World Journal of Bioscience and Biotechnology 2025, 1 (1):49-63

Journal homepage: https://socibiotech.com/world-j-biosci-biotechnol

ORIGINAL RESEARCH ISSN: 3061-8185

# Assessing the impact of $CO_2$ concentration on growth, biomass productivity, and $CO_2$ fixation rate in a mixed *Scenedesmus* culture

# Evaluación del impacto de la concentración de $CO_2$ en el crecimiento, la productividad de biomasa y la tasa de fijación de $CO_2$ en un cultivo mixto de *Scenedesmus*

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## ABSTRACT

The $CO_2$ accumulation in the atmosphere requires sustainable biotechnological mitigation strategies, such as biofixation by microalgae. This study evaluated the effect of $CO_2$ concentration (0% - 20% v/v) on the growth parameters, biomass productivity, and $CO_2$ fixation efficiency of a *Scenedesmus* consortium (*S. quadricauda, S. obliquus,* and *S. dimorphus*). The cultures were carried out in a bubble column photobioreactor, and growth parameters were determined by fitting the experimental data to the Gompertz model ($R^2 \geq 0.988$). The results showed that the highest stationary phase biomass concentration ($X_\infty$) was achieved at 15% $CO_2$ (0.650 g $L^{-1}$), whereas the highest specific growth rate ($\mu$ = 0.548 $d^{-1}$) was observed in the control without added $CO_2$. The maximum biomass productivity (0.054 g $L^{-1}$ $d^{-1}$) and $CO_2$ fixation rate (0.102 g $L^{-1}$ $d^{-1}$) occurred within the range of 10%–15% $CO_2$. The carbon balance indicated that the overall $CO_2$ capture efficiency was low, reaching a maximum of 0.131% at 10% $CO_2$. The results indicate that the principal limitation is not biological

tolerance, but mass transfer. Therefore, optimizing the system configuration is essential to develop efficient phototrophic technologies for biological $CO_2$ capture.

**Keywords:** $CO_2$ biofixation, Gompertz model, mass transfer limitation, microalgae consortium, *Scenedesmus*

## RESUMEN

La acumulación de $CO_2$ en la atmósfera requiere estrategias biotecnológicas sostenibles de mitigación, como la biofijación por microalgas. Este estudio evaluó el efecto de la concentración de $CO_2$ (0% - 20% v/v) sobre los parámetros de crecimiento, la productividad de biomasa y la eficiencia de fijación de $CO_2$ de un consorcio de *Scenedesmus* (*S. quadricauda*, *S. obliquus* y *S. dimorphus*). Los cultivos se realizaron en un fotobiorreactor de columna de burbujeo y los parámetros de crecimiento se determinaron ajustando los datos experimentales al modelo de Gompertz ($R^2 \geq 0.988$). Los resultados mostraron que la concentración de biomasa en fase estacionaria ($X_\infty$) más alta se alcanzó con 15% de $CO_2$ (0.650 g $L^{-1}$), mientras que la tasa máxima de crecimiento específico (μ = 0.548 $d^{-1}$) se observó en el control sin $CO_2$ añadido. La productividad máxima de biomasa (0.054 g $L^{-1}$ $d^{-1}$) y la tasa de fijación de $CO_2$ (0.102 g $L^{-1}$ $d^{-1}$) ocurrieron en el rango de 10%–15% de $CO_2$. El balance de carbono indicó que la eficiencia global de captura de $CO_2$ fue baja, alcanzando un máximo de solo 0.131% a 10% de $CO_2$. Los resultados indican que la limitación principal no es la tolerancia biológica, sino la transferencia de masa. Por lo tanto, optimizar la configuración del sistema es esencial para desarrollar tecnologías fototróficas eficientes para la captura biológica de $CO_2$.

***Palabras clave*:** Biofijación de $CO_2$, consorcio de microalgas, limitación de transferencia de masa, modelo Gompertz, *Scenedesmus*.

Received: 6 May 2025 / Received in revised form: 15 June 2025 / Accepted: 25 June 2025 / Published online: 30 June 2025.
https://doi.org/10.29267/wjbb.2025.1.1.49-63

## 1. INTRODUCTION

According to the Scripps Institution of Oceanography, the atmosphere has reached alarming $CO_2$ levels (~420 ppm) due to anthropogenic activities that intensify global warming and the greenhouse effect. As a result, various physical, chemical, and biological $CO_2$ fixation methods have been developed (Wang *et al.,* 2008). Among these, biological methods stand out, with microalgae considered important agents in $CO_2$ fixation, as they are estimated to fix approximately 50% of the planet's total $CO_2$ (Jacob-Lopes *et al.,* 2008; Mora Salguero *et al.,* 2018).

Among the main $CO_2$-fixing microalgal species are *Spirulina platensis* (de Morais & Costa, 2007), *Chlamydomonas reinhardtii* (Mora Salguero *et al.,* 2018), and *Scenedesmus* spp. (de Morais & Costa, 2007; Wang *et al.,* 2008; Tebbani *et al.,* 2014). Species of *Scenedesmus*, supports $CO_2$ concentrations of up to 80%, positioning it as one of the most promising biofixers. These species exhibit high biomass productivity, elevated lipid content, and broad tolerance to high nitrogen and phosphate concentrations, making them suitable for biodiesel production and wastewater treatment (Ho *et al.,* 2010; Boonma *et al.,* 2015; Assunção *et al.,* 2017; Tapia-López *et al.,* 2025).

