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EFFECT OF DIFFERENT TYPES OF SOIL AREA, SOIL DEPTH AND PRETREATMENT ON ELECTRICITY GENERATION USING MEMBRANE-LESS MICROBIAL FUEL CELL

Nurul Atiqah Shamsuddin[1], Muhammad Najib Ikmal Mohd Sabri[1], Nor Asniza Ishak[2], Salwa Mohd Zaini Makhtar[3], Nor'Izzah Zainuddin[4], Mohamad Danial Shafiq[5] and Muaz Mohd Zaini Makhtar[1,6]*

[1]*School of Industrial Technology, Bioprocess Technology Division, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[2]*School of Educational Studies, Universiti Sains Malaysia, 11800 Penang, Malaysia*
[3]*Faculty of Civil Engineering Technology, Universiti Malaysia Perlis, 01000 Kangar, Perlis*
[4]*Indah Water Konsortium, Lorong Perda Utama 13, 14300 Bukit Mertajam, Penang, Malaysia*
[5]*School of Materials and Mineral Resources Engineering, Universiti Sains Malaysia, 14300 Nibong Tebal, Malaysia*
[6]*Fellow of Center for Global Sustainability Studies, Universiti Sains Malaysia, 11800 Penang, Malaysia*

*Corresponding Author: muazzaini@usm.my

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Abstract

The membrane-less microbial fuel cell (ML-MFC) is an innovative renewable energy technology that can overcome the global energy crisis by generating electricity, mitigating greenhouse gas emissions, and bioremediating pollutants through utilization of plentiful renewable resources such as soil organic carbon. Typically, the presence of proton exchange membrane (PEM) (typically Nafion) has to boost the overall cost of MFC. In this study, the soil will act as a pseudomembrane to separate the anode and cathode. The MFC-based soil is inexpensive to construct and maintain. The soil that was collected at random from five different places around Parit Buntar areas acted as an organic substrate. These areas were chosen based on different community activities: residential (A & B), non-residential (C & D) and agricultural areas (E). The effect of different soil pretreatments (supplemented with tap water or POME sludge) and soil depths (1 to 5 inch) was also analyzed. Results showed that the power generated from palm oil plantation soil (agricultural areas) of depth 5 inches (topsoil) with the addition of tap water and POME activated sludge both generated 83.83 mW/m2 and 495.13 mW/m2, respectively. This showed that long-term operation of the ML-MFC using these complex lignocellulosic compounds as a direct substrate for electricity generation is feasible, though their slow degradation. The profiling of *Bacillus subtilis* (BS) biomass and the power density showed that MFC had a trend of associated growth-product where the growth of BS biomass reflected to the increment of power density. It was also proved that additional of POME had increased the population of BS as phylogenetic analysis determined the present of BS strain.

INTRODUCTION

Today's global economic growth and energy demand are accelerating and will continue to do so in the coming decades. However, billions of people lacked access to basic energy, electricity [1]. It was predicted that this problem would continue steadily until 2030 [1]. As reported by Jollands et al., (2010), about 1.4 billion people worldwide lacked electricity in which 2.7 billion of them depend on the traditional method using biomass [2]. Most of them live in Sub-Saharan Africa, India and other developing Asian countries [3]. Sub-Saharan Africa had become the greatest challenging region where only 31 % of the population had

access to electricity, which was the lowest level in the world [1] and most of them were from the rural areas.

The government of Malaysia has formulated various energy-related policies in order to guarantee the long-term reliability and security of energy supply for sustainable social economic development in the country. Among them are National Energy Policies 1979, National depletion Policy 1980 and Fuel Diversification Policy 1981 and 1999 [4]. These policies aimed to ensure the provision of adequate, secure and cost-effective energy supply by developing indigenous energy resources (both non-renewable and renewable) using least cost options and to diversify supply source [1]. In this regard, a new research area on the production of electricity using microbial fuel cells (MFC) has been geared forward.

MFC is a bio-electrical device that utilizes microbial metabolism to generate electricity [5]. Microbes in MFC consume sugars and other nutrients in their surrounding environment and liberate a portion of the energy contained in that food in the form of electricity., the MFC is high-potential technology that, thanks to its large-scale process provided free electricity to the poor people. However, an exploration of material and resources that are cheap to be used in MFC to produce electricity is urgently needed [2]. Typical MFC's configuration requires anode/cathode electrodes, anodic/cathodic chambers, proton exchanges membrane (PEM) and electrode catalyst. These requirements are high in cost especially the cost to fabricate the chambers in order to separate the oxic (cathodic) and anoxic (anode) environment [5]. Besides that, the expense for aeration supply system plus the electricity need to power the MFC therefore make it not economical. So, the alternative solution is needed to overcome the problems. With this view, the feasible, low capital cost, simple configuration and economical MFC are needed; ML-MFC based soil.

