Impact of salinity on the kinetics of CO₂ fixation by *Spirulina platensis* cultivated in semi-continuous photobioreactors

Javier Christian Ramirez-Perez¹,²*, Harry William Janes²

1. University of São Paulo, Institute of Physics, Department of Applied Physics, São Paulo, São Paulo, Brazil.
2. Rutgers The State University of New Jersey, Biotechnology Center for Agriculture and the Environment, Department of Plant Biology and Pathology, New Brunswick, New Jersey, United States.

*Corresponding author: Javier Christian Ramirez-Perez, Phone: +55 11 98291-6169, Email address: jperez@if.usp.br*

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**ABSTRACT:** In this research, the physiological response of the microalgae *Spirulina platensis* to salinity stress (1 and 100 g L⁻¹) was investigated. *Spirulina platensis* and *S. platensis* (adapted to high salt concentration) were operated at laboratory scale in a semi-continuous photobioreactors. The responses examined were within 0.5 to 10% CO₂ concentration, temperatures from 10 to 40 °C, light intensities from 60 to 200 µmol m⁻² s⁻¹ and presented better results in terms of all kinetic parameters. The highest rate of CO₂ biofixation for *S. platensis* was 25.1 g CO₂ m⁻³ h⁻¹, and the maximum specific growth (μₒ) achieved was 0.44 d⁻¹ - 0.67 d⁻¹ at 2.5% CO₂, 150 µmol m⁻² s⁻¹ at 25 °C. Corresponding determined values of *S. platensis* adapted were 18.2 g CO₂ m⁻³ h⁻¹, 0.31 d⁻¹ - 0.58 d⁻¹ at 2.5% CO₂, 60 µmol s⁻¹ m⁻² and 28 °C. However, both microalgae exhibited experimental limiting growth factors, CO₂ 10%, 40 °C and 200 µmol m⁻² s⁻¹, conditions under which photosynthetic CO₂ biofixation may be inhibited and photoinhibition of photosynthesis may be enhanced by salinity. The efficiency of 2.5% CO₂ removal by *S. platensis* achieved 99%, whereas *S. platensis* adapted to 96%, respectively. The kinetic parameters estimated for *S. platensis* can be used to improve photobioreactor design for reducing of atmospheric carbon dioxide.
1. Introduction

Global warming is generally attributed to greenhouse gases (GHG) increase in the atmosphere, particularly carbon dioxide (CO₂), for which atmospheric concentration has already achieved 387 ppm and needs to get down to 350 ppm or less in order to avoid global climate change consequences. By 2100, 26 billion tons of CO₂ are estimated to be released into the atmosphere from anthropogenic sources. Photosynthetic organisms such as microalgae species are potent producers of value-added bioactive compounds such as pigments, vitamins and long-chain polyunsaturated fatty acids, when grown under stress conditions can accumulate significant quantities of total lipids. Recent studies indicated that improvements in culture conditions are needed to obtain adequate productivity of lipid, protein, carbohydrate content. It is well known that numerous parameters influence the growth of these compound content in microalgae: CO₂ addition, light, temperature, salinity, nutrient addition, inoculation size, stirring, pH, etc. The National Aeronautics and Space Administration (NASA) was the first institution to become interested in microalgae *Spirulina* for oxygen production, CO₂ reduction and proposed it as one of the primary foods to be cultivated in a future bioregenerative life support system for long-term manned space missions' scenarios such as Moon and Mars bases. The cyanobacterium *Spirulina platensis* (*S. platensis*) is commercially produced as a nutrient source in health food, feed and pharmaceutical industries, especially in developing countries. *S. platensis* has shown ability of adaptation to quite different habitats and colonizes harsh environments, where life is exceedingly difficult for other organisms. For example, in the lakes containing salt concentrations > 30 g L⁻¹, the cyanobacterial population became practically monospecific and *Spirulina* was the only organism present in significant quantities. Indeed *S. platensis* was found in waters containing from 20 to 270 g L⁻¹ of salt, but growth seemed to be optimal at salt concentrations ranging from 20 to 70 g L⁻¹ and it is possible that the population of *S. platensis* found at the highest salt concentrations, such as in temporary ponds just before drying, was that of the cyanobacterial biomass established when the concentration of salts was much lower. *S. platensis* are thermophilic algae with optimal growth temperature between 35 to 37 °C. When *S. platensis* was cultivated outdoor under high natural sunlight and salinity-stress, its production was usually accompanied by photosynthesis photoinhibition. Furthermore, it was suggested that salinity-stress enhances photosynthesis photoinhibition in green alga *Chlamydomonas reinhardtii*. Thus, *S. platensis* incorporates into a suitable photo-bioreactor configuration that can enhance photosynthesis by increasing growth conditions and controlling exposure of *S. platensis* to environmental factors, as well as suitable for greenhouse gases attenuation, particularly converting CO₂ into biomass in which carbon is biofixed and incorporated into carbohydrates, lipids and proteins. The microalga biomass produced can also be used for various applications, such as biofertilizer, soil conditioner, and biofuels production. However, more research and development are necessary on strain of microalgae selection, acclimation, and adaptation with regards to salt tolerance and the impact of other environmental parameters. In this paper, we studied the impacts of light intensity, temperature, and inlet CO₂ concentration on the specific growth rate of *S. platensis* and *S. platensis* adapted to salinity-stress during biofixation of CO₂ in photobioreactors at laboratory scale.

