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Bioelectricity Generation using Carbon Felt Electrode in Microbial Fuel Cell (MFC) Inoculated with Mixed Cultures

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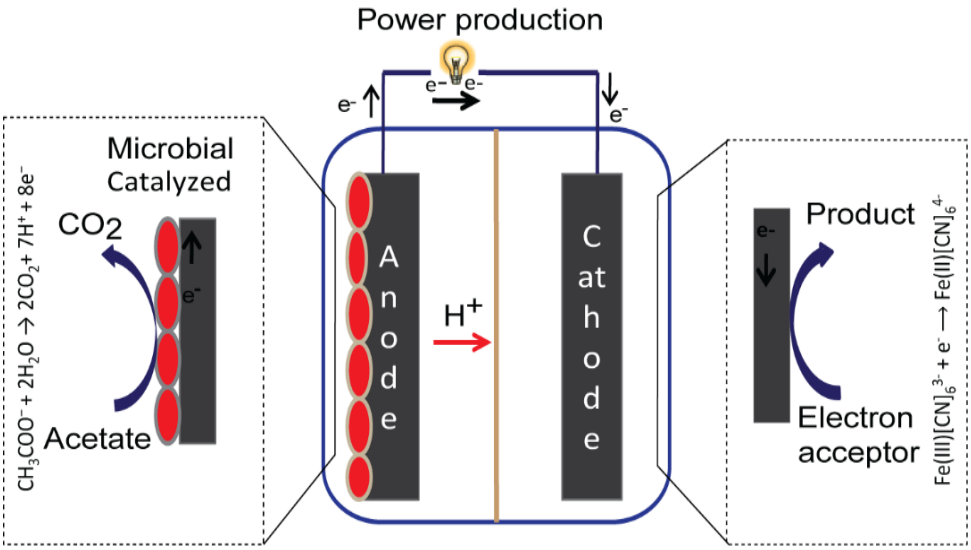

Microbial fuel cell (MFC) that was configured with the carbon felt electrode and the cation exchange membrane, and inoculated with mixed culture was demonstrated to yield bioelectricity. The cell was operated under four external loads with pHs ranging from 4 to 10 and the total cell operation was monitored up to 25 days. The presented results revealed that the potentiality of maximum current and power production was achieved while hexacyanoferrate(III) used as a cathodic reaction and at neutral pH condition of media. The maximum current density 2.5 Am^{-2} and power density 1410 mWm^{-2} were observed on the 25th day at an anode potential of -378 mV. Stable and steady power was produced by MFC on the day 22nd to 25th when cell operated at 250 Ω external load. The internal resistance of the fuel cell was decreased with the increase of the operation time. Coulombic efficiency (CE %) was found 22.70 % at the stable phase of fuel cell operation.

Keywords: MFC; Bioelectricity; Carbon felt; Mixed culture microorganisms; Power density

Introduction

In the recent years, green energy production utilizing renewable resources is becoming an active area of research in the research fraternity. Ethanol, bio-diesel, bio-hydrogen, and bioelectricity production from waste materials are finding prominence in this direction [1-2]. Now, the efforts are devoted in developing alternative electricity production methods. New electricity production from renewable resources is much indispensable [3-4]. In this regard, microbial fuel cell (MFC) is a promising technology that can convert biodegradable materials *e.g.* organic materials present in wastewater, into clean and renewable electricity. In MFC, bacteria can be used to convert the energy stored in the chemical bonds of organic compounds into electrical energy [5].

Electrochemically active microorganisms play a crucial role in the generation of electricity through MFC by oxidizing different biodegradable materials to CO_2 and protons for their growth while transferring the electrons towards a solid electrode [5]. Electron transfer from the microorganisms to the electrode was explained by several proposed mechanisms [6-7]. In MFC, anodic oxidation is accompanied by a cathodic reduction that is enclosed in a separate compartment [8]. An external electrical circuit is used with a resistor or by the power user to transfer electrons from the anode to the cathode. Protons and other cations are transferred from the anode to the cathode through a cation exchange membrane in order to close the circuit and maintain electroneutrality in both anodic and cathodic compartments.

