

In Situ Electrooxidation of Photobiological Hydrogen in a Photobioelectrochemical Fuel Cell Based on *Rhodobacter sphaeroides*

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In this paper, we present a photobiological fuel cell that utilizes the metabolic activity of living cells of *Rhodobacter sphaeroides* for the generation of electricity based on the in situ oxidation of photobiological hydrogen. Organic acids and alcohols contained in synthetic media as well as in fermented media of *Escherichia coli* K 12 served as the proton donor source for the photobiological hydrogen production by *R. sphaeroides*. We demonstrate that the photobiological hydrogen is efficiently oxidized in the microbial medium at electrocatalytic electrodes coated with a platinum–poly(3,4-ethylenedioxythiophene) (Pt–PEDOT) bilayer composite. The experimental results are discussed in terms of current and power output, substrate, and solar conversion efficiency.

Introduction

Since the discovery of biological hydrogen production in the first half of the twentieth century, scientists have dreamed of renewable energy generation using microorganisms. Two major approaches are followed—the direct electricity generation in microbial fuel cells (1–3) and the production of biohydrogen and biogas (4–7) combined with the subsequent conversion to electricity. Biofuel cells mostly exploit heterofermentative and metal-reducing microbes or cyanobacteria as biocatalysts (see, i.e., refs 8–13). For biogas and biohydrogen production, dark fermentative and methanogenic microorganisms as well as phototrophic hydrogen producing microorganisms (green algae, non-sulfur purple bacteria, and cyanobacteria) are used (4–6). The following study aims to combine these approaches by means of a photobiological fuel cell exploiting the non-sulfur purple bacterium *Rhodobacter sphaeroides* for electricity generation based on in situ oxidation of the photobiological hydrogen. This integration shows a number of potential advantages. Thus, the combustion of biohydrogen in a conventional fuel cell requires the gas to be separated from the biomass, as well as its extensive purification (4). The separation of the hydrogen gas from microbial cultures is considered to be an important issue since a large fraction of the gas remains dissolved in the microbial medium—promoting the growth of hydrogen consuming microorganisms and thus lowering the hydrogen yield. Additionally, high hydrogen partial

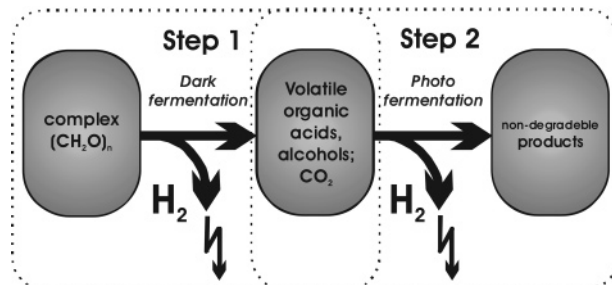


FIGURE 1. Schematic illustration of the application of photoheterotrophic substrate utilization in a two-step microbial fuel cell system.

pressures are known to limit the microbial hydrogen synthesis, which is subject to negative feedback inhibition at high partial pressure levels (5, 14). A proposed method for the hydrogen removal is the purging with an inert gas (e.g., nitrogen). This procedure, however, would be expensive and produces highly diluted hydrogen. With the concept of the direct electrochemical depletion of hydrogen from the bacterial solution by the implementation of electrocatalytic anodes into the microbial cultures, these issues can be avoided. It requires, however, the development of electrocatalytic anodes resistant against biofouling and deactivation in the complex microbial environments. This succeeded in 2003 with the presentation of a novel microbial fuel cell concept that, based on the anaerobic decomposition of glucose by *Escherichia coli* (15), reached current densities above all known systems that far. In principle, the achievement was based on an anode system composed of a highly electrocatalytic electrode overlaid by a conductive polymer that prevents poisoning and that may also show catalytic activity toward fuel oxidation. Further research proved the feasibility of using complex carbon substrate digestion (starch, molasses, cellulose) by *Clostridium* biocatalysts and the effective electricity generation from the produced hydrogen by means of the novel anode materials (16, 17).

One disadvantage using dark fermentation for hydrogen production is the incomplete substrate oxidation combined with the formation of a number of reduced fermentation byproducts, especially organic acids (e.g., lactate, acetate, formate, succinate, propionate, and butyrate) and alcohols. This limits the maximum substrate conversion efficiency to 4 mol of hydrogen per mol of glucose. Photoheterotrophic non-sulfur purple bacteria may help solve this problem and increase the substrate conversion efficiency since they use the remaining organic acids as a resource for photobiological hydrogen production. Hence, *R. sphaeroides*, being well-known for distinctive photoheterotrophic hydrogen production, should be a promising candidate for a second step utilization of dark-fermentation products (as illustrated in Figure 1).

