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From Dynamic Measurements of Photosynthesis in a Living Plant to Sunlight Transformation into Electricity

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We propose here a new method for the direct and continuous measurement of O_2 and glucose generated during photosynthesis. Our system is based on amperometric enzyme biosensors comprising immobilized redox enzymes (glucose oxidase (GOx) and bilirubin oxidase (BOD)) and redox hydrogels "wiring" the enzyme reaction centers to electrodes. We found that these electrodes, implanted into a living plant, responded in real time to visible light as an external stimulus triggering photosynthesis. They proved to be highly selective and fast enough and may be a valuable tool in understanding photosynthesis kinetics. Furthermore, we demonstrate that with our electrodes we could harvest glucose and O_2 produced during photosynthesis to produce energy, transforming sunlight into electricity in a simple, green, renewable, and sustainable way.

Photosynthesis is one of the most important and complex chemical processes in nature. Measurement of photosynthesis rate and evolution of the photosynthetic reactions are of vital importance to understand the photosynthetic apparatus and to follow crop production in agriculture since photosynthesis is the way in which the plant fixes carbon and hence a measure of its growth rate. Because solar energy is in abundant supply and because photosynthesis is one of the most efficient photovoltaic devices developed by nature, numerous artificial biophotovoltaic and biophotoelectronic devices have been developed, incorporating native proteins extracted from higher plants,[1−3] whole living photosynthetic organisms,[4−6] and synthetic biomimetic molecules,[7] however with low quantum efficiency.

Photosynthesis is the conversion of solar energy into chemical energy. In the presence of visible light, carbon dioxide and water form glucose ($C_6H_{12}O_6$) and O_2 in a series of reactions. Many nondestructive techniques have been employed for the study of photosynthesis *in vivo*. Chlorophyll fluorescence is the most widely used spectroscopic technique in photosynthesis and plant research.[8−10] Chlorophyll fluorescence yield is inversely proportional to the rate of photosynthesis. An alternative is to measure the changes in oxygen and CO_2 concentrations in a closed chamber.[11−13] Earlier, Tsionsky et al.[14] also showed that O_2 evolution in photosynthesis could be measured by SECM microscopy. However, to the best of our knowledge, *in vivo* glucose production profiles have not yet been reported.

A plant leaf is a highly complex media. Photosynthesis involves a complex set of physical and chemical reactions that occur in a coordinated manner to accomplish the synthesis of carbohydrates. To produce sugar molecules, plants require nearly 30 different proteins that work within a complicated membrane structure.[8] Different sugar forms, including small intermediate molecules of the glucose synthetic pathway and also oligo and polysaccharides used for stocking fuel, can be found within a leaf. Therefore highly selective and sensitive systems are needed to detect and quantify individual chemical compounds, such as glucose and O_2. Redox enzymes immobilized in a hydrogel matrix are ideal components to build biosensors, because of their high specificity. Enzyme electrodes have been proposed and are already in use for applications such as glucose concentration monitoring for diabetic people, as electrocatalysts in potentially implantable biofuel cells and in bioelectromechanical transducers.[15]

As a proof of concept, we propose a new method for direct and continuous measurement of O_2 and glucose generated during photosynthesis. Our system is based on amperometric enzyme biosensors comprising immobilized redox enzymes (glucose oxidase (GOx) and bilirubin oxidase (BOD)) and

