



Construction of conducting polymer/cytochrome C/thylakoid membrane based photo-bioelectrochemical fuel cells generating high photocurrent via photosynthesis

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ABSTRACT

In this study, a photo-bioelectrochemical fuel cell was constructed for photocurrent generation by illuminating the electrodes within an aqueous solution. In this purpose, gold electrode was coated with poly 4-(4H-Dithieno [3,2-b:2';3'-d]pyrrol-4-yl) aniline, P(DTP-Ph-NH₂) conductive polymer film by using electrochemical polymerization. Then, P(DTP-Ph-NH₂) conductive polymer film coated surface was electrochemically modified with cytochrome C which covalently linked onto the surface via bis-aniline functionality of the polymer film and formed crosslinked-structure. The thylakoid membrane was attached on the surface of this electrode by using bisulfosaxinimidyl suberate (BS₃) and used as photo-anode in photo-bioelectrochemical fuel cell. The photo-cathode of the photo-bioelectrochemical fuel cell fabrication was followed by the modification of conductive polymer poly[5-(4H-dithieno [3,2-b:2';3'-d]pyrrol-4-yl) naphthalene-1-amine] film coating, glutaraldehyde activation, and bilirubin oxidase enzyme immobilization. During the photosynthesis occurring in thylakoid membrane under the light, water was oxidized and separated; while oxygen was released in anode side, the cathode side was reduced the oxygen gas into the water via a bio-electro-catalytic method. The cytochrome C was used for binding of thylakoid membrane to the electrode surface and play an important role for transferring of electrons released as a result of photosynthesis.

1. Introduction

Photosynthesis is an efficient, sustainable and complex process converting the light energy into chemical energy in fuel cells (Renger, 2008; Buchner and Ewingen, 2009). In recent years, many studies are carried out in developing the photo-electro-chemical or solar cells mimicking the photosynthesis or implementation of natural photosystems to run the electrochemical cells (Kalyanasundaram and Graetzel, 2010; Bard and Fox, 1995). Thylakoid membranes and photosystems isolated from plants or cyanobacteria are frequently used as a source for converting the light into electrical energy. Besides the light conversion, electron transport from reaction centers to the electrode having another important point for the photocurrent generation. Efforts have been increased by the researchers to produce enhanced photocurrents using the biological and synthetic materials for the system architecture

(Tender et al., 2002; Rosenbaum et al., 2010).

Thylakoid membrane is a structure existing in eukaryotic cells and some photosynthetic bacteria which functioning in light-dependent photosynthesis reactions. Thylakoid membrane has many integral protein complexes take place in absorbing the light and luminous stage reactions of photosynthesis. Under favor of these protein complexes named Photo-system I (PSI) and Photo-system II (PSII), by using the light energy, the chemical energy (ATP and NADPH) is obtained. The photons absorbed by the PSII reaction center, known as P680, the electron in this molecule raise to a higher energy level. Electrons captured by the electron acceptors called plastoquinone (Qa and Qb) molecules and transferred to another complex (Cytochrome *b₆f*) and then to the PSI. Photons reaching the PSI reaction center (P700) at different wavelengths, where electrons jump in higher energy and transfer to the enzyme NADPH oxidoreductase via Phylloquinone and Ferredoxine.

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These electrons combine with the NADP + molecule to form NADPH and the electron flow is terminated (Haehnel, 1977).

In order to satisfy the energy needs in future from solar energy, significant efforts are made in recent years to develop photo-electrochemical fuel cells based on the integration of natural photosynthetic reaction centers. For instance, the photocurrents generated by Photosystem I (PSI) and/or Photosystem II (PSII) when connected to Os complex conducting polymers (Hasan et al., 2015) and hydrogel (Badura et al., 2011), structures consisting of the integration of multiple surfaces (Ciesielski et al., 2010; Frolov et al., 2008) and self-integrated ones (Das et al., 2004; Ciesielski et al., 2008; Terasaki et al., 2009), p-added silicon (LeBlanc et al., 2012), bisaniline cross-linked plating nanoparticles (Yehezkeili et al., 2010) and gold nanoparticles (Yehezkeili et al., 2011) have been reported. Many studies that have been carried out on photosynthesis based photo-electrochemical cells have been obtained as a result of extracting the photosystems (PSI and PSII) insulated from thylakoid membrane. The thylakoid membranes, which are used in photo-electrochemical fuel cell systems and can be immobilized via simple methods, allow the electron transfer through various ways. For this reason, they are more advantageous than insulated reaction centers in studies of translating the light into the electrical energy. They can be easily utilized for the photo-electrochemical system offering high-efficiency of the energy cycle and electricity production (Fuhrmann et al., 2009).

Electrical conductivity of cytochrome C (Cyt C) molecule, which is a molecule that is similar to plastoquinone and plastocyanin conveying electron, has been reported in many studies such as bio-electrocatalytic transformations (Katz et al., 2004), electrochemical biosensors (Katz et al., 2001) and biofuel cell production (Katz et al., 1999). Under favor of these properties of it, Cyt C can be used as used as active components in the tailoring of biofuel cells. As a functional material, the ability to connect proteins (Willner, 2002) to the electrodes allows the production of photo-electrochemical cells that can be formed by using thylakoid membrane. Some of the studies in recent years have shown that Cyt C used in biofuel cells to generate the photocurrent (Efrati et al., 2013). Bilirubin oxidase (BOD) is a well-known enzyme which oxidizes some natural compounds while reducing O₂ directly to H₂O. The catalytic activity of BOD is based on the activation of four Cu ions split in the active sites. While one site bind and oxidize the organic compounds and another site bind and reduce O₂ (Mano, 2012). Because of this featured structure, BOD is a suitable enzyme for production of biocathodes in biofuel cell applications. The oxygen reduction capacity of BOD immobilized cathode depends on the enzyme kinetics of BOD and the amount of immobilized BOD. The rate of reduction at BOD cathode is limited by the mass transfer of oxygen (Mano, 2012).

