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Highlights

- Influence of CO_2 on the growth rate of microalgae is investigated.
- Influence of CO_2 on the growth rate of microalgae cultured in industrial wastewater is investigated.
- The carbon fixation ability of *Scenedesmus* sp. was evaluated in BG-11 and wastewater media.
- Microalgae production in wastewater could be an excellent alternative to forced CO_2 capture.

Abstract

Greenhouse gases (GHG) emissions are widely related to climate change, triggering several environmental problems of global concern and producing environmental, social, and economic negative impacts. Therefore, global research seeks to mitigate greenhouse gas emissions. On the other hand, the use of wastes under a circular economy scheme generates subproducts from the range of high to medium-value, representing away to help sustainable development. Therefore, the use of wastewater as a culture medium to grow microalgae strains that biocapture environmental CO_2, is a proposal with high potential to reduce the GHG presence in the environment. In this work, *Scenedesmus* sp. was cultivated using BG-11 medium and industrial wastewater (IWW) as a culture medium with three different CO_2 concentrations, 0.03%, 10%, and 20% to determine their CO_2 biocapture potential. Furthermore, the concomitant removal of COD, nitrates, and total phosphorus in wastewater was evaluated. *Scenedesmus* sp. achieves a biomass concentration of 1.9 g L^{-1} when is grown in BG-11 medium, 0.69 g L^{-1} when is grown in a combination of BG-11 medium and 25% of industrial wastewater; both cases with 20% CO_2 supplied. The maximum CO_2 removal efficiency (8.4%, 446±150 mg CO_2 L^{-1} day^{-1}) was obtained with 10% CO_2 supplied and using a combination of BG-11 medium and 50% IWW (T2). Also, the highest removal of COD was reached with a combination of BG-11 medium and T2 with a supply of 20% CO_2 (82% of COD removal). Besides, the highest nitrates removal was achieved with a combination of BG-11 medium and 75% IWW (T3) with a supply of 10% CO_2 (42% of nitrates removal) and the maximum TP removal was performed with the combination of BG-11 medium and 25% IWW (T1) with a supply of 10% CO_2 (67% of TP removal). These results indicate that industrial wastewater can be used as a culture media for microalgae growth and CO_2 biocapture can be performed as concomitant processes.

Keywords: CO_2 biocapture, Phycocapture, Phycoremediation, Microalga, Greenhouse gases mitigation, Climate change, Circular economy, Wastewater

1. Introduction

The accelerated increase of greenhouse gas emission in the last years has an anthropogenic origin such as industrial activities, electricity generation, land-use change, and forestry. CO_2 is one of the principal greenhouse gases, with an emission rate of 94,340 thousand metric tons of CO_2 per decade and linked to climate change and the global warming phenomenon (IPCC, 2014; Mahmoud and Gan, 2018). Climate change is a global problem with serious environmental, social, and economic consequences that directly affect the human lifestyle. The principal results of climate change are the rise of sea level, increase in natural disasters, intensify the transmission of diseases in human populations, mass extinction of species, the vulnerability increase of mangrove areas, and change of water cycle, among others (Chu et al., 2019; Dinan, 2017; Lazo-Cancino et al., 2019; Losada et al., 2019; Nagy et al., 2019; Robert et al., 2019; Sousa et al., 2019). Due to the above, there is a particular interest to reduce CO_2 emissions through different alternatives to capture the CO_2 generated by industrial activities (UN, 2019; UNFCCC, 2015).

Some capturing CO_2 methods are methods based on physical and chemical processes such as adsorption and absorption. For instance, zeolite 13 can efficiently capture CO_2 (95–99% purity), an approach that can be useful in some industries (Cloete et al., 2019; Jiang et al., 2019; López-Bautista and Flores-Tlacuahuac, 2020; Zhang et al., 2019). Another method to capture CO_2 is the use of living organisms, mostly those that perform photosynthesis, such as plants, trees, algae, and microalgae; this method is known as biocapture, biosequestration, or biofixation (Jain et al., 2012; Oliveira et al., 2020; Pham et al., 2017; Xiaoyan et al., 2019).

Microalgae are photosynthetic organisms of high interest in the CO_2 biocapture process because the generated biomass can produce compounds such as nutritional supplements, biofuels or pigments (Durmaz et al., 2020; Priharto et al., 2020; Suarez Ruiz et al., 2020; Wei et al., 2013). Several microalgae species have been tested for biocapture CO_2 purposes. For example, *Chlorella* sp. can reach a CO_2 biocapture rate of 96 mg CO_2 L^{-1} day^{-1} when 15% CO_2 is supplied (Kassim and Meng, 2017) and *Scenedesmus obliquus* biocapture 94–95% of the CO_2 supplemented (10% CO_2 gas inlet) (Kang and Wen, 2015; X. Liu et

Table 1 Biocapture of CO_2 by microalgae growth in wastewater.

Type of wastewater	CO_2 influx (%)	Microalga	Biocapture of CO_2 (mg CO_2 L^{-1} day^{-1})	Removal (%) COD	TN	PO_4^{-3}	Reference
Palm oil mill effluent	10	*Chlorella* sp.	828	12	75	33	Hariz et al., 2019
Brewery wastewater	15	*Chlorella* sp. UTEX1602	–	44	81	97	Song et al., 2020
Brewery wastewater	15	*Scenedesmus* sp. *336*	–	73	75	95	Song et al., 2020
Chemical wastewater	2	*Chlorella pyrenoidosa*	82[a]	–	–	–	Yang et al., 2020
Chemical wastewater	10	*Chlorella pyrenoidosa*	91[a]	–	–	–	Yang et al., 2020
Kitchen wastewater	6	*Chlorella* sp.	–	32	–	75	P. K. Kumar et al., 2019
Soybean wastewater	5	*Chlorella* sp.	–	78	96	95	Hu et al., 2020
Soybean wastewater	10	*Chlorella* sp.	–	38	89	92	Hu et al., 2020

a. %

al., 2020). These previous studies show the potential of microalgae in the CO_2 biocapture.

