAP. The addition of Cu^{2+} to CAP-containing wastewater selectively enriched the host bacteria of *cmlA*. As the initial concentration of Cu^{2+} increased, the relative abundance of *cmlA* gradually increased, but the relative abundance of *floR* decreased significantly. This phenomenon is due to the *floR* host, including Methyloversatilis Methylophilus, Brevundimonas, Methylobacillus, Pseudoxanthomonas and Hydrogenophaga, is metabolically inhibited in the presence of Cu^{2+}. The increased selection pressure also leads to an increase in gene copy numbers of *intI1* and *sul1* (Ma et al., 2019). With the increase of salinity, the corresponding tolerant bacteria are screened out, and the ARGs that are the main host are enriched by HGT under selective pressure, resulting in an increase in relative abundance, while the relative abundance of ARGs in salinity-sensitive microorganisms decreases.

4.7. Temperature and pH

To ensure the stable operation of MFC in cold regions or areas with significant temperature fluctuations, it is crucial to consider the impact of temperature and temperature changes on the performance of the reactor. Liang et al. (2016) found that

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lowering the temperature of the biocathode reactor from 25℃ to 10℃, resulted in a significant reduction in the degradation rates of CAP and COD. However, the addition of 2% sodium chloride slowed down the effect of this temperature. This phenomenon was also confirmed in the study by Guo et al. (2018). The author identified 2421 functional genes at 25℃ that could not be detected at 10℃. These unique functional genes mainly belonged to the categories of carbon cycling, metal resistance, stress response, and bioremediation of organic pollutants. The relative abundances of ARGs and *intI*1 under 15℃ and 10℃ were similar, both of which were higher than those at 30℃ in the BES. Additionally, continuous electrical stimulation at low temperature can also increase the stability of the biocathode against environmental changes.

The removal of CAP was found to be significantly affected not only by changes of temperature but also by changes of pH value. Zhang et al. (2017) conducted experiments involving three pH values (6.0,7.0, and 8.0) using orthogonal experimental designs. The optimal pH value was 7.12, and the degradation rate of CAP reached 96.53% under favorable conditions. However, in the study by Yang et al. (2015)，it was observed that the adsorption capacity of TC decreased as the pH decreased. At the solution pH of 3, the adsorption capacity was 35.1 mg/g, and it decreased gradually with decreasing pH until reaching 19.0 mg/g at pH 11. This phenomenon could be explained by the presence of TC in the form of THC^{3+} in solution at a pH below 3.3, making it more conductive to adsorption due to the negative charge on the activated carbon surface. However, the percentage of negative charge increases with the

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increase of pH, resulting in a decrease in the possibility of adsorption and ultimately reducing the removal efficiency.

5. Fate and mitigation of ARGs in MFC coupled systems

5.1. MFCs coupled with Fenton system

The Fenton process is widely employed in wastewater treatment. Under acidic conditions, the presence of ferrous ions (Fe^{2+}) enables hydrogen peroxide (H_2O_2) to generate highly oxidizing hydroxyl groups (·OH), which further initiate the production of other reactive oxygen species. This process facilitates the degradation of organic matter. Currently, the main obstacle in promoting the Fenton method is the production and utilization of H_2O_2 (Li et al., 2022). The integrated MFCs and fenton (MFC-Fenton) system consisted of an anaerobic anode chamber filled with a biodegradable organic matrix and an aerated cathode chamber filled with biorefractory pollutants. In the MFC-Fenton system, electrons are released from the biological reaction at the anode and transported to the cathode via an external loading circuit to generate H_2O_2 via two-electron reduction of cathode oxygen, which is then combined with Fe^{2+} to produce ·OH for pollutant oxidative degradation, as shown in equations (1) and (2) (Feng et al., 2010).

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH \quad (2)$$

A number of studies have been published using MFCs coupled to electrofenton systems for degradation of pollutants, such as dye wastewater (de Dios et al., 2013),

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latex wastewater (Selvaraj et al., 2020) and bactericide (Zhao et al., 2018). Fenton method is also commonly used for the treatment of antibiotics in wastewater, and the photo-Fenton and electro-Fenton are highly superior to theconventional treatments without altering the structure of antibiotics (Liu et al., 2018). Antibiotics can be effectively degraded in a few minutes (Ma et al., 2021). The solar photo-Fenton process ensures effective disinfection of ARBs and elimination of ARGs in wastewater, and this process is very effective to induce permanent disinfection on ARBs (Ahmed et al., 2020; Giannakis et al., 2018). However, the disadvantages commonly observed in all these studies are high processing costs and the difficulty of storing and transporting hydrogen peroxide.

