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Enhancement of Bioelectricity Production from Soil Microbial Fuel Cell (SMFC) by Additional Glucose, Nutrient Broth and *Escherichia coli* bacteria

(Peningkatan Pengeluaran Bioelectrik dari Sel Bahan Api Mikrob Tanah (SMFC) dengan Penambahan Glukosa, Nutrien Broth dan *Escherichia coli* Bakteria)

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ABSTRACT

Soil Microbial Fuel Cell (SMFC) is a device that using bacteria in soils as a biocatalyst. These bacteria, called exoelectrogenic bacteria are oxidizing organic substrates to release electrons, which then harvested in an external circuit to produce bioelectricity. Despite all the potential, the bioelectricity production from soils is still low and its relation with SMFC conditions is uncertain. Hence, the main objective in this study is to enhance and stabilize the bioelectricity production of SMFC by additional glucose, nutrient broth and Escherichia coli (E. coli) as exoelectrogenic bacteria. A number of factors of SMFC performance were first identified to be preliminary investigated, that is the type of electrode, water addition to soil and distance between anode to cathode. It has been established in this study to use SMFC with the configuration of 9.5 cm in diameter and 15 cm height of the plastic container, with the 12 cm distance between carbon felt of anode and cathode. The electricity produced was measured by using a multimeter in term of voltage reading (mV). From this study, the highest bioelectricity produced was obtained from SMFC using nutrient broth with a maximum voltage of 700 mV. It has found that the additional E. coli bacteria did not increase the bioelectricity production. The use of E. coli needed to be combined with nutrient broth in order to achieve high and stable bioelectricity. It can be suggested that the indigenous bacteria that exist in the soils possibly played the role in producing bioelectricity.

Keywords: Microbial Fuel Cell; Soil Microbial Fuel Cell; Bioelectricity; Exoelectrogenic bacteria, Escherichia coli

ABSTRAK

Sel Bahan Api Mikrob Tanah (SMFC) ialah alat yang menggunakan bakteria dalam tanah sebagai biopemangkin. Bakteria ini dinamakan sebagai bakteria exoelektrogenik bertindak mengoksidasikan substrat organic untuk melepaskan elektron, yang kemudiannya menghasilkan litar luaran untuk menghasilkan bioelektrik. Walau bagaimanapun, penghasilan bioelektrik daripada tanah tetap rendah dan kaitannya dengan keadaan SMFC masih tidak dikenalpasti. Oleh itu, objektif utama di dalam eksperimen ini ialah untuk meningkatkan dan mengstabilkan penghasilan bioelektrik SMFC dengan menambah glukosa, kaldu nutrien dan bakteria Escherichia coli (E. coli) sebagai exoelektrogenik bakteria. Faktor- faktor penyumbang kepada prestasi SMFC dikenalpasti dahulu iaitu jenis elektrod, tambahan air ke atas tanah dan jarak antara anod dan katod. SMFCdengan konfigurasi diameter 9.5 cm dan ketinggian 15 cm bekas plastik, dengan 12 cm jarak antara felt karbon anod dan katod telah dikenalpasti. Elektrik yang dihasilkan telah diukur dengan menggunakan multimeter dalam bacaan voltan (mV). Daripada eksperimen ini, bioelektrik tertinggi yang dihasilkan daripada SMFC ialah SMFC dengan tambahan 'nutrient broth' dengan voltan maksimum sebanyak 700 mV. Didapati, penambahan bakteria E. coli tidak meningkatkan penghasilan bioelektrik. Penggunaan bakteria E. coli perlu dikombinasikan dengan 'nutrient broth' untuk mencapai penghasilan bioelektrik yang tinggi dan stabil. Boleh dirumuskan juga bahawa bakteria indigenen yang wujud di dalam tanah mungkin memainkan peranan dalam penghasilan bioelektrik.

Kata kunci: Microbial Fuel Cell; Soil Microbial Fuel Cell; Bioelektik; Bakteria Exoelektrogenik, Escherichia coli

INTRODUCTION

Bioelectricity helps to reduce the emission of greenhouse effect compared to other forms of renewable energy. Bioelectricity can be an advantage in reducing municipal waste if waste is utilized, and thus give effect in the environment. For examples, and Microbial fuel cell (MFC) concept has been used to remediate the polluted wastewater sludge (Logan et al. 2006; Rahimnejad et al. 2015; Wolinska et al. 2014) and sediment (Min & Logan, 2004) and has been identified as potential bioelectricity sources. Also, in order to remediate the soil contaminated by phenol, MFC was implemented by Huang et al. (2011).

