Assessment of Power Generation from Dewatered Sludge using Membrane-Less Microbial Fuel Cell

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Abstract. Water and energy security are gaining high interest of many researchers and intensive exploration took place around the global. Membrane-less microbial fuel cell (ML-MFC) has been emerging as one of the popular wastewater treatment-based technology to provide clean water and green energy. MFC are bio-electrical devices that harness the natural metabolism of electrogenic bacteria (EB) to produce electrical energy. In this study, Bacillus subtilis (BS) was used to catalyst the transformation of carbon source in dewatered sludge into renewable energy. From the study, the MFCs were tested to see the robustness of the MFC by exposing them to the ambient temperature (25± 1°C) with the parameter of pH (6), electrode distance (6 cm), initial moisture content (30 % vol/wt) were set as constant. The result focused on the performance of the ML-MFC during noon (8-10 am and 4-6 pm) as these were the periods which BS recorded actively growth (increment of ±5.167 × 10⁻³mg/L of biomass per day). The ML-MFCs were carried out for 7 days incubation period and the BS growth reflected significantly on the voltage and power generated. The highest voltage and power density were recorded which were 90 mV and 8.793 Watt/m² (at morning on 6th day), respectively. Moreover, observation gram staining of BS under a light microscope indicated a purple appearance due to thick peptidoglycan layer of the cell wall. Obviously, this study could be the bench mark of the practicality of the MFC technology which projected to be implemented in remote area where the natural environment condition is the surrounding parameter of the MFC same like in current study.

1. Introduction
Issues involving competing land use as well as environmental and political may cause sustainable development for the extraction of natural resources in most of regions to be restricted [1]. Thus renewable energy discharged to integrate global concerns, such as climate change for increase substantially the share of renewable energy in the global energy mix [2]. The microbial fuel cell (MFC) is a technology that uses electrochemically active bacteria as a catalyst to oxidize organic and inorganic matter to generate current [3]. The MFC consists of an anode compartment where the electrons are released by electrochemically active bacteria [4] and transferred to the electrode [5]. The electron transferred to the electrode in the anode flow via an electrical circuit with a power harvester towards the cathode compartment where the electrons are consumed by a reduction process [4]. There
are varieties of MFC architecture; for example, single chamber, double chamber, and tubular membrane MFC had been used to produce energy generation [4]. The Nafion membrane used as proton exchange membrane (PEM) and ferricyanide solution which function as protons transfer and electron mediator respectively, also to separate the oxidation and reduction process. As eloquently stated by Bruce Logan, (2009) and Li (2008), both ferricyanide and PEM are not economical and non-environmental friendly in fabrication of the MFC [8][6]. Membraneless MFC implemented in this experiment can enhance and support the usage of eco-friendly material in conducting the mechanism plus electrical energy generation simultaneously. Common and familiar strain such as Rhodoferax ferrireduces, Shewanella putrefaciens and Clostridium butyricum. Rhodoferax ferrireduces were used in two chambers MFC vessel connected with cation-selective membrane which was inoculated into anaerobic condition, while Shewanella putrefaciens as metal–reducing bacteria was set up with ferricyanide as a mediator and both MFC configuration resulted a slow output of power and voltage [9][10]. While in other research study by Schröder (2003) highlighted Clostridium butyricum species was set up in form of immobilized cells and substrates were lactate which reported as form of hydrogen evolution [11]. It has been reported that hydrogen evolution; platinum as electrocatalyst for hydrogen oxidation had caused low power densities also poison the MFC media [12]. However, there was lack information on study and research of membrane – less single chamber MFC using Bacillus subtilis as a biocatalyst and dewatered sludge which end product of wastewater treatment used as the substrate. Bacillus subtilis is a bacteria which commonly used in the fermentation production due to easier cultivation [9]. Moreover this bacterial reaction, easily carried out over several different temperature ranges depending on the tolerance of the bacteria toward moderate or room – temperatures (15 – 26°C) [14].

2. Materials and Methods

2.1 Sample collection

Dewatered sludge was collected from MWTP Kerian Indah Water Treatment Plant Parit Buntar, Malaysia to conduct ML-MFC. The sample was extracted from drying bed at MWTP Kerian Indah Water Treatment Plant Parit Buntar, Malaysia. Then the sample was kept under precaution method proposed by Muaz et al [15] to maintain its freshness.