Photobioreactors (PBRs) are the most widely used systems for cultivating microalgae, primarily due to their ability to control key parameters and optimize growth conditions for specific applications. For instance, in biofuel production, regulating temperature, pH, light intensity, and nitrogen concentration are essential, as these factors significantly influence lipid accumulation in microalgae (Robles-Heredia *et al.,* 2016; Navarro-Peraza *et al.,* 2017; Méndez *et al.,* 2020). Among PBR configurations, airlift and bubble column systems are the most employed due to their efficient mixing, reduced shear stress on cells, and have a flexible design, which facilitates effective biomass recovery without compromising productivity (Robles-Heredia *et al.,* 2016).

A critical factor in PBR operation is the mass transfer and $CO_2$ equilibrium in the aqueous medium, as this directly affects photosynthesis and biomass production. When $CO_2$ is introduced into water, it undergoes a series of equilibrium reactions that determine the availability of inorganic carbon for microalgae (Equation 1):

$$CO_{2(g)} + H_2O_{(l)} \leftrightarrow H_2CO_{3(aq)} \leftrightarrow H^+_{(aq)} + HCO^-_{3(aq)} \quad \text{(Eq. 1)}$$

This equilibrium determines the partitioning of dissolved inorganic carbon and is influenced by operational and environmental variables such as pH, temperature, $CO_2$ concentration, mixing intensity, and light availability. All these factors affect the speciation of dissolved inorganic carbon and its assimilation by microalgae during cultivation (Ji *et al.,* 2017; Fu *et al.,* 2019; Pruvost *et al.,* 2022).

While bioprocesses involving microalgae are constantly evolving, culture systems at the industrial level still face limitations, such as high costs and low productivity. These challenges derive from an incomplete understanding of carbon assimilation pathways in photosynthesis and

of the metabolic pathways involved in the biosynthesis of valuable products (Pignolet *et al.,* 2013; Sivakaminathan *et al.,* 2018; Sun *et al.,* 2018).

Therefore, the aim of this study was to evaluate the effect of $CO_2$ concentrations on a mixed *Scenedesmus* culture within a bubble column photobioreactor (BC-PBR). The evaluation focused on quantifying key performance parameters, specifically biomass productivity, $CO_2$ fixation rate, and the mathematical characterization of the culture's growth responses using kinetic models.

## 2. MATERIALS AND METHODS

### 2.1. Inoculum characterization and maintenance

A 50 mL water sample was obtained from a domestic freshwater aquarium (Hermosillo, Sonora, México) and used as the initial inoculum to establish a mother culture. The culture was maintained and adapted in a 1 L glass bubble column photobioreactor (BC-PBR) containing 750 mL of Guillard's F/2 medium (Guillard, 1975). The maintenance conditions were 20°C (1 atm), with continuous aeration and constant illumination provided by three 4.0 W LED lamps, operating 24 h $d^{-1}$.

Once the culture reached stability, morphological identification was performed using a Zeiss PrimoStar optical microscope equipped with an AxioCam ERc5c and ZEISS software. The analysis revealed that the culture was not a pure strain, but a stable consortium composed of three *Scenedesmus* species: *S. quadricauda, S. obliquus,* and *S. dimorphus.* All subsequent experiments were conducted using this characterized mixed culture as the inoculum.

Different concentrations of $CO_2$-enriched air (0%–20% v/v) were supplied to evaluate the growth performance, biomass productivity, and $CO_2$ fixation rate of the culture. Unless otherwise stated, growth, productivity, and $CO_2$ fixation metrics refer to the mixed *Scenedesmus* culture at the community level. For each reactor, samples were collected in triplicate at each time point, and results were reported as the mean ± standard deviation (SD) of technical replicates.

### 2.2. Experimental setup and operating conditions

The experiments were conducted in 4.5 L vertical glass bubble column photobioreactor (BC-PBR) with a cylindrical geometry. The reactor was equipped with an acrylic cover fitted with ports for aeration and sampling. It measured 32.5 cm in height and 14 cm in internal diameter. Each reactor was inoculated with 5% (v/v) of the adapted *Scenedesmus* mixed culture described in 2.1.

Five experimental groups were established based on the $CO_2$ concentration supplied: a control (ambient air, ~0.04% $CO_2$) and four enriched treatments (5, 10, 15, and 20% v/v). The gas mixtures were prepared by mixing ambient air and high-purity $CO_2$ using a mass flow controller

(SHELDON) to ensure accurate and stable dosing, maintaining a total gas flow rate of 1.0 L $min^{-1}$.

All cultures were operated under continuous illumination (24 h $day^{-1}$), provided by three 4.0 W white LED lamps positioned externally and equidistantly around the reactor. The temperature was maintained at 20 °C, consistent with the conditions used during the adaptation phase.

### 2.3. Sampling, biomass quantification, and statistical analysis

Culture samples (15 mL) were collected at 8-hour intervals to quantify biomass. Sampling at each time point was performed in triplicate (technical replicates) to ensure measurement precision. Samples were vacuum filtered through pre-dried and pre-weighed Millipore membranes (0.45 µm pore size, 47 mm diameter). The retained biomass was dried in a convection oven at 80 °C for 6 hours, and dry weight was determined gravimetrically.

Descriptive statistics were performed for the growth parameters. The results are expressed as means ± standard deviation (SD).