Many factors could influence electricity generation in ML-MFC. Temperature [6], pH [4], moisture [7], light [8], medium compositions are among the factors affecting growth of microorganisms and power production. However, limited work is reported on the impact of soil as a growth substrate on ML-MFC performance. In fact, the kinetic growth of bacteria that produces electron from the soil substrate has never been studied, much less exploited. In view of that, a study on the effect of different soil location, soil depth and soil pre-treatment on the growth of microorganisms and power generation using microbial fuel cell (MFC) was carried out in this study.

MATERIALS AND METHODS

Soil Samples Collection

Soil samples were randomly collected from five different places in Parit Buntar, Perak, Malaysia (5.1474 ˚N, 100.4212 ˚E) areas and served as an organic substrate. These areas were chosen based on different community activities: residential, non-residential and agricultural areas. The extracted samples were then kept in boxes at room temperature and analysed for nutrient composition. The soil was taken on the day the experiments were conducted to ensure the freshness and originality of the soil for maintaining the MFC high performance. In order to identify the significant effect of power generated by the MFC, three parameters were studied which include: a) soil locations (5 areas), b) soil depth (5-25 inch), and c) soil pre-treatment is done by treated the soil with tap water and activated sludge.

Construction and Operation of MFC

Membrane-less Microbial Fuel Cell (ML-MFC) as 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, as described by Muaz et al. (2020) [9]. The anode electrode was installed on the bottom of the vessel. The anode was then covered dewatered sludge with the setup pretreatment, up to a depth of 6 cm, and allowed to settle for a few minutes. The cathode electrode was put on the soil's surface (top). The chamber was then closed using a lid and incubated for 6 days at room temperature (27^{o}C). Electricity generation was measured using a digital multimeter. Experiments were carried out in a chamber containing an anode, a cathode electrode, a circuit board, and all experimental runs were done in triplicates at room temperature.

Microorganisms

Microorganisms present in the tested soil of MFC were used to generate electricity. Soils contained a lot of electrogenic bacteria (EB) which can generate electricity [10]. From research done by Muaz et al. (2021) and the team successful extracted *Bacillus subtilis* (BS) from soil sample and implemented in the current MFCs studies [11].

Analytical Method

Proximate Analysis of Soil

The soil samples were analyzed for its macro-, micro-nutrients and trace element that affected the growth of the BS. Availability of iron (Fe), zinc (Zn), cadmium (Cd), manganese (Mn), and nickel (Ni) present in the sludge was analyzed using flame atomic adsorption spectrophotometer (AAS) with acetylene-air fuel (GBC model 903, Australia) after digesting the sludge using the acid digestion technique (9). Total carbon (C), hydrogen (H), nitrogen (N), and sulfur (S) content in the sludge was measured using an elemental analyzer. While for the determination of phosphorus (P), standard reagent method using a spectrophotometer (DR

5000, HACH) at 420 nm was used as described by Shamsuddin et al.(2021)[12].

Determination of Power

The electrical power generation was calculated using Ohm's law

$$V = I.R \quad (1)$$
$$\mathrm{P} = \mathrm{VI} \quad (2)$$

Where V is the potential difference between the anode and the cathode (in Volts), I is the current (in Amps), R is the resistance applied and P is power.

Determination of Biomass

The biomass concentration (mg/L) was determined using the method as described by Zheng and Shetty et al. (1998) [13] with slight modification. All the samples were checked with absorbance at 650 nm using a spectrophotometer (Thermo Scientific Evolution 201).

Phylogenetic Analysis

In the pretreatment process using POME activated sludge, 0.05 g/mL POME activated sludge was dissolved in a stock deionized water solution. A series of dilutions (10^3 dilutions) were carried out to reduce the concentration stock mixture. To culture the electrogenic bacteria (EB), agar plates were made by dissolving 25 g of agar in 1 L of deionized water and autoclaving at 120 ^{o}C for 30 minutes. After that, the autoclaved agar solution was divided evenly among 50 petri dishes. One milliliter of the diluted stock mixture was added to each of the 20 dishes and gently swirled to ensure that the EB growths were distributed evenly. These 20 petri dishes were incubated for 24 hours at 30 ^{o}C. Individual EB colonies that grew in the agar were separated and cultivated separately in the remaining 30 petri dishes after 24 hours. The major population of isolated EB was chosen using an inoculation loop and streaked in an agar slant after another 24 hours in the incubator to allow the EB to proliferate. After a 24-hour incubation period at 30^{o}C, the slant was stored in the refrigerator for subsequent molecular and phylogenic analysis.

Cell Molecular and Phylogenic Analysis

Microbial identification was performed on the isolated samples (agar slants) in Macrogen company located at South Korea. To determine the taxonomic hierarchy of the sequences, the National Centre for Biotechnology Information's (NCBI) online nucleotide BLAST programmed and the ribosome database-II (12) were used.

RESULTS AND DISCUSSIONS

Effect of Different Soil Locations

Tables 1 and 2 show the places where the samples were taken as well as the nutritional contents. The concentration of micro-element in agricultural soil (E) were found higher than that of soil samples from other places possibly due to high fertiliser content in the soil. It also had a higher nitrogen, phosphorus, and potassium content [14] stated that this palm plantation soil was essentially peat land, which held a lot of carbon and provided a good environment for microorganisms to develop. According to Rao et al. (2005) [19] microorganisms could assimilate diverse chemical substances to create new cell wall components. All the nutrients that were observed played a significant role for the metabolism of the bacteria cells that is summarized in Table 3.

