Voltage optimization and antibiotics removal in a microbial electrolysis cell using concentrated sludge as substrates

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Abstract: Microbial electrolysis cells (MECs) were often used for anaerobic wastewater treatment, but research on MECs using concentrated sludge as substrates was still in its infancy. Meanwhile, sewage sludge was an important source of antibiotics pollution in the environment. In this study, batch experiments were conducted to investigate the removal of antibiotics in MECs treating concentrated sludge under different applied voltages. The electrochemical performance and redox reactions of the system, removal of antibiotics, and the responses of microorganisms to different applied voltages were analyzed. The removal efficiencies of antibiotics were 18.1-78.4%, among which chlortetracycline and azithromycin were hardly removed. The removal efficiencies of antibiotics under the applied voltages of 0.6V and 1.0V was significantly higher than other groups (0V, 0.3V and 1.5V). Furthermore, the viability, activity and microbial community structure of the suspended sludge were less affected by the applied voltages. Applied voltages mainly influenced the enrichment and electrocatalytic activity of the electrode microorganisms, and thus the enhanced removal of antibiotics in MECs was mainly due to the electrode bioaugmentation.

Keywords: Microbial electrolysis cells, Antibiotics, Concentrated sludge, Electrode bioaugmentation

1. Introduction

Microbial electrolysis cell (MEC) is one of the emerging bioelectrochemical technologies[1]. The exoelectrogenic microbes on the anode consume organic matters and transfer electrons to the cathode under the external voltage to achieve reduction reaction. The introduction of bioelectrochemical reaction can accelerate the reaction rate and improve the system stability of traditional biological processes[2]. Some researchers proposed to introduce MEC into the traditional anaerobic sludge digestion system, to overcome shortcomings such as long sludge retention time, low methane yield [3,4], acid inhibition, sludge hydrolysis restriction and digestion substrate limitation[5]. However, the research on MEC anaerobic digestion system treating concentrated sludge is still limited. Concentrated sludge as high solid substrates may exhibit different responses and performances to bioelectrochemical systems, which require in-depth exploration.

Previous studies on MEC treating concentrated sludge have focused on organic matters removal and energy recovery in the form of methane [6-8]. However, the fate of antibiotic pollutants in the MEC system has been rarely reported. Antibiotics have received wide attention as emerging pollutants, and the pollution of antibiotics in sewage sludge was serious with the concentration up to 4328–21,335 μg/kg[9,10]. Antibiotics removal efficiency in traditional anaerobic sludge treatment processes was very limited[9], especially the removal efficiencies of fluoroquinolones was only 12-49%[11]. The working conditions and microorganism composition of MEC are different from traditional sludge anaerobic digestion process. The gradient partitioning of redox potential and pH caused by electron directed transport promotes the diversification of chemical reactions [12] and provides potentials for the enhancement of antibiotics removal. In addition, specific environmental niche created by

bioelectrodes can enrich new functional bacteria, symbiotic bacteria and related enzymes[13,14], which might be useful for the degradation of antibiotics. As one of the most important exoelectrogenic microbes, *Geobacter* can utilize a wide range of organics as substrate, including some special trace organic pollutants such as alcohols, phenols and benzene[15]. At present, only a few studies have investigated the antibiotics removal in bioelectrochemical systems treating wastewater [16-18]. Liang et al. [16] and Chen et al. [17] investigated the co-metabolism removal and metabolites of chloramphenicol and oxytetracycline under synthetic wastewater conditions with glucose as the sole carbon source. However, studies on the removal of antibiotics in MEC processes with concentrated sludge as substrates are almost blank.

As one of the important parameters of MEC, applied voltage not only affects the electrochemical performance of the system, biochemical reaction conditions and kinetics[2,19], but also has a significant impact on the microbial composition and distribution. Applied voltage has a significant effect on the enrichment of bioelectrodes microorganisms and its concomitant bioaugmentation[2]. On the other hand, applied voltage can cause irritation or inhibition to suspended sludge microorganisms in the MEC [20]. It was reported that excessively high applied voltage will inhibit activity and even kill microorganisms in the suspended sludge [21,1]. Luo et al.[22] found that an electric current of 20 mA could result in increase of surface hydrophobicity and cell apoptosis, while others found that microbials can be stimulated at 10mA to show better biodegradation[1]. Although several studies had investigated the effects of different applied voltages on the performance of MEC treating sewage sludge, the mechanism has not been fully and deeply revealed, and the results were still inconsistent.

