ISSN 2176-5480

22nd International Congress of Mechanical Engineering (COBEM 2013)
November 3-7, 2013, Ribeirão Preto, SP, Brazil

PHOTOBIOREACTOR FOR MICROALGAE CULTIVATION WITH CO_2 ADITION

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Abstract. *This paper presents the experiences of microalgae "Dunaliella tertiolecta" cultivation, with different concentration of CO_2 in photobioreactor, as feedstock oil for biodiesel production. The advantage of using photobioreactor for the growth of microalgae is the possibility of process control parameters. The effect of addition of dissolved CO_2 in atmospheric air at concentrations of 2%, 4% and 6% CO_2/air as a carbon source in the growth of microalgae was also evaluated by varying time of culture in each experiment. These were also compared to experiments without the addition of CO_2. The results indicate maximize growth with added CO_2 concentration of 2%. Also other parameters were controlled, such as light intensity, temperature, gas mixture, among others, to maximize the growth of microalgae and oil yield. The numbers of microalgae were counted by the method of Neubauer chamber. The results show the applicability of the experiment.*

Keywords: *photobioreactor; microalgae; biodiesel; Dunaliella tertiolecta; biofuel.*

1. INTRODUCTION

A proposal to reduce the fossil fuels exploration and its fire has been encouraged by the biodiesel use, and its production has origin on vegetable oils. The biodiesel has interesting properties, such as the absolute compatibility with the petroleum diesel. Other advantage is the low generation of polluting compounds in the exhaust gases, as well as its raw material is from renewable sources (Krahl and Knothe, 2005). Microalgae have been considered a potential and useful sources for the of biodiesel production's in the future, because several species have high concentrations of lipids, with high energies (Lawrence, 2006).

Different studies show that microalgae have a higher performance for extracting oil than oilseeds, such as colza, soybean, palm, sunflower, etc. Therefore, microalgaes could, in thesis, produce more oil per hectare than others cultures, not impact with foods oils supplies and make reduce the cost of biofuels and (Chisti, 2007).

The microalgae's cells growth, in the culture medium where they are inserted, is affected by the combinations of various parameters, among of these parameters can be cited: quantities and types of nutrients, light intensity, photoperiod, temperature, etc. (Tang *et al.*, 2010).

The best design of the photobioreactor chose's, is very important, and also is target of several studies. A photobioreactor project made priority the light exposition and its contact with de microalgae, to maximize the photosynthesis performance. The gases exchange between the cell and the culture environment and a good gas and liquid mixture phases, as well as the contact between the CO_2 and the cells is also crucial (Carlozzi, 2008).

Based on the acquaintance of the intensive microalgae photosynthesis', several studies suggest the injection of CO_2 in higher concentrations than present in atmospheric air. Suggesting a CO_2 capture and reducing their presence in the atmosphere concentrations. At the same time, increasing cell growth, and increasing the oil production (Muradyan *et al.* 2004).

2. MATERIALS AND METHODS

For the present study, was used a laboratory tubular photobioreactor unit, with a total volume of 5L. It may be disassembling the construction in four stages: Mechanical Structure; Electrical Installation, Gases lines Installation (CO_2 and compressed air), and Photobioreactor Installation.

2.1 The experimental unit

The mechanical support structure is made up of profile bars "L", 100 cm length, 70 cm width and 64 cm depth. The illumination system consists of 6 white fluorescent lamps with 40 W each. To prevent an external light influence, the walls were protected with Styrofoam plates, wrapped in a metal reflexive foil.

The gases (CO_2 and compressed air) valves connections were installed in a galvanized sheet and fixed to the side of the structure. The CO_2 supply was done by CO_2 cylinder, Praxair brand of 7 liters in volume and 99.99% of purity. To working pressure, it was $1{,}013 \times 10^5$ Pa, being regulated in the cylinder pressure gauge. To regulate the CO_2 flow, was

ISSN 2176-5480

installed in the gas line a valve with manual adjustment. Compressed air, was supplied by online laboratory, and installed a micrometric valve for flow regulation, with also regulated to 1,013 x10^5 Pa. The calibration of both gases was made in a graduated manual flow meter.