MFC which employed agricultural soil from palm oil plantations and residential areas yielded high power output (Figure 1). This was due to the high number of nutrients available for BS in MFC to ingest for growth activity, which was reflected in the generation of power. Non-residential soil, on the other hand, had a poor electrical generation due to the soil's characteristics. Bacteria thrive in slightly moist environments, making it easier for them to proliferate and use more carbon sources. In order to simplify the carbon source, moisture also contributes to the efficiency of the hydrolysis process. As a result, BS was able to consume the carbon source in a more efficient manner.

Figure 2 also revealed that the location site with the highest nutritional content had a higher population of electrogenic bacteria. After 40 hours of incubation, the biomass of bacteria in the MFC employing agricultural soil was 1.4 mg/kg, whereas the biomass from the residential area was 1.15 mg/kg. In fact, until the completion of the study, the bacteria population remained constant. Despite the fact that the biomass in non-residential soil was around 1.2 mg/kg, the value plummeted dramatically to 1.0 mg/kg. This was due to a lack of nutrients in the soil and increased competition among bacteria, which caused the microorganisms to die. Figure 3 shows a plot of voltage and power of MFC against time at different locations, and was obtained using the Eq (2). Similar trends of electrogenic bacteria were observed as in Figure 2.

Table 1. Type of soil location.

Name of place	Symbol	Area	Condition of the soil
Jaya College	A	Non-residential	- Far from water source - Dry - Open place (expose to sunlight) - Light in colour
Lembaran College	B		
Taman Ilmu	C	Residential	- Nearby plant oil plantation - Shaded area - Moderately moist
Taman Pekaka	D		
Palm oil plantation	E	Agriculture	- Peat land - Shaded area - Pretty dark in colour

Table 2. Nutrient composition for each location.

Element		Soil location (Area)				
		Non-residential		Residential		Agricultural
		A	B	C	D	E
Copper	(ppm)	0.078	0.093	0.134	0.119	5.216
Iron		0.507	0.61	0.742	0.643	0.405
Manganese		0.096	0.101	0.201	0.301	0.196
Nickel		9.831	8.756	8.635	7.837	7.023
Zinc		0.926	1.025	1.34	1.953	4.725
Phosphorus	(mg/L)	7.7	8.1	23.9	29.0	120
Potassium		5.3	4.2	15.2	13.2	80.1
Carbon	%	9.1	10.1	13.7	15.7	28.3

Table 3. Macro-, micro-nutrients and trace elements needed for the metabolism of bacterial cell (Li. et al., 2008).

Elements	Function
	Macronutrients
Carbon	Main constituent of cellular material
Nitrogen	Constituent of amino acids, nucleic acids nucleotides, and coenzymes
Hydrogen	Constituent of organic compounds and cell water. Also important in energy generation as protons.
Phosphorus	Constituent of nucleic acids, nucleotides, phospholipids, LPS, teichoic acids
	Micronutrients
Potassium	Main cellular inorganic cation and cofactor for certain enzymes
Magnesium	Inorganic cellular cation, cofactor for certain enzymatic reactions
Calcium	Inorganic cellular cation, cofactor for certain enzymes reactions
Iron	component of cytochromes and cofactor for some enzymatic reactions
	Trace elements
Ni	Several different enzymes including some involved in carbon monoxide metabolism, urea metabolism and methanogenesis
Mn	Required by a number of enzymes in catalytic sites.
Cu	Catalytic role in some enzymes that react with oxygen for example cytochrome oxidase.
Zinc	Structural role in many enzymes including DNA polymerase

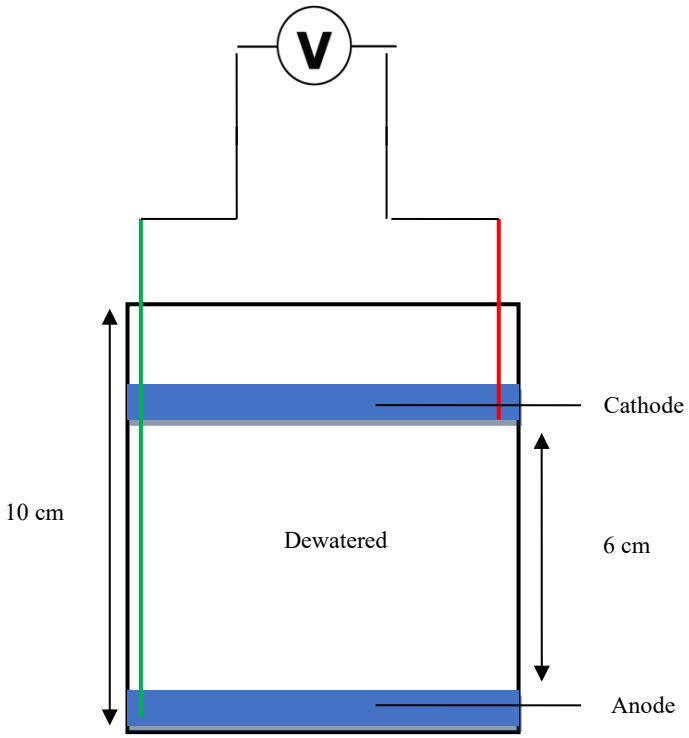

Figure 1: ML-MFC configuration setup.

