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# Bio-Solar Cells Incorporating Catalase for Stabilization of Thylakoid Bioelectrodes during Direct Photoelectrocatalysis

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Thylakoid membranes have been proposed for electrochemical solar energy conversion, but they have been plagued with short term instability. In this paper, thylakoid membranes extracted from *Spinacia oleracea* were physically adsorbed onto Toray paper electrodes with and without catalase, followed by entrapment in a vapor deposited silica matrix. The bioelectrodes were tested using voltammetry and amperometry and tested in a complete photobioelectrochemical cell. Upon subsequent polarization experiments, a significant decrease in the maximum current density from 1.53 ± 0.13 $\mu Acm^{-2}$ to 0.75 ± 0.14 $\mu Acm^{-2}$ was observed without catalase present. When catalase was included in the anode, this current decrease was not observed, showing the importance of catalase to scavenge reactive oxygen species produced by the thylakoids during photoelectrocatalysis.
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Thylakoid membranes contain the redox complexes responsible for the light-dependent reactions of photosynthesis found in cyanobacteria and the chloroplasts of plants. They are composed of two photosystems along with several other enzymes and cofactors. Light is absorbed by photosystem II to oxidize water to dioxygen. The electrons produced in the reaction are shuttled to plastoquinone, then cytochrome $b_6f$, plastocyanin, and finally photosystem I where they are excited by absorbed photons.[1] They are then used in the reduction of ferredoxin by ferredoxin reductase with the simultaneous conversion of $NADP^+$ to NADPH.[2] The protons generated form a proton gradient which allows for the production of ATP, the unit of energy currency in the living cell, by ATP synthase.[1] As the quantum yield for photosynthesis in certain components of thylakoids, specifically photosystem I, is almost 100%,[3] developing a method for using these membranes for photoelectrocatalytic energy conversion in a bio-solar cell is highly desirable.

Photovoltaic devices or solar cells are defined as a device capable of converting light (photons) into electrical energy. They are generally constructed of semiconductors and come in a number of varieties, ranging from organic thin film (19.6 ± 0.6% efficiency) and dye sensitized (11.0 ± 0.3%) solar cells (DSSC) to more efficient silicon based (25.0 ± 0.5%) and III-V semiconductor (28.3 ± 0.8%) solar cells.[4] Biological and bio-inspired photoelectrocatalysts have been proposed for solar energy conversion.[5] The bio-inspired photoelectrocatalysts are mostly focused on organometallic complexes with structure inspired by photosystem I or photosystem II,[5a,6] whereas the biological photoelectrocatalysts have focused on either the use of photosystem I[5b,7] or II[8] or the intact thylakoid membranes[5c,9] or chloroplasts.[10] The majority of the literature on photosystem I or II photobioelectrocatalysis and thylakoid photobioelectrocatalysis has employed the use of mediators.[5c,5d,9b] However, these mediators typically result in a voltage loss as well as issues associated with their own light, temperature, and long term stability. Therefore, there has been research on direct photobioelectrocatalysis,[5b,7a,8] but primarily focused on photosystems and not thylakoid immobilization.

In this work, a bio-solar cell was developed which incorporated a thylakoid anode to generate photobioelectrocatalytic current. The thylakoid membranes were entrapped in a silica matrix using vapor deposited tetramethyl orthosilicate (TMOS) developed by Atanassov et al.[11] The anodes were connected with an air-breathing cathode (although other cathodes could be easily employed) and tested electrochemically using a combination of amperometry, cyclic voltammetry, and current-voltage curves. Catalase, an enzyme that is commonly employed to reduce the damaging effects due to reactive oxygen species production by biocatalysts,[12] was incorporated in the bioanodes. It is hypothesized that addition of this enzyme will lead to greater stability for the bio-solar cell by consuming any reactive oxygen species that may be formed by the thylakoid membranes.

## Results and Discussion

Experimental procedures for all of the reported experiments are detailed in the Supplementary Material.[13] Thylakoids purified from spinach were immobilized onto Toray paper electrodes with vapor deposited TMOS, as shown in Figure 1. SEM micrographs of immobilized thylakoids with and without the TMOS layer were obtained and are shown in Figure S1 in the Supplementary Material.[13] With no TMOS layer, the thylakoids look similar to previously reported thylakoid layers in the literature.[14] However, after the TMOS layer is added, a more 3-dimensionally structured surface is observed, as shown in Figure S1b. Elemental analysis confirmed an increase in Si after TMOS deposition to 3.47 atomic%, which corresponds to 6.78 wt%, and confirms the silicate layer formation.

Activity assays of immobilized thylakoids with no catalase showed an increase in oxygen production of 219 ± 22 μmol/min when exposed to light compared to dark. This activity was not stable and decreased to the background value after less than 10 minutes. When catalase was included with the immobilized thylakoids, the activity in light showed the same increase in oxygen production and remained constant for approximately 30 minutes and then gradually decreased to 50% activity after 2 hours. These results indicate that the addition of catalase does increase the stability of the thylakoids. Additionally, the amount of peroxide generated by the immobilized thylakoids was determined using a peroxidase assay. Thylakoids with no catalase produced 1.2 ± 0.4 mmol/min while thylakoids immobilized with catalase showed no detectable peroxide generation, indicating that the peroxide is being consumed by the catalase.

