Hydrogen production by native species of microalgae isolated from México

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ABSTRACT

Hydrogen production from biological processes as biophotolysis is an environmental friendly alternative for biofuel production. It has been reported that some microalgae are able to produce hydrogen under stress conditions induced by a sulfur lack in the culture medium. In this work, microalgae isolated from Mexico were grown anaerobically under sulfur deficiency to define their potential as hydrogen producers. Four green microalgae were used for the bioscreening, *Chlamydomonas mexicana, C. gloeopara, Chlorella vulgaris* and *Scenedesmus obliquus.* The strains were maintain in 125 mL Erlenmeyer flasks with medium 'f' modified at 100 and 300 μE $m^{-2}s^{-1}$ and 20°C. For anaerobic induction of gas production, biomass was recovered and transferred into sealed containers with a sulfur-limited medium and a septum for gas recollection. The cultures were monitored daily for cellular growth, specific growth rate, duplication time and gas production. At the first stage for biomass generation, *Scenedesmus obliquus* developed the highest biomass. However in the second stage for gas production at the end of 3 days, the higher gas producers were in descending order *Chlorella vulgaris* > *Chlamydomonas gloeopara* > *Scenedesmus obliquus* > *C. mexicana.*

Key words: hydrogen, microalgae, biophotocatalysts.

1. INTRODUCTION

The world of microorganisms comprises billions of living beings classified in a great number of species with a wide diversity of functions and shapes [1]. These microorganisms include bacteria, fungi, yeast, protozoa, virus and microalgae [2]. Marine and freshwater microalgae form the base of the food chain in sea, rivers and lakes, making the fixation of nutrients which are intermediates through the food chain. Microalgae are photoautotrophic cells with capacity to transform inorganic compounds (CO_2 y N_2) into organic matter with high nutritional value (lipids, proteins and carbohydrates) powered by the sun[3]. All this, thanks to their cellular structures with special functions called chloroplasts (chlorophylls, phycobilins, carotenoids) which have several pigments capable to capture the light. The light is one of the factors most important in the metabolism of microalgae, since changes in quantity (irradiance) and quality (spectral distribution of light) have a direct effect on growth, biochemical composition and formation of organelle [4].

Currently, microalgae have a high potential in many areas such as aquaculture, pharmacology, genetic, biochemistry, biotechnology and currently for biofuel production mainly hydrogen. Some microalgae species show high photosynthetic efficiency, high biomass production and growth rates [5]. To this respect, some species of microalgae have been isolated from the North of Mexico which could show potential for biohydrogen production. However, studies measuring the effect of irradiance on growth and cell size in these species that then will be cultured for H_2 production are scarce. Thus, it is important to study this environmental factor with the purpose to obtain microalgae cultures with a high biomass density for H_2 production.

2. METHODOLOGY

2.1 Strains

Chlamydomonas gloeopara CMGO [6], *C. mexicana* CMM [7], *Chlorella vulgaris* CLV [8] and *Scenedesmus obliquus* SCO [9] were obtained from the Culture Collection at the Laboratory of Biology and Culture of Microalgae in the CICESE, Mexico.

2.2 Growth conditions

Microalgae cultures were maintained routinely in 125 mL Erlenmeyer flaks containing 100 mL of "f" medium [10]. Exponential-phase cultures were growth by triplicate at irradiances 100 and 300 μE $m^{-2}s^{-1}$.Experiments were performed under light-dark periods of 24 h at 20 ± 1°C and pH 7.0.The initial inocula were 275,000, 175,000, 650,000 and 800,000 cells ml^{-1} for CMGO, CMM1, CLV1 and SCO, respectively. Cell counting (by use of the Neubauer chamber) was performed with 1 ml of culture for each test every 24 h during 7 days. Results were expressed as number of cells (cells ml^{-1}), specific growth rate (SGR), duplication time (td) and number of cell divisions (d).

Cell size was determined at the end of the 7^{th} day. The cells were observed in an inverted microscope (Zeiss LSM 510). The images were capture with an Axio CAM HRc camera (512x 512pixels) and edited with Axio Ver 200 ®version 4.

2.3 Anaerobic induction of gas accumulation

Liquid cell cultures were concentrated by centrifugation and resuspended in 100 ml of a sulfur-deprived "f" medium. Concentrated cells were transferred to glass serum vials sealed with butyl rubber septa and purged with nitrogen to establish anaerobic conditions. Cells were incubated at 20°C under continuous white light (100 μE $m^{-2}s^{-1}$). Gas evolution was daily measured by the displacement method in brine solution (NaCl).

