Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2023.29618

297 (2023) 131–137
June

Inhibiting the growth of microbes on the air-cathode in the microbial fuel cell by using an antimicrobial agent

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Received 21 February 2023; Accepted 20 May 2023

ABSTRACT

The accumulation of aerobic microorganisms on the air-cathode in a single-chamber-microbial fuel cell can suppress the activity of the catalyst, and result in the reduction of the efficiency of the system. Therefore, a suitable mitigation measure is essential to control the growth of biofouling on the cathode. This study aims to investigate the effectiveness of chloramphenicol as an anti-biofouling agent on Pt-coated cathode. Two microbial fuel cells (MFCs) were used where the first (MFC-1) was a conventional Pt-coated air-cathode as a control, while the other one (MFC-2) with antibiotic mixed Pt-coated air-cathode. Results demonstrate that the maximum generated cell potential of 0.82 V in the control MFC-1 was reduced to 0.62 V after three months of operation due to biofouling development on the Pt-coated air-cathode. Besides, the control system achieved 95% chemical oxygen demand (COD) removal after 5 d of hydraulic retention time (HRT). However, using the newly developed cathode in MFC-2, the system achieved a maximum cell potential of 0.85 V with 95% of COD removal by 10 d HRT. The maximum columbic efficiency (CE) achieved in control MFC-1 and MFC-2 were 8 and 12.5%, respectively. The increment in CE was due to the absence of cathodic aerobic biofilm as well as the removal of COD was solely accomplished by the anodic biofilm. This result shows that the application of the antibiotic coating on the cathode can suppress the growth of cathodic biofouling without interfering with the other bio-electrochemical properties (COD removal, power recovery, pH change) of the system.

Keywords: Aerobic anode; Anti-biofouling; Antibiotic cathode; Single-chamber-microbial fuel cell

1. Introduction

Microbial fuel cell (MFC) is a promising biotechnological technique to produce energy through biodegradable organic matter using microorganisms [1,2]. The mechanism of MFC is to break down organic materials and generate electricity using bacteria as a fuel source. After the oxidation of organic compounds which are electron donors, the anodic biofilm will receive the electron [3]. The main products of the oxidation reaction are carbon dioxide (CO_2), electrons, and protons. Proton is diffused to cathode by a proton exchange membrane (PEM), while electrons pass

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through an external electric circuit. At the cathode, oxygen is reduced to form water through the combination of electrons and diffused protons [3]. The oxidation–reduction reaction (ORR) is explained by Eqs. (1) and (2) [4].

Anodic reaction (oxidation):

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \quad (1)$$

Cathodic reaction (reduction):

$$6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \quad (2)$$

The air-cathode microbial fuel cell is the famous model of (SC-MFC) [5]. The general layout of SC-MFC is shown in Fig. 1.

Since the cathode is exposed directly to air, the required energy for the system is reduced [6]. Moreover, no internal resistance exists, thus, it can produce high power as compared to a double chamber [7]. When the distance between the cathode and anode is reduced, it resulted in reducing the distance of proton traveling [8], and in turn, it will increase the produced density by 67% [9,10].

One of the issues related to the SC-MFC is the biofouling of the cathode. It is a result of an accumulation of heterotrophic aerobic bacteria on the cathode surface, and negatively affects the produced power density [11]. Biofouling can block the access of protons and charged ions to cathode catalyst sites. In addition, it blocks hydroxide transport out of the cathode resulting in a pH imbalance within the electrolyte and catalyst layer [12]. Aerobic bacteria may also consume some parts of diffused oxygen and reduce the rate of oxygen reduction reaction on the cathode as well as increase the internal resistance [13]. Substrate competition of anodic and cathodic biofilms is another drawback of biofilm formation on the cathode. As a result, columbic efficiency drops, and power generation reduces [14].

Incorporation of an anti-microbial agent in the cathode of a microbial fuel cell is one of the biofouling mitigation strategies [9]. Enrofloxacin (ENR) was tried as an antimicrobial agent. The cathode was treated with enrofloxacin to suppress microbial growth. The result shows the biomass quantity decreased in the treated cathode compared with the control after 91 d. In addition to that, the dissolved oxygen (DO) near the cathode was higher in the treated one if less biofilm consumed compared with the control which has more biofilm [7,15]. From cycle 4 to cycle 58 (91 d) the performance reduces by 33.7 mV compared with 84.7 mV in control. The maximum power density of the treated cathode is 1,069.7 ± 10.5 mW/m^2 while 841.2 ± 14.0 mW/m^2 for the control. The result indicates the stability of the cathode performance compared with the control.

