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Photocurrent Production with Thermophilic Cyanobacterial Strain under Electrochemical Treatment without Adding of Mediators

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A thermophilic cyanobacterial strain, *Thermosynechococcus sp.* CL-1 (TCL-1) was examined to investigate photocurrent generation under illumination without the adding of artificial mediators in an H-type two-compartment electrolysis cell. Dissolved inorganic carbon (DIC) was used as a nitrogen source at 0.2 V (vs Ag/AgCl). Additionally, cyclic voltammetric experiments were used to verify the presence of oxidative compound excreted from TCL-1. Results indicated that the relationship between photocurrent generation and TCL-1 cell mass is a positive linear. No apparent differences on TCL-1 growth between the case of control and 0.2 V (vs Ag/AgCl) indicated the electron uptake from TCL-1 at 0.2 V has no effect on its growth. The presence of redox compound excreted from TCL-1 is supported through the cyclic voltammetric experiment and plastoqunione, cytochrome b6/f, and plastocyanin between 0.2 and 0.7 V are the most probable mediators in electron transportation.

Keywords: Thermophilic cyanobacterial strain, Photocurrent, Cyclic Voltammetric, Photosynthesis, Electron Transporation.

1. INTRODUCTION

The combustion of fossil fuel causes an increase in CO_2 that intensifies global warming. The U.S. Department of Energy in a report of 2005 indicated that one hour of sunlight provides one year of usable energy based on average rates of consumption [1]. This has thus contributed to the development

and application of solar cells as a dominant clean energy strategy worldwide. Photosynthesis fixes CO_2 in the Calvin cycle using sunlight as its power supply (creating adenosine triphosphate, ATP and reduced nicotinamide adenine dinucleotide, NADH). This production of electric power during CO_2 can potentially be developed as a method of eliminating energy crises and global warming. Microorganisms have advantages over higher plants for this purpose because of their higher level of photosynthetic efficiency. Microbial fuel cells (MFCs) are devices can generate electrical energy using microorganisms as biocatalysts. In the recent years, they have utilized organic compounds in wastewater. MFCs specifically containing photosynthetic microorganisms (PMFCs) should be developed as advanced energy-capturing devices to harness the critical supply available from sunlight. Chiao indicated that cyanobacteria (Phylum Cyanophyta) have greater potential to produce electricity compared with *Saccharomyces* cerevisiae (brewer's yeast) with M-blue as the mediator at 10 Ω external resistances [2]. Several studies have modelled current production in photosynthetic microorganisms by adding mediators for electron transport [2-8]. Yagishita demonstrated that the electrons are produced by photosynthetic oxygenation in the light and glycogen degradation in the dark with 2-hydroxy-1, 4-naphthoquinone (HNQ) as the mediator [5-6]. The current response resulted from the transformation of HNQ and ferrocyanide. The reactions in the anode and cathode occurred as follows:

Anode: HNQ (Red) → HNQ (Ox) + $2H^+$ + $2e^-$

Cathode: ferricyanide (Fe (III)) + e^- → ferrocyanide (Fe (II))

When Synechococcus sp. UTEX2380 was used, the current increased with cell mass and the conversion efficiency was approximately to 3% [6]. Generally, artificial mediators are typically required, such as phenolic compounds that are commonly used for electron transportation, but their high cost and toxicity have limited the range of applications of photosynthetic microbial fuel cells (PMFCs). In previous studies regarding mediator-free MFCs, several bacteria have demonstrated the ability to excrete endogenous mediators for electron transfer from cells to electrodes. Expanding on this premise, Strik tested an open-type bioreactor with a mixture of microalgae and bacteria to produce endogenous mediators for electron transportation [8]. The source of endogenous mediators in this experiment was concluded to be bacteria, not microalga. One study investigated PMFCs using a mediator-free, pure culture, with attached, *Spirulina platensis* cyanobacteria as the anode [9]. The production of electric power was much greater in the dark (1.64 mW m^{-2}) than in the light (0.132 mW m^{-2}), revealing that a heterotrophic metabolism dominated electrical production and that photosynthesis provided for only the accumulation of glycogen. Recently, Pisciotta demonstrated that direct light-dependent electrogenic activity exits in "mesophilic" cyanobacteria [10]. Thermophilic cyanobacteria have not yet been found to directly produce photocurrent without the adding of artificial mediators. However, the possibility still merits further investigation as high-temperature; sun-exposed environments become increasingly common.

