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Short communication

Hydrogen photosynthesis by *Rhodobacter capsulatus* and its coupling to a PEM fuel cell

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Abstract

Four different mutant strains of *Rhodobacter capsulatus* (IR1, IR3, IR4 and JP91), a photosynthetic purple non-sulfur bacterium, were tested for their ability to produce hydrogen in a 3 L volume photobioreactor coupled to a small PEM fuel cell. The four mutants, together with the wild-type strain, B10, were grown at 30 °C under illumination with 30 mmol L^{-1} DL-lactate and 5 mmol L^{-1} L-glutamate as carbon and nitrogen source, respectively. Bacterial growth was measured by monitoring the increase in absorbance at 660 nm, and hydrogen yield, and substrate conversion efficiency were measured under the same conditions. The hydrogen production capability of the five strains was then compared and shown to be in the order: IR3 > JP91 > IR4 > B10 > IR1. The most preferment strain, IR3, showed a substrate conversion efficiency of 84.8% and a hydrogen yield of 3.9 L L^{-1} of culture. The biogas produced by these photobioreactor cultures was successfully used as feed for a small PEM fuel cell system, with the mutant IR3 showing the most sustained hydrogen and current production. The maximum current was similar to that obtained using pure hydrogen produced by a small electrolysis cell (High-Tec Inc.).

Keywords: Photoproduction; Hydrogen; *Rhodobacter capsulatus*; Lactate; PEM fuel cell

1. Introduction

Hydrogen is a clean and efficient fuel, considered as a potential and more sustainable energy substitute for fossil fuels. It has been predicted that the contribution of hydrogen to global energy consumption will increase dramatically, to approximately 50%, by the end of the 21st century due to the development of efficient end-use technologies, possibly becoming the main final energy carrier. Also, it is undoubted that hydrogen will play a strategic role in the pursuit of a low-emission energy source for environmental demand [1,2].

To this end, it will be necessary for hydrogen to be produced renewably and on a large scale. The global hydrogen production system, initially fossil-fuel based, is shifting progressively toward renewable sources. The following technologies for the conversion of secondary and primary fuels into hydrogen are being investigated extensively: electrolysis, coal gasification, steam methane reforming of natural gas, partial oxidation of fuel oil, solar thermal cracking, biomass gasification and photobiological synthesis [1–5]. Biological hydrogen production stands out as an environmentally harmless process carried out under mild operating conditions with renewable resources. Currently, much research on hydrogen production is carried out with laboratory-scale or pilot-scale

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reactors using photosynthetic microorganisms [3–11]. Phototrophic purple non-sulfur bacteria, such as *Rhodobacter capsulatus*, are commonly utilized for hydrogen production from various carbon sources [12–20]. However, the production rate and the yield vary greatly depending on the carbon source used and the experimental, physiological conditions, such as light intensity or pH [15,21]. On the other hand, several studies have shown that mutant strains can be isolated and show improved hydrogen producing capabilities compared to the wild-type [22,23].

Four different mutants of *R. capsulatus* (IR1, IR3, IR4 and JP91), isolated in previous studies, as well as the wild-type strain, B10, were checked for photohydrogen production in a large culture volume (3 L) with 30 mmol L^{-1} DL-lactate provided as the carbon source and 5 mmol L^{-1} L-glutamate as the nitrogen source. The growth characteristics of these five strains were determined by monitoring the absorbance of the cultures at 660 nm and calculating the cell dry weight. The cultures were incubated at 30 °C and illuminated by two 120 W incandescent lamps placed at a distance of 1 m. The hydrogen yield and substrate conversion efficiency of each strain were measured and used to compare the hydrogen production capabilities of these four mutants and the wild-type B10.

We also checked the practicability of coupling the photohydrogen produced these bacterial cultures to the operation of a fuel cell. It is well known that fuel cells have significant potential to become an important element of the portfolio of options to meet ever-increasing demands for energy services while responding to more stringent reliability and power quality standards, mounting environmental constraints, cost-effectiveness pressures and other challenges that energy systems will face in the future [24]. In the present work, a small polymer electrolyte membrane fuel cell (PEMFC) was selected for further evaluation. Hydrogen was applied without purification and generated an efficient current response, indicating the potential of this system for future applications.

