

Production of microalgae biomass and biohydrogen in solar bioreactors

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Abstract: Only water hydrolysis with renewable energies is a sustainable process for hydrogen production. Biohydrogen production is an interesting alternative that is being explored at the scientific level. The microalgae *Chlamydomonas reinhardtii* has been extensively studied and used as a model for the photo-production of H₂. The aim of this proposal is to develop a sustainable bioprocess for the production of H₂ from *C. reinhardtii* and just preliminary results are shown here. For the first step, solar bioreactors have been tested. The biomass is recovered and suspended in another culture media with restricted concentrations of sulphur. The culture is maintained in a closed photobioreactor with magnetic stirring and 24-h illumination with fluorescent lamps. Hydrogen is produced continuously reaching maximal durations of about 20 days. Solar light should be tested in order to avoid energy requirements from artificial illumination during hydrogen production. Coupling the production system to a hybrid electric station, the process would be more sustainable. However, a lot of research must be developed before this technology would allow scale the hydrogen production to a pilot plant in order to be used in rural communities as a source of energy and as an alternative economic activity.

Keywords: Microalgae, *Chlamydomonas reinhardtii*, Biohydrogen, Photobioreactor, Sustainability

1. Introduction

At this moment, none of the sustainable ways to produce energy can completely replace fossil fuels [1]. Nuclear and hydroelectric processes have been proposed some decades ago to solve some energetic demands, but they are controversial regarding sustainability. Alternatively, solar and wind systems are starting to offer technologic solutions at different energy consumption levels. In addition, biofuels are intended to slowly replace fossil fuels, but our knowledge still requires an improvement in the massive methods to produce them. On the other hand, hydrogen (H₂) has been proposed as an alternative fuel with a number of advantages [2]. Being a gas, molecular hydrogen is an attractive substance since it can be transformed to mechanical, thermal or electrical energies involving free-carbon processes. A clean combustion is possible with hydrogen, which involves environmental advantages over the common fossil fuels and even the biofuels. Moreover, future technologies for nuclear fusion could transform hydrogen to clean energy, emulating the processes in the Sun and the other stars.

Hydrogen is the most abundant element in the Universe; in the Earth, water and organic matter include hydrogen as a part of their composition [2]. Nevertheless, molecular hydrogen is not present in Nature and all its commercial production is not only more expensive than fossil fuels but also produce more pollution [3-5]. More than 90 % of the hydrogen production in the world depends on carbon compounds, principally fossil fuels, and its use as energy carrier is only justified in some applications as fuel for spaceships or demonstrative buses or cars. Renewable energies are being proposed to produce sustainable hydrogen in commercial processes: electrolysis units coupled to wind turbines, photovoltaic modules or hydropower systems [6]. Nevertheless, biohydrogen production is also an interesting alternative that is being explored at the scientific level, focusing on three microbial systems: bacterial fermentation, nitrogen fixation in photosynthetic cyanobacteria and photoproduction in green microalgae [7]. Another interesting bioprocess is the use of *in vitro* enzymatic cocktails [8].

The microalgae *Chlamydomonas reinhardtii* have been extensively studied and used as a model for the photo-production of H₂. Light is used through the photosynthesis as the primary energy source for biomass and bio-hydrogen production. The biomass is normally produced in aerobic systems through the well-known photosynthetic pathway. However, after a period of anaerobiosis, the electrons normally generated in the photosystem II are redirected to produce molecular hydrogen in a step catalyzed by a Fe-hydrogenase. Both the genetic expression of the enzyme and its activity are importantly inhibited with small quantities of oxygen [9]. However, only experiments with temperature control and fluorescent illumination are reported for these processes, resting sustainability to the hydrogen production.

The aim of this work is to develop a sustainable bioprocess to produce H₂ from *C. reinhardtii*. The process is separated in three steps: (i) aerobic cultures for biomass production, (ii) recuperation of the biomass and (iii) anaerobic systems for biohydrogen production. The production of biomass and biohydrogen should be performed in bioreactors with natural solar illumination and at environmental temperature. The integration of these steps with a hybrid electric station is planned in order to add sustainability to the process. However, it should be noted that only preliminary results are shown in this work, since more research is being performed before proposing energy balances or economical feasibility.