In this study, bioelectrochemical performance, antibiotics removal and microbials responses at different positions (electrode-attached microorganisms and suspended sludge microorganisms) of MEC treating concentrated sludge under different applied voltages were investigated. The enhanced removal mechanism of antibiotics in MEC was revealed and the optimal applied voltage of concentrated sludge in MEC was also evaluated.

2. Materials and methods

2.1 Raw sludge and seed sludge

Raw sludge used in the experiment was obtained from the secondary sedimentation tank of a municipal wastewater treatment plant in Tianjin, China. Sludge was first screened by using a 40-mesh sieve to remove the larger particles. After absolute-rest precipitation for 24 h, sludge supernatant was removed and sludge was concentrated to about 4%. Raw sludge was stored at 4℃ prior to use. The seed sludge was collected from an anaerobic digester operated in mesophilic anaerobic digestion. The seed sludge was also screened using a 40-mesh sieve and diluted prior to use. Before the operation of MEC, the raw sludge was mixed with the seed sludge with a VS ratio of 1:1.

2.2 MECs startup and operation

Single-chamber MEC was constructed and operated with a total and working volumes of 300 and 200 ml, respectively. Anodes and cathodes were both made of graphite felt (4cm×4cm×0.5cm) with space of 2cm and cathodes were coated with Pt/C catalyst. Electrodes were connected to DC power sources with copper wires, and the applied voltages were 0.3, 0.6, 1.0 and 1.5 V. Reactors with no applied voltage (0 group) and without electrodes (Control) were set as controls, and a mesophilic anaerobic digestion (AD) group was set to compare the antibiotics removal efficiencies. The voltages across the external resistance (10Ω) were recorded by the Keithley 2700 data system at a regular time

interval of 10 min and then calculated to current. Ag/AgCl was used as a reference electrode (+196 mV vs. SHE) for measuring cathode potential. A silica tube across the cap of reactors was connected to the gasbag. The MECs were purged with nitrogen for 15 min to eliminate oxygen. All reactors were operated in fed-batch mode at room temperature (20~25℃) for 20d and stirred at 100 rpm with magnetic stirrers. After the successful startup of MEC (the fourth operation cycle), effluent and electrodes of MECs were sampled for further analysis and characterization. All the experiments were carried out in triplicate and results were presented as the mean value.

2.3 Analysis of suspended sludge

Norfloxacin (NOR), ofloxacin (OFL), ciprofloxacin (CIP), tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC), azithromycin (AZI), dehydrated erythromycin (ERY-H_2O) and roxithromycin (ROX) were determined and their removal efficiencies in concentrated sludge was calculated. Quantification of antibiotics in influent and effluent of MECs were the same as previous reported[11]. Flow cytometry (BD FACSCalibur, USA) was used to characterize the viability of suspended sludge microorganisms under different applied voltages. 5 ml of homogenized sludge samples were diluted 10-fold with 0.22 μm filtered phosphate-buffered saline solution (PBS, pH 7.2, 3 g K_2HPO_4, 1 g KH_2PO_4, 8.5 g NaCl per liter, sterilized)[23] and dispersed for 10min. Then, it was diluted 50 times with PBS, and passed through a 20 μm filter membrane. LIVE/DEAD ® BacLightTM Bacterial Viability and Counting Kit was used for staining. SYTO9 and Propidium iodide (PI) were used in double staining procedure to identification viable and dead cells, and microsphere standard was used for absolute quantification. The staining procedure follows the manufacturer's instructions. Adenosine triphosphate (ATP) is essential for microbials energy storage, which can be used as an indicator for microbial growth and metabolism activity[1]. ATP in suspended sludge microorganisms was measured by Lumitester PD-30 (Kikkoman, Japan) using LuciPac A3 Water Kit. Samples were collected from the suspended sludge of MECs under different applied voltages. The initial mixed sludge was analyzed for comparison. Suspended sludge microorganisms DNA was extracted using FastDNA Spin Kit for Soil (MP Biomedicals Inc., USA) according to the manufacturer's instructions. High-throughput Sequencing was carried out on IonS5™XL platform (Novogene Co. Ltd., Beijing, China).

