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Minireview

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The impact of environmental factors on carbon dioxide fixation by microalgae

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One sentence summary: Cultivation conditions have a direct impact on CO_2 fixation by microalgae.
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ABSTRACT

Microalgae are among the most productive biological systems for converting sunlight into chemical energy, which is used to capture and transform inorganic carbon into biomass. The efficiency of carbon dioxide capture depends on the cultivation system configuration (photobioreactors or open systems) and can vary according to the state of the algal physiology, the chemical composition of the nutrient medium, and environmental factors such as irradiance, temperature and pH. This mini-review is focused on some of the most important environmental factors determining photosynthetic activity, carbon dioxide biofixation, cell growth rate and biomass productivity by microalgae. These include carbon dioxide and O_2 concentrations, light intensity, cultivation temperature and nutrients. Finally, a review of the operation of microalgal cultivation systems outdoors is presented as an example of the impact of environmental conditions on biomass productivity and carbon dioxide fixation.

Keywords: carbon dioxide biofixation; microalgae; outdoor cultivations; pH; temperature; irradiance

INTRODUCTION

The accumulation of greenhouse gases in the atmosphere, from human activities and industrialization, has been considered as the main driver of climate change and is recognized as being among the major global problems facing mankind (IPCC 2014). While strong steps are being taken to reduce these emissions, carbon dioxide capture is a viable alternative (Mikkelsen, Jørgensen and Krebs 2010). A microalgal contribution to carbon dioxide capture was suggested in the 1960s by Oswald and Golueke (1960) and much attention has been drawn due to the higher solar energy yield and environmental tolerance of microalgae compared with higher plants, and to the possibility of year-round cultivation (Chisti 2007; John *et al.* 2011). Microalgae can also contribute to reducing global warming emissions by recycling carbon dioxide into biofuels (Chisti 2007), or through production of value-added products from flue gases. Furthermore, microalgae can also reduce carbon dioxide emissions from wastewater treatment by lowering the energy needed for aeration (Razzak *et al.* 2013), or during biogas upgrading (Meier *et al.* 2015). Nevertheless, worldwide carbon dioxide emissions (≈40 Gt year^{-1}, IPCC 2014) are too high compared with 13 600 t (IEA 2017) of commercialized microalgal biomass (around 27 200 t of carbon dioxide). This extremely low contribution of engineered processes emphasizes the need to increase productivity, as well as to optimize and scale up the existing technologies, in order to produce larger amounts of microalgal biomass. Economic

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viability of microalgal cultivation requires natural sunlight for photosynthetic carbon fixation, and an increasing number of scientists consider that large-scale outdoor cultivation remains the only viable option for cell and biofuel production at a commercial scale (Singh and Gu 2010; Chen *et al.* 2011; de Vree *et al.* 2015; Eustance *et al.* 2016; Ho *et al.* 2017). It has been reported that carbon dioxide fixation by microalgae might become economically viable when production costs fall below \$500 t^{-1} ha^{-1} $year^{-1}$ (Bilanovic and Holland 2012).

Efficient microalgal carbon dioxide mitigation depends on microalgal species, light, carbon dioxide source and bioavailability, as well as photobioreactor design and operating conditions (Cheng *et al.* 2006), and very exhaustive reviews have reported the carbon dioxide removal efficiencies, biomass productivities and carbon dioxide fixation ability of a broad spectrum of microalgae growing at different operational conditions (Farrelly *et al.* 2013; Singh and Singh 2014; Zhao and Su 2014; Cuellar-Bermudez *et al.* 2015).

A key parameter influencing biomass productivity is the photosynthetic conversion efficiency, representing the luminous energy transformed to chemical energy, which is at the base of the anabolic reactions leading to biomass production. The maximum theoretical efficiency of solar energy conversion into biomass is around 12% (Weyer *et al.* 2010; Williams and Laurens 2010; Ooms *et al.* 2016). This value is calculated on the basis that only 47% of the total solar spectrum is available for photosynthesis and a maximum absorption of only 27% is achieved by the photosynthetic system from the photosynthetic active radiation. Finally, an additional 10% reduction is considered due to photo-transmission losses. However, photosynthetic conversion efficiency values between 1.5%, for open raceway ponds, and 3.8–5% for tubular or flat panel photobioreactors have been reported in outdoor conditions (Norsker *et al.* 2011; de Vree *et al.* 2015) and of 6% under laboratory conditions (Norsker *et al.* 2011). Higher photosynthetic conversion efficiency should allow higher productivities and lower costs (Wijffels, Barbosa and Eppink 2010; Wijffels and Barbosa 2010).