The mixture of CO_2 and compressed air was injected after the pump and immediately prior to the photobioreactor, thereby ensuring the mixture between the gas and the liquid phases.

The photobioreactor was constructed in one piece, using glass tubing to light collector made in borosilicate. The connections used are of PVC (Poly Vinyl Chloride) and the degasser is also in plastic material. The pump used is typical for use in home aquariums, it's a submerged pump, model S300 Sarlobetter, with maximum flow of 280 L/h. The photobioreactor was deposited on steel coated with aluminum foil, just below the lamps. An overview of the system can be seen in Fig. 1.

This photobioreactor model follows a tubular reactor in "scale-down", as suggested by Rosello *et al.* (2007), and the works reference of Acién Fernandez *et al.* (2001).

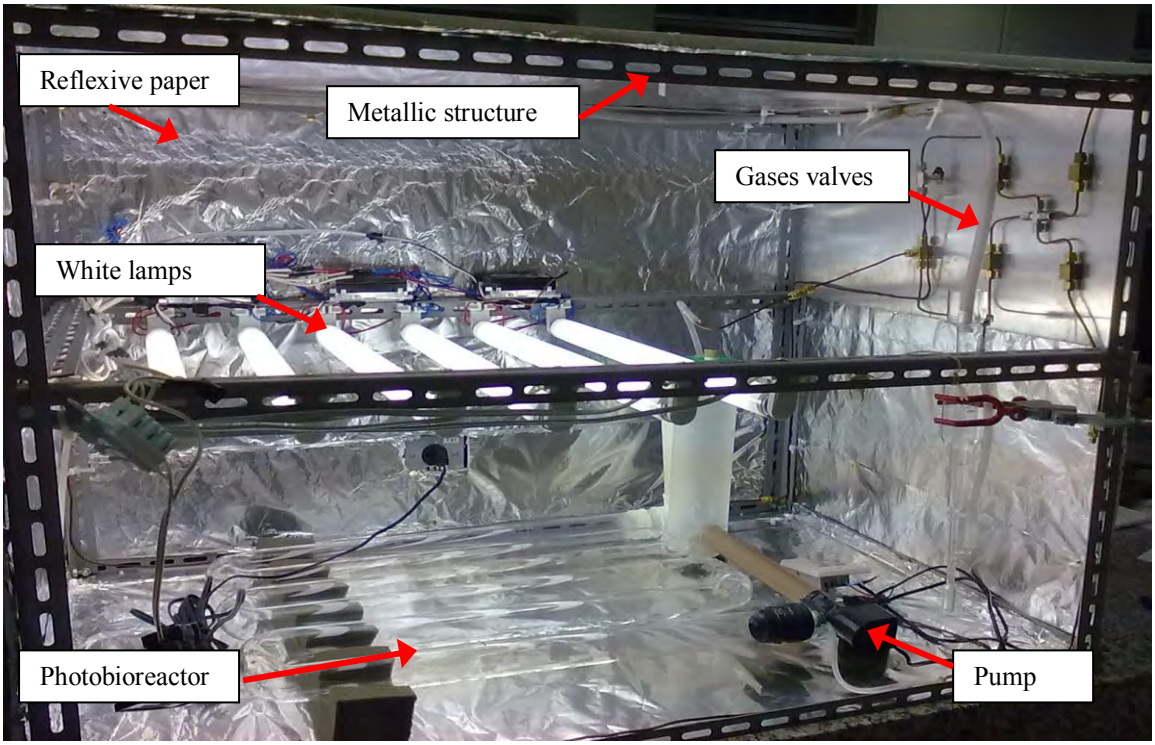

Figure 1. Experimental unit and components.