2. Experimental

2.1 Algal strain and cultivation conditions

*Spirulina platensis* from the American Type Culture Collection (ATCC) strain 53844 was cultivated in Zarrour’s culture fresh medium described in Tab. 1, adjusted (autoclave medium) to a final pH 9.0 ± 0.2. The stock culture was maintained in a 250 mL Erlenmeyer flask containing 50 mL of the medium at 20 °C under 60 µmol m⁻² s⁻¹ of light intensity, and 16/8 h day/night cycle. Every week the culture was transferred to a 500 mL flask containing the respective fresh medium and acclimatized to 0.5% of CO₂ mixed with air. Then, for further tests, the acclimated culture was transferred into each photobioreactor and the CO₂ concentration was increased gradually by bubbling CO₂ (2.5%, 5%, 7.5% and 10%) for 24 h before starting the test at a flowrate of 0.05 L m⁻¹.

Adaptation of *S. platensis* to high salinity, an inoculum of microalgae *S. platensis* were cultivated in a modified Zarrour’s medium (Tab. 2).
Table 1. Composition of stock solutions used to prepare nutrient solution for *S. platensis*.

| Stock Solution            | Composition                                                                 | Stock solution / g L⁻¹ |
|---------------------------|-----------------------------------------------------------------------------|------------------------|
| Nutrient Solution         | NaHCO₃                                                                      | 16.8                   |
|                           | K₂HPO₄                                                                      | 0.5                    |
|                           | NaNO₃                                                                       | 2.5                    |
|                           | K₂SO₄                                                                       | 1                      |
|                           | NaCl                                                                        | 1                      |
|                           | MgSO₄.7H₂O                                                                  | 0.2                    |
|                           | CaCl².2H₂O                                                                  | 0.04                   |
|                           | FeSO₄.7H₂O                                                                  | 0.01                   |
|                           | EDTA                                                                        | 0.08                   |
|                           | H₂BO₃                                                                       | 2.86                   |
| Trace metals mix A5       | MnCl₂.4H₂O                                                                  | 1.81                   |
|                           | ZnSO₄.7H₂O                                                                  | 0.222                  |
|                           | NaMoO₄.2H₂O                                                                 | 0.39                   |
|                           | CuSO₄.5H₂O                                                                  | 0.079                  |
|                           | Co(NO₃)₂.3H₂O                                                               | 0.0494                 |
| Trace metals mix B6 modified | NH₄NO₃                                                                     | 0.23                   |
|                           | K₂Cr₃(SO₄)₄.24H₂O                                                          | 0.096                  |
|                           | NiSO₄.7H₂O                                                                  | 0.0478                 |
|                           | Na₂WO₄.2H₂O                                                                 | 0.0179                 |
|                           | Ti(SO₄)₃                                                                   | 0.040                  |

*Preparation: Combine salt solution ingredients with 1 mL trace metals A5 mix and 1 mL trace metals B6 to prepare 1 L, adjust medium for final pH 9.0, and autoclave at 121 °C for 15 min. Adapted from “Carbon dioxide sequestration by *Spirulina platensis* in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 67.

Table 2. Composition of stock solutions used to prepare nutrient salt solution for *S. platensis* (adapted).

| Stock Solution            | Composition                                                                 | Stock solution / g L⁻¹ |
|---------------------------|-----------------------------------------------------------------------------|------------------------|
| Salt nutrient solution    | NaCl                                                                        | 100.0                  |
|                           | Seawater (Aquarium salt)                                                    | 16.0                   |
|                           | NaNO₃                                                                       | 0.51                   |
|                           | Na₂SO₄                                                                      | 1.23                   |
|                           | MgCl₂                                                                       | 0.033                  |
| Trace metal mix¹ Vitamin solution³ | EDTA.2H₂O                                                                 | 4.36                   |
|                           | CoCl₂.6H₂O                                                                  | 0.010                  |
|                           | FeCl₂.6H₂O                                                                  | 3.15                   |
|                           | MnCl₂.4H₂O                                                                  | 0.018                  |
|                           | CuSO₄.5H₂O                                                                  | 0.010                  |
|                           | Na₂MoO₄.2H₂O                                                                | 0.0063                 |
|                           | ZnSO₄.7H₂O                                                                  | 0.022                  |
|                           | NaH₂PO₄.2H₂O                                                                | 4.0                    |
|                           | Thiamine-HCl                                                                | 2.0                    |
| TV solution⁷              | Biotin                                                                      | 0.005                  |
|                           | Vitamin B₁₂                                                                  | 0.005                  |

Preparation: Trace Metal mix solution¹: Dissolve EDTA first in hot water and then combine with the other ingredients to 1L. Vitamin solution³: After combining the ingredients adjust to pH 6 filter sterilized (Do not autoclave). TV solution⁷, prepare the ingredients in 100 mL. Filter sterilized (Do not autoclave). Adapted from “Carbon dioxide sequestration by *Spirulina platensis* in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 68.

