Research Article

Simultaneous bioelectricity generation, desalination, organics degradation, and nitrogen removal in air–cathode microbial desalination cells

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Abstract
In this study, a three-chamber air–cathode microbial desalination cell (AMDC) was constructed using activated sludge as the inoculating microbial source and carbon cloth as the electrode. The simultaneous bioelectricity generation, desalination, degradation of organic compounds, and nitrogen removal in the AMDC having different electrode spacings (16, 12, and 8 cm) provided an insight into the microbial community. The experimental results showed that with a gradual decrease in the electrode spacing, electricity production and desalination performance first increased and then decreased. While AMDC1 with 16 cm electrode spacing became active faster than other cells and reached a peak voltage of 598 mV on the tenth day, AMDC2 with 12 cm electrode spacing showed the best start-up performance with a maximum output voltage of 645 mV, maximum power density of 214.7 mW/m^3, and minimum resistance of 516.9 Ω. AMDC3 with 8 cm electrode spacing reached a peak voltage of 598 mV, which was between those of AMDC1 and AMDC2. The three AMDCs were run until the end of the cycle, and there was no significant difference in their ammonium removal rate and chemical oxygen demand. For AMDC1–3, the desalination rates were 84.86%, 87.71%, and 83.43%, and the Coulomb efficiencies were 17.29%, 18.60%, and 17.95%, respectively. Further, scanning electron microscopy showed that a large number of microorganisms could attach to the surface of the anode carbon cloth electrode; 16S rRNA sequencing showed that the typical electrogenic microbial communities were *Bacillus* (11.6%) and *Arcobacter* (9.6%). As the poor performance of the AMDC was primarily due to a lack of electrogenic microorganisms in the active function of the anode chamber, screening the functional microorganisms provided a reference for optimizing and amplifying the application of microbial desalination cells in practical research.

Keywords Air–cathode microbial desalination cell · Electrode spacing · Desalination · Organics degradation · Nitrogen removal · Microbial community

1 Introduction

Water is crucial for human survival, and water shortage and pollution problems have increased significantly worldwide in recent years [1, 2]. The total volume of water on the Earth is ~ 1.386 billion cubic kilometers. However, freshwater accounts for only 2.53% of the total water reserves. Freshwater resources available for human use (including rivers, freshwater lakes, shallow groundwater, etc.) merely account for 0.3% of the total freshwater reserves. Therefore, desalination and wastewater treatment are effective ways to increase the amount of water

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resources. The nexus of energy, water, and environment is crucial for future seawater desalination sustainability, research in desalination methods contributing to reduce specific energy consumption and protect the environment [3]. Several seawater desalination technologies, such as reverse osmosis (RO) [4], multi-cascade evaporators [5], multi-stage flashing (MSF) [6], multi-effect distillation (MED) [7], seawater thermocline-driven MED (ST-MED) [8], multi-effect distillation, and an adsorption cycle (MED-AD) [9, 10], have been reported in the literature. The recent hybridization trend of different desalination technologies has been to overcome the individual technological limitations. The economics of desalination technologies has been improving continuously owing to the expansion of the desalination market. Consequently, high-efficiency and low-consumption methods of desalination and wastewater treatment, and improvements of the performance ratios of these technologies have become the focus of research and development in the field of environmental engineering [11–14].

Microbial desalination cells (MDCs) are a new type of technology developed from microbial fuel cells (MFCs) for the first time at Tsinghua University for power production, desalination and wastewater treatment. The concept of operation of MDCs is similar to water electrodialysis, but the MDC is a device that uses bacteria as a catalyst to transform the chemical energy that exists in wastewater into electricity through electrochemical reactions. MDCs employ an additional chamber that contains salty water between the anode and cathode chambers of the MFCs. Ion exchange membranes separate the three chambers and permit the migration of salt ions to achieve desalination [15–17]. After the invention of MDCs by Cao et al. [18], researchers have developed recirculation microbial desalination cells (rMDCs) that avoid pH imbalance and accelerate bacterial respiration to increase the power of MDCs and achieve efficient desalination [19]. Desalination and treatment of chromium-containing wastewater have been achieved using a new three-chamber MDC [20]. Stacked resin-packed microbial desalination cells (SR-MDCs) filled with mixed ion exchange resins have also been implemented for secondary desalination of domestic sewage [21]. Microbial capacity desalination cells with the capacity to regenerate through exchange of the connection between the electrode and activated carbon cloth assemblies have been reported [22]. MDCs show great potential in bioelectricity generation, wastewater purification, and desalination owing to their excellent stability. They have been widely studied because of advantageous attributes such as low cost, good stability, wastewater treatment capacity, and desalination ability. However, most studies have so far only been conducted with laboratory-scale designs, and there are no reports of their practical applications.

Studies have shown that electrode spacing is an important parameter that can influence the performance of MFCs owing to its role in determining the internal resistance of the cell, electrode potential, power production, extent of organic removal, conductivity, etc. For further study on scale-up devices and broadening the practical application of MDCs, it is worth investigating their performance and efficiency with different electrode spacings.

Based on the traditional MDCs, in this experiment, a three-chamber air–cathode microbial desalination cells (AMDC) was designed and initiated. The effect of different electrode spacings (16, 12, and 8 cm) on the performance of the AMDC was investigated by using carbon cloth as the electrode material. Meanwhile, salt, organics, and nitrogen removal efficiency of the AMDC were evaluated, and 16S rRNA sequence was used to assess the structure and function of microbial community. The long-term operation stability of the AMDC was also examined in terms of current output, power generation, columbic efficiency (CE), internal resistance, and polarization to further evaluate their performance.