2.2 Preservation of microalgae's inoculum

For the initial maintenance of microalgae used in this study, was used a germination chamber with alternating temperature and photoperiod, brand Cientec, in a photoperiod of 12 h: 12 h (light: dark) and temperature of 19ºC. The Erlenmeyer flasks were initially subjected to autoclaving to sterilization.

In each experiment were prepared 5.0 (five) liters of culture medium solution type Gillard- "f/2" modified, and subsequently 4.0 L were transferred into the photobioreactor, after sterilization. The remaining 1L was used to make a new inoculum to future assays, being held weekly.

The seawater used was from the Institute of Eco-development of the bay of Ilha Grande, located in Angra dos Reis, RJ, whose treatment consists in a filtration system and ultraviolet disinfection.

2.3 Operating System

Strains were chosen from *Dunaliella tertiolecta* for each of the experiments, according to which showed the most promising of cell viability analysis, in a Neubauer chamber. In each new batch, the tubular photobioreactor was washed with water and 10% of commercial bleach, rinse, with drinking water and sterilized in ultraviolet radiation chamber for 45 minutes.

All of the assays were evaluated in a simple batch, where no permission of reactants entrance or exit products during the process (Fogler, 2002). Were also subjected to the same light intensity, which was measured with a lux meter, Icel brand, model LD-520.

ISSN 2176-5480

For each batch start, the inoculum was removed from microalgae germination chamber, previously counted to the water sea dilution and calculated to contain 10^5 cells/mL inside photobioreactor. The solution was added to the photobioreactor by degasser.

As the aim is evaluate the effect of different CO_2/air concentrations and their influence on the microalgae growing, as a carbon source for the microalgae metabolism, it was evaluated on 3 different concentrations, being: 2%, 4% and 6%.

The routine of tests were 5 days, duration of each batch. After that, was not observed significant growth of cells numbers, this period was established because it's enough to arrive at the stationary phase. The photobioreactor was emptied and cleared to other batch.

During the operation, daily, a rate of about 10 ml was withdrawn from the tubular photobioreactor to make the pH measurement and counting of cells. After reaching the stationary phase, after five days, or being evident the population of microalgae decline, all the contents in the photobioreactor was removed, so that, it could start a new test. The cell counting, was done using an Improved Neubauer Chamber, of Optik New, were also made measurements of pH, on Quimis pH meter.

The Neubauer counting chamber were done to achieve approximately the number of cells 400, and then through the multiplying factor conversion, provided by the method. The results heave an error of 10%, in number of cells/ml, it was judged acceptable for this study (Lawrence, 2006).

3. RESULTS AND DISCUSSION

The results follow, from tests at 2%, 4% and 6% CO2/air concentrations, and then they were compared with the best test conducted by Soares (2010), who used the same photobioreactor, but just with atmospheric air injection.

All the assays were subjected to the same light intensity, whose value presented 4,880 lux, on the photobioreactor surface (average of 7 points). The temperature range during the tests oscillated from 24ºC to 26ºC. The initial pH of the culture medium was the same as seawater, about 7.2, and was alkalinized with de CO_2 in seawater dilution.

3.1 Testing performed by injecting a 2% CO_2/air mixture.

The results presented in Fig. 2 were repeated feeding the photobioreactor with 10^5 cells/mL, and then CO_2/air mixture was injected in a concentration of 2%.