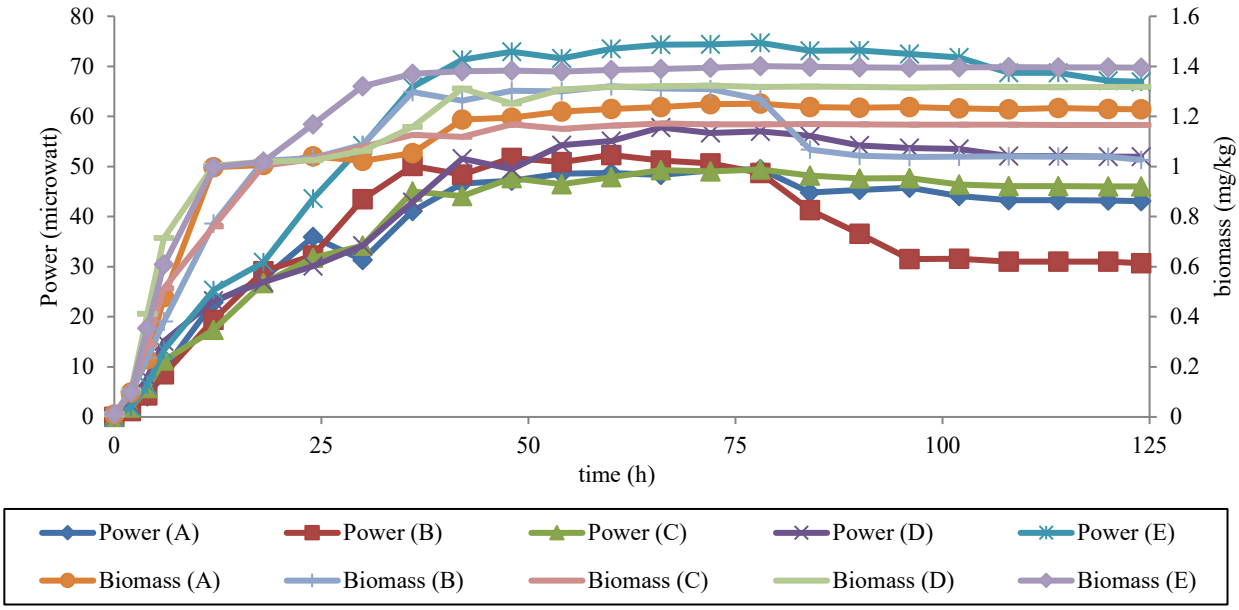

Figure 2: Power and biomass profiles using soils at different location. (A - Jaya College, B – Lembaran College, C – Taman Ilmu, D- Taman Pekaka, and E – Palm oil plantation).

