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Impact of salinity on the kinetics of CO_2 fixation by *Spirulina platensis* cultivated in semi-continuous photobioreactors

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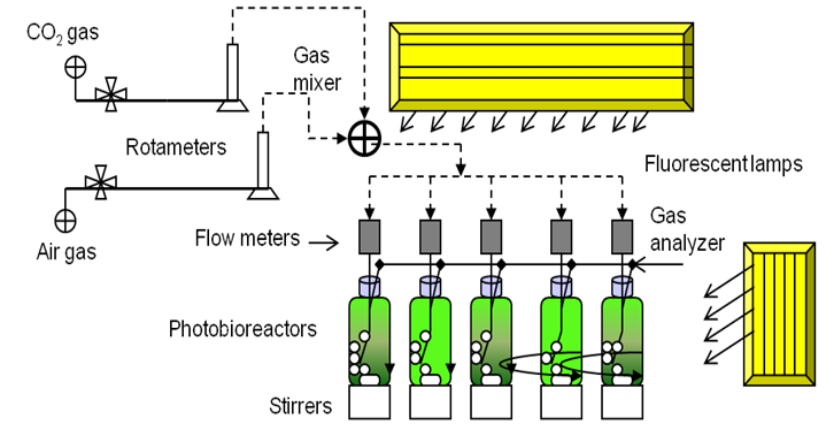
ABSTRACT: In this research, the physiological response of the microalgae *Spirulina platensis* to salinity stress (1 and 100 g L^{-1}) was investigated. *Spirulina platensis* and *S. platensis* (adapted to high salt concentration) were operated at laboratory scale in a semi-continuous photobioreactors. The responses examined were within 0.5 to 10% CO_2 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 CO_2 biofixation for *S. platensis* was 25.1 g_{CO2} m^{-3} h^{-1}, and the maximum specific growth (μ_{max}) achieved was 0.44 d^{-1} - 0.67 d^{-1} at 2.5% CO_2, 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% CO_2, 60 µmol s^{-1} m^{-2} and 28 °C. However, both microalgae exhibited experimental limiting growth factors, CO_2 10%, 40 °C and 200 µmol m^{-2} s^{-1}, conditions under which photosynthetic CO_2 biofixation may be inhibited and photoinhibition of photosynthesis may be enhanced by salinity. The efficiency of 2.5% CO_2 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.



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1. Introduction

Global warming is generally attributed to greenhouse gases (GHG) increase in the atmosphere, particularly carbon dioxide (CO_2), 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[1,2]. By 2100, 26 billion tons of CO_2 are estimated to be released into the atmosphere from anthropogenic sources[3]. 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[4-6]. 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: CO_2 addition, light, temperature, salinity, nutrient addition, inoculation size, stirring, pH, etc.[4,7]. The National Aeronautics and Space Administration (NASA) was the first institution to become interested in microalgae *Spriulina* for oxygen production, CO_2 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[4-6]. The cyanobacterium *Spirulina platensis* (*S. platensis*) is commercially produced as a nutrient source in health food, feed and pharmaceutical industries, especially in developing countries[8]. *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[7]. *S. platensis* are thermophilic algae with optimal growth temperature between 35 to 37 °C[8]. 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 *Chlamydomobas reinhardtii*[9]. 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 CO_2 into biomass in which carbon is biofixed and incorporated into carbohydrates, lipids and proteins[10,11]. The microalgae biomass produced can also be used for various applications, such as biofertilizer, soil conditioner, and biofuels production[8,12]. 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, were studied the impacts of light intensity, temperature, and inlet CO_2 concentration on the specific growth rate of *S. platensis* and *S. platensis* adapted to salinity-stress during biofixation of CO_2 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 Zarrouk's culture[10] 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 CO_2 mixed with air. Then, for further tests, the acclimated culture was transferred into each photobioreactor and the CO_2 concentration was increased gradually by bubbling CO_2 (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 Zarrouk's medium[10] (Tab. 2).

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Table 1. Composition of stock solutions used to prepare nutrient solution for *S. platensis**.

