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Demobilizing Antibiotic-Resistant Bacteria and Antibiotic Resistance Genes by Electrochemical Technology: New Insights

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Abstract

Taking into account its merits in terms of high efficiency and low energy consumption, electrochemical (EC) technology especially bioelectrochemical system (BES) has been applied largely in reducing different antibiotics from wastewater. BES averts the spread of antibiotic resistance genes (ARGs) via forming less quantity of sludge compared with wastewater treatment plants. Nevertheless, transmembrane permeability and membrane potential could be influenced by the electrical stimulation, conducting to augmentations in the antibiotic-resistant bacteria (ARB) and ARGs in BES. This work discusses the utilization of EC technology especially BES for antibiotic reduction and the fate of ARB and ARGs in such systems. BES can effectively remove antibiotics. Nevertheless, low electric current promotes vertical and horizontal ARGs transfer during the treatment of antibiotics in BES. ARB and ARGs could be inhibited by a higher electric current. Questions regarding the potential role of BES in antibiotic removal and the consequent fate of ARGs and ARB in wastewater are presented. Further research is needed to elucidate the primary ARG transfer mechanism and to fully understand the advantages of BESs.

Subject Areas

Chemical Engineering & Technology, Electrochemistry

Keywords

Antibiotic-Resistant Bacteria (ARB), Antibiotic Resistant Genes (ARGs), Bioelectrochemical System (BES), Disinfection, Electrochemical (EC) Technology

1. Introduction

The immoderate utilization of multiple antibiotics conducted to the extensive diffusion of antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in numerous environment matrices like water, sludge [1], soil [2], sediment, etc. [3] [4] [5] [6]. Such a resistance decreases the effectiveness of antibiotics in dealing with infectious diseases, which provokes more than 23,000 deaths per year in the U.S., nearly 25,000 deaths per year in Europe, and even more in less-developed countries [7] [8] [9]. It turned into a worldwide problem for human and animal health [10]. The antibiotic resistance could be diffused by sharing ARGs among microorganisms through horizontal gene transfer (HGT) [11]. Plasmids, integrons, and transposons are the mobile genetic elements frequently implied in the ARG sharing phenomena [11]. In nature, ARGs could remain even after the bacteria are dead [12]. Both intracellular and extracellular ARGs are all set to adapt to novel hosts [13]. The effluents from wastewater treatment plants (WWTPs) and livestock production, frequently with elevated levels of ARGs, are regarded to be important sources of ARB and ARGs in nature [4] [14] [15].