In conductive polymers field that is gradually growing, the syntheses and implementations of conductive polymers having π -conjugated systems have drawn significant attention in recent years. Among the conductive polymers with these π -conjugated systems, the highest level of attention has been drawn by conductive polymers having dithiopyrrole (DTP) structure. Dithiopyrrole (DTP) is a term referring to the adjacent ring series consisting of two thiophene rings combined to the pyrrole ring. The compounds with π -conjugated systems draw attention of researchers because of their implementations in optic, electronic, and photovoltaic devices, light-emitting diodes, sensors and field-effect transistor properties (Yassin et al., 2010; Evenson et al., 2011; Dervisevic et al., 2016; Azak et al., 2013; Udum et al., 2014).

Herein, a gold electrode (GE) surface is first coated with poly 4-(4H-Dithieno[3,2-b:2',3'-d]pyrrole-4-yl)aniline, P(DTP-Ph-NH₂) and then Cyt C was cross-linked to bisaniline as a result of electro-polymerization. Finally, thylakoid membranes were cross-linked to the Cyt C via bisulfosuccinimidyl suberate (BS3) (bioanode). This structure generated high-degree photocurrent because of very fast transfer of electrons, which arises as a result of the oxidation of water in thylakoid membrane via photosynthesis under the visible light, from the thylakoid membrane to the electrode. To complete the pBFC, another gold electrode

(used as biocathode) is modified by cross-linking the BOD enzyme with poly [5-(4H-dithieno[3,2-b:2',3'-d] pyrrole-4-yl) naphthalene-1-amine], P(DTP-Naphthyl-NH₂). While oxygen will be released as a result of the oxidation of water in the photo-anode of the fuel cell, the cathode side will reduce this oxygen gas into the water via a bio-electrocatalytic method.

2. Materials methods

2.1. Materials

The synthesis of monomers 4-(4H-Dithieno [3,2-b:2',3'-d]pyrrol-4-yl) aniline, (DTP-Ph-NH₂), and 5-(4H-dithieno [3,2-b:2',3'-d]pyrrol-4-yl) naphthalene-1-amine, (DTP-Naphthyl-NH₂) were reported in our previous studies (Udum et al., 2014). Cytochrome C from bovine heart purchased from Sigma Aldrich. Bilirubin oxidase, BOD (E.C. 1.3.3.5) from *Myrothecium verrucaria* was purchased from Sigma Aldrich and was used with no additional purification (stored at -18 °C). The concentration of BOD in a stock solution was determined by absorbance measurement at 600 nm using a ϵ of 4800 M⁻¹ cm⁻¹ (Shimizu et al., 1999; Shleev et al., 2004; Christenson et al., 2006). The final specific activity was found to be 40 U per mg of protein. Bis(sulfosuccinimidyl) suberate and N-(maleimidocaproyloxy)- sulfosuccinimide ester were purchased from Pierce, USA. All other chemicals were purchased from Sigma/Aldrich and were used as supplied. Ultrapure water from a Nanopure (Barnstead) source was used throughout this work.

2.2. Methods

2.2.1. Fabrication of bioanode and biocathode electrodes for photo-bioelectrochemical fuel cell

Prior to any modification, GEs were mechanically cleaned with alumina slurry (0.5 μ) and washed with distilled water. After that electrochemical cleaning were performed scanning the electrodes between + 1.5 V to - 1.5 V at a scan rate of 100 mV/s in 0.08 M H₂SO₄ solution. Fabrication of bioanode was initiated by coating the GE surface with P(DTP-Ph-NH₂) film. After that, the P(DTP-Ph-NH₂) film covered GE was modified with thioaniline-functionalized Cyt C via electro-polymerization using 100 mV/s cyclic voltammetry in a potential range of - 0.1 V to + 1.1 V, performed in 0.1 M phosphate buffer solution (PBS). In the electro-polymerization experiment, a graphite electrode (d = 5 mm) was used as the auxiliary electrode, and the standard calomel electrode was used as a reference electrode. After the electro-polymerization process, electrodes were washed with PBS. Then 100 μ L of thylakoid membrane (750 mg mL⁻¹) solution was added to the electrode surface. Finally, the electrodes were incubated for 30 min by adding 0.001 mg mL⁻¹ bis (sulfosuccinimidyl) suberate (BS3) solution. Cross-linking between Cyt C and thylakoid membrane was obtained and the bioanode preparation was completed (Fig. S5).

The biocathode was subjected to the same mechanical and electrochemical cleaning processes before the electrode was prepared. Electro-polymerization of DTP-Naphthyl-NH₂ monomer onto gold electrodes was performed using cyclic voltammetry at a scan rate of 10 mV/s in a medium consisting of DTP-Naphthyl-NH₂ monomer and TBAPF₆ (0,1 M)/CH₂Cl₂. Finally, bilirubin oxidase (1 mg mL⁻¹) and 10 μ L 1% glutaraldehyde were dropped onto the electrode surface (Fig. S8). Before in use, all electrodes were rinsed with distilled water to remove the unbound materials.

