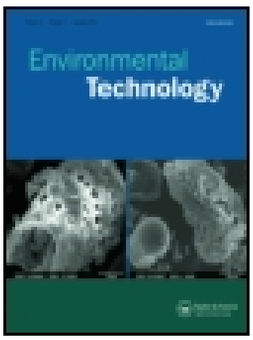

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Carbon biofixation and lipid composition of an acidophilic microalga cultivated on treated wastewater supplied with different CO_2 levels

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Carbon biofixation and lipid composition of an acidophilic microalga cultivated on treated wastewater supplied with different CO_2 levels

This study evaluated productivity, CO_2 biofixation, and lipid content in biomass of the acidophilic microalga *Chlamydomonas acidophila* LAFIC-004 cultivated with five different carbon dioxide concentrations. The influence of carbon dioxide concentration on nutrient removal and pH was also investigated. Treated wastewater (secondary effluent) was used as culture medium. Five experimental setups were tested: T-0% - injection of atmospheric air (0.038% CO_2), T-5% (5% CO_2), T-10% (10% CO_2), T-15% (15% CO_2) and T-20% (20% CO_2). The T-5% and T-10% experiments showed the highest values of productivity and CO_2 biofixation, and maximum biomass dry weight was 0.48 ± 0.02 and 0.51 ± 0.03 g L^{-1}, respectively. This acidophilic microalga proved to be suitable for carbon biofixation and removal of nutrients from secondary effluent of wastewater treatment plants with high CO_2 concentration. All assays were performed without pH control. This microalga species presented high lipid content. However, fatty acid methyl esters (FAME) are not suitable for biodiesel use.

Keywords: biofixation; *Chlamydomonas*; wastewater; biodiesel; microalga

Introduction

Researchers in the field of environmental biotechnology have shown that microalgae cultivation is a potential way to biofix atmospheric CO_2 emitted by natural and anthropogenic sources, including wastewater recovery and starting materials used to make biofuels [1-7]. In fact, use of autotrophic microalgae production to control CO_2 emissions can be envisioned as an important step toward the reduction of industrial carbon emissions [8]. However, high concentrations of CO_2 injected into photobioreactors may also lead to acidification of the culture medium [9].

Some of the most produced microalgae in the world are extremophiles, including, for example *Dunaliella salina* adapted to high salinity and *Arthrospira* platensis adapted to high pH. The cultivation of such extremophiles could present some advantages owing to the easier culture maintenance and reduced problems with microorganismal contamination when production is scaled up [10].

Tang et al. [7] studied the cultivation of the microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa* with CO_2 concentration input varying from 0.03 to 50%. These authors observed higher microalgae productivity using a CO_2 concentration between 5 and 10%. However, low pH values were observed when CO_2 concentration was increased above 20%, resulting in inhibited algae growth. In contrast, acidification of the culture medium could be envisioned as an advantage to scale up production of some microalgae species by reducing the incidence of other competing organisms and predators [11]. Nevertheless, it is necessary to explore the use of extremophile species adapted to acidic pH (acidophilic microalgae) that could provide adequate productivity, CO_2 biofixation rate and biomass with commercial potential.

Accordingly, acidophilic species, such as *Chlamydomonas acidophila*, have been used as a model for physiological studies related to the mechanisms of tolerance and detoxification. These species present high adaptability to environments with extreme acidity and high heavy metal concentrations [12-14]. These mechanisms could also allow for metal biosorption in acidophilic species, as demonstrated for other species of microalgae [15]. In addition, some authors have reported relatively high levels of lipids for *C. acidophila*, especially when grown to stationary phase [13, 16], suggesting a potential use of acidophilic microalgae for the development of bioproducts and bioremediation of urban or industrial wastewater.

Therefore, this study aimed to evaluate the productivity, CO_2 biofixation, and fatty acids composition in biomass of the acidophilic microalga *C. acidophila* LAFIC-004 cultivated in treated wastewater (secondary effluent) with injection of different CO_2 content.

Materials and Methods

Microalga strain

The strain of microalga used in this research was isolated from acid mine drainage (AMD) of the coal mining region of Santa Catarina State in southern Brazil (Lat. 28°35'S, Long. 49°27'W) and held in the Laboratory of Phycology (LAFIC) at the Federal University of Santa Catarina (UFSC) as *Chlamydomonas acidophila* LAFIC-004. The pH in this environment varies from 1.85 to 3.55, turbidity varies from 28 to 70 NTU, salinity varies from 0.31 to 0.80 ppt, and Dissolved Oxygen varies from 0.0 to 6.5 mg L^{-1}.

