UKM2 Chlorella sp. Strain Electricity Performance as Bio-anode under Different Light Wavelength in a Biophotovoltaic Cell  
(Prestasi Elektrik Strain UKM2 Chlorella sp. sebagai Bio-anod di bawah Gelombang Cahaya Berbeza dalam Sel Biofotovoltan)

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ABSTRACT

A biophotovoltaic cell (BPV) is an electrobiochemical system that utilises a photosynthetic microorganism for instance is algae to trap sunlight energy and convert it into electricity. In this study, a local algae strain, UKM2 Chlorella sp. was grown in a BPV under different trophic conditions and light wavelengths. Once the acclimatisation phase succeeded, and biofilm formed, power generation by UKM2 algae at the autotrophic mode in synthetic Bold’s Basal media (BBM) under white, blue and red lights were tested. Polarisation and power curves were generated at these different conditions to study the bioelectrochemical performance of the system. Later, the condition switched to algal mixotrophic nutritional mode, with palm oil mill effluent (POME) as substrate. Maximum power generation obtained when using UKM2 in BBM under red light where a power density of 1.19 ± 0.16 W/m^2 was obtained at 25.74 ± 3.89 A/m^2 current density, while the open circuit voltage OCV reached 226.08 ± 8.71 mV. UKM2 in POME under blue light recorded maximum power density of 0.85 ± 0.18 W/m^2 at current density of 16.75 ± 3.54 A/m^2, while the OCV reached 214.05 ± 23.82 mV. Chemical oxygen demand (COD) removal reached an efficiency of 35.93%, indicating the ability of wastewater treatment and electricity generation in BPV at the same time.

Keywords: Algae; bioelectricity; biophotovoltaic; monochromatic light

INTRODUCTION

World energy demand will be experiencing high surge attributes to the upbeat industrial revolution and population growth. By the year 2040, world energy demand expected to rise to almost 30% from 575 quadrillion BTU in 2015 (EIA 2017). Currently, 91% of world energy supply is from non-renewable resources, mainly fossil fuels (Jones & Warner 2016). The problems associated with the reliance on fossil fuels for energy supply are its rising price, the release of greenhouse gas emissions and its risk of depletion. Researchers hence have focused on greener and cleaner technologies for energy production in the future like solar, hydro-energy, biomass and wind power, which are renewable. However, each form of renewable energy...
is associated with a different problem such as expensive semiconductors for solar, destruction of habitat through hydro-energy, crop competition with biomass and unsteady weather for wind (Jones & Warner 2016).

A fuel cell is an emerging technology in the field of clean energy production due to its absence of combustion and minimal pollution (Jafary et al. 2018; USGS 2018). A branch of the fuel cell, microbial fuel cell (MFC) mainly is being deeply explored for electricity production. An MFC consists of an anode and a cathode in a single unit whereby a living microorganism used as biocatalyst at the anode. The microorganism oxidises substrates in the anode chamber to liberate electrons. These electrons will travel to the cathode via an external circuit, thus generating power (while releasing CO$_2$ in the anode chamber (Daud et al. 2018; Shamsuddin et al. 2018). At the cathode, an electron acceptor such as oxygen would accept the electrons and become reduced to water (Rusli et al. 2018). Wastewater is usually used as an organic substrate for microorganism at the anode, consequently coupled with wastewater treatment (Rahimnejad et al. 2014).

