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Research Article

Bioelectricity Generation From Single-Chamber Microbial Fuel Cells With Various Local Soil Media and Green Bean Sprouts as Nutrient

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ABSTRACT. In this experiment, seven single-chamber microbial fuel cells (MFCs) were made and filled with various types of local agricultural soil and sediments found in irrigation channels, which were mixed with glucose and green bean sprouts mashed as nutrients for microbial survival. MFC electric power was measured every day for 35 days. Every time low electric power indicated weak microbial activity, green bean sprouts were added. The highest electric power of 118 µW (23.4 µW/m²) was observed in fuel cells filled with agricultural land planted with rice. Power density reached the range of 120–140 mW/m², whereas the incubation time showed a maximum of 35 days. This study found that adding green bean sprouts can increase the length of the MFC cycle and strengthen the generated power up to 122 µW. ©2020. CBIORE-IJRED. All rights reserved

***Keywords*:** Local soil, redox, media, microbial fuel cell, incubation

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1. Introduction

Producing energy from organic waste is becoming increasingly important because energy production from fossil fuels has become more expensive and not environmentally friendly, and this causes climate change problems. Microbial fuel cells (MFCs) present as an alternative environmentally friendly technology to produce bioelectricity directly from materials that naturally decompose, such as organic acids, proteins, and carbohydrates (Chaturvedi & Verma, 2016), (Parkash, 2016). However, there are still some obstacles in the commercial production of these cells, such as the cost of fuel cell construction, the problem of increasing size, and the problem of increasing the ability to produce power density and contribute to an increase in output voltage. In MCF systems, the configuration of the anode and cathode catalyst can play a crucial role in improving the power density. Other important technical parameters in the design of MFCs include the distance of the two electrodes from the fuel cell (Logan & Rabaey, 2012; Pant *et al.*, 2011; Choudhury *et al.,* 2017), the anode and the cathode membrane separator (Biffinger *et al.,* 2007), the use of membrane-electrode assembly (Park, 2000), cathode type (Cheng *et al.,* 2011),(Rahimnejad *et al.,* 2015), (Ömeroğlu & Sanin, 2016), and the operating conditions of the system being operated, including pH (Zhang *et al.*, 2013) as well as the sustainability of batch versus flow system operation. Research on power density values in the range of 1–2.4 W/m² has been published with regard to the design of the MFC, with the addition of several parameters (Logan *et al.*, 2015; Helder *et al.*, 2012). The production of MFCs has several advantages when compared with material cell production, such as low cost and the absence of toxic mediators. For instance, a good system can be successfully made without the use of expensive selective membranes. The use of mixed membrane materials has been investigated in a number of articles about MFCs, and more recently, bioelectricity has been produced using energy sources from wastewater (Tharali *et al.*, 2016). With this substrate, the power density that can be produced by MFCs is in the range of 10–14 mW/m², whereas MCFs that use mixed media are reportedly producing slightly larger power density values from 0.3 to 3600 mW/m². Several researchers conducted a study by inserting artificial waste water into a reactor (He *et al.*, 2016; Xu *et al.*, 2016; Asai *et al.*, 2017) with mud media, and they observed a 20-fold increase in current

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density over 30 days, which made it possible to select beneficial organisms, even though the power density produced was not large enough at 1.3 mW/m^2.

Microbial fuel cells have previously used a two-compartment system, where aerated cathode compartments contain chemical solutions of iron and oxygen and anode compartments contain bacterial cells, electron mediators, and reduced substrates (Ucar *et al.*, 2017; Song *et al.*, 2019). Other soil substrate materials, such as paddy soil and river sediments, have been reported (Aziz, 2015; Xia *et al.*, 2017).

In MFCs, the substrate is one of the most critical factors that can influence bioelectricity generation because it functions as a source of nutrition and energy for the growth of the microorganisms involved. In most MFC studies, pure compounds, such as glucose (Rabaey et al., 2003), amino acids from cysteine (Logan *et al.*, 2005), and ethanol (Kim *et al.*, 2007), have been used for electricity generation. Other compounds that have been used include complex substrates, such as domestic wastewater (Liu *et al.*, 2009), starch processing wastewater (Min & Logan, 2004), and marine sediments (Min & Logan, 2004). The mixture of organic compounds in wastewater has provided information that a diverse microbial community plays an essential role in oxidizing organic matter because most exo-electrogenic bacteria can only survive if supported by various types of substrates (Wrighton *et al.,* 2010).