2.2.2. Whole fabrication of photo-bioelectrochemical fuel cell

The thylakoid membrane attached to the surface of the anode (GE/P (DTP-Ph-NH₂)/CytC/thylakoid membrane) oxidizes water to oxygen under visible light. The surface of the cathode (GE / P (DTP-Naphthyl-NH₂)/BOD) located at a certain distance from the anode was modified with BOD. Oxygen released from the anode surface is reduced by the cathode in the reaction chamber and converted into water. The

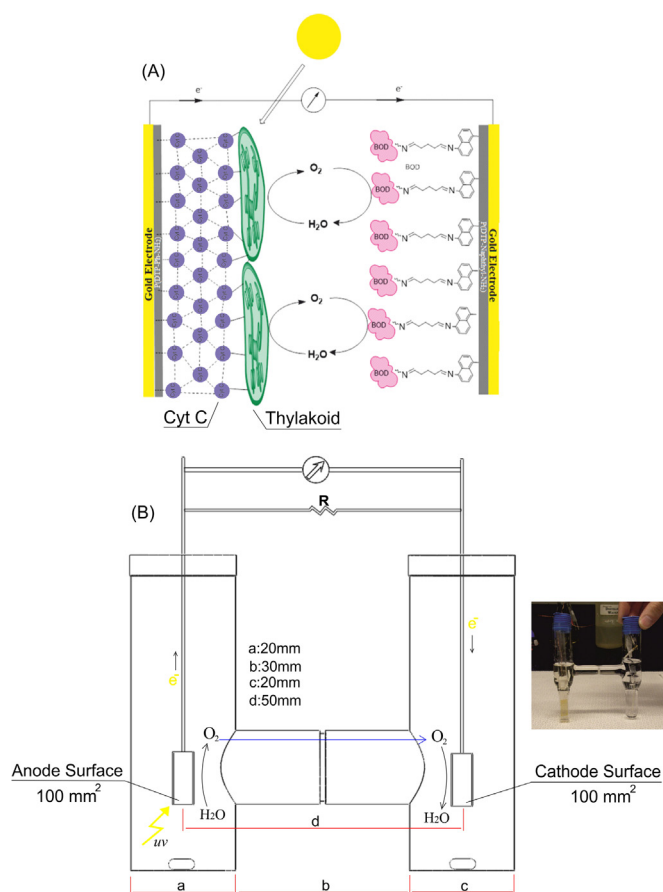


Fig. 1. A) Schematic representation of photo-bioelectrochemical fuel cell (pBFC). B) Illustration of H-type (pBFC) used in photocurrent measurements.

schematic representation of the reaction chamber and the electrodes are represented in Fig. 1A. The anode and cathode electrodes were serially connected to each other and placed in an H type reaction cell. Gold electrodes having a surface area of 100 mm^2 were placed in the centers of the tubes in two opposing cells with a tube diameter of 20 mm. The distance between the anode and the cathode in the reaction chamber was measured as 50 mm (Fig. 1B). It has been reported that oxygen released during photosynthesis easily reacts with electrons to form radicals, thereby limiting electron transfer in the anode and reducing power output in BFCs (McCormick et al., 2015). The electroreduction of O_2 to water is a diffusion-controlled process (Tsujiyama et al., 2003) which plays an important role in the production of photocurrent using an enzyme (BOD) adsorbed on the cathode (Mano et al., 2002), reducing the limiting effect of oxygen on the anode. During the measurements, an optimum stirring (1000 rpm) was applied to both tubes to achieve the highest photocurrent density by increasing the oxygen diffusion rate. A potentiostat device was serially connected to the system with different resistors and additionally, in order to measure the voltage passing through the resistance, a multimeter was placed against the resistor. The voltage values were read with a multimeter by applying a different load of resistors to the pBFC. Using the obtained voltage values and resistors Ω , current density and power density were calculated according to Ohm's law and polarization curves were drawn.

2.3. Photocurrent experiments and experimental variables

The photocurrent experiments were performed using a solar simulator involving a special photochemical system. This system consists of 300 W Xe lamp, a monochromator, and a specialized separator. During all photocurrent measurements, the H-type cell presented in Fig. 1B was

used. Electrodes (bioanode and biocathode) were modified and placed in the tubes having a distance of 50 mm between each other. In all chronoamperometry (CA) experiments 10 mM phosphate buffer, including 10 mM NaCl and 5 mM MgCl_2 at pH 7.4 (PBS) was used as electrolyte. All CA measurements were performed by using a potentiostat upon a cyclic on-off illumination at a light intensity of 1400 W/m^2 measured at the electrode surface. Before conducting a measurement, electrolyte solutions were degassed with Ar gas for 15 min. The pBFC was serially connected with different resistors (100Ω – $10 \text{ k}\Omega$) and voltage readings were performed with a multimeter under regular CA experiments.

In cyclic voltammetry experiments, 3-electrode cell setup was used at a scan rate of 100 mV/s , if not stated otherwise. A constant applied potential (0 V) was used in CA measurements vs Ag/AgCl in 10 mM pH 7.4 PBS. All measurements were performed at room temperature ($20 \pm 2^\circ\text{C}$). All data reported here are averages based on three independent experimental replicas.

The bioanode performance experiments were conducted to determine the oxygen concentration (in mole). Measurements were evaluated in a 3-electrode cell configuration under illumination in 10 mM pH 7.4 PBS 0 V vs Ag/AgCl. The amount of releasing oxygen by the time-dependent illumination was measured. The experiments to determine the performance of the biocathode, the amount of reduced oxygen was determined based on the amount of BOD. Different amounts of BOD-containing electrodes were used in the cell and time-dependent decrease in the amount of was measured. Polymeric film thicknesses were optimized by applying the different coating cycle in electro-polymerization and also measured with a micrometer.

3. Results and discussion

A control experiment was performed to demonstrate the photocurrent flow from GE/P (DTP-Ph-NH₂)/CytC/thylakoid membrane. Under the illumination of the visible light range (1400 W/m^2), a regular experiment was conducted in ethanol instead of water. However, no remarkable photocurrent formation has been observed. This experiment revealed that the system is sensitive only to water, and it generates photocurrent through the electron transfer as a result of the photosynthesis-caused water oxidation.

