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Damage of anodic biofilms by high salinity deteriorates PAHs degradation in single-chamber microbial electrolysis cell reactor

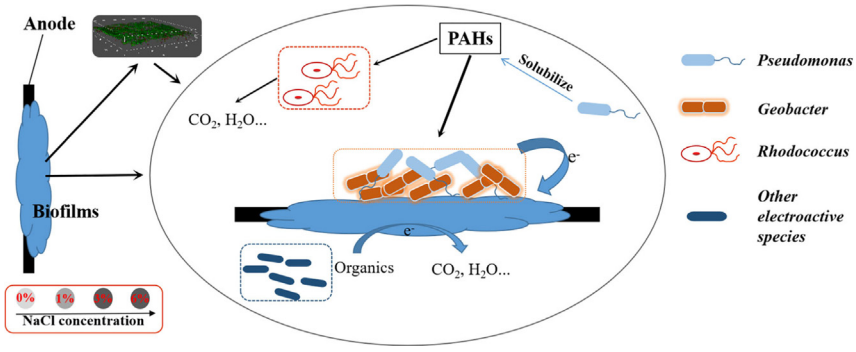
Peng Ding [a], Ping Wu [a], Zhang Jie [a], Min-Hua Cui [a,b,c,*], He Liu [a,b,c,*]

[a] School of Environmental and Civil Engineering, Jiangnan University, Wuxi 214122, China
[b] Jiangsu Key Laboratory of Anaerobic Biotechnology, Wuxi 214122, China
[c] Jiangsu Collaborative Innovation Center of Water Treatment Technology and Material, Suzhou 215011, China

HIGHLIGHTS

- High salinity deteriorated the degradation of naphthalene by MEC.
- High salinity reduced the thickness of anodic biofilms.
- The thickest biofilm was obtained under 10 g/L NaCl concentration in MEC.
- The live/dead bacteria ratio decreased with increasing salinity.
- *Pseudomonas* played an important role in MEC with high salinity.

GRAPHICAL ABSTRACT



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ABSTRACT

The anaerobic biodegradation of polycyclic aromatic hydrocarbons (PAHs) in high salinity wastewater is rather hard due to the inhibition of microorganisms by complex and high dosage of salts. Microbial electrolysis cell (MEC), with its excellent characteristic of anodic biofilms, can be an effective way to enhance the PAHs biodegradation. This work evaluated the impact of NaCl concentrations (0 g/L, 10 g/L, 30 g/L, and 60 g/L) on naphthalene biodegradation and analyzed the damage protection mechanism of anodic biofilms in batching MECs. Compared with the open circuit, the degradation efficiency of naphthalene under the closed circuit with 10 g/L NaCl concentration reached the maximum of 95.17% within 5 days. Even when NaCl concentration reached 60 g/L, the degradation efficiency only decreased by 10.02%, compared with the MEC without additional NaCl. Confocal scanning laser microscope (CSLM) proved the superiority of the biofilm states of MEC anode under high salinity in terms of thicker biofilms and higher proportion of live/dead bacteria cells. The highest dehydrogenase activity (DHA) was found in the MEC with 10 g/L NaCl concentration. Moreover, microbial diversity analysis demonstrated the classical electroactive microorganisms *Geobacter* and *Pseudomonas* were found on the anodic biofilms of MECs, which have both PAHs degradability and the electrochemical activity. Therefore, this study proved that high salinity had adverse effects on the anodic biofilms, but MEC alleviated the damage caused by high salinity.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants composed of two or more aromatic rings fused together. Coal chemical wastewater is one of the main sources of PAHs pollution, which also contains high content of inorganic salts (e.g., chloride,

* Corresponding authors at: Jiangsu Key Laboratory of Anaerobic Biotechnology, Wuxi 214122, China.
E-mail address: liuhe@jangnan.edu.cn (H. Liu).

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sulfate, nitrate) (Ji et al., 2016). This typical refractory high-hazard organic wastewater with high salinity is a serious threat to human living environment and the beneficial development of industry (Xiong and Wei, 2017). Microbial electrolysis cell (MEC) has been shown to be effective to degrade recalcitrant organic matter by the supply of the external electric field to reduce the energy barrier. It has been confirmed that an ion exchange membrane (IEM)-free MEC with pre-acclimated bioanode could be achieved 2,4-dichloronitrobenzene wastewater treatment (Liu et al., 2020a). Luo et al. found the removal efficiency of nitrobenzene (50 mg/L) in MEC can reach 98% in 36 h with 0.8 V applied voltage (Luo et al., 2019). It is cost-effective to provide a small external voltage delivered by a power source for circumventing the thermodynamic reaction barrier of PAHs degradation (Cheng et al., 2006; Sun et al., 2015). At present, most studies focus on promoting the degradation of PAHs by MEC, but few studies concentrate on the high salinity of PAHs wastewater in complex environments.

Microbial biofilm is an important form of microbial aggregation and growth, which plays an essential role in controlling pollutant migration, transforming and degrading pollutants, and has been a research hotspot in recent years (Liu et al., 2020b). Similarly, the stability of biofilms is the hinge to the efficient operation of MEC. It was found that the number of typical hydrogenotrophic denitrifiers in the biofilms of MEC integrated gas diffusion membrane increased during the remediation of nitrate contamination (Liang et al., 2020). Factors such as temperature, pH and concentration of salt ions, substrate and others will affect the microorganisms in the anodic biofilms and thus affect the operation of MEC (Xu et al., 2014; Wang et al., 2010; Carmona-Martinez et al., 2015; Yu et al., 2020). However, only investigating changes in microbial populations and populations in previous studies was not enough to explore the state of anodic biofilms in dealing with hazards (Zakaria et al., 2019; Zhang and Li, 2020). Therefore, in this study, we evaluate the impact of NaCl on the stabilization of anodic biofilm in various aspects during long-term operation.

The aim of this research was to explore the influence of high salinity on PAHs degradation in simulated chemical industry wastewater by a single-chamber MEC. Naphthalene was selected as the model pollutant of PAHs for their relatively high content and typical structure in the coal industrial wastewater. The removal efficiency of naphthalene by MEC and anodic electrochemical properties under different NaCl concentrations were analyzed, the biofilm homeostasis and microbial activity in anode were studied at the same time. The degradation mechanism of PAHs wastewater with high NaCl by MEC is revealed, which provided a basis for MEC process to treat high salinity industrial wastewater.

