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Microbial Fuel Cells: The Effects of Configurations, Electrolyte Solutions, and Electrode Materials on Power Generation

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Abstract This objective of this study is to conduct a systematic investigation of the effects of configurations, electrolyte solutions, and electrode materials on the performance of microbial fuel cells (MFC). A comparison of voltage generation, power density, and acclimation period of electrogenic bacteria was performed for a variety of MFCs. In terms of MFC configuration, membrane-less two-chamber MFCs (ML-2CMFC) had lower internal resistance, shorter acclimation period, and higher voltage generation than the conventional two-chamber MFCs (2CMFC). In terms of anode solutions (as electron donors), the two-chamber MFCs fed with anaerobic treated wastewater (AF-2CMFCs) had the power density 19 times as the two-chamber MFCs fed with acetate (NO_3^-2CMFCs). In terms of cathode solutions (as electron acceptors), AF-2CMFCs with ferricyanide had higher voltage generation than that of ML-2CMFCs with nitrate (NO_3^-ML-2CMFCs). In terms of electrode materials, ML-2CMFCs with granular-activated carbon as the electrode (GAC-ML-2CMFCs) had a power density 2.5 times as ML-2CMFCs with carbon cloth as the electrode. GAC-ML-2CMFCs had the highest columbic efficiency and power output among all the MFCs tested, indicating that the high surface area of GAC facilitate the biofilm formation, accelerate the degradation of organic substrates, and improve power generation.

Keywords Electrogenic bacteria · Microbial fuel cell · Power generation · Internal resistance · Wastewater treatment

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Abbreviations

MFC	Microbial fuel cell
2CMFC	Two-chamber microbial fuel cell
ML-MFC	Membrane-less microbial fuel cell
NO_3^--2CMFC	Two-chamber microbial fuel cell with nitrate (NO_3^-) as the electron acceptor
AF-2CMFC	Two-chamber microbial fuel cell with anaerobic treated wastewater in anode and ferricyanide in cathode
NO_3^--ML-2CMFC	Membrane-less two-chamber microbial fuel cell with nitrate (NO_3^-) as electron acceptor
GAC-ML-2CMFC	Membrane-less two-chamber microbial fuel cell with granular-activated carbon as electrode
PEM	Proton exchange membrane
COD	Chemical oxygen demand
ORP	Oxidation–reduction potentials
OCP	Open circuit potential
$\Delta OCP_{A\text{-}C}$	The OCP difference between anode and cathode electrodes ($\Delta OCP_{A-C} = OCP_{anode} - OCP_{cathode}$)
$\Delta ORP_{C\text{-}A}$	The ORP difference between anode and cathode solutions ($\Delta ORP_{C-A} = OCP_{cathode} - OCP_{anode}$)
P_{Cat}	Power density
I_{Cat}	Current density
ε_C	Coulombic efficiency
W	Total experimental energy generation measured over the MFC operational period
W_{theo}	Total theoretical energy generation
R_{in}	Internal resistance
R_{ext}	External resistance

Introduction

Microbial fuel cell (MFC) is a promising biotechnology to generate electricity from the degradation of organic contaminants in wastewater treatment processes. Conventional two-chamber MFCs (2C-MFCs) consist of anaerobic anode and aerobic cathode separated by a proton exchange membrane (PEM). In the anode chamber, substrates (e.g., glucose, organic contaminants in wastewater) are oxidized by anaerobic bacteria, through which electrons are produced. The electrons are then transferred to the anode surface and eventually flow to the cathode surface through the external circuit. Protons (H^+) generated as a result of the substrate oxidation in the anode chamber migrate through the PEM and combine with the electrons and oxidants (e.g., oxygen [1–3], ferricyanide [4–6], nitrate [7]) in the cathode chamber. There are two advantages of MFCs for wastewater treatment. First, the contaminants in wastewater provide the endless carbon sources for the MFCs. Second, electricity generated from contaminant removal could be sufficient to power the wastewater treatment processes and thus reducing the energy consumption.

Due to the great potential of the application of MFCs in wastewater treatment processes, tremendous efforts have been invested to improve the power output of MFCs. MFCs with catalysts coated on electrodes (e.g., platinum [8], cobalt tetramethoxyphenyl-porphyrin [9], Mn (IV), and Fe (III) [10]) have been developed for higher power densities. Electrogenic bacterial strains (e.g., *Geobacter sulfurreducens* [11], *Shewanella putrefaciens* [12], *Rhodoferax ferrireducens* [13]) have been found to directly transfer electrons to anode surfaces without adding artificial mediators. Various mixed cultures of bacteria (e.g., activated sludge [2, 14], wastewater [15]) have been studied for electricity generation from organic substrates. Several novel MFC configurations (e.g., graphite fiber brush anode [16], stacked MFC [17], and upflow MFC [18]) have been designed. Furthermore, MFCs have been tested for nitrogen removal in wastewater treatment by reducing nitrate (NO_3^-) to nitrogen gas (N_2) in the cathode chambers [7]. Traditional nitrogen removal includes aerobic nitrification and anoxic denitrification, which requires long sludge retention time, extra carbon sources, and high operational cost [19]. Using NO_3^- as the electron acceptor in MFCs can simultaneously achieve nitrogen removal and power generation.

