Algae Biofilm on Indium Tin Oxide Electrode for Use in Biophotovoltaic Platforms

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Abstract
Algae are amongst the most photosynthetically efficient organisms harnessing solar energy for all its metabolic life-supporting activities. The solar energy is transformed into chemical energy in a normally wasteful process. In this study, this excess wasted energy may be directed towards electricity generation in a biophotovoltaic platform. This is a new approach in renewable energy production from algae. As an initial step, algal biofilms are established on indium tin oxide (ITO) anodes. Two microalgae, the unicellular \textit{Chlorella} UMACC 313 and the filamentous \textit{Spirulina} UMACC 159 were used to form biofilm on ITO anodes under three different treatments (T\textsubscript{1}: unmodified smooth surface, T\textsubscript{2}: modified surface etched with interval of 2.5 mm between lines and T\textsubscript{3}: modified surface etched with interval of 1 mm between lines). Results show significantly higher biofilm coverage on the etched anodes compared to the smooth ones. Anodes of T\textsubscript{3} registered the highest biofilm coverage of 99.46\% for \textit{Chlorella}. For \textit{Spirulina}, highest biofilm coverage (80.70\%) was observed on T\textsubscript{2} anodes. The increase in biofilm coverage successfully resulted in increase of photosynthetic efficiency for both strains. \textit{Spirulina} registered the highest maximum relative electron transport rate at 153.507 µmol electrons m\textsuperscript{-2}s\textsuperscript{-1} compared to \textit{Chlorella} (140.796 µmol electrons m\textsuperscript{-2}s\textsuperscript{-1}). This was correlated to pigment content. Biofilms established on the ITO anodes and the resulting high rate of photosynthetic efficiency achieved in these experiments are expected to enable electrical energy production from biophotovoltaic platforms.

INTRODUCTION
In photosynthesis, charge separation takes place in the electron transport chain with the energy directed for use in biomass production and the rest being wasted. Radiant energy absorbed by chlorophyll can undergo one of three fates: (i) used for photosynthesis (ii) dissipated as heat or (iii) re-emitted as chlorophyll fluorescence [1]. Hence, by measuring the yield of chlorophyll fluorescence, information about efficiency of photochemistry and heat dissipation can be generated using a pulse amplitude modulation (PAM) fluorometer (Diving PAM, Walz, Germany) [2]. In our current work, the capture and utilization of this excess and wasted energy is converted to electricity using a proposed biophotovoltaic (BPV) platform.

Cyanobacteria have been used for hydrogen generation [3] and electricity generation using 2-hydroxy-1,4-naphthoquinone as an electron shuttle between the algae cells and a carbon-cloth anode [4]. A bioreactor with an air cathode and a graphite-felt anode coated by a biofilm of bacteria.
and algae, generated electricity when irradiated [5]. Nanoprobes have also been developed to directly extract the photosynthetic electrons from single algal cells [6]. Indium Tin Oxide (ITO) will be utilized as the potential anode material in this experiment for the algae biofilm growth. ITO was an early favorite for hole injection cathode with good transparency and conductivity [7]. Previously, BPV studies have utilised various exogenous soluble mediator compounds to facilitate electron transfer. In a more recent study [8], the use of exogenous mediators was avoided with the development of biofilms. Biofilms are composed of microorganisms attached to surfaces forming a hydrated polymeric matrix composed of polysacharides, protein and nucleic acids [9].