Stock Solution	Composition	Stock solution / g L^{-1}
Nutrient Solution	$NaHCO_3$	16.8
	K_2HPO_4	0.5
	$NaNO_3$	2.5
	K_2SO_4	1
	NaCl	1
	$MgSO_4.7H_2O$	0.2
	$CaCl^2{\cdot}2H_2O$	0.04
	$FeSO_4.7H_2O$	0.01
	EDTA	0.08
Trace metals mix A5	H_3BO_3	2.86
	$MnCl_2{\cdot}4H_2O$	1.81
	$ZnSO_4{\cdot}7H_2O$	0.222
	$NaMoO_4.2H_2O$	0.39
	$CuSO_4{\cdot}5H_2O$	0.079
	$Co(NO_3)^2{\cdot}6H_2O$	0.0494
Trace metals mix B6 modified	NH_4NO_3	0.23
	$K_2Cr_2(SO_4)_4{\cdot}24H_2O$	0.096
	$NiSO_4{\cdot}7H_2O$	0.0478
	$Na_2WO_4{\cdot}2H_2O$	0.0179
	$Ti(SO_4)_3$	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 plantensis* 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
	$NaNO_3$	0.51
	Na_2SO_4	1.23
	$MgCl_2$	0.033
Trace metal mix† Vitamin solution†	$EDTA.2H_2O$	4.36
	$CoCl_2.6H_2O$	0.010
	$FeCl_3.6H_2O$	3.15
	$MnCl_2.4H_2O$	0.018
	$CuSO_4.5H_2O$	0.010
	$Na_2MoO_4.2H_2O$	0.0063
	$ZnSO_4.7H_2O$	0.022
	$NaH_2PO_4.2H_2O$	4.0
	Thiamine-HCl	2.0
TV solution*	Biotin	0.005
	Vitamin B_{12}	0.005

Preparation: Trace Metal mix solution†: Dissolve EDTA first in hot water and then combine with the other ingredients to 1L. Vitamin solution†: After combining the ingredients adjust to pH 6 filter sterilized (Do not autoclave). TV solution*, prepare the ingredients in 100 mL. Filter sterilized (Do not autoclave). 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. 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.

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2.2 Photobioreactors and experiments

Figure 1 shows the graphical abstract scheme of the experimental set up at laboratory scale. The input CO_2 gas concentration was controlled by mixing CO_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_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 *S. platensis* and filled with 1800 mL nutrient solution prepared and mixed 24 h in advance to reach about 0.5 g L^{-1} concentration of suspension biomass. An experimental factorial design was proposed to study the three factors: CO_2 concentration, temperature and light intensity, ranging at five levels using the same experimental procedure, first for *S. platensis* culture followed by *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^{-1}, the photobioreactors operated at the same temperature and light intensity, but the cell biomass in each photobioreactor received different CO_2 concentrations (0.5, 2.5, 5.0, 7.5 and 10%) under continuous bubbling of CO_2 at a rate of 0.5 L m^{-1}, 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^{-2} s^{-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), 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_2 was provided by Airgas Specialty Gases New Jersey, 200 and 300 cubic feet volume cylinders of CO_2 concentrations of 0.5, 2.5, 5.0, 7.5 and 10% balance with air, the concentrations certified by the vendor.

Figure 1. Experimental setup of algal bioreactors at small scale.
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. 67.

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 (*S. platensis*) as absorbance, using a spectrophotometer (UV-VIS Shimadzu- 1700). The cell dry weight of *S. platensis* and optical density (OD_{680}) were established by linear regression (dry cell biomass, g L^{-1} = 0.477 × OD_{680} + 0.376; R^2 = 0.957; p = 0.01). Likewise, for *S. platensis* adapted and cell biomass g L^{-1}=2.35 × OD_{680} + 0.32; R^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^{-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_2 input/output of each bioreactor was measured with a Gas Chromatograph Shimadzu 17A

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with TDC detector, gas samples were taken in plastic bags. The CO_2 concentration was also monitored analyzing CO_2 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:

$$dy/dt = \mu X \quad (1)$$

Integrating and re-arranging Eq. 1, the growth coefficient also called specific growth rate (μ, d^{-1}) can be calculated using the Eq. 2:

$$\mu = \frac{\ln(X_2 / X_1)}{t_2 - t_1} \quad (2)$$

where X_1 and X_2 were the microalgae concentration (g L^{-1}) on days t_1 and t_2, respectively[13]. The biomass productivity rate, also called linear growth (p), is estimated according to Eq. 3:

$$p = \frac{X_2 - X_1}{t_2 - t_1} \quad (3)$$

where p (g L^{-1} d^{-1}). Since no organic carbon source is available in culture medium, the CO_2 biofixation rate can be indirectly calculated by the carbon content and biomass productivity rate, according to Eq. 4[13]

$$R_{CO2} = Cc \times p \times (M_{CO2}/M_C) \quad (4)$$

Therefore, the rate of CO_2 biofixation per initial inoculation mass of microalgae can be determined by Eq. 5

$$r_{CO2} = R_{CO2} / Xo \quad (5)$$

where R_{CO2} [g_{CO2} L^{-1} d^{-1}] is the biofixation rate and r_{CO2}[g_{CO2} g^{-1} dry cell].

