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RESEARCH PAPER

Biotechnological potential of *Chlorella* sp. and *Scenedesmus* sp. microalgae to endure high CO_2 and methane concentrations from biogas

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Abstract
Biogas, a gaseous effluent from the anaerobic digestion of organic waste, is considered an important source of energy, since it has a composition mainly of methane (CH_4; 55–75%) and CO_2 (20–60%). Today, CO_2 from biogas is an excellent carbon source to induce high microalgal biomass production; however, each microalga strain can have different optimal CO_2 concentrations for maximizing their bio-refinery capacity as well as different ability to endure stressful conditions of industrial effluents. This study assessed the bio-refinery capacity of *Chlorella* sp. and *Scenedesmus* sp., native of Lago de Chapala, Mexico, from biogas, as well as the effect of high CO_2 and methane concentrations on the physiological performance to grow, capture CO_2 and biochemical composition of both microalgae cultured under different biogas compositions. The results show that both microalgae have the biotechnological potential to endure biogas compositions of 25% CO_2–75% CH_4. Under this condition, the biomass production attained by *Chlorella* sp. and *Scenedesmus* sp. was 1.77 ± 0.32 and 2.25 ± 0.20 g L^{-1}, respectively, with a biochemical composition mainly of carbohydrates and proteins. Overall, this study demonstrates that both microalgae have the ability to endure the stressful biogas composition without affecting their physiological capacity to capture CO_2 and biosynthesize high-value metabolites. Moreover, it is worth highlighting the importance of screening wild-type microalgae from local ecosystems to determine their physiological capacity for each biotechnological application.

Keywords Anaerobic digestion · Bio-refinery · Bio-remediation · CO_2 fixation · Flue gases

Introduction

The supply of CO_2 from industrial gases to microalgae culture is a bio-refinery approach used with different purposes, such as increasing biomass production and biosynthesizing high-value metabolites, and reducing production costs and CO_2 emissions to the atmosphere [1, 2]. Specifically, biogas, a gaseous effluent from the anaerobic digestion of organic waste, is considered an important source of energy, since it has a composition of methane (CH_4; 55–75%), CO_2 (20–60%), and sulfidic acid (H_2S; 0.005–2%) [3]. To date, several studies have demonstrated that CO_2 from biogas is an excellent carbon source to induce high microalgal biomass production and simultaneously perform CH_4 upgrading, since CO_2 content reduces the calorific value of CH_4 preventing meeting the specifications of fuel gas [3–9]. Nevertheless, this dual purpose varies in each microalga strain, because the potential to endure high CO_2 and CH_4 concentrations is strain-dependent; besides, the high concentration of both metabolites causes stress on several strains decreasing their physiological activity [3, 10]. Thus, selecting the appropriate strain is the main factor to ensure success of biomass production and cell compound accumulation from CO_2 content from biogas [2, 3, 10, 11].

According to Varshney et al. [12], an ideal microalgal strain for CO_2 fixation from industrial flue gases must have specific traits, for example, (1) tolerance to high CO_2 concentrations and other toxic components that are typically found in flue gases; (2) tolerance to environmental conditions that are present in cultivation systems; and (3) high commercial or calorific value. Although microalgal CO_2

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fixation can be improved by genetic engineering [4, 13], the use of genetically modified microalgae is limited by safety regulations to prevent environmental contamination and human consumption [2, 11, 14, 15]. In this context, several studies have focused on isolating and identifying wild-type microalgae from local habitats with this novel physiologic and biotechnological performance [11, 12, 14, 15]. Since native microalgae are already adapted to the environmental conditions prevailing in a specific geographical location, they are preferred for bio-refinery and bio-remediation purposes rather than microalgae of collection banks [14] or genetically modified [15]. Specifically, the microalgae of the genus *Chlorella* and *Scenedesmus* have been widely used to produce biomass from biogas [3, 5–8] because of their ability to tolerate high CO_2 concentrations [16]. However, microalgae are a very diverse group, and strains from the same genus can have different optimal CO_2 concentrations for maximizing their bio-refinery capacity, as well as their ability to tolerate high CO_2 and CH_4 concentrations from industrial effluents [2, 11, 14]. In this context, *Chlorella* sp. and *Scenedesmus* sp. were isolated from Lago de Chapala, Jalisco, the largest lake of Mexico and well known for high phytoplankton diversity [17] to integrate the anaerobic digestion process of agro-industrial waste and microalgal biomass production. To our knowledge, no assessment has been performed on these native microalgal strains for their bio-refinery capacity from CO_2 content from biogas and biotechnological potential to endure stressful composition of this effluent.