A biophotovoltaic (BPV) cell has a similar setup as that of the MFC. The difference lies when the BPV utilises anoxygenic photosynthetic microorganism such as the algae or cyanobacteria at the anode (McCormick et al. 2014). During photosynthesis, the photosynthetic microorganism generates electrons through water photolysis to produce oxygen at the anode (Dolai 2016; Ucar et al. 2017) and later reduces to water at the cathode. Therefore, BPV deemed as more carbon-neutral than MFC for not requiring a continuous supply of organic substrates (McCormick et al. 2014). Studies of BPV utilising algae focuses on the following species: Chlorella vulgaris, Chlamydomonas reinhardii, and Dunaliella tertiolecta with most studies utilising Chlorella vulgaris as the biocatalyst. They reported that Dunaliella tertiolecta achieved higher power output (7 mW/m$^2$) compared to Chlorella vulgaris (0.45 mW/m$^2$); however, Chlorella vulgaris (Thong et al. 2019) was far superior in terms of biomass density and biofilm attachment on the anode (McCormick et al. 2011). In an open-air cathode BPV, Chlorella vulgaris achieved 0.014 mW/m$^2$ of power generation in autotrophic conditions (De Caprariis et al. 2014). Up until now, limited information discusses on algal electricity generation in mixotrophic growth, combining both autotrophic and heterotrophic modes of growth (Ng et al. 2018; Saar et al. 2018). Algae is a microorganism that can utilise both modes of growth, as seen in studies involving algal wastewater treatment (Baicha 2016). Light intensity and type of light exposed to algae in BPV also has an impact on its electricity generation (Anderson et al. 2016). Algal contains chlorophyll and chlorophyll b, both of which are light-harvesting pigments that are sensitive to red and blue lights. A report shows that algal appears growing under red and blue lights (Singh & Singh 2015). In a study involving Chlorella vulgaris in a BPV with domestic wastewater, red light had the best impact on algae growth and simultaneously, the current generation (Yan et al. 2013).

The purpose of this research was to compare the performance of Chlorella vulgaris as a catalyst in BPV when in autotrophic and mixotrophic conditions and to determine the best monochromatic light for algal bioelectricity generation in the BPV. Therefore, this study highlights the capability of the algae species to produce clean electrical energy through BPV system while complementing the UKM2 Chlorella sp. application in wastewater treatment (Hariz et al. 2019; Hazman et al. 2018).

**MATERIALS AND METHODS**

**MEDIUM AND CULTIVATION OF MICROALGAE**

UKM2 Chlorella sp., a local microalgae strain isolated from palm oil mill effluent (POME) was selected for this study, and it was obtained from Carbon Capture Laboratory of Faculty of Engineering and Build, Universiti Kebangsaan Malaysia (UKM). UKM2 was cultivated in two different media; Bold’s Basal Media (BBM) for autotrophic and POME for mixotrophic growth study. The compositions for both media were as provided in Tables 1 and 2. The cell cultivation was using a setup, as shown in Figure 1, at 25°C and under 24 h exposure to a fluorescent lamp. UKM2 was transferred to the BPV once it reached the exponential growth phase.
LIGHT SOURCE

LED USB strip lights (Ledener, Canada) capable of emitting red, green, blue, and white light through a remote, was used as the light source for UKM2. White light (wavelength of 390 - 700 nm) was used during the acclimatisation phase. However, either blue (wavelength of 450 - 495 nm) or red light (wavelength of 620 - 750 nm) was applied during further parameter testing. The LED strips taped to the wall of a black box containing the BPV cells. The cells were placed at about 1 cm distance from the wall. The distance provides the UKM2 in the anode with a light source of 1000 lux.

| TABLE 1. Composition of Bold’s Basal Media (BBM) |
|-----------------------------------------------|
| **Solution** | **Component** | **Quantity (g)** |
| Main solution (in 10 mL) | NaNO₃ | 0.25 |
| | CaCl₂·2H₂O | 0.025 |
| | MgSO₄·7H₂O | 0.075 |
| | K₂HPO₄ | 0.075 |
| | KH₂PO₄ | 0.175 |
| | NaCl | 0.025 |
| | H₃BO₃ (in 1 mL) | 0.01142 |
| EDTA solution (in 1 mL) | EDTA | 50 |
| | KOH | 31 |
| Acidified metal solution (in 1 mL) | FeSO₄·7H₂O | 0.498 |
| | H₂SO₄ (96%) | 0.1 mL |
| | ZnSO₄·7H₂O | 8.82 |
| Trace solution (in 1 mL) | MnCl₂·4H₂O | 1.44 |
| | MoO₃ | 0.71 |
| | CuSO₄·5H₂O | 1.57 |
| | Co(NO₃)₂·6H₂O | 0.49 |