The performance of the MFC mainly relies on the materials and the reactor configuration, and the microorganisms that required to produce the current [5, 9]. Power output from the MFC is thus affected by the variations in these operating conditions. Thus, based on these factors, different mediators and their different configurations, wide variety of substrates and anode inoculum were studied to increase the efficiency in the conversion of electricity from substrate [1, 10-11]. In the MFC, microorganisms act as a catalyst in the transfer of electrons from the substrate to the anode, thus, high performing microbial consortium (either pure or mixed culture) is very important to enhance MFC performance [12-13]. Up until now, it has been reported that the mixed cultures used in MFC have greater potentiality to produce power densities than those using pure cultures [9, 14-15]. Wastewater is often considered as a rich source of a variety of exoelectrogenic bacteria and thus it could be used in the MFC to increase its performance [16-17]. A mixed culture of bacteria often consists of different type of exoelectrogenic bacteria including *Geobactor sp.*, *Pseudomonas sp.*, *Bacillus sp*., *Shewanella sp*., *Brevibacillus sp.*, and so on, which serve as the bacterial inoculum for the formation of primary electrochemically active biofilm on carbon electrode [9,15]. The mixed culture electroactive biofilms were consisted of these different types of bacteria that vary in morphology from spherical, rod, and oval shape. Bacteria, such as spherical and rod shape, shows the presence of nanowires (pili), which can be responsible for extracellular electron transfer (EET). For producing the bioelectricity, the electrons generated by the bacteria upon the oxidation of organic substrate could be transported effectively through these living nanowires to an electrode [9].

Porous carbon-based materials such as graphite granules, graphitic felt, carbon cloth, and reticulated vitreous carbon (RVC) have been used currently as the anode of the MFC to make the entire process more economically feasible [18]. Relatively cheap and porous nature of carbon-based materials are becoming attractive because their high specific surface areas lead to the high volumetric activity. To increase the power production,

different strategies have been reported, for example, precipitating iron oxide onto carbon electrodes [19], adding Mn^{4+} [20], Fe_3O_4, or Fe_3O_4 and Ni^{2+} to graphite anodes [21], ammonia treatment of carbon cloth anodes [22]. However, inefficient attachment of bacterial nanowires to carbon cloth electrodes could be the cause of limiting power production.

The eventual aim of this study was to investigate bioelectricity production as well as power production by mixed culture MFC, in which the plain carbon felt was used as the electrode materials. The overall MFC performance was evaluated in terms of maximum power based on polarization and power density curves, internal resistance, and columbic efficiency (CE).

Materials and Methods

Microbial fuel cell design and setup

The experiments were conducted in the same electrochemical cell as described previously [23]. MFC consists of two plexiglass plates containing a single flow channel, two electrodes, and two plexiglass support plates (Figure 1A). The two plates with the flow channel were separated by the cation exchange membrane (Fumasep FKB, Fumatech, St. Ingbert, Germany). The other side of the flow channel faced the electrode. Both the anode and the cathode were made with the carbon felt. The projected surface area of the both electrodes in contact with the solution was 22 cm^2, and the volume of the flow channel was 33 mL (11.2 cm length × 2.0 cm width × 1.5 cm height). Both inlet and outlet of the anode and cathode chambers were connected to a 600 mL glass reservoir. The cation exchange membrane was pre-treated subsequently in 30% H_2O_2, deionized water, 0.5 M H_2SO_4, and deionized water (for 1 h each) to increase porosity. All electrochemical experiments were carried out in a three-electrode cell arrangement that consists of a working electrode, the reference electrode, and the cathode counter electrode. To measure the anode, cathode, membrane and cell potential, both anode and cathode compartments were equipped with an Ag/AgCl (3M KCl, +0.205 V vs NHE) reference electrode. A schematic overview of the experimental setup is presented in Figure 1B.