Considering the above, the aims of this study were to assess both microalgae *Chlorella* sp. and *Scenedesmus* sp., on their bio-refinery capacity from biogas, as well as the effect of high CO_2 and CH_4 concentrations from biogas on their biochemical composition and physiological capacity to grow and capture CO_2 cultured under different biogas compositions.

Materials and methods

Microorganisms and culture conditions

Chlorella sp. and *Scenedesmus* sp. (Fig. 1) were isolated from Lago de Chapala (Jalisco, Mexico; 20°15′27″N, 103°02′33″W) according to the methodology described by Smith et al. [18]. Both microalgae were maintained in C30 + M medium [9] at 27 ± 2 °C, 200 µmol photons m^{-2} s^{-1}, and stirred at 120 rpm for 14 days.

Experimental growth conditions

The experiment was set up by adding 50 mL of each microalga previously pre-cultured in 450 mL of C30 + M medium, using a 1-L flask with 500 mL of working volume. Both microalgae were maintained at 27 °C, 200 µmol photon m^{-2} s^{-1} and stirred at 120 rpm in an incubator shaker (Innova 43, New Brunswick Scientific, Nürtingen, DE) for 8 days. During all incubation time, a gas mixture was

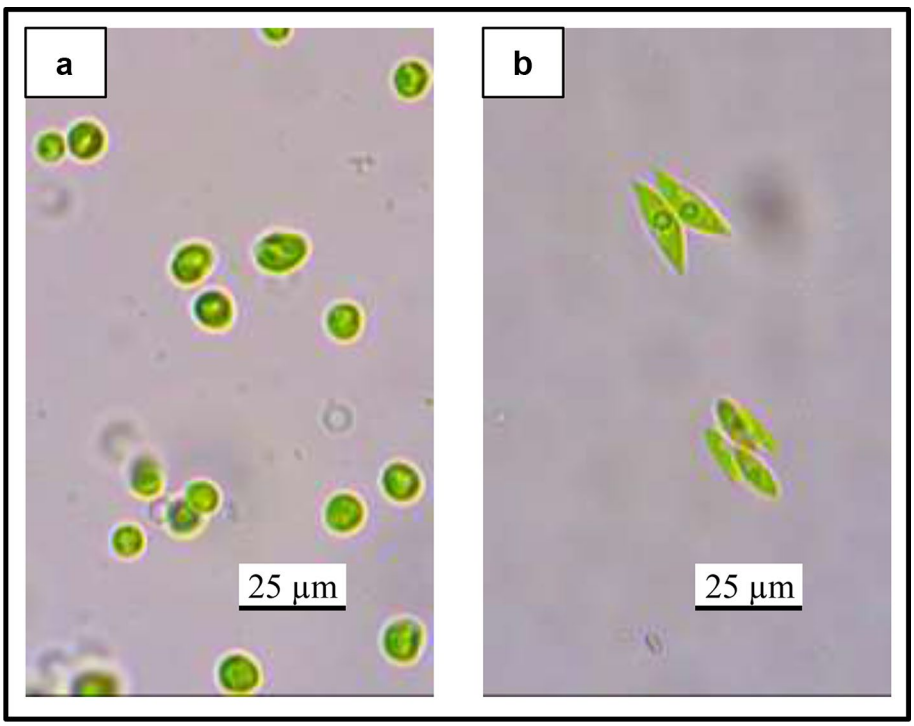

Fig. 1 Photomicrographs illustrating the morphological features of *Chlorella* sp. (**a**) and *Scenedesmus* sp. (**b**) strains isolated from Lago de Chapala, Jalisco, Mexico. Images were obtained using a microscope Leica DM750 model with a camera ICC50 E (×100) and software Leica LAS Interactive

bubbled continuously at the bottom of each flask with a flow rate of 0.006 vvm. Five different gas mixtures were utilized in this study: (1) 75% CH_4–25% CO_2; (2) 50% CH_4–50% CO_2 (synthetic biogas; treatments); (3) 75% Argon–25% CO_2; (4) 50% Argon–50% CO_2; and (5) hydrocarbon-free air (as control). Argon (Ar) was used to avoid the CH_4 content from biogas and evaluate the effect of CH_4 concentration on each microalga. Each gas mixture was acquired from Praxair, Mexico and sterilized with acrodisc filters of 0.2 µm (Millipore, MA, USA) before feeding each microalgal culture.