This study investigated the ability of a thermophilic cyanobacterial strain, *Thermosynechococcus sp.* CL-1 (TCL-1), to assimilate CO_2 and generate photocurrent under illumination without the adding of artificial mediators. The experiments were conducted in an H-type two-compartment electron-photo bioreactor. Dissolved inorganic carbon (DIC) was used with nitrate

as a nitrogen source at 0.2 V (vs Ag/AgCl). Photocurrent generation was quantified as a function of carbon assimilation and changes in level of light with a no-voltage scenario as the control.

2. EXPERIMENTS

2.1. Microbial species

The *Thermosynechococcus sp.* CL-1 (TCL-1) strain was isolated from Chin-Lun hot spring (pH 9.3, 62°C) in Taiwan as described previously [11-12]. A Modified Fitzgerald medium was adopted as the growth culture consisting of (in mg L^{-1}) 496 $NaNO_3$, 39 K_2HPO_4, 75 $MgSO_4 \cdot 7H_2O$, 27 $CaCl_2$, 58 Na_2SiO_3, 6 $FeC_6H_5O_7$, 6 citric acid, 1 EDTA, and 1 mL L^{-1} Caffron solution with distilled water [13]. The medium was used for pre-culture and further tests under various conditions.

2.2. Set-up procedure

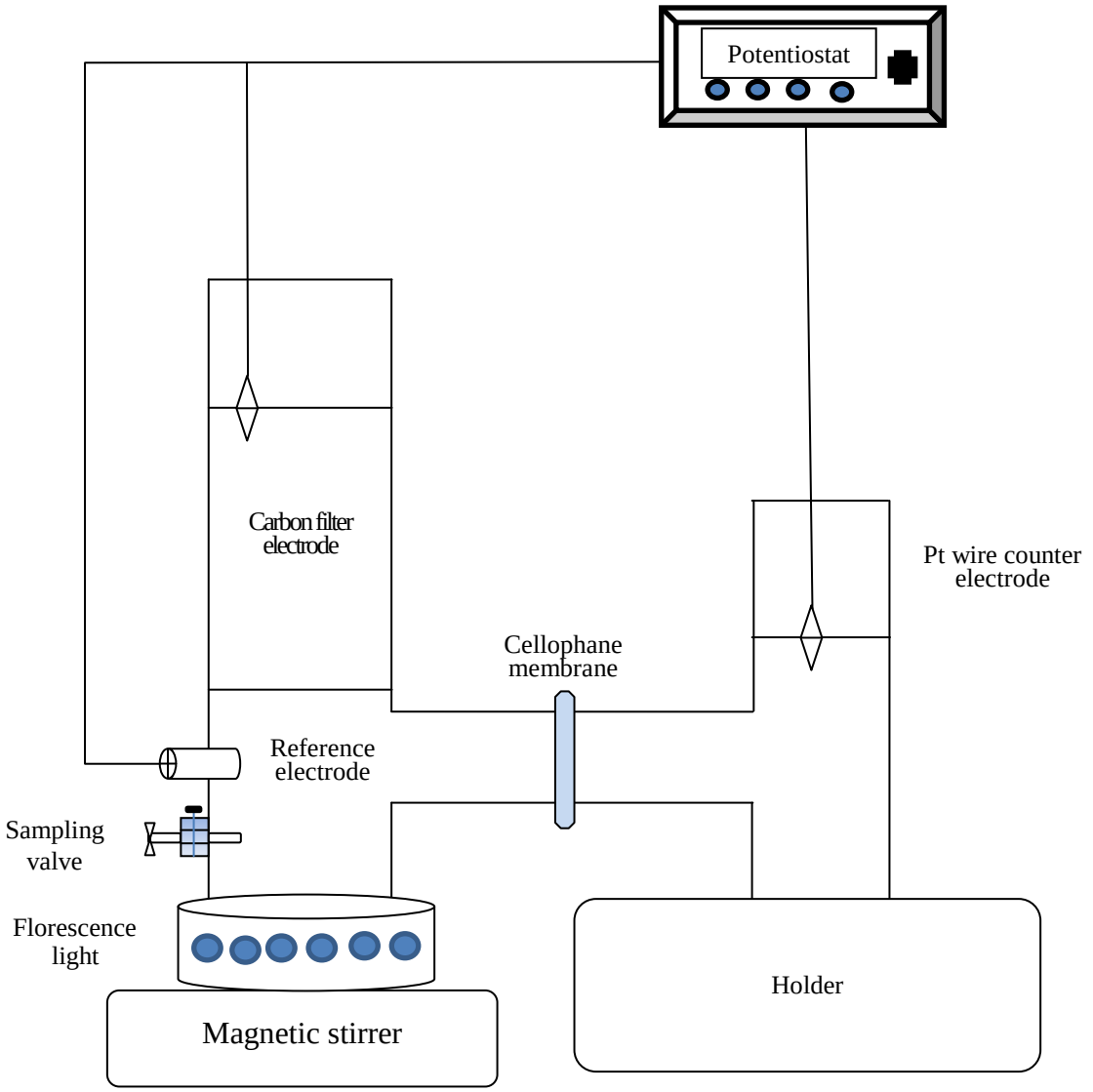

Figure 1. Experimental schematic of an H-type two-compartments electrolysis cell in this study.

An H-type two-compartment electro-photo bioreactor cell was used in this study (Fig. 1). The 250 ml anodic chamber was filled with a 200 mL working volume of culture medium containing DIC concentration of 47 mM at an initial pH 9.5 which was prepared with the mixture of $NaHCO_3$ and Na_2CO_3. Carbon felt (B0050, Toray, Japan) was fixed inside the anodic chamber with a given area elastic accessory and connected with a potentiostat (HA-151A, Japan) with a Pt wire for electron transfer at a given voltage. The pre-treated procedures on the carbon felt included (1) washing in 0.1 N HCl solution, (2) washing in 0.1 N NaOH solution overnight for cleaning, and (3) drying at 60°C overnight, for avoiding impurities existing. Ag/AgCl submerged in saturated KCl solution as the reference electrode was also connected with potentiostat for voltage reference. The counter electrode in cathodic chamber containing 20 ml medium was also connected with the potentiostat for electron balance. The anodic and cathodic chambers were separated by a cellophane membrane, thus ions could be transported each other.

