Hydrogen Production from Chlamydomonas Reinhardtii by Solar Irradiation

Andrea Nicolini
CIRIAF
University of Perugia
Via G.Duranti, 67 – 06125 Perugia, Italy
nicolini.unipg@ciriaf.it

Abstract: *Hydrogen is considered a fundamental vector for clean energy. For a large-scale use, the production of a great amount of hydrogen is economically essential. From a strictly economical point of view, it is difficult to expect in the next few decades that biologically produced hydrogen may match against chemically-synthesized hydrogen. However, hydrogen production from algae and bacteria is a topic which is interesting due to the potential practical application of the process in renewable fuel production. In order to find the better conditions to optimize this process, the influence of many different physical and physiological factors have to be considered. Microscopical green algae like Chlamydomonas reinhardtii can produce hydrogen using solar energy as energy source also if only solar radiations in the 400-700 nm wavelength range ("Photosynthetic Active Radiation"--PAR) may be used. This paper deals with an experimental investigation on hydrogen production by means of Chlamydomonas reinhardtii in different working conditions. In particular, tests were made for keeping constant the hydrogen production versus time by restoring the culture TAP medium. Different kinds of TAP medium were tested in order to determine the best one both in terms of hydrogen production and culture growth performances. Hydrogen produced by Chlamydomonas reinhardtii will be tested as fuel for a MCFC at the University of Perugia.*

Keywords: Hydrogen production; algae; solar energy; TAP restoration.

Introduction

The economical growth in the last decades was strongly dependant on fossil fuels as energy sources. These resources are limited in the long term, and environmental concerns have led to researches of clean energy sources. Fuel cells are a well-known method for energy production with low environmental impact but they need hydrogen as input fuel. In particular, for a large-scale use, the production of great quantities of hydrogen is economically essential. Photo-biological hydrogen production can be obtained by biological systems under sun irradiation. Some algae and bacteria can produce hydrogen under suitable conditions [1-2]. Algae pigments absorb solar energy; enzymes in the algal culture act as catalysts to split water into hydrogen and oxygen [3-4]. Many research activities show that the efficiency (energy produced from hydrogen divided by solar energy) of such systems can be estimated to be approximately 10%. This value has to be increased for a large scale hydrogen production. The proposed paper deals with an investigation on novel methods to increase and keep constant the hydrogen production by green algal cultures. The effect of different artificial solar irradiation conditions on hydrogen production was studied for a green algal culture: Chlamydomonas reinhardtii [5-6]. The algal cultures were kept under continuous irradiation by means of fluorescent lamps and xenon lamps in three photo-bioreactors made by aluminum reflecting wall boxes. Preliminary results showed that the highest hydrogen production occurs when cultures are grown on a TAP (Tris-acetate-phosphate) medium without Sulphur. The maximum total hydrogen production was got thanks to a xenon lamp (which simulates the solar irradiation) after 20 days. Later, hydrogen production diminishes. Besides, algae growth improves when Tap medium with Sulphur is used. Thus, these results are used in the design of a TAP restoration facility in the next experimental tests. In fact, the preliminary tests showed that TAP medium with Sulphur improves the growth of algae, while TAP medium without Sulphur improves the production of hydrogen. Two kinds of experiments were conducted to test the effect of a TAP medium restoration on the hydrogen production by Chlamydomonas reinhardtii under different kinds of light irradiation: the first one by replacing the algal suspension with TAP without sulphur; the second one by replacing the algal suspension alternatively with TAP without sulphur and TAP with sulphur. The experiment results show that the maintenance of algal cultures in constant metabolic condition through the restoration with TAP without sulphur (the first experiment) produces a constant high increase of hydrogen production with respect to a no-restoration test. Also an alternating restoration of algal suspension with TAP with and without sulphur produces a constant production of hydrogen but smaller than the one obtained by restoring only with TAP without sulphur.

1. Experimental tests on hydrogen production by Chlamydomonas reinhardtii

The cultures: Chlamydomonas reinhardtii was grown in TAP (Tris-acetate-phosphate) medium with and without sulphur. The algal cultures were grown at about 28 °C in

500 ml graduated borosilicate glass bottles; the bottles are characterized by 5 ground necks closed by plastic screw tops with openings with porous butyl-teflon septa. These septa are characterized by a low gas permeability and allow to sample the inner gas from the bottle using a syringe. The bottles were selected for the optimal capacity to transmit the solar wavelength radiation. The glass absorption is negligible in the [310-2200] nm wavelength range. Six samples were prepared (3 using TAP medium with sulphur and 3 using TAP medium without sulphur) using 200 ml of TAP and 15x106 cells of Chlamydomonas reinhardtii. The algal cultures were injected in the bottles. The headspace volume of each bottle was 435 cm^3.