Therefore, this study aims to evaluate the potential of local rice fields and river sediments mixed with plastic soil in enhancing the bioelectricity produced by MFCs and maintain the survival of MFCs by adding softened mung bean sprouts as a nutrient. We chose to use mung bean soy sauce as a nutrient in the MFC system because it contains vitamin C compounds, ß-carotene, free amino acids, and reduced levels of flatulence-causing oligosaccharides and mineral-binding phytic acids (Liu, 2008).

2. Materials and Methods

2.1 Cultivation and activation of bacteria

Bacterial growth stages, bacterial cell preparation by *Escherichia coli*, and metabolite measurements were carried out according to Cao *et al.* (2006). *E. coli* bacteria were anaerobically planted for 24 hours in a media at 37 °C. The media consisted of 10.00 g/L of D-glucose (≥ 99.5% (GC) Sigma-Aldrich), 5.00 g/L of yeast extract (Granulated Millipore), 8.50 g/L of NaH_2PO_4 (BioXtra, ≥ 99.0% Sigma-Aldrich), and 10.00 g/L of $NaHCO_3$ (≥ 99.7% Sigma-Aldrich) under nitrogen–carbon dioxide gas (85:15) in a 250-ml bottle, which resembles 100% atmospheric nitrogen in a fuel cell system whose pH value is controlled (Zhang *et al.*, 2007). Glucose is added to the media after being treated in an autoclave. The results of this suspension are obtained by taking a phase-stationary culture at 4 °C through centrifugation at a speed of 5500 rpm (Rolfe *et al.*, 2012). The suspension that contains the cells is washed twice with a phosphate buffer solution that has a pH level of 7.0. Then, these cells are washed again with the same solution (pH 7.0)

The research consisted of several stages: (i) preparing samples of various soil types soil from rice paddies and local rivers around paddy fields located in the Kranjingan region, Sumbersari, Jember; (ii) inserting multiple types of soil media into the MFC unit and electrode placement; and finally (iii) measuring the time of incubation and power density of a single-chamber MFC with various types of soil media mixed using glucose at optimal concentrations (30 g/L) as a substrate (Jafary *et al.,* 2000).

2.2 Procedure of single-chamber MFC

Fuel cells are made of plastic in the form of a tube with a diameter of 5 cm and a height of 7 cm. This fuel system has a pair of electrodes, namely, a cathode and an anode, which are made of carbon fiber. The anode is connected with titanium wire and wrapped in green, whereas the cathode is connected with copper wire. Inside a container containing the substrate, the anode is positioned at a height of 1 cm from the bottom of the container, whereas the cathode is at a distance of 4.5 cm from the anode, such that the two electrodes are separated by the soil which serves as a microbial growth medium. Both electrodes are connected to the circuit boards that measure the current and voltage. Figure 1 shows the structure of a single-chamber fuel cell system.

Figure 1(c) shows the placement of an electronic measuring board (MudWatt, Keego Technologies, LLC, USA) on the cover of the MFC container. This board has eight pins that have different functions, as shown in Figure 1(c). The positive sign must be properly linked to the cathode electrode and the negative sign to the anode electrode, whereas pins 1 and 2 should be connected to the capacitor that functions as the LED light indicator (Misto *et al.*, 2019). The other pin is connected to the LED light.

2.3 Single-Chamber MCF system

The substrate is a main factor in electricity production in an MCF system. The type of substrate used was an organic compound (i.e., glucose) that can help the growth of active microbes. The types of soil media that were used include organic soil and plastic waste, soil covered with water spinach, fertile soil overgrown with rice, waste soil or land on the irrigation door, protected soil for chili plants, soil found 1 m away from organic soil + plastic waste, and soil found on a pile of rubbish.

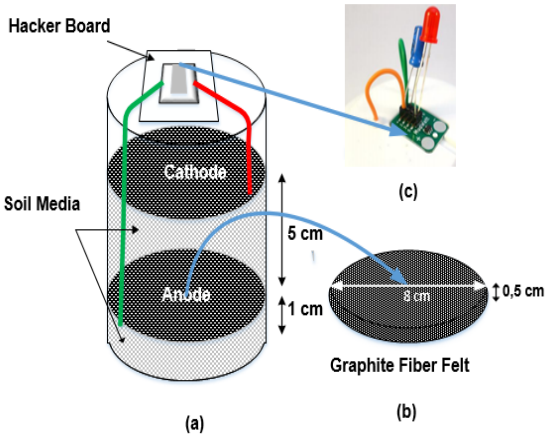

Fig 1. (a) Structure of single-chamber fuel cell with anode and cathode; (b) dimension of graphite felt anode; (c) MudWatt board for measuring MFC power.