There already exists a number of ideas including two-step biohydrogen production (4) and mixed culture systems (18), in which dark fermentative microorganisms are used for the first and photoheterotrophic microorganisms for the second step; however, to the best of our knowledge, none of these processes is successfully used for direct electricity generation. The basis of this presentation is an extensively study of *R. sphaeroides* for an in situ application in a two-step microbial fuel cell system, as illustrated in Figure 1. We have developed an anode that allows optimum performance for the conditions of the photobiological hydrogen production. Data are presented describing substrate and light

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conversion efficiencies, fuel cell power output characteristics, and the performance of the studied anode materials.

Materials and Methods

Bacterial Growth. The *R. sphaeroides* 94.84 (DSMZ, Germany) strain maintenance was realized with the recommended DSMZ medium 27 (19) (*Rhodospirillaceae* medium). For photobioelectrochemical experiments, 3 mL of the precultured medium was inoculated into standard medium (20) containing glutamic acid as the sole nitrogen source and various organic acids as the carbon source and proton donor for hydrogen production. The cultures were grown unstirred at 32 °C in 100 mL volume rubber septum-sealed injection bottles, illuminated by tungsten lamps (40 W).

E. coli K12 was kindly provided by the Division of Applied Microbiology (Prof. Schauer), Institute of Microbiology and Molecular Biology, University of Greifswald. The aerobic cultivation was performed in standard medium containing 10 g of glucose, 5 g of yeast extract, 10 g of sodium hydrogen carbonate, and 8.5 g of sodium dihydrogen phosphate per liter. For anaerobic cultivation as a pre-step of *R. sphaeroides* photofermentation, the amount of yeast extract was reduced to 2 g/L to decrease the content of unspecified nitrogen sources. After *E. coli* fermentation, the medium was centrifuged, enriched with 7 mM glutamic acid, vitamins, and trace elements as described for the medium by Rechenberg (20), and sterilized.

For all experiments in this study, the bacterial cultures were grown in batch systems running over one cycle of life of the culture solutions, which is between 1 and 4 days depending on culture and growing conditions.

Metabolic Analysis. Metabolic substrate diminution and nongaseous fermentation product formation were followed applying HPLC analysis. The HPLC (Knauer, Berlin, Germany) was equipped with a Rezex ROA-Organic Acid column in combination with the SecurityGuard cartridge AJO-4490 (Phenomenex, Aschaffenburg, Germany). The chromatograms were recorded at a column temperature of 55 °C with 0.005 N sulfuric acid as the eluent; the detector was a differential refractometer. The quantification of the gaseous products CO₂ and H₂ was performed volumetrically, the separation of H₂ from CO₂ being achieved by absorption of CO₂ in the NaOH solution.

Electrochemical Instrumentation and Methods. Two types of electrochemical experiments have been used in this study—potentiostatically controlled and fuel cell experiments. Potentiostatic experiments allow a precise control of electrode potentials and provide the means for precise potential variation. For the potentiostatic experiments, 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 (Elektrokohle Leipzig, Germany), 19 cm² surface area, separated from the bacterial solution by a Nafion 117 perfluorinated membrane (purchased from Aldrich). Silver/silver chloride electrodes, saturated KCl, 0.195 V versus SHE (Sensortechnik Meinsberg, Germany), served as reference electrodes. All potentials given in this paper refer to the silver/silver chloride reference electrode. The experiments were carried out using PGSTAT10 and PGSTAT20 potentiostats (Ecochemie, Utrecht, Netherlands). For the current measurement, a permanent potential of 0.2 V was applied to the working electrode to in situ oxidize microbial hydrogen at a potential similar to that present under short circuit conditions in a microbial fuel cell. Control experiments confirm that varying the applied potential between 0.1 and 0.3 V did not noticeably affect the anodic current density.

Fuel cell experiments were carried out using a self-made simplified fuel cell model (Figure 2). It was composed of two

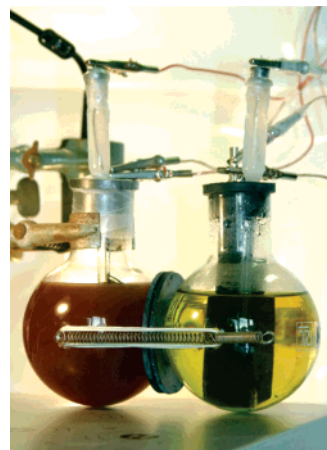


FIGURE 2. Model fuel cell for the photobiological electricity generation using the purple bacterium *Rhodospirillum rubrum*.