The traditional disinfection techniques (e.g., chlorination [16] [17] [18], UV irradiation [19] [20] [21], and ozonation [22]) in water and wastewater treatment [23] [24] [25] have been found efficient in demobilizing ARB effectively [3] [11] [26]. Nevertheless, most of the ARGs endured even when the ARB are fully demobilized during the disinfection techniques [12] [27] [28]. Rather than all of the time, the disinfection technologies could demolish bacterial deoxyribonucleic acid (DNA) or the cellular structure [29] [30] [31]; however, ARGs could remain in the cell debris and the extracellular ARGs are still causing continuing danger [32] [33] [34]. Lately, techniques for eliminating intracellular ARGs have been to a great degree studied comprising enhanced disinfection [35] [36] [37], constructed wetland [38] [39] [40], and advanced oxidation process (AOPs) [3] [41] [42] [43]. Zhang *et al.* [44] illustrated a positive relationship between the demobilization of ARGs and the Cl_2 injection and residence period; further, they proved that consecutive UV/chlorination [45] [46] [47] could ameliorate demobilization considerably. To reduce both ARB and ARGs efficiently, Oh *et al.* [48] found that an injection of Cl_2 as high as 30 mg/L or an injection of 3 mg/L O_3 is needed. As the dose of UV irradiation augmented, the abundance of ARGs reduced exponentially [49]. Elevated doses of UV irradiation (>10 mJ/cm^2) reduced ARB and ARGs greatly but considerably augmented the frequency of ARGs transfer together for the higher pressure [50]. Constructed wetlands, particularly those possessing a surface flow pattern, have demonstrated acceptable ARGs removal performances; however, the danger of augmented ARGs transfer still endured [51] [52] [53]. For that reason, novel substitutional methods with elevated reduction performance and low hazard of ARGs transfer are highly required [54] [55] [56]. Lately, AOPs (like Fenton reaction [57], TiO_2 photocatalysis, and UV/H_2O_2 [19]) have demonstrated elevated capacity to demobilize ARB and ARGs [19] [20] [57]. In the Fenton treatment and UV/H_2O_2 process, the hydroxyl radicals could reduce ARGs efficaciously (2.3 - 3.8 logs of decrease) and the Fenton treatment achieved better than UV/H_2O_2 process [58] [59]. Guo *et al.* [60] proved that photocatalysis by TiO_2 has the potential to decrease ARB by 4.5 - 5.8 logs and ARGs by 4.7 - 5.8 logs. For their elevated reduction performance, AOPs are encouraging manners for decreasing ARB and ARGs [61] [62] [63]. However, there are still some gaps in expertise [64] [65] [66]. As an illustration, even if electrochemical (EC) disinfection has been largely employed in killing different bacteria [67] [68] [69], viruses [70] [71] [72], and microalgae [67] [73] [74], its capability in reducing ARGs has not been explored pointedly [75] [76] [77]. Moreover, all the previous researches have concentrated on eliminating intracellular ARGs; however, there is no data about reducing extracellular ARGs performances.

This work discusses the inactivation of ARB and ARGs by EC oxidation/electro-Fenton process. Further, it compares electro-Fenton and photo Fenton like process UV-C/H_2O_2/IDS-Cu method with other AOPs techniques. A special focus is accorded to decreased *Klebsiella michiganensis* strain LH-2 viability and corresponding ARG abundance in bioelectrochemical reactors (BERs). The fate of ARGs during bioelectrochemical treatment of high-salinity pharmaceutical wastewater is discussed. Finally, dares and prospects for bioelectrochemical systems (BESs) are reviewed.

2. Inactivation of Antibiotic-Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARGs) by Electrochemical (EC) Oxidation/Electro-Fenton Process

Recently, Chen *et al.* [3] assessed the capability of EC oxidation and electro-Fenton method as substitutional treatment processes for demobilizing ARB and ARGs in both intracellular and extracellular forms. They proved that EC oxidation technique was efficacious in dealing with chosen ARB; however, not in treating intracellular ARGs or extracellular ARGs. The electro-Fenton method was more performant in eliminating both intracellular and extracellular ARGs. Reducing efficacy following 120 min of electro-Fenton application under 21.42 mA/cm^2 was 3.8 logs for intracellular *tet*A, 4.1 logs for intracellular *amp*C, 5.2 logs for extracellular *tet*A and 4.8 logs for extracellular *amp*C, respectively in the occurrence of 1.0 mmol/L Fe^{2+} (**Figure 1**). They concluded that EC oxidation was a performant disinfection technology for ARB and the electro-Fenton process is an encouraging process for eliminating both intracellular and extracellular ARGs in wastewater.

3. Comparing Electro-Fenton and Photo Fenton Like Process UV-C/H_2O_2/IDS-Cu Method with Other AOPs Techniques

AOPs are extremely performant in demobilizing ARGs more than traditional disinfection techniques (e.g., chlorination, UV, and ozonation). Zhang *et al.* [58]