Biomass production

Biomass production (g L^{-1}) was quantified by cell dry weight. Briefly, 20 mL of microalgal culture were sampled each 48 h and centrifuged at 10,000 rpm for 10 min; the microalgal pellet was washed twice with distilled water and dried at 80 °C in Thermo Scientific Heratherm™ OGS100 Lab oven (Waltman, MA, USA) for 12 h. Biomass productivity (P; g L^{-1} day^{-1}) was calculated with Eq. 1 where X_f and X_i corresponded to biomass production (g L^{-1}) at initial (t_i) and final time (t_f) [9]:

$$P = (X_f - X_i)/(t_f - t_i). \quad (1)$$

Specific growth rate (μ; day^{-1}) was calculated with Eq. 2 where X_i and X_f were the biomass production (g L^{-1}) at the initial (t_i) and final time (t_f) of the exponential growth phase:

$$\mu = \ln\left(\frac{X_i}{X_f}\right)/(t_f - t_i). \quad (2)$$

Determination of CO_2 fixation from biogas

Dissolved inorganic carbon (DIC) was quantified at the end of experimental time (8 days) in culture media by a total organic carbon analyzer (Shimadzu-VCSN, Tokyo, JP); the pH in culture medium was determined with a pH meter (Thermo-Orion Model 720A, MA, USA); CO_2 fixation rate (R_{CO_2}; g L^{-1} day^{-1}) was determined with Eq. 3, according to Tang et al. [19] considering the typical molecular formula of microalgal biomass, $CO_{0.48}$ $H_{1.83}$ $N_{0.11}$ $P_{0.01}$ [20]:

$$R_{CO_2} = C_c P(M_{CO_2}/M_c), \quad (3)$$

where P is biomass productivity; C_c is carbon content of the microalgal cell; M_c is carbon molecular weight; and M_{CO_2} is the CO_2 molecular weight.

Microalgal biomass characterization

Microalgal biomass was measured at the end of each experiment. Total carbohydrates were quantified by the phenol–sulfuric method [21], while protein content was determined by the Lowry method [22]. Total lipids were extracted by the procedure established [23] and quantified in a Rotovapor IKA RV-10 (Staufen, DE).

Experimental designs

To determine biomass production, CO_2 fixation rate, biochemical composition, as well as simultaneously evaluate the effect of high CO_2 and CH_4 concentrations from biogas on both microalgae, each experiment consisted of five gas mixtures: (1) 75% CH_4–25% CO_2; (2) 50% CH_4–50% CO_2 (synthetic biogas; treatments); (3) 75% Ar–25% CO_2; (4) 50% Ar–50% CO_2; and (5) hydrocarbon-free air (as control). Each experiment was performed in triplicate and repeated twice; the results obtained from each experiment were analyzed using ANOVA and then LSD post hoc analysis. Significance was set at $P < 0.05$, using Statistica 6.0 software (StatSoft, Tulsa, OK).

Results

Carbon dioxide fixation rate by microalgae cultured under different biogas composition

Chlorella sp. recorded the highest CO_2 fixation rates at 8 days when it was supplied with 25% CO_2–75% CH_4 (0.28 ± 0.036 g L^{-1} day^{-1}) and 25% CO_2–75% Ar (0.29 ± 0.018 g L^{-1} day^{-1}), which was significantly similar when fed with these two gas mixtures (Fig. 2a, lowercase letters). However, when it was provided with 50% CO_2, either balanced with 75% CH_4 or Ar, the CO_2 fixation rate of this microalga significantly decreased, recording 0.18 ± 0.023 and 0.14 ± 0.022 g L^{-1} day^{-1}, respectively, and it did not show significant differences between both gas mixtures (Fig. 2a, lowercase letters). This same pattern was found in *Scenedesmus* sp. at eight days, reaching 0.39 ± 0.015 and 0.35 ± 0.037 g L^{-1} day^{-1} when it was supplied with 25% CO_2–75% CH_4 or 25% CO_2–75% Ar, respectively, showing to be significantly similar when fed with these gas mixtures (Fig. 2b, lowercase letters). Similarly, at the end of experimental time (8 days), CO_2 fixation rate of *Scenedesmus* sp. also significantly decreased when it was supplied with 50% CO_2–50% CH_4 (0.28 ± 0.036 g L^{-1} day^{-1}) and 50% CO_2–50% Ar (0.28 ± 0.036 g L^{-1} day^{-1}), although the results were similar between these two gas mixtures (Fig. 2b, lowercase letters).

