INTRODUCTION

The significant increase in world energy consumption along with the expanding human population is leading to excessive carbon emissions to the atmosphere and climate change. Various clean energy alternatives including algal biophotovoltaic (BPV) devices have been proposed to tackle the carbon emission problem. Hammarström\(^1\) stated that 0.02% of solar energy that arrives on the earth’s surface would produce sufficient energy to satisfy the world’s energy demand, if the device has 15%-20% energy conversion efficiency. A BPV device contains photosynthetic microorganisms that absorb and convert solar energy into chemical energy with minimal carbon footprint.\(^2\) BPV platforms consisting of live...
photosynthetic organisms such as microalgae have demonstrated the feasibility of bioelectricity generation through photosynthesis.\textsuperscript{3-7} Instead of releasing carbon dioxide (CO₂), microalgae remove CO₂ from the surroundings for biomass production. Bioelectricity generation in algal BPV devices begins when light impinges on the algal cells. Microalgae have multiple protection mechanisms that shield the cells from sudden exposure to high irradiance but the deleterious consequences of excessive illumination over extended period of time can render the photoprotective mechanisms ineffective. Although most photoautotrophic organisms including microalgae will recover from minor photo-induced damages, the cessation in photosynthetic energy conversion under high irradiance will debilitate power output from algal BPV devices.\textsuperscript{8}

In microalgae, chlorophyll binding proteins harvest light energy from light sources during photosynthesis.\textsuperscript{9,10} The energy oxidizes water molecules into molecular oxygen, protons, and electrons in the Photosystem II (PSII) oxygen-evolving complex (OEC). Some of these electrons are attracted to the anode of an algal BPV device and flow to the cathodic side through an external circuit for a complete electrical circuit. Electrons from the cells are ferried to the electrode through one of the following pathways: (a) direct electron transfer (DET), (b) an endogenous electron transfer mediator such as Flavin, or (c) an exogenous electron transfer mediator such as polypyrrole and polyaniline.\textsuperscript{7,11,12} Despite the various electron shuttling pathways available, Bombelli et al\textsuperscript{3} explained that algal BPV devices without redox mediators were simpler, more efficient, and cheaper in upscaled applications. In the recent years, DET from biofilms has been linked to enhanced electron transfer as direct contact with the anode minimizes internal potential loss.\textsuperscript{4} Nevertheless, the magnitude of electric current generated is still circumscribed by the monolayer algal cells that are in direct contact with the anode.\textsuperscript{13}

Some of the earliest algal BPV devices contained algal biofilms in the anodic chamber for bioelectricity generation.\textsuperscript{4} The feasibility of growing algal biofilms directly on anodes made from various materials was validated by Thorne et al\textsuperscript{14} who used fluorine-doped tin oxide (FTO)-coated ceramics, FTO-coated glass and carbon felt in their study, although only FTO-coated ceramics enabled strong 	extit{Chlorella vulgaris} biofilm attachment and promising power output from their photo-microbial fuel cell. When Ng et al\textsuperscript{6} developed algal biofilm on ITO anode, there was a 10-fold increase in power output (3.13 × 10⁻⁴ W m⁻²) compared to the power output (24.8 μW m⁻²) reported by Inglesby et al.\textsuperscript{15} When the ITO anode was replaced with reduced graphene oxide (rGO) anode, Ng et al\textsuperscript{5} reported a promising 119% increase in power output compared to the power output from their previous study using ITO anode. Today, power generation from microalgae has evolved from using algal biofilms to using immobilized algal cultures. The idea of increasing power output from algal BPV devices using algal immobilization technique was validated when alginate was used as the immobilization medium for 	extit{Chlorella} cells to generate 18% more power than suspension cultures.\textsuperscript{7} Since microalgae are biological cells, the immobilization material should be inert, stable, cost effective, and not interfere with cell metabolism.\textsuperscript{16} Alginate has been reported to possess these characteristics.\textsuperscript{17} Hence, it is commonly used to encapsulate algal cells for biomass retention and resistance against reaction with toxic materials.\textsuperscript{18} Alginate is a biopolymeric chain made up of α-L-guluronic and (1,4)-linked β-D-mannuronic acid.\textsuperscript{19-25} The presence of divalent ions such as Ca\textsuperscript{2+} leads to formation of biopolymer from alginate with high mechanical strength.\textsuperscript{23} Immobilization with alginate provides a protected environment for microalgae cells from deleterious conditions that could affect cell growth.\textsuperscript{16} The transparency of the alginate gel ensures that the cells receive sufficient amount of light with minimal light attenuation.