2 Materials and methods

2.1 Experimental materials and AMDC construction

The three chambers of the AMDC, namely anode, desalination, and cathode, were constructed using an acrylic cube cut into 6 cm, 10 cm, and 20 cm pieces, respectively, and separated with ion exchange membranes. The three chambers had a total working volume of 3.6 L (Fig. 1). Both the anion exchange membrane (AEM, AMI-Grion0011V; Lvhe Co., Ltd., China) and cation exchange membrane (CEM, CMI-Grion0011V; Lvhe Co., Ltd., China) exchange membranes had a cross-sectional area of 60 cm^2. Both AEM and CEM were prepared as described in a previous study [23], and in accordance with the manufacturer's instructions, the membranes were left in 5% NaCl solution for 48 h to allow hydration and expansion before installation. Both the anode and cathode were made from carbon cloths (6 cm × 12 cm, B1B, E-Tech, USA), and pre-treated with 15% v/v acetone solution for 24 h before use to remove the grease stain on the machined surface. The electrodes were immersed in distilled water, and before the experiment, rinsed with deionized water and then placed in an oven to dry [24]. A closed-circuit was set up by connecting a titanium wire coupled with an external resistance (1000 Ω). Three groups of AMDCs with different electrode spacings (16, 12, and 8 cm) were designed as AMDC1,

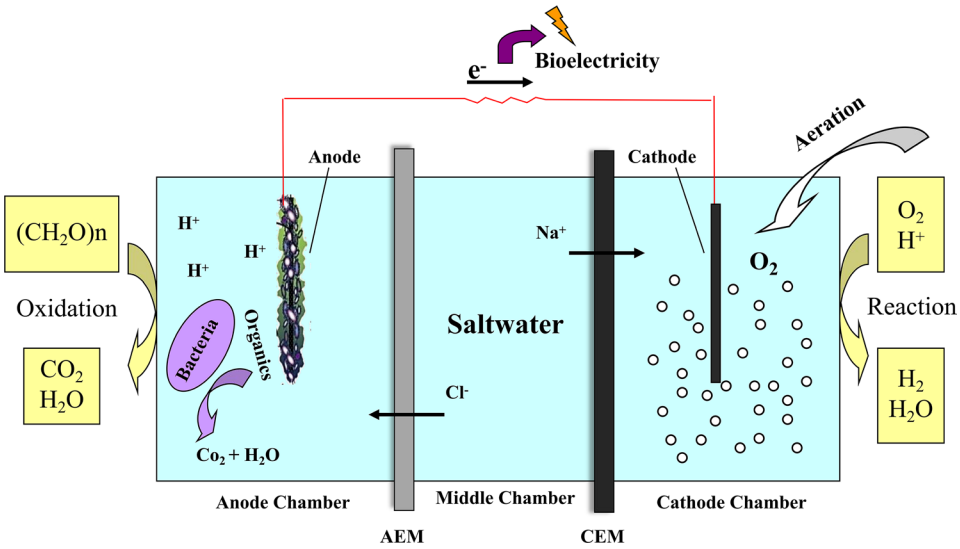

Fig. 1 Schematic diagram of the air–cathode microbial desalination cells

AMDC2, and AMDC3, respectively. The reactor was operated intermittently.

2.2 System inoculation and culture solution

To further reduce the start-up period and operating costs of the AMDC, the cathode chamber was directly inoculated with aerobic sludge collected from second sedimentation in a local wastewater treatment plant (Lingang New City, Shanghai). The ratio of aerobic sludge and nutrient solution was 1:1, and it was sealed and subjected to constant temperature (30 °C) oscillation in a water bath for a few days to obtain anaerobic sludge as an anode chamber inoculation of microorganisms [25]. To ensure an adequate electron donor, the anode chamber was fed with glucose nutrient medium as the fuel containing $C_6H_{12}O_6$ (0.56 g/L), KH_2PO_4 (4.40 g/L), K_2HPO_4 (3.40 g/L), and NH_4Cl (0.32 g/L). The anode chamber was stirred using a magnetic stirrer (40–50 rpm) to mix the components thoroughly. The cathode chamber was treated with sodium bicarbonate as the nutrient substance containing KH_2PO_4 (4.40 g/L), K_2HPO_4 (3.40 g/L), NH_4Cl (0.32 g/L), and $NaHCO_3$ (1.92 g/L). Continuous aeration was achieved by an aerating pump to increase dissolved oxygen (DO), and the cathodic DO concentration was controlled at greater than or equal to 6.0 mg/L by adjusting the flow aerator. Further, 12.5 g/L of trace metal and 5 g/L of vitamin solutions were separately added to both chambers [21, 26]. The desalination chamber was filled with artificial salt water containing 35 g/L of NaCl solution. After inoculation, the anolyte, catholyte, and salt solution were not changed until the voltage reached 100 mV. During the start-up and operational stages, the cathode chamber was refilled with new solution every 48 h.

2.3 Measurements and analysis

To assess the performance of the AMDC, conventional water quality indicators such as chemical oxygen demand (COD), ammonia, and nitrate nitrogen were determined and referenced to the standard methods [27]. The solution pH and NaCl concentration were monitored using a multi-parameter water quality analyzer (HQ40; Hach, USA).