Experimental setup

The effect of different CO_2 levels injected into the *C. acidophila* LAFIC-004 culture was evaluated. Five experimental treatments were tested. The experimental setup with constant aeration flow of 0.1 vvm (volume of gas per volume of medium per minute) using atmospheric air (0.038% CO2 v v^{-1}) was denominated T-0%. Four other experimental setups were evaluated. Atmospheric air was enriched with CO_2 (provided by a cylinder), resulting in carbon dioxide concentrations of 5, 10, 15 and 20% (v v^{-1}) with 0.1 vvm flow, in turn denoted as T-5%, T-10%, T-15% and T-20%, respectively.

Each experimental setup had three replicates, randomly distributed, with a total of 15 experimental units. Each experimental unit consisted of a square column photobioreactor (dimensions: 70 mm long, 70 mm wide and 1,200 mm high). The cultivation volume was 5,000 mL (1,020 mm of water column height). The experimental units were arranged in a

room with controlled temperature of 25 ± 2 ^{o}C. The lighting was constant with luminous intensity of 150 μmol m^{-2} s^{-1}. The pH of each experimental unit was monitored daily.

Culture medium

Treated domestic wastewater (secondary effluent) was used as an alternative culture medium. This alternative medium was collected at the end of a wastewater treatment plant located in the municipality of Florianópolis in the state of Santa Catarina, Brazil. The treatment station is composed of an upflow anaerobic sludge blanket reactor (UASB), followed by an activated sludge system and final disinfection with sodium hypochlorite. The station operates at an average flow of 50 L s^{-1} and is designed to serve a population of 36,000 inhabitants. The chemical composition of the treated wastewater used as culture medium is shown in Table 1. The Standard Methods for the Examination of Water and Wastewater protocols were followed to perform these analyses [17]. [TABLE 1 NEXT HERE]

The *C. acidophila* LAFIC-004 strain was acclimated to the treated wastewater a week before the beginning of the experiment cultured with 1% CO_2 ($v.v^{-1}$)-enriched air injection. Each experimental unit was started with an inoculum of exponential growing culture with a dry mass of 0.09 ± 0.01 g L^{-1}.

Growth Parameters

To obtain the growth curve, daily aliquots were collected to quantify the cell density of each experimental unit. Cell counting was performed under a microscope with the assistance of a Neubauer chamber.

The specific growth rate (μ, day^{-1}) was calculated as Equation (1):

$$\mu = \left(\frac{LnX_t - LnX_0}{t - t_0} \right) \quad (1)$$

where

X_0 = initial cell density (cells mL^{-1}) at time t_0 (day);

X_t = maximum cell density (cells mL^{-1}) at time t (day); and

t= time (day) that maximum cell density was reached.

Biomass dry weight was calculated at the beginning of the experiment and every two days. Samples were collected, filtered and dried in an oven at 50 ^oC until reaching constant weight, according to Arredondo-Vega and Voltolina [18]. The productivity in g L^{-1} day^{-1} was calculated from the biomass dry weight data as a function of time.

Carbon Biofixation Evaluation

Carbon biofixation was evaluated through CO_2 biofixation rate (RF_{CO2}), the amount of CO_2 removed by the cultivation process, and the amount of CO_2 biofixed by microalgae.

The gas mixture introduced in each experimental unit was provided by an air compressor and pure CO_2. In order to achieve the desired carbon dioxide concentration in each experimental setup, both air and pure CO_2 streams were controlled by rotameters. The gas mixture was adjusted to 0.1 vvm with a rotameter positioned just before the air diffuser in each experimental unit. The enriched air was injected at the bottom of the photobioreactors with an air diffuser 1.5 cm in diameter.

The concentrations of carbon dioxide present in the injected air were quantified and adjusted daily after the beginning of the experiment using the CO_2 meter equipment GEM 2000 (Landtec; 0.1% accuracy).

