

Effect of Nitrogen Content and CO₂ Consumption Rate by Adding Sodium Carbonate in the Lipid Content of *Chlorella vulgaris* and *Neochloris oleoabundans*

Nain Elvira-Antonio^{*1}, Alejandro Ruíz-Marín², Yunuen Canedo-López³

^{1, 2, 3}Universidad Autónoma del Carmen, Campeche. DES-DACQYP. Facultad de Química.

Calle 56 x Av. Concordia. Col. Benito Juárez. C.P. 24180. México.

^{1*}nelvira@pampano.unacar.mx; ²aruiz@pampano.unacar.mx; ³ycanedo@pampano.unacar.mx

Abstract- New alternatives for the production of fuels have led to considering the use of microalgae to obtain biofuel. Studies have reported that *Chlorella vulgaris* (59%), *Nannochloropsis* (68%) and *Neochlorisoleoabundans* (54%) had high content of lipid under nitrogen limitation. The present study evaluated the effects on growth and lipid content of *Chlorella vulgaris* and *Neochlorisoleoabundans* under reduced nitrogen content and enrichment with sodium carbonate (Na₂CO₃). Both *C. vulgaris* and *N. oleoabundans* were cultivated in medium with NH₄Cl and KNO₃ as only source of nitrogen, respectively. The nitrogen initial content was of 30 mg l⁻¹ at 32 °C and light intensity of 100 μmol m⁻² s⁻¹. The maximum cell density obtained was of 21.80 x 10⁶ cells ml⁻¹ and 28.12 x 10⁶ cells ml⁻¹ for both microalgae, where higher growth rate was obtained for *N. oleoabundans* of 0.219 d⁻¹ that *C. vulgaris* of 0.183 d⁻¹ with similar lipids content (65.20-69.31%). In culture with Na₂CO₃ at concentrations of 1, 2.5 and 5 g l⁻¹, the highest lipid content (69.5%) for *C. vulgaris* in culture with Na₂CO₃ of 1 g l⁻¹ was obtained during 144 h of culture, whereas that for *N. oleoabundans* the 57.7% lipid content was obtained at 120 h. The lipid content and growth for both microalgae decreases at higher concentration of Na₂CO₃; caused probable by inhibition processes. The consumption rate of carbon dioxide showed that *N. oleoabundans* had a greater capacity and tolerance for using carbon dioxide and carbonate (112.8-115.2 mg l⁻¹ d⁻¹) with respect to *C. vulgaris* 95.76-105.75 mg l⁻¹ d⁻¹.

Keywords- *Chlorella vulgaris*; *Neochloris oleoabundans*; Nitrogen; Lipid Content; CO₂ Consumption Rate

I. INTRODUCTION

A mitigation strategy of CO₂ that has been studied in the last years is the use of microalgae, which has received major attention as a viable alternative for the biomass production and energy [1].

The mitigation CO₂ can be performed by plants and photo synthetic microorganisms. In particular attention by microalgae, which correspond to a group of unicellular microorganisms with rapidly growing and ability to fix CO₂ while capturing solar energy [2].

Microalgae are cells that use sunlight to convert carbon dioxide to potential biofuels [3], and different types of renewable biofuels, including methane produced by the anaerobic digestion of algal biomass, biodiesel and biohydrogen photobiological [4, 5]. An important feature is that the oil accumulated in the case of the microalgae is constituted mainly of triglycerides (> 80%) which can be used for the production of biodiesel [6].

The microalgae culture systems show a versatility which allows them to be used in different processes such as in waste water treatment, production of animal feed, fertilizer production and production of other chemical compounds [7]. The utilization of nutrients present in waste water or conventional culture media is incorporated by microalgae such as proteins, carbohydrates and lipids. The ability to accumulate oil microalgae depends on growing conditions, such as temperature, light intensity, pH, salinity, mineral, nitrogen inputs and CO₂ enrichment. Studies have reported that the limitation of nitrogen can increase the lipid content of the strains of *Chlorella* [8]. The species *Neochlorisoleoabundans* under nitrogen limiting conditions may accumulate 35% to 54% lipids of biomass dry weight and triglycerides by 80% of total lipids [9], while *C. vulgaris* can present up to 59.7% lipids [8].

