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Effect of Pretreatment of Anodic Inoculum and Presence of Algae on the Performance of Microbial Fuel

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Abstract

The performance of two identical dual chambered microbial fuel cells (MFCs) was investigated by inoculating mixed anaerobic sludge with sonication and heat pretreatment. The MFC with sonicated inoculum produced more power (3.79 W/m^3) and chemical oxygen demand (COD) removal (83.24 %), as compared to the MFC inoculated with heat pretreated sludge in which corresponding values were 2.99 W/m^3 and 69.07 %, respectively. MFCs with earthen pot anode chamber were installed in the oxidation pond having mixed algal growth in cathodic chamber (MFC-AT). This MFC-AT produced more power (4.5 W/m^3) than the MFC having only tap water in the cathodic chamber (MFC-TW) which produced power of 3 W/m^3. The COD removal efficiency was 90 % in MFC-AT and 85.3 % in MFC-TW. With algal growth in the anode chamber, power production was increased to 4.7 W/m^3 and 3.3 W/m^3 and COD removal decreased to 85.3 % and 83.4 % in MFC-AT and MFC-TW, respectively. Using *Spirulina* in the cathode chamber power production of 3.8 W/m^3 and the COD removal of 85.5 % was observed in MFC-AT. While growing Tilapia fish in cathodic chamber, the specific growth rate of fish was observed to be higher in the presence of MFC.

Keywords: Microbial fuel cell. Sonication. Oxidation pond. Algal growth. Power density. Aquaculture.

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Introduction

Microbial fuel cell (MFC) is a device that converts chemical energy directly to the electrical energy with the aid of catalytic reaction of microorganisms (Allen & Bennetto, 1993). MFC provides new opportunity for the sustainable production of energy in the form of direct electricity from biodegradable compounds while simultaneously treating wastewater (Logan, 2005; Min et al., 2005). The substrate is oxidized in the anode chamber producing carbon dioxide, protons and electrons (Rabaey & Verstraete, 2005). These electrons and the protons meet at the cathode, via the external electrical circuit and through the proton exchange membrane (PEM), respectively. In the cathodic chamber, an oxygen is being reduced producing water and generating electricity (Jadhav & Ghangrekar, 2008).

Anaerobic sewage sludge is a good source of fermentative bacteria, methanogens, sulphate reducers (Kim et al., 2005) and electrochemically active strains of bacteria (Logan & Regan, 2006). There is a possibility of non-electricity producing bacteria occupying space on the electrode during the initial inoculation, preventing efficient power generation in the MFC (Kim et al., 2005). Sonication is the more effective method compared to other pretreatments to enhance electrogenic microbial growth from mixed anaerobic culture and to restrict methanogenesis process by encouraging electrogenesis as the end process in the metabolic pathway (Min et al., 2005, More & Ghangrekar, 2009). Gram-negative bacteria were reported to survive in the sewage samples even after ultrasonication treatment (Kesari & Behari, 2008). Ultrasonication can suppress the activity of gram-positive methanogens by retaining gram-negative bacteria. Majority of the methanogens are reported to be gram-positive (Zeikus, 1977). Heat treatment is another alternative pretreatment method to suppress the methanogens, which is generally used for screening hydrogen producing bacteria.

Algal biomass like *Spirulina maxima*, *Chlamydomonas reinhardtii* and *Pseudokirchneriella subcapitata* (green algae) were used as a substrate for anaerobic digestion (Schamphelaire & Verstraete, 2009). Bioelectricity production using algae in MFCs is useful as a low temperature method of power generation, but it needs to be further improved in order to make it competitive with alternative energy technologies (Velasquez-Orta et al., 2009). Some algae studied are *Synechococcus sp*. (Yagishita et al., 1993) and *Anabaena variabilis* (Tanaka et al., 1985) in biofuel cells. *Phylum Cyanophyta* was used to produce electrons by a photosynthetic reaction under light (Chiao et al., 2006) and *Chlorella vulgaris* (microalgae) and *Ulva lactuca* (macroalgae) were used by Velasquez-Orta et al. (2009). A novel photosynthetic microbial cathodic half cell, employing the microalgae species *Chlorella vulgaris* as the direct electron acceptor was developed earlier (Powell et al., 2009). In a recent study, the photosynthetic microorganism *Spirulina platensis* was used for electricity generation in both photosynthetic MFC and MFC under the dark and light conditions (Fu et al., 2009).