Irradiance is one of the most crucial parameters that regulates the physiology of microalgae as the energy required for photosynthesis and biomass production comes from the sun. As irradiance level constantly fluctuates throughout the day, microalgae regulate the amount of energy absorbed through various regulatory mechanisms in PSII. Any excess light energy is removed via three different pathways: reemission as fluorescence, thermal dispersion, and conversion of chlorophyll-\textit{a} (Chl-\textit{a}) into its triplet state.\textsuperscript{26} Overexposure to light induces over-reduction of the electron transport chain and formation of reactive oxygen species (ROS) that could lead to cellular death.\textsuperscript{27} Carotenoids act as the first line of defense against photooxidative damage by quenching effective singlet oxygen near light harvesting complexes (LHCS) and photosynthetic reaction centers in PSII.\textsuperscript{28} While reshuffling of light harvesting apparatus under high irradiance is a vital photoprotective mechanism in microalgae cells, chlorophyll antenna size reduction in response to increasing irradiance was correlated to increased photosynthetic productivity in microalgae.\textsuperscript{29} Nonphotochemical quenching (NPQ), on the other hand, is a heat dissipation mechanism that comprises of individual quenching mechanisms such as energy-dependent dissipation in the PSII antenna known as qE.\textsuperscript{30} State transition quenching, qT, photoinhibitory quenching, qI\textsuperscript{26} and zeaxanthin-based quenching, qZ\textsuperscript{28} which work simultaneously to prevent overexcitation of PSII under high irradiance. Despite the existence of these protective mechanisms, light absorption beyond the photosynthetic capacity of the cells leads to reduction of the maximum photosynthetic rate, $P_{\text{max}}$.\textsuperscript{31} In fact, continuous illumination under intense light leads to photoinhibition in which synthesis of D1 protein for PSII recovery lags behind the rate of PSII degradation.\textsuperscript{32-34}

Microalgal growth can proceed in both light and dark conditions; autotrophic growth is subjected to the presence of light whereas energy-dependent heterotrophic growth occurs
when light availability becomes limited. Studies on effect of varying irradiance on oxygenic photosynthesis are essential because ATP and NADPH production as well as biomass manufacturing will not take place in the cells without the vibrational effect from electromagnetic wavelengths to initiate electron excitation. Simultaneously, oxidation of water molecules during photosynthesis requires substantial amount of energy to break the bonds between hydrogen and oxygen. Since bioelectricity generation from algal BPV devices is mainly reliant on the water splitting reaction, absence of light will result in much lower power output from the devices. Hence, the present study is aimed at profiling the power output of the algal BPV devices under increased irradiance.

2 | MATERIALS AND METHODS

2.1 | Algal culture

The algal strain selected for this project was the *Chlorella* sp. UMACC 313 from the University of Malaya Culture Collection (UMACC). UMACC 313 was isolated from a treatment pond of palm oil mill effluent. The methods of preparing and maintaining the algal cultures were adapted from Ng et al. and Ng et al. The inoculum size used was 20%, prepared from exponential phase algal cultures that were standardized at OD$_{620nm}$ = 2.0. The algal cultures, grown in Bold’s Basal Medium in 500 mL conical flasks, were placed on an incubator shaker (130 rpm) at a temperature of 25 ± 1°C and an irradiance level of 30 μmol photons m$^{-2}$ s$^{-1}$ on a 12:12 light–dark cycle.

2.2 | Immobilization of algal culture

Sodium alginate powder (purchased from Natural Colloids Industries Pte. Ltd.) was used to immobilize the *Chlorella* cells. The algal cells were immobilized in 2% sodium alginate. 4 g of sodium alginate powder was added into 190 mL of sterile distilled water to prepare 2% sodium alginate solution. The sodium alginate solution was continuously stirred for 24 hours.

The algal culture containing cells from the logarithmic growth phase was centrifuged at 604 g for 10 minutes. The supernatants were removed, and the concentrated algal cells were resuspended in Bold’s Basal Medium to prepare an algal suspension of OD$_{620nm}$ = 2.0. 10 mL of the algal suspension was added to the 190 mL sodium alginate solution to form an algal alginate suspension. Three mL of the algal alginate suspension was pipetted and spread onto ITO-coated glass slides (KINTEC) of dimension 3.5 cm × 3.5 cm and layer thickness of 100 nm. The ITO-coated glass with the algal–alginate layer was set aside for a minimum of 15 minutes to allow the algal–alginate suspension to settle on the glass surface. The gelation process of the algal–alginate suspension was completed by spraying 0.5 mL sterile calcium chloride, CaCl$_2$ (0.1 mol L$^{-1}$) solution on its entire surface. Sterile distilled water was used to rinse the surface of the gel film to remove the CaCl$_2$ solution after the culture immobilization process was completed (Figure 1).

