DOI 10.7603/s40682-016-0004-y

Revista Latinoamericana de Biotecnología Ambiental y Algal
Vol. 7 No. 2 p. 42-56.

Artículo Original

Monitoring the CO_2 consumption of *Monoraphidium* sp. microalgae: Characterization of algal biomass produced

Figuera, A[1*].; Reyes, Y[1].; González, R[1].; Paula, R[1].; Basto, L[2].; Aranda, D[1].

[1.]Greentec Laboratory, School of Chemistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil (*Corresponding author: figuera.andreina@gmail.com)
[2.]ECO 100 Company, Rio de Janeiro Brazil (luciano@ivig.coppe.ufrj.br).

Abstract

Microalgae are unicellular organisms capable of photosynthesis, turning sunlight and carbon dioxide (CO_2) into rich biomass, precisely because of this definition in recent years, various sectors have been targeting the ability to reduce CO_2 emissions and the capacity of simultaneously synthesize biomass which can later be used to produce bio-fuels. In respect to the capturing and utilization of CO_2 emitted by different industries (cement, generation of electric energy, cellulose and ethanol production by fermentation of sugars) for the cultivation of microalgae destined to the production of bio-fuels, for example, biodiesel, ethanol or bio-oil. However, this research was developed in the Green Technologies Laboratory - Greentec / EQ / UFRJ, which focussed on the monitoring of CO_2 consumption in microalgae *Monoraphidium* sp. cultivated in closed-window type photobioreactor, as well as, the characterization of the microalgal biomass produced in relation to the total lipid content (TL), convertible lipids into biodiesel (CLB), carbohydrates and proteins.The overall procedure involved the evaluation of the following parameters: injection of CO_2, temperature (°C), lighting (μE m^{-2} s^{-1}), pH, cell density (cells ml^{-1}) and dry biomass. From the results of this study, it was observed that at the beginning of the culture (day 0) 0.79 g of CO_2 were consumed per each gram of biomass produced. On the last day (12), 0.3 g of CO_2 were consumed per gram of culture. On the sixth day of cultivation, the consumption of CO_2 per gram of biomass increased, resulting in a CO_2 consumption of 0.61 g. The best result was obtained on the second day of cultivation, when for each gram of biomass produced approximately 1.2 grams of CO_2 were consumed. The biomass *Monoraphidium* sp. produced, contained 17,37 ± 3,27% of total lipid content, approximately 8.36 ± 2.69% of convertible lipids into biodiesel, 32% ± 3.37 of carbohydrates and 34.26% ± 0.41 of protein. The analysis performed by -GC-MS Gas chromatography showed the following composition of saturated fatty acids (SAFAs) mainly the C16:0 (palmitic), as in monounsaturated acids (MUFAs), in high quantity C18:1 (oleic) and polyunsaturated acids (PUFAs) mainly represented by C18:2 (Linoleic) and C18:3 (linolenic).

Keywords: *Microalgae*, *Photobioreactor*, *CO_2 capture*, *Greenhouse effect gas*.

1. INTRODUCTION

Many factors, some of which are related to industrial development and diverse agricultural practices (forest burning), are responsible for large quantities of carbon monoxide and dioxide in the atmosphere. The most visible effect of the increasing amount of carbonic emissions is global warming and the reduction of the polar ice caps, lately growing at a faster pace.

The Emissions Gap Report 2015 published by the United Nations Environment Programme (UNEP). According to data compiled by the document, the efforts made will reduce emissions of six gigatons a year by 2030, bringing the conclusion that the world has come half way towards the necessary cuts in emissions to limit global temperature rise to 2 °C by the year 2100.To meet that goal, experts state that a reduction of over 12 gigatons of carbon dioxide is required. This document represents the evaluation of a team of global scientists about the promises of cuts in gas emissions, called National Voluntary Contributions made by 146 countries, including Brazil. These nations together are responsible for over 88% of all greenhouse gas emissions in the world.

In Brazil, during the United Nations (UN) Summit on Sustainable Development in September 2015, President Dilma Rousseff had already announced that the country plans to reduce by 37% the emissions that cause the greenhouse effect until the year2025, compared to levels recorded in 2005, reaching 43% by 2030.

In this context, this research has developed aims in capitalizing on the CO_2 removed in the biogas purification process, from its bio-fixation in microalgae cultivation for the production of renewable hydrocarbons.

According to the literature, one kilogram of dry biomass microalgae uses approximately 1.83 kilograms of CO_2 (Razzak *et al*, 2013; Sanchez-Voltolina & Saavedra, 2005). These organisms can produce fatty acids, oils and hydrocarbons. In some conditions, microalgae can produce high concentrations of lipids, on average 20-40% of their dry weight, reaching concentrations of up to 85% (Richmond, 1988; Sánchez-Saavedra &Voltolina, 2005).

Lipids are one of the main components of microalgae. Depending on the species and growth conditions, microalgae contain around 2-60 % lipids of the total cell dry weight. Lipids derived from microalgae have been the focus of considerable interest because these oils contain fatty acid and triglyceride compounds that can be esterified to obtain alcohol and esters. The resulting so-called ester fuels have proven suitable for blending with diesel stocks, up to 30% without affecting engine performance (Brindley C, *et al* 2002).

2. MATERIALS AND METHODS

2.1. Microorganism

The lineage of chloroficea gender: *Monoraphidium* sp. (Fig. 1A) courtesy of the Laboratory of Ecophysiology and Toxicology of Cyanobacteria (LETC) of the Institute of Biophysics Carlos Chagas Filho, UFRJ. The microalgae were kept in maintenance medium ASM-1 at about 23 °C and 12 hours of photoperiod. The microalgae in the pilot photobioreactor used the same medium.