Representative cyclic voltammograms of the modified electrodes were obtained in the presence and absence of light, as shown in Figure S2 in the Supplementary Material.[13] No significant differences were observed in these experiments. Amperometric measurements were conducted by measuring the current at 0.3 V vs. SCE, as shown in Figure 2. Because the process being observed is an oxidation, it is

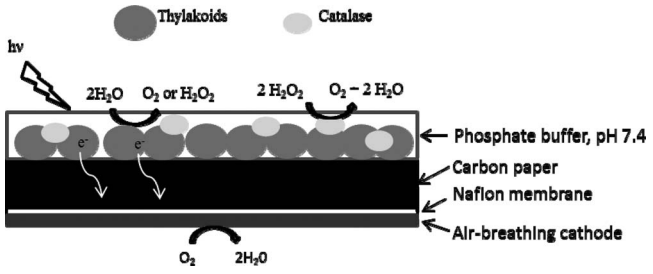


**Figure 1.** Schematic of bio-solar cell.

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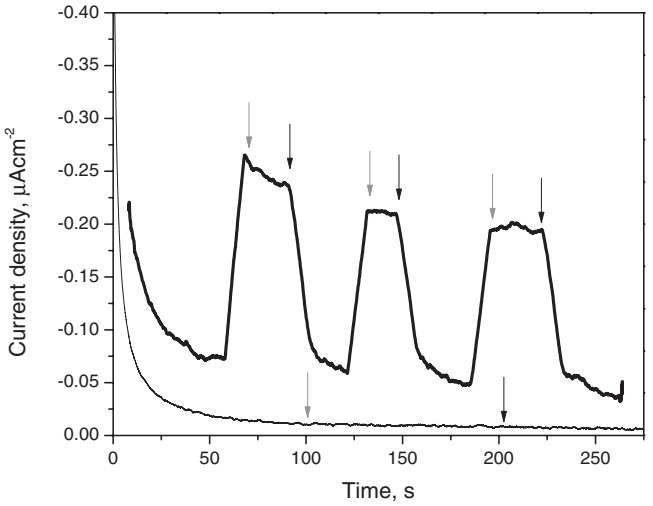


**Figure 2.** Current measured with a thylakoid-modified electrode (thick line) at 0.3 V vs. SCE in 0.1 M phosphate buffer with 0.1 M nitrate. Gray arrows indicate when the light was turned on and black arrows indicate when the light was turned off. Data from a control experiment with an electrode containing TMOS and catalase only is also included (thin line).

expected that the current will increase in the negative direction when the light is turned on. This result is observed and the current returned to background levels when the light was turned off. The increase was approximately 150 nA/cm$^2$. Control experiments performed with electrodes modified with catalase and TMOS, but no thylakoids showed no current change in the presence of light, as shown as the control plot in Figure 2.

The thylakoid-modified electrodes were connected to an air-breathing platinum cathode separated by a Nafion membrane and the resulting bio-solar cells were tested. The average open circuit voltage (OCV) was 0.46 ± 0.03 V. Current-voltage plots (I-V plots) were obtained with and without light, as shown in Figure 3. When no catalase was included in the thylakoid layer, the currents decreased with each subsequent test, showing photoelectrocatalysis but instability. The average maximum current for the first test in the presence of the lamp was 1.53 ± 0.13 μA/cm$^2$, but decreased to 0.90 ± 0.14 μA/cm$^2$ for the second test in the presence of the lamp. The performance of the biosolar cell continued to decrease with each subsequent lamp test.

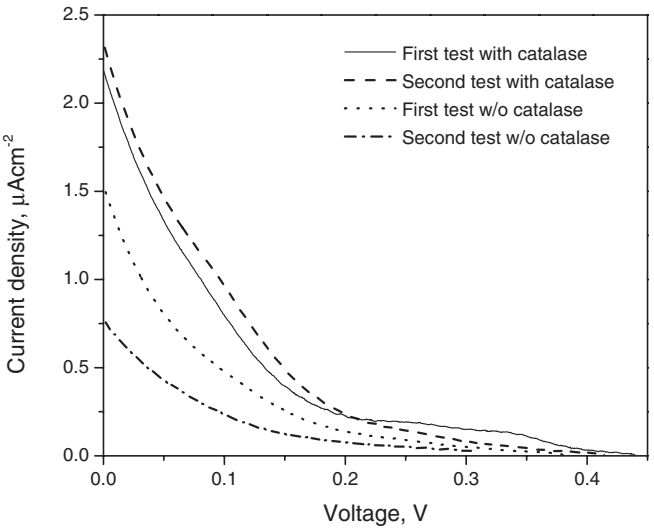


**Figure 3.** Current-voltage plots for a thylakoid bio-solar cell. Each cell was tested twice while exposed to light. Anodes containing no catalase and anodes with catalase are compared.