2.3 | Algal BPV devices and experimental set-up

Each algal BPV device consists of a cathode and an anode made of platinum-coated glass and ITO-coated glass, respectively. The BPV device with suspension algal cultures was prepared by loading 3 mL of the *Chlorella* UMACC 313 culture (at exponential phase; OD$_{620nm}$ = 2.0) onto the anode through the open inlet in the middle of the algal BPV device (Figure 2) and was then sealed with “Smith & Nephew clear, sterile waterproof film dressing.” For the immobilized algae BPV device, the anode had the algal–alginate film (prepared as described in Section 2.2) attached to its surface (Figure 1) and was sealed with polydimethylsiloxane (PDMS) before 2 mL Bold’s Basal Medium was loaded to the device. The cathode and the anode were separated by a Perspex piece. Crocodile clips and copper wires were used to connect the anode and cathode to the external circuit. To investigate the effect of irradiance on algal growth and power output, the devices were placed in an incubator under conventional phosphor converted LED white lights with irradiance levels of 30, 90, 150, and 210 μmol photons m$^{-2}$ s$^{-1}$ throughout three different sets of experiments. Set 1 consisted of the control irradiance of 30 μmol photons m$^{-2}$ s$^{-1}$ compared with 90 μmol photons m$^{-2}$ s$^{-1}$. Set 2 consisted of the control irradiance compared with 150 μmol photons m$^{-2}$ s$^{-1}$. Set 3 consisted of the control irradiance compared with 210 μmol photons m$^{-2}$ s$^{-1}$. The temperature in the incubator was monitored with a temperature and light data logger (HOBO Pendant®) and was maintained at 25 ± 1°C.
2.4 Determination of chlorophyll-a, carotenoids, specific growth rate, and carbon fixation

The chlorophyll-a (chl-a) content of the algal cells was used to estimate biomass. Chl-a was determined on days 0, 4, 8, and 12. The suspension culture was filtered through glass-fiber filter papers (Whatman GF/C, 0.45 μm) to separate the algae from the medium. The filter papers with suspended algae from the medium. The filter papers with suspended algae were then ground into small pieces with a tissue grinder (Kimble) before being transferred into 10 mL of analytical grade 100% acetone. The centrifuge tubes were immediately wrapped with aluminum foil and stored in the freezer at 4 °C for 24 hours. After 24 hours, the samples were centrifuged at 604 g for 10 minutes. The chl-a content was determined through spectrophotometry at wavelengths of 630 nm (OD630nm), 645 nm (OD645nm), and 665 nm (OD665nm). The chl-a content of immobilized algal cells was determined by first releasing the cells from alginate gel film with a tissue grinder. The remaining procedures to analyze the chl-a content in suspension cultures were carried out for the immobilized algae cells. The formula used to calculate chl-a content was as follows:

\[
\text{chl-a} (\text{mg m}^{-3}) = \frac{(C_a - V_a)}{V_c} \tag{1}
\]

where \(C_a = 11.6 \times \text{OD}_{665\text{nm}} - 1.31 \times \text{OD}_{645\text{nm}} - 0.14 \times \text{OD}_{630\text{nm}}, \)
\(V_A = \text{Volume of acetone (mL) used for chlorophyll extraction; }\)
\(V_C = \text{Volume of algal culture (L); chl-a (mg L}^{-1} = \text{chl-a (mg m}^{-3})/1000.\)

A correlation analysis between chl-a, biomass, and OD750nm was carried out based on the suggestion by the reviewer.

Carotenoid content was estimated using the same extract used for the chl-a content estimation as described above. Carotenoid content was also determined using the spectrophotometry method at the wavelength of 452 nm. The formula used to calculate carotenoids content was as follows:

\[
\text{Carotenoids (mg L}^{-1}) = \frac{\text{OD}_{452\text{nm}} \times 3.86 \times V_c}{V_e} \tag{2}
\]

where \(V_e = \text{Volume of acetone (mL) used; } V_c = \text{Volume of algal culture (L).}\)

The specific growth rates, SGR (μ), of the algal cultures were determined from the chl-a content of the algal cells at exponential growth phase and were calculated with the following formula:

\[
\text{Specific Growth Rate, } \mu (d^{-1}) = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \tag{3}
\]

where \(N_2 = \text{chl-a content at } t_2; \ N_1 = \text{chl-a content at } t_1; \ t_2 - t_1 = \text{duration when exponential growth phase occurred.}\)

The carbon fixation and CO2 fixation rate were estimated according to Fulke et al40 using the following formula:

\[
\text{CO2 fixation rate, g L}^{-1} d^{-1} = 0.50P \times \frac{44}{12} \tag{4}
\]

where \(P = \text{biomass productivity; } P = (\text{biomass at } t_2 - \text{biomass at } t_1)/(t_2 - t_1); \ 44 = \text{Molecular weight of carbon dioxide (g mol}^{-1}); \ 12 = \text{Atomic weight of carbon (g mol}^{-1}).\)

2.5 Electrical measurements

Power output measurements were taken using a multimeter (Agilent U1251B) in terms of millivolts (mV). Resistors of different resistance loads (10 MΩ, 5.6 MΩ, 2 MΩ, 560 kΩ, 240 kΩ, 62 kΩ, 22 kΩ, 9.1 kΩ, 2.7 kΩ, and 910 Ω) were applied to the external circuit and by applying Ohm’s Law, and polarization curves were generated for determination of maximum current density and maximum power density. The second set of BPV devices using suspension algal cultures with the same cell concentration as the immobilized algae were used for comparison. All experiments were conducted in three individual replicates. Power density generated by the algal BPV devices comprising BBM (without the algae) and alginate (without the algae) was determined to observe whether power was generated by these abiotic components.

