

Miriam Rosenbaum · Uwe Schröder · Fritz Scholz

Utilizing the green alga *Chlamydomonas reinhardtii* for microbial electricity generation: a living solar cell

Received: 2 December 2004 / Revised: 5 January 2004 / Accepted: 5 January 2005 / Published online: 5 February 2005
© Springer-Verlag 2005

Abstract By employing living cells of the green alga *Chlamydomonas reinhardtii*, we demonstrate the possibility of direct electricity generation from microbial photosynthetic activity. The presented concept is based on an in situ oxidative depletion of hydrogen, photosynthetically produced by *C. reinhardtii* under sulfur-deprived conditions, by polymer-coated electrocatalytic electrodes.

Introduction

Gaffron and Rubin (1942) described the hydrogen metabolism of unicellular eukaryotic algae. After anaerobic induction for several hours in the dark, hydrogenase activity could be measured for a short time when the algae were brought back into the light. This discovery marked the laying of a cornerstone for a long tradition of scientific work on biohydrogen production by phototropic microorganisms. To date, a great number of phototropic hydrogen-producing species have been found, both eukaryotic (green algae) and prokaryotic (cyanobacteria, photosynthetic bacteria). Although photosynthetic hydrogen production seemed to be a promising way for the biological conversion of sunlight into fuel, there are hardly any biotechnological applications. The major problem is the extreme sensitivity of the hydrogenase enzyme towards molecular oxygen. Oxygen both suppresses the expression of the hydrogenase genes and within minutes completely and irreversibly inactivates the hydrogenase (Ghirardi et al. 1997). Using *Chlamydomonas reinhardtii*, a model organism that is able to express a Fe-hydrogenase enzyme shortly after anaerobic adaptation (Happe and Kaminski 2002),

different strategies have been followed to overcome this problem. Thus, extensive work has been dedicated to genetically engineer mutants of *C. reinhardtii* that possess a better O₂ tolerance (Flynn et al. 2002). The second concept uses so-called indirect photobiolysis by temporarily separating the water-splitting and oxygen-evolving reactions from the hydrogen production process. This concept was realized by Melis et al. (2000). The authors achieved a sustained photobiological hydrogen production by transferring grown cells of *C. reinhardtii* into sulfur-deprived nutrient conditions. The lack of sulfur leads to a fast diminution of photosystem II activity—the oxygen-evolving part of the photosynthetic apparatus—and to a decrease in the oxygen production rate below the rate of mitochondrial respiration (Melis 2002).

Our research is dedicated to the utilization of microbial metabolic activity for the direct generation of electricity. Recently, we proposed a novel microbial fuel cell concept that allowed boosting the power output of microbial fuel cells to reach current densities far above those described before (Schröder et al. 2003). The new approach is based on an anode design that allows the efficient electrocatalytic oxidation (and thus depletion) of microbially synthesized hydrogen—the superior fuel for energy generation in fuel cells—in the microbial medium and under the physiological conditions of microbial growth. The design comprised the electrocatalyst platinum and an overlay of a conductive polymer, like polyaniline or its fluorinated derivatives. The polymer coating not only preserves the electrocatalyst from deactivation, it also improves the efficiency of hydrogen oxidation (Nießen et al. 2004). We showed that, using the new concept, electricity generation is possible from the decomposition of complex carbohydrates by exploiting the catabolism of chemotropic microorganisms (Nießen et al. 2004).

In this communication, we propose the direct generation of electricity using living cells of the alga *C. reinhardtii*. We demonstrate that, by combining the photosynthetic activity of hydrogen-producing green algae and a new electrode concept, the direct conversion of the energy of sunlight into electricity is possible by means of a living, green solar cell.

M. Rosenbaum · U. Schröder (✉) · F. Scholz
Institut für Chemie und Biochemie, Universität Greifswald,
Soldmannstrasse 16,
Greifswald, 17489, Germany
e-mail: uweschr@uni-greifswald.de
Tel.: +49-3834-864330
Fax: +49-3834-864451

Materials and methods

Algal growth

C. reinhardtii strains CC125 (mt+); (Chlamydomonas Genetic Center, Duke University, Durham, N.C.) and SAG 83.81 (SAG, Göttingen, Germany) were grown photoheterotrophically in tris-acetate-phosphate (TAP) medium, pH 7.2. For that, the liquid cultures (100 ml) were grown temporarily shaken at room temperature (25°C) in 250-ml Erlenmeyer flasks under continuous illumination with fluorescence light (compact fluorescent light lamps, 15 W, 2,700 K). To transfer the cultures into the sulfur-deprived, hydrogen-producing state, the dilution method described by Laurinavichene et al. (2002) was used. For that, the cultures, grown in sulfur-rich medium, were diluted 1:10 (v/v) into TAP medium with all sulfur-containing ingredients replaced by the respective chloride salts (TAP-S).