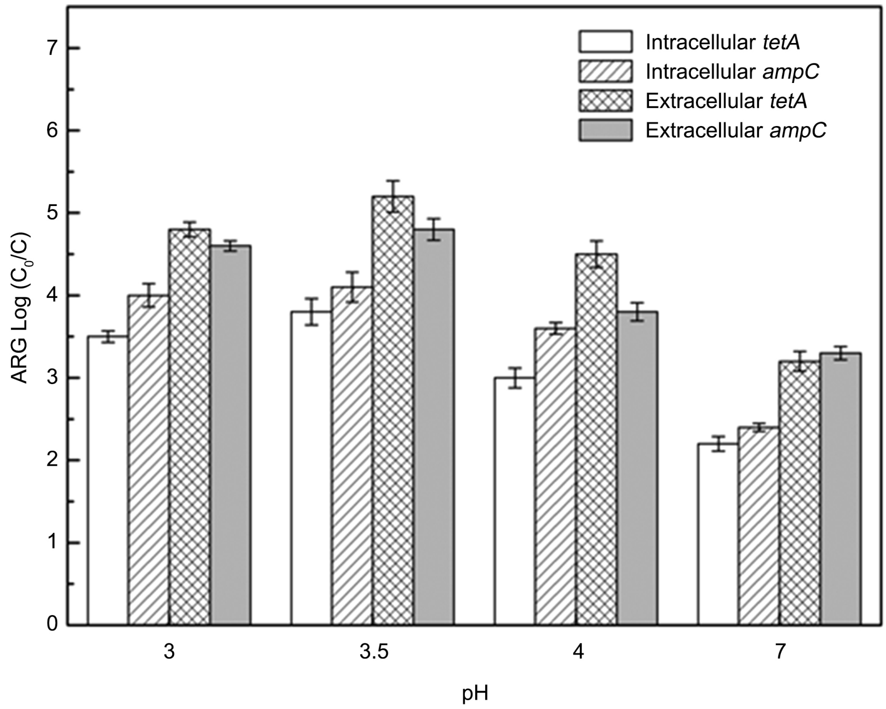

Figure 1. Effect of initial pH on the removal of ARGs after 120 min of electro-Fenton treatment (current density: 21.42 mA/cm^2; Fe^{2+} concentration: 1.0 mmol/L) [3].

illustrated that the maximum reduction of ARGs by the UV/H_2O_2 method and the Fenton technique was 2.8 - 3.5 logs and 2.6 - 3.8 logs, respectively. Whilst Guo *et al.* [60] noted that a reduction of 5.2 logs intracellular *mec*A, 3.3 logs extracellular *mec*A, 4.4 logs intracellular *amp*C and 2.6 logs extracellular *amp*C were attained by UV/H_2O_2/TiO_2 photocatalysis (**Table 1**). The electro-Fenton method appears as an encouraging method for eliminating both intracellular and extracellular ARGs in wastewater.

Recently, Di Cesare *et al.* [78] juxtaposed the performance of a novel AOP, namely the photo Fenton like process UV-C/H_2O_2/IDS-Cu, in eliminating determinants of antibiotic resistance and pathogenic bacteria to a consolidated AOP (namely UV-C/H_2O_2) in a secondary treated municipal wastewater. Tests were realized in both, human pathogens favorable conditions (HPC, in rich medium and 37˚C) and in environmental mimicking conditions (EMC, original wastewater and 20˚C). UV-C/H_2O_2/IDS-Cu method resulted to be more efficient than the UV-C/H_2O^2 in demobilizing bacterial cells in the EMC post-treatment regrowth tests. Both AOPs were efficaciously abating potential human pathogenic bacteria and ARGs in the HPC regrowth tests, even if such a tendency cannot be detected in the measurements taken immediately following the disinfection. In comparison with the UV-C/H_2O_2, the UV-C/H_2O_2/IDS-Cu technique did not clearly provide considerable amelioration in decreasing the tried parameters in the wastewater effluent. By estimating the findings of the regrowth trials it was however easy to extrapolate more complex tendencies, suggesting opposite performances that are visible only after a few hours. Di Cesare *et al.* [78]

Table 1. Juxtaposing electro-Fenton with other AOPs techniques for eliminating ARGs [3].