2.4 Statistical analysis

The growth parameters were subjected of statistical analysis using a Student's t-test with a significance level of 5%. STATISTICA® version 6.0 (Stat Soft Inc., 2002) was used to perform the analysis.

3. RESULTS

3.1.Microalgae growth

Figure 1 shows the microalgae growth at two irradiances. The cultures showed a short adaptation time (1 day) and then the exponential phase was clearly recognized. In all species, the behavior at the applied irradiances was very close but differences in the biomass density were observed: SCO reached the highest value after 6 days of incubation with 4.5 million cells ml^{-1} in both irradiances; CMM1 showed the lowest biomass density. The levels of irradiances studied did not show a significant effect on cellular growth ($p>0.05$).

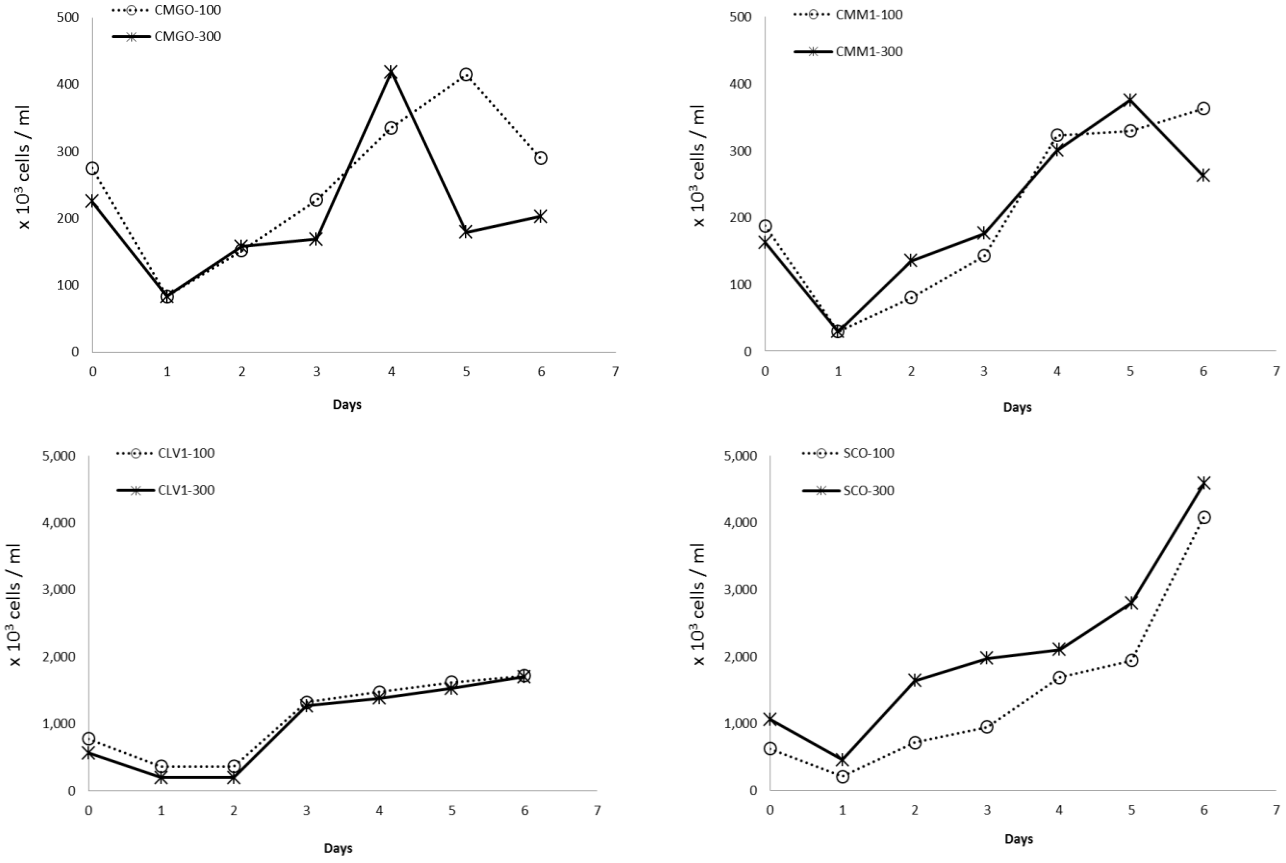

Figure 1. Cell growth of A) *Chlamydomonas gloeopara* CMGO, B) *C. mexicana* CMM, C) *Chlorella vulgaris* CLV, D) *Scenedesmus obliquus* SCO grown at 100 and 300 µE $m^{-2}s^{-1}$.