2.2. Preliminary study

2.2.1. Characteristics of dewatered sludge. Atomic absorption spectrophotometry (AAS)(GBC model 903, Australia) was used to detect trace elements such as nickel (Ni), zinc (Zn), magnesium (Mg), iron (Fe) and Manganese (Mn). Preliminary treatment on the dewatered sludge using acid digestion should be handled as described in Muaz’s method [15]. 1 g of dewatered sludge was added with nitric acid (HNO₃) by ration 1:1 in the digestion vessel. The vessel was watch glass covered and heated at 95 ± 5°C and refluxed for 10 – 15 minutes without boiling. Then, the sample was cooled down to room temperature. 5 mL of concentrated HNO₃ was added to the sample and the reflux process was repeated for 30 minutes. If there was a brown fume generated HNO₃ been added and this step was repeated until no brown fume generated and this step was repeated until no brown fume discharge the sample.

2.3. Analytical method

2.3.1 COD analysis. The organic compounds in dewatered sludge had been analyzed by a COD digester (Checkit Direct, Lovibond). The dewatered sludge sample weight 1 g was diluted in 10 mL of distilled water then was vortexed for 3 minutes. Later the vortexed sample centrifuged at 4k rpm for 5 minutes. Syringe filter (MF – Millipore Millex GS syringe filter with pore size 0.22 μm) was used to filter the sample. The COD vials consist of premixed chemicals (K₂Cr₂O₇, AgNO₃, HgSO₄, Potassium
hydrogen phthalate, H$_2$SO$_4$) was added with 2 mL of the filtrate. The COD vials were digested at 150 °C for 2 hours. After 2 hours, the content of the vials are cooled down to room temperature. The COD kit (Checkit Direct, Lovibond) was used to measure the COD value. A blank sample was prepared by adding 2 mL of distilled water into the vials.

2.3.2. *Macro and micro-nutrient.* The characterization of macro-nutrients, micro-nutrients in dewatered sludge was carried out using an elemental analyzer (PerkinElmer 2400 Series II) as described in Muaz’s method [15].

2.3.3. *Biomass of Bacillus Subtilis species.* Sludge with mass of 10 g would be taken from the ML-MFC. Based on the standard method [16], the growth of BS in the ML – MFC can be measured. In order to know the BS specific growth rate ($\mu$), the equation was represented in equation (1):

$$\mu = \frac{\ln (X_2 - X_1)}{(t - t_0)}$$

Where $X_2$ is final biomass concentration, $X_1$ is initial biomass concentration, and $t$ is time recorded. Meanwhile doubling time can be calculated based on cell numbers and the net specific rate of replication. Thus, the doubling time, $T_d$ can be calculated by using equation (2) below:

$$T_d = \frac{\ln 2}{\mu}$$

where $T_d$ is doubling time when the cell starts to multiply itself which resulting cell number density and cell mass increase exponentially with time.

The biomass of BS that growth in the ML-MFC was obtained from UV-Vis spectrometer at 600 nm. The value then was correlated to the sludge calibration curve ($y = 0.529x - 0.024$) to get the actual biomass value.

2.4. *Biochemical Test by Gram-Staining of Bacillus Subtilis*  
1 loop of *Bacillus subtilis* (BS) was taken out from stock culture media using inoculation loop. The culture smear onto glass slide in laminar flow to minimize contamination. Briefly 1 drop of crystal violet was applied to the glass slide for 10 to 60 seconds at room temperature, and slide were briefly rinsed with water to remove excess crystal violet. 1 drop of iodine was applied onto slide with BS culture for 60 seconds and washed with water. Decolorizer or alcohol solvent was applied to the slides for 5 second in order to remove any non-specific crystal violet staining, when the solvent was no longer colored as it flows over the slide. Then 1 drop of safranin was introduced in order to counterstain for 40 to 60 seconds. Final step, the slide was washed with running water [17].