However, there are three major challenges for increasing the power generation in MFCs. First, the high internal resistance ($R_{in.}$) consumes the power generated inside MFCs and causes low energy conversion efficiency [20]. The R_{in} consists of ohmic resistance (i.e., electrode resistance, PEM and electrolyte resistance), charge (electron and proton)–transfer resistance, and diffusion resistance [18, 21, 22]. Reducing R_{in} is crucial to improve power output. Second, the low efficiency of electrons transfer from bacterial cells to anode surfaces limits the power generation. Although several mechanisms of electron transfer have been studied (i.e., direct electron transfer to anode [14], indirect electron transfer with soluble mediators [23]), there is no efficient method to improve the electron transfer efficiency inside MFCs. Third, the high costs of electrodes materials, PEM, and electron donors/acceptors hinder the real-world application of MFCs. Cost-effective materials should be explored to improve power generation of MFCs as well as accelerate the engineering operation of MFCs in wastewater treatment plants.

Many studies have focused on the individual aspects of MFCs, such as configuration [6, 16–18], electrode materials [8–10], inoculums [11–13], and operational conditions [5, 18, 21]. In order to improve the power generation of MFCs, it is critical to elucidate the limiting factor for power generation. One efficient approach to understand the limiting factor is to systematically compare these factors for MFC performance. Therefore, the objective in this study was to compare the effects of configurations, electrolyte solutions, and electrode materials on the electrochemical (i.e., power generation, columbic efficiency) and biochemical characteristics (i.e., organic substrate removal, the acclimation of bacteria) of MFCs. Two MFC configurations (two-chamber MFC (2CMFC), membrane-less two-chamber MFC (ML-2CMFC)) were examined to reduce R_{in} and increase power generation. Two anode inoculums (domestic wastewater and anaerobic treated wastewater) and two cathode solutions (nitrate and ferricyanide) were studied for the capacity of electron generation and acceptance in MFCs. Two electrode materials (granular-activated carbon and carbon cloth) were compared for the effects of electrode surface area on biofilm formation and power generation.

Methods

MFC Setup

Four types of MFCs (the effective volume, 200 mL) with different configurations, electrolyte solutions, and electrode materials were examined in this study. A summarized specification of these four MFCs is listed in Table 1, and related schematics are shown in Fig. 1. The first type was the conventional 2CMFCs with acetate (Fisher Scientific, NJ,

Table 1 Specification of MFCs configurations, materials, inocula, and electrode solutions.

List number	MFCs name abbreviations	Anode materials	Inocula	PEM	Electrode distance (cm)	Components in cathode
A	NO_3^--2CMFCs	Carbon cloth	Municipal wastewater	+	10	NO_3^-
B	AF-2CMFCs	Carbon cloth	Anaerobic treated wastewater	+	10	Ferricyanide
C	NO_3^--ML-2CMFCs	Carbon cloth	Municipal wastewater	−	5	NO_3^-
D	GAC-ML-2CMFCs	GAC	Municipal wastewater	−	5	NO_3^-

A is conventional MFC as contrast, while B, C, and D are the improved MFCs. Related schematics are shown in Fig. 1

+ PEM present, − PEM absent

USA) as the electron donor (organic substrate) in the anode chamber and nitrate (Fisher Scientific) as the electron acceptor in the cathode chamber (termed as NO_3^-2CMFC, Fig. 1a). This type of MFC was examined for the simultaneous electricity generation and nitrogen removal in wastewater treatment. A PEM membrane (Nafion, Ion Power Inc.) was used to separate the anode and cathode chambers. The anode and cathode electrodes were made of carbon cloth (non-wet and wet proofing, E-TECH) with the surface area of 6 cm^2. The distance between these two electrodes was 10 cm. The second type was the 2CMFC with anaerobic treated wastewater (containing acetate, ethanol, and butyrate) as the organic substrate in the anode chamber and ferricyanide (Orgnics, NJ, USA) as the electron acceptor in the cathode chamber (termed as AF-2CMFC, Fig. 1b). The third type was a membrane-less 2CMFC (termed as NO_3^-ML-2CMFC, Fig. 1c). There was no PEM