2.6 Pulse amplitude modulation (PAM) fluorometer measurements

Evaluation on the effects of irradiance levels on the photosynthetic activity of microalgae in BPV devices was conducted using chl-a fluorescence data; observations on the
changes in chl-a fluorescence were performed using the Pulse Amplitude Modulated (PAM) Fluorometry method. The photosynthetic parameters that were investigated in this study include maximum quantum efficiency \( F_v/F_m \), maximum relative electron transport rate \( \text{rETR}_{\text{max}} \), photoadaptive index \( E_k \), and nonphotochemical quenching (NPQ). All these parameters were measured with a Diving-PAM (Walz). The minimum fluorescence value \( F_o \) during the dark adaptation process and the maximum fluorescence value \( F_m \) when the reaction centers are closed after absorption of the energy of a photon were both measured with a PAM Fluorometer. The variable fluorescence, \( F_v \), is the difference between \( F_m \) and \( F_o \). The maximum quantum efficiency, \( F_v/F_m \), can then be calculated with the following formula:

\[
\text{Maximum quantum efficiency} = \frac{F_v}{F_m} = \frac{(F_m - F_o)}{F_m} \quad (5)
\]

where \( F_m \) = Maximum fluorescence value; \( F_o \) = Minimum fluorescence value.

Rapid light curves (RLC) were generated when the algae cells were exposed to actinic light emitted by LEDs at different irradiance levels. The initial slope of RLC, \( \alpha \) determined the maximum photosynthetic efficiency whereas the product of irradiance and quantum yield measured at the end of the interval determined rETR. \( E_k \) was calculated with the formula \( \text{rETR}_{\text{max}}/\alpha \) where \( \text{rETR}_{\text{max}} \) is the maximum photosynthetic rate. Excess light energy from photosynthesis will not be stored by photosynthetic plants but it will be converted into heat energy to be released. This phenomenon is expressed in the form of NPQ which can be calculated with the formula \( (F_m' - F_o')/F_m' \). All statistical analyses were conducted using the Statistica 8 program.

### Results and Discussion

#### 3.1 Growth of Chlorella UMACC 313 cultures in algal biophotovoltaic devices

In this study, the growth of both suspension and immobilized cultures was estimated using algal biomass (Chl-a content), as shown in Figures S1-S3. Although exposure to different irradiance levels may affect the pigment content of the algal cells, strong correlations between chl-a and biomass \((r = 0.976, P < .01, \text{between } \text{OD}_{500\text{nm}} \text{ and biomass } (r = 0.980, P < .01))\) and between chl-a and \( \text{OD}_{700\text{nm}} \) \((r = 0.951, P < .01))\) were found. The specific growth rate, SGR, of the Chlorella cultures was determined from the exponential growth phase of the algal cells. When SGR of the suspension cultures was compared across the irradiance levels of 30, 90, and 150 μmol photons m\(^{-2}\) s\(^{-1}\), the SGR at 150 μmol photons m\(^{-2}\) s\(^{-1}\) was significantly higher (ANOVA, \( P < .05 \)) than the lower irradiance levels. The SGR of suspension cultures was observed to increase in the following order of irradiance levels: 30 μmol photons m\(^{-2}\) s\(^{-1}\) < 210 μmol photons m\(^{-2}\) s\(^{-1}\) < 90 μmol photons m\(^{-2}\) s\(^{-1}\) < 150 μmol photons m\(^{-2}\) s\(^{-1}\). The increase in SGR with respect to increasing irradiance is due to larger amount of energy provided at higher irradiance for higher biomass production through increased metabolism. Effective utilization of higher amount of light promotes ATP and NADPH production which in turn stimulated cell growth. Below light saturating limit, microalgae responds to increasing irradiance with additional chlorophyll pigment protein complexes to readily absorb the light available. The resulting increase in chlorophyll content (data not shown here) supports faster electron transfer from water to NADP\(^+\) for NADPH formation which along with ATP are then used to manufacture sugar through the Calvin–Benson cycle. Nevertheless, increased biomass productivity with respect to increasing irradiance is only pertinent to a certain extent. At 210 μmol photons m\(^{-2}\) s\(^{-1}\), the decrease in SGR in both suspension and immobilized cultures (Refer to Figure 3) indicated that the algal cells were experiencing photo-induced stress, as the energy level received by the algal cells exceeded the amount of energy required for optimum biomass production. Hence, the SGR at 210 μmol photons m\(^{-2}\) s\(^{-1}\) was lower than the SGR at 90 μmol photons m\(^{-2}\) s\(^{-1}\) and 150 μmol photons m\(^{-2}\) s\(^{-1}\), respectively.