2.5. *Construction of ML-MFC*  
ML-MFC was built using cylindrical glass reactors (diameter: 10 cm; height: 10 cm) (Fig. 1) with the radiuses, thicknesses and surface areas of the graphite felt electrodes (anode and cathode) were 3.6 cm, 0.65 cm, and 0.00407 m$^2$, respectively. The initial pH (6), electrode distance (6 cm), and initial moisture content (30 % vol/wt) were set as constant and the configuration was setup as used by [15].
2.5.1. **Determination of power using polarization curve technique.** MFC performance can be evaluating by polarization or voltage-current curve. The ML-MFC was connected to a multimeter to record cell voltage at different external resistances (47, 100, 220, 470 and 1000 Ω) and the power was determined based on Ohm’s law as presented on both equation (3) and (4) as a function of power (Watt). The polarization curve was plotted throughout the voltage and current measurements. The peak of the power curve was the maximum power of the ML-MFC [18][19].

\[ R = \frac{V}{I} \]  
\[ P = VI \]  
\[ P = \frac{VI}{A} \]

Equation (3) and (4) are Ohm’s law equation meanwhile equation (5) is power density equation where \( R \) is resistance \( V \) is voltage, \( I \) is current, \( P \) is power and \( A \) is area.

3. **Experimental Design**

3.1. **Operation of ML-MFC**

The distance between electrode, moisture content, and temperature were set at single chambered at the bottom, 6 cm on top of anode as sketched in Fig. 1. The voltage generation and COD removal of ML-MFC were studied. The temperature of the ML-MFC was set at ambient temperature(25± 1℃). Throughout the experiment, the ML-MFCs were incubated for 7 d and the samples were collected for COD analysis and the voltage was recorded every 12 h using digital multimeter.

4. **Result and Discussion**

4.1. **ML-MFC Performance**

The electricity generated by the ML-MFC with the dewatered sludge as carbon source later converted to a simple substrate was determined by polarization curve as stated above on equation (3) to equation
According to Huang (2014) and Larrosa-Guerrero (2010) research outcomes, by having open circuit voltage (OCV) system the faster electrogentic bacteria (EB) would grew compared to the MFC under close circuit (CC) [21][20]. Thus, the study focused on the impact of OCV and the voltage profiling results (Fig.2).

![Figure 2](image_url)

**Figure 2.** Average open circuit voltage of *Bacillus subtilis* profile in ML-MFC.

*Figure 2* showed that the voltage having an increment drastically about 65mV on second day. These could be due to the adaptation process by BS into the new environment in ML-MFC, thus the carbon source in the ML-MFC was slowly consumed. Then the voltage started to increase steady and linearly from third day until fourth day with 66 mV and 79 mV respectively. Increment of voltage increased at peak on day 5 (88mV). Then the voltage in ML-MFC declined on seventh day (80 mV).

This reflected that BS reacted well for six day in the ML – MFC thus acceleration of the metabolic rate of BS results in rapid growth of the bacteria and high voltage output [20]. Furthermore, the rate of electrochemical reactions on electrodes and the rate of proton transfer were increased with increasing temperature [22]. Salam(2020) gave an evidence that a small amount of entropy due to rise of temperature indicates better and stable performance in a fuel cell [23]. Equation (6) provides an overview of reduction reaction occurred at the cathode with involved of complex constituents of dewatered sludge which is complex sugar to simple sugar [24] by aided of microorganism thus this reaction also represent the phenomenon that occur inside ML-MFC itself. Sugar reduction by BS culture occurs inside ML-MFC significantly described by equation (6).

\[
C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2
\]  

(6)

Where \(C_6H_{12}O_6\) is glucose, \(CH_3CH_2OH\) is ethanol and \(CO_2\) is carbon dioxide.

Result of gram staining test proved the research done by Nimje et al. (2009) which reported that aerobic gram-positive BS species had the ability to produce biofilm in the surface of anode electrode in MFC [25]. Maximum COD removal up to 84% and 90% was obtained for ML-MFC which inoculated with BS and dewatered sludge was achieved at steady-state condition. Steady-state condition defined as the subject of many MFC studies for understanding proper anode potential thus preferred to keep high biomass where implemented for high power density [26].
Table above proved that BS had the ability to produce electrogens which are reasonable and important bacterial strains for electricity production where able to utilize a wide range of substrate because they have ability to produce electric power in mediator less [27][28]. The morning session (25±1°C) was an effective surrounding effect as BS reacted well (0.11 mg/L biomass), thus tally with research by Komanthi (2017) which found MFC operated well when the temperature not exceed35°C [29]. Figure 4 showed BS more energetic on morning session than evening session with 74mV on third day to 92mV on sixth day. This may due to high intensity of light catalyzed the metabolism of BS, thus increase dramatically the carbon consumed by BS. As the result, it increased the voltage and recorded higher voltage at morning session (25±1°C) compared to evening session (23±1°C). This proved that temperature had a very significant effect on voltage generation and this tally with the research by Min et (2008) that stated increment of temperature boosted the intracellular biochemical reaction rate and the growth rate of EB [30]. It was also influencing the rates of enzymatically catalyzed reactions and increased the rate of diffusion of organics in the dewatered sludge to the cells (31).
Figure 4. Open circuit voltage profiling for morning and evening of *Bacillus subtilis* in ML-MFC.

