HYDROGEN FROM MICROALGAE

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Abstract. *The growing demand for biofuels results in more investments in research and development for renewable energy sources. In this way, the hydrogen production by biological pathways proves to be a promising alternative in this area. This study describes the efforts to produce H_2 from the cultivation of the microalgae Chlamydomonas chlorastera through the measurement in the laboratory of the growth curves of cultures to be used in the near future for the development of mathematical models aiming at the modeling, simulation and optimization of the entire industrial scale process of producing hydrogen from microalgae.*

Keywords*: biofuels, hydrogen, microalgae, photobioreactors, green energy.*

1. INTRODUCTION

The modern society is eager to find alternative energy sources in order to cope with the depleting fossil fuel sources scenario. Also, the global energy matrix based on the use of fossil fuels is now recognized as the major cause of most global environmental problems. Kruse *et al*. (2005) point out the emergence in the pursuit and development of fuels with zero emissions of CO_2 due to the rapid depletion of oil reserves, which requires the replacement of infrastructure in exploitation models.

In this scenario, microalgae have been shown to be a promising alternative when it comes to developing alternative energy sources, with particular attention to the production of biodiesel from microalgae oil, which has been extensively studied by the Sustainable Energy Research and Development Center (NPDEAS) at Federal University of Parana (UFPR), Curitiba, Brazil.

One possibility regarding the use of microalgae refers to the use of their photosynthetic apparatus for the production of H_2, which results in the production of highly energetic fuel that does not contribute to environmental pollution and to the aggravation of the problems related to climate change on a global scale (Melis *et al*., 2006).

The process of H_2 producing from the cultivation of microalgae does not occur spontaneously in nature, therefore, proper conditions must be created to the development process. At first, the cultures need to pass through a phase of growth and cell multiplication, so that microalgal biomass is generated. Next, the microalgae are transferred to a system in which the cultures are deprived of sulfur nutrients in anaerobic conditions (i.e., without oxygen) in the dark, as the enzyme responsible for catalyzing the reaction of H_2 production is suppressed in presence of O_2 (Kosourov *et al*., 2012). As this process leads to a reduction in energy reserves and consequent physiological stress to the cell, their maintenance is possible only for a short period of time.

Most studies addressing H_2 production from the photosynthetic apparatus of microalgae ars based on the use of the microalgae species *Chlamydomonas reinhardtii*, which has been extensively studied as a model organism in many areas in biology. In this study, we used the species *Chlamydomonas chlorastera*, which is closely related to the model organism and shows potential for the production of H_2.

Thus, the objective of this study is to evaluate the potential for generating H_2 from microalgae and collect experimental data for the future development of a mathematical model aimed at the modeling, simulation and optimization of the entire industrial scale process of producing hydrogen from the microalgae *Chlamydomonas chlorastera*, and possibly from other species.

2. MATERIALS AND METHODS

All stages of the experiment were performed in the NPDEAS laboratories at UFPR, in an environment with constant light and controlled temperature, with the duration of 14 days from inoculation until maximum growth was achieved.

As inoculums, strains of the microalgae species *Chlamydomonas chlorastera* were used, through a courtesy of the Federal University of São Carlos, and maintained by NPDEAS.

2.1 Conditions of cultures

The experiment was performed in triplicate in Erlenmeyer flasks with final volume of 1.5 L and TAP medium (Tris-Acetate-Phosphate) under the following conditions: a) constant lighting without photoperiod, using 2 lamps with 40 watts and light intensity of about 5,000 Lux; b) stirring was provided constantly by injection of atmospheric air sent by

a compressor, with an average volumetric flow rate of 3 L.min^{-1}; c) temperature was kept at about 22º C throughout the duration of the experiment.

2.2 Determining the growth of microalgae

In order to evaluate the growth of microalgae in culture, the cell concentration was measured by direct counting with an optical microscope (Lourenço, 2006) daily over 14 days of the experiment.

Daily, samples were collected from 1.5 mL per flask culture. After collecting the three samples, they were mixed to correct homogenization of rates relating to individual vials. After the process of collecting and mixing, the counts were made in triplicate in a Neubauer chamber.

The results of the counts were used to describe the increased cellular concentration during the cultivation period.

3. RESULTS AND DISCUSSION

Using the daily measurements of the cellular concentration, it was possible to construct a graph that expressing the increase in cell number along the time of the experiment, as shown in Fig. 1.

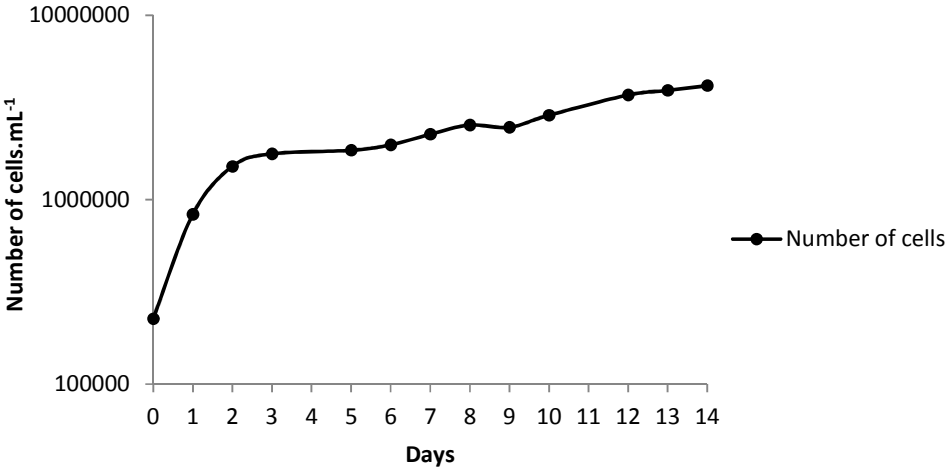

Figure 1. Growth curve of *Chlamydomonas chlorastera* along the period of the experiment

Table 1 shows the cellular concentration data obtained during the 14 days of cultivation.