2.4 Characterization of bioelectrodes

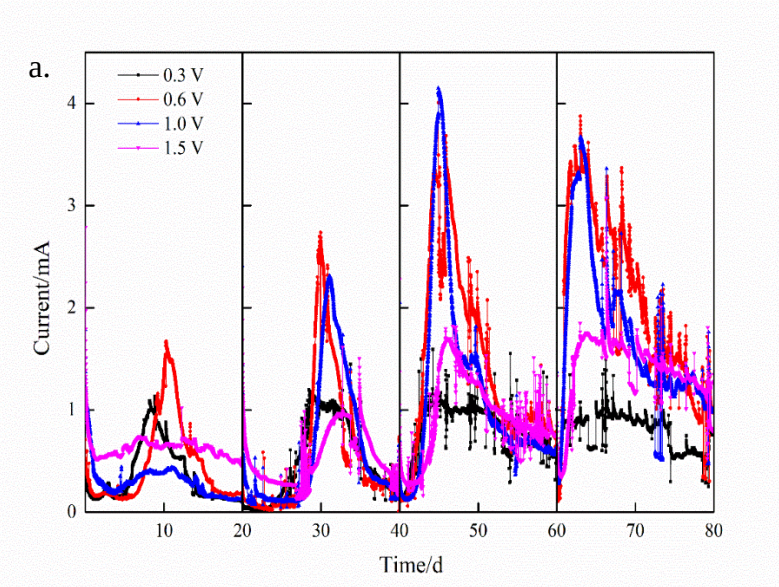
The enrichment of electrode biofilm was characterized by cyclic voltammograms (CV) and scanning electron microscopy (SEM). CV of the anodic biofilms were carried out with a CHI660E system (CH Instruments, Inc.,) in a three-electrode conventional cell[24]. Anode, cathode and Ag/AgCl electrode were connected to working, counter and reference electrodes, respectively, with a scan rate of 1 mV/s in the potential range from -0.8 to 0.2V(vs. Ag/AgCl)[25]. CV curves were determined in-situ conditions at the end of the operation cycle and in PBS solution, respectively[26]. Total coulombs were calculated by integrating current with time. The JSM-7800F field emission SEM was used to visualize the microstructural morphology of electrode surface, and bioelectrodes samples were pretreated in accordance with previously reported method[27].

3. Results and discussion

3.1 Current and cathode potential development during startup

The current and cathode potential development of the MECs during the startup period under different applied voltages were shown in Fig.1a and b, respectively. Current increased with consecutive

cycles of operations, which proved the development and accumulation of electroactive biofilms during the startup. The stable peak current was noticed after the third cycle of operation, which indicated the maturity of the bioelectrode electrochemical properties and the successful startup of the MECs. After successful startup (the fourth operating cycle), the average currents at 0.3, 0.6, 1.0 and 1.5V were 0.79, 2.03, 1.71 and 1.37 mA, respectively, which increased by 2.2-5.7 times compared to the average current in the first cycle. The change trend of current in a single operation cycle was first increased and then decreased, which was the same as that of other studies on MECs treating sludge[28,6]. The time of peak current was also significantly shortened with the startup process. The peak currents of 0.3, 0.6 and 1.0 V in the first cycle were 1.13, 1.67 and 0.44 mA, respectively, and the corresponding peak times were 8.8, 10.3 and 11d, respectively, and there was no obvious peak current at 1.5V. Until the fourth cycle, the peak time of all MECs were 3.0 d, and the peak currents at 0.3, 0.6, 1.0, and 1.5V were 1.76, 3.87, 3.67 and 3.08 mA, respectively. Both average current and peak current were the highest at 0.6V, followed by 1.0V, 1.5V and 0.3V. In the process of sludge hydrolysis, more readily available substrates for exoelectrogenic microbes on anode were produced, such as acetate, and with the progress of biochemical reaction, residual substrates available for the anodic oxidation was reduced[6]. It was reported that MECs fed with acetate, butyrate, and propionate achieved peak current density of 6.0, 2.5 and 1.6 ± 0.14 A/m^2[29]. The mutual promotion of sludge hydrolysis and anodic oxidation gradually shortens the peak time during the startup process. Moreover, the total coulombs of 0.3, 0.6, 1.0, and 1.5V were 1,360, 3,507, 2,965 and 2,376 C, respectively, higher than the 1,354 C at 1.0V in previous study[30].