The bioreactor systems: the bottles containing the algal cultures were kept under continuous artificial solar wavelength radiation in three photo-bioreactors made by aluminum reflecting wall boxes. A different type of lamp was installed in each photo-bioreactor. In particular, the following radiation sources were used in order to establish different irradiation conditions:

- a mercury vapor fluorescent lamp with a 5.600 K color temperature. This lamp is characterized by a solar distribution concentrated in the [400-630] nm wavelength range with three main emission stripes (440, 550 and 580 nm);
- a mercury vapor fluorescent lamp with a 2.700 K color temperature. This lamp is characterized by a solar distribution concentrated in the [550-650] nm wavelength range with two main emission stripes (550 and 610 nm);
- a Xenon lamp with a 6.800 K color temperature. This lamp is characterized by a spectral distribution very close to the solar one.

The distance between the lamps and the bottles containing the algal cultures were settled in order to have the same power irradiation for each photo-bioreactor.
In Figure 1 the three experimental photo-bioreactors are shown.

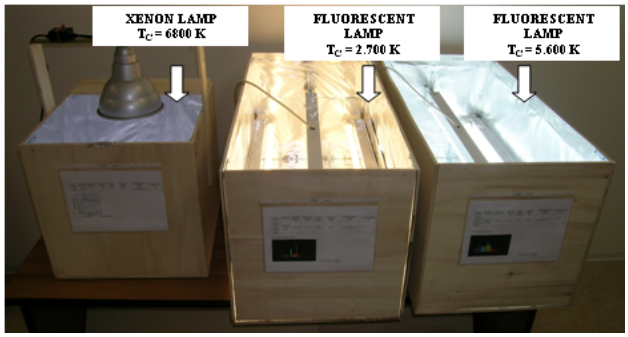

Figure 1. The experimental photobioreactors

Figure 2 shows the absorption spectrum of green algae (solid line) compared to the solar spectrum (dotted line). X-axis represents the wavelength, with the visible wavelength range showed in the left upper bar and the relative light intensity shown on the Y-axis. Thus, part of the sunlight energy is not absorbed by the green algae. For this reason, the efficiency of transformation of sunlight energy into hydrogen energy can never be 100%. The maximum absorption was obtained in the [400-500] nm and [650-700] nm wavelength ranges.

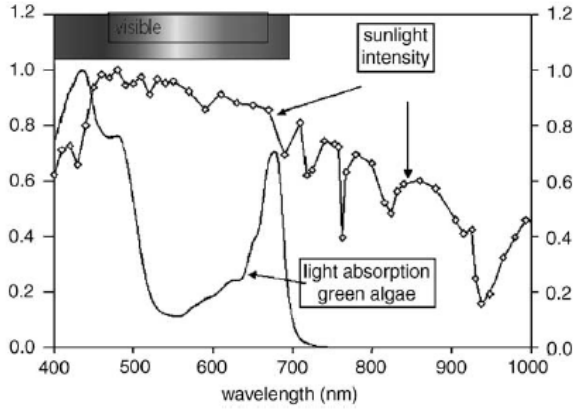

Figure 2. Sunlight intensity and light absorption by green algae

2. Measurements results

Hydrogen produced by each algal culture was measured by sampling gas from the bottle headspace with a gas tight syringe. A gas chromatograph (model CP4900, Varian) with its data analysis software (Star 6.41, Varian) was used to determine the concentration (%vol) of H_2 in the headspace of each bottle. A molecular sieve column (MS-5A) was used to separate O_2, N_2 and H_2. Signals were generated by the thermal conductivity detector of the instrument. The signals were calibrated by injection of known amounts of compounds. During the whole test period (32 days) 11 measurements were made. The first one was made for time t = 0 when the algal cultures were injected in the bottles. The total volume of the hydrogen produced at the i-th time of measurement is given by:

$$V_{iH_2 produced} = \sum_{i=1}^{n-1} x_i \cdot v_i + V_{H_2 before\ the\ analysis\ i-th}$$

Where:
n = number of measurements
x_i = %vol of hydrogen measured
v_i = volume of sampled gas

$$V_{H_2 before\ the\ analysis\ i-th} = x_i \cdot (V_{headspace} - \sum_{i=1}^{n-1} v_{i-1}) .$$

Thus, the total volume of the hydrogen produced at the i-th time of measurement may be written as follows:

$$V_{iH_2 produced} = \sum_{i=1}^{n-1} x_i \cdot v_i + x_i \cdot (V_{headspace} - \sum_{i=1}^{n-1} v_{i-1})$$

The code used for the different kinds of algal culture samples is reported in the table 1.