Sewage can be treated effectively using MFC recovering power and the treated sewage can be used for aquaculture to enhance the revenue further. A major factor in sewage fed aquaculture is selection of fish species according to the load of nutrients in wastewater. The species suitable for wastewater aquaculture are: Indian carps, Israeli carps, Chinese carps, silver carps, bighead carps, grass carps, common carps, hybrid buffalo, catfish, largemouth bass, tilapia, freshwater prawn, etc. (Moav et al., 2007).

This study was aimed to investigate the performance of MFC, using easily available mixed anaerobic sludge as an inoculum subjected to sonication and heat pretreatment. In addition, the aim was to quantify the growth of fish in the wastewater treated by MFC. Studies were conducted to evaluate the suitability of using oxidation pond, exposed to natural environment, as a cathode chamber for MFC, to rely on photosynthetic oxygen as final electron acceptor. Performance of MFC has been evaluated in terms of wastewater treatment efficiency and power output of MFC.

Materials and Methods

MFC- construction

Two chambered MFC made up of poly-acrylic plastic with identical sizes were used for experiments on the effect of sonication and heat pretreatment on the performance of MFC. The working volumes of anode and cathode chambers of these MFCs were 1310 mL each and they were separated with proton exchange membrane (PEM) having an area of 25 cm^2 (0.007 inches thickness, PEM, Nafion ® 117, Aldrich). Anodes and cathodes were graphite rods in roll form having a surface area of 59.73 cm^2 and 182.01 cm^2, respectively, in each MFC. The electrodes were connected externally with copper wire through a constant external resistance of 50 Ω throughout the experiment.

Two identical earthen-pot MFCs were used for the experiments to study the effect of growth of algae in cathode chamber. The working volume of earthen pot used as anode chamber was 400 mL. Two glass tanks of size 60 cm x 30 cm x 30 cm were used as the cathodic chamber. Stainless steel (SS) mesh having projected surface area of 190 cm^2 was used as anode and two graphite plates having total surface area of 231 cm^2 were used as cathode. The SS mesh anode was wrapped inside the earthen pot whereas the graphite plate cathodes were placed outside the pot, touching the walls of the pot. Wall of the earthen pot itself was used as PEM. Electrodes were connected externally through concealed copper wire through external resistance of 100 Ω. Two poly-acrylic plastic tanks of size 60 cm x 30 cm x 30 cm were used for Tilapia fish culture. In one of these tanks, an earthen-pot MFC was used as the anode and it was covered with paraffin film to make the anode chamber anaerobic. So this tank was used as the cathode for the MFC as well as fish rearing tank.

MFC Inoculation and operation

Synthetic wastewater with sucrose as a carbon source having chemical oxygen demand (COD) of around 500 mg/L and pH of 7.91 to 8.15 was used throughout the sonication and heat pretreatment experiments as suggested by Ghangrekar et al. (2005). Mixed anaerobic sludge used for the inoculation was collected from septic tank bottom. The volatile suspended solids (VSS) concentration in the sludge was 33.6 g/L. The sludge loading rate to the anode chamber was maintained by 0.25 kg COD/kg VSS.day with influent COD of 500 mg/L and hydraulic retention time (HRT) of 12 h. Sonication pretreatment (40 kHz, 5 min) was given to the sludge before adding to the anodic chamber of MFC-1. Heat pretreatment was given to the sludge (100 °C for 15 minutes) and added to the anodic chamber of the MFC-2 (More & Ghangrekar, 2009). The MFCs were operated in continuous mode by pumping the wastewater from the bottom port of the anodic chamber using a Peristaltic pump (Miclins, India) and the effluent came out from the top port provided in the anodic chamber. Oxygen was used as terminal electron acceptor by aerating tap water in cathodic chamber.

Oxidation ponds were used as the cathodic chamber in both the earthen pot MFCs. One of the earthen pot MFC's cathodic chamber contained wastewater having the initial COD concentration of 86 mg/L and a mixed-culture of algae collected from the rice field was added to it (MFC-AT). The other earthen pot MFC's cathodic chamber was filled with tap water (initial COD = 7 mg/L), without any addition of algal culture (MFC-TW). 50 mL of anaerobic mixed inoculum sludge was added in the anodic chamber after heat pretreatment at 100 °C for 15 minutes. 350 mL of synthetic wastewater of COD around 5000 mg/L was added to it. The earthen pot was covered with paraffin film to make the anode chamber anaerobic. No artificial aeration was given in the cathodic chambers. Apart from the experiment with mixed algae, experiments were carried out using pure culture algae (*Spirulina platensis*) in cathodic chamber. The previous earthen pot MFCs were cleaned thoroughly. The cathode chamber of MFC-AT was replaced with pure culture of algae and wastewater. In MFC-TW, the cathodic chamber was inoculated with pure culture of algae and tap water. Earthen pot MFCs were operated in batch fed mode. The top of the earthen-pot MFC was made non-transparent to know the effect of algae in the cathodic chamber only.

