HYDROGEN PRODUCTION FROM BIOLOGICAL SYSTEMS UNDER DIFFERENT ILLUMINATION CONDITIONS: PHOTOBIOREACTOR PROPOSAL

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ABSTRACT
Photobiological hydrogen production can be obtained by biological systems under sun irradiation.
Certain algae and bacteria can produce H_2 under suitable conditions.
Algae pigments absorb solar energy; enzymes in the cell act as catalysts to split water into hydrogen and oxygen. There are many research activities studying hydrogen production from biological systems. These studies show that the efficiency (energy produced from hydrogen divided by solar energy) of such systems can be estimated to be 10%. This value has to be increased for a large scale hydrogen production. The effect of different artificial illumination conditions on H_2 production was studied for green algae cultures (*Chlamydomonas reinardtii*). Results will be used to design an high efficiency photobioreactor for a large scale hydrogen production.

INTRODUCTION
Economic growth in the last decades was strongly dependant on fossil fuels as sources of energy. These resources are not illimited in the long run , and environmental concerns have led to the search of clean energy sources. Hydrogen (H_2) only produces water when burned with oxygen, and is therefore an interesting fuel. To large-scale use, the production of significant quantities of hydrogen is economically essential.
From a strictly economic point of view, it is difficult to expect in the next few decades that hydrogen biologically produced can compete with chemically-synthesized hydrogen.
However, hydrogen production from algae and bacteria is a subject that attracts interest due to the potential practical application of the process in renewable fuel production (Melis et al. 2000). In effort to find the better conditions to optimize this process the influence of many different physical and physiological factors must be considered. Microscopical green algae like *Chlamidomonas reinhardtii* can produce H_2 using sunlight as an energy source. Only radiations between 400 and 700 nm can be used by microalgae. This part of the solar spectrum is called 'Photosynthetic Active Radiation' (PAR). On an energy basis, 43% of the solar radiation is in the PAR region (Thimijan RW, Heins RD 1983; Pottier et al. 2005).
Biological hydrogen production from *Chlamidomonas reinhardtii* cultures under continuous artificial illumination has been investigated. Lamps with different emission spectra and different colour temperature have been utilized in effort to individuate the best illumination conditions for H_2 production.

MATERIALS AND METHODS

Cultures
Chlamydomonas reinardtii was grown in TAP (Tris-acetate-phosphate) medium with and without sulphates.
Liquid cultures were grown at about 28 °C (Kosourov S et al 2002) in 500 ml graduated borosilicate glass bottles with 5 ground necks closed by plastic screw taps with apertures with porous buthile/teflon septa. These septa have a low gas permeability and permit to sample the inner gas from the bottle using a syringe.
The bottles were selected for the optimal capacity to transmit the sunlight. In spectral range from approx. 310 to 2200 nm the absorption of glass is negligibly low.
Six samples were prepared (3 using TAP with sulphate and 3 using TAP without sulphate) using 200 ml of TAP and $15x10^6$ cell of *Chlamidomonas reinhardtii* The cultures were injected in the bottles. The volume of headspace of each bottle $(V_{headspace})$ was 435 cc.

Bioreactor systems
The cultures were maintained under continuous illumination in three photobioreactors made up aluminium reflecting wall boxes.
In each photobioreactors was installed a particular type of lamp.
In particular, two types of vapours fluorescent lamps were used with 5.600 and 2.700 K colour temperatures and a Xenon lamp with 6.800 K colour temperature in order to establish different illumination conditions. In Fig.3 are shown the three experimental photobioreactors and Tab. 1 shows the technical informations of the installed lamps.

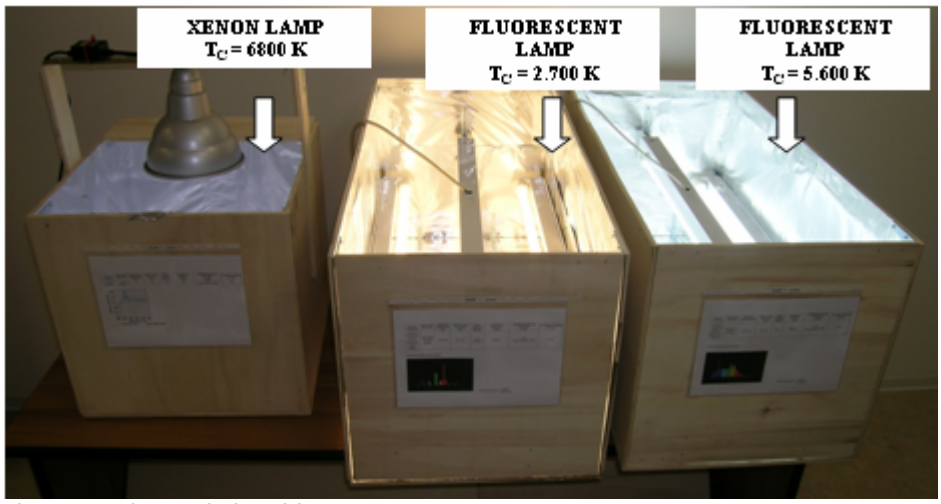

Fig.3 Experimental photobioreactors.