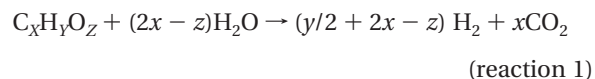
250 mL round-bottomed flasks pressed together at laterally inserted windows. A Nafion membrane (Ø 2.2 cm) was clamped between the windows separating the anode and cathode compartments. The cathode compartment was filled with 100 mM K₃[Fe(CN)₆] in 50 mM KH₂PO₄/K₂HPO₄ buffer, pH 7 ± 0.2 (15, 21). It was equipped with the cathode (graphite foil (70 mm × 30 mm × 1 mm; Chempur, Karlsruhe, Germany) wrapped by carbon cloth (NCBE Microbiology, The University of Reading, UK) or carbon rod (26 cm² surface area) and a silver/silver chloride reference electrode (Sensortechnik Meinsberg, Germany).

The anode compartment contained the bacterial solution, the anode (for preparation, see next), and a silver/silver chloride reference electrode. It was tightly sealed and was illuminated by two light bulbs (40 W tungsten) placed on its opposite sides. A common assumption is that for a conventional light bulb 10% of total energy uptake is converted to visible light—in our case, this corresponds to 8 Wh (2 bulbs with 40 W each). Taking into account the actually used geometrical solid angle, the anode chamber was illuminated with 320 mWh. Both compartments were stirred. The monitoring of redox potentials and currents under different external resistance loads was performed with a data acquisition system (Keithley Integra 2700 digital multimeter equipped with 7700 multiplexer, Keithley Instruments, Inc., Cleveland, OH) interfaced to a personal computer. The fuel cell setup was thermostatically controlled.

Via the time integral of the $I(t)$ function, and provided that the fuel cell current results exclusively from hydrogen oxidation, the electrochemical (photobiological) hydrogen yield (mol of hydrogen being electrochemically oxidized) and thus the efficiency of the microbial fuel cell can be calculated using eq 1 and formula 1

$$V_{\text{H}_2} = \frac{\int_{t_0}^{t_{\text{end}}} I dt}{zF} V_{n/32^\circ\text{C}} \quad (1)$$

where V_{H_2} is the volume of the electrochemically oxidized hydrogen, I is the current, t represents the time of the experiment, F represents the Faraday constant, z is the number of transferred electrons, and $V_{n/32^\circ\text{C}}$ is the molar volume of ideal gases at 32 °C,



where eq 1 follows from the Faraday law and reaction 1 describes the maximum amount of hydrogen achievable by full substrate degradation.

From the amount of oxidized hydrogen calculated from the integration of the current curves over time and by using eq 1, and from the calculated theoretical hydrogen amounts (Table 1), the substrate conversion efficiency can be determined following eq 2.

$$\text{substrate conversion efficiency} = \frac{Q_{\text{real}}}{Q_{\text{theor.}}} \approx \frac{V_{\text{H}_2, \text{oxidized}}}{V_{\text{H}_2, \text{theoretical}}} \quad (2)$$

It has to be distinguished between the gross conversion efficiency and the net conversion efficiency. The gross conversion efficiency is related to the total amount of substrate (C_0), while the net conversion efficiency gives the hydrogen yield referring to the amount of actually depleted substrate.

Short circuit experiments are usually performed to determine the maximum substrate conversion efficiency of the microbial fuel cell. Energy related efficiencies, however, are determined using defined external loads allowing a defined cell potential to establish. The resulting power analysis (polarization curve) enables the determination of the optimum resistance for a maximum power output.

