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BIOFIXATION OF AIR EMISSIONS AND BIOMASS VALORIZATION – EVALUATION OF MICROALGAL BIOTECHNOLOGY

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ABSTRACT

This research appraised the simultaneous biofixation of carbon dioxide (CO_2) and nitric oxides (NO_x) by microalgae species *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Scenedesmus subspicatus*. The experimental design was established by five treatments with gas concentrations between control – 0.04% of CO_2, 5 to 15% of CO_2, and 30 to 100 ppm of NOx. Parameters such as pH, growth, productivity, lipids, protein, carbon/ nitrogen ratio, and astaxanthin were evaluated. For all species, the maximal growth was achieved with 5% of CO_2 and 30 ppm of NO_x. Regarding protein content, for all the three species, better results were obtained at higher concentrations of CO_2 and NOx. These results prove the microalgae capacity for CO_2 and NO_x biofixation and reuse of biomass as a source of high value-added products, such as lipids, proteins, and astaxanthin. These findings support the indication of these species for flue gas treatment process and use in biorefineries systems.

Keywords: Greenhouse gases; Biomitigation; *Chorella vulgaris*; *Haematococcus pluvialis*; *Scenedesmus subspicatus*.

1. Introduction

The energy production to meet the demands of industries and the global population has increased, over the years, the emission of greenhouse gases (GHG). This is mainly due to combustion in thermoelectric plants, whose emissions are around 10 to 15% of CO_2 and 100 to 300 ppm of NO_x and SO_x (1). Activities like that, which use non-renewable natural resources, are one of the major anthropogenic causes of GHG emissions (2).

As a result of increased emissions, global warming and climate changes are reported (3, 4). Furthermore, there are other consequences such as impairment of environmental quality due to air, water, and soil pollution, changes in the incidence of heat waves, drought, soil disruption, rising sea levels, acid rain, and consequently, damage to human health, such as respiratory diseases, cancer, and death (5, 6).

Faced with this perspective, strategies have been developed for the mitigation of GHG emissions (2). Among these strategies, biofixation is highlighted, which is characterized by the use of biological activity to fix gases such as CO_2 and its conversion into biomass (2).

Along with biofixation, the use of microalgae stands out due to its characteristics such as nutrient assimilation from diversified sources, and its conversion into high-value biomass. In addition, the microalgae present a biofixation potential from 10 to 50 times highest than terrestrial plants species (7), may utilize wastewater for growth (8), and presents potential to concomitant use in environmental treatment (biofixation) and production of high value-added products (9).

Chlorella, Haematococcus, and *Scenedesmus* are the genera of microalgae that stand out for their applicability in environmental treatments such as biofixation (10), wastewater treatment and biofuels production (8); biofertilizers (11); food and feed supplementation (12, 13) and medical applications (14). Therefore, promoting microalgae growth by the disposal of specific nutrients sources of carbon and nitrogen, like CO_2 and NO_x from potentially polluting activities, may constitute a biotechnological solution (15, 16) to GHG mitigation (17).

This research emerged from the need for the development and application of an alternative for GHG mitigation in a thermoelectric power plant. Thus, it aimed to appraise *Chlorella vulgaris, Haematococcus pluvialis*, and *Scenedesmus subspicatus* for simultaneous biofixation of CO_2 and NO_x at different concentrations.

2. Material and methods

The microalgae species used in this research were *Chlorella vulgaris* Beyerinck (Algal Biotechnology Laboratory - UFSCar, Brazil), *Haematococcus pluvialis* Flotow (Botany Department - UBC, Canada), and *Scenedesmus subspicatus* Chodat (Algal Biotechnology Laboratory - UFSCar, Brazil). They were maintained in Bold's Basal medium (BBM) (18).

2.1 Cultivation conditions

To appraise microalgae tolerance, the cultures were carried out in glass photobioreactors with a liquid volume of 2 L, conditioned at 22 °C and a photoperiod of 10 hours light and 14 hours dark, that was supported by fluorescent lamps of 40 w (1, 10,

19) at an exposition of 150 µmol photons m^2 s^{-1}, approximately. The initial concentration of the inoculum was 0.1 g L^{-1} (20). The aeration was carried out by compressed air and simulated gas, with mixing of CO_2 and NO_x at different concentrations, arranged in industrial cylinders, according to the different treatments protocols.

It was established five treatments: CT: control treatment – air (0,04% of CO_2); Injection Treatments (IT) 1 – 5% of CO_2 and 30 ppm of NO_x, IT 2 – 7% of CO_2 and 40 ppm of NO_x, IT 3 – 10% of CO_2 and 60 ppm of NO_x, and IT 4 –15% of CO_2 e 100 ppm of NO_x. Each treatment was performed in triplicate.

Cultures aeration was carried out by air injection utilizing a compressor (Schultz model 20/250). The flow inlet was 1 L min^{-1}. The simulated gas injections were performed with the same flow (1 L min^{-1}). This was controlled by solenoid valves during the light period (adapted from 10). The duration of gas injection was one minute every 19 minutes. The tests lasted 16 days. In the end, the biomass was recovered by filtration and used to measure elementary contents, stoichiometry, lipids, protein, and astaxanthin (Figure 1).

Figure 1

2.2 Analytical methods

Culture pH was measured every 24h with a digital pHmeter (Hanna, HI 2221 model). The growth was measured by absorbance in a spectrophotometer (Kasuaki, IL 226 model) at 670 nm (10), which was used for biomass determination (g L^{-1}), through a correlation calculation.

Productivity was calculated with the biomass values obtained gravimetrically. Every 48 hours samples of 10 mL were filtered through pre-weighed glass microfiber

filters (GF-1, 25 mm), which were dried and weighted (21). The obtained values were applied on the formula:

$$P = (X_t - X_0)/(T - T_0)$$

X_t is the final biomass concentration, X_0 is the initial biomass concentration, (T) is the final day, and (T_0) is the initial day of culture (10). The analysis was performed in triplicate and it was obtained n=9. All samples were taken before the first gas injection of the day.

