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Green electricity production by Epipremnum Aureum and bacteria in plant microbial fuel cell

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ABSTRACT

Due to high energy demand worldwide, finding an alternative renewable and sustainable energy source is of great interest. Plant microbial fuel cell (P-MFC) is one of the most promising methods to generate green energy. In P-MFC, a plant is placed into the anode compartment. Mutual interaction between plant root rhizodeposits and bacterial community results in the biofilm formation at the vicinity of the rhizosphere area in plant root could be utilized to generate electricity. Indeed, in P-MFC, bacteria metabolize rhizodeposits into electrons and protons. These electrons could be then converted into green electricity. The objectives of this research are to utilize *Epipremnum aureum* plant collected from Kota Tinggi's lake to generate electricity and observe current generation by different resistors, to characterize immobilized bacteria attached on the anode surface then identify the optimum growth temperature for isolated bacteria. Five plant microbial fuel cells were constructed in a H-shape (dual- chambers) configuration in the plastic container. Maximum current density for 20 days for P-MFC by external resistance of 100k Ω was 0.1 μA/cm^2 with maximum power density of 0.85 μW/cm^2 and the open circuit voltage (OCV) was measured at 195 mV. Besides, fresh biomass averages increased 5g after 20 days of experiments below and above ground as compared to the initial fresh biomass. Five isolated bacterial strains from the graphite felt surface found on the anode were screened by nine biochemical tests such as catalase, TSI (triple sugar iron agar), gelatin and etc. The immobilized bacteria attached to anode electrode in P-MFC were further examined with Fast Electron Scanning Electron Microscopy (FESEM). The isolated bacterial growth curves were determined at two different temperatures of 25 °C and 37 °C. The optimum growth temperature predominantly for them was 37 °C.

Keywords:
Plant-microbial fuel cell, Epipremnum aureum, Power generation, Bacterial consortium

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1. Introduction

Generation of power with less emission and high efficiency is highly demanding. Introducing sustainable, new and renewable energy could be the best solution to reduce emission of greenhouse gases. Furthermore, this is a new challenge between nations to exploit. Recently, fuel cells are considered as a high potential clean energy technology, due to the high energy conversion efficiency through the chemical degradation process. Microbial fuel cells are one of the most studied fuel cells, due to its potential application to generate electricity from wastewater treatment processes. Various types of bacteria and yeast involved in the system have been investigated. The electron transformation mechanism and microorganism behavior have been studied in some articles [1-3]. The plant microbial fuel cell (P-MFC) is a bioreactor that generates green electricity from the interaction between microorganisms of rhizosphere and root organic which released compounds such as sugars, organic acids, polymeric carbohydrates, enzymes, dead cell materials and etc. [4]. Some parts of these organic compounds are then oxidized; donated electrons are then transferred to suitable electrodes which are located at the anode compartment [5]. On the other hands, protons are transferred through the membrane and undergo reduction in the cathode chamber producing water. The P-MFCs was primarily implemented and they achieved maximum power production of 67 $mV.m^{-2}$ anode surface area [4]. They designed dual-chamber set up for P-MFC which was connected by a membrane (proton exchange membrane), while other study represents sediment P-MFC without employing membrane between cathode and anode compartment [6].

Although electricity generation by MFCs has increased indefinably at lab scale, but scaling up this system is still a big problem. In addition, high cost of proton exchange membrane and its fouling problem is a vital upcoming problem which could lead to the increase of the internal resistance and reduction of power output as well [7]. From the energy demand and cost aspect, providing external artificial illumination increase the cost of constructing this system as well [8, 9]. Besides non compatibility of this system with food production it could be united with agricultural products [10,11]. Therefore, this system has the potential to be implemented in inappropriate locations such as green roofs and wetlands for crop production. One of the biggest disadvantages in applying this system is the request for large surface area of electrodes. On the other hand, topsoil excavation for integration of this system could hinder the fertility of the soil. Therefore, in order to remain the top soil from weakening and also remaining soil fertility aquatic plant could be the better option [12]. A usual problem which normally happens in the MFCs is the pH gradient between the membranes. Due to the degradation of substrates the pH in the anode converts to the acidic. While, in the cathode alkaline environment is produced [13]. This problem could be overcome by applying different techniques such as utilizing buffers [14] and membraneless microbial fuel cell [7]. However, these methods dramatically decline the fuel cell energy recovery [15]. Therefore, further development need to be achieve in order to reduce the pH gradient [13].