The turbulence of the cultivation solution in anodic chamber was controlled by a magnetic stirrer at a constant speed to enhance the mixing of the reactor content, thus avoiding the biomass settling. The cultivation temperature was also controlled within an illuminated incubator (FH-130w, Taiwan) at 50°C. Light intensity, measured at the nearest distance from the light source with 99 light-emitting diode (LED) lamps under the bottom of the anodic chamber by a Lux meter (TM 50000, TOMEI), was 20 klx.

2.3. Start-up of the electro-photo bioreactor and analysis on the photocurrent production

After fabricating the H-type two-compartment electro-photo bioreactor, the steady state of blank current response, about 5 μA, was obtained after 1 hr at a 0.2 V with a Potentiostat. 5 ml medium containing TCL-1 was injected into the anodic chamber for seeding and then recorded half-hour data of current response until the end of each run. The $OD680_{\ nm}$ (optical density at 680nm) was used for throughout the experiments. The final $OD_{680\ nm}$ was about 0.2. The $OD_{680\ nm}$ was measured with a spectrophotometer (Lambda 35, Perkin Elmer, USA) twice a day.

2.4. Cyclic voltammetry analysis

The cyclic voltammetry analysis of carbon felt electrode before and after cultivation in the anodic chamber was carried out with an electrochemical analyzer (CHI611A, ALS/CH Instruments, USA) at 50°C. The scan rate was controlled at 1 or 5 mV/s between 0.7 to -0.1 V under quiet conditions.

3. RESULTS AND DISCUSSION

3.1. Effect of cell mass on the photocurrent production in the light

The photocurrent production was experimentally examined at a 0.2 V under the illumination of 20 klx in an H-type two-compartment electro-photo bioreactor using a thermophilic cyanobacterial species, TCL-1 in the anodic chamber without adding an electron transfer mediator.