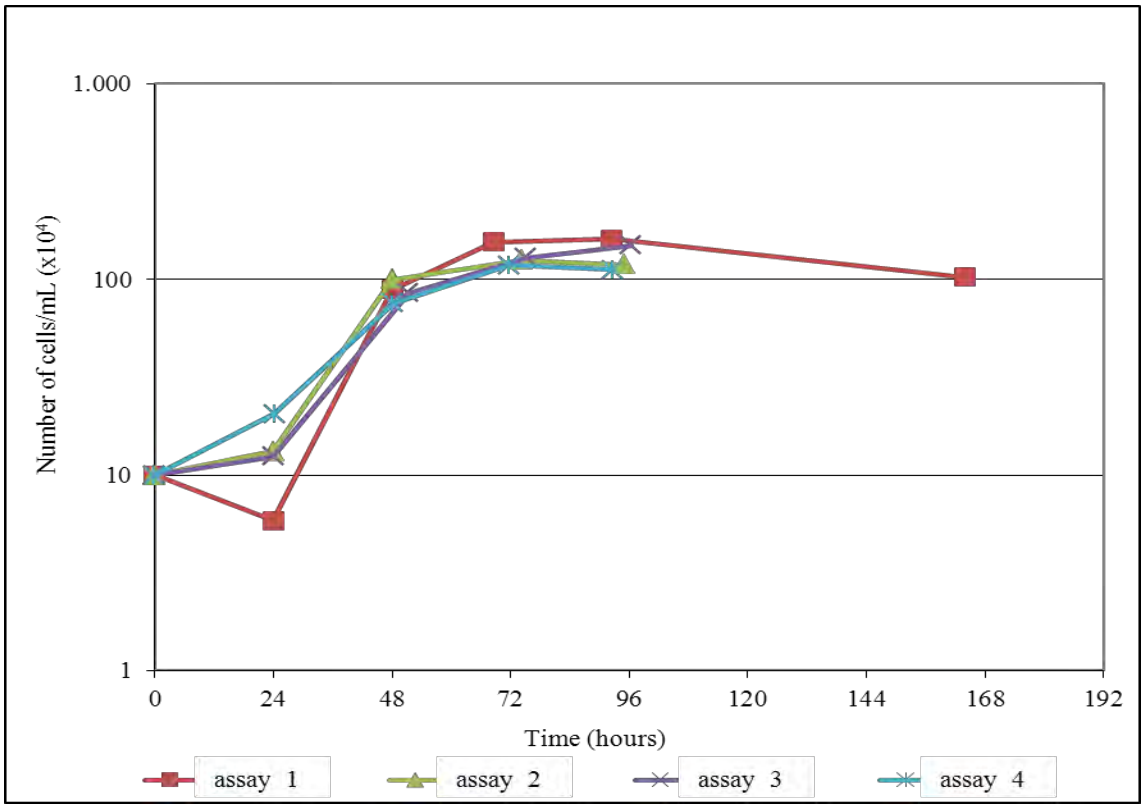

Figure 2. Test of microalgae growth in the photobioreactor with 2% of CO_2/air.

ISSN 2176-5480

Evaluating the Fig. 2, it can be observed that after 24 hours, cells exponential growth in trial 1 and had very intensive up to 72 hours. Cell density has a little difference in the time between 72 and 96 hours, when it reached its maximum value. It was the best result of this concentration of CO_2/air, the cells number was $1.55x10^6$ cells/mL.

The remaining trials 2, 3 and 4 are replicas to better understand the profile in these culture conditions. Values resulted in the same growth profile trial 1 and the difference between them is not significant. This further indicates that after 96 hours of growing, it was entered in the stationary phase.

3.2 Testing performed by injecting a mixture of 4% CO_2/air

There was an exponential growth within the first 48 hours for most tests, as shown in Fig. 3. The maximum value was for the time of 72 hours.