Table 1. Lamps installed – technical informations.

TYPE OF LAMP / CHARACTERISTICS	*Mercury vapours Fluorescent lamp mod 765 BASIC-OSRAM*	*Mercury vapours Fluorescent lamp mod LUMINUX-OSRAM*	*Xenon lamp*
Tonality	Daylight	Warm white	Ultra white
Nominal power (w)	3x30 =90	3x30 =90	35
Chromatic yield index ra	70…79	80…89	>90
Luminous flux ϕ (lm)	1900 (63 lm/W)	2400 (80 lm/W)	2.500 (70 lm/W)
Lenght x Diameter (mm)	895x26	895x26	40x7
Colour temperature T_c (k)	5.600	2.700	6.800
Illuminance E_v (lux)	12.600	15.300	3.400
Boxes dimensions length x width x heigth (mm)	100x50x50	100x50x50	50x50x50

Illuminance E_V and colour temperature T_C were measured with a colorimeter CHROMA METER CL – 200 Konica Minolta.
Fig 5 A) and B) shows the spectral distribution of fluorescent lamps with 5.600 K e 2.700 K colour temperatures.
Fig.5 C) show the Xenon lamp spectral distribution compared with natural middey sunlight spectra.

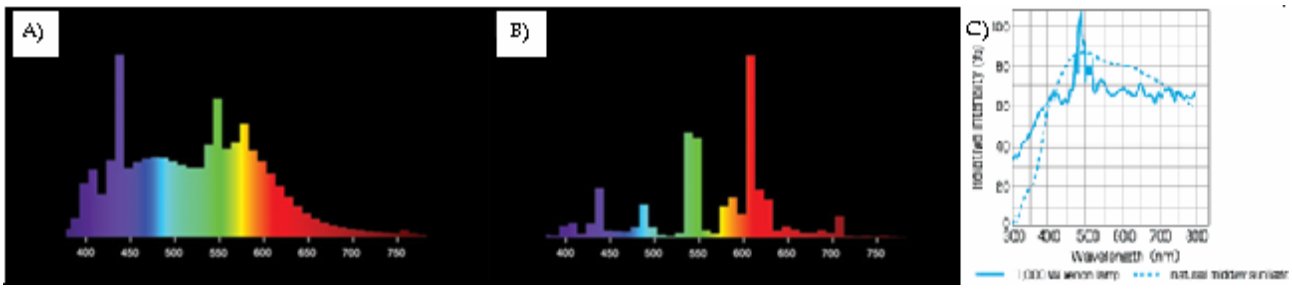

Fig.5 Spectral distribution of fluorescent lamps with A) 5.600 K and B) 2.700 K colour temperatures.
$\left(\text{figure height} = \frac{400mW}{1.000x10nm}\right)$ C) Xenon lamp spectral distribution compared with natural middey sunlight spectra.

Three types of lamps were selected considering the light absorption by green algae. Fig. 7 shows the light absorption spectrum of green algae (solid line) compared to the sunlight (dotted line) spectrum (Akkerman I et al. 2002).
The X -axis represents the wavelength, with the visible part indicated by the coloured bar, and the relative light intensity shown on the Y -axis. Part of the sunlight energy from the spectrum 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 got with wavelengths between 400 and 500 nm and between 650 and 700 nm.

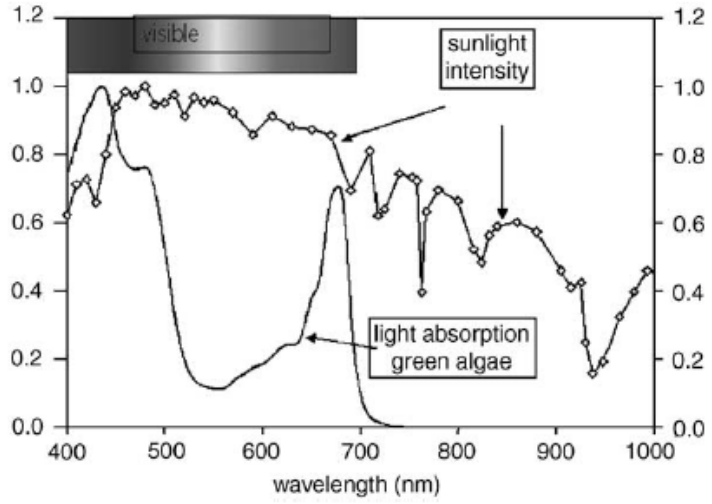

Fig.7 Sunlight and light absorption by green algae.