Five numbers of Tilapia fish having body length of 5.6 cm to 5.8 cm were added to two fish tank, one containing earthen pot MFC anode, operated in batch mode and fed with synthetic wastewater of 5000 mg COD/L, and second without MFC. Artificial pelleted feed (35 % crude protein and 12 % fat) was given two times (equal amount each time) per day (3-4 times the body weight of the fish). The study was carried out for 50 days. The depth of aerated tap water in the fish tanks was maintained at 20 cm during the experiments. Artificial aeration was provided in each tank.

Analyses and calculation

The performance of MFC was studied in terms of COD removal efficiency and electricity harvesting. Current and voltage were recorded using a data acquisition/switch unit (Agilent Technologies, Malaysia) and converted to power according to $P = I * V$; where P = power, I = current, and V = voltage (V). The power density was calculated by dividing the power by total surface area of the anode and power per unit volume was expressed by dividing the power by working volume of the anodic chamber. The parameters pH, VSS, influent and effluent COD concentration were monitored as per the Standard Methods (APHA et al., 1998). Polarization studies were carried out by varying the external resistances from 5000 to 10 Ω. Internal resistance of the MFC was measured from the slope of the line from the plot of voltage versus current (Picoreanu et al., 2007). The Coulombic Efficiency (CE) of the system was calculated by integrating the measured current relative to the theoretical current possible based on the observed COD removal as described by Logan et al. (2006). The specific growth rate of the fish cultivated was determined by the following formula (Elliot &Hurley, 1995)

$$SGR = \frac{\ln W_2 - \ln W_1}{D} * 100$$

Where;

SGR = specific growth rate, d^{-1}; W_1 = initial weight; W_2 = final weight; D = days of culture.

Results and Discussion

Effects of sonication and heat pretreatment of sludge inoculum

Wastewater treatment

On the first day after inoculation these MFCs were kept in batch mode to acclimatize the bacteria to the operating conditions. From the next day, they were operated in continuous mode with external circuit load of 50 Ω. The MFCs were fed with synthetic wastewater of COD varying from 470 to 500 mg/L. The MFC-1 with sonicated inoculum showed significant removal of substrate giving average COD removal at the steady state of 83.24 % and COD removal in MFC-2, with heat pretreated inoculum, was 69.07 %. A gradual improvement in the current generation was observed with days of operation in both MFCs.

Power production

The voltage production during the initial days was more in case of MFC-2 but with the passage of time, it became lower than that of MFC-1. This may be due to the microorganisms taking time to overcome the effect of sonication. The short circuit current (SC) increased in the early days of operation. After reaching the maximum, it started decreasing in both the MFCs. The maximum SC was found to be 6.39 mA in MFC-1 and 4.95 mA in MFC-2. The open circuit voltage (OCV) decreased in both the MFCs with the days of operation. The maximum OCV in MFC-1 and MFC-2 was observed to be 0.77 V and 0.79 V, respectively. The maximum operating voltage and operating current observed in MFC-1 and MFC-2 were 0.155 V and 0.139 V, and 3.1 mA and 2.78 mA, respectively. Powers obtained in MFC-1 and in MFC-2 were 4.96 mW and 3.91 mW, respectively. The maximum power density, normalized to anode surface area, of 0.83 W/m^2 and 0.654 W/m^2 was obtained in MFC-1 and MFC-2, respectively; and the respective power per unit volume was 3.79 W/m^3 and 2.99 W/m^3. Sonication of the inoculum demonstrated better pretreatment than heat pretreatment in terms of power generation.