In MFC-Fenton system, antibiotics are oxidized and decomposed in the anode chamber of MFC. The generated protons and electrons then transferred into the cathode chamber through the membrane and external circuit, where they combine with O_2 to form H_2O_2 required for Fenton reaction (Li et al., 2020a). The produced H_2O_2 combine with Fe^{2+} to form hydroxyl group, while generating electric energy. This system can control the reaction process well, and avoid the storage and transportation of hydrogen peroxide (Wang et al., 2019). Li et al. (2020b) verified the feasibility of the MFC-Fenton system (Fig 2), and indicated that the removal rate of most ARGs was above 90% after one cycle. Changes in antibiotic concentration and pH during the reaction process can also in turn affect ARGs degradation. However, in the bio-electro-Fenton processes studied by Li et al. (2022), although the removal rate of

NOR was close to 80%, a high relative abundance and diversity of ARGs can still be detected in the effluent, and the most widespread resistance gene type was the fluoroquinolone antibiotic gene (*adeF*), an efflux resistance gene. After further adding NOR, the relative abundance of *adeF* decreased, while that of *basS* and *macB* increased, indicating that NOR might affect the integrity of cell membrane. Meanwhile, the relative abundance of Proteobacteria decreased from 68.3% to 66.8%, while that of actinobacteria increased from 3.1% to 7.5%, both of which are major ARGs carriers, but the mechanisms of antibiotic resistance were significantly different in different species.

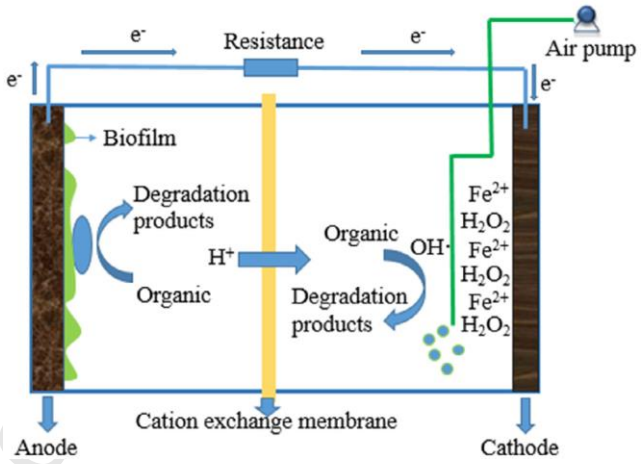

Fig 2. Schematic of a bio-electro-Fenton system (Li et al., 2020b)

5.2. MFCs coupled with constructed wetland

Constructed wetlands (CWs) are a well-known and cost-effective wastewater treatment technology that is often utilized for the treatment of antibiotic wastewater due to its ease of maintenance and environmentally friendly characteristics (Liang et al., 2018). Antibiotics' removal in CWs is mainly carried out through three pathways, including substrate adsorption, plant uptake, and degradation. MFCs operates under

anaerobic conditions at the anode, while the cathode is exposed to air, which can naturally occur in CWs (Wang et al., 2020b). The coupling of MFCs with CWs enhances the pollutant removal efficiency in wastewater by increasing the abundance and activity of electroactive bacteria through MFCs. The coupling of the two technologies has the advantages of efficient wastewater treatment and electrical energy recovery at low cost. Electrical stimulation of microorganisms in MFCs also alleviated the increase in ARGs (mainly *sul* and *tet*-related genes) abundance caused by antibiotic exposure in CW to a certain extent (Song et al., 2018). In recent years, extensive studies have been conducted for the removal of antibiotics and ARGs in MFC-CWs coupling system (Doherty et al., 2015).

In the MFC-CW configuration, the anode and cathode are placed on the plant rhizosphere and surface, respectively, to harness the reaction energy from the wetland. The anaerobic region at the bottom serves as the anode, while the aerobic region at the top serves as the cathode. Guo et al. (2023) compared the treatment efficiency of the vertical flow constructed wetlands-Microbial fuel cell (VFCW-MFC) and the vertical flow constructed wetlands (VFCW) (Fig 3), and found that VFCW-MFC had higher average removal rates of COD, ammonia nitrogen, total nitrogen, and total phosphorus than the VFCW. The SDZ removal rate in VFCW-MFC reached 82.55%, which was higher than that of VFCW (76.60%). Both open- and closed-circuit MFC-CWs have a high removal rate for NH_4^+-N, total inorganic nitrogen, COD, and TP. The MFC-CWs in closed-circuit mode showed better removal efficiency (<99.25%) than that of the

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open-circuit mode (<97.5%). The possible reason is that electric field stimulation can enhance microbial activity and enhance the utilization of SDZ in microbial metabolism, whereas, higher relative abundance of ARGs (*sul1* and *sul2*) in the anode region and cathode effluent might be produced along with the removal of SDZ (Liu et al., 2023).