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(1) Evans, B. R.; O'Neill, H. M.; Hutchens, S. A.; Bruce, B. D.; Greenbaum, E. *Nano Lett.* **2004**, *4*, 1815–1819.
(2) 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.; Schnur, J.; Bruce, B. D.; Zhang, S.; Baldo, M. *Nano Lett.* **2004**, *4*, 1079–1083.
(3) Lebedev, N.; Trammell, S. A.; Spano, A.; Lukashev, E.; Griva, I.; Schnur, J. *J. Am. Chem. Soc.* **2006**, *128*, 12044–12045.
(4) Kruse, O.; Rupprecht, J.; Mussgnug, J. H.; Dismukes, G. C.; Hankamer, B. *Photochem. Photobiol. Sci.* **2005**, *4*, 957–970.
(5) Furukawa, Y.; Moriuchi, T.; Morishima, k. *J. Micromech. Microeng.* **2006**, *16*, S220–S225.
(6) Logan, B. E.; Hamelers, B.; Rozendal, R.; Schroder, U.; Kellers, J.; Freguia, F.; Alterman, P.; Verstratete, W.; Rabaey, K. *Environ. Sci. Technol.* **2006**, *40*, 5181–5192.
(7) Amao, Y.; Takeuchi, Y. *Int. J. Hydrogen Energy* **2008**, *33*, 2845–2849.
(8) Hopkins, W. G.; Huner, N. P. A. *Introduction to plant physiology*; Hoboken, NJ, 2009.
(9) Baker, N. R. *Annu. Rev. Plant Biol.* **2008**, *59*, 89–113.
(10) Maxwell, K.; Johnson, G. N. *J. Exp. Bot.* **2000**, *51*, 659–668.
(11) Boardman, N. K. *Annu. Rev. Plant Physiol.* **1977**, *28*, 355–377.
(12) Berry, J.; Björkman, O. *Annu. Rev. Plant Physiol.* **1980**, *31*, 491–543.
(13) Takahashi, M.; Ishiji, T.; Kawashima, N. *Sens. Actuators* **2001**, *77*, 237–243.
(14) Tsionsky, M.; Cardon, Z. G.; Bard, A. J.; Jackson, R. B. *Plant Physiol.* **1997**, *113*, 895–901.
(15) Heller, A. *Curr. Opin. Chem. Biol.* **2006**, *10*, 664–672.

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redox hydrogels "wiring" the enzyme reaction centers to electrodes.[15−17] In this work we show that these electrodes, implanted into a living plant, responded dynamically in real time to visible light as an external stimulus triggering photosynthesis. They proved to be highly selective and fast enough and may be a valuable tool to understand photosynthesis kinetics and to better understand the photosynthetic machinery. Furthermore, we demonstrate that with our electrodes we could harvest glucose and O_2 produced during photosynthesis to produce energy, transforming sunlight into electricity in a simple, green, renewable, and sustainable way. The power density produced by this small biofuel cell is highly dependent upon illumination of the leaf.

EXPERIMENTAL SECTION

Electrochemical measurements were performed using a bipotentiostat (CH Instruments, Austin, TX, model CHI760C). All potentials reported in this paper are quoted with respect to the Ag/AgCl reference electrode. BOD (EC 1.3.3.5) from *Trachyderma tsunodae* and GOx (EC 1.1.3.4) from *Aspergillus niger* were purchased from AMANO and SIGMA, respectively, and purified according to published procedures.[16,18] Synthesis of polymers I (PVP-[Os(*N*,*N*′-dialkylated-2,2′-bis-imidazole)$_3$]$^{2+/+3}$ $E^0 = -0.19$ V vs Ag/AgCl) and polymer II (copolymer of polyacrylamide and poly(*N*-vinylimidazole) complexed with [Os (4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$, $E^0 = 0.36$ V vs Ag/AgCl) and electrode preparation have been previously reported.[18,19] Glassy carbon electrodes (3 mm diameter) (BAS) were used. GOx modified electrodes were composed of 60% polymer I, 35% enzyme, and 5% PEGDGE(400) with a total loading of 305 μg cm^{-2}. BOD modified electrodes were composed of 77% polymer II, 15.5% enzyme, and 7.5% PEGDGE(400) with a total loading of 470 μg cm^{-2}.