The Fig. 2A shows the photocurrent experiment of the GE coated with thylakoid membrane and a photocurrent of 18 nA was observed under the illumination of 1400 W/m^2 visible range light in 10 mM pH 7.4 PBS. This result shows that the electrons formed by photosynthesis are transferred directly to the electrode without using any mediator. All control experiments were performed at 0 V constant potential and 500 mg mL^{-1} thylakoid membranes. The gold electrode was coated with 40 cycles of P(DTP-Ph-NH₂) conductive polymer using the electro-polymerization method, then 500 mg mL^{-1} thylakoid membrane was immobilized on the electrode. The electrode is then immediately placed in 10 mM pH 7.4 PBS and a visible light of 1400 W/m^2 under the constant potential of 0 V is applied to the system. The amount of photocurrent was increased from the system up to 107 nA (Fig. 2B). This demonstrates that increasing the photo-conductivity is achieved by the P (DTP-Ph-NH₂) polymer, which is effective in transferring electrons.

In order to optimize the polymer thickness of P (DTP-Ph-NH₂), the electrodes were made with polymers having different coating cycles. A significant photocurrent of 0.346 nA can be clearly seen from P (DTP-Ph-NH₂) with 60 cycles (highest photocurrent level). When the electro-polymerization cycles were increased to 80 and 100 cycles, a decrease in the photocurrent values was observed. This shows that the polymer film has optimum thickness and conductivity for electron transfer. At the cycles, higher than 60 the polymer film becomes too thick for electron transfer and the sufficient number of electrons cannot be reached to the electrode (Fig. 3A). To increase the photocurrent generation, Cyt C was immobilized to the GE/P(DTP-Ph-NH₂) electrode before cross-linking the thylakoid membrane by using electro-

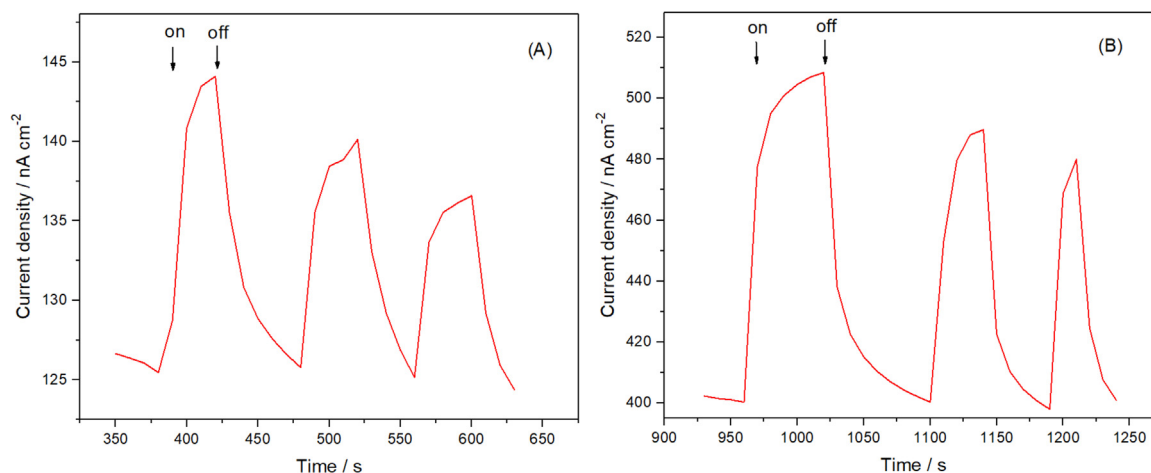


Fig. 2. A) The Chronoamperometry (CA) of a thylakoid membrane modified gold electrode (GE) in 10 mM pH 7.4 PBS at an applied potential; 0 V vs Ag/AgCl. B) The CA of P(DTP-Ph-NH₂)/membrane modified gold electrode in 10 mM pH 7.4 PBS at an applied potential; 0 V vs Ag/AgCl.

polymerization. The functional properties of the Cyt C, GE/P(DTP-Ph-NH₂)/Cyt C and GE/P(DTP-Ph-NH₂)/Cyt C/thylakoid membrane has been analyzed by CV (Fig. 3B). The characteristics of the reversible redox couple Fe⁺³/Fe²⁺ in Cyt C molecules well observed (represented by the green line) and anodic peak potential (E_{pa}) and cathodic peak potential (E_{pc}) were clearly observed at around 0 V, at a scan rate of 100 mV/s. A sharp decrease in the E_{pc} and E_{pa} were observed after modification of polymer (P(DTP-Ph-NH₂/Cytc) (shown in black line). A further assembly step continues with thylakoid membrane (shown in

the red line) caused a decrease in the oxidation and reduction peaks. Formation of the layers has negative effects on the redox potentials of Cyt C, indicating that the blocking the electrical contact with the electrode. However, the resulting electrode system, GE/P(DTP-Ph-NH₂)/Cyt C/thylakoid membrane, exhibits the sufficient oxidation and reduction peaks mean that Cyt C molecules are cross-linked and well oriented in the electrode architecture (Feifel et al., 2016).

After that, to evaluate the influence of Cyt C on the electron transfer pathway, CA measurements are performed (shown in Fig. 3C). The use