Table 1. Sample codification

Cultures	*Lamps - T_c (K)*		
	2700	*5600*	*6800*
Chlamydomonas reinhardtii in TAP without sulphur	A	B	C
Chlamydomonas reinhardtii in TAP with sulphur	AS	BS	CS

Figure 3 shows the results of the measurements obtained by testing the algal culture grown in a TAP medium without sulphur. Figure 4 shows the results of the measurements obtained by testing the algal culture grown in a TAP medium with sulphur.

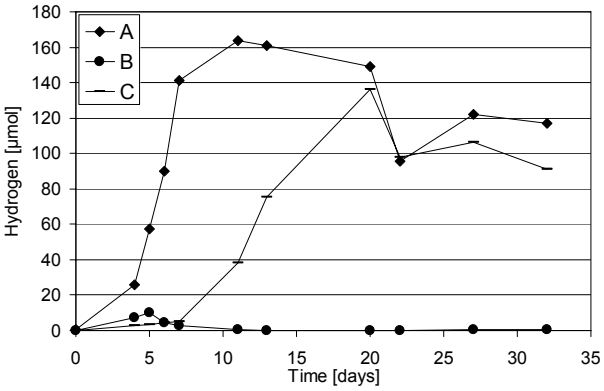

Figure 3. Total amount of the hydrogen produced by cultures in TAP without sulphur

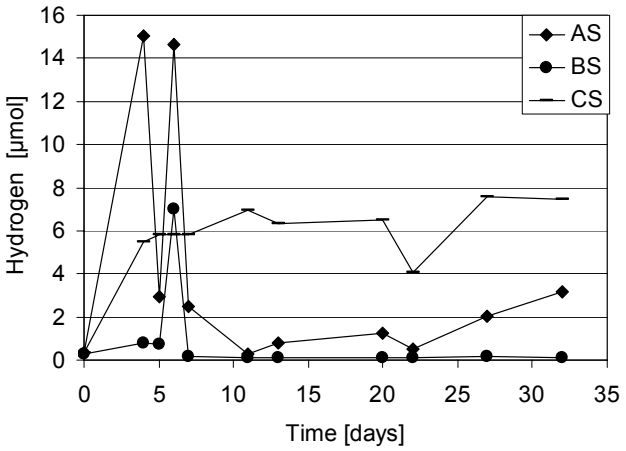

Figure 4. Total amount of the hydrogen produced by cultures in TAP with sulphur

The algal culture sample which showed the best results is the sample A that is Chlamydomonas reinhardtii in a TAP medium without sulphur under continuous irradiation with the fluorescent lamp with T_c (color temperature) = 2700 K. Also sample C (Chlamydomonas reinhardtii in TAP without sulphur under continuous irradiation with xenon lamp) gives good results in terms of hydrogen production. Sample B (Chlamydomonas reinhardtii in TAP without sulphur under continuous irradiation with the fluorescent lamp with T_c = 5600 K) gives no relevant results. The total amount of the hydrogen produced by sample A increased till the 11th day with a maximum value of 164 µmol. From the 11th till the 22nd day the total amount of the produced hydrogen decreased till 96 µmol. From the 22nd till the 32nd day the trend is unstable. The total amount of the hydrogen produced by sample C increased till the 20th day with a maximum value of 177 µmol. From the 20th till the 22nd day the total amount of the produced hydrogen decreased till 105 µmol. From the 22nd till the 32nd day the trend is unstable. Cultures in TAP with sulphur showed an oscillating trend of the total amount of the produced hydrogen during the first 11 days. In this period the maximum values of the produced hydrogen were 15 µmol for the sample AS (Chlamydomonas reinhardtii grown in TAP medium with sulphur under continuous illumination with fluorescent lamp with T_c = 2700 K) and 7 µmol in sample BS (Chlamydomonas reinhardtii grown in TAP medium with sulphur under continuous illumination with fluorescent lamp with T_c = 5600 K). Sample CS (Chlamydomonas reinhardtii grown in TAP medium with sulphur under continuous illumination with xenon lamp with T_c = 6800 K) shows an approximately increasing trend during the whole test period; the maximum value of the produced hydrogen is 7,6 µmol after 27 days. Both for algal cultures grown in TAP medium without sulphur and with sulphur, the main problem of this technique is the fact that Chlamydomonas reinhardtii grows in TAP medium for approximately 35 days; after this period, the hydrogen production is greatly reduced. Thus, a solution may be a TAP restoration which can allow the algal culture to have a continuous growth.