In chronoamperometry experiments, both the glucose and O_2 electrodes were poised at +0.1 V, and data points were measured every 0.1 s (10 points per second). A piece of cactus skin was carefully removed with a scalpel, and the enzyme electrode was dipped inside the leaf together with a Ag/AgCl reference and a Pt counter electrodes. A conventional desk lamp able to yield light of two different intensities (160 and 250 W m^{-2}) was used as a light source and placed at 14 cm from the leaf. The light intensity was measured with a power meter (Spectra Physics SP 404) and a filter transmitting only photosynthetically active radiation (PAR light, 400−700 nm). The cacti were kept in darkness for at least 24 h before experiments. Electrochemical measurements always began in the dark state (light OFF). The current value reaches a stable baseline after a continuous measurement period of about 15 min. The plant was illuminated only when the baseline current had been stable for at least 5 min. Each enzyme electrode was use only once and discarded afterward. For new experiments on a plant already tested, electrodes were inserted in a new position far from previous tests.

Five different types of cacti were tested, from genus *mytillocactus*, *echinocereus*, *tephrocactus*, *gymnocalcium*, and *opuntia*. The experiments depicted in Figures 2 and 3 panels a and b and Figure 6 were performed on a plant from the genus *mytillocactus*; the ones in Figure 3c,d in a *tephrocactus*; the one in Figure 4 in a *gymnocalcium*; and that in Figure 5 in an *echinocereus*. Experiments were repeated at least 3 times. The shape of CVs and chronoamperometry profiles were always reproducible. The CVs peak height and the area under the peak (both under dark) varied around 30% between different experiments, probably due to partial film damage during introduction of the electrode in the leaf. The value of the current change under illumination also varied around 30% when using the same cacti and different enzyme electrodes, the change followed the trend of the peak height of the CVs (lower peak height, lower current change).

Thermostatized experiments were performed on a fresh cut leaf immersed in a 2 L beaker full of water.

Calibration curves were measured for BOD and GOx electrodes *in vitro* in a 50 mM Na phosphate buffer, 0.1 M NaCl, pH

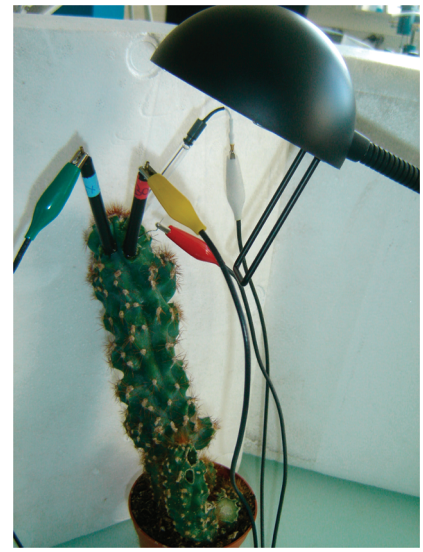

Figure 1. Experimental setup. Two working, counter, and reference electrodes dipped inside the cactus leaf and a light source.

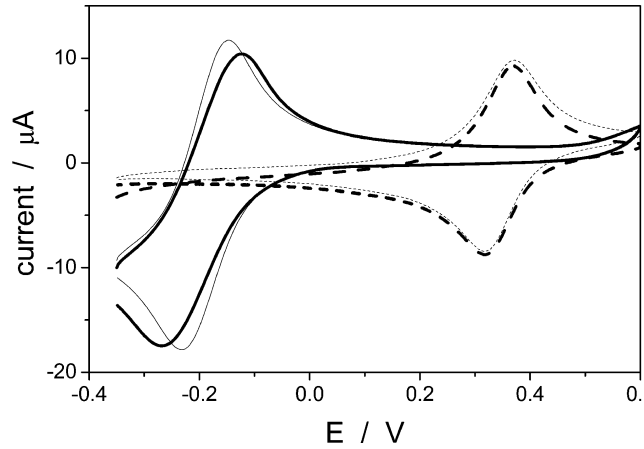

Figure 2. Cyclic voltammograms of a glucose electrode (solid lines) and an O_2 electrode (dashed lines). Thick lines: experiment performed on a cactus kept in the dark state for 24 h. Thin lines: experiment performed after 2 illumination/darkness cycles (final step was a 1200 s darkness period). Scan rate: 25 mV s^{-1}. 3 mm diameter electrodes.

