

Harvesting Bioelectricity from Microbial Fuel Cells (MFCs) Powered by Electroactive Microbes

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ABSTRACT

The application of microbial fuel cells is still facing some challenges due to its low power output and high internal resistance. It is desirable to obtain a stable and consistent power output from an MFC to support practical real-world applications. Five electroactive bacteria (isolate LGf1, LGf11, LGf15, LGf20, and LGf22) isolated from the sediment of Waduk Saguling were exploited as the potential anodic biocatalyst for MFC, and the performance of these MFCs were studied in terms of voltage generation (open and close circuit), power density and the losses (polarization technique), and efficiencies (coulombic and energy). MFC biocatalyst by isolate LGf11 performed the best electrochemical performances, including highest OCV (open circuit voltage) value (804 mV) and power output (0.043 W/m2), lowest ohmic resistance (475 Ω), and highest coulombic efficiency (75.79%) and energy efficiency (88.36%) among all anodic biocatalysts. Nevertheless, all the five isolates were potential to be exploited as active biocatalyst for MFC due to their high OCV values and the stability of voltage generations, both in open circuit and close circuit mode. The development of system configuration and the use of more suitable substrate for different electroactive microbes in order to harvest more power output was recommended for further study. Utilization of these potential microbes for other applications in MFC (such as wastewater treatment etc.) was also suggested for further research.

1. INTRODUCTION

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Microbial fuel cell (MFC) has attracted many attentions as one of the environmentally friendly technology that exploited microbes and their cellular complex biochemical reactions for converting chemical energy into electricity. Research and development on MFC technology are increasing along with the increasing of awareness toward various environmental issues, such as the need for renewable energy and electricity production in

remote areas (Rahimnejad *et al.*, 2015), bioremediation (Kubota *et al.*, 2019), wastewater treatment (Gude, 2016), detoxification of polluted soils (Rodrigo *et al.*, 2014), and environmental sensors (Adekunle *et al.*, 2021).

The phenomenon of bioelectricity was first explored by Potter in 1908 during the study of microbial degradation. Later, by utilizing *Saccharomyces*, he discovered the first microbial fuel cell in 1911. After this discovery, the idea of MFC was under shade until Cohen introduced stacked MFCs in 1931 that generated a substantial amount of voltage, 35 V. A new path of MFC development for another application was introduced by DelDuca *et al.* (1963). They introduced *Clostridium butyricum* as a biocatalyst for hydrogen production. A year later, the production instability faced by DelDuca was resolved by Suzuki group. Another main issue regarding MFC was associated with the cost of mediator that created a hurdle in its commercialization. In 1999, the first mediator-less MFC was introduced by Kim *et al.* (1999) to resolve the issue. MFC was then became a hot topic among researchers and underwent development in various forms, such as microbial wetland cells, microbial desalination cells, microbial waste treatment cells, and microbial metal extraction cells (Naseer *et al.*, 2021).

Microbial fuel cells are similar to batteries or other fuel cells which consist of two electrodes (anode in anodic chamber and cathode in cathodic chamber) separated by an electrolyte. The difference between MFC and other fuel cells lies in the use of organic compounds as substrates to generate electricity and the use of microbes as active biocatalysts. Electroactive or electrogenic microbes are, among all microbial groups, most often exploited as biocatalysts in MFC due to their capability in degrading substrates anaerobically into CO_2 , proton (H⁺), and electron (e⁻), and their ability to subsequently transfer electrons to extracellular electrodes (Lovley, 2006).

Extracellular electron transfer (EET) is part of electrogenic microbial respiration which allows electrons to be transferred from the microbial cell to solid materials outside the cell. The existence of this EET mechanism distinguishes electrogen from other microbial groups, in which electrons cannot be transferred extracellularly outside the cell (Kato *et al.*, 2012). In the MFC system, the protons generated by the electrogen will diffuse into the cathodic chamber through the proton exchange membrane, while the electrons will be transferred to the electrode of anodic chamber and then flow through the external electrical circuit to the cathodic chamber (Rabaey & Verstraete, 2005). These electrons flow can be harvested as electrical energy.