### 2.4. Determination of growth parameters

Kinetic parameters were determined using the modified Gompertz model (Eq. 2), with experimental data fitted by non-linear least squares regression.

$$X = X_0 + X_\infty e^{-e^{-\mu(t-t_{lag})}} \quad \text{(Eq. 2)}$$

where: µ, expresses the specific growth rate ($d^{-1}$); $X_0$, dry weight biomass concentration in initial phase (g $L^{-1}$); $X_\infty$, is the maximum dry weight biomass concentration in the stationary phase (g $L^{-1}$); $t$, time (d) and $t_{lag}$, lag phase or adaptation time (d).

### 2.5. Analysis of $CO_2$

Biomass productivity ($P_x$) was calculated during the exponential phase using Equation 3:

$$P_x = \frac{\Delta X}{\Delta t} \quad \text{(Eq. 3)}$$

where: $\Delta X$, is the change in biomass concentration (g $L^{-1}$) over culture period. $\Delta t$ (d).

The $CO_2$ fixation rate ($R_{CO_2}$) was then calculated from biomass productivity ($P_x$) according to Basu et al. (2015), using Equation 4:

$$R_{CO_2} = 1.88 \cdot P_x \quad \text{(Eq. 4)}$$

The factor 1.88 (g $CO_2$ $g^{-1}$ biomass) is a stoichiometric coefficient derived from the semi-empirical molecular formula of microalgal biomass, $CH_{1.83}O_{0.48}N_{0.11}P_{0.01}$ proposed by Chisti (2007).

### 2.6. Carbon balance and efficiency

The total mass of $CO_2$ fixed into biomass ($m_{CO_2,fix}$) over the entire cultivation period was calculated using Eq. 5:

$$m_{CO_2,fix} = 1.88 \cdot \Delta X \cdot V \quad \text{(Eq. 5)}$$

where: $\Delta X$, is the change in dry biomass concentration (g $L^{-1}$) from the first to the last sampling point, V is the working volume (L), and 1.88 is the stoichiometric factor (g $CO_2$ $g^{-1}$ biomass) (Chisti, 2007).

The total mass of $CO_2$ supplied ($m_{CO_2,sup}$) was calculated based on the total gas flow rate, $CO_2$ fraction, molar properties, and cultivation time, according to Eq. 6:

$$m_{CO_2,sup} = Q \cdot Y_{CO_2} \left(\frac{M_{CO_2}}{V_m}\right) \cdot t \quad \text{(Eq. 6)}$$

where: $Q$, is the total gas rate (1.0 L $min^{-1}$); $Y_{CO_2}$, is the volumetric fraction of $CO_2$ in the inlet gas (0.05, 0.10, 0.15, 0.20); $M_{CO_2}$, is the molar mass of $CO_2$ (44.01 g $mol^{-1}$); $V_m$, is the molar volume of an ideal gas at operating conditions (20°C, 1 atm; approximately 24.06 l $mol^{-1}$); and $t$, is the total cultivation time (minutes). Note: the ambient $CO_2$ fraction (0.0004) was included in the $Y_{CO_2}$ for the calculation.

The overall $CO_2$ capture efficiency ($\eta_{fix}$) was calculated as the ratio of fixed to supplied $CO_2$, using Eq. 7:

$$\eta_{fix}(\%) = \frac{m_{CO_2,fix}}{m_{CO_2,sup}} \cdot 100 \quad \text{(Eq. 7)}$$

## 3. RESULTS

### 3.1 Identification of the mixed culture

It was observed an evident change in culture pigmentation and the formation of biofilm on the inner walls in the photobioreactor (PBR) after four days of inoculation, indicating active biomass accumulation. Microscopic analysis confirmed the presence of microalgae (Fig. 1), revealing three distinct morphotypes. Two of these exhibited ellipsoidal unicellular forms measuring 15 to 40 µm in length, each containing a central pyrenoid.

The first morphology corresponded to a coenobium of four cells, each bearing outward spine-like projections (Fig. 1A). The second morphology consisted of unicellular forms with a central circular pyrenoid, and chloroplasts distributed toward the cell poles (Fig. 1B). The third morphology comprised curved unicellular cells (~30 µm) with a partially straight ventral margin, a central pyrenoid, and chloroplasts located at both ends (Fig. 1C).

These structural characteristics were consistent with members of the Scenedesmaceae family, particularly the genus *Scenedesmus*, which includes species such as *S. quadricauda, S. obliquus*, and *S. dimorphus*, as reported in AlgaeBase.org (Gour *et al.,* 2016).

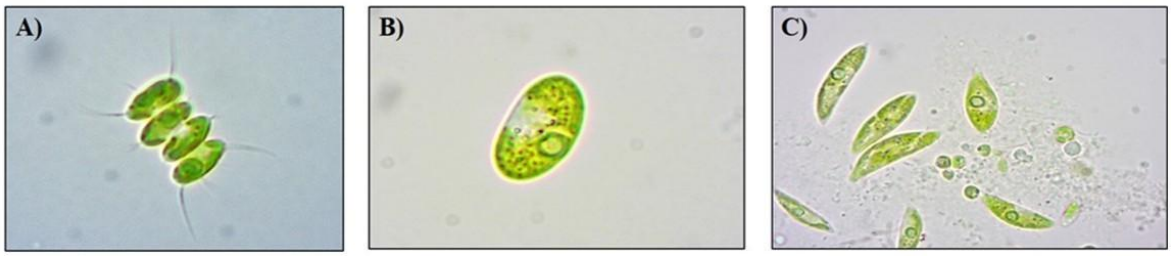


**Fig. 1.** Original micrographs of the mixed consortium identified in the simple. The three dominant morphologies were identified as: A) *Scenedesmus quadricauda* (coenobium), B) *Scenedesmus obliquus* (unicellular), and C) *Scenedesmus dimorphus* (curved unicellular).