(16) Gao, F.; Courjean, O.; Mano, N. *Biosens. Bioelectron.* **2009**, *25*, 356–361.
(17) Mano, N.; Mao, F.; Heller, A. *J. Am. Chem. Soc.* **2003**, *125*, 6588–6594.
(18) Mano, N.; Fernandez, J. L.; Kim, Y.; Shin, W.; Bard, A. J.; Heller, A. *J. Am. Chem. Soc.* **2003**, *125*, 15290–15291.
(19) Mao, F.; Mano, N.; Heller, A. *J. Am. Chem. Soc.* **2002**, *125*, 4951–4957.

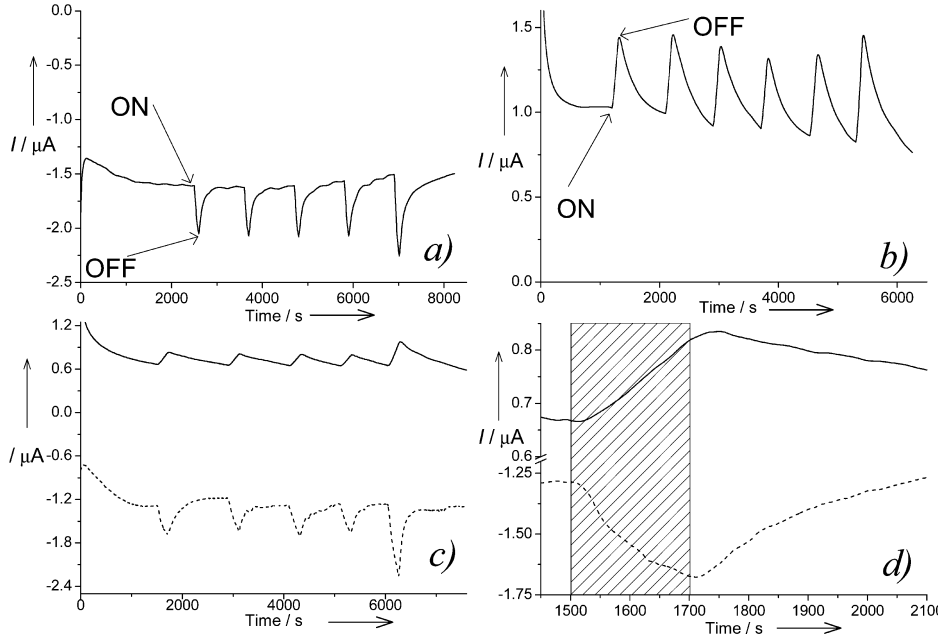

Figure 3. Measurements of the photosynthesis evolution. a) BOD electrode; b) GOx electrode; and c) BOD (dashed line) and GOx (full line) electrodes measured simultaneously. Last cycle light intensity: 250 W m^{-2}, otherwise 160 W m^{-2}. d) Zoom of the first cycle of the experiment depicted in c), dashed area corresponds to light ON.

