Electricity Generation by Locally Isolated Electroactive Bacteria in Microbial Fuel Cell

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Abstract Electroactive bacteria can transfer electrons to electrodes to generate electricity in the microbial fuel cell (MFC). Electroactive bacteria can generate energy for growth via the oxidation of organic compounds and transfer electrons to the electrodes that serve as the terminal electron acceptor. In this study, electricity generation in a double chamber evaluated MFC by four newly isolated electroactive bacteria strains (ESPK 22, ESPK 26, KP20, and KP22). ESPK22 and ESPK26 were previously identified as gram-positive Bacillus genera, while KP20 and KP22 belong to gram-negative Klebsiella genera. Among all the strains tested, the gram-negative KP20 strain shows the highest electricity generation value is 222.08 mV and the lowest electricity generation was ESPK26 of 44.82 mV.

1. Introduction
Recently, most attention has been given to renewable energy in conjunction with green materials as an alternative to providing the future potential increasing demand of consumption [1-6]. Microbial Fuel Cell (MFC) has gained intensive attention in the last decade due to the improved performance in generating electricity. MFC is a system that generates power from electroactive bacteria and conversion of energy, releasing metabolic reactions into electrical energy [7-9]. This system defines an environmentally friendly process because MFC produces electricity without the combustion of fossil fuels. MFC technology potential applications are in water treatment, biosensors, waste disposal, and electronic devices as summarized via infographic in Figure 1 [10, 11].
Indeed, MFC technology has taken the technical community's consideration for the opportunity to convert electroactive bacteria direct into electricity. Commonly, the electroactive bacteria found in food waste and sewage [12]. Electroactive bacteria are utilized due to the bacteria can move or transfer the electrons to extracellular electron acceptors and have the possibility to be used in devices such as bio-electrochemical systems (BES). BES is a unique system that can exchange chemical to electrical energy while forming microbes as the catalyst. In MFC, BES permit bacteria to do a respiration, oxidize, and reduce organic molecules [13].

Application of extracellular electron transfer in microorganisms for BES synthesis applying microbes to catalyze an anodic and cathodic biochemical reaction. While the anodic reaction is electron transfer from microbe to the anode are used for current manufacture. Indeed, cathodic is electron transfer from cathode to microbe recently applied for current consumption for valuable biochemical production. In microorganisms, extracellular electron transfer is utilized in the microorganism's metal reduction reaction, and in MFC, the metal is used as an electron acceptor [14-16].

A study of electroactive bacteria such as Shewanella and Geobacter has shown that both directions of moving for electron transfer would be possible. Mainly, Geobacter and Shewanella species are the two most extensively studied bacteria for extracellular electron transfer. Geobacter and Shewanella are metal-reducing gram-negative bacteria that can extracellularly transfer electrons, applied for BES [17].

MFC has a dual-chamber system consisting of an anode and a cathode detached by a proton exchange membrane. As a primary reactor, a dual-chamber system is used to study the electrode material, microbial activity, and parameter optimization. Generally, MFC used a conventional H-type reactor with an effortlessly maintained anaerobic environment for the anode but such a reactor had a higher internal resistance [18]. The previous research shows that S. oneidensis is the right candidate as an electroactive bacteria that can generate electricity thus, other bacteria from the genus Shewanella and Klebsiella are used to optimize electricity generation [19]. In this work, the discovered are on evaluating the electricity in MFC potential generated from genus Bacillus and Klebsiella.

2. Methodology
2.1 Electroactive Bacteria.
The new isolated electroactive bacteria strains were used in the study from Kuantan Port in Pahang and South China Sea. Shewanella oneidensis MR-1 strain was obtained from American Type Culture Collection (ATCC). Table 1 reveals the List of Electroactive bacteria isolated from Kuantan Port, Pahang, and the South China Sea as shown in Table 1.
Table 1. Electroactive bacteria sources and classification.

| Code    | Closely Related Strains               | Isolated Location    |
|---------|---------------------------------------|----------------------|
| ESPK22  | *Bacillus licheniformis* the South China Sea |                      |
| ESPK26  | *Bacillus velezensis*                  | South China Sea      |
| KP20    | *Klebsiella pneumonia*                 | Kuantan Port, Pahang |
| KP22    | *Klebsiella variicola*                 | Kuantan Port, Pahang |