The number of cell division per day for all strains was lower in the first days of incubation as was expected (Table 1). Then, the values increased throughout the incubation period. All strains, except *Chlorella vulgaris* (CLV1), exhibited their highest value at 300 μE $m^{-2}s^{-1}$. Respect to the specific growth rate (SGR) and the duplication time, these growth parameter were similar under the irradiances conditions studied for all strains (Figure 2).Cell size was determined in an inverted microscope, cells of CMGO were the largest while the cells of SCO were the smallest (Table 2; Figure 3). It was not found significant differences either in length or width at the two tested irradiances.

Irradiance is one of the most important parameters during the microalgae culture [11]. The high levels of this parameter, especially during the first days of incubation could result in photoinhibition. This phenomenon was evident in all the cultures in which the number of cells and divisions per day were affected in a negatively way. This negative effect was stronger in CMM since at the first day of incubation only between 15 to 17 percent of the initial inoculum survived. Following recommendations of previous reports [12], the strains were grown at a higher irradiance (300 μE $m^{-2}s^{-1}$) expecting a higher cell density, however this effect was not clearly observed (except for SCO). This could be to the fact, that the irradiancy applied was not enough to do damage to the photosynthetic system in the strains tested.

Table 1. Number of cell division per day of *Chlamydomonas gloeopara* (CMGO), *C. mexicana* (CMM), *Chlorella vulgaris* (CLV) and *Scenedesmus obliquus* (SCO) grown at 100 and 300 μE $m^{-2}s^{-1}$.

Days	CMGO 100	CMGO 300	CMM1 100	CMM1 300	CLV1 100	CLV1 300	SCO 100	SCO 300
0	-0.75	-0.62	-1.17	-1.08	-0.47	-0.65	-0.68	0.53
1	0.38	0.40	0.63	0.96	0.00	0.00	0.77	0.80
2	0.25	0.04	0.36	0.17	0.81	1.16	0.18	0.12
3	0.24	0.57	0.51	0.33	0.07	0.05	0.36	0.04
4	0.13	-0.53	0.01	0.14	0.06	0.06	0.09	0.18
5	-0.22	0.08	0.06	-0.22	0.04	0.07	0.47	0.31
6	-0.24	-0.53	-0.41	-0.37	0.12	0.00	-0.22	-0.20