This adapted culture followed the same experimental procedure for *S. platensis* as explained above. Experiments were designed in parallel photobioreactors and operated under the same experimental conditions to study the adaptation process of *S. platensis* to salinity.
2.2 Photobioreactors and experiments

Figure 1 shows the graphical abstract scheme of the experimental set up at laboratory scale. The input CO\textsubscript{2} gas concentration was controlled by mixing CO\textsubscript{2} and air directly to each photobioreactor of 2 L working volume (WV) glass (Pyrex). The input gas mixture was connected by PVC tube (d=0.15 cm). The flowrate of CO\textsubscript{2} was measured by a flowmeter (Colepalmer) and a sintered sparge (porous air diffuser) placed into the photobioreactor for bubbling the into the biomass. The exhaust gas from each photobioreactor was connected by a PVC tube of 0.15 cm diameter and the flowrate measured by a flowmeter. Each photobioreactor was inoculated with 200 mL of precultured \textit{S. platensis} and filled with 1800 mL nutrient solution prepared and mixed 24 h in advance to reach about 0.5 g L\textsuperscript{-1} concentration of suspension biomass. An experimental factorial design was proposed to study the three factors: CO\textsubscript{2} concentration, temperature and light intensity, ranging at five levels using the same experimental procedure, first for \textit{S. platensis} culture followed by \textit{S. platensis} adapted to high salt concentration. Five parallel photobioreactors were set up in the same chamber, the initial concentration of the cell biomass was approximately 0.5 g L\textsuperscript{-1}, the photobioreactors operated at the same temperature and light intensity, but the cell biomass in each photobioreactor received different CO\textsubscript{2} concentrations (0.5, 2.5, 5.0, 7.5 and 10%) under continuous bubbling of CO\textsubscript{2} at a rate of 0.5 L m\textsuperscript{-3}, and all run lasted 12 days. The following experiments were examined at varied temperatures of 15, 20, 25, 30 and 40 °C and then light intensities of 60, 80, 100, 150 and 200 µmol m\textsuperscript{-2} s\textsuperscript{-1}.

The photobioreactors temperature was maintained constant by immersing the photobioreactors in acrylic open water baths (0.46 x 0.25 x 0.8 m) with immersion circulator analog controller (Isotemp 2100). The light intensity was generated by cool white fluorescent tubes (General Electric 40w to 80w) and measured using a quantum sensor (model LI-190SA\textsuperscript{2}) connected to a quantum/radiometer/photometer light meter (model LI-250A, Li Cor Inc. Lincon, NE, USA). The quantum sensor was configured to make measurements of photon flux density (PDF) in the PAR (Photosynthetic Active Radiation, 400-700 nm). The source of CO\textsubscript{2} was provided by Airgas Specialty Gases New Jersey, 200 and 300 cubic feet volume cylinders of CO\textsubscript{2} concentrations of 0.5, 2.5, 5.0, 7.5 and 10% balance with air, the concentrations certified by the vendor.

2.3 Analytical determinations

The concentration of microalgae was measured by the method of filtration, being 10 mL suspension of cell biomass filtered on membrane filter (GF/C filters 1.2 µm, d=47 mm). After dried filters at 80 °C for 24 h, cell biomass weights were determined until achieved constant weight over time. The ratio of carbon in the dry cell biomass [Cc] was determined by ignition, dried cell biomass was ignited at 500 °C in a Thermolyne Furnace (model 62700, Thermolyne Corp. Dubuque, IA) to estimate the dry weight biomass. The cell biomass concentration for both microalgae was also determined as the changes in optical density (OD). The OD of the suspension algal biomass was measured at 680 nm (\textit{S. platensis}) as absorbance, using a spectrophotometer (UV-VIS Shimadzu- 1700). The cell dry weight of \textit{S. platensis} and optical density (OD\textsubscript{680}) were established by linear regression (dry cell biomass, g L\textsuperscript{-1} = 0.477 × OD\textsubscript{680} + 0.376; R\textsuperscript{2} = 0.957; p=0.01). Likewise, for \textit{S. platensis} adapted and cell biomass g L\textsuperscript{-1}=2.35 × OD\textsubscript{680} + 0.32; R\textsuperscript{2}=0.94, p=0.01. Triplicate samples of the cell biomass were collected every day until reached maximum microbial growth (4-5 g L\textsuperscript{-1}), depending upon the experimental conditions some photobioreactors were shut down earlier than others. The pH of the suspension of cell biomass was measured with a pH meter (Acumet ABIS Plus) calibrated with standard pH solutions of 4, 7 and 11. Analysis of CO\textsubscript{2} input/output of each bioreactor was measured with a Gas Chromatograph Shimadzu 17A.

Figure 1. Experimental setup of algal bioreactors at small scale.
Adapted from “Carbon dioxide sequestration by \textit{Spirulina platensis} in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, \textit{Habitation}, 12(1), p. 67.
with TDC detector, gas samples were taken in plastic bags. The CO₂ concentration was also monitored analyzing CO₂ directly in the gas stream off the bioreactor using colorimetric gas detection tubes RAE systems.

2.4 Determination of growth rate and kinetic parameters

Assuming that the microalgae growth can be modeled by a first order dynamic equation:

\[ \frac{dy}{dt} = \mu X \]  

Integrating and re-arranging Eq. 1, the growth coefficient also called specific growth rate (\( \mu \), d⁻¹) can be calculated using the Eq. 2:

\[ \mu = \frac{\ln(X_2 / X_1)}{t_2 - t_1} \]  

(2)

where \( X_1 \) and \( X_2 \) were the microalgae concentration (g L⁻¹) on days \( t_1 \) and \( t_2 \) respectively. The biomass productivity rate, also called linear growth (p), is estimated according to Eq. 3:

\[ p = \frac{x_2-x_1}{t_2-t_1} \]  

(3)

where \( p \) (g L⁻¹ d⁻¹). Since no organic carbon source is available in culture medium, the CO₂ biofixation rate can be indirectly calculated by the carbon content and biomass productivity rate, according to Eq. 4:\n
\[ R_{\text{CO}_2} = C_c \times p \times (M_{\text{CO}_2}/M_c) \]  

(4)

Therefore, the rate of CO₂ biofixation per initial inoculation mass of microalgae can be determined by Eq. 5:

\[ R_{\text{CO}_2} = R_{\text{CO}_2} / X_0 \]  

(5)

where \( R_{\text{CO}_2} \) [g CO₂ L⁻¹ d⁻¹] is the biofixation rate and \( R_{\text{CO}_2} [g CO₂ g⁻¹ dry \ cell] \).