Electrochemical instrumentation

Sealed and illuminated glass jars served as electrochemical cells hosting a working electrode, a reference and a counter electrode in a conventional three-electrode arrangement. The counter electrodes were graphite rods. Silver/silver chloride electrodes (saturated KCl, 0.195 V vs standard hydrogen electrode) served as reference electrodes. All potentials given in this communication refer to the silver/silver chloride reference electrode. The counter electrodes were separated from the algal solution by a Nafion 117 perfluorinated membrane. Experiments under potentiostatic control were carried out using PGSTAT10 and PGSTAT20 potentiostats (Ecochemie, Netherlands). To measure the electricity generation, a permanent potential of 0.2 V was applied to the working electrode, in order to in situ oxidize microbial hydrogen under the conditions present in a microbial fuel cell. To determine the redox potential in the algal solutions, the open circuit potential was measured between a working electrode (see next section) and a silver/silver chloride reference electrode, using a data acquisition system (Integra 2700 digital multimeter equipped with 7700 multiplexer; Keithley Instruments, Cleveland, Ohio, USA) interfaced to a personal computer.

Electrode preparation

The working electrodes were platinum mesh electrodes (50×90 mm) electrochemically platinized in a stirred acidic solution containing 20 mM H₂PtCl₆ (Fluka) at a potential of -0.6 V for 500 s. The electrodes were subsequently covered by conductive polymers, such as poly(2,3,5,6-tetrafluoroaniline) or poly(2-fluoroaniline), using oxidative electropolymerization at fixed potentials. Poly(2,3,5,6-tetrafluoroaniline) was polymerized from a stirred solution of 50 mM 2,3,5,6-tetrafluoroanilin (Acros Organics) in 2 M HClO₄ at a potential of 1.6 V for 500–1,000 s. Poly-2-

fluoroaniline was prepared from 0.1 M 2-fluoroaniline (Fluka) in 0.1 M NaClO₄/0.1 M HClO₄. A polymerization potential of 1.3 V was applied for 1,000 s.

Similar to the fluorinated polyanilines, the unsubstituted parent compound, polyaniline, can serve as an electrode modifier. The electrode preparation is described by Schröder et al. (2003). The results described in this communication, however, refer to studies involving the fluorinated polymers.

Results

The inset in Fig. 1 shows the course of the redox potential measured in a freshly inoculated culture of sulfur-deprived *C. reinhardtii* cells in TAP-S medium over 87 h. The positive potential of about 300 mV at the beginning illustrates the aerobic, oxygen-producing state of the culture. With depletion of residual amounts of sulfur in the medium, the potential of the solution slowly decreases. After 30 h, the transition to negative potentials indicates that the algal culture becomes anaerobic, marking the point where the photosynthetic oxygen production rate falls below the rate of mitochondrial respiration. After becoming anaerobic, the potential of the culture quickly decreases to values below -600 mV. Such a highly negative potential is characteristic for the presence of hydrogen in the culture.

The potentiometric measurements are a valuable indicator giving information on: (1) the metabolic state (i.e., the redox state) of the microbial culture and (2) the ability of the utilized electrode material to translate the redox potential of the biological system into an electrical potential, a major prerequisite for the efficient transfer of electrons for electricity generation. For most compounds (even hydrogen) present in biological systems like algal solutions, the equilibration at conventional unmodified electrode materials like carbon is too slow or even fully inhibited. However, the polymer-modified electrodes used here allow a quick equilibration, the major redox couple being H₂/2H⁺.

To study the performance and capacity of the algal cultures to generate electricity, the current at the working electrode was measured when the electrode was held at a constant oxidation potential of 0.2 V, leading to the oxidation of the photosynthesized hydrogen in the culture. In order to assure the complete depletion of hydrogen from the algal solution, large mesh electrodes (50×90 mm) were used, capable of achieving a current density up to 1.5 mA cm⁻² from the oxidation of dissolved hydrogen. Figure 1 shows the current generation at a polytetrafluoroaniline-modified platinum electrode that was immersed in a TAP-S culture solution of *C. reinhardtii* CC-125. The experiment was carried out in a 550-ml glass cell for 90 h. Due to the absence of hydrogen in the algal solution and in accordance with the potentiometric results, no significant current was detected during the first 24 h. After about 30 h, the current increased rather quickly to a maximum value of 9 mA. Based on the Faraday law (see also Eq. 1) and assuming the complete electrochemical oxidation of the microbially formed hydrogen, the current values were equivalent to the rate of the photosynthetic hydrogen production. Thus,