#### 3.2 Photosynthetic performance of suspension and immobilized Chlorella UMACC 313 cultures in algal biophotovoltaic devices

Tables 1-3 show the photosynthetic performance of the suspension and immobilized cultures in the algal BPV devices at
30, 90, 150, and 210 μmol photons m$^{-2}$ s$^{-1}$ in terms of maximum quantum efficiency. The maximum quantum efficiency of PSII, denoted as the variable $F_{v}/F_{m}$, measures the concentration of open reaction centers in PSII that are actively utilizing the light quanta absorbed for photosynthesis to proceed. In suspension cultures, $F_{v}/F_{m}$ ranged between 0.510 ± 0.116 and 0.748 ± 0.012 and was highest at the beginning of the experiment for all irradiance levels. In the study conducted by Parkhill et al. a $F_{v}/F_{m}$ value of 0.6 was determined to be the minimum value for identifying healthy algal cells. At 210 μmol photons m$^{-2}$ s$^{-1}$, $F_{v}/F_{m}$ was below the threshold value of 0.6 on Days 4, 8, and 12, thus suggesting that the Chlorella cells were unable to acclimate to high irradiance. In general, the $F_{v}/F_{m}$ values in immobilized cultures began declining after Day 8 due to nutrient depletion in response to cell growth. At exponential growth phase, the algal cells are actively multiplying, thus nutrient uptake will be high. Once nutrient starts depleting, the cells enter stationary growth phase and the effect of nutrient deficiency is revealed in terms of declining maximum quantum efficiency. $F_{v}/F_{m}$ could indicate lower photosynthetic response due to morphological change in algal cells. Ciniciato and collaborators explained that the presence of electric field generated from the potential across an algal BPV cell could transform algal cell proteins and lipids and affect photosynthesis, reflected in terms of low $F_{v}/F_{m}$ values. Since no detailed structural analysis was performed on the Chlorella cells in the algal BPV devices used in this study, there is no evidence that the decreasing $F_{v}/F_{m}$ in the present study was due to modified cell components, but such possibility will be taken note in future studies to ascertain the main factor that affects the maximum quantum yield of the algal cells.

**TABLE 1** Maximum quantum efficiency, $F_{v}/F_{m}$ for suspension and immobilized cultures at irradiance levels of 30 and 90 μmol photons m$^{-2}$ s$^{-1}$; data as means ± SD (n = 3)

| Day | 30 Suspension | 30 Immobilized | 90 Suspension | 90 Immobilized |
|-----|---------------|----------------|---------------|----------------|
| 0   | 0.748 ± 0.012$^{a}$ | 0.519 ± 0.021$^{def}$ | 0.715 ± 0.013$^{ab}$ | 0.478 ± 0.056$^{def}$ |
| 4   | 0.510 ± 0.116$^{def}$ | 0.708 ± 0.037$^{abc}$ | 0.644 ± 0.048$^{abde}$ | 0.606 ± 0.105$^{abcde}$ |
| 8   | 0.652 ± 0.041$^{abcde}$ | 0.666 ± 0.001$^{abcd}$ | 0.635 ± 0.040$^{abcde}$ | 0.620 ± 0.055$^{abcde}$ |
| 12  | 0.654 ± 0.031$^{abcde}$ | 0.641 ± 0.028$^{abcde}$ | 0.638 ± 0.025$^{abcde}$ | 0.595 ± 0.113$^{abcde}$ |

Note: Difference between alphabets indicates significant differences between different irradiance levels (ANOVA, Tukey HSD test, $P < .05$).

**TABLE 2** Maximum quantum efficiency, $F_{v}/F_{m}$ for suspension and immobilized cultures at irradiance levels of 30 and 150 μmol photons m$^{-2}$ s$^{-1}$; data as means ± SD (n = 3)

| Day | 30 Suspension | 30 Immobilized | 150 Suspension | 150 Immobilized |
|-----|---------------|----------------|---------------|----------------|
| 0   | 0.680 ± 0.018$^{abcd}$ | 0.575 ± 0.050$^{abcdde}$ | 0.685 ± 0.009$^{abcd}$ | 0.412 ± 0.046$^{f}$ |
| 4   | 0.645 ± 0.034$^{bcde}$ | 0.630 ± 0.016$^{abcd}$ | 0.623 ± 0.025$^{abde}$ | 0.536 ± 0.105$^{cdef}$ |
| 8   | 0.623 ± 0.009$^{bcde}$ | 0.555 ± 0.097$^{bcde}$ | 0.560 ± 0.049$^{bcde}$ | 0.612 ± 0.014$^{abcde}$ |
| 12  | 0.654 ± 0.007$^{bcde}$ | 0.632 ± 0.096$^{abcd}$ | 0.593 ± 0.052$^{bcde}$ | 0.583 ± 0.065$^{abdef}$ |

Note: Difference between alphabets indicates significant differences between different irradiance levels (ANOVA, Tukey HSD test, $P < .05$).