2. Experimental

Five strains of *R. capsulatus*, B10 (wild-type), IR1, IR3, IR4 and JP91, were tested in this study. The preparation of these mutants has been described before [22,23]. Precultures were grown photosynthetically at 30–32 °C in a mineral salts (RCV) medium supplemented with 30 mmol L^{-1} DL-malate and 7.5 mmol L^{-1} $(NH_4)_2SO_4$ as described previously [22,23]. The culture for absorbance measurements and the photohydrogen production contained 30 mmol L^{-1} DL-lactate as carbon source and 5 mmol L^{-1} L-glutamate as nitrogen source. The medium was autoclaved (120 min, 120 °C, 1.2 bar) before use. Rubber-stoppered glass bottles of 10 mL volume were used for cell growth of different strains of *R. capsulatus*. A water-jacketed glass reactor of 3.5 L liquid volume was used for hydrogen production. The volume of culture was 3 L. The schematic figure of the experimental setup is shown in Fig. 1. The temperature of the photobioreactor was controlled at 30 °C in a glass-sided water bath. Illumination was provided by two 120 W incandescent lamps placed at a distance of 1 m. To initiate growth of the culture, 20–30 mL pre-culture was inoculated into the bioreactor.

The flow rates of photohydrogen produced by the photobioreactor were measured with a mass flow controller coupled to a digital multi-meter, which was connected via RS232C to a compatible PC. The yields of biogas were determined by integrating the curves of flow rates against time. The bacterial cell concentration was determined spectrophotometrically, it was found that an absorbance at 660 nm of 1.0 is equivalent to a cell density of 0.45 g dry weight L^{-1} culture under our experimental conditions.

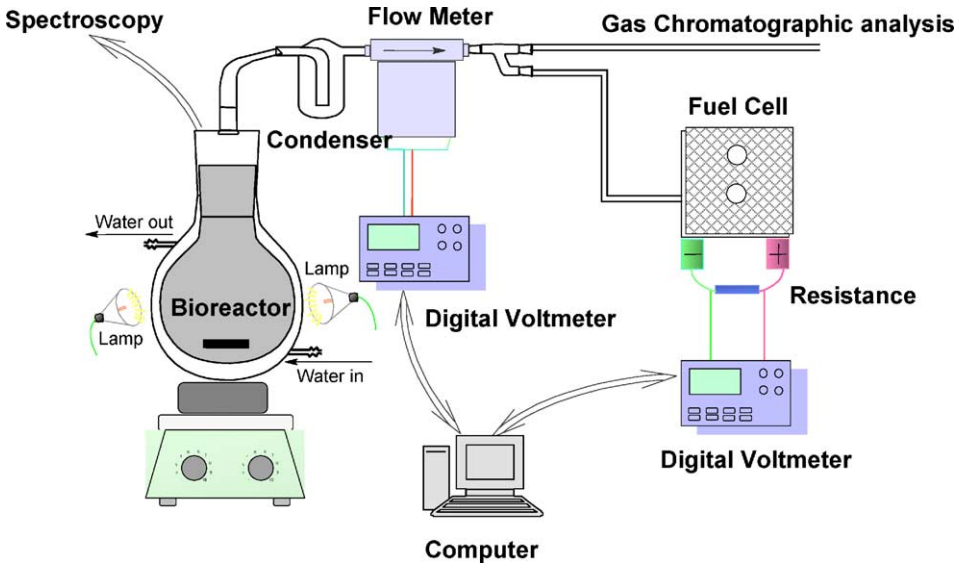

Fig. 1. Schematic diagram of the photohydrogen production and application system by *R. capsulatus*.

The composition of the evolved biogas was determined by gas chromatography. The GC (HP6890) was equipped with a thermal conductivity detector and PORAPAK Q and a molecular sieve heated at 80 °C, which enabled the separation of CO_2, H_2, O_2, N_2 and H_2O. The pH of the culture medium was measured with a standard combination pH electrode connected to a GLP21 pH meter.

The small PEMFC (PEMFC-KIT, ref. 1919, High-Tec Inc.) was fed directly with the biogas produced by different strains of *R. capsulatus*. The membrane electrode assembly has a surface area of 16 cm^2 and works under open outlet mode running on a constant load. Air was used for the cathodic reaction; a 10 Ω resistance was used as the load of the PEM fuel cell. The current curves were measured by HP3478A multi-meter connected to a PC via IEEE 488.

3. Results and discussion

3.1. The growth characteristics of different strains

The absorbance (A_{660}) of cultures was measured at 660 nm so as to monitor bacterial growth. The cell dry weight (CDW, g L^{-1}) of bacteria in the culture is proportional to the absorbance at 660 nm and thus can be calculated according to the following relationship:

$$\mathrm{CDW_{bacteria}} = 0.45A_{660} \quad (1)$$

The growth curves of different strains in 3 L cultures are shown in Fig. 2. During the first 24 h, strains JP91, IR4 and IR1 grew more rapidly than strain B10. These results agree well with the results obtained in smaller volume culture (55 mL) [23]. However, it can be seen that strain IR3 grew more slowly than other strains, although after 40 h the growth rates of the five strains were very similar.