The main problem associated with outdoor algal cultivation is the limited productivity resulting from (i) light attenuation along the path in the cultivation system; (ii) excessive light impinging on the superficial layers causing photoinhibition (Janssen and Jack 2016); (iii) non-optimal temperatures due to climatic or seasonal conditions; (iv) respiratory losses of biomass; (v) salinity variations driven by water evaporation; (vi) inhibitory high oxygen concentration (Cohen, Koren and Arad 1991); and (vii) cultivation failure by bio-contamination with fungal or bacterial species, competing algae, predators, parasites, viruses, etc. In the case of biofuel production, strains should be found with high lipids or carbohydrates for biodiesel or biogas (hydrogen or methane) that are capable of maintaining fast growth rates under the nutrient limitation required to trigger the accumulation of those compounds. While some key parameters, such as pH, may be maintained, others, including temperature and irradiance, cannot be controlled to the same extent as under laboratory conditions (Wolf *et al.* 2016). Some of the most important environmental factors affecting growth rate, biomass productivity and carbon fixation will be reviewed here.

PHOTOSYNTHESIS AND CARBON DIOXIDE CONCENTRATING MECHANISM

Photosynthesis allows microalgal carbon dioxide fixation and the production of life-sustaining organic molecules. In the light-dependent cycle, photons from sunlight provide energy to split water into molecular oxygen, and chlorophylls and carotenoids in the photosystems (I and II) are the key light-harvesting molecules that funnel the absorbed energy into the electron transport chain to create the high-energy and reducing molecules, ATP and NADPH, respectively. These are further used in the Calvin–Benson cycle to capture carbon dioxide through ribulose bisphosphate carboxylase–oxygenase (Rubisco) enzyme mediation. (Information about the time scale of these processes can be found in Falkowski and Raven (1997) and Luo and Al-Dahhan (2011).) The low Rubisco specificity is compensated through a carbon dioxide concentrating mechanism by enhancing the carbon dioxide concentration up to 1000 times with respect to the carbon dioxide in the liquid medium (Price *et al.* 2008) and by decreasing photorespiration (Zhao and Su 2014).

When carbon dioxide dissolves in water, it can be found as carbon dioxide, HCO_3^-, CO_3^{2-} and H_2CO_3. Among these, carbon dioxide and HCO_3^- are the major species used by microalgal cells. Carbonic anhydrases play a key role in the carbon dioxide concentrating mechanism and are found in the periplasm, cytosol and chloroplasts, having different functions, such as maintaining the carbon dioxide and HCO_3^- equilibrium or fostering transport through membranes. Once Rubisco acts, carbon dioxide is transformed into the carbohydrate precursors used for cell metabolism and growth (Zhao and Su 2014).

MICROALGAL GROWTH

The simple structure of microalgae permits fast cell growth with doubling times of 1 or 2 days or less (a few hours) and higher carbon dioxide fixation efficiencies than terrestrial plants (Richmond 2004). Microalgal growth is influenced by both biotic and abiotic factors that include, among others, temperature, pH, carbon dioxide, dissolved O_2, light intensity and nutrients present in the medium. Additionally, the chemical composition of microalgal biomass also depends on the microalgal species, the growth phase and even on harvest time (Sánchez-Mirón *et al.* 2003; Juneja, Ceballos and Murthy 2013). Therefore, it is important to know the single and combined effect of the above-mentioned key parameters to improve the productivity of biomass and its products.

Nutrients

The molecular formula of microalgal biomass has been reported as $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti 2007), which can help to determine the nutritional requirements for cell growth. Table 1 summarizes the main nutrients constituting microalgal cells. As can be observed, nutrients include nitrogen (N), phosphorus (P), magnesium (Mg), sulfur (S) and trace element. The major cell component is carbon at around 50%, so to produce 1 ton of algal biomass, 1.8 tons of carbon dioxide need to be fixed (Chisti 2007; Alcántara, García-Encina and Muñoz 2013). carbon dioxide can be supplied from the atmospheric air, industrial gaseous streams (e.g. flue gas) (Pires *et al.* 2012; Cuellar-Bermudez *et al.* 2015; Duarte *et al.* 2016; Fistarol, Farias and Salomon 2016; Thomas, Mechery and Paulose 2016) or even soluble carbonates (e.g. $NaHCO_3$ and Na_2CO_3) (Abinandan and Shanthakumar 2016; Mokashi *et al.* 2016; Wang *et al.* 2008). Atmospheric carbon dioxide concentration is low (around 400 ppm) and cannot sustain an intensive production of microalgal biomass; therefore, streams with higher carbon dioxide content, such as those found in flue gases, are preferred. Nevertheless, certain aspects of flue gases have to be considered, including their temperature and the presence of other gaseous components, such as SO_x

Table 1. Nutrient functions in microalgal cultivation (adapted from Zeng *et al.* 2011).