MATERIALS AND METHODS

Growth of Biofilms. Fourteen strains from the University of Malaya Algae Culture Collection (UMACC) [10] and two strains from the Culture Collection of Marine Phytoplankton (CCMP), USA were screened for growth rate and photosynthesis efficiency. The freshwater Chlorella UMACC 313 and brackish-water Spirulina UMACC 159 were selected. Chlorella was grown in Bold’s Basal Medium while Spirulina was grown in Kosaric Medium [11]. 100 ml culture medium were placed into 200 ml glass staining jar and autoclaved. 50 ml of microalgae inoculum from exponential phase was added into the jar. ITO coated glass slides measuring 20 x 20 mm with three different treatments were prepared as follows: T1: unmodified surface (Smooth), T2: modified surface (etched with interval of 2.5 mm between lines) and T3: modified surface (etched with interval of 1 mm between lines). Etching was carried out using a diamond tipped pencil. The ITO slides were placed in the staining jar with the microalgae culture and transferred into an incubator at 24°C illuminated by cool white fluorescent lamps (30 µmol m⁻² s⁻¹) on a 12:12 hour light-dark cycle to allows for algae biofilm formation on the slides. This was done in triplicates. Every two days the biofilm growth was monitored by photographing the slide surface with a Sony Cyber-shot DSC-WX30 Camera until the slides were completely covered by the biofilm. The surface area coverage (%) of the biofilm on ITO was calculated using ImageJ software [12]. At the end of the experiment (day 18), the biofilm of each slide was removed by washing with jets of distilled water from a pipette in to a sterile beaker. The microalgae cells were then harvested by millipore filtration using filter paper (Watman GF/C, 0.45 µm) and the pigments of Chlorella and Spirulina were extracted using acetone [13] and phosphate buffer [14].

Pulse Amplitude Modulation (PAM) Fluorometer measurement. Photosynthetic parameters were measured fluorometrically using a Diving-PAM (Walz, Germany) [2, 15]. Rapid light curves (RLC) were obtained under software control (Wincontrol, Walz). Red light emitting diodes (LEDs) provided the actinic light used in the RLC at the level of 0, 307, 426, 627, 846, 1267, 1829, 2657 and 4264 µmol photons m⁻² s⁻¹. The biofilm of each ITO slide on day 18 was exposed to each light level for 10 seconds. Fv/Fm (maximum quantum yield), a parameter to indicate physiological state of phytoplankton was used to assess nutrient status and health. It is an indicator of stress and defined as: Fv/Fm = (Fm-F0)/Fm, where Fm is maximum fluorescence and F0 is minimum fluorescence resulting in variable fluorescence Fv. The maximum photosynthetic efficiency is determined from the initial slope (α) of RLC. The relative electron transport rate (rETR) was calculated by multiplying the irradiance by quantum yield measured at the end of that interval. Light saturation coefficient (Ek) are obtained from the curve fitting model [16]. The value is the interception point of the alpha value with the maximum photosynthetic rate (rETRmax) and defined as: Ek = rETRmax/α. Non Photochemical Quenching (NPQ) reflects the ability of a cell to dissipate excess light energy harvested during photosynthesis as heat and used as an indicator of photoprotection. NPQ is calculated as (Fm’).NPQ = (Fm’ - Fm’’)/ Fm’. All statistical analyses were performed using the Statistica 8 programme.
RESULTS AND DISCUSSION

Optimum biofilm coverage was observed after 18 days (Figure 1). From day 0 to day 18, a biofilm-like growth was evident for both cultures. Biofilms of *Chlorella* formed faster and covered more of the ITO slide. Biofilm coverage with *Chlorella* was best with T<sub>3</sub> > T<sub>2</sub> > T<sub>1</sub>. For *Spirulina*, biofilm coverage was best with T<sub>2</sub> > T<sub>3</sub> > T<sub>1</sub>. Etched surface provided better substrate for biofilm formation.

The unicellular (2-10 µm) *Chlorella* preferred the finer etched T<sub>3</sub> (1mm) surface but the filamentous *Spirulina* grew better on the T<sub>2</sub> surface with lines etched further apart (2.5 mm).

Table 2: Comparison of biofilm coverage (%), pigment content (µg/L) and photosynthetic efficiency values of *Chlorella* UMACC 313 and *Spirulina* UMACC 159 in the different treatments. Data as means ± S.D. (n=3). Differences between alphabets indicate significant difference between different strains and treatments (ANOVA, Turkey HSD test, p < 0.05).