2. Materials and methods

2.1. Chemicals

Naphthalene at >99% purity was obtained from Aladdin Chemistry Co. Ltd. (Shanghai, China). Acetone, methanol, n-hexane and acetonitrile are high-performance liquid chromatography (HPLC) grade, which were purchased from Sigma-Aldrich (St. Louis, USA). Other chemicals were reagent grade and bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. MECs set-up and acclimatization

Six lab-scale reactors were built and operated simultaneously. These 400-mL single-chamber reactors used in MEC experiments are shown in Fig. S1, which were constructed with acrylics. Carbon brushes (50 mm in diameter and 80 mm in length, produced by Toho tenax Co., Ltd., Japan) in the center and nickel meshes (80 mm in width and 250 mm in length, produced by Anping kaian metal mesh Co., Ltd., China) around the reactors were used for anode and cathode respectively and pretreated to remove the interference of impurities (Cui et al., 2017). Carbon brushes were immersed in acetone and hydrochloric acid (1 M) for 24 h respectively then rinsed with running water for 1 h; the next operation was washing with deionized water for three times; after drying in 105 °C, carbon brushes were heat-treated in muff furnace at 450 °C for 30 min. Nickel screen was soaked in acetone overnight then rinsed with running water and washed by deionized water, finally, dried at 105 °C. The applied voltage was set at 800 mV by DC power source to overcome electrodes overpotentials and avoid water electrolysis. Reactors were operated with external 10 Ω resistance. The current was converted by monitoring potential data. Titanium wire (0.2 mm in diameter, Baoji LiXing Titanium Group Co., Ltd., China) was used to stretch out the reactor and connect external circuit. The initial pH was adjusted to 7.0 ± 0.1 by sodium hydroxide aqueous solution (1 M) and hydrochloric acid (1 M) and all reactors were placed in constant temperature room (35 ± 0.5 °C).

The sewage and sludge from biochemical pool in this experiment stemmed from a coal chemical industry in Xuzhou (Jiangsu province, China), which used as microbial inoculation source. The quality of actual wastewater was not suitable for the growth of microorganisms, but the microbial in high-salinity wastewater with complex chemical has a potential ability for the degradation of refractory organic matter. Therefore, sewage and synthetic wastewater were domesticated in a ratio of 1:10, different contents of naphthalene and sodium acetate were also added. The components of synthetic wastewater are shown in Table S1. The sewage and synthetic wastewater were aerated with nitrogen to remove the oxygen in the solution and then inoculated into the reactor with sludge. After 30 days stabilization, the anodes of MECs were successfully covered with the microorganisms, which output voltage were also stable.

2.3. Experimental design

After domestication stage, the reactors were operated for 10 periods in sequencing batch mode, which were shown in Table 1. A total of 90 days elapsed from domestication to the end of the reactions. In each period, R_O and R_{O60} were operated in open circuit and respectively added NaCl concentration is 0 g/L and 60 g/L, while R_{C0}, R_{C10}, R_{C30} and R_{C60} were operated in closed circuit with different NaCl concentration (0 g/L, 10 g/L, 30 g/L and 60 g/L). For each cycle, 400 mL synthetic wastewater containing 10 mg/L naphthalene without oxygen were added into each reactor by nitrogen protected. Each cycle operation time was 5 days according to the decreased current. After the end of each cycle, all the liquid in the reactors was drawn out, and replaced with new synthetic wastewater for the next cycle.

Table 1
The characteristics of the MEC reactors.

Reactor	Circuit	NaCl concentration	PAHs concentration	Phenol concentration
R_O	Open circuit	0 g/L	10 mg/L	150 mg/L
R_{C0}	Closed circuit	0 g/L	10 mg/L	150 mg/L
R_{C10}	Closed circuit	10 g/L	10 mg/L	150 mg/L
R_{C30}	Closed circuit	30 g/L	10 mg/L	150 mg/L
R_{C60}	Closed circuit	60 g/L	10 mg/L	150 mg/L
R_{O60}	Open circuit	60 g/L	10 mg/L	150 mg/L

2.4. Analytical methods

The water sample was collected from each reactor once every 24 h, respectively, then filtered through syringe filter (0.22 μm, Nantong haizhixing experimental equipment Co., LTD, China). The COD were measured three replicates according to standard methods. Liquid sample was pretreated according to the literature (Zhou et al., 2020) for the determination of naphthalene and phenol. 5 mL of n-hexane, used as an extraction agent, was added to the water sample, and the upper portion was collected after mixing thoroughly and layering. The extraction operation was repeated three times. After drying under nitrogen, dissolve the extract with 2 mL methanol and filtered through 0.22 μm organic filter (0.22 μm, Nantong haizhixing experimental equipment Co., LTD, China). Naphthalene and phenol were quantified by a HPLC (Ultimate 3000 Series, Thermo Fisher Scientific, USA). The HPLC was equipped with Acclaim™ 120 C_{18} (4.6 mm × 250 mm, 5 μm, Thermo Fisher, USA), and mobile phase consisted of 75% acetonitrile and 25% water by volume ratio. The injection volume was 10 μL and column temperature was 30 °C with a flow rate of 1.0 mL/min. Naphthalene and phenol were detected at the wavelength of 220 nm and 270 nm, respectively.

2.5. Dehydrogenase activity

Dehydrogenase activity (DHA) of microorganisms was measured according to the literature (Reddy et al., 2010), which based on the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC). Since taking biofilm samples during the reaction cycle would affect the stability of the reactor, the anode biofilms were only sampled in the last cycle. The free microorganisms in the liquid were sampled and tested daily from a stable period.

2.6. Electrochemical analysis

Cyclic voltammetry (CV) was conducted with electrochemical workstations (CHI604E, CH Instruments, INC., China) to assess electrocatalytic activity of anode-attached biomass (Yin et al., 2019). The anode was set as the working electrode, the counter electrode was nickel net and the reference electrode was a standard calomel electrode. The anode potential was applied a potential ramp with time at a scan rate of 10 mV/s in a cyclic manner from +0.6 V to −0.8 V.

2.7. Biofilm characterization

At the end of the last reaction cycle, the biofilms were characterized. The cell viability assessment in anode biofilms was evaluated by confocal laser scanning microscopy (CLSM, Leica TCS SP8, Leica Microsystems, Germany). LIVE/DEAD® *Bac*Light™ Bacterial Viability Kits (L7012, Thermo Fisher Scientific, USA) was used to stain the biofilms on anodes. The thickness of the biofilms was obtained through the software of Leica and the percentage of live cells was calculated by counting pixels of layer-scanned images with ImageJ.

2.8. Microbial diversity analysis

After the last cycle of the reaction, the anode carbon brushes were detached from the reactors for microbial community analysis. The anode was first softly washed with sterile phosphate buffer to remove free microorganism and impurities. Then the total DNA of electrode biofilms was extracted with the PowerSoil DNA Isolation Kit (MoBio, Shanghai, China). After the DNA samples met the requirements, high-throughput sequencing based on Illumina Miseq PE was used to analyze the microbial community. The universal primers 338F (5′-ACTCCTACG GGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used.

2.9. Date calculation

Naphthalene removal efficiency (RE, %) was defined as:

$$RE = \frac{C_0 - C_1}{C_0} \times 100\% \quad (1)$$

where the C_0 (mg/L) and C_1 (mg/L) represent the naphthalene concentrations in the initial and real-time, respectively.

The current (I, A) was calculated according to the ohm law:

$$I = \frac{U}{R} \quad (2)$$

where U (V)represents the voltage of the resistor, and R (Ω)represents the resistance of the external resistor.

Anodic coulombic efficiency (CE, %) of the MEC was calculated according to the following formula (Zhao et al., 2016)

$$CE = \frac{It}{nF(C_0 - C_1)V/M} \times 100\% \quad (3)$$

I (A) is the current, t (s) is the time of running time of the reactor, n is the number of electrons ($n = 4$), F is faradays constant (96,485C/mol), V (L) is the volume of the reactors, M is the molecular weight (32,000 mg/mol), C_0 and C_1 are the COD in the initial and real-time respectively (mg/L).