Electrode Preparation. For anode preparation, platinum mesh electrodes (100 cm² geometrical surface), platinum sheet electrodes (3.2 cm²), or paraffin impregnated graphite electrodes, PIGE (3.65 cm² surface area), were 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 coated with conductive polymers (e.g., polyaniline or poly(3,4-ethylenedioxythiophene) (PEDOT)) using oxidative electropolymerization. Polyaniline was polymerized from a stirred solution of 100 mM aniline in 100 mM H₂SO₄ by potential cycling between -0.1 and 1.2 V at a scan rate of 50 mV s⁻¹. The preparation of the fluoro-substituted form, poly(2,3,5,6-tetrafluoroaniline), has been described before (22). Poly(3,4-ethylenedioxythiophene) was synthesized from an aqueous microemulsion containing 50 mM 3,4-ethylenedioxythiophene (Aldrich), 30 mM polyoxyethylene-10-lauryl ether (Sigma), and 0.5 M LiClO₄ as described by Tsakova et al. (23). A constant polymerization potential of 0.8 to 0.95 V (depending on electrode surface) was applied for a period of 100 s. Higher polymerization potentials enhance the polymerization process; however, they lead to the decrease of the redox activity of the polymer due to overoxidation (23).

Results and Discussion

Anode Design and Performance. In previous studies, we have demonstrated that platinum-polyaniline (Pt-PANI) and platinum-polyfluoroaniline (Pt-PFA) bilayer composites efficiently catalyze the in situ oxidation of biohydrogen from dark fermentation (15) and photosynthesis (24). These anode materials, however, appear to be unsuitable for the application of purple bacteria like *R. sphaeroides*. In experiments involving these materials, the performance of the electrodes and of the microbial hydrogen synthesis itself collapses after only 5–10 h of fuel cell operation. Visual control of the electrode surface revealed the degradation of the polymer, explaining the loss of the electrode performance. A possible explanation could be the degeneration of the polymer layer by the bacteria or by their fermentation products. Such polymer degradation by purple bacteria has not yet been reported in the literature; however, growth experiments give evidence that *R. sphaeroides* is able to grow on small amounts of aniline (2 mM) as the sole nitrogen source. Higher concentrations (5 mM) of aniline seemed to be toxic. An aniline utilization by purple bacteria has not been observed so far, but it has, however, been well-investigated for a number of other bacteria (*Burkholderia*

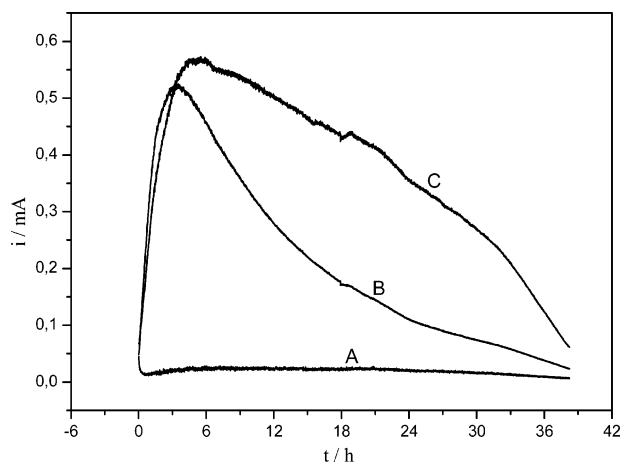


FIGURE 3. Photobiological fuel cell experiment (external load 100 Ω) with three differently modified graphite electrodes. (A) Graphite-PEDOT; (B) Graphite-Pt-PANI; and (C) Graphite-Pt-PEDOT. *R. sphaeroides* was grown in 40 mM lactate in the anode compartment. The cathode was a graphite rod (26 cm²); the experiment was performed under batch conditions.

spec., *Pseudomonas* spec.). The formation of ammonium would explain the decreasing hydrogen production, as ammonium utilization is a well-known competitive process to nitrogenase activity (25). The clarification of the pathway of the aniline degradation (simple deamination, oxidative deamination to catechol, and metabolization, etc.) requires further investigation, which, however, was beyond the scope of this study.

To overcome the problem of the polymer degradation, a polymer had to be found that could not serve as a nitrogen source. This succeeded with the application of the sulfur based PEDOT, poly(3,4-ethylenedioxythiophene). Figure 3 shows the current generation at differently modified graphite electrodes in a photobiological fuel cell experiment. The experiment was performed under batch conditions, leading to the almost complete decrease of the current after 36 h due to substrate depletion and a decreasing penetration depth of the light into the anode chamber caused by increased cell density. Curves B and C represent graphite electrodes covered by Pt-PANI (B) and Pt-PEDOT (C). At the beginning of the experiment, both electrodes show a very similar activity toward hydrogen oxidation. After approximately 6 h, the activity of the polyaniline coated electrode (Figure 3B) decreases, while the performance of Pt-PEDOT (curve C) remains stable. In contrast to polyaniline (15, 22), no catalytic activity was observed for an electrode with PEDOT coating alone (curve A). Generally, Pt-PEDOT modified electrodes were allowed to perform experiments for a long period of time (up to several days) and for multiple applications without a decreasing electrode activity.