Another factor that has been reported and that affects the growth and accumulation of lipids is carbon dioxide. Various CO₂ mitigation strategies have been investigated, which can be generally classified into two categories: (1) chemical reaction-based approaches and (2) biological CO₂ mitigation. A popular chemical reaction-based CO₂ mitigation approach is achieved by cyclic carbonation/de-carbonation reactions in which gaseous CO₂ reacts with solid metal oxide (Represented by MO) to yield metal carbonate (MCO₃). The reaction can be represented by equation 1.



Once the metal oxide reaches its ultimate, metal carbonate may be thermally regenerated to metal oxide and CO₂ by

heating the metal carbonate beyond its calcination temperature. As this method for CO₂ capture is relatively expensive and high energy consumption, the advantage of mitigating becomes marginal. Therefore it is necessary to develop cost-effective and sustainable alternatives to fix CO₂ emissions.

However, the carbonate produced could present an opportunity to be added to the culture medium for algal biomass production. This would provide a high concentration of CO₂, which would be beneficial as long as microalgae are able to tolerate high levels of CO₂ [10]. Microalgae species have the capacity to use carbonate such as Na₂CO₃ and NaHCO₃ for cell growth [11-13]. Some of these species typically have a high extracellular carbon hydrazase activity, which is responsible for the conversion of carbonate to free CO₂ and thereby facilitate the assimilation. Thus some species of microalgae can fix CO₂ released from the carbonates and may be advantageous in many aspects: a) CO₂ released from industrial facilities could be converted to carbonate salt by chemical reactions, b) a limited number of microalgae species growth on media containing high concentrations of carbonate, therefore the control of other invading species is relatively simple and, c) a majority of these species have high optimum pH (9 -11).

The present study evaluated the growth and lipid accumulation by *Chlorella vulgaris* and *Neochloris oleoabundans* in cultures to different nitrogen limitation and concentration of carbonate, which was possible to estimate the consumption rate of carbon dioxide. Combining CO₂ mitigation and nitrogen limitation as a strategy for lipid accumulation in microalgae may provide an innovative alternative to current carbon-reduction and biofuel-production strategies.

II. MATERIALS AND METHODS

A. The Microalga Strain and Growth Medium

Microalgae *C. vulgaris* and *N. oleoabundans* were growing in sterile artificial wastewater prepared with the following concentrations: 7 mg l⁻¹ NaCl; 4 mg l⁻¹ CaCl₂; 2 mg l⁻¹ MgSO₄·7H₂O; 15 mg l⁻¹ KH₂PO₄ [14]. Trace metals and vitamins were added in reference to the f/2 medium by Guillard and Ryther [15], the nitrogen source used for *C. vulgaris* was NH₄Cl (115 mg l⁻¹) and for *N. oleoabundans* was KNO₃ (217 mg l⁻¹). Both microalgae were maintained in artificial wastewater medium for acclimatization in 250 ml flasks at 32 ± 1 °C and continuous illumination at 100 μmol m⁻² s⁻¹ with cool white fluorescent lamps.

B. Culture in Photobioreactors

Both microalgae, once acclimated in artificial wastewater medium, a volume of culture with initial inoculum of 1 x 10⁶ cell ml⁻¹ was transferred to bioreactors, which consisted in closed containers of polyethylene terephthalate (PET) of 3 liters with operating volume of 2.5 liters. The bioreactors were aerated to keep the cells in suspension and in completely mixed conditions. The air diffuser device was placed 1 cm from the base of the bioreactor. Another outlet was adapted as sampling system. The bioreactors were disinfected before each culture by appropriate washing with water and chlorine solution.

The study consisted in cultures with nitrogen limitation used for *C. vulgaris* 30 mg N-NH₄ l⁻¹ and for *N. oleoabundans* of 30 mg N-NO₃ l⁻¹. For each culture microalgae were added concentrations of Na₂CO₃ of 1, 2.5 and 5 g l⁻¹. In addition, a reactor was installed as a control for each algal species, which consisted of medium without addition of Na₂CO₃. All treatments were run in triplicates. The reactors were maintained at 32 ± 1 °C with continuous illumination. In each reactor was transferred initial cell density of 1 x 10⁶ cells ml⁻¹ previously determined by counting in Neubauer hemacytometer chamber depth of 0.1 mm.