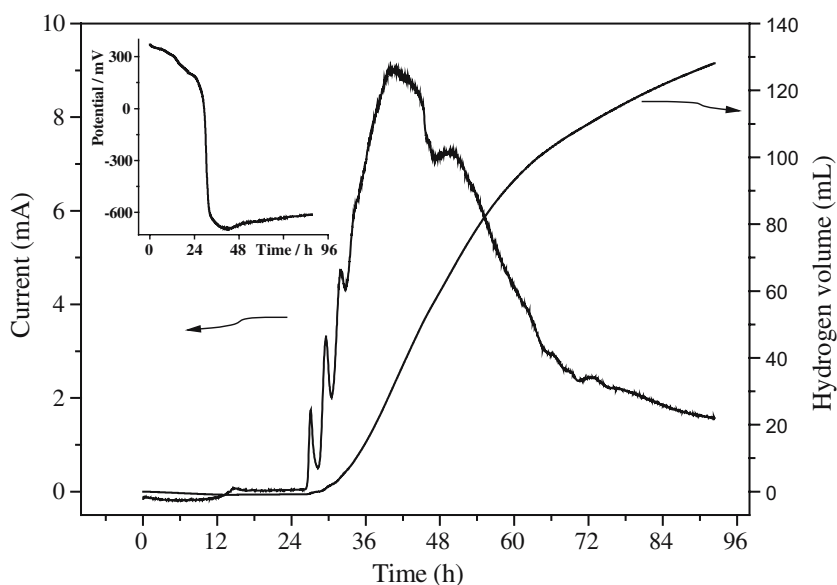


Fig. 1 Current generation and hydrogen production of a culture of *C. reinhardtii* CC125, grown in TAP medium and transferred (1:10, v/v) into TAP-S. Current generation was measured at a polytetrafluoroaniline-modified platinum mesh electrode. The electrodes were potentiostatically poised at a potential of 0.2 V. The culture (550 ml) was sealed and constantly illuminated with fluorescent light. The experiment was performed at room temperature. The total volume of

microbially produced and electrochemically oxidized hydrogen was calculated from the integration of the current curve. mL milliliters. *Inset* Redox potential of a culture of *C. reinhardtii* CC125, grown in TAP medium and transferred (1:10, v/v) into TAP-S, measured at a poly(2-fluoroaniline)-modified platinum electrode. All other conditions as above

the maximum current of 9 mA corresponds to a maximum hydrogen production rate of 4.1 ml h^{-1} , which, normalized to the culture volume, corresponds to a value of $7.6 \text{ ml hydrogen l}^{-1} \text{ culture h}^{-1}$. After reaching its maximum at about 40 h, the hydrogen production rate (and thus the current) slowly decreases to reach a value of 0.8 ml h^{-1} (1.6 mA) at the end of the experiment.

By integrating the recorded current over time, the electric charge can be determined. Since this charge is almost exclusively caused by the oxidation of the photosynthetic hydrogen, it can be used to determine the total amount of hydrogen photosynthetically produced by the algae and electrochemically oxidized at the polymer-modified electrodes. The volume of hydrogen was determined according to Eq. 1:

$$V_{H_2} = \frac{\int Idt}{z \cdot F} \cdot V_{n/25^\circ\text{C}} \quad (1)$$

V_{H_2} is the volume of the electrochemically oxidized hydrogen, I is the current, t is the time of the experiment, F is the Faraday constant, z is the number of transferred electrons and $V_{n/25^\circ\text{C}}$ is the molar volume of ideal gasses at 25°C .

For the current generation experiment documented in Fig. 1 (left axis) the integrated curve (right axis) depicts the amount of electrochemically oxidized hydrogen, the final yield being 129 ml.

