Outstanding Photo-bioelectrochemical Cell by Integrating TiO$_2$ and Chlorophyll as Photo-bioanode for Sustainable Energy Generation

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Abstract. Photosynthesis is a technique for converting light energy into chemical energy that is both efficient and sustainable. Chlorophyll in energy-transducing photosynthetic organisms is unique because of their distinctive structure and composition. In photo-bioelectrochemical research, the chlorophyll's quantum trapping efficiency is attractive. Chlorophyll from Spirulina platensis is demonstrated to communicate directly with TiO$_2$-modified Indium Thin Oxide (ITO) to generate electricity without the use of any mediator. TiO$_2$-modified ITO with a chlorophyll concentration of 100 % generated the greatest power density and photocurrent of approximately 178.15 mW/m$^2$ and 596.92 mA/m$^2$ from water oxidation under light among all the other materials. While the sensitivity with light was 0.885 mA/m$^2$.lux, and Jmax value was 1085 mA/m$^2$. Furthermore, the power and photocurrent density as a function of chlorophyll content are studied. The polarizability and Van der Waals interaction of TiO$_2$ and chlorophyll are crucial in enhancing electron transport in photo-bioelectrochemical systems. As a result, this anode structure has the potential to be improved and used to generate even more energy.

Keywords: photo-current; Indium Thin Oxide; Van Der Waals interaction; polar interaction; light sensitivity

1. Introduction

Chlorophyll is widely utilized in the manufacture of fuels, hydrogen, and other high-value compounds. Chlorophyll discovered in higher plant and in microalgae are one of the greatest ways to collect light since all the subunits were optimized in a way maximum for photoconversion (Kirchofer et al., 2012). For these uses, chlorophyll's comparatively basic cellular architecture and ease of use have given it a competitive advantage over their photosynthetic equivalent. Direct energy production using chlorophyll has better benefits than algae or other dissimilar metals microorganisms, as chlorophyll can work for electricity production utilizing the photosynthesis electron transport chain without any outside fuels. Although technology for photosynthetic electrochemical cells is not developed enough for practical applications, ongoing progress in the area can make that technology a viable alternative to photovoltaics or comparable technologies today. In terms of biodegradability, low costs and no dependence on inorganic material, photosynthetic electrochemical cells provide distinct benefits than any similar technology (Sekar et al., 2014).

Photosynthesis-based energy conversion in the anode of photo-electrochemical cells has been studied utilizing isolated photosynthetic reaction centers such as thylakoids or chlorophyll Chlorophyll's photocatalytic characteristics can be adjusted to structural alterations (Wang et al., 2006; Wang et al., 2007). Chlorophyll-driven photosynthesis just requires light and water without an extra organic carbon source and so has a great potential to produce a clean, sustainable and environmentally acceptable alternative energy source (Sekar and Ramasamy, 2015). The further benefit of chlorophyll molecules is that J-type aggregates can form, directed by n-n stacking with neighboring molecules, which increases the mobility of their charging carrier (Duan et al., 2017; Amao & Komori, 2004). There are serious limitations to employing whole cell of algae since they need costly, labor demanding isolation processes, use sophisticated immobilization methods, low redox activity and have a lower long-term stabilization for practical use. Using chlorophyll in electrochemical cells as a photo-biocatalyst might circumvent such precautions. The extra light energy used by chlorophyll gives the electrical surface a higher stability.

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Due to its low cost, high stability, non-toxicity, biological inertness and redox capability, and environmentally friendly, TiO$_2$ was extensively investigated as the potential photocatalyst for carbon conversion, water splitting and degradation of hazardous contaminants in the aqueous and gaseous system (Rossettu et al., 2015; Venkatkarthick et al., 2015; Chen et al., 2016; Chen et al., 2015; Ferrari-Lima et al., 2015). TiO$_2$ is employed in several areas, including as solar cells, water and air purification, carcinoma treatment, and antibacterial self-cleaning materials due to the great stability redox characteristics (Liu et al., 2014; Luan et al., 2012). In case of TiO$_2$, the electron-holes are generated by irradiation by solar-light. The electrons in the conduction band (CB) convert the H$^+$ ions to H$_2$ and oxidize H$_2$O to create O$_2$ in the valence band (Gao et al., 2015).