| TABLE 2. Composition of palm oil mill effluent at pH 7.5 |
|-----------------------------------------------|
| **Parameter** | **Value (mg/L)** |
| BOD₅ | 809 |
| COD | 2370 |
| TN | 97.22 |
| NH₃-N | 197.5 |
| Oil & Grease | 0.63 |
| Copper (Cu) | 0.08 |
| Zinc (Zn) | 0.04 |
| Cadmium (Cd) | 0.011 |
| Phosphorus (P) | 8.734 |
A double-chambered BPV constructed from a light penetrable ‘Perspex’ material with a total volume of 0.1 L in each chamber. Long graphite brushes of 7.5 cm twisted around titanium wires were used as the anodes and cathodes while Nafion cationic exchange membranes (CEM) placed in between the two chambers. Each open-end anode chamber sealed with a glass panel (96 × 56 × 2 mm) to allow penetration of the light source.

At the beginning of the experiments, the anolyte volume filled with 50% UKM2 Chlorella sp. culture and 50% of BBM. Potassium hexacyanoferrate (III) together with potassium phosphate buffer at 100 mM became the catholyte. The distance between the anode and cathode electrodes was about 5 cm. The BPV components and configuration is, as shown in Figure 2. The anolytes were agitated at a rate of 400 rpm. The voltage across the electrodes was monitored continuously by an online data acquisition system (Keithley 2700 Multimeter, Keithley, America). All experiments ran in triplicates.

For the first 20 days, UKM2 in the BPVs were exposed to constant 24 h illumination of 1000 lux white light (as control). An external load of 1000 ohm applied to each cell, and the voltage data from each cell recorded continuously. Fresh BBM media replaced half of BBM anolyte once the voltage dropped to 45 - 60 mV at each cycle. The anodic chamber was sparged with nitrogen gas by bubbling for 30 s to displace the oxygen produced by algal photosynthesis. Once stable voltage cycles over time were observed, the algae considered as successfully attached to the surface of the electrodes, forming the biofilm.

Once the biofilm formed, the LED light strip then switched to blue colour and the cells were exposed to 24 h blue light at 1000 lux over four days. Electrogenic activity assessment was performed at the end of the fourth day to test the performance of the cells under the blue light condition. The same procedure repeated when operated in red light. About 50% of BBM media from the anode was replaced with POME media. The replacement of media was after analyzing the performance of the cells under the illumination of blue and red lights. The POME media centrifuged twice at 8000 rpm for 10 min prior to use. Later, the POME was autoclaved at 121 °C to ensure the elimination of microorganism in the POME. The cells again exposed to the blue and red lights, and their electrogentic activities recorded.

For the electrogenic assessment, open-circuit voltage (OCV), closed-circuit voltage (CCV) and current at 1000 Ω external load were measured using the data acquisition system. Polarisation and power curves were generated through linear sweep voltammetry technique using the Autolab Metrohm potentiostat (Metrohm, Sweden), with scan rate of 1 mV/s and range from 0 mV to system open circuit potential detected by the measuring instrument. The densities normalised according to the volume of the anolyte, which was 0.1 L. Each time the media was replaced, COD, pH, and dissolved oxygen (DO) readings were taken from the residual media. The COD measured by using high range COD vials (Hach, USA) according to the manufacturer’s manual while the DO
was measured using the Multiparameter HI2040-01 DO Meter (Hanna Instruments, USA). Cyclic voltammetry analysis was conducted on the anolytes and anodes using the potentiostat with Ag/AgCl as a reference electrode. The potential scan range was between -1 and 0.1 V at a scan rate of 0.025 V/s. The Columbic efficiency (CE) of the cells were calculated using the formula used by Rodrigues (2014):

$$CE = \frac{I \cdot t \cdot M}{n \cdot F \cdot v \cdot An \cdot \Delta COD}$$

where I is current (A); t is batch time (h); M is molecular weight of substrate in media (g/mol); n is number of electrons exchanged per mole of oxygen; F is 96,485 C/mol (Faraday’s constant); vAn is the anolyte volume and is the change of COD during that batch time.

ALGAL BIOFILM IMAGING
At the end of the study, a 0.5 cm cut of the graphite brush was extracted from the system for imaging under Variable Pressure Scanning Electron Microscope (VPSEM) LEO1450VP (Zeiss, German). Before the graphite brush cut-out analysed under VPSEM, it was immersed inside ethanol of different concentration for 10 min each: 30, 50, 70, 80, 90, and 100%. The sample was dried again via a critical point dryer before viewing using VPSEM.