**TABLE 3** Maximum quantum efficiency, $F_{v}/F_{m}$ for suspension and immobilized cultures at irradiance levels of 30 and 210 μmol photons m$^{-2}$ s$^{-1}$; data as means ± SD (n = 3)

| Day | 30 Suspension | 30 Immobilized | 210 Suspension | 210 Immobilized |
|-----|---------------|----------------|----------------|----------------|
| 0   | 0.719 ± 0.022$^{ab}$ | 0.602 ± 0.057$^{abcdde}$ | 0.729 ± 0.003$^{ab}$ | 0.639 ± 0.027$^{abcde}$ |
| 4   | 0.655 ± 0.015$^{bcde}$ | 0.571 ± 0.104$^{abcddef}$ | 0.577 ± 0.064$^{abcde}$ | 0.660 ± 0.017$^{abcd}$ |
| 8   | 0.664 ± 0.057$^{bcde}$ | 0.676 ± 0.018$^{abcd}$ | 0.595 ± 0.032$^{bcde}$ | 0.638 ± 0.025$^{abcde}$ |
| 12  | 0.660 ± 0.077$^{abcd}$ | 0.674 ± 0.020$^{abcd}$ | 0.517 ± 0.009$^{def}$ | 0.602 ± 0.082$^{abcd}$ |

Note: Difference between alphabets indicates significant differences between different irradiance levels (ANOVA, Tukey HSD test, $P < .05$).
Maximum relative electron transport rate, \( r_{ETR_{\text{max}}} \), defines the rate at which electrons are mobilized across the photosynthetic electron transport chain. \( r_{ETR_{\text{max}}} \) values in this study ranged between 16.996 ± 0.850 \( \mu \text{mol electrons m}^{-2} \text{s}^{-1} \) and 244.069 ± 7.964 \( \mu \text{mol electrons m}^{-2} \text{s}^{-1} \) (Refer to Figure 4A,B). A decreasing trend was observed in the \( r_{ETR_{\text{max}}} \) of suspension cultures throughout the duration of the experiment, but \( r_{ETR_{\text{max}}} \) was significantly higher (ANOVA, \( P < .05 \)) in immobilized cultures on Day 4 compared to Day 0. When the algal cells were immobilized on Day 0, sudden restriction on mobility led to cellular stress and affected the photosynthetic performance as well as electron transport in the cells. In suspension cultures, the algal cells are constantly floating in media, adjusting to the height where optimum irradiance level for cell metabolism is detected. If a biofilm is formed from settled algal cells, the contrast in light absorbed by the cells at different biofilm thickness levels will be large. The cells closer to the light source will receive more light compared to the cells lying underneath. As a result, light attenuation could possibly render some of these cells photosynthetically inactive.\(^{50}\) Similarly, immobilized algal cells can only absorb the limited photons available when irradiation quality is poor. Hence, immobilized \textit{Chlorella} cells suffered a setback in electron transfer on Day 0 when the cells were unable to reposition for better light harvesting. Once the cells had adapted to being encapsulated within the alginate gel matrix on Day 4, improvement in light utilization efficiency followed by increased electron transport rate in the cells was observed.

Light harvesting efficiency, \( \alpha \), is another parameter that supplements \( F_v/F_m \) in determining photosynthetic efficiency. It translates the ability of algal cells to efficiently harvest and convert light energy into a numerical value.\(^{51}\) Figure 5A,B illustrate \( \alpha \) of both suspension and immobilized cultures at the different irradiance levels investigated. The range of \( \alpha \) in suspension cultures fell between 0.210 ± 0.004 and 0.769 ± 0.017 whereas \( \alpha \) in immobilized cultures ranged from 0.179 ± 0.007 to 0.843 ± 0.033. The maximum \( \alpha \) values in both suspension and immobilized cultures were found on Day 8 at 210 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \). The high \( \alpha \) values were results of high radiant flux received by the algal cells at 210 \( \mu \text{mol photons m}^{-2} \text{s}^{-1} \). However, not all the light...
impinged on the surface of the cells could be utilized for photosynthesis as low $F_v/F_m$ values at 210 μmol photons m$^{-2}$ s$^{-1}$ indicated that the algal cells were stressed at high irradiance. Despite effective harvesting of light, regulation of photons was rather ineffectual as saturation of light in the algal cells could have dampened the number of functional reaction centers in PSII for efficient photosynthetic activity to occur.