Typically, the cultivation of microalgae for CO_2 biocapture assays can be costly, partly because to the cost of the growing medium (Banerjee et al., 2016). Therefore, the use wastewater that can allow the growth of microalgae is sought, encouraging a circular economy scheme (Chang, 2018; López-Pacheco et al., 2021; Yadav et al., 2020). Thus, wastewater can be used as a culture medium for microalgae growth, obtaining favorable results while treating wastewater in a bioremediation process (Fernández-Linares et al., 2017; Kumar et al., 2018; López-Pacheco et al., 2019; Lv et al., 2018; Onyshchenko et al., 2020; Ren et al., 2017; Solimeno et al., 2019).This tendency moved to performed CO_2 biocapture while bioremediation of wastewater process co-occurred, making the process even more sustainable by treating two different wastes and generating high value subproducts. Some of these studies can be shown in **Table 1**.

Therefore, to highlight the potential of microalgae as biocatalyst for the bioremediation of wastewater and CO_2 biocapture, this study researched the growth of *Scenedesmus* sp. in BG-11 medium mixed with different proportions of industrial wastewater (IWW) and exposed at different concentrations of CO_2 in order to determine its CO_2 biocapture potential. Also, chemical oxygen demand (COD), nitrates, and total phosphorus (TP) of wastewater were monitored to obtain the wastewater biodegradation profile performed by this algae strain.

2. Materials and methods

2.1. Reagents and equipment

The reagents used to prepare the culture medium were purchased from Sigma-Aldrich. The following instruments were used to measure: (1) pH with a Thermo Scientific™ Orion™ 3-Star Benchtop pH Meter, (2) settleable solids with Imhoff cones (USEPA, 1983) and total solids (TS) with the method of total solids dried at 103° - 105 °C (USEPA, 2001), (3) Nitrates was analyzed Dimethyl phenol Method (Nitrate, Nitrogen TNTplus™, LR, HACH, CO, USA) (EPA, 2018), (4) Total Phosphorus with Ascorbic Acid Method (Total phosphorus, Total phosphorus TNTplus™, LR, HACH, CO, USA) (EPA, 1978) and (5) Chemical Oxygen Demand with Reactor Digestion Method (COD TNTplus™, LR, HACH, CO, USA) (USEPA, 1980). The spectrophotometric measurements were taken on a DR 5000™ UV–Vis Laboratory Spectrophotometer.

2.2. Conditions of microalgae growth

The microalga used was *Scenedesmus* sp. from UTEX (UTEX, Austin, TX, USA). Erlenmeyer flasks were placed with a brand OPTIMA 4.5-W pump with an air filter of 0.20 μm. The stock cultures were kept at 20 ± 1 °C with continuous light at 80 μmol photons m^{-2} s^{-1}. The BG- 11 culture medium composition is as follow: $NaNO_3$ 1.5 g L^{-1}, K_2HPO_4 40 mg L^{-1}, $CaCl_2{\cdot}2\ H_2O$ 36 mg L^{-1}, $MgSO_4{\cdot}7\ H_2O$ 75 mg L^{-1}, Citric Acid$\cdot H_2O$ 6 mg L^{-1}, $C_6H_8FeNO_7$ 6 mg L^{-1}, $Na_2EDTA{\cdot}2\ H_2O$ 1 mg L^{-1}, Na_2CO_3 20 mg L^{-1}, H_3BO_3 2.86 mg L^{-1}, $MnCl_2{\cdot}4\ H_2O$ 1.81 mg L^{-1}, $ZnSO_4{\cdot}7\ H_2O$ 0.22 mg L^{-1}, $Na_2MoO_4{\cdot}2\ H_2O$ 0.39 mg L^{-1}, $CuSO_4{\cdot}5\ H_2O$ 0.079 mg L^{-1} and $Co(NO_3)_2{\cdot}6\ H_2O$ 0.04 mg L^{-1}.

2.3. Growth measure of Scenedesmus *sp. in BG-11 medium at different concentration of* CO_2

A diagram of the methodology followed in the present study is shown in **Fig. 1.** A volume of 100 mL of microalgae culture at 1 g L^{-1} of *Scenedesmus* sp. was used as seed culture and was inoculated into

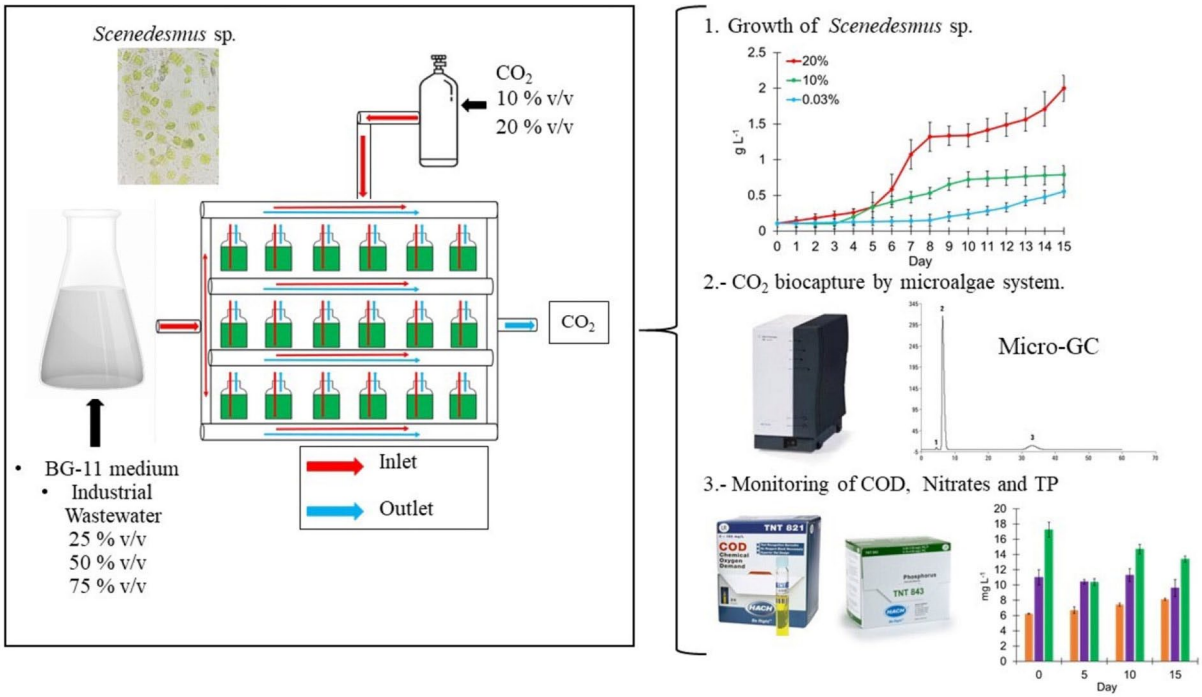

Fig. 1. Global scheme of the methodology. 1) Growth behavior of *Scenedesmus* sp. grow with BG-11 medium and Industrial wastewater with CO_2 supply (10–20% CO_2). 2) Biocapture of CO_2 by *Scenedesmus* sp. grow with BG-11 medium and industrial wastewater with CO_2 supply (10–20% CO_2). 3) Monitoring of COD, nitrates, and TP in *Scenedesmus* sp. culture with industrial wastewater and with CO_2 supply (10–20% CO_2).