Discussion

In order to evaluate the efficiency of the presented “living solar cell”, the amount of electrochemically oxidized hydrogen determined from the electric charge can be compared with values obtained from volumetric experiments. Table 1 summarizes the final yields of hydrogen produced by sulfur deprived *C. reinhardtii* cells from experiments performed by different scientific groups. Melis et al. (2000) reported a final volume of $140 \text{ ml hydrogen l}^{-1}$ alga suspension over 130 h, after centrifugation of TAP-grown cells and re-suspension in TAP-S. These results were improved by adding small amounts of sulfur to the TAP-S medium; and a maximum hydrogen yield of 241 ml from 1.2 l

Table 1 Normalized data for hydrogen production by *C. reinhardtii* under different conditions (25°C , 1.013 bar, 1 l culture volume)

Supply of sulfur	Duration of experiment (h)	Final H_2 -yield (ml l^{-1} culture)	Reference
1:10 v/v aerobic culture in TAP-S	90	235 ^a	This work
TAP-S, centrifugation	130	114 ^b	(Melis et al. 2000)
TAP-S, centrifugation, $+25 \mu\text{M SO}_4^{2-}$; synchronized	140	162 ^b	(Kosourov et al. 2002)

^aCalculated from electrochemical data

^bVolumetric data

synchronized culture solution over 140 h was reached by adding 12.5 μM sulfate to the medium (Kosourov et al. 2002). In our experiments, we applied the dilution method described by Laurinavichene et al. (2002) as a superior technique.

Comparing the hydrogen yields calculated from our electrochemical experiments with values from the literature shows that, by utilizing the described in situ electrochemical H_2 oxidation method, hydrogen yields are achieved that may lay at the upper limits of hydrogen evolution by *C. reinhardtii*. These promising results may be attributed to the use of the dilution method, although a positive influence of the in situ hydrogen depletion may also be possible. Thus, keeping the hydrogen pressure in the algal solution at a low level may stimulate hydrogenase activity and increase hydrogen production. This explanation, however, requires further experimental investigation.

The presented results show that the performance of the polymer-modified electrocatalytic electrodes allows efficient utilization of the hydrogen produced by *C. reinhardtii* for electricity generation. The presented concept is aimed to deliver a means to directly exploit the photosynthetic activity of green algae for electricity generation in a living, green solar cell, without the detour of complex gas separation and purification. Certainly, there is still a long way is to go, which may involve biochemical and genetic work to improve the practicability and productivity of algal hydrogen production.

Other steps may involve the immobilization of algal cells at the electrodes, making the exchange of culture medium and the supply of nutrients more practical. Thus, we observed cells of *C. reinhardtii* SAG 83.81 aggregating into small beads and immobilizing quite tightly at the mesh electrodes during the course of the experiments.

Acknowledgements U.S. gratefully acknowledges the support by the United States Office of Naval Research (ONR project N00014-03-1-0431) and by the Fonds der Chemischen Industrie.

References

- Flynn T, Ghirardi ML, Seibert M (2002) Accumulation of O_2 -tolerant phenotypes in H_2 -producing strains of *Chlamydomonas reinhardtii* by sequential applications of chemical mutagenesis and selection. *Int J Hydrogen Energy* 27:1421–1430
- Gaffron H, Rubin J (1942) Fermentative and photochemical production of hydrogen in algae. *J Gen Physiol* 26:219–240
- Ghirardi ML, Togaasaki RK, Seibert M (1997) Oxygen sensitivity of algal H_2 -production. *Appl Biochem Biotechnol* 63:141–151
- Happe T, Kaminski A (2002) Differential regulation of the Fe-hydrogenase during anaerobic adaptation in the green alga *Chlamydomonas reinhardtii*. *Eur J Biochem* 269:1022–1032
- Kosourov S, Tsygankov A, Seibert M, Ghirardi ML (2002) Sustained hydrogen photoproduction by *Chlamydomonas reinhardtii*: effects of culture parameters. *Biotechnol Bioeng* 78:731–740
- Laurinavichene TV, Tolstygina IV, Galiulina RR, Ghirardi ML, Seibert M, Tsygankov A (2002) Dilution methods to deprive *Chlamydomonas reinhardtii* cultures of sulfur for subsequent hydrogen photoproduction. *Int J Hydrogen Energy* 27:1245–1249
- Melis A (2002) Green alga hydrogen production: progress, challenges and prospects. *Int J Hydrogen Energy* 27:1217–1228
- Melis A, Zhang L, Forestier M, Ghirardi ML, Seibert M (2000) Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga *Chlamydomonas reinhardtii*. *Plant Physiol* 122:127–135
- Nießen J, Schröder U, Rosenbaum M, Scholz F (2004) Fluorinated polyanilines as superior materials for electrocatalytic anodes in bacterial batteries. *Electrochem Commun* 6:571–575
- Schröder U, Nießen J, Scholz F (2003) A generation of microbial fuel cell with current outputs boosted by more than one order of magnitude (*Angew Chem Int Ed* 42:2880–2883). *Angew Chem* 115:2986–2989