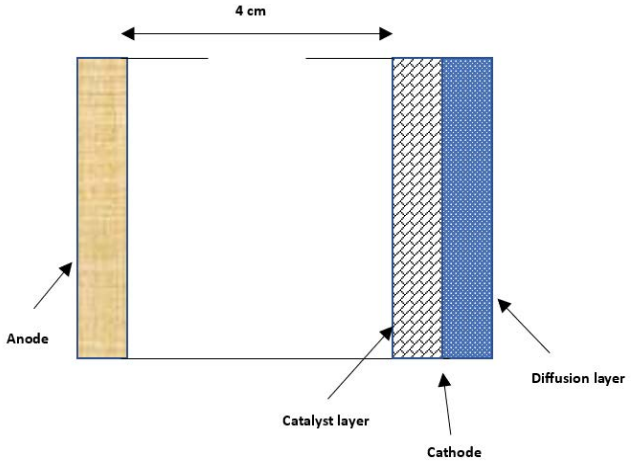

Fig. 1. General layout of air-cathode SC-MFC.

The main objective of this study is to investigate the effectiveness of antimicrobial agents in biofouling mitigation. Chloramphenicol is used in this study. A cathode of MFC is treated with chloramphenicol, while another reactor will be considered as a control. Both reactors will be run for 115 d. Different tests and measurements were done for the reactors to compare the performance and stability of the reactor.

2. Experiment section

2.1. MFC experimental set-up

Air-cathode single-chamber microbial fuel cell (SC-MFC) was used in this study. Two similar MFC experimental setups were used where the first set-up was considered as the control while the second set-up was considered as the modified setup. The cathode of the control setup was free of any antibiotic while the cathode of the modified setup was incorporated with antibiotic (chloramphenicol). The reactor was made up of an acrylic cylinder tube with a diameter of 9 cm and a length of 7 cm glued to two acrylic sheets as holders. Carbone fiber brush (2.8 cm of outer diameter and 12.5 cm of length) was used as the anode where a titanium wire was inserted as an electron collector. The air-cathode of the control system was made of carbon cloth coated with carbon black and polytetrafluoroethylene (PTFE) as the gas diffusion layer on the air side. In addition, an 0.5 mg/cm^2 of Pt was used as the catalyst layer on the electrolyte side with an active surface area of about 63 cm^2. Two strips of titanium sheet were attached to the cathode serving as the current collectors. In case of modified SC-MFC, a similar cathode as the control system was used besides incorporating the antibiotic (chloramphenicol) to the catalyst layer. The anode and cathode were connected with a 1,200 Ω external resistance (R_{ext}). The anode was inserted from one side of the reactor (Fig. 2a) and the cathode was placed at the other side of the reactor (Fig. 2b) providing a 2-cm distance between the electrodes. Rubber gaskets were used to make a watertight seal in the SC-MFC. The SC-MFC was bolted together using two acrylic sheets on each side of the reactor as shown in Fig. 2. The SC-MFC had an active volume of 350 mL after assembly.

2.2. Coating procedure

The incorporation method was conducted by dissolving 180 mg of chloramphenicol in 45 mL of methanol as the organic solvent. 4.5 mL of the prepared solution was then dispersed evenly over the surface area of the cathode catalyst layer followed by drying for 10 min at 80°C.

(a) (b) (c)

Fig. 2. Schematic of the SC-MFC set-up (a) air-cathode view, (b) anode view, and (c) side view of the SC-MFC.

The process was repeated until all the solution was applied on the cathode [16].

2.3. Medium

The SC-MFC was inoculated (50% v/v) with a pre-acclimated mixed culture of exo-electrogenic microorganisms from a functioning and enriched MFC system for three months. The anode chamber of the SC-MFC was fed with a media consisting of real domestic wastewater collected from a wastewater treatment plant (Haya WWTP, Oman) and 2 g/L of sodium acetate to have sufficient carbon nutrients for biofilm growth and anodic biocatalytic activity. Electrolyte refreshment was carried out every 5 d.