a 1000 mL glass bottle. The cultures were kept at the same conditions mentioned in the previous section and sparged at 0.2 vvm with 0.03% (concentration of CO_2 in the air), 10%, and 20% *v*/v CO_2 mixed with N_2 (This supply was continuous throughout the experiment). The cultures were performed for 15 days. The pH was not adjusted in all the experiments, the initial pH of both media was 7. These experiments were conducted by triplicate. The biomass concentration of *Scenedesmus* sp. cultures was evaluated every 24 h, for this, 5 mL of culture were sampled, and cell growth was measured by cell count in a Neubauer chamber. The concentration of biomass expressed as g L^{-1} was obtained by Eq. (1) (R^2 =0.911):

$$g\ L^{-1} = 0.0591\ (Cell\ mL^{-1} \times 10^6) + 0.0538 \quad (1)$$

2.4. CO_2 removal efficiency by Scenedesmus *sp.*

The amount of CO_2 removed was determined from the samples taken in Tedlar polyvinyl fluoride (PVF) gas bags (Supelco Inc., USA) from the inlet and outlet gaseous phase of the system in the cultures supplied with 10% and 20% of CO_2. The CO_2 concentration presented in the samples was determined and compared with a standard by gas chromatography (490 Micro GC, Agilent Technologies) adapted with thermal conductivity detectors (TCDs) and a 10-m PPU column. In the analysis, helium was used as the carrier gas. The temperature of the column was 80 °C, and the temperature of the injector was 110 °C. Also, the CO_2 removal efficiency was determined using Eq. (2) (Guo et al., 2019; Sayedin et al., 2020):

$$CO_2 \text{ removal efficiency } (\%) = \frac{C_{in} - C_{out}}{C_{in}} \times 100 \quad (2)$$

where C_{in} and C_{out} represent the CO_2 concentration in the inlet and outlet gaseous phase, respectively.

2.5. Industrial wastewater as a culture media for Scenedesmus *sp. with different concentration of CO_2*

The industrial wastewater was obtained after the primary treatment (Solids separation) from a local industrial WWTP (Sistema Ambiental Industrial S.A. De C.V.) located in Nuevo León, México. The IWW pH was adjusted to 7 and stored at 4 °C. Nitrates were analyzed by the Dimethyl phenol Method, total phosphorus (TP) by the ascorbic acid method, chemical oxygen demand (COD) by the reactor digestion method, settleable solids with Imhoff cones, and the total solids (TS) with the method of total solids dried at 103–105 °C. All samples were analyzed in triplicate.

A volume of 100 mL of 1 g L^{-1} of *Scenedesmus* sp. was used as seed culture to inoculate 1000 mL glass bottle. A Factorial design was used in this experiment (**Table 2**). The sparging of both cultures was kept at the same conditions as Section 2.3. These experiments were conducted by triplicate. The biomass concentration of *Scenedesmus* sp. cultures was evaluated every 24 h. It was sampled 5 mL of culture and was measured the cell growth by cell count in a Neubauer chamber. The

Table 2 Factorial design of experiments.

Run order	% v/v CO_2	% IWW
1	1	T3
2	2	T3
3	2	T1
4	2	T2
5	1	T2
6	1	T1
7	1	T1
8	2	T2
9	1	T3
10	2	T3
11	2	T1
12	1	T2
13	1	T3
14	1	T2
15	1	T1
16	2	T2
17	2	T3
18	2	T1

% v/v CO_2: 1(10% v/v CO_2) & 2(20% v/v CO_2)

% IWW: T1 (25% IWW: 75% bidistilled water), T2 (50% IWW: 50% bidistilled water) and T3 (75% IWW: 25% bidistilled water). In none of the experiments with IWW was a micronutrient supplementation performed.

concentration of biomass expressed as g L^{-1} was obtained by Eq. (1). The pH was not adjusted in all the experiments. The amount of CO_2 removed was determined in the previous section.

2.6. Monitoring of nitrates, total phosphorus and chemical oxygen in industrial wastewater used as a culture media for Scenedesmus *sp. with different concentration of* CO_2

The experiments were sampled every five days (15 mL) the whole experiment to obtain the concentration of nitrates, total phosphorus (TP), and chemical oxygen demand (COD) of each of the flasks. All the samples were centrifuged at 4000 rpm for 20 min. Then, the supernatants were filtered with a 0.45 μm filter before analysis. After filtration, the samples were analyzed for nitrates, phosphorus total, and COD using the same methods as the previous section.

2.7. Data analysis

The logit regression model for each strain was done with STATISTICA software (StatSoft Inc., Tulsa, USA).

3. Results and discussion

3.1. Growth measure of Scenedesmus *sp. in BG-11 medium with different concentration of CO_2 and CO_2 removal efficiency*

The growth behavior of *Scenedesmus* sp. in BG-11 medium with different concentrations of CO_2 (0.03, 10, and 20%) and CO_2 removal were determined (**Fig. 2**). The CO_2 concentration range (10–20% CO_2) was established because to flue gas general characteristics (Rodas-Zuluaga et al., 2021; Song et al., 2004). From the experiments, we elucidate that the increase of CO_2 concentration increases *Scenedesmus* sp. biomass (Fig. 2a). The biomass concentration obtained were 1.9 g L^{-1} with 20% CO_2, 0.9 g L^{-1} with 10% CO_2, 0.72 g L^{-1}, and with 0.03% CO_2. Our microalga growth profile with 0.03% CO_2 were similar to the results obtained in previous experiments where a biomass concentration of 0.7–1.5 g L^{-1} was achieved (Raeisossadati et al., 2020; Sha et al., 2019).

