

Photobioreactor of Microalgas for CO₂ Biofixation

Yordanka Reyes Cruz¹, Gisel Chenard Díaz¹,
Andreina Z. Figuera Leonett¹, René Gonzalez Carliz¹,
Vinicius Rossa^{2*}, Luciano Basto Oliveira³,
Maurílio Novais da Paixão¹, Donato Alexandre Gomes Aranda¹

¹School of Chemistry, Federal University of Rio de Janeiro (EQ/UFRJ), Rio de Janeiro, Brazil

²Faculty of Chemical Engineering, Federal University of Uberlândia (FEQUI/UFU), Minas Gerais, Brazil

³International Virtual Institute for Global Change (IVIG/COPPE/UFRJ), Rio de Janeiro, Brazil

Email: *vinnyrossa@gmail.com

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Abstract

Microalgae are unicellular organisms capable of photosynthesis, turning sunlight and carbon dioxide (CO₂) into rich biomass. Precisely because of this definition, in recent years various sectors have been targeting their ability to reduce CO₂ emissions and the capacity of simultaneously synthesize biomass which can be later used to produce bio-fuels. Besides being considered fast-growth microorganisms, microalgae have a diverse biochemical composition with similar characteristics to traditional biomass. In this context, the present work aimed to evaluate the biofixation of CO₂ by the microalgae *Monoraphidium* sp., cultivated in a closed-window type photobioreactor, as well as characterization of microalgal biomass produced in relation to the total lipid content (TL), lipids converted into biodiesel (LCB), carbohydrates and proteins. The results achieved showed that the best result was obtained after 24 h of cultivation, where for each gram of biomass produced approximately 1.2 g of CO₂ were consumed. In the growth phase the average biomass productivity in the Janela photobioreactor was 58 mg·L⁻¹·day⁻¹ concluding that microalgae culture systems could be coupled to the chimneys of large industries emitters CO₂ using this gas, resulting from combustion processes, in the process of photosynthesis. The biomass *Monoraphidium* sp. produced had a content of lipids converted into biodiesel of approximately 8.36% ± 2.69%, carbohydrates 32% ± 3.37% and proteins 34.26% ± 0.41%.

Keywords

Biomass, CO₂ Capture, Cultivation, Microalgae, Photobioreactor

1. Introduction

Microalgae are unicellular organisms, photosynthesizers, capable of using light energy more efficiently than higher plants and are excellent carbonic gas fixers [1].

In principle, microalgae were investigated as a source of protein for human consumption. The initial focus was shifted to its use in CO₂ capture and in parallel to take advantage of the biomass synthesized in the production of biofuels, such as renewable hydrocarbons (bioquerosene or green diesel), biodiesel or second-generation ethanol, as well as several other products such as hydrogen, food products, pharmaceuticals and cosmetics [1] [2].

Among the microalgae characteristics, the fact is that they grow rapidly, synthesize and accumulate large amounts of lipids, carbohydrates, proteins and pigments, such as carotenoids and chlorophyll. Selection of the appropriate strain and the best growing conditions is essential to make the most of microalgae culture. Growing conditions involve: water quality; pH; temperature; nutrients and CO₂ dosage in a controlled manner. Nutrients should be added to ensure the growth of microalgae (nitrogen, phosphorus, minerals and vitamins) as well as a source of carbon (CO₂) and solar energy.

Phototrophic cultivation is the most widely used, mainly in large scale production. The variation of lipids in this condition is between 5% and 60%, according to the type of microalga [3]. This type of culture uses light as a source of energy and inorganic carbon (CO₂) from atmospheric or injected air from pressurized cylinders as a source of carbon to produce chemical energy through the process of photosynthesis. In closed systems the efficiency of CO₂ removal and fixation depends on the microalgae species, CO₂ concentration, photobioreactor design and operating conditions [4].

Por outro lado, em tratamentos com 12% de CO₂, a eficiência de fixação foi de 7% a 17% pela *Spirulina sp.* e 4.9% por *S. obliquus* [5]. Em outras palavras, as espécies de microalgas influenciam na eficiência e remoção de CO₂, isto está relacionado a condições fisiológicas, como o potencial de crescimento celular e o metabolismo de CO₂.

According to Meldon (2011) [6], it is possible to increase the economic use of microalgae using a two-stage process. In the first phase, CO₂ from power plants or from other sources is first purified (exemplo: remoção com aminas) and concentrated by conventional processes. After this first phase, the CO₂ is transported to the production of microalgae. This process can be compared economically with other conventional CO₂ capture methods involving separation, transport, and finally disposal in deep oceans or depletions of gas wells.