### 3.2. Growth curve analysis and Gompertz model parameters

The growth behavior of the mixed *Scenedesmus* culture was monitored under 0, 5, 10, 15 and 20% of $CO_2$ concentrations, and the experimental data were successfully fitted to the modified Gompertz model (Fig. 2). The model provided an excellent description of the sigmoidal growth curves for all treatments, achieving a fit of $R^2 \geq 0.988$. The key kinetic parameters obtained from this analysis are summarized in Table 1.

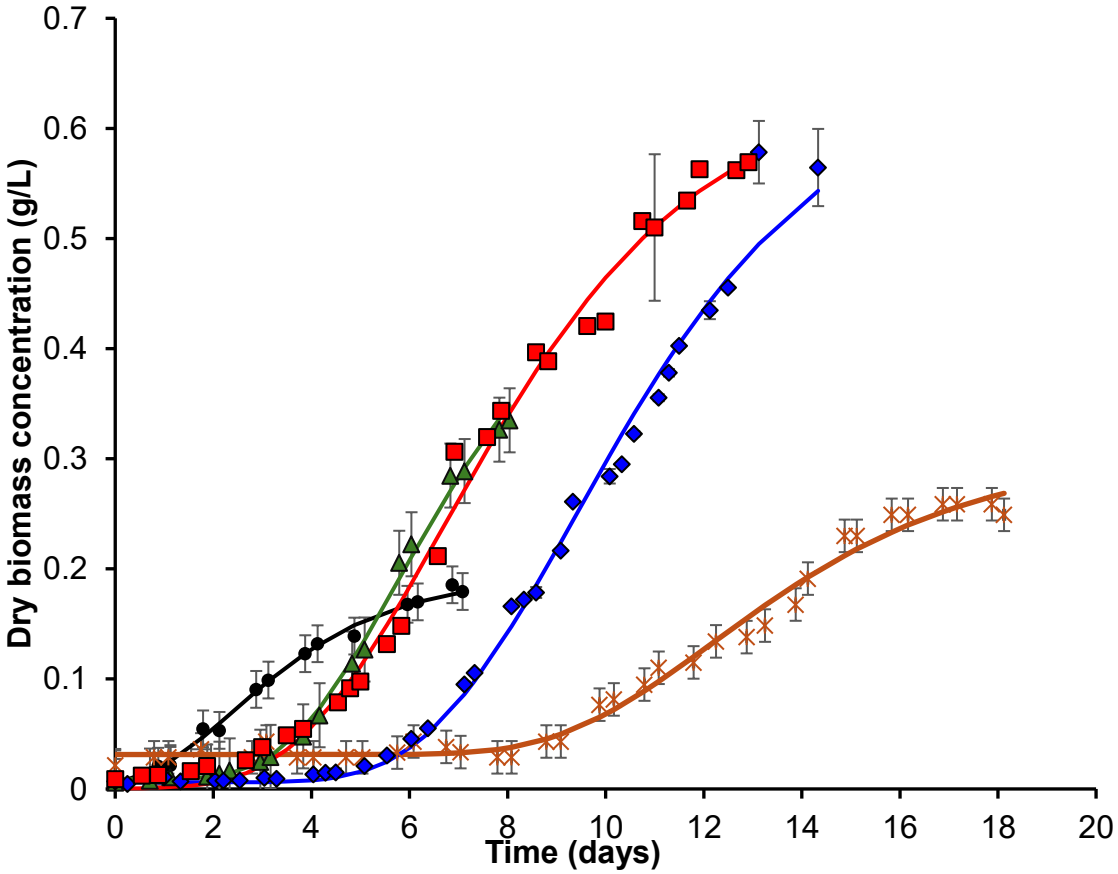


**Fig. 2**. Growth curves of the mixed *Scenedesmus* culture at different $CO_2$ concentrations: (●) air + $CO_2$ 0%, (x) air + $CO_2$ 5%, (▲) air + $CO_2$ 10%, (◆) air + $CO_2$ 15% and (■) air + $CO_2$ 20%. (–) Solid lines represent fits to the experimental data (symbols) by the Gompertz model.

**Table 1.** Growth parameters for the mixed *Scenedesmus* culture derived from the modified Gompertz model.

| Parameter | $CO_2$ concentration in air (v/v%) | | | | |
|---|---|---|---|---|---|
| | **0** | **5** | **10** | **15** | **20** |
| $\mu$ ($d^{-1}$) | 0.548 | 0.321 | 0.442 | 0.333 | 0.334 |
| $X_\infty$ (g $L^{-1}$) | 0.193 | 0.275 | 0.489 | 0.650 | 0.647 |
| $t_{lag}$ (d) | 2.418 | 12.178 | 5.764 | 9.348 | 6.699 |
| $t_d$ (d) | 1.264 | 1.567 | 1.567 | 2.084 | 2.077 |
| $R^2$ | 0.994 | 0.988 | 0.996 | 0.990 | 0.993 |

The model parameters revealed that $CO_2$ concentrations had a strong but complex influence on the growth dynamics. The theoretical maximum biomass concentrations ($X_\infty$), or carrying capacity, was directly and positively affected by the $CO_2$ availability. $X_\infty$ increased progressively as the $CO_2$ concentration closed from 0% (0.193 g $L^{-1}$) to its peak at 15% $CO_2$ (0.650 g $L^{-1}$), remaining nearly constant at 20% $CO_2$ (0.647 g $L^{-1}$).