Every 24 hours a water sample (150 ml) was collected for analysis of N-NH₄ and N-NO₃ according to standard methods (AOHA, 1995). The number of cells (cells ml⁻¹) was determined by counting in Neubauer hemacytometer chamber depth of 0.1 mm. To determine dry-weight biomass, a volume of 20 ml were filtered through a Whatman GF/C glass fiber filters (2.5 cm diameter), previously rinsed with distilled water, and incinerated at 470 °C for 4 h to constant weight. The samples were dried at 120 °C to constant weight, and placed at 450 °C in a muffle furnace. Total lipids were extracted using the method of Bligh and Dyer [16] and its quantification was estimated by the method of Pande et al. [17] using tripalmitin (99%) as standard.

The effect caused by the nitrogen limitation and carbonates concentration on the growth, nitrogen removal and lipid content for the two species of microalgae was analyzed by analysis of covariance (ANCOVA) at P ≤ 0.05 using Statistical software (StatSoft Inc., Tulsa, OK, USA). The Tukey test was applied when results showed significant differences.

C. Determination of the CO₂ Consumption Rate

The increase of biomass concentration (X; g l⁻¹) was used to calculate the maximum specific growth rate (μ_{max}, d⁻¹). The maximum biomass concentration achieved in culture was designated as X_{max} (g l⁻¹), and the biomass productivity (P, mg l⁻¹d⁻¹) was calculated using Equation 2 [18].

$$P = \Delta X / \Delta t \quad (2)$$

Where ΔX is the variation of biomass concentration (mg l⁻¹), during the culture time Δt (d). Moreover, the CO₂ consumption rate (P_{CO₂}, mg l⁻¹d⁻¹) was derived with Equation 3 [18].

$$P_{CO_2} = 1.88 \times P \quad (3)$$

Where P is the biomass productivity biomass ($\text{mg l}^{-1} \text{d}^{-1}$)

III. RESULTS AND DISCUSIONS

A. Growth and Lipid Content

There exists a great challenge when it comes to maximizing oil production in microalgae. A high lipids concentration is achieved when the algae are under environmental stresses, in particular nutrients limitation, but it has been associated that while the lipid concentration can be increased under nitrogen limiting, the growth rate and biomass production are reduced, which is not recommended when the intended has a high productivity of oil [19].

Many autotrophic microalgae such as *Chlorella vulgaris*, *Botryococcus braunii*, *Navicula pelliculosa*, *Scenedesmus acutus*, *Cryptocodinium cohnii*, *Dunaliella primolecta*, *Monallanthus salina*, *Neochloris oleabundans*, *Phaeodactylum tricornutum*, y *Tetraselmis suecica* can accumulate lipids [20, 21] and the accumulation of lipid depend on growing conditions, such as temperature, light intensity and quality, pH, salinity and nitrogen sources. All the parameters should be considered simultaneously in the selection of the species or strain more suitable for lipid accumulation. It has been reported that nitrogen limitation may increase the lipid content of the strains of *Chlorella* [8, 22]. Other studies suggest that a high light intensity and nitrogen deficiency are important for lipid accumulation for *Chlorella sp.* [23]. On the other hand, the microalga *Parietochlorisincisa* increased lipid content (35% dry weight) with high light intensities of $400 \mu\text{E m}^{-2} \text{s}^{-1}$ [24].

In this context, studies had reported a lipid content in some strains as *Chlorella emersonii* (63%), *C. minutissima* (56%), *C. vulgaris* (59.7%), *C. luteoviridis* (28.8%), *C. capsulata* (11.4%), and *C. pyrenoidosa* (29.2%) [25]. In some other species as *N. oleabundans* has been reported under nitrogen limiting conditions an accumulation of lipid of 35%-54% of biomass dry weight and triglycerides 80% of total lipids [9].

Moreover, the microalgae *Botryococcus braunii* under nitrogen limiting conditions showed a decrease in the growth rate and biomass production, consequently this was accompanied by changes in the biochemical composition of the cell as low content of nitrogen compounds and carbohydrates, and improvement in lipid synthesis [26].