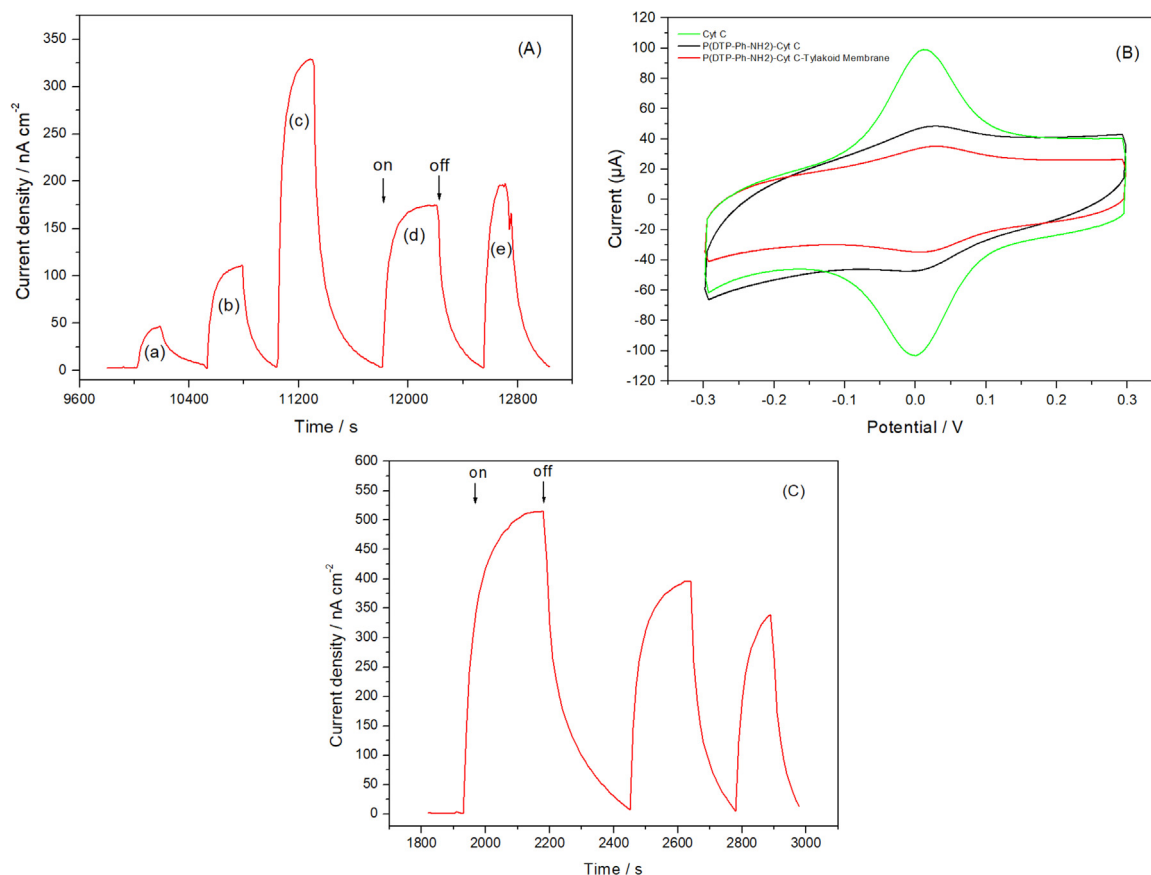


Fig. 3. A) CAs of thylakoid membrane modified GE/P(DTP-Ph-NH₂) electrodes with different cyclic voltammetry cycles a) 20, b) 40, c) 60, d) 80, e) 100 applied for P(DTP-Ph-NH₂) polymer coating. B) Cyclic voltammograms (CVs) of Cyt C, P(DTP-Ph-NH₂)/Cyt C / thylakoid membrane at a scan rate of 100 mV/s in 10 mM PBS pH 7.4. C) CA of P(DTP-Ph-NH₂)/Cyt C/thylakoid membrane PBS. All CAs were performed upon a cyclic on-off illumination of light at an intensity of 1400 W/m², applied potential; 0 V vs Ag/AgCl in 10 mM pH 7.4 PBS.

of Cyt C as an electron shuttle between the electrode and thylakoid membrane enhances the amount of obtained photocurrent increased up to 550 nA. This result shows that all thylakoid membranes previously immobilized are not in electrical contact with the electrode prior to immobilization of Cyt C. Immobilization of Cyt C molecules increases the amount of thylakoid membrane electrically connected with the electrode (Stieger et al., 2014).

One of the studies on the characterization of GE modified with P (DTP-Ph-NH₂) / CytC / Thylakoid membrane is the study on the optimization of the number of cycles to attach the Cyt C to the polymer film. In this optimization study, aniline functionalized Cyt C (100 mM) was coated on the conductive polymer film with oligoaniline bonds at 20, 30, 40, 50, 60, 70 and 80 electro-polymerization cycles. After binding of the 500 mg mL⁻¹ thylakoid membrane, CA measurements of the modified electrodes were performed with P(DTP-Ph-NH₂) / CytC / Thylakoid membrane. The cyclic on-off illumination (1400 W/m², applied potential: 0 V) was performed ~5 min on and 5 min off depending on the fuel cell reaching the pseudo-steady-state.

As the number of electro-polymerization cycles used to bind Cyt C onto the polymer film increased, the amount of photocurrent observed increased. These trends continued up to 60 cycles, and after 60 cycles, this increase in photocurrent tends to stop. The increase in photocurrent until 60 cycles can be explained by an increase in the amount of Cyt C, which results in an increase in electron transfer. In addition, the number of Cyt C increased the association of thylakoid membranes with the electrode, which was observed to increase photocurrent. On the other hand, after the photocurrent reached saturation, the increase in Cyt C (70 and 80 cycles) caused a decrease in photocurrent. It is understood that the increase in the number of Cyt C causes the decrease in the conductivity and the electron transfer rate, and the optimum number of cycles for immobilization of Cyt C to the anode surface is determined as 60 (Fig. 4).