2. Methodology

2.1. Algal cultures

The strain CC-124 of *Chlamydomonas reinhardtii* (Chlamydomonas Center, USA) was used in this work. Three culture media have been tested for biomass production [10]: (i) Sueoka's High Salt (SHS) medium, as recommended by the strain provider, (ii) Tris-Acetate-Phosphate (TAP) medium, commonly used for hydrogen bioproduction [9], and (iii) a modified TAP medium without acetates (TP) in order to avoid bacterial contaminations during algae growth. The media were sterilized by vacuum filtration (Whatman, GFC, 0.2 µm) but the containers were only washed with household bleach (commercial sodium hypochlorite solution) and distilled water. Stocks are maintained with the SHS medium in Roux bottles with 24-h fluorescent illumination. Pre-cultures for inoculation in reactors were prepared with the corresponding medium during three to five days in Roux cultures with continuous artificial illumination and with bubbling from small air pumps.

2.2. Biomass characterization

Cellular counts were performed with an optical microscope (Olympus, CH-2) and correlated with optical density and with dry weight. Optical density was measured through a portable spectrophotometer (StellarNet, EPP2000) at 640 nm of wavelength. Dry weight was obtained with vacuum filtration of 10 ml of culture sample through membranes (Whatman, GFC 0.2 µm) and the same volume of culture medium to wash. Before and after filtration, the membranes were dried in a microwave oven (15 min, 10 W) and stabilized in a desiccator for 30 min in order to quantify the mass with an analytical balance (Ohaus Adventurer, ARx). Chlorophyll *a* was quantified directly on cellular samples with a fluorometer (Varian, CARY Eclipse), with an excitation wavelength at 432 nm and an emission wavelength at 668 nm. Calibration was previously performed with stock chlorophyll solutions at different concentrations.

2.3. Biomass production

2.3.1. Reactors

Two designs were tested for production of biomass in solar photobioreactors (Fig. 1). Vertical tubular reactors (VTR) were made on acrylic tubes (wall-thick: 25 mm, height: 90 cm) of different internal diameters: 70, 95 and 120 mm. The low part of the tubes was sealed with acrylic, allowing the air inlet through small holes (~1 mm i.d.). The operation volumes of the VTR were 3, 6 and 9 L, respectively. A flat panel reactor (FPR) was also made with acrylic walls (thick: 50 mm), and dimensions of 50 cm length, 50 cm height and 10 cm thick. The operation volume of the FPR was 20 L with air inlet in the low part of the box through porous stones. Both VTR and FPR have acrylic taps with an air outlet. The air inlet comes from a blower and it is passed through a humidifier before going into the reactors; air flux is controlled at 1 L·min⁻¹ per liter of culture. Natural illumination was used, with solar radiation marked by days and nights. At least 14 batch experiments were performed with the VTR and two with the FPR.



Fig 1. Pictures showing two different types of solar photobioreactors for the production of microalgal biomass at external conditions: flat panel reactor and vertical tube reactors.

2.3.2. Off-line and on-line monitoring

Daily samples were analyzed with optical density, dry weight and fluorometry. Dry weight was used to evaluate the biomass growth kinetics and the maximal cellular density. Automatic measurements of temperature and illuminance were performed with programmable data loggers (Onset, HOBO Pendant UA-002-64). In addition, on-line monitoring of the pH and pO₂ was possible in the FPR with the corresponding probes (Sensorex) connected to a power supply and an interface (National Instruments, cDAQ-1972 chassis, NI9203 and NI9205 modules). The interface is connected to a personal computer in which data are automatically registered with the Signal Express (National Instruments) software.

2.3.3. Batch algae growth experiments

A number of 12 experiments were performed in the VTR with different conditions: two culture media (SHS and TP), three tube diameters (70, 95 and 120 mm) and two annual seasons (Winter and Spring). Later, one experiment was performed in two VTR with the same diameter (95 mm) and the same meteorological conditions, but with two culture media (SHS and TP). Finally, one experiment more was performed with three VTR at the same conditions (SHS, 95 mm) in order to verify the reproducibility of the growth. Two more experiments were performed with the FPR and the HSS medium at similar meteorological conditions. Other experiments are planned with different concentrations of CO₂ mixed with air.

2.4. Biomass separation

In order to change the culture medium of the microalgae, two low-cost processes have been tested. A separation column with packed cotton wool showed good results with the retention of the biomass. A number of packing densities and designs were essayed in order to optimize the biomass separation. After being washed with the new medium TAP-S (TAP with minimal concentrations of sulphur), the cotton wool is pressed in order to recover the biomass, which is re-suspended in the TAP-S medium at a defined cellular density. The second process uses the same VTR in which the biomass is grown. Polymeric hydrogel is added to the culture with air bubbling and the medium is absorbed and retained in the hydrogel structure. The concentrated culture is re-suspended in the TAP-S medium at a defined cellular density. The hydrogel does not absorb algal cells, it can be dried in a solar system and reused for subsequent culture absorption processes.