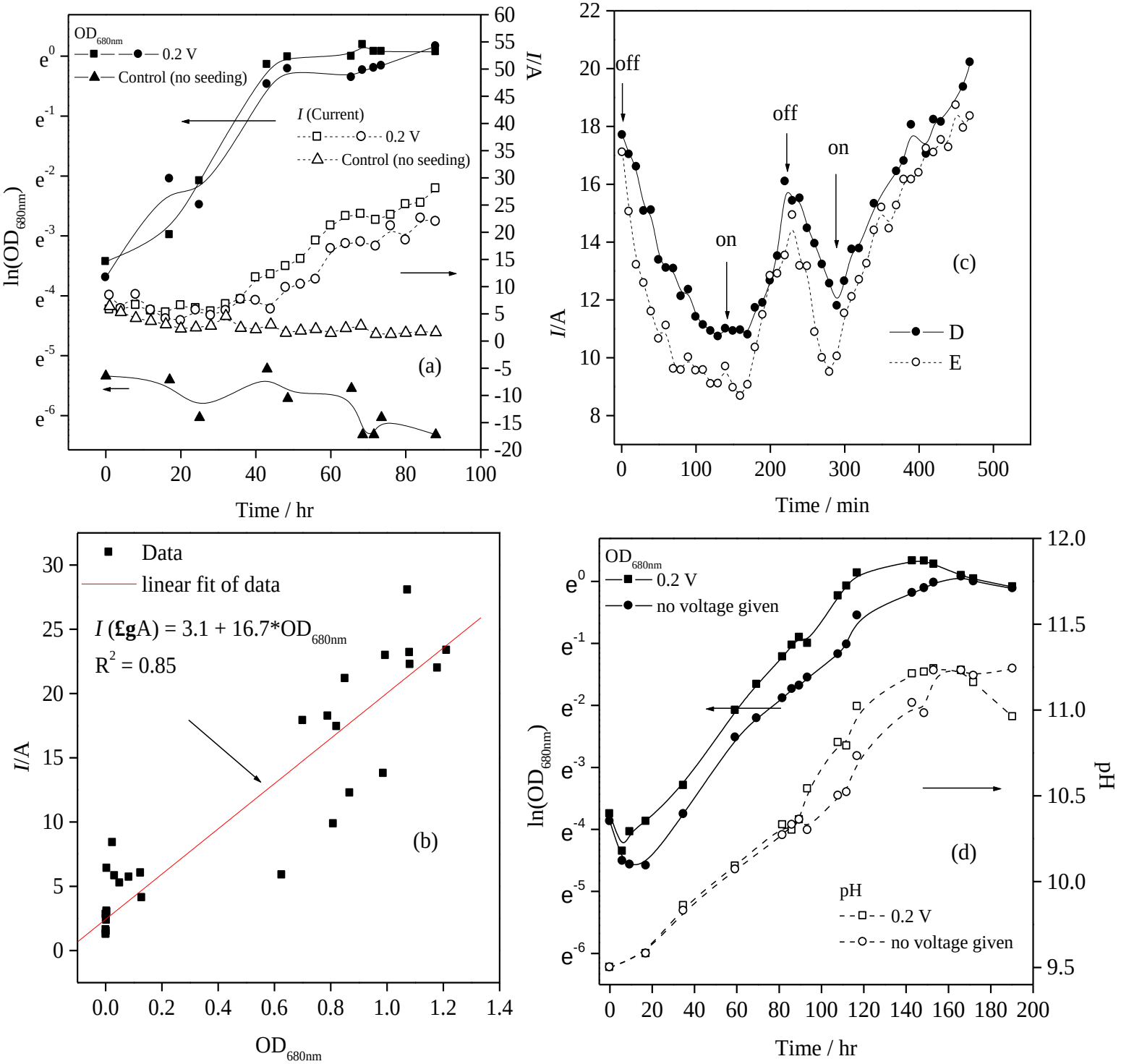

Figure 2. Interaction between current and photosynthesis (a) current response at 0.2 V; (b) the relationship between optical density and current response; (c) The current response under light on and off; (d) Optical density and pH curves of time course under 0.2 V and no voltage given.

Results showed that the current response was a function of the cell mass under illumination (Fig. 2a), signifying that the current source was most likely from the electron flow excited by photosynthesis in TCL-1 cells. It is expected that endogenous mediators would be produced by TCL-1 for electron transportation (passing through the cells to electrodes) because no artificial mediators were used in this system. On the other hand, a control was carried out by replicating the experiment without seeding TCL-1 and no current response or cell growth was observed within 90 h under experimental conditions. As shown in Fig. 2a, after the seeding of TCL-1, the current response increased from 5 to 25 μA and the cell mass increased from 0.1 to 1.2 (OD_{680nm}). This finding was consistent with the result obtained by other reports, in which their researches shown that the current production is a function of cell mass and bioelectrocatalysis plays important factor for electron transfer from microorganisms to anode in microbial fuel cells [14-16]. Note that a long growth phase was obtained for the period of 20 to 80 h after seeding, which indicates that the growth of TCL-1 was not inhibited under 0.2 V. Although the TCL-1 growth curve revealed slight vibration, the current response was still a function of the cell mass. Figure 2(b) reveals a positive linear relationship between the cell mass and the current response with an R^2 of 0.85, indicating that the electrons originated from the TCL-1 cyanobacteria. However, turbulence attached some TCL-1 cells that attached to the anodic carbon felt and created dual mechanisms for the transfer of electrons from microorganisms to the anode [17]. This renders it difficult to confirm whether the dominant source of electrons was contact between TCL-1 and the anodic carbon felt or the majority of the TCL-1 cells, which were suspended in the medium.