Table 1. Number and average of cells along the experiment.

Days	Number of cells (x10^4).ml^{-1}			Average
0	26	24	18	22.66667
1	88	84	78	83.33333
2	155	151	149	151.6667
3	187	172	173	177.3333
5	188	182	186	185.3333
6	201	202	192	198.3333
7	230	230	219	226.3333
8	252	263	247	254
9	253	272	269	264.6667
10	283	288	291	287.3333
12	364	375	370	369.6667
13	388	398	388	391.3333
14	396	421	429	415.3333

Using the data obtained by direct microscopic counting, we calculated the growth rates of crops, the number of duplications per day and generation time, which expresses the estimated time to double the population of microalgae.

Table 2 presents data that will be used in a follow up study for the mathematical model of the hydrogen from microalgae process.

Table 2. Values calculated for growth rate, number of duplication per day and time of generation of cultures.

Time interval (days)	**(0-1)**	**(1-2)**	**(2-3)**	**(3-5)**	**(5-6)**	**(6-7)**	**(7-8)**	**(8-9)**	**(9-10)**	**(10-12)**	**(12-13)**	**(13-14)**
Growth rate	1.30	0.5988	0.156	0.022	0.068	0.132	0.11	0.041	0.08	0.12	0.056	0.06
Duplication per day	1.88	0.8639	0.225	0.032	0.098	0.19	0.167	0.06	0.119	0.18	0.082	0.08
Generation time	0.53	1.1574	4.43	31.41	10.22	5.25	6.01	16.86	8.43	5.50	12.16	11.64

In general, microalgae cultures have different growth phases that consist of different responses of cells to the conditions provided by the medium.

The *lag* phase is the period of adaptation to the new culture medium and in general depends on the different media used, and might not occur if the conditions are similar to the original medium the microalgae came from. In our experiments this phase was not observed, because the cultures were pre-adapted to TAP medium.

The *log* phase is represented by the period of exponential growth of cultures. This phase was observed since the experiment starting point, and lasted until the third day of culture which is clearly seen through the high rates of cell growth in Fig. 1, as well as the daily number of duplications and generation time.

In a recent work (Geier *et al*., 2012) obtained similar results, reaching cellular concentrations of approximately 10^7 cells / mL in around 60 hours under similar conditions to our experiment.

From the third day of the experiment until the end of cultivation, the stationary phase was characterized, in which growth rates remain low and the cell multiplication is similar to the number of dead cells in the period. The phase of decline and death occurs when there is a decrease in cell number due to depletion of nutrients present in the culture medium. This phase was not observed in this experiment because the cultures were stopped before they reached that point.

Based on the scientific literature, and in the planning for the further steps of the development of the hydrogen from microalgae process at NPDEAS/UFPR, Fig. 2 proposes a flowchart for the entire hydrogen production process. The procedure starts with the growth of microalgae in aerobic conditions, followed by inoculation in an anaerobic system producer of H_2, where the production is checked by chromatography, and the physiological state of algae is analyzed by optical microscopy. At the end of this phase, the residual biomass will be used by a biodigestor for methane generation or for extracting lipids and subsequent use in biodiesel production.

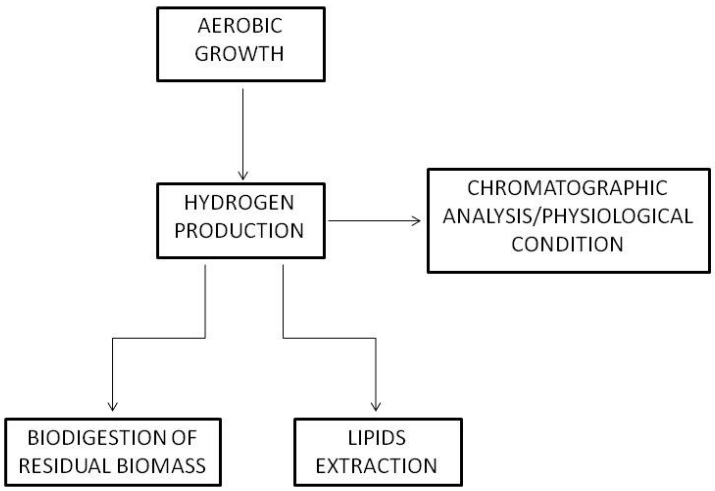

Figure 2. Flowchart of the H_2 production and the possible uses for the residual biomass.

Although the production of H_2 from microalgae cultivation appears to be an attractive alternative for energy production, the group believes there is a need to improve the process as a whole.

Following this line of reasoning and the work done so far, the next steps consist of quantifying the production of H_2 under the proposed conditions, make changes or substitutions in the culture media seeking to reduce costs, development

of the reactor design for the anaerobic phase and the identification of the bioproducts from the residual biomass in the end of the process. In parallel, a mathematical model of all processes is under development, so that other microalgae species could have their potential for hydrogen production evaluated, and the individual components and steps could be optimized for maximum global thermodynamic performance.

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