2.2. Maintenance and propagation of cultures

The strain or standard culture used in the study was stored under controlled conditions in a screw-top test tube containing ASM-1medium (pH 7) kept in an incubator BOD 411D, with temperature controlled at 23 ° C and illuminated by four (4) 8 watts (W) fluorescent lamps providing a light intensity of 500 µE $m^{-2}s^{-1}$ (Fig. 1B).

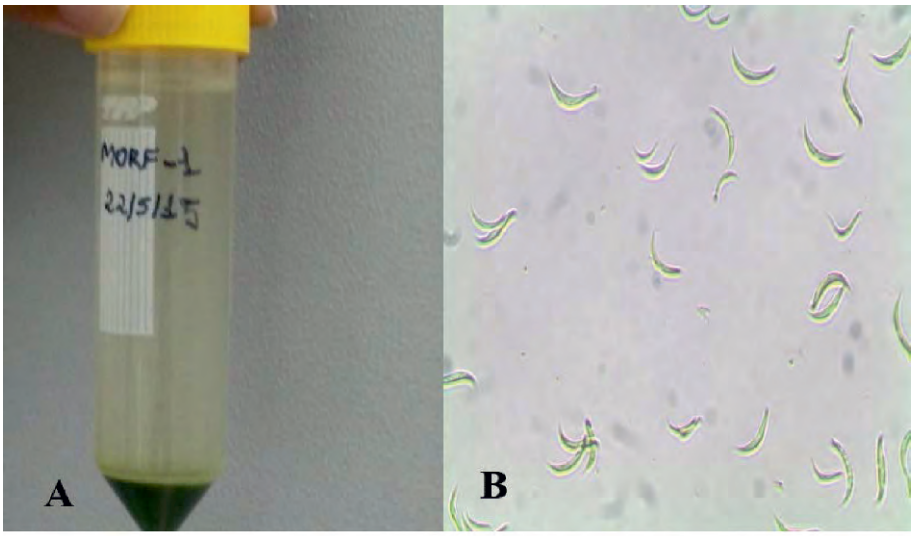

Figure 1. (A) Inoculum lineage *Monoraphidium* sp. **(B)** Image of microalgae *Monoraphidium* sp. observed through the light microscope Olympus BX increase with 100 X.

2.2.1. Inoculum in 250 mL Erlenmeyer flask

In the preparation of the inoculum of 250 mL a ratio of 1:10 was used (inoculum: culture medium sterilized). The flask used in the culture was kept at a controlled temperature of 23 ± 2 °C, light intensity of 314.77 µE m^2s^{-1}, 12 hours of photoperiod and pH of 8.0 (Fig. 2).

2.2.2. Inoculum in the flask of 2L

After five (5) days of growth in a 250 mL erlenmeyer flask, 10% of the algal culture from 200 mL of inoculum were transferred to the 2 L conical flask contain 1.6 L of the same culture medium (Fig. 3). The amount of algal culture in the 2 L conical flask was held for a period of five days (5) at temperature of 23 ± 2 ºC, illumination of approximately 314.77 µE m^2s^{-1}(measured using photosynthetically active radiation PAR) Biospherical Instruments Inc. model 2101 QSL logger 2100 software with 12-12 hours photoperiod (light/dark), air flow rate of 4 mL min^{-1} and the final cell concentration of 1.435 x 10^8 mL cel^{-1}, monitoring was carriedout directly bycounting in Fuchs-Rosenthal chamber, made of special optical glass, spare cover glass 20 x 26 x 0.4 mm and depth 0.2 mm.

2.2.3. Inoculum in the transparent bottle of 20 L

The cultivation conducted in a 2 L erlenmeyer flask was used as an inoculator in the transparent bottle of 20 L, which already contained 18 L of medium ASM-1, and the final cell concentration was 1.28 x10^7 mL cel^{-1}, temperature of 22 to 23 °C, illumination 350 µE $m^{-2}s^{-1}$, air flow of 5 L min^{-1} and photoperiod of 12 hours, (Fig. 4). The culture was incubated for 5 days.

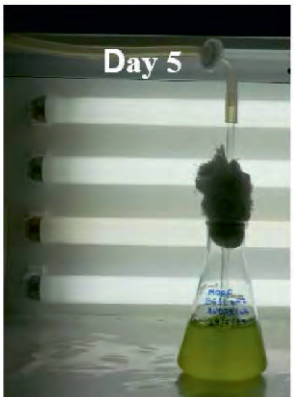

Figure 2. Culture of microalgae *Monoraphidium* sp. in a 250 mL Erlenmeyer flask, duration of five days.

Figure 3. Culture of microalgae *Monoraphidium* sp. in a 2L Erlenmeyer flask, duration of five days.

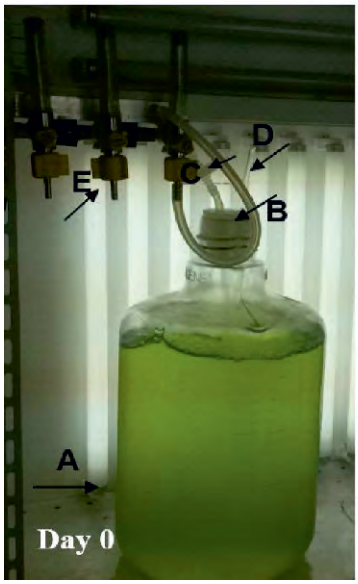

Figure 4. Inoculum of *Monoraphidium* sp. and its components in a transparent bottle of 20 L: (A) transparent bottle; (B) silicone stopper; (C) hose for air intake and filter; (D) air outlet; (E) Rotameter.

2.2.4. Culture in a pilot-scale photobioreactor

The study was conducted in a pilot–scale photobioreactor type closed-window with a capacity of 110 L, installed on the roofing of the Center of Excellence for Oil and Biofuels in the EQ / UFRJ.