MEASURAMENTS

The amount of hydrogen produced by each culture was measured by sampling gas from the bottle headspace with a gas tight syringe. A gas chromatograph (model CP4900, Varian) with 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 t = 0 when the culture were injected in the bottles.

The total volume of the hydrogen produced at the time of measurement *i* is given by:

$$Vi_{H_2 produced} = \sum_{i=1}^{n-1} x_i \cdot v_i + V_{H_2 before\ the\ analysis\ i} \quad (1)$$

Where:

n = number of measurements

x_i = $\%_{vol}$ of hydrogen measured

v_i = volume of gas sampled

$$V_{H_2 before\ the\ analysis\ i} = x_i \cdot (V_{headspace} - \sum_{i=1}^{n-1} v_{i-1}) \quad (2)$$

$$Vi_{H_2 produced} = \sum_{i=1}^{n-1} x_i \cdot v_i + x_i \cdot (V_{headspace} - \sum_{i=1}^{n-1} v_{i-1}) \quad (3)$$

The codification used for the different kinds of samples is indicated in the table 4.

Table 4. sample codification.

CULTURES	*Lamps - Tc (K)*		
	2700	*5600*	*6800*
Chlamydomonas reinhardtii in TAP without sulphate	A	B	C
Chlamydomonas reinhardtii in TAP with sulphate	AS	BS	CS

Values in mmol of the total volume of the hydrogen produced at the time of each measurement are reported in table 5.

Table 5. Amounts of hydrogen produced at the time of each measurement.

INCUBATION TIME (DAYS)	*Total hydrogen produced (mmol)*					
	A	*B*	*C*	*AS*	*BS*	*CS*
0	0,3558	0,2490	0	0,3380	0,2668	0,3380
4	26,0010	7,0760	1,5033	15,0353	0,7974	5,4847
5	57,2128	9,8894	1,4154	2,9199	0,7095	5,8364
6	89,9913	4,6449	1,5902	14,6153	7,0030	5,8364
7	141,4267	3,0862	2,1098	2,4924	0,1570	5,8364
11	163,8995	0,3260	58,1543	0,2623	0,1210	6,9686
13	160,7561	0,2309	110,8779	0,7891	0,0959	6,3569
20	149,0079	0,2392	176,7008	1,2556	0,0939	6,5236
22	95,5864	0,1902	104,8186	0,5368	0,0890	4,0730
27	121,7943	0,3183	137,7186	2,0578	0,1418	7,5952
32	117,0738	0,2869	131,9630	3,1398	0,1261	7,4697

On equal terms of illumination, the production of hydrogen is greater in cultures grown in TAP without sulphate (samples A, B and C) than in cultures grown in TAP with sulphate (samples AS, BS and CS).

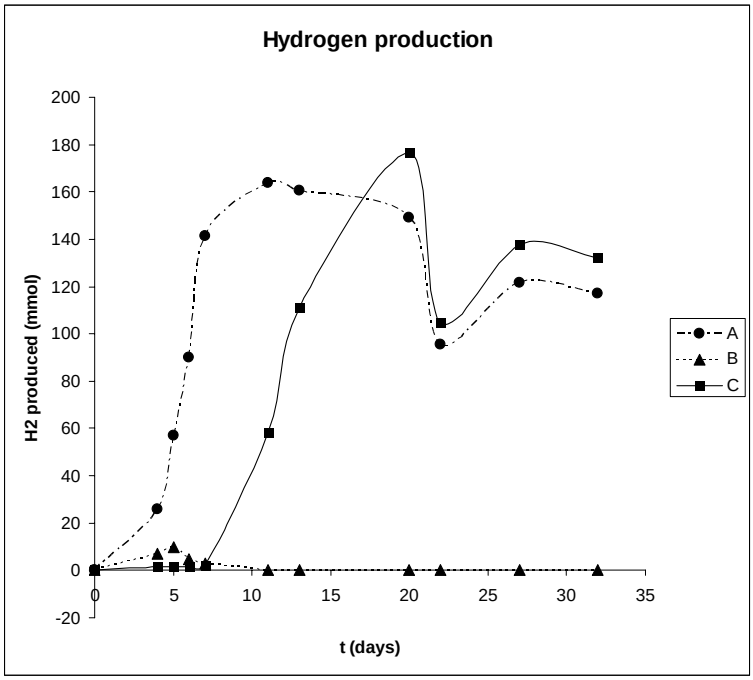

Fig. 8 Total amount of hydrogen produced by cultures in TAP without sulphate.