Polarization and internal resistance

Polarization curves were obtained by varying the external resistance between the anode and cathode from 5000 to 10 Ω for MFC-1 and MFC-2 (Fig 1a & 1b). Comparatively less current generation along with rapid stabilization of voltage was observed at higher external load and vice versa. Effective electron discharge observed at lower resistances might be the probable reason for further potential drop and slow stabilization of the voltage at lower resistances. Maximum power densities of 111.67 mW/m^2 (at 200 Ω) and 91.79 mW/m^2 (at 250 Ω) were observed in MFC-1 and in MFC-2, respectively, during polarization at steady state condition. The internal resistances were 198 and 257 Ω in MFC-1 and in MFC-2, respectively. A power curve that describes the power (or power density) as the function of the current (or current density) is calculated from the polarization curve. As no current flows in open circuit conditions, no power is produced. From this point onward, the power increases with current to a maximum power point, MPP (0.68 mW in MFC-1 and 0.55 mW in MFC-2). Beyond this point, the power drops due to the increasing ohmic losses and electrode overpotentials to the point where no more power is produced (Logan et al., 2006).

Performance of earthen pot MFC having algal growth in cathode chamber

The earthen-pot MFCs removed considerable amount of COD from the wastewater in the anode chamber along with production of power. Influent COD concentration was around 5000 mg/L. The average COD removal in the anaerobic compartment was 90 % in MFC-AT and 85.8 % in MFC-TW. The lowest value of COD removal was observed during the initial adaptation of anaerobic biomass. In the third feeding cycle, the COD removal was 89 % in MFC-AT and 85 % in MFC-TW. Though there was considerable COD reduction, the biofilm growth on the surfaces blocked, the transportation of electrons and protons to the cathode surface resulting in low power production during this feed cycle. The Coulombic efficiency of MFC-AT was found to be 4.3 % and in MFC-TW, it was 3.4 %. The COD of cathodic wastewater in the beginning was 86 mg/L and after the addition of algae and its subsequent growth, it increased with time to a COD value of 168 mg/L after two weeks, reaching the maximum of 198 mg/L at the end of the next feed cycle. The initial COD of tap water was 7 mg/L and with time, it showed an increase in value due to deposition and precipitation of dust and some algal growth. The COD was 52 mg/L after two weeks. Then the total water was exchanged after cleaning the cathodic chamber. After this, the COD decreased to 10 mg/L.

MFC-AT and MFC-TW were operated in batch fed mode. The working voltage (WV) was produced on the second day of operation. The WV produced in the MFC-AT was higher compared to the MFC-TW. This might be due to the availability of more photosynthetic oxygen in the cathodic chamber of MFC-AT. The maximum WV was 0.24 V in MFC-AT and 0.19 V in MFC-TW in the first feeding cycle. In the next feed cycle, the maximum WV was 0.25 V and 0.2 V in MFC-AT and MFC-TW, respectively. The WV decreased in both the MFCs and after two weeks the values were 0.18 V and 0.14 V in MFC-AT and MFC-TW, respectively. This might be due to the algal growth observed on the surfaces of the earthen-pot and cathodes. Fig 2a shows that WV in both the MFCs started increasing after the feed was given, subsequently reached maximum level and then decreased. This occurred possibly due to the microbes using the feed rapidly causing the WV as well as the power to increase rapidly. After reaching the maximum level the feed in the anode chamber started to decrease, as a result there was not much substrate to degrade. Therefore, the WV decreased after reaching the maximum value. Fan et al., 2008 reported that the feed was given when the WV reached the value of 30 mV. The open circuit voltage (OCV) was very low on the same day of addition of mixed culture of inoculum and synthetic wastewater to the MFCs. The OCV was 0.21 V in MFC-AT and 0.12 V in MFC-TW. The maximum OCV was recorded on the third day of the first feed cycle of both the MFCs and it was 0.45 V in MFC-AT and 0.41 V in MFC-TW. Like the WV, the OCV also decreased in both the MFCs due to the same reason of algal growth on the outer surfaces of earthen-pot and cathodes. The OCV decreased to 0.41 V in MFC-AT and 0.39 V in MFC-TW (Fig 2b). The short circuit current was generated on the second day of operation of MFCs. The SC followed the same trend as OCV. The maximum SC of 4 mA and 3 mA was found in both the MFCs at second feed cycle. After two weeks, the SC decreased to 3.3 mA and 2.5 mA in MFC-AT and MFC-TW, respectively.