The average cell carbon content \( C_c \) [g C g⁻¹ dry cell] ratio measured experimentally was 0.59 g carbon g⁻¹ dry cell weight, according to the measurement using an elemental analyzer, and \( M_{\text{CO}_2} \) and \( M_c \) represents the molecular weight of CO₂ and C, respectively.

The efficiency of CO₂ removal was calculated as follows: 100 × (CO₂ input - CO₂ output) / (CO₂ input), where CO₂ input is the initial and CO₂ output the stream gas off the bioreactor.

Empirical microbial growth kinetic models were explored to describe the impact of environmental factors on specific microbial growth of \( S. \ platensis \) and \( S. \ platensis \) adapted. Mônod model (Eq. 6).

\[ \mu = \mu_{\text{max}} \frac{S}{K_s + S} \]  

(6)

where \( K_s \) is the Mônod kinetic constant and \( S \), CO₂ concentration or light intensity. When the substrate inhibits microbial growth at high concentrations, an optimum at which the highest specific growth rate occurs in this case, the Mônod model can be modified by Andrews model (Eq. 7).

\[ \mu = \mu_{\text{max}} \frac{S}{K_s + S + S^2 / K_i} \]  

(7)

where \( K_s \) and \( S \) are the same meaning as Mônod, and \( K_i \) is the inhibition constant.

The effect of the temperature on the maximum specific growth rate is based on Arrhenius model (1889), which implies an exponential increase in growth rate of the cells with rising the temperature. However, it is well recognized that the Arrhenius model fails once the temperature approaches the value of optimum activity, because it cannot represent the decline in rates at higher temperatures. Due to this limitation, alternative models have been proposed, which can predict the decline in rate following the optimum. Mayo model (Eq. 8) modified the Arrhenius equation based on the premise that the active fraction of the enzymes involved in the growth limiting reaction deceases when the temperature exceeds the optimum, this expression is also able to predict a decline in the maximum specific growth rate when the temperature exceeds the optimum:

\[ \mu = \frac{A' e^{(-E_1/RT)}}{1 + k e^{(-E_2/RT)}} \]  

(8)

where \( A' \) and \( k \) are constants, \( E_1 \) is the activation energy for cellular multiplication, and \( E_2 \) is the activation energy for the thermal denaturalization process.

2.5 Statistical analysis

The experimental results were evaluated by comparing the specific growth rates of CO₂ biofixation by \( S. \ platensis \) and \( S. \ platensis \) adapted to salinity.
under different environmental conditions in photobioreactors and analysis of variance (ANOVA) of the kinetic parameters, significance was tested by Tukey at \( p < 0.05 \), using R software.

3. Results and discussion

3.1 Effect of salinity on the dry cell mass growth of \( S. \) platensis under different environmental conditions

Figure 2 gives the growth curve of dry cell mass of \( S. \) platensis cultivated in photobioreactors at normal salinity concentration for 12 days cultivation period at 2.5% \( \text{CO}_2 \) concentration, 25 \( ^\circ \text{C} \) and \( \mu \text{mol m}^{-2} \text{s}^{-1} \) dry cell mass achieves 4.3 g L\(^{-1} \), which slowly declines when cultivation conditions change by increasing \( \text{CO}_2 \) concentration (5%), temperature (30 \( ^\circ \text{C} \)) and light intensity (150 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). The dry cell mass growth of \( S. \) platensis continued to decline even more and photosynthesis of \( S. \) platensis is inhibited at cultivation conditions of \( \text{CO}_2 \) concentration (10%), temperature (40 \( ^\circ \text{C} \)), and light intensity (200 \( \mu \text{mol m}^{-2} \text{s}^{-1} \)). Figure 3 shows the impact of salinity when \( S. \) platensis adapted to 100 g L\(^{-1} \) \( \text{NaCl} \) (1.71 mol L\(^{-1} \)) is cultivated in the same way of \( S. \) platensis, for example, dry cell mass achieves 3.2 g L\(^{-1} \) for a 12 days cultivation period at 2.5% \( \text{CO}_2 \) concentration, 25 \( ^\circ \text{C} \) and 100 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), salinity-stress is usually accompanied by photoinhibition of photosynthesis. Figure 4 illustrates the UV-visible spectrums of \( S. \) platensis and \( S. \) platensis adapted. An analysis of the spectrum from 600 to 800 nm shows the strongest band centered near 680 nm for \( S. \) platensis, which decreases and displaces the maximum to 675 nm for \( S. \) platensis adapted, due to the impact of salinity.

### Figure 2
Dry cell mass of \( S. \) platensis grown under different \( \text{CO}_2 \) concentrations, temperatures and light intensities.
Adapted from “Carbon dioxide sequestration by \( Spirulina \) platensis in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, Hartitation, 12(1), p. 69.

