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Carbon dioxide fixation and lipid storage by *Scenedesmus obtusiusculus*

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HIGHLIGHTS

- ▶ CO_2 fixation by *Scenedesmus obtusiusculus* is among the highest values for Scenedesmaceae.
- ▶ *S. obtusiusculus* tolerates gas stream with 10% of CO_2 v/v.
- ▶ The microalga stores a high lipid content (55.7%) under nitrogen starvation.
- ▶ High lipid productivities (200 g m^{-3} d^{-1}) are achieved with *S. obtusiusculus*.

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ABSTRACT

An indigenous microalga was isolated from the springs in Cuatro Ciénegas, México. It was morphologically identified as *Scenedesmus obtusiusculus* and cultivated in bubble-column photobioreactors in batch operation mode. This microalga grows at 10% of carbon dioxide (CO_2) showing a maximum CO_2 fixation rate of 970 g m^{-3} d^{-1}. The microalga, without any nutrient limitation, contained 20% of nonpolar lipids with a biomass productivity of 500 g m^{-3} d^{-1} and a maximum biomass concentration of around 6,000 g m^{-3} at 5% CO_2 and irradiance of 134 μmol m^{-2} s^{-1}. Furthermore, it was observed that the microalga stored 55.7% of nonpolar lipids when 5% CO_2 was fed at 0.8 vvm and 54.7 μmol m^{-2} s^{-1} under nitrogen starvation. The lipid profile included C16:0, C18:0, C18:1n9t, C18:1n9c, C18:3n6 with a productivity of 200 g lipid m^{-3} d^{-1}. Therefore, the microalga may have biotechnological potential producing lipids for biodiesel.

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1. Introduction

The increase of carbon dioxide concentration in the atmosphere is considered the main cause of global warming. CO_2 emissions have grown exponentially at a rate of 6.5% per year (CDIAC, 2011) and the increase in deforestation has limited the capacity of the environment to absorb this extra CO_2 load. Emissions sources are mostly from the combustion of fossil fuels used in power generation, transport and industry. Also, gaseous discharges from power plants are responsible for more than 7% of the global release and contain about 15% v/v of CO_2 concentration (Matsumoto et al., 1997).

Some techniques have been developed for CO_2 mitigation including sequestration and storage, mineral carbonation and biological fixation by plants and algae. Biological CO_2 fixation with microalgae is 10 times more efficient than terrestrial plants and microalgae can grow about 10 to 50 times faster (Chen et al., 2011a). In addition, microalgae are a source of high-value products such as polyunsaturated fatty acids, natural colorants, biopolymers, therapeutic substances and biofuels. It is currently accepted that a combination of CO_2 fixation with biofuel production could yield a sustainable process for CO_2 mitigation.

Biodiesel obtained by transesterification of lipids from microalgae may provide a renewable and environmentally friendly fuel source. Despite the massive investments currently being made into algal biofuels research, the scalability, sustainability and cost-efficiency of full-scale algae cultivation for energy production remain unproven and controversial.

CO_2 fixation by microalgae has been positively correlated with its cell growth rate and light utilization efficiency, since it involves photoautotrophic growth (Jacob-Lopes et al., 2009). Some of the physicochemical parameters that affect CO_2 fixation include: temperature, medium composition, pH, light intensity, and CO_2 concentration (Ho et al., 2011). Hence, efficient CO_2 uptake coupled to the production of lipids requires extensive exploration for

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microalgae strains with high growth rate, lipid content, tolerance to high CO_2 levels and temperature (Yoo et al., 2010) as well as tolerance to NOx and SOx (Matsumoto et al., 1997).

Therefore, in this study, a microalga with high potential for application on CO_2 fixation was isolated. First, the effect of three key factors influencing cell growth: CO_2, aeration and light intensity were evaluated and then the accumulation of lipids assessed under nitrogen limitation.

2. Methods

2.1. Microorganisms and culture medium

The microalga was harvested from Poza Churince located 19.5 km southwest from the town of Cuatro Ciénegas, Coahuila, México which is located in the Chihuahuan Desert of Coahuila in north central Mexico. The basin is surrounded by high mountains and is a system of springs, streams, and pools. These ecosystems support several endemic species, living stromatolites and other microbial communities representing a desert oasis of high biodiversity (Souza et al., 2006). Endemic species are adapted to live in extreme conditions, such as lack of phosphorus and high incidence of solar radiation.

Water samples were distributed in 500 mL flasks capped with cotton plugs and with 100 mL of sterile BG11 medium containing in g L^{-1}: $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4·7H_2O$, 0.075; magnesium disodium EDTA, 0.001; $CaCl_2·2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; Na_2CO_3, 0.02; and the following salts in mg L^{-1}: H_3BO_3, 2.86; $MnCl_2·4H_2O$, 1.81; $ZnSO_4·7H_2O$, 0.222; $NaMoO_4·2H_2O$, 0.39; $CuSO_4·5H_2O$, 0.079; $Co(NO_3)_2·6H_2O$, 0.494; at pH 7.5 and incubation temperature of 30 °C under continuous illumination of 60 μmol m^{-2} s^{-1}.