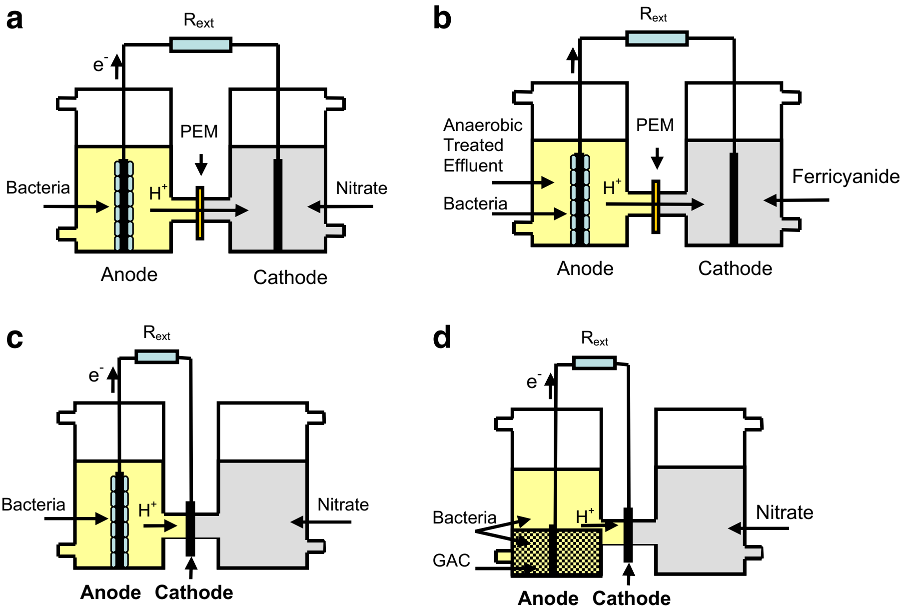

Fig. 1 The schematics of NO_3^--2CMFCs (**a**), AF-2CMFCs (**b**), NO_3^--ML-2CMFCs (**c**), and GAC-ML-2CMFCs (**d**). **a**, **b**, **c**, and **d** are the MFCs list number referred in Tables 1, 2, 3, and 4

between the anode and cathode chambers. The distance between anode and cathode electrodes was 5 cm. NO_3^- was used as the electron acceptor. The fourth type was the membrane-less 2CMFC with granular-activated carbon (GAC; General Carbon Corporation) as the anode electrode to increase the surface area (termed as GAC-ML-2CMFC, Fig. 1d). NO_3^- was used as the electron acceptor. GAC particles weight of 35 g were put into the anode chamber, and a graphite rod inserted in the GAC bed was used to connect the GAC particles with the external circuit. The R_{ext} was 1,000 Ω in all MFC tests. MFCs were operated in a temperature control room at 30 °C.

Inocula and Growth Media

Wastewater collected from University of Connecticut Wastewater Treatment Plant was used as the inocula to cultivate the anaerobic electrogenic bacteria in the anode chambers of NO_3^-2CMFCs, NO_3^-ML-2CMFCs, and GAC-ML-2CMFCs [10, 14, 24]. Anaerobic treated wastewater (containing acetate of 328 mg/L, ethanol of 191 mg/L, butyrate of 29 mg/L) collected from an anaerobic biofermentor was used as the inocula for AF-2CMFCs. The wastewaters were mixed with growth media solution at the ratio of 1:1 and then inoculated in the anode chambers of MFCs. Growth media solution provided nutrients for anaerobic electrogenic bacteria. The constituents of the growth media solution per liter were: 1 g sodium acetate, 310 mg NH_4Cl, 130 mg KCl, 5.09 g NaH_2PO_4, 7.89 g Na_2HPO_4, and 12.5 mL mineral and 12.5 mL vitamin solutions [14, 24]. The chemical oxygen demand (COD) of the growth media solution was 1,000 mg/L. The growth media solution was refilled in the anode chambers when the voltage output dropped below 50 mV during the MFC operation. For the cathode solutions, ferricyanide (35 g/L, for AF-2CMFC) and NO_3^- (50 mg/L, for NO_3^-2CMFC, NO_3^-ML-2CMFC, and GAC-ML-2CMFC) were tested as the electron acceptors.

Electrochemical Measurement

The voltage (V) across the external resistance (R_{ext}) was recorded at 2-h intervals using a data acquisition system (Keithley 2700, Keithley Instruments Inc.). The current (I) of the R_{ext} was determined by Ohm's law ($I = VR^{-1}$). Polarization curve measurement was performed to evaluate the maximum power output of MFCs, in which the R_{ext} was adjusted from 50 to 1,500 Ω, and the voltages were recorded using a digital multimeter (Radioshack Co.). The power generation ($P = V^2R^{-1}$, W) were calculated and plotted against current densities (I_{Cat}; $I_{Cat} = VR^{-1}S^{-1}$, where S is the surface area of electrodes) to obtain the polarization curves. The open circuit potential (OCP) is the maximum value of anode potential and the minimum value of cathode potential in the absence of current [25]. The OCP difference between anode and cathode ($\Delta OCP_{A-C} = OCP_{anode} - OCP_{cathode}$) was used to indicate the maximum voltage output of MFCs. The OCP was measured weekly using a potentiostat (Gamry Instruments Inc.) with Ag/AgCl as the reference electrode. The oxidation–reduction potentials (ORP) and pH of the anode and cathode solutions were measured weekly using Accumet probes and meters (Accumet AP72, Fisher Scientific Inc.). All the measurements were performed in an anaerobic chamber (Bactron, Sheldon Manufacturing, Inc).