2.5. Biohydrogen production

Just preliminary experiments have been performed to test the production of hydrogen from microalgae in VTR with 24-h fluorescent illumination ($\sim 100 \mu\text{E}$) in a laboratory with controlled temperature (298-300 K) and constant magnetic stirring (Fig. 2). The VTR are made in acrylic tubes (70 mm i.d.) as described previously, with a high of 30 cm and an operation volume of 1 L. The base of the tube is sealed in acrylic and the tap is made in Teflon with an o-ring to seal the system and with a gas outlet. This outlet is connected to a hydrogen PEM-FC (Polymeric Electrochemical Membrane Fuel Cell), which uses oxygen from air to produce water and electricity. The pH and the voltage of the fuel cell are registered on-line with the interface described in section 2.3.2. and related with the production of hydrogen in the system. The temperature and the illuminance in the reactor are automatically registered with the data logger described in the same section. At the moment, four experiments have been performed with different biomass concentrations: 48, 71, 164 and 248 mg/L. Experiments with light/dark cycles and with natural solar illumination are planned.



Fig. 2. Pictures showing the reactors for biohydrogen production with microalgae under laboratory conditions.

2.6. Hybrid electric station

The electrical requirements to perform the three processes, basically for air bubbling in the biomass production and separation, and for mixing in the biohydrogen production, may be obtained from a hybrid electric station. At the moment (Fig. 3), four polycrystalline photovoltaic panels (Yingli Solar, 110 W) and one wind turbine (Whisper 200, 1000 W) are installed with four acid lead batteries (Rolls Surette, 6 V) and an electrical inverter (Xantrex, DR1524) from DC (24 V) to AC (120 V). This installation is placed in the Cinvestav Marine

Station at Telchac Port, Yucatan (5 m AMSL, LAT: 21°20'28" N, LONG: 89°18'21" W). Electricity production from the hybrid station will be correlated with the meteorological conditions. The meteorological system (Davis, Vantage Pro 2 Plus) is installed in the Marine Station and has an automatic data registration every 10 min. Wind speed and orientation, solar radiation and temperature are some of the data which are obtained. The energetic fluxes in the hybrid plant will be registered in real time through an interface (National Instruments, Field Point).



Fig. 3. Pictures showing the hybrid electric station installed in Telchac Port, Yucatan, Mexico

3. Results

A typical biomass growth curve for the microalgae in the solar photobioreactors is shown in Fig. 4. The kinetics of the growth does not fit the Monod model; actually, the Gompertz model fits better with the experimental results (Fig. 5) [11,12]. In Table 1, 12 experiments in the VTR are compared in relation with the maximal cellular density, the specific growth rate and the residence time as a function of the medium (SHS or TP), the reactor diameter (70, 95 or 120 mm) and the annual season (winter or spring). The specific growth rate was computed as the slope from a linear correlation with the corresponding Gompertz equation as a function of the time; the linear correlation had always a value $r^2 > 0.9$. The residence time was calculated as the surface below the curve in a graphic with the inverse of the instant growth rate *versus* the biomass concentration for everyday during the experiments [13]. Experiments 1 to 6 were performed during the Winter, the coolest season in Yucatan (Mexico), with an average temperature of 298 K and illuminance average values below 10 klux. In contrast, the experiments 7 to 12 were performed during the Spring, the hottest period in the year, with an average temperature of 303 K and illuminance averages values over 10 klux. Similar results were obtained in other experiments with both VTR and FPR. In addition, pH and pO_2 do not change importantly during the biomass growth.

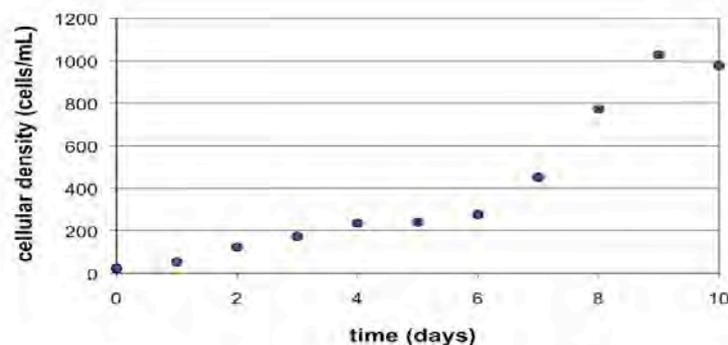


Fig. 4. Typical biomass growth of the microalgae in solar photobioreactors as followed by optical density.