3.3 Power output from suspension and immobilized Chlorella UMACC 313 cultures in algal BPV devices

The highest power densities from all irradiance levels were shown in Figure 6A,B,C, respectively. In this study, the highest power density generated from suspension cultures under light and dark conditions was 0.345 ± 0.056 mW m$^{-2}$ and 0.258 ± 0.033 mW m$^{-2}$, respectively, at 90 μmol photons m$^{-2}$ s$^{-1}$. On the other hand, the highest power output from immobilized cultures under light and dark conditions was 0.456 ± 0.026 mW m$^{-2}$ and 0.430 ± 0.126 mW m$^{-2}$, respectively, at 150 μmol photons m$^{-2}$ s$^{-1}$. At 210 μmol photons m$^{-2}$ s$^{-1}$, the maximum power density was low in both suspension and immobilized cultures, producing only 0.098 ± 0.012 mW m$^{-2}$ and 0.103 ± 0.002 mW m$^{-2}$, respectively, under light condition (Figure 6C). Previously, Ng et al$^7$ reported maximum power output from suspension and immobilized cultures under light condition as 0.245 ± 0.019 mW m$^{-2}$ and 0.289 ± 0.004 mW m$^{-2}$, respectively. Hence, the power generated in this study improved by 40.8% in suspension cultures and 57.8% in immobilized cultures. When analyzed in terms of power density normalized to Chl-a, the maximum power density per Chl-a from suspension cultures and immobilized cultures was 23.445 ± 1.586 mW m$^{-2}$/mg Chl-a at 30 μmol photons m$^{-2}$ s$^{-1}$ and 326.278 ± 28.033 mW m$^{-2}$/mg Chl-a at 150 μmol photons m$^{-2}$ s$^{-1}$, respectively.

The highest power output from suspension cultures at 90 μmol photons m$^{-2}$ s$^{-1}$ was relatable to high photosynthetic capacity of the algal cells. In a study conducted by Parkhill et al$^{47}$ the minimum threshold for microalgae to be considered healthy was 0.6. Masojidek and collaborators,$^9$ on the other hand, reported $F_v/F_m$ values of up to 0.8 as indication of healthy microalgae. On Day 8, the $F_v/F_m$ value for suspension cultures at 90 μmol photons m$^{-2}$ s$^{-1}$ was 0.635 ± 0.040.
The healthy cells acclimated to the amount of light energy supplied at 90 μmol photons m\(^{-2}\) s\(^{-1}\) and effectively utilized most energy for photolysis of water, resulting in high electron turnover in PSII and eventually high power output from the algal BPV devices.

Maximum power density in immobilized culture was observed at a higher irradiance level compared to suspension culture, mainly because the alginate gel matrix offers a conducive environment for cellular growth by protecting the cells from adverse conditions.\(^16\) Besides, cell immobilization in alginate leads to larger deposition of algal cells on the base layer of the alginate gel matrix which enhances electron mobility across the cell–anode interface. The close contact between the lowest layer of algal cells and the ITO anode promotes dynamic electrochemical activities on the electrode,\(^52\) thus leading to high power density from the algal BPV devices. Observation on the voltage against current density curve in Figure 7 shows that the activation loss and ohmic loss in the immobilized system occurred at a consistent trend with respect to increasing current density. Activation loss is understood as the charge transfer resistance stemming from low initiation rate of anodic and cathodic activities.\(^4\) Since the alginate layer is in direct contact with the anodic surface, the reduced distance between immobilized cells and the electrode leads to faster reaction rate and subsequently, lower charge transfer resistance and activation loss. In immobilized cultures, ohmic loss is minimalized by adding minimal BBM to the device chamber to provide essential nutrients to the algal cells as large volume of medium contains high concentration of ions that will increase ionic resistance in the algal BPV device, besides attenuating light.

When the algal BPV devices were exposed to a higher irradiance level of 210 μmol photons m\(^{-2}\) s\(^{-1}\), the significant decrease (ANOVA, \(P < .05\)) in maximum power density compared to the lower irradiance levels of 30, 90, and 150 μmol photons m\(^{-2}\) s\(^{-1}\) (as shown in Figure 6C) was attributed to photoinhibition in the algal cells. Photoinhibition stems from formation of ROS that renders the PSII reaction centers dysfunctional.\(^26\) Excessive photon absorption induces disequilibrium between the rate of D1 protein degradation in PSII and the rate of repair of PSII.\(^53\) The consequential decline in photosynthetic activity reduces electron transport rate along PSII and delays turnover of the electron transfer chain for the subsequent round of electron flow to ensue.\(^53\) Hence, the reduced electron donation from the water-oxidizing complex in PSII became the limiting factor in high power generation from the algal BPV devices at high irradiance in this study.

In the present study, higher power output was obtained from immobilized cultures compared to suspension cultures. This finding was expected as the improved cellular contact between immobilized cells lays a truncated pathway for electron flow from the cells to the anode.\(^7\) The cutdown on electron transmission distance increases electron transfer efficiency,\(^50\) as charge transfer resistance is reduced at the electron-electrode interface when the electrons are nearer to the anode.\(^54\) In immobilized cultures, close contact between the alginate-immobilized algal cells and the anode was established from the moment when the algal–alginate film was attached to the surface of the anode during the preparation process whereas suspension cultures are free to move within the medium in the device, and only a portion of the cells make contact with the anode. The alginate film has a denser number of cells compared to the suspension culture. The immobilized algae grow faster than the suspension algae.\(^55\) This results in higher electron transfer in the immobilized system resulting in higher power output. Besides, the higher cell density in immobilized cultures protected the algal cells from overexposure to irradiation as mutual cell shading prevents the cells from absorbing excessive light energy that results in formation of ROS and impaired reaction centers in PSII.