The power generated from microbial fuel cell (MFC) providing new opportunities for the sustainable production from organic and inorganic sources (Logan et al. 2006). Soil microbial fuel cell (SMFC) is one type of fuel cells that use an active microorganism (exoelectrogenic type bacteria) in the soil as a biocatalyst in an anaerobic anode for bioelectricity production (Rahimnejad et al. 2015) and it is considered renewables as the soil is renewable and easy to access. The fundamental knowledge on the relationship between the numbers of electricity generating bacteria or exoelectrogenic bacteria present in the soil still uncertain to maximize and stabilize SMFCs power output.

SMFC used microorganism in the soil as biocatalyst for the production of electricity. There can be billions of microorganism number in a single gram of soil. Good quality of soil supports healthy populations of soil bacteria. Bacterial counts in soil ranged from 4x 10^6 to 2 x10^9 g-1 dry soil (Whitman et al. 1998). Aelterman et al. (2006) confirm the importance of Proteobacteria as a pool of electricity-producing bacteria. Bacteria move from the natural electron acceptor to an insoluble acceptor which is MFC anode electrode (Rabaey & Verstraete 2005). Sediment MFC is designed which the anode is placed into the anaerobic sediment, rich with the exoelectrogenic bacteria and the cathode is placed into the overlying water containing oxygen (Liu et al. 2005).

The basic equation below shows the chemical reaction produces by microorganism in MFCs (Das and Mangwani. 2010). Generally, in anaerobic condition (lack of oxygen) at the anode, the type of anaerobic microbes decomposed sugars produces carbon dioxide, proton and electron in Equation (1). These electrons produced will transfer to the anode, it then travels to the cathode and reacts with proton and oxygen to form water at the cathode in Equation (2). The electron flow from the anode to the cathode will generate current and voltage to produce electricity (Prathipa & Karthikeyan. 2015).

$$C_{12}H_{22}O_{11} + 13H_2O \rightarrow 12CO_2 + 48H^+ + 48e \quad (1)$$

$$O_2 + 4e + 4H^+ \rightarrow 2H_2O \quad (2)$$

The main objective in this work is to enhance and stabilize the bioelectricity production of SMFC by additional glucose, nutrient broth, and *E. coli* as exoelectrogenic bacteria. The bioelectricity collected in SMFC was measured by voltage reading. The microbial counting was measured to monitor the presence of the bacteria in the soils.

Exoelectrogenic bacteria were mostly found in the anaerobic environment in soil. It was expected that increasing bacterial number by adding exoelectrogenic bacterial type into soil might increase and stabilize the voltage reading. Recent researches focus more on isolated and characterize the bacterial community contained in anode that may help in transporting the electron for producing electricity in MFC (Schroder et al. 2003; Xing et al. 2008). In this experiment, *E. coli* bacteria were added into the soil to observe whether these exoelectrogenic type bacteria were increased up and also stabilize the voltage reading.

E. coli are one type of gram-negative bacteria. They are known for their ability to produce electricity at higher power densities in MFC (Logan et al. 2008; Logan et al. 2009). Recent research shows that these bacteria give advantage for studying power generation in MFCs (Schroder et al. 2003; Xing et al. 2008).

Hence, in this study, soil in the SMFCs not only was treated with the bacteria but also the combination with substrates. As the additional bacterial may compete with existing bacteria in the soil for readily available source in the soil, substrate treatment may give additional supply to all bacterial communities to sustain their activity. Hence, enhance and stabilize the voltage production.