In the present study, the maximum cell density obtained for *Chlorella vulgaris* was of 22×10^6 cells ml^{-1} , with a growth rate of 0.183 d^{-1} . Similar results were reported for *C. vulgaris* of 0.128 d^{-1} a 30°C and illumination of $27 \mu\text{E m}^{-2} \text{s}^{-1}$ [27]. Other studies have reported a lower cell density of 6.4×10^6 cells ml^{-1} and rate growth of 0.377 d^{-1} for this microalga cultivated in artificial medium with concentrations of 30 mg N l^{-1} after two days of culture at 25°C and $135 \mu\text{E m}^{-2} \text{s}^{-1}$ [14]. This difference is probably due to various factors involved in the experimental stage to the production of biomass, such as temperature and light intensity (Figure 1).

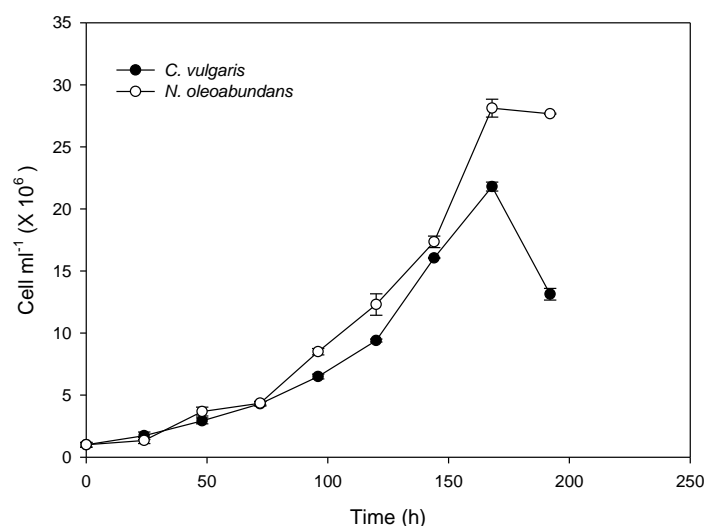


Fig. 1 Algal growth (cell ml^{-1}) in artificial wastewater without addition of carbonate (Na_2CO_3) with 30 mg l^{-1} of nitrogen

The microalgae *Neochloris oleabundans* showed a preference for nitrate, reached a maximum cells density of 28.12×10^6 cells ml^{-1} and growth rate of 0.219 d^{-1} (Figure 1). Similar to *C. vulgaris*, the microalgae *N. oleabundans* presented a similar growth rate, which suggest that a higher concentration of N causes increase in the growth of the microalgae. Cultures of *N. oleabundans* in medium with 10 mM NaNO_3 , (approximately 140 mg l^{-1} of N) may reached maximum biomass production of $0.63 \text{ g l}^{-1} \text{d}^{-1}$ [2]. The authors argue that probably increasing the nitrate concentration to 15 mM (210 mg l^{-1} of N) the biomass production decreases and causes inhibition on growth at high concentrations. However, a lipid content of 40% to 3 mM NaNO_3 (42 mg l^{-1} N) was obtained. These results clearly show that the potassium nitrate is a preferential source for the growth and lipid production for this microalgae.

Although the temperature may be a factor limiting the growth of microalgae, it is considered as optimum temperature of 30 °C. However, other factors must be considered as the light intensity and CO₂ concentration. That is, the effective temperature regulates various metabolic processes and interaction between CO₂ concentrations, being that the temperature is related to the growth and algal biomass production. Studies suggest that *Chlorella vulgaris* has the optimum growth to 30 °C and this can be significantly improved when CO₂ levels are increased to 6% [27]. Studies indicate that the CO₂ present in the air and supplied within culture could not support the growth of *C. vulgaris* at 50 °C while high CO₂ concentrations significantly improve growth. Studies for *Chlorella sp.* isolated from hot springs in Japan have reported favorable growth at 42 °C when the supply air containing 40% CO₂ [28].

The lipid content obtained in this study for *C. vulgaris* and *N. oleoabundans* (69.3% and 65.2%, respectively) were similar to a concentration of 30 mg l⁻¹ over a period of 4 and 6 days, respectively (Table 1 and 2: Control). It was clear that at this time, the lipid content showed no significant difference ($P \geq 0.05$). This could be attributed to the effects of stress to which the cells are subjected, particularly nitrogen limitation. Some studies suggest that if light, carbon dioxide and other nutrients are not limiting, cells may continue to increase cell density, but less rapidly.