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The substrate that was filled into the single-chamber MFC compartment was incubated for 7, 14, and 21 days. The time needed by microbes to form a biofilm at the anode is around 7 days (Sultana *et al.*, 2015; Baranitharan *et al.*, 2015). During this period, measurements of current, potential difference, and power output were carried out from the first day to the 37th day (Park, 2000; Aelterman *et al.*, 2008). At 7 days and above, it is expected that the current is strong and the voltage will increase, whereas at 21 days, it is expected that the values will be stable. All measurements were carried out in closed circuit mode with external resistance.

2.4 Polarization test and data recording

To determine the polarization characteristics, we needed an external resistance ranging from 10 kΩ to 10 Ω mounted between the anode and the cathode to produce strong current and voltage between the two electrodes, which are recorded every 60 s (Koók *et al.*, 2020). From these two quantities, the power output from the MFC will be monitored in real time using an NI USB-6211 multichannel data acquisition unit (National Instrument) every 5 min. This measurement is carried out at room temperature (or 29 °C).

The voltage of the fuel cell system is measured using an NI USB DAQ 6211 that has an ADC shield (16 bytes). Similarly, the electricity generated by the system was measured using the NI USB DAQ. Then, the value of this electric quantity was processed so that the electric power (mW) is obtained for each anode surface area. Power density was calculated using Eq. 1 (Marashi & Kariminia, 2015).

$$\text{Power (mW)} = \text{V (volt)} \times \text{I (mA)} \quad (1)$$

3. Results and Discussions

3.1 Effect of soil type on power at the beginning of incubation

Seven single-chamber MFCs were operated for 3 days with each type of soil. Using a circuit board, power measurements were taken every day at the beginning of incubation. As shown in Figure 2, the soil from plastic and organic waste and the fertile soil overgrown with rice produce the highest electricity compared to the other types of soil. These are followed by the type of soil on the irrigation door, the soil piled up in garbage, and the soil found within 1 m from the mixture of plastic and organic waste. Meanwhile, the remaining soil types produce power below 20 µW (3.9 mW/m²).

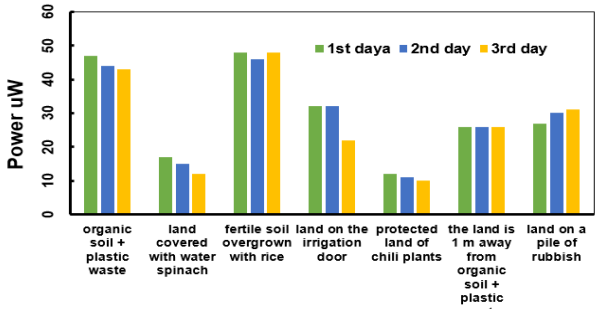

Fig. 2 Electricity produced by various types of soil at the beginning of incubation.

The different soil types were examined together with pure soil, as a reference. Soil that was mixed with waste will produce greater bioelectricity, whereas the reference soil as a control does not produce electricity. The type of pure reference soil is tested to determine that the electric power generated actually comes from the presence of microbial biodegradation activity and not from ionization or redox reactions between elements and minerals in the soil (Totsche *et al.*, 2018). Such results are supported by Fosso-kankeu *et al.*, (2015), who studied the "impact of soil type on electricity generation from a microbial fuel cell." They stated that the low power produced by the configuration of pure clay without compost (organic material) is mainly from microbial activity, although it is slightly increased by the soil. In their research, the results of the power density produced by MFCs reached the highest value of 0.09 mW/m². In this study, the results reached approximately 50 µW (0.992 mW/m²).