Water Quality Analysis

The water quality analysis was conducted at the beginning and the end of each cycle. The COD of the anode solutions were measured using the Hach COD system (Method 8000, Hach Co., CO, USA). Acetate concentration was analyzed using a gas chromatograph (Agilent 6890N) equipped with a DB-FFAP column (Agilent Technologies). Nitrate concentration in the cathode cambers of NO_3^-2CMFC, NO_3^-ML-2CMFC, and GAC-ML-2CMFC was measured using the Hach Nitrate system (Method 8038, Hach Co.).

The Evaluation of Power Generation by Electrogenic Bacteria in MFCs

Coulombic efficiency (ε_C) was used to evaluate the power generation from the degradation of substrates in MFCs. ε_C is the ratio of the actual coulombs to the theoretical coulombs generated from COD removal [25–27], described by Eq. 1:

$$\varepsilon_C = \frac{M \int_0^t I \mathrm{d}t}{F b v_{An} \Delta \mathrm{COD}} \tag{1}$$

where $M=32$ is the oxygen molecular weight, $\int_0^t I \mathrm{d}t$ is the actual coulombs generated over the time period (t), F is the Faraday constant (9,6485 C/mol electrons), $b=4$ is the mole number of electrons generated per mole of oxygen, ν_{An} (liter) is the volume of the anode chamber, and ΔCOD (gram per liter) is the amount of COD removed over time period (t).

Total theoretical energy generation (W_{theo}, joule) generated from COD removal was calculated based on by Eq. 2:

$$W_{theo} = \frac{\Delta \mathrm{COD}_{in-eff}}{M_{O_2}} \times 4 \times F \times V \tag{2}$$

Where $\Delta \mathrm{COD}_{\text{in-eff}}$ is the amount of COD removed in MFCs, 4 is the mole number of electrons generated per mole of oxygen, F is the Faraday constant (9,6485 C/mol electrons), V is the voltage output, and M_{O_2} is the molar mass of oxygen (32 g/mole).

Total experimental energy generation (W, joule) measured on R_{ext} during the MFC operational period (t) was calculated based on Eq. 3:

$$W = \int_0^t \frac{V^2}{R_{ext}} \mathrm{d}t \times \frac{R_{ext} + R_{in}}{R_{ext}} \tag{3}$$

Where $\int_0^t \frac{V^2}{R_{ext}} \mathrm{d}t$ was the actual energy generated over the time period (t), and V is voltage output measured over R_{ext}.

Results and Discussion

The Influence of MFC Configuration on Power Generation and COD Removal

The physical characteristics of the MFCs (i.e., the size of electrodes, electrode spacing, and the presence or absence of PEM) represent the MFC configuration. The modification of MFC configuration was aimed at reducing R_{in} and increasing power generation. Two types of MFC configurations were compared in this study: 2CMFCs and ML-2CMFCs. In the NO_3^-2CMFCs, the distance between electrodes was 10 cm. The R_{in} was 310 Ω and the ε_C was only 18% (Table 2). About 24% of power generated in 2CMFCs was consumed by the R_{in}. The power density (P_{Cat}) was 69 mW/m^2 (Table 3). In the NO_3^-ML-2CMFCs, the distance between electrodes was reduced to 5 cm, which led to 35% reduction of R_{in}. As a result, the ε_C increased to 19% and the P_{Cat} increased to 230 mW/m^2. About 17% of the power generated being consumed by the R_{in}. The difference between 2CMFCs and

Table 2 The major electrochemical characteristics of the MFCs tested.

List number	MFCs	COD removal (%)	Internal resistance (R_{in}, Ω)[a]	External resistance (R_{ext}, Ω)	Voltage output (mV)	Total theoretical energy generation ($W_{theo.}$, J)[b]	Total experimental energy generation (W, J)[c]	Coulombic efficiency (ε_C, %)[d]	Ratio of power consumed on R_{in} (%)[e]
A	NO_3^--2CMFCs	67±24	310±65	1,000	199±15	209±115	38±0.5	18±6	24±1
B	AF-2CMFCs	89±1	155±5	1,000	584±20	367±158	176±0.5	43±25	14±0.5
C	NO_3^--ML-2CMFCs	88±7	200±50	1,000	265±30	386±94	74±9	19±9	17±12
D	GAC-ML-2CMFCs	88±2	162±24	1,000	431±27	457±110	292±19	64±21	14±6

[a] The average R_{in} was measured by the polarization curve approach

[b] Total theoretical energy generation (W_{theo}) was calculated from Eq. 2

[c] Total experimental energy generation (W) was calculated from Eq. 3. The integral time was the duration period

[d] Coulombic efficiency (ε_C) was calculated from columns W and W_{theo}

[e] Ratio of power consumed on R_{in} (percent) was calculated from columns R_{in}, R_{ext}, and W

Table 3 The acclimation/duration periods and current/power densities of the MFCs tested.