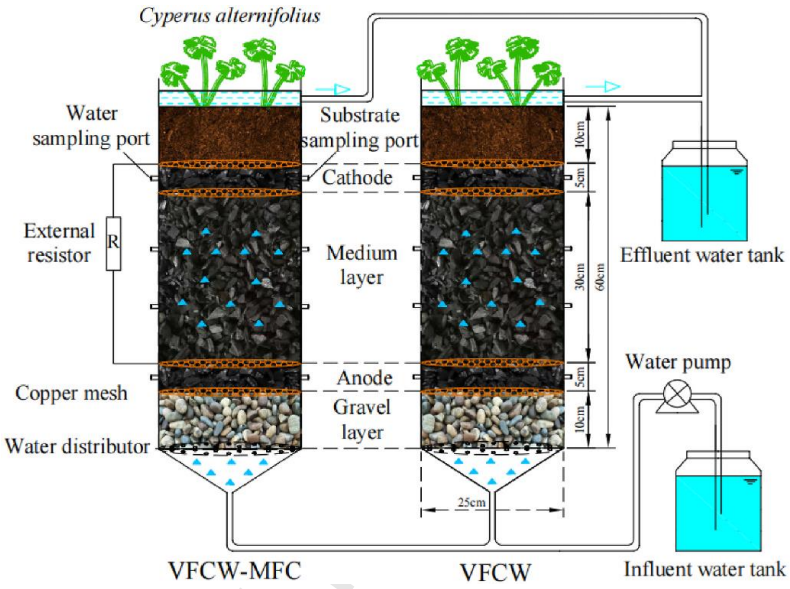

Fig 3. Schematic diagram of VFCW and VFCW-MFC (Guo et al., 2023)

The antibiotic concentration and operating parameters could affect the removal of ARGs in the integrated MFC-CW systems. For example, Zhang et al. (2018b) demonstrated that the removal rate of SMX could reach more than 99.29% in a stacked microbial fuel cell-constructed wetland coupled biofilm electrode reactor system (MFC-CW-BER) and the microbial stress resulting from SMX degradation would be enhanced under high load of SMX caused by the high HRT, and thus the risk that *sul* genes might be present in the system would be increased. Li et al. (2018b) also constructed a continuous flow MFC-CW coupled with a biofilm electrode reactor (BER) system, the total SMX removal rate in the coupled system was over 99%, the copy

number of *sul1* gene was positively correlated with SMX concentration and negatively correlated with HRT.

5.3. MFCs coupled with membrane bioreactor

The membrane bioreactors (MBRs) technology combines membrane filter process with the activated sludge process, which performed high treatment efficiency, low sludge yield and good effluent quality in comparison with the traditional activated sludge technology (Jeong et al., 2010; Kim et al., 2011; Pretel et al., 2015). It is reported that the MBR can effectively inhibit the diffusion of ARGs, and is often used for antibiotic wastewater treatment (Cheng et al., 2018; Li et al., 2023c; Qiu et al., 2013). However, one of the biggest drawbacks of MBR is the problem of membrane fouling, which leads to a decrease in permeation flux or an increase in transmembrane pressure after long-term operation, thereby, shortening the membrane life and hindering the promotion and application of the MBR technology (Meng et al., 2009). Recent studies have shown that in MFCs and MBR coupling systems, the negatively charged activated sludge is far away from the membrane surface due to electrophoresis and rarely accumulates on the membrane (Li et al., 2018a). Moreover, under the synergic action of aeration and micro-electric field, the charged particles are migrated and neutralized, which further reduces the flocculation of sludge. The current can also stimulate the degradation rate of microorganisms, jointly reducing the membrane pollution problem (Hou et al., 2022; Zang et al., 2023).