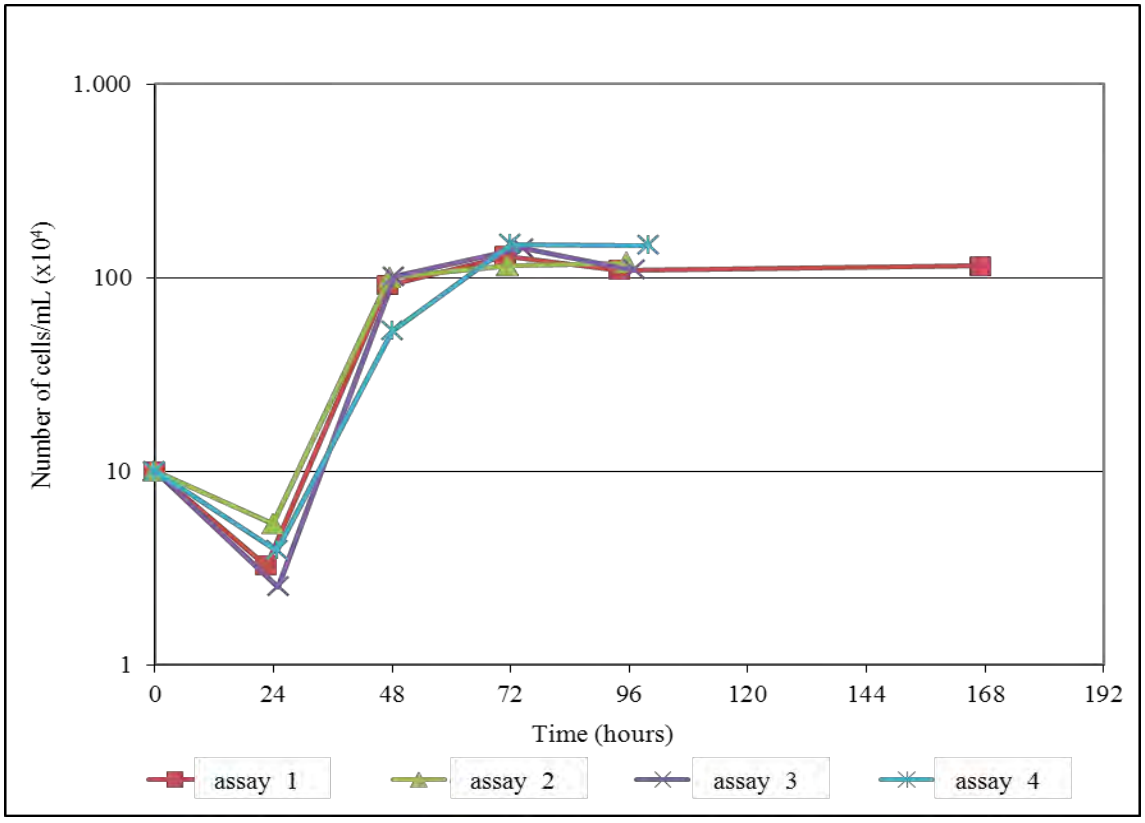

Figure 03. Test of microalgae growth in the photobioreactor with 4% of CO_2/air.

The profile of experiments at 4% was similar as 2%, but the cells growing were not so good than that concentration. The best value was $1.48x10^6$ cells/mL.

The trial 4 had a result outside the profile of the other three tests up to 48 hours. However, a final balance in the cells number it was higher than the others one. Considering the test 4 as showed, it presented a lower performance in the first 48 hours, and this fact can be explained by relating of the growth with the pH, which in the first 24h was the lowest in the whole experiments, about value of 6.2.

3.3 Testing performed by injecting a mixture of 6% CO_2/air

The concentration of 6% CO_2 in air was the highest concentration evaluated in this study. The inoculum was used in order to start the tests with 10^5 cells, as usual, in the tubular photobioreactor.

Figure 4 shows that the curves have similar behavior, with exponential growth after 24 hours and reached its maximum in 96 hours of operation of the photobioreactor.