2.3 Biomass analysis and CO_2 Biofixation

For elementary analysis of carbon, hydrogen, and nitrogen (CHN) from microalgal biomass, samples were sent to the Instrumental Analytical Center of São Paulo University. Therefore, 5 mg was processed in an elementary analyzer (Perkin Elmer – CHN 2400 series II), which performed biomass combustion in an environment of pure oxygen. The gases obtained were automatically measured. These analyzes were performed in duplicate and the values were used to calculate the carbon/nitrogen (C/N) ratio.

Nitrogen values obtained were used to calculate the protein of the biomass, for which was adopted the conversion value of 4.44 proposed by López et al. (22).

With the values of carbon content, it was calculated the CO_2 biofixation rate according to the formula proposed by Duarte and Costa (23):

$$Biofixation = Px.Xcbm.(Mco_2/Mc)$$

Px is productivity in mg L^{-1} d^{-1}, Xcbm is the fraction of carbon contained in biomass, Mco_2 and Mc are the molecular weights of carbon dioxide and carbon.

The lipids content analysis was performed by the Bligh and Dyer (24) based method. Samples of 50 mg of microalgal biomass were weighed and destined for the cold extraction of lipids. The biomass was treated with 3 mL of chloroform/ methanol (2:1, v/v) and 10 µL of butylhydroxytoluene (BHT) 1% in methanol. The samples were placed in Falcon tubes covered with aluminum for light protection. These tubes were destined for an ultrasonic bath (3 times x 15 min.). At the end of the cycles, the samples were conditioned at 4 °C for approximately 24 hours. After this period, samples were again sonicated (3 times x 15 min.), centrifuged at 5000 rpm for 3 min. Then, the supernatant was recovered and reserved, and to the pellets in the Falcon tubes were added 1.5 mL of chloroform/ methanol solution and centrifuged in the same conditions. The supernatant was recovered over again and added to the reserved ones. In the recovered liquid were added 2 mL of reverse osmosis water and 1 mL of chloroform. It was performed one more cycle of centrifugation in the same conditions previously established. The recovered liquids were conditioned in glass flasks and dried on the stove at 50 °C for solvents evaporation. Then, the flasks were cooled under vacuum until constant weight and the lipids content were determined gravimetrically.

Astaxanthin extraction from *Haematococcus pluvialis* biomass was performed by an acidic extraction (HCl and Acetone). The extracts analysis was carried out by high-performance liquid chromatography (HPLC - LC 10AD, Shimadzu, Japan) equipped with a C18 column. For carotenoids separation, it was used a gradient of eluents concentration (A – acetone and B – methanol: water 9:1 v/v), under the following conditions: B 80 to 20% by 25 min.; 20% by 10 min. and 20 to 80% by 5 min. The flow rate was 0.8 mL min^{-1} and the temperature at 40 °C (25). The detection was carried out at 476 nm. For

astaxanthin identification and quantification, an astaxanthin standard was used (purity 96-98.2%, Carbosynth, United States). Astaxanthin was quantified by the formula:

$$y = 3.10^8 x + 10124$$

Y is the area of the astaxanthin curve obtained for each treatment. This formula was obtained by a linear correlation (R^2= 1).

2.4 Statistical analysis

Using Statistica 7 software, distribution analysis was performed using the Shapiro-Wilk method. The significant differences were identified by Tukey and Kruskal-Wallis tests, and the level of significance was set at $p \leq 0.05$.

Biomass, productivity, and biofixation were used to comparisons between time intervals (1, 4, 8, 12, and 16 days) and concentrations of simulated gas (treatments). For pH, protein, lipids, C/N ratio and astaxanthin, comparisons were performed between treatments. Correlations analyses were carried out using Spearman's test and data about gas concentration, biomass, protein, lipids, and astaxanthin. The correlation coefficient considered was: null (0), weak (0 to 0.3); regular (0.3 to 0.6); strong (0.6 to 0.9); and very strong (0.9 to 1.0), based on Callegari-Jacques (26).

3. Results and discussion

3.1 pH

The values of pH presented in CT and IT treatments by the three species of microalgae followed linearity, with a variance of 6.7 to 10.1 during the 16 days of cultivation. For *C. vulgaris* and *S. subspicatus*, the mean between treatments do not present statistical differences, while for *H. pluvialis* the differences were observed

between the treatments IT 3 (7.7±0.4) and IT 2 (7.8±0.4), which presented the lowest pH averages (Tab. 1).

Table 1

It was identified that the lower pH was not a limiting factor for growth, since the values of biomass (Fig. 2) and productivity (tab. 2) of the treatment IT 2 presented equivalency to the control CT. However, between the treatments with gas injection, those with lower pH (IT 3 and IT 2), were also the ones with the lowest growth.

The average pH range for *C. vulgaris*, *H. pluvialis*, and *S. subspicatus* was, respectively, 8.8, 8.4, and 8.7, and are associated with higher growth rates. These values were obtained from the treatment IT 1 (5% of CO_2 + 30 ppm of NO_x).

Radmann et al. (10) evaluated the tolerance of *Spirulina* sp., *Scenedesmus obliquos*, *S. nidulans*, and *Chlorella vulgaris* to the exposure of 12% of CO_2, 60 ppm of SO_2, and 100 ppm of NO_x, concentrations similar to the treatment IT 4, reported a pH range between 6.0 and 10.0. These authors also concluded that the highest biomass was related to a pH range between 8.0 and 9.0, which corroborated with the results obtained for *C. vulgaris*, *H. pluvialis*, and *S. subspicatus*.

Injections of gases such as CO_2 and NO_x in the culture medium alters the pH, which interferes in the microalgae development by decreasing the bioavailability of some nutrients such as phosphorus and inorganic carbon (27, 10) and change the solubility of CO_2 in the medium (27, 28). The data obtained for pH during cultivation proves that the gas injection did not interfere with the pH in order to prevent microalgae growth.

3.2 Growth

The biomass measurement by absorbance (OD_{670nm}) demonstrated that *C. vulgaris* has the biggest values for IT 1, followed by the treatments 2, 3, and 4, consecutively. To appraise these results, it was observed a strong and negative correlation (-0.65) between increased simulated gas concentration and decreased growth (Fig. 2).