The objectives of this research are to utilize *Epipremnum aureum* plant to generate electricity and observe current generation by different resistors then characterize immobilized bacteria attached on the anode surface.

2. Material and method

2.1 Sub-section

2.1. Preparations

2.1.1. Hoagland solution

1 ml/L of prepared micronutrient solution with 0.25 ml/L of iron stock along with the nutrient solution was used to make the final Hoagland Solution [16].

2.1.2. Micronutrient stock

Following amount of chemicals was mixed in a total volume of one liter of distilled water: 5. 2.86g/L of H_3BO_3, 1,81 g/L of $MnCl_2$. $4H_2O$, 0.22 of $ZnSO_4$.$7H_2O$, 0.08 g/L of $CuSO_4$. $5H_2O$, 0.02 of H_2MoO_4. H_2O.

2.1.3. Iron stock

This stock was prepared with the following compositions (in g/L): EDTA 26.1; KOH 19; FeSO4.7H2O 24.9. EDTA was dissolved in 286 mL water which includes KOH. Then FeSO4.7H2O was dissolved in about 500 mL water. Iron sulfate solution was added to the potassium EDTA solution and it was aerated overnight with stirring. Prepared final solution in 1L volume with red wine colour and high pH about 7.1 covered with aluminum foil and was kept in room temperature.

2.2 Experiment set up

The five prepared plant microbial fuel cells were constructed in a H-shape (dual- chambers) configuration in the plastic container with capacity of 500 ml. The anode compartments were covered with aluminum foil and were filled with Hoagland solution and graphite felt in diameter 4 cm × 4cm ×0.5 cm. The cathode electrodes consist of graphite felt (40 cm^2) attached to the copper rods and were placed in the cathode chamber. Cathode compartment were filled with distilled water and 2 mL/L phosphate buffer. PVC tubes at the length of 5cm were prepared as salt bridges containing 10% w/v agar and 1M NaCl. This salt brigde were fixed between anode and cathode compartments. The electrical circuit was completed by connecting graphite rods to the external resistors with value ranging between 1kΩ to 10kΩ. The plant microbial fuel cells were kept in the greenhouse with temperature between 26-32 °C and uncontrolled humidity.

2.3 Plant microbial fuel cell operations

Epipremnum Aureum was collected from Kota Tinggi's lake in Johor Bahru. For the experiment, stems of *E.aureum* were separated precisely from the soil and roots were washed to become free from soil particles. One or two stem(s) of *E.aureum* (fresh weight 12–20 g and height 36 to 50 cm) were placed in the anode compartment of the microbial fuel cells. Control plants were grown in the pot soil and were watered regularly with tap water 2-3 times per week. For the startup of the plant-MFC, the anode compartments were filled with Hoagland solution which was neutralized at pH 7. After two weeks of startup, the graphite felts were taken out to characterize immobilized bacteria attached on the anode surface by streaking on nutrient agar plates. Naturally occurring micro-organisms were already present on the roots of the plants at the time of replacement into the plant-MFC anode. Therefore, the plant-MFC contains a whole range of micro-organisms. The cathode

compartments during start-up and operation were filled with demineralized water containing 2 ml/L, 1M phosphate buffer (K_2HPO_4: 132.7 g/L; KH_2PO_4: 168.5 g/L). Also, they were capped to facilitate the anaerobic condition. Water loss occurred in the anode compartments due to the water evaporation and root uptake. To compensate it, the anode compartments were replenished with Hoagland solution from (day 14-20). Water loss was not noticeable in cathode compartments.

2.4 Analytical methods

Cell voltage was measured off-line with a digital multimeter. The circuit was determined by loading different resistors from 10 Ω -100 kΩ. Current was calculated from the voltage (v) at a resistance (R) by Eqn. 1. Power (P) also calculated by Eqn. 2.

$$I = V/R \quad (1)$$

$$P = V.I \quad (2)$$

2.5 Identification test

The isolates were subjected to the Gram Staining Technique and various biochemical tests to identify the bacteria like Catalase Test, Triple Sugar Iron Agar (TSI) Test, Simmons Citrate Agar Test, Motility Test (Motility Medium), Gelatin Test, Urease Test, Starch Hydrolysis Agar Plate and Oxidation-Fermentation (OF) Test.