On the contrary, the dissolved inorganic carbon concentration (DIC) in culture media was significantly higher when both microalgae were supplied with 50% CO_2 balanced either with CH_4 or Ar. In contrast, pH in culture media was significantly higher in the two gas mixtures composed of 25% CO_2 balanced either with CH_4 or Ar (Table 1).

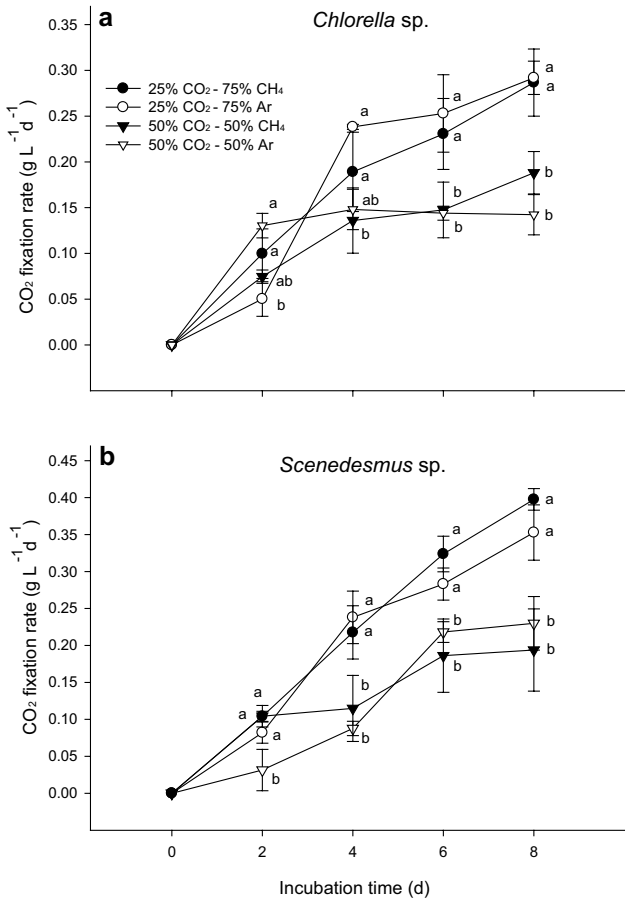

Fig. 2 CO_2 fixation rate by *Chlorella* sp. (**a**) and *Scenedesmus* sp. (**b**) supplied with different gas mixtures. Points at each time interval denoted by different lowercase letters differ significantly when each microalga grew supplied with different gas mixtures. Statistical analyses were performed using ANOVA and LSD post hoc analysis ($P < 0.05$). Bars represent standard error

Biomass production by microalgae cultured under different biogas composition

Similarly, *Chlorella* sp. recorded the highest biomass production at 8 days, attaining 1.77 ± 0.32 and 1.81 ± 0.17 g L^{-1} when it was supplied with 25% CO_2–75% CH_4 and 25% CO_2–75% Ar, respectively; it did not show significant differences when supplied with both gas mixtures (Fig. 3a, lowercase letters). Likewise, *Scenedesmus* sp. also showed the highest biomass production at 8 days, recording 2.25 ± 0.20 and 2.04 ± 0.13 g L^{-1}, respectively, when it was provided with these two gas mixtures; the results were statistically similar (Fig. 3b, lowercase letters). Nonetheless, biomass production in each microalgae significantly decreased when supplied with 50% CO_2 either balanced with CH_4 or Ar (Fig. 3a, b, lowercase letters), while the two microalgae supplied with air showed the lowest biomass production (Fig. 3a, b, lowercase letters).