As proposed before (15), the anode performance can be improved by applying a regenerative potential program that retains the long-term catalytic activity of the electrocatalyst. The procedure may either be based on an oxidative removal of chemisorbed species from the electrode by applying short oxidative potential pulses (26). Alternatively, it can be achieved by reduction—by means of the reducing power of the hydrogen dissolved in the anaerobic culture solution (16). For the here described experimental conditions, the oxidative reactivation was found to be superior to the reductive method.

In Figure 4, the effect of an electrode regeneration procedure is demonstrated. To evaluate the anode performance separate from the performance of the biocatalyst, the anode size was small as compared to the volume of the anolyte (21.3 cm² L⁻¹), which contained the substrate and the biocatalyst. The right y-axis refers to the current normalized

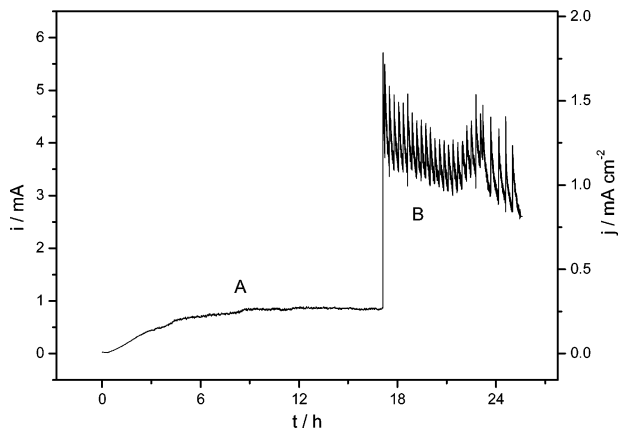


FIGURE 4. Current generation at a Pt-PEDOT electrode (size: 3.2 cm^2) in a freshly inoculated anaerobic solution containing *R. sphaeroides* and 40 mM lactate as the carbon and proton source. The experiment was performed under illumination and under potentiostatic control: cell volume = 150 mL and $\vartheta = 32 \text{ }^\circ\text{C}$. (A) Constant potential (0.2 V) and (B) constant potential (0.2 V) with regular regenerative potential pulses (1 V , 10 s , every 1000 s).

to the surface area of the electrode. In further sections of this paper, we also refer to data normalized to the volume of the anolyte solution. These data are based on electrode surface to reactor volume ratios of $400 \text{ cm}^2 \text{ L}^{-1}$ (electrode surface area 100 cm^2) that give a better picture of the overall performance of the biological fuel cell and of the microbial cultures. A combination of both data sets can help evaluate fuel cell design parameters such as the optimum ratio of electrode size and anolyte volume.

Section A, Figure 4, illustrates the current generation under constant potential conditions ($E_a = 0.2 \text{ V}$). The current density reaches $270 \mu\text{A cm}^{-2}$, a value that lies considerably under that expected for hydrogen saturated solutions—showing that the electrocatalyst does not reach its full catalytic activity. By means of a regular application of oxidative potential pulses, the current density can be increased to 1.2 – 1.5 mA cm^{-2} (section B, Figure 4). These current densities are expected as they correspond to data that are achieved in chronoamperometric measurements at platinum electrodes immersed in stirred, hydrogen saturated buffer solutions.

Photobiological Fuel Cell Experiments Utilizing Synthetic Fermentation Media. One major disadvantage of using dark fermentation for electricity generation via biohydrogen is the limited hydrogen yield. Large fractions of chemical energy remain unexploited in the form of volatile organic acids and alcohols. As these compounds are the favorite substrates of purple bacteria, it should be desirable and feasible to combine dark fermentation and photofermentation in two-step microbial fuel cell systems to achieve high Coulombic yields combined with a high substrate utilization efficiency. For this purpose, we studied the possibility of using the products of the mixed acid fermentation of *E. coli* for a photobioelectrochemical electricity generation by *R. sphaeroides*. To study the basic biological and electrochemical parameters and to identify the bottlenecks of our photobioelectrochemical fuel cell, we performed experiments with synthetic substrates containing the main constituents of the mixed acid fermentation of glucose by *E. coli*. In Figure 5, results of a fuel cell experiment are presented that was recorded under short circuit conditions. For the experiment, *R. sphaeroides* cells were grown on a synthetic fermentation medium containing lactate, acetate, formate, succinate, and ethanol—with individual concentrations of 7 mM —as the carbon and proton source. A summary of the maximum hydrogen yields for the supplied as well as for the actually utilized substrate amounts is given in Table 1. A Pt-PEDOT