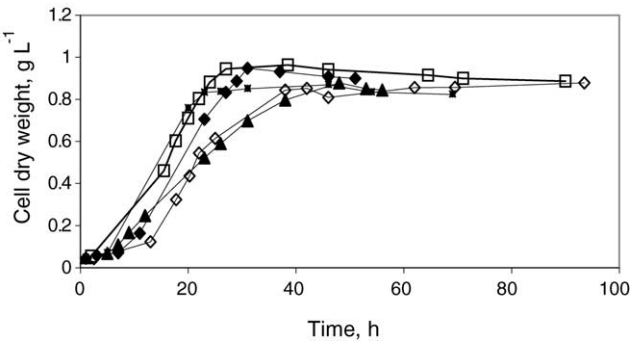

Fig. 2. Bacterial growth kinetic of *R. capsulatus* strains (B10, IR1, JP91, IR3 and IR4) in 3 L culture (30 °C, pH 6.8). 30 mmol L^{-1} DL-lactate and 5 mmol L^{-1} L-glutamate were used as carbon source and nitrogen source, respectively. (◊) corresponds to B10; (□) IR1; (▲) IR3; (♦) IR4 and (■) JP91.

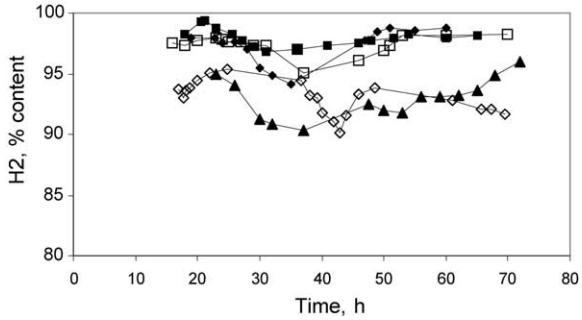

Fig. 3. Variations of the relative content of hydrogen in the biogas produced from different strains: (◊) corresponds to B10; (□) IR1; (▲) IR3; (♦) IR4 and (■) JP91.

3.2. Composition of the biogas produced by different strains

Phototrophic purple bacteria can convert carbon substrates, such as Lactate, into H_2 and CO_2, using light as energy source [25,26]. The composition of the biogas produced by the five strains under the same experimental conditions was determined by gas chromatography. Fig. 3 shows the variations of the content of hydrogen during the cultivation in a batch experiment. It can be seen that the content varies with the growth of bacteria. Overall, the relative contents of hydrogen in the biogas (H_2 + CO_2) of strains JP91, IR1 and IR4 were higher than those of strains B10 and IR3, the highest value being observed with strain JP91. The average values for the different strains are listed in Table 1.

3.3. Kinetics of photohydrogen production

R. capsulatus grew well in the chosen culture medium under photosynthetic conditions and the quantity of bacteria increased during cultivation. Hydrogen production began once the bacterial concentration reached a threshold value. However, the bacterial growth time and the kinetics of hydrogen photoproduction varied from strain to strain. Hydrogen flow rates of five strains were monitored by the flow controller. It can be seen that initially, for the first 8 h, no hydrogen was produced, although the bacterial strains were growing in the culture. After this lag period, hydrogen was produced rapidly, and the maximum flow rate was reached after about 30 h. The maximum hydrogen production rates

Table 1
Average relative contents of hydrogen in biogas produced from different *R. capsulatus* strains

Strain	Average relative content of hydrogen (%)
B10	93.0 ± 1.4
IR1	97.4 ± 0.8
IR3	93.2 ± 1.8
IR4	97.3 ± 1.5
JP91	98.0 ± 0.7

differed greatly between strains, being 0.60, 0.57, 0.97, 0.77 and 0.71 mL min^{-1}, for strains B10, IR1, IR3, IR4 and JP91, respectively. After this peak, the hydrogen production rates decreased with time, presumably due to the consumption of substrate in culture batch. Nevertheless, average H_2 production rates could be calculated by integration of the curves, and were found to be 18.6, 14.7, 34.4, 20.6 and 23.6 mL h^{-1} L^{-1} culture for strains B10, IR1, IR3, IR4 and JP91, respectively.

3.4. Hydrogen production yields and substrate conversion efficiency

The yields of hydrogen production reflect the capability of different bacterial strains to convert the carbon substrate into biogas. The yields of hydrogen were obtained by integrating the time courses of flow rates for the five strains. Another useful parameter for characterizing microbiological hydrogen production is the substrate conversion efficiency, which is the ratio of the actual amount of hydrogen evolved to the amount expected through stoichiometric conversion of the substrate. For lactate, which is the primary carbon substrate used in this study, 6 mol of hydrogen are expected to be produced per mole of lactate utilized according to Eq. (2):

$$C_3H_6O_3 + 3H_2O \rightarrow 6H_2 + 3CO_2 \quad (2)$$

Therefore the substrate conversion efficiency (η) can be calculated as a percentage of the theoretical maximum for the complete conversion of lactate and glutamate to H_2 and CO_2 from the Eq. (3) [1]:

$$\eta = \frac{V}{6(24.47M_0)} \times 100\% \quad (3)$$

where V is the volume of H_2 in L, 24.47 is the volume (L) of H_2 at Standard Laboratory Conditions (25 °C, 1 atm) and M_0 is the initial concentration of lactate.