The rate of CO₂ fixation is directly related to the efficient use of light and the cellular density of the microalga which involves photoautotrophic growth. Therefore, biomass measurements or growth rate assessments are fundamental to determine the potential of the microalgae system and culture in the direct CO₂ removal [7].

Shrivastav *et al.* (2015) [8] monitored the growth of the microalga *Monoraphidium sp.* in BG 11 medium under two conditions, using an air + 2% CO₂ and 0.035% CO₂ air mixture for 16 days of cultivation. The purity of the CO₂ used was 99%. Thus, rapid growth was observed with (air + CO₂) obtaining a biomass and lipid yield of $241.61 \pm 1.417 \text{ mg}\cdot\text{L}^{-1}$ and $28.9\% \pm 0.008\%$, respectively. When air was used, only the total biomass and lipid content was $117.16 \pm 1.417 \text{ mg}\cdot\text{L}^{-1}$ and $22.40\% \pm 0.153\%$, respectively.

Thus, to enrich the production of lipids and reduce CO₂ emissions, artificial sources of CO₂, such as gas flow produced by coal-fired power plants, can be used and mixed with atmospheric air after pretreatment.

According to Herzog and Golomb (2004) [9], CO₂ capture can be divided into three categories: 1) Gas absorption separation based on chemical absorption using monoethanolamine (MEA), diethanolamine (DEA) and monodiethanolamine (MDEA) [10], 2) by oxygen combustion and 3) by biological CO₂ fixation, focus of the present work.

In Brazil the microalga *Monoraphidium sp.* is cultivated in the northeast of the country, in the city of Natal. Large-scale production techniques include the use of raceways, which are large open tanks (cultivators of the Petrobras/UFRN pilot plant in Extremoz/RN). Also, the Grande Valle Bio Energia Ltda. Biodiesel plant, located in the state of Rio de Janeiro, together with the Rio de Janeiro State Research Foundation—FAPERJ, has made some investments in the development of technology for the cultivation and use of the microalga *Monoraphidium sp.* as an alternative raw material for biodiesel production [11].

There are few published studies related to the capture of carbon dioxide (CO₂) by the microalga *Monoraphidium sp.* Most of the studies are focused on producing biodiesel. Shrivastav *et al.*, (2015) observed that in the addition of CO₂ and air, the microalga *Monoraphidium sp.* has the capacity to mitigate CO₂, due to its high capacity to fix carbon. And because of its high productivity of lipids and biomass, it represents a potential raw material for biofuels [8]. Although the species *Monoraphidium sp.* to adapt very well to regions of low temperature and of little light incidence, presenting good growth in waste water and with lipid levels like soybean oil [12], *Monoraphidium sp.* is able to grow at a temperature of 35°C [6] [13] [14] [15].

According to the literature, one kilogram of dry microalgae biomass uses approximately 1.83 kg of CO₂ [16]. Studies have been developed to validate microalgae as biofilters to purify combustion gases from the main generating industries such as: thermoelectric, cement, agricultural and other industries. Thus, microalgae are being used to improve the biogas quality by reducing the CO₂ content, which would lead to an increase in methane (CH₄) concentration.

Although there are still a number of technological challenges to be overcome in order for microalgae to become competitive in the biofuels market and as CO₂ sequesters, their yield potential is significant and therefore new R&D investments are needed.

In this context, this work was developed to study the biofixation of CO₂ by cultivating the microalga *Monoraphidium sp.*, Cultivated in a closed-window type photobioreactor, and characterizing the microalgal biomass produced in relation to: total lipid content, lipids convertible in biodiesel—LCB, carbohydrates and proteins.

2. Materials and Methods

For the accomplishment of this work, was used the strain of the microalga *Monoraphidium sp.* as shown in **Figure 1**, maintained in the collection of the cultures bank of the Laboratory of Green Technologies (Greentec) of the School of Chemistry/UFRJ.

2.1. Scheduling of Microalgae Cultivation *Monoraphidium sp.*

For the staggering of the cultivation of the microalga *Monoraphidium sp.*, four transfers were made (MORF strain, 250 mL, 2 L, 20 L) as shown in **Figure 2**, in order to obtain the amount of inoculum necessary for cultivation in the a closed-window type photobioreactor of 110 L. It was started with the inoculum of the strain, previously maintained in ASM-1 medium, under pH = 7, illumination of 170 $\mu\text{photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and temperature of 23°C, in erlenmeyer of 250 mL (activation culture), followed by transfer to a second 2 L erlenmeyer flask and then to a carboy transparent 20 L. At the end, the culture of the carboy is used as inoculum in a closed-window type photobioreactor of 110 L where CO₂ consumption and cell growth are monitored.