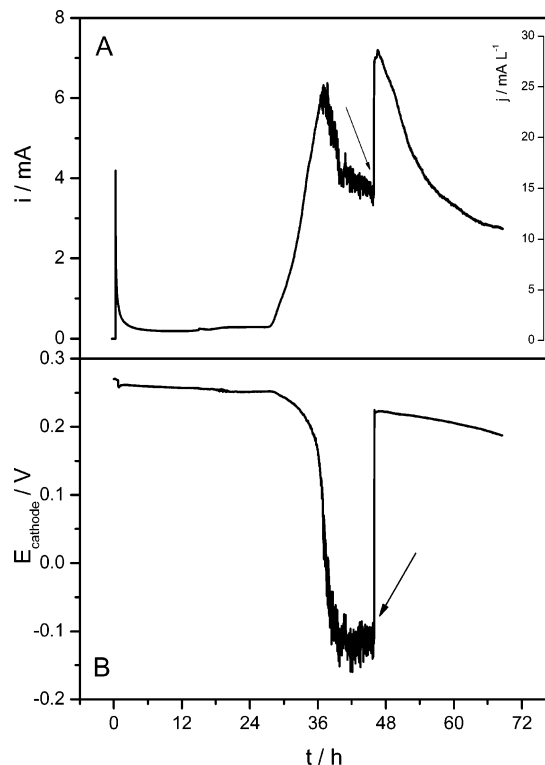


FIGURE 5. Photobiological fuel cell experiment. Biocatalyst: *R. sphaeroides*. Synthetic fermentation medium containing succinate, lactate, formate, acetate, and ethanol (individual concentrations: 7 mM). Anode: Pt-PEDOT modified mesh electrode, 100 cm^2 surface area. Cathode: carbon cloth electrode, 3.8 cm^2 surface area, placed in front of the Nafion membrane window. The arrows indicate the insertion of a second graphite rod cathode (26 cm^2 surface area) into the cathodic compartment. Panel A depicts the short circuit current recorded during the experiment (right axis: current per culture volume). Panel B: cathode potential referring to Ag/AgCl. The experiment was performed under batch conditions.

TABLE 1. Theoretical Amounts of Molecular Hydrogen, Achievable by Total Substrate Degradation (According to Reaction 1) Calculated for the Total Substrate Concentration and for Amount of Utilized Substrate

substrate ^a	hydrogen (mmol)	hydrogen (mL L^{-1})	substrate		
			utilization (mM^b)	hydrogen (mmol)	hydrogen (mL L^{-1})
lactate	42	1000	4.5	27.1	675
succinate	49	1225	2.4	16.7	420
acetate	28	700	6.1	24.2	605
formate	7	175	c		
ethanol	42	1000	0.6	3.7	90
sum		4100	sum		1790

^a The initial substrate concentrations were 7 mM for all substrates.

^b Determined via HPLC analysis of the metabolic products. ^c No net depletion—formate is a substrate of fermentation as well as a product of fermentation of other substrates.

modified mesh electrode with a geometrical surface of 100 cm^2 area served as the anode. The large anode size was chosen to maximize the current generation. About 28 h after inoculation, the hydrogen production and thus the current flow began. As illustrated in Figure 5B, the current increase is accompanied by a steadily increasing drop of the cathode potential. After the potential decreased from 250 mV to approximately 50 mV , the short circuit current stopped increasing at 6.2 mA . The current dropped to a value of about 4 mA as the cathode potential further decreased to a potential of -125 mV . This bottleneck—the insufficient performance

TABLE 2. Summary of Experimental and Calculated Data from Different Fuel Cell Experiments, Given as Absolute Values and Relative to the Culture Volume

expt	max. current (mA/mA L ⁻¹)	Coulombic yield (C/C L ⁻¹)	hydrogen yield (mM/ mM L ⁻¹)	hydrogen yield (mL/mL L ⁻¹)	substrate conversion efficiency (%) (gross/net ^a)	energy output (mWh)	light conversion efficiency (%)
1 ^b	7.2/28.8	600/2400	3.1/12.4	77.8/311	8.4/17.4	c	c
2 ^d	3.6/14.4	355/1420	1.8/7.4	46.2/185	5.1/13.3	27	8.4
3 ^e	5.9/23.6	525/2100	2.7/10.9	68.1/272	5.1/6.4	c	c

^a Gross conversion efficiency: related to input amount of substrate and net conversion efficiency: related to the amount of utilized substrate. ^b Data from the short circuit fuel cell experiment presented in Figure 5. ^c Short circuit conditions—no calculation of electric energy output. ^d Fuel cell experiment similar to experiment 1 but using 7 mM lactate and succinate and 14 mM acetate; resistance: 100 Ω. ^e Data experiment Figure 7.