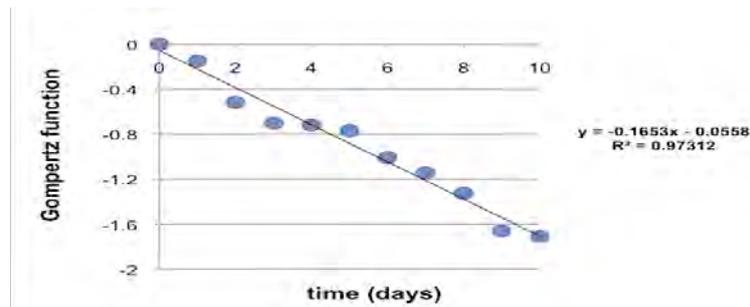


Fig. 5. Fit of the Gompertz model to the experimental cellular growth followed with dry biomass.

Table 1. The compared results for 12 experiments in vertical tubular reactors with three different diameters (d), two culture media and two annual seasons. Maximal cellular density as dry weight (D), specific growth rate (μ) and residence time (T) are reported.

Experiment number	Annual season	Culture medium	d (mm)	D (g/L) ± 0.01	μ (day^{-1})	T (day) ± 1.0
1	Winter	SHS	70	0.63	0.22 ± 0.02	8.5
2	Winter	SHS	95	0.64	0.30 ± 0.02	7.8
3	Winter	SHS	120	0.60	0.21 ± 0.01	9.8
4	Winter	TP	70	0.31	0.42 ± 0.05	5.2
5	Winter	TP	95	0.47	0.49 ± 0.03	3.4
6	Winter	TP	120	0.45	0.41 ± 0.04	4.1
7	Spring	SHS	70	0.34	0.09 ± 0.01	9.2
8	Spring	SHS	95	0.52	0.17 ± 0.01	7.2
9	Spring	SHS	120	0.44	0.16 ± 0.01	8.0
10	Spring	TP	70	0.33	0.49 ± 0.03	5.0
11	Spring	TP	95	0.39	0.48 ± 0.01	3.8
12	Spring	TP	120	0.35	0.41 ± 0.03	4.5

For the hydrogen production, the calibration of the fuel cell is being performed, where the voltage change is related with the hydrogen produced by the microalgae. In a graphic of measured voltage *versus* time, the surface below the curve is proportional to the total hydrogen production in the experiments. It is possible to see some preliminary results in Table 2 at different initial biomass concentrations. In general, the hydrogen production rate grows gradually during the first 30 hours and then it is maintained more or less constant for a certain period before going down to the base line. The length of this stationary production is directly related with the concentration of the initial biomass concentration with a maximal duration of 20 days for the biggest value of biomass. However, the production rate does not change between 71 and 439 mg/L of initial biomass concentration. The pH neither does change importantly during the experiments. However, the culture illuminances inside the reactor were found to decrease with time, and temperatures were found to be always lower than room temperature, principally at the beginning of the experiments when the biomass concentration is minimal.

Table 2. Biohydrogen production as a function of initial biomass concentration, at $T = 298\text{ K}$ and atmospheric pressure. The maximal hydrogen production rate (ΔV_{\max}) and the total hydrogen production (THP) are reported. The experiment with initial biomass concentration of 164 mg/L was cut before finishing the hydrogen production.

Initial biomass concentration (mg/L)	Final biomass concentration (mg/L)	ΔV_{\max} (mV)	THP (V.h)
48	79	134	10,166
71	219	210	195,524
164	-	197	-
349	835	220	614,030

4. Discussion

The maximal concentration biomass has been obtained after 6 to 12 days of external cultures in solar photobioreactors. The VTR are easy to construct and manipulate, allowing to compare different culture conditions; they are very useful for research work. In general, for the experiments reported in Table 1, the reactor with 95 mm of internal diameter show a better performance for the production of biomass. The meteorological conditions influence significantly on this production, being Winter much better than Spring. Finally, it is possible to see that the TP medium gives the biggest values of the specific growth rate and the lowest values of the residence time, while the SHS medium gives the biggest cellular yields. These results show that the composition in the medium is important to regulate both the kinetics and the maximal biomass production. In particular, the SHS medium has important limitations of calcium and manganese, while the phosphate concentration is extremely low in the TP medium.