Technique	Optimal conditions	Reduction of targeted ARGs	Reference
Electro-Fenton	Current density: 21.42 mA/cm^2; Fe^{2+}: 1.0 mmol/L; pH: 3.5; time: 2 h.	3.8 - 4.1 logs (intracellular); 4.8 - 5.2 logs (extracellular)	[3]
UV/H_2O_2	H_2O_2: 0.01 mol/L; pH: 3.5; time: 30 min.	2.8 - 3.5 logs (intracellular)	[58]
Fenton	Fe^{2+}/H_2O_2 (mol): 0.1; H_2O_2: 0.01 mol/L; pH: 3.0; time: 2 h.	2.6 - 3.8 logs (intracellular)	[58]
UV/H_2O_2/TiO_2 photocatalysis	UV fluence dose: 120 mJ/cm^2; H_2O_2: 0.1 mol/L.	4.4 - 5.2 logs (intracellular); 2.6 - 3.3 logs (extracellular)	[60]

presented a detailed discussion of the elimination performance of microbiological/genetic parameters for the UV-C/H_2O_2/IDS-Cu technique, calling for technical adjustments for this extremely encouraging method. Moreover, Di Cesare *et al.* [78] comprehensibly proved the inadequacy of currently applied methodologies in the estimation of specific parameters (e.g. determinants of antibiotic resistance and pathogenic bacteria) in wastewater.

4. Decreased *Klebsiella michiganensis* Strain LH-2 Viability and Corresponding Antibiotic Resistance Gene (ARG) Abundance in Bioelectrochemical Reactors (BERs)

Researchers demonstrated that the electrolytic stimulation method in a bioelectrochemical reactor (BER) could accelerate the growth of sulfadiazine (SDZ) antibiotic-resistant bacteria (ARB) in nutrient broth medium [79]. Nevertheless, the effect of various medium nutrient richness on the fate of ARB and the relative abundance of their corresponding ARGs in such technique is little known. Precisely, it is not known if the fate of ARB in minimal nutrition simulated wastewater is the same as in nutrient broth under electrolytic stimulation [79]. Thus, Li *et al.* [79] compared nutrient broth medium and the simulated wastewater to determine differences in the relative abundance of *Klebsiella michiganensis* LH-2 ARGs in response to the electrolytic stimulation process, as well as the fate of the strain in simulated wastewater. They obtained lower biomass, specific growth rates, and viable bacterial counts in response to the application of increasing current to simulated wastewater medium (**Figure 2**). In addition, the percentage of ARB lethality, which was reflected by flow cytometry analysis, augmented with the current in the medium. An important positive correlation of *sul* genes and *intI* gene relative abundance versus current was also noted in nutrient broth. Nevertheless, an important negative correlation was noted in simulated wastewater due to the higher metabolic burden, which may have conducted to reduced ARB viability. The reduction in ARGs abundance was responsible for reduced strain tolerance to SDZ in simulated wastewater. Minimal nutrition simulated wastewater may decrease ARB and ARGs propagation in BER.