2.2. Cultivation System Used in Monitoring the Consumption of CO₂

For the absorption of CO₂ by the microalga *Monoraphidium sp.*, the culture was carried out in a 110 L window-type photobioreactor installed in the Greentec Laboratory pilot plant, located in the cover of the Nucleus of Excellence in Petroleum and Biofuels of the School of Chemistry/UFRJ (**Figure 2**). The culture was started on ASM-1 medium; temperature controlled between 20°C - 35°C;

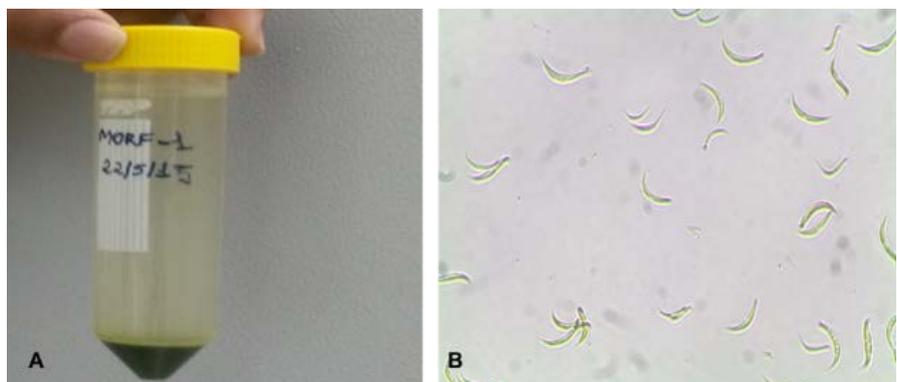


Figure 1. (A) Inoculum of the strain *Monoraphidium sp.* (MORF-01); (B). Image of the microalga observed through the Olympus BX optical microscope with 100× magnification.



Figure 2. Cultivation of the microalga *Monoraphidium sp.* in the 110 L window photobioreactor.

concentration of 6.05×10^6 cel·mL⁻¹; air injection around 1 to 7 L·min⁻¹ through a horizontal stainless steel tube installed at the bottom of the photobioreactor, with the aim of increasing turbulence, avoiding the deposition of the cells and increasing the contact of the liquid with CO₂ and automated pH control system between 7.0 and 8.0 by injecting CO₂ at three points inside the photobioreactor using diffusers that ensure the injection of the gas in the form of micro bubbles, facilitating their dilution in the water.

The cultivation of the microalga *Monoraphidium sp.* was performed during a period of 12 days, since the focus was to measure the absorption of CO₂. Therefore, in batch cultures, the increase in CO₂ consumption occurs in the first days of cultivation, after which the consumption starts to decrease.

The monitoring of CO₂ consumption, considering that it is consumed at the times when the cells are performing the process of photosynthesis, was performed in the interval between 8:00 am to 8:00 pm, quantifying in this interval the number of times the system injected CO₂ and the time of injection. Thus, since CO₂ was always injected with a constant flow rate (1 L·min⁻¹), it was possible to determine its consumption during the monitored period. In addition to CO₂ consumption (g·day⁻¹), other parameters were measured daily during growing time, such as: temperature (°C), illumination (μphotons·m⁻²·s⁻¹), pH, cells density (cell·mL⁻¹) and dry biomass (g), the latter analyzed in triplicate.

2.3. The Growth of the Culture Monitored Using the Cell Density and Dry Weight Methodologies

2.3.1. Cellular Density

The number of cells (or cell density, in cell·mL⁻¹) present in the samples collected daily during the culture was determined by direct counting under an optical microscope (Olympus SC30 model) (40-fold increase), with the aid of a chamber of Fuchs-Rosenthal. The cell density was expressed in cell numbers per milliliter of culture (cell·mL⁻¹).

2.3.2. Determination of Dry Biomass

The dry weight was determined according to the methodology defined by CENPES/PETROBRAS (2013). A membrane was calcined at 575°C for 1 h for removal of moisture; cooled it in desiccator and recorded its initial mass (m_i); 15 mL (V_f) of microalgae culture was filtered into the membrane; then the membrane was oven-conditioned at 65°C for 1 h; cooled it in a desiccator and recorded its final mass (m_f) for determination of the dry weight and, consequently, the dry biomass and moisture contents, according to Equations (1)-(3).