When the nitrogen is exhausted from medium culture, the cell-nitrogen is used in enzymes and essential cellular structures and a portion of the carbon dioxide content is converted to lipid and carbohydrates [29]. Other studies suggest that the depleted of external nitrogen, causes an increase of carotenoid/chlorophyll, and this could be related to partial degradation of the chloroplast membrane to obtain the cell requires energy, which causes a decline in the content polar lipids during the culture period, this suggests that nitrogen depletion in the culture medium causes that the cell uses other energy sources as chlorophyll and carotenoids cell [26].

In the present study, the nitrogen content after the fourth day declines significantly, however, it was observed that growth continued until day 8, probably related with the mentioned previously. The algal-culture with lower nitrogen content maintained a low cell density in the reactor, which allows the diffusion of light, and results in more metabolic flux generated from the photosynthesis to the formation or accumulation of lipid, however, because chlorophyll is a nitrogen-rich compound, it is possible to conclude that the nitrogen exhausted from medium causes that the cells use chlorophyll-nitrogen to the synthesis of cell material for further division and/or growth. Similar results were reported, suggesting that chlorophyll is one of the easy accessibility of intracellular materials and cells begin to use it when the external nitrogen becomes depleted [2]. However, it should be noted that the chlorophyll is the essential component of the photosynthetic mechanism of green algae, which is responsible for the capture of CO₂ to generate energy and metabolic fluxes for both growth and accumulation of lipids, therefore, low critical levels of chlorophyll may affect the algal-growth.

For purposes of comparing, the production of biomass and growth rate, with a similar nitrogen concentration, *Chlorella vulgaris* reached a maximum cell density of 11.5×10^6 cells ml⁻¹ and growth rate of 0.54 d⁻¹ using KNO₃ as nitrogen source [30]. Other studies, reported a lower density of 16×10^6 cells ml⁻¹ with similar inoculum size (1×10^6 cells ml⁻¹) [31]. Similarly, Lau et al. [32] reported a growth rate for *C. vulgaris* of 0.364 d⁻¹, and Lau et al. [33] reported a cell density of 26.5×10^6 cells ml⁻¹ and growth rate of 0.362 d⁻¹, both studies used Bristol medium with 40 mg l⁻¹ NO₃-N, which in terms nitrogen content is similar to the used for *C. vulgaris* in the present work.

Probably, this difference in the maximum cell density can be attributed to the types of nitrogen inputs and concentration. In the present study it was used NH₄Cl equivalent to 30 mg N l⁻¹, higher to the reported by Jeanfils et al. [30] with 12 mM NO₃ equivalent to a concentration of 140 mg N l⁻¹. The difference is related to the different inputs of nitrogen in water or culture medium (ammonia, urea, nitrite and nitrate), the concentration of these compound can affect the assimilation of inorganic nitrogen [34]. A fact is that both microalgae showed favorable growth to 30 mg l⁻¹ with ammonium and nitrate, which induces both high lipid content. An alternative approach to minimize the effect of low biomass production is the use of a new system combining a nitrogen-enriched photo bioreactor with subsequent transfer to nitrogen limitation medium (two-stage method), the process allows higher biomass production algal and high lipid content [19].

B. Lipid Content in Carbonated Salts Culture

One of the forms in which it can perform CO₂ capture is by chemical reactions occurring soluble carbonate (NaHCO₃ and Na₂CO₃), which may subsequently be added to the culture medium for algal biomass production. This would provide a high concentration of CO₂, which would be beneficial as long as microalgae are able to tolerate high levels of CO₂ [10]. Studies indicate that many species of microalgae can be tolerant to high levels of CO₂ as is the case of marine microalgae *Chlorococcum littorale* that showed tolerance to CO₂ concentrations by more than 40% [35], similarly, *Chlorella* and *Scenedesmus kessleriobliquus* reached tolerance up to 18% of CO₂ dissolved in the medium [36].