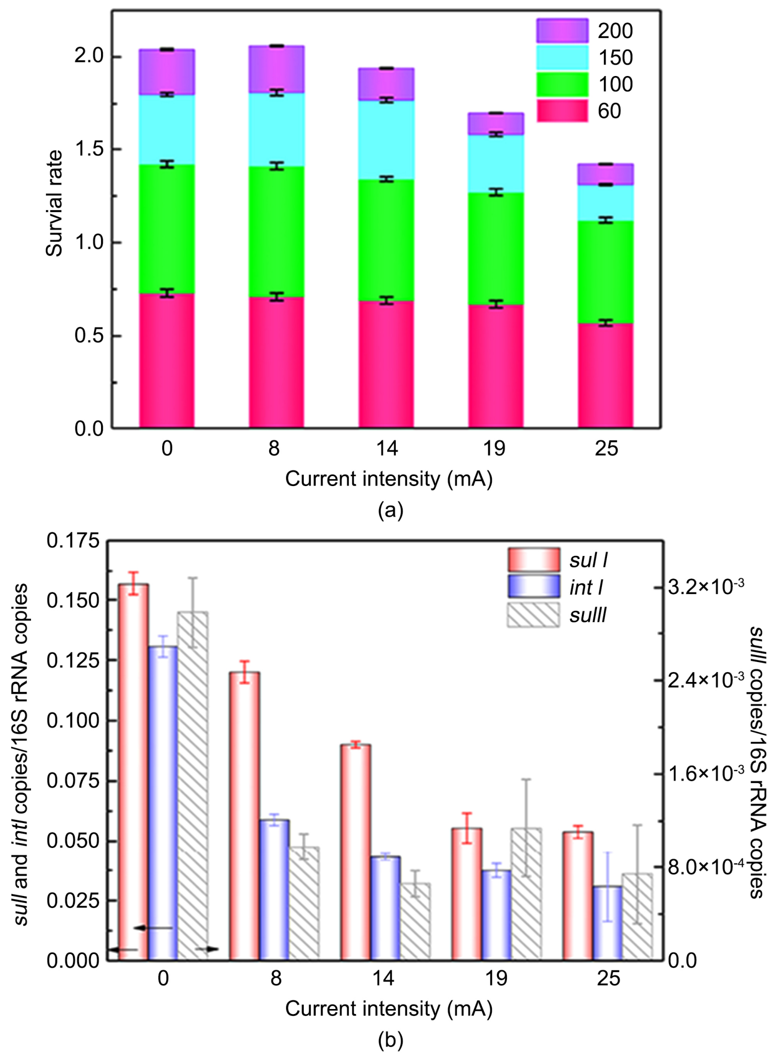

Figure 2. Effects of different current intensities on changes of *K. michiganensis* LH-2 resistance characteristic to sulfadiazine. (a) Survival rate of the strain exposed to different concentration sulfadiazine in simulated wastewater medium without glucose after electrolytic stimulation. (b) *Sul* genes and *intI* gene relative abundance change of *K. michiganensis* LH-2 in simulated wastewater medium without glucose after electrolytic stimulation [79].

5. The Fate of Antibiotic Resistance Genes (ARGs) during Bioelectrochemical Treatment of High-Salinity Pharmaceutical Wastewater

Pharmaceutical wastewaters carrying antibiotics and high salinity could harm conventional biological treatment and conduct to the spread of ARGs [80] [81] [82]. Bioelectrochemical system (BES) is an encouraging method for treating pharmaceutical wastewater. Nevertheless, the fate of ARGs in BES and their correlations with microbial communities and horizontal genes transfer stay unknown. Guo *et al.* [83] examined the response of ARGs to bioelectrochemical treatment of chloramphenicol (CAP) wastewater and their potential hosts below various salinities. Three ARGs encoding efflux pump (*cml*A, *flo*R and *tet*C), one class 1 integron integrase encoding gene (*intI*1), and *sul*1 gene (associate with *intI*1) were followed. Correlation analysis between the microbial community and ARGs showed that the abundances of potential hosts of ARGs were greatly influenced by salinity, which further determined the modification in ARGs abundances below diverse salinities. There were no important correlations between ARGs and *intI*1, showing that horizontal gene transfer was not related to the considerable modifications in ARGs (**Figure 3**). Further, the CAP reduction performance was improved under a moderate salinity, attributed to the altered microbial community driven by salinity. Consequently, microbial community shift is the major factor for the changes of ARGs and CAP removal efficiency in BES under different salinities. Guo *et al.* [83] suggested novel insights on the mechanisms underlying the change of ARGs in BES treating high-salinity pharmaceutical wastewater.