To discard ash values from the final dry weight, the membranes with dry biomass were calcined at 575°C for 1 h, cooled and weighed. The determination of dry weight was performed in triplicate.

$$X = \frac{(mf - mi) * 1000 * FD}{Vf} \quad (1)$$

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

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

where:

X = concentration of biomass or dry weight (gL^{-1});

m_i = mass of calcined membrane without the filtered microalgae (initial mass, g);

m_f = membrane mass with dry microalgae (final weight, g);

V_f = volume of culture of microalgae filtered (mL);

FD = dilution factor (mL);

DM = dry matter content (g);

CM = moisture content (%).

Figure 3 illustrates the different steps of the process.

With the experimental data of cell density, and dry weight were developed graphs as a function of time (days). After 12 days following the cultivation the biomass was concentrated using Hitachi centrifuge model CR22N. For this, a speed of 8000 rpm was used for a time of 10 min. For quantification of the moisture content in the microalgae biomass obtained by the centrifugation process, 2 g of biomass were weighed in a Shimadzu model MOC63U electronic scale, with a temperature of 40°C to 50°C.

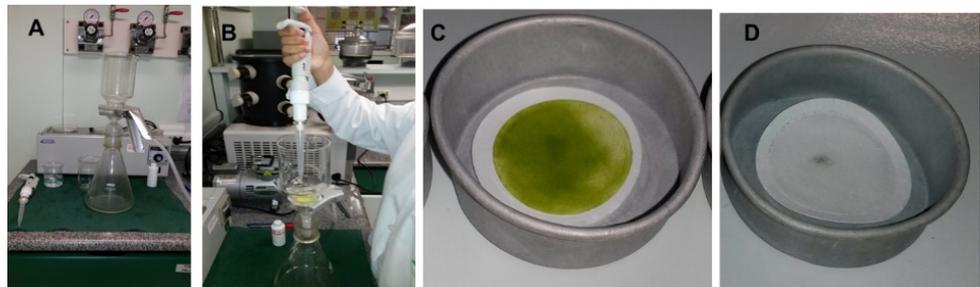


Figure 3. Determination of dry weight. (A) Membrane washing; (B) Filtration system; (C) Drying in the greenhouse; (D) Calcined biomass.

2.4. For the Characterization of the Microalgal Biomass the Following Methodologies

To determine the content of lipids, carbohydrates and proteins present in the biomass cultured in the photobioreactor, the microalgae biomass after centrifugation was dried by lyophilization. This step was performed using freeze drier—Labconco model of 4.5 L capacity, operating at a temperature of -45°C and a vacuum of 0.220 bar.

2.4.1. Lipid Content (CL)

The lipid content in biomass of microalgae was determined following the method of Bligh and Dyer (1959) modified for the extraction of lipids [17]. The lipid extraction is done in two steps according to the following procedure (Figure 4): at the first the sample was subjected to a pre-treatment with hydrochloric acid to break through the wall cellular and at the second the lipids are extracted with the mixture of solvents. After extraction, the excess solvent was removed, and the lipid quantification was performed gravimetrically.

In the first step based in the digestion of the sample with hydrochloric acid, was weighed 400 mg of the sample directly into the falcon tube. Were added 5 mL of 8 M hydrochloric acid solution and agitated to promote the dispersion of the material. Then the sample was heated in a water bath at 80°C for 1 hour to complete digestion of the cells. Posteriorly leave the flask to rest for 10 minutes at 25°C and centrifuged at 2000 rpm for 15 minutes to separate the biomass and the acid residue. After digestion remove the residual acid and continue with the flask which contains the biomass to step the extraction of lipids.

In the second step were added to the falcon tube the mixture of solvents, methanol (4 mL), chloroform (4 mL), water (3.6 mL), ratio = 1:1:0.9 (v/v/v), respectively. And stirred for 10 minutes and centrifuged at 2000 rpm for 15 minutes. After centrifugation were obtained 3 phases containing off the lower phase where the lipids are dissolved and chloroform (which we must recover) the middle phase containing biomass extracted and the upper phase composed of water and methanol.

Pour the ethereal phase (inferior) to the balloon previously weighed and repackage it in thermostatic bath at 65°C until complete evaporation of the solvent. In the phases remaining in the falcon tube (biomass, methanol, water) do re-extraction of lipids who are still in biomass by adding 4 mL of 10% solution



Figure 4. CL extraction process (A) Pretreatment acid; (B) Lipid extraction; (C) Samples with lipids; (D) Recovery of the solvent.

v/v methanol in chloroform, shake and centrifuge at 2000 rpm for 15 min. This step is essential for migration of lipids withheld in the residue of microalgae for the solvent. Repeat the procedure for re-extraction one more time and remove off the lower phase where are lipids and join with the first extract (ethereal phase). Finally leave the balloon in a water bath at 65 °C, for the solvent evaporation. After removal of the solvents by evaporation, put the balloon in oven at 102 °C ± 2 °C for 1 h. Cool down in desiccator and weigh. Repeat drying and weighing until constant mass. The content of lipids (CL) as determined in triplicate is calculated by the Equation (4), by gravimetric analysis (Gravimetry by Extraction)

$$CL = 100 * \frac{(m3 - m1)}{m2} \quad (4)$$

where:

CL = lipid content;

m1 = mass of the balloon (g);

m2 = mass of sample (g);

m3 = mass of the balloon with the extract (g).