Fig. 8 and 9 show the results of such measurements in culture grown in TAP without sulphate and for the cultures gown in TAP with sulphate respectively.
The samples that showed the best results are sample A (*Chlamydomonas reinhardtii* in TAP without sulphate under continuous illumination with fluorescent lamp with T_c = 2700 K) and sample C (*Chlamydomonas reinhardtii* in TAP without sulphate under continuous illumination with xenon lamp with T_c = 6800 K). In sample B

(*Chlamydomonas reinhardtii* in TAP without sulphate under continuous illumination with fluorescent lamp with T_c = 5600 K) no relevant results were got.

- In sample A the total amount of hydrogen increased until the 11th day with a maximum value of 164 mmol. From the 11th until the 22nd day the total amount of hydrogen decreased until 96 mmol. From the 22nd until the 32nd day the trend is oscillating.
- In sample C the total amount of hydrogen increased until the 20th day with a maximum value of 177 mmol. From the 20th until the 22nd day the total amount of hydrogen decreased until 105 mmol. From the 22nd until the 32nd day the trend is oscillating.

Cultures in TAP without sulphate show an oscillating trend of the total amount of hydrogen during the first 11 days. In this period the maximum values raised. These values were 15 mmol for the sample AS (*Chlamydomonas reinhardtii* in TAP with sulphate under continuous illumination with fluorescent lamp with T_c = 2700 K) and 7 mmol in sample BS (*Chlamydomonas reinhardtii* in TAP with sulphate under continuous illumination with fluorescent lamp with T_c = 5600 K). Sample CS (*Chlamydomonas reinhardtii* in TAP with sulphate under continuous illumination with xenon lamp with T_c = 6800 K) shows an approximately increasing trend during the whole test period; the maximum value raised to 7,6 mmol after 27 days.

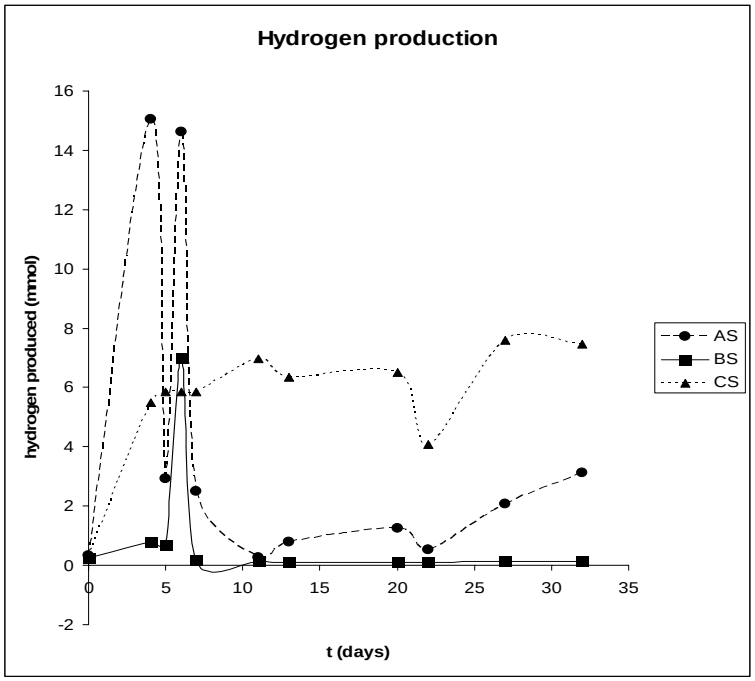

Fig. 9 Total amount of hydrogen produced by cultures in TAP with sulphate.

CONCLUSIONS

On equal illumination terms the best culture medium was TAP without sulphate.

The best illumination conditions were obtained with the fluorescent lamps with T_c = 2700 K and with the xenon lamp. The maximum total hydrogen production (177 mmol) was got thanks to this lamp after 20 days.

These information may be useful in the design of algal H_2-production photobioreactor system.

Next experimental tests will carry out with *Chlamydomonas reinhardtii* cultures in TAP without sulphate under continuous illumination using the individuated lamps. For some sample will be established anaerobic conditions. The same types of sample will be prepared and placed under natural illumination conditions to value the effect of artificial illumination on hydrogen production. Microscopical analysis will be carry out to monitoring the biological cycle of the cultures.

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