Another characterization of the bioanode is the optimization of the number of thylakoid membranes used in the photocurrent generation. In all photocurrent dependent characterization studies so far, 500 mg mL⁻¹ thylakoid membrane was attached to GE / P(DTP-Ph-NH₂) / Cyt C electrodes and photocurrent measurements were performed. In this study, GE was first coated with the P (DTP-Ph-NH₂) polymeric film using 40 electro-polymerization cycles. The aniline functionalized Cyt C was then attached to the polymer coated electrode surface via oligoaniline bonds using a 60 cycle electro-polymerization. After that, solutions containing 250, 500, 750 and 1000 mg mL⁻¹ thylakoid membranes were then immobilized on electrodes coated with P(DTP-Ph-NH₂) / CytC. The photocurrent measurements of different

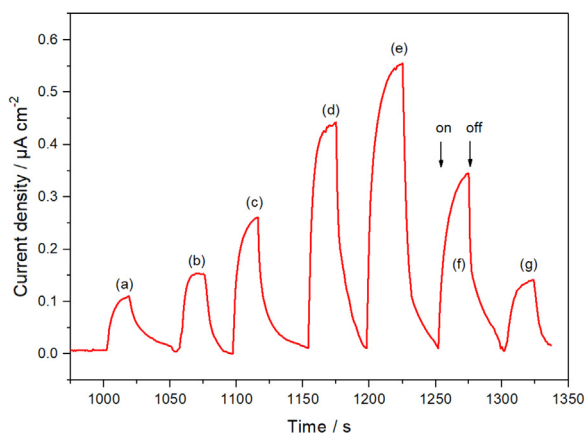


Fig. 4. The CAs of GE / P(DTP-Ph-NH₂) / (Cyt C) / thylakoid membrane electrodes including different CV cycles applied for Cyt C immobilization (a) 20, (b) 30, (c) 40, (d) 50, (e) 60, (f) 70, (g) 80 amount of Cyt C in 10 mM pH 7.4 PBS upon cyclic on-off illumination of visible light at an intensity of 1400 W/m², applied potential; 0 V vs Ag/AgCl.

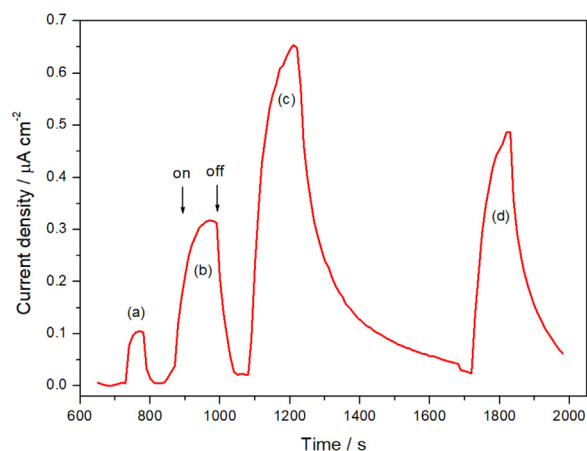


Fig. 5. The CAs of different concentration of Thylakoid membrane (a) 250 mg mL⁻¹, (b) 500 mg mL⁻¹, (c) 750 mg mL⁻¹, (d) 1000 mg mL⁻¹ modified P(DTP-Ph-NH₂)/Cyt C electrodes in 10 mM pH 7.4 PBS upon a cyclic on-off illumination of visible light at an intensity of 1400 W/m², applied potential; 0 V vs Ag/AgCl.

amounts of thylakoid membrane immobilized electrodes were performed upon a cyclic on-off illumination at a light intensity of 1400 W/m² under a constant potential of 0 V. The cyclic on-off illumination was performed, depending on the fuel cell reaching the pseudo-steady-state regardless of the time.

The photocurrent generation of the pBFC was observed to increase together with thylakoid membrane concentration. The maximum current response was obtained in a concentration of 750 mg mL⁻¹ thylakoid membrane and a further increase in membrane concentration was found to cause a decrease in photocurrent. It was observed that the reason for this was the increase in the concentration of the biocomponent on the surface of the electrode, which caused the surface to be thickened, blocking the electron transfer (Fig. 5). Thus, 750 mg mL⁻¹ was determined as the optimum concentration of thylakoid membrane to be used for bioanode fabrication.

The performance of the biocathode in pBFC was evaluated by analyzing the oxygen concentration in the chamber. The increasing on the reduction of oxygen amount is observed when the amount of BOD was increased, indicating the system performance depends on the biocatalytic function of the BOD. The amount of oxygen was gradually decreased and reached equilibrium (after 20 min, data not shown) at a concentration of 40 Units mL⁻¹ of BOD which shows the maximum oxygen reduction on the biocathode (Fig. 6A). The measurements were performed in conditions for bioanode: (P(DTP-Ph-NH₂):40 cycles, Cyt C:60 cycles, TM: 750 mg mL⁻¹ and biocathode: P(DTP-Naphtyl-NH₂):20 cycles, under illumination of 1400 W/m² visible light. The photocurrent generation with thylakoid membrane results in oxidation of water into oxygen. The performance of bioanode was tested in terms of oxygen production vs. time under illumination of 1400 W/m² visible light in 10 mM pH 7.4 PBS 0 V vs Ag/AgCl (conditions: P(DTP-Ph-NH₂):40 cycles, Cyt C:60 cycles, thylakoid membrane: 750 mg mL⁻¹. In Fig. 6B the maximum produced oxygen amount in the fuel cell was calculated as ~3.2 mol at an illumination time of 25 min. It is possible to say that the amount of oxygen in the fuel cell is directly reduced to a water molecule on the cathode surface. This increase the power output of the system and lifetime by eliminating higher oxygen saturation dependent stress on the membrane. The power generation of the biofuel cell was obtained by polarization curve (black line Fig. 6C) with external resistors were serially connected to pBFC during the measurements under illumination of visible light 1400 W/m². The increase in the power outputs was calculated at a light on condition from 1.5 and 4.9 mW m⁻². The maximum power generation of biofuel cell was reached at pseudo-steady state as 4.9 mW m⁻² at a current density of