Source	Main nutrient	Example of function	Concentration range
Carbon	CO_2, HCO_3^-, CO_3^{2-}, etc.	Synthesis of carbohydrates, protein, lipids of the whole cell	1–10 g L^{-1}
Nitrogen	NO_3^-, urea, ammonia, N_2, etc.	Synthesis of nucleic acids, proteins, pigments such as chlorophyll and phycocyanin	10–2000 mg L^{-1}
Phosphorous	Hydrophosphate, phosphate, etc.	Part of the backbone of DNA and RNA, ATP, phospholipids	10–500 mg L^{-1}
Sulfur	Sulfate	Formation of the amino acids methionine and cysteine	1–200 mg L^{-1}
Inorganic salts	K, Ca, Na, Mg, etc.	Maintenance of cell structure and activity	0.1–100 mg L^{-1}
Trace elements	Fe, Zn, Mn, Pb, Cd, etc.	Coenzyme factors	0.01–10 mg L^{-1}
Vitamins	B, C, E, etc.	Aid to cell division	0.01–1000 μg L^{-1}

Table 2. Common optimum conditions for microalgae growth.

Microalgae	Temperature (°C)	Irradiance (μmol m^{-2} s^{-1})	pH	CO_2 tolerance (%)	Mixing	Reference
Chlorella sp.	27	100	7	40	Aeration	Hanagata *et al.* (1992), Kim *et al.* (2012)
Dunaliella sp.	25	100		10	Aeration	Kim *et al.* (2012)
Dunalliella salina DCCBC2	27	80	8	5	Aeration	Kim *et al.* (2012)
Haematococcus pluvialis	25–28	90	7	34	Aeration	Fan, Vonshak and Boussiba (1994), Sarada, Tripathi and Ravishankar (2002), Huntley and Redjalje (2007)
Euglena gracilis	27–31	100	–	45	Fermenter	Nakano *et al.* (1996), Kitaya, Azuma and Kiyota (2005)
Phaeodactylum tricornutum	20.4	10	7.8	15	Aeration	Bitaubé, Caro and Peréz (2008)
Scenedesmus almeriensis	35	200	7–8	–	Aeration	Costache *et al.* (2013)
Scenedesmus obtusiusculus	35	300	7–8	15	Aeration	Cabello, Morales and Revah (2015)
Spirulina platensis	30	330	9–10	10	Aeration	Kebede and Ahlgren (1996), Oliveira *et al.* (1999), Richmond (2004), Colla *et al.* (2007), Kumar *et al.* (2010)
Spirulina maxima	35	–	9–10	–	Aeration	Oliveira *et al.* (1999), Richmond (2004)

and NO_x (Yen *et al.* 2015; Duarte-Santos *et al.* 2016; Sun, Zwolinska and Chmielewski 2016) or metals (Napan *et al.* 2015; Hess *et al.* 2017) that could be toxic to microalgae. Nitrogen is the second major component in importance for microalgal growth and may interact with the carbon source. Bilanovic *et al.* (2009) used the response surface methodology to define carbon dioxide and N concentrations to maximize both biomass production and carbon dioxide fixation capacity and found that nitrogen concentration for freshwater algae should be between 285 and 427 mg_N L^{-1} for carbon dioxide concentrations from atmospheric to 70%. Phosphorous is necessary to synthesize amino acids, phospholipids and energy carriers and it is generally provided as phosphates. Nutrient limitation (mainly N or P) shifts the cell metabolism from growth to storage of high energy content molecules, such as lipids and carbohydrates (Takagi *et al.* 2000; Khozin-Goldberg and Cohen 2006; Rodolfi *et al.* 2009; Xin *et al.* 2010; Juneja, Ceballos and Murthy 2013), but the specific carbon capture rate is generally lowered. Oligo- and trace elements are also required in low amounts and sodium chloride (NaCl) affects carbon dioxide and O_2 solubility and helps to regulate biomass growth and product formation (Ben Amotz, Tornabene and Thomas 1985; Takagi, Karseno, Yoshida 2006). In conclusion, medium formulation is a necessary step to increase growth rates and productivity and, therefore, carbon dioxide capture, considering both the cell composition and process requirements (Danquah *et al.* 2010).