The CO_2 biofixation rate ($RFco_2$) in g L^{-1} day^{-1} was calculated using Equation (2) [3].

$$RF_{co_2} = C_C \cdot \left(\frac{X_{t_1} - X_t}{t_1 - t} \right) \cdot \left(\frac{M_{CO_2}}{M_C} \right) \quad (2)$$

where

C_c = organic carbon content in microalgae biomass (%);

X_{t1} = biomass dry weight at day t + 1, g L^{-1};

X_t = biomass dry weight at day t, g L^{-1};

M_{co2} = carbon dioxide molecular weight; and

M_c = carbon molecular weight.

The organic carbon content in microalgae biomass was adopted as 50% [2]. To determine the concentrations of carbon dioxide at the exit of the experimental units, a sample of gas was collected every 24 hours and quantified using the GEM 2000. These analyses only occurred in experimental setups T-5 %, T-10%, T-15% and T-20%.

In order to obtain the amount of carbon dioxide removed by cultures, CO_2 removal efficiency was calculated daily following Jacob-Lopes et al. [19] as expressed in Equation 3.

The average of removal efficiencies and the total amount of fixed CO_2 (in kilograms per experimental unit) were calculated at the end of the experiments.

$$ER_{co_2} = \left(\frac{[CO_{2i}] - [CO_{2f}]}{[CO_{2i}]} \right) \quad (3)$$

where

ER_{CO2} = CO_2 removal efficiency (%);

$[CO_{2f}]$ = CO_2 concentration at reactor exit (%); and

$[CO_{2i}]$ = CO_2 concentration at reactor inlet (%).

With accumulated biomass dry weight data, the amount of CO_2 biofixed per experimental unit was determined.

Lipid content and fatty acid methyl ester profile (FAME) evaluation

Culture samples were collected at the end of the experiment and centrifuged in a refrigerated centrifuge (New Technique, Model: 250) at 3,500 rpm and temperature at about 10 ^oC for 10 minutes. The biomass was washed twice with distilled water to remove residual salts from the culture medium. Biological replicas were individually freeze-dried.

Direct transesterification of the biomass was performed according the Hartman and Lago method, adapted for microscale, as described by Menezes et al. [20]. The fatty acids analysis was determined by gas chromatography in a GC (Agilent 7890) equipped with a flame ionization detector (FID) and a split/splitless injector, using a DB-WAX capillary column (30 m× 0.25 mm× 0.25 μm). The oven was operated with an initial temperature of 70 °C, heated at 10 °C min^{-1} until 240 °C, remaining at this temperature for 13 minutes, and then heated 5 °C min^{-1} to 250 °C. The injector temperature was maintained at 310 °C with an injection volume of 2 μL, in the split mode, with a split ratio of 10:1. The detector temperature was kept at 310 °C. Hydrogen was used as the carrier gas at a linear velocity of 42 cm s^{-1}, and nitrogen was used as the auxiliary make-up gas at 20 mL min^{-1}. The FAME profile was identified by direct comparison with oil samples of known composition (soybean, canola, and crambe) through the FAME reference standards (Nu-ChekPrep®) and analyses via high-resolution gas chromatography coupled with mass spectrometry (HRGC-MS), as described by Menezes et al. [20].

Nutrient analysis

Aliquots were collected from the culture medium at the beginning (treated wastewater before microalgae inoculation) and at the end (after microalgae separation) of the experiment to determine the initial and final concentrations of ammonia ($N\text{-}NH_4^+$), nitrite ($N\text{-}NO_2^-$), nitrate ($N\text{-}NO_3^-$) and orthophosphate ($P\text{-}PO_4^{3-}$). These procedures followed the Standard Methods for Water and Wastewater Survey protocols [17].

Statistical analysis

The results were submitted to analysis of variance (ANOVA, $\alpha < 0.05$). When significant differences were detected, Tukey's test was applied to compare the average values of experimental treatments [21].