The electrochemical characteristics of the AMDC were studied as follows. Cell voltage was recorded every minute by an 8-channel data acquisition system (RTKINS Co., Ltd., Wuhan, China) connected to a computer, and the averages were calculated every hour. The steady state reading was recorded at each resistance value. Power generation, current density (A/m^3), and power density (mW/m^3) were calculated and analyzed by the net total working volume. The polarization curves were obtained by varying the external resistance from 1000 to 50 Ω as described by Qu et al. [28]. The cell internal resistance was obtained from the polarization curve as described by Logan et al. [29]. Finally, the coulombic efficiency (CE) was used to characterize the electrical capacity of the anode microorganisms.

2.4 Scanning electron microscopy and microbial community structure analysis

At the end of the experiment, the anode carbon cloth electrodes of the AMDC with the best effect were immediately stored at − 20 °C after being taken out from the chambers. The structures of the carbon cloths and microbial community were observed and evaluated using scanning electron microscopy (SEM). The primer sets used were 338F (ACTCCTACGGGAGGCAGCA) and 806R (GGACTACHVGGG TWTCTAATAT). The V3–V4 regions of the bacterial 16S rRNA gene were analyzed on an Illumina MiSeq platform

(Personal Biotechnology Co., Ltd., Shanghai, China) to identify the dominant electrochemically active bacteria [30].

3 Results and discussion

3.1 Bioelectricity generation characteristics of the AMDC

3.1.1 Start-up of the AMDC under diverse electrode spacing

The reactor was operated intermittently at a temperature of 25 ± 1 °C. The cathode used oxygen as an electron donor, the desalination chamber was filled with 35 g/L of NaCl solution, and the anode chamber was fed with glucose nutrient medium as a fuel. The dynamic changes in the output voltage with time during the operation of the AMDC are shown in Fig. 2. The output voltage exceeded 600 mV for two consecutive periods and tended to be stable. The reactor start-up phase was complete after 30 days of culture, and the maximum voltage was ~ 645 mV, which is similar to the voltage generated by traditional MFCs [31].

The voltage was low at the beginning, likely because there was an insufficient amount of sludge inoculation, which led to low accumulation of electrogenic microorganisms on the anode surface. Consequently, the inoculated microorganisms needed to adapt to the new environment and showed slow metabolism. As a result, the output voltage of the three AMDCs showed considerable fluctuations. As domesticated microorganisms gradually adapted to the new environment, constant absorption of the substrate nutrients accelerated their growth and metabolism led to a rapid increase in the voltage [32]. The output voltages of the three AMDCs reached a peak on and around day 10, 12, and 12, respectively, and the microbial adhesion reached the highest level and remained stable. The reactor entered a stable period and continued to operate for 5 days. On day 17, owing to nutrient consumption and accumulation of metabolites in the reactor, microbial growth was restricted and the output voltage gradually decreased. On day 20, the initial period in the second cycle of substrate solution replacement was shorter than in the first cycle. A possible reason is that the metabolism of electricity-producing microorganisms accelerated and the substrate nutrition was consumed rapidly, which is consistent with the change in the COD.

As shown in Fig. 2, the maximum voltages of AMDC1–3 were 598, 645, and 610 mV, respectively. Under the same conditions of anode electrode materials, AMDC2, with its electrode spacing of 12 cm, showed the best effect, followed by AMDC3 with its electrode spacing of 8 cm, and the AMDC1 with the electrode spacing of 16 cm has the worst effect. As the electrode spacing decreased, the maximum voltage first increased and then declined. The reason may be that the different electrode spacing changed the internal resistance of the AMDC, which is consistent with the change in power density.

The anode chamber used glucose as the sole carbon source while the cathode chamber used oxygen as the sole electron donor. The changes in COD and NaCl concentration in the AMDC open- and closed-circuit systems are shown in Fig. 3.

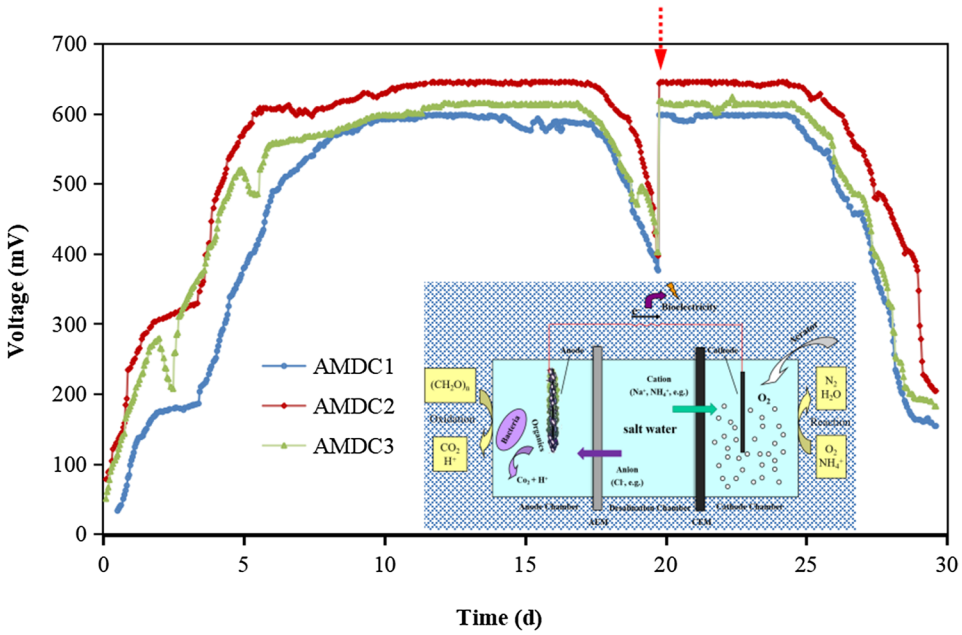

Fig. 2 Output voltage during the start-up period (arrow indicates anolyte replacement)