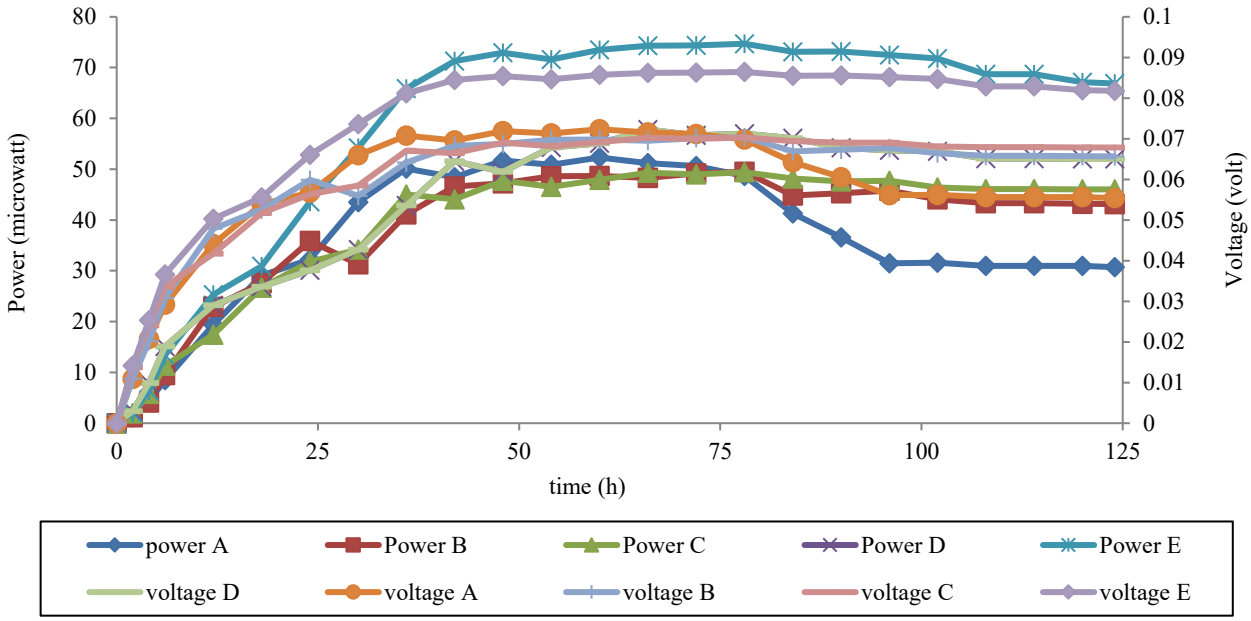

Figure 3: Voltage and power against time profiles of MFC at different soil locations. (A - Jaya College, B – Lembaran College, C – Taman Ilmu, D- Taman Pekaka, and E – Palm oil plantation).

Effect of Soil Depth

Figure 4 depicts the impact of various soil depths on electricity generation. It turned out that the deeper the earth, the lower the power generated. After 60 hours of incubation, the highest power generated was 74.4 µW resulted from 5-inches depth of palm oil soil. The power reduced beyond this point. Figure 4 also shows current and voltage patterns derived just from the palm oil plantation at various soil depths. The amount of electrogenic bacteria present in the soil was generally proportional to the generation of power. (Figure 4).

According to Bullen et al. (2006), the more bacteria there were, the more electrons were transferred between the electrodes of the MFC [15]. As a result, more electricity is produced. Fierer et al. (2003) found that the topsoil at a depth of 1 to 7 cm had the highest concentration and diversity of bacteria [16]. The bacterial concentration and variety decreased as the soil depth increased after the topsoil layer. The presence of organisms in deep soil layers was highly influenced by the moisture content, temperature, and organic matter of the soil. Fierer et al. (2003) stated clearly on the distribution of microorganisms at various soil depths and the later reported that the soil microbes were greatly influenced by the biogeochemical processes throughout the soil profiles [16]. However, large studies focusing primarily on the top 15 cm of the soil column have constrained our understanding of the organisation and diversity of soil microbial communities.

Effect of Soil Pre-Treatment

There were several nutrients contained in the POME activated sludge that could be utilized by the bacteria for their growth as presented in Table 3. The nutrients were divided into two groups which were macronutrient and micronutrient. The macronutrient could be broken into two more groups which were primary and secondary nutrients. The primary nutrients are nitrogen (N), phosphorus (P) and potassium (K). These major nutrients usually were deficient from the POME activated sludge because the large used from the bacteria for their growth and survival. This opportunity being used by the microorganism to grab the nutrient and used them for their metabolic pathway. The secondary nutrients of the macronutrients are calcium (Ca), magnesium (Mg) and sulfur (S) [7]. There were usually nutrients in the dewatered sludge for development cofactor for certain enzymatic reactions. While for micronutrients are those elements essential for microorganism growth which are needed in only very small quantities. These elements are sometimes called minor elements or trace element. The micronutrients are copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn). The micro- and macro-nutrients, and trace-elements are very important elements not only as co-factors for enzymes functions but also for building compounds and protons for energy generation in the bacteria cell.

Palm oil plantation soil was supplemented with tap water and POME for a better electrogenic process in MFC. As a control, soil that had not been pre-treated was used. The soil