### Figure 3
Salinity effect on dry cell mass of \( S. \) platensis (adapted) grown at 100 mg L\(^{-1} \) \( \text{NaCl} \) and under different \( \text{CO}_2 \) concentrations, temperatures and light intensities.
Adapted from “Carbon dioxide sequestration by \( Spirulina \) platensis in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, Hartitation, 12(1), p. 69.
3.2 Effect of Salinity on the specific growth rate ($\mu$) and CO$_2$ biofixation rate ($R_{CO_2}$)

Table 3 shows that during the 12 days cultivation period, the highest $\mu$ and R values of S. platensis (0.278 d$^{-1}$, 25.1 gCO$_2$ m$^{-3}$ h$^{-1}$) cultivated at 2.5% CO$_2$ was significantly higher ($p < 0.0012$) than 5% CO$_2$ or higher CO$_2$ concentration runs. In the same cultivation mode, the $\mu$ and R of S. platensis adapted decreases (0.15 d$^{-1}$, 13.9 gCO$_2$ m$^{-3}$ h$^{-1}$) because of the effect of salinity, in addition to the high CO$_2$ concentration of 5 to 10% runs, according to the results of Tab. 3. There was no significant difference between $\mu$ and R values of S. platensis between 20 and 25 °C runs, but there were significant differences ($p < 0.0012$) between $\mu$ and R values of S. platensis adapted in the same range of temperatures, these values were significantly lower when the cells were exposed to higher temperatures from 30 to 40 °C, respectively. When S. platensis were exhibited to 150 µmol s$^{-1}$ m$^{-2}$ $\mu$ and R values achieved higher values elevated light intensities e.g. 0.18 d$^{-1}$, 16.2 gCO$_2$ m$^{-3}$ h$^{-1}$ than corresponding values at 100 and 60 µmol s$^{-1}$ m$^{-2}$. But there was no significant difference between $\mu$ and R of S. platensis for 100 and 150 µmol s$^{-1}$ m$^{-2}$ runs.

| Treatment                  | $\mu$ / d$^{-1}$ | R / gCO$_2$ m$^{-3}$ h$^{-1}$ | $\mu$ / d$^{-1}$ | R / gCO$_2$ m$^{-3}$ h$^{-1}$ |
|----------------------------|------------------|-------------------------------|------------------|-------------------------------|
| ANOVA                      |                  |                               |                  |                               |
| S. platensis               | 2.90e$^{-11}$*** | 4.45e$^{-2}$*                 | S. platensis     | 3.00e$^{-11}$***              | 4.47e$^{-2}$*                 |
| S. platensis Adapted       | 0.1149 c         | NA                            | S. platensis     | 10.36 c,d                     | NA                            |
| Carbon dioxide, %, Pr(>F)  |                  |                               |                  |                               |
| 0.5                        | 0.2783 a         | 0.1540 b                      | 25.08 a          | 13.88 b,c                     |
| 2.5                        | 0.1592 b,c       | 0.1105 b,c                    | 14.34 b,c        | 10.38 c,d                     |
| 5.0                        | 0.0597 c,d       | 0.1045 b,c                    | 5.38 d,e         | 9.42 d,e                      |
| 7.5                        | 0.0549 c,d       | 0.0501 d                      | 4.94 e           | 4.53 e                        |
| 10.0                       |                  |                               |                  |                               |
| Temperature, °C, Pr(>F)    |                  |                               |                  |                               |
| 12                         | 0.0260 e         | NA                            | 2.32 e           | NA                            |
| 15                         | 0.0477 d         | 0.0297 e                      | 4.28 d,e         | 2.70 e                        |

Figure 4. Impact of salinity on the UV-vis absorption of S. platensis and S. platensis adapted. Adapted from “Carbon dioxide sequestration by Spirulina platensis in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.
Conversely, the μ and R values of S. platensis adapted—increased as the cells were exposed to lower light intensity from 60 μmol s⁻¹ m⁻² (0.15 d⁻¹, 13.2 g CO₂ m⁻³ h⁻¹) to 200 μmol s⁻¹ m⁻² (0.05 d⁻¹, 4.96 g CO₂ m⁻³ h⁻¹). But there were no significant differences between μ and R values of S. platensis adapted cultivated with 60 and 100 μmol s⁻¹ m⁻², respectively, indicating that S. platensis adapted to salinity stress is less tolerant to growth at elevated light intensity and temperature, suggesting a decrease in photosynthetic activity of S. platensis adapted (Tab. 2).

3.3 Effect of salinity on the kinetics of S. platensis and S. platensis adapted

The relationship between the μ value and environmental cultivation parameters such as CO₂ concentration, temperature and light intensity in terms of experimental kinetic models (Monôd, Andrews and Mayo) and the impact of salinity is illustrated for S. platensis (Tab. 3) and S. platensis adapted (Tab. 4), respectively. The impact of light intensity on μ of S. platensis, in principle is described by Monod well in the range of 60 to 150 μmol m⁻² s⁻¹ (Eqs. 9 and 10 (Tab. 4). The maximum specific growth (μ_max) estimated (0.44 d⁻¹) is depleted as CO₂ concentration and temperature were increased. Under these conditions, μ_max decreased to 0.22 d⁻¹ and Andrew’s model described better the kinetics of S. platensis exposed to higher light intensity (Eqs. 11, 12 in Table 4 and Fig. 5). In fact, a decline in the photosynthetic activity was observed after four days of experiment run under 10% CO₂, 200 μmol m⁻² s⁻¹, and 40 °C, suggesting these values as potential limiting factors of microalgae growth and photoinhibition. The influence of light intensity on the kinetics of S. platensis adapted was stronger than the impact on the μ of pure culture S. platensis (see Tab. 5: Eqs. 19, 20 and Fig. 5), Andrew’s model described this impact, μ_max dropped from 0.31 d⁻¹ for 5% CO₂ and 25 °C to 0.22 d⁻¹ for light intensities higher than 150 μmol m⁻² s⁻¹ and photoinhibition occurred when CO₂ and temperature increase to 10% and 40 °C, suggesting that in addition to light intensity salt stress affect its photosynthetic activity and may inhibit completely at 10% CO₂ and 40 °C. These results agree with Zeng et al. who reported that the effect of salinity stress was stronger when cells of S. platensis were grown under higher light intensity 200 μmol m⁻² s⁻¹ and showed lower capacity of recovery in the photosynthetic activity after photoinhibition than lower light intensity (100 μmol m⁻² s⁻¹) grown cells. This was attributed as a result of the fact that stressed cells have lower protein synthesis capacity and thus a slower repair mechanism.