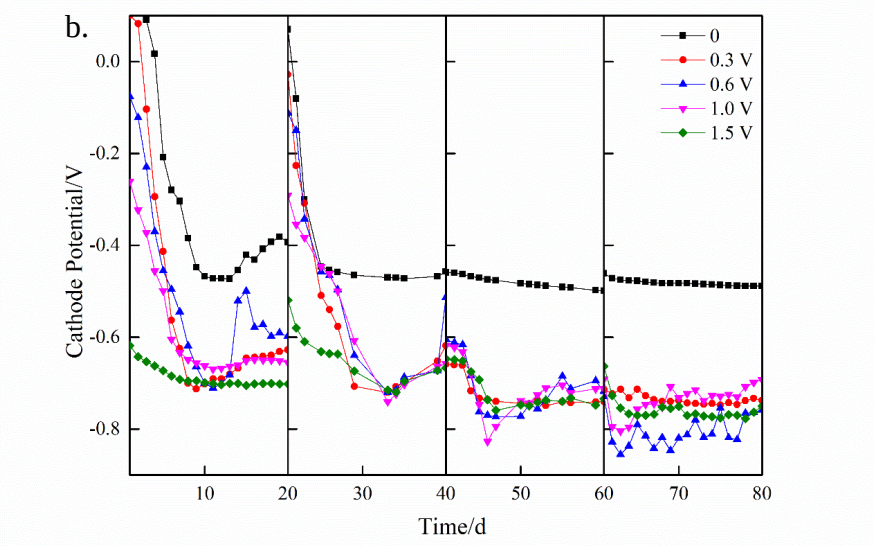


Fig.1 Current development (a), and cathode potential development (b) during the startup of MECs under different applied voltages

Cathode potential increased with consecutive cycles of operations, the average cathode potential in the fourth operation cycle of 0, 0.3, 0.6, 1.0 and 1.5V were stable at -0.481V, -0.735, -0.804, -0.735 and -0.758V, respectively. The change of cathode potential was inconsistent with applied voltages, and the cathode potential was maximum at 0.6V, indicating its enhanced cathodic reduction reaction. Similar to the results of this study, the cathode potential of MECs treating sludge under 0.3，0.5 and 0.7 V were -0.51，-0.73 and -0.96V (vs. Ag/AgCl), respectively[31,8]. On the other hand, the anode potential was consistent with the applied voltage. At the fourth operation cycle, the anode potentials of 0, 0.3, 0.6, 1.0, and 1.5V were -0.479, -0.450, -0.294, 0.235 and 0.697V, respectively.

3.2 Variation of antibiotics under different applied voltages

The concentrations of antibiotics in the initial mixed sludge were shown in Table 1. The pollution of fluoroquinolones (FQs including NOR, CIP and OFL) were the most serious, followed by tetracyclines (TCs including TC, OTC and CTC), and finally macrolides (MLs including AZI, ERY-H_2O and ROX), which was the same as the previous studies [9,11]. The removal efficiencies of antibiotics in MECs were shown in Fig.2 to investigate the influence of applied voltages and also compared with mesophilic anaerobic digestion. FQs and TCs were removed in the same range of 18.1%-47.7% in all experimental groups, except that CTC as well as AZI showed negative removal in some groups. ERY-H_2O and ROX were removed by 52.7%-78.4% with no significant difference between the experimental groups ($p > 0.05$). TCs and MLs removal in AD group were lower than previous research that reached up to 59%-100%[32,11]. While FQs and ERY-H_2O were better degraded compared with previous anaerobic removal efficiencies that was lower than 42% and even negative removal[11]. In this study, the antibiotic removal effect of MECs system at room temperature was comparable to that of AD, except for CTC and AZI ($p < 0.05$), which had greater advantages and potential from the perspective of energy conservation. For FQs and TCs, antibiotic removal efficiencies at 0.6V and 1.0V were significantly higher than other applied voltages ($p < 0.05$), which increased by 16.7%-26.6%. CTC and AZI were removed by 31.4% (1.0 V) and 36.1%-42.7% (0.6 V and 1.0 V), respectively, compared with negative removal under other applied voltages. Antibiotics in this study was mainly oxidative degraded proved by previous studies[33-35]. Considering the anode potential was

positively correlated with the applied voltage, indicating that its anodizing ability was enhanced with the increase of the applied voltage, but the oxidation removal of antibiotics was not improved. Therefore, the electrochemical reaction in MECs had negligible effect on the enhanced removal of antibiotics, and the main role was biodegradation, which was similarly concluded for methanogenesis[13].