Table 2 gives the yields and the substrate conversion efficiencies for all the strains. It is observed that the yields ranged between 1.82 and 3.93 L, the values of the substrate conversion efficiencies between 44.6% and 84.8%. In both cases, the highest values were observed for strain IR3 and the lowest for strain IR1. These parameters for strain IR3 were 80–90% higher than for the wild-type, confirming the enhanced hydrogen producing capability of the mutant strain under our experimental conditions.

Table 2
Hydrogen yields and substrate conversion efficiency of *R. capsulatus* strains in 3 L photobioreactor culture (30 °C, pH 6.8)

Strain	Hydrogen yield (L)	Substrate conversion efficiency (η) in (%)
B10	2.16	44.6
IR1	1.83	40.6
IR3	3.93	84.8
IR4	2.36	52.5
JP91	2.53	56.2

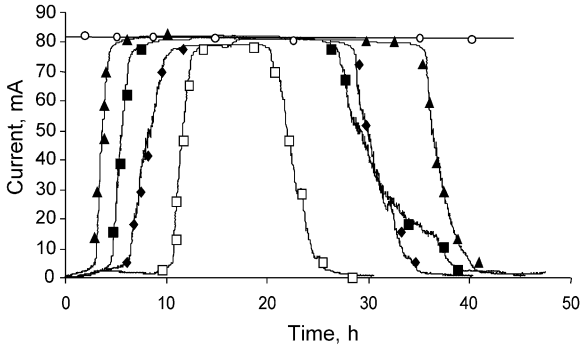

Fig. 4. Current vs. time response of a PEMFC running on a constant load at a cell potential of about 0.8 V feed by photohydrogen produced from strains JP91, IR1, IR3 and IR4 in 3 L culture (30 °C, pH 6.8). 30 mmol L^{-1} DL-lactate and 5 mmol L^{-1} L-glutamate were used as carbon source and nitrogen source, respectively. Two 120 W incandescent lamps placed at a distance of 100 cm were utilized as light source. (◯) corresponds to a small electrolysis cell; (□) IR1; (▲) IR3; (♦) IR4 and (■) JP91.

3.5. The current response of the small PEM fuel cell

The biogas produced by different strains of *R. capsulatus* was directly introduced in a small PEM fuel cell, without prior purification. The current curves obtained with B10, JP91, IR1, IR3 and IR4 bacterial cultures associated with PEMFC are illustrated in Fig. 4. After a period of no H_2 production, which depended of the bacterial strain, the current rapidly increased due to the production of H_2. The maximal current value, closed to 80 mA for an electrode area of approximately 16 cm^2, was similar to the value obtained with pure H_2 produced by water electrolysis (81 mA) under the same experimental conditions. At this working point, the cell potential was about 0.8 V. The hydrogen utilization in the PEMFC, which is the ratio of the hydrogen consumed by the fuel cell to the inlet flow rate of hydrogen, could be estimated from the current by using Faraday's law and the hydrogen production rate of the bioreactor. Ratios larger than 50% were obtained, indicating that a large quantity of hydrogen was converted into electricity by the PEMFC.

The delay for obtaining the maximal current via PEMFC depended of the bacterial strains. So, *R. capsulatus* IR3 exhibited the best efficiency in terms of hydrogen production and delay for obtaining the maximal current. At the opposite, *R. capsulatus* IR1 revealed the worst efficiency. The maximal current was maintained during a period of 11–32 h, depending to the bacterial strains.

These results showed that an efficient conversion of hydrogen into electricity can be performed over a long time period using a batch photobioreactor. The excellent performance of the PEM fuel cell was due to the low CO_2 content of the biogas. The influence of carbon dioxide on PEM fuel cell performance remains rather small and has been demonstrated previously [27,28].

4. Conclusions

The various strains used in this study grow well photosynthetically in culture [22,23] medium provided with DL-lactate and L-glutamate and produce hydrogen at high rates. Summing up the above results, the order of the hydrogen production capability, the H_2 production rate and the substrate conversion efficiency were IR3 > JP91 > IR4 > B10 > IR1. The relative hydrogen content of the biogas was greater than 90%, so high purity H_2 can be generated by the "*R. capsulatus* + lactate" system. The utilization of a PEM fuel cell showed that the hydrogen produced by this photobiological process could be used successfully as the gas source to provide electricity.

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