3. Results and discussion

3.1. Effect of NaCl on naphthalene degradation

The removal of naphthalene reflects the performance of MEC and the living state of microorganisms on the anode. The half-life of naphthalene can effectively reflect the degradation efficiency of naphthalene, which was calculated as reported by Zhou et al. (2020). Changes of naphthalene concentration in the reactors are shown in Fig. 1a. The naphthalene degradation efficiency of R_O (open circuit) on day 5 was 85.88%, lower than that of R_{C0} (closed circuit, 95.17%). Similarly, the half-life of naphthalene in R_{C0} was 1.25 d, compared with 2.37 d for R_O. Therefore, the MEC can significantly improve the ability of naphthalene degradation efficiency compared with anaerobic biodegradation. According to the risk of anodic biofilms exposed in the PAHs environment, the superiority of MEC in the process of PAHs degradation was demonstrated (Venkidusamy et al., 2016). High NaCl concentration has great influence on PAH degradation, in which some bacteria are insufficient to function availably at salinities of seawater or above (Ghosal et al., 2016). When NaCl concentration increased to 60 g/L (R_{C60}), the degradation efficiency of naphthalene in MEC decreased to 85.15% and the naphthalene half-life in R_{C60} increased to 2.18 d. The degradation of naphthalene was significant under high salinity conditions, but it was encouraging that even when the NaCl concentration was as high as 10 g/L, the degradation effect of MEC on naphthalene was still positive. This is undoubtedly acceptable for the treatment of high-salinity PAHs wastewater. The naphthalene degradation efficiency in R_{O60} was significantly lower than that in closed-circuit (R_{C60}), and the half-life also increased to the highest 3.70 d in each reactor. The applied potential can improve the activity of enzyme and promote the metabolism of microorganisms, which can improve the abundance of microorganisms and enhance the degradation effect of PAHs (Kronenberg et al., 2017). The kinetics followed a pseudo first-order rate law ($R_{C0} > 0.889$), and the apparent rate constants (k) under different NaCl content were determined (Fig. 1b). R_{C0} exhibited a superior performance, and the k was more than 1.61 times larger than R_O. Notably, R_{C30} at salinities that of seawater also showed a higher k value than R_O. This suggested that MEC still has advantages due to the impact of relatively high salinity.

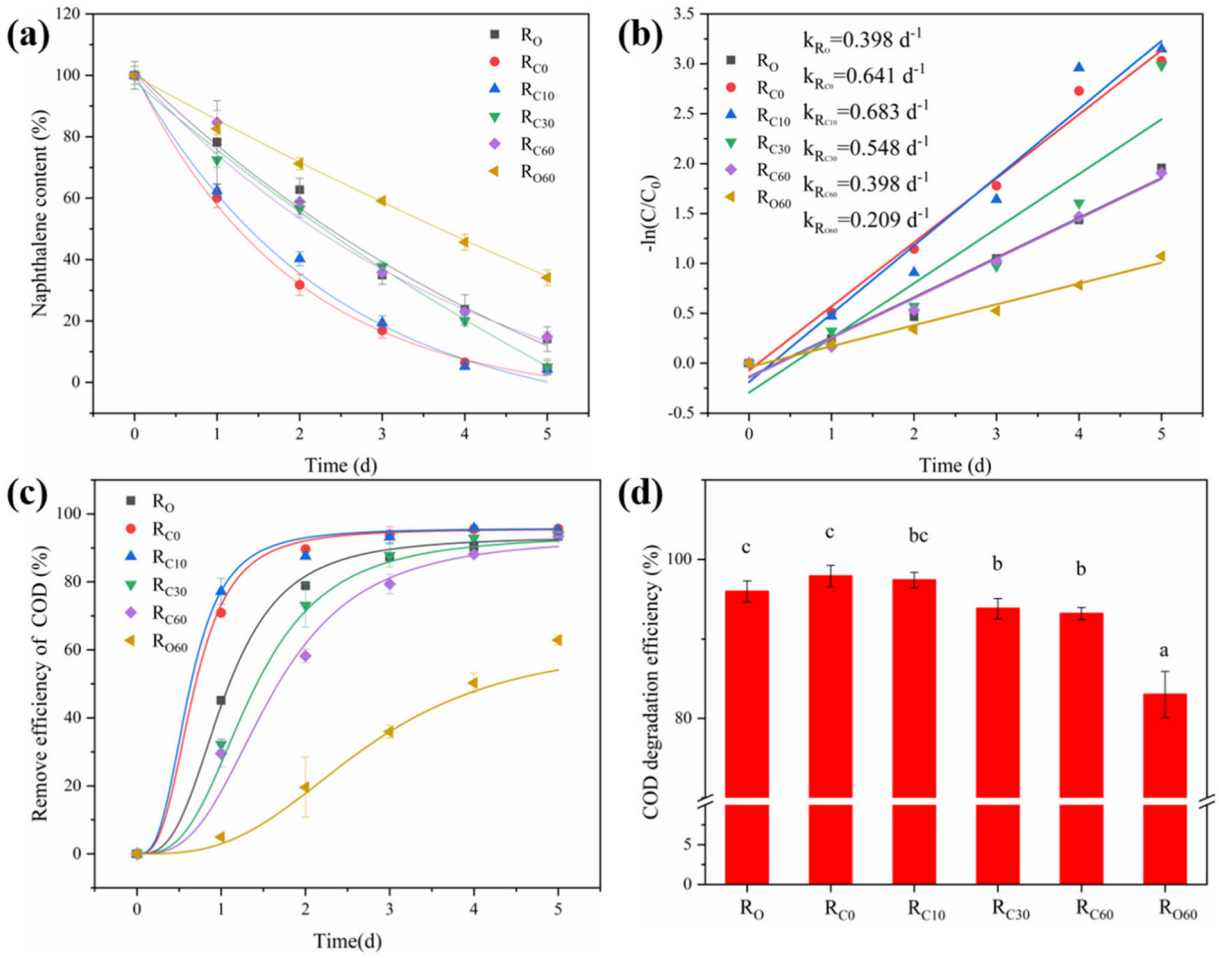

Fig. 1. The performance of naphthalene and COD degradation in MEC: (a) naphthalene concentration content in 5 days and; (b) corresponding linearized pseudo first-order kinetic profiles of naphthalene degradation (C_0 denotes the initial COD concentration and C represents the COD concentration measured in the reactor); (c) the COD removal efficiency; (d) the average degradation efficiency of COD. Error bar represents the standard error, *P*-value of significance difference analysis was less than 0.05, $n = 3$.

The COD removal efficiency in the reactor also varied with the change of NaCl concentration and whether the reactor was charged or not. Naphthalene half-life computational method was used for the calculation of COD half-life. The COD concentration in the MEC declined more rapidly than simple anaerobic reactor, COD half-life of R_{C0} was 0.40 d earlier than R_O, this implied the superiority of MEC for substrate degradation. However, final removal rate of COD did not have significant differences between R_O and R_{C0} as shown in Fig. 1d. The COD removal efficiency of the MECs at different salt loads were demonstrated in Fig. 1c. The COD degradation efficiency was the highest when NaCl concentration was 10 g/L. In non-BES systems, this salinity may inhibit anaerobic degradation (Pereira et al., 2019). Adding salt to bioelectrochemical reactor can reduce ohm resistance in MECs and may be an efficient solution to enhance MEC performance. Miyahara et al. (2015) reported that 0.1 M (5.8 g/L) NaCl facilitated the growth of microorganism in BES anode biofilms. These findings have highlighted the potential of MEC as an alternative high-salinity wastewater treatment technology. For instance, when treating coal chemical wastewater, a high concentration environment of salt solution may facilitate refractory organic removal. Apparently, high NaCl concentration still affected the removal of COD by MEC, but it was still far superior to the high-salinity control group (R_{O60}). This is inconceivable in conventional anaerobic degradation, because the presence of high salt significantly inhibits enzyme activity associated with degradation and also changes the microbial community (Guo et al., 2016).