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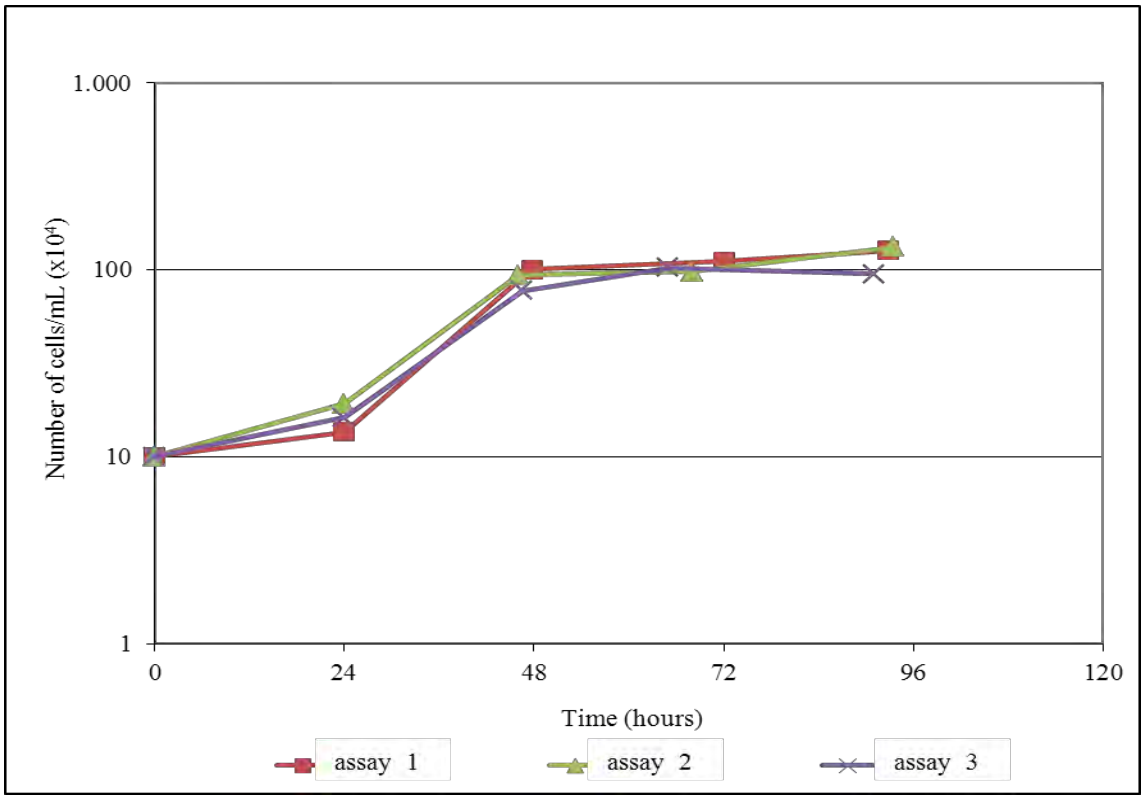

Figure 4. Test of microalgae growth in the photobioreactor with 6% of CO_2/air.

3.4 Comparison between just atmospheric air and CO_2 mixture injection, on photobioreactor.

The comparison is useful because it reveals considerable increase in cell growth, increasing biomass production and consequently oil for future conversion into biodiesel. Thus, Fig. 5 shows the results of the best performance in tests conducted with the injection of CO_2 in concentrations of 2%, 4% and 6%.

The values were compared with the Soares (2011) assay, which had not performed experiments with CO_2 injection, but citing experiments Tang *et al.* (2010) highlights a considerable increase in the cells growth of obtained by this using low concentration of CO_2. The results are showed in Fig. 5.