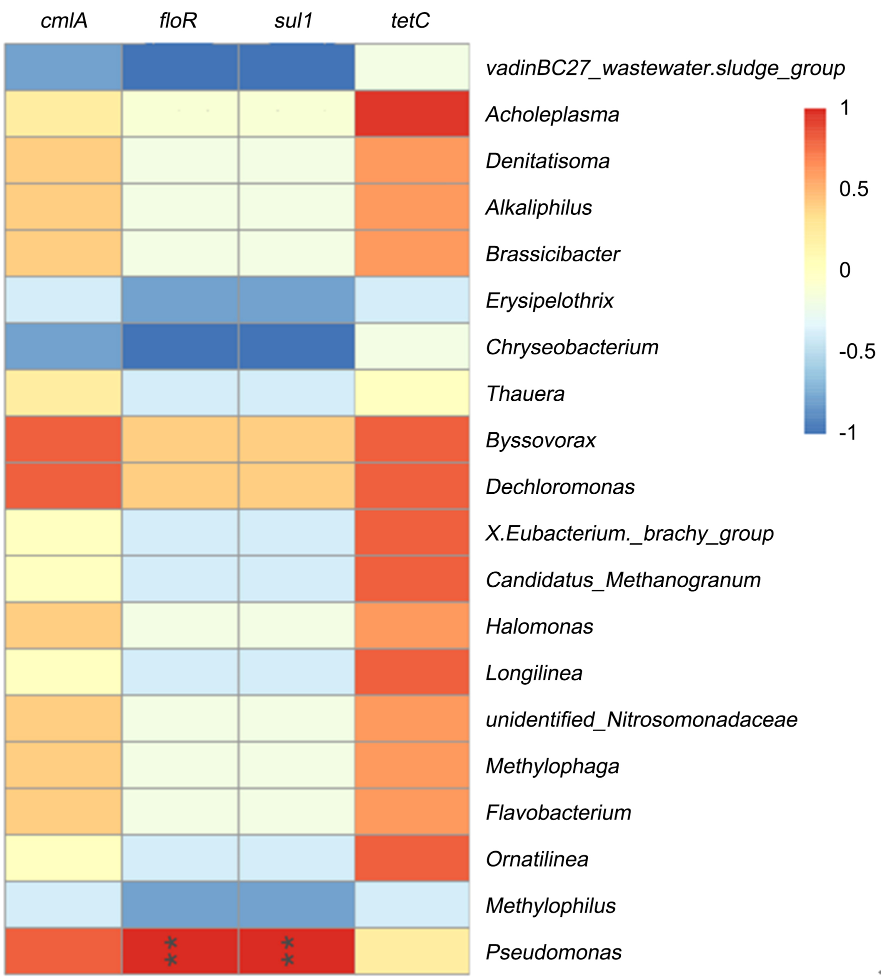

Figure 3. Quantitative correlation of the bacterial genera and ARGs in the samples collected from BESs under different salinities. The correlation analysis was performed using SPSS software, and the heatmap was drawn by R language. The scale bar shows the Spearman Indices (R). Red represents the positive correlation, and blue represents the negative correlation. An asterisk (*) indicates Significant Correlation ($P < 0.05$), and two asterisks (**) indicate Extreme Significant Correlation ($P < 0.01$) [83].

6. Dares and Prospects for Bioelectrochemical Systems (BESs)

By merging microbial metabolism and EC redox reduction, BESs are adopted as an emerging environment-benign and encouraging handling for emerging contaminants, particularly antibiotics. Yan *et al.* [84] discussed the impact of different environmental agents on the BESs' efficiency, functional microbes, and ARGs. Nevertheless, the present pieces of literature mostly focused on searching functional bacteria but not further to discover the biocatalyst pathway about functional genes. Further, numerous researches were dedicated to examining the elimination potential of conventional BESs for wastewater carrying antibiotics but not participate in developing BESs to deal with antibiotics contaminants concentrated in solid matrixes. Consequently, taking into account the diversity of antibiotic contaminants and the complexity of realistic pollutant environments, numerous dares in terms of the amelioration of the elimination capability, a revelation of biocatalyst pathway, development of BESs, and ARGs research stay to be addressed and require more focus (**Table 2**).