Table 1. The concentration of antibiotics in the initial mixed sludge

Antibiotics	Concentration (μg/kg)
NOR	1534.51 ± 30.51
CIP	452.81 ± 0.54
OFL	2894.71 ± 37.40
TC	120.12 ± 2.56
OTC	850.49 ± 4.63
CTC	15.88 ± 0.39
AZI	445.33 ± 35.66
ERY-H_2O	9.22 ± 1.61
ROX	10.92 ± 0.12

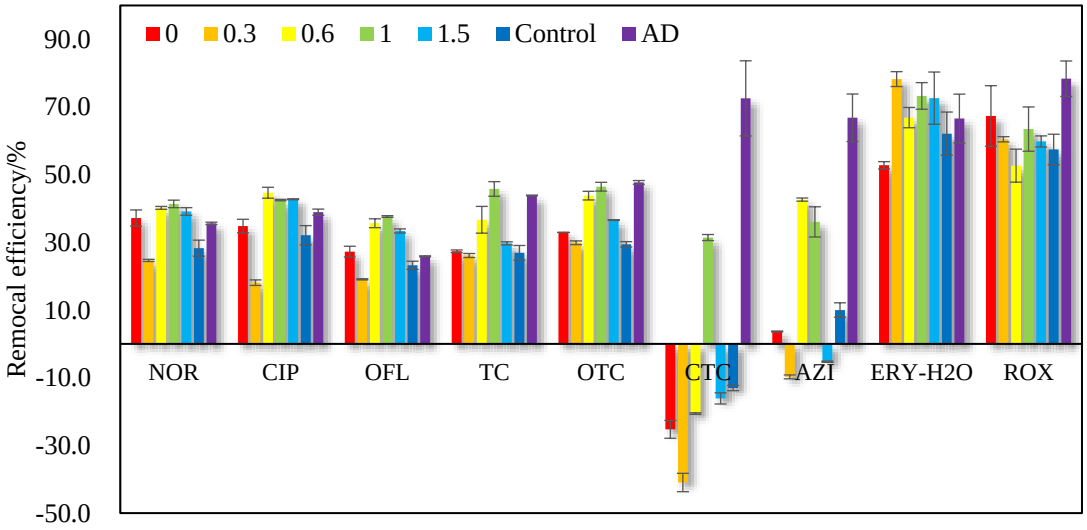


Fig.2 Removal efficiencies of antibiotics in MECs under different applied voltages

3.3 Viability, activity and composition of suspended sludge microorganisms

The viability and activity of suspended sludge microorganisms in MECs under different applied voltages were shown in Fig.3a and b and compared with AD. As shown in Fig.3a, the digestion of suspended sludge was more thorough under mesophilic anaerobic digestion, and the number of live bacterial cells was significantly reduced to 4.72×10^8 cells/ml ($p < 0.05$). Different from AD, the number of live cells in MECs was higher than the dead cells, and the introduction of voltage had a stimulating effect on microbial growth compared with no applied voltage ($p < 0.05$). However, there was no significant difference in microbial viability under different applied voltages ($p > 0.05$). The average live and dead cell numbers were 8.07×10^8 cells/ml and 6.80×10^8 cells/ml, respectively. ATP, as an important role in the material and energy cycle, was used to characterize the activity of suspended

sludge microorganisms in this study. Suspended sludge microorganisms activity at 1.0 V-MECs and AD was significantly higher than other MECs ($p < 0.05$). The ATP at 1.0V-MECs was 1.7-2.0 times as much as other MECs. Ding et al. investigated cell rupture and microbial activity of suspended sludge microorganisms under applied voltages from 0 to 2.0V in two-chamber MEC treating synthetic wastewater[1]. Their research suggested that high voltages (1.0 V and 2.0 V) led to plasmarrhexis and slow growth and metabolism. While in this study, sludge substrates can withstand higher voltages up to 1.5V. 0.3-1.5 V and 1.0 V stimulated the growth and metabolic activity of microorganisms, respectively.