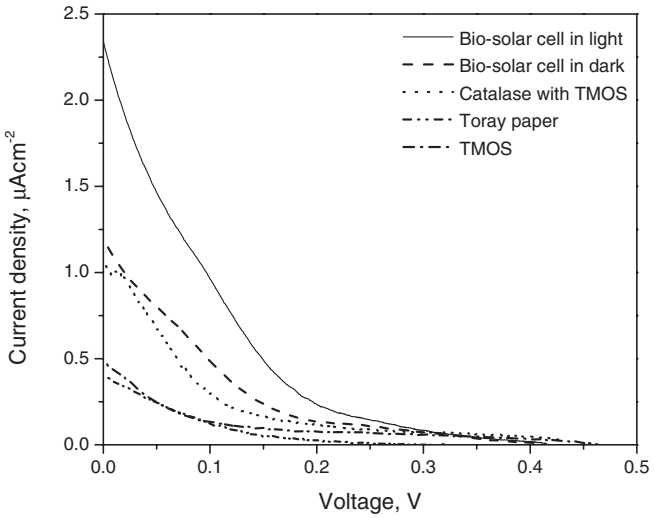


**Figure 4.** Control experiments: bare Toray paper, TMOS only, catalase covered with TMOS, and a complete thylakoid solar cell in the dark and light.

Literature has previously reported that reactive oxygen species are produced by photosystem I during cellular function, but these reactive oxygen species are consumed by ascorbate peroxidase in intact chloroplasts.[15] However, the purified thylakoids do not contain this peroxidase, which likely results in a buildup of reactive oxygen species after thylakoid isolation, which is detrimental to the functioning of enzymes in the thylakoid system. Additionally, it has been shown that heat can cause photosystem II to produce reactive oxygen species as well.[16] In the system being used for testing the bio-solar cell, the lamp generates significant heat with extended use and results in an equilibrium temperature of 38°C in the light box when operated in a room temperature environment (25°C). We hypothesized that the addition of catalase to consume the reactive oxygen species should minimize the issues associated with reactive oxygen species degradation in thylakoid photoelectrocatalysis. When catalase was added to the thylakoids before deposition on the Toray carbon electrode, an increase in current is observed for the bio-solar cell instead of a decrease in current. The average maximum current was 2.14 ± 0.11 μA/cm$^2$ for the first test in the presence of light and 2.46 ± 0.10 μA/cm$^2$ for the second test in the presence of light. Both tests were done at the same environmental temperature of 38°C. These results show that the catalase helps to minimize the effective of reactive oxygen species before and during photon exposure.

In order to verify direct photobioelectrocatalysis, a number of controls were performed to confirm that the current observed is due to the reactions from the thylakoids. As seen in Figure 4 and Table I, the

**Table I. Average maximum current values and open circuit voltages for thylakoid bioanodes utilized in bio-solar cells with and without catalase along with results from control experiments. The results of three different electrochemical cells were averaged for each.**

| | Maximum Current Density (μAcm$^{-2}$) | Open Circuit Potential (V) |
|---|---|---|
| Thylakoids w/ catalase bioanodes | | |
| First light treatment | 2.14 ± 0.11 | 0.46 ± 0.03 |
| Second light treatment | 2.46 ± 0.10 | 0.45 ± 0.02 |
| Thylakoids w/o catalase bioanodes | | |
| First light treatment | 1.53 ± 0.13 | 0.47 ± 0.05 |
| Second light treatment | 0.90 ± 0.14 | 0.42 ± 0.01 |
| Bare Toray paper | 0.29 ± 0.07 | 0.44 ± 0.02 |
| TMOS coated Toray paper | 0.41 ± 0.01 | 0.31 ± 0.02 |
| Catalase and TMOS coated Toray paper | 0.58 ± 0.05 | 0.39 ± 0.04 |

individual components of the modified electrodes (i.e. Toray carbon paper alone, Toray carbon paper with a TMOS coating layer, Toray carbon paper with catalase in a TMOS coating layer) show little to no photocurrent. Additionally, a solar cell containing a thylakoid-modified anode showed significantly smaller currents in the dark than when exposed to light, showing solar photobioelectrocatalysis. These background experiments confirmed direct photobioelectrocatalysis and the ability of the thylakoids to transfer electrons to the electrode without the presence of an external mediator.

## Conclusions

Thylakoids were successfully entrapped in a vapor deposited silica matrix on a Toray carbon paper electrode to make an anode for a bio-solar cell capable of direct photobioelectrocatalysis. When combined with an air-breathing cathode, the cell was able to produce a maximum current density of $2.46 \pm 0.10$ $\mu A/cm^2$ with an open circuit voltage of $0.46 \pm 0.3$ V. The current was not stable due to the formation of reactive oxygen species. The addition of the enzyme catalase to the anode corrected this problem allowing for a stable current density and extending the feasible lifetime of this bio-solar cell.

## Supporting Information

Experimental procedures and results of additional experiments, including scanning electron micrographs of bioanodes with and without the addition of the TMOS immobilization layer and representative cyclic voltammograms of the immobilized thylakoids bioanodes.

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