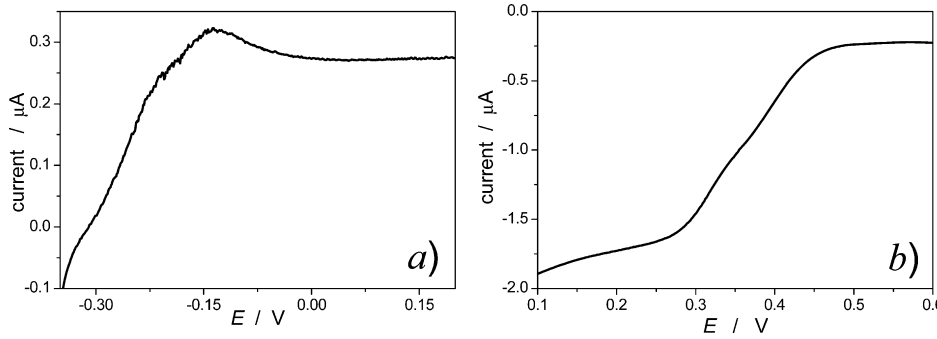

Figure 4. Linear voltammetry measurements with electrodes implanted into the cactus leaf after 5 min of continuous illumination. The graphs have been background corrected (current in the absence of illumination has been subtracted). 5 mV s^{-1} scan rate, 160 W m^{-2} (a) "wired" GOx electrode and (b) "wired" BOD electrode.

= 4.5 (the pH measured inside the cactus leaf), at room temperature.

For the herbicide control experiment, 2×10^{-4} M DCMU solutions were prepared in tap water + 2% ethanol. The cactus soil was watered, the cactus leaf was sprayed with this solution, and the experiment was performed 5 h later.

RESULTS AND DISCUSSION

Modified electrodes comprised of GOx and polymer I (PVP-[Os(*N*,*N*′-dialkylated-2,2′-bis-imidazole)$_3$]$^{2+/3+}$[19] or BOD and polymer II (PAA-PVI-[Os (4,4′-dichloro-2,2′-bipyridine)$_2$Cl]$^{+/2+}$[17,18] were used to monitor glucose and O_2 concentrations, respectively. Figure 1 displays the experimental setup. We worked with cacti because it is experimentally easier to dip macroelectrodes into a thick leaf and because their natural high water content insures good conduction of ionic species. The thick lines in Figure 2 show the cyclic voltammograms (CVs) of the electrodes modified with "wired" GOx (solid line) and "wired" BOD (dashed line) when implanted in the cactus leaf (under dark). The CVs were taken at a scan rate fast enough as to minimize the catalytic signal. The scan can be repeated several times yielding almost superimposable signals. The magnitude and the shape of the CVs are similar to those obtained with the same electrodes dipped in buffer solution indicating good conduction of ionic species within the leaf. The thin lines in Figure 2 show the CVs after a 4000 s illumination/darkness cycles experiment (the final step was a 1200 s darkness period) and are almost superimposed to the thick lines (before photosynthesis experiment, see below). The redox signals of the Os polymers remain essentially unmodified after more than an hour and light exposure. This reproducibility shows that the modified electrodes are stable within the experimental time, i.e. the polymer-enzyme film does not leak into the leaf, and it is neither inactivated nor contaminated by the chemical species present in the leaf.

Figure 3 shows current responses upon illumination/darkness cycles. In the first series of experiments, we independently measured the evolution of O_2-electroreduction current (panel a) and of glucose electroxidation current (panel b) during 100 s illumination periods (marked as ON) followed by darkness periods. Upon illumination, the O_2 signal increases returning

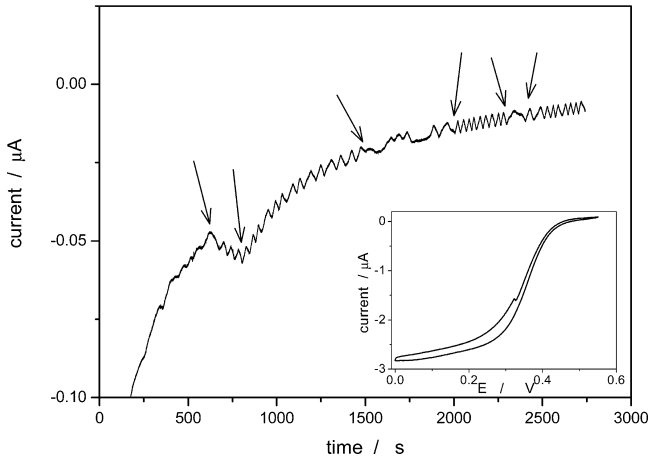

Figure 5. Chronoamperometry experiment for "wired" BOD electrode dipped in a cactus pretreated with DCMU. The arrows signal the time intervals when the lamp was ON. Note the difference in the *y* axis scale as compared to Figure 2 panels a, c, and d. Inset: CV experiment for the same electrode in a phosphate buffer under air right after the chronoamperometry experiment, showing that the enzyme on the electrode is still active.

to its baseline value (within 7%) after the light was turned OFF. The first 4 cycles show a remarkable reproducibility (within 5%).