3.2. Dehydrogenase activity

Dehydrogenase is one of the indispensable intracellular enzymes for microorganisms to degrade organic pollutants and obtain energy. Dehydrogenase activity (DHA) reflects the living state of microorganism, which used to characterize the ability of cells to degrade substrates. The daily DHA in the effluent of the reactors was found to increase gradually their peak value in the first 2 days and then decreased rapidly until the end of cyclic operation (Fig. 2a), which was consistent with the degradation rule of COD and naphthalene in the reactors. The maximum DHA in MEC was R_{C0} without extra NaCl at the second day. This highest DHA at MEC can be attributed to the activation of voltage-sensitive channels of the electrode microorganisms, which leads to the enhancement of the biocatalyst at this condition (Semenov et al., 2015; Modestra et al., 2015). With the increase of NaCl concentration, the DHA in liquid phase decreased. This was predictable because of the inhibition of enzyme activities by high salinity. The maximum DHA in liquid phase with 60 g/L NaCl concentration (R_{C60}) was only 55.17% of the R_{C0}, but it was still 1.94 times higher than the open-circuit control group (R_{O60}) under the same NaCl concentration conditions. The DHA in R_{O60} remained at a low level throughout the cycle. On the anodic biofilms, the DHA was higher in R_{C0} and R_{C10} than other groups (Fig. 2b). Although the DHA in R_{C10} was slightly higher than that of R_{C0}, there was no significant difference between the two groups. Surprisingly, 10 g/L NaCl concentration did not adversely affect the DHA of the anode biofilms, while the total dissolved solids (TDS) of most coal gasification wastewater was below 10 g/L. This

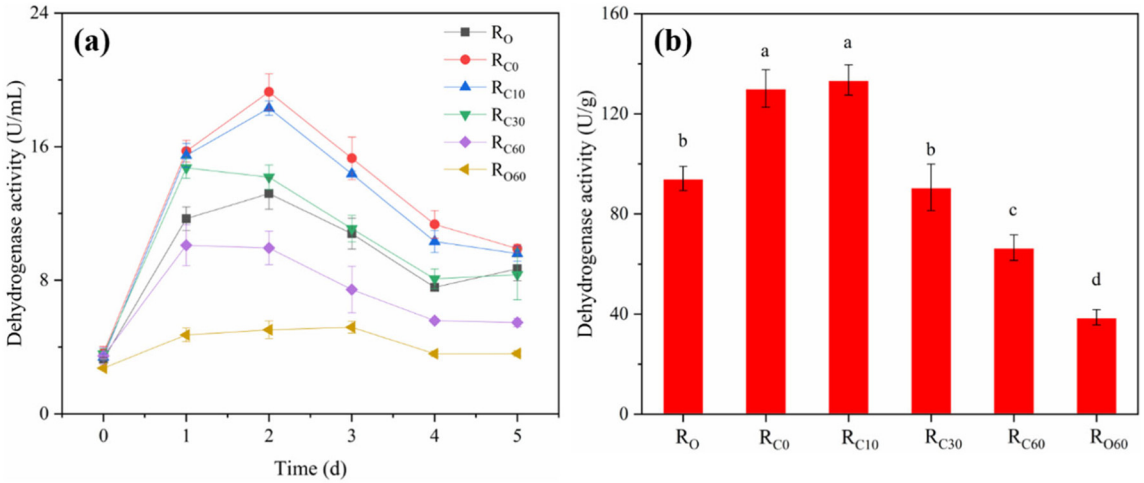

Fig. 2. (a) Dehydrogenase activity of microorganisms in the reactors; (b) Dehydrogenase activity of microorganisms on the anode of MEC and open circuit control group in different NaCl concentrations (Mean ± SD, P-value of significance difference analysis was less than 0.05, $n = 5$).

may be affirmative for the application of MEC in the treatment of high-salinity coal chemical wastewater. Under the condition of 60 g/L NaCl concentration, the DHA in closed-circuit reactor (R_{C60}) was 1.72 times than that of the open-circuit (R_{O60}). It was indicated that MEC can effectively protect the microorganisms loaded on the anode to some extent (10–30 g/L) when the NaCl concentration is high, even high salinity damage to anode biofilms is visible.

3.3. Biofilm characterization

The control groups and the MECs were subjected to different NaCl concentrations. As shown in Fig. 3, it compared the CLSM images of the anode biofilms obtained from the different cases, differentiating the living cells (green) and inactive cells (red). It was apparent that more living cells ratio appeared on the anode surface in R_{C0}, while more damaged cells were observed on the anode surface in open-circuit control group (R_O) during long-term operation. The thickness of biofilms in R_O was only 66.68 μm, approximately 63.49% of the thickness in R_{C0} (105.02 μm) (Table 2). This intuitively indicated that the growth of biofilms was enhanced by a weak electrical stimulation. Similarly, MEC also enhanced microbial activity on the biofilms. The live/dead bacteria ratio (r_{ld}) was the highest in R_{C0}, more than 5 times of R_O. With the increase of NaCl concentration, the r_{ld} rapidly decreased, and it was the lowest in R_{O60}. The influence of NaCl on the biofilms of MEC anode was significant. When the concentration of NaCl increased from 10 g/L to 60 g/L, the thickness of the biofilms on anode decreased by 56.99%. The biomass on the anode of the control under 60 g/L NaCl concentration (R_{O60}) was significantly reduced, remaining only sporadic spotted parts. This clearly shows that MEC can reduce the damage of microorganisms in biofilms at high salinity. As Puyen et al. (2012) observed, the reduction in both the number of cells and their biovolume as the salt concentration increased from 0 g/L to 10 g/L, because of the high osmotic pressure caused by high salinity, the growth of microorganisms was inhibited. It was arguable that the maximum biofilm thickness appeared in R_{C10} rather than R_{C0}, although R_{C0} has the highest r_{ld}. Although it has been reported that high salinity would take place ionic strength effect, resulting in a serious reduction of membrane permeability, but the 10 g/L NaCl concentration did not reach the limit threshold (Sun et al., 2010). On the other hand, the presence of NaCl in the solution improves MEC performance by increasing the conductivity and therefore decreasing the solution resistance between electrodes (Merrill and Logan, 2009). It was reported that the applied low voltage (<0.9 V) can promote activity of bacteria by expand membrane permeability and promote enzymatic reactions (Chen et al., 2016). Therefore, the optimum MEC operation state may also be positively correlated with the thickness of the anodic biofilms. The enhancement of anodic biofilms is bound to improve the efficiency and stability of high-salinity PAHs wastewater treatment by MEC.