Results and Discussion

Biomass Growth

Cell density over the cultivation period is shown in Figure 1. Cultures with 5 and 10% CO_2 (v v^{-1})-enriched air injected (T-5 % and T-10%) reached higher maximum cell density. No significant differences were noted between them. The experimental setup that did not receive CO_2 enrichment (T-0%) reached a maximum cell density statistically lower compared to T-5% and T-10%, but a statistically higher cell density than either T-15% or T-20% treatments. These last two did not differ statistically from each other. [FIGURE 1 NEXT HERE]

The growth parameters achieved in *C. acidophila* LAFIC-004 culture using treated wastewater as culture medium were dependent on the CO_2 levels injected into the photobioreactors (Table 2). It is worth highlighting the existence of an optimal range within which carbon dioxide is not considered a limiting factor to microalga development. However, the present research does not exceed the threshold concentration supported by the species. Concentrations of 5 and 10% (v v^{-1}) can be considered within this optimal range. A similar result is reported by Tang et al. [7] with the microalgae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*. According to Cheng et al. [22], the concentration of CO_2 in the culture medium should not be less than the concentration required for maximum microalga productivity, nor more than the maximum level tolerated by the organism.[TABLE 2 NEXT HERE]

The CO_2 concentrations present in emissions from fossil fuel combustion are generally around 10 and 20% [23]. The results presented in this research demonstrate that the acidophilic microalga is able to grow with injection of air containing these CO_2 concentrations.

Values of biomass dry weight are presented in Figure 2. At the end of the experiment, T-5% and T-10% presented the highest biomass dry weight, reaching averages of 0.48 ± 0.02 and 0.51 ± 0.03 g L^{-1}, respectively. These values did not present significant differences. The

T-0% setup reached a biomass dry weight of 0.42 ± 0.03 g L^{-1}. This value was lower than the T-10% treatment, but with no significant difference from the value reached in the T-5% setup. Still, the T-15% and T-20% experiments reached the lowest averages (0.20 ± 0.04 and 0.16 ± 0.02 g L^{-1}, respectively). [FIGURE 2 NEXT HERE]

Evaluation of Carbon Fixation

Figure 3 shows the CO_2 biofixation rates (RF_{CO2}) during cultivation. The CO_2 biofixation rate (RF_{CO2}) through the cultivation period for the T-5% and T-10% experimental setups were 0.092 and 0.094 g L^{-1} day^{-1}, respectively. These experimental setups presented the maximum values on the fourth day of cultivation (0.16 and 0.15 g L^{-1} day^{-1}, respectively). The T-0% treatment presents an RF_{CO2} through cultivation of 0.074 g L^{-1} day^{-1}, followed by T-15% with 0.048 g L^{-1} day^{-1} and T-20% with 0.014 g L^{-1} day^{-1}. The T-0% and T-15% treatments showed maximum RF_{CO2} values of 0.11 g L^{-1} day^{-1} between the fourth and sixth day of culture, while the T-20% treatment reached the maximum RF_{CO2} value of 0.05 g L^{-1} day^{-1} on the sixth day of culture. [FIGURE 3 NEXT HERE]

Factors such as light intensity, cell density and carbon dioxide concentration may influence photosynthetic activity, inhibit CO_2 biofixation and decrease microalgae productivity [22]. According to Costa et al. [24], a shading effect can occur when autotrophic cultivation presents high cell density. This last effect reduces the light intensity available to cells inside the culture, reducing CO_2 biofixation. In the present research, cultures were carried out in batch systems; therefore, it would be hypothetically possible to fix approximately 34 kilograms of carbon dioxide per cubic meter of culture medium per year by cultivating *C. acidophila* in treated wastewater with 5 or 10% (v v^{-1}) of CO_2 injection. This amount is lower than that reported in cultures of *Chlorella vulgaris*, *Botryococcus braunii*, *Dunaliella tertiolecta* [25], *S. obliquus* and *C. pyrenoidosa* [7]. Management strategies to increase light intensity and perform semicontinuous cultures may increase CO_2 biofixation

rate [26-28].

The highest CO_2 biofixation rates were reached around the fourth day of cultivation when the microalgae were in exponential growth phase. As the cell density increases, it is possible dilute the cultivation periodically by removing a certain amount of biomass in order to avoid productivity reduction and, consequently, decrease the carbon dioxide biofixation rate [24]. Therefore, considering a semicontinuous cultivation process with dilutions performed every two days from the fourth day of cultivation, it would be possible to biofix approximately 55 kg m^{-3} $year^{-1}$ when the cultures are injected with air containing 5 or 10% (vv^{-1}) CO_2.

The use of gaseous emissions from anthropogenic activities (e.g., manufacture of cement, power plants, landfills and other industries) as an economical alternative to increase microalga biomass production is justified only at 5 and 10% (v v^{-1}) CO_2 concentrations, essentially because productivity would be decreased at higher levels. However, the use of gaseous emissions containing CO_2 concentrations around 15% may be an alternative to mitigate carbon emission impact when cultivated up to six days, or, as discussed above, in semicontinuous systems.