<table>
<thead>
<tr>
<th>Surface</th>
<th>Biofilm coverage (%)</th>
<th>Pigment (µg/L)</th>
<th>Alpha(α)</th>
<th>rETR&lt;sub&gt;max&lt;/sub&gt; (µmol electrons m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>E&lt;sub&gt;k&lt;/sub&gt; (µmol photons m&lt;sup&gt;-2&lt;/sup&gt; s&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>F&lt;sub&gt;v&lt;/sub&gt;/F&lt;sub&gt;m&lt;/sub&gt;</th>
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<td><em>Chlorella</em> T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>90.50 ± 7.58&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.55 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.25 ± 12.51&lt;sup&gt;e&lt;/sup&gt;</td>
<td>179.39 ± 26.02&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.59 ± 0.02&lt;sup&gt;e&lt;/sup&gt;</td>
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<tr>
<td><em>Chlorella</em> T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>92.41 ± 6.44&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.95 ± 0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.49 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>118.82 ± 5.53&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>241.32 ± 14.31&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.65 ± 0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td><em>Chlorella</em> T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>99.46 ± 0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.72 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.64 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>140.80 ± 2.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>219.37 ± 12.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.64 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td><em>Spirulina</em> T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>58.59 ± 10.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.35 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>104.17 ± 12.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>235.16 ± 43.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.67 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><em>Spirulina</em> T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>80.70 ± 1.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.89 ± 0.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>153.51 ± 5.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>258.25 ± 12.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.67 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Spirulina</em> T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>66.66 ± 5.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.84 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>133.06 ± 5.84&lt;sup&gt;d&lt;/sup&gt;</td>
<td>243.90 ± 14.86&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.63 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
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Pigment content increased with biofilm coverage (%) but results showed that although biofilm of *Chlorella* was higher than in *Spirulina*, the latter had higher pigment content. This may be due to thicker biofilms formed by the filamentous *Spirulina*. Alpha(α), rETR<sub>max</sub> (µmol electrons m<sup>-2</sup> s<sup>-1</sup>), E<sub>k</sub> (µmol photons m<sup>-2</sup> s<sup>-1</sup>) and F<sub>v</sub>/F<sub>m</sub> derived from PAM readings indicate photosynthesis efficiency. *Spirulina* had higher photosynthetic efficiency than *Chlorella* (Table 2). Photosynthetic efficiency increased with pigment content following the percentage of biofilm coverage. The F<sub>v</sub>/F<sub>m</sub> data of all the biofilms showed healthy condition without any indication of stress. White et al. (2010) reported...
PAM fluorometry as a tool to assess *Chlorella* sp. from Kwazulu Natal, South Africa. *Chlorella* was reported to have the following photosynthetic parameters: Alpha(α): 0.34, $\text{rETR}_{\text{max}}$ (µmol electrons m$^{-2}$s$^{-1}$): 45, $E_k$ (µmol photons m$^{-2}$s$^{-1}$): 528 and $F_v/F_m$: 0.84 [17]. In our study, the range of photosynthetic parameters were: Alpha(α): 0.42-0.64, $\text{rETR}_{\text{max}}$ (µmol electrons m$^{-2}$s$^{-1}$): 76.25-153.51, $E_k$ (µmol photons m$^{-2}$s$^{-1}$): 179.39-258.25 and $F_v/F_m$: 0.59-0.67. This shows that our algal strains have higher photosynthesis efficiency than the South African strain indicating the potential of the Malaysian algae for use in biophotovoltaic platforms.

When the actinic light exceeds the plant photosynthetic requirements, the microalgae will undergo physiological regulation, including Non-photochemical quenching (NPQ) by diversion of excess energy from the photosystem reaction centres [18]. This is an important short term process for the photoprotection of microalgae against light induced damages. NPQ reflects the ability of a cell to dissipate excess light energy harvested during photosynthesis as heat [2]. Figure 2 showed that higher biofilm coverage provided higher photoprotection. *Chlorella* biofilm grown on ITO with 1 mm line spaces showed a higher capacity to generate NPQ with NPQ coefficient approaching 0.94 for the highest light level experienced during the RLCs. *Spirulina* biofilm grown on ITO with smooth surface meanwhile showed low ability to generate NPQ implying an inability to cope with greater irradiance.

Further studies of the biofilms were carried out in assembled biophotovoltaic devices. A series of studies were carried out to establish the biofilm coverage as an amount of photocurrent being generated and overall performance of the device. Figure 3 shows a typical power curve and polarisation curve observed for a *Chlorella* biofilm grown on ITO.
CONCLUSIONS

In this work, local algal strains were shown to be good candidates for utilization in future biophotovoltaic platforms. Modified anode surfaces meanwhile enabled increased biofilm coverage on anode materials as well as enhanced photosynthetic performance. Higher biofilm coverage and rate of photosynthetic efficiency achieved in these experiments are expected to enable increased electrical energy production from the proposed biophotovoltaic platforms.

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