To show that we could monitor not only O_2 but also glucose as a product of the natural photosynthetic process, we performed similar experiments with a GOx modified electrode. As depicted in Figure 3b, the glucose electroxidation current increases upon illumination returning to its baseline value after the light was turned OFF. The reproducibility is similar to that shown for the O_2 electrode. However, the baseline is not as stable as for the O_2 electrode. Decay in background current is not attributable to film degradation (otherwise the current change upon illumination would be different from cycle to cycle) but might be due to other processes occurring within the leaf once the photosynthesis reaction is triggered, such as equilibrium changes between the different sugars (for example, conversion of glucose into sucrose).[8]

To demonstrate that glucose and O_2 were simultaneously generated upon illumination, we recorded both signals in parallel (panel 3c). Panel 3d is a zoom of the first illumination cycle in 3c. The dashed area shows the precise period when the lamp was ON. Upon illumination, the O_2 and glucose signals increased after 7 and 23 s, respectively. When the lamp was turned OFF, the O_2 current still increased for ~9 s and then decreased. The glucose electrooxidation current still increased for ~38 s, then remained stable for ~20 s, and finally decreased.

O_2 and glucose concentration profiles were measured on 5 different cacti types (see the Experimental Section). Qualitatively the results were the same for all species, and the value of the current change for same period of time and same illumination intensity was of the same order of magnitude, though the exact value depended upon the plant under study. The value of the current change for the *opuntia* genus was the lowest, but in that case experiments were performed on a leaf that had been cut from the plant.

For all experiments, exposure to higher illumination intensity (last cycle) increased the catalytic current by 55%, for both glucose and O_2. The linear portions of the current increase curves were fitted. The slopes for the last cycle of Figure 3a, b,c were ~50% higher than the slopes of the first cycles. This is in agreement with literature values.[14] For both 160 and 250 W m^{-2} light intensities, photosynthesis rate is light-limited and increases linearly with light intensity.

Current decays under darkness were fitted to exponential decays. Characteristic decay times were of 140 ± 45 s and 1000 ± 300 s for O_2 and glucose, respectively. Decay values and the shape of the concentration profiles were reproducible for all experiments performed, for every cycle, for different cacti species and for different illumination intensities. This behavior is consistent with the theory of the photosynthetic cycle: O_2 production stops almost immediately when the light is turned OFF and its concentration decreases quickly since it is liberated outside of the leaf. However, glucose production will keep on going as long as there is NADPH produced by photosystem II.[20,21]

To check that the catalytic current was only generated by the "wired" enzymes in the presence of their substrates, and not by other parasite processes or chemical species, different control experiments were performed. We first performed linear voltammetry within the cactus after illumination. The characteristic catalytic responses (plateau shape) of mediated enzymatic systems in the presence of substrate were observed. Figure 4a,b clearly shows that O_2 and glucose are generated upon illumination. Because GOx is highly selective for β-D-glucose as compared to other sugars (relative activity less than 2%), and BOD only accepts O_2 as electron acceptor, the electrocatalytic currents observed in Figures 3 and 4 can only be related to the oxidation of glucose and reduction of O_2. Then, we performed experiments with implanted electrodes made of redox polymer and cross-linker only (without enzymes, one electrode for each polymer I and II). Upon illumination, no electrooxidation (glucose) or electroreduction (O_2) were observed (see Figure S-1 in the Supporting Information). This control clearly shows that the current change observed in Figures 3 and 4 is directly related to the presence of the enzymes. During exposure cycles, we noticed that the temperature of the leaf changed about 1 °C. Results obtained in a thermostatized cactus leaf (not shown) were similar to those previously obtained, ruling out an increase of the current because of heating. Finally, we tried to measure O_2 generation on a cactus that had been treated with DCMU. This is a herbicide well-known to block photosystem II and to stop photosynthesis.[22] As seen is Figure 5, upon illumination almost no electroreduction current was observed. The BOD electrode was used afterward in an *in vitro* experiment to check that the absence of signal in the cactus was not due to enzyme inactivation by DCMU. The inset in Figure 5 shows the enzyme was still fully active and clearly indicates that our electrode implanted in the leaf is specific and selective for O_2. All these experiments showed