3. Results and discussion

3.1 Plant microbial fuel cell

Plant microbial fuel cell open circuit voltage (OCV) increased steadily from day one and reached the maximum of 195 mV at day 10 as shown in Fig. 3.1(a). During the operation period in total 20 days, maximum obtained close circuit voltage by loading external resistance of 100k Ω was 63 mV (Fig. 3.1.b). OCV (cell voltage) from day one started to increase steadily until day 10 and reached the maximum 195 mV at day 11. After day 10, OCV decreased about 32% of the maximum value. In order to improve the OCV at day 14, 2 mL phosphate buffer (1M) was added to the cathode and OCV increased approximately 29.5% from value taken at day 10. This is due to the increasing number of cations concentration in the cathode. Indeed, lower buffer capacity of phosphate buffer increased the acidity of catholyte.

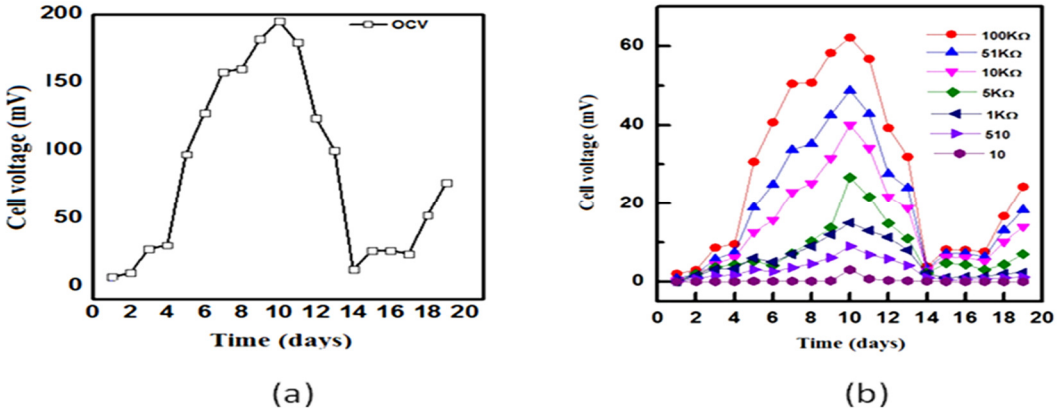

Fig. 3.1. Plant microbial fuel a: Open circuit cell voltage (OCV) and b: Close circuit voltage (CCV). Consequently. The arrow indicates 2 mL 1 M phosphate buffer addition to the cathode chamber.

Therefore, cell voltage by adding the phosphate buffer started to improve and increase. This value later followed by a decline. This is probably due to the salt bridge fouling which may reduce the potential ability of the membrane to transmit the protons to the cathode. This decline could not be caused by the bio-anode vitality, as the plant showed normal vitality. This outcome indicates that cathode requires more improvements, based on polarization curve analysis. In addition, improvement of P-MFC configuration by bio-cathode combination could increase the current output [17]. On the other hand, utilizing the high concentration of phosphate buffer which possess the high buffer capacity 50 mM could be better option to increase the final voltage and power output as well [18]. Usually fericyanide used in the cathode, but since oxygen is sustainable and environmentally friendly in this study, fericyanide was not utilized [19,20,21].

3.2 Polarization curve in P-MFC

According to Ohms law power was calculated based on this equation and polarization curve plotted based on Eqn. 1, 2 and 3. Based on the results obtained by varying the external loads, maximum current generation was achieved at day 10. Maximum power production was 0.85 $\mu W/cm^2$ anode surface areas. Even after declining current generation plant was keep growing afterward, without being lethal.