The average cell carbon content Cc [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_{CO2} M_C^{-1} represents the molecular weight of CO_2 and C[4,14], respectively.

The efficiency of CO_2 removal was calculated as follows: 100 × $(CO_2)_{input}$.- $(CO_2)_{output}/(CO_2)_{input}$, where CO_{2input} is the initial and $(CO_2)_{output}$ the stream gas off the bioreactor[4].

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. Mönod model (Eq. 6).

$$\mu = \mu_{max} \frac{S}{Ks + S} \quad (6)$$

where *Ks* is the Mönod kinetic constant and *S*, CO_2 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 Mönod model can be modified by Andrews model (Eq. 7).

$$\mu = \mu_{max} \frac{S}{Ks + S + S^2 / K_I} \quad (7)$$

where *Ks* and *S* are the same meaning as Mönod, 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[15].

$$\mu = \frac{A' e^{(-E_1 / RT)}}{1 + k e^{(-E2 / RT)}} \quad (8)$$

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 CO_2 biofixation by *S. platensis* and *S. platensis* adapted to salinity

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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[16].

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% CO_2 concentration, 25 °C and µmol m^{-2} s^{-1} dry cell mass achieves 4.3 g L^{-1}, which slowly declines when cultivation conditions change by increasing CO_2 concentration (5%), temperature (30 °C) and light intensity (150 µmol m^{-2} 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 CO_2 concentration (10%), temperature (40 °C), and light intensity (200 µmol m^{-2} s^{-1}). Figure 3 shows the impact of salinity when *S. platensis* adapted to100 g L^{-1} 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% CO_2 concentration, 25 °C and 100 µmol m^{-2} s^{-1}, salinity-stress is usually accompanied by photoinhibition of photosynthesis[10]. 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.

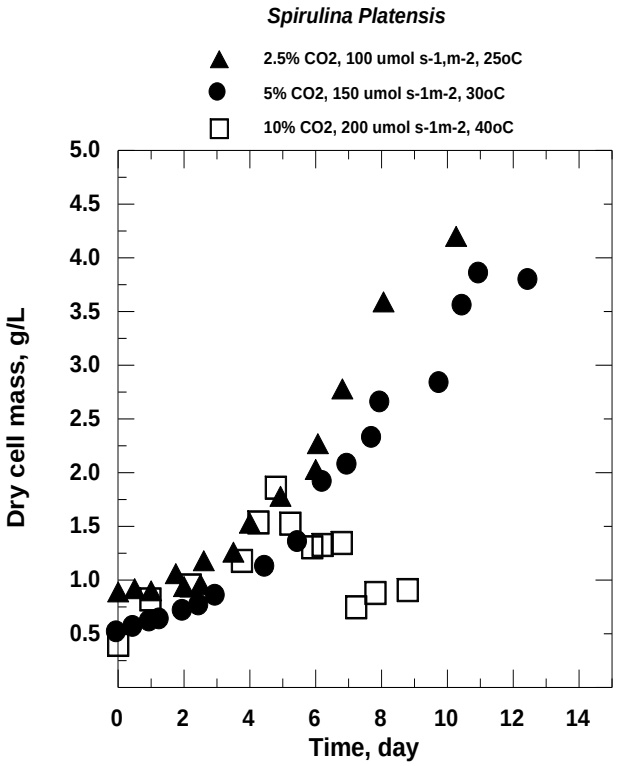

Figure 2. Dry cell mass of *S. platensis* grown under different CO_2 concentrations, temperatures and light intensities.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.