2.4. Analyses and calculations

2.4.1. Electrochemical analyses for the major parameters

The voltage (V) of each reactor across the external resistance was measured at 30 min intervals using a multimeter (Omega, USA). Current density (I_D)was calculated using Eq. (3), which was normalized by the cathode surface area (A_{cat}). R_{ext} is the external energy.

$$I_D = \frac{V}{R_{ext} \times A_{cat}} \tag{3}$$

$$P_D = \frac{V}{I_D} \tag{4}$$

The polarization and power analyses were performed at different stages of the experiments to monitor the effect of biofouling on the SC-MFC performance by applying the linear sweep voltammetry (Squidstat Plus 1132, Admiral Instruments, USA) at a scan rate of 5 mV/s. Power density (P_D) was calculated using Eq. (4) which was normalized by the cathode surface area (A_{cat}). The linear sweep voltammetry tests were performed in open circuit voltage (OCV) status of the system under a two-electrode configuration (anode as the working electrode and cathode as the counter and reference electrode) at different cycles of the SC-MFC. Anolyte samples were taken at the beginning chemical oxygen demand (COD_{in}) and at the end of each cycle (COD_{out}) to analyze COD using the standard method as shown in Eq. (5).

$$COD_{removal(\%)} = \frac{COD_{in} - COD_{out}}{COD_{in}} \times 100 \tag{5}$$

Columbic efficiency (CE) was calculated for each cycle using Eq. (6).

$$CE = \frac{8\int_{t_1}^{t_2} Idt}{Fv\Delta COD} \tag{6}$$

where t_1 and t_2 were the initial time and final time of each cycle, respectively, I is the current, F is Faraday's constant, v represented the volume of the anode, and ΔCOD is the changes of COD over a cycle [16]. Electrical conductivities (EC) and pH of the media in the anode chamber were monitored daily using benchtop EC and pH meter (HI5521, Hanna, USA).

3.4.2. Release of antibiotic

The release of antibiotics from the modified cathode to the electrolyte after each of the first three sequent cycles was measured by using a spectrophotometer. To establish the standard curve, 0.1 g of the chloramphenicol solution was mixed with glacial acetic and distilled water and added to different known concentrations of the samples. The absorbance of all samples was measured at λ_{max} = 440 nm and the calibration curve was extracted. The release of chloramphenicol to the SC-MFC electrolyte was measured by adding 2 mL of anode samples to the mixture of glacial acetic and distilled water by using the developed standard curve [17].

3.4.3. Characterization of biofouling

The morphology of the biofilms formed on both the catalyst layer (electrolyte side) and gas diffusion layer (air side) of the air-cathode was analyzed by scanning electron microscopy (SEM). A 2 cm^2 × 2 cm^2 piece of the electrode was cut from the cathode after three months of operation to analyze the morphology of the biofilms formed on

catalyst layers. The biofilms formed on both sides of the cathode were fixed initially by using glutaraldehyde and osmium. The samples were then dehydrated using different concentrations of ethanol (5%, 10%, 30%, 50%, 80% and 100%) for 10 min. The samples were then dried in an automatic critical point dryer, coated with gold and observed by SEM.

3. Results and discussions

3.1. Cell potential generation during biofilm enrichment period

Two reactors were run in batch mode using the same external resistance of 1.2 KΩ. The volume of the two reactors was 368 and 355 mL, for MFC-1 and MFC-2, respectively. 2 g of sodium acetate was used as a substrate for microbes in each liter of electrolyte, which was prepared by mixing 50% of raw wastewater with 50% of effluents of the enriched MFC as seeding microbes. Fig. 3 shows the generated voltage and current in MFC-1. On the first day of operation, the results showed that the generated voltage was dropped slightly due to bacterial adaptation to the new substrates and no biofilm attachment on the anode electrode occurred. However, within a very short period, the cell voltage started to increase and reached a maximum value of 0.66 V during the second day of operation. Thereafter, it started to drop gradually due to the reduction of substrate concentration. The substrate concentration was refreshed in the reactor every 5 d. At each refreshment of a substrate, and due to the increase in biofilm density over the anode electrode, the stable maximum cell voltage and current were increased gradually and reached the maximum value of 0.82 V and 0.68 mA, respectively at the 9th cycle (i.e., after 41 d of operation). However, the maximum stable voltage generation was reduced from 0.82 V to 0.61 V for 3 months of operation. This observation is most likely due to the adverse effect of the development of the aerobic cathodic biofouling layer. As mentioned earlier, MFC-2 was operated with the same condition similar to MFC-1 to enrich the anodic biofilm during the first two months. The Pt coated cathode in MFC-2 was replaced with a newly developed antibiotic (chloramphenicol) coated Pt-cathode. Fig. 4 shows that the voltage and current generation in MFC-2 due to suppressed growth of cathodic aerobic biofilm and the presence of anodic biofilm only, the COD removal was slow. Moreover, the antimicrobial agents might release from the newly developed cathode which negatively affects the performance of anodic biofilm