Conversely, the maximum specific growth rate (μ) showed the opposite trend. The highest μ value was identified in carbon-limited control (0% $CO_2$) at 0.548 $d^{-1}$. All $CO_2$-enriched cultures exhibited lower specific growth rates, with the 5% $CO_2$ treatment showing the slowest modeled rate (0.321 $d^{-1}$).

These adaptation restrictions were also reflected in the lag phase ($t_{lag}$), which was shortest in control (2.418 d) but markedly prolonged at 5% $CO_2$ (12.178 d). The longest doubling time ($t_d$) calculated as 1/μ was for the 15% and 20% $CO_2$ treatments (approximately 2.08 d), which also achieved the highest $X_\infty$values.

Therefore, the model parameters (Table 1) indicated that while $CO_2$ supplementation was critical for maximizing final biomass accumulation ($X_\infty$), this occurred at the expense of a reduced specific growth rate (μ) and extended doubling time ($t_d$) compared with the control.

### 3.3. $CO_2$ fixation rate and productivity

The effect of $CO_2$ concentration on the culture's performance was evaluated, revealing distinct trends across growth rates, final biomass accumulation, and $CO_2$ capture efficiency (Table 2).

**Table 2.** Biomass concentration, productivity and $CO_2$ fixation rate of the mixed *Scenedesmus* culture at different $CO_2$ concentrations. Values represent the mean ± SD of technical replicates.

| % $CO_2$ | Biomass concentration (g $L^{-1}$) | SD | Biomass productivity (g $L^{-1}$ $d^{-1}$) | $CO_2$ fixation rate (g $L^{-1}$ $d^{-1}$) |
|---|---|---|---|---|
| 0 | 0.185 | 0.00105 | 0.027 | 0.052 |
| 5 | 0.259 | 0.02195 | 0.022 | 0.041 |
| 10 | 0.335 | 0.00000 | 0.054 | 0.102 |
| 15 | 0.578 | 0.03506 | 0.054 | 0.101 |
| 20 | 0.563 | 0.00191 | 0.050 | 0.093 |

The analysis of growth rates demonstrated a clear optimum concentration. The highest $CO_2$ fixation rate (0.102 g $L^{-1}$ $d^{-1}$) was detected at the 10% $CO_2$ treatment. This concentration, along with the 15% $CO_2$ treatment, also yielded the highest biomass productivity (0.054 g $L^{-1}$ $d^{-1}$). At 20% $CO_2$, both productivity (0.050 g $L^{-1}$ $d^{-1}$), and fixation rate (0.093 g $L^{-1}$ $d^{-1}$) began to decrease, indicating the beginning of the inhibition or saturation effect in the growth culture.

In contrast to the rate parameters, the final biomass concentration continued to increase at higher $CO_2$ levels. The maximum final biomass (0.578 g $L^{-1}$) was achieved at 15% $CO_2$, a value significantly higher than that obtained at 10% $CO_2$ (0.335 g $L^{-1}$).

A key observation was the poor performance at 5% $CO_2$. This treatment has the lowest productivity (0.022 g $L^{-1}$ $d^{-1}$), even less than the ambient air control (0.027 g $L^{-1}$ $d^{-1}$).

The carbon balance further contextualized these findings. The highest overall $CO_2$ capture efficiency (0.131%) was observed at 10% $CO_2$. At higher concentrations (15% and 20%), capture efficiency declined markedly. This indicates that although high $CO_2$ levels (15 – 20%) promoted greater final biomass accumulation, the process became less efficient because most of the supplied $CO_2$ was not assimilated into biomass.

## 4. DISCUSSION

### 4.1. Growth characteristics of the mixed consortium

The maximum biomass productivity achieved in this study, 0.054 g $L^{-1}$ $d^{-1}$ at 10% $CO_2$ represents a fundamental result. This value aligns with those reported in the literature for laboratory scale microalgal cultures under comparable conditions (de Morais & Costa, 2007; Zhang *et al.,* 2022; Pandey *et al.,* 2023), although it differs from the benchmarks typically observed in high-intensity or large-scale photobioreactor systems (0.2–0.4 g $L^{-1}$ $d^{-1}$) (Tang *et al.,* 2011; Xu *et al.,* 2023). This distinction suggests that while the culture demonstrated strong biological performance, the overall bioprocess efficiency was due to the operational configuration of the bubble column reactor. Similar limitations have been reported in closed systems with restricted gas–liquid mass transfer (Ho *et al.,* 2011; Singh & Sharma, 2012).

The Gompertz model parameters (Table 1) provided key insights into the biological response of the consortium to $CO_2$ enrichment. A clear physiological trade-off was observed: the carbon-limited control (0% $CO_2$) achieved the highest specific growth rate (μ = 0.548 $d^{-1}$), but the lowest stationary-phase biomass concentration ($X_\infty$ = 0.193 g $L^{-1}$). Conversely, higher $CO_2$ concentrations (15%) were required to reach maximum biomass accumulation ($X_\infty$= 0.650 g $L^{-1}$), although this occurred at the expense of a slower specific growth rate. This pattern illustrates a physiological trade-off, where an improvement in one performance trait (biomass accumulation) occurs at the expense of another (growth rate). Similar compensatory effects have been documented in microalgal cultures under carbon-rich or high-$CO_2$ stress conditions (Hu *et al.,* 2008; Zhang *et al.,* 2022).