The addition of granular activated carbon (GAC) to the reactor as a fluidized

particle has proved to be a good control of membrane contamination (Lin et al., 2011). In the combined treatment process of MFCs and anaerobic fluidized bed membrane bioreactor designed by Ren et al. (2014), the membrane was operated at a constant high permeate flux of 16 L/m^2/h over 50 days without cleaning. Li et al. (2017) designed and tested the MBR/MFC coupling system with FeOOH/TiO_2 doped GAC as a dynamic layer on the cathode membrane (Fig 4). The MFC was placed on top of the MBR, and the two chambers were separated with multi holed clapboard, using polyvinylidene fluoride coated carbon fiber cloth as the cathode film, which removed more than 90% of the TC. Under the action of gravity, oxygen diffuses to the cathode film, and the COD is converted to electricity on the GAC through $4e^-$ pathway redox process, which not only reduces the energy consumption required for maintaining aeration, but also reduces the membrane pollution through the micro-electric field in the MFC. The concentration of antibiotics was also greatly affect the cleaning cycle and service life of the membrane, and the higher the concentration of antibiotics could also increase the thickness and density of the membrane pollution (Zhu et al., 2018).

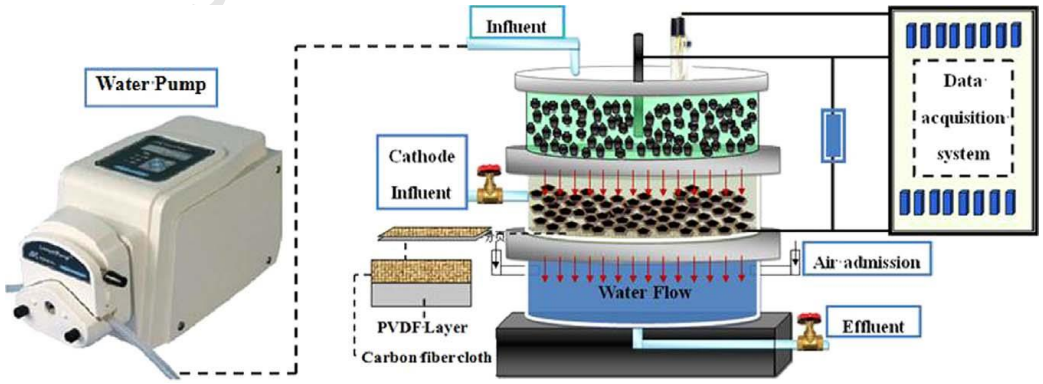

Fig 4. The schematic diagram of MBR/MFC coupled system (Li et al., 2017)

5.4. Others

A BER-MFC-CW (Mn) system was constructed by Li et al. (2023a) to successively remove SDZ and CIP under Zn^{2+} exposure. In this system, the effluent of the BER was used as the influent of MFC-CW system, meanwhile, the electric energy generated by MFC-CW system was supplied to the BER, while the common carbon substrate MFC-CW was replaced by Mn ore substrate. Under Zn^{2+} exposure, the removal of SDZ was significantly inhibited, but the removal of CIP had little effect. This may be because CIP has better adsorption performance and more adsorption pathways than SDZ (electrostatic attraction， cation exchange and bridging, π-π interaction and hydrogen bond) (Zhang et al., 2019). SN are mainly removed by microbial degradation in the MFC-CW system, because the hydroxyl group on the surface of Mn ore can be replaced by antibiotics. This enriched functional bacteria, especially antibiotic degrading bacteria and electrochemical active bacteria, which reduced the internal resistance of the reactor and improved the conversion efficiency of organic matter to electricity by disintegrating metal reduction. The richness of bacterial communities in different reactors indicated that the effect of electric field on BER cathode biofilm was less than that of Zn^{2+} exposure. Proteobacteria, as the main host bacteria, had low resistance to Zn^{2+}. Therefore, exposure to high concentration of Zn^{2+} could reduce the number of host bacteria by reducing bacterial diversity. The absolute abundance of total ARGs in MFC-CW effluent was significantly lower than that in BER effluent, due to the inhibition of high concentration of Zn^{2+}. The BER-MFC-CW

(Mn) coupled system can realize energy recycling and has great potential in reducing the spread of antibiotics and ARGs by heavy metal inhibition.

Photocatalytic degradation is also commonly used to couple with MFCs for the removal of antibiotic contaminants from aqueous environments to reduce ARGs. Compared with traditional chemical disinfection technology to remove ARGs, photocatalytic technology consumes less energy and produces no secondary pollution, which is a sustainable and eco-friendly method for advanced oxidation. Photocatalysts are a major part of photocatalytic technology, typically made from semiconductor materials that are irradiated in wastewater to produce reactive oxygen species (ROS) and hole-electron pairs (O'Dowd et al., 2021). They undergo Redox reactions with substances adsorbed on the surface of the catalyst, in which electrons are captured by dissolved oxygen in water to generate superoxide ion free radicals, holes and OH^- etc. to form ·OH, as shown in equations (3)-(6)(Xu et al., 2021).