METHODOLOGY

EXPERIMENTAL SETUP

In this experiment, soils used were sampled form Nursery soil, located in Tampoi, Johor Bahru. The physical-chemical analysis of soil showed the following characteristics: pH 5.7, moisture 35%, Po (mg/L) 2.6, K (mg/L) 4.2, Br (mg/L) > 7.7, Fe (mg/L) < 1.0, Mn (mg/L) 0.24, Mg (mg/L) 5.6, Mo (mg/L) 0.30 and Na (mg/L) > 300. The different substrate which is nutrient broth (GLifeTech) showed the following characteristics: pH 7.4, peptone (mg/L) 5, NaCl (mg/L) 5, HM peptone beef extract (mg/L) 1.5, yeast extract (mg/L) 1.5. Other substrates are 10% and 20% glucose (GLifeTech) and *Escherichia coli (E. coli)* (ATCC 10799) were treated into soils. Mc conkey agar (MERCK 10546) was used for differentiate the negative strain colonies in plates.

SMFC CONFIGURATION

Different type of electrodes (aluminium, copper, steel, and carbon felt) was used as anode and cathode in the cookies beaker which had 15 cm height and 9.5 cm diameter. The sizes of electrodes for both anode and cathode were 9 cm in diameter.

Anode and cathode were separated by soils. Both anode and cathode were connected to the multimeter by using red and black copper wire respectively. The different conditions of SMFCs were described in Table 2 testing for 30 days of electricity production. Then, the experiment proceeded with determining the different distance of electrodes (4 cm, 8cm and 12 cm) over electricity production for about 21 days. Hence, the basic design of soils MFC in this work has been decided before proceeding to the next objectives. The output voltage was measured and collected daily by using a multimeter (Digital Multimeter ECS 820C). The component design of SMFCs used in this study is described in Figure 1.

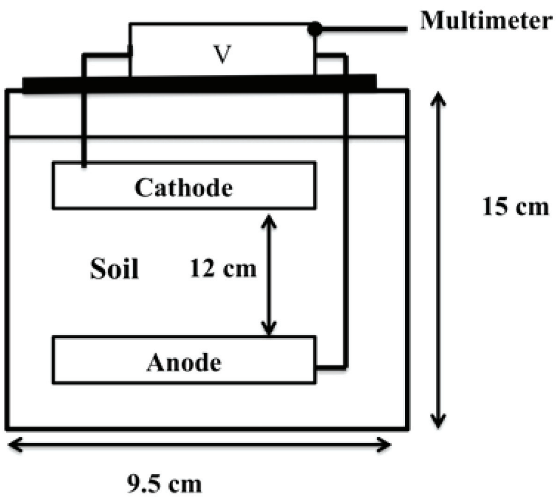

FIGURE 1. Component of Soil Microbial Fuel Cell (SMFC)

GLUCOSE, NUTRIENT BROTH AND *E. COLI* TREATMENT INTO THE SOIL

The bacterial with ~ 0.66 OD *E. coli* was treated into the soil. Other than that, the different substrates of Nutrient Broth (NB) and glucose with different concentration of 10% and 20% also were treated into the soil. Table 1 indicates the different treatment in the soil of SMFC.

In this study, the negative strain colony forming units per grams of soil (CFU/ g soil) were determined in the soil sample using plating method. Recent researches show most of the exoelectrogenic bacteria are known as the negative strain bacterial that contributes to the bioelectricity production (Feng et al).

RESULTS AND DISCUSSION

Table 2 described the different conditions of SMFCs set up. Water was added until submerged the cathode in SMFC1, SMFC2, SMFC3, and SMFC4 whereas for SMFC5 was dried cathode and SMFC6 was cathode in a moist condition. The different type of anode condition and electrode type in the SMFC were firstly determined in this study. No electricity produced on SMFC except for SMFC6 contained carbon felt with the moist condition**.** The reading produces on SMFC6 was 490 mV during 13th days.

Whereas Table 3 shows the maximum voltage produced within 21 days. The highest values of voltage which is 650 mV shown in 12 cm distance which is slightly higher than 590 mV for 8 cm distances. Similar to previous results reviewed in the literature Jang et al (2004), the higher distance between electrodes resulting in high voltage reading.

From the results in Table 2 and Table 3, it has been established in this study to use SMFC with the configuration of 9.5 cm in diameter and 15 cm height of the plastic container, with the 12 cm distance between carbon felt of anode and cathode. The amount of 1352 g soil was placed into SMFC. Hence, the basic design of SMFC in this work has been decided.