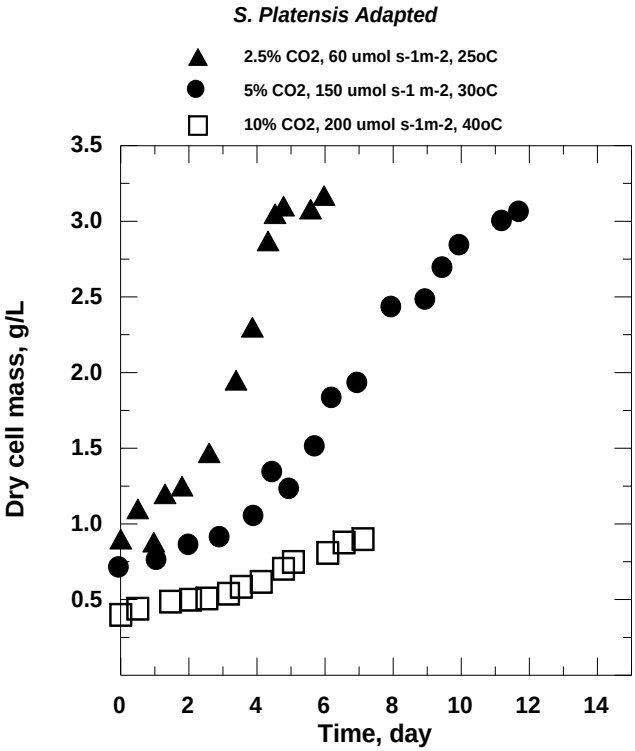

Figure 3. Salinity effect on dry cell mass of *S. platensis* (adapted) grown at 100 mg L^{-1} NaCl and under different CO_2 concentrations, temperatures and light intensities.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.

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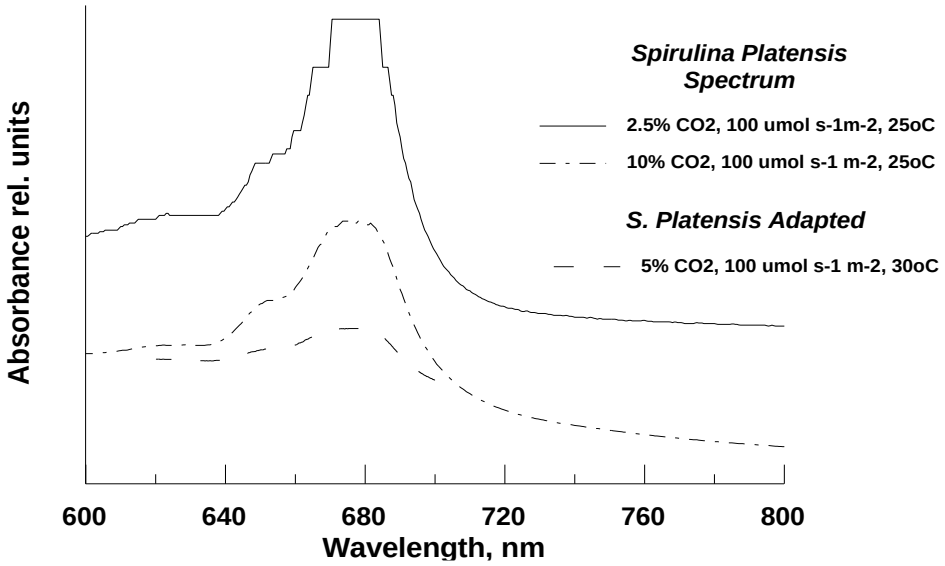

Figure 4. Impact of salinity on the UV-vis absorption of *S. platensis* and *S. platensis* adapted.Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 69.

3.2 Effect of Salinity on the specific growth rate (μ) and CO_2 biofixation rate (RCO_2)

Table 3 shows that during the 12 days cultivation period, the highest μ and R values of *S. platensis* (0.278 d^{-1}, 25.1 g_{CO2} 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 μ and R of *S. platensis* adapted decreases (0.15 d^{-1}, 13.9 g_{CO2} 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 μ and R values of *S. platensis* between 20 and 25 °C runs, but there were significant differences ($p < 0.0012$) between μ 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} μ and R values achieved higher values elevated light intensities e.g. 0.18 d^{-1}, 16.2 g_{CO2} m^{-3} h^{-1} than corresponding values at 100 and 60 μmol s^{-1} m^{-2}. But there was no significant difference between μ and R of *S. platensis* for 100 and 150 μmol s^{-1} m^{-2} runs.

Table 3. Specific growth rate (μ, d^{-1}); CO_2 biofixation rate (R, g_{CO2} m^{-3} h^{-1}) for *S. platensis* and *S. platensis* adapted growing in photobioreactors under different CO_2 concentrations, temperatures and light intensities.

Treatment	**μ / d^{-1}**		**R / g_{CO2} m^{-3} h^{-1}**	
ANOVA	*S. platensis*	*S. platensis Adapted*	*S. platensis*	*S. platensis Adapted*
Carbon dioxide, %, Pr(>F)	$2.90e^{-11}$***	$4.45e^{-2}$*	$3.00e^{-11}$***	$4.47e^{-2}$*
0.5	0.1149 c	*NA*	10.36 c,d	*NA*
2.5	0.2783 a	0.1540 b	25.08 a	13.88 b,c
5.0	0.1592 b,c	0.1105 b,c	14.34 b,c	10.38 c,d
7.5	0.0597 c,d	0.1045 b,c	5.38 d,e	9.42 d,e
10.0	0.0549 c,d	0.0501 d	4.94 e	4.53 e
Temperature, °C, Pr(>F)	$1.67e^{-5}$***	$8.68e^{-13}$***	$1.68e^{-5}$***	$8.64e^{-13}$***
12	0.0260 e	*NA*	2.32 e	*NA*
15	0.0477 d	0.0297 e	4.28 d,e	2.70 e