One of the key factors for the development of MFC technology lies in the variety of microbial species that can be exploited, which are generally the group of electrogenic bacteria, also known as exoelectrogen (Oh & Logan, 2007), electricigen (Lovley, 2006), anodophilic (Logan, 2009), electrochemically active bacteria (Chang *et al.*, 2005), anode -respiring bacteria (Torres *et al.*, 2007), electrotrophs (Lovley, 2008), cathode-oxidizing bacteria (Martin *et al.*, 2011), or electroactive bacteria (Sydow *et al.*, 2014). A total of 120 identified electrochemically active bacteria have been extensively utilized in MFC, including the phyla of *Actinobacteria*, *Proteobacteria*, *Firmicutes*, and *Archaea* (Sreelekshmy, 2020).

The various species of microbes that have electrochemical activity open the opportunities for the exploration of electrogenic microbes from various potential ecosystems, including nutrient-rich sediments or soils. Important factors affecting the existence of electroactive microbes in nature include: (1) suboxic/anoxic environmental conditions that allow anaerobic respiration; (2) the presence of electron donors, for example C-organic, dissolved organic carbon, humic acids, ammonium (Wang & Ren, 2013; Weber *et al.*, 2018), or inorganic compounds (Schamphelaire *et al.*, 2008), and (3) the presence of extracellular electron acceptors, for example Fe or Mn oxides

(Lovley, 2013). Therefore, various aquatic and soil/sediment environments are habitats of electrogens (Kato *et al.*, 2010), including river sediments (Lyautey *et al.*, 2011), mangrove sediments (Kristensen *et al.*, 2008), marine sediments (Nercessian *et al.*, 2012), tidal sediment (An *et al.*, 2010), swamp sediments (Miceli *et al.*, 2012; Rousseau *et al.*, 2014), as well as various wastewaters (Cercado *et al.*, 2013; Zhao *et al.*, 2015). The terrestrial ecosystems also have high microbial diversity due to the wide distribution of organic and inorganic materials (Schamphelaire *et al.*, 2008). These conditions open the chance in utilizing electrogenic microbes coupled with various species of plants in plant-MFC technology, which enable the bioelectricity being harvested on the site (in situ) where MFC reactors are installed (Cahyani *et al.*, 2020).

The application of MFC is still facing some challenges due to its low power output and high internal resistance. It is desirable to obtain a stable and consistent power output from an MFC to support practical real-world applications. However, MFC often experiences electrical output fluctuation due to the dynamics of microbial metabolic activity, the decrease of catalytic activity of enzymes, accumulation of cell debris, etc. (Sun *et al.*, 2016). Hence, in this present work, performance of MFCs biocatalyst by electroactive microbes in electricity generation was evaluated to assess the potency of this technology to be developed. Five selected electroactive bacteria isolated from the sediment of Waduk Saguling were chosen for this work, and the performance of these anodic biocatalysts were evaluated in terms of voltage generation (open circuit and close circuit), power density and the losses (polarization technique), and the efficiencies (coulombic and energy).

2. MATERIALS AND METHODS

2.1. Electroactive Microbes and Culture Medium

Five electroactive bacteria (named isolate LGf1, LGf11, LGf15, LGf20, and LGf22) were used as anodic biocatalysts for MFCs. These isolates were five among 23 electroactive microbes isolated from the sediment of a part area of Waduk Saguling located in Galanggang village, Batujajar Subdistrict, Bandung, West Java, Indonesia (latitude: - 6.91, longitude: +107.49), using thioglycollate medium enriched with FeCl₃ 0.1% w/v. These five isolates were chosen due to their high electrogenic capability among all 23 isolates in preliminary study (Indrivani *et al.*, 2023). These five selected isolates were then solely inoculated in five milliliters fluid thioglycollate culture medium composed of dextrose (5.5 g/L), sodium thioglycollate (0.5 g/L), triptone (15 g/L), yeast extract (5 g/L), NaCl (2.5 g/L), and resazurin (0.001 g/L); and subsequently incubated overnight. These 5 mL bacterial suspensions (10^8 CFU/mL) were then transferred to the anolyte mixture in the anodic chamber.