2.2 Medium and Cell Culture.

Luria-Bertani (LB) media that were used in this experiment consist of 10 g l⁻¹ of tryptone, 5 g l⁻¹ yeast extract, and 5g l⁻¹ Sodium chloride (NaCl). Media were sterilized at 121°C for 1 hour for sterilization process that required in aseptic technique. In this experiment, *Shewanella oneidensis* MR-1 was selected as control and for comparison with the electricity generation with other electroactive bacteria. Electroactive bacteria were started by streaking cells directly from a frozen glycerol stock into LB agar on a plate. The plate that had been streaked was put in the incubator overnight at 30°C for the growth of bacteria which the bacteria from a single colony. The single colony of electroactive bacteria strains was transferred into 5 mL of LB broth in test tubes for preparing seed culture. Then, the culture was incubated overnight in an incubator shaker 30°C without the presence of oxygen. Autoclaved 50 mL of LB broth was prepared for inoculum that was used for anode compartment. 1 mL of the overnight culture was transferred into 50 mL of LB broth for inoculation and leave the inoculum overnight. Optical density (OD) was required to determine the growth of bacteria. OD reading for electroactive bacteria that involved in MFC was a range 0.8–1.0 which was that the mild log phase where the bacteria was still fresh to generate the electricity.

2.3 Double Chamber Microbial Fuel Cell.

Double chamber MFC was operated using two glass jars with a diameter 20 mm were occupied and notice electroactive bacterial as cathode and anode. The double chamber consists of two holes of diameter 6 mm and 1.5 mm on each of the lids for the insertion of the electrodes. In the anode compartment 40 mL of the inoculum was used and for both anode and cathode compartment, fill the compartment up until 400 mL of volume with the substrate. Both chambers of MFC possessed an arm ending in a joint where a proton exchange membrane (PEM) such as Nafion which this PEM soaked in 1% of HCl solution for 24 hours before use in the MFC. Nafion was wrapped between the linkages and clamped with a pinch clamp. The upper opening of each chamber was closed with cotton and wrapped using aluminum foil. The electrode materials that used in this experiment were a graphite plate where this electrode was cut into 5 cm X 5 cm dimensions. The whole of the MFC was autoclaved at 121°C for 1 hour to sterilize all the compartments so that this method can reduce contamination for the MFC. The substrate was poured into the anode and cathode compartment while for the anode part, inoculum was transferred which this MFC was run for 24 hours to indicate the electricity generation. All the steps were repeated for all type of Electroactive bacteria and abiotic to detect the electric generation. By using the true root mean square (TRMS) multimeter, the reading of electricity generation voltage
from MFC was recorded. Interpret data of generating electricity was transferred via the wireless device then was interpreted with the software EX540 that was installed on a laptop. The voltage values that are transferred to the laptop can differentiate the amount of electricity generated. The measurement of voltage was recorded in millivolts (mV) which the reading was taken for 24 hours. TRMS multimeter was connected to MFC using crocodile clips.

3. Results and Discussion
The current generated by electroactive bacteria and abiotic control was conducted for 24 hours. LB broth acted as the substrate in this experiment to indicate current generation from *Shewanella oneidensis* MR-1 which acts as the control followed by *Klebsiella pneumonia* (KP20), *Klebsiella varicola* (KP20), *Bacillus licheniformis* (ESPK22), *Bacillus velezensis* (ESPK26), and abiotic. The data measured was recorded using a TRMS meter.

Figure 2 shows the result of the electrical generation in double chamber MFC. The generated electricity of electroactive bacteria strains is MR-1, KP20, KP22, ESPK22, and ESPK26, while abiotic measured the generation of electricity without the presence of electroactive bacteria in the substrate. Overall values of generating electricity for both electroactive bacteria and abiotic are in positive value. Based on the five electroactive bacteria, KP20 has the highest electricity generation value of 222.08 mV while abiotic the highest value of 51.54 mV. The lowest value of 44.82 mV obtained for electricity generation among five types of electroactive bacteria strains is ESPK26. The abiotic value showed the lowest value of 44.19 mV.