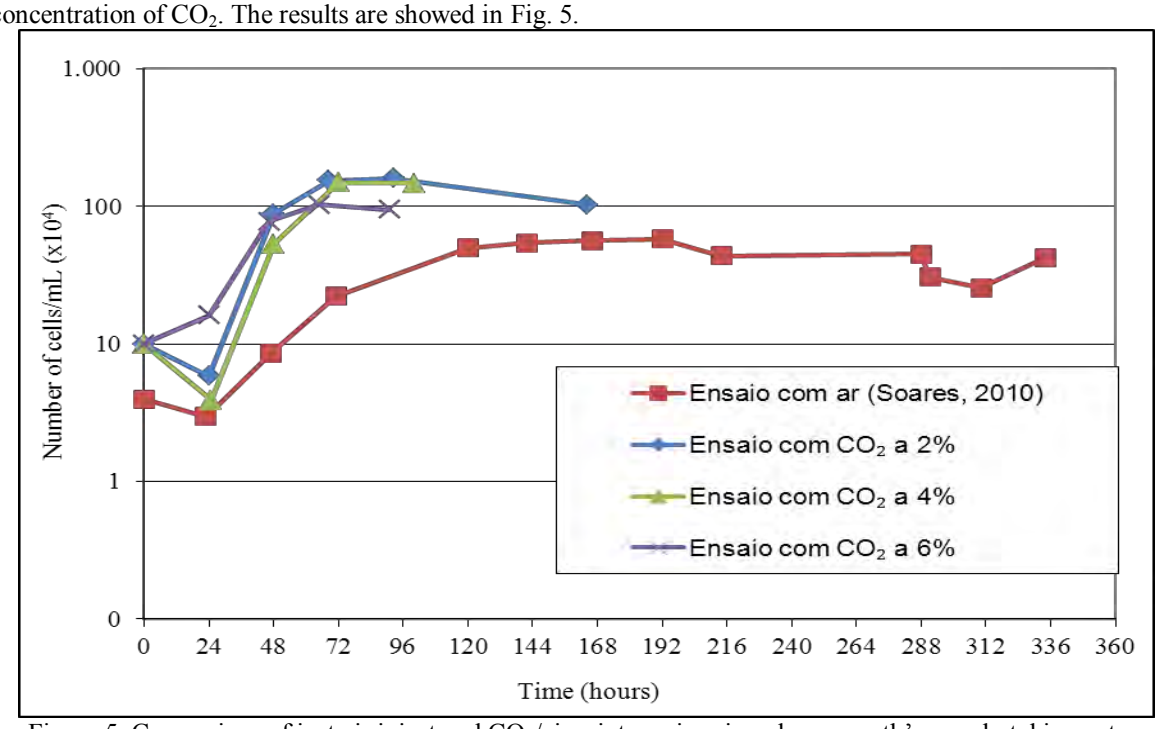

Figure 5. Comparison of just air inject and CO_2/air mixtures in microalgae growth's, on photobioreactor.

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Analysis of cell growth in *Dunaliella tertiolecta* culture, Soares (2010) with just atmospheric air injection, that obtained cell concentration reached its maximum at time of 192 hours (after 8 days of culturing), corresponding to $5.81x10^5$ cells / mL in the photobioreactor. After 120 hours of the beginning, when the cell concentration was $4.99x10^5$ cells / mL, there was less growth.

When considering the CO_2 concentration of 2%, there is an intense cell growth 24h from reaching a cell density of 1.55×10^6 cells / mL in a 72 hour period, or in just 3 days of onset FBR operation.

For the test with an injection of 4% CO_2, it was found that cell growth curve was very similar to the growth profile of 2% CO_2, however, the cell concentration reached was less than this, about 1.48×10^6 cells / mL for 72 hours.

In the case of the mixture of 6% CO_2, the results showed maximum 1.02×10^6 cells / mL after 3 days of culture.

4. CONCLUSIONS

It may concluded that the addition of CO_2/air mixtures for microalgae cultivation's *Dunaliella tertiolecta* using of tubular photobioreactor, has great benefits if compared to without CO_2 for further growth reaches individuals with values above the stationary phase and lower time beyond that in closed systems, as in the photobioreactor used, it is possible to control the process parameters.

The study showed that, for the conditions of the experiment, the optimum concentration of CO_2/air was 2%. The addition of different concentrations of CO_2 can be extended widely, and the light intensity over culture medium, among others.

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6. RESPONSIBILITY NOTICE

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