Furthermore, this study shows that applying fertile rice soil at the beginning of incubation (for 3 days) for energy generation resulted in the maximum effect of energy produced in the MFC (see Figure 3). The next lower maximum energy occurs in the application of soil that was covered by organic waste and plastic. This plastic-covered organic soil is thought to be a suitable environment for microbial growth. The next lower maximum energy was observed in the application of pile soil, which shows a continued increase from the first day to the third day. The next maximum energy occurs in soil applications that come from near the trash (1 m from the place), which showed consistent results at the incubation phase (days 1 to 3). However, in the other two soil applications, the results were quite low (less than 20 µW) and were not what you would expect at this phase. In this study, a trend of increasing power can be expected in soil use from phase 1 to phase 2, producing power above 40 µW (7.9 mW/m²).

3.2 Determination of the duration of bacterial incubation cycle in various types of soil

The amount of electric power produced by a fuel cell is largely determined by the type and amount of bacteria. The more bacteria that are active on the surface of the anode electrode, the faster the oxidation–reduction (redox) reaction. The rate of this redox reaction has an impact on the amount of electricity produced (Rabaey & Verstraete, 2005). According to Faraday's law, the amount of electric current produced depends on the transfer of electrons or the speed of reaction (Logan *et al.*, 2006).

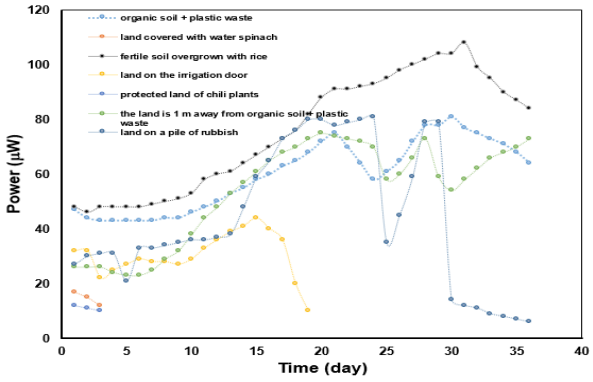

Fig. 3 Effect of incubation time on the power produced.

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Figure 3 shows two types of soil, namely, soil overgrown with water spinach and soil overgrown with chili plants, producing very little power. Bacteria in an MFC unit filled with these two types of soil can only survive for 3 days. The electricity produced is very small, which means that the number of bacteria active on the surface of the electrode is very small. On the other hand, the life span of only 3 days is caused by the very low nutrient content available. Figure 3 also shows the average power generated by the reactor during the study. Observations show that the maximum power values occur from the 15th day to the 24th day for each soil type, except the land on the irrigation door, which shows a continuous decline. After the 24th day, three soil types show a decrease except for one type (*i.e.* fertile soil overgrown with rice) which shows an increase until the 31st day.

Unlike other types of soil, the soil found 1 m away from organic soil and plastic waste and the soil on a pile of rubbish had a much longer cycle time of 35 days. Of the four types of soil, the unit filled with fertile soil overgrown with rice produced the largest electric power of 118 µW (23.4 mW/m^2), followed by units filled with organic and plastic waste soil and soil media piled up with trash, which produced the same power of 80 µW (15.8 mW/m^2**)**. On the other hand, a unit filled with soil found 1 m away from the organic soil and plastic waste produced power below 80 µW. Meanwhile, for units filled with soil found on the irrigation door, this unit only produced approximately 40 µW (7.9 mW/m^2) of power, with a cycle period of 19 days or half the period of the four types above.

Using various types of soil as MFC media where bacteria are active, as well as the diffusion of protons (Du *et al.*, 2007) leading to the cathode, produced variations in the incubation period (*i.e.* 3–35 days) and the power density (2.2–22.4 mW/m^2 when measured directly with MudWatt and 140 µW/m^2 when measured using the polarization method) as shown in Table 1. However, other research results show an incubation period of 6–9 days (Goto & Yoshida, 2016). When compared with the incubation periods in the literature, those found in this study have a very significant difference, where values can reach up to 1156%. However, measurements of power density show almost the same results (*i.e.* approaching 23 µW/m^2) despite using different types of soil media for the MFC units. The four types of soil produced large power density values as compared to the three types of soil media that used microorganisms, ranging between 29% and 88%.

3.3 Effect of adding green bean sprouts waste

From the seven MFC units that showed bacterial activity, only two units can become fuel cell candidates. The first candidate was an MFC unit that was filled with soil media originating from fertile soil planted with rice. Figure.4 shows that this MFC unit produced more electric power after adding nutrients. The power produced was only 108 µW before any nutrients were added, but after adding nutrients, power increased to 122 µW (24.2 mW/m^2), indicating a power increase of 14 µW (2.8 mW/m^2). Then, after 10 days of adding nutrients to generate electric power, power decreased to a value of 20 µW, with an incubation period of approximately 50 days. Meanwhile the second candidate was an MFC unit that was filled with soil media from land that was stacked with organic waste. This unit produced 104 µW of power, with an incubation period of 20 days, indicating that the life cycle of bacteria in this unit is much shorter compared to the first MFC unit.