RESULTS AND DISCUSSION
AUTOTROPHIC GROWTH: ELECTROGENIC ACTIVITY OF ALGAE DURING ACCLIMATISATION PHASE
During the acclimatisation phase, CCV and current generated from the cells under an external load of 1000 ohm were recorded and plotted in Figure 3. Each cycle indicates the replacement of BBM media in the anolyte and hexacyanoferrate in the cathode. All cells reached a homeostatic state from the acclimatisation phase by the 12th day (at the end of Cycle 3) and a stable pattern of electricity generation. The acclimatisation period is slightly longer than that achieved by De Caprariis et al. (2014) who obtained acclimatisation of Chlorella vulgaris within six days, however, with a different setup and media for algae growth. The voltage recorded increased each time the media was replaced, indicating the ability of UKM2 adapting to the environment. In Cycle 1, carbon dioxide supplied to all systems as a carbon source for oxygenic algal photosynthesis; however, a variation of voltage recorded was very small up to 9 ± 7 mV. This voltage could also reflect noise or variation from the data logger. The peak voltage values recorded were much lower than that obtained from another study which was 50 mV under an external load of 100 Ω (Subhash et al. 2013) with mixed algae culture in sewage water at the anode. In Cycle 2, an increased concentration of BBM only resulted in a decline of power (Ghoreyshi et al. 2011). The concentration used in Cycle 3 later was doubled from Cycle 2 and managed to increase the average voltage up to 63% from Cycle 1. At the end of Cycle 3, all cells indicated a steady pattern of voltage raised and declined during media replenishment, indicating a stable UKM2 community development in the anode. However, the voltage values in all systems need to be standardised in order to proceed with the next parameter.

![FIGURE 3. Closed circuit voltage of UKM2 Chlorella sp. under 1000 Ω resistance](image-url)
The deviation in power production among the replicate was due to one of the anodes had healthy algae growth, that appeared through the bright green colour. The healthy growth could contribute to its low CCV values. McCormick et al. (2011) and Saratale et al. (2017) stated that oxygen supplied by algae through its oxygenic photosynthesis develops a limitation within the system that in turn, causes a low power generation. Thus, in succeeding cycles, nitrogen gas had to be supplied to the anode for one minute at 60 cm$^3$/min in all cells for each feeding.

Sparging carbon dioxide for almost a minute into the BPV systems during each feeding time, might not be sufficient. There was a possibility that the carbon dioxide did not dissolve well in the anolyte, and little might have reached UKM2 for photosynthesis. Hence, in Cycle 6, sodium bicarbonate was chosen as an inorganic carbon source substitute as it could dissolve in the anolyte better than carbon dioxide. Sodium bicarbonate can increase algal biomass (Mokashi et al. 2016). In Cycle 7, the cells achieved peak voltages in the range of 40-53 mV and considered as a standardised and ready for the next testing parameter.

AUTOTROPHIC GROWTH: ELECTROGENIC ACTIVITY OF ALGAE UNDER DIFFERENT MONOCHROMATIC LIGHT

The power and polarisation curves were generated at the end of 26 days acclimatisation phase under white light. Then, the light was switched to blue light for four days, followed by a red light for another four days. Polarisation and power curves were generated at the end of each period. The internal resistance of the cell was determined by taking the linear gradients of the polarisation curves (De Caprariis et al. 2014; Logan 2008). A maximum power density of 1.19 ± 0.16 W/m$^3$ was acquired when UKM2 in BBM exposed to red light, which is 29% more compared to white light exposure (0.85 ± 0.02 W/m$^3$) and 26% more compared to blue light (0.88 ± 0.19 W/m$^3$) exposure (Figure 4). These results comply with the superior current production and internal resistance shown by the red light followed by the blue and the white lights (Table 3). Through observation, UKM2 in BBM media under autotrophic growth showed maximum peak power density when placed under red light, followed by blue light and white light (Figure 4). The same result obtained by Lan et al. (2013) when exposing *Chlamydomonas reinhardtii* to a red light and McCormick et al. (2011) when *Chlorella vulgaris* was under red light, where improvement of power generation was recorded.