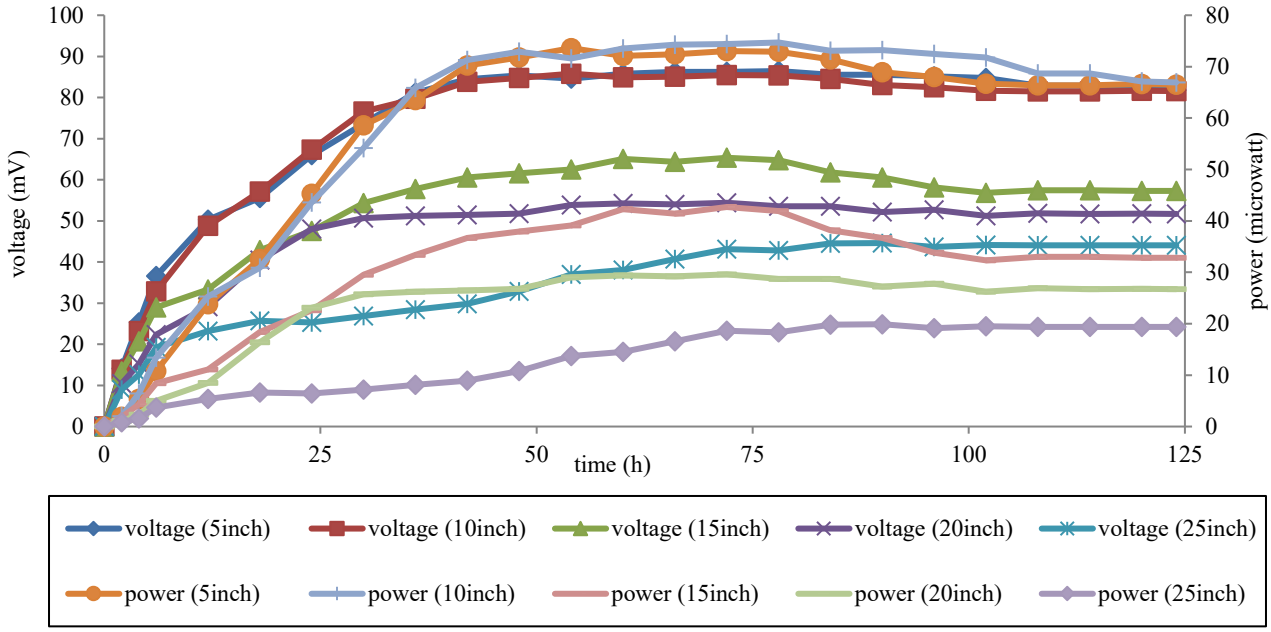

Figure 4: Voltage and power generated by MFC using soil of different depth from the palm oil plantation.

mixed with activated sludge resulted highest power generation as depicted in Figure 7 and Table 4. The power generated for each pre-treatment were 2015.2 µW (soil with activated sludge), 341.2 µW (soil with tap water) and 74.7 µW (soil alone) after 96 hr, 90 hr, and 78 hr of the incubation period, respectively. From the phylogenetic analysis, the POME activated sludge also recorded to contain rich of the *Bacillus subtilis* (BS) (Figure 5), thus this was the key reason why the high current and power are generated from soil supplemented with POME activated sludge. The enrichment of BS accelerated the degradation of abundant carbon compound in POME sludge, thus the electron released from the BS consumption reflects to a high of electricity generation (Figure 6). BS has the an ability to secrete amylase, α-amylase, protease, alkaline protease, lipase, phytase, cellulose, β-glucanase, xylanase and α-galactosidase. Thus, abundant carbon sources presence in the peat soil and POME activated sludge could be utilised in MFC to get the high-power generation.

The BS started to replicate and digested organic matter present once it being moisturize. Having a suitable moisture level help with hydrolysis of the organic compounds, makes it easier for bacteria to consume organics and donates electron for electricity generation. Low moisture content in soil would slow down the movement of protons being transferred to the cathode during the redox potential process in MFC, thus lowering the electricity generation. Figure 7 and Table 4 shows the MFC with high nutrient compositions of POME activated sludge that has accelerated the amount of power by 26 folds as compared to the MFC containing only palm plantation soil alone (untreated).

Yarie et al. (1990) reported that the microbial population fluctuated with moisture content as water logging caused development of anoxic condition [7]. This was due to the short burst respiratory activities by aerobic bacteria to tolerate with very low O_2. Similar observation was also reported by Wagener and Schimer et al. (1998) who studied on microbial community structures and activities in the forest at different level of moisture [17][18]. As in the present study, under a control condition in which the soil was taken directly from the palm plantation, without any addition of tap water or activated sludge, the power showed less than 100 microwatt (Figure 2 - 4). With the addition of tap water, higher increments of the power generated were observed. Figure 8 represented the power and current generated using palm oil soil supplemented with POME activated sludge and tap water. It clearly showed that increasing of the current value was due to the amount of carbon degradation [10]. The population of BS proved that they played a significant effect for the power generation as high population of BS easily helped the breakdown of the carbon and release more electrons from the oxidation process then it passed to the anode electrode. The MFC shows the growth-product shown that the increment of biomass reflected to the power density generation (Figure 7).

Table 4. Maximum electrical power generated at different soil location and soil pre-treatment.

Soil location	Maximum electrical power generated (microwatt)		
	Soil pre-treatment		
	Control*	Soil supplemented with tap water	Soil supplemented with palm oil mill activated sludge
A	52.3	129.3	273.8
B	49.5	106.8	281.1
C	57.8	110.1	295.7
D	49.4	149.0	297.2
E	68.7	341.2	2015.2
Normalize power generation to surface area of anode electrode (0.00407 m^2) for evaluation of power density			
	Maximum electrical power generated (mW/m^2)		
A	12.85	31.76	67.27
B	12.16	26.24	69.06
C	14.20	27.05	72.65
D	12.13	36.60	73.02
E	16.87	83.83	495.13