These results demonstrated that the treatments with gas injection IT 1 (5% of CO_2 + 30 ppm of NOx) and IT 2 (7% of CO_2 + 40 ppm of NOx) presented higher growth than the control. For IT 1, the maximum biomass concentration obtained was 32% higher than the control treatment. This corroborates with the hypothesis that *C. vulgaris* presents the capacity to grow in culture systems with different concentrations of CO_2 and NO_x.

H. pluvialis presented the highest biomass in treatments IT 4 (15% of CO_2 + 100 ppm of NOx) and IT 1 (5% of CO_2 + 30 ppm of NOx) (Fig. 2). When comparing the maximum biomass concentration obtained, the differences were, respectively, 12% and 11% higher than the control treatment. The lowest growth was observed during the IT 2 (7% of CO_2 + 40 ppm of NOx), which was also observed with a low pH value (7.8) (Tab. 1). For *H. pluvialis* it has not established a link between increased simulated gas concentration and decrease of biomass. Furthermore, this species demonstrated tolerance to different concentrations of CO_2 and NO_x.

Chekanov et al. (29) states that moderate concentrations of CO_2 (5%) are beneficial for cultures of *H. pluvialis*, which advantage the carbon accumulation and biomass production. However, CO_2 injections at level ≥ 10% are harmful and result in decreased growth. This data corroborates with the results obtained in IT 1, which was the treatment with the highest growth. On the other hand, it is not consistent with the results

of IT 4 (15% of CO_2 + 100 ppm of NOx), which presented a maximum growth 12% higher than the control treatment (air – 0.04% CO_2).

Cheng et al. (30) demonstrated the tolerance of *H. pluvialis* to exposure at 15% of CO_2 and reported that this concentration improved the culture yield when compared to air injection. According to these authors, this response is associated with the increase of photosynthetic activity and CO_2 competition.

S. subspicatus obtained approximate values of biomass between the treatments with gas injections (IT), which presented higher growth than the control treatment (Fig. 2). The biggest growth was registered in IT 1 (5% of CO_2 + 30 ppm of NOx) and IT 3 (10% of CO_2 + 60 ppm of NOx), which presented a maximum biomass production 23% higher than the control treatment. Thereafter, the treatment IT 4 increased 21% more than control. The lower growth was observed in IT 2 (7% of CO_2 + 40 ppm of NOx). The data presented indicated a regular and positive correlation (0.51) between simulated gas concentration and growth.

These results prove that *S. subspicatus* is tolerant to exposure to different concentrations of CO_2 and NOx. However, contrary to what was observed for *C. vulgaris*, increased concentrations of simulated gas did not promote significant decreases in the growth of *S. subspicatus*. It should be noted that the treatments with gas injections presented growth similar to each other and higher than the control.

Nayak et al. (8), when appraised the growth of *Scenedesmus* sp. at different concentrations of CO_2 did not find a correlation between gas concentration and microalgae growth. Li et al. (21) also obtained similar results for *Scenedesmus raciborskii* at exposure to 7% of CO_2 and to simulated gas with 15% of CO_2, 200 ppm of SO_2 and 100 ppm of NO. There were no significant differences between these two treatments.

Figure 2

The results of biomass production confirm the tolerance of the three microalgae species at different concentrations of CO_2 and NO_x. In addition, the best scenario for growth was achieved during the concentration IT1 (5% CO_2 + 30 ppm NO_x).

3.3 Productivity

When the biomass productivity was evaluated, it was verified that each microalgae species presented a different behavior during the cultivation. However, for the three of them, the highest productivity was obtained in IT 1 (5% of CO_2 + 30 ppm of NO_x).

C. vulgaris presented the highest productivity on day 8 for the tests IT 1, IT 2 and IT 4, respectively (Tab. 2). For IT 3, the better result observed was 0.05 g L^{-1} d^{-1} at days 12 and 16 (Tab. 2). Radmann et al. (10) appraised *C. vulgaris* and reported maximum productivity of 0,05 g L^{-1} d^{-1} with an injection of 12% of CO_2, 60 ppm of SO_2, and 100 ppm of NO. This value is the same that was obtained in the treatment IT 4 (15% of CO_2 + 100 ppm of NOx).

For *H. pluvialis*, the maximal biomass productivity was 0.05 g L^{-1} d^{-1} obtained in IT 1 (5% of CO_2 + 30 ppm of NOx) between time intervals 8-12 days, while the lower biomass productivity was registered in the treatment IT 2, which had a larger adaptation phase, as is shown in Table 2. These results indicate that a trend related to gas concentration and biomass productivity has not been established. Nevertheless, the treatments IT 1 and IT 4 promoted a productivity improvement.

Li et al. (31) reported that *H. pluvialis*, at an exposure of 15% of CO_2, obtained maximum productivity of 0.66 g L^{-1}. According to these authors, aeration with 15% of CO_2 significantly increases photosynthesis and carbon assimilation.

When the productivity of *S. subspicatus* was evaluated, the maximum values were obtained at day 8 for the treatments IT 1 and IT 2, with a productivity of 0.09 g L^{-1} d^{-1} for both. For the treatment IT 4, the biggest productivity was 0.07 g L^{-1} d^{-1} registered on day 4 (Tab. 2). The IT 3 presented the lowest productivity between the gas injections treatments. However, all treatments with gas injection (IT 1, 2, 3, and 4) had an improvement in productivity in comparison with the control treatment (Tab. 2).

Table 2

Radmann et al. (10) obtained the maximum productivity of 0.04 and 0.05 g L^{-1} d^{-1}, for *S. obliquus* and *C. vulgaris*, respectively. The values presented by *C. vulgaris* were the same, while for *S. subspicatus* were higher (0.07 g L^{-1}) during IT 4 (15% of CO_2 + 100 ppm of NOx).

Radmann et al. (10) assume that the exposure at time zero to gases containing molecules such as nitrogen oxides (NO_x), can inhibit the growth of microalgae. However, as observed by Vaz et al. (2016), the microalgae appraised in this research were not inhibited for the exposure to gases. Contrarily, it presented higher growth in the treatments with gas injection than control treatment (air).

From obtained data, the three microalgae species presented maximal biomass productivity for the treatment IT 1 (5% of CO_2 + 30 ppm of NOx). When comparing the biomass productivity between microalgae species, *S. subspicatus* stands out with its productivity, which values up to 0.09 g L^{-1} d^{-1}, after 8 days from cultivation.