EFFECT OF ENVIRONMENTAL FACTORS ON carbon dioxide BIOFIXATION

In this section, the main environmental factors affecting photosynthesis and, consequently, the growth rate, biomass productivity and carbon fixation are analyzed and Table 2 summarizes the optimum values for some species.

Irradiance

Light intensity is essential for photosynthesis, microalgal growth and, therefore, carbon dioxide fixation (Fernández *et al.* 2012; Costache *et al.* 2013). During photosynthesis, the products of light reactions, ATP and NADPH, are used to fix carbon dioxide by Rubisco in the Calvin–Benson cycle. Both molecules are provided from light-dependent reactions; therefore, a good light supply is necessary. On the one hand, low light conditions lead to low biomass concentration and carbon dioxide fixation. On the other hand, excess light increases the production of reactive oxygen species that are responsible for abnormal physiological reactions due to oxidative stress (Seo *et al.* 2017). They damage the essential proteins required for electron transfer during photosynthesis and, consequently, carbon dioxide fixation is reduced.

The empirical relationship between irradiance and photosynthesis is graphically depicted by photosynthesis-irradiance

(*P–I*) curves (Béchet, Shilton and Guieysse 2013). These curves are species-dependent and need to be experimentally determined under uniform light intensity (Béchet, Shilton and Guieysse 2013). Generally, three regions are identified: (i) a region limited by the rate of capture of photons where the photosynthetic rate is directly proportional to irradiance; (ii) the 'light-saturation' region where photosynthesis is irradiance-independent—here, photosynthesis is limited by the reaction rates achieving maximum value (Béchet, Shilton and Guieysse 2013); and (iii) the photoinhibition region at high irradiances where a decrease in photosynthesis is observed (Camacho-Rubio *et al.* 2003). However, an important issue relating to *P–I* curves is that they are determined under uniform light intensity, contrary to the situation found during cultivation in photobioreactors, where dense cultures can be achieved. Consequently, *P–I* curves cannot be used directly to predict productivity in dense cultures, where the light regime must be known or some valid correlations must be used to predict light attenuation based on depth, biomass concentration and mixing patterns.

Photo-acclimation allows microalgae to respond to light changes, balancing the light reactions, the energy demand for carbon dioxide fixation and other metabolic reactions (Vonshak and Torzillo 2004). The photo-acclimation periods vary according to species (Falkowski and Chen 2003; Nikolaou *et al.* 2016). In the short term (time scales of seconds to minutes), adaptation includes adjustments in the delivery of light energy to photosystem II through reorganization of photosynthetic complexes (Mooij *et al.* 2017). Long-term acclimation comprises changes in enzyme activity and gene expression affecting both the structure and the stoichiometry in the photosystems (Cabello, Morales and Revah 2014). Photoinhibition occurs in systems exposed to high irradiances, just above the value where maximum photosynthetic activity occurs (Camacho-Rubio *et al.* 2003; Pulz and Gross 2004), and involves deactivation of proteins in photosynthetic units (Camacho-Rubio *et al.* 2003; Fan *et al.* 2007). This process is time-dependent and a half of the photosynthetic system might be irreversibly damaged after a period of 10–20 min of exposure (Pulz and Gross 2004).

Different light sources are used for microalgal cultivation, including natural sunlight and artificial light. Kommareddy and Anderson (2003) compared different light sources, including incandescent, halogen, fluorescent, AlInGaP II (aluminum indium gallium phosphide with a peak at a wavelength of 643 nm) and light emitting diodes. They found that light emitting diodes and AlInGaP II were the most efficient and economical light sources. Recent advances include illumination with selected wavelengths, dye compounds to lower the energy photons, fluorescent paints and nanoparticles to filter the light (Ramanna, Rawat and Bux 2017). Although many studies are performed indoors with artificial light under controlled conditions, large scale cultivation systems operate outdoors with solar light depending on the circadian cycle, location, season of the year and climatic conditions. Furthermore, a successful cultivation system with high biomass productivity must have high cell concentrations to minimize costs (Molina-Grima *et al.* 2003); however, this high biomass content produces light gradients in the medium and cells are exposed to different light intensities (Béchet, Shilton and Guieysse 2013).