3.4. Analysis of electrochemistry

The current and anode potential development of the MECs under different salinity were shown in Fig. S2. The current and potential gradually stable with consecutive operation cycles, which proved the development of anodic biofilms. The current variation characteristics in MECs was firstly increased to a peak current and then decreased gradually. The current became stabilized after 2 d when salinity less than 30 g/L (R_{C0}, R_{C10}, R_{C30}), because the substrate in MECs were almost consumed within 2 days. In R_{C60}, the current gradually smoothed after 3 d, which was related to the slow degradation of the substrate at high salinity.

Cyclic voltammetry (CV) experiments were conducted to explore electroactivity of biofilms attached to the anode caused by voltage imposition and NaCl addition. R_{C0} had the largest redox peak, followed by R_{C10}, which indicated anode biofilm had better electrochemical activity than the other groups (Fig. 4). The area of the closed area indicated the strength of the charge and discharge performance of the biofilms, which can be used to characterize the electron transfer ability. Therefore, the electron transfer ability of R_{C0} and R_{C10} anodic biofilms were the best. Obviously, the electron transfer performance of the anode biofilm was negatively correlated with the NaCl concentration when the concentration was higher than 10 g/L.

Coulombic efficiency (CE) can be used to assess the fraction of available electrons from substrate that ends up as electrical current (Sleutels et al., 2011). During the 24 h in R_{C0}, COD in the reaction system decreased rapidly by 70.92%, while the CE of the anode was as high as 89.82%. The 24-h CE of R_{C10}, R_{C30} and R_{C60} were 79.9%, 58.13%, and 50.05% respectively, decreasing with the increase of NaCl concentration. The CE of each reactor decreased as the reaction progresses. This indicated that the increase of salinity significantly inhibited the electroactive microorganisms in the anodic biofilms. It was also possible that other microorganisms degraded the COD, leading to electronic loss. Even though the DHA of R_{C0} and R_{C10} was not significantly different, the differences of the microbial composition in the anodic biofilms may exist. In addition, the decrease of the r_{ld} in the anodic biofilms with the increase of salinity was also positively correlated with the decrease of CE. At the end of the 5th day, the CE of R_{C0}, R_{C10}, R_{C30} and R_{C60} were 38.56%, 17.19%, 16.64% and 13.81%, indicating that the anodic reaction only contributed to only a part of organic degradation. According to the CE calculation, it was implied that other anaerobic reaction participated into the organic catabolism.

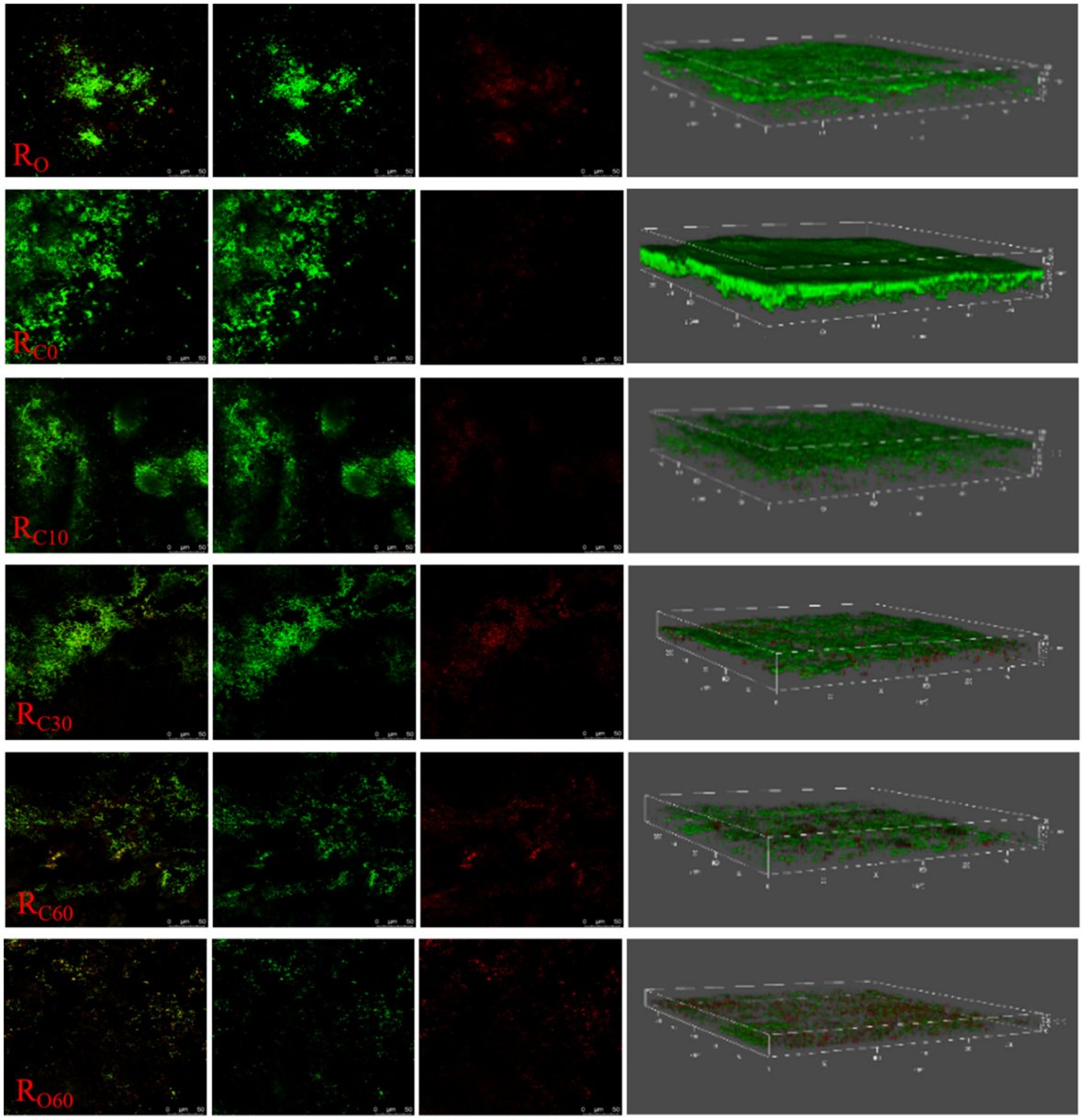

Fig. 3. Planar and three-dimensional CLSM images of anode biofilm (red represents dead cells, green represents living cells).

3.5. Microbial community

The microbial communities in the reactors were responsible for the degradation of organic compounds into energy to drive the orderly operation of the whole system, which play important roles. Therefore, microbial community taxonomic compositions at different levels were analyzed. Ten bacterial phyla were viewed from 6 libraries (Fig. 5a). The majority of sequences belonged to *Proteobacteria* and *Actinobacteria* that together accounted for 40.74% ~ 76.63% of the total reads in

Table 2
Biofilm thickness and Live/dead bacteria ratio under different conditions.