3.2. Effect of illumination on the current response

Because the current response under illumination is based on electrons produced by the splitting of water in photosynthesis, we investigated whether the current response would be different under conditions of darkness. The light source was repeatedly turned off and on in intervals of 150 min and the current response was recorded. The light source was switched off after the current response reached 18 μA; it decreased from 18 to approximately 10 μA within 90 min and remained at 10 μA for 60 min (Fig. 2c). Subsequently, the light source was switched on again and the current response increased to 16 μA within 40 min. Repetitions of this procedure revealed similar responses, confirming the relationship between the current response and TCL-1 photosynthesis. The current response decreased and remained stable during the first period of darkness, possibly because of heterotrophic electron production using endogenous glycogen with existing endogenous mediator [5, 9]. In the second period of darkness, the current response decreased more quickly indicating that the endogenous glycogen had already been consumed. Although Fu et al. also indicated that cyanobacteria, Spirulina platensis, attached to the anode can produce electrical power without artificial mediators [9]. The current response in their study was dominated by a heterotrophic metabolism, in contrast to the autotrophic metabolism in this study. Because the current response is a function of the cell mass under illumination (Fig. 2b) and is higher in light than in the dark (Fig. 2c), electrons excited by photosynthesis should be the dominant current response source in this study. Pisciotta et al. indicated that “mesophilic” cyanobacteria also possess “electrogenic” activity without the addition of artificial

mediators [10]. By comparison the current response found in this study, it is more applicable to power production because of the thermophilic cyanobacteria used in this study and the contemporary increase in hot and sunny environments. Consequently, we conclude that current response in thermophilic cyanobacteria occurs mainly under illumination without additional mediators, similar to findings in mesophilic cyanobacteria [10].

3.3. Effect of oxidative voltage on the cell growth of TCL-1

The results mentioned in the previous section address the correspondence between the current response and cell mass under illumination at 0.2 V (Fig. 2b). The electron output at 0.2 V may damage TCL-1 by causing a shortage of electrons in the dominant metabolisms of the Calvin cycle. The case of 0.2 V produced a higher growth curve than that of the control (Fig. 2d). The sensitivity of the current response to illumination (Fig. 2c) suggests that the majority of electron outflow derives from photosynthesis in photosystems II and I (PSII and PSI). Electrons produced by photosynthesis are commonly used for the production of NADPH or ATP with residual electrons producing free radicals that harm the cells. Many studies have examined protection responses against photo-damage at high level of radiation, such as the xanophyll cycle, and decrease the growth rate for energy consumed for metabolisms [18]. The electron output from the cell at 0.2 V (vs. Ag/AgCl) thus seems to be an effective strategy for automatic electron outflow regulation during cell growth under high radiation and various other forms of stress.

3.4. Voltammetric characterization of TCL-1 electrode

Artificial mediators, such as potassium ferricyanide, 2, 3-dimethoxy-5-methyl-1, 4-benzoquinone, sodium 2,6-dichloroindophenol, and 2-methyl-1,4-naphthoquinone, have been commonly used to enhance electron transfer from microbial cells to electrodes. Unfortunately, their high cost and inhibitory effect on cell activity limited the application in pilot scale [19].

However, some microorganisms have been shown to use electrodes as electron acceptors without exogenous mediators [20]. Cyanobacteria can produce photocurrent heterotrophically, using endogenous glycogen for either the entirety or the majority of the process. This method involves an exogenous mediator for electron transfer under conditions of darkness.

Findings of this type of autotrophic (photosynthetic) power production have been published for mesophilic cyanobacteria, but not for the thermophilic cyanobacteria, which were our focus [5, 10]. To elucidate the phenomenon of electron transfer from microbial cells to the electrode, the cyclic voltammetry was carried out to confirm the voltammetric characterization of the endogenous mediator in the anodic chamber.