List number	MFCs	Acclimation period (h)	Duration period (h)	Current density (I_{Cat}, mA/m^2)	Power density[a] (P_{Cat}, mW/m^2)
A	NO_3^--2CMFCs	500±100	100±40	340±8	69±17
B	AF-2CMFCs	430±100	300±50	974±1	1,292±69
C	NO_3^--ML-2CMFCs	50±10	160±50	599±78	230±37
D	GAC-ML-2CMFCs	150±20	400±30	784±33	557±50

[a] The maximum power output obtained by polarization curve at the highest voltage output when R_{ext} was equal to R_{in}

ML-2CMFCs indicated that removing PEM and shortening the distance between anodes and cathodes significantly reduced R_{in} and increased power generation. However, the ε_C of NO_3^-ML-2CMFCs were still lower than the highest reported value (90%, Logan et al. [28], Kim et al. [29]). There are two reasons for the low ε_C in this study. First, although the distance between electrodes were reduced to 5 cm in ML-MFCs, it was still 20% longer than those in the study of Logan et al. [28], which caused higher energy loss inside MFCs. Second, the R_{ext} (1,000 Ω) used in this study was much higher than the R_{ext} (10 Ω) used by Kim et al. [29], which led to a higher resistance of electron transfer from anode to cathode.

In addition, NO_3^-ML-2CMFCs exhibited a shorter acclimation period (50 h) and a longer duration period (160 h) than NO_3^-2CMFCs (Table 3), indicating that bacteria grew better in the anode chamber of NO_3^-ML-2CMFCs. NO_3^-ML-2CMFCs also had a higher I_{Cat} (599 mA/m^2) and higher P_{Cat} (230 mW/m^2) than those (340 mA/m^2 and 69 mW/m^2) of NO_3^-2CMFCs, which could be explained by the lower anode potential (−464 mV) in NO_3^-ML-MFCs than that (−352 mV) in NO_3^-2CMFCs (Table 4). These results were in agreement with the previous findings that anaerobic electrogenic bacteria grow faster at negative anode potentials in the vicinity of −400 mV [28].

Since the chemical energy stored in organic substrates is converted into electrical energy by anaerobic electrogenic bacteria in MFCs, a correlation between COD removal and power generation should be established. The results showed that COD removal efficiencies (88–89%) of ML-2CMFCs and AF-2CMFCs were higher than that (67%) of NO_3^-2CMFCs (Table 2), and their P_{Cat} values were three to 19 times as that of NO_3^-2CMFCs (Table 3). The high R_{in} (310 Ω) of NO_3^-2CMFCs caused the low efficiencies of COD removal and ε_C. The calculation of the total energy generation during the MFC operational period showed that the experimental energy generation (W, 38 J) was much lower than the theoretical energy generation (W_{theo}, 209 J) in NO_3^-2CMFCs (Table 2), which demonstrated the high R_{in} reduced the electron transfer capability and inhibited the microbial activity of decomposing organic substrates [2].

Table 4 Comparisons of anode potential in acclimation period and duration period.

List number	MFCs	Anode potential in acclimation period	Anode potential in duration period
A	NO_3^--2CMFCs	−55±36	−352±102
B	AF-2CMFCs	83±45	−466±16
C	NO_3^--ML-2CMFCs	104±40	−464±3
D	GAC-ML-2CMFCs	−220±40	−467±7

The Influence of Electrolyte Solutions on Power Generation and COD Removal

A variety of electrolyte solutions were examined for their effects on MFC performance. The NO_3^-2CMFCs (acetate in anode chamber and nitrate in cathode chamber) and the AF-2CMFCs (anaerobic treated wastewater in anode chamber and ferricyanide in cathode chamber) were compared. The results showed that the R_{in} (155 Ω) of AF-2CMFCs was much lower than that (310 Ω) of NO_3^-2CMFCs (Table 2). Correspondingly, the voltage generation (584 mV) of AF-2CMFCs was almost three times as that (199 mV) of NO_3^-2CMFC, and the COD removal (89%) was 25% higher than that (67%) of NO_3^-2CMFCs, indicating that more chemical energy stored in anaerobic treated wastewater was converted into electricity. There were two reasons for the better performance of AF-2CMFCs. First, the anaerobic treated wastewater used in AF-2CMFCs contains multiple substrates (i.e., acetate, ethanol, and propionate) for bacteria to utilize. The anaerobic treated wastewater also contains diverse anaerobic bacteria, which have synergetic interactions to conduct electrochemical reactions [30, 31] and possess a higher capability of electron generation in the anode chamber. Second, ferricyanide was more efficient as the electron acceptor than NO_3^-. The biological standard potential of ferricyanide (+771 mV, Fe^{3+}/Fe^{2+}) is higher than that of NO_3^- (+421 mV, NO_3^-/NO_2^-) [32], which led to a higher ORP value (281 mV) in the cathode chamber of AF-2CMFCs than that (−29 mV) of NO_3^-2CMFCs (Fig. 2a). An electron acceptor solution with a higher ORP value is helpful for electron transfer from anode to cathode. Nitrite (NO_2^-) was detected as the reductive product in the cathode of NO_3^-2CMFCs, but there was no clear relationship between nitrate/nitrite concentrations with power generation.