CO_2 utilization efficiency

The average values of introduced, removed and biofixed CO_2 in each photobioreactor (experimental unit) for each setup during the cultivation period are shown in Table 3.

[TABLE 3 NEXT HERE]

The percentage of removed CO_2 varied from 2.97 to 17.60%. As the proportion of injected CO_2 increased, the percentage of removed CO_2 decreased, and, consequently, more CO_2 was emitted throughout the photobioreactors. This could be explained by the culture medium already saturated with dissolved CO_2. The T-5% treatment presented a higher amount of removed CO_2 and lower emitted quantity. Meanwhile, cultures with CO_2 injection

only biofixed a small amount of carbon in relation to the total introduced. The percentage of carbon biofixed in these experimental treatments varied between 0.05 and 0.62%. As the proportion of CO_2 level increased, the percentage biofixed by microalgae decreased. However, the T-0% treatment biofixed around 75% of the total injected carbon present in the natural atmospheric air.

The results obtained by Zheng et al. [28] also demonstrate that CO_2 biofixation efficiency decreased when CO_2 concentration increased in the inlet flow. These authors found a percentage of CO_2 biofixation between 0.60 and 3.72% for cultures with 5, 10 and 15% of CO_2. Therefore, Zheng et al. [28] suggest reducing the proportion of CO_2 in the inlet air to 2.9% to avoid CO_2 losses. It is also recommended that cultivation be started with a high cell concentration. Therefore, when using gaseous emissions that contain a high CO_2 concentration, such as those emanating from an industrial source with 15 or 20% concentration, for example, it may be interesting to dilute the gas emitted to a 5% concentration prior to the injection in microalgae cultures. Then, it would be possible to improve the productivity and CO_2 biofixation, as our results have shown.

Another alternative for increasing CO_2 biofixation efficiency is the injection of this gas for a specific time, as proposed by Morais and Costa [3], who reported the injection of CO_2 enriched air for 15 minutes every two hours. Also, according to the same authors, another alternative is to recirculate the outlet gas for CO_2 not retained by the system's reutilization.

The percentage of biofixed CO_2 relative to the total amount of CO_2 removed by the photobioreactors varied between 1.71 and 6.38%. The lowest value was found in the T-20% experimental setup and the highest in the T-10% setup (Table 3). Similar results were found by Jacob-Lopes et al. [29], who reported other CO_2 transformation routes through microalgae cultivation. Among these routes, we note mineralization, biomineralization, and excretion of organic compounds, such as polysaccharides, as well as biotransformation of CO_2 into

volatile organic compounds [29-31]. According to Gonzáles-Lópes et al. [32], polymers, such as polysaccharides, are excreted in greater amounts as the concentration of CO_2 increases. This fact may explain why the T-15% and T-20% treatments presented adequate CO_2 removal compared to the T-10% experiment, even with lower productivity and carbon dioxide biofixation.

It is possible that different CO_2 removal routes occurred through the cultivation of *C. acidophila* LAFIC-004. However, future studies are needed to elucidate, both qualitatively and quantitatively, which routes, either physicochemical or biological, occur, with the aim of determining the mass balance of carbon through the photobioreactors.

Biomass lipid content and FAME profile

The CO_2 levels injected in the cultures influenced the biochemical composition of microalgae. Table 4 shows the biomass lipid content of *C. acidophila* LAFIC-004 in each experimental setup performed. As CO_2 levels increased, biomass lipid content tended to decrease. In all experiments evaluated, lipid content was considered high in relation to that reported in the literature [7, 25]. Microalgae biomass lipid content is usually related to the nitrogen availability. Upon nitrogen starvation in the microalgae culture, biomass typically presents lipid accumulation. Therefore, the nitrogen available in all treatments tested in the present research was similar (Table 5).