The next cycle for the MFC unit containing land on pile of rubbish can still produce a power value of 109 µW (21.6 mW/m^2), which is slightly larger than the first cycle. The second bacterial life cycle takes longer than the first cycle because of the availability of more nutrients. However, the power produced remains lower than that of the first MFC unit. One possible reason would be the different types of bacteria that are active on the surface of the electrode, despite the fact that the amount of nutrients given to the MFC unit is the same. If Figure 4 is carefully analyzed, we will be able to map the cycle power density, as shown in Table 2.

Among the various types of soil that can be used as bacterial growth media in MFCs after maximum incubation, three types of soil are suitable media candidates for making MFCs. The three types of soil are organic soil and plastic waste, land on a pile of rubbish, and fertile soil overgrown with rice. Table 1 shows that land on a pile of rubbish gives the result of a more stable power profile compared to the other types of soil (Goto & Yoshida, 2016). The repetition cycle of adding nutrients produces the best reproducibility (22.4–23.4 mW/m^2) in electric power. This means that this type of environment provides a suitable environment with organic material reducing bacteria as an electron producer.

Table 1
Comparison of observations of the incubation period and power density to the literature (Khater *et al.*, 2017)

No	Type of Soil	Incubation Period (Day)	Power (µW)	Percentage of Incubation Period (%)	Power Density (mW/m^2)
1	Organic soil + plastic Waste	35	80	789	15.9
2	Land covered with water spinach	3	13	44	2.6
3	Fertile soil overgrown with rice	35	113	1156	22.4
4	Land on the irrigation door	18	42	367	8.3
5	Protected land chili plants	3	11	22	2.2
6	The land is 1 m way from organic + plastic waste	35	75	733	14.9
7	Land on a pile of Rubbish	30	80	789	15.9

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Table 2
Effect of adding green bean sprout waste

No	Type of soil	Power /Power Density	Number of cycles (20 days)
1	Organic Soil + Plastic Waste	75 uW (14.9 mW/m²)	1
2	Land on a pile of rubbish	113–18 uW (22.4–23.4 mW/m²)	> 3
3	Fertile soil overgrown with rice	80–122 uW(15.8–24.2 mW/m²)	2

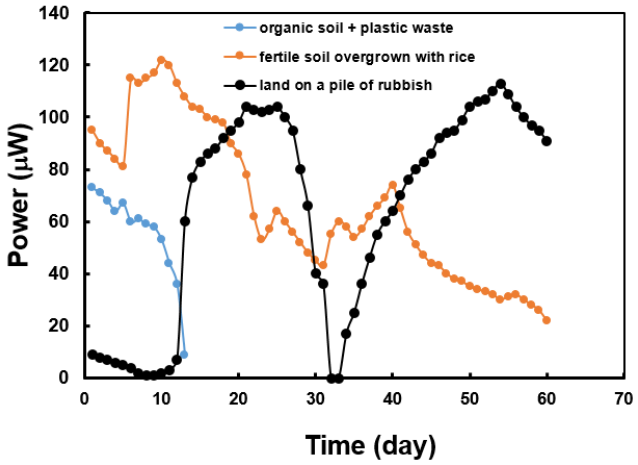

Fig 4. Effect of adding nutrients to bacterial activity in MFCs

Furthermore, the relationship between potential and current density can be explained by the maximum power density that can be produced by MFCs, as shown in Figure 5. Figure 5 shows the characteristics of voltage, current density, and power density of MFCs with a single chamber. A felt-type graphite electrode with a thickness of 1 cm is used as a cathode, and felt-type graphite electrode with a thickness of 0.5 cm is used in the anodic space. Figure 5 shows the current density and maximum power density in MFCs with soil media from river sediments that are larger than those using paddy soil media. The difference between the two values is close to 20 mW/m², at a voltage of about 0.5 V.