(20) Mehta, N.; Panda, A.; Sengupta, S.; Datta, S. N. J. *J. Phys. Chem. B* **2006**, *110,* 10951–10961.
(21) Taiz, L.; Zeiger, E.; *Plant Physiology*; Sinauer Associates, Inc.: Sunderland, 2006.
(22) Shimazaki, K.-I.; Gotow, K.; Kondo, N. *Plant Cell Physiol.* **1982**, *23*, 871–879.

that the "wired" enzyme electrodes are selective enough to dynamically monitor glucose and O_2 generated during the photosynthesis.

Our enzymes electrodes sense O_2 and glucose total concentrations for each data point. Therefore, they allow the measurement of the kinetics of products that are being generated and accumulated during the photosynthesis. The response of the biosensors in the cactus leaf is fast enough to follow glucose and O_2 accumulation, as illustrated in Figure 3 where a clear signal change can be seen from point to point showing concentration changes in time intervals of 0.1 s.

To correlate the current values measured with volume concentrations, calibration curves were measured for BOD and GOx electrodes *in vitro* in 50 mM Na phosphate buffer, 0.1 M NaCl, pH = 4.5, the pH measured inside the cactus leaf, at room temperature (see Figure S-2 in the Supporting Information). The signal for the BOD electrode showed only minor changes when tested in solutions of different pH and ionic strength. Therefore we could compare the signal measured inside the cactus with the *in vitro* experiment at pH = 4.5 to assess the O_2 concentration changes inside the leaf. This is not the case however for the GOx electrode, whose signal changes considerably with the pH of the solution and ionic strength. Exact composition of the aqueous media inside the leaf is unknown, and therefore the *in vitro* calibration measured at pH 4.5 is used to estimate *in vivo* concentrations. The value given below for the glucose concentration should be regarded as an estimated value, indicating an order of magnitude and not a precise value. Despite this limitation, because in the *in vitro* experiment the GOx electrode showed a linear response in the range of concentration of interest and because the K_{MS} for GOx is 25 mM (much higher than the concentration values considered in this work) a current increase can be correlated with a glucose concentration increase. Based on the calibration plots and the above considerations, we calculated the concentration of O_2 and glucose generated upon 200 s illumination (Figure 3c) to be 0.1 mM and ≈20 μM, respectively. Considering the surface of the cactus exposed to PAR radiation (approximately 4 cm^2) and assuming there are not major limitations to glucose and O_2 diffusion toward the electrodes, we find that the experimental concentration values are in agreement with literature values for photosynthetic CO_2 assimilation, in the order of 20 μmol m^{-2} s^{-1} [20,21] (the exact value varies with plant variety, light intensity, and CO_2 concentration). Even though it may be a rough estimation, it allows us to compare our results with known rates of O_2 evolution and glucose formation. For a more precise calculation we would need to be able to correlate the illuminated area with mass transport processes in the leaf, which so far is beyond our scopes.

Earlier, Tsionsky et al.[14] showed that O_2 evolution in photosynthesis could be measured by SECM microscopy. Our method cannot measure localized concentrations as SECM does. However, the use of enzymes improves the specificity which allows us to introduce the electrodes in the leaf itself instead of next to it[14] and to monitor not only O_2 but also glucose concentration. To the best of our knowledge, *in vivo* real time glucose concentration profiles have not been shown so far.