A distinctive aspect of this study is the use of a stable mixed consortium composed of *S. quadricauda*, *S. obliquus*, and *S. dimorphus*. While monocultures are common in laboratory experiments, mixed consortia are increasingly recognized for their ecological robustness and higher stress tolerance (Lindehoff *et al.,* 2024). Several studies have shown that multi-species cultures can outperform axenic systems under dynamic or $CO_2$-rich environments, providing a more stable platform for carbon capture and biomass production (Farronan *et al.,* 2021; Garrido *et al.,* 2025). This adaptive capacity represents a clear advantage for industrial applications, where maintaining axenic cultures is typically impractical and economically demanding.

### 4.2. Implications of the mixed *Scenedesmus culture*

A central feature of this study is the use of a stable mixed consortium composed of *S. quadricauda*, *S. obliquus*, and *S. dimorphus*. While monocultures are commonly employed in laboratory studies, co-cultivation is emerging as a promising strategy, as mixed consortia often display enhanced physiological resilience and can outperform monocultures in several key performance metrics (Ramanan *et al.,* 2016; Lindehoff *et al.,* 2024).

The co-growth of these *Scenedesmus* species likely contributed to the observed robustness of the culture. Mixed communities tend to exhibit greater tolerance to environmental fluctuations, including elevated $CO_2$ concentrations, due to interspecific metabolic complementarity and resource partitioning (Farronan *et al.,* 2021). In this study, the high tolerance to $CO_2$ (up to 15% before significant inhibition) may be directly attributed to cooperative interactions among the constituent species within the consortium.

This resilience provides a distinct advantage for large-scale and industrial applications, where maintaining axenic cultures is operationally challenging and economically demanding. Similar findings have been reported in recent pilot scale studies employing multi-species photobioreactors, where microbial diversity enhanced both process stability and carbon capture efficiency (Singh & Sharma, 2012; Garrido *et al.,* 2025). Therefore, the development and optimization of stable mixed cultures could play a key role in the design of more robust, efficient, and sustainable $CO_2$ biofixation systems.

### 4.3. $CO_2$ captures efficiency and biotechnological relevance

While biomass productivity is a standard metric, the net $CO_2$ capture efficiency is the fundamental parameter for evaluating the biotechnological relevance and viability of a mitigation system. Therefore, a carbon balance analysis was performed using an empirical approach, since

the carbon content of the biomass was not experimentally determined. This estimation was applied to provide an approximate evaluation of the overall process efficiency.

This analysis revealed that the total capture efficiency was limited, reaching a maximum of 0.131% at the 10% $CO_2$ optimum, a key finding of this study. Although the culture biologically tolerated elevated $CO_2$ levels (up to 20%), achieving its highest $X_\infty$ at 15% $CO_2$, the net capture efficiency derived from this empirical estimation decreased from 0.131% to 0.083% as $CO_2$ increased from 10% to 15%. This clearly indicates that most of the supplied $CO_2$ was not biologically fixed.

The large discrepancy between tolerance and fixation efficiency demonstrates that the primary process delay is not biological, but engineering-related, specifically, a gas-liquid mass transfer limitation. In standard bubble column photobioreactors (BC-PBRs) without specialized spargers or high-dispersion systems, increased gas flow rates can lead to poor $CO_2$ dissolution and excessive stripping, preventing effective uptake and conversion by the microalgal consortium.

This finding holds strong implications for industrial applications focused on the mitigation of this pollutant. Flue gases typically contain $CO_2$ concentrations within the 12–15% range (Songolzadeh *et al.,* 2014). The results of this study indicate that, although the mixed *Scenedesmus* consortium demonstrates high biological tolerance up to 20% $CO_2$, the practical success of large-scale mitigation systems will depend primarily on bioprocess engineering improvements, particularly those aimed at enhancing gas, liquid transfer efficiency and reactor hydrodynamics (Ho *et al.,* 2011; Singh & Sharma, 2012; Zhang *et al.,* 2023).

## 5. CONCLUSION

This study highlights the strong adaptive capacity of a mixed *Scenedesmus* consortium exposed to high $CO_2$ levels, sustaining growth up to 20%. The consortium reached its highest biomass productivity and $CO_2$ fixation rate within the 10–15% $CO_2$ range, confirming its suitability for intensive carbon utilization systems. Although the biological performance was promising, the overall $CO_2$ capture efficiency revealed that the main limitation lies in gas-liquid mass transfer rather than metabolic tolerance. These findings emphasize that further progress in photobioreactor engineering and process optimization will be decisive for transforming this robust biological system into a scalable technology for sustainable $CO_2$ mitigation and bioresource generation.

## ACKNOWLEDGEMENTS

The author M.G.R gratefully acknowledges the Secretary of Science, Humanities, Technology and Innovation (SECIHTI) via the Mexican Government for the scholarship awarded.

## AUTHOR CONTRIBUTION

Magly García: Conceptualization, methodology, software, formal analysis, investigation, data curation, writing-original preparation, writing-review and editing and visualization; Esther Carrillo: Methodology and supervision; Francisco Javier Zavala: data curation; Juan Antonio Noriega: Conceptualization, software, writing-review and editing, project administration, funding acquisition, data curation and supervision.