In the photobioreactor, the culture inoculums used was from transparent bottle of 20 L which was added to ASM-1 medium and swelled with treated water from a microfiltration system. The culture started with an inoculum of $6.05x10^6$ cellules mL^{-1}.

During the culture days (12) remained under constant aeration by injection of compressed air with a flow rate between 5 and 7 L min^{-1}, internal temperature controlled between 25 - 35 °C and pH measured and adjusted in an automatic manner between 7.5 - 8.5 by CO_2 injection. This injection was carried out at a constant flow of 1 L min^{-1} through three porous stones placed at different points in the bottom of the photobioreactor and monitored during the period of 8:00 to 16:00 (Fig. 5).

As source of CO_2 gas, a cylinder of 30 kg with pressure of 1.5 kg-f / cm^2 was used, where the daily consumption of CO_2 and cell growth cultivation was monitored through the analysis of cell density and dry weight. Besides the CO_2 consumption (g day^{-1}) other parameters were measured daily for twelve (12) days of cultivation at: 8:00, 10:00, 12:00, 16:00 18:00 and 20:00 hours.

2.2.5. Temperature

The temperature in the experiment was measured in degrees Celsius (°C) through a thermocouple that is connected to the control panel on the photobioreactor (Fig. 5).

2.2.6. pH

The pH was measured by a pH meter submerged in the culture and coupled to an automatic control system that released CO_2 injection (1 L min^{-1}) with solenoid valve whenever the pH was above 8.5. The pH reading is done on the control panel (Fig. 5).

2.2.7. Light intensity

The ilumination was measured employing a photosynthetically active radiation (PAR) Biospherical Instruments Inc. model QSL 2101, 2100 LOGGER software, the absorption of light is in unit of micro Einsteins per square meter per second (µE $m^{-2}s^{-1}$). The equipment was calibrated before taking measurements.

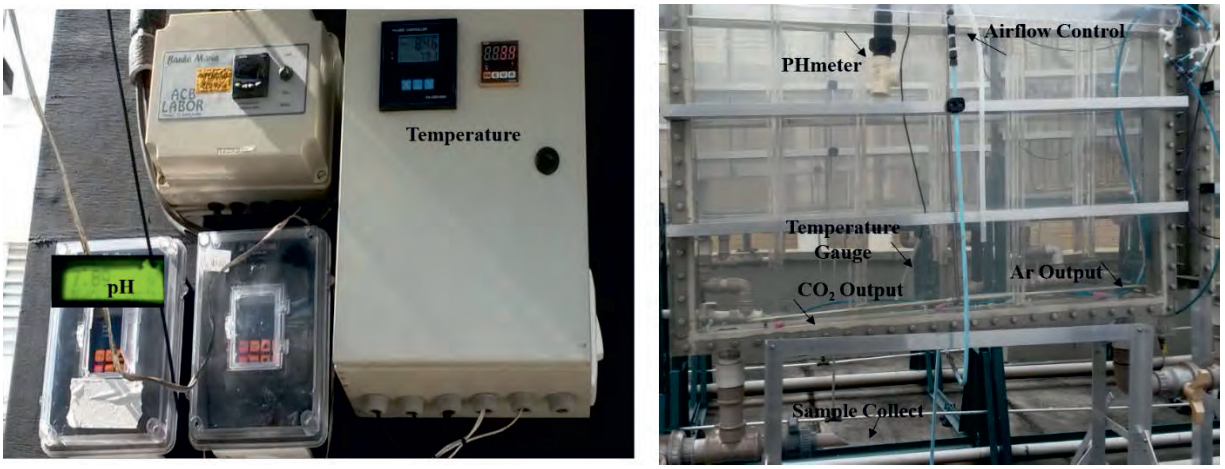

Figure 5. Control panel used for monitoring the cultivation. Components of the photobioreactor.

2.2.8. Monitoring of growth parameters of the culture

Cell growth of cultures performed in different scales was monitored by the following parameters:

2.2.9. Cell density

The cell density of the microalgae *Monoraphidium* sp., throughout the cultivation was monitored daily by counting cells under a microscope (Olympus Model BX 53) (40-fold increase), with the aid of a Fuchs-Rosenthal chamber. Cell density was expressed as number of cells per milliliter of culture (cel.mL^{-1}) and determined in triplicates. Graphs were plotted with the experimental data cell density versus time (days).

2.2.10. Analysis of dry weight

The following protocol developed by technicians intending to assist in the management of bio-products from Cenpes/Petrobras was utilized to determine the dry weight of the biomass: a membrane was calcined at 575˚C for 1h to remove humidity and cooled to note the initial mass (mi); 10 mL (Vf) of the microalgae culture were filtered in the membrane, then placed in a stove at 65˚C for 1 h; cooled to register final mass (mf) for determination of dry weight.

Consequently, the content of dry biomass and moisture content, according to equations (1)-(2)-(3), was used to calculate the values of ash and the final dry weight, the membranes with dry biomass were calcined at 575˚C for 1 hour, cooled and weighed. The dry weight determination was performed in triplicate.

$$X = \frac{(mf - mi)x1000xFD}{Vf} \quad (1)$$

$$DM = \frac{X}{10} \quad (2)$$

$$CM = 100 - DM \quad (3)$$

Where:
X = concentration of biomass or dry weight in g/L;
mi = mass of calcined membrane without the filtered microalgae (initial mass);
mf = membrane mass with dry microalgae (final weight);
Vf = volume of culture of filtered microalgae;
FD = dilution factor;
DM = dry matter content;
CM = moisture content.

2.3. Biomass concentration

After 12 days, the culture biomass is concentrated by centrifugation using centrifugal Hitachi model CR22N. The operating parameters used were 8000 rpm for 10 minutes (Fig. 6). Then the biomass was freeze-dried for characterization.