7. Conclusions

This work discussed the inactivation of ARB and ARGs by EC oxidation/electro-Fenton process and compared electro-Fenton and photo Fenton like process UV-C/H2O2/IDS-Cu method with other AOPs techniques. A special focus is accorded to decreased *Klebsiella michiganensis* strain LH-2 viability and corresponding ARG abundance in bioelectrochemical reactors and the fate of ARGs during bioelectrochemical treatment of high-salinity pharmaceutical wastewater. Dares and prospects for bioelectrochemical systems (BESs) are suggested. The main points drawn from this work are listed below:

1) The CA and Cu^{2+} removal ability of a BES was studied and the fate of the ARGs (*cml*A, *flo*R, *tet*C, and *sul*1) and *intI*1 was followed [112], and the bacterial community's structure when the cathode was exposed to different initial concentrations of Cu^{2+}. The efficiency of the BES for CAP removal was inhibited when Cu^{2+} and CAP coexisted and the inhibition effect increased with increasing Cu^{2+} concentration. Further, the various concentrations of Cu^{2+} dramatically changed the relative abundances of the ARGs and the bacterial community structure in the BES. The shift of the potential host bacteria mainly contributed to the changes in the ARGs (except for *sul*1).

2) Laboratory-scale EC disinfection tests were performed to examine its reduction performance for 23 ARGs that confer against eight classes of antibiotics and its effects on the antibiotic resistance of surviving bacteria [113]. EC treatments were realized at varying current densities (D treatment) and with different reaction times (T treatment). Prolonged electrolysis conducted to a higher demobilization rate than an augmented current density, while the former was less efficient in the removal of ARGs. As an illustration, the demobilization ratios for the T20 and D80 treatments were both >99%, while the decrease in the relative

Table 2. Dares and prospects for bioelectrochemical systems (BESs) [84].