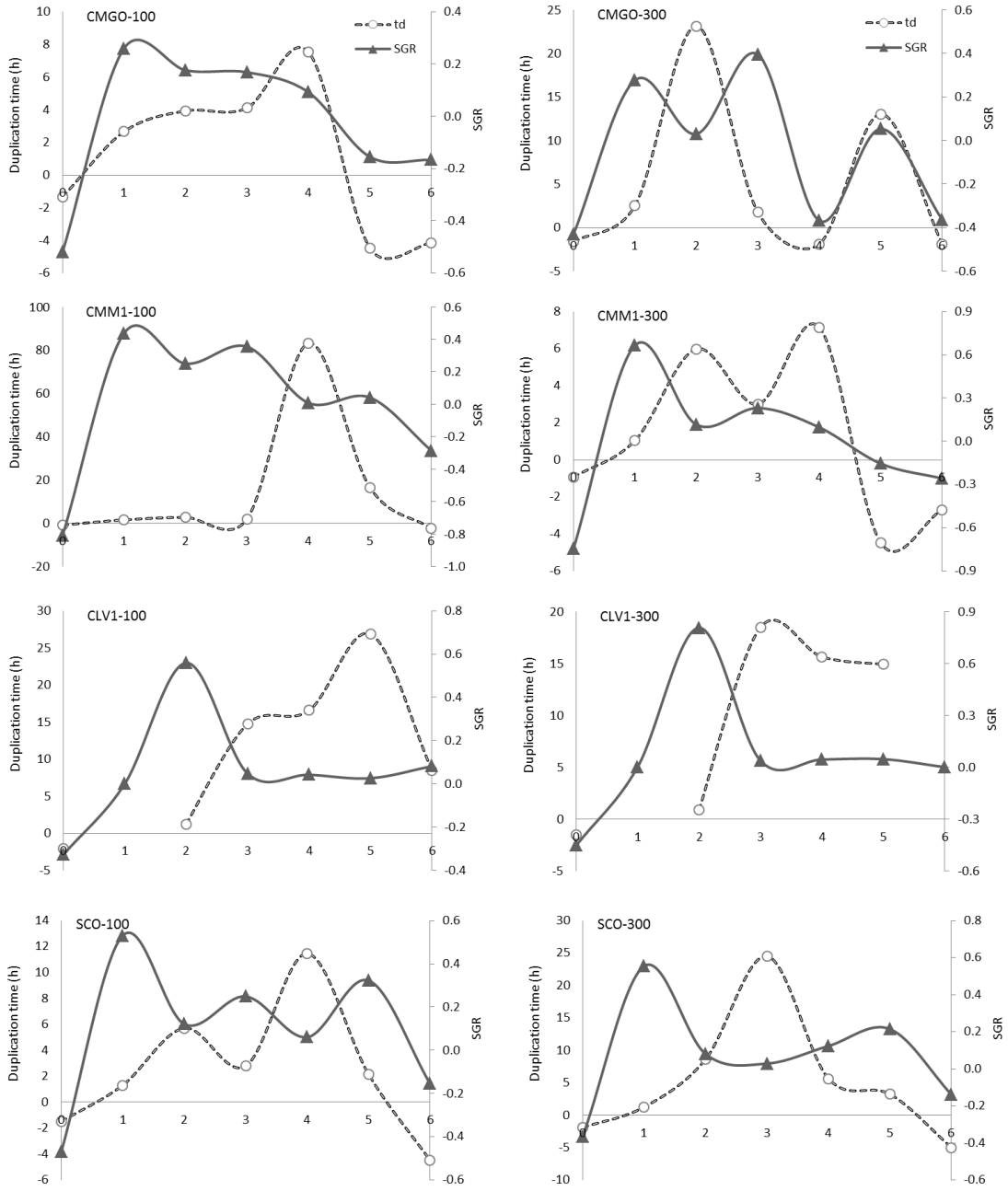

Figure 2. Specific growth rate (SGR), duplication time (td) of *Chlamydomonas gloeopara* (CMGO), *C. mexicana* (CMM), *Chlorella vulgaris* (CLV) and *Scenedesmus obliquus* (SCO) grown at 100 and 300 μE $m^{-2}s^{-1}$.

Table 2. Cell size (length and width) of *Chlamydomonas gloeopara* (CMGO), *C. mexicana* (CMM), *Chlorella vulgaris* (CLV) and *Scenedesmus obliquus* (SCO) grown at 100 and 300 $\mu Em^{-2}s^{-1}$.

Strain	Length (µm)	St. Dev.	Strain	Wide	St. Dev. (µm)
CMGO-100	8.34	0.42	CMGO-100	5.20	0.80
CMGO-300	7.80	0.63	CMGO-300	6.13	1.29
CMM1-100	6.44	1.49	CMM1-100	6.67	1.61
CMM1-300	7.96	1.57	CMM1-300	7.24	1.36
CVL1-100	7.80	1.26	CVL1-100	7.23	0.80
CVL1-300	7.23	0.61	CVL1-300	7.08	0.73
SCO-100	7.96	0.77	SCO-100	3.55	0.88
SCO-300	7.84	0.58	SCO-300	2.92	0.46