The Influence of MFC Configuration and Electrolyte Solutions on Acclimation Period and Duration Period

The MFC configurations had significant effects on the acclimation period and duration period. In this study, the acclimation period was termed as the time needed to achieve the peak voltage generation after inoculation, and the duration period was termed as the time length of the peak voltage in a MFC operational cycle. The desirable MFC operation should have short acclimation period and long duration period, which were clearly achieved by modifying MFC configurations. In term of the acclimation period, the NO_3^-2CMFCs required the longest acclimation period (500 h), while ML-2CMFCs (NO_3^-ML-2CMFCs and GAC-ML-2CMFCs) had much shorter acclimation periods (50–150 h; Table 3). Correspondingly, the pH of the cathode chamber in the NO_3^-2CMFCs was much higher than the rest of MFCs (Fig. 2a), indicating that less amounts of protons (H^+) were transferred from anode to cathode chambers in the NO_3^-2CMFCs. The results were in agreement with the previous findings that the biofilm formation on the PEM in 2CMFCs blocked the proton transfer from anode to cathode and subsequently delayed the acclimation [33]. In terms of the duration period, the NO_3^-2CMFCs only had 100 h of peak voltage output, while the ML-2CMFCs had much longer duration period with the peak voltage lasting for 400 h in GAC-ML-2CMFCs. This can be explained by the reduction of R_{in} from the 310 Ω in the NO_3^-2CMFCs to 162–200 Ω in the ML-2CMFCs (Table 2). Less amount of power was consumed inside the MFCs with lower R_{in} and thus leading to a longer duration period.

Besides MFC configurations, the electrolyte solutions also affected the acclimation and duration periods. In terms of anode solution, the ML-MFCs (NO_3^-ML-2CMFCs and GAC-ML-2CMFCs) fed with acetate had the acclimation periods of 50–150 h, while the