Dares and prospects	Description
Capacity improvement	Despite the main signs of progress, the elimination potentials of antibiotics in BESs are comparatively low taking into account their future utilization in realistic polluted waters. Enhancing the electron transfer capability remains crucial to deal with such trouble [85] [86] [87]. Electrodes are the habitats of exoelectrogens and dictate the activity of microorganisms and the global efficiency of BESs [88] [89] [90]. Consequently, low-cost and durable electrode materials with superior conductivity and biocompatibility remain to be sophisticated [91] [92] [93]. Biochar and its modification materials as well as other cost-effective carbon-based electrodes could be excellent solutions [94] [95] [96]. Juxtaposing diverse electrodes in terms of their impact on microbial community and electron transfer has to be examined in the next years [97] [98] [99]. Moreover, adding electron transfer mediators could increase the elimination capability, and the interplay of electrochemically active microbes, electrodes, and electron transfer mediators warrants further studies [100] [101] [102].
Biocatalyst mechanism	The functional species matching with numerous representative antibiotics were well illustrated by Yan *et al.* [84]. However, the pathway implied in the electron transfer between pathogens and electrodes and among mixed bacteria stays vague and the metabolic mechanisms of functional genes for antibiotics detoxification request to be illustrated in the next years [103] [104] [105]. Omics techniques, like metagenomics, metaproteomics, and metabolomics, might be conducive for the exploration of functional genes concerning the electron transfer and metabolic routes of antibiotics to reveal the potential proteins mediating electron transport and to define the potential enzymes catalyzing metabolic reactions of antibiotics. Further, how to efficaciously dominate the generation of functional biofilms and to improve the expression of relevant functional genes under various working factors have to attract more attention in the next studies.
ARG investigation	Considering the diversity of ARGs and the difference of ecological parameters of diverse ARGs, more examinations of more types of ARGs and their interaction in BESs are needed [106] [107] [108]. High-throughput quantitative polymerase chain reaction (PCR) stays a powerful procedure that could be employed to simultaneously quantify nearly 300 types of ARGs [109] [110] [111]. Further, functional metagenomics possess an essential contribution in detecting obscure ARGs. Consequently, high-throughput quantitative PCR and functional metagenomics are predictable to light scientific troubles about biofilms in BESs comprising the abundance and diversity of ARGs, route of horizontal gene transfer, and dissimilarities of ARG expression under single and multi-antibiotics during long-term operation. The co-existence of antibiotics and additional emerging contaminants (like pharmaceuticals and personal care products) should be noticed in the realistic environment. As a result, besides the feasibility of BESs for eliminating co-existent contaminants, the influence of co-existent contaminants on ARGs in electroactive biofilms should be considered.
System development	Numerous investigations were realized in simulative aquatic mediums; however, the emerging contaminants of antibiotics are omnipresent and frequently established to be readily concentrated in solid matrixes (like sediments, sludge, and soil). The development of sediment MFCs and plant MFCs or other solid BESs might be an important basis to examine such problems. Specific BESs remain to be suggested for numerous surroundings implying the study of the elimination capability and crucial parameters of antibiotics-containing solid matrixes. Furthermore, the coexistence of antibiotics pollutants and other contaminants (like heavy metals) is pandemic. Heavy metals pose a co-selective pressure on ARGs. Biocathodes have proven to be a good platform for the reduction of metals and antibiotics. Thus, it requires to be examined if biocathodes are a good choice for the treatment of matrixes carrying both antibiotic pollutants and heavy metals and for the release of the co-selective pressure through a rapid transformation.

abundance of ARGs with D80 (from 0.54 to 4.1) was greater than that with T20 (from 5.4 to 5.2). The detection frequency of bacteria resistant to the tested antibiotics decreased by 9% - 100% after EC treatment. This was mainly attributed to a change in bacterial composition. The proportion of bacteria with high antibiotic resistance frequencies decreased (like *Escherichia*), while that with low resistance frequencies (such as *Acinetobacter* and *Pseudomonas*) increased. Further, fewer multi-antibiotic-resistant bacteria survived EC disinfection, which also contributed to the significant decrease in the frequency of ARB as well as in the multi-antibiotic-resistance indices of wastewater samples (from 0.47 to 0.35) after EC treatment ($P < 0.05$). In total, EC disinfection not only reduced the relative abundance of ARGs but also impaired the antibiotic resistance of surviving bacteria. Therefore, it might be a promising disinfection method for controlling the dissemination of antibiotic resistance.

3) For removing antibiotics, BES has numerous advantages and disadvantages [114]. The efficient removal of antibiotics occurs mainly due to faster oxidation through co metabolic degradation or direct oxidation by the anode in which the antibiotic is served as a sole electron donor in microbial fuel cells (MFCs). For the microbial electrolysis cells (MECs), a cathode can provide continuous electrons for the reduction of antibiotics. The most abundant phylum in BES is *Proteobacteria*. Antibiotics and electric current affect the microbial communities and their relative abundances. Antibiotics can be used as the sole carbon source for electricity generation in MFCs, but antibiotics could inhibit the electricity-generating activity of the microbial community. Therefore, the relationship between antibiotics and electricity generation requires further investigation. In addition, a low electric current could promote ARG transfer through vertical gene transfer (VGT) and horizontal gene transfer (HGT) during antibiotic degradation in BES. ARB and ARGs are eliminated with the high electric current. Questions regarding the potential role of BES for antibiotic removal and the reduction of ARGs and ARB are raised [115] [116] [117]. Further research is needed to elucidate the primary ARG transfer mechanism and to fully understand the advantages of BESs.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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