Green algae, an oxygenic photosynthesis microorganism contains chloroplast in its cellular structure. Inside the chloroplast are two photosystems recognised as Photosystem I and Photosystem II, both of which contain chlorophyll and carotenoid pigments for light absorption (Lan et al. 2013). The maximum absorption peak for chlorophyll is at the red light wavelength while for carotenoid pigments is at the blue light wavelength.
Exposure to either of these lights proven to increase the efficiency of photosynthesis, resulting in an overall better algal growth (Singh & Singh 2015). The increase in photosynthetic efficiency indicates an improvement in electron generation, which leads to an improvement in electricity generation.

Since carotenoid pigments are secondary agents to photosynthesis/electron generation compared to chlorophyll, exposure to a red light, however, had shown to cause the highest power generation.

The OCVs recorded when operating the BPVs in different lights showed blue light was 1% more than the white, while the red light was almost 5% more than the white (Table 3). The obtained OCVs in this study, however, was higher than that reported by Zou et al. (2009) (reported OCV up to 130 mV), which used an air-cathode reactor while much smaller than that reported by Jadhav et al. (2017) (reported up to 570 mV), which used clayware reactor as a separator with lower overpotential.

The DO profile presented in Figure 5 signifies an almost constant level of DO at 2.5 – 5 ppm, with no apparent rise in oxygen content. The low stable oxygen content is attributed to the purging of nitrogen to the anode chamber of all systems during feeding time to avoid the accumulation of photosynthetic oxygen from interrupting in power generation. The profile, however, shows a slight increase in pH over time due to intake of sodium bicarbonate in BBM algal autotrophic growth (Kim et al. 2014).

MIXOTROPHIC GROWTH: ELECTROGENIC ACTIVITY OF ALGAE UNDER DIFFERENT MONOCROMATIC LIGHT

The experiment was repeated by switching UKM2 feeding mode from autotrophic to mixotrophic by changing the BBM media to POME and placing it under blue and red light for four days each. In mixotrophic growth, there was no apparent difference in power generation by UKM2 when using blue or red light. UKM2 in POME under both lights recorded a maximum power density of 0.85 W/m² (Table 4). A plausible explanation for the low power generation could be the anoxicogenic mixotrophic feeding mode carried by UKM2 in POME. The anoxicogenic environment created each time POME was replenished involving purging of nitrogen into the anode creating an oxygen-poor environment. Thus, UKM2 most likely switched to anoxygenic photosynthesis in this environment. In an autotrophic mode where algae would perform oxygenic photosynthesis, it would utilise photosystem I, photosystem II and the b-f cytochrome complex in an electron transport chain that would generate oxygen in the end. However, in the anoxygenic photosynthesis process, green algae use the bacteriochlorophyll molecules to trap sunlight energy as an energy source, additionally using organic carbon or inorganic carbon such as carbon dioxide as electron source (Ng et al. 2018). The pigment molecules that capture the sunlight would direct it to bacteriochlorophyll a, and after a series of photochemical reactions, negative and positive charges penetrate through the membrane without the formation of oxygen. According to Connolly et al. (1982), the maximum absorption peak for bacteriochlorophyll is the light wavelength in the range of 300 - 400 nm. Since blue light and red light are both out of these ranges, the exposure to either colour of the light was not able to increase the efficiency of anoxygenic photosynthesis by UKM2. Thus, there was no notable difference in power generation under these two lights. The red light, however, seems not compatible with mixotrophic growth. The internal resistance increased up to 86% with a standard error of the mean (σM) distribution increased up to 368% compared when in autotrophic growth (Tables 3 & 4). The result agrees with the OCV, increased up to 235% of σM in mixotrophic growth compared to autotrophic growth due to overpotential within the replicates.
As time progressed, DO, and pH levels showed a declining trend (Figure 6). The result is related to the mixotrophic growth whereby oxygen was used as the final electron acceptor in the dark aerobic respiration process, resulting in the production of carbon dioxide. The carbon dioxide generation contributes to a more acidic environment, hence a lower pH.
MIXOTROPHIC GROWTH: COD REDUCTION IN PALM OIL MILL EFFLUENT MEDIA