Maximum power produced during first feed cycle was on the third day after the feeding in both the MFCs and it was 1.45 mW in MFC-AT and 1.12 mW in MFC-TW. In the next feed cycle, the power increased to 1.8 mW and 1.2 mW in MFC-AT and MFC-TW, respectively. In the third feed cycle, the power produced decreased to 1.32 mW in MFC-AT and 0.92 mW in MFC-TW. The maximum power per unit volume of 4.5 W/m^3 and 3 W/m^3 was observed in MFC-AT and MFC-TW, respectively. The microbes took some time to stabilize and produce power. The power production increased slowly with time in both the MFCs. The voltage and current increased with days of operation due to an increase in bacterial density. The bacterial density changes the amount of metabolites in the solution, which in turn influences the conductivity and capacity. However, after few weeks of operation the power dropped, possibly due to growth of algae on cathode surface, limiting cathodic reactions.

Performance of MFC having algal growth in both the anode and cathode chamber

After the third feeding cycle, there was algal growth inside the anode chambers of both the MFCs. This study was carried out for four feeding cycles. The COD reduction decreased during this phase of algal growth in both the MFCs. The COD removal in the first feeding cycle was 84.5 % and 83.2 % in MFC-AT and MFC-TW respectively. The average COD removal during this study of four feeding cycle was 85.3 % in MFC-AT and 83.4 % in MFC-TW. The Coulombic efficiency was more in this case than when there was no algal growth in the anode chamber. The Coulombic efficiency of MFC-AT was found to be 4.35 % and in MFC-TW was 3.31 %. The increase in CE might be due to the additional substrate available because of algal growth in anodic chamber and subsequent dead cells acting as a substrate, which is not reflected in the CE calculation based on the substrate supplied.

A significant drop in WV was not observed after algal growth in the MFCs. Maximum WV was 0.23 V in MFC-AT and 0.18 V in MFC-TW (Fig 2a.). However, the OCV increased more when compared to the previous results and reached the maximum of 0.47 V in MFC-AT and 0.438 V in MFC-TW (Fig 2b.). The generated voltage remained constant in the range of 0.45-0.47 V in MFC-AT and 0.42-0.43 V in MFC-TW. The maximum short circuit current observed was the same as before. A maximum of 4 mA was found in MFC-AT and 3 mA in MFC-TW. The power production increased in the MFCs after algal growth in the anodic chamber. The maximum power was 1.88 mW and 1.32 mW in MFC-AT and MFC-TW, respectively. The maximum power density produced was 4.7 W/m^3 and 3.3 W/m^3 in MFC-AT and MFC-TW, respectively. Fu et al. (2009) reported that algae were fed to anode and they help in the transfer of electrons to the anode. The algae also produced carbohydrates during photosynthesis and the dead cells of the algae added substrates for degradation by the microbes. This result showed that the growth of algae in anodic chamber and carbohydrates production compensated the photosynthetic oxygen produced during day light hours and did not affect the MFC performance adversely.

Performance of MFC with pure culture of algal growth in cathode chamber

The performance of MFC was evaluated by the growth of pure culture of algae (*Spirulina platensis*) in the cathode chamber of the MFC-AT and MFC-TW. The WV and OCV was increased in both the MFCs. The WV increased from 0.22 V (1st feeding cycle) to 0.24 V (3rd feeding cycle) in MFC-AT. In MFC-TW, the WV increased from 0.19 V to 0.22 V (Fig. 2a). The OCV was in the range of 0.440 V to 0.450 V in MFC-AT and 0.42 V to 0.44 V in MFC-TW (Fig. 2b). The short current also increased with time in both the MFCs. The maximum short circuit current in the MFC-AT increased from 2.98 mA to 3.38 mA and 2.5 mA to 2.8 mA in MFC-TW from first feeding cycle to third feeding cycle. The maximum power produced in the first feeding cycle after introducing pure algal culture in the cathodic chamber was 1.31 mW and 1.05 mW in MFC-AT and MFC-TW respectively. The power production increased with time and the maximum power produced in the third feeding cycle was 1.52 mW in MFC-AT and 1.23 mW in MFC-TW. The maximum power per unit volume of 3.8 W/m^3 and 3.07 W/m^3 was produced in MFC-AT and MFC-TW, respectively. The power produced increased with the increase in growth of algal culture in the cathode chamber of the MFCs. This showed that the photosynthetic algae played a major role in the power production. COD removal also increased with time. The maximum COD removal was 85.5 % in MFC-AT and 81 % in MFC-TW. The CE of MFC-AT was found to be 3.74 % and in MFC-TW, it was 3.15 %.