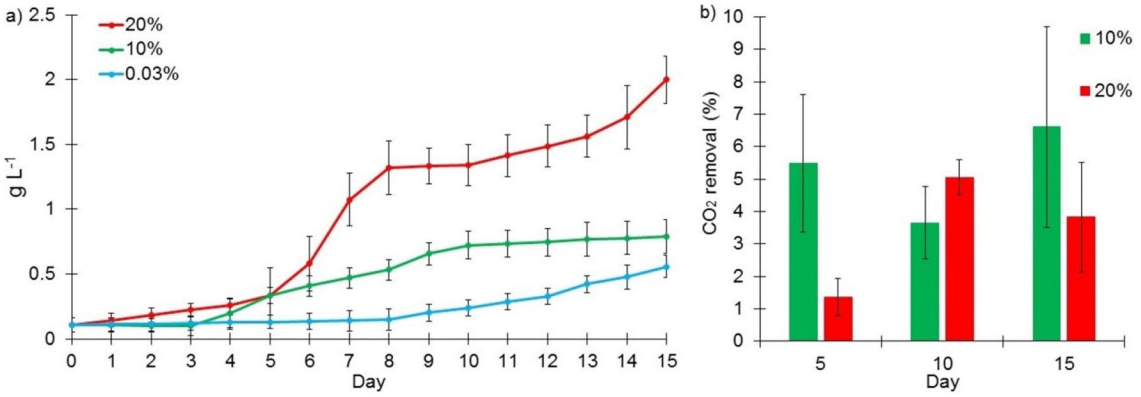

Fig. 2. a) Cell growth (g L^{-1}) of *Scenedesmus* sp. with different concentrations of CO_2 in BG-11 medium. The samples were taken every day. b) CO_2 removal efficiency (%) by *Scenedesmus* sp. at 10% and 20% CO_2. The samples were taken every five days. All points were sampled by triplicate.

Similarly, *Scenedesmus* sp. 336 growth with 15% CO_2 obtained a biomass growth of 1 g L^{-1} (Song et al., 2020). This result is found in the range of the biomass concentration obtained in our experiments with 10% and 20% of CO_2 (0.9–1.9 g L^{-1}). This CO_2 concentrations clearly improved *Scenedesmus* sp. growth. Also, *Scenedesmus obliquus* SJTU-3 culture in BG-11 showed higher growth when CO_2 concentration is increased from 0.03% to 10%; this trend aligned with our results. In contrast, exposing *S. obliquus* SJTU-3 to concentrations in the range of 20–50% CO_2 a decrease in biomass production is found. Furthermore, the highest biomass produced was 1.84 g L^{-1} with 10% CO_2 in contrast to our biomass production which was 1.95 g L^{-1} with 20% CO_2 (Tang et al., 2011). *Scenedesmus* has been reported to be resistant to high CO_2 concentrations, It has obtained 2.75 g of biomass per liter at 70% CO_2 and 3.92 g of biomass per liter at 10% CO_2. Alongside with our results, we can found that *Scenedesmus* is able to increase its growth at high CO_2 concentrations (Huang et al., 2020). These results suggest that *Scenedesmus* sp. have great potential for CO_2 capture, such as flue gas from industrial emissions where CO_2 concentration is usually higher than 10%.

The CO_2 capture potential by *Scenedesmus* sp. were evaluated at 10% and 20% CO_2 concentrations (In the case of the concentration of 0.03% CO_2, this removal was not done because to the low concentration of CO_2 in the air and the sensitivity of the gas chromatograph used in the experiment). In these experiments, the highest CO_2 removal efficiency was observed on the 15th day (6.60%), and on the 10th day (5.06%), at 10% and 20% CO_2 concentrations, respectively (Fig. 2b). The more significant capture obtained when 10% CO_2 was injected may be due to the low solubility of CO_2 in the culture medium. It has been determined before that with less CO_2 solubility, it would be less fixation of CO_2 by microalgae (Song et al., 2019). Also, this behavior has been observed in other studies where an increase of the CO_2 concentration in the culture medium reduces the carbon dioxide capture by microalgae (Chiu et al., 2009; Guo et al., 2019). Our results are similar to previous experiments where *Scenedesmus* sp. grown with a 12% CO_2 concentration shows removal efficiencies between 4 and 8% (de Morais and Costa, 2007). Also, Ma et al. (2019) cultivated *Scenedesmus obliquus* PF3 obtained a maximum CO_2 removal efficiency of 10.45% at 10% CO_2. These results clearly show that CO_2

enhances *Scenedes*mus sp. growth as result of the CO_2 captured (X. Liu et al., 2020). Regarding the CO_2 capture rate, our *Scenedesmus* sp. performed a range capture from 192±58 to 347± 183 mg CO_2 L^{-1} day^{-1} and 392±77 to 2177±22 mg CO_2 L^{-1} day^{-1} at 10% and 20% of CO_2, respectively. In the process of CO_2 biocapture by microalgae, it has been determined that 1.8 Kg of CO_2 is needed for the production of 1 Kg of microalga (Adamczyk et al., 2016; Dineshbabu et al., 2017; Hariz et al., 2019). Thus, from the above, and taking this as a basis, the CO_2 removal obtained in this study microalgae biomass production range between 0.10 and 0.19 g L day^{-1} at 10% of CO_2 and 0.21–1.2 g L day^{-1} at 20% of CO_2. These results are similar to the biomass productivity obtained from experimental results listed in **Table 3**; concluding that microalgae growth is related to the ability to biocapture CO_2. The capture of CO_2 by microalgae depends on microalgae's physiological conditions, such as the potential of cell growth and the ability of its metabolism (Chiu et al., 2008). Also, it has been highlighted that high CO_2 capture by microalgae produces an overproduction of ROS and an upregulation of antioxidant systems, such as CAT and SOD2 (Huang et al., 2020). Furthermore, other different processes have been recognized to help to capture CO_2, in addition to the metabolic approach performed by the microalgae. For instance, abiotic CO_2 removal by the equilibrium of dissolved carbon or CO_2 capture by the exopolysaccharides (EPS) produced by some microalgae strains (**Fig. 3**) (Bhola et al., 2014; Cheng et al., 2006; Delattre et al., 2016; M. Kumar et al., 2019; Sivaramakrishnan et al., 2020).

Table 3 Logit regression model of cell growth of *Scenedesmus* sp. culture in BG-11 medium with different concentration of CO_2.

