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# PRELIMINARY STUDIES ON POWER GENERATION BY *Bacilli E1* USING DUAL CHAMBER MICROBIAL FUEL CELL

(Kajian Awal Penghasilan Elektrik oleh *Basilli El* Menggunakan Sel Bahan Api Mikroorganisma Dwi-kebuk)

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**Abstract**

Microbial fuel cell (MFC) is a developed technology to utilize microbial degradation ability and turned the degradation products to electricity. One of the limiting factor that contributes to the performance level of MFC is the microorganism used in the MFC. In the present research, *Bacilli E1* has been tested for its ability to utilize glucose and converted it to electricity in dual chamber MFC. The MFC operated using E1 produced a maximum average of open circuit voltage (OCV) of 0.8 V. Meanwhile, by inserting a 1000 Ω resistance in the MFC circuit, produced a stable voltage of 0.1 V and calculated current and power were 0.2 ± 0.017 mA and 0.1 $Wm^{-2}$ Comparison of glucose based voltage production between individual and mixed culture shows similar pattern of voltage profile and since individual CC did not show any significant increase of OCV, it was concluded that *Bacilli E1* plays major role in the present MFC for power production.

**Keywords**: microbial fuel cell, *bacilli*, glucose

**Abstrak**

Sel bahan api berasaskan mikrob (MFC) adalah satu teknologi terbangun yang menggunakan keupayaan mikroorganisma mendegradasi dan menukarkan produk degradasi kepada elektrik. Antara yang menjadi faktor penghad yang menyumbangkan kepada tingkat prestasi MFC adalah mikroorganisma yang digunakan di dalam MFC. Dalam kajian ini, *Bacilli E1* telah diuji keupayaannya untuk menggunakan glukosa dan menukarkannya kepada elektrik dalam dwikebuk MFC. MFC yang dijalankan dengan menggunakan E1, telah menghasilkan voltan litar terbuka (OCV) sebanyak 0.8 V. Manakala, dengan memasukkan rintangan sebanyak 1000 Ohm dalam litar MFC menghasilkan voltan yang stabil berjumlah 0.1 V serta jumlah arus dan kuasa yang dikira adalah masing-masing 0.2 ± 0.017 mA dan 0.1 $Wm^{-2}$. Perbandingan antara penghasilan voltan daripada glukosa menggunakan individu dan kultur campuran adalah hampir serupa dan memandangkan CC secara individu tidak menunjukkan sebarang kenaikan dalam OCV, maka kesimpulan dibuat bahawa *Bacilli E1* memainkan peranan besar dalam MFC yang dijalankan untuk penghasilan kuasa..

**Kata kunci:** sel bahan api berasaskan mikrob, *bacilli*, glukosa

## Introduction

Microorganism(s) which has the ability to oxidize organic compounds to carbon dioxide, protons and electrons which the electrons are then been transferred to electrodes is called 'electricigens' [1, 2]. Electricigens are known to convert organic waste into protons and electrons directly from the waste itself or with help of different alternative mechanism(s). However, in the advances in microbiology and biotechnology, some of the microorganisms have been identified as 'super bug' that mainly referred as unique microbes that can perform electron transfer directly with the aid of appendages equipped on the microorganisms or some natural surface proteins on the microbes. For example, *Geobacter* sp. [3] has been reported to possess appendage-like accessory called nanowire that can attach to electrodes and directly transferred electrons without any aid of other chemical substance or physical means. Recently, *Geobacter sulfurreducens* KN400 mutants were discovered to produce high current densities in pure cultures [4]. The addition of *Shewanella* sp. and *Pseudomonas* sp. diversified the types of unique microorganisms that each of one of them possesses different capacity of power generating mechanism(s).

Naturally, microorganism(s) competes to use substrate available in order to survive in their micro-environment and this may be a challenge in bioreactor like an MFC. However, there were reports of more than two microbes present in an MFC and able to produce way better or more or less similar power value to individual microbes [5, 6]. Previously, it was conducted by our research team on immobilized cells which were individual microbes been immobilized separately and then mixed in a single chamber MFC and produced comparable power to other MFC trials to date [7].

In the present research, a bacilli species has been isolated from anaerobic digester of a local waste water treatment plant (designated as E1) and been tested for its ability to initiate electricity production in a MFC. In addition, the *Bacilli E1* was mixed with another microorganisms which is known not able to conduct any voltage production as a control. The data will be presented on its open circuit and closed-circuit condition using a structurally designed MFC which was based on the principle of a dual chamber reactor separated by a proton-exchange membrane.