Power Density = Power / Surface area (3)

The Maximum current density for 20 days for P-MFC by external resistance of 100k Ω was 0.1 $\mu A/cm^2$. This results to the maximum power density of 0.85 $\mu W/cm^2$ (Figure 3.2b). The reduction in current generation was not due to the declining of the plant vitality, as the plants were observed growing well. Most probably the acidification of bio-anode reduced the bio-film community regeneration. The potential of anode remained constant and cathode potential declined at higher currents [8]. This outcome indicates that cathode requires more improvements. In addition, improvement of P-MFC configuration by bio-cathode combination could increase current output [17] in which could minimize the internal resistance. High resistance of catholyte, anolyte and CEM, salt bridge are some factors that could increase the resistance as discussed by [22]. Reducing the distance between electrodes power density could increase under different external resistances [23].

Internal resistance is a critical issue for achieving the power density, which is caused by the proton exchange membrane in two chamber MFCs. Salt bridge has been found to have the high internal resistance in comparison with the Nafion. Therefore, produced power could support this outcome by indicating the lower power in salt bridge. For improving the power production, the proton transport rate needs to be increased. Also oxygen transport should be declined to reduce the internal resistance of MFC. To prepare salt bridge in this work 10 % concentration of agar was selected due to the high performance of it to generate current and power as well. This will lower the oxygen diffusion rate to the anode compartment. One of the most effective methods to reduce the oxygen diffusion inside the anode chamber by applying the mixed culture of aerobic bacteria in the anode compartment as their oxygen uptake could reduce the internal resistance as well. So, this will help to maintain an anaerobic- conditions in closed anode MFC [24].

Moreover, increasing the anode surface area are proven to effective in reducing the internal resistance [8,24]. This type of electrode material in this work was selected. Graphite felts have the ability to generate the power 15 times higher than the graphite granules according to their mass [12]. The constant cell voltage behaviour between day 6 and 8 is probably due to the light cycle [8] and fluctuation of the temperature. Rhizodeposits composition is quite complex. It consists of various

components. Rizhodeposition rate is closely related to the life cycle of the plant root [26]. Elevating the photosynthesis during the day might increase the root exudates inside the MFC.

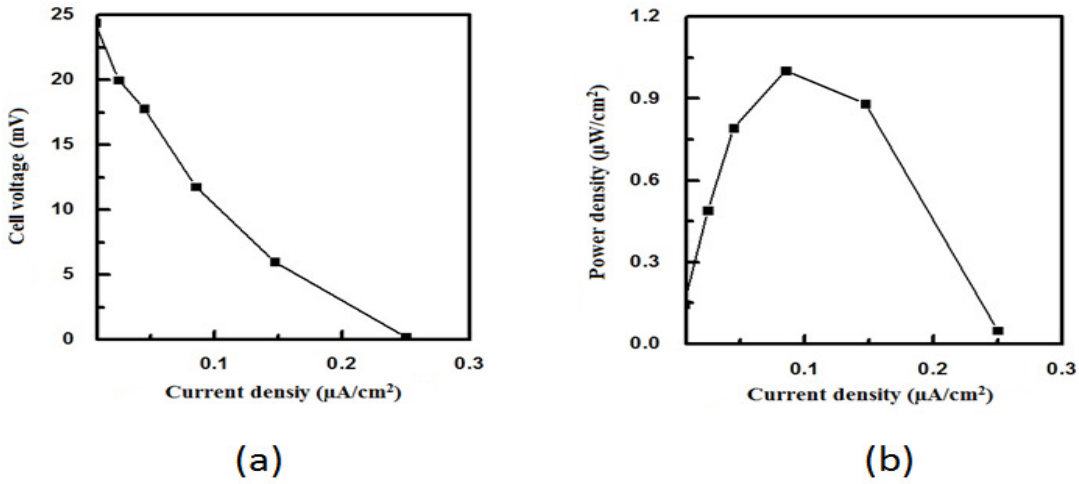

Fig. 3.2. (a) Polarization curve with cell voltage, (b) with Plant MFC.

In additions, the formations of bio film microbial activity could be increase by the rising of temperature. Therefore, it leads to the increase of the cell voltage as well [8]. Effectiveness of sunlight on root exudates was also proven [27]. By increasing the quantity of one common exudates component in the anode current could be increased up to the maximum of 50 %. So, high rate of photosynthesis and temperature could elevate the exudates release, in addition anode potential is reduced.