TABLE 1. SMFCs set-up

SMFC label	Treatment types	Weight soil SMFC (g)
SMFC-C	No treatment (Control)	1352
SMFC-NB	Nutrient Broth	1352
SMFC-10%G	1 mL of 10% glucose	1352
SMFC-20%G	1 mL of 20% glucose	1352
SMFC-E1	1 mL E. coli	1352
SMFC-E2	1 mL E. coli + 1 mL Nutrient Broth	1352
SMFC-E3	1 mL E. coli + 1 mL 10% Glucose	1352
SMFC-E4	1 mL E. coli + 1 mL 20% Glucose	1352

TABLE 2. Various SMFC first trial set-up testing for 30 days

SMFC label	Material type of anode	Material type of Cathode	Water addition	Voltage reading
SMFC1	Aluminium	Steel	Yes	NO
SMFC2	Aluminium	Copper	Yes	NO
SMFC3	Steel	Copper	Yes	NO
SMFC4	Carbon felt	Carbon felt	Yes	NO
SMFC5	Carbon felt	Carbon felt	No	NO
SMFC6	Carbon felt	Carbon felt	No (Originally moist condition)	YES

TABLE 3. The maximum voltage produced against the different distance between anode and cathode

SMFC	Maximum voltage (mV)
4 cm	90 ± 0.001
8 cm	590 ± 0.001
12 cm	650 ± 0.289

Table 4 shows /g soil with the maximum and final voltage production. SMFC-NB produced maximum 700 mV voltage after 23 days with 300 x 10^4 CFU/g negative strains. This was the maximum microbial counting CFU/g soil compared to other treatments. Both SMFC 20% Glucose and SMFC-E2 gives similar bioelectricity production which is 650 mV at 18 and 23 days respectively. The maximum counting microbial CFU/g soil for both of them was slightly similar which was >220 x 10^4 CFU/g soil. The maximum voltage obtained from SMFC-E3 which was 15.5 mV at 14th days, the lowest among others. It only contains 170 x 10^2 CFU/g soil. After 30 days, the voltage dropped to only 5.1 mV with less than 20x 10^2 CFU/g soil.

It can be seen from the result, the CFU/g soil increased with the increase of voltage number (mV) except for SMFC-10%G and SMFC-E1. Meaning that, there were a positive correlation between the abundance of negative strain bacterial in soil and production of electricity. However, it stills could not indicated that all the communities of this negative strain influenced the higher production of voltage. The microbial count dropped most of the experiment after 30 days. This might due to the lack of nutrient for microbial activity. Reasonably, bacteria will grow quickly in the food-rich environments. The lack of nutrients in the soil was one of the factors that inhibited the bacterial activity. Hence, reduced the voltage productivity and stability. The simpler substrate for bacterial growth has been suggested for the production of high power density which includes glucose and acetate compared to the complex substrate which hard for the microbes to digest resulting the low power production (Wolinska et al. 2014; Baranitharan et al. 2013).

TABLE 4. Voltages generated and microbiological CFU counting for negative strains in SMFCs

SMFCs	Maximum voltage			Days of result obtained after 30 days	
	Voltage (mV)	Days of the result obtained	Maximum microbial counting (CFU/g soil)	Final voltage (mV)	Final microbial counting (CFU/g soil) after 30 days
SMFC-C	560 ± 0.578	18	115 x 10^4	390	125 x 10^2
SMFC-NB[a]	700 ± 0.577	23	300 x 10^4	683	118 x 10^4
SMFC-10%G[b]	590 ± 0.000	17	150 x 10^2	490	143 x 10^2
SMFC-20%G[c]	650 ± 0.000	18	232 x 10^4	550	118 x 10^4
SMFC-E1	300 ± 0.000	29	151 x 10^2	300	150 x 10^2
SMFC-E2[a]	650 ± 0.000	23	225 x 10^4	630	200 x 10^4
SMFC-E3[b]	15.5 ± 0.000	14	170 x 10^2	5.1	>20 x 10^2
SMFC-E4[c]	620 ± 0.000	18	170 x 10^4	320	115 x 10^2

Note: The letter (a ,b ,c) showed a significant difference between two groups based on paired t-test statistical analysis

In this study, the glucose (10% and 20%) added into the soil was purposely to stimulate the bacterial activity. The nutrient broth used to treat the soil also contained additional composition ingredient of beef extract and peptone was also useful for bacterial growth. Beef extract and peptone ingredient included component of carbohydrates, organic nitrogen compounds, and salts. All these compositions have the ability to support the growth of bacteria in soil including *E. coli.*