Table 1 Dissolved inorganic carbon (DIC) and pH by *Chlorella* sp. and *Scenedesmus* sp. supplied with different gas mixtures

Microalga	Gas mixture	Dissolved inorganic carbon (DIC; mg L^{-1})	pH
Chlorella sp.			
	Air	–	7.87 ± 0.50a
	25% CO_2–75% Ar	57.89 ± 5.98b	6.34 ± 0.55b
	25% CO_2–75% CH_4	65.10 ± 9.16b	5.88 ± 0.29b
	50% CO_2–50% Ar	136.61 ± 8.55a	4.32 ± 0.65c
	50% CO_2–50% CH_4	148.42 ± 5.87a	3.56 ± 0.35c
Scenedesmus sp.			
	Air	–	8.67 ± 0.72a
	25% CO_2–75% Ar	76.63 ± 7.49b	7.64 ± 0.15b
	25% CO_2–75% CH_4	60.39 ± 13.09b	6.25 ± 0.47c
	50% CO_2–50% Ar	141.75 ± 11.5a	4.62 ± 0.36d
	50% CO_2–50% CH_4	124.67 ± 6.77a	4.16 ± 0.61d

Values denoted by different lowercase letters differed significantly when each microalga grew supplied with different gas mixtures. Statistical analyses were performed using ANOVA and LSD post hoc analysis ($P < 0.05$); ± represents standard error

On the other hand, both microalgae also showed the highest specific growth rates and biomass productivities when they were supplied with 25% CO_2 rather than 50% CO_2 balanced either with CH_4 or Ar (Table 2).

Biochemical composition of microalgae cultured under different biogas composition

Under our experimental conditions, the two microalgae evaluated accumulated mainly carbohydrates and proteins, while lipids were not detected. Both microalgae recorded the highest accumulation of both compounds supplied with 25% CO_2 balanced with CH_4 or Ar (Fig. 4). In this condition, *Chlorella* sp. showed a biochemical composition of 24.42 ± 1.10% and 29.10 ± 1.33% of carbohydrates when it was supplied with 25% CO_2 balanced with 75% CH_4 and Ar, respectively (Fig. 4a), while the protein content was 33.36 ± 1.60% and 27.89 ± 0.74%, respectively (Fig. 4b). Similarly, *Scenedesmus* sp. recorded 33.41 ± 1.07% and 26.33 ± 1.39% of carbohydrates supplied with 25% CO_2–75% CH_4 and Ar, respectively (Fig. 4c), and a protein content of 32.96 ± 2.26% and 28.36 ± 1.55%, respectively (Fig. 4d). Likewise, cell composition of both microalgae showed significantly higher differences when supplied with