*soil taken directly from the site, without any pre-treatment

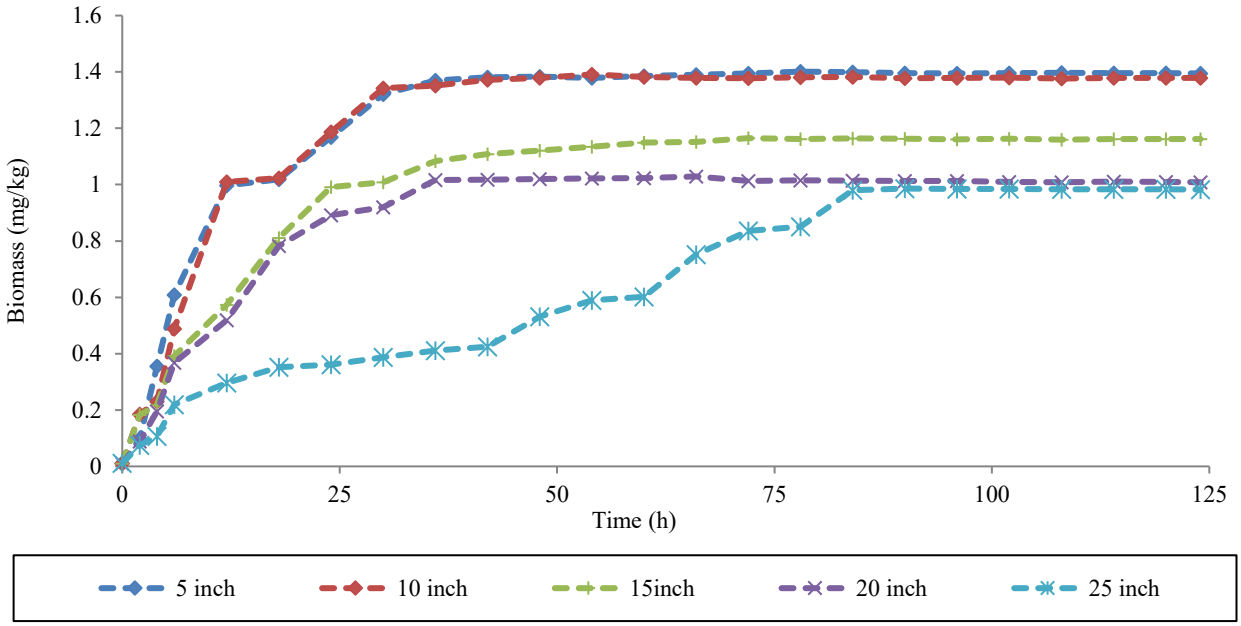

Figure 5: Growth profiles of electrogenic bacteria using palm oil plantation soil extracted at different depth.

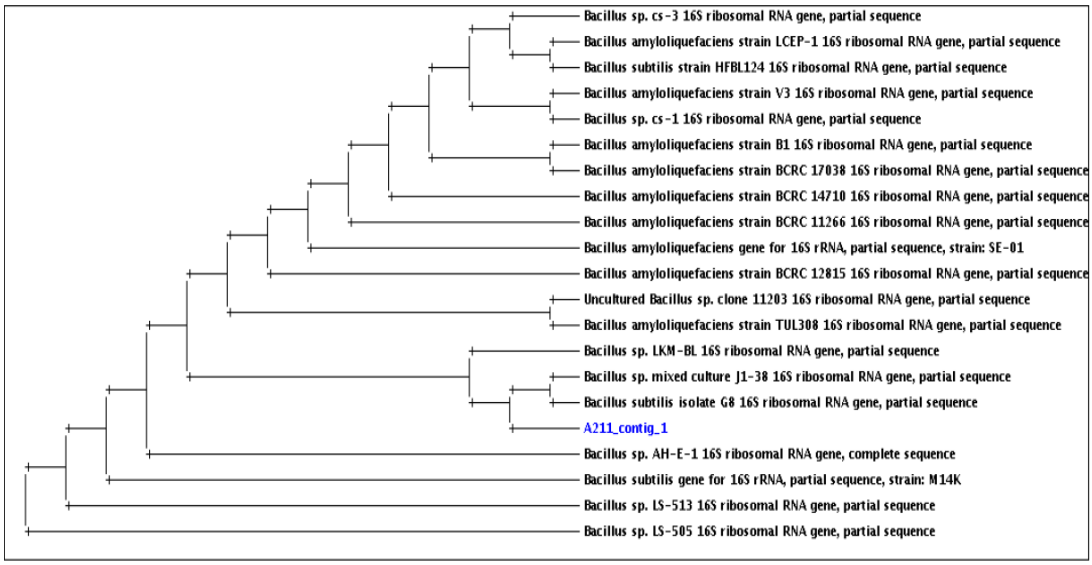

Figure 6: Phylogenetic trees for *Bacillus subtilis* species in MFC.