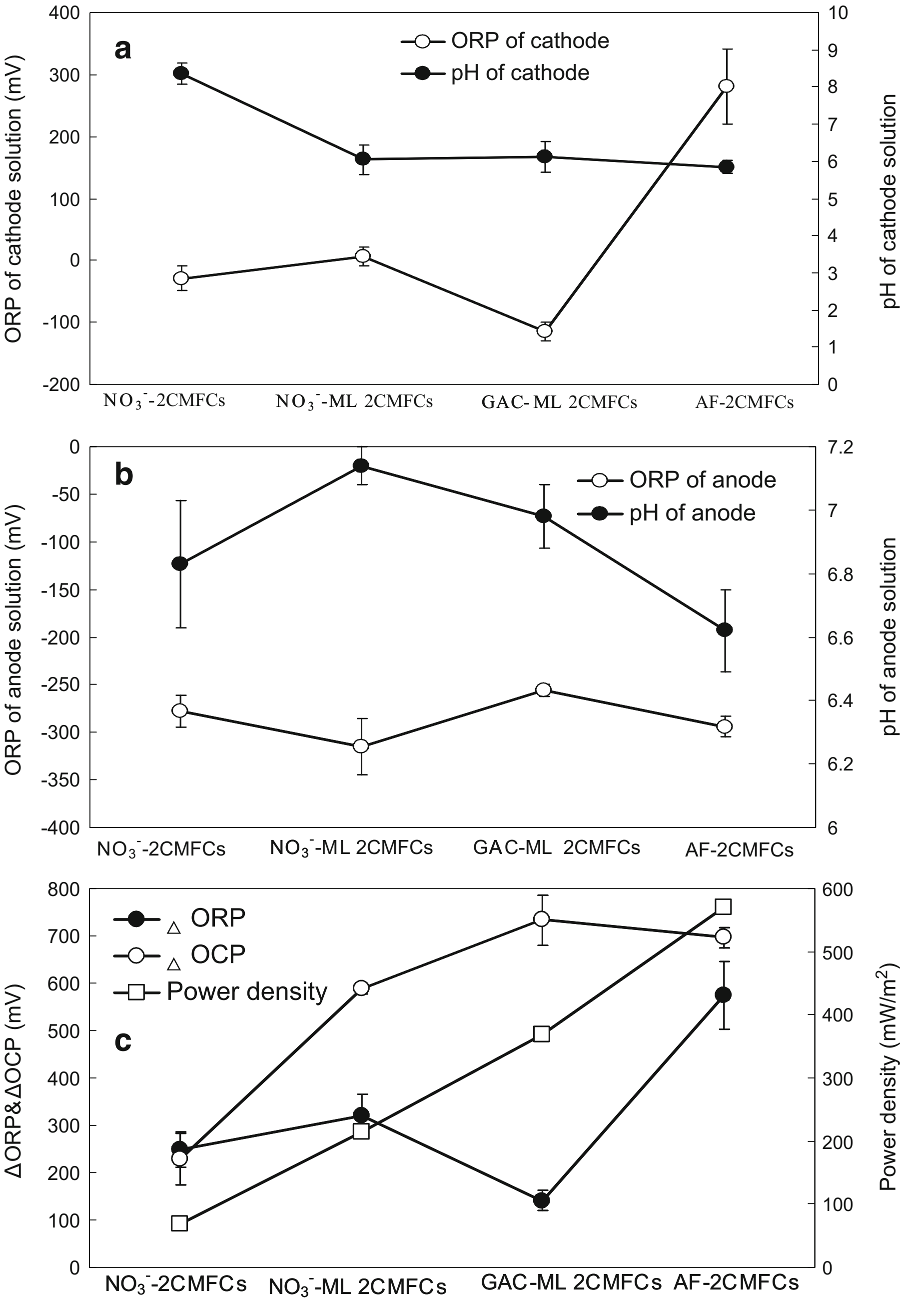

Fig. 2 The correlations of oxidation–reduction potential (*ORP*), pH, open circuit potential (*OCP*), and power density among four types of MFCs tested. **a** Correlation of ORP and pH in cathode. **b** Correlation of ORP and pH in anode. **c** Correlation of ORP difference between electrode solutions (ΔORP_{C-A}), OCP difference between anode and cathode (ΔOCP_{A-C}), and power generation

AF-2CMFCs fed with anaerobic treated wastewater had the acclimation period of 430 h (Table 3). There are two possible reasons for the long acclimation period in AF-2CMFCs. First, protons directly transferred from anode to cathode in ML-MFCs, while protons needed to transfer through PEM in AF-2CMFCs. After being operated for a period of time, the biofilm formation on the PEM reduced the proton transfer from anode to cathode in AF-2CMFCs [33]. Second, the anaerobic treated wastewater was taken from an anaerobic biofermentor, which had a low pH (<5.0). Electrogenic bacteria needed long time to get used to the low pH condition in the anode chamber, which resulted in a longer acclimation period. However, once the electrogenic bacteria became dominant after 430 h, they maintained the stable voltage generation for 350 h, the second longest duration period among the MFCs tested (Table 3). The synergistic interactions (e.g., the electron transfer through different biochemical reactions, the uptake of interspecies metabolites) among microorganisms in anaerobic wastewater prolonged the stable power generation in AF-2CMFCs [30]. In terms of cathode solutions, ferricyanide was used as the electron acceptor in AF-2CMFCs, and nitrate was used as the electron acceptor in the other three types of MFCs (NO_3^-2CMFCs, NO_3^-ML-2CMFCs, and GAC-ML-MFCs). AF-2CMFCs had a much longer duration period (300 h) than those (100–160 h) of NO_3^-2CMFCs and NO_3^-ML-2CMFCs. This can be explained by the high ORP (281 mV) in the cathode solution of AF-2CMFCs than in the other MFCs (Fig. 2a), which improved electron transfer from anode to cathode and prolonged the duration period.

The duration periods were directly correlated with I_{Cat} and P_{Cat} in MFCs. NO_3^-2CMFCs had the shortest duration period, and its I_{Cat} and P_{Cat} were the lowest among the MFC tested (Table 3). The I_{Cat} and P_{Cat} values of ML-2CMFCs steadily increased to 599–784 mA/m^2 and 230–557 mW/m^2 as the duration periods increased to 160–400 h. GAC-ML-2CMFCs and AF-2CMFCs had the longest duration periods (400 and 300 h), the highest I_{Cat} values (784 and 974 mA/m^2), and P_{Cat} values (557 and 1292 mW/m^2). The high surface area of GAC electrode significantly improved the electron transfer from bacterial cells to electrode surfaces. For AF-2CMFCs, diverse anaerobic bacteria in anaerobic treated wastewater thoroughly degrade the organic substrates and led to a longer duration period and a higher I_{Cat} value [30].