![Voltage vs Time Graph](image)

**Figure 2.** Electricity generation of Electroactive bacteria in the double chamber of MFC for 24 hours.

Overall electroactive bacteria show the increasing value for electricity generation. These bacteria are gradually increasing the electricity generation when approaching 24 hours. The trend for electricity generation of KP20 steadily increases from 1 until 20 hours. For KP22, the graph's pattern slightly increases from 1 until 19 hours with the growth of KP22 was in the range of 4 mV per hour. The value
at 19 hours is the highest for KP22. Then, it gradually dropped in value for electricity generation. The iron reduction previous studies focused on dissimilatory iron, reducing bacteria to elucidate the specific mechanisms responsible for microbial metal reduction. To generate the energy throughout the anaerobic condition, dissimilatory iron reduction used iron (III) (Fe^{3+}) as a terminal acceptor. *Shewanella* and *Geobacter* are model organisms for discovering the biochemistry that enables the dissimilatory reduction of extracellular electron acceptors.

The generation of electricity can be explained by the effectiveness of electroactive bacteria to transfer electrons out of the cell to produce electricity. For the greatest portion of consuming the same external membrane cytochromes which can reduce Cr (VI), resting cells of *Shewanella oneidensis* MR-1 can also metabolically reduce ferrie iron [20]. Hence, all electroactive bacteria for this work can reduce iron (III) to iron (II) to generate energy for electricity production. Reduction of Fe (III) minerals can be microbiologically by firmly anaerobic or facultative electroactive material where the electroactive bacteria used Fe (III) as a terminal electron acceptor to produce electricity. A previous study indicates that *Geobacter* necessitates direct interaction with insoluble Fe (III) oxide surfaces for dissimilatory growth on Fe (III) minerals [21].

KP20 indicates the highest value for electricity generation due to the higher reduction of iron. KP20, Gram-negative, and facultative anaerobic has the higher potential to be the newly isolated bacteria that can generate electricity such as *Shewanella* and *Geobacter*. On the other hand, ESPK26 (negative-positive bacteria) indicates the lower electricity generation because of the lower iron reduction.

The dissimilatory iron reduction that can generate electricity can apply extracellular electron transfer (EET) in this process. All organisms can transfer electrons out of the cell from electroactive bacteria strains that electrons will accept by the electrode. Although electroactive bacteria strains can produce energy using diverse strategies, a growing number comprises electron transfer to or from extracellular substrates. Electron transfer between microbes and minerals such as iron and manganese hydroxides can define EET. EET is related to using a substrate either in solid or liquid where that substrate can interact with the cellular electron transport chain through a protein that resides on the cell surface and the extracellular substrate.

Figure 3 explains the mechanism of the extracellular transfer of electrons. As electroactive bacteria are metal iron reduction, bacteria can transfer electrons out of the cell to generate electricity. Metal such as Uranium, Vanadium, Arsenic, Chromium, Technetium, and Selenium are soluble in the substrates for anaerobic respiration. An analogy for extracellular respiration where the substrate such as iron (hydr)oxide and it reduce product, ferrous iron where a protein that resides on the cell surface (orange oval) directly interacts with the extracellular substrate to transfer an electron from the cell to the substrate.

![Figure 3. Mechanism of metal iron reduction by bacteria.](image)

The nutrients contained in the substrate (LB broth) can detect the flow of electrical generation. Moreover, the iron reduction bacteria transfer electrons out of the cell where the electron is moving towards the electrode as an electron acceptor. The electron will move from anode to cathode so that the electricity generation can be detected. Then, electron transfer from the electroactive bacteria and
reaction of an electron with the electrode can define for extracellular respiration for this process. During extracellular respiration, the substrate is used as an electron donor, and because of that substrate can detect electricity generation.

4. Conclusion
For genus Klebsiella sp., KP20 indicates the higher electricity generation with maximum electricity is 222.08 mV compare to KP22 of 115.72 mV. While Genus Bacillus sp. powered the maximum generation of electricity of ESPK26 and ESPK22 are 107.92 mV and 105.43 mV, respectively.

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