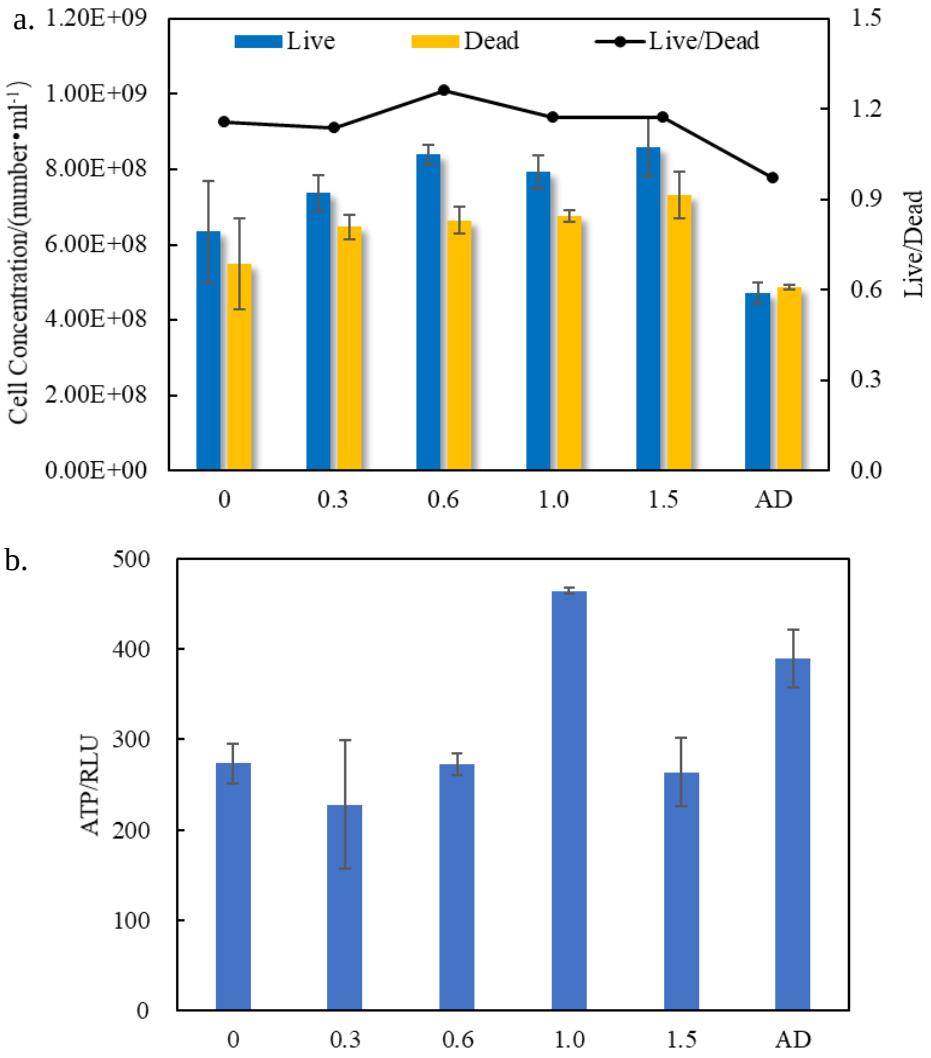


Fig.3 Suspended sludge microorganisms viability (a) and ATP (b) in different applied voltages MECs and anaerobic digestion (AD)

Fig. 4 showed the unweighted unifrac distance and relative abundance of initial mixed sludge and suspended sludge under different applied voltages MECs. As can be seen from the unweighted unifrac distance of Fig. 4, the microbial composition of initial mixed sludge was different from the suspended sludge microorganisms of MECs. The microbial composition of MECs suspended sludge under different applied voltages (0.3-1.5 V) was not significantly different ($p > 0.05$), which was different from previous research[31]. While the 0 group (MEC with no applied voltage) showed somewhat different from other MECs. At the phylum level, the most abundant bacterial populations were

Proteobacteria, Bacteroidetes, Firmicutes, Actinobacteria and Chloroflexi, which was similar to previous reports[31,36]. Proteobacteria was 39.8% in the initial mixed sludge, while it decreased to 18.2%-22.1% in MECs suspended sludge and approached to the reference[27]. Accordingly, Bacteroidetes and Firmicutes increased from 18.7% and 6.5% to 31.8%-35.5% and 18.5%-22.0% in different applied voltages MECs. Bacteroides and Firmicutes enriched in MECs are the main fermentative bacteria and syntrophic bacteria that can degrade VFAs, respectively.