Study of fish culture along with power production by earthen-pot MFC

An earthen-pot MFC was released to a fish tank to know the effect of existence of electric potential on fish behavior and fish growth. The earthen-pot MFC was started in batch fed mode. Voltage and current were produced after one day of addition of heat pretreated mixed anaerobic inoculum and wastewater of 5000 mg/L of COD to the earthen-pot. The power production in the initial days was lower. This might be due to the bacteria taking some time to acclimatize themselves and to attach themselves to the anode surfaces. The short circuit current increased in the earlier days and remained almost constant in the further feedings. A maximum value of short circuit current of 2.98 mA was observed on the 17th day of operation. The OCV was measured after removing the resistance and allowing the MFC to stabilize for 15-20 minutes. The maximum OCV was found to be 0.440 V on the 17th day of operation.

The WV with the presence of a 100 Ω resistance was 0.240 V. The maximum operating current was found to be 2.4 mA. The COD removal increased with time. The average COD removal and the maximum COD removals were found to be 83.42 % and 85.5 %, respectively. The CE of the MFC was 3.2 %. The maximum power density normalized to anode surface area was 69 mW/m^2 and the power per unit volume was 3.28 W/m^3.

The average final weight of the fishes in the fish tank with MFC was found to be more than that of the fish tank with no MFC. The average weight of fish in fish tank without MFC was 14.94 gm and with MFC it was 17.76 gm. The specific growth rate (SGR) of fish was also higher in case of fish tank with MFC (2.28) than fish tank without MFC (2.01).

Conclusion

Attempts were made in this study to develop MFCs for effective wastewater treatment and energy harvesting. The experimental results indicate that sonication pretreatment gave a better result in terms of power production (3.79 W/m^3) and organic matter removal compared to heat pretreatment (2.99 W/m^3). The earthen-pot MFC having algal growth in cathode chamber produced better result, due to photosynthetic oxygen yield, than an identical MFC having only tap water in the cathode chamber. After algal growth in the anode chamber, the power produced was increased and organic matter removal was lowered in both the MFCs. Pure culture of *Spirulina platensis* in cathodic chamber produced similar power production and COD removal as compared to mixed culture algal growth in cathodic chamber. The fish growth was not limited by the electric potential produced by the MFC. The observation that the fishes remained nearer to the MFC, showed that the latter was not a hindrance in the fish growth. The power produced in low cost earthen pot MFC in oxidation pond is comparable with many of the sophisticated MFCs. Further studies are needed to establish this technology as a wastewater treatment and onsite energy harvesting device.

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Figure 1. Polarisation curve of (a) MFC-1 (b) MFC-2

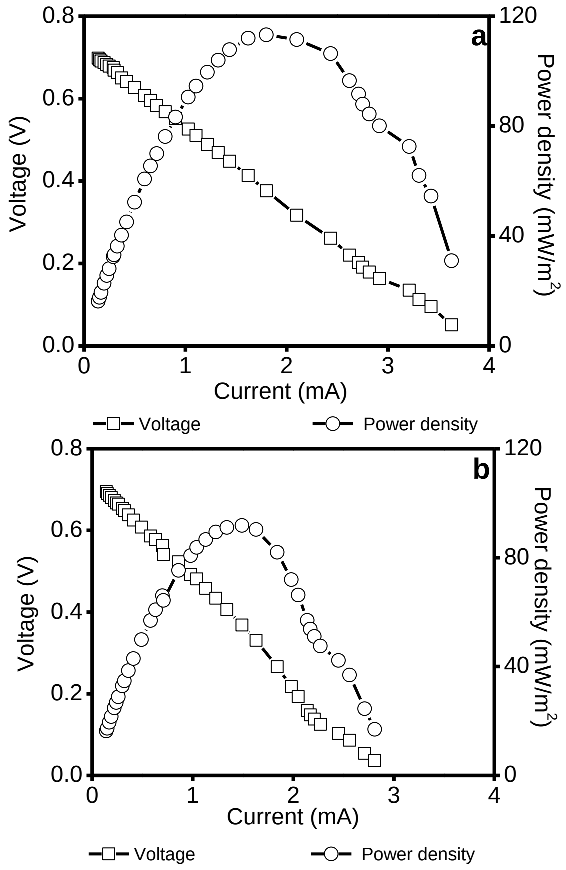

Figure 2. Variation of (a) working voltage (b) open circuit voltage with respect to days of operation of earthen-pot MFC in oxidation pond