Growth Performance of Microalgae Exposed to CO_2

Alessandro Minillo, Hania Cardamoni Godoy, and Gustavo Graciano Fonseca

Abstract—**The increase of CO_2 emission and other gases of greenhouse effect have caused global debates concerning climatic alterations, stimulating the development of mitigative strategies. Researches in this area include CO_2 kidnapping through aquatic microalgae production, as well as their use in the production of biofuels. The aim of this work was to determine the growth kinetics of microalgae (*Chlorella* sp, *Scenedesmus spinosus*, *Scenedesmus acuminatus* and *Coelastrum* sp.) exposed directly to CO_2. Measurements of microalgae growth and pH from medium were taken weekly. The results showed that carbon dioxide promoted growth inhibition in most microalgae. This condition should be considered for the developing of operational strategies and in projects of photobioreactors for the biological conversion of CO_2.**

Index Terms—**Microalgae, carbon capture, biofuel, kinetic.**

I. Introduction

With the global population growth and industrialization increase, energy consumption has consequently shown an expressive rise in recent decades. In this scenario, about 85% of global energy demand is supported by the burning of fossil fuels [1].

Due to the uncontrolled consumption of fossil fuels as the primary source, it becomes necessary the search for new renewable and clean energy matrices to amortize the effects of economic dependence of natural resources. However, it is evident that the requirements for fossil fuels, mainly for power generation and industrial production, will be still necessary in the future. Burning these fossil fuels releases large amounts of CO_2 into the atmosphere, which affects the balance in the presence of these gases, leading directly to the greenhouse effect. Although CO_2 emissions through anthropogenic activities are relatively low if compared with the natural flows of carbon, e.g. the photosynthesis flow, the greater release is due by the influences of the global climate in a very short period of time [2]. CO_2 levels in the atmosphere have increased from 260 to 380 ppm in the past 100 years [3].

The increased concentration of CO_2 in the atmosphere influences the balance of input and output energy on its system, leading to an increase in the average temperature of the earth's surface, known as global warming, which is currently a major environmental problem [4] Thus, CO_2 has often been cited as the main greenhouse gas (GHG), as well as the cause of climate change. Although there are uncertainties on this subject, in recent years have been published many studies on the need to reduce CO_2 emissions so as not to promote climate change [5].

Several alternatives have been studied in order to promote the reduction of emissions, sequestration and biological fixation of carbon dioxide emitted by stationary sources. One of them is the use of reforestation of photosynthetic organism with the capacity to absorb carbon dioxide from the atmosphere [4], but currently the alternative that has been more studied is the cultivation of microalgae. Microalgae are the main responsible for the biological uptake of atmospheric CO_2 in the oceans that cover ¾ of the globe surface, once that they are present in large numbers in the water column [6]. A portion of the CO_2 absorbed by microalgae is transferred to the deep ocean in a process known as "biological pump" [7]. This process, along with direct diffusion of CO_2 into the water, prevents that the buildup of "greenhouse effect" gases became even greater.

Besides having the capacity to fix carbon dioxide from air, using it as a carbon source, microalgae have the capacity of cycling the organic matter and may offer during its growth a number of mineral elements, vitamins, lipids, pigments and proteins, presenting thus large industrial and commercial applicability [8].

Microalgae can be utilized to produce hydrogen from methane by anaerobic digestion of biomass and biodiesel from intracellular lipid content [9]. According to Teixeira and Morales [10], the microalgae oils possess physical and chemical characteristics similar to that found in the vegetable oils and therefore can be considered as potential raw material for biodiesel production.

In the production of biodiesel, microalgae have several advantages when compared to plants. Microalgae grow at higher rates, have greater oil yield and productivity and can be cultivated on land unsuitable for agriculture. In addition, they are able to remediate wastewater by consuming its nitrogen and phosphorus [8] or even heavy metals [10]. Moreover, they can grow in brackish and/or salted water [4] and have a wide tolerance to environmental extremes, being able to growth in intensively cultivated small spaces [11] using much less water for its cultivation when compared to the culture of plants [12], and also has high efficiency of carbon dioxide fixation [13].