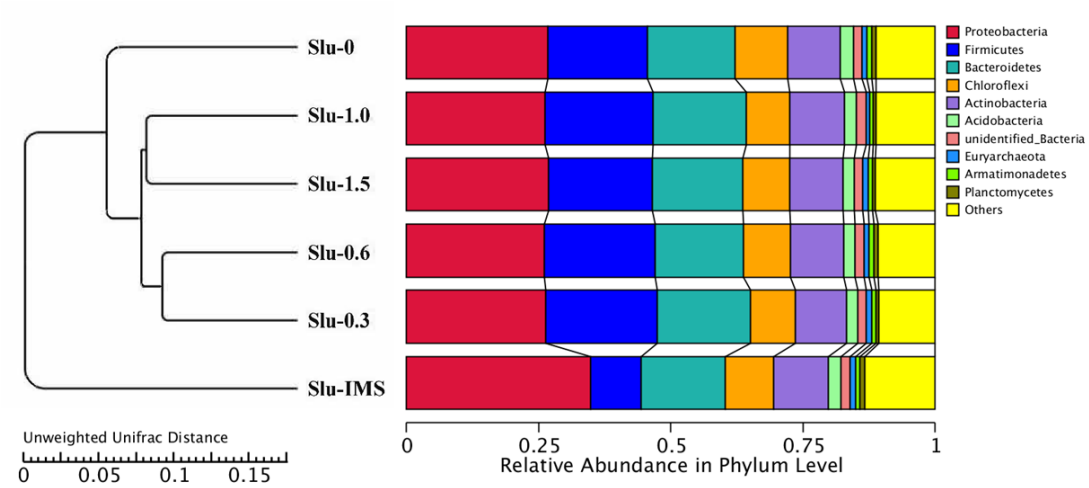


Fig.4 Unweighted unifrac distance and relative abundance at phylum level of suspended sludge microorganisms in raw sludge and different applied voltages MECs

The responses of MCEs suspended sludge microorganisms to different applied voltages were investigated from microbials viability, microbials metabolic activity and microbials composition. The introduction of voltage in the range of 0.3V-1.5V can stimulate the growth of microorganisms. But there was no significant difference in the viability, metabolic activity (except for the enhancement at 1.0 V) and the microbials composition of different applied voltages MECs. Considering that the removal of antibiotics, especially FQs and TCs, was significantly affected by the applied voltage in MECs (analyzed as 3.2), it can be speculated that the enhanced removal of antibiotics under optimized voltage was not caused by suspended sludge microorganisms, but by bioaugmentation derived from electrode biofilms.

3.4 Bioelectrochemical properties and characterizations of bioelectrodes

CV profiles of bioanodes at different applied voltages MECs at the end of a batch cycle as readily degraded substrates were depleted (in-situ) were shown in Fig. 5a. Cyclic voltammetry analysis helped in understanding the bioelectrochemical activity of the anode electrodes[30,37]. CV revealed enhanced oxidation and reduction current of bioelectrochemical active anodes at 0.6V and 1.0V in comparison to others. When applied voltages were 0V, 0.3V and 0.6V, the oxidation peaks were noticed at -96, -89 and -47 mV (vs Ag/AgCl) with a peak current of 3.6, 5.32 and 9.39 mA, respectively, which demonstrated that biocatalyst formation and appropriate voltages facilitated electron transfer. When substrate-depleted solution was replaced by PBS without substrate as Fig. 5b, redox peaks remined present at the constant potential, indicating that they depend on biofilm-bound redox compounds (e.g. outer-membrane bound cytochromes) rather than soluble electron shuttles[25]. Although no peak occurred on the CV curve at 1.0V, it still showed enhanced bioelectrocatalytic activity, which can be exhibited by the maximum redox current[38,37]. The maximum oxidation currents increased with

biocatalyst development on the bioanode in the order of 1.5V (1.37mA), 0V (2.42mA), 0.3V (3.65mA), 0.6V (5.9mA) and 1.0V (9.15mA). In addition, charge distribution observed from voltammetric profiles also increased with the same order of 1.5V (1.34C), 0V (3.24C), 0.3V (4.58C), 0.6V (9.26C) and 1.0V (10.46C). These results showed the highest bioelectrocatalytic activity and electron transfer of the anode at 0.6V and 1.0V, indicating the effective enrichment of exoelectrogenic microbes on the surface of anodes. While the excessive applied voltage (1.5V) was not conducive to the enrichment of electroactive microorganisms, leading to a sharp decline in electrocatalytic activity.