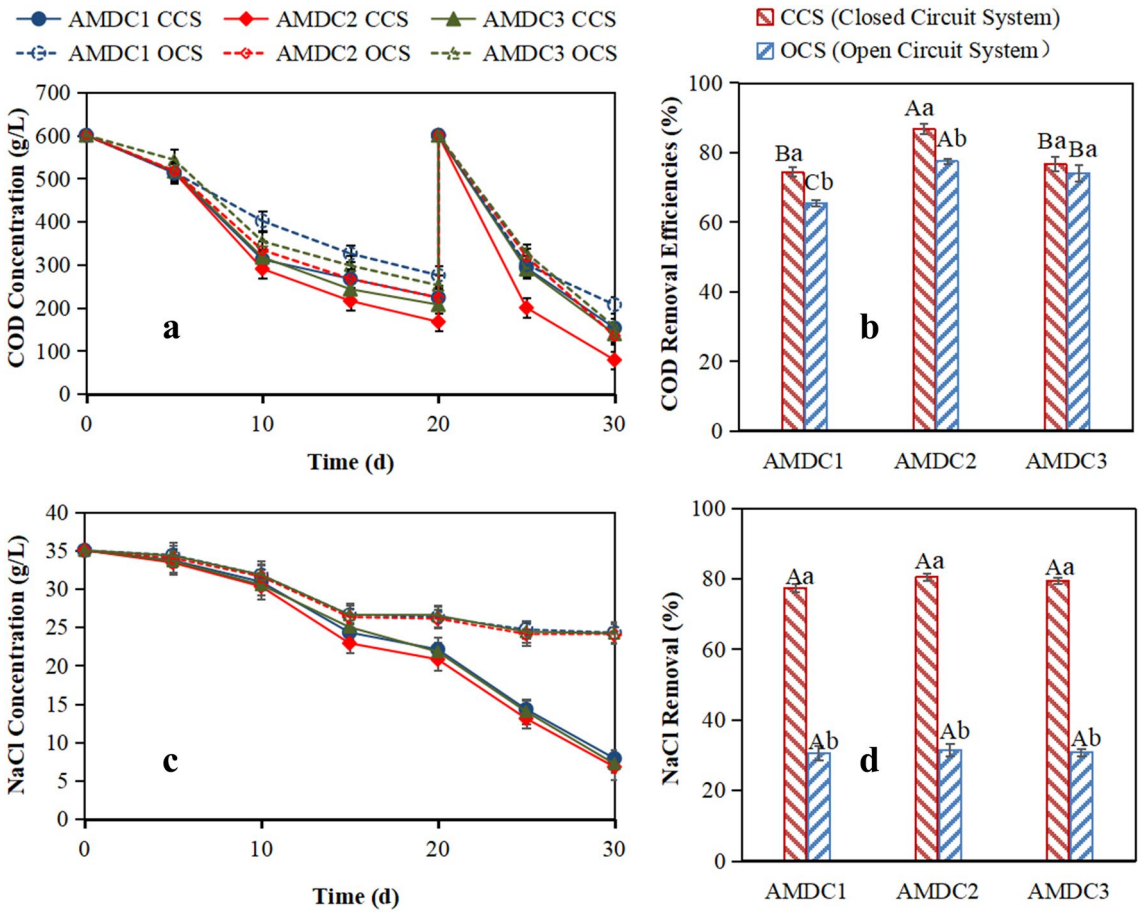

Fig. 3 Changes in COD (**a**), NaCl concentration (**c**), COD removal efficiency (**b**), and NaCl removal efficiency (**d**) for the open-circuit system (OCS) and closed-circuit system (CCS) under different electrode spacings. In **b**, **d**, different uppercase letters indicate significant differences between the same system at different electrode spacings ($p < 0.05$). Different lowercase letters indicate significant differences between the different systems at the same electrode spacing ($p < 0.05$)

The difference of the COD degradation rates in OCS and CCS was not significant ($p > 0.05$). The anode microorganisms in the three AMDCs had the same effect on the degradation of organic matter, and the results of inoculation of the same kind of sludge were consistent. Furthermore, there was no significant effect on the COD degradation rate between the OCS and CCS on changing the electrode spacings. Voltage output under the closed-circuit system indicated that the anode microorganisms oxidized organic matter to produce electron transfer to the cathode, which constituted a closed loop. Under the action of an electric field, the salt ions in the desalination chamber were pushed to migrate to both ends, which significantly promoted the NaCl concentration change caused by single concentration differential dialysis in the open-circuit system [33, 34]. There was a significant effect of promoting the change in NaCl concentration in the CCS concentration was significantly different ($p < 0.01$). Under identical system conditions, the difference in NaCl concentration in the three AMDCs was not significant ($p > 0.05$). In summary, the AMDC with an electrode spacing of 12 cm had a higher COD degradation rate and changes in NaCl concentration than other AMDC.