3.4 CO_2 Biofixation

C. vulgaris presented a CO_2 biofixation range between 40.3±7.7 and 146.2±68.3 (mg L^{-1} d^{-1}). The highest ranges were observed for treatments IT 1 (146.2±68.3) and IT 4 (124.7±22.6), both at day 4. In this interval, these averages were significantly higher than treatments IT 2 and IT 3. This indicates that, for these treatments, the biggest biofixation has occurred in the initial days of cultivation. On days 8, 12, and 16 were observed higher values for IT 2 and IT 3 (Tab. 3).

Table 3

Biofixation performance by *H. pluvialis* presented a range between 15.0±6.9 and 101.6±13.3 mg L^{-1} d^{-1}. Better averages were observed on treatments IT 1 and IT 4 at day 12 (101.6±13.3 mg L^{-1} d^{-1}) and day 4 (53.5±18.2 mg L^{-1} d^{-1}) (Tab. 3). In treatments IT 2 and IT 3, maximum averages were obtained at day 12.

Regarding CO_2 biofixation by *S. subspicatus,* its values were between 68.8±5.5 and 156.7±19.6 mg L^{-1} d^{-1}. The highest significant averages were registered during IT 1 and IT 2 on day 8 (Tab. 3). For IT 3, the maximum average was achieved at day 12 (88.2±8.3 mg L^{-1} d^{-1}) and for IT 4 at day 4 (116.7±18.0 mg L^{-1} d^{-1}). *S. subspicatus* presented the highest biofixation between days 4, 8, and 12.

Yadav et al. (20) evaluated *Chlorella* sp. cultivated with the flue gas (10% CO_2, 0.554% CO, 8.33% O, 61 ppm NO_x, 0.3% SO_x ,and 9 ppm hydrocarbons) and they obtained a CO_2 fixation range of 175±10 (mg d^{-1}). Duarte and Costa (23) cultivated *Synechococcus nidulans* at 10% of CO_2, 100 ppm of NO, and 60 ppm of SO_2, and registered a value of 155.8±13.0 mg L^{-1} d^{-1}. These results are close to those obtained for

C. vulgaris and *S. subspicatus*. Chekanov et al. (29) reported a reduction in CO_2 fixation mediated by *H. pluvialis* when CO_2 concentration increased to 10% and 20%. According to these authors, this may be a negative effect on photosynthetic carbon fixation. This was not observed in this research, since *H. pluvialis* presented a different behavior, with a better biofixation on IT 4 (15% CO_2) than IT 3 (10% CO_2).

Between the microalgae species evaluated in this research, *S. subspicatus* presented the highest rates of CO_2 biofixation (up to 156.7±19.6 mg L^{-1} d^{-1}) and these rates were related with the highest growth (Fig. 1). This corroborates with Adamczyk et al. (32) which affirm that the highest biofixation capacity is associated with the highest productivity and growth.

3.5 Biomass analysis

Protein

The total protein levels obtained at the end of cultures presented averages with differences between treatments for the three microalgae species. *C. vulgaris* treatments IT 3 and IT 4 presented the highest significant values 15.1% and 15.7%, respectively. The lower value was observed for treatment IT 1 (5% CO_2 + 30 ppm NO_x) (Tab. 4). Thus, there was registered a strong and positive correlation (0.68) between increased simulated gas concentration and protein production, and a strong and negative correlation (-0.82) between the decrease of growth and high level of protein.

Concerning *H. pluvialis* in treatments with gas injection, the highest level of total protein was obtained for IT 3 (25%) followed by IT 2 (22%). The treatment IT 1 was presented with the lowest protein content (Tab. 4). From this, a regular and negative correlation (-0.43) between growth and protein content was registered.

When evaluating *S. subspicatus*, treatments IT 3 and IT 2 presented averages of the protein content of 14.1% and 11.7%, respectively. These values were significantly higher than treatment IT 1 (5% CO_2 + 30 ppm NO_x), which presented the lowest value (8.2 %) (Tab. 4).

The three microalgae species presented a similar response, the lowest protein content associated with IT 1 (5% of CO_2 + 30 ppm of NO_x). In addition, it was found that for *C. vulgaris*, the protein content increased with lowest growth at higher concentrations of gas. For the other species, this behavior was not observed. *H. pluvialis* and *S. subspicatus* presented the same standard response for protein production, with the higher protein content presented by IT 3, IT 2, IT 4, and TI 1, consecutively (Tab. 4).

Moreover, when comparing the three microalgae species, *H. pluvialis* presented the highest value of protein content, which was between 10.0 and 25.0%, followed by *C. vulgaris* (9.9 – 15.7%), and *S. subspicatus* (8.2 – 14.1%). This finding suggests that the increase in protein content was improved with NO_x injection and, between the three microalgae species, *H. pluvialis* presented better NO_x fixation.

Yadav et al. (20) also observed a significant increase in protein content of *Chlorella* sp. cultivated at different CO_2 concentrations. According to Li et al. (21), simulated gas injection (15% CO_2 + 200 ppm SO_2 + 100 ppm NO) promotes protein biosynthesis in *Scenedesmus raciborskii*. For microalgae metabolism, NO_x serves as an additional source of nitrogen (33, 21).

Lipids

Content of total lipids from biomass obtained at the end of cultivations was demonstrated that *C. vulgaris* registered the highest values at treatments IT 1 and IT 4,

with lipids concentrations of 25% and 23%, respectively. Between the injection treatments, the lowest lipid content (19.6%) was observed for IT 3 (10% CO_2 + 60 ppm NOx) (Tab. 4). Therefore, these values were higher than presented by Yadav et al. (20) when they appraised *Chlorella* sp. and obtained 12.7±1.7 and 4.5±1.0% for concentrations of 5% of CO_2 and flue gas (10% CO_2, 0.554% CO, 8.33% O, 61 ppm NO_x, 0.3% SO_x, and 9 ppm of hydrocarbons).