2.4.2. Lipid Content Convertible into Biodiesel (LCB)

Determination of the lipid content convertible into biodiesel was performed using the Schmid-Bonzynski-Ratzlaff (modified) method [18]. Based on the digestion of microalgae biomass with hydrochloric acid, followed by extraction of the lipid fraction with ethyl alcohol, ethyl ether and petroleum ether. After extraction, the excess solvent was removed and the LCB quantification was performed gravimetrically. The method was performed in triplicate according to the following procedure (Figure 5).

Weighed 1 g of lyophilized biomass was into a 100 mL Erlenmeyer with previously identified lid, was added 10 mL of 8 M hydrochloric acid solution (HCl) was added and homogenized. Samples were heated directly on heating plate at 70 °C for 10 minutes. After the time of digestion, the microscopic cell structure of the microalga was observed in the microscope, confirming that the cells were ruptured. Afterwards, 10 mL of ethyl alcohol was added, shaken manually for 30 seconds; 25 mL of ethyl ether and 25 mL of petroleum ether were added, stirring for an additional 1 min. The sample was then centrifuged at 3000 rpm for 15 min.

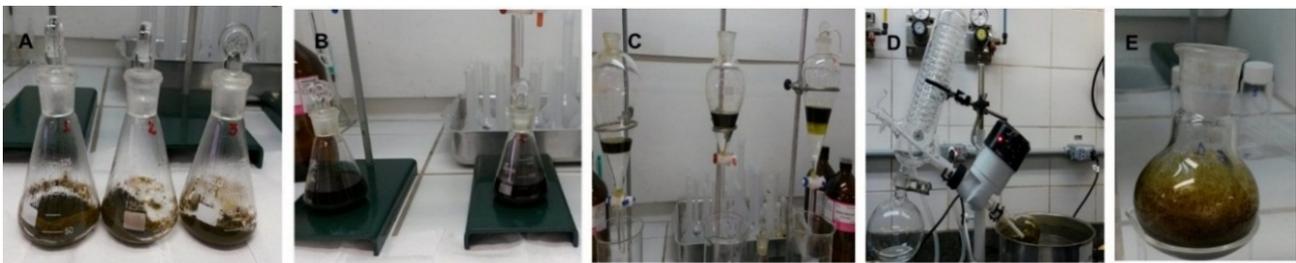


Figure 5. LCB extraction process (A) Rupture of biomass with HCl; (B) Addition of solvents for separation of the ethereal phase; (C) Separation of the ethereal phase; (D) Rotaevaporation of the ethereal phase with oil; (E) Dry oil.

The ether phase was transferred to a separatory funnel and washed with deionized water to remove the HCl until it reached a pH between 6 - 7, then was transferred to a pre-weighed balloon. The solvents were rotoevaporated at 65°C and the balloon with the oil (extract) were oven dried at 60°C to constant weight. The content of convertible lipids in biodiesel was determined by means of Equation (5), by gravimetric analysis (Gravimetry by Extraction)

$$LCB = 100 * \frac{(m3 - m1)}{m2} \quad (5)$$

where:

LCB = lipids convertible into biodiesel;

m1 = mass of the balloon (g);

m2 = mass of sample (g);

m3 = mass of the balloon with the extract (g).

2.4.3. Lipid Profile of Microalgae Biomass

For the identification of the lipid profile of the extracted oil, as described in item 2.4.2, it was necessary to convert these lipids to methyl esters in order to facilitate the identification of the components through gas chromatography.

In this initial stage a methanolysis of the lipid extract was carried out, according to the methodology described by YOO with modifications [19], using 2 mL of methanol and 5% of HCL at 75°C for 10 minutes in a Maria bath. This step was performed in a closed bottle to avoid evaporation.

Next, the phase containing the methylated fatty acids was separated with the addition of 2 mL of distilled water and 2 mL of hexane PA. The higher hexane phase was collected with an automatic pipette and transferred to a glass bottle, then placed in an oven at 60°C for solvent evaporation and concentration of the sample.