Startup and MFC operation

Anode chamber was inoculated with the enriched mixed bacterial culture from another MFC run on acetate. The source of inoculums was wastewater, which served as the bacterial inoculum for the formation of a primary electrochemically active biofilm on a potentiostatically positive poised carbon electrode (0.4 V vs. SHE (the standard hydrogen electrode)). Acetate (20 mM) served as the substrate in the growth medium, whose pH was adjusted to 6.8 with 20 mM phosphate buffer solution at pH 7. The bacterial growth medium solution contained following chemicals (per liter): 10 mL/L of a macronutrient solution containing 28 g/L NH_4Cl, 10 g/L $MgSO_4 \cdot 7H_2O$, and 0.57 g/L $CaCl_2 \cdot 2H_2O$; 2 mL/L of micronutrient solution containing 2 g/L $FeCl_2 \cdot 4H_2O$, 1 g/L $CoCl_2 \cdot 6H_2O$, 0.5 g/L $MnCl_2 \cdot 4H_2O$, 0.05 g/L $ZnCl_2$, 0.05 g/L H_3BO_3, 0.04 $CuCl_2.2H_2O$, 0.07 g/L $(NH_4)6Mo7O_{24} \cdot 5H_2O$, 1 g/L $NiCl_2 \cdot 6H_2O$, 0.16 g/L $Na_2SeO_3 \cdot 5H_2O$, and 2 mL/L 37% HCl; and 2 mL/L of a vitamin solution as reported previously by Ter Heijne *et al.*(2008) [23]. In order to ensure anaerobic conditions, the substrate and buffer solutions were purged with nitrogen for 30 min before use. The anolyte and catholyte (volume 550 mL) were continuously recirculated at a rate of 100 mL/min using a peristaltic pump. MFC was

operated in batch mode at the temperature of (27 ± 2ºC) under anaerobic conditions. During a feeding event, the anode chamber was purged with N_2 gas for 30 min to create the anaerobic microenvironment in the cell.

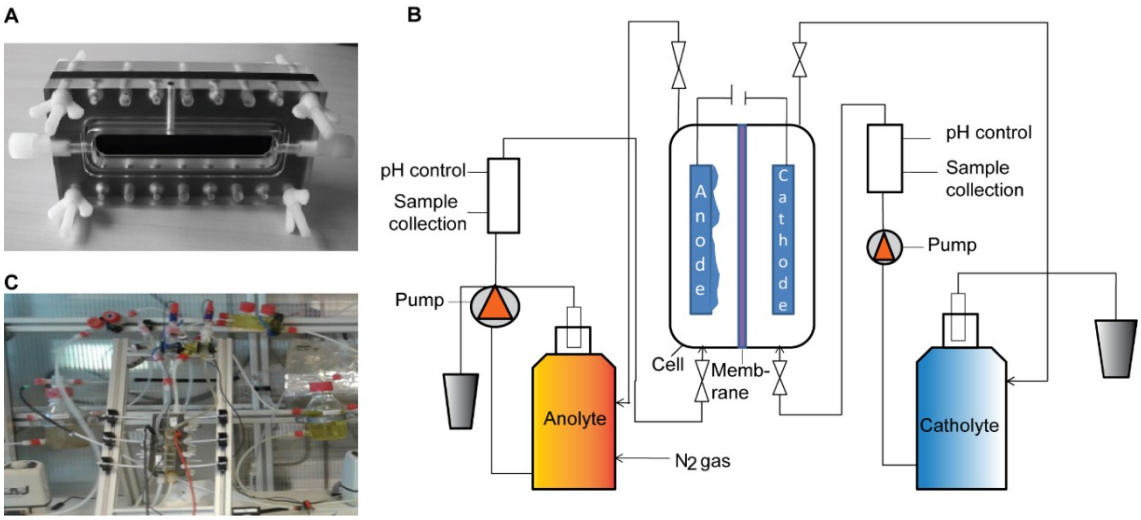

Figure 1. (A) MFC design: the assembly of flow channel, carbon felt electrode, and support plate of one side of the MFC; (B) Schematic of MFC operation to produce bioelectricity; (C) Photograph of the experimental setup.