On the other hand, a different response was observed for *H. pluvialis*. Treatment IT 2 (7% CO_2 + 40 ppm NOx) presented 22.6% of lipid content, a value significantly higher than IT 3, IT 4 and IT 1, which presented respectively 15.8%, 13.8%, and 7.7% (Tab. 3). From these results, was found a lipid content decrease of 7% from IT 2 to IT 3. Cheng et al. (30) also obtained similar results for *H. pluvialis*, with a lipid decline of 6% due to an increase of CO_2 concentration. According to these authors, lipid production may be strongly affecting astaxanthin accumulation.

S. subspicatus did not present a significant difference between treatments, which presented a range between 27.8% and 31.3%. Therefore, the highest averages of lipid content were observed for treatments IT 1 and IT 2, with 31.2 and 31.3% (Tab. 4). In addition, *S. subspicatus* presented the highest lipid content, followed by *C. vulgaris* and *H. pluvialis*, consecutively.

The difference in lipid accumulation may be a biological response of microalgae to a stress factor, such as greenhouse gas injections in the culture medium. According to results obtained by Nayak et al. (8), the lipid accumulation may decrease due to an increase in CO_2 concentration. When these authors appraised *Scenedesmus* sp. at 10% of CO_2, the lipid content was 16.6%. This value is 40% lower than that obtained for *S. subspicatus* in treatment IT 3 (10% of CO_2 + 60 ppm of NO_x) (Tab. 4).

Table 4

Carbon:Nitrogen Ratio

The stoichiometry of main elements (C/N) was demonstrated that the treatment IT 1 (5% CO_2 + 30 ppm NO_x) stands out with the highest C/N ratios for the three microalgae species (Tab. 4). Chekanov et al. (29) also observed this response for *H. pluvialis*, with a rise of C/N ratio due to the 5% CO_2 concentration in the medium.

Otherwise, results of lowest C/N ratios were related with high concentrations of gas injections, as 15% CO_2 + 100 ppm NO_x for *C. vulgaris* and 10% CO_2 + 60 ppm NO_x for *H. pluvialis* and *S. subspicatus*. These treatments also presented the highest protein and astaxanthin content (Tab. 4). According to Chekanov et al. (29), the lowest C/N ratios were associated with microalgae physiological conditions such as cells with high content of nitrogen compounds like protein, nucleic acids, and chlorophyll.

Astaxanthin

Astaxanthin profiles were obtained by chromatography and the results indicate that, for *H. pluvialis*, astaxanthin was identified in all treatments (0.04 – 15% of CO_2 and 0 – 100 ppm de NO_x). Astaxanthin, as well protein and lipids content, may be useful to assess microalgae response to stress conditions.

When performing the astaxanthin quantification, it was observed that the highest astaxanthin concentration was in IT 3 (10% CO_2 + 60 ppm NOx), which also had the highest protein content in injections treatment. A correlation was established weak and positive (0.26) between increase of gas concentration and astaxanthin content. These results demonstrated that the injection of simulated gas at concentrations between 10 and

15% of CO_2 + 60 and 100 ppm of NO_x did not inhibit astaxanthin biosynthesis, once this can raise its production (Tab. 5).

Table 5

Chekanov et al. (29) related that concentrations up to 20% of CO_2 did not induce inhibition in astaxanthin biosynthesis of *H. pluvialis*. Nevertheless, according to Cheng et al. (30), the accumulation of lipids is one of the essential factors for the astaxanthin synthesis.

4. Conclusions

Chlorella vulgaris, *Haematococcus pluvialis,* and *Scenedesmus subspicatus* presented tolerance to growth under simultaneous injections of CO_2 and NO_x. These species were capable of simultaneous GHG biofixation and production of proteins, lipids, and astaxanthin when cultivated under gas injections at concentrations near to emissions of coal and natural gas thermoelectric plants. *S. subspicatus* presented the highest potential for CO_2 biofixation and *H. pluvialis* for NO_x fixation. This raises its applicability for the flue gas treatment process. Thus, *Chlorella vulgaris*, *Haematococcus pluvialis,* and *Scenedesmus subspicatus* are indicated for use in biorefinery systems with the production of high-value added bioproducts.

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Declarations

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Conflicts of interest/Competing interests (include appropriate disclosures)

We have no conflicts of interest to disclose.

Availability of data and material (data transparency)

Yes.

Code availability

Not applicable.

Authors' contributions

Not applicable.

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Tables

Table 1 – Average pH range presented by *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Scenedesmus subspicatus* during cultivations under different concentrations of simulated gas.

TREATMENTS	*Chlorella vulgaris*	*Haematococcus pluvialis*	*Scenedesmus subspicatus*
CT (AIR)	8.9±0.6[a]	8.7±0.4[b]	9.1±0.7[a]
IT 1 (5% + 30 ppm)	8.8±0.7[a]	8.4±0.7[ab]	8.7±0.8[a]
IT 2 (7% + 40 ppm)	8.9±1.0[a]	7.8±0.4[a]	8.6±1.0[a]
IT 3 (10% + 60 ppm)	7.7±0.6[a]	7.7±0.4[b]	8.2±0.7[a]
IT 4 (15% + 100 ppm)	8.5±0.9[a]	8.3±0.5[ab]	8.6±0.9[a]

Lowercase letters on the same column represent differences between treatments for each species. Significance level of 95% ($p \leq 0.05$) by Tukey's test.

Table 2 – Biomass productivity (g L^{-1} d^{-1}) by *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Scenedesmus subspicatus* at different time intervals and gas concentrations.