The moisture content of the concentrated microalgae biomass is determined on an electronic scale Shimadzu MOC63U model, with temperature 40-50 °C. The moisture content is shown in item 3.2 in the results section. The wet biomass of microalgae was placed in a glass beaker and frozen at -5 °C in a Brastemp BRB39AB refrigerator for 72 hours. Later, it was lyophilized, using the equipment model Labconco 4.5 liters capacity, the temperature of -45 °C and vacuum pressure of 0.220 bar (Fig. 7).

Figure 6. Concentration of the biomass by centrifugation.

Figure 7. Lyophilized biomass.

2.4. Chemical characterization of the biomass

2.4.1. Total lipid content (TL)

The method of Bligh & Dyer (1959) was used for the extraction of TL. The acid pre-treatment procedure was performed as follows: an amount corresponding to 400 mg wet biomass dry weight was weighed on an analytical balance; 5 mL of HCl 2 M was added to the biomass and homogenized with the aid of vortex model MS3 basic IKA; the tube was placed in a thermostated bath at 80 °C for 1h; centrifuged at 2500 rpm for 15 minutes to separate the biomass acidic residue; the acid residue was removed and discarded in an appropriate vial. Further, in the pre-treatment, a total lipids extraction procedure was carried out as described below:

4 mL of methanol was added to the biomass hydrolyzate and homogenized; 2 mL of chloroform were added to the suspension and stirred by vortex for 2 minutes; an additional 2 mL of chloroform was added and stirred by vortex for 2 minutes; 3.6 mL of Milli-Q® water was added, stirred by vortex for 2 minutes and centrifuged at 2500 rpm for 15 minutes.

The phase of chloroform in lipids was transferred to a flask already weighed and reserved. For the re-extraction of the lipids that were still in the biomass, 4 mL of solution of 10% v/v methanol in chloroform was added to the remaining phases and stirred by vortex for 2 minutes. The mixture was centrifuged at 2500 rpm for 15 minutes; the lipid phase was removed and placed in the flask along with the first extract.

The procedure of re-extraction was repeated once and excess chloroform was evaporated at 65 °C and atmospheric pressure. The flask was oven dried at 60 °C until constant weight. The lipid content was calculated in triplicate with the following equation:

$$TL = 100x\frac{m_3 - m_1}{m_2} \quad (4)$$

Where:
TL= total lipid;
m_1 = weight of the flask (g);
m_2 = weight of the sample (g);
m_3 = weight of the flask with extract.

2.4.2. Convertible lipids into biodiesel (CLB)

The content of convertible lipids into biodiesel was determined in triplicate by method J: Bondzynski-Schmid-Ratzlaff (modified, IDF, 1986). The procedure is described below:
In the test tube, 1.0 g of lyophilized biomass was weighed, 10 mL of 8 M hydrochloric acid (HCL) solution was added and homogenized by vortex basic IKA MS3. The samples were heated directly on a hotplate at 70 °C for 10 minutes. After the digestion time, a microscopic observation was realized in cell wall structure of microalgae to confirm the disruption. Subsequently, 10 mL of ethanol were added and was homogenized by manually shaking for 30 seconds; 25 mL of ethyl ether and 25 mL of petroleum ether were added, stirring for 1 minute. The sample was then centrifuged at 3000 rpm for 15 min. The ethereal phase was transferred to a separation funnel and washed with Milli-Q® water to remove HCl to reach a pH of 6-7, which was then transferred to a flat bottom flask. The solvent was stirred in a rotary evaporator at 65 °C and ambient pressure and the flask with the oil was dried in an oven Med Clave model 3 at 60 ° C to constant weight. The determination of the oil content was performed by the following equation:

$$CLB = 100x\frac{m_3 - m_1}{m_2} \quad (5)$$

Where:
CLB= the oil content;
m_1 = weight of the flask (g);
m_2 = weight of the sample (g);
m_3 = weight of the flask with oil.

2.4.3. Lipid profile of microalgae biomass

To analyze the profile and the conversion of fatty acids into FAME (fatty acids methyl esters) Tests were performed in triplicate. The lipid fractions were subjected to methanolysis reaction (Yoo *et al*., 2010), using 0.0752, 0.0762, and 0.0995 g of sample. Then the saponification was carried out with 2 mL of saturated solution of KOH-CH_3OH (alcoholic potassium hydroxide) at 75 °C for 10 min in a water bath.
The sample was subjected to methanolysis with 4 mL methanol with 5% HCl at 75 °C for 10 min. These steps were carried out in a closed bottle to prevent evaporation. Next, the phase containing fatty acids was separated by adding distilled water and hexane PA. The upper phase which is of hexane was collected with a pipette and transferred to glass jars and were placed in an oven at 60 °C for the evaporation of solvent and sample concentration.
In the end, the samples were weighed, injected and analyzed by Gas Chromatography with Flame Ionization Detector (GC-FID) in the chromatograph Shimadzu, GC-2014 model, injecting 0.1 µL in chromatographic column Carbowax (0.07 CW, 30 M x 0.32 mm ID x 0.25 UM), split 20, with a column flow rate of 3 mL min^{-1}at isothermal 200 °C. The identification of fatty acids was performed by comparison with the retention time of

fatty acid patterns. Identification was by means of standard for methyl esters (ME) Supelco component 37 FAME mix.

2.4.4. Determination of protein content

The protein determination in this study was performed according to the Kjeldahl classic method (AOAC, 1995), which is based on the determination of the total organic nitrogen, which protein will be converted by means of a conversion factor. The conversion factor used was 6.25, used for food in general. The analysis began with digestion of 0.5 g of frozen dried biomass in 0.05 M sulfuric acid in Erlenmeyer flask. Assays were performed for all growing conditions. The sample was collected in form of ammonium, with 0.033 M boric acid, and then titrated with ammonium hydroxide solution with the 0.05 M sulfuric acid solution.