The MFC was started with an external load of 16 kΩ and a 0.020 M phosphate buffer at pH 7 in the cathode, and air was continuously circulated through the catholyte. Firstly, the system was stabilized overnight to reach a steady state. Four resistors with a range of 0–16 kΩ were used, and thereupon potential was measured two times a day with constant interval using a Keithley 2700 multimeter (Keithley Instruments, Cleveland, OH, USA). Polarization and power density curves were obtained by varying the external resistance applied to the circuit. Here, all electrode potentials were given as vs Ag/AgCl (3M KCl, + 0.205 V vs NHE (the normal hydrogen electrode)) and all the current density values were normalized to the geometric surface area.

After 25th day of operation, the catholyte was replaced with a Fe (III) $[CN]_6^{3-}$ solution (0.050M) in 0.020M buffer (pH 7) for a fast cathode reaction (reduction of Fe(III)$[CN]_6^{3-}$ to Fe(II)$[CN]_6^{4-}$). In this case, the MFC was also operated at three different resistances with first R = 16 kΩ during the first 8 days, second R = 2 & 0.5 kΩ during the next 5 days, and third R=250 Ω during the last 12 days. After reaching stable performance, power output was monitored by measuring voltage using an external resistor (250 Ω) connected across the electrodes.

To characterize pH effects on the MFC performance, media with pHs ranging from 4.0 to 10 at 0.5 pH unit increments were created with 5 M solutions of HCl or NaOH.

Analysis

Cell voltage across an external resistor was recorded using a multimeter. Polarization curves were obtained by varying the external resistance applied to the circuit (in a decreasing order) and using the average voltage obtained after stabilization (2 times in a day). Current density was calculated using I = V/R, where I (mA/m^2) is the current, V (mV) is the measured voltage, and R (Ω) is the applied resistance, and A (m^2) is the

geometric surface area of the anode electrode. Power densities (mWm^{-2}) were calculated using P= IV, and normalized by the projected anode surface area [5].

Coulombic efficiency was calculated as CE (%) = (C_{Ex}/C_{Th}) × 100, where C_{Ex} is the total Coulombs calculated by integrating the current over time, C_{Th} is the theoretical number of Coulombs available from the oxidation of acetate calculated as, C_{Th} = FbMv, F is the Faraday's constant (96,485 C/ mole, b is the number of moles of electrons available per mole of substrate (8 mol e^-/ mol acetate), M is the acetate concentration ($molL^{-1}$), and v is the volume of liquid in the anode chamber (L) [5].

The energy losses of MFC were measured in three parts, which were anode, cathode, and membrane losses. These energy losses therefore led to the internal resistance of the system. Internal resistance can be split into partial internal resistances, for instance, anodic resistance, cathodic resistance, and membrane resistance. Anodic and cathodic resistances were calculated as overpotential divided by current density.

Anodic resistance calculated according to R_{an}= (E_{an}-$E°_{an}$)/I, where, R_{an} = anodic resistance ($\Omega.m^2$), E^0_{an}= Theoretical anode potential (V), E_{an} = measured anode potential at certain external load (V), I = current density (A/m^2). Assumed that theoretical anode potential is open circuit potential (at zero current). Cathodic resistance, R_{cat} = ($E°_{cat}$-E_{cat})/I, where R_{cat} = cathodic resistance ($\Omega.m^2$), E^0_{cat} = theoretical cathode potential (V), E_{cat} = measured cathode potential, I = current density (A/m^2) [24].

Results and Discussion

Reactor performance

Following inoculation of the anode, the operation of MFC were started with a 0.020 M phosphate buffer at pH 7 in the cathode, in which oxygen was circulated continuously for reduction reaction ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$). Firstly, to obtain polarization and power density curve, MFC was started with a 16 kΩ external resistor, and stabilized overnight to reach a steady state. Afterward, the polarization test was performed (every day two times) to evaluate the development of activity of electrochemically active microorganisms in time that means the performance of the bioanode. Higher current density accompanying the lowest anode overpotential is indicating best performance of MFC [5]. During the polarization test, the current density was extracted from the maximum current densities of the batch experiments. At the beginning of the experiment, negligible current was observed, and this is due to the lack of biocatalysts at the electrode surface. About 12 day after the initial inoculation, the current rose significantly, indicating the formation of an electrochemically active biofilm. The biocatalytic current density and the power density reached the maximum values of about 51.5 mAm^{-2} and 11.6 mWm^{-2}, respectively, on day 25^{th} after inoculation at 250 Ω for operation.