Reactor	Biofilm thickness (μm) thickness(μm)	Live/dead bacteria ratio
R_O	66.68 ± 10.27 cd	1.73
R_{C0}	105.02 ± 4.08 b	8.69
R_{C10}	155.03 ± 17.80 a	4.28
R_{C30}	93.35 ± 18.86 bc	2.02
R_{C60}	66.68 ± 9.43 cd	1.26
R_{O60}	43.34 ± 4.71 d	0.71

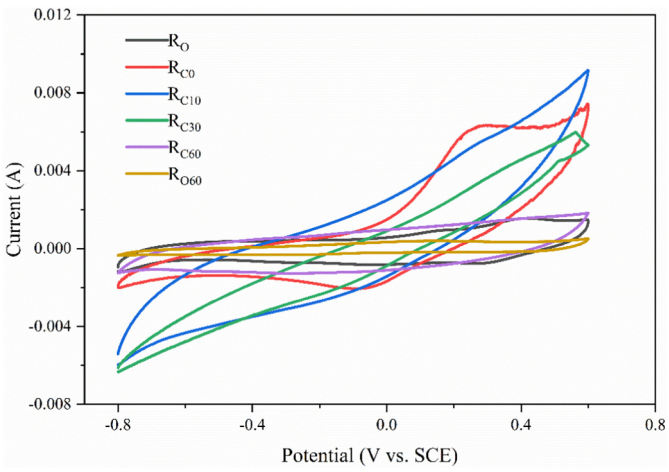

Fig. 4. Cyclic voltammetry curve of the anodic electrodes.

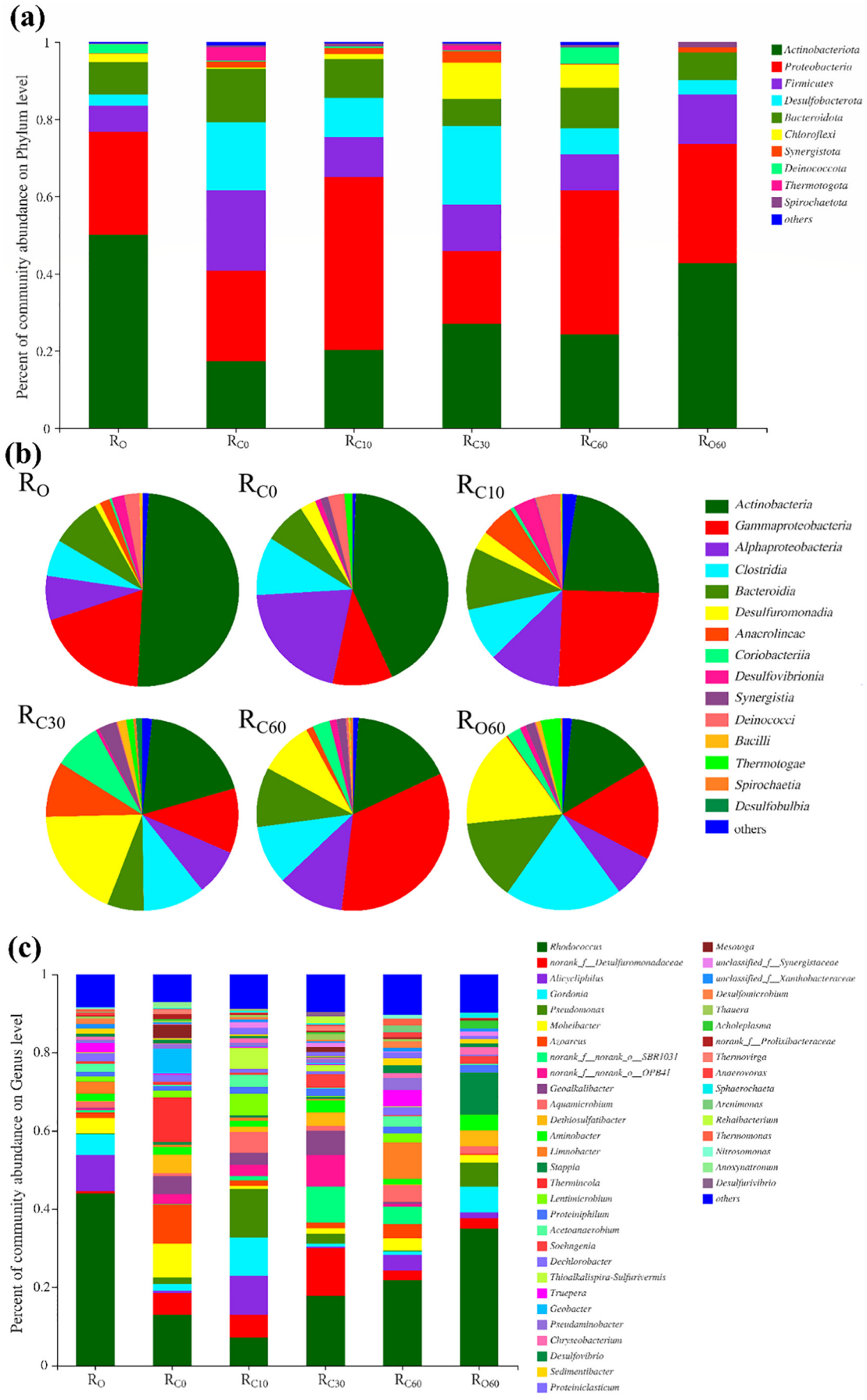

Fig. 5. Taxonomic composition of bacterial communities at three levels (a) phyla, (b) classes, (c) genera.

bacterial communities. Specifically, in the control groups without power supply, the proportions of *Proteobacteria* and *Actinobacteria* together reached 76.63% (R_O) and 73.51% (R_{O60}). However, in the MECs, the proportions were significantly reduced. The investigation of relevant indicates that along with numerous biotechnological applications, halophilic microorganisms have greater catabolic generality than previously thought about (Ghosal et al., 2016). *Actinobacteria* was the most important component in the bacterial communities under the condition of high salinity, and the percentage of *Actinobacteria* was as high as 42.66% in R_{O60}. The significant increase of *Desulfuromonadia* at the class level (Fig. 5b) in MECs implied the correlation of electron transfer. *Bacteroidia* were frequently founded in the anode biofilm in MECs, which reported to involve electricity generation (Ha et al., 2012). *Proteobacteria* were also important in MEC because *Gamma*- and *Alpha*-proteobacteria were also detected in a certain proportion, which are well-known microbial communities in utilizing organic substrates (Ariesyady et al., 2007). In addition, *Thermotoga* may also play a potential role in bioelectrochemical systems due to its increased proportion in MEC, this found might be novel (de Barros et al., 2017).

In genus level (Fig. 5c), it seemed that relatively high bacterial diversities in all MECs. This proves that applied voltage would perform a beneficial role in anodic microbial community, which also strengthens the biodegradation ability of MEC for refractory organic matter. The bacterial diversity in the high-salinity control group (R_{O60}) appeared to be low, which indicated that high-salinity was harmful to microorganisms. Obvious variations were observed, *Rhodococcus*, which proven to play an active role in degrading PAHs (Ivshina et al., 2016), was found to be a dominant group in all groups, especially in the control groups without applied voltage. Although the proportion of *Rhodococcus* was still high, the content of MECs group was significantly lower than that of the control groups without power supply. *Geobacter*, a typical electrogenic microbe, has been detected in samples of all MECs, especially in R_{C0} (6.36%). Interestingly, Zhou et al. (2016) isolated *Geobacter sulfurreducens* from petroleum hydrocarbons as a sole carbon source, which can initiate the degradation of benzene and naphthalene respectively. This indicates that *Geobacter* can be utilized for producing electricity with PAHs. It is beneficial for the removal of PAHs in BESs. The recognized exoelectrogenic bacteria and hydrocarbon degraders *Pseudomonas* was also a part of the anode biofilm in MECs, especially in reactor R_{C10} (12.46%). *Pseudomonas* has also been reported to be able to produce surfactants to solubilize hydrophobic organic substance, increasing the likelihood of PAHs being degraded (Singh and Tripathi, 2013; Patowary et al., 2017). In addition, the proportion of *Pseudomonas* in high NaCl control group (R_{O60}) was also higher (6.14%), which may be due to the vigoroso salt tolerance of *Pseudomonas* (Kastner et al., 1998; Pereira et al., 2019).