3.1.2 Power density curves and polarization curves of the AMDCs

In the CCS, the current density and volume power density were calculated from the effective volume of the reactor, and the polarization curves were determined by the steady state discharge method [33]. During the stable power production stage of the experiment, polarization measurements were made using a variable resistor box, and the external resistance was reduced stepwise from 1000 Ω to 50 Ω (the external resistances were, respectively, 1000, 500, 300, 200, 100, 50 Ω). The power density curves and polarization curves are shown in Fig. 4. The maximum power densities of the three AMDCs were 184.0, 214.7, and

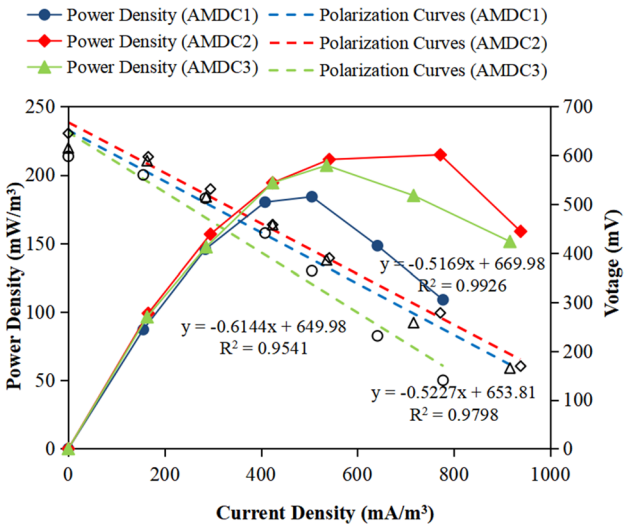

Fig. 4 Power density curves and polarization curves of the AMDC under diverse electrode spacing

206.9 mW/m^3, respectively, which was slightly lower than that of the conventional reactor. This may be attributed to the lack of electricity-generating microorganisms in the inoculation sludge function, or the slow metabolism of microorganisms with insufficient substrate nutrients that resulted in the poor electricity-generating effect [26]. In addition, the reactor internal resistance was large and the reduction rate of the cathode oxygen electron donor on the electrode was slow, which limited the overall power output of the reactor that resulted in a low power density.

The NaCl concentration in the desalination chamber gradually decreased with the operation of the reactor, and the conductivity of the low-concentration solution declined, which increased the internal resistance of the AMDC. At a certain stage, the power density of the three AMDCs showed a decreasing trend. Thus, decreasing the electrode spacing effectively reduced the internal resistance of the reactor and increased the output power. In the CCS, when the electrode spacing was changed from 16 to 8 cm, the power density increased from 184.0 to 214.7 mW/m^3, and then decreased to 206.9 mW/m^3, and the internal resistance of the three AMDCs was 614.4, 516.9, and 522.7 Ω, respectively. The results showed that reducing the electrode spacing effectively reduced the resistance to ion migration in solution, which was beneficial to the degradation of organic matter by microorganisms, and sped up the mass transfer of organic matter and products, thus reducing the resistance of mass transfer in the reactor and increasing the output power of the reactor. In conclusion, the three-chamber AMDC had a good start. With the same electrode materials, the AMDC with an air–cathode spacing of 12 cm showed the best starting effect.

3.2 Analysis of desalination, organics degradation, and nitrogen removal

3.2.1 Organics and nitrogen removal efficiency

After start-up, the anode substrate of the AMDC was updated and the operation began. The three AMDCs lasted 12.5 days from the end of the cycle, and the removal effect of major pollutants is shown in Table 1. During the operation of the three AMDCs, the organic matter in the substrate was decomposed by the catalytic activity of the anaerobic microorganisms. As a result, the COD concentration of the anode substrate solution decreased. As calculated in Table 1, the COD degradation rates of the anode substrate solution of the three AMDCs were 69.03, 71.69, and 69.79%, respectively, i.e., not significantly different

Table 1 Influent and effluent concentrations of the AMDC with different electrode spacings

Class	Location	Mode	pH	COD (mg/L)	Ammonium (mg/L)	Nitrate nitrogen (mg/L)
AMDC1	Anode chamber	Influent	6.67	600.00	42.72	2.39
		Effluent	6.04 ± 0.10	185.82 ± 9.64	11.20 ± 1.23	2.06 ± 0.06
	Cathode chamber	Influent	6.92	–	18.67	0.97
		Effluent	7.51 ± 0.15	–	3.24 ± 0.08	1.24 ± 0.04
AMDC2	Anode chamber	Influent	6.67	600.00	42.72	2.39
		Effluent	5.87 ± 0.19	169.86 ± 10.25	10.36 ± 1.20	1.99 ± 0.02
	Cathode chamber	Influent	6.92	–	18.67	0.97
		Effluent	7.97 ± 0.24	–	2.98 ± 0.14	1.26 ± 0.07
AMDC3	Anode chamber	Influent	6.67	600.00	42.72	2.39
		Effluent	5.98 ± 0.08	181.26 ± 8.50	10.97 ± 0.98	2.14 ± 0.08
	Cathode chamber	Influent	6.92	–	18.67	0.97
		Effluent	7.62 ± 0.21	–	2.77 ± 0.08	1.27 ± 0.04

($p > 0.05$). These results indicate that microorganisms have the same catalytic ability to decompose organic matter, and the change of electrode spacing has no significant effect on microbial degradation. The reasons for this COD removal effect that may be the resistance of anode material and reaction rate of cathode. As the resistance of the anode material decreased, the removal amount of COD increased considerably, while the changes in the cathode reaction rate had little impact on the removal effect of COD.