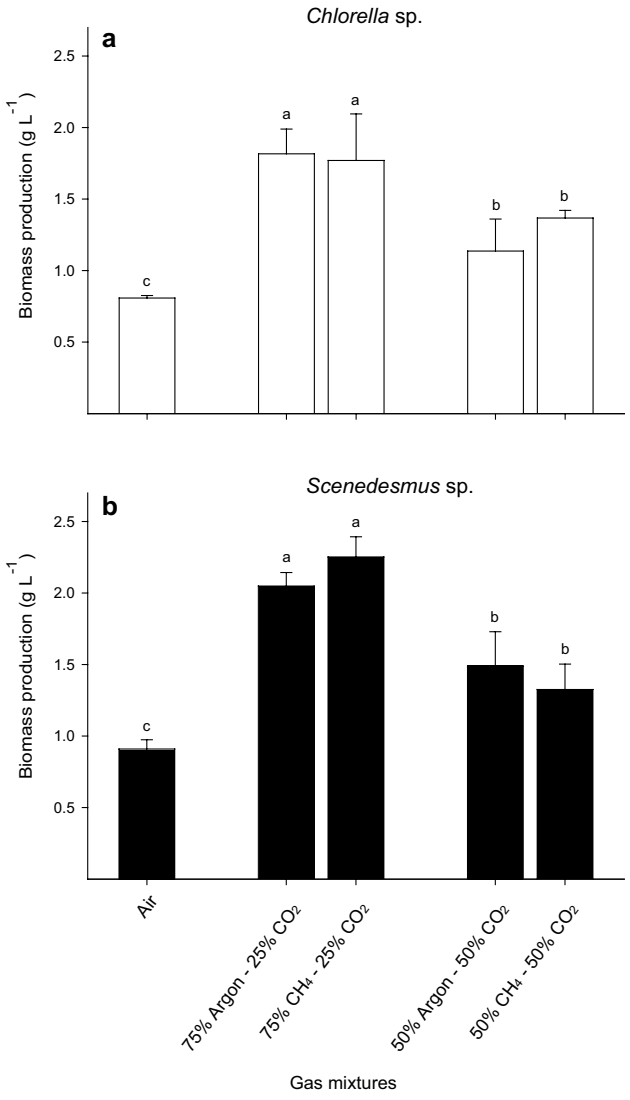

Fig. 3 Biomass production by *Chlorella* sp. (**a**) and *Scenedesmus* sp. (**b**) supplied with different gas mixtures. Columns denoted by different lowercase letters differed significantly when each microalga grew supplied with different gas mixtures. Statistical analyses were performed using ANOVA and LSD post hoc analysis ($P < 0.05$). Bars represent standard error

25% CO_2 rather than 50% CO_2 balanced either with Ar or CH_4 (Fig. 4, lowercase letters).

Discussion

Strains from the same group of microalgae can have different ability to tolerate high CO_2 and CH_4 concentrations, produce biomass, and accumulate cell compounds [2, 11, 14]. Thus, our study hypothesis was that *Chlorella* sp. and *Scenedesmus* sp., isolated from and native to Lago de Chapala, might have the biotechnological capacity to endure the stressful biogas

Table 2 Biomass productivity and specific growth rate of *Chlorella* sp. and *Scenedesmus* sp. supplied with different gas mixtures

Microalga	Gas mixture	Specific growth rate (μ; day^{-1})	Biomass productivity (P; $g\ L^{-1}\ day^{-1}$)
Chlorella sp.			
	Air	0.06 ± 0.02c	0.04 ± 0.01c
	25% CO_2–75% Ar	0.18 ± 0.05a	0.16 ± 0.02a
	25% CO_2–75% CH_4	0.16 ± 0.03a	0.15 ± 0.04a
	50% CO_2–50% Ar	0.10 ± 0.02b	0.08 ± 0.04b
	50% CO_2–50% CH_4	0.12 ± 0.02b	0.10 ± 0.01b
Scenedesmus sp.			
	Air	0.09 ± 0.04c	0.05 ± 0.02c
	25% CO_2–75% Ar	0.19 ± 0.03a	0.20 ± 0.03a
	25% CO_2–75% CH_4	0.20 ± 0.02a	0.22 ± 0.01a
	50% CO_2–50% Ar	0.15 ± 0.05b	0.13 ± 0.03b
	50% CO_2–50% CH_4	0.13 ± 0.03b	0.11 ± 0.04b

Values denoted by different lowercase letters differed significantly when each microalga grew supplied with five different gas mixtures. Statistical analyses were performed using ANOVA and LSD post hoc analysis ($P < 0.05$); ± represents standard error

composition, capture CO_2, produce biomass, and synthesize high-value cell compounds starting from biogas. Therefore, the aims of this study were to assess both *Chlorella* sp. and *Scenedesmus* sp., microalgae on their bio-refinery capacity, as well as the effect of high CO_2 and CH_4 concentrations of this effluent on their physiological capacity and biochemical composition to grow and capture CO_2 cultured under different biogas compositions.

Our results demonstrated that *Chlorella* sp. and *Scenedesmus* sp. have biotechnological potential to endure high CO_2 (25%) and CH_4 (75%) concentrations, as well as bio-refinery capacity to produce biomass and high-value metabolites from this effluent. These results might be attributed to the ability of both endemic microalgae to tolerate acid pH induced by CO_2 solubility in culture media in the form of dissolved inorganic carbon (DIC: HCO_3^-, CO_3^{-2}, and $H_2CO_3^-$) [11], since the CH_4 concentrations evaluated in this study did not negatively affect their physiological performance. According to Solovchenko and Khozin-Goldberg [16], the mechanisms that allow microalgae to tolerate high CO_2 concentrations are (1) ability to prevent acidification of the chloroplast stromal compartment and cytoplasm to maintain ribulose bisphosphate carboxylase–oxygenase activity (Rubisco; EC 4.1.1.39) and (2) ability to rapidly and reversibly shutdown the CO_2 concentrating mechanism (CCM), operating under atmospheric CO_2 levels but facilitating the drop of pH in cells under elevated CO_2 concentrations. Particularly in microalgae, CCM plays a vital role during the carbon fixation process, because it can enhance CO_2 level at the Rubisco active site by transporting inorganic carbon into