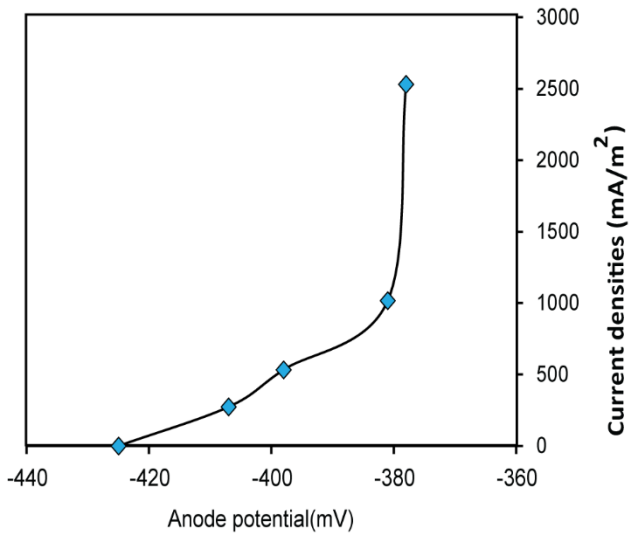

Figure 2. Polarization test gave insight into the performance of a bio-anode at operation 250 Ω in time using potassium hexacynonoferrate(III) reduction at the cathode on 25th day.

To investigate the effect of the catholyte on the performance of MFC (in terms of power generation), the catholyte was replaced by Potassium hexacyanoferrate(III) (Fe (III) $[CN]_6^{3-}$ solution, 0.050M). In this case, the MFC was started with freshly inoculated anolyte. With the cathodic reaction of potassium hexacyanoferrate(III), a higher current density as well as a higher power density was observed (current density 2.5 Am^{-2}, power density 1410 mWm^{-2}) at the anode potential of -378 mV on day 25 (see in Figure 2), while the MFC was operated at 250 Ω. From the polarization test, Figure 3 is depicted an overview of obtained cell voltage, cathode potential, and anode potential during maximum power generation.

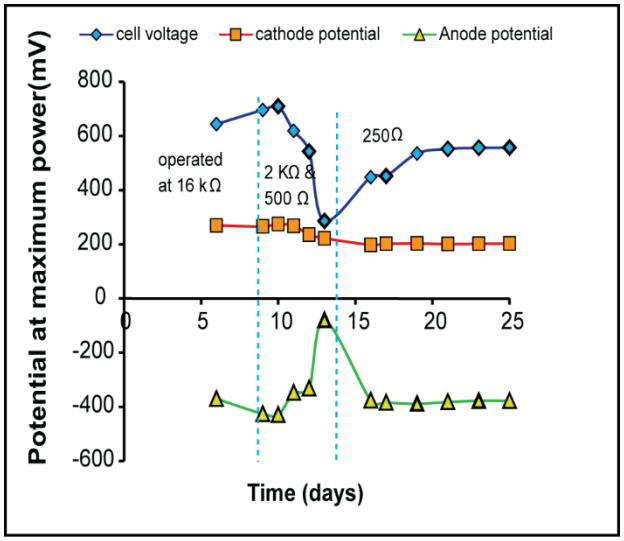

Figure 3. Cell voltage, cathode potential, and anode potential were recorded from the polarization test while maximum power produced by MFC, using Hexacyanoferrate (III) as the catholyte. First 8 days, the cell was operated with a 16 kΩ external resistor, next 9-13 days, cell was operated at 2 KΩ & 500 Ω and last 14-25 days, cell was operated at 250 Ω.