% CO_2	Model equation	r^2	μ_{max} (est)	Biomass productivity (mg L^{-1} day^{-1})
0.03%	$y = \frac{(0.873 \times 0.11) \times e^{(0.21 \times t)}}{(0.873 \times 0.11) \times e^{(0.21 \times t)} - 1}$	0.986	0.21	0.08
10%	$y = \frac{(0.981 \times 0.17) \times e^{(0.34 \times t)}}{(0.981 \times 0.17) \times e^{(0.34 \times t)} - 1}$	0.997	0.34	0.13
20%	$y = \frac{(0.987 \times 0.05) \times e^{(0.57 \times t)}}{(0.987 \times 0.05) \times e^{(0.57 \times t)} - 1}$	0.987	0.57	0.42

Model equation: equation of logit regression model. Time was considered at 0–15 days. μ_{max} (est): Maximum growth rate estimated in the model. The biomass productivity expressed is the maximum value obtained in each treatment in the fifteen days of cultivation.

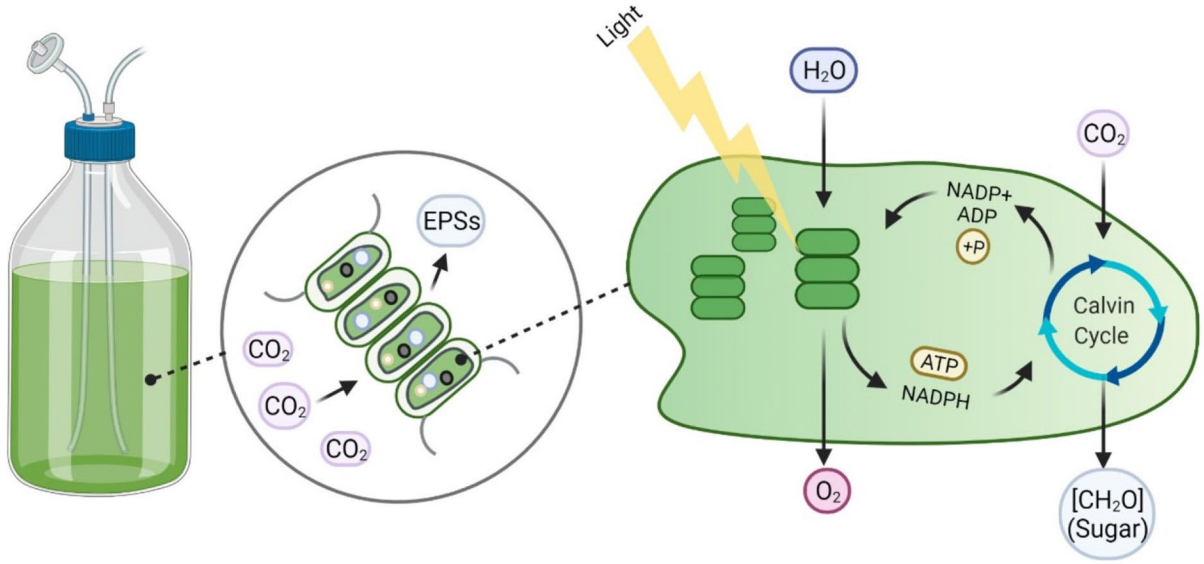

Fig. 3. General diagram of the CO_2 capture process by microalgae.

3.2. *Industrial wastewater as a culture media for* Scenedesmus *sp. with different CO_2 concentrations*

3.2.1. Characterization of industrial wastewater

The characterization of the IWW used in this experiment is shown in **Table 4**. This results are similar to previously reported results, where pH was found from 7.8 to 10, nitrates from 0.4 to 31 mg L^{-1}, COD from 2100 to 28,180 mg L^{-1}, total solids at 5.84–25 g L^{-1}, TP from 7 to 15 mg L^{-1} (Jatto et al., 2020; W. Liu et al., 2020; Papadopoulos et al., 2020; Peñafiel et al., 2016; Schneider et al., 2021; Song et al., 2020). These results indicate that IWW can be considered a perfect waste to be reused as a culture medium for microalgae growth and CO_2 capture.

Table 4 Characterization of industrial wastewater.

Parameter	Value	Unit
pH	7.3 ± 0.05	–
TP	39.4 ± 2.3	mg L^{-1} PO_4
Nitrates	5.06 ± 0.57	mg $L^{-1}NO_3$
COD	4395.6 ± 341	mg L^{-1}
Settleable solids	89.3 ± 6.02	mL L^{-1}
TS	3.9 ± 0.7	g L^{-1}

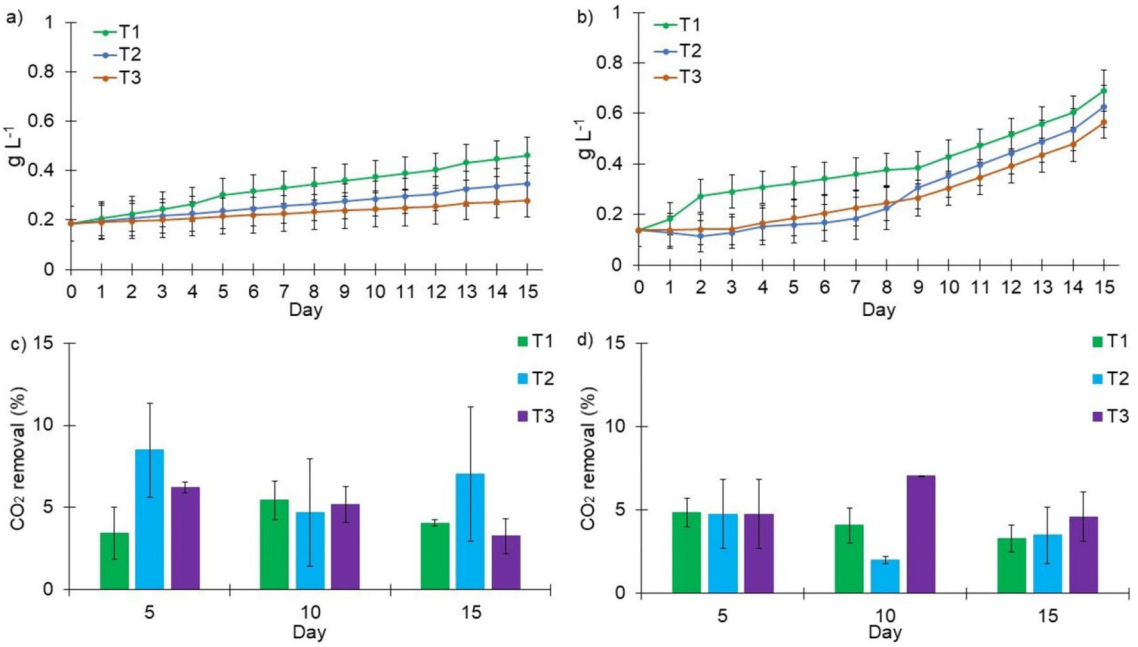

Fig. 4. a) Cell growth (g L^{-1}) of *Scenedesmus* sp. with different concentrations of industrial wastewater with 10% CO_2. b) Cell growth (g L^{-1}) of *Scenedesmus* sp. with different concentrations of industrial wastewater with 20% CO_2. The samples were taken every day. c) CO_2 removal efficiency (%) by *Scenedesmus* sp. with different concentrations of industrial wastewater with 10% CO_2. d) CO_2 removal efficiency (%) by *Scenedesmus* sp. with different concentrations of industrial wastewater with 20% CO_2. The samples were taken every five days. All points were sampled by triplicate.