Ammonia nitrogen (NH_3–N) exists in the anode substrate solution in the form of free ammonia (NH_3) or ammonium (NH_4^+). When the pH is high, it exists in the free form, and vice versa as ammonium. During the operation of the AMDC, denitrifying ammonia oxidizers under hypoxia conditions, NH_4^+ was denitrified into N_2, thereby reducing the NH_4^+ content in the solution. Meanwhile, with the operation of the AMDC, the pH of the anode chamber decreased and free ammonia escaped as a gas, which is also one of the reasons for the decreasing concentration of ammonia nitrogen in the anode chamber. The ammonium removal rates of the anode substrate solutions of the three AMDCs were 73.78, 75.74, and 74.32%, respectively, with no significant difference ($p > 0.05$), which was the same as COD degradation. The ammonium removal rates of the cathode chamber of the three AMDCs were higher than that of the anode chamber, which were 82.64, 84.03, and 85.16%, respectively. This was attributed to the aeration of the cathode chamber and conversion of ammonium (NH_4^+) into nitrate nitrogen (NO_3^-–N) under aerobic conditions, which was consistent with the increase in nitrate nitrogen concentration in the cathode chamber. The results showed that the AMDC can degrade inorganic nitrogen pollutants effectively, and the change of electrode spacing had no significant effect on the degradation of inorganic nitrogen pollutants.

The initial pH values of the AMDC anode and cathode electrolytes were 6.67 and 6.92, respectively. At the end of operation, pH values of the AMDC1–3 anode electrolytes had decreased, while those of the cathode electrolytes had increased; this variation was the most obvious for the AMDC2 reactor with an electrode spacing of 12 cm. During the operation of the AMDC, the pH decreased and increased for the anolyte and catholyte, respectively, probably because the organics in the anode chamber were degraded, H^+ were produced, and e^- were released. The e^- reached the cathode through the external circuit and combined with O_2 to produce H_2O. The H^+ generated in the anode chamber and OH^- generated in the cathode chamber accumulated continuously, leading to a change in the pH value. The extremely low and high pH values of the anolyte and catholyte, respectively, adversely affected the reactor performance. The pH change in the AMDC2 reactor was the most obvious. As mentioned above, with the reduction of electrode spacing, the internal resistance of the reactor decreased, which was conducive to the decomposition of anaerobic microorganisms. Therefore, the combined effect of pH change and electrode spacing had no significant effect on the degradation of organics in the reactor, which is consistent with the removal effect of COD.

3.2.2 Analysis of desalination and energy recovery efficiency

The three AMDCs were operated until the end of the cycle, and the desalination effect under diverse electrode spacing conditions was studied (Fig. 5). The NaCl concentration in the three AMDCs decreased gradually with time, and the desalination efficiencies were 84.86%, 87.71%, and 83.43%, respectively. As described in Sect. 2.1, the desalination effect of the electricity generation was affected by the electrode spacing. The AMDC with an electrode spacing of 12 cm showed good electrical desalination characteristics compared with the other two AMDCs. Coulomb efficiency is used to measure the ability of anode microorganisms to convert organic matter into electrical energy [35, 36]. The calculated Coulombic efficiencies of the three AMDCs were 17.29, 18.60, and 17.95%, respectively. Under the same operating conditions, the difference in the Coulombic efficiencies of the three AMDCs was significant ($p < 0.05$) on changing the electrode spacing, which showed that the utilization rate of organic matter was similar. The possible reasons for this result are the following: (1) the influence of electrode spacing on the internal resistance of the reactor; (2) the change of pH of the anode and cathode chamber; and (3) the loss of energy of organic

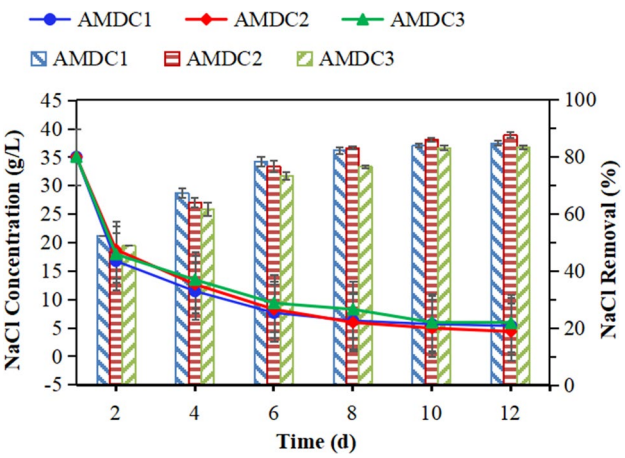

Fig. 5 Desalination rate versus time of the AMDC under diverse electrode spacing

substrate hydrolysis, and other human factors, such as the error of current intensity measurement.

3.3 Growth and microbial community analyses

3.3.1 SEM images of anode electrode of the AMDC

To further analyze the microbial adhesion growth of the anode carbon cloth electrode on the AMDC under different electrode spacing conditions, the morphology of the carbon cloth electrode was analyzed by SEM, and the results were shown in Fig. 6. The carbon cloth electrode pre-treated by acetone immersion had a uniform surface, loose texture, and a membrane surface with a large number of voids, which were conducive to the adhesion and growth of microorganisms. After a period of operation, the three AMDCs were observed using a scanning electron microscope under × 250 SE and × 500 SE, respectively. Microorganisms were attached to the surface of the anode electrode, effectively utilizing the void structure of the membrane surface to enable the microorganisms to degrade organic matter smoothly.