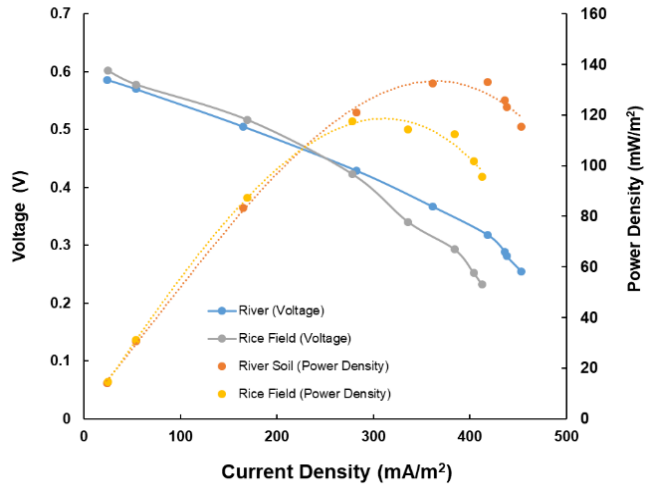

Fig 5. Relationship between polarization and power density to the current density produced by MFCs using soil media from river and rice field.

The results of this study indicate that the maximum power density values that were obtained were higher (120–140 mW/m²) than those achieved by Lorenso (80.3 mW/m²), Liu *et al.*, 2004 (25 mW/m²), and Vilas Boas *et al.* (5.04 mW/m²). These results will show that certain soil types produce high power density values.

Unique fuel cell electrochemical resistance models can be applied to explain MFC models, which can be expressed as follows (Zaidi & Rauf, 2009):

$$E = E^{o} - (\alpha + \beta \ln i) - R_{\Omega} i \qquad (2)$$

where E^{o} is the standard potential; E is the potential of cell electrodes, α and β are the terms for system or internal resistance, respectively; and *i* is the current density based on the anode area.

Figure 5 was further analyzed, and the final results that were obtained are shown in Figure 6. We divided the polarization curve area into three: activation loss, ohmic loss, and concentration loss (Kadivarian & Karamzadeh, 2020).

The existence of these three regions affects the effective voltage of the MFC. Furthermore, the three values from the area result in a reduction in the value of the voltage. By comparing the two curves above, results show that the effective voltage (V_{eff}) of the MFC with river ground media (0.27 V) is greater than that of the MFC with paddy soil media (0.20 V). The higher the V_{eff}, the higher the power generated. To calculate the V_{eff} of the MFC, we use the following equation:

$$V = OCV - \eta_{act} - \eta_{ohm} - \eta_{concen}, \qquad (3)$$

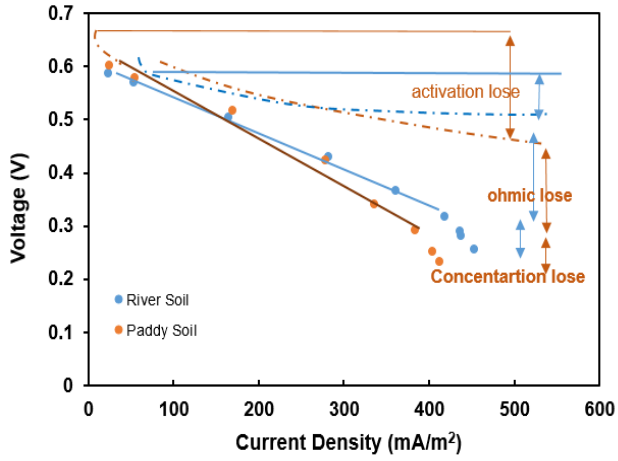

Fig 6. Analysis results of the polarization curve of a single-chamber MFC

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These terms represent the open-circuit voltage, activation voltage loss on the electrodes, voltage loss due to soil media resistance (soil conductivity), and voltage loss due to changes in substrate concentration consumed by microbes, respectively (Yasri *et al.*, 2019), (Kadivarian & Karamzadeh, 2020).

4. Conclusion

The type of soil media can greatly influence the electric power generated and the incubation period of the MFC unit. Soil types that contain organic matter produce more power density than ordinary soil types that have low organic matter content. An MFC can be regenerated to produce more power than previously untreated land. The maximum power density of 22.4–23.4 mW/m^2 was produced by an MFC with rice field soil, which contains a lot of organic waste with a life cycle > 3 (20 days) after the addition of glucose nutrition. More research are still needed to study various types of substrate or types of agricultural waste to get greater power density values and longer life cycles.

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