This way, considering the crescent demand for new alternatives that enable the provision of more green energy sources, a strong enthusiasm has been generated around the great potential offered by microalgae either in the production of biomass or biofuels, which represents a promising proposal of reducing the dependence for fossil fuels, without compromising the environment.

The aim of this work was to evaluate the growth performance of four different isolated microalgae strains of

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the species *Chlorella* sp., *Scenedesmus spinosus*, *Scenedesmus acuminatus* and *Coelastrum* sp. grew in medium containing CO_2 as the sole carbon source.

II. Materials and Methods

A. Microalgae Collection, Isolation and Identification

Microalgae used in this study were collected using a phytoplankton net (20 µm mesh opening), in a mesocosmos located in the university campus. After collection they were taken to the laboratory for isolation and culture.

For the microalgae isolation were utilized excavated blades with the aid of a stereoscope microscope, where the cells were isolated one by one by using glass capillary needles, being subsequently inserted and maintained in test tubes (5 ml) containing synthetic cultivation medium (Chu-12). After isolation, consecutive samplings of the biological material were carried out until achieve the complete purification of microalgae.

In parallel, the identification of the isolated microalgae was performed in test tubes using appropriate laminas and laminules with the assistance of a binocular optical microscope, according to morphological and morphometric characteristics for analysis achieved at the lowest possible taxonomic level based on literature [14]. These cultures were kept in BOD incubator in synthetic medium (Chu-12) at 25 ºC, 1klux light, 12h photoperiod and constant stirring.

B. Microalgae Growth Experiments

Growth experiments were carried out in 500 ml Erlenmeyer flasks, sealed, fed with 400 ml synthetic medium (Chu-12). Cultivations started with the addition of 2.5 ml of microalgae inoculum (density of approximately 8 - 25 x 10^4 cells / ml). Each treatment was represented by a single species, which was cultivated in triplicate. The tests were operated in a BOD incubator at 25 ºC, light intensity of 10 klux, 12 h light photoperiods, with carbon dioxide provided under direct injection from a pressured cylinder. The control treatment was performed at the same conditions described above, except by the aeration, maintained by aquarium pumps.

Samplings were carried out at intervals of 4 days. Algae growth and pH were monitored. For the cell count was used a Neubauer chamber. All assays were accomplished after verified the stationary growth phase of the microalgae cultures.

C. Analysis of Results

All results were analyzed with a software "Statistica for Windows" version 5.1. Regression analysis (r^2) and the Spearman correlation test (R) we performed, where significance was considered for $p \geq 0.05$. Figures were elaborated with the software Origin 8.0. Parameters considered for the results' analysis were microalgae growth and pH.

III. Results and Discussion

After isolation, it was obtained different species of microalgae, being selected four species (*Chlorella* sp, *Scenedesmus spinosus*, *Scenedesmus acuminatus* and *Coelastrum* sp) due its high potential described in literature for producing oils. Growth kinectis of these four microalgae species in treatments with and without CO_2 are presented in Fig. 1 and Fig. 2, respectively.

According to Fig. 1, there was distinction in the growth rates between the species in the presence of CO_2. The species that obtained best growth performance was *S. spinosus*, followed by *S. acuminatus*, *Chlorella* sp. and *Coelastrum* sp. In this experiment it can be seen that the treatments with *Chlorella* sp. and *Coelastrum* sp. reached its maximums in the 16th day, while the *S. spinosus* growth apex was found on the 24th day and for *S. acuminatus* on the 28th day. *S. spinosus* showed higher density among all species analyzed when directly exposed to CO_2 injection.