3.3.2 Analysis of microbial community structure characteristics

Microorganisms, as the core of the MDCs, play an important role in the biocatalytic reaction for power generation and pollutant removal. Experimental results show that the performance of the AMDC needs to be improved compared to previous studies. To further verify the hypothesis that poor performance was due to a lack of functional electricity-producing microorganisms, we conducted an in-depth study of the microbial communities. Through the 16S rRNA sequencing analysis, the anode microbial community structure of the AMDC with the best electrode spacing of 12 cm was analyzed and divided according to 97% homology. A total of 1512 OUT classification

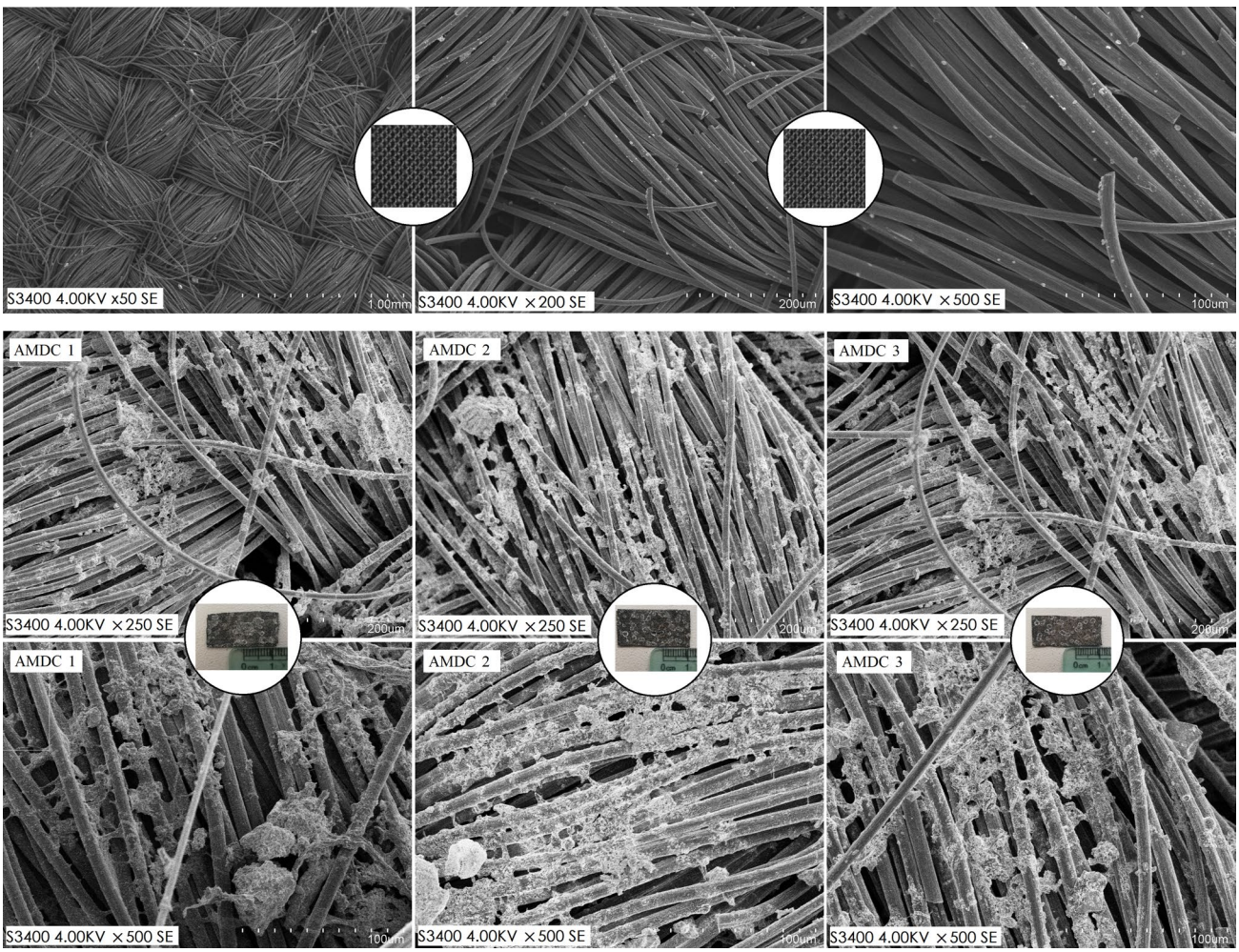

Fig. 6 SEM images of anode carbon cloth electrode of the AMDC under diverse electrode spacing

operation units were obtained. The total number of Chao1, Ace, and Simpson indicators were 1621, 1733, and 0.9678, respectively, with high microbial richness and diversity, and the Shannon index was 7.5. The bands flattened out when the number of bands read was more than 10,000, indicating that the sequencing of bands was reasonable, and it was less likely that new species would be discovered at greater sequencing depth.

The microbial community structure in the anode of the AMDC was analyzed using the Illumina MiSeq sequencing platform, and the results are shown in Fig. 7.

As shown in Fig. 7, at phylum level, the *Bacteroidetes* accounted for the highest proportion in the anode chamber (33.9%), followed by *Firmicutes* and *Proteobacteria*, accounting for 30.7% and 26.1%, respectively. Studies by Cabezas show that *Proteobacteria* and *Bacteroidetes* contain a variety of electrogenic microorganisms [37, 38]. *Proteobacteria* were enriched in the MDCs, and *Bacteroidetes* could metabolize some easily degraded organic matter and improve the metabolism of co-plasma to co-substrate [39]. *Firmicutes* are facultative oxygen microorganisms, and as an important component of the microbial community of MDCs, they were capable of electron transfer outside the cell [40].