3.2.2. Cell growth and CO_2 removal efficiency of Scenedesmus *sp. with different CO_2 concentrations in culture medium with IWW*

The growth behavior of *Scenedesmus* sp., in industrial wastewater as a culture medium (25, 50, and 75%) with different concentrations of CO_2 (10 and 20%) and CO_2 removal efficiencies was determined (**Fig. 4**). Superior results were obtained with the microalga grown with 20% of CO_2 being cultivated in the culture medium with IWW (Fig. 4a-b). In the experiment supplemented with 20% CO_2, the biomass concentrations obtained on the 15th day was 0.69 g L^{-1} with T1, 0.62 g L^{-1} with T2, and 0.56 g L^{-1} with T3. On the other hand, the cultures supplemented with 10% CO_2 produced a biomass concentration of 0.46 g L^{-1} with T1, 0.34 g L^{-1} with T2, and 0.27 g L^{-1} with T3. Although these results were lower than those obtained with the use of BG-11 medium without IWW (10 and 20% CO_2), culture medium

added with IWW stands still considered a good option for microalgae growth. We highlight this because in the case of the use of T1 with 20% CO_2, similar growth results were obtained compared to the growth of *Scenedesmus* sp. grown in BG-11 medium supplemented with air.

Previously, it had been found that >1 g L^{-1} of *Scenedesmus* biomass can be obtained using artificial brewery wastewater. Despite this, our study did not obtain such good results regarding microalgae growth with IWW (Song et al., 2020). In another study, where treated IWW was used for *Scenedesmus* sp. growth, 0.11 g L^{-1} of biomass was obtained, which is lower than our results. Likewise, wastewater has been used as a culture medium for the growth of other microalgae strains such as *Chlorella*, with a biomass production of 4.9 g L^{-1}; concluding that the use of residual water as a microalgae culture medium is feasible (Cho et al., 2011). This fact is also reflected in our results. Furthermore, it was observed that increasing the percentage of IWW, the growth of *Scenedesmus* sp. was affected. Thus, the recommended residual water percentage for microalgae growth should be less than 50% (Lópezpacheco et al., 2019; López-Pacheco et al., 2021).

Some other studies have shown microalgae's ability to perform CO_2 capture using IWW as a culture medium (Ding et al., 2020). For example, *Chlorella* sp. was grown using treated IWW and supplied with 10% CO_2. In that study, biomass productivity was 0.44 g L^{-1} day^{-1} (Hariz et al., 2019); these data are comparable to our results for the treatment labeled as T1 and with a concentration of 10% of CO_2. *Scenedesmus obliquus* has a biomass growth of 0.42–0.44 g L^{-1} being cultivated using food wastewater (0–2%) with a supply of 10 and 14.1% CO_2 (Ji et al., 2015). These results are less favorable than our results with 20% of CO_2 in all the concentrations of IWW used.

In addition to cell growth behavior, CO_2 capture was determined by our microalgae systems to know their capture CO_2 potential at 10% and 20% CO_2 concentration using culture medium added with IWW (Fig. 4c-d). The results obtained in the experiment with 20% CO_2 supplied shows a CO_2 removal efficiency of 4.8% with T1 and 4.7% with T2 on the 5th day and 7% on the 10th day with T3. On the other hand, the experiment with 10% CO_2 supplied shows a CO_2 removal efficiency of 5.4% with T1 on 10th day and 8.4% with T2 and 6.2% with T3 on 5th day of culture. Similarly, to the experiments carried out with BG-11

medium, an outstanding capture of CO_2 was performed when CO_2 was injected at 10% concentration. This may be as result of the low solubility of CO_2 in the culture medium at higher CO_2 concentrations (Song et al., 2019). Our results show inferior efficiency compared with other previously reported results where a high removal efficiency (91%) was obtained by *Chlorella pyrenoidosa* grown in treated wastewater as culture medium with a CO_2 of 10% (Yang et al., 2020). Nevertheless, it is important to highlight that our study uses untreated wastewater as culture medium.

The rates of CO_2 capture for *Scenedesmus* sp., in the experiment with 20% CO_2 supplied, were ranged from 347±84 to 509±92 mg CO_2 L^{-1} day^{-1} in T1 conditions, 209±24 to 498±217 mg CO_2 L^{-1} day^{-1} with T2, and 481±155 to 738±21 mg CO_2 L^{-1} day^{-1} with T3. On the other hand, in the experiment with 10% CO_2 supplied, a rate of CO_2 capture range from 180±82 to 285±60 mg CO_2 L^{-1} day^{-1} with T1, 246±172 to 446 ± 150 mg CO_2 L^{-1} day^{-1} with T2, and 171±53 to 326±17 mg CO_2 L^{-1} day^{-1} with T3. Some studies present high rates of CO_2 capture. For example, *Chlorella minutissima* obtained a rate of CO_2 capture of 51.51 g $L^{-1}d^{-1}$ with a CO_2 supply of 5% (De Bhowmick et al., 2019). On the other hand, studies with similar results (368 mg L^{-1}) to ours have been obtained with a CO_2 supply of 2.5% (Nayak et al., 2016). Thus, concluding the feasibility of capture CO_2 by microalgae using wastewater as culture medium.