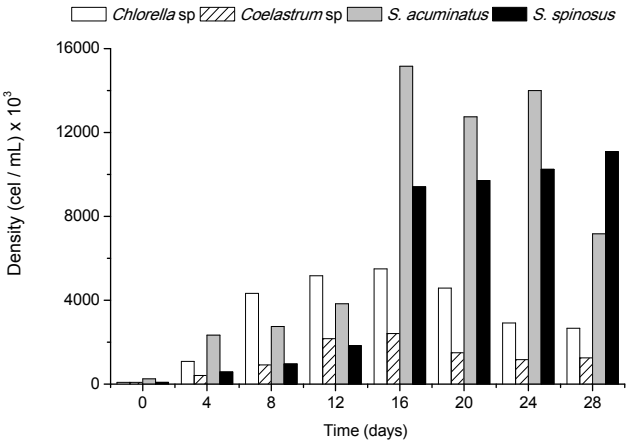

Fig. 1. Growth kinetics of four microalgae species in treatments with CO_2.

When air was injected without CO_2 supplementation, there was a change in the profile among the favored species. Growth was higher for *S. acuminatus*. *Chlorella* sp., *S. spinosus* and *Coelastrum* sp. followed respectively in growth. In this assay, Coelastrum sp and *S. spinosus* showed their highest densities on the 16th day, while *Chlorella* sp. recorded its maximum growth on the 20th day and *S. acuminatus* on the 28th day. *S. acuminatus* was the species that had the highest number of reproduced cells (Fig. 2).

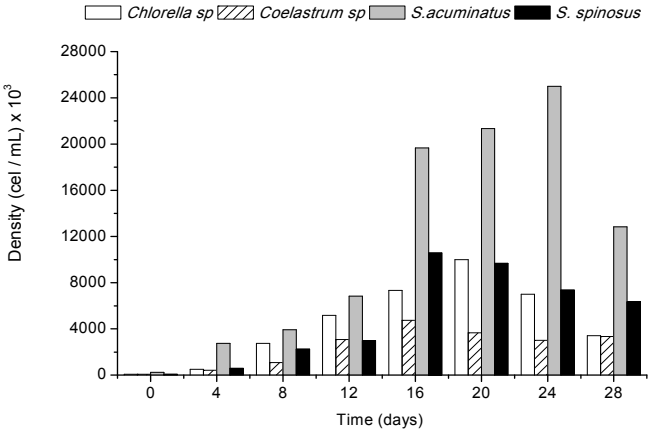

Fig. 2. Growth kinetics of four microalgae species in treatments without CO_2.

According to Table I, it was demonstrated by the regression analysis that almost all treatments ($p \geq 0.05$) were significant, except for *Chlorella* sp. when maintained in the presence of CO_2. However, the highest correlation with the

density and pH was obtained with *Chlorella* sp. with O_2 (without CO_2) and *S. spinosus* with CO_2. Regarding the Spearman correlation test, the only significant results were obtained with *Chlorella* sp. with O_2 (without CO_2) and *S. spinosus* with CO_2.

TABLE I: REPRESENTATION BY SPECIES OF THE REGRESSION ANALYSIS (R^2) AND THE SPEARMAN CORRELATION TEST (R).

Species	R (CO_2)	R (O_2)	r^2 (CO_2)	r^2 (O_2)
Chlorella sp.	0.12	0.81*	0.016	0.54*
Coelastrum sp.	0.45*	0.45*	0.21	0.15
S. spinosus	0.81*	0.74*	0.59*	0.28
S. acuminatus	0.54*	0.74*	0.19	0.26

(*) VALUES CONSIDERED SIGNIFICANT BETWEEN DENSITY AND PH FOR SPECIES, CONSIDERING $P \geq 0.05$.

Growth curves and the relationship between the development of microalgae in relation to pH of the medium in treatments with or without CO_2 are presented for *Chlorella* sp. (Fig. 3), *Coelastrum* sp. (Fig. 4), *S. spinosus* (Fig. 5) and *S. acuminatus* (Fig. 6).