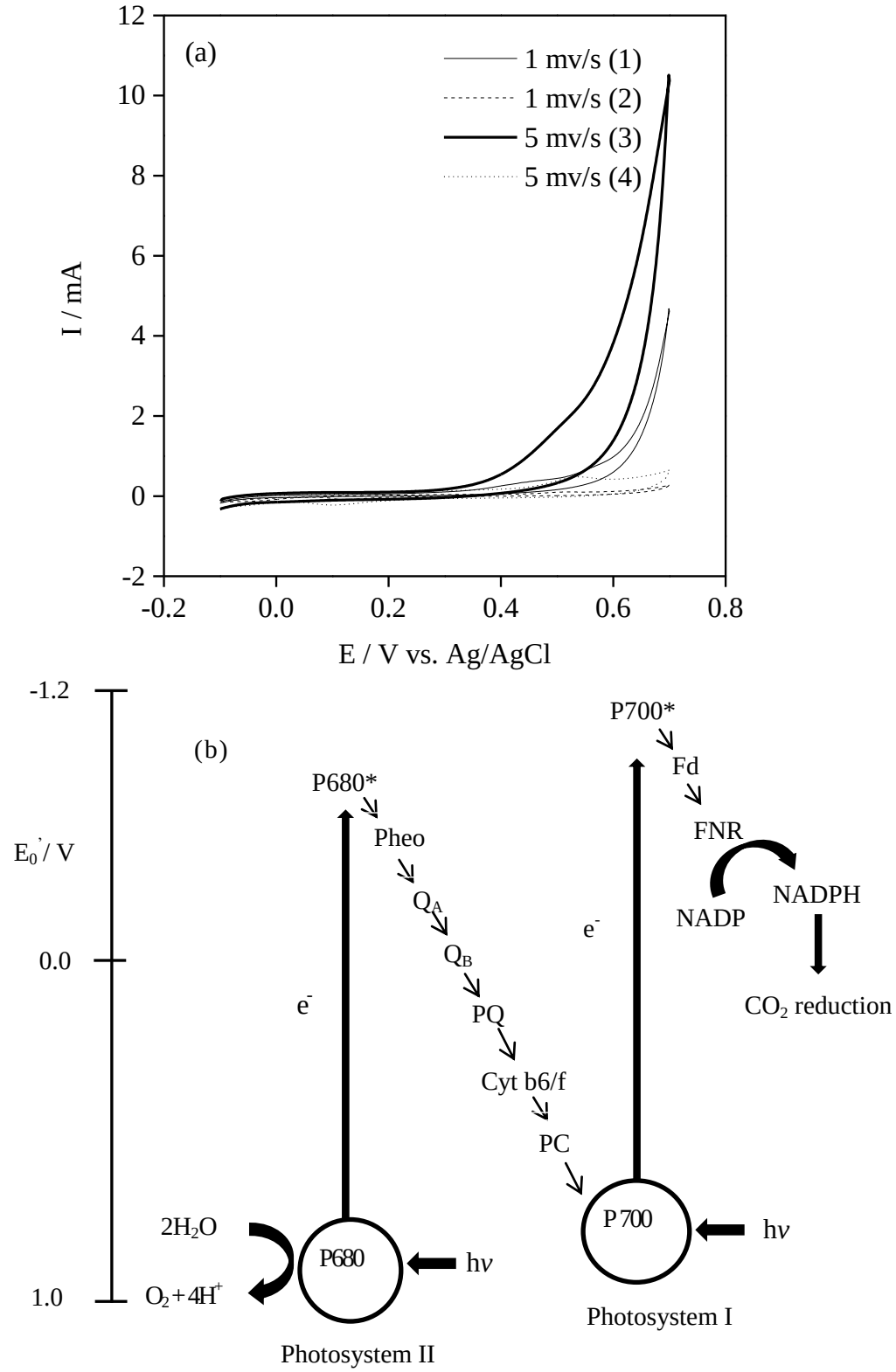

Figure 3. The potentials of mediators (a) cyclic voltammograms of graphite carbon fiber electrode in the anodic chamber under quiet conditions at 50°C. All the electrodes were submerged into the medium buffer completely. The dotted line represents the background current response of electrode in the culture medium only (pH 9.5). The slim and broad curves show the current responses of electrode with the scan rate of 1 and 5 mV/s, respectively, after the cell cultivation (The final pH was 11); (b) Electron transport chain and the potentials of the mediators between PSII and PSI.