Light supply may be continuous or with intermittent photoperiods. Cycles of 12:12 or 18:6 dark–light cycles have been reported as adequate to achieve good carbon dioxide fixation (Ramanna, Rawat and Bux 2017); however, Jacob-Lopes *et al.* (2009) found that during continuous illumination, carbon dioxide fixation by *Aphanothece microscopica* Nägeli microalgae reached 99.69% of elimination efficiency. Furthermore, algal growth and carbon dioxide fixation are affected not only by light–dark cycle, but also by light intensity. Thawechai *et al.* (2016) found that the optimal conditions of 60 μmol photons m^{-2} s^{-1} and continuous illumination allowed a carbon dioxide fixation rate of 0.729 mg L^{-1} day^{-1}. Contrary to those results, Sun and Duan (2007) found that for the microalga *Tetraselmis tetrathele*, light cycles ranged from 6 to 18 h, whereas the cell density, chlorophyll *a* and protein content reached high levels suggesting the importance of an optimized light cycle to maximize metabolism and algal growth.

Temperature

Temperature influences both physicochemical carbon dioxide availability to the cells and the metabolic processes within the cell (Ördög *et al.* 2016; Paliwal *et al.* 2017). carbon dioxide solubility depends on temperature; it is known that higher temperatures (>20°C) lower the carbon dioxide solubility. On the other hand, increases in temperature decrease Rubisco's affinity for carbon dioxide (Kumar *et al.* 2011). However, the temperature effect on the reaction metabolic rate is strain dependent. Light and temperature were the most important factors affecting the biomass productivity and carbon dioxide fixation in a long-term experiment in which *Scenedesmus obliquus* was grown in outdoor photobioreactors with N and carbon dioxide maintained at a constant level (Hindersin *et al.* 2014).

Ras, Steyer and Bernard (2013) performed a review of the effect of temperature on different species, emphasizing the biochemical effect and the adaptation to high temperatures. Microalgae usually grow in a temperature range between 15 and 30°C with optimal values at 20–25°C. It has also been reported that temperatures below 16°C affect particularly mechanisms of carbon fixation (Ras, Steyer and Bernard 2013) and metabolic activity in general. On the other hand, high temperatures lower carbon dioxide solubility (Zhao and Su 2014) and temperatures above 35°C are normally lethal for many species (Briassoulis *et al.* 2010), decreasing the growth rate and increasing both respiration and photorespiration. Temperature is especially important for treatment of flue gases (temperatures around 120°C) or large-scale outdoor cultivation with circadian variations between 10 and 45°C. In these cases, the use of temperature-tolerant organisms could result in savings related to cooling costs. Some of the reported photosynthetic organisms include *Chlorella pyrenoidosa* (optimum around 39°C and tolerance up to 45°C), *Chlorogleopsis* sp. (isolated from a hot spring in the Yellowstone National Park; grows at 50°C; Ono and Cuello 2007) and *Synechococcus* sp. (Miller and Castenholz 2000; 42–75°C), which exhibits the thermal maximum for photosynthetic life (Brock 1967; Castenholz 1969).

pH

The pH regulates, on the chemical level, the distribution of different dissolved inorganic species (carbon dioxide, HCO_3^- and CO_3^{2-}; de Morais and Costa 2007). It is also one of the important factors for algal growth because it affects the activity of different enzymes (Zhang *et al.* 2016). Its value has a strong influence on cell growth and the optimum and tolerance are species-dependent. The optimum pH is normally in the neutral to slightly alkaline range (6–8.3; Moss 1973; Azov 1982; Olaizola 2003; Pandey, Pathak and Tiwari 2010; Ying, James Gilmour and Zimmerman 2014). However, some organisms bloom in extreme conditions. *Spirulina* grows in highly alkaline conditions (pH 9–11; Pandey, Pathak

and Tiwari 2010; Koru 2012; Sharma et al. 2014). Alkaline conditions, at pH above 11.0, could help to maintain microalgal cultures free of contaminants, such as protozoa and other competing microalgae (Touloupakis et al. 2016), and also promote neutral lipid accumulation in microalgal cells (Zhang et al. 2016). Other microalgae thriving in more acidic conditions include the autotrophic Chlorophyta (*Chlamydomonas acidophila*, *Dunaliella acidophila*), Chrysophyta (*Ochromonas* sp.) and Euglenophyta (*Euglena mutabilis*), filamentous algae identified as *Zygnema circumcarinatum* and *Klebsormidium acidophilum* (Sung et al. 1998; Novis and Harding 2007; Rowe et al. 2007), and *Chlorella protothecoides* var. *acidicola* isolated from mine-impacted water bodies, which grows optimally at pH 2.5 under heterotrophic conditions (Nancucheo and Johnson 2012).