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20	0.1283 a,b	0.1070 b,c	11.56 b,c	9.70 c
25	0.1471 a,b	0.2022 a	13.26 b	18.23 a
				Continue…
30	0.1109 b	0.0978 c	9.99 b,c	8.83 c,d
40	0.0278 e	0.0493 d	2.51 e	4.45 d,e
Light, µmol $s^{-1} \times m^{-2}$, Pr (>F)	$2.49e^{-2}$*	$4.42e^{-3}$**	$2.40e^{-2}$*	$4.22e^{-3}$**
60	0.1129 b,c	0.1467 a,b	10.18 b,c	13.22 b
100	0.1413 a,b	0.1111 b,c	12.73 a,b	10.03 b,c
150	0.1800 a	0.1120 b,c	16.24 a	10.1 b,c
200	0.0734 d	0.0549 e	6.61 d	4.96 d,e

NA Not available
Signif. codes: 0 `***' 0.001 `**' 0.01 `*' 0.05 `.' 0.1 ` ' 1
Tukey multiple comparisons of means 95% family-wise confidence level

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 CO_2 concentration, temperature and light intensity in terms of experimental kinetic models (Monöd, 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 Mönod 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 CO_2 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% CO_2, 200 µmol m^{-2} s^{-1}, and 40 °C, suggesting these values as potential limiting factors of microalgae growth and photoinhibition[10]. 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% CO_2 and 25 °C to 0.22 d^{-1} for light intensities higher than 150 µmol m^{-2} $s^{-1}$and photoinhibition occurred when CO_2 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% CO_2 and 40 °C. These results agree with Zeng *et al.*[10], 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	
$\mu = 0.0183\frac{I}{72.3+I}$; (25 °C, 2.5%,)	(9)
$\mu = 0.0119\frac{I}{80.2+I}$; (25 °C, 5.0%)	(10)
$\mu = 0.012\frac{I}{92.3+I+I^2/74.0}$; (30 °C, 7.5%)	(11)

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$\mu = 0.009\frac{I}{98.3 + I + I^2/62.3}$; (40 °C, 10%)	(12)
Continue…	
Effect of CO_2 Concentration	
$\mu = 0.028\frac{CO_2}{1.76 + CO_2 + CO_2^2/3.43}$; (25 °C, 150 μmol s^{-1} m^{-2})	(13)
$\mu = 0.021\frac{CO_2}{1.37 + CO_2 + CO_2^2/1.19}$; (30 °C, 100 μmol s^{-1} m^{-2})	(14)
$\mu = 0.017\frac{CO_2}{1.77 + CO_2 + CO_2^2/3.16}$; (40 °C, 200 μmol s^{-1} m^{-2})	(15)
Effect of Temperature	
$\mu = \frac{0.029e^{(-0.19/RT)}}{1 + 288.3e^{(-1.55/RT)}}$; (2.5%, 60 μmol s^{-1} m^{-2})	(16)
$\mu = \frac{0.020e^{(-0.20/RT)}}{1 + 304.2e^{(-1.50/RT)}}$; (5%, 100 μmol s^{-1} m^{-2})	(17)
$\mu = \frac{0.014e^{(-0.20/RT)}}{1 + 310.2e^{(-1.60/RT)}}$; (10%, 200 μmol s^{-1} m^{-2})	(18)

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

Effect of light intensity	
$\mu = 0.013\frac{I}{94.3 + I + I^2/134.2}$; (25 °C, 5%)	(19)
$\mu = 0.009\frac{I}{100.83 + I + I^2/111.5}$; (40 °C, 10%)	(20)
Effect of CO_2 Concentration	
$\mu = 0.024\frac{CO_2}{2.05 + CO_2 + CO_2^2/2.55}$; (25 °C, 60 μmol s^{-1} m^{-2})	(21)
$\mu = 0.010\frac{CO_2}{2.08 + CO_2 + CO_2^2/2.27}$; (40 °C, 200 μmol s^{-1} m^{-2})	(22)
Effect of Temperature	
$\mu = \frac{0.028e^{(-0.19/RT)}}{1 + 327e^{(-1.59/RT)}}$; (2.5%, 60 μmol s^{-1} m^{-2})	(23)