Table 4. Experimental kinetic models and kinetic parameters of S. platensis.

| Effect of light intensity | \( \mu = \frac{I}{72.3 + I} \); (25 °C, 2.5%) | (9) |
|--------------------------|------------------------------------------|----|
| \( \mu = \frac{I}{80.2 + I} \); (25 °C, 5.0%) | (10) |
| \( \mu = \frac{I}{92.3 + I + I^2/74.0} \); (30 °C, 7.5%) | (11) |
\[
\mu = 0.009 \frac{I}{98.3 + I + I^2/62.3}; \text{ (40 °C, 10%)}
\] (12)

**Effect of CO\textsubscript{2} Concentration**

\[
\mu = 0.028 \quad \text{CO}_2 \quad 1.76 + \text{CO}_2 + \text{CO}_2^2/3.43; \text{ (25 °C, 150 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (13)

\[
\mu = 0.021 \quad \text{CO}_2 \quad 1.37 + \text{CO}_2 + \text{CO}_2^2/1.19; \text{ (30 °C, 100 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (14)

\[
\mu = 0.017 \quad \text{CO}_2 \quad 1.77 + \text{CO}_2 + \text{CO}_2^2/3.16; \text{ (40 °C, 200 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (15)

**Effect of Temperature**

\[
\mu = \frac{0.029e^{(-0.19/RT)}}{1 + 288.3e^{(-1.55/RT)}}; \text{ (2.5%, 60 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (16)

\[
\mu = \frac{0.020e^{(-0.20/RT)}}{1 + 304.2e^{(-1.50/RT)}}; \text{ (5%, 100 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (17)

\[
\mu = \frac{0.014e^{(-0.20/RT)}}{1 + 310.2e^{(-1.60/RT)}}; \text{ (10%, 200 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (18)

**Table 5.** Experimental kinetic models and kinetic parameters of S. platensis adapted.

**Effect of light intensity**

\[
\mu = 0.013 \frac{I}{94.3 + I + I^2/134.2}; \text{ (25 °C, 5%)}
\] (19)

\[
\mu = 0.009 \frac{I}{100.83 + I + I^2/111.5}; \text{ (40 °C, 10%)}
\] (20)

**Effect of CO\textsubscript{2} Concentration**

\[
\mu = 0.024 \quad \text{CO}_2 \quad 2.05 + \text{CO}_2 + \text{CO}_2^2/2.55; \text{ (25 °C, 60 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (21)

\[
\mu = 0.010 \quad \text{CO}_2 \quad 2.08 + \text{CO}_2 + \text{CO}_2^2/2.27; \text{ (40 °C, 200 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (22)

**Effect of Temperature**

\[
\mu = 0.028e^{(-0.19/RT)}; \text{ (2.5%, 60 \mu mol s\textsuperscript{-1} m\textsuperscript{-2})}
\] (23)

The effect of CO\textsubscript{2} concentration on the kinetics of S. platensis, Eqs. 13, 14 and 15 (Tab. 4) and S. platensis adapted Eqs. 21 and 22 (Tab. 5), is depicted by Andrew’s kinetic model and is illustrated in Fig. 6. The \(\mu_{max}\) value estimated for 2.5% CO\textsubscript{2}, 25 °C and 150 \mu mol m\textsuperscript{-2} s\textsuperscript{-1} declined from 0.67 d\textsuperscript{-1} to 0.41 d\textsuperscript{-1} when CO\textsubscript{2} concentration increased in the range of 5 to 10% and the temperature and light intensity rose to 40 °C and 200 \mu mol m\textsuperscript{-2} s\textsuperscript{-1}, respectively, suggesting that CO\textsubscript{2} is a limiting factor that inhibited S. platensis growth particularly at 200 \mu mol m\textsuperscript{-2} s\textsuperscript{-1} and 40 °C. An optimal \(\mu_{max}\) value for S. platensis adapted to salinity stress of 0.58 d\textsuperscript{-1} determined under the following environmental parameters 2.5 % CO\textsubscript{2} (25 °C and 60 \mu mol m\textsuperscript{-2} s\textsuperscript{-1}) declined to 0.24 d\textsuperscript{-1} when CO\textsubscript{2} concentration rose more than 5% (40 °C and 200 \mu mol m\textsuperscript{-2} s\textsuperscript{-1}), suggesting that CO\textsubscript{2} is a limiting factor of CO\textsubscript{2} biofixation in combination with high salt concentration. The effect of temperature on the kinetics of S. platensis (Eqs. 16, 17 and 18 in Tab. 4) and S. platensis adapted to salinity stress (Eq. 23 in Tab. 5) and depicted by Mayo’s model (Fig. 7), the optimum temperature for cultivation of S.
S. platensis occurred around 25 °C, 150 µmol s⁻¹ m⁻² and 2.5% CO₂ but it declined slowly as temperature increased along with CO₂ concentration and light intensity to a minimum µ values 10% CO₂ and 200 µmol m⁻² s⁻¹. Likewise, as temperature decreased less than 15 °C, µ values decreased, indicating that the temperature is a limited growth factor under the influence of high CO₂ concentration and light intensity in spite the fact that S. platensis was characterized as thermophilic microalgae. The optimum temperature of S. platensis adapted occurred around 25 °C, for 60 µmol m⁻² s⁻¹ and 2.5% CO₂ but declined slowly as the temperature increased. Despite the fact that S. platensis has been characterized as a thermophilic microalgae, the high salt concentration may have caused strong impact on the cells stress supported by high CO₂ concentration (10%) and light intensity (200 µmol m⁻² s⁻¹) minimize µ values. Likewise, as temperature decreased less than 15 °C, µ values decreased, suggesting that the temperature is a limiting growth factor. In addition, other factors were shown, such as CO₂ concentration and light intensity in combination with high salt concentration affect CO₂ biofixation rate.