2.4.5. Determination of carbohydrates

The extraction of total carbohydrates from the microalgae *Monoraphidium* sp. was realized according to the method described by Myklestad and Haug (1972). The method called phenol-sulfuric allows extraction of water soluble carbohydrates that are present in the dry biomass.

Initially, 10 to 20 mg of lyophilized biomass was weighed to which 1 mL of sulfuric acid 80% was added. The samples were maintained at rest for 20 hours. During the acid addition and the subsequent 4 hours the vials with samples were kept in an ice bath at 8 °C in order to avoid carbonization of biomass. Samples were diluted with 10 mL of distilled water and filtered through glass fiber of 0.47 μm pore and diameter 25 mm, previously heated in muffle furnace for 1 hour at 575 °C. The filtrate was transferred to 0.1 mL test tubes, in which 0.5 mL of 5% phenol solution and 2.5 mL of concentrated sulfuric acid were added, followed by vigorous stirring. The samples were allowed to repose for 30 minutes immediately after the addition of sulfuric acid to achieve full extraction of carbohydrates, the absorbance reading was taken at 485 nm using a spectrophotometer model 1105 Bel photonics.

The method is simple, rapid, sensitive and with reproducible results, it is not a selective analysis, and it involves any type of carbohydrate. To quantify the carbohydrates, for the spectrophotometer, a standard glucose curve was constructed with concentrations of 0-60 $\mu g\ mL^{-1}$.

3. RESULTS & DISCUSSION

3.1. Pilot-scale cultivation of *Monoraphidium* sp.

Table 1 shows the results of the monitoring of cell growth cultivation and daily consumption of CO_2. Theoretically, according to the dry weight value obtained, when collected in the reactor there was 53.03 g of wet biomass. After centrifuging and lyophilizing 44.74 g of dry biomass were obtained. Considering the theoretical value as a reference, there was a loss of approximately 15% of the biomass collection and concentration steps (centrifugation), mainly in the transfer of biomass from one container to another.

According to the results in Table 1, at the beginning of the culture (day 0) 0.79 g of CO_2 were consumed for each gram of biomass produced. On the last day (12), 0.3 g CO_2 were consumed per gram of culture. On the sixth day of cultivation the consumption of CO_2 per gram of biomass increased, resulting in a CO_2 consumption of 0.61 g. The best result was however obtained on the second day of cultivation, where for each gram of biomass were consumed approximately 1.2 grams of CO_2.

Table 1. Monitoring of cell growth, cell count, dry biomass and CO_2 consumption.

Days Of culture	Cell count (cell mL^{-1})	Dry weight (mgL^{-1})	Quantity of biomass in the reactor (g)	CO_2 consumption (L day^{-1})	CO_2 consumption (g day^{-1})
0	6,05 x 10^6	84,44 ± 0,10	7,56	3,03	**6,00**
1	7,70 x 10^6	88,22 ± 0,01	7,94	4,82	**9,50**
2	8,05 x 10^6	122,22 ± 0,03	11,00	5,30	**10,50**
3	1,10 x 10^7	177,33 ± 0,01	15,96	5,63	**11,10**
4	1,88 x 10^7	264,00 ± 0,00	23,76	8,13	**16,10**
5	2,06 x 10^7	310,67 ± 0,00	27,96	9,55	**18,80**
6	2,14 x 10^7	346,00 ± 0,06	31,14	9,63	**19,00**
7	2,62 x 10^7	400,67 ± 0,00	36,06	9,02	**17,80**
8	2,71 x 10^7	423,78 ± 0,01	38,14	8,53	**16,90**
9	2,75 x 10^7	520,44 ± 0,02	46,84	8,38	**16,60**
10	3,19 x 10^7	525,11 ± 0,02	47,26	8,33	**16,50**
11	4,72 x 10^7	569,11 ± 0,01	51,22	7,95	**15,70**
12	4,39 x 10^7	589,33 ± 0,03	53,03	7,92	**15,70**

This behavior is in complete agreement with the results of the culture described in the literature (Holbrook *et al*, 2014), in which during the cell growth phase, the process of photosynthesis is much more intense and consequently the consumption of CO_2 is higher. As the number of days increased, the cells being counted in the analysis "cell count" or dry weight they do not consume CO_2 because there are dead.

It is important to note that, the CO_2 consumption was recorded over a period of eight hours, covering a range of 8:00 to 16:00 hours, corresponding to the most sunlight hours and therefore greater photosynthesis. The CO_2 injection time was approximately 11 to 15 seconds. During the first 6 days of culture, an increase in the number of CO_2 injection was observed during a daily monitored period. After the seventh day the number of daily injections decreased and remained constant until the last day of culture.

In Fig.8, the CO_2 consumption versus amount of biomass in the photobioreactor is correlated. A graphic view of the behavior is described using the results of Table 1. Analyzing the growth curve of the crop in closed-window type photo-bioreactor as shown in Fig. 9 it can be observed a lag phase or adaptation for the first two days of cultivation, followed by an exponential growth phase which starts from the third day, with high growth rate until the seventh day. From the eighth day the biomass production continues but at a lower growth rate. In this case the cultivation was concentrated before reaching the stationary phase, which is prevalent by cell death (Kambourova R, *et al*, 2006). In Fig. 10 an observation in the increase of cell concentration in the culture can be seen, if perceiving a change in color.