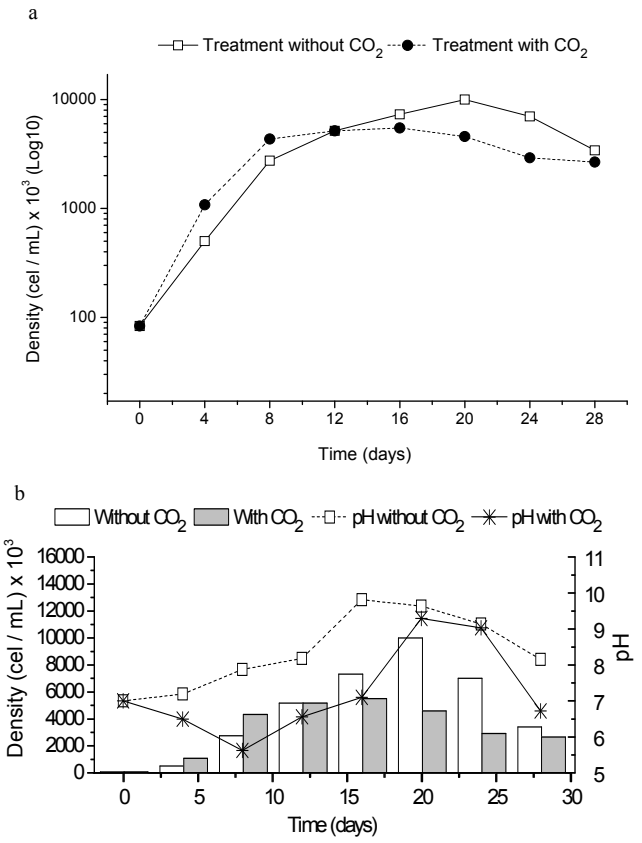

Fig. 3. *Chlorella* sp.: (a) growth curve and (b) relationship between the development of microalgae in relation to pH of the medium in treatments with or without CO_2

According to Fig 3a, initially *Chlorella* sp. developed more rapidly in the treatment with CO_2, reaching the log phase in 8 days, while its stationary phase started from the 12th day. The same species when maintained in O_2 began its development more slowly, attaining the log phase on the 14th day, with its stationary phase beginning after the 20th day. It was still observed that on the 12th day, the microalgae *Chlorella* sp. contained the same density in the medium for both treatments whereas from this period there was an inversion in the cells growth rate.

With respect to pH, *Chlorella* sp. in medium without CO_2 had developed with higher pH. With CO_2 there was no correlation between these two variables (Fig. 3b).

The microalgae *Coelastrum* sp. produced the same number of cells in both treatments until the 8th day. From the 12th day, this species reproduced more actively in medium with O_2, where the log phase was achieved both at 16th day and its stationary phase has initiated from the 24th day (Fig. 4a). In both treatments the growth curve had the same behavior, but cell production was higher in treatments maintained with O_2.

Regarding the pH, *Coelastrum* sp. presented biomass increase for both treatments with and without CO_2. There was a relation between the biomass and pH, being that after the 4th day there was a fall, then a peak, followed by a slight reduction in their values on the 20th day (Fig. 4b).

From Fig. 5a, it was observed that *S. spinosus* presented a growth behavior very similar for both treatments. Which has differed was that in medium without CO_2 was reproduced more cells than in medium with CO_2, but the log phase began on 4th day. *S. spinosus* also had its maximum production at the 16th day for both treatments, but it had better reproduced in the absence of CO_2. Depending on the pH, there was a decrease in pH on the 4th, increasing their growth until the 24th day, where there was a fall again (Fig. 5b).