After the methanolysis, the percentage of fatty acid methyl esters was determined by Gas Chromatography using the EN 14103 method. For this purpose, the samples were diluted in heptane at the ratio of 0.05:1 (m/m). 1 µL of this sample was injected into the Shimadzu gas chromatograph, model GC-2014 with split/splitless Injector, flame ionization detector (FID), Carbowax 20 M column (30 m × 0.25 mm × 0.25 µm), Quadrex brand, with the following conditions: column flow of 3 mL/min⁻¹, 200°C isothermal, injector: 250°C, detector: 250°C, drag gas pressure of 1.9 mL/min⁻¹. The analyses were performed for 30 min.

The qualitative identification of the fatty acid methyl esters (fatty acid profile,%) is carried out by comparing the retention time of the constituents of the sample with a mixture consisting of 37 external patterns of methyl esters of fatty acids from Sigma (C4:0-C24:0). The composition is determined by the percentage of the relative areas of each characteristic peak of fatty acid methyl ester (EMAG) with the total peak area of the chromatogram (normalization method).

2.4.4. Carbohydrate Content

The extraction of total carbohydrates was performed according to the method

described by Myklestad and Haug (1972) [20]. The so-called phenol-sulfuric method allows to extract the water-soluble carbohydrates that are present in the dry biomass.

Initially, 10 - 20 mg of lyophilized biomass were weighed in which 1 mL of 80% sulfuric acid was added. Samples were kept at rest for 20 hours. During addition of the sulfuric acid and in the next 4 hours the flasks with the samples were kept in an ice bath at 8°C to avoid carbonization of the biomass. Subsequently, the samples were diluted with 10 mL of distilled water and filtered through 0.47 µm pore and 25 mm diameter glass fiber membranes previously treated in the muffle for 1 h at 575°C. From the filtrate, 0.1 mL was transferred into long 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. After 30 minutes, counted from the addition of sulfuric acid, the reading was carried out at 485 nm using the Bel Photonics 1105 spectrophotometer. The concentration of the samples was determined through the standard glucose curve with concentration between 0 - 60 µg mL⁻¹. For the calculation of concentrations, Equation (6) was used.

$$V1 = \frac{(C2 * V2)}{C1} \quad (6)$$

where:

C1: Concentration of the concentrated solution (stock solution), 400 mg L⁻¹;

V1: Volume of the concentrated solution (mL);

C2: Concentration of diluted solution (mg L⁻¹);

V2: Volume of diluted solution, 1 mL.

2.4.5. Total Protein Content

The protein determination in this study was performed according to the Kjeldahl modified A.O.A.C, (1995) method [21], 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 [22].

The digestion step was performed with sulfuric acid (H₂SO₄), where 0.1 g of dry microalgae biomass was weighed in a digestion tube, added 5 mL H₂SO₄ and selenium catalyst. Then the tube was placed in a Marconi model MA-4025 digester block until reaching the temperature of 300°C (biomass digestion, totally clear solution). The sample was distilled in Marconi distiller model MA-036. Before the distillation of digested samples, was added 60% sodium hydroxide (NaOH) to neutralize them. The distillate was collected in a solution containing 2% boric acid (HBO₃), bromocresol green indicator solutions and 0.2% m/m methyl red. The color change indicated the presence of nitrogen, so the process continued until all the nitrogen had been distilled. At the end, the sample was titrated using a 0.05 M hydrochloric acid (HCl) solution. The volume of sample titration solution spent indicates the nitrogen content recovered. For the determination of proteins, Equation (7) was used.

$$Proteins = \frac{V * 0.14 * F}{P} \quad (7)$$

where:

V = volume of HCL 0.05 M spent on the titration;

P = number g of the sample;

F = conversion factor (6.25)

3. Results and Discussion

Analyzing the growth curve of the crop in the window photobioreactor (**Figure 6**) a lag phase or adaptation during the first two days of cultivation, followed by an exponential growth phase starting from 48 hours of cultivation and going through the eleventh day, when the concentration of cells begins to decrease. Subsequently, after verifying the onset of the cell death stage, culture concentration was started using Hitachi centrifuge model CR22N.

According to **Figure 7**, the highest CO₂ consumption occurred during the first six days of cultivation and then began to decrease. It is observed that during the cultivation the growth of the biomass is greater in these first six days. For this ration the cultivation was carried out until the 12th day because the focus of this work was to monitor the CO₂ consumption in the microalga *Monoraphidium sp.*

This behavior is totally in agreement with the results of cultures described in the literature [12], where during the cell growth phase the photosynthesis process is much more intense and consequently the CO₂ consumption is higher.