In photosynthesis, chlorophyll pigment plays an essential role in transforming sunlight into chemical energy. The presence of TiO$_2$ in the chlorophyll structure plays an important part in light absorption. Using a TiO$_2$, we eliminate the need for costly metal-based catalytic to immobilize photosynthetic machinery on electrode surfaces and increase the efficiency of photocatalytic activity (Li et al., 2018; Qi et al., 2018; Omar and Abd Rahim, 2020). Chlorophyll absorbs light in different wavelengths and achieves a green wavelength color. In photo-bioelectrochemical cells, the study into chlorophyll dyes has attracted significant interest.

We intend in this work to modify TiO$_2$ with chlorophyll which has been isolated from microalgae *Spirulina platensis* and afterwards coated in ITO surface as a photo-bioelectrochemical cell photoanode for sustainable energy generation. Polarization and voltage would be studied and chlorophyll-TiO$_2$ bonding would be chemically investigated. Similar structures including chlorophyll and TiO$_2$ are commonly employed in the fabrication of solar cells, not photo-bioelectrochemical cells, therefore this is an academic novelty of this research. It will therefore be able to contribute even more to the development of photo-bioanodes for photo-bioelectrochemical cells, allowing for the generation of more sustainable and environmentally friendly electrical energy.

### 2.1 Materials and Methods

#### 2.1.1 Chlorophyll Extraction

Extraction of *Spirulina* using the technique introduced by Tong et al., (2012) with slight modifications. *Spirulina* powder was purchased from Nealgae Indonesia Makmur (Sukoharjo, Indonesia). A glass bottle containing 10 grams of *Spirulina* was filled with 100 mL of ethanol (Sigma Aldrich, St. Louis, USA). The mixture was then sonicated for 1 hour to aid in the extraction process (Christwardana et al., 2021). The mixture was then filtered, and the filtrate was collected. To avoid auto-oxidation, the filtrate is placed in a dark bottle. The UV-Vis spectra of chlorophyll extract can be shown in Figure 1 and its concentration was 120.72 ppm, obtained by using equation (1) to (3) from Arnon (1949).

\[
C_{a} (\frac{mg}{L}) = 12.74A_{663} - 2.69A_{645} \quad (1)
\]

\[
C_{b} (\frac{mg}{L}) = 22.9A_{645} - 4.68A_{663} \quad (2)
\]

\[
C_{\text{total}} (\frac{mg}{L}) = C_{a} + C_{b} \quad (3)
\]

where the $A_{663}$ and $A_{645}$ are the absorbance of chlorophyll at wavelength of 663 and 645 nm, respectively. While $C_{a}$, $C_{b}$, and $C_{\text{total}}$ are the concentration of chlorophyll-a, chlorophyll-b, and total chlorophyll, respectively.

#### 2.2 Photo-anode Modification

In the first stage, commercial ITO glass was sterilized for 15 minutes by sonication with ethanol as the medium. The ITO glass was then put on tissue paper. Each edge of the ITO glass was sealed, ensuring that the TiO$_2$ paste forms properly in the middle during the coating process. During the TiO$_2$ coating preparation procedure, 0.25 g TiO$_2$ (Merck, Darmstadt, Germany) was blended with 2 mL ethanol. The liquid was mixed until it becomes a paste, then poured over ITO glass that has been isolated on all four edges. Using a ruler, TiO$_2$ paste was uniformly spread on the surface of the ITO glass. The ITO glass was then annealed in the oven for 15 minutes at 240 °C. The ITO glass was then cooled to room temperature before being dripped with various concentration of chlorophyll as shown in Table 1, and let to stand for one day. TiO$_2$-chlorophyll on the surface of the ITO glass was washed with ethanol before to use as a photo-bioanode to eliminate unbound chlorophyll. The reaction mechanism between TiO$_2$ and chlorophyll was illustrated in Figure 2a and the photograph of photo-bioanode can be shown in Figure 2b.

<table>
<thead>
<tr>
<th>Table 1: The concentration of Chlorophyll-a, Chlorophyll-b, and Total Chlorophyll from chlorophyll extract of <em>Spirulina platensis</em></th>
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<td>Chlorophyll Concentration (% v/v)</td>
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2.3 Photo-bioelectrochemical Cell Configuration

The anode was ITO/TiO₂ containing chlorophyll, whereas the cathode was ITO/C placed a specific distance away from the anode. The reaction chamber's distance between the anode and cathode was measured to be 5 cm, as illustrated in Figure 3. The cathode in the reaction chamber reduces the oxygen emitted from the anode surface and converts it to water. The anode and cathode electrodes were serially linked and put in a reaction cell made of a beaker glass. In this experiment, water acts as the electrolyte solution for photo-bioelectrochemical cell. The photocurrent experiments were carried out with the help of a solar simulator and a special photo-biochemical system consisting of a modified light chamber equipped with a desk LED lamp of varying light intensity as a light source and a digital light meter LX1010B to measure the real-time light intensity inside the light chamber. The photo-bioelectrochemical was then placed within a modified light chamber and closed so that no light from the surroundings could enter. This analysis is performed in duplicate, and the findings presented are the averages.