4. Conclusion

This study characterized the effect of high salinity on MEC anode biofilms during PAHs degradation. The naphthalene degradation efficiency at 60 g/L NaCl concentration in open circuit on 5 days was 65.84%, significantly lower than that in closed circuit (85.15%) with the same salt concentration. Similar results appeared in the degradation of COD. The DHA increase of anodic biofilms at high salinity in MEC was demonstrated to have higher substrate degradation performance by microorganisms, which may be responsible for the promotion of naphthalene degradation. Visually, CLSM showed the protective effect of MEC on biofilms at high salinity. The microbial community analysis suggested that *Geobacter* and *Pseudomonas* were selectively enriched in the biofilms of anode, which was considered to be closely related to electron transfer and PAHs degradation. Our findings indicated a novel insight into the PAHs degradation by MEC in high salinity wastewater.

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CRediT authorship contribution statement

Peng Ding: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Ping Wu:** Conceptualization, Formal analysis, Writing – review & editing. **Zhang Jie:** Methodology, Validation, Investigation. **Min-Hua Cui:** Resources, Writing – review & editing, Supervision, Funding acquisition. **He Liu:** Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

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References

Ariesyady, H.D., Ito, T., Okabe, S., 2007. Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. Water Res. 41 (7), 1554–1568. https://doi.org/10.1016/j.watres.2006.12.036.

Carmona-Martinez, A.A., Trably, E., Milferstedt, K., Lacroix, R., Etcheverry, L., Bernet, N., 2015. Long-term continuous production of H-2 in a microbial electrolysis cell (MEC) treating saline wastewater. Water Res. 81, 149–156. https://doi.org/10.1016/j.watres.2015.05.041.

Chen, Y., Yu, B., Yin, C.K., Zhang, C., Dai, X.H., Yuan, H.P., Zhu, N.W., 2016. Biostimulation by direct voltage to enhance anaerobic digestion of waste activated sludge. RSC Adv. 6 (2), 1581–1588. https://doi.org/10.1039/C5RA24134K.

Cheng, S., Liu, H., Logan, B.E., 2006. Increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing. Environmental Science & Technology 40 (7), 2426–2432. https://doi.org/10.1021/es051652w.

Cui, M.H., Cui, D., Gao, L., Wang, A.J., Cheng, H.Y., 2017. Evaluation of anaerobic sludge volume for improving azo dye decolorization in a hybrid anaerobic reactor with built-in bioelectrochemical system. Chemosphere 169, 18–22. https://doi.org/10.1016/j.chemosphere.2016.11.034.

de Barros, V.G., Duda, R.M., Vantini, J.D., Omori, W.P., Ferro, M.I.T., de Oliveira, R.A., 2017. Improved methane production from sugarcane vinasse with filter cake in thermophilic UASB reactors, with predominance of Methanothermobacter and Methanosarcina archaea and Thermotogae bacteria. Bioresour. Technol. 244, 371–381. https://doi.org/10.1016/j.biortech.2017.07.106.

Ghosal, D., Ghosh, S., Dutta, T.K., Ahn, Y., 2016. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Front. Microbiol. 7, 1369–1395. https://doi.org/10.3389/fmicb.2016.01369.

Guo, G., He, F., Tian, F., Huang, Y., Wang, H., 2016. Effect of salt contents on enzymatic activities and halophilic microbial community structure during phenanthrene degradation. Int. Biodeterior. Biodegradation 110, 8–15. https://doi.org/10.1016/j.ibiod.2016.02.007.

Ha, P.T., Lee, T.K., Rittmann, B.E., Park, J., Chang, I.S., 2012. Treatment of alcohol distillery wastewater using a bacteroidetes-dominant thermophilic microbial fuel cell. Environmental Science & Technology 46 (5), 3022–3030. https://doi.org/10.1021/es203861v.

Ivshina, I., Kostina, L., Krivoruchko, A., Kuyukina, M., Peshkur, T., Anderson, P., Cunningham, C., 2016. Removal of polycyclic aromatic hydrocarbons in soil spiked with model mixtures of petroleum hydrocarbons and heterocycles using biosurfactants from Rhodococcus ruber IEGM 231. J. Hazard. Mater. 312, 8–17. https://doi.org/10.1016/j.jhazmat.2016.03.007.

Ji, Q.H., Tabassum, S., Hena, S., Silva, C.G., Yu, G.X., Zhang, Z.J., 2016. A review on the coal gasification wastewater treatment technologies: past, present and future outlook. J. Clean. Prod. 126, 38–55. https://doi.org/10.1016/j.jclepro.2016.02.147.

Kastner, M., Breuer-Jammali, M., Mahro, B., 1998. Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Appl. Environ. Microbiol. 64 (1), 359–362. https://doi.org/10.1128/AEM.64.1.359-362.1998.

Kronenberg, M., Trably, E., Bernet, N., Patureau, D., 2017. Biodegradation of polycyclic aromatic hydrocarbons: using microbial bioelectrochemical systems to overcome an impasse. Environ. Pollut. 231, 509–523. https://doi.org/10.1016/j.envpol.2017.08.048.

Liang, D., He, W., Li, C., Wang, F., Crittenden, J.C., Feng, Y., 2020. Remediation of nitrate contamination by membrane hydrogenotrophic denitrifying biofilm integrated in microbial electrolysis cell. Water Res. 188, 116498. https://doi.org/10.1016/j.watres.2020.116498.

Liu, Y., Wang, C., Zhang, K.J., Zhou, Y.R., Xu, Y.L., Xu, X.Y., Zhu, L., 2020a. Rapid degradation of 2,4-dichloronitrobenzene in single-chamber microbial electrolysis cell with pre-acclimated bioanode: a comprehensive assessment. Science of the Total Environment, 724 https://doi.org/10.1016/j.scitotenv.2020.138053.

Liu, Y.N., Lv, Z.T., Lv, W.L., Liu, X.W., 2020b. Plasmonic probing of the adhesion strength of single microbial cells. Proceedings of the National Academy of Sciences of the United States of America 10, 1073–1078. https://doi.org/10.1073/pnas.2010136117.

Luo, H.P., Hu, J., Qu, L., Liu, G.L., Zhang, R.D., Lu, Y.B., Qi, J.X., Hu, J.P., Zeng, C.P., 2019. Efficient reduction of nitrobenzene by sulfate-reducer enriched biocathode in microbial electrolysis cell. Sci. Total Environ. 674, 336–343. https://doi.org/10.1016/j.scitotenv.2019.04.206.