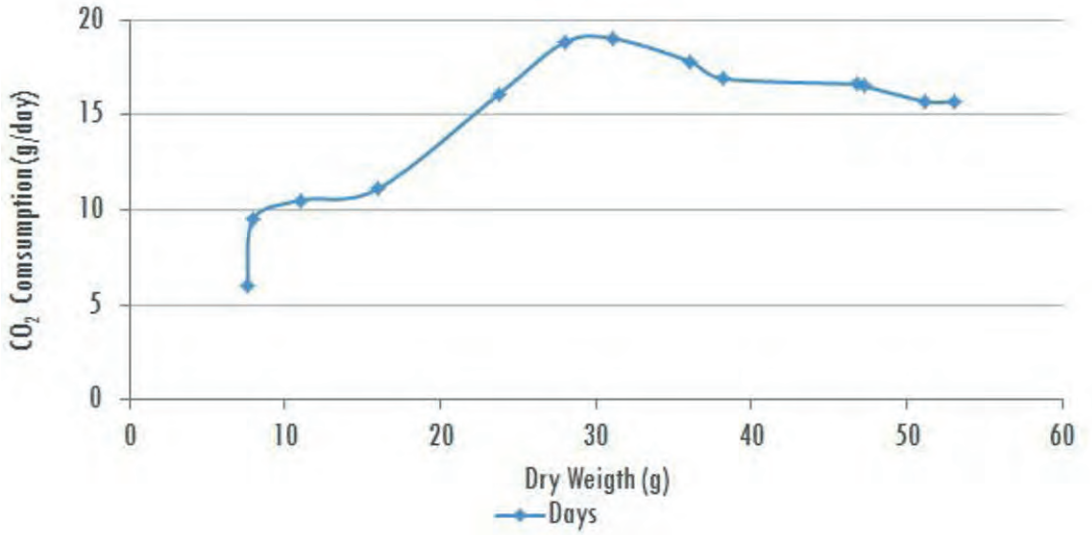

Fig 8. CO_2 consumed by microalgae *Monoraphidium* sp. during the growth period in the pilot photobioreactor.

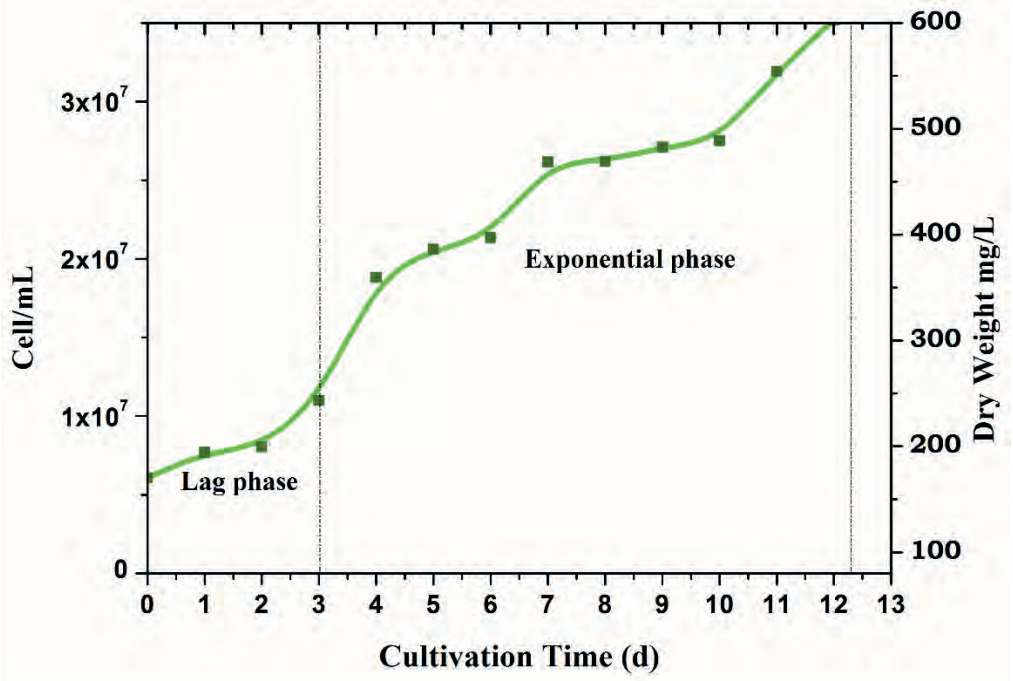

Fig 9. Profile of growth of *Monoraphidium* sp. cultivated in closed-window type photobioreactor