Higher current density as well as higher power density was obtained for Potassium hexacyanoferrate(III) reduction than the oxygen reduction at the cathode. This lower current density for oxygen reduction at the cathode could be the results of (i) oxygen diffusion through the membrane from cathode to anode leading to a parasitic reaction at the anodic compartment, therefore limiting the number of electrons available for electricity production. The parasitic side reaction that leads to the formation of mixed potential, may substantially lower the anode potential and reduce the cell voltage as well as current density and power density. Furthermore, this side reaction may not only reduce the cell voltage but also reduce the columbic efficiency of the electrode reaction [25]. (ii) Competing reduction reaction may be occurred during oxygen reduction at the cathode. (for instance, not only formation of water, but often considerable extent of hydrogen peroxide formation). This competing reaction can lead to the formation of mixed cathode potential (reduce cell voltage), which limits the overall performance of MFC. (iii) Due to limited diffusion of oxygen in the electrode surface, the higher cathodic partial internal resistance produced (3050 $m\Omega.m^2$ at maximum current density 51.5 mAm^{-2}, on day 25) can limit the performance of the cell.

Improvisation of the MFC performance by operating at a lower external resistor was revealed through the polarization test. Figure 4 depicts that the MFC operation at a lower external resistor caused higher current densities. The potential difference in the anode (between the electron donor (acetate) and the electron acceptor (the anode) became larger due to lower resistance of the MFC that helped the electrochemically microorganisms to attain more energy from the substrate. This energy gaining for the growth of microorganisms in turn helped produce higher current that reflected the maximum bioelectrocatalytic activity of the biofilm [23].

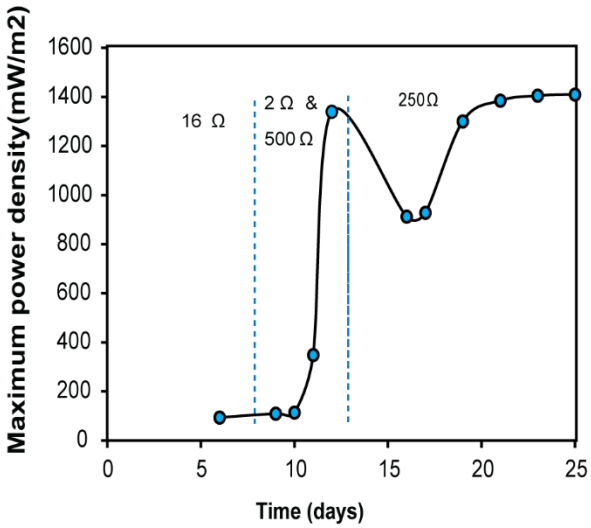

Figure 4. Maximum power evolution was recorded from days 6th to 25th during cell operated at different external loads 16 KΩ, 2 KΩ, 500 Ω, and 250 Ω. (Hexacyanoferrate(III) used as a catholyte).

To determine the performance of a microbial fuel cell, the internal resistance (R_{int}) has been recognized as an important factor [16]. Thus, the internal resistance profile was observed over the operation period in order to envisage the changes in performance of MFC, while hexacyanoferrate (III) was reduced at the cathode (hexacyanoferrate (III) used

as a catholyte). Figure 5 depicts the anode and cathode resistances over the period of operation.

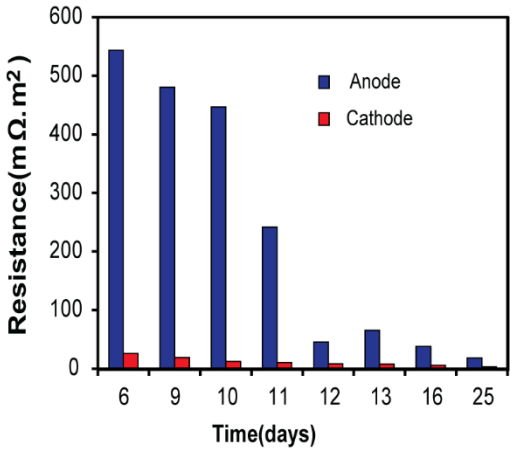

Figure 5. Internal resistances profile of anode and cathode over the period of MFC operation.