The biomass productivity, from the CO_2 captured by the microalgae, in the experiment with 20% CO_2 supply was 0.19–0.28 g L day^{-1} with T1, 0.11–0.27 g L day^{-1} with T2, and 0.26–0.41 g L^{-1} day^{-1} with T3. Regarding the experiment with 10% CO_2 supplied, biomass productivity rates of 0.10–0.15 g L day^{-1} with T1, 0.13–0.24 g L day^{-1} with T2, and 0.09–0.18 g L day^{-1} with T3 were found. Although these results are not very similar to the biomass productivity obtained from experimental results expressed in **Table 5**, it is considered that the system can capture at least 35% of the CO_2 injected into the system (Hariz et al., 2019). Similar to the previous section, in these experiments there are some process in the system that can help to capture CO_2 in addition to the biological process of the microalgae (Bhola et al., 2014; Cheng et al., 2006; Delattre et al., 2016; Hariz et al., 2019; M. Kumar et al., 2019; Sivaramakrishnan et al., 2020). The mechanisms of microalgae metabolism when they are exposed to elevated CO_2 concentration are

Table 5 Logit regression model of cell growth of *Scenedesmus* sp. culture in IWW with different concentration of CO_2.

Treatment	Model equation	r^2	μ_{max} (est)	Biomass productivity (mg L^{-1} day^{-1})
10% CO_2				
T1	$y = \frac{(0.54 \times 0.18) \times e^{(0.14 \times t)}}{(0.54 \times 0.18) \times e^{(0.14 \times t)} - 1}$	0.994	0.14	0.03
T2	$y = \frac{(0.783 \times 0.188) \times e^{(0.06 \times t)}}{(0.783 \times 0.188) \times e^{(0.06 \times t)} - 1}$	0.996	0.06	0.02
T3	$y = \frac{(0.835 \times 0.185) \times e^{(0.03 \times t)}}{(0.835 \times 0.185) \times e^{(0.03 \times t)} - 1}$	0.996	0.03	0.01
20% CO_2				
T1	$y = \frac{(0.523 \times 0.186) \times e^{(0.15 \times t)}}{(0.523 \times 0.186) \times e^{(0.15 \times t)} - 1}$	0.994	0.15	0.09
T2	$y = \frac{(1.591 \times 0.078) \times e^{(0.16 \times t)}}{(1.591 \times 0.078) \times e^{(0.16 \times t)} - 1}$	0.986	0.16	0.09
T3	$y = \frac{(2.178 \times 0.104) \times e^{(0.12 \times t)}}{(2.178 \times 0.104) \times e^{(0.12 \times t)} - 1}$	0.988	0.12	0.08

Model equation: equation of logit regression model. Time was considered at 0–15 days. μ_{max} (est): Maximum growth rate estimated in the model. The biomass productivity expressed is the maximum value obtained in each treatment in the fifteen days of cultivation.

not fully understood in the literature. Nevertheless, some hypothesis indicate that several metabolites serve as buffer compensating the acidification caused by the CO_2 supply and causing an overproduction of some genes (Guo et al., 2017; Huang et al., 2020).

3.3. Monitoring of nitrates, total phosphorus and chemical oxygen in industrial wastewater used as a culture media for Scenedesmus *sp. with different concentration of* CO_2

To know the bioremediation of industrial wastewater performed by *Scenedesmus* sp. during the CO_2 capture process, Nitrates, TP, and COD concentrations were monitored. Regarding the COD, the highest removal from the experiments was shown on 15th day of culture with the medium labeled as T1 with a supply of 10% CO_2 (39% of COD removal) and on the 10th day of culture with T2 with a supply of 20% CO_2 (82% of COD removal). In the other hand, a higher COD removal was obtained in the treatments supplied with 20% CO_2 (**Fig. 5**a-b).