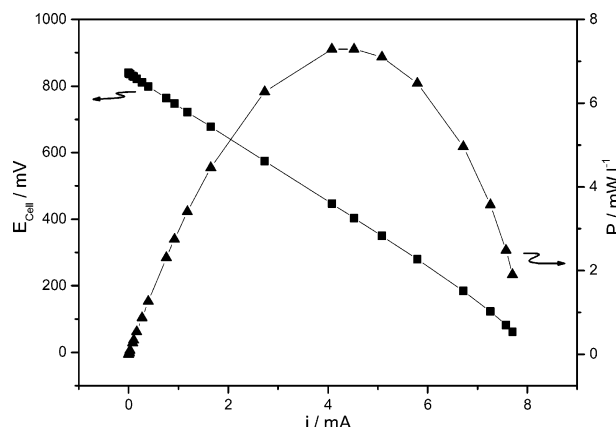


FIGURE 6. Fuel cell power analysis: independent potential and current measurement at variable resistances between 0 and 1000 kΩ in a 250 mL volume fuel cell with *R. sphaeroides* growing in 40 mM lactate. A Pt–PEDOT modified mesh electrode (100 cm² surface area) served as the anode, and the cathode was a graphite rod (26 cm²). The left axis shows the cell potential as a function of the current, and the right axis represents the resulting power per volume of microbial culture.

of the fuel cell cathode, could not be eliminated by the increase of the catholyte concentration; however, the increase of the size of the cathodic surface area by insertion of a second cathode (graphite rod) immediately stabilized the potential at about 220 mV—leading to the recovery of the current flow with a maximum value of 7.2 mA. At this point, it becomes obvious that even at the comparably low current densities of microbial fuel cells a careful dimensioning of the fuel cell setup has to be taken into account. In this case, the insufficient current density at the fuel cell cathode required a larger cathode surface area to compensate for the low performance.

A summary of the experimental results and of the calculated data for the experiment presented in Figure 5 is given in Table 2, line 1. As it can be seen, a gross conversion efficiency of 8.35% and a net conversion efficiency of 17.4% were achieved. This differentiation is not usual in the literature dealing with photobiological hydrogen production. Generally, the comparison of substrate conversion efficiencies of purple bacteria with literature values is difficult since conversion efficiencies, if given at all, are based on very different standards of determination. However, the literature values for different strains of purple bacteria, substrates, and illumination energies range from below 10 up to 80% (27).

In Figure 6, the polarization experiment for a photobiological fuel cell based on *R. sphaeroides* is illustrated. For the described fuel cell setup, the maximum power of 7.3 mW L⁻¹ was achieved using an external load of 100 Ω. At very high external loads, the current flow and thus the associated power output tend to zero, whereas under short circuit conditions (with the fuel utilization approaching a value of 1), the cell potential (and thus the power output) collapses.

Line 2, Table 2, represents the data of an experiment similar to the short circuit experiment illustrated in Figure

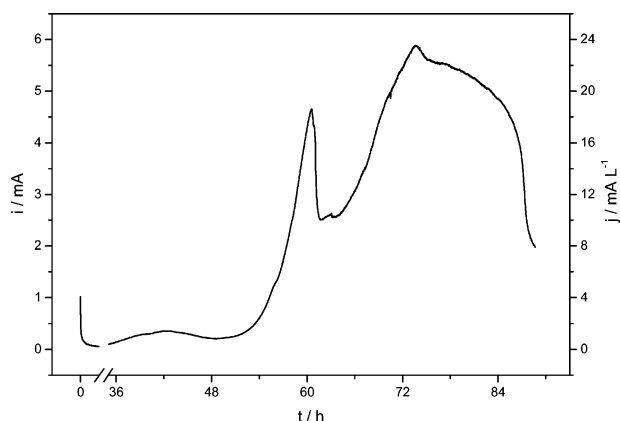


FIGURE 7. Current generation (short circuit) in a photobiological fuel cell experiment. Biocatalyst: *R. sphaeroides*. Pre-fermented *E. coli* medium (for composition, see Table 3). Anode: Pt–PEDOT modified mesh electrode, 100 cm² surface area. Right axis: current normalized to culture volume. The experiment was performed under batch conditions; the cathode was a graphite rod (26 cm²).