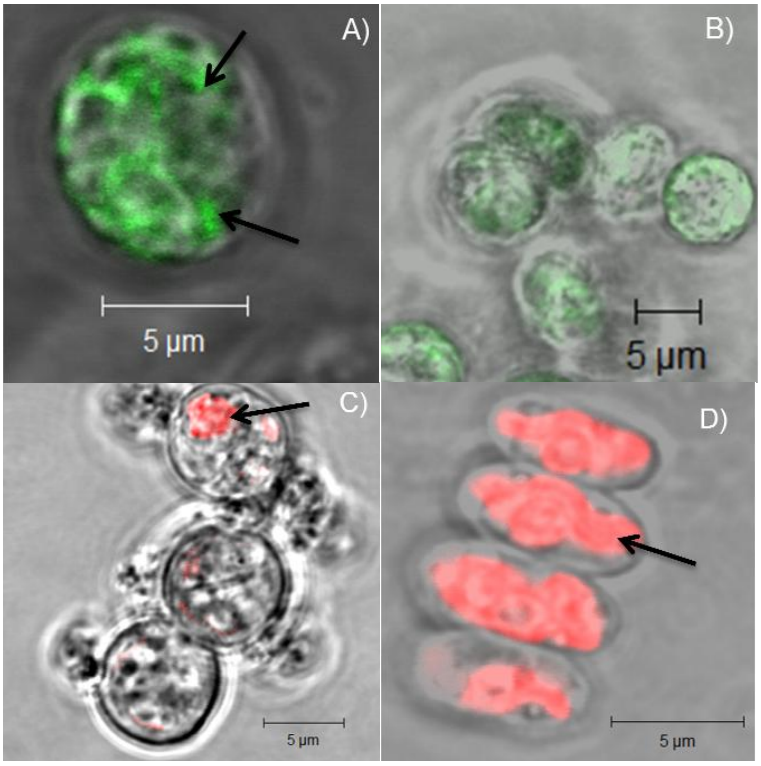

Figure 3. Cells of A) *Chlamydomonas gloeopara* (CMGO), B) *C. mexicana* (CMM), C) *Chlorella vulgaris* (CLV) and D) *Scenedesmus obliquus* (SCO) grown at 100 and 300 $\mu E\ m^{-2}s^{-1}$. Arrows indicate autofluorescence.

The microalgae culture obtained at two irradiances were recovered and resuspended a sulfur-deprived medium and grown under anaerobic conditions. This methodology guarantees the hydrogen evolution after a few days of incubation in light since the photosynthetic activity decreases below the respiratory activity [13]. Figure 4 shows the gas evolution after incubation in anaerobic conditions for the strains tested. It was noticeable that *Scenedesmus obliquus* had the highest gas evolution rate (1.522 ml d^{-1}) and also the smallest adaptation time, however it was not the highest producer and the gas evolution stopped at the 10^{th}day with a total volume of 8 ml. *Chlorella vulgaris* displayed a gas evolution rate of 1.229 ml d^{-1} and reached a gas volume of 10 ml at the end of the incubation time. *Chlamydomonas gloeopara* and *C. mexicana* had similar gas evolution rate (1.042 and 1.017 ml d^{-1}, respectively) and total volume (8.5 and 7.5 ml, respectively).

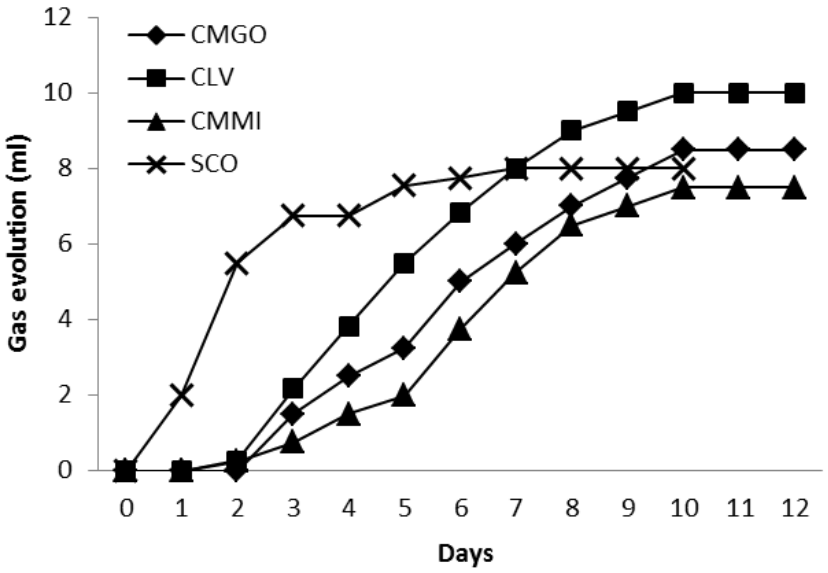

Figure 4. Gas evolution by *Chlamydomonas gloeopara* (CMGO), *C. mexicana* (CMM), *Chlorella vulgaris* (CLV) and *Scenedesmus obliquus* (SCO) by anaerobic induction in a sulfur-deprived medium.

4. Conclusions

Irradiances of 100 and 300 μE $m^{-2}s^{-1}$ were tested to study their effects on different growth parameters in strains of microalgae isolated in Mexico; *Chlamydomonas mexicana, C. gloeopara, Chlorella vulgaris* and *Scenedesmus obliquus*. The results showed that photoinhibition was only observed at the first days of incubation, and then the growth parameters were very similar in all strains. This allows suggesting that the irradiancies applied were not enough to do damage to the photosynthetic system in the strains tested. On the other hand, all strains displayed gas evolution after their incubation under anaerobic conditions in a sulfur-deprived medium; *Scenedesmus obliquus* showed the highest gas evolution rate followed by *Chlorella vulgaris, Chlamydomonas gloeopara* and *C. mexicana*. But, the highest gas producer was *Chlorella vulgaris* reaching 10 ml at the end of 10 days of incubation.

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