Fourteen fatty acids were identified as present in the biomass of *C. acidophila* LAFIC-004 in all experiments, except the T-15% experiment where the fatty acid myristoleic (C14: 1 c9) was not identified (Table 4). As reported by Tang et al. [7], the presence of fatty acids with 16 and 18 carbons (C16 - C18) was also identified in all cultures, representing more than 97% of the total. According to Miao et al. [33], these fatty acids are favorable for the production of biodiesel. [TABLE 4 NEXT HERE]

Saturated fatty acids represent a significant portion of the total fatty acids present in the biomass. According to Tang et al. [7], a rise in CO_2 levels may increase the degree of fatty acid unsaturation. The results reported for *C. acidophila* LAFIC-004 follow this trend, as shown in Table 4. Among the unsaturated fatty acids, triunsaturated fatty acids predominated. In addition, the presence of polyunsaturated fatty acids (PUFA) was verified in all cultures. As lipid content decreased with the increase of CO_2 concentration, PUFA content tended to increase. According to Paliwal et al. [34], under abiotic stress condition, microalgae produce a large array of compounds, including PUFAs, in order to survive in the extreme environmental conditions through adaptation. It is possible that these results express a stress condition owing to the high concentration of CO_2. These PUFAs may be of economic interest.

Regarding the potential of *C. acidophila* LAFIC-004 biomass as biodiesel feedstock, the European standard EN 14214 that describes the requirements and test methods for FAME stipulates that the maximum acceptable limit for linolenic acid is 12%, and for fatty acids with more than three double bonds, it is 1% [2]. Therefore, all experimental setups produced biomass without characteristics compatible with the European standard. *C. acidophila* LAFIC-004 biomass presented values of triunsaturated (TUFA) and PUFA superior to the limits determined by norm EN14214.

Nitrogen, phosphorus, and pH in treated wastewater used as culture medium

The stress generated by extremely acidic pH results in high energy consumption since acidophilic microalgae tend to maintain neutral intracellular pH despite external values [35]. In addition, in highly acidic waters, inorganic carbon occurs almost exclusively in the form of dissolved CO_2 without a bicarbonate pool [36]. Consequently, inorganic carbon concentrations are so low that they can limit photosynthetic growth [37]. The average pH values monitored daily during cultivation period are shown in Figure 4. As CO_2 levels supplied to the cultures increased, pH markedly decreased. However, in all experimental

setups, the tendency was toward stabilization of this parameter. The lowest value was 6.14 ± 0.05 in the T-20% treatment. [FIGURE 4 NEXT HERE]

The high solubility of CO_2 and the high concentrations used in this research led to a rapid decrease of pH values [9], which were rapidly stabilized by the high alkalinity found in the treated wastewater used as culture medium. The T-0% experimental setup did not receive CO_2 enriched air, resulting in the absence of pH acidification, which could have negatively influenced the productivity of the acidophilic microalga *C. acidophila* LAFIC-004 compared to other experimental setups.

The results of nitrogen and phosphorus concentrations, both at the beginning before inoculum addition and end of the cultivation period after separation of the biomass produced, as well as the efficiency of removing these nutrients, are presented in Table 5. [TABLE 5 NEXT HERE]

Before the acclimation of *C. acidophila* LAFIC-004 to start this research, this strain was cultured at TAP medium [38] which presents 93 mg L^{-1} of ammoniacal nitrogen (N-$NH_4^{+)}$ and 81.7 mg L^{-1} of orthophosphate (P-PO_4^{3-}) [39]. This culture medium has nitrogen and phosphorous in abundance, without these macronutrients being considered as limiting factors. Nevertheless, the use of domestic wastewater as an alternative culture medium for microalgae cultivation has been shown to be efficient in providing balanced amounts of macro- and micronutrients, bringing environmental and economic benefits by minimizing the demand for water and fertilizers [40]. The N:P ratio found at the beginning of the experiment was approximately 38:1. This ratio is considered high for microalgae cultivation, since the ratio is generally around 16: 1 [41]. Therefore, it is assumed that phosphorus limits productivity and, consequently, the removal of ammonium. However, it is possible that the enrichment of culture medium used in this research with phosphate increased microalgae productivity, CO_2 biofixation and nitrogen removal.

Conclusions

The acidophilic microalga *Chlamydomonas acidophila* LAFIC-004 presented better productivity when cultivated with 5 and 10% CO_2 injected in photobioreactor. However, higher CO_2 levels are tolerated by this species. This microalga has potential for CO_2 biofixation present in atmospheric emissions, as well as for removing nutrients present in treated wastewater. In order to increase CO_2 utilization efficiency by cultures, it is recommended that enriched air be injected with no more than 5% (v v^{-1}) CO_2 concentration. The acidophilic microalga presented high concentrations of lipids. However, the composition is not suitable as a biodiesel feedstock.