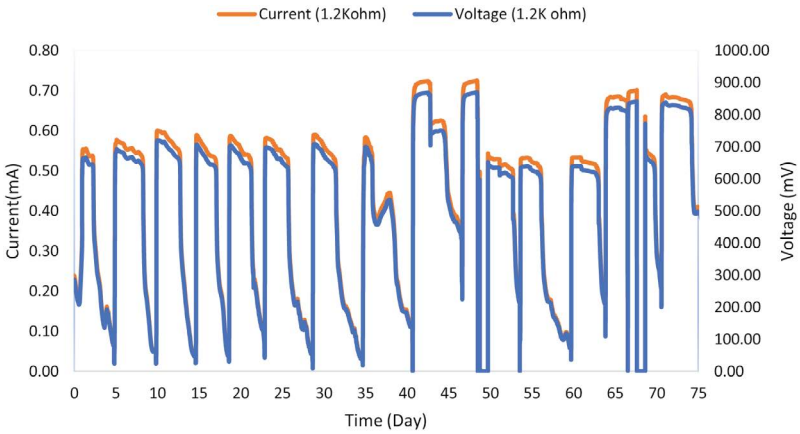

Fig. 3. Voltage and current generation in MFC-1 (control).

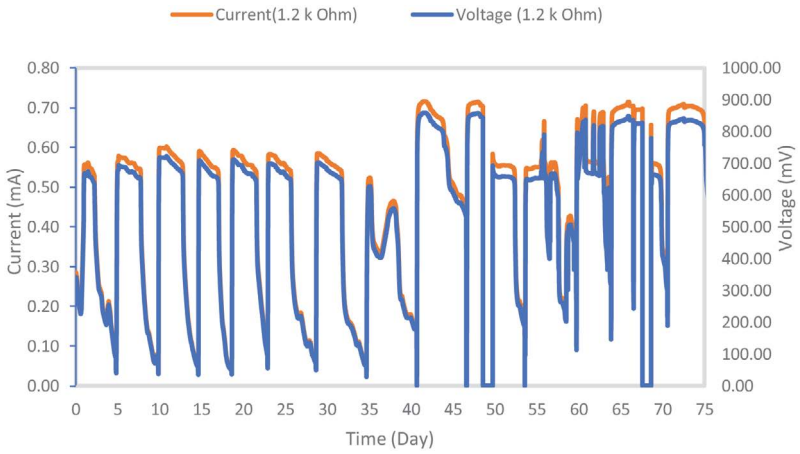

Fig. 4. Voltage and current generation in MFC-2 (with antibiotic coated cathode).

and subsequently the COD removal. This observation resulted in increasing the duration of substrate refreshment to 10 d. The maximum cell voltage generated was 0.74 V in the first cycle, and then it decreased gradually to 0.13 V for a 10-d period. After 3 cycles, the reactor was adapted with a new cathode and generated the maximum cell voltage up to 0.85 V, which is approximately equal to the maximum stable cell potential generation without applying the antibiotic coating. These results showed that antibiotic coating did not affect the stable voltage generation in MFC-2, however, it only affects the COD removal rate and substrate refreshment duration. By applying chloramphenicol on the cathode, the system becomes more stable in terms of voltage generation as compared to that without chloramphenicol coating.