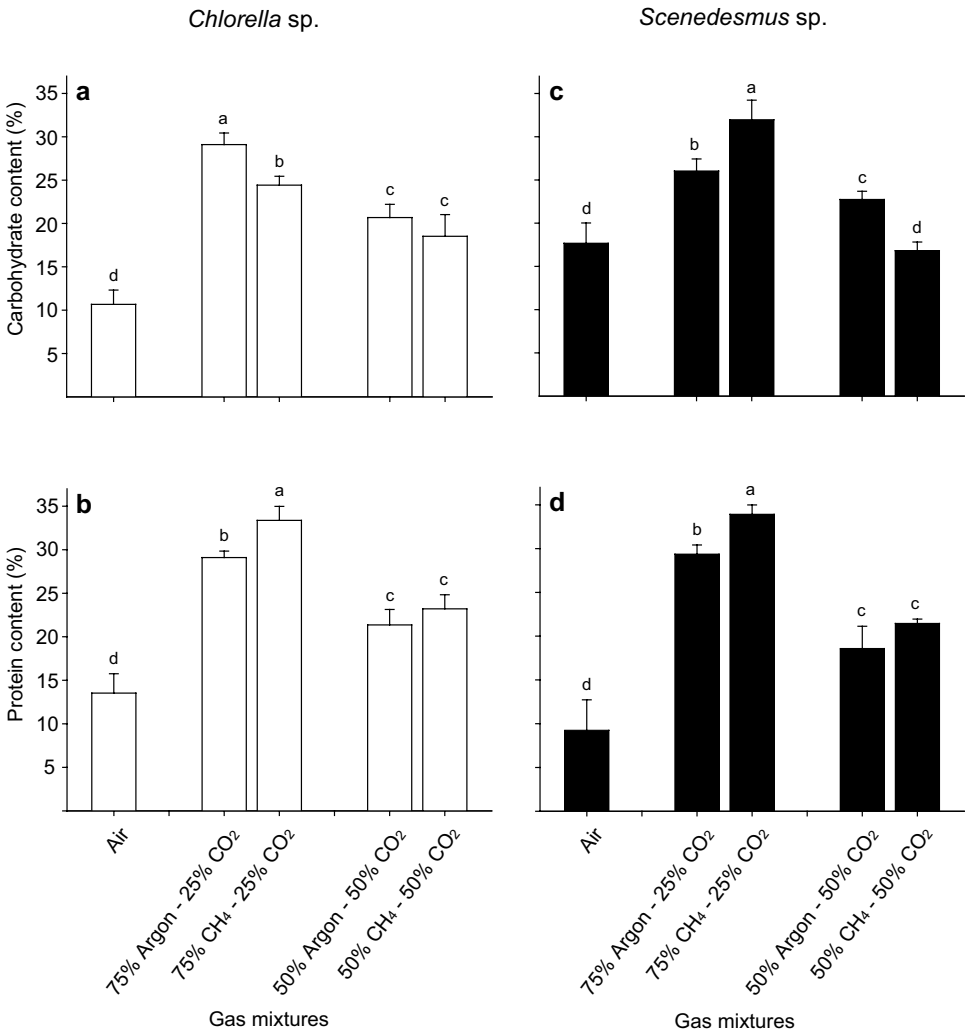

Fig. 4 Biochemical composition by *Chlorella* sp. (**a**, **b**) and *Scenedesmus* sp. (**c**, **d**) supplied with different gas mixtures. Columns denoted by different lowercase letters differed significantly when each microalga grew supplied with different gas mixtures. Statistical analyses were performed using ANOVA and LSD post hoc analysis ($P < 0.05$). Bars represent standard error