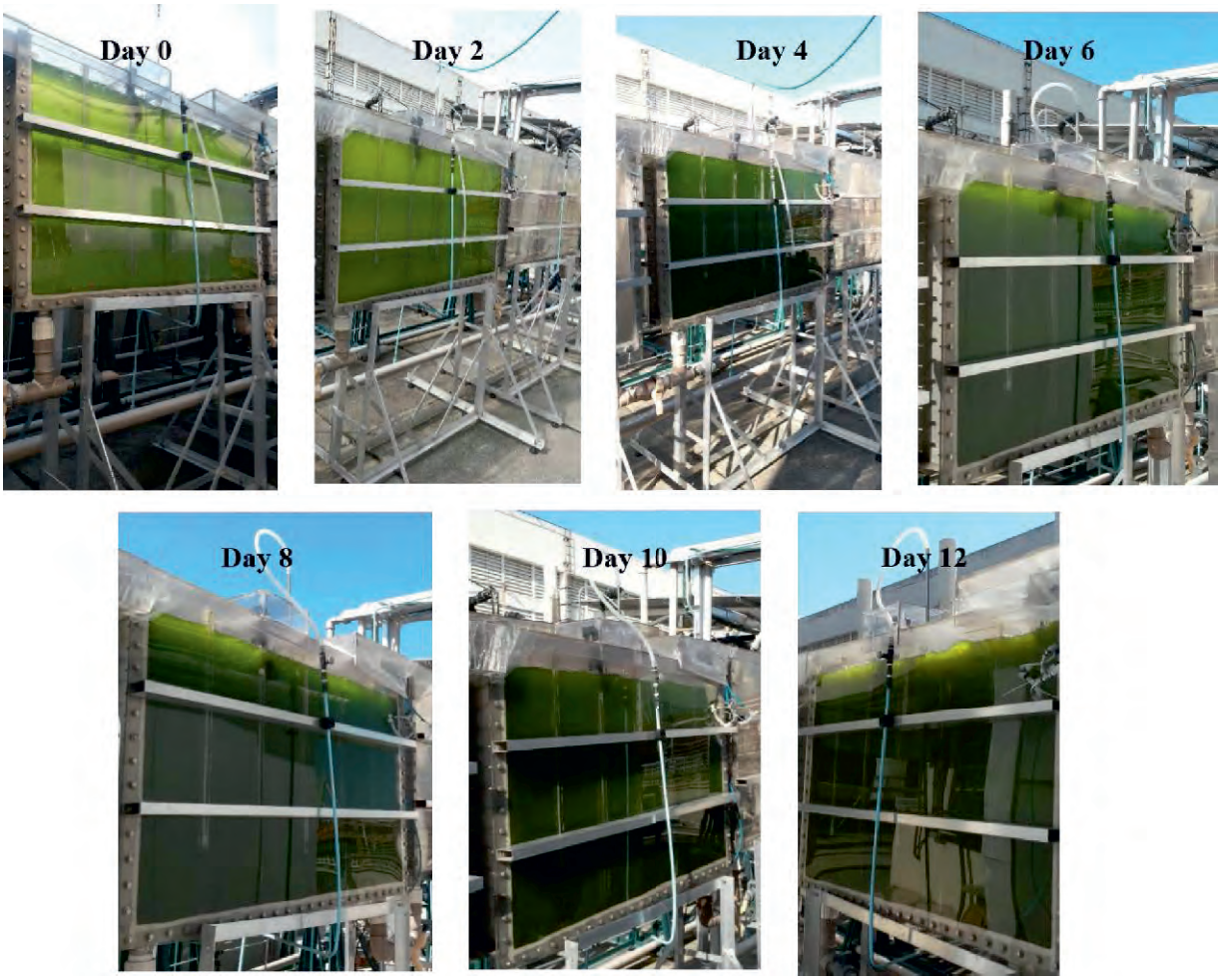

Fig 10. Production of biomass *Monoraphidium* sp. in photobioreactor type window during 12 days of cultivation.

3.2. Biomass concentration and determination of moisture content

The biomass of microalgae concentrated by centrifugation under the conditions described in section materials and methods, presented a high moisture content (91.45%). Aresta *et al* (2005), mentioned that one of the most important issues for the use of algae as an energy proposal is its high water content between 80-90%, however, to acquire energy from algal biomass the viability of the process increases with the reduction of the moisture content of biomass.

3.3. Chemical characterization of the biomass

The biomass of the microalgae *Monoraphidium* sp. cultivated in the photo bioreactor was characterized according to item 2.5 described under materials and methods. The characterization results are shown in Table 2.

Table 2. Biochemical composition of the biomass of microalgae *Monoraphidium* sp.

Specie	Protein %	Carbohydrates %	TL %	LCB %
***Monoraphidium* sp.**	34,26±0,41	32±3,37	17,33±3,27	8,36± 2,79%

According to the literature, the microalgae *Monoraphidium* sp. usually shows levels of total lipids, proteins and carbohydrates in amounts between 19 - 35%, 28 - 45% and 17 - 25%, respectively (Diaz *et al*, 2015). The lipid content of microalgae of the genus *Monoraphidium* sp. can reach 56% depending on the conditions and means of cultivation used, a number 25.5% higher than that found in other species (Yu, *et.al*, 2012).

The TL content obtained in this biomass is close to the value reported in the literature (Diaz *et al*., 2015), since the carbohydrate content was higher.

Analysis of the microalgal biomass *Monoraphidium* sp. performed by Tavares *et al*., (2014), indicated that this strain has a desirable profile for biodiesel production because the thermogravimetric analysis at the dehydrated microalgae showed 34.24% m/m of total lipids.

According to the lipid values, proteins and carbohydrates present in this biomass, this microalgae builds interest to the food industry and for the production of second and third generation biofuels.

3.3.1. Lipid profile

The lipid profile of microalgae *Monoraphidium* sp. collected in closed-window type photobioreactor is represented in Table 3. The microalgae presented the following distribution of saturated fatty acids (SAFA) and monounsaturated acids (MUFA) and polyunsaturated acids (PUFA): content SAFA>PUFA>MUFA. Refering to SAFAs mainly the C16:0 (palmitic).In relation to the PUFAs it was observed mainly represented by C18:2 (Linoleic) and C18:3 (linolenic).As to the MUFAs was also identified in high amounts C18:1 (oleic). According to Yu *et al* (2012), the *Monoraphidium* sp. FXY-10 grown in Yunnan, China had 23.80% of SAFA and 68% of PUFAs.

Table 3. Lipid profile of microalgae *Monoraphidium* sp. collected in closed-window type photobioreactor.