3.2. Electrochemical performance of MFC

3.2.1. Polarization and power density

A linear voltammetry test was carried out to calculate the voltage polarization and power density of the reactors. It was conducted by using a two-electrode configuration for the reactors under OCV conditions at different cycles. The test was done for MFC-1 at 42 and 115 d. The maximum OCV was 0.84 V on day 42, which indicated the enrichment of anodic biofilm. However, after three months of operation (at day 115), the maximum OCV decreased by 6% and reached 0.79 V, which indicated the growth of aerobic biofouling on the cathode that negatively affected cell potential generation. A similar voltage polarization was conducted for MFC-2 on day 115. The result showed that the maximum OCV was 0.83 V which is equal to the maximum OCV generated by MFC-1. Therefore, the data demonstrated that dispersed antibiotics on the catalyst layer of the air-cathode can inhibit the growth of aerobic biofilm as well as maintain the stable performance of voltage generation.

The maximums power density achieved by MFC-1 was 0.654 W/m^2 at day 42 during the enrichment period when the cathode was free of aerobic biofilm. As a result of cathodic biofouling, the power density decreased to 0.602 W/m^2 on day 115. However, at day 115, the MFC-2 generated less power as compared to MFC-1 by 39%. Coating the cathode with antibiotics suppressed the growth of aerobic biofilm on it and reduced the active sites of catalysts on the cathode by growing a layer of antibiotics on catalysts particles. In addition, the released antibiotics from the cathode could hinder the performance of anodic biofilm, which ultimately reduced t power generation. A similar observation was reported by Liu et al. [16].

3.2.2. Columbic efficiency

The CE represents the fraction of substrate solely utilized to harvest the electrical current as compared to the total amount of COD oxidized in the MFC reactor. In MFC-1, the CE started to increase from 4.5% (in the first cycle) and reached the maximum value of 8% (in cycle 9). The increase of CE during the first 9 cycles indicated that the growth of electrogenic biofilm was presented on the anode. After three months of operation (i.e., at cycle 23), the CE values started to reduce and reached 2.5%. Such reduction of CE indicated the growth of aerobic cathodic biofilm which utilized the major fraction of substrates (COD) without producing electricity. However, by applying chloramphenicol antibiotic on the cathode in MFC-2, the CE values increased to 12.5% in the first cycle and then reached 10%. The increase of CE in MFC-2 happened due to no biofilm on the cathode, which indicated that the major fraction of COD was removed by the anodic electrogenic microbes.

3.3. COD removal efficiency

COD was measured by the reaction of titrant ferrous ammonium sulfate ($Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$) with the compound of potassium dichromate ($K_2Cr_2O_7$) and electrolyte. The COD was measured at the beginning and end of each cycle. Results demonstrated that the initial value of COD was higher than 1,000 mg/L and it was dropped to 200 mg/L by the consumption of biofilm to generate protons and electrons in all cycles. The maximum COD removal in MFC-1 was 413 mg/L after 17 cycles. The system achieved 95% removal of the COD in 5 d, which was shown by the linear trend line shown in Fig. 5a. After coating the cathode with antibiotic, the COD removal was reduced to 107 mg/L by 10 d hydraulic retention time (Fig. 5b). This result was expected since the COD was oxidized by only one group of microbes on the anode and the released antibiotic might affect the microbial activity on the anode [17].