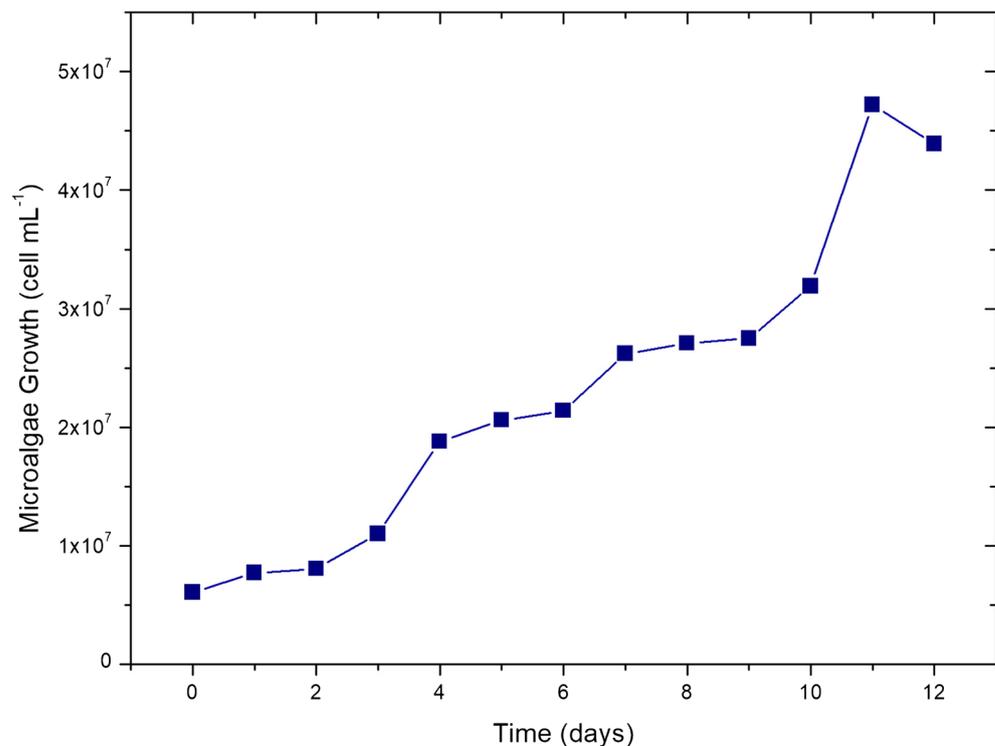


Figure 6. Growth curve of the microalga *Monoraphidium sp.* cultured in 110 L window type photobioreactor.

As days go by, some of the cells being counted in the cell count or dry weight tests do not consume CO₂ because they are dead. This behavior is observed in batch cultures. In continuous crops, as is usually the case on a commercial scale, it is possible to maintain the level of CO₂ absorption always at high levels. The results allowed to define that, at the start of cultivation (day 0), for each gram of biomass produced, 0.79 g ± 0.05 g of CO₂ were being consumed. Already on the last day (day 12), for each gram of cultivation, 0.3 g ± 0.01 g of CO₂ were consumed. On the sixth day of cultivation, for each gram of biomass, 0.61 g ± 0.08 g of CO₂ was consumed. The best result was obtained after 24 h of cultivation, in which, for each gram of biomass, approximately 1.2 g ± 0.03 g of CO₂ was consumed. It is worth mentioning that the consumption of CO₂ was recorded in a period of 12 h, contemplating the interval between 8:00 am to 8:00 pm, including the time of greatest solar incidence and therefore greater photosynthesis. The time of each CO₂ injection averaged 11 - 15 seconds.

The microalgae biomass after centrifugation and lyophilized was characterized following the procedures described in the item of materials and methods. The results of the characterization are shown in **Table 1**.

The protein and carbohydrate contents obtained in this study correspond to the values reported in the literature for this microalgae species that are between 28% - 45% of proteins and 17% - 35% of carbohydrates [23]. According to these