On the other hand, the FPR allow bigger production volumes and can soften temperature variations or extreme illuminances. A previous work reports that the raise of the temperature inside the FPR can be diminished when the biggest walls of the reactor are not exposed directly to solar radiation [11]. In general, both VTR and FPR show similar results for the biomass growth. This biomass production must be increased in order to find the economical feasibility and the sustainability of the process. This can be reached improving different operation conditions. By instance, a better system for gas/liquid interchanges, a different air bubbling flux or adding carbon dioxide to the air, could increase the yield of the production [12]. Additionally, it is necessary to reduce costs of the culture media and new compositions should be tested using alternative sources as wastewaters. With respect to the reactors, cheaper materials, as plastic bags, should be used instead of acrylic. It was also found that carbon dioxide in the air is minimally used when bubbled and the height of the reactors may be considerably increased. It is also important to test with a continuous or semi-continuous operation instead of the batch reactors.

For the biomass separation, the filtration with cotton wool is technically complicated and the risk of contamination is important. The medium absorption with hydrogel is much easier and cleaner, although the kinetics of the process should be standardized. The recycling of both hydrogel and medium is important to gain sustainability in the process. In relation with the biohydrogen production, much more work should be performed. It is very important to calibrate the performance of the fuel cell in order to have a true representation of the hydrogen production in the systems. The influence of the initial biomass concentration in the hydrogen production should be completely specified, as well the influence of the illuminance and the light/dark cycles, in order to scale the process in a solar photobioreactor.

5. Conclusions

Only partial results are shown here and more work is being performed to complete the project. It has been demonstrated that *Chlamydomonas reinhardtii* can be grown at external conditions in Yucatan (Mexico) using solar photobioreactors, both VTR and FPR. The production of biohydrogen in external reactors with solar illumination and no control of the temperature must be tested. The integration of all the processes with the hybrid electric station would allow knowing the feasibility of the hydrogen production as a sustainable process.

References

- [1] B. Sorensen, Renewable energy; its physics, engineering, environmental impacts, economics & planning. Elsevier, 3 ed, 2004, 928 pp.
- [2] B. Sorensen, Hydrogen and fuel cells: emerging technologies and applications. Elsevier, 450 pp.
- [3] L. Barelli, G. Bidini, F. Gallorini, and S. Servili, Hydrogen production through sorption-enhanced steam methane reforming and membrane technology: A review, Energy 33, 2008, pp. 554-570.
- [4] M. Balat, Potential importance of hydrogen as a future solution to environmental and transportation problems, International Journal of Hydrogen Energy 33, 2008, pp. 4013-4029.
- [5] M. Balat, and M. Balat, Political, economic and environmental impacts of biomass-based hydrogen, International Journal of Hydrogen Energy 34, 2009, pp. 3589-3603.
- [6] P. A. Pilavachi, A. I. Chatzipanagi, and A.I. Spyropoulou, Evaluation of hydrogen production methods using the Analytical Hierarchy Process, International Journal of Hydrogen Energy 34, 2009, pp. 5294-5303.
- [7] M. L. Ghirardi, A. Dubini, J. Yun, and P. Maness, Photobiological hydrogen-producing systems, Chemical Society Reviews 38, 2009, pp. 52-61.
- [8] X. Ye, Y. Wang, R. C. Hopkins, M. W. Adams, V. R. Evans, J. R. Mielenz, and Y.-H. P. Zhang, Spontaneous high-yield production of hydrogen from cellulosic materials and water catalyzed by enzyme cocktails, ChemSusChem 2, 2009, pp. 149-152.
- [9] A. Hemschemeier, A. Melis, and T. Happe, Analytical approaches to photobiological hydrogen production in unicellular green algae, Photosynthesis Research 102, 2009, pp. 523-540.
- [10] Chlamydomonas Center, Recipes for commonly used culture media, 2010, www.chlamy.org.
- [11] P. Castorina, P.P. Delsanto and C. Giot, Classification scheme for phenomenological universalities in growth problems in physics and other sciences, Physical Review Letters 96, 2006, pp. 188701(1-4).
- [12] P. Castorina, T.S. Deisboeck, P. Gabriele and C. Giot, Growth laws in cancer: implications in radiotherapy. Radiation Research Society 168, 2007, pp. 349-356.
- [13] J.M. Lee. Biochemical engineering, Prentice-Hall, 2001, Chapter 6.
- [14] L. Pantí, P. Chávez, D. Robledo and R. Patiño, A solar photobioreactor for the production of biohydrogen from microalgae, Solar Hydrogen and Nanotechnology Proc. of SPIE, 2007, pp. 66500Z1-66500Z9.
- [15] H.J. Ryu, K.K. Oh and Y.S. Kim, Optimization of the influential factors for the improvement of CO₂ utilization efficiency and CO₂ mass transfer rate, Journal of Industrial and Engineering Chemistry 15, 2009, pp.471-475.