Figure 5 illustrated polarization curve that can be divided into three main region; (i) activation losses, (ii) ohmic losses and (iii) mass transport effects [32]. This curve was the most common method of representing the performance of ML-MFC. Based on Fig 5 (i) activation losses occurred when bacterial surface on the electrode transferred energy for oxidation/reduction reaction. The slowness of the reaction seems to fairly distribute over temperature which affected both current and voltage. (ii) ohmic losses or voltage drop may cause by ions resistance that occurred in the ML-MFC where dewatered sludge as the medium that contributed to complete the electrical circuit assisted by anodic and cathodic electrolyte respectively, and (iii) mass transport effect also called as concentration loss happen when reduction in concentration is the result of a failure to transport sufficient reactant to the electrode surface.

The polarization curve as a function of current, potential and power measured at variable external resistance $R_{ext}$ between (47 – 1000 $\Omega$). The dominance of activation loss was observed from the initial step decrease in the voltage from value at 81 mV to 52 mV. The subsequent slope of the voltage decreased linearly which might indicate the dominance of ohmic losses. The polarization experiment showed that ML – MFC was able to supply a maximum power for 57.5mWatt, 52 mV under load of 47 $\Omega$ (Figure 5). The curve depicts a maximum power density of 7.068 Watt/m$^2$ at resistance 47 $\Omega$. 
Figure 5. Polarization curve of *Bacillus subtilis* in ML-MFC on sixth day.

Figure 6 illustrates comparison voltage and power density on different session aided by polarization curve as a function of current, potential and power measured at variable external resistance $R_{ext}$ between (47 – 1000 Ω). The dominance of activation losses for both morning and evening session were observed from the initial step decreased in the voltage from 90mV to 83mV (Fig 6A) and 72mV to 69mV (Fig 6B) respectively. The results obtained from the activation losses for both morning and evening session occur when energy is lost as heat for initiating oxidation and reduction process. Figure 6A shows the ohmic losses on morning session; 0.09 to 0.377 mA, almost linear with subsequent slope of the voltage decreased similarly to evening session (Fig 6B) with 0.072 to 0.305 mA. The low voltage drop from 66 to 58 mV (Fig 6A) and 60 to 46mV (Fig 6B) and maximum current obtained at lower resistance which is 47 Ω for both Fig 6A and Fig 6B were revealed that a lower potential drop and lower mass transfer losses at the electrode. The effective electron discharge observed at the lower resistances was due to further potential drop and slow stabilization of voltage at lower resistance. Based on [33] maximum voltage output of MFC $V_{max}$ can be obtained proportionally with maximum power also when the external resistance equals to internal resistance based on calculation as follows:

$$R_{int} = R_{ext} \quad (7)$$

Where $R_{int}$ is internal resistance and $R_{ext}$ is external resistance.

Both (Fig 6A) and (Fig 6B) were showed a curve depicts a maximum power density of 8.793 Watt/m² and 5.531 Watt/m² respectively under load of 47 Ω. As conclusion, power density produced on morning (high temperature) session was indicated as the optimum power generation compared to evening session (low temperature).
Figure 6. Comparison of polarization curve of *Bacillus subtilis* culture (A) morning, and (B) evening session on sixth day.

Figure 7 displayed overall power density that was generated by BS for seven days. The power generated by BS started initially at 5.416 Watt/m². Second day seemed to drop about 2.821 Watt/m² due to adaptation of BS in ML-MFC later on the sixth day the highest power density generated was 7.162 Watt/m².
Figure 7. Optimum power density of *Bacillussubtilis* culture in ML-MFC.

Table above showed the specific growth and doubling time for BS are 0.287 g/l/h and 2.42 h respectively. These results confirmed the association between specific growth rate and doubling time which quick doubling time proportionally maximum biomass. These results further support the idea which by having higher value of $\mu$ will achieve higher biomass, thus increase the rate of COD consumption and it reflected to the power generation. Low value of doubling time was required as it represented the effectiveness of the EB growth in order to achieve maximum biomass.