2.2. Microbial Fuel Cells Construction

The reactors of MFCs with dual chamber design were constructed following (Indriyani, 2017), and modification was made in the dimension of volume. A working volume of 200 mL anolyte dedicated for the anodic chamber, composed of the mixture solution of glucose 0.1 M, *thioglycollate* 0.5% w/v, and phosphate buffer 0.1 M (pH ±6.5-7.0); and a working volume of 200 mL catholyte was dedicated for the cathodic chamber, containing the mixture solution of potassium permanganate 0.01 M and phosphate buffer 0.1 M (pH ±6.5-7.0). The post-treated of Nafion membrane 117[®] (Lyntech, United States) with a surface area of 18 cm² was used to separate anodic and cathodic chamber were carbon fibers (length x width of 6x6 cm or the total surface area of 72 cm² for both

sides, per electrode); both electrodes were connected using a Cu-wire and placed in the center of each chamber (the distance between both electrodes is ± 6 cm). The schematic representation of the constructed MFC system and reactor is presented in Figure 1.



Figure 1. (a) A schematic representation of dual chamber MFC system separated by proton exchange membrane (PEM) (modified from Logan, (2009)), (b) a 6x6 cm carbon fiber as electrode, (c) A schematic representation of dual chamber MFC reactor, (d) A dual chamber MFC reactor constructed using acrylic material, consist of an anodic chamber and a cathodic chamber (dark colored). Carbon fibers were used as electrodes (anode and cathode) and inserted until they touched the bottom part of the chambers

2.3. Evaluation of Microbial Fuel Cells Performance

Electrochemical performance of MFCs powered by five electroactive microbes were evaluated 30 minutes after the MFCs system was installed. A multi-channels Arduino UNO based data logging system was used as the monitoring system for MFCs potentials generation (Figure 2). This developed microcontroller-based data logger has absolute error and relative error of 1.21 mV and 1.26%, respectively. The evaluation of MFC performances were conducted in terms of voltage generation (open and close circuit) (Figure 3), power density and internal resistance/ohmic losses, and the efficiencies (coulombic and energy).



Figure 2. (a) Monitoring of voltage generation of ten MFC bioreactors powered by five electroactive bacteria in open circuit mode by using multi-channels Arduino Unobased data logger. The experiment was conducted in duplicate, (b) Schematic representation of the system configuration



Figure 3. A schematic representation of monitoring MFC voltage generation using data logger: (a) in open circuit mode (without any resistance connected to the electrodes of MFC), and (b) in close circuit mode (with resistance connected to the electrodes)

Voltage generation of MFCs fueled with glucose 0.1 M in open circuit mode (known as open circuit voltage or OCV) was evaluated for lasted 75 hours. Voltage production in close circuit mode was measured during 125 hours of operation under external resistance of 1000 Ω and with various concentrations of glucose as electron donor: 2, 4, 6, 8, and 10 g/L. The current density (I) and power density (P) were calculated following Ohm's law (I=(V/R)/A and P=(I.V)/A), with A is the surface area of anode electrode (72 cm²).

The close circuit evaluation was also aimed to calculate the efficiencies of the system in terms of coulombic efficiency and energy efficiency. Coulombic efficiency describes the energy contained in the substrate which is converted into electrical energy. Energy efficiency describes the amount of energy that can be obtained from the MFC system compared to the total amount of energy contained in the substrate. Coulombic and energy efficiency are calculated with equations (1) and (2), respectively:

$$C_{\rm E} = \frac{M_S \int_0^{L^D I} dt}{F \, b_{es} V_{an} S} \, x \, 100\% \tag{1}$$

$$E_{\rm E} = \frac{M_i \int_0^{tb} P \, dt}{\Delta H \, S_i \, V} \times 100\% \tag{2}$$

where C_E = coulombic efficiency, M_S = molecular mass of O_2 , I = current (A), F = Faraday constant (96.485 C mol⁻¹ e⁻), b_{es} = mole e⁻ per mole O_2 , V_{an} = volume of anolyte (L), S = concentration of substrate in a single batch cycle (g/L). E_E = energy efficiency, M_i = molecular mass of substrate (g/mol), ΔH = enthalpy reaction (-2806 kJ/mol for glucose), S_i = concentration of substrate (g/L), and V = volume of anolyte (L).