COD readings of the POME media was recorded before and after feeding it to UKM2 at the anode. As expected, a reduction of COD observed each time replacing the POME (Table 5). UKM2 utilised the organic carbon in its mixotrophic growth, which explains the COD reduction from the initial COD in the system, 2370 mg/L. The COD results within the four days of operation at different light sources in the mixotrophic growth environment recorded the total COD reduction at blue light (106%) compared to the red (38%) (Table 5). The obtained results support the conundrum of the increased in overpotential of the mixotrophic growth BPV when operating under red light.

| Parameter | Blue light (mg/L) | Red light (mg/L) |
|-----------|------------------|-----------------|
| Day 2     | 905 ± 18.72      |                 |
| Day 4     | 1323.67 ± 21.67  |                 |
| Day 6     | 1833.67 ± 39.07  | 2012 ± 33.98    |
| Day 8     |                  |                 |

MIXOTROPHIC GROWTH: COLUMBIC EFFICIENCY OF BPV

CE of the system refers to the comparison of electrons generated as a result of substrate degradation in anolyte. The substrate degradation causes electrons to move to the anode and through the external circuit of a cell. The higher its CE value, the higher efficiency of electricity generation of that system (Rodrigues 2014). For POME, acetate became the calculation basis for substrate (Hariz & Takriff 2017), and the following data was used in determining the CE using formula (1); where $M = 59$ g/mol, $n = 8$ electrons/mole and $t = 48$ h. CE value was calculated for BPV under red and blue lights in POME (Table 6). A higher CE was obtained under blue light (30.36%) when compared to red light (25.98%). The recorded value is much higher than Cui et al. (2014) and Zhou et al. (2012); both of which utilised algae as bio-cathode. The results in this study agree that biofilm growth of UKM2 Chlorella sp. on graphite brush has formed well (Rodrigues 2014).

| Parameter                      | Blue light | Red light |
|--------------------------------|------------|-----------|
| Maximum current density (A/m²) | 19.57 ± 3.85| 16.75 ± 3.54|
| Coulombic efficiency (CE) (%)  | 30.36      | 25.98     |

MIXOTROPHIC GROWTH: CYCLIC VOLTAMMETRY TEST ON BPV ANODE

The CV result obtained for anodic biofilm shows a bulge at the current (current produced when electrode donates an electron towards the electrolytes) around voltage of -395 to -195 mV (vs SHE), which was absent for the system without biofilm. Referring to the standard reduction potential for a biological system, at pH 7 and temperatures
between 25 - 37 °C, the voltage recorded should refer to the reaction of -ketoglutarate towards carbon dioxide, proton, and electrons for citrate production, while b-type cytochrome in Chlorella nitrate reductase is -168 mV (Barber et al. 1987). As this system operated at a pH of 8, it is unknown whether a similar reaction has occurred. However, VPSEM analysis performed was able to determine the presence of UKM2 biofilm on the anode.

Generally, for a CV test, a brush with biofilm layer on it will show a redox catalytic current value indicating the existence of oxidation-reduction reactions performed by algal biofilm layer. However, from Figure 7, it was observed that current from the anode of BPV with biofilm was lower than the one without biofilm (control) and vice versa with the current from the anode. This result signifies that for electrode biofilm in which CV performed in-situ; it is easier for the electrode to donate its electrons towards surroundings. Small current from the anode means the existence of high electrolyte resistance to donate electrons towards the cathode.

![Image](image.png)

**FIGURE 7.** Cyclic voltammetry test of BPV

**MIXOTROPHIC GROWTH: COMPARISON OF ELECTRICITY GENERATION WITH OTHER STUDIES**

The results of this study were compared with those obtained from other studies (Table 7). However, the studies utilising microalgae in the anode of BPV using graphite brush is still lacking, and most researches would normalise the current generation obtained in their systems with the surface area of the electrode. However, in this study, the current and power obtained were normalised using the volume of anolyte in the BPV. The results then are compared with studies that utilised brush electrodes in their BPV and where the data was normalised using the volume of the reactor.