During carbon dioxide fixation and microalgal growth, pH variations occur in relation to carbon dioxide dissolution, equilibrium of the dissolved inorganic carbon species and the uptake of carbon dioxide and nitrate. However, these changes also depend on the carbon dioxide input concentration. When HCO_3^- is used, the carbonic anhydrase converts it to carbon dioxide resulting in the release of the hydroxyl ion (OH^-) and consequently an increase in pH. This situation indicates carbon dioxide limitation affecting the system performance and carbon dioxide supply and allows the control of culture pH at its optimal value. Recently, Duarte-Santos et al. (2016) have used the strategy of pH control with injection of carbon dioxide-rich gases on demand. This allowed them to meet the carbon requirements of microalgal cultures and to optimize biomass productivity and carbon dioxide fixation. That study was performed in a pilot scale- raceway pond, to produce high value-added compounds, such as phycocyanin (Chen et al. 2016). The same strategy was also reported to control the protozoa (ciliates or amoebae) (Ma et al. 2017). High carbon dioxide concentrations, similar to those found in flue gases (10–25%), acidify the cultivation medium when continuously fed (Zhao and Su 2014; Choi et al. 2017). Furthermore, other chemicals, such as SO_2 or NO_x from flue gases, reduce the pH when they dissolve in water (Kao et al. 2014; Cuellar-Bermudez et al. 2015; Yen et al. 2015; Fistarol, Farias and Salomon 2016). Consequently, pH control by addition of neutralizing agents, by designing buffers or by defining strategies of carbon dioxide supply is critical to avoid growth inhibition.

carbon dioxide concentration

carbon dioxide fixation involves mass transfer and solubilization from the gaseous stream to the liquid phase, attaining the ionic equilibrium between carbon dioxide, HCO_3^- and CO_3^{2-}, and carbon assimilation by the microalgal cells. Tolerance and optimal carbon dioxide concentration are strain-dependent (Solovchenko and Khozin-Goldberg 2013) and most microalgae generally grow well at 2% carbon dioxide, but levels above 5% carbon dioxide (Cheng et al. 2006) may inhibit their growth (Yun et al. 1997; Chiu et al. 2008). This effect may be related to the acidification of the stroma in the chloroplast (Solovchenko and Khozin-Goldberg 2013) causing the inactivation of key enzymes of the Calvin–Benson cycle. Singh and Singh (2014) reviewed the influence of carbon dioxide levels on different microalgal species including *Scenedesmus obliquus*, *Botryococcus braunii*, *Chlorella vulgaris* and *Nannochloropsis oculata*. They reported that the highest carbon dioxide concentration was fixed by *S. aquatilis*, *B. braunii*, *C. vulgaris* and *Synechococcus* species. However, according to Singh and Ahluwalia (2013), although the most carbon dioxide-tolerant strains were *Chlorella* sp. T-1 (100% carbon dioxide), *Scenedesmus* sp. (80% carbon dioxide) and *Euglena gracilis* (45%), their maximum biomass productivity was found at carbon dioxide concentrations of 10%, 10–20% and 5%, respectively. Likewise, *Chlorella* sp. KR-1 showed the maximum growth at 10% carbon dioxide and tolerated up to 70% carbon dioxide (Sung et al. 1998). The above-mentioned range is important because some microalgae can grow with 10–15% carbon dioxide, the range normally found in flue gases, which are an important greenhouse gas source of pollution, but may be an excellent carbon dioxide source for microalgae.

Dissolved O_2 concentration

Dissolved oxygen is an indicator of photosynthetic activity by microalgae, and registered values are higher than those found in equilibrium with air. Low dissolved oxygen indicates problems with the microalgal growth and possibly dissolved oxygen consumption by heterotrophic microorganisms. The build-up of O_2 in microalgal cultivation systems is one of the major problems in obtaining high biomass productivity and carbon dioxide fixation rate. Dissolved oxygen may exceed 250% of saturation during daytime (Fernández et al. 2012; Raso et al. 2012; Costache et al. 2013; Bilanovic et al. 2016). High oxygen concentration leads to the oxidative stress of reactive oxygen species and also competes with carbon