Maximum power output or maximum power point (MPP) and internal resistance were measured with polarization technique which was made by stepwise the adjusted external resistance ranging from 68000 to 1.2Ω (68 k Ω , 50.5 k Ω , 40.5 k Ω , 38 k Ω , 12 k Ω , $2.1 k\Omega$, $1 k\Omega$, 560 Ω , 220 k Ω , 83 k Ω , 6.5 k Ω , and $1.2 k\Omega$) at the stable OCV value. The voltage data were read manually by using a multimeter digital (SANFIX DM-888D), five minutes after the resistance installed. Internal resistance ($R_{int.}$) was then calculated using equation (3):

$$R_{int.} = \frac{v_{emf} - v_{max}}{i_{max}} \tag{3}$$

Where V_{emf} is electromotive force and correspondents to OCV value, V_{max} and i_{max} are the highest voltage and current at the polarization curve, respectively.

3. RESULTS AND DISCUSSION

3.1. Voltage Production at Open Circuit and Close Circuit Mode

OCV is the potential difference between the anode and cathode electrodes, and does not depend on the presence of resistance. OCV measurement is performed to evaluate the maximum voltage of MFC (Erensoy et al., 2022), as there are no current and losses. Figure 4 (a) shows the curve of OCV value versus time for each MFC biocatalyst by five electroactive microbes isolated from the sediments of Waduk Saguling, and (b) the highest OCV value attained by these five bacteria. Figure 4 also confirms that these five isolates were electrogenic microbes capable of generating relatively high and stable electricity in the MFC system, that was above 600 mV in voltage generation. This result indicated that isolate LGf1, LGf11, LGf15, LGf20, and LGf22 have high electrochemical activity and fast adaptation in the MFC system fueled with glucose as the electron donor. Liu (2008) reported that conventionally OCV value of MFC ranging from 500 to 800 mV.



Figure 4. (a) Voltage in open circuit mode vs time, generated by five electroactive bacteria isolated from sediment of Waduk Saguling during 75 h of MFC operation, and (b) the highest OCV (*open circuit voltage*) value attained by these five isolates

Figure 4a shows that each isolate has different electrogenic capacity and electrochemical process. Isolate LGf1 and LGf11 could produce high electricity in relatively short of time in the MFC system, but then subsequently suffered a declining before obtained a new stability at its lower voltage values. Meanwhile, isolate LGf15, LGf20, and LGf22 probably need longer time to adapt to the new environmental condition (in the anodic chamber of MFC) and attained a certain number of cells that enable them to carry out higher electrochemical activity and achieved more stable electricity production.

As can be seen in Figure 4b, the highest OCV values attained by these five selected isolates were relatively higher than electrogenic microbes in other MFC studies, which also utilized glucose as electron donor and pure cultures as biocatalysts, such as *Rhodoferax ferrireducens* (Chaudhuri & Lovley, 2003), *Bacillus* sp., *Pseudomonas* sp., *Citrobacter* sp. (Saravanakumari & Angel, 2015), *Pseudomonas aeruginosa* (Ali *et al.*, 2017), *Staphylococcus saprophyticus* ICBB9554, *Acinetobacter baumannii* ICBB9557, and *Micrococcus* sp. ICBB9556 (Indriyani, 2017). Some electroactive bacteria are known to be unable to utilize glucose as a donor electron and prefer acetate or other

carbon sources. Hence, the use of various electron donors or carbon sources are recommended in further studies.

The application of microbial fuel cell is still facing some challenges due to its low power output and high internal resistance. It is desirable to obtain a stable and consistent power output from an MFC to support practical real-world applications. However, MFC often suffered fluctuations in electrical output due to the dynamic of microbial metabolic activities, the decreased of enzymatic catalytic activity, the accumulation of cell debris, and so on (Sun *et al.*, 2016). Electroactive microbes that can produce high and relatively stable electrical output are very potential to be developed in MFC technology, along with the enhancement of system design that allows the expected electrical output to be harvested. Figure 5 shows the CCV (close circuit voltage) production of the five selected bacteria on MFC with various concentrations of glucose in the anodic solution, under external resistance of 1000Ω . Zhang *et al.*, (2018) found that MFCs connected to external resistance of 1000Ω had a shorter start-up time than the lower resistance values (250, 50 and 10Ω). Hence, in this present work, the external resistance of 1000Ω was chosen.