No faradaic current was observed (the dotted line in Fig. 3a) indicating that no redox compounds existed to react with the electrode in the culture buffer within the potential range between 0.7 and -0.1 V. After 3 days of cultivation, when the current response had reached its maximum value (approximately 25 μA), cyclic voltammetry was introduced again and a remarkable catalytic current occurred at approximately 0.3 V (vs. Ag/AgCl). This result suggests that some redox-active compounds were present in the buffer after cultivation and were oxidized by the electrode. There are a few possibilities or redox compounds. Researchers have suggested that the pilus on the surface of a microbe is capable of carrying out the role of electron transfer as a type of electric wire, not an oxidized compound as in our case (Fig. 3a) [21]. Additionally, there is no appendage resembling a pilus on the surface a TCL-1 cell. Studies have indicated that hydrogen production by microbes and oxidization on the surface of electrodes are the main generators of photocurrent during electron transfer.

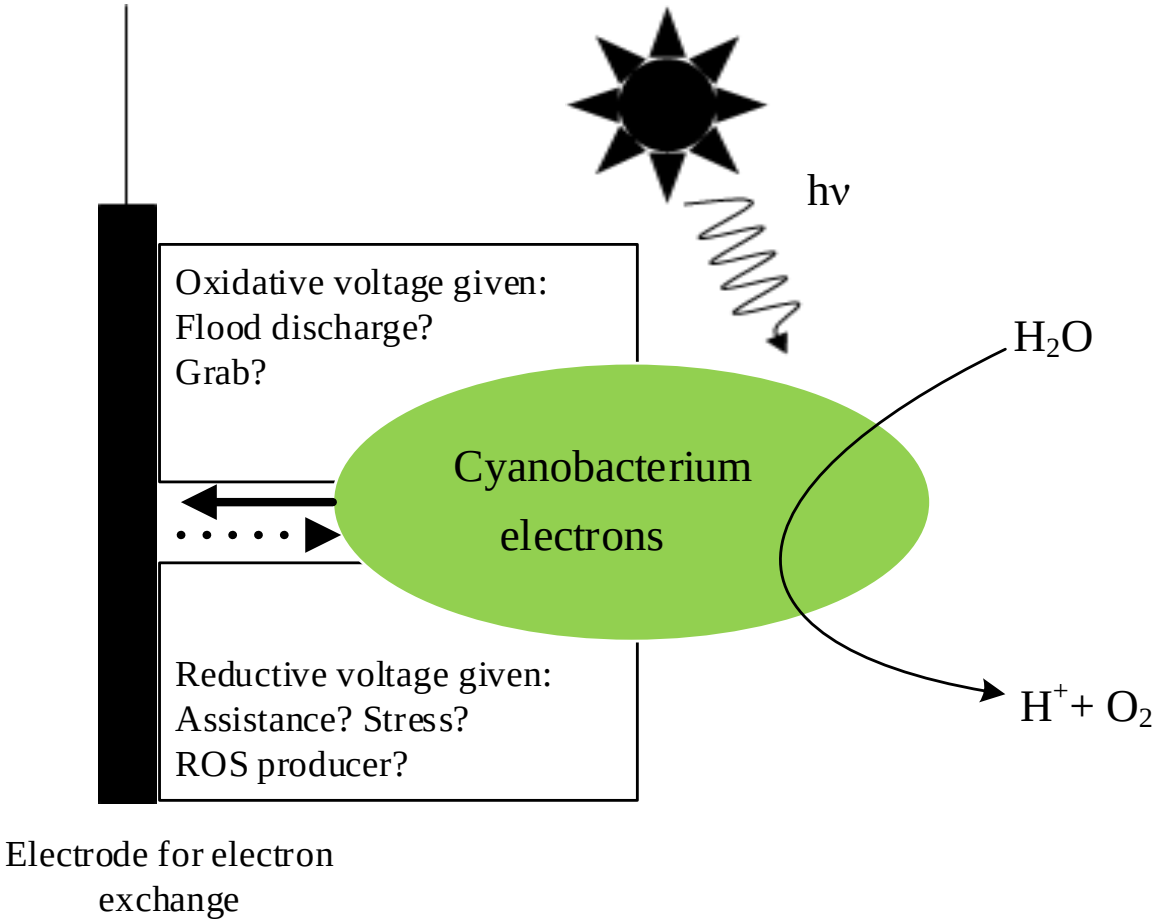

Figure 4. Schematic diagram of effect on TCL-1 under positive and negative voltage.