3.3 Identifications of bacteria

Five isolated bacterial strains from the graphite felt surface found on the anode were designated as BF1, BF2, BF3, BF4 and BF5. Further work had been proceeding with these isolates. These strains were identified using biochemical test. On the microscopic observation strains BF1 strain is gram negative and cocobacillus, BF2 strain is gram positive and cocobacillus, BF3 strain and BF4 revealed as the coccus, BF4 strain is gram positive, BF4 strain is gram negative, and BF5 strain is gram positive and bacillus. From Catalase test result, BF2 and BF3 belong to the *Micrococcaceae* strain and specifically to the Aerococcus *urinae* species since they are gram positives and revealed the positive result from catalase test. BF5 strain is gram positive isolate which revealed the negative result belongs to the *Aerococcus viridians*. BF1 and BF4 gram-negative maybe belong to the one of these species such as *Campylobacter fetus*, *Campylobacter jejuni*, and *Campylobacter coli* which have positive result and can be distinguished from other *Campylobacter* species [30,31]. According to Triple Sugar Iron (TSI) test, bacteria isolates BF1, BF2, BF3, and BF5 belong to the Enterobacteriaceae family [32,33] because all four isolates could fermentate lactose as well as glucose, whereas isolates BF4 was not able to fermentate lactose.

During the Simmons citrate test only isolates BF5 showed the positive result and could belong to the *Enterobacteriaceae* family [34]. Furthermore, this isolate could potentially use the citrate as its carbon source other than glucose or lactose for growth and metabolism. Other four isolates did not reveal any changes and considered as citrate negative organisms. Besides, the motility test result shown that the isolates BF1 and BF2 are motile organisms. While, the isolates BF3, BF4, BF5 indicates non-motile results. Based on the motility test results isolates BF1, BF2 and BF3 belong to the *Enterobacteriaceae* family [35-37]. Furthermore, isolates BF1, BF2, BF3, BF4 in gelatin hydrolyses test

indicated positive results. This liquefaction of media proved that those 4 isolates have the extracellular gelatinase enzyme which could hydrolyze gelatin into the poly peptides and amino acids subsequently. Whereas, the BF5 isolate revealed the negative result, this isolate does not have this enzyme to break down the gelatin to small components. According to the gelatin test isolates BF1, BF2, BF3, and BF4 belongs to the pathogenic *Staphylococcus aureus,* while, isolates BF5 belongs to the nonpathogenic *Staphylococcus epidermidis* as it showed negative result in this test [38].

The result from urease test indicates that strain BF3 revealed the positive result. Other strains indicated negative results. According to the results, the strain BF3 contains urease enzyme which could metabolize the urea to the ammonia rapidly. This group of bacteria are also considered as a rapid urease-positive organism. Long-time incubation of other strains did not show any positive result. Therefore, BF3 have the potential to degrade and detoxify the waste products by its soluble urease enzyme. This isolate is a good candidate to be applied in waste water treatment purposes with high ammonia content. Based on the urease test, the isolate BF3 belongs to the *Proteeae* which can be distinguished from other family of *Enterobacteriaceae*. Based on the starch test analysis, strains BF3, BF4, BF5 displayed the positive results. These strains have extracellular enzymes such as α-amylase and oligo-1, 6-glucosidase that could hydrolyze the starch to the small molecules that could let small molecules enter to the cell. While other two strains (BF1 and BF2) indicated these two strains do not have the extracellular enzyme to break down the starch. So, isolates BF3, BF4 and BF5 belong to one of these species, such as *Streptococcus, Clostridium, Corynebacterium, Fusobacterium, Enterococcus, Pseudomonas*, and *Bacillus* [30,39].

OF test result indicates that all isolates do not have the ability to metabolize the glucose either by fermentation or aerobic respiration in aerobic and anaerobic conditions as the media colour which did not change after incubation in two different conditions. During the glucose fermentation anaerobically acid production could convert the colour indicator (bromthymol blue) to yellow from green colour. Table 3.3 shows biochemical test analysis summary for the five isolated bacteria strains.