Algae are capable of using CO₂, carbonate (CO₃²⁻) and bicarbonate (HCO₃⁻). The most preferred source under normal conditions is CO₂ gas. CO₂ transporting through the cell membrane is dependent on the energy and its accumulation in cells is performed — HCO₃⁻. CO₂ is later converted by carbonic anhydrase localized in the chloroplast-pyrenoids [37].

It has been shown that carbonic anhydrase is essential to use organic carbon and this capacity is stimulated to low concentrations of CO₂ gas and alkaline pH. This activity decreases or disappears in eukaryotic microalgae enriched air under 1-

5% CO₂. However, it has been reported that the levels of susceptibility are dependent on the algal-species [38, 39].

In this study, we observed that the cell density of *C. vulgaris* produced in cultures at different concentrations of Na₂CO₃ (Figure 2), showed significant differences ($P \leq 0.05$), whereas in the cultures of *N. oleoabundans* no significant differences (ANCOVA, $P = 0.324$) in cultures with 30 mg N-NO₃ l⁻¹ to the same conditions as for *C. vulgaris* (Figure 2).

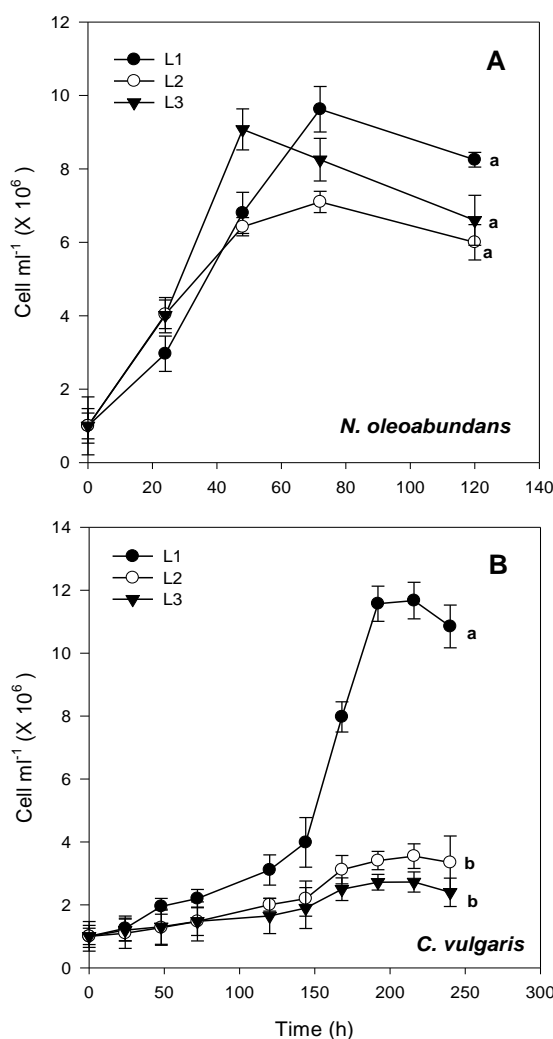


Fig. 2 Growth of *N. oleoabundans* (A) and *C. vulgaris* (B) and to different concentrations of carbonate (Na₂CO₃); L₁: 1 g l⁻¹; L₂: 2.5 g l⁻¹ and L₃: 5 g l⁻¹. Different letters show significant differences between treatments.

During growth, *C. vulgaris* showed greater acclimation phase in those cultures with carbonate (1, 2.5 and 5 g l⁻¹) compared to control culture, but to concentrations of 1 g l⁻¹ sodium carbonate was reached higher productivity of 11.67 x 10⁶ cells ml⁻¹. Unlike *C. vulgaris*, the microalgae *N. oleoabundans* showed no adaptation phase, a greater biomass production at concentrations of 1 g l⁻¹ of Na₂CO₃ was obtained. This suggests that the microalgae *N. oleoabundans* had greater ability to grow in alkaline media compared with *C. vulgaris*.

Li et al. [2] reported for *N. oleoabundans* in cultures with ammonium bicarbonate the half of biomass produced in comparison with the obtained using sodium nitrate, suggesting that high concentrations of salts cause stress which produces an inhibition of cell growth. Serpa et al. [40] mentioned that various species of *Dunaliella salina* shows an inverse relationship between the average cell density and salinity. For this species, the minimal cell density obtained was 40.9 x 10³ cells ml⁻¹ with 4.5 M industrial salt (261 g l⁻¹ of NaCl). The factor salinity may decrease the metabolic and cell activity in many microalgae species.