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Tables

Table 1. Average values obtained in the treated wastewater used as an alternative culture medium.

Parameter	
Temperature (^{o}C)	25.8
BOD_5 (mg L^{-1})	14.0
QOD (mg L^{-1})	78.0
Total Phosphorous (mg L^{-1})	3.94
Suspended Solids (mg L^{-1})	53.0
Nitrogen-NH_4 (mg L^{-1})	57.9
Nitrogen-NO_2^- (mg L^{-1})	1.7
Nitrogen-NO_3^- (mg L^{-1})	6.2
Total Alkalinity (mg $CaCO_3$ L^{-1})	165.4
pH	7.8

Table 2. Maximum cell density (MCD), specific growth rate (μ) and productivity of *Chlamydomonas acidophila* LAFIC-004 cultivated in different levels of CO_2 injection.

Growth parameter	T-0%	T-5%	T-10%	T-15%	T-20%
MCD (10^4 cells mL^{-1})	793±137 b	1347±32 a	1305±55 a	489±68 c	335±65 c
μ - (d^{-1})	0.80±0.07 a	0.92±0.06 a	0.87±0.01 a	0.76±0.03 ab	0.61±0.11 b
Productivity (g L^{-1} day^{-1})	0.04±0.01 ab	0.06±0.01 a	0.06±0.01 a	0.03±0.01 bc	0.02±0.01 c

Table 3. Amount of introduced, removed and biofixed CO_2 by the experimental units (E.U.) with different levels of CO_2 injection after the cultivation period.

Parameter	Introduced CO_2 amount	Removed CO_2 amount		Biofixed CO_2 amount		
Treatment	(kg/E.U.)	(kg/E.U.)	(%)	(kg/E.U.)	(%)	($\%_{CO2\ remov.}$)
T-0%	0.005	-	-	0.00378	75.60	-
T-5%	0.70	0.123	17.60 ± 7.7 a	0.00432	0.62	3.51
T-10%	1.41	0.072	5.09 ± 0.5 b	0.00459	0.33	6.38
T-15%	2.12	0.088	4.15 ± 0.5 b	0.00180	0.08	2.05
T-20%	2.82	0.084	2.97 ± 1.7 b	0.00144	0.05	1.71

Table 4. FAME composition and total lipid content in the biomass of *Chlamydomonas acidophila* LAFIC-004 cultivated with different levels of CO_2 injection.

Fatty Acid Methyl Ester (FAME)	Shorthand Notation	FAME Content (%)				
		T-0%	T-5%	T-10%	T-15%	T-20%
Myristic	C14:0	0.2	0.3	0.5	0.4	0.4
Myristolic	C14:1 c9	0.5	0.2	0.5	-	0.6
Palmitic	C16:0	38.3	32.7	30.9	35.1	32.2
Palmitoleic	C16:1 c9	0.3	0.3	0.5	0.2	0.4
7,10-Hexadecadienoic	C16:2 c7,10	1.1	1.1	1.1	1.7	2.0
7,10,13-Hexadecatrienoic	C16:3 c7,10,13	3.6	5.9	7.5	7.7	8.7
Stearic	C18:0	1.6	1.2	1.1	1.2	1.0
Oleic	C18:1 c9	21.9	17.5	14.4	10.9	9.4
Vaccenic	C18:1 c11	7.3	7.6	7.9	10.0	12.2
Linoleic	C18:2 c9,12	10.6	11.3	9.5	5.1	5.2
Gamma-linolenic	C18:3 c6,9,12	0.4	0.4	0.5	0.6	0.5
Linolenic	C18:3 c9,12,15	11.1	16.7	19.7	19.8	20.3
5,9,12,15-Octadecatetraenoic	C18:4 c5,9,12,15	1.6	3.1	4.5	5.3	5.4
Nonadecanoic	C19:0	0.6	0.5	0.4	0.3	0.1
Polyunsaturated	-	0.9	1.2	1.0	1.7	1.6
Saturated Fatty Acids (%)		40.7	34.7	32.9	37.0	33.7
Monounsaturated Fatty Acids(%)		30.0	25.6	23.3	21.1	22.6
Diunsaturated Fatty Acids (%)		11.7	12.4	10.6	6.8	7.2
Triunsaturated Fatty Acids (%)		15.1	23.0	27.7	28.1	29.5
Polyunsaturated Fatty Acids (%)		2.5	4.3	5.5	7.0	7.0
Total Lipid Content		50.6	37.9	25.5	27.6	27.6

Table 5. Values of ammonia, nitrite, nitrate and orthophosphate of treated wastewater utilized as culture medium for *Chlamydomonas acidophila* LAFIC-004 in different levels of CO_2 injection.