	Identification		**FAME%**
Lauric Acid	C12:0	SAFA	0.19
Tridecanoic Acid	C13:0	SAFA	-
Myristic Acid	C14:0	SAFA	1.65
Pentadecanoic Acid	C15:0	SAFA	0.35
Cis-10 Pentadecanoic	C15:1:0	MUFA	0.03
Palmitic Acid	C16:0	SAFA	**41.71**
Palmitoleic Acid	C16:1	MUFA	0,78
Stearic Acid	C18:0	SAFA	**6.75**
Oleic Acid	C18:1	MUFA	**16.09**
Linoleic Acid	C18:2	PUFA	**10.19**
Linolenic Acid	C18:3	PUFA	**16.50**
Arachidonic Acid	C20:0	SAFA	2.04
Arachidonic Acid	C20:4	PUFA	0.16
Eicosapentaenoic Acid	C20:5	PUFA	0.46
Erucic Acid	C22:1	MUFA	0.32
Cis-13,16-Docosadienoic Acid	C22:2	PUFA	1.76
Lignoceric Acid	C24:0	SAFA	1.02

4. Conclusions

1. Under the conditions used in the pilot photobioreactor, the microalgae *Monoraphidium sp*. showed efficiency in CO_2 fixation, consuming during cell growth over 1.2 g CO_2 per gram of biomass produced.
2. The microalgae culture systems can be coupled to large chimneys of industries that emits CO_2 ,gas resultant of combustion, capitalising on the benefits of this gas in the process of photosynthesis.
3. The biomass grown in the photobioreactor accumulated high levels of protein and carbohydrates which can be used in the production of ethanol through fermentation.
4. The content of convertible lipids into biodiesel accumulated in the biomass was not very high. If the focus of this biomass was for biodiesel production, growing conditions should be modified with a view of accumulating the lipids of interest.

5. Acknowledgements

The authors acknowledge the support in terms of infrastructure provided by Greens Technologies Laboratory Greentec / EQ / UFRJ and for financing this research. The support of the National Counsel of Technological and Scientific Development (CNPQ) due to a scholarship offered. Also to the Food Technology Laboratory / EQ / UFRJ which provided support on some tests performed, and the National Center of Technology (CNTQ) in Venezuela which promotes the development of new technologies in the area of renewable energy.

6. References

Aresta, Michele, Angela Di benedetto, Maria Carone, Teresa Colonna, and Carlo Fragale. 2005. “Production of Biodiesel from Macroalgae by Supercritical CO_2 Extraction and Thermochemical Liquefaction,” 136–39.

Association of Official Analytical Chemists (A.O.A.C). 1995. Official methods of analysis of the Association of Official Analytical Chemists (method 991.20). 33. p. 10-12.

Bligh, G. and Dyer, W. 1959 A Rapid Method for Total Lipid Extraction and Purification. Canadian Journal of Bio-chemistry and Physiology, 37, 911-917.

Brindley C, Garcia-Malea MC, Acien FG, Fernandez JM ,Garcia JL, Molina E. 2002 Influence of power supply in the feasibility of Phaeodactylum tricornutum cultures. Biotechnology and Bioengineering; 87:723–33.

Díaz, Gisel Chenard, Yordanka Reyes Cruz, René González Carliz, Rosa C Vitorino De Paula, Donato A Gomes Aranda, Marcellus A G Dario, Gustavo Saraiva Marassi, and Nelson C Furtado. 2015. Cultivation of Microalgae *Monoraphidium* sp, in the Plant Pilot the Grand Valle Bio

Energy , for Biodiesel Production.376, 370–378.

Holbrook, Gabriel P., Zachary Davidson, Robert a. Tatara, Norbert L. Ziemer, Kurt a. Rosentrater, and W. Scott Grayburn. 2014. "Use of the Microalga *Monoraphidium* sp. Grown in Wastewater as a Feedstock for Biodiesel: Cultivation and Fuel Characteristics." *Applied Energy* 131: 386–93.

Kambourova R, Petkov G, Bankova V. 2006. Extracellular polar substances in cultures of the green alga Scenedesmus. 119:155–62.

Myklestad, s. &Haug, a. 1972.Production of carbohydrates by the marine Chaetocerosaffinis var. O Willei (Gran.)Hustedt. I. Effect of the concentration of nutrients in the cu1ture medium. J. exp mar. Biol. Ecol (9):125-136.

Razzak, Shaikh A., Mohammad M. Hossain, Rahima A. Lucky, Amarjeet S. Bassi, and Hugo de Lasa. 2013. "Integrated CO_2 Capture, Wastewater Treatment and Biofuel Production by Microalgae culturing—A Review." *Renewable and Sustainable Energy Reviews* 27. Elsevier.630, 643: 622–53.

Richmond, A. (Ed), 1988. CRC Handbook of microalgal mass culture. Florida: CRC. 528p.

Sánchez-Saavedra. M. P &Voltolina, D., 2005. The growth rate, biomass production and composition of Chaetoceros sp. grown with different light sources. Aquacultural engineering 35: 161–165.

Schmid-Bondzynski-Ratzlaff. 2012. Extração e quantificação de lipídeos em microalgas. Modificado pela Universidade Federal de Viçosa. Departamento de Tecnologia de Alimentos.

Tavares, V.R; Costa, M; Medeiros, S.I.G and Souto, C.R.O. 2015. Analises de microscopia eletrônica e termogravimetria para acompanhamento da extração de lipídios da *Monoraphidium* sp. Congresso Brasileiro de Química. p 1.

The Emissions Gap Report 2015. A UNEP Synthesis Report. ISBN: 978-92-807-3491-1. http://uneplive.unep.org/media/docs/theme/13/EGR_2015_301115_lores.pdf.

Yoo, C., Jun, S.Y. and Lee, J.Y. 2010.Selection of Microalgae for Lipid Production under High Levels Carbon Dioxide. Bioresource Technology, 101, S71-S74. http://dx.doi.org/10.1016/j.biortech.2009.03.030

Yu, Xuya, Peng Zhao, Cian He, Junjun Li, Xianhua Tang, Junpei Zhou, and Zunxi Huang. 2012. "Bioresource Technology Isolation of a Novel Strain of *Monoraphidium* sp. and Characterization of Its Potential Application as Biodiesel Feedstock." Bioresource Technology 121. Elsevier Ltd: 256–62.