The effect of CO_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 μ_{max} value estimated for 2.5% CO_2, 25 °C and 150 μmol m^{-2} s^{-1}, declined from 0.67 d^{-1} to 0.41 d^{-1} when CO_2 concentration increased in the range of 5 to 10% and the temperature and light intensity rose to 40 °C and 200 μmol m^{-2} s^{-1}, respectively, suggesting that CO_2 is a limiting factor that inhibited *S. platensis* growth particularly at 200 μmol m^{-2} $s^{-1}$and 40 °C. An optimal μ_{max} value for *S. platensis* adapted to salinity stress of 0.58 d^{-1} determined under the following environmental parameters 2.5 % CO_2 (25 °C and 60 μmol m^{-2} s^{-1}) declined to 0.24 d^{-1} when CO_2 concentration rose more than 5% (40 °C and 200 μmol m^{-2} s^{-1}), suggesting that CO_2 is a limiting factor of CO_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.*

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platensis occurred around 25 °C, 150 µmol s^{-1} m^{-2} and 2.5% CO_2 but it declined slowly as temperature increased along with CO_2 concentration and light intensity to a minimum µ values 10% CO_2 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 CO_2 concentration and light intensity in spite the fact that *S. platensis* was characterized as thermophilic microalgae. The optimum temperature of *S. platensis* adapted occurred at around 25 °C, for 60 µmol m^{-2} s^{-1} and 2.5% CO_2 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 CO_2 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 CO_2 concentration and light intensity in combination with high salt concentration affect CO_2 biofixation rate.

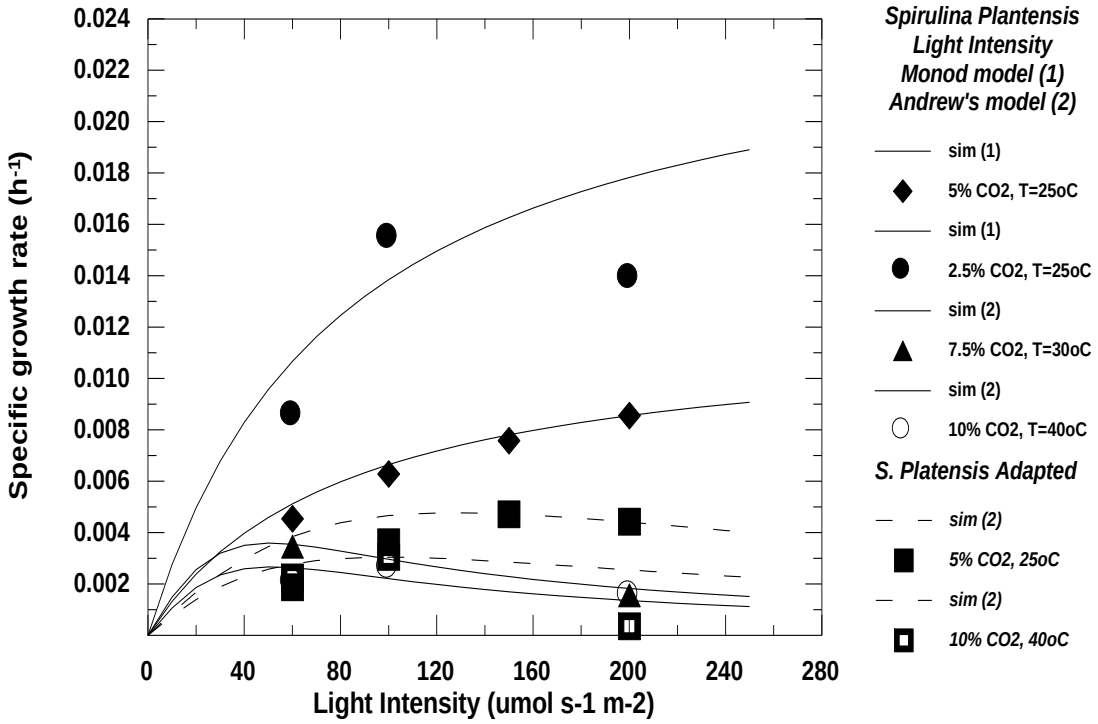

Figure 5. Effect of light intensity on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different CO_2 concentrations and temperatures.
Mönod model fit (solid line), Andrew's model fit (dash line). Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 71.