Analyzed Parameter	Initial Concentration (mg L^{-1})	Treatment	Final Concentration (mg L^{-1})	Removal Efficiency (%)
$N\text{-}NH_4^+$	57.90	T-0%	22.24 ± 6.22 a	61.58 ± 10.75 a
		T-5%	23.04 ± 1.67 a	60.20 ± 2.88 a
		T-10%	23.81 ± 0.17 a	58.86 ± 0.31 a
		T-15%	32.41 ± 4.29 a	44.02 ± 7.42 a
		T-20%	26.14 ± 4.18 a	54.84 ± 7.23 a
$N\text{-}NO_2^-$	1.68	T-0%	0.09±0.03 a	94.44 ± 1.92 a
		T-5%	0.08±0.06 a	95.00 ± 3.33 a
		T-10%	0.11±0.06 a	93.33 ± 3.85 a
		T-15%	0.17±0.10 a	90.00 ± 5.77 a
		T-20%	0.15±0.03 a	91.11 ± 1.92 a
$N\text{-}NO_3^{-2}$	6.2	T-0%	0.29 ± 0.21 a	95.38 ± 3.45 a
		T-5%	0.25 ± 0.17 a	95.97 ± 2.74 a
		T-10%	0.30 ± 0.18 a	95.16 ± 2.96 a
		T-15%	0.71 ± 0.41 a	88.55 ± 6.66 a
		T-20%	0.51 ± 0.29 a	91.83 ± 4.76 a
$P\text{-}HPO_4$	3.94	T-0%	0.95 ± 0.29 a	75.91 ± 5.8 a
		T-5%	0.63 ± 0.12 a	83.99 ± 3.81 a
		T-10%	0.79 ± 0.13 a	79.91± 3.25 a
		T-15%	1.21 ± 0.15 a	69.23 ± 3.05 a
		T-20%	1.22 ± 0.57 a	69.08 ± 14.41 a

Means ± standard deviation followed by the same lowercase letters do not differ at the 5% level of probability by the Tukey test.

Figure captions

Figure 1. Increase of cell density (cells mL^{-1}) during cultivation period. Each line refers to the average of three replicates. Conventions: T-0% -introduction of 0.03% (v v^{-1}) of CO_2; T-5% -introduction of 5% (v v^{-1}) of CO_2; T-10% -introduction of 10% (v v^{-1}) of CO_2; T-15% -introduction of 15% (v v^{-1}) of CO_2; T-20% -introduction of 20% (v v^{-1}) of CO_2.

Figure 2. Increase of biomass dry weight (g L^{-1}) during cultivation period. Each line refers to the average of three replicates. Conventions: T-0% -introduction of 0.03% (v v^{-1}) of CO_2; T-5% -introduction of 5% (v v^{-1}) of CO_2; T-10% -introduction of 10% (v v^{-1}) of CO_2; T-15% -introduction of 15% (v v^{-1}) of CO_2; T-20% -introduction of 20% (v v^{-1}) of CO_2.

Figure 3. CO_2 biofixation rate during cultivation period. Each point refers to the average of three replicates. Conventions: T-0% -introduction of 0.03% (v v^{-1}) of CO_2; T-5% -introduction of 5% (v v^{-1}) of CO_2; T-10% -introduction of 10% (v v^{-1}) of CO_2; T-15% -introduction of 15% (v v^{-1}) of CO_2; T-20% -introduction of 20% (v v^{-1}) of CO_2.

Figure 4. pH variation during cultivation period. Each line refers to the average of three replicates. Conventions: T-0% -introduction of 0.03% (v v^{-1}) of CO_2; T-5% -introduction of 5% (v v^{-1}) of CO_2; T-10% -introduction of 10% (v v^{-1}) of CO_2; T-15% -introduction of 15% (v v^{-1}) of CO_2; T-20% -introduction of 20% (v v^{-1}) of CO_2.