Table 3.3
Biochemical Test Results Summary

Tests / Isolates	Gramm reaction	Shape	Motility	Gelatine	Starch	TSI	Simmons citrate	Urease	OF (aerobic)	OF (anaerobic)	catalase
BF1	-	cocobacillus	+	+	-	yellow	-	-	-	-	+
BF2	+	cocobacillus	+	+	-	yellow	-	-	-	-	+
BF3	+	coccus	-	+	+	Yellow	-	+	-	-	+
BF4	-	coccus	-	+	+	Yellow /red	-	-	-	-	+
BF5	+	bacillus	-	-	+	yellow	+	-	-	-	_

3.4 FESEM analysis

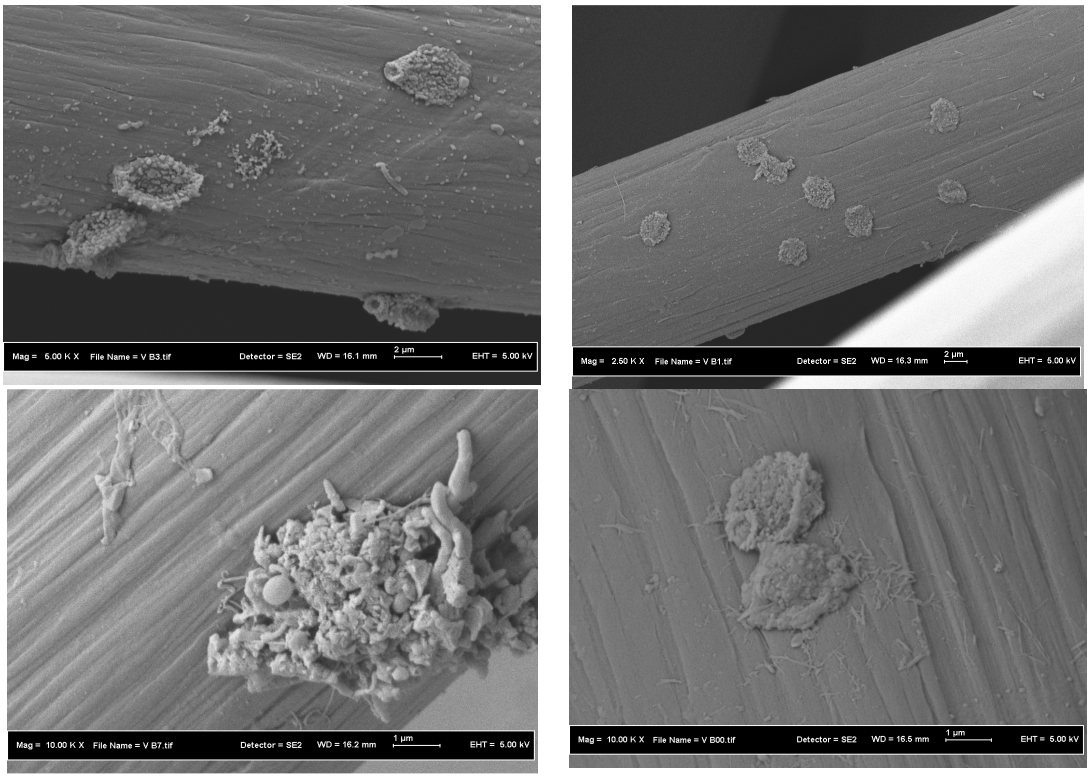

Fig. 3.4. FESEM image of bacteria attachment to graphite felt electrode

4. Conclusion

Green electricity and biomass can be produced simultaneously in P-MFCs. Therefore, it can be concluded that the consumption of rhizodeposits by the electroactive bacteria can produce sustainable and non-destructive bio energy. *Epipremnum aureum* seems to have the potential to grow and generate electricity in P-MFC. Maximum OCV achieved in this P-MFC was 195 mV. However, further research need to be carried out to increase power and current density. It can be concluded that the isolated bacteria strains from P-MFC mostly belong to the *Enterobacteriaceae* family. Indeed, these five isolates diversity of microorganism are involved in the P-MFC during the current generation process. Bacterial community interaction and their concentration are closely related to the environmental condition. FESEM analysis used to demonstrate the bacteria interaction and communication on the biofilm. Further bacteria analysis by the molecular tests needs to be carried out to investigate the exact species names which are involved in the current generation.

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