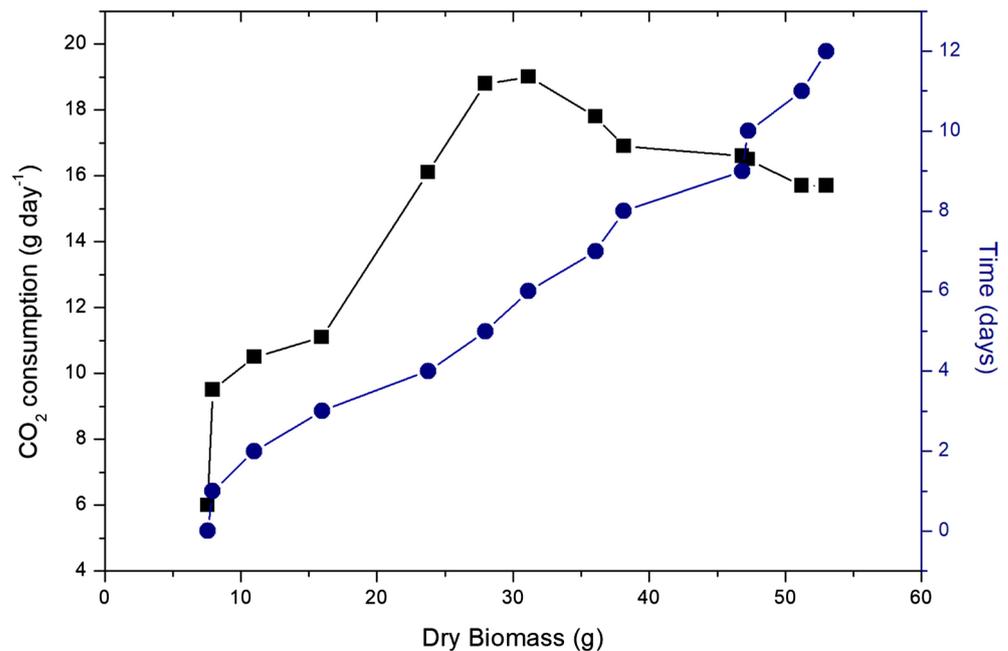


Figure 7. Amount of dry biomass vs CO₂ consumption (■) per day in *Monoraphidium* cultivation and Amount of biomass (g) vs. time (days) (●) of culture of *Monoraphidium*.

Table 1. Composição da biomassa da microalga liofilizada.

Proteins % ± SD	Carbohydrates % ± SD	TL % ± SD	LCB % ± SD
34.26 ± 0.41	32.00 ± 3.37	17.33 ± 3.27	8.36 ± 2.79%

results, the biomass of this microalgae is of interest to the food industry and to ethanol production from carbohydrates [24] [25] [26].

The total lipid content (CL) in this biomass, obtained by the methodology of Bligh & Dyer (1959), was 17.33%. According to the literature, the lipid content in the dry biomass of *Monoraphidium sp.* is between 15% and 35%, so the result obtained in the present work is within the range of expected values [22]. The lipid concentration may vary depending on the medium and the culture conditions used [27] [28] [29].

The content of biodiesel convertible lipids (LCB) was 8.36%, a value considered low when compared to other studies that appear in the literature that report values between 15% and 20% [30]. Some studies also deal with the possible manipulation of culture media, aiming at maximizing the content of convertible lipids in biodiesel, through the depletion of nutrients such as nitrogen and phosphorus [31].

In this case, the low content of LCB present in the biomass may be related to the composition of the ASM-1 culture medium, used mainly for the maintenance of strains in laboratory scale and consequently with low concentration of nutrients, when compared with other culture media commonly used in larger scale as BG-11 or Bolds Basal Medium (BBM) [32] [33].

From the oil extracted in the quantification of the lipid content convertible into biodiesel, the lipid profile was identified by gas chromatography. The results show the following distribution of saturated fatty acids (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA): content of SAFA>PUFA> MUFA. Within the SAFAs, the C18:0 (stearic) was outstanding in relation to the PUFAs C18:1 (Linoleic) and C18:3 (Linolenic), and the MUFA that presented itself in greater quantity was the C18:1 (oleic) [34] [35] [36].

4. Conclusions

In the culture conditions used in the window type photobioreactor, the microalga *Monoraphidium sp.* showed to be efficient in the biofixation of CO₂, consuming on the day of greatest cellular growth 1.2 g of CO₂ per gram of biomass produced. Microalgae culture systems could be coupled to the chimneys of large industries emitters CO₂ using this gas, resulting from combustion processes, in the process of photosynthesis. The CO₂ extracted in the biogas purification stage could be injected into microalga photobioreactors to give an ecological destination for this greenhouse gas responsible for the greenhouse effect. The lipid profile of the oil present in the biomass of the microalga *Monoraphidium sp.*, cultivated in the window type photobioreactor, showed the following behavior: SAFA > PUFA > MUFA. Highlighting the SAFA C18:0 (stearic), PUFAs C18:1 (Linolenic) and C18:3 (Linolenic) and MUFA C18:1 (oleic).

In batch cultures an increase in the consumption of CO₂ is observed only in the first days of cultivation, after that consumption begins to decrease. In continuous cultures, as is usually the case on a commercial scale, it is possible to

maintain the level of CO₂ absorption always at high levels.

In the growth phase, the average biomass productivity in the Janela photobioreactor was 58 mg·L⁻¹·day⁻¹.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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