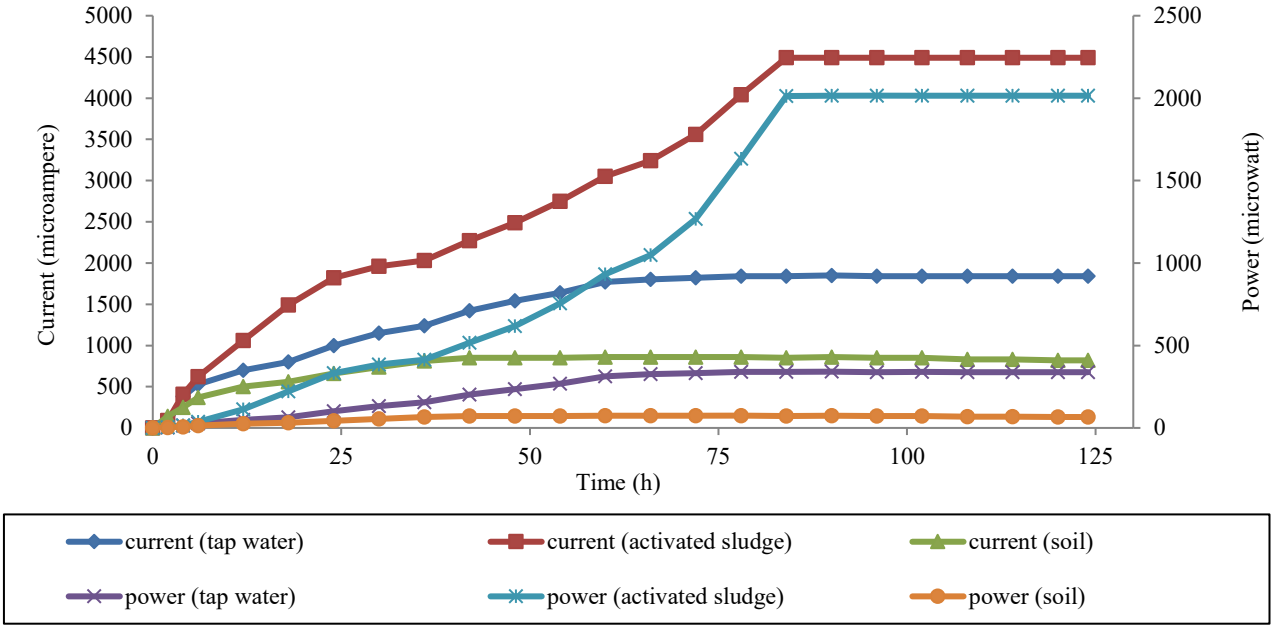

Figure 7: Power and current generated using palm oil plantation soil supplemented with POME activated sludge and tap water.

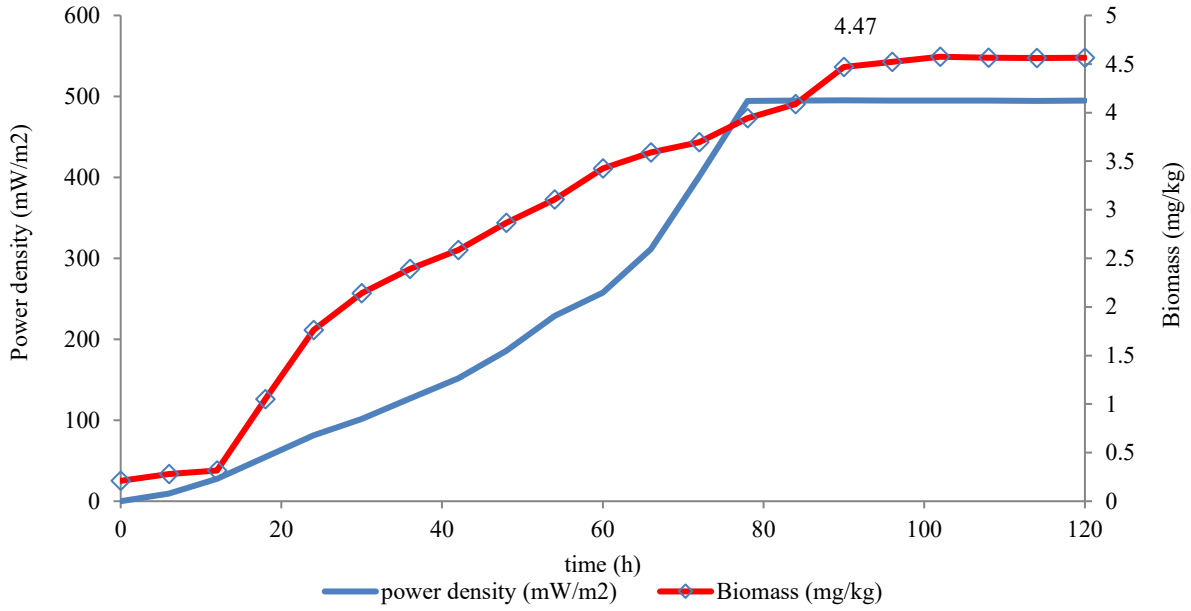

Figure 8: Relation between biomass and power generation of optimum ML-MFC and incubation time.

CONCLUSION

In the present study, MFC generated the highest power with the soil extracted from the palm oil plantation at 5inch depth. Then this soil was further treated with different pre-treatment which were supplemented with tap water and POME activated sludge and both generated 83.83 mW/m^2 and 495.13 mW/m^2, respectively. Nutrient composition of each soil played an important role as it was the factor that affected the growth of BS. The POME activated sludge had rich in term of macro, micro-nutrient and trace element which accelerated the growth of BS. From the phylogenetic analysis, the additional of POME also contributed to the enrichment of BS thus population of BS had increased thus easier for them to degrade the carbon source present. This proved that BS was a biocatalyst that worked well in MFC for the electricity generation.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest for the manuscript.

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