## Conflict of Interest

The authors declare no conflict of interest.

## References


Assunção, J., Batista, A. P., Manoel, J., Silva, T. L. da, Marques, P., Reis, A. & Gouveia, L. (2017). $CO_2$ utilization in the production of biomass and biocompounds by three different microalgae. Engineering in Life Sciences. 17 (10), 1126-1135. https://doi.org/10.1002/elsc.201700075

Basu, S., Sarma Roy, A., Ghoshal, A. K. & Mohanty, K. (2015). Operational strategies for maximizing $CO_2$ utilization efficiency by the novel microalga *Scenedesmus obliquus* SA1 cultivated in lab scale photobioreactor. Algal Research. 12, 249-257. https://doi.org/10.1016/j.algal.2015.09.010

Boonma, S., Chaiklangmuang, S., Chaiwongsar, S., Pekkoh, J., Pumas, C., Ungsethaphand, T., Tongsiri, S. & Peerapornpisal, Y. (2015). Enhanced Carbon Dioxide Fixation and Bio-oil Production of a Microalgal Consortium. CLEAN – Soil, Air, Water. 43 (5), 761-766. https://doi.org/10.1002/clen.201400171

Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology Advances. 25 (3), 294-306. https://doi.org/10.1016/j.biotechadv.2007.02.001

De Morais, M. G. & Costa, J. A. V. (2007). Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. Journal of biotechnology. 129 (3), 439-445. https://doi.org/10.1016/j.jbiotec.2007.01.009

Farronan, B., Carrasco, R., Flores, J.W.V, Olivera, C.C., Lopez, J., & Alfaro, E. G.B. (2021). Microalgae *Scenedesmus* sp. as a clean technology in reducing greenhouse gas carbon dioxide. Chemical Engineering Transactions. 86, 445–450. https://doi.org/10.3303/CET2186075

Fu, J., Huang, Y., Liao, Q., Xia, A., Fu, Q., & Zhu, X. (2019). Photo-bioreactor design for microalgae: a review from the aspect of $CO_2$ transfer and conversion. Bioresource technology. *292*, 121947. https://doi.org/10.1016/j.biortech.2019.121947

Garrido, L., Romero, C., & Méndez, J. (2025). Microalgae *Scenedesmus* sp. as a potential inoculum in a $CO_2$ capture device against change in environmental temperature**.** Processes. 13(8), 2479. https://doi.org/10.3390/pr13082479

Gour, R. S., Chawla, A., Singh, H., Chauhan, R. S. & Kant, A. (2016). Characterization and Screening of Native *Scenedesmus* sp. Isolates Suitable for Biofuel Feedstock. PLoS ONE. 11 (5). https://doi.org/10.1371/journal.pone.0155321

Guillard, R. R. L. (1975). Culture of Phytoplankton for Feeding Marine Invertebrates. En W. L. Smith & M. H. Chanley (Eds.), Culture of Marine Invertebrate Animals: Proceedings—1st Conference on Culture of Marine Invertebrate Animals Greenport (pp. 29-60), Springer. US. https://doi.org/10.1007/978-1-4615-8714-9_3

Ho, S. H., Chen, W. M. & Chang, J.S. (2010). *Scenedesmus obliquus* CNW-N as a potential candidate for $CO_2$ mitigation and biodiesel production. Bioresource technology. 101 (22), 8725-8730. https://10.1016/j.biortech.2010.06.112

Ho, S.-H., Chen, C.-Y., Lee, D.-J., & Chang, J.-S. (2011). Perspectives on microalgal $CO_2$-emission mitigation systems—A review. Biotechnology Advances. 29(2), 189–198. https://doi.org/10.1016/j.biotechadv.2010.11.001

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., & Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. The Plant Journal. 54(4), 621–639. https://doi.org/10.1111/j.1365-313X.2008.03492.x

Jacob-Lopes, E., Cacia Ferreira Lacerda, L. M. & Franco, T. T. (2008). Biomass production and carbon dioxide fixation by *Aphanothece microscopica Nägeli* in a bubble column photobioreactor. Biochemical Engineering Journal. 40 (1), 27-34. https://doi.org/10.1016/j.bej.2007.11.013

Ji, C., Wang, J., Li, R., & Liu, T. (2017). Modeling of carbon dioxide mass transfer behavior in attached cultivation photobioreactor using the analysis of the pH profiles. Bioprocess and biosystems engineering. 40(7), 1079-1090. https://doi.org/10.1007/s00449-017-1770-6

Lindehoff, E., Mattsson, L., Olofsson, M., Svensson, F., Farnelid, H., & Legrand, C. (2024). Biomass performance and stability of 5-year outdoor microalgal cultivation for $CO_2$ removal from cement flue gas. Bioresource technology reports. 25, 101730. https://doi.org/10.1016/j.biteb.2023.101730

Méndez, A. S., Quintero, M. M., de la Rosa, E. O., Lozano, D. C. & Del Bianchi, V. L. (2020). Study of agitation, color and stress light variables on *Spirulina platensis* culture in a vertical stirred reactor in standard medium. Revista Mexicana de Ingeniería Química. 19 (1), 481-490. https://doi.org/10.24275/rmiq/Bio616

Mora Salguero, D. A., Fernández-Niño, M., Serrano-Bermúdez, L. M., Páez Melo, D. O., Winck, F. V., Caldana, C. & González Barrios, A. F. (2018). Development of a *Chlamydomonas reinhardtii* metabolic network dynamic model to describe distinct phenotypes occurring at different $CO_2$ levels. PeerJ. 6, e5528. https://doi.org/10.7717/peerj.5528

Navarro-Peraza, R., Soto-León, S., Contreras-Andrade, I., Piña-Valdez, P., Viveros-García, T., Cuevas-Rodriguez, E. & Nieves-Soto, M. (2017). Effects of temperature and nitrogen limitation on growth kinetics, proximate composition and fatty acid profile of *Nannochloropsis* sp. Revista Mexicana de Ingeniería Química. 16 (2), 359-369.