## Materials and Methods

### Microorganism characterization

*Bacilli E1* was isolated from an anaerobic digester of wastewater treatment system in a local palm oil mill in Pasir Gudang, Johor. Briefly, wastewater samples were diluted several times with sterile distilled water and dilutions were plated onto nutrient agar and incubated in an anaerobic jar (Thermo scientific Oxoid) supplemented with a commercially available Anaerogen sachet (Oxoid) which provides anaerobic conditions. Emerging microbes were then re-isolated in the same conditions and selected for MFC trials. Meanwhile, *Clostridium cellulolyticum* ATCC35319 (designated as CC) a strictly anaerobic microorganism has been purchased from American Type Culture Collection (ATCC).

Both microbes were subjected to standard simple staining and gram staining. Briefly, for simple staining, the microbes were heat-fixed onto a glass slide and one drop of methylene blue stain (Merck) was added to the glass slide for staining. Meanwhile, gram staining was conducted by fixing individual microbes onto a glass side and stained with sequential steps of different solutions (crystal violet, iodine, and safranin) which was purchased as kit from Merck Milipore.

### MFC preparation

Stock *Bacilli E1* and *CC* cell suspensions were maintained in -80 $^{\circ}$C previously and thawed prior to use. An amount 12.5 mL each of the cell suspension were transferred into a freshly prepared MFC containing 10% glucose in 0.1 M Potassium phosphate (Kpi) buffer with a pH of 7. All chemicals were analytical grade and sterilized through autoclaving prior to use.

### MFC operation

An acrylic dual chamber MFC was designed with a total volume of 250 mL for each chamber. In addition, anodic chamber was filled with 0.1 M Kpi, pH 7.0 mixed with cell suspension *Bacilli E1* and glucose as described previously or otherwise mixed of *E1* and *CC* for comparison. As for the cathodic chamber, 0.005 M of potassium permanganate in 0.1 M Kpi, pH 7, was added. Both chamber was equipped with rectangular shaped reticulated carbon cloth with a surface area of 20 $cm^2$. Meanwhile, a Nafion membrane (117) was pre-treated and set in between the chambers to separate them. The MFC was operated for three days to analyze the MFC performance in electrical production or voltage values with no resistant applied in between anode and cathode or indicated as open

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circuit voltage (OCV). In addition, after OCV values were stable, different resistant values were applied in between the anode and cathode and voltage values were measured or indicated as closed-circuit voltage (CCV). Both OCV and CCV were monitored and recorded using an auto-logged multimeter.

**Closed-circuit voltage analysis**
CCV obtained was then calculated for power and current using the general equation (1 and 2) by Ohm's Law;

$$V = IR \quad (1)$$

$$P = V^2R \quad (2)$$

where V is the voltage obtained, I is the current calculated from V and resistant applied designated as R. P is the calculated power. All MFC trials were done in duplicates.

## Results and Discussion

**General characterization**
Simple and gram staining reveals that the microbe designated as E1 was bacilli in shape as shown in Figure 1. In addition, the gram staining shows that *Bacilli E1* was gram negative. In the group of 'electricigens' so far, there is no generalization that 'electricigens' are gram negative in nature but it is known from literature that generally in the MFC, these microbes tend to form electroactive biofilms on the anodic electrode [7, 8, 9] and it was also observed that after a period of time, biofilm formed on the anodic surface (data not shown). It was shown in several MFC trials that these electricigens within the attached biofilm on the anodic electrodes, capable of donating electrons or receiving electrons from electrodes, via extracellular electron transfer within the MFC environment [10]. However, it was difficult to explore how the electron transfer occurs in the vicinity of *Bacilli E1* without further dissections of the electron transfer process. In the next section, *Bacilli E1* capability in producing electricity will be discussed.