Table 7 shows that the power generation of UKM2 *Chlorella* sp. in this study is still low compared to other studies. However, the difference is because of other studies utilised algae as bio-cathode, while this study used algae as bio-anode. Therefore, electricity generation of microalgae in BPV relies entirely on the photosynthetic efficiency of algae compared to other conventional MFCs, which utilises non-photosynthetic bacteria for power. According to McCormick et al. (2011), the electron transfer ability of oxygenic photosynthetic microorganism is lower than the non-photosynthetic bacteria in MFCs. Besides, the accumulation of oxygen at the BPV anode due to photosynthesis contributes to low electricity generation as time passes. These factors, added with other factors like BPV design, may explain, why the power generated by UKM2 as bio-anode in this study was much lower than other systems that utilising algae as bio-cathode.
TABLE 7. Comparison of electricity generation of UKM2 Chlorella sp. with other studies

| Parameter                | This Study | Cui et al. (2014) | Zhou et al. (2012) | Wang et al. (2010) | Ng et al. (2014) |
|--------------------------|------------|------------------|-------------------|-------------------|-----------------|
| Algae species            | UKM2 Chlorella sp. | Chlorella vulgaris | Chlorella vulgaris | Chlorella vulgaris | UMACC 313 Chlorella sp. |
| Type of cell             | Double chamber | Double chamber | Double chamber | Double chamber | Single chamber |
| Algae usage              | Bio-cathode | Bio-cathode | Bio-cathode | Bio-cathode | Air-cathode |
| Electrode                | Anode: Graphite brush, Cathode: Graphite brush | Anode: Carbon brush, Cathode: Carbon cloth | Anode: Carbon felt, Cathode: Carbon fibre cloth | Anode: Carbon brush, Cathode: Carbon cloth | Anode: Indium tin oxide (ITO) coated glass, Cathode: Platinum coated glass |
| Power density (W/m$^3$)  | 1.19       | 8.77             | 2.49             | 4.10 – 5.60       | 0.29 × 10$^{-3}$ W/m$^2$ |
| Current density (A/m$^2$)| 25.74      | -                | 7.9              | -                 | 5.35 × 10$^{-3}$ A/m$^2$ |
| COD reduction (%)        | 35.93      | -                | 84.8             | -                 | - |
| Columbic efficiency, CE (%)| 28.17     | 6.5              | 9.40             | -                 | - |

ALGAL BIOFILM FORMATION ON ANODIC SURFACE

The biofilm layer formation of UKM2 on anodic graphite brush was observed by comparing a small cut sample of graphite brush immersed in UKM2 culture to a clean graphite brush using VPSEM imaging. Figure 8(a) shows the VPSEM images obtained under 5000× magnifications obtained for graphite brush cut samples, both clean and with UKM2 biofilm. Based on Figure 8(b), the graphite brush with the UKM2 biofilm, has clumps of microorganism on the surface of the brush. Figure 8(b) especially shows the presence of spherical shape UKM2 cells forming a biofilm layer on the brush (Hariz & Takriff 2017).

(a) (b)

FIGURE 8. (a) Clean graphite brush and (b) graphite brush with UKM2 biofilm (Image taken with VPSEM at magnification of 5000×)
CONCLUSION
In this study, UKM2 Chlorella sp. was grown in two media, which are BBM and POME for inoculation inside the BPV. UKM2 culture showed good growth in both media and showed quick adaptation in the environment and was able to enter the exponential phase almost immediately. Once UKM2 inoculum introduced into the BPV anode, on average UKM2 was able to acclimatise in 12 days. Afterwards, a stable electricity generation (rise and decline over time) observed each time replacement of a new media. Overall UKM2 showed maximum power generation when grown in BBM and placed under red light, whereby a power density of 1.19 W/m² obtained at a current density of 25.74 A/m². Although the results obtained are lower than other studies, this difference is attributed to the usage of alga as bio-cathode in the other studies compared to bio-anode used in this study. More research is necessary for optimising the power generation of algae in BPV due to its potential as a carbon-neutral energy source with wastewater treatment integration.

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