The correlation between biomass of BS and amount of sludge decomposed was analyzed using calibration curve as stated on Fig 8 below. Correlation of biomass engaged with amount of carbon that held in dewatered sludge revealed in both Fig 8 and Fig 9. Vanita (2009) mentioned that BS made reduction of carbon and utilized the nutrients from dewatered medium [34]. At the beginning of the experiment on 1 day, the initial biomass ($X_i$) was 0.079 mg/L. The strong relationship between both parameters (biomass and voltage) was also shown when an exponential increase in bacterial biomass took place from first day to sixth day: the biomass value rose from 0.079 mg/L to 0.11 mg/L (an approximately 0.72 fold increase), the power density/voltage increased from 59 mV to 92 mV/0.428 Watt/m² to 0.995 Watt/m² respectively due to increment of biomass growth in ML-MFC.
5. Conclusions
This study demonstrates that operation of a ML-MFC in the morning (25 ± 1°C) contributed a higher voltage and power density (90mV and 8.792Watt/m² at sixth day) compared to the ML-MFC which operated in the evening (23 ± 1°C)(72mV and 5.531Watt/m² at sixth day). The increment of temperature in MFC would accelerate the intracellular biochemical reaction rate and the growth rate of the bacteria (µ = 0.287g (L.h)−1 and Td = 2.42 h) with the BS maximum biomass was 0.11 mg/L. BS also recorded as gram positive microorganism had distinctive purple appearance (due to thick peptidoglycan layer of the cell wall) when observed under alight microscope following gram staining. Many other researchers just recorded once in a day for the power generation, so by having twice measurement (morning – high temperature and evening – low temperature) the power generation being averaged and it would obtain more precise value of the power generation from the ML-MFC. The study clearly shows that ML-MFCs could be implemented in rural area as BS was robust EB which suited to the ambient temperature and managed to generate promising electricity.
References

[1] United Nations, Department for Economic and Social Affairs (UN DESA) 2013 World economic and social survey 2013: sustainable development challenges (New York: UN Publications) 1–181.

[2] Gielen D, Boshell F, Saygin D, Bazilian MD, Wagner N and Gorini R. The role of renewable energy in the global energy transformation 2019 Energy Strateg. Rev. 24 38–50.

[3] Muaz M Z M, Don MM and Tajarudin H A 2018 Microbial fuel cell (MFC) development from anaerobic digestion system Anaerobic Digestion Processes (Green Energy and Technology) eds N Horan, A Yaser and N Wid (Singapore: Springer) p31-9

[4] Muaz M Z M and Vadivelu VM. Membraneless microbial fuel cell: characterization of electrogenic bacteria and kinetic growth model 2019 Environ. Eng. 145(5) 1–7.

[5] Mohd Zaini Makhtar M and Tajarudin HA. Electricity generation using membrane-less microbial fuel cell powered by sludge supplemented with lignocellulosic waste 2020 Int J Energy Res. 44(4)3260-5.

[6] Li Z, Yao L, Kong L and Liu H. Electricity generation using a baffled microbial fuel cell convenient for stacking 2008 Bioresour Technol. 99(6) 1650-5.

[7] Debabrata D 2018 Microbial Fuel Cell: A bioelectrochemical system that converts waste to watts (India: Springer)

[8] Logan BE. Exoelectrogenic bacteria that power microbial fuel cells 2009 Nat. Rev. Microbiol. 7(5) 375-81.

[9] Chaudhuri SK and Lovley D R 2003 Electricity generation by direct oxidation of glucose in mediatortless microbial fuel cells Nat. Biotechnol. 21(10) 32 – 1229.

[10] Kim HJ, Park HS, Hyun MS, Chang IS, Kim M and Kim B H2002 A mediator-less microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. Enzyme Microb Technol. 30(2) 52 - 145.

[11] Schröder U, Nießen J and Scholz F 2003 A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude Angew Chemie Int Ed 42(25) 2880-3.

[12] Schröder U. Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency 2007 Phys Chem Chem Phys. 9(21) 2619-29.

[13] Ismail Z Z and Jaeel A J 2013 Sustainable energy generation in microbial fuel cell catalyzed with bacillus subtilisspecies. Scientific World Journal. 31–5.