the cell and inducing an increase in photosynthetic rate [10, 11, 16]. The previous information can explain the high CO_2 fixation rates obtained by both microalgae evaluated when they were fed with biogas, since microalgae can perform the photosynthetic process under toxic CO_2, ammonia, cyanic acid, water vapor, and other toxic gases [24], although their tolerance level to CO_2 or CH_4 from biogas is dependent on the microalga strain [10, 14]. For instance, Kao et al. [4] claimed that *Chlorella* sp. MB-9 tolerated high CO_2 (20%) and CH_4 (80%) concentrations from biogas, while Thiansathit et al. [25] stated that a biogas composition of 40% CO_2–60% CH_4 was an optimum carbon source for *S. obliquus* TISTR 8522. Yan et al. [26] reported that the best CH_4 concentration during CO_2 fixation from biogas by *Chlorella* sp. was 45–55% (v/v), while Kao et al. [13] stated that growth rate of *Chlorella* sp. MM-2 decreased proportionally increasing CH_4 concentration from 20 up to 80%. In this study, CO_2 capture and biomass production were not inhibited when *Chlorella* sp. and *Scenedesmus* sp. were fed with biogas composed of 25% CO_2 balanced either with 75% CH_4 or Ar, indicating that CH_4 did not affect them negatively. However, both microalgae decreased their physiologic activity when they were provided with biogas composed of 50% CO_2–50% CH_4 or Ar, which suggested that this CO_2 concentration was adverse for both. Nonetheless, both microalgae assessed can be considered as CO_2 tolerant according to the division of CO_2-tolerant microalgae as established by Solovchenko and Khozin-Goldberg [16].

The above can be supported by DIC culture media uptake, since its concentration was lower when each microalga was provided with biogas composed of 25% CO_2 rather than 50%. It was happened, because the solubility of high CO_2 concentration in culture medium decreased pH significantly and inhibited the Rubisco and Carbonic anhydrase (EC 4.2.1.1) activities, vital during CO_2 capture by microalgae [19, 27]. For example, Meier et al. [6] demonstrated that the growth of *Nannochloropsis gaditana* CCMP-527 was completely inhibited with a pH less than 5.00 induced by the high CO_2 concentrations supplied. In this study, the pH of culture media decreased to 3.56 and 5.88 when supplied

with biogas composed of 50% and 25% CO_2, respectively. According to Razzak et al. [27] and Tang et al. [19], a pH of 5–7 is an optimum range for freshwater microalgae and Rubisco and carbonic anhydrase enzymatic activities. This result explains the higher CO_2 fixation rates, biomass productivities, and growth rates as recorded by both microalgae provided with 25% CO_2, since CO_2 capture by microalgae is directly correlated with parameter growth [27, 28], confirming that the 50% CO_2 provided in this study was detrimental for the physiological performance of both microalgae. The previous information highlights the importance of assessing and determining the optimal CO_2 and CH_4 concentrations for each microalga used.

On the other hand, both microalgae assessed in this study also showed the highest metabolite biosynthesis, mainly carbohydrates and proteins, supplied with biogas composed of 25% CO_2 balanced either with 75% CH_4 or Ar, while lipid accumulation was not detected under the experimental conditions of this study. Supplying the optimal CO_2 concentration to the microalga culture increased Rubisco activity and CO_2 fixation rate, inducing greater biomass production and high-value compound biosynthesis. However, cell compound biosynthesis is dependent on each microalga strain [29]. Previously, Choix et al. [30] demonstrated that *Chlorella* sp. and *Scenedesmus* sp. assessed in this study have the ability to synthesize mainly carbohydrates and proteins but no lipids. Particularly, carbohydrate biosynthesis by microalgae starting from CO_2 fixation is more viable energetically than lipid synthesis [31]. Moreover, the 3-phosphoglycerate produced during photosynthesis is a signal for high carbon and energy content within the microalga cell, activating the ADP-glucose pyrophosphorylase enzyme, key in starch biosynthesis by microalgae [32]. In addition, Cheng et al. [33] demonstrated that protein content reflected high metabolic activity, and cells grew constantly; which confirms the biorefinery capacity of both microalgae assessed in this study starting from CO_2 content from biogas and their commercial value.

Conclusions

Overall, our results show that *Chlorella* sp. and *Scenedesmus* sp. microalgae, native of Lago de Chapala-Mexico, are CO_2-tolerant and both have the biotechnological potential to endure stressful biogas composition (25% CO_2 and 75% CH_4) without affecting their physiological capacity to capture CO_2, grow and biosynthesize high-value metabolites. Besides, these results exhibit the bio-refinery capacity and commercial value of both microalgae accumulating mainly carbohydrates and proteins, useful for obtaining biofuels as ethanol or biogas. Although this study was performed at laboratory scale, it is important to highlight that both wild-type microalgae could be cultured to large scale to produce biomass and biosynthesizing high-value metabolites from secondary effluents generated during anaerobic digestion process of agro-industrial wastes. Finally, this study shows the importance of isolating and identifying microalgae from local ecosystems to determine their physiological capacity for each biotechnological application.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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