According to the analysis of the genus level, *Bacteroides* are present in the highest proportion of 18.1%, *Bacteroides* belongs to the order *Bacteroidales*, the class *Bacteroidia*. As mentioned above, *Bacteroides* can accelerate the metabolism of the co-matrix by the metabolism of organic matter and increase the activity of electrogenic bacteria. The known functional electrogenic bacteria were mainly *Bacillus* and *Arcobacter*, constituting 11.6% and 9.6% of the AMDC, respectively. *Bacillus* (order *Bacillales* and class *Bacilli*) are one of the most common native microorganisms in natural water [41] and have been shown to be associated with electricity generation as a typical electrogenic microbe. *Arcobacter* belongs to the class *Epsilonproteobacteria*, and the results show that *Arcobacter* is a dominant flora in municipal sewage and chemical bioflocculation activated sludge. Nuria et al. [42] improved the recovery of halophilic *Arcobacter halophilus* in seawater by using NaCl medium, which provided a possibility for the study of *Arcobacter* in the high-salt environment of MDCs.

It was found that the lack of electrogenic microorganisms in the active function of the anode chamber (< 30%) was an important factor in the poor performance of the AMDC, which was consistent with the hypothesis. To improve the effect of the AMDC, follow-up studies to

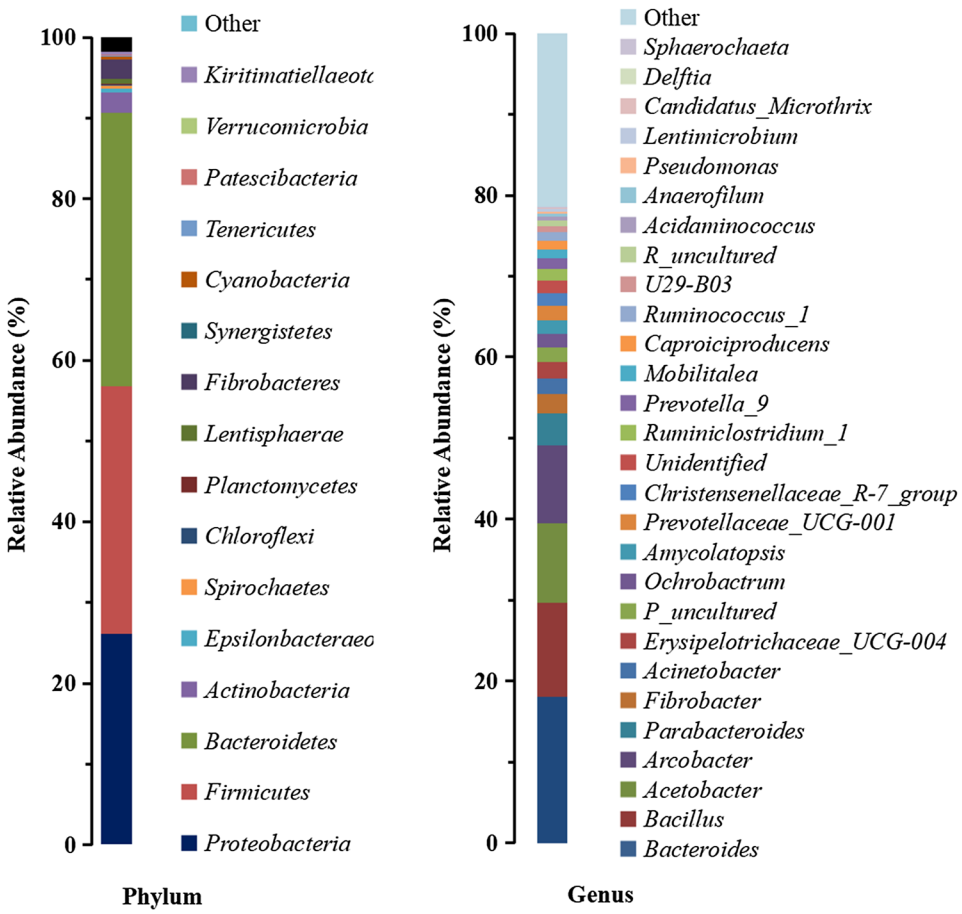

Fig. 7 Microbial communities based on the relative abundance of 16S rRNA sequence of the AMDC anode biofilms in the phylum and corresponding genus levels

improve the reactor and screen, isolate and purify new electrogenic microorganisms are required.

4 Conclusion

Microbial desalination cells constitute a promising environment-friendly desalination technology, and understanding their performance under different operating conditions and modes is helpful for their practical applications. In this study, the effect of electrode spacing on AMDC was studied.

- It was found that under identical system conditions, the AMDC with an electrode spacing of 12 cm exhibited the best starting effect. When the three AMDCs (with different electrode spacings) were run to the end of the cycle, a stable working state was maintained in terms of COD degradation rate and inorganic nitrogen removal. Interestingly, changes in the electrode spacing did not have any significant impact on the ability of the microorganisms to degrade organic matter and remove inorganic nitrogen. However, during the operation of the reactor, both electricity generation and desalination performance were affected by the electrode spacing. The AMDC with 12 cm electrode spacing showed the best performance.
- The overall output of the three AMDCs was poor. SEM images showed that the microbes were attached to the surface of the anode, and 16S rRNA microbial community structure analysis revealed that the functional electrogenic bacteria in the AMDC were *Bacillus* and *Arcobacter*. The lack of active functional electrogenic bacteria is the primary reason for the poor performance of the AMDCs.
- It has been suggested that the efficiency of these cells may be improved by upgrading the reactor and screen, isolating and purifying new electrogenic microorganisms, and applying it to the actual sewage treatment process, which would provide invaluable guidance for future research and development of this technology.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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