PSI and PSII, are also called plastocyanin-ferredoxin oxidoreductase and water-plastoquinone oxidoreductase, respectively. PSI is the second photosystem in the photosynthetic light reactions of algae, plants, and some bacteria. It is an integral membrane protein complex that

uses light energy to yield the high energy carriers ATP and NADPH. PS II is the first protein complex in the light-dependent reactions of oxygenic photosynthesis. It is located in the thylakoid membrane of plants, algae, and cyanobacteria. Within the photosystem, enzymes capture photons of light to energize electrons that are then transferred through a variety of coenzymes and cofactors to reduce plastoquinone to plastoquinol. The energized electrons are replaced by oxidizing water to form hydrogen ions and molecular oxygen. By replenishing lost electrons with electrons from the splitting of water, PS II provides the electrons for all of photosynthesis to occur. The hydrogen ions (protons) generated by the oxidation of water help to create a proton gradient that is used by ATP synthase to generate ATP. The energized electrons transferred to plastoquinone are ultimately used to reduce $NADP^+$ to NADPH or are used in cyclic photophosphorylation.

However, in our experiments the main photosynthetic products of TCL-1 in the anodic chamber were oxygen and hydrogen. This demonstrates that endogenous mediators should be present and anticipates oxidative or reductive reactions between 0.3 and 0.6 V. Because this endogenous mediator must carry electrons from the inside to the outside of the cell, processes involving PS II and PSI should be discussed on the basis of their potential. Electrons in a photosynthetic electron transport chain (ETC) were produced by splitting water with radiation energy and Zn coordinated catalyst and transported by several electron carriers under various oxidative and reductive potentials (Fig. 3b). PQ, Cyt b6/f, and PC between 0.2 and 0.7 V are the most probable mediators in electron transportation from the inside to the outside of cyanobacterial cells in our voltammetric characterization of the TCL-1 electrode. In a series of experiments related to inhibitors in PS II and PS I, Pisciotta indicated that the mesophilic cyanobacteria also possess “electrogenic” activity and that PQ is the major mediator, but the difference between thermophilic and mesophilic cyanobacteria means that we should not conclude that PQ is also the main mediator in our case [10]. The presented approach is only a preliminary estimation. For a detailed understanding of the structure of the redox compound, additional studies must involve the purification and identification of the redox compound. Moreover, tolerance of high temperatures is crucial for electron production under solar radiation; the electrogenic activity of thermophilic cyanobacteria should therefore be screened in the future.

Because an electrode with 0.2 V (vs. Ag/AgCl) provided an alternative pathway for electron flooding, the effect of various voltages on the growth and cellular components of TCL-1 should be considered for further study. An expected strategy is to the regulation of the oxidative and reductive states at several fixed potentials via endogenous mediators (Fig. 4). At the positive voltages given, electrons flow from the inside to the outside of cells, resulting in reactions such as electron flood discharges or electron grabs in the photosynthetic ETC (oxidative voltage given in Fig. 4). To our knowledge, this strategy as an additional pathway for electron flow in the photosynthetic ETC may reduce the heat formation and fluorescence emission, but if too many electrons are obtained in this manner, the results are bound to be similar to those obtained under lower light intensity photosystem inhibition. Negative voltage functioned as an ROS inducer to enhance the possible reactions to electron flow from the outside to inside of cells, such as assistance or form of stress (reductive voltage given in Fig. 4). Endogenous mediators may provide electrons if poor manifest at a lower light intensity. However, when the electron flow is sufficient, negative voltage may create stress or become an ROS inducer. This implies that energy can be obtained not only from radiation but also from the electric

power produced in photosynthesis. Regulation through the voltages described in this paper should be applicable in ETC optimization.

4. CONCLUSIONS

A thermophilic cyanobacterial strain, *Thermosynechococcus sp.* CL-1 (TCL-1) purified from Taiwan hot spring was used to examine the photocurrent generation under illumination without the adding of artificial mediators in an H-type two-compartment electrolysis cell. The current response was found to be a function of the cell mass under illumination and revealed a positive linear relationship with an R^2 of 0.85. The majority of electron outflow derives from photosynthesis in photosystem II and I was attributed to the sensitivity of the current response to illumination. The presence of redox compound excreted from TCL-1 is supported through the cyclic voltammetric experiment and plastoqunione, cytochrome b6/f, and plastocyanin between 0.2 and 0.7 *V* are the most probable mediators in electron transportation.

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