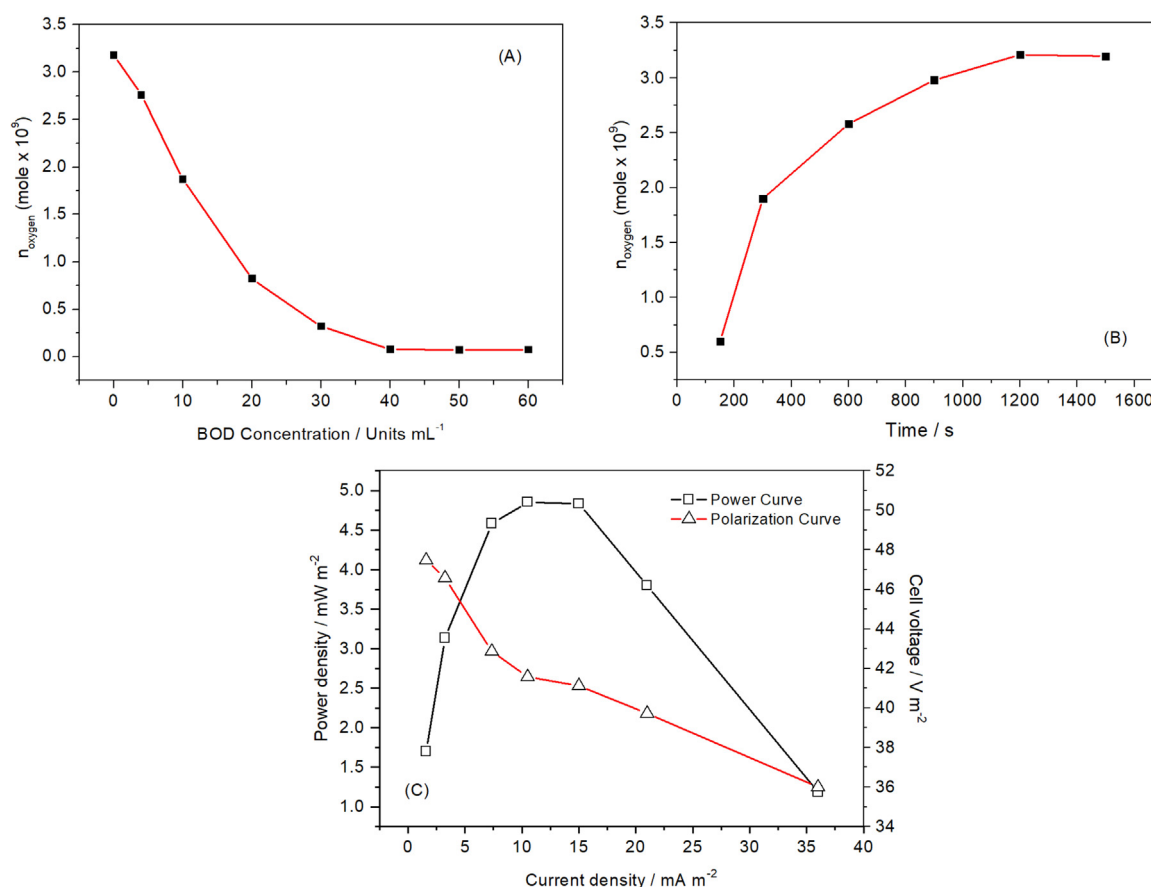


Fig. 6. A) Optimization of biocathode according to BOD concentration / Units mL^{-1} vs. oxygen concentration / moles. B) Optimization of bioanode performance in terms of oxygen concentration / moles vs time. C) Polarization curve (red line) and dependence of power output density (black line) of the fuel cell on the cell voltage. Measurements were performed with external resistors ranging from 100 Ω to 10 k Ω in 10 mM pH 7.4 PBS under light conditions.

15 mA m^{-2} . A growth media containing fuel cell, but no thylakoid membrane was used as a control and showed no considerable current response to illumination of light. As expected, the power increase by the light was found to be directly related to the changes in the oxygen in the device. The oxygen concentration in the reaction chamber is increased as predicted by the function of the thylakoid membrane (seen in the increase in Fig. 6B). At the cathode surface, the oxygen concentration in the reaction chamber is reduced by the catalytic action of adsorbed BOD, and this reduction is seen as in the curve shown in Fig. 6A. The power output in the chamber is more dependent on the anodic interactions, but stabilization of the system for a longer period of maximum current density can be explained by balancing the oxygen concentrations between the anode and the cathode depending on the illumination.

4. Conclusions

In this work, we have constructed a photocurrent generator from the electrode system based on the construction of a conducting polymer (P (DTP-Ph-NH₂)). The electrodes were designed via layer by layer method and the fully cross-linked of the conducting polymer, Cyt C and thylakoid membrane was obtained. The CV studies have been performed at each step of the modification of electrodes in order to find out the system which generates highest photocurrent. The prominent feature of the system is the monitor ability of surface modification electrodes by CV due to use of conducting polymer and Cyt C. CA studies demonstrated the electron transfer efficiency of the conducting polymer modified electrode during photocurrent generation is six-fold greater than the electrode without using conducting polymer. Another distinctive point in achieving enhanced photocurrent is that the use of Cyt

C in the electrode architecture. Because the results clearly show that the highest photocurrent measured from the electrodes having Cyt C (optimum coating cycle: 60) compared to the other electrodes (without Cyt C). Since Cyt C is a natural protein, it serves a very suitable surface for the attachment of thylakoid membrane. It also functions as an electron-transporting protein because of its unique structure having an iron complex. Furthermore, it is understood from the photocurrent values that Cyt C actively binds more thylakoid membrane to the electrode surface. On the other hand, O₂ is released after photosynthesis in thylakoid membrane and rapidly reduced to water molecules by the BOD enzyme located on the cathode surface, resulting in a cyclic pBFC. This system shows how to obtain high efficiency from photo-bio-fuel cells and its importance; good electrical communication and the use of natural components. At the same time, in such bio-hybrid systems, achieving high photocurrent (light dependent) is dependent on the well-organized electrode platforms which allow electron transportation.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2018.04.055>.