2.4 Electrochemical Characterization

A multimeter device UNI-T UT-61E was systematically linked to the electrode of a photo-bioelectrochemical cell without the inclusion of any external resistance, which may be related to Open Circuit Voltage (OCV). After that, chronoamperometry (CA) measurement was determined and all CA measurements were taken using a multimeter under cyclic on-off illumination at a light intensity of 1600 lux within the light chamber. Various light intensity between 900 to 1600 lux was used to measure photocurrent of cell for sensitivity determination purpose. Various resistor values (10 MΩ – 100 Ω) were utilized to calculate the polarization and power curves under Closed-Circuit Voltage (CCV). The voltage values were read, and the electrical current was calculated by dividing the voltage by the resistance load, while the power density was calculated using Ohm's law and polarization curves were drawn following equation (4), (5) and (6) (Christwardana et al., 2020; Hadiyanto et al., 2021).

\[
I = \frac{V}{R} \quad (4)
\]

\[
J = \frac{I}{A} \quad (5)
\]

\[
P = V \times J \quad (6)
\]

where I and J are current (mA) and current density (mA/m²), respectively, V is voltage (V), R is resistance (Ω), A is electrode area (m²), and P is power density (mW/m²).

2.5 FTIR and Molecular Dynamics Characterization

FTIR measurements have been performed using a spectrometer Shimadzu IRAffinity-1S (Kyoto, Japan). The molecular dynamic of photo-bioanode was modeled using Marvinsketch 21.3 software while it undergoing molecular interaction such as polarizability and Van der Waals surface area. Menu calculations → charge → polarizability and calculations → geometry → molecular surface area (3D) was used to analyze the polarizability and Van der Waals surface area, respectively, on the structures of chlorophyll, TiO₂, and chlorophyll/TiO₂ composites.

3. Results and Discussion

3.1 Molecular Dynamic Simulation Analysis

The interaction of chlorophyll with TiO₂ is very interesting to investigate at the molecular level. Non-polar bonds dominate the bonding that happens in chlorophyll (left picture) in Figure 4a. Meanwhile, the TiO₂ bond (middle image) clearly demonstrates the polar connection between Ti and O. When chlorophyll interacts with TiO₂, some portions of the chlorophyll functional group bind to O in TiO₂. According to the results of molecular dynamic simulation, the interaction formed between TiO₂ and chlorophyll is dominated by polar bonds (right figure) with a more compact shape than chlorophyll's structure. This polarizability is critical because it is associated to TiO₂-chlorophyll conductivity in transferring electrons to the external circuit. As a result, more TiO₂-chlorophyll in the photo-bioanode will improve the electron transfer rate in the oxidation process at the anode side, improving the photo-bioelectrochemical cell's performance.

Fig. 2 a) Bonding mechanism between chlorophyll and TiO₂, while b) is photographs of TiO₂-Chlorophyll photo-bioanode in the surface of ITO glass

Fig. 3 The schematic diagram of anode and cathode of photo-bioelectrochemical cell
In addition to the polarizability of the interaction between TiO$_2$ and chlorophyll, the Van der Waals surface area was estimated to further understanding of the two materials’ interactions. The Van der Waals surface area was discovered to be linearly related to polarizabilities. In Figure 4b, the Van der Waals surface area of chlorophyll (left picture) is 1439.86 Å$^2$, whereas TiO$_2$ (middle image) has a surface area of 91.51 Å$^2$, which includes 60.64 Å$^2$ on O (30.32 Å$^2$ each) and 30.87 Å$^2$ on Ti. Meanwhile, the Van der Waals surface area of chlorophyll modified TiO$_2$ (right image) is 2375.81 Å$^2$, which is approximately 65% higher than chlorophyll. This demonstrates that the interaction of TiO$_2$ with chlorophyll increases the surface area of the photo-anode indirectly, which affects the performance of the photo-bioelectrochemical cell.