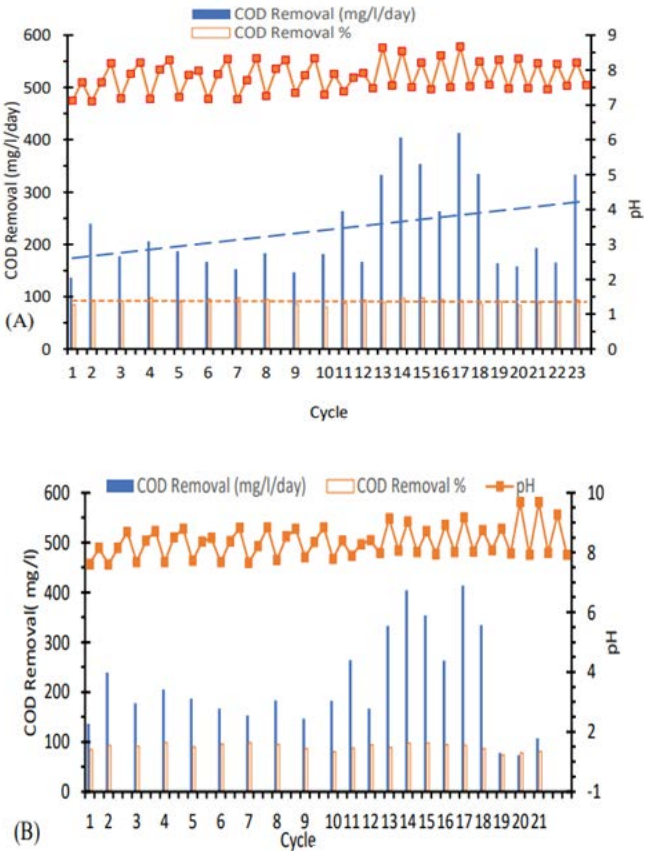

Fig. 5. Chemical oxygen demand removal efficiency of the (A) control MFC and (B) MFC-2 with antibiotic coated cathode.

pH was measured at the beginning and end of each refreshment cycle. pH should be in the range of 6.5–7.5 at the beginning of the cycle which is a required range for bacterial growth. Oxidation–reduction reactions occurred near the cathode, where biofouling prevents OH ion to transport out of the cathode that led to raising pH value to more than 8. However, in the case of MFC-2 where the cathode was coated and inhibit biofouling, the rate of reaction was high and produce more OH ions (Yuan et al., 2013). Therefore, pH was increased to 9 at the end of each cycle as shown in Fig. 5b.

3.4. Releasing of antimicrobial agent on the system

A spectrophotometer (Thermo Fisher Scientific/EVO300 PC) was used to quantify the amount of released chloramphenicol from the newly developed antibiotic coated cathode to the electrolyte of MFC-2. It was clear that the released amount of chloramphenicol was high in the first cycle with a value of 0.276 ppm. However, in the third cycle, it was reduced to 0.137 ppm. A similar coating method was used by Liu et al. [16] and the same result was detected with enrofloxacin as an antimicrobial agent. As the antibiotic solution was adsorbed on the surface of the catalyst layer, the release was expected. Following another procedure for coating such as mixing the antibiotic with a binder of air-cathode could prevent realizing of the antibiotic in the electrolyte.

3.5. SEM analysis of cathodic biofilm without antibiotic application

After three months of operation, the cathode of MFC-1 was separated and analyzed using SEM. Results showed that the cathode was covered with a thick layer of aerobic biofilm. The cathode was pretreated through a few steps to fix the biofilm and dehydrated in a critical point drier.

SEM results showed that a thicker layer of biofilm development existed on the electrolyte side, whereas a thin

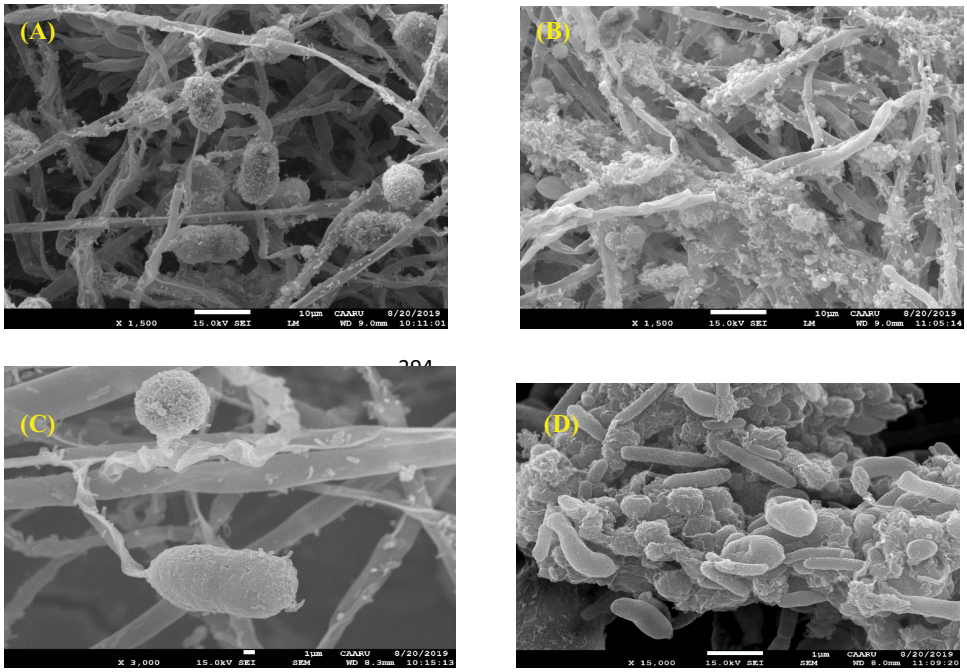

Fig. 6. Scanning electron microscopy image of the air-cathode from the (a,b) outer side and (c,d) inner side.