Figure 5. Voltage production over time of MFCs biocatalyst by five electrogenic bacteria and fueled with various concentrations of glucose in close circuit mode during 125 h of operational time

The highest and relatively stable voltage output were achieved by MFC utilized isolate LGf11 as anodic biocatalyst, followed by isolate LGf22 and isolate LGf20, at glucose concentrations of 6 and 10 g/L, respectively. These results showed that the increase of substrate (glucose) concentration did not always enhance the MFC's electricity production. Isolate LGf11 produced its highest electricity (±350-400mV) during the first 80 hours of MFC operation at a glucose concentration of 6 g/L. Isolate LGf22 produced its highest electricity at the same glucose concentration but after 70 hours of MFC operation. The highest electricity production of isolate LGf20 was achieved at a glucose concentration of 10 g/L, after 100 hours of the MFC operational time with fluctuating voltage values in the range of 40-70 mV. Meanwhile, at glucose concentrations of 2, 4, and 8 g/L, the voltage output obtained by all bacteria had a relatively similar pattern, that was stable at low voltage values. This indicated the relatively stable electrochemical activity of these five isolates in the MFC system.

An increase in the glucose concentration which is not always positively correlated with an increase in electricity output was also found by Lee *et al.*, (2008) who studied the use of fermentable substrates (ie. glucose) and non-fermentable substrates (ie. acetate) for electricity generation in MFCs. They found that there is a diversion of electron flow to non-electricity sinks on fermentable substrates, which affects electricity production and energy efficiency in MFCs. Increasing the concentration of glucose resulted in a higher density of biomass and more organic residues. This leads to a slow electron transfer kinetics to the anode and causes loss due to the substrate concentration gradient in the anodic biofilm at the electrode surface.

Optimizing the utilization of various substrates or carbon sources that are more suitable for different microbes is a recommendation for further research. This is because some electrogenic microbes are known to be unable to metabolize fermentable substrates (i.e. glucose) as a carbon source and prefer non-fermentable carbon sources (i.e. acetate) (Lee *et al.*, 2008).

3.2 Power Density and Losses of the System

Ohmic losses were the dominant factor that affects the voltage drop from the highest OCV value could be obtained by MFCs biocatalyst by LGf1, LGf11, LGf15, LGf20, and LGf22. Some parameters of MFCs performance evaluation were shown in Table 1.

Anodic biocatalyst	OCV (mV)	R _{ext} (Ω)	V _{max} (mV)	i _{max} (mA)	I _{max} (mA/m ²)	R _{int} (Ω)	MPP
							(mw/m)
LGf1	688	2100	234	0.111	15.48	3846	3.62
LGf11	804	560	424	0.746	103.67	475	43.33
LGf15	750	2100	270	0.129	17.86	3733	4.82
LGf20	725	560	354	0.632	87.80	587	31.08
LGf22	502	2100	188	0.090	12.43	3507	2.34

Table 1. Bio-electrochemical activity of electrogenic bacteria and MFCs performance

 evaluated from the polarization technique

Note: OCV = open circuit voltage, R_{ext} = external resistance (external resistance value during polarization test that give the highest current and power output), V_{max} = maximum voltage output on close circuit mode with certain value of R_{ext} , I_{imax} = maximum current obtained at certain value of R_{ext} , R_{int} = internal resistance, MPP = maximum power point or maximum power output.

MFC biocatalyst by isolate LGf11 performed the highest MPP and lowest internal resistance among the five isolates, followed by isolate LGf20 with the second highest power density and the second lowest internal resistance. Meanwhile, the MPP values

of the other three bacteria were below 5 mW/m and their internal resistance were higher, that were above 3500Ω . These results showed that the losses in MFCs system were dominated by the large of internal resistance (ohmic losses). Moreover, greater the ohmic losses lower will be the power density due to a substantial amount of electrons will be lost to overcome the internal resistance (Khater *et al.*, 2015).