Fig. 4 a) Polar interaction in the molecule of chlorophyll (left), TiO$_2$ (middle), and TiO$_2$-Chlorophyll (right), while b) its Van der Waals surface area. Red and blue color show polar area, while grey color show non-polar area

3.2 FTIR Analysis

Identifying the chemical bonding development between TiO$_2$ and chlorophyll during anode synthesis is critical as shown in Figure 5. In the undoped TiO$_2$ photo-anode, the peak at 2863 - 2931 cm$^{-1}$ and 3228 - 3331 cm$^{-1}$ were attributed to the stretching vibration of the O–H bond or Ti-OH, as well as different interactions between hydroxyl groups and the TiO$_2$ surface (Lv et al., 2017). While the peak at 1640 cm$^{-1}$ corresponds to the bending H–O–H bond vibration of water molecules absorbed on the TiO$_2$ surface (Lv et al., 2017), the peak at 1631 - 1635 cm$^{-1}$ results in the C=O stretching vibration. At 425 - 721 cm$^{-1}$, a broad peak O-Ti-O or Ti-O-Ti stretching adsorption band in the TiO$_2$ lattice appeared (dang et al., 2009). When chlorophyll was added to TiO$_2$, the peak intensity of hydroxyl groups at 2869 - 2934 cm$^{-1}$ decreased as increased in chlorophyll concentration. In the FTIR spectra of chlorophyll-sensitized TiO$_2$, a new absorption peak at 2338 - 2359 cm$^{-1}$ showed that hydroxyl groups were depleted owing to chemical interactions between chlorophyll molecules and the surface of TiO$_2$ (Saikia & Parthasarathy, 2010). A small peak at 1317 cm$^{-1}$ appeared, corresponding to species with C-N bonds or nitrogen oxide species (Lv et al., 2017). While C=O stretching was seen in conjugated ketone and carbonyl groups at 1631 - 1635 cm$^{-1}$, CN stretching in chlorophyll was observed at 1405 cm$^{-1}$ (Dhafina et al., 2020; Nan et al., 2017). These observations therefore clearly show that Spirulina chlorophyll is strongly bound to the surface of TiO$_2$.

3.3 Voltage and Current Density of Photo-bioelectrochemical Cell

As shown in Figure 6a, the performance of a photo-bioelectrochemical cell incorporating chlorophyll was studied in pulse mode. The stability of the OCV at 0.85 V in light-on mode corresponds to the completely charged state. The inclusion of visible light might significantly improve the system’s power output. Contrary, in light-off mode, the OCV drops to 0.65 V and grows up to 0.75 V in the fully charged state. Because the OCV values in the four samples are almost identical, determining the optimal performance of a photo-bioelectrochemical cell based on voltage analysis is extremely challenging. Taking into consideration that operating the anode at the OCV value, which generates low anodic current densities, may result in the photo-bioanode degrading quickly owing to photo-oxidative damage to photosystem II (Pankratova et al., 2017). A change in the voltage decline to lower OCVs during the pulsing of the discharge is due to the rise in the chlorophyll redox potential in electrolyte (Heller, 2006). CA measurements are used to evaluate the influence of chlorophyll concentration on the electron transfer pathway, as illustrated in Figure 6b. The use of chlorophyll as an electron generator increases the amount of photocurrent produced, which ranges between 3200 and 4000 mA/m². The greatest resulting current is from ITO/TiO$_2$ with 100% chlorophyll with a value of 4000 mA/m², followed by 50% with a value of 3800 mA/m², which is slightly lower. The average photocurrent produced by ITO/TiO$_2$ with 25% and 0% chlorophyll was 3500 and 3100 mA/m², respectively. This means that
ITO/TiO$_2$ at 50% was optimum since the photocurrent result was comparable to chlorophyll at 100%. Under dark condition, the CA can achieve only 2500 mA/m$^2$. This finding demonstrates that increasing chlorophyll up to a certain extent can increase the photocurrent produced by the cell.