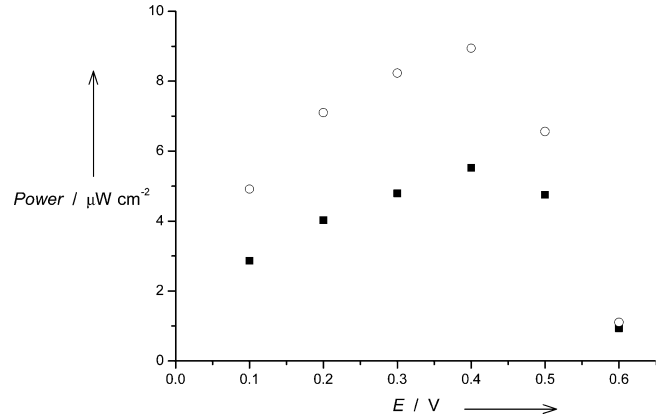

Figure 6. Dependence of the power density on the cell voltage before (full squares) and after 4 min illumination at 250 W m^{-2} (empty circles).

Finally, we demonstrate that we could transform the products of natural photosynthesis into electrical power with efficiency proportional to the intensity of the illumination at which our simple device is exposed to. We used the electrodes previously described as biosensors to construct a membrane-less biofuel cell.[16,17] The redox potentials of polymers I and II were tailored to be slightly oxidizing and reducing respectively to GOx and BOD, allowing an electron flow from anode to cathode (from glucose to O_2). The use of enzymes provides high specificity and its coimmobilization with redox mediators avoids leaking of the catalysts and unwanted reactions of the redox mediators in the other electrode; therefore, avoiding the need of a membrane between electrodes.

Figure 6 represents the dependence of the power density on the cell voltage before and after 4 min of continuous illumination of 250 W m^{-2}. Glucose is electrooxidized at the anode

$$\textit{Anode}: 2C_6H_{12}O_6 \rightarrow 2C_6H_{10}O_6 + 4H^+ + 4e^- \quad (1)$$

and O_2 is reduced to water at the cathode

$$\textit{Cathode}: O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (2)$$

resulting in the net bioelectrochemical power generating reaction

$$\textit{Biofuel Cell}: 2C_6H_{12}O_6 + O_2 \rightarrow 2C_6H_{10}O_6 + 2H_2O \quad (3)$$

At +0.4 V, the power density (9 $\mu W \cdot cm^{-2}$) was 70% higher under illumination than it was under darkness showing that the power output generated by such a biofuel cell implanted into a living plant is proportional to the light at which the plant is exposed to. The increase in power density can be attributed to the higher current flow achievable due to the accumulation of fuel (glucose and O_2) produced by photosynthesis. Earlier, we demonstrated the generation of electrical power in a grape, but no attempt was made to measure changes in power density upon illumination.[17] We believe our new setup improves that idea: a fruit cut out of the plant will eventually exhaust its fuel content, not being able to produce more glucose since it is not photosynthetically active, whereas a living plant will ideally

keep on generating fuel to make the biofuel cell work for as long as the anode and cathode are active.

CONCLUSIONS

Current understanding of the structure and function of the components of the photosynthetic apparatus is highly advanced. However, the quantitative understanding of the cooperation of these components *in vivo* under physiological relevant conditions is more limited.[23] We believe that the method proposed here together with simultaneous applications of other nondestructive techniques might help plant researchers in addressing these questions. This method can be extended to other plant varieties other than cacti by using microelectrodes, such as 7 μm thick carbon fiber electrodes.

(23) Baker, N. R.; Harbinson, J.; Kramer, D. M. *Plant Cell Environ.* **2007**, *30*, 1107–1125.

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SUPPORTING INFORMATION AVAILABLE

Chronoamperometry experiments with control electrodes made of polymer+PEGDGE (in the absence of enzymes) and *in vitro* calibration curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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