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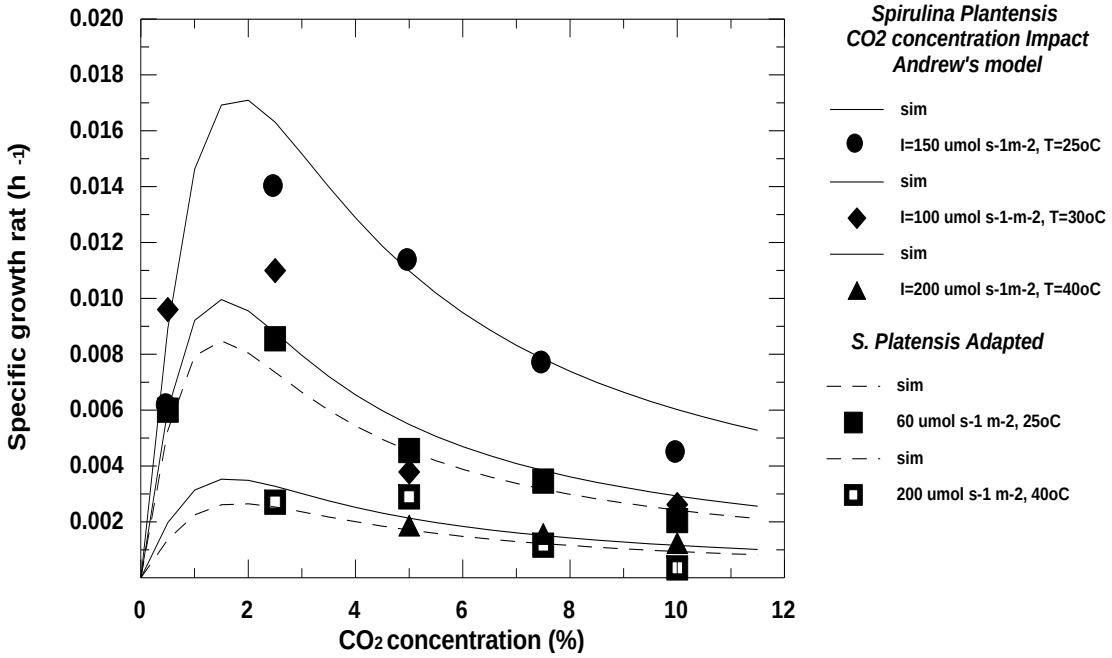

Figure 6. Effect of CO_2 concentration on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different light intensities and temperatures.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 71.

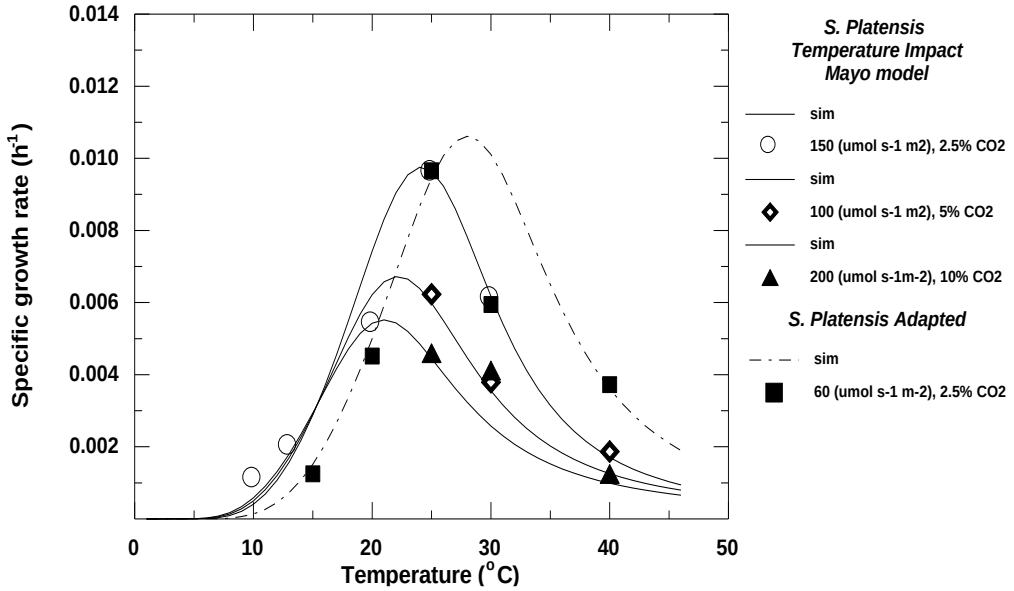

Figure 7. Effect of temperature on the kinetics of *S. platensis* and *S. platensis* adapted to salinity stress at different light intensities and CO_2 concentrations.
Adapted from "Carbon dioxide sequestration by *Spirulina plantensis* in photo-bioreactors", by J. C. Ramirez-Perez and H. W. Janes, 2009, *Habitation*, 12(1), p. 72.