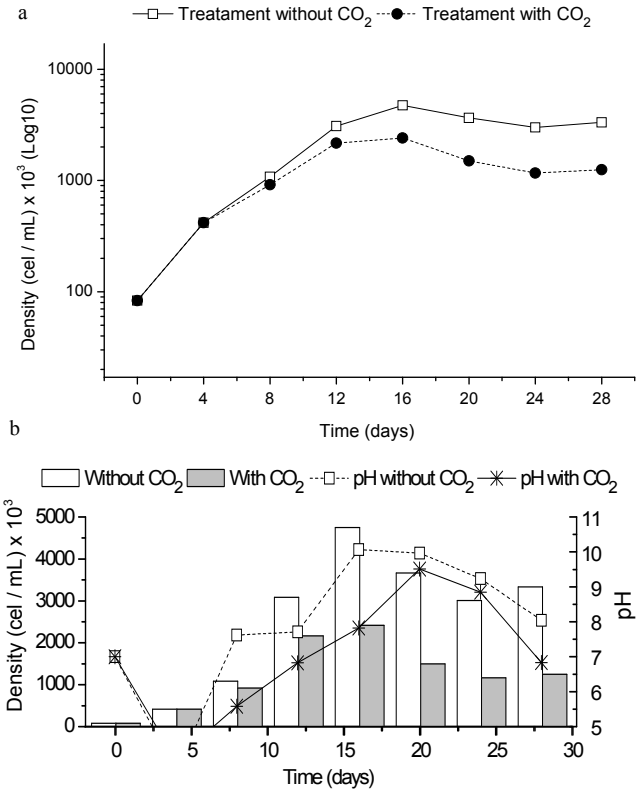

Fig. 4. *Coelastrum* sp.: (a) growth curve and (b) relationship between the development of microalgae in relation to pH of the medium in treatments with or without CO_2

Analyzing the growth of *S. acuminatus*, it observes that this species showed higher values for treatments with only natural air without CO_2 supplementation, represented at 28th day (Fig. 6a), where the pH was approximately 7.0 (Fig. 6b). This microalga also presented similar growth profile for both treatments, showing a slight decline in growth in medium with CO_2 between 4th and 12th days. From the 12th day there

was a quick lag phase where at the 16th day the number of cells produced in medium with CO_2 reached approximately the same number as that produced in medium without CO_2. Moreover, from the same 16th day, there was a slight decrease in medium without CO_2, reaching the stationary phase even before that the *S. acuminatus* produced with CO_2.

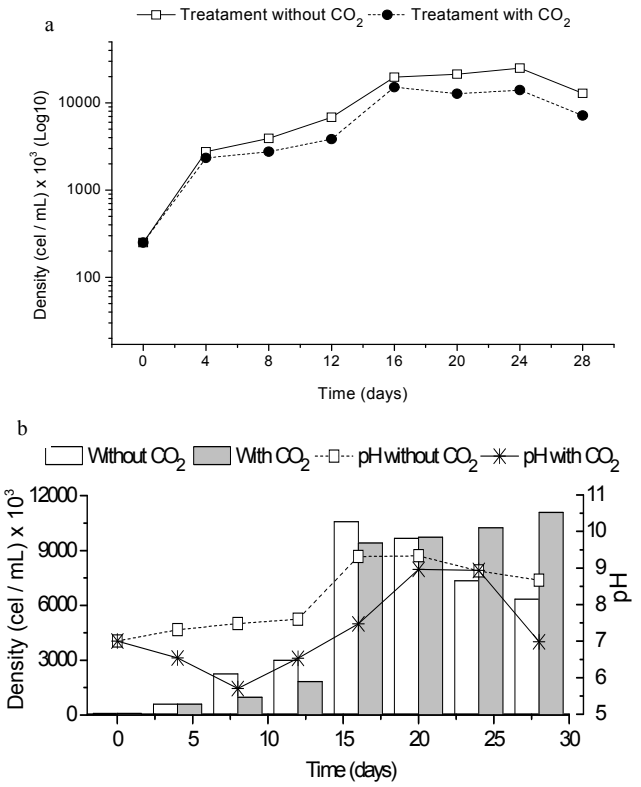

Fig. 5. *S. spinosus*: (a) growth curve and (b) relationship between the development of microalgae in relation to pH of the medium in treatments with or without CO_2