Gram-negative bacteria have an outer membrane that involved in electron transfer to or from iron/manganese hydroxides. The final electron acceptor of an exoelectrogen is found extracellularly and can be a strong oxidizing agent in aqueous solution or a solid conductor/electron acceptor. Two commonly observed acceptors are iron compounds (specifically Fe(III) oxides) and manganese compounds (specifically Mn (III/IV) Oxides)

Figure 2 shows the voltage generated for all SMFCs within 30 days. There was zero voltage produce in day 1st for all SMFCs, it might because the bacterial was tried to adapt to a new environment. SMFC-NB started rapidly increase to above 100 mV at day 2 higher than other treatment, followed by SMFC-10% and SMFC-20% which were above 50 mV. Majority of SFMC (SMFC-20%, SMFC-NB, SMFC-E2, SMFC-E4) produced more than 400 mV after 13 days.

The highest voltage obtained was 700 mV shown in SMFC-NB at day 23, followed by SMFC-E2 which was treated with mixed *E. coli* and nutrient broth which was 620 mV. The statistical analysis showed a significant difference between the mean population of SMFC-NB and SMFC-E2. Treatment mixed bacterial and 10% glucose shown in SMFC-E3 produce lower voltage might be caused by the limitation substrate consumption for bacteria in the soil. The data for both SMFC-E3 and 10% glucose does show a significant difference. The voltage shown in SMFC-NB remains at steady state at 680 mV from 24th till 30th days compared with other treatment. The other treatments showed a decreasing voltage until the end of days.

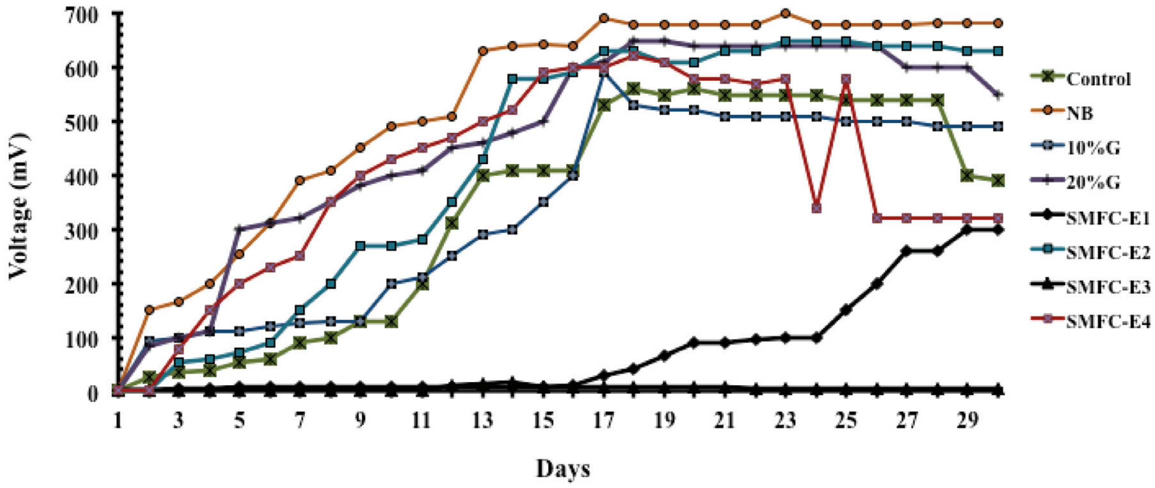

FIGURE 2. Voltage generated for all SMFC

From the results, it can be seen that by treated soil with nutrient broth was produce 700mV electricity highest than other treatment. Besides, it showed the most stable electricity compared to other treatment. This can be concluded that bacteria in the soil required other components instead of carbon in glucose only for stimulates their activity.

CONCLUSIONS

This study shows that the simpler substrate which is glucose-contain-carbon is required for microbial activity. However, by the addition of the other ingredient such as ingredient contained in the nutrient broth will enhance more bioelectricity production. In this experiment, SMFC treated with nutrient broth shows the highest voltage which is 700 mV compared to other treatment. Hence, nutrient broth treatment also can stabilize the bioelectricity production.

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