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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% CO_2 and air in a closed space such as a space station or a submarine < 1% CO_2[2]. It has been reported that microalgae present one of the few technologies for the capture and utilization of CO_2[17]. The results indicate that both *S. platensis* and *S. platensis* adapted to high salt concentration can be used for CO_2 biofixation of flue gas emitted from stationary sources. In fact, maximum daily evaluation of the efficiency CO_2 removal by *S. platensis* during experiments up to ten days, achieved 92% at 0.5% CO_2, 99% at 2.5% CO_2, 84% at 5% CO_2, 89% at 7.5% and 88% at 10% CO_2, respectively. Corresponding values for *S. platensis* adapted being 96% at 2.5% CO_2, 90% at 5% CO_2, 78% at 7.5% CO_2 and 73% at 10% CO_2, respectively (See Appendix).

Small pH changes of *S. platensis* cell suspension observed during experiments from an initial pH (8.9) in the presence of $CO_2 \leq 2.5\%$ 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 $CO_2 > 5\%$ and temperatures > 25 °C the pH increased to ~~e~~ 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 CO_2 because both microalgae were previously acclimatized to CO_2. *S. platensis* and *S. platensis* adapted were able to metabolize CO_2 because both microalgae were previously acclimatized to CO_2. Therefore, neither was formation of carbonic acid nor decline in pH.

In this study, the maximum mean CO_2 biofixation rate recorded for *S. platensis* was 25.1 g_{CO2} m^{-3} h^{-1} cultivated at 2.5% CO_2, 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% CO_2, 60 µmol m^{-2} s^{-1} and 25 °C.

In a three-stage serial tubular photobioreactor were cultivated *Scenedesmus obiquus* and *Spirulina* sp. at 30 °C. It was found that, for *Spirulina* sp. the μ_{max} was 0.44 d^{-1}, 9.2 g m^{-3} h^{-1} with 6% CO_2, maximum daily CO_2 removal efficiency was 53.3% for 6% CO_2 and 45.6% for 12% CO_2, the corresponding values for *S. obliquus* being 28.1% for 6% CO_2 and 13.6% for 12% CO_2 runs[14]. Yun *et al.*[18] estimated 26.0 g_{CO2} m^{-3} h^{-1} biofixation rate value when *Chlorella vulgaris* was cultivated after adaptation in 5% CO_2 in wastewater supplemented with nutrients and without pH control at 15% CO_2, this value is comparable with the findings of this research and suggests that elevated CO_2 concentration may exert effects on the photoinhibitory behavior of the microalgae to different extents according to species[19]. The efficiency of CO_2 biofixation by these microalgae strains may have been due to its physiological conditions, such as potential of cell growth and ability of CO_2 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[10]. 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 CO_2 concentrations (7.5 and 10%) inhibit microalgae grow, show low photosynthetic activity and consequently photoinhibition[20]. 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[10].

4. Conclusions

This study shows the potential CO_2 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 CO_2 biofixation was achieved by *S. platensis* as indicated by its kinetic parameters and efficiency CO_2 removal, when compared to *S. platensis* adapted. The impacts of light intensity, CO_2 concentration and temperature on the specific growth rate followed the Mönod, Andrews and Mayo kinetic models. For *S. platensis* the highest dry cell mass concentration was 4.1 g L^{-1}, cultivated at 2.5% CO_2, 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 (μ_{max}) 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% CO_2, 25 °C and 60 µmol s^{-1} m^{-2}, 18.2 g_{CO2} m^{-3} h^{-1}, and μ_{max}, 0.32 d^{-1} – 0.58 d^{-1}. This suggests that the impact

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of salinity in combination with environmental grow factors such as elevated CO_2 concentration, light intensity and temperature may exert effects on the photoinhibitory behavior of *S. platensis* adapted, provoking a CO_2 biofixation rate depletion. Therefore, the photosynthetic biofixation of CO_2 *S. platensis* and *S. platensis* adapted showed optimum values at 2.5% CO_2 and 25 °C, and more sensitivity to light intensity than *S. platensis*, suggesting that salinity enhanced photoinhibition of photosynthesis. The efficiency of CO_2 removal by *S. platensis* achieved 99%, whereas for *S. platensis* adapted 96%, both at 2.5% CO_2 concentration and small pH changes exhibited both microalgae cell suspension.

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Appendix

Table 1A. *Spirulina platensis* efficiency of CO_2 removal.

R^{2*}	$[CO_2]_{in}$ (%)	$[CO_2]_{out}$ (%)	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 CO_2 removal.

R^{2*}	$[CO_2]_{in}$ (%)	$[CO_2]_{out}$ (%)	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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