The power output of the MFC could be increased by reducing the internal resistance of the system, which can be achieved by increasing the electrolyte conductivity, surface area of the electrodes and membranes (or even eliminating the use of membranes), pH controlling, also by reducing the distance between both electrodes in anodic and cathodic chamber. Ohmic losses or internal resistance occur due to the present of some resistances in the transfer of charge/electricity as a consequent of resistances from the electrodes, electrolytes, membranes, and the distance between the anode and cathode electrodes (Rismani-Yazdi *et al.*, 2008).

Electrogenic microbes metabolize organic substrates in anoxic or anaerobic conditions and generate CO₂, electrons, and protons. The flow of electrons can then be harvested as electrical energy. Figure 6 shows the correlation between the concentration of glucose supplemented to the anodic medium toward the production of power density. In MFCs powered by isolate LGf1 and LGf20, an increase in glucose concentration have positive correlation to the increase of power density. In the case of isolate LGf11, LGf15, and LGf22, the increasing of glucose concentration did not correlate to the increase of power density (Figure 6). This showed the uniqueness of metabolic process and electrochemical abilities of different electroactive bacteria in converting certain carbon sources as their electron donors.



Figure 6. Correlation between various concentrations of glucose and the production of power output in the MFCs powered by different electroactive bacteria

3.3. Coulombic and Energy Efficiency

MFC performances were also evaluated in the terms of efficiency parameters, such as the percentage of substrate converted into electricity (coulombic efficiency) and the percentage of substrate that can be harvested as energy (energy efficiency). Figure 7 shows the coulombic efficiency and energy efficiency of MFCs powered by the five electroactive isolates at various concentrations of glucose. The highest coulombic efficiency (CE) and energy efficiency (EE) were achieved by MFCs utilized isolate LGf11 as anodic biocatalyst, with CE and EE values of 75.79% and 88.36%, respectively, in an anodic solution containing 6 g/L glucose. It means that 75.79% and 88.36% of substrate were converted into electricity and energy, respectively, by isolate LGf11 and could be harvested in MFC system.

The highest CE value next to LGf11 was performed by LGf1 with 39.84% (in 2 g/L glucose), followed by LGf15 with 20.7% (in 2 g/L glucose), LGf22 with 20.19% (in 6 g/L glucose), and LGf20 with 11.08% (in 4 g/L glucose). In the term of energy efficiency, the highest EE value next to LGf11 was obtained by LGf1 with 9.34% (in 2 g/L glucose), followed by LGf22 with 6.2% (in 6 g/L glucose).



Figure 7. Coulombic efficiency (CE) and energy efficiency (EE) of five electroactive bacteria in various concentration of glucose as electron donor and carbon source

As shown in Figure 6 that the increase of glucose concentration did not always have positive correlation with the increase of power density production in MFCs powered by different electroactive microbes, as goes with the coulombic and energy efficiency. Figure 8 shows the correlation between concentration of glucose and the efficiency of MFC system biocatalyst by five different electroactive microbes. This result showed the different electrochemical abilities of these five electroactive bacteria in converting glucose into electricity and energy. It is also showed that different substrate concentration delivers a highest efficiency for different electroactive microbes.





Figure 8. Correlation between concentration of glucose and the efficiency (coulombic efficiency [CE] and energy efficiency [EE]) of MFC system biocatalyst by five electroactive microbes.

4. CONCLUSIONS

The potency of microbial fuel cell as a green technology for harvesting bioelectricity was studied in this present work, exploited five electroactive microbes isolated from nutrient-rich sediment of Waduk Saguling. Among five bacteria, isolate LGf11 was performed the best electrochemical activity as anodic biocatalyst of MFC for electricity generation: highest OCV value of 804 mV, power output of 0.043 W/m, ohmic resistance of 475 Ω , coulombic efficiency of 75.79%, and energy efficiency of 88.36%. All five isolates were potential to be exploited as active biocatalysts for MFC due to their high OCV values and the stability of voltage generations, both in open circuit and close circuit mode, with fermentable substrate (glucose) as anolyte. The development of system configuration and the use of more suitable substrate (for example nonfermentable substrate like acetate, propionate, or butyrate) for different electroactive microbes in order to harvest more power output was recommended for further study. Utilization of these potential microbes for other applications in MFC (such as wastewater treatment etc.) was also suggested for further research.

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