Pandey, A., Srivastava, S., & Kumar, S. (2023). Carbon dioxide fixation and lipid storage of *Scenedesmus* sp. ASK22: A sustainable approach for biofuel production and waste remediation. Journal of Environmental Management. *332*, 117350. https://doi.org/10.1016/j.jenvman.2023.117350

Pignolet, O., Jubeau, S., Vaca-Garcia, C. & Michaud, P. (2013). Highly valuable microalgae: Biochemical and topological aspects. Journal of industrial microbiology and biotechnology. 40 (8), 781-796. https://doi.org/10.1007/s10295-013-1281-7

Pruvost, J., Le Gouic, B., & Cornet, J. F. (2022). Kinetic modeling of $CO_2$ biofixation by microalgae and optimization of carbon supply in various photobioreactor technologies. ACS Sustainable Chemistry & Engineering, *10*(38), 12826-12842.

Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., & Kim, H.-S. (2016). Algae–bacteria interactions: Evolution, ecology and emerging applications. Biotechnology Advances. 34(1), 14–29. https://doi.org/10.1016/j.biotechadv.2015.12.003

Robles-Heredia, J. C., Sacramento-Rivero, J. C., Ruiz-Marín, A., Baz-Rodríguez, S., Canedo-López, Y. & Narváez-García, A. (2016). Evaluación de crecimiento celular, remoción de nitrógeno y producción de lípidos por *Chlorella vulgaris* a diferentes condiciones de aireación en dos tipos de fotobiorreactores anulares. Revista Mexicana de Ingeniería Química. 15 (2), 361-377.

Singh, R. N., & Sharma, S. (2012). Development of suitable photobioreactor for $CO_2$ sequestration through microalgae – A review. Renewable and Sustainable Energy Reviews. 16(4), 2347-2353. https://doi.org/10.1016/j.rser.2012.01.026

Sivakaminathan, S., Hankamer, B., Wolf, J. & Yarnold, J. (2018). High-throughput optimization of light-driven microalgae biotechnologies. Scientific reports. 8 (1), 11687. https://doi.org/10.1038/s41598-018-29954-x

Songolzadeh, M., Soleimani, M., Takht Ravanchi, M., & Songolzadeh, R. (2014). Carbon Dioxide Separation from Flue Gases: A Technological Review Emphasizing Reduction in Greenhouse Gas Emissions. The Scientific World Journal. 2014(1), 828131. https://doi.org/10.1155/2014/828131

Sun, H., Zhao, W., Mao, X., Li, Y., Wu, T. & Chen, F. (2018). High-value biomass from microalgae production platforms: Strategies and progress based on carbon metabolism and energy conversion. Biotechnology for biofuels. 11 (1), 227. https://doi.org/10.1186/s13068-018-1225-6

Tang, D., Han, W., Li, P., Miao, X., & Zhong, J. (2011). $CO_2$ biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different $CO_2$ levels. Bioresource Technology. 102(3), 3071–3076. https://doi.org/10.1016/j.biortech.2010.10.047

Tapia-López, L., Chairez, J. I. & Fernandez-Linares, L. C. (2025). Optimizing nitrogen source and concentration for enhanced biomass and bioproducts in *Scenedesmus dimorphus*. Mexican Journal of Biotechnology 2025. 10 (3), 48-65. https://doi.org/10.29267/mxjb.2025.10.3.48-65

Tebbani, S., Filali, R., Lopes, F., Dumur, D. & Pareau, D. (2014). $CO_2$ Biofixation by Microalgae: Modeling, estimation and control. John Wiley & Sons. https://doi.org/10.1002/9781118984475

Wang, B., Li, Y., Wu, N. & Lan, C. Q. (2008). $CO_2$ bio-mitigation using microalgae. Applied Microbiology and Biotechnology. *79* (5), 707-718. https://doi.org/10.1007/s00253-008-1518-y

Xu, P., Li, J., Qian, J., Wang, B., Liu, J., Xu, R., & Chen, P. (2023). Recent advances in $CO_2$ fixation by microalgae and its potential contribution to carbon neutrality. Chemosphere. 319, 137987. https://doi.org/10.1016/j.chemosphere.2023.137987

Zhang, M., Wang, H., & Chen, F. (2023). Time-resolved transcriptome analysis of *Scenedesmus obliquus* HTB1 under 10% $CO_2$ condition. Microbial Biotechnology. 16(2), 448-462. https://doi.org/10.1111/1751-7915.14100

Zhang, X., Wei, X., Hu, X., Yang, Y., & Chen, X. (2022). Effects of different concentrations of $CO_2$ on Scenedesmus obliquus to overcome sludge extract toxicity and accumulate biomass. Chemosphere. 305, 135514. https://doi.org/10.1016/j.chemosphere.2022.135514