Productivity g L^{-1} d^{-1}	Time intervals (d)				
Chlorella vulgaris	1	4	8	12	16
CT (0.04% CO_2)	0.00 ± 0.00^{aA}	0.04 ± 0.01^{abA}	0.05 ± 0.01^{bBC}	0.05 ± 0.01^{bAB}	0.05 ± 0.00^{bBC}
IT 1 (5% + 30 ppm)	0.00 ± 0.00^{aA}	0.08 ± 0.04^{bcB}	0.08 ± 0.01^{cC}	0.07 ± 0.01^{bcB}	0.06 ± 0.01^{abC}
IT 2 (7% + 40 ppm)	0.00 ± 0.00^{aA}	0.05 ± 0.00^{abcAB}	0.06 ± 0.01^{bcBC}	0.06 ± 0.01^{cB}	0.05 ± 0.01^{abB}
IT 3 (10% + 60 ppm)	0.00 ± 0.00^{aA}	0.02 ± 0.00^{abA}	0.03 ± 0.00^{bcA}	0.05 ± 0.00^{cA}	0.05 ± 0.00^{cBC}
IT 4 (15% + 100 ppm)	0.00 ± 0.00^{aA}	0.07 ± 0.01^{cB}	0.05 ± 0.01^{bcAB}	0.04 ± 0.01^{abA}	0.04 ± 0.01^{abA}
Haematococcus pluvialis	1	4	8	12	16
CT (0.04% CO_2)	0.00 ± 0.00^{aA}	0.04 ± 0.01^{cB}	0.02 ± 0.00^{bcAB}	0.02 ± 0.00^{abA}	0.02 ± 0.00^{bA}
IT 1 (5% + 30 ppm)	0.00 ± 0.00^{aA}	$0.04\pm0.^{01abB}$	0.05 ± 0.01^{bcC}	0.05 ± 0.01^{cC}	0.04 ± 0.02^{bcB}
IT 2 (7% + 40 ppm)	0.00 ± 0.00^{aA}	0.01 ± 0.00^{abA}	0.01 ± 0.00^{bcA}	0.02 ± 0.00^{cAB}	0.02 ± 0.00^{bcA}
IT 3 (10% + 60 ppm)	0.00 ± 0.00^{aA}	0.03 ± 0.00^{cAB}	0.02 ± 0.00^{abAB}	0.03 ± 0.00^{cBC}	0.02 ± 0.00^{bcAB}
IT 4 (15% + 100 ppm)	0.00 ± 0.00^{aA}	0.03 ± 0.01^{bB}	0.03 ± 0.00^{bBC}	0.02 ± 0.00^{abAB}	0.02 ± 0.00^{abBA}

Scenedesmus subspicatus	1	4	8	12	16
CT (0,04% CO_2)	0.00 ± 0.00^{aA}	0.05 ± 0.01^{cB}	0.04 ± 0.00^{bcA}	0.04 ± 0.00^{bcA}	0.04 ± 0.00^{abA}
IT 1 (5% + 30 ppm)	0.00 ± 0.00^{aA}	0.08 ± 0.01^{cD}	0.09 ± 0.00^{cB}	0.07 ± 0.01^{bcB}	0.07 ± 0.00^{abC}
IT 2 (7% + 40 ppm)	0.00 ± 0.00^{aA}	0.08 ± 0.01^{bcCD}	0.09 ± 0.01^{cB}	0.08 ± 0.00^{bcB}	0.06 ± 0.00^{abBC}
IT 3 (10% + 60 ppm)	0.00 ± 0.00^{aA}	0.04 ± 0.00^{abA}	0.04 ± 0.00^{aA}	0.05 ± 0.00^{bA}	0.05 ± 0.00^{bAB}
IT 4 (15% + 100 ppm)	0.00 ± 0.00^{aA}	0.07 ± 0.01^{cC}	0.05 ± 0.01^{bcAB}	0.06 ± 0.00^{bcAB}	0.05 ± 0.00^{abAB}

Lowercase letters on the same line represent differences between time intervals for the same treatment.

Capital letters in the same column represent differences between treatments for the same time interval. Significance level of 95% ($p\leq 0.05$).

Kruskal-Wallis. (n= 9).

Table 3 – CO_2 biofixation (mg L^{-1} d^{-1}) by *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Scenedesmus subspicatus* cultivated with simulated gas containing CO_2 and NOx at different concentrations and time intervals.

CO_2 biofixation	Time intervals			
Chlorella vulgaris	4	8	12	16
IT 1 (5% + 30 ppm)	146.2 ± 68.3^{Bb}	132.3 ± 20.0^{Bb}	121.5 ± 14.8^{Cab}	95.5 ± 12.1^{Ca}
IT 2 (7% + 40 ppm)	80.2 ± 5.1^{Aa}	95.9 ± 10.3^{BCb}	104.4 ± 14.1^{BCb}	76.5 ± 12.5^{Ba}
IT 3 (10% + 60 ppm)	40.3 ± 7.7^{Aa}	54.3 ± 5.4^{Ab}	74.6 ± 6.2^{Abc}	78.7 ± 6.2^{Bc}
IT 4 (15% + 100 ppm)	124.7 ± 22.6^{Bb}	79.8 ± 11.2^{ABab}	68.4 ± 9.3^{Aa}	62.5 ± 10.5^{Aa}
Haematococcus pluvialis	4	8	12	16
IT 1 (5% + 30 ppm)	69.7 ± 20.8^{Ba}	91.5 ± 13.3^{Cb}	101.6 ± 13.3^{Bb}	88.0 ± 13.3^{Bab}
IT 2 (7% + 40 ppm)	15.0 ± 6.9^{Aa}	24.3 ± 5.6^{Ab}	37.2 ± 2.0^{Ac}	32.3 ± 5.9^{Ac}
IT 3 (10% + 60 ppm)	53.3 ± 8.4^{Bc}	32.8 ± 4.5^{Aa}	47.4 ± 3.3^{ABbc}	42.1 ± 3.1^{Ab}
IT 4 (15% + 100 ppm)	53.5 ± 18.2^{Ba}	52.2 ± 6.9^{BCa}	41.9 ± 8.6^{Aa}	41.3 ± 3.7^{Aa}
Scenedesmus subspicatus	4	8	12	16
IT 1 (5% + 30 ppm)	148.1 ± 14.1^{Bc}	156.5 ± 5.0^{BCc}	130.1 ± 9.4^{Bb}	115.5 ± 6.2^{Ba}
IT 2 (7% + 40 ppm)	141.1 ± 22.9^{Bb}	156.7 ± 19.6^{Cb}	135.7 ± 2.7^{Bb}	112.2 ± 3.5^{Ba}
IT 3 (10% + 60 ppm)	72.0 ± 7.6^{Aa}	68.8 ± 5.5^{Aa}	88.2 ± 8.3^{Ab}	85.5 ± 6.7^{Ab}
IT 4 (15% + 100 ppm)	116.7 ± 18.0^{ABb}	88.6 ± 12.4^{ABa}	95.9 ± 4.7^{Aab}	83.5 ± 5.3^{Aa}

Lowercase letters on the same line represent differences between time intervals averages. Capital letters in the same column represent differences between treatment averages. Significance level of 95% ($p \leq 0.05$). Kruskal-Wallis (n= 9).