3.4 Sensitivity of Photo-bioelectrochemical Cell

The impact of the light intensity on the electrical current produced by photo-bioelectrochemical cells should be studied very importantly, given that the cell’s performance is one indicator for the sensitivity between biocatalyst and substrate. The Michaelis-Menten plot was therefore calculated for every photo-bioanode catalyst to get the apparent-Michaelis-Menten constant value (Figure 7). The difference in maximum photocurrent density ($J_{\text{max}}$) of ITO/TiO$_2$ with 0, 25, 50, and 100% chlorophyll were 889, 1009, 1041, and 1085 mA/m$^2$. While, the lowest $K_m$ values were ITO/TiO$_2$ with 100% chlorophyll with values of 908 lux, followed by 50, 25, and 0% chlorophyll with 1907, 2054, and 2257 lux values, respectively. The lower the $K_m$ value, the more sensitive the catalyst photo-bioanode is to light. The amount of chlorophyll connected to TiO$_2$ undoubtedly underlies this sensitivity, so that, the more chlorophyll is attached, the more protons and electrons in the presence of light are produced. The slope of each curve is determined to support the sensitivity. Among the four samples with values of 0.885 mA/m$^2$.lux, the sensitivity of ITO/TiO$_2$ with 100% chlorophyll continues to dominate, followed by 50% chlorophyll with value of 0.488 mA/m$^2$.lux, then 25 and 0% chlorophyll with value of 0.437 and 0.345 mA/m$^2$.lux, correspondingly.
3.5 Performance Photo-bioelectrochemical Cell

The performance of the photo-bioelectrochemical cell was further examined by performing discharge tests with varied external resistances, as shown in Figure 8a. Under the light, a maximum power output of around 178.15 mW/m² was obtained when 100% chlorophyll concentration was loaded in TiO₂, followed by 0% chlorophyll concentrations with values of 130.39 mW/m², or 27% lower than with 100% chlorophyll. Power generation in the dark condition was also tested (Figure 8b), with power density values of 75.55 and 89.46 mW/m² obtained from TiO₂ coupled without and with 100% chlorophyll concentrations, respectively. This result is comparable or higher than others which can be shown in Table 2.

3.6 Limitation and Future Outlook

This article explores the use of chlorophyll derived from the cyanobacterium Spirulina platensis as an anode photo-biocatalyst in photo-bioelectrochemical cells by altering it with TiO₂ on ITO substrates. The binding process between TiO₂ and chlorophyll, which affects electron transport, is the important process in this study. Because the quantity of chlorophyll linked to TiO₂ influences the performance of photo-bioelectrochemical cells, optimization is required. Binding chlorophyll to TiO₂ may be accomplished in numerous methods, including I dipping followed by immersion and (ii) dripping chlorophyll on TiO₂ that has been cast on the substrate. Advances in the usage of chlorophyll and TiO₂ as photo-anodes of photo-bioelectrochemical cells suggest that man-made photonic materials that mirror the notion of chlorophyll photosynthesis may be created in a viable manner. To improve electron transport, the electrode must be modified by boosting its conductivity. Lastly, the integration of modified anodes may result in not only photo-bioelectrochemical cells that convert light energy into electric power, but also chemical fuels (e.g., hydrogen) simultaneously. Furthermore, for increasingly complicated photo-bioelectrochemical cell applications, photocatalyst printing on flexible substrates is desirable.

4. Conclusion

TiO₂ loaded with chlorophyll extracted from Spirulina platensis and coated in ITO glass was created as visible-light-driven photocatalysts for energy production in aqueous electrolyte solutions in this study. By using bioelectrodes, this coupling photoanode-modified anodes to cathode generates increases the energy generation of a photo-bioelectrochemical cell. OCV can achieve 0.85 V under light conditions, whereas OCV can only achieve 0.65 V under dark conditions. Under light conditions, the current density may reach 4000 mA/m², which is 1.3 times higher than the 3100 mA/m² without chlorophyll, and 2500 mA/m² in the absence of light. The Michaelis-Menten constant was 908 lux and the sensitivity with light was 0.885 mA/m².lux, while the 10% value was 1085 mA/m². The power density generated from polarization curve was 178.15 mW/m² at current density of 596.92 mA/m², which is 1.3 times lower than the 385 mW/m². Under the condition, the current density may reach 4000 mA/m², which is 1.3 times higher than the 3100 mA/m² without chlorophyll, and 2500 mA/m² in the absence of light. The Michaelis-Menten constant was 908 lux and the sensitivity with light was 0.885 mA/m².lux, while the 10% value was 1085 mA/m². The power density generated from polarization curve was 178.15 mW/m² at current density of 596.92 mA/m². The Michaelis-Menten constant was 908 lux and the sensitivity with light was 0.885 mA/m².lux, while the 10% value was 1085 mA/m². The power density generated from polarization curve was 178.15 mW/m² at current density of 596.92 mA/m².