The higher lipid content (65.0 %) for *C. vulgaris* was obtained in culture with solution of 1 g l⁻¹ Na₂CO₃ and 30 mg N l⁻¹ during 144 h of culture. We can conclude on the basis of results for the lipid content *C. vulgaris* decreases at higher salt concentration Na₂CO₃ (Table 1), this could present because inhibition processes as *N. oleoabundans* which showed lipid content on 120 h of 66.1% with the same concentration of salts. In both microalgae was a decrease in the lipids accumulation with the increase the carbonates content, which can be attributed to the effects caused by salts on both growth and lipid content (Table 2).

Similar trend was reported for *N.oleoabundans* lipid containing about 25% of the dry weight [41], in a culture medium MD

[42], in which the salt concentrations are above the levels of salts seawater. This suggests that high concentrations of salts, cause salt stress which can bring about a change in the lipids percentage.

The analysis of the rate of consumption of carbon dioxide for both microalgae confirms that *N. oleoabundans* has a greater capacity utilization of carbon dioxide with an estimated range of 112.8-115.2 mg l⁻¹ d⁻¹ (Table 1), which is greater than that obtained for *C. vulgaris* 95.76-105.75 mg l⁻¹ d⁻¹ (Table 2).

TABLE 1 AVERAGE REMOVAL OF NITROGEN (%), RATE OF GROWTH AND UTILIZATION OF CO₂
FOR *C. VULGARIS* AT DIFFERENT CONCENTRATIONS OF Na₂CO₃

Treatments	% removal (N)	% lipids	μ (d ⁻¹)	Consumption rate of CO ₂ (mg l ⁻¹ d ⁻¹)	Biomass productivity (mg l ⁻¹ d ⁻¹)
Control	87.36	69.3	0.18	95.7	50.93
L1	85.13	65.0	0.12	105.7	56.25
L2	80.77	57.0	0.06	54.1	25.41
L3	79.02	52.0	0.04	54.1	25.41

TABLE 2 AVERAGE NITROGEN REMOVAL(%),RATE OF GROWTH AND UTILIZATION OF CO₂
FOR *N. OLEOABUNDANS* TO DIFFERENT CONCENTRATIONS OF Na₂CO₃

Treatments	% removal (N)	% lipids	μ (d ⁻¹)	Consumption rate of CO ₂ (mg l ⁻¹ d ⁻¹)	Biomass productivity (mg l ⁻¹ d ⁻¹)
Control	88	65.2	0.21	115.2	61.25
L1	86	66.1	0.14	108.1	62.0
L2	80	46.0	0.12	112.8	60.0
L3	80	45.2	0.12	71.44	38.0

IV. CONCLUSIONS

Both microalgae showed favorable growth in media with 30 mg l⁻¹ of ammonium and nitrate, which induces both a high lipid content under nitrogen limitation. Therefore in the present study, the nutrient limitation was one of the factors that promotes the increase of lipid content. An alternative approach to minimize the effect of low biomass production is the use of a new system combining a nitrogen enriched photo bioreactor with subsequent transfer to nitrogen limitation medium (two-stage method), the process allows a higher biomass production algal and high lipid content.

During growth in cultures at different concentrations of Na₂CO₃, *C. vulgaris* showed greater acclimation phase in culture with carbonate; compared with the microalgae *N. oleoabundans* showed no adaptation phases, with a greater biomass production at concentrations of 1 g l⁻¹ of Na₂CO₃. This suggests that the microalga *N. oleoabundans* had greater ability to grow in alkaline media compared with *C. vulgaris*. It can be concluded based on the results that the higher concentration of Na₂CO₃, in the cultures of *C. vulgaris* and *N. oleoabundans* could present decrease lipid contents. For both microalgae the lipid content was high to 0.1% Na₂CO₃. Therefore, the combining CO₂ mitigation and nitrogen limitation as a strategy for lipid accumulation in microalgae may provide an innovative alternative to current carbon-reduction and biofuel-production strategies.

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