Merrill, M.D., Logan, B.E., 2009. Electrolyte effects on hydrogen evolution and solution resistance in microbial electrolysis cells. J. Power Sources 191 (2), 203–208. https://doi.org/10.1016/j.jpowsour.2009.02.077.

Miyahara, M., Kouzuma, A., Watanabe, K., 2015. Effects of NaCl concentration on anode microbes in microbial fuel cells. AMB Express 5 (1), 123–131. https://doi.org/10.1186/s13568-015-0123-6.

Modestra, J.A., Babu, M.L., Mohan, S.V., 2015. Electro-fermentation of real-field acidogenic spent wash effluents for additional biohydrogen production with simultaneous treatment in a microbial electrolysis cell. Sep. Purif. Technol. 150, 308–315. https://doi.org/10.1016/j.seppur.2015.05.043.

Patowary, K., Patowary, R., Kalita, M.C., Deka, S., 2017. Characterization of biosurfactant produced during degradation of hydrocarbons using crude oil as sole source of carbon. Front. Microbiol. 8, 279–292. https://doi.org/10.3389/fmicb.2017.00279.

Pereira, E., Napp, A.P., Allebrandt, S., Barbosa, R., Reuwsaat, J., Lopes, W., Kmetzsch, L., Staats, C.C., Schrank, A., Dallegrave, A., Peralba, M.D.R., Passaglia, L.M.P., Bento, F.M., Vainstein, M.H., 2019. Biodegradation of aliphatic and polycyclic aromatic hydrocarbons in seawater by autochthonous microorganisms. Int. Biodeterior. Biodegradation 145, 89–99. https://doi.org/10.1016/j.ibiod.2019.104789.

Puyen, Z.M., Villagrasa, E., Maldonado, J., Esteve, I., Sole, A., 2012. Viability and biomass of Micrococcus luteus DE2008 at different salinity concentrations determined by specific fluorochromes and CLSM-image analysis. Curr. Microbiol. 64 (1), 75–80. https://doi.org/10.1007/s00284-011-0033-z.

Reddy, M.V., Srikanth, S., Mohan, S.V., Sarma, P.N., 2010. Phosphatase and dehydrogenase activities in anodic chamber of single chamber microbial fuel cell (MFC) at variable substrate loading conditions. Bioelectrochemistry 77 (2), 125–132. https://doi.org/10.1016/j.bioelechem.2009.07.011.

Semenov, I., Xiao, S., Kang, D., Schoenbach, K.H., Pakhomov, A.G., 2015. Cell stimulation and calcium mobilization by picosecond electric pulses. Bioelectrochemistry 105, 65–71. https://doi.org/10.1016/j.bioelechem.2015.05.013.

Singh, D.N., Tripathi, A.K., 2013. Coal induced production of a rhamnolipid biosurfactant by Pseudomonas stutzeri, isolated from the formation water of Jharia coalbed. Bioresour. Technol. 128, 215–221. https://doi.org/10.1016/j.biortech.2012.10.127.

Sleutels, T.H.J.A., Darus, L., Hamelers, H.V.M., Buisman, C.J.N., 2011. Effect of operational parameters on Coulombic efficiency in bioelectrochemical systems. Bioresour. Technol. 102 (24), 11172–11176. https://doi.org/10.1016/j.biortech.2011.09.078.

Sun, C., Leiknes, T., Weitzenbock, J., Thorstensen, B., 2010. Salinity effect on a biofilm-MBR process for shipboard wastewater treatment. Sep. Purif. Technol. 72 (3), 380–387. https://doi.org/10.1016/j.seppur.2010.03.010.

Sun, R., Zhou, A.J., Jia, J.N., Liang, Q., Liu, Q., Xing, D.F., Ren, N.Q., 2015. Characterization of methane production and microbial community shifts during waste activated sludge degradation in microbial electrolysis cells. Bioresour. Technol. 175, 68–74. https://doi.org/10.1016/j.biortech.2014.10.052.

Venkidusamy, K., Megharaj, M., Marzorati, M., Lockington, R., Naidu, R., 2016. Enhanced removal of petroleum hydrocarbons using a bioelectrochemical remediation system with pre-cultured anodes. Sci. Total Environ. 539, 61–69. https://doi.org/10.1016/j.scitotenv.2015.08.098.

Wang, A.J., Liu, W.Z., Ren, N.Q., Zhou, J.Z., Cheng, S.A., 2010. Key factors affecting microbial anode potential in a microbial electrolysis cell for H-2 production. Int. J. Hydrog. Energy 35 (24), 13481–13487. https://doi.org/10.1016/j.ijhydene.2009.11.125.

Xiong, R.H., Wei, C., 2017. Current status and technology trends of zero liquid discharge at coal chemical industry in China. Journal of Water Process Engineering 19, 346–351. https://doi.org/10.1016/j.jwpe.2017.09.005.

Xu, L.J., Liu, W.Z., Wu, Y.N., Lee, P., Wang, A.J., Li, S., 2014. Trehalose enhancing microbial electrolysis cell for hydrogen generation in low temperature (0 degrees C). Bioresour. Technol. 166, 458–463. https://doi.org/10.1016/j.biortech.2014.05.018.

Yin, C.K., Shen, Y.W., Yuan, R.X., Zhu, N.W., Yuan, H.P., Lou, Z.Y., 2019. Sludge-based biochar-assisted thermophilic anaerobic digestion of waste-activated sludge in microbial electrolysis cell for methane production. Bioresour. Technol. 284, 315–324. https://doi.org/10.1016/j.biortech.2019.03.146.

Yu, B., Liu, C.L., Wang, S.Y., Wang, W.D., Zhao, S.Y., Zhu, G.B., 2020. Applying constructed wetland-microbial electrochemical system to enhance NH_4^+ removal at low temperature. Science of the Total Environment, 724 https://doi.org/10.1016/j.scitotenv.2020.138017.

Zakaria, B.S., Lin, L., Dhar, B.R., 2019. Shift of biofilm and suspended bacterial communities with changes in anode potential in a microbial electrolysis cell treating primary sludge. Sci. Total Environ. 689, 691–699. https://doi.org/10.1016/j.scitotenv.2019.06.519.

Zhang, X.Y., Li, R.Y., 2020. Electrodes bioaugmentation promotes the removal of antibiotics from concentrated sludge in microbial electrolysis cells. Science of the Total Environment, 715 https://doi.org/10.1016/j.scitotenv.2020.136997.

Zhao, Z.S., Zhang, Y.B., Quan, X., Zhao, H.M., 2016. Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell. Bioresour. Technol. 200, 235–244. https://doi.org/10.1016/j.biortech.2015.10.021.

Zhou, L., Deng, D.D., Zhang, D., Chen, Q., Kang, J.Q., Fan, N.J., Liu, Y., 2016. Microbial electricity generation and isolation of exoelectrogenic bacteria based on petroleum hydrocarbon-contaminated soil. Electroanalysis 28 (7), 1510–1516. https://doi.org/10.1002/elan.201501052.

Zhou, Y.K., Zou, Q.P., Fan, M.J., Xu, Y., Chen, Y.W., 2020. Highly efficient anaerobic co-degradation of complex persistent polycyclic aromatic hydrocarbons by a bioelectrochemical system. J. Hazard. Mater. 381, 945–955. https://doi.org/10.1016/j.jhazmat.2019.120945.