Experimental data in the present study indicated that maximum power density was generated from suspension cultures (BBM with algae) at 90 μmol photons m\(^{-2}\) s\(^{-1}\) and immobilized cultures (alginate-BBM with algae) at 150 μmol photons m\(^{-2}\) s\(^{-1}\). When the algal BPV devices were investigated using only BBM (without algae) at 90 μmol photons m\(^{-2}\) s\(^{-1}\) and alginate-BBM (without algae) at 150 μmol photons m\(^{-2}\) s\(^{-1}\), it was observed that 0.066 ± 0.010 mW m\(^{-2}\) and 0.115 ± 0.007 mW m\(^{-2}\) were generated, respectively (Figure S4). However, the power generated by the suspension cultures and the immobilized cultures in all replicates was significantly higher (ANOVA, \(P < .05\)) than the power generated by the BBM and the alginate-BBM alone, indicating that the Chlorella UMACC 313 cells were the main contributing factor in power generation from the algal BPV devices.

Apart from determining power output from the algal BPV devices, carbon fixation in the algal BPV devices was also computed on Day 8 when power output from

![FIGURE 7](image.png)  
**FIGURE 7** Polarization curve of immobilized culture that produced the maximum power density at 150 μmol photons m\(^{-2}\) s\(^{-1}\). Data are presented as mean ± SD in three replicates (n = 3).
both suspension and immobilized cultures was found to be highest at 90 and 150 μmol photons m\(^{-2}\) s\(^{-1}\), respectively. Carbon fixation by suspended and immobilized algal cells was estimated to be 0.012 mg carbon and 0.021 mg carbon, respectively. On the other hand, CO\(_2\) fixation rate was estimated four days from Day 4 to Day 8 of the experiments. The CO\(_2\) fixation rate for suspension and immobilized cultures was determined to be 58.10 mg CO\(_2\) L\(^{-1}\) d\(^{-1}\) and 217.11 mg CO\(_2\) L\(^{-1}\) d\(^{-1}\), respectively. When expressed in terms of anode surface area, the CO\(_2\) fixation rate for suspension and immobilized cultures was 10.71 mg CO\(_2\) m\(^{-2}\) d\(^{-1}\) and 12.23 mg CO\(_2\) m\(^{-2}\) d\(^{-1}\), respectively. It must be noted that these values are based on very small BPV devices with anode surface area of 0.0009 m\(^2\). Although the carbon fixation rates have been extrapolated from BPV devices with small surface areas and volumes, they show potential when the systems are scaled up. These values are expected to be higher when the algal BPV devices are eventually scaled up, as Ho and co-authors\(^\text{56}\) reported that some Chlorella sp. cultures were able to fix carbon at a rate ranging between 800 and 1000 mg L\(^{-1}\) d\(^{-1}\). The higher carbon fixation rate in immobilized cultures at higher irradiance (150 μmol photons m\(^{-2}\) s\(^{-1}\)) was reasonable, considering that a linear correlation between the quantity of light absorbed by microalgae and the cells’ carbon fixation rate was drawn\(^\text{56,57}\). During photosynthesis, carbon dioxide is utilized in the Calvin–Benson cycle through regulation of the enzyme ribulose bisphosphate carboxylase-oxygenase (Rubisco); declining Rubisco level induces higher CO\(_2\) uptake by up to magnitudes of three.\(^\text{58}\) When required, the enzyme carbonic anhydrase (CA) converts bicarbonate, HCO\(_3^-\) to CO\(_2\) for Rubisco-driven metabolic activities.\(^\text{59}\) Hence, the regulation of Rubisco activity by shifting amounts of active and inactive forms of Rubisco in response to varying irradiance\(^\text{60}\) directly affects the carbon fixation rate by microalgae. In an operational system, modules of the devices will be stacked to provide the required power.

## 4 CONCLUSION

Power output from our algal biophotovoltaic devices has increased steadily from the change of anode material from ITO to rGO.\(^\text{5}\) The use of suspension algal cultures to biofilms\(^\text{6}\) and finally alginate-immobilized systems\(^\text{7}\) resulted in further increase in power density. Results from the present study gave a 32% higher power output from the immobilized algal cultures compared to the suspension cultures. The effective range of irradiance was determined, with highest power density being observed at 90 μmol photons m\(^{-2}\) s\(^{-1}\) and 150 μmol photons m\(^{-2}\) s\(^{-1}\) for suspension and immobilized algal cultures, respectively, while the highest irradiance level of 210 μmol photons m\(^{-2}\) s\(^{-1}\) resulted in reduced power due to photoinhibition of the photosynthetic process. The algal BPV devices have added value in performing carbon removal, thus its promise to be a new, sustainable, environment-friendly energy providing system.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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