3. TAP restoration tests

A second experimental test was carried out to determine the effect of an alternating restoration of TAP medium with sulphur (improve the growth performance of algae) and TAP without sulphur (improves the production of hydrogen) on the hydrogen production obtained by Chlamydomonas reinhardtii. Preliminary experiments showed that it is possible to obtain the resumption of hydrogen production from the algal cultures in which a new TAP is restored in the experimental reactor. In fact, the new TAP allows to keep constant the culture metabolism and anaerobic conditions reached during the first stage of cultivation and needed for the activation of enzymes for hydrogen production. The restoration of algal cultures with new TAPs carried out after the fall of the hydrogen production allows to regenerate the culture and, consequently, to increase the production hydrogen. The reintegration of TAP medium in the culture could be combined with a stable production of hydrogen, overcoming the mentioned problem of the reduction of hydrogen production. The new experiment was carried out as follows: Chlamydomonas Reinhardtii was suspended in a TAP medium with sulphur; the cultures were grown at laboratory environmental temperature and subjected to intermittent irradiation with a xenon lamp, with a rate

comparable to the sunlight one (16 hours of light phase and 8 hours of darkness). These experimental tests were carried out only by the xenon lamp, because it gave good results in terms of hydrogen production in the previous tests and however it is the only source which may represent the solar natural irradiation.

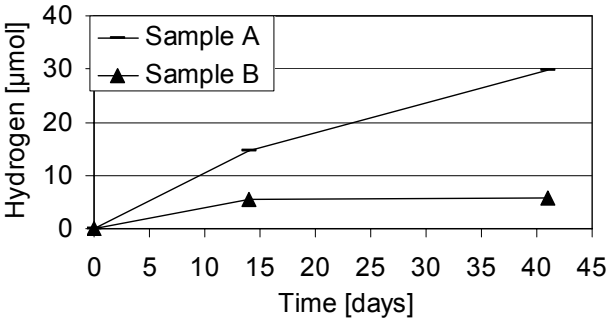

Figure 5. Total amount of hydrogen production by TAP restoration tests

Then, tests continued as follows: 150 ml of algal suspension were weekly replaced with 150 ml of TAP medium without sulphur (sample A) or, alternatively, with TAP with and without sulphur (sample B). Before the restoration of the algal suspension with the new TAP, the produced hydrogen was measured by gas chromatography method. The results show that, in the tested experimental conditions, the regular and constant restoration of TAP medium without sulphur is a necessary and sufficient condition to obtain an increase in the hydrogen production. Besides, in these experimental tests, the cultures were kept in the anaerobic condition. The increase in the hydrogen production occurred also where TAP medium with sulphur and TAP medium without sulphur were restored alternately (see Figure 5). The lower hydrogen production by sample B is to be related to the algae cultivation in conditions that, as shown by previous experiments, are associated to lower hydrogen production (TAP with sulphur). Thus, the maintenance of algal cultures in constant metabolic condition through the restoration with TAP without sulphur (sample A conditions) may induce a constant increase of hydrogen production. This fact is very important for the future development of a chemo-state for the cultivation algae.

Conclusions

Experimental tests were carried out to determine the hydrogen production obtained by Chlamydomonas reinhardtii grown in TAP medium with and without sulphur under artificial solar irradiation. Preliminary results showed that the best culture in terms of hydrogen production was the one grown in a TAP medium without sulphur. These results are used in the design of a TAP restoration facility for the next experimental test. Thus, TAP restoration tests were led to improve and keep continuous the hydrogen production. The effect of an alternating restore of TAP medium with sulphur (facilitates the growth of algae) and TAP medium without sulphur (improves the production of hydrogen) on the hydrogen production obtained by Chlamydomonas reinhardtii were also carried out. The experimental results showed that the maintenance of algal cultures in constant metabolic condition through the restoration with TAP without sulphur produces a constant increase of hydrogen production; this solution is better than the alternating restoration of TAP with and without sulphur. Results will be used to develop an automatic controlled bioreactor for hydrogen production by Chlamydomonas reinhardtii. Besides, hydrogen produced by Chlamydomonas reinhardtii is going to be tested as fuel for a Molten Carbonate Fuel Cell which is produced in the Terni Fuel Cell Laboratory of the University of Perugia.

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