[14] Rezaei F, Richard TL and Logan B E 2008 Enzymatic hydrolysis of cellulose coupled with electricity generation in a microbial fuel cell Biotechnol. Bioeng. 101(6) 1163–9.

[15] Muaz MZ, Abdul R and Vadivelu VM. 2019 Recovery of energy and simultaneous treatment of dewatered sludge using membrane-less microbial fuel cell. Environ Prog. Sustain. Energy. 38(1) 208-19.

[16] APHA (American Public Health Association). 2005. Standard Methods for the Examination of Water and Wastewater. 23 ed. Washington, DC: APHA.

[17] Smith AC and Hussey M A 2005 Gram stain protocols. Am Soc Microbiol114.

[18] Logan B E et al. 2006 Microbial fuel cells: methodology and technology. Environ Sci Technol. 40(17) 5181-92.

[19] Logan B E 2012 Essential data and techniques for conducting microbial fuel cell and other types of bioelectrochemical system experiments. Chem. Sus. Chem. 5(6) 988-94.

[20] Larrosa-Guerrero A, Scott K, Katuri KP, Godinez C, Head IM and Curtis T 2010 Open circuit versus closed circuit enrichment of anodic biofilms in MFC: effect on performance and anodic communities Appl Microbiol Biotechnol. 87(5) 1699-713.

[21] Huang J, Wang Z, Zhu C, Ma J, Zhang X and Wu Z 2014 Identification of microbial communities in open and closed circuit bioelectrochemical MBRs by high-throughput 454 pyrosequencing. PLoS One 9(4).

[22] Buie C R et al. 2006 Water management in proton exchange membrane fuel cells using integrated electroosmotic pumping. J. Power Sources 161(1) 191–202.
[23] Salam M A et al. 2020 Effect of temperature on the performance factors and durability of proton exchange membrane of hydrogen fuel cell: A narrative review. Mater. Sci. Res. India 17(2) 91 - 179.

[24] Gude VG 2016 Microbial fuel cells for wastewater treatment and energy generation Microbial Electrochemical and Fuel Cells (Mississippi: Elsevier) p 247-285

[25] Nimje V R et al. 2009 Stable and high energy generation by a strain of Bacillus subtilis in a microbial fuel cell. J. Power Sources. 190(2) 258-63.

[26] Li W W, Sheng G P, Liu X W and Yu H Q 2011 Recent advances in the separators for microbial fuel cells. Bioresour. Technol. 102(1)244-52.

[27] Konovalova E Y, et al. 2018 The microorganisms used for working in microbial fuel cells. AIP Conf. Proc. 3-12.

[28] Yoganathan K and Ganesh P 2015 Electrogenicity assessment of Bacillus subtilis and Bacillus megaterium using microbial fuel cell technology. Int. J. Appl. Oed. Res. 1(13) 8-435.

[29] Komathi P 2017 Electricity generation from Bacillus Subtilis isolated from aerobic sludge using microcal fuel. Pharmacy and Pharmaceutical Sciences. 6(8) 82-1277.

[30] Min B, Román ÓB and Angelidaki I 2008 Importance of temperature and anodic medium composition on microbial fuel cell (MFC) performance Bioresour. Technol. 30(7) 8-1213.

[31] Liang C, Das KC and McClendon R W 2003 The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. Bioresour. Technol. 86(2) 7-131.

[32] Verstrate W and Rabaey K 2006 Critical review microbial fuel cells: methodology and technology. Environmental Science and Technol. 40(17) 92-5181.

[33] Manohar AK, Bretschger O, Nealson KH and Mansfeld F 2008 The use of electrochemical impedance spectroscopy (EIS) in the evaluation of the electrochemical properties of a microbial fuel cell. Bioelectrochemistry. 72(2) 54-149.

[34] Vanita R, et al. 2009 Stable and high energy generation by a strain of Bacillus subtilis in a microbial fuel cell. Power Sources. 190 63-258.

Acknowledgments
The authors would like to thank the UniversitiSains Malaysia for the financial support of this study via the Research University Grant Short Term Grant (304/PTEKIND/6315353) and the support from the Ministry of Higher Education Malaysia under Fundamental Research Grant Scheme (FRGS)(203/PTEKIND/6711823). The authors have declared no conflict of interest for the manuscript.