GAC as the Biofilm Carrier and Anode Material

Besides the configurations and electrolyte solutions, the anode material is also important for power generation in MFCs. Biofilms growing on anode have been found to play an important role to transfer electrons to anode surfaces [34]. A good anode material should have good conductivity and high compatibility for biofilm growth. Carbon cloth has been widely used as the electrode materials in MFCs [2, 28, 35]. In this study, GAC, a filtration packing material for water and wastewater treatment, was examined as a novel electrode material to improve biofilm formation and electron transfer in MFCs. The performance of carbon cloth and GAC was evaluated by comparing the power output and ε_{C} values. The results showed that the R_{in} (162 Ω) of the GAC-ML-2CMFCs was 19% lower than that (200 Ω) of NO_3^-ML-2CMFCs (Table 2). Correspondingly, the P_{Cat} value increased to 557 mW/m^2 and the ε_{C} value increased to 64%, more than three times as that of NO_3^-ML-2CMFCs (Tables 2 and 3). There are two main reasons for the improvement. First, GAC is a good conductive material with high surface area, which significantly improves bacterial adhesion and facilitates the electron transfer from bacterial cells to GAC surfaces. Second, the GAC-ML-2CMFCs had a relatively stable anode potential (−467 ~ −220 mV) during acclimation and duration periods compared with the large fluctuation of anode potential

(−464 ~ 104 mV) in the NO_3^-ML-2CMFCs (Table 4), which provided a suitable condition for electrogenic bacteria growth [36].

The Correlations of pH, OCP, and ORP in Anode and Cathode Chambers

In MFCs, the current is generated by the simultaneous transfer of protons (H^+) and electrons from anode to cathode. This process leads to the reduction of pH in the cathode and the difference of OCP between anode and cathode ($\Delta OCP_{A\text{-}C}$). The results showed that the pH of the cathode solution declined with the increase of the $\Delta OCP_{A\text{-}C}$ (Fig. 2a and c), which indicated that $\Delta OCP_{A\text{-}C}$ is the driving force for proton transfer. With the distance between anode and cathode electrodes being reduced and the electrode materials and electrolyte solutions being improved in the modified MFCs, the $\Delta OCP_{A\text{-}C}$ increased and the electric force was strengthened, which can be explained by Eqs. 4 and 5:

$$F = qE \tag{4}$$

$$E = \frac{U}{D} \tag{5}$$

where F (newton) is the electric force for the charge, q (coulomb) is the charge, E (volt per meter) is the electric field intensity wherein the charge is located, U (volt) is the electric field voltage, and D (meter) is the distance between anode and cathode electrodes. With a shorter D in the ML-MFCs than in 2C-MFCs, E was increased and a higher electric force (F) was achieved.

ORP of anode/cathode solutions are closely related with bacterial metabolic activities. Bacteria gain energy from respiration, and the electrons generated are transferred to the external electron acceptors via the respiratory chain [37, 38]. The energy is released when the electrons move from a lower ORP to a higher ORP. Thus, a greater ORP difference ($\Delta ORP_{C\text{-}A}$) between the anode and cathode solutions can increase power output [39]. The results showed that NO_3^-2CMFCs and AF-2CMFCs had higher $\Delta ORP_{C\text{-}A}$ than NO_3^-2CMFCs (Fig. 2c). Correspondingly, these two MFCs had higher P_{Cat} than NO_3^-2CMFCs (Table 3). One exception was GAC-ML-MFCs, in which the ORP of the anode solution was the highest among the MFCs tested (Fig. 2b), and thus its $\Delta ORP_{C\text{-}A}$ was the lowest, even though it still had a high P_{Cat}. There was one possible reason for the high ORP in the GAC-MFCs. The high surface area of GAC particles greatly improved the biofilm formation on the surfaces. The electrons generated from the reactions inside biofilms were directly transferred to the GAC particles, which prevented a decrease in the ORP of the anode solution.

Conclusions

The influences of MFC configurations (2CMFCs and ML-2CMFCs), anode solutions (acetate and anaerobic treated wastewater), cathode solutions (ferricyanide and nitrate), and electrode materials (carbon cloth and GAC) on the power generation of MFC were extensively investigated in this study. There are three major conclusions as follows. First, with the absence of the PEM and the short distance between anode and cathode electrodes, ML-2CMFCs had three to eight times increase in power density and 31% increase in COD removal efficiencies when compared with NO_3^-2CMFCs. ML-2CMFCs had the short acclimation period and the long duration period. Second, electrolyte solutions affected the capabilities of electron generation and acceptance. MFCs inoculated with anaerobic treated wastewater had the highest power density of 1,292 mW/m^2 and the long duration period of 300 h. Due to the migration of protons and electrons from anode to cathode, a high OCP in the cathode chamber was correlated with a low pH. Third, with a high surface area and a good conductivity, GAC substantially enhanced the power generation and COD removal in MFCs. GAC-ML-2CMFCs had a high power density of 557 mW/m^2 and COD removal of 88%, indicating the suitability of GAC to improve the MFC performance.

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