Table 4 – Content of protein and lipids % w/w on a dry basis and stoichiometry from biomass of *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Scenedesmus subspicatus* cultivated at different concentrations of CO_2 and NOx.

Chlorella vulgaris	Protein* (%)	Lipids# (%)	C:N Ratio#
CT (0.04% CO_2)	13.2 ± 0.2^{b}	20.1 ± 2.0^{ab}	15.5 ± 0.1^{bc}
IT 1 (5% + 30 ppm)	9.9 ± 0.6^{a}	24.7 ± 1.9^{c}	21.2 ± 1.0^{d}
IT 2 (7% + 40 ppm)	12.0 ± 0.1^{b}	19.7 ± 1.9^{a}	16.8 ± 0.0^{c}
IT 3 (10% + 60 ppm)	15.1 ± 0.3^{c}	19.6 ± 0.7^{a}	13.3 ± 0.1^{ab}
IT 4 (15% + 100 ppm)	15.7 ± 0.0^{c}	23.1 ± 1.1^{bc}	13.1 ± 0.0^{a}
Haematococcus pluvialis	Protein* (%)	Lipids# (%)	C:N Ratio#
CT (0.04% CO_2)	26.8 ± 1.6^{d}	4.4 ± 0.5^{a}	8.3 ± 0.4^{a}
IT 1 (5% + 30 ppm)	10.0 ± 0.4^{a}	7.7 ± 1.0^{ab}	24.0 ± 0.7^{b}
IT 2 (7% + 40 ppm)	22.4 ± 0.5^{bc}	22.6 ± 1.3^{c}	10.0 ± 0.0^{a}
IT 3 (10% + 60 ppm)	25.0 ± 0.0^{cd}	15.8 ± 4.1^{bc}	8.5 ± 0.0^{a}
IT 4 (15% + 100 ppm)	21.2 ± 0.2^{b}	13.8 ± 1.1^{bc}	10.2 ± 0.0^{b}
Scenedesmus subspicatus	Protein* (%)	Lipids# (%)	C:N Ratio#
CT (0.04% CO_2)	12.4 ± 0.3^{b}	29.0 ± 1.7^{a}	17.4 ± 0.6^{ab}
IT 1 (5% + 30 ppm)	8.2 ± 0.1^{a}	31.2 ± 3.8^{a}	26.3 ± 0.1^{c}
IT 2 (7% + 40 ppm)	11.7 ± 1.4^{b}	31.3 ± 4.0^{a}	18.5 ± 2.1^{ab}
IT 3 (10% + 60 ppm)	14.1 ± 0.7^{b}	27.8 ± 4.4^{a}	15.1 ± 0.5^{a}
IT 4 (15% + 100 ppm)	10.8 ± 0.0^{ab}	29.3 ± 5.0^{a}	19.6 ± 0.0^{bc}

Lowercase letters in the same column represent differences between treatment averages for each microalgae species. Significance level of 95% ($p \leq 0.05$). *Tukey and #Kruskal-Wallis (n=9).

Table 5 – Astaxanthin concentration in biomass of *Haematococcus pluvialis* cultivated at different concentrations of CO_2 and NOx.

Astaxanthin	µg mL
CT (0.04% CO_2)	0.23 ± 0.14^{abc}
IT 1 (5% + 30 ppm)	0.14 ± 0.04^{abc}
IT 2 (7% + 40 ppm)	0.14 ± 0.08^{b}
IT 3 (10% + 60 ppm)	0.27 ± 0.11^{c}
IT 4 (15% + 100 ppm)	0.24 ± 0.12^{abc}

Lowercase letters represent differences between treatment averages. Significance level of 95% ($p\leq 0.05$). Kruskal-Wallis (n=12).

Figure captions

Fig. 1 Experimental design of microalgae cultivation.

Fig. 2 Growth curves by time intervals (g L^{-1} d^{-1}) from *Chlorella vulgaris,* *Haematococcus pluvialis,* and *Scenedesmus subspicatus* during cultivations with gas injections at different concentrations. Biomass on a dry basis.