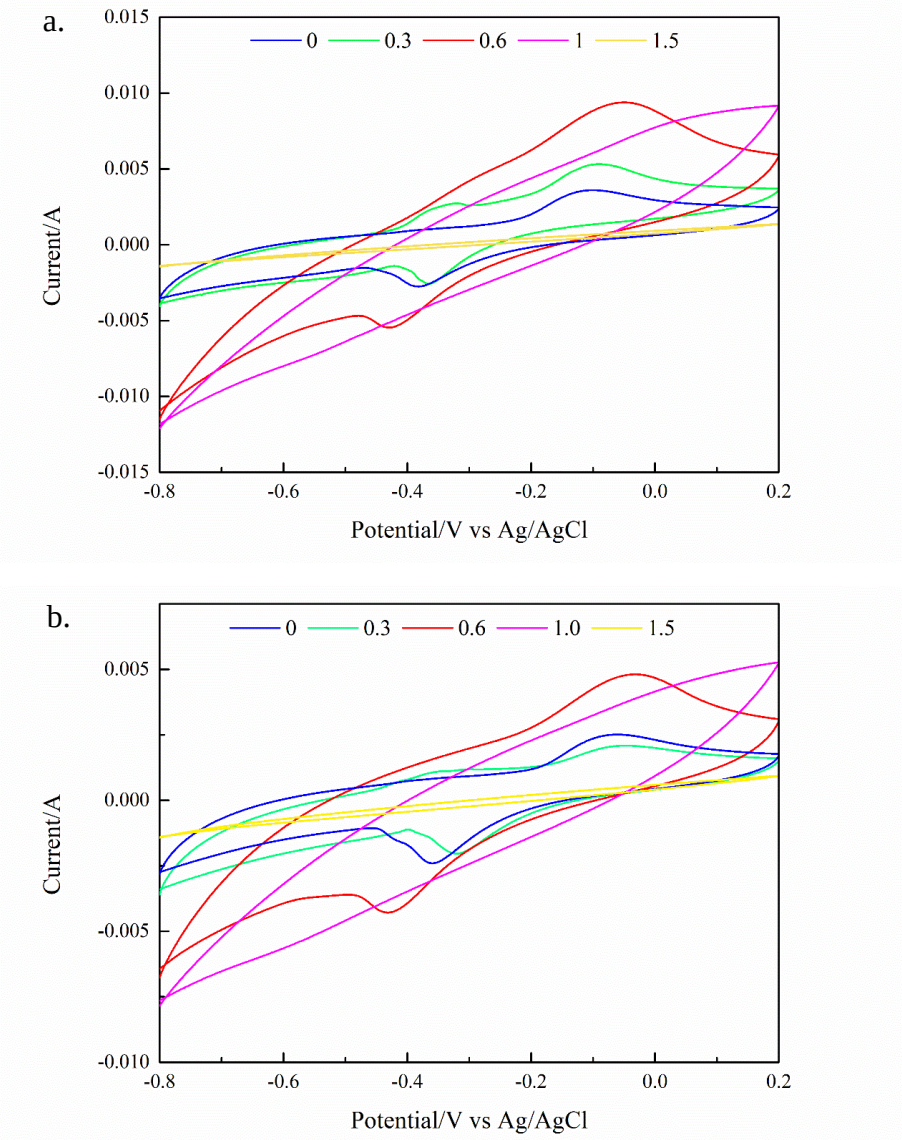


Fig.5 Cyclic voltammetry (CV) profile of bioanodes under different applied voltages in in-situ (a) and PBS (b)

To further verify the enrichment effect of electrode electroactive microorganisms reflected by CV curves, SEM was conducted to visualize the microbial biofilm growth on anodes and cathodes surface. Graphite felt had plenty of carbon fiber silk on the surface, which provided sufficient space for the growth and enrichment of electroactive microorganisms. Bioanodes successfully enriched a large number of rod-shaped exoelectrogenic microbes at 0.3V, 0.6V and 1.0V, but the growth was sparse at 0V and 1.5V. Electrogenic bacteria stacked and formed zoogloea at 0.6V, with some gel-like

components that possibly belonging to the extracellularly metabolic products of the microorganisms[27]. Irregular deposits were formed on the surface of biocathodes, and cathode deposits at 0.6V were interspersed with rod-shaped and cocci-shaped bacteria.

According to the previous analysis, neither electrochemical reaction nor the suspended sludge microorganisms was the driving force for the enhanced removal of antibiotics. Both CV and SEM analysis confirmed the enhanced biofilm enrichment and bioelectrochemical properties of the electrodes at 0.6V and 1.0V, and were consistent with the removal of antibiotics. Thus, the bioaugmentation of electrodes with effective enriching biofilm on the removal of antibiotics in the sludge was confirmed.

4. Conclusions

Effects of applied voltages on antibiotics removal from concentrated sludge in a MEC system were studied, and the mechanism was analyzed from the aspects of electrochemical performance, metabolism and composition of suspended sludge microorganisms, and enrichment of electrode microbials. The antibiotics removal efficiencies of 18.1-78.4% in MECs at room temperature were comparable to that in mesophilic AD. The antibiotics removal, electrocatalytic activity and electrode microbials enrichment were significantly improved at 0.6V and 1.0V. The enhancement of antibiotics removal in MECs was mainly contributed by the bioaugmentation of bioelectrodes.

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