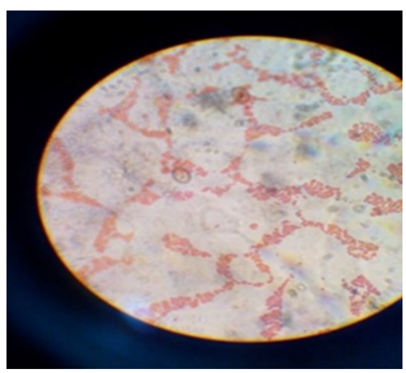

Figure 1. *Bacilli* E1 stained as gram negative

**Open circuit voltage**
Basically, OCV cannot be evaluated as indicator of the MFC performance but merely indicates of potential existence. Figure 2 (a) shows that a maximum average of 0.8 (V) was produced using *Bacilli E1* but *CC* did not show any changes in OCV values and produced less potential difference. Furthermore, similar maximum average of 0.8 (V) was observed for using a mixture of both microbes in the same MFC. This observation proved that the presence of E1 is needed to ensure the possibility of electrical activity initiated in the MFC. However, the OCV start-up seems to shift rapidly for mixed culture which an indication of possible fast degradation though there was no test done in access of the degradation products content. In addition, thermodynamically, the OCV value can be evaluated as the cell electron motive force ($E_{mf}$) which is a maximum achievable voltage without taking account on the losses due internal resistance in the system [11, 12]. From the present results, $E_{mf}$ = 0.8 V was achieved after 5

to 6 hours of operation and stabilized for almost 50 hours as shown Figure 2a. In contrast, as later shown, with current presents, the maximum voltage produced was much less than 0.8 V.

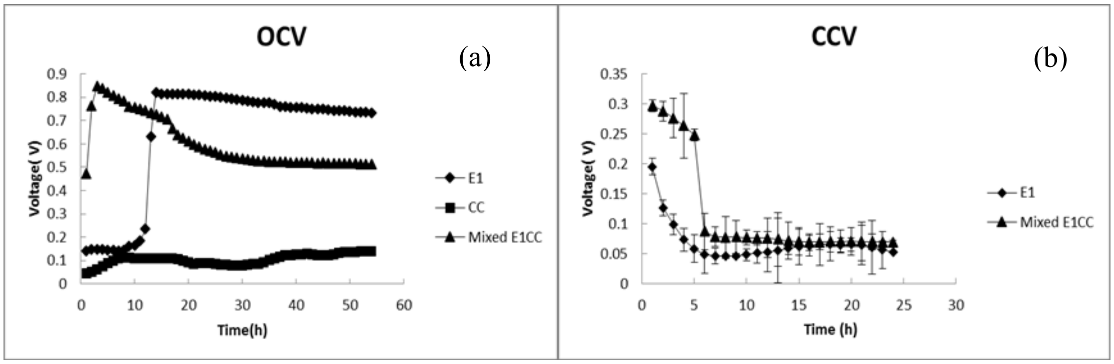


Figure 2. Voltage production in open and close circuit conditions (a) the open circuit voltage (OCV) profile and (b) shows the closed-circuit settings with 1000 Ohm as the resistant applied

**Closed circuit voltage**

The presence of load or resistant, at 1000 Ohm showed a rapid voltage drop (from 0.3 V to approximately 0.1 V) using the mixed culture which an indication of higher current densities produced (based on Ohm's Law) with maximum average calculated value of 0.2 ± 0.017 mA. In contrast, more stable voltage was activated after approximately 5 hours, mainly after large internal resistant and external resistant were equal [13]. It was reported previously more than 50% glucose carbon was discharged as carbon dioxide gas, acetate and various soluble products [14]. Meanwhile, it was also reported that the glucose metabolism within the fuel cell was a combination of both aerobic and anaerobic conditions which also means that the varieties of ionic components produced would probably lead to high internal resistance [12, 13, 14]. However, to synthesize the true source of high internal resistance that occurs in a particular MFC, further comparison in terms of type of membranes, electrolyte used and electrodes type should be conducted [15].

Meanwhile, Table 1 shows comparison of some established lab-scale MFC with the present tested MFC. Based on calculation, the presence dual chambered MFC for mixed culture used produced approximately 0.1 $Wm^{-2}$. The power density obtained was comparable to most sugar-based MFC but the differences in value are due to different materials of electrodes, the microbes used and independent design (anode surface area, volume, etc.) [16] which influence the performance of the MFC.

Table 1. Comparison of different studies of sugar-based MFC

| Type of MFC | Substrate or Fuel | Mode of Operation | Power ($Wm^{-2}$) | References |
|---|---|---|---|---|
| Dual chamber | Date syrup | Batch | 0.065 | [17] |
| Dual chamber | Date syrup | Batch | 0.052 | [18] |
| Dual chamber | Glucose | Fed-batch | 3.6 | [19] |
| Dual chamber | Glucose | Batch | 0.1 | Current work |

## Conclusion

It was revealed that *Bacilli E1* was able to be used for electricity production using microbial fuel cell (MFC). The highest OCV obtained by single microorganism *Bacilli E1* was 0.8 V and the CCV value with 1000 Ohm resistant produced a stable 0.1 V with calculated current of 0.2 ± 0.017 mA.

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