Figures

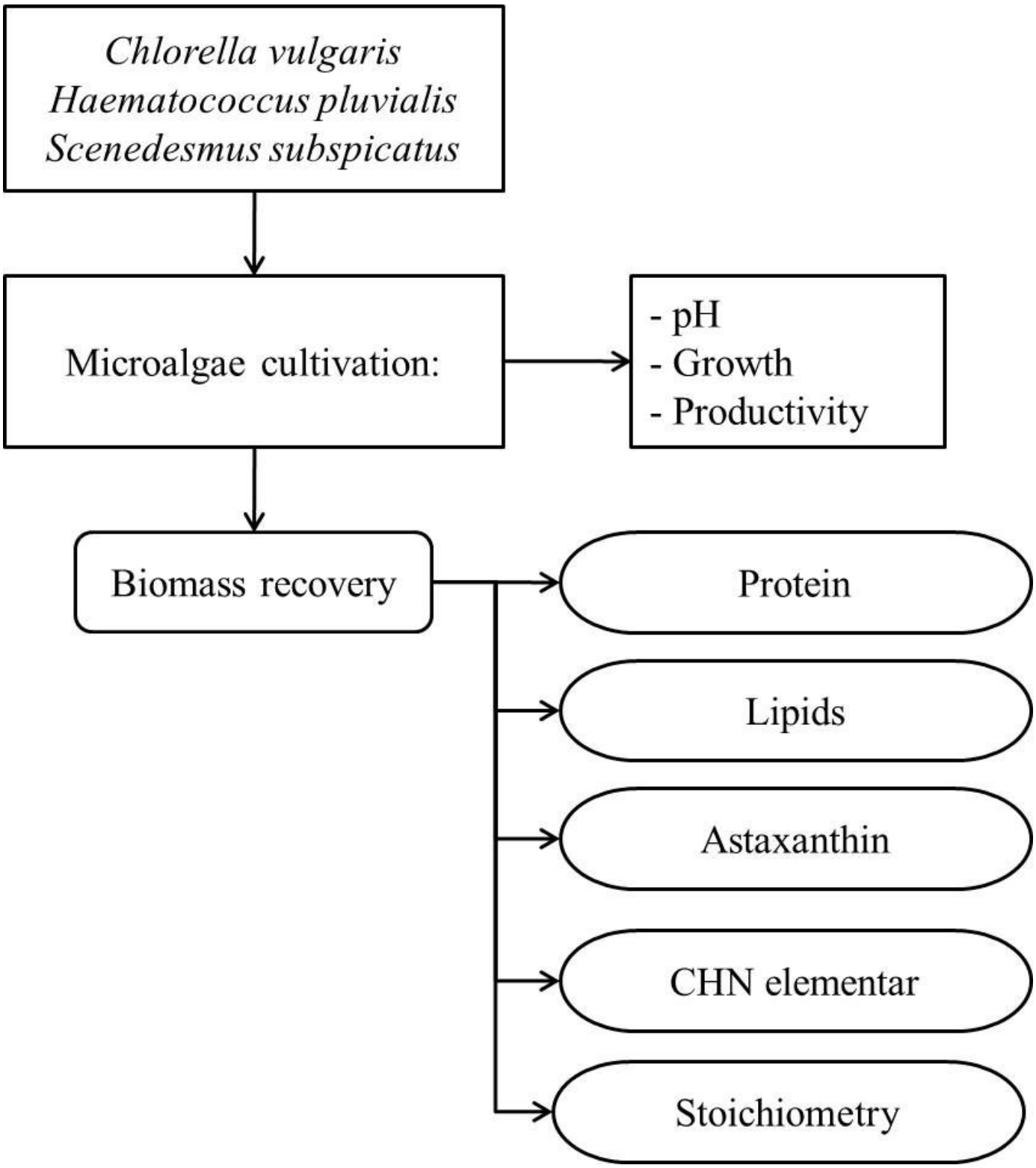

Figure 1

Experimental design of microalgae cultivation.

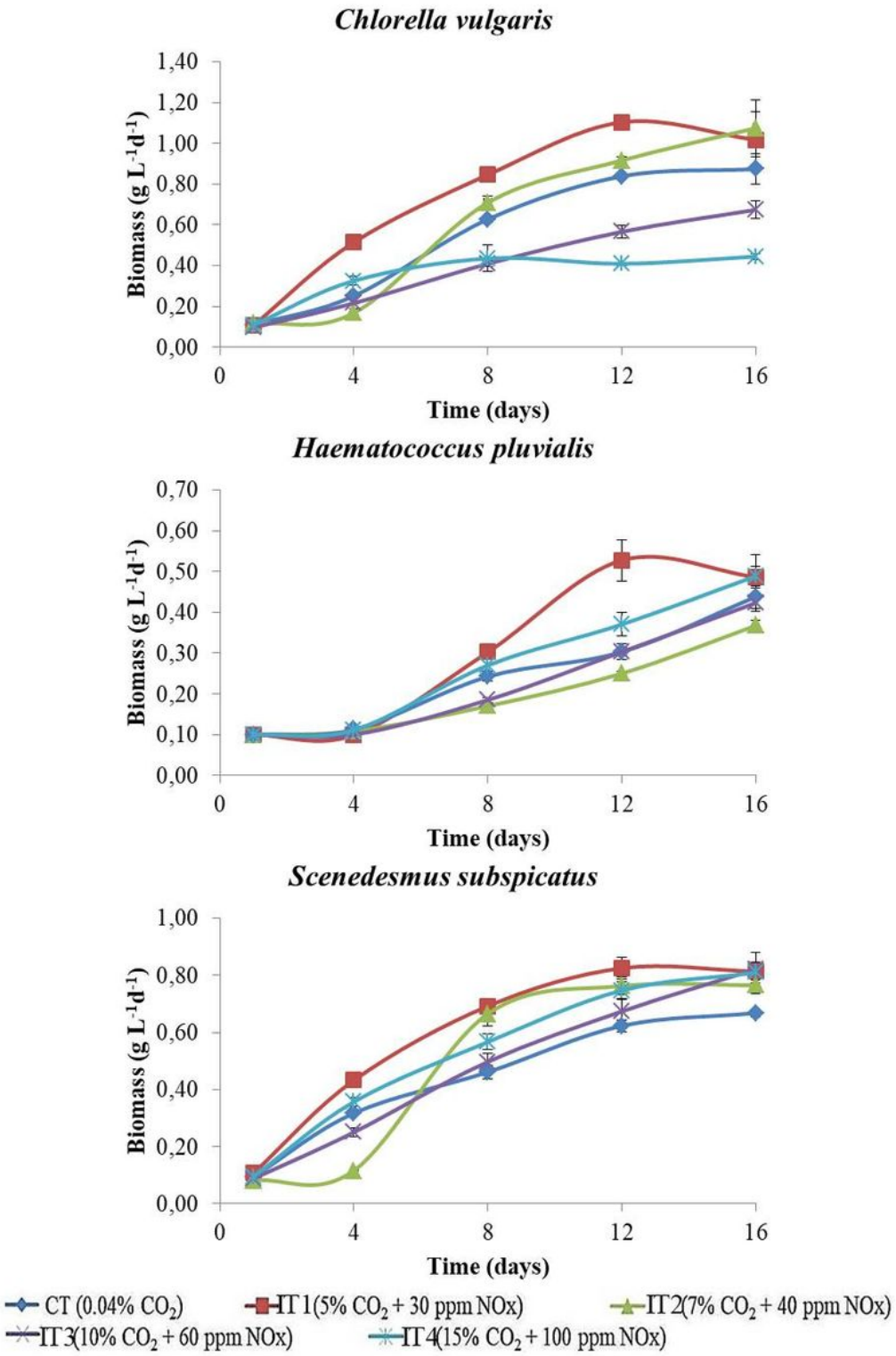

Figure 2

Growth curves by time intervals (g L-1 d-1) from Chlorella vulgaris, Haematococcus pluvialis, and Scenedesmus subspicatus during cultivations with gas injections at different concentrations. Biomass on a dry basis.