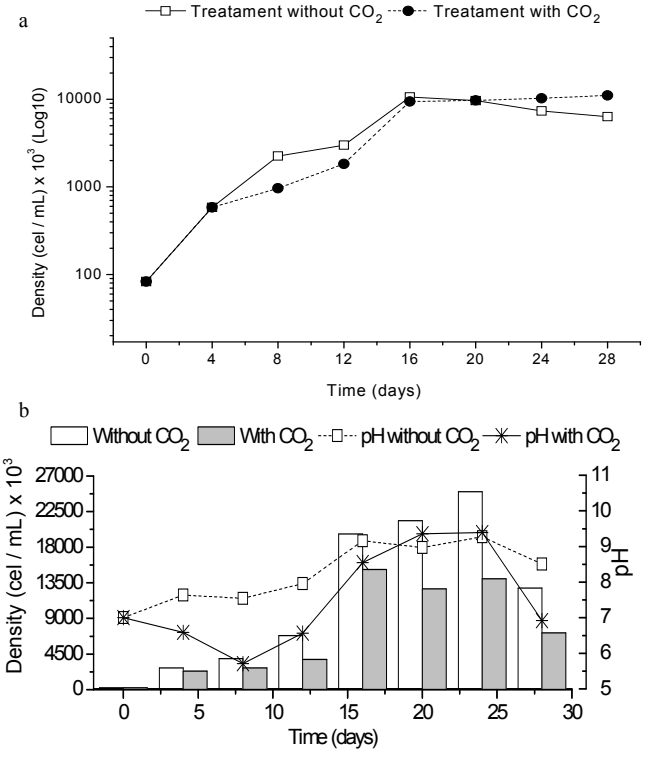

Fig. 6. *S. acuminatus*: (a) growth curve and (b) relationship between the development of microalgae in relation to pH of the medium in treatments with or without CO_2

It is evident, if we analyze the growth performance of *Chlorell*a sp., *Coelastrum* sp. and *S. spinosus* with and without CO_2, that all grew better in absence of CO_2 supplementation. This may have occurred because these species are sensitive to the presence of CO_2 because the gas in contact with the medium promotes greater concentration of carbonic acid (H_2CO_3) [15], promoting acidification of the medium. Possibly these three microalgae species showed lower tolerance limits under acidic environment to survive, therefore grew better in medium maintained with natural air, where the pH of medium is more alkaline.

The microalga *S. acuminatus* when compared individually to the other species, presented a better growth performance, based on the higher growth rate observed in medium without CO_2 supplementation, but if compared with other microalgae species analyzed here, it was observed that for treatments, i.e. with or without CO_2 supplementation, the maximum number of cells attained was always superior.

Probably the nutrient available in the medium without CO_2 became scarce than in the medium with CO_2, due to its high growth rate. However, if compared to the larger amount of cells reproduced, it has that the *S. spinosu*s obtained the larger number than *S. acuminatus*, even that the first had presented better development in the presence of O_2. A plausible explanation for it is the fact that the reproduction rate of these cells is slower for *S. spinosus* than for *S. acuminatus*, which was observed at the time that the analyses were performed.

IV. Conclusion

Among the four microalgae species studied, *S. acuminatus* was the one that presented better performance in the growth of cells for both treatments tested, especially in medium with aeration without CO_2 supplementation. *S. spinosus*, although having obtained a smaller number of cells produced, deserves attention due their better developed on medium with CO_2.

The results indicated that the four isolated microalgae strains showed different profiles in their growth when exposed to CO_2, which is directly linked to the adaptive mechanisms that each species has for better absorption of atmospheric CO_2. These microorganisms present great potential for future studies related to the development of the clean mechanism, which may assist in the remediation of the problems associated with the greenhouse gases.

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