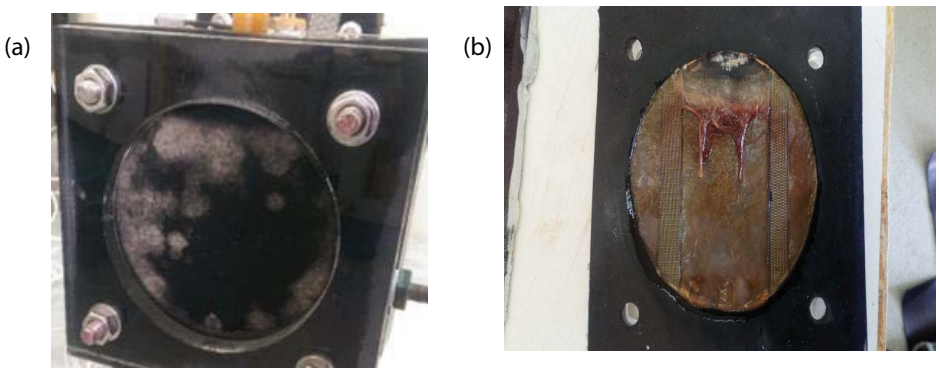

Fig. 7. Images of the air-cathode from (a) outer side and (b) inner side.

layer of biofilm spotted at a few points of the electrode in the open-air side was developed as in Fig. 6.

Diversified types of bacteria were grown in the air-cathode of the MFC-1 due to the availability of nutrients and air. Exposing the cathode to wastewater for a long time provided a good environment for microbes to grow and enhanced the accumulation of biofilm on the cathode that resulted in biofilm thickness increasing (Fig. 7). As the air-cathode contained pores that allowed oxygen and nutrients to pass through both sides, the biofilm could be seen on both sides. Because of limitations of nutrients on the air side, the biofilm thickness and diversity were less as compared to the electrolyte side as shown in Fig. 7.

4. Conclusion

MFC is a bio-electrochemical system, which offers wastewater treatment while producing clean electrical energy. However, the cathodic biofouling issue affects the performance of MFC as well as its stability. Results showed that the stable cell potential in MFC-1 was reduced from 0.82 to 0.61 V during three months of operation due to cathode biofouling. The control system achieved 95% COD removal by 5 d hydraulic retention time (HRT). The newly developed antibiotic coated cathode (MFC-2) generated a stable cell potential of 0.85 V with 95% COD removal by 10 d HRT. Moreover, it achieved 12.5% of CE as compared to 8% CE in MFC-1. The higher CE and HRT of MFC-2 demonstrated better bioelectricity recovery without having biofouling of the Pt catalysts on the cathode. The results were promising and demonstrated that the antibiotic coating on Pt cathode could achieve a considerable inhibition of aerobic biofouling on the cathode, and hence, improve the performance and stability of the MFC system.

However, the release of antibiotic chemicals from the cathode to the electrolyte could negatively affect the anodic biofilm performance in the long-term operation. Therefore, more studies were needed for long-term investigation with this antibiotic coated cathode or exploring the application of other antibiotics on cathode to make the system cost-effective and eco-friendly.

The main issue for large-scale SC-MFC implementation was biofouling of Pt-coated cathode that negatively affects the MFC performance. Hence, to make the system cost-effective and eco-friendly, further studies are essential for long-term investigation of the current antibiotic coated cathode or exploring the application of other antibiotics on the cathode to suppress the growth of biofilm on air-breathing cathode.

Exploring the replacement of Pt catalysts with activated carbon on the air-cathode to investigate the effect of the activated carbon layer on biofouling is recommended. In addition, exploring diversified types of coating methods to maximize the active site of the catalyst layer could highly enhance the rate of biochemical reactions. Moreover, the release of antibiotics in electrolytes could negatively affect the exaelectron biofilm on the anode. Therefore, the incorporation of antimicrobial agent on the cathode could use a binder chemical to achieve a better and more effective coating. By combing two or three above-mentioned methods, the best results could be achieved in biofouling mitigation.

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