**Figure 5.** Effect of light intensity on the kinetics of S. platensis and S. platensis adapted to salinity stress at different CO₂ concentrations and temperatures. Mónod model fit (solid line), Andrew’s model fit (dash line). Adapted from “Carbon dioxide sequestration by Spirulina plantensis in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 71.
Figure 6. Effect of CO₂ concentration on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different light intensities and temperatures. Adapted from “Carbon dioxide sequestration by *Spirulina platensis* in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 71.

Figure 7. Effect of temperature on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different light intensities and CO₂ concentrations. Adapted from “Carbon dioxide sequestration by *Spirulina platensis* in photo-bioreactors”, by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 72.
Waste stream flue gases emitted from stationary sources such as power plants, industrial boilers, refineries and others, using fossil fuels for combustion and energy production produce from 4 -14% CO₂ and air in a closed space such as a space station or a submarine < 1% CO₂. It has been reported that microalgae present one of the few technologies for the capture and utilization of CO₂. The results indicate that both S. platensis and S. platensis adapted to high salt concentration can be used for CO₂ biofixation of flue gas emitted from stationary sources. In fact, maximum daily evaluation of the efficiency CO₂ removal by S. platensis during experiments up to ten days, achieved 92% at 0.5% CO₂, 99% at 2.5% CO₂, 84% at 5% CO₂, 89% at 7.5% and 88% at 10% CO₂, respectively. Corresponding values for S. platensis adapted being 96% at 2.5% CO₂, 90% at 5% CO₂, 78% at 7.5% CO₂ and 73% at 10% CO₂, respectively (See Appendix).

Small pH changes of S. platensis cell suspension observed during experiments from an initial pH (8.9) in the presence of CO₂ ≤ 2.5% and temperatures ≤ 25 °C, after 24 h pH rose to 9.4 ± 0.1 and S. platensis adapted from an initial pH of 8.5 increased to 8.9 ± 0.1. However, in cultivation of S. platensis at CO₂ > 5% and temperatures > 25 °C the pH increased to 9.1 ± 0.2 and S. platensis adapted to 9.2 ± 0.2, after 24 h. These results indicate that there was no formation of carbonic acid but no abrupt decline in pH due to S. platensis and S. platensis adapted were able to metabolize CO₂ because both microalgae were previously acclimatized to CO₂. S. platensis and S. platensis adapted were able to metabolize CO₂ because both microalgae were previously acclimatized to CO₂. Therefore, neither was formation of carbonic acid nor decline in pH.

In this study, the maximum mean CO₂ biofixation rate recorded for S. platensis was 25.1 g CO₂ m⁻³ h⁻¹ cultivated at 2.5% CO₂, 150 µmol m⁻² s⁻¹ and 25 °C, corresponding values for S. platensis adapted being 18.2 g CO₂ m⁻³ h⁻¹ for 2.5% CO₂, 60 µmol m⁻² s⁻¹ and 25 °C.

In a three-stage serial tubular photobioreactor were cultivated Scenedesmus obliquus and Spirulina sp. at 30 °C. It was found that, for Spirulina sp. the µmax was 0.44 d⁻¹, 9.2 g m⁻³ h⁻¹ with 6% CO₂, maximum daily CO₂ removal efficiency was 53.3% for 6% CO₂ and 45.6% for 12% CO₂, the corresponding values for S. obliquus being 28.1% for 6% CO₂ and 13.6% for 12% CO₂ runs. Yun et al. estimated 26.0 g CO₂ m⁻³ h⁻¹ biofixation rate value when Chlorella vulgaris was cultivated after adaptation in 5% CO₂ in wastewater supplemented with nutrients and without pH control at 15% CO₂, this value is comparable with the findings of this research and suggests that elevated CO₂ concentration may exert effects on the photoinhibitory behavior of the microalgae to different extents according to species. The efficiency of CO₂ biofixation by these microalgae strains may have been due to its physiological conditions, such as potential of cell growth and ability of CO₂ metabolism. S. platensis was cultivated under different light intensities (100-200 µmol m⁻² s⁻¹) at 35 °C and adapted to salinity stress up to 0.75 mol L⁻¹. It was reported that the cells grown in higher light intensity are less tolerant to salinity stress than those grown in lower light intensities, suggesting that salt stress enhances photoinhibition of photosynthesis through a direct effect on PSII reaction center. The results demonstrated that S. platensis adapted to high salinity media 1.71 mol L⁻¹ cultivated at 200 µmol s⁻¹ m⁻², 40 °C and high CO₂ concentrations (7.5 and 10%) inhibit microalgae grow, show low photosynthetic activity and consequently photoinhibition. Photoinhibition occurs when the photon flux absorbed by chloroplasts is extremely high, so the concentration of high energy electrons in the cells is too elevated to be consumed in the Calvin cycle. These electrons react with water to form hydrogen peroxide, which is highly harmful to sub-cellular structures and the cell itself, indicating that salinity enhances photoinhibition of photosynthesis through a direct effect on PSII reaction center, the reason for declination of PSII activity of cells under salinity stress remains open. Moreover, they believed that salinity stress induced damage or inactivation of PSII reaction center as it is in the case of photoinhibition of photosynthesis.