$$h^+ + OH^- \rightarrow \cdot OH \quad (3)$$

$$O_2 + e^- \rightarrow \cdot O_2^- \quad (4)$$

$$2 \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (5)$$

$$H_2O_2 + e^- \rightarrow \cdot OH + OH^- \quad (6)$$

These strong oxides damage the bacteria cell wall, membrane, RNA and DNA, reducing the abundance of ARGs rapidly. However, some eARG survived and continued to spread after bacterial recovered (Ahtesham et al., 2023). Light-assisted MFCs can improve the efficiency and performance of MFCs. In a photo-MFC system

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constructed by Yuan et al. (2023), the maximum output voltage and power were 18.8% and 46% higher than that under the dark condition. The removal rate of TC (50 mg/L) by photocatalytic MFCs could reach 95.2% within 3 hours. The system was significantly improved by Photocatalysis, but the weakness of Geobacter and the enrichment of Proteobacteria made ARGs uncontrollable.

6. Future perspectives

Despite the significant progress in removing antibiotics and ARGs through MFCs, controlling the fate of ARGs remains challenging due to multiple influencing factors, particularly the transmission of ARGs and the control of MGEs. While current studies primarily focus on the impacts of individual factors on the fate of ARGs, it is necessary for more comprehensive research to understand how the MFC system responds to multiple stressors simultaneously. To achieve the stable and effective removal of antibiotics and ARGs in MFCs, further research is required to explore the effects of various influencing factor like environmental factors and operating conditions, as well as the related influence mechanism.

1) The fate of different types of ARGs in MFCs can vary significantly even when exposed to the same external conditions. For instance, the relative abundance of certain ARGs like *cmlA* and *floR* may be inhibited with increasing salinity, while others like *tetC*, which encodes an efflux pump gene, could increase due to the proliferation of their main host in salt-tolerant environments. Several types of antibiotics and ARGs are

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present in the antibiotic contaminated wastewater, it is significant to investigate the performance of MFCs in the treatment of wastewater containing different types of antibiotics and ARGs.

2) Additionally, In the same MGEs, there may be various genetic elements that can facilitate the evolution of microbes, such as ARGs and heavy metal resistance genes (HRGs). When bacteria face selection pressure against heavy metals, their genetic mobile elements not only enrich HRGs but also enrich ARGs. This is a co-selection mechanism between antibiotics and heavy metals. As heavy metals can persist in the environment for a long time, the selection pressure exerted by heavy metals in the co-selection of antibiotic resistance and the spread of ARGs should also be taken seriously.

3) Microorganisms play a crucial role as both the creators and carriers of ARGs. The composition of microbial communities influences the relative abundance of various ARGs. Changes in experimental conditions, such as the introduction of antibiotics, heavy metals, or other additives, can directly influence ARGs dynamics by altering the microbial community structure. The abundance of ARGs corresponding to the host directly affects the abundance of ARGs. Exploring the tolerance levels of microorganisms under different conditions and correlating these with changes in ARGs abundance can enhance our understanding and

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prediction ARGs development.

4) The integration of MFCs with other systems represents a promising strategy for the treatment of antibiotics and ARGs. However, most MFC and MFC-coupled systems have only been studied on a lad scale. To achieve large-scale application of MFC systems, more studied are required in the future.

7. Conclusion

This article provides a comprehensive review of the mechanisms involved in the production and removal of ARGs in MFCs, highlighting the roles of various influencing factors in shaping this process. In MFCs, the efficient degradation and oxidation of antibiotics as electron donors, along with the lower sludge yield, reduce the selection pressure of ARGs, thereby limiting the VGT and HGT of ARGs. Different reaction conditions and additive concentrations can select for tolerant microbial communities, affecting the abundance of ARGs carried by different microbes as hosts. In MFCs, electrical stimulation can increase cell membrane porosity, thereby increasing the diffusion risk of ARGs. By altering the carbon source structure, reducing selective pressure, and modifying reaction conditions to control tolerant microbial communities, the removal efficiency of MFCs can be enhanced. Furthermore, MFCs can be integrated with existing processes, such as Fenton systems, artificial wetlands, and membrane reactors, to achieve efficient removal of antibiotics and ARGs. This integration allows for the utilization of energy generated by MFCs, leading to energy

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saving in the overall treatment process. The influence of ARGs within MFCs is intricate and multifaceted, with interdependencies among various factors necessitating a deeper understanding for comprehensive and accurate elimination of ARGs within an MFC system.

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