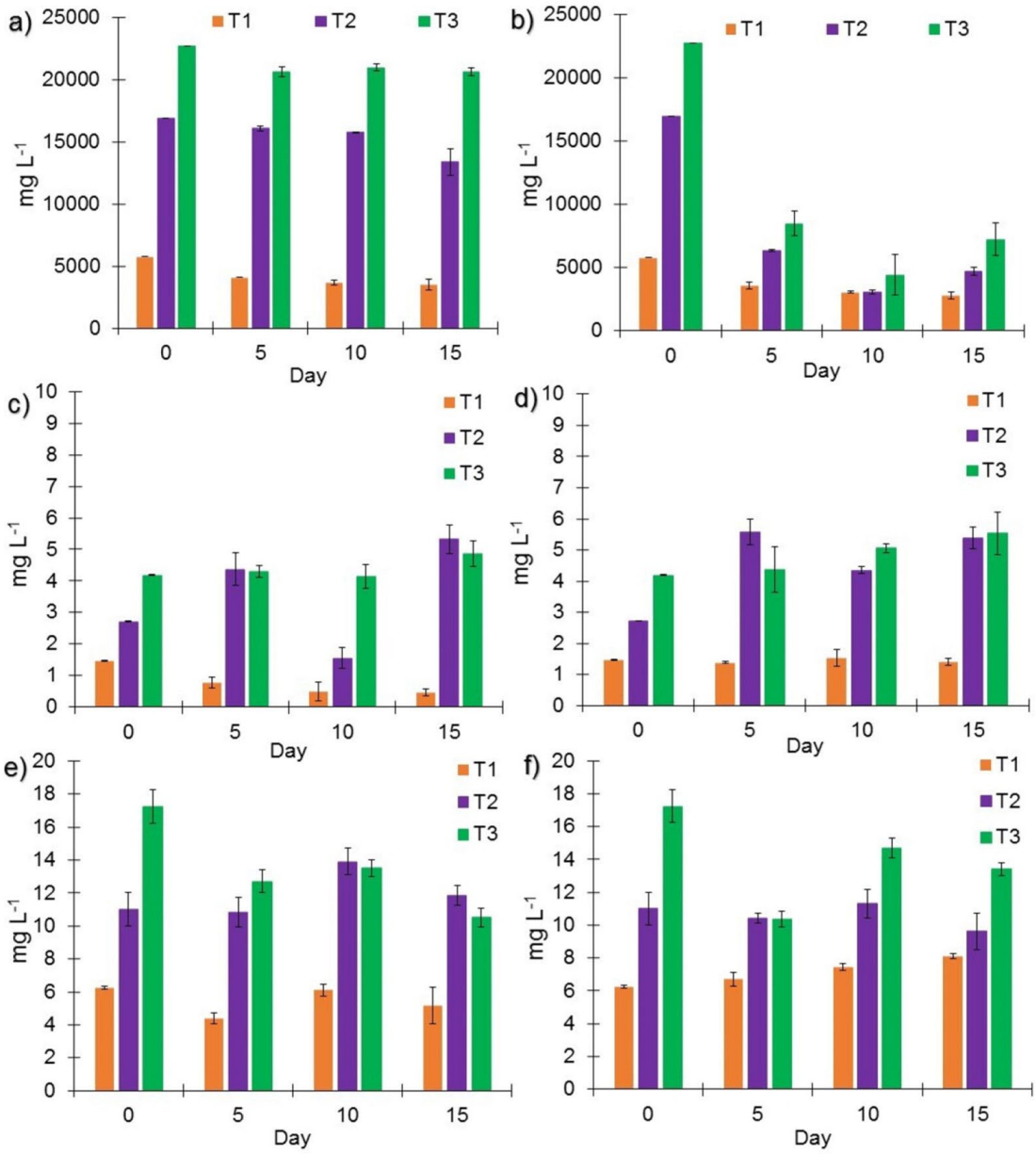

Fig. 5. COD, TP and nitrates in industrial wastewater used as a culture media for *Scenedesmus* sp. with different concentration of CO_2 supply. a) Quantification of COD (mg L^{-1}) of industrial wastewater, in different proportions with 10% CO_2. b) Quantification of COD (mg L^{-1}) of industrial wastewater, in different proportions with 20% CO_2. c) Quantification of TP (mg L^{-1}) of industrial wastewater, in different proportions with 10% CO_2. d) Quantification of TP (mg L-1) of industrial wastewater, in different proportions with 20% CO_2. e)Quantification of nitrates (mg L^{-1}) of industrial wastewater, in different proportions with 10% CO_2. f) Quantification of nitrates (mg L-1) of industrial wastewater, in different proportions with 20% CO_2. The samples were taken every five days. All points were sampled by triplicate.

The removal of COD in these systems are related to some bacteria in the wastewater since it did not go through a sterilization process and also to chemical oxidation caused by the aeration of the system (Mezzomo et al., 2010).

These results can be compared to previous researches, where a 47% of COD removal in wastewater using *Chlorella* sp. was obtained (Hariz et al., 2019). Also, *Scenedesmus* sp. performed a COD removal of 92–86% using domestic wastewater as culture medium and supplied with 5–10% CO_2. These results are in agreement with our results presented in the experiments with 20% of CO_2 (Nayak et al., 2016). Likewise, it is observed that microalgae performance on COD removal depends on the type of wastewater used for the process (De Bhowmick et al., 2019).

Regarding the TP, the highest removal from the experiments was shown on the 15th day of culture with T1 and 10% CO_2 (67% of TP removal). In the case of the experiments performed with a supply of 20% CO_2, a decrease in TP concentration were not obtained in most of the treatments (Fig. 5c-d). These results can be compared to previous researches, where it was obtained 33% of phosphate removal in wastewater using *Chlorella* sp. for CO_2 capture purposes (Hariz et al., 2019), *Scenedesmus obliquus* removes 73–84% of TP in food wastewater with 5–14% CO_2 supplied to the medium, however, that study was carried out with only 2% of wastewater (Ji et al., 2015). These results lead us to consider that the growth of microalgae in industrial wastewater can help removing TP; however, the cultivation time must be considered as a key factor to improve this contaminant's removal.

The behavior of the increase of TP in the experiments can be given because microalgae exhibit this type of behavior when exposed to large amounts of pollutants present in wastewater because the N:P ratio is inadequate, causing an increase in TP concentration in the medium and influences the decrease in the TP removal (Gardner-Dale et al., 2017; Patel et al., 2012; Xin et al., 2010). Besides, this increase can be by a rupture process of the microalgae cell wall, thus releasing phosphorus to the medium. This process was confirmed by microscopic analysis in previous experiments, where it was showed bleaching in the microalgae. The rupture of the cell wall, as well as the presence of dead microalgae, contribute to TP concentration in

the medium. Regarding to our study, cell death was not detected in the growth curves, but this does not mean that there was no presence of dead cells during the experimental process (Droop, 1975; Martínez, 2000).

In the case of nitrates monitoring, the highest removal from the experiments was shown on the 15th day of culture using the culture medium labeled as T3 with a supply of 10% CO_2 (42% removal) and on the 5th day of culture using the same culture medium but with a supply of 20% CO_2 (39%) (Fig. 5e-f). *Scenedesmus* sp. removed nitrates in the range of 56–65%, in domestic wastewater with a supply of 5–10% CO_2; which can be compared to our results with 10% of CO_2 (Nayak et al., 2016). In this study, nitrogen was monitored in the form of nitrate since nitrate is the most thermodynamically stable form of inorganic nitrogen and not has nitrogen losses due to volatilization. Therefore, it was considered that this could be the optimal way to measure the behavior of nitrogen in this study (Gonçalves et al., 2014). The nitrates increase concerning to the initial value in some treatments with both percentages of CO_2 supply (10% and 20%). This result may have occurred because partial nitrification performed by the microalgae since IWW used has ammonia in its composition (España-Gamboa et al., 2018; Rada-Ariza et al., 2017; Song et al., 2020). In particular, this process can be observed as the treatments with higher cell growth increased in nitrates concentration in IWW and in the treatments where there was less cell growth, there was more effective removal of this variable. As was observe in this study, the removal of COD, nitrates, and TP in IWW by microalga depended on the culture time, cell growth, IWW concentration, and CO_2 supply.

4. Conclusions

In this study, the growth of *Scenedesmus* sp., COD, nitrate, and TP removal was evaluated in a system that combined the use of wastewater (T1, T2, and T3) as a culture medium and CO_2 supply (10 and 20%) for 15 days. The highest growth occurred in the treatments that contained a lower concentration of residual water (T1), both using a CO_2 supply of 10% (0.46 g L^{-1}) and 20% (0.69 g L^{-1}). Likewise, it can be seen that the increase in CO_2 supply allowed to obtain a higher concentration of

biomass. The maximum CO_2 removal efficiency (8.4%, 446 ± 150 mg CO_2 L^{-1} day^{-1}) was obtained at 10% CO_2 with T2. The highest removal of nitrates and TP was obtained with 10% CO2 supply, with T3 (42% of nitrates removal) and T1 (67% of TP removal), respectively. Regarding COD, the highest removal obtained was with T2 supplied with 20% CO_2 (82% of COD removal).

CRediT authorship contribution statement

Itzel Y. López-Pacheco: Conceptualization, Methodology, Formal analysis, Writing—original draft.
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Damià Barceló: Writing—review and editing.
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Roberto Parra-Saldívar: Conceptualization, Supervision, Project administration, Funding acquisition.

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