4. Conclusions

This study shows the potential CO₂ biofixation by S. platensis and S. platensis adapted to high salinity, 1.71 mol L⁻¹ NaCl at laboratory scale in photobioreactors. In general, a better rate of CO₂ biofixation was achieved by S. platensis as indicated by its kinetic parameters and efficiency CO₂ removal, when compared to S. platensis adapted. The impacts of light intensity, CO₂ concentration and temperature on the specific growth rate followed the Mónod, Andrews and Mayo kinetic models. For S. platensis the highest dry cell mass concentration was 4.1 g L⁻¹, cultivated at 2.5% CO₂, 25 °C and 150 µmol m⁻² s⁻¹, the highest rate of biofixation 25.1 g CO₂ m⁻³ h⁻¹ and the maximum specific growth (µmax) was 0.44 d⁻¹ - 0.67 d⁻¹. The corresponding values for S. platensis adapted were 3.2 g L⁻¹ at 2.5% CO₂, 25 °C and 60 µmol s⁻¹ m⁻², 18.2 g CO₂ m⁻³ h⁻¹, and µmax, 0.32 d⁻¹ - 0.58 d⁻¹. This suggests that the impact
of salinity in combination with environmental grow factors such as elevated CO₂ concentration, light intensity and temperature may exert effects on the photoinhibitory behavior of *S. platensis* adapted, provoking a CO₂ biofixation rate depletion. Therefore, the photosynthetic biofixation of CO₂ *S. platensis* and *S. platensis* adapted showed optimum values at 2.5% CO₂ and 25 °C, and more sensitivity to light intensity than *S. platensis*, suggesting that salinity enhanced photoinhibition of photosynthesis. The efficiency of CO₂ removal by *S. platensis* achieved 99%, whereas for *S. platensis* adapted 96%, both at 2.5% CO₂ concentration and small pH changes exhibited both microalgae cell suspension.

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Table 1A. *Spirulina platensis* efficiency of CO2 removal.

| R²   | [CO₂]_{in} (%) | [CO₂]_{out} (%) | Eff (%) |
|------|----------------|-----------------|---------|
| 1.00 | 0.5            | 0.02            | 96      |
| 1.00 | 0.5            | 0.03            | 94      |
| 0.92 | 0.5            | 0.04            | 92      |
| 0.87 | 0.5            | 0.05            | 90      |
| 0.89 | 0.5            | 0.05            | 90      |
| 0.97 | 2.5            | 0.05            | 98      |
| 0.97 | 2.5            | 0.02            | 99.2    |
| 0.97 | 5.01           | 0.7             | 86      |
| 0.94 | 5.13           | 1               | 80      |
| 0.98 | 5.13           | 0.65            | 87      |
| 0.89 | 5              | 0.95            | 81      |
| 0.94 | 5.01           | 0.7             | 86      |
| 0.84 | 5.01           | 1               | 80      |
| 0.85 | 5.12           | 0.65            | 87      |
| 0.85 | 7.5            | 0.95            | 87      |
| 0.25**| 7.5            | 0.7             | 91      |
| 0.96 | 10             | 0.65            | 94      |
| 0.85 | 10             | 0.95            | 91      |
| 0.88 | 10             | 0.7             | 93      |
| 0.40**| 10             | 1               | 90      |
| 0.56**| 10             | 2.5             | 75      |
| 0.86 | 10             | 2.2             | 78      |
| 0.72**| 10             | 0.7             | 93      |

* p-value = 0.001. ** p-value = 0.01.

Table 2A. *Spirulina platensis* adapted efficiency of CO2 removal.

| R²   | [CO₂]_{in} (%) | [CO₂]_{out} (%) | Eff (%) |
|------|----------------|-----------------|---------|
| 0.944| 2.5            | 0.10            | 96      |
| 0.944| 5.0            | 0.10            | 98      |
| 0.965| 7.5            | 0.30            | 96      |
| 0.828| 10.0           | 2.00            | 80      |
| 0.792| 10.0           | 3.00            | 70      |
| 0.969| 2.5            | 0.10            | 96      |
| 0.504**| 5.0          | 0.05            | 99      |
| 0.6236**| 7.5          | 3.00            | 60      |
| 0.971| 10.0           | 3.00            | 70      |
| 0.856| 5.0            | 0.10            | 98      |
| 0.906| 5.0            | 0.15            | 97      |
| 0.837| 5.0            | 0.60            | 88      |
| 0.897| 5.0            | 1.50            | 70      |
| 0.6236**| 5.0          | 0.10            | 98      |
| 0.977| 5.0            | 0.15            | 97      |
| 0.912| 5.0            | 0.95            | 81      |
| 0.970| 5.0            | 1.00            | 80      |
| 0.805| 5.0            | 0.10            | 98      |

* p-value = 0.001. ** p-value = 0.01.

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**Appendix**

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