References

- Azak, H., Guler, E., Can, U., Odaci, D.D., Yildiz, H.B., Talaz, O., Timur, S., 2013. *RSC Adv.* 3, 19582.
- Badura, A., Kothe, T., Schuhmann, W., Rögner, M., 2011. *Energy Environ. Sci.* 4, 3263.
- Bard, A.J., Fox, M.A., 1995. *Acc. Chem. Res.* 28, 141.
- Buchner, T.B., Ewingen, H.N., 2009. Nova Science Publishers.
- Ciesielski, P.N., Scott, A.M., Faulkner, C.J., Berron, B.J., Cliffl, D.E., Jennings, G.K., 2008. *ACS Nano* 2, 2465.
- Ciesielski, P.N., Faulkner, C.J., Irwin, M.T., Gregory, J.M., Tolk, N.H., Cliffl, D.E., Jennings, G.K., 2010. *Adv. Funct. Mater.* 20, 4048.
- Christenson, A., Shleev, S., Mano, N., Heller, A., Gorton, L., 2006. *Biochim. Biophys. Acta (BBA)* 1757 (12), 1634.
- Das, R., Kiley, P.J., Segal, M., Norville, J., Yu, A.A., Wang, L., Trammell, S.A., Reddick, L.E., Kumar, R., Stellacci, F., Lebedev, N., Schmur, J., Bruce, D., Zhang, S., Baldo, M., 2004. *Nano Lett.* 4, 1079.
- Dervisevic, M., Dervisevic, E., Azak, H., Cevik, E., Şenel, M., Yildiz, H.B., 2016. *Sens. Actuat. B: Chem.* 225, 181–187.
- Efrati, A., Tel-Vered, R., Michaeli, D., Rachel, N., Willner, I., 2013. *Energy Environ. Sci.* 6, 2950.
- Evenson, S.J., Mimm, M.J., Pohodya, K.I., Ramussen, S.C., 2011. *Macromolecules* 44, 835.
- Feifel, S.C., Stieger, K.R., Kapp, A., Weber, D., Allegrozzi, M., Piccioli, M., Turano, P., Lisdat, F., 2016. *ACS Omega* 1, 1058.
- Frolov, L., Wilner, O., Carmeli, C., Carmeli, I., 2008. *Adv. Mater.* 20, 263.
- Fuhrmann, E., Gathmann, S., Rupperecht, E., Golecki, J., Schneider, D., 2009. *Plant Physiol.* 149, 735.
- Haehnel, W., 1977. *Biochim. Biophys. Acta* 459, 418.
- Hasan, K., Cevik, E., Sperling, E., Michael, A.P., Donal, L., Gorton, L., 2015. *Adv. Energy Mater.* 5, 1–11.
- Kalyanasundaram, K., Graetzel, M., 2010. *Curr. Opin. Biotechnol.* 21, 298.
- Katz, E., Lioubashevski, O., Willner, I., 2004. *J. Am. Chem. Soc.* 126, 11088.
- Katz, E., Bückmann, F., Willner, I., 2001. *J. Am. Chem. Soc.* 123, 10752.
- Katz, E., Willner, I., Kotlyar, A.B., 1999. *J. Electroanal. Chem.* 479, 64.
- LeBlanc, G.C., Gizzie, E.A., Jennings, G.K., David, E.C., 2012. *Adv. Mater.* 24, 5959.
- Mano, N., 2012. *Appl. Microbiol. Biotechnol.* 96, 301.
- Mano, N., Kim, H.-H., Zhang, Y., Heller, A., 2002. *J. Am. Chem. Soc.* 124, 6480–6486.
- McCormick, A.J., Bombelli, P., Bradley, R.W., Thorne, R., Wenzel, T., Howe, C.J., 2015. *Energy Environ. Sci.* 8, 1092–1109.
- Renger, G., 2008. *RSC Publishing*, p. 109.
- Rosenbaum, M., He, Z., Angenent, L.T., 2010. *Curr. Opin. Biotechnol.* 21, 259.
- Shimizu, A., Kwon, J.H., Sasaki, T., Satoh, T., Sakurai, N., Sakurai, T., Yamaguchi, S., Samejima, T., 1999. *Biochemistry* 38, 3034.
- Shleev, S., El Kasm, A., Ruzgas, T., Gorton, L., 2004. *Electrochem. Commun.* 6, 934.
- Stieger, K.R., Feifel, S.C., Lokstein, H., Lisdat, F., 2014. *Phys. Chem. Chem. Phys.* 16, 15667.
- Tender, L.M., Reimers, C.E., Stecher, H.A., Holmes, D.E., Bond, D.R., Lowy, D.A., Pillobello, K., Fertig, S.J., Lovely, D.R., Wang, W., 2002. *Nat. Biotechnol.* 20, 821.
- Terasaki, N., Yamamoto, N., Hiraga, T., Yoshinori, Y., Tetsu, Y., Hiroshi, N., Tsutomu, O., Makoto, S., Masaaki, F., Akihiko, T., Masako, I., Yasunori, I., Satoshi, Y., Makoto, M., Isao, M., 2009. *Angew. Chem. Int. Ed.* 48, 1585.
- Tsujimura, S., Kawaharada, M., Nakagawa, T., Kano, K., Ikeda, T., 2003. *Electrochem. Commun.* 5, 138–141.
- Udum, A., Yildiz, H.B., Azak, H., Sahin, E., Talaz, O., Cirpan, A., Toppare, L., 2014. *J. Appl. Polym. Sci.* 130, 40701.
- Willner, I., 2002. *Science* 298, 2407.
- Yehezkeli, O., Wilner, I., Tel-Vered, R., 2010. *J. Phys. Chem. B* 114, 14383.
- Yehezkeli, O., Tel-Vered, R., Raichlin, S., Willner, I., 2011. *ACS Nano* 5, 2385.
- Yassin, A., Leriche, P., Roncali, J., 2010. *Macromol. Rapid Commun.* 31, 1467.