The results revealed that the anode resistance declined from 543.9 mΩ.m^2 to 18.5 mΩ.m^2 (from day 6 to 25), indicating the enhancement in activity of the electroactive biofilm. Cathode resistance also decreased from 25.9 mΩ.m^2 to 3.1 mΩ.m^2 (from day 6 to 25). As both anode and cathode resistances decreased with time, it indicted that the performance of MFC increased with time. The total cell resistance (sum of the anode, cathode and membrane internal resistances) decreased from 582.7 mΩ.m^2 to 27.48 mΩ.m^2 (from 6 day to 25 day). At the maximum performance on the day 25th, the anode, the cathode, and the membrane contributed to 67%, 11%, and 20% of the total resistance, respectively.

When MFC was operated with potassium hexacyanoferrate(III) for cathodic reaction, the Coulombic efficiencies (CE %) 22.70 % of MFC was observed at the stable phase of fuel cell operation.

Effect of pH on MFC performance

Physiologically permissive medium is crucial for the growth of a viable biocatalyst on an electrode. To enhance the MFC operation and performance, an improved understanding of the bioanode process as a function of medium pH conditions is of crucial importance. Therefore, MFC performance was examined by using media with pHs ranging from 4.0 to 10 at 0.5 pH unit increments. MFC performance was characterized based on current densities as well as power densities produced using the polarization test.

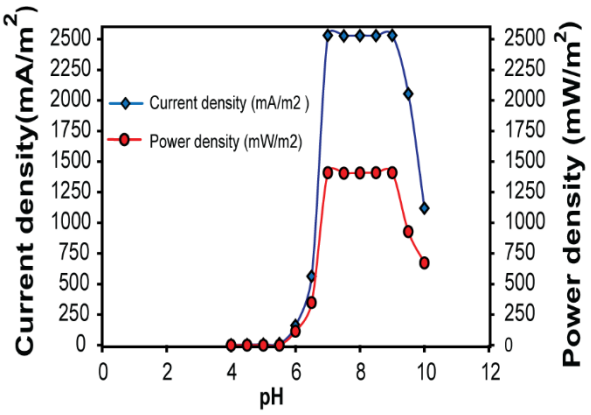

Figure 6. Obtained current density and power density correspond to cell operated with media of pHs ranging 4 to 10. The maximum current density and power density were extracted from 25 day when MFC operated with 250 Ω external load.

Figure 6 shows that MFC performance was enhanced as pH became neutral, and this result is consistent with a previous study [26]. However, at low pH condition, the current densities and power densities decreased. The acidification of the anode biofilm affected current generation, because microbial activity is inhibited in low pH [27]. The produced current densities and power densities were remained almost steady from pH 7 to 9. In alkaline medium and high buffer concentration, bioanode performance was enhanced by increasing flux of proton shuttles out of the anode biofilm [28]. However, the maximum power density (P_{max}) decreased at pH 9.5, showing that neutral pH was the optimum pH for attaining the highest power density in this system.

Conclusion

In this study, we have shown that the bioelectricity yield at neutral pH condition from microbial fuel cell (MFC), where untreated carbon felt was used as both the anode and the cathode, and the anolyte was inoculated with mixed culture. The performance of MFC was enhanced by replacing continuous air cathode with potassium hexacyanoferrate (III). Potassium hexacyanoferrate (III) reduced at cathode that increased current density by addressing the limited diffusion of the substrate into the electrode surface. The increase in power density to 1410 mWm^{-2} resulted in the improved performance of the system at higher current densities (51.5 mAm^{-2} to 2500 mAm^{-2}). Power generation using MFC with low cost electrodes and mixed culture was considered as cost-effective and environmentally sustainable process which will also provide a great potentiality for other applications like handy power supplies for remote sensors using native fuels.

Declarations

Availability of data and materials

Data and materials related to this work are available upon request.

Authors' contribution

All authors contribute equally during the research study and preparation of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Consent for publication
All authors approve the manuscript for publication.

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