TABLE 3. Metabolite Concentrations after Dark Fermentation and Photofermentation in a Two-Step MFC Experiment^a

secondary substrate ^{b,c}	end products ^{b,c}
16.7 mM lactate	7.9 mM acetate
14.3 mM acetate	2.7 mM succinate
2.4 mM succinate	15.1 mM ethanol
25.8 mM ethanol	32.4 mM <i>n</i> -butyrate
6.7 mM <i>n</i> -butyrate	
formate	
glucose	
22.8 mmol/L hydrogen	10.9 mmol/L hydrogen

^a The primary substrate (glucose, 55 mM) was fermented by *E. coli* K12. After sterilization, the fermentation products were used as substrates for photofermentation by *R. sphaeroides*. ^b Nongaseous products were analyzed via HPLC analysis. ^c Hydrogen was determined from the electric charge of the fuel cell experiment using eq 1.

5 but using a constant external load of 100 Ω. From the resulting energy output of 27 mWh, the effective light conversion efficiency can be calculated. The resulting value of 8.5% is an excellent result keeping in mind the common hypothesis that a value of 10% total solar efficiency is the maximum for photobiological hydrogen production (28). Photobiological fuel cell experiments utilizing fermented *E. coli* media.

In a two-step experiment, the primary substrate glucose, 10 g L⁻¹ (55 mM), was decomposed by *E. coli* K12 via mixed acid fermentation. The nongaseous fermentation products were then used as the secondary substrate for photofermentation by *R. sphaeroides*. The corresponding current curve and the experimental data are presented in Figure 7, in Table 2, line 3, and in Table 3.

Growing on the fermentation medium, *Rhodobacter* required a considerably longer lag-time to adapt to the new

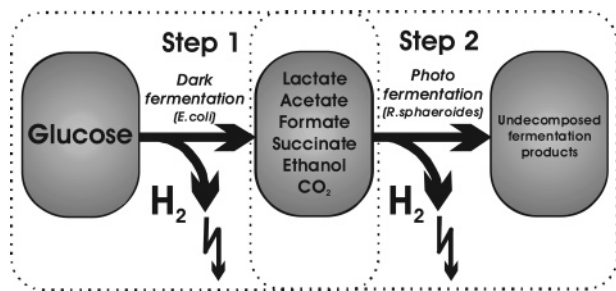


FIGURE 8. Schematic illustration of the substrate conversion in the two-step MFC process presented in this paper.

substrate conditions. While during the first step, the dark fermentation, 22.8 mmol L⁻¹ hydrogen, was produced by *E. coli*, the second step, photofermentation, yielded 10.9 mmol L⁻¹ hydrogen. Thus, although under the experimental conditions the organic acids were not completely utilized, the hydrogen yield from the primary substrate glucose was increased by nearly 50%. The most efficient substrate utilization was observed for lactate (complete depletion), ethanol (10.8 mmol L⁻¹), and acetate (6.4 mmol L⁻¹). Succinate meanwhile was accumulated, probably due to digestion of residual amounts of glucose in the medium. Although sufficient substrate was still left in the medium, the hydrogen production decreased clearly after about 80 h. This may be due to a lack of nitrogen after the long duration of the experiment. A more complete substrate degradation and thus increased electric yield and conversion efficiency can probably be achieved under optimized experimental conditions (e.g., in flat panel cells and under semi-batch or continuous flow conditions).

The experimental results of our study demonstrate that phototrophic hydrogen producing microorganisms such as the purple bacterium *R. sphaeroides* can be used for electricity generation and substrate decomposition in a photobiological fuel cell. As schematically depicted in Figure 8, such a biofuel cell can increase the efficiency and the yield of microbial fuel cells when used in two-step microbial fuel cell systems. However, to exploit the potential of such a system, further studies have to be focused on the improvement of the fuel cell efficiency. One emphasis is the enhancement of the dark fermentative and photofermentative hydrogen yield by using more efficient biocatalysts (e.g., bacterial consortia (29) and purple bacteria (20)). Further steps will be the examination of realistic substrates as wastewater and under the conditions of natural sun light illumination.

Acknowledgments

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