The microalgal culture was then enriched in illuminated flasks and samples were taken for morphological identification. Single cells were separated into several 5 mL sterile tubes with modified Bristol medium containing in g L^{-1}: $NaNO_3$, 1.5; K_2HPO_4, 0.075; KH_2PO_4, 0.175; $MgSO_4·7H_2O$, 0.075; $CaCl_2·2H_2O$, 0.025; NaCl, 0.025; at pH 7.5, and cultivated at 20 °C under 12/12 h (light/dark) illumination of 42.85 μmol m^{-2} s^{-1}. Clonal cultures of microalgae growth with atmospheric CO_2 after 20 days. Then, morphological characteristics of the microalgae were observed with a microscope with differential interference contrast (Nikon Optiphot 1990, Japan).

2.2. Photobioreactor

Bubble column photo-biorectors (BCR) were located inside a chamber equipped with sixteen 40W-fluorescent tube lamps (OSRAM universal 40W day-light type, Brazil). The photobioreactors were built using 4 mm thick glass with an inner diameter of 0.105 m, height of 0.705 m and a nominal volume of 0.003 m^3. Air or CO_2 enriched air was supplied through a 0.105 m diameter sintered glass plate located at the bottom of the column.

2.3. Cultivation conditions

The microalga was cultivated in the BCR maintained at 30 °C with a working volume of 0.0025 m^3 and an inoculum of 20% v/v of a concentrated microalgal suspension. Various operating conditions were assayed including non-sterile gas flow rates of 0.4 or 0.8 vvm (volume of air per volume of reactor per minute), inlet CO_2 concentrations of 5% or 10% v/v and light intensities of 54.7, 94.4 or 134 μmol m^{-2} s^{-1} these were adjusted by switching on/off some of the lamps. CO_2 and air flow rates were measured with rotameters (Cole Parmer, USA) and the light intensity with a light meter (Extech Instruments 407026sp model 2.2, USA). Temperature and pH of the culture medium were monitored daily. Table 1 shows the conditions for each experiment. They were performed only once and lasted 13 days.

Microalgal cultivation with limited nitrogen source was performed to promote lipid accumulation. In these assays, two CO_2 concentrations were tested: 0.04% (air without CO_2-enrichment) and 5% CO_2. Also a two-stage process was implemented. In the first stage, the microalga was grown on complete medium to allow cell growth. In the second stage, the biomass was transferred to a nitrogen-limited medium (10% and 28% of the original nitrogen in mineral medium) to enhance the lipid content. In both cases, the microalga was cultivated in the BCR with BG11 medium, a light input of 54.7 μmol m^{-2} s^{-1} and a gas flow rate of 0.8 vvm.

2.4. Analytic determinations

2.4.1. Biomass, carbon content and nitrate concentration

Twenty mL of culture medium were collected daily and replaced with non-sterile distilled water. Biomass was quantified by filtering a known volume of culture medium through a 0.45 micron membrane and dried at 50 °C for 24 h. The carbon content of the biomass was determined using a CHNS analyzer (Series II 2400 CHNS/O Perkin Elmer, Boston, USA).

For nitrate determination, 2 mL of a sample previously filtered through 0.22 microns pore size membrane, were mixed with 1 mL of HCl 1 N and read at wavelength of 220 nm. A calibration curve was prepared using $NaNO_3$.

Total biomass after each run was recovered by flocculation. The pH of the culture medium was first adjusted to 7.5 with HCl or $CaCO_3$. Then, mixed in a 4:1 proportion with a solution containing chitosan/HCl/H_2O (100 mg of chitosan were dissolved into 10 mL of 0.1 M HCl solution and diluted with distilled water to 100 mL) and stirred by 30 min. The flocculated biomass solution was vacuum filtered with Whatman filter paper (no. 50). Recovered biomass was dried at 60 °C for 48 h. Dry biomass was pulverized in a mortar and sieved at 1 mm mesh. Hexane was used to extract the nonpolar lipids by the Soxhlet method.

Nile red (7-diethylamino-3,4-benzophenoxazine-2-one) (N3013 Sigma Aldrich) was used to determine daily the nonpolar lipid content. A 20 μL sample of the culture medium was mixed with 100 μL of dimethyl sulfoxide and shaken for 1 min. It was then heated in a microwave oven (100% power 1650 W) for 50 s, then 20 μL of Nile Red (0.25 mg L^{-1} in acetone) were added and diluted to 1 mL with distilled water. The mixture was shaken for 1 min and heated again for 60 s. The sample was maintained for 10 min in the dark and then transferred to a 2 mL vial with 1 mL of distilled water (total volume of the mixture was 2 mL) and analyzed in a fluorometer (Turner Designs Instrument model 7200-000, Sunnyvale CA, USA) (Chen et al., 2011b). The excitation and emission band on the equipment were 485 and 585 nm, respectively. The reading units given as Reference Fluorescent Units (RFU) were transformed to grams of lipid per liter of culture medium using a reference curve

Table 1
Parameters obtained at different light intensities and CO_2 inlet concentrations. gas inlet flow was 0.4 vvm.

CO_2 concentration (% v/v)	5	5	5	10	10	10
CO_2 load (kg $m^{-3}d^{-1}$)	40	40	40	80	80	80
Irradiance (μmol m^{-2} s^{-1})	54.7	94.4	134	54.7	94.4	134
X_{max} (g m^{-3})	3,370	3,120[a]	6,000	3,100[a]	2,700	5,700
P_{max} (g m^{-3} d^{-1})	250	320	500	260	310	520
μ_{max} (d^{-1})	0.18	0.23	0.37	0.18	0.34	0.38
P_{CO_2} (g m^{-3} d^{-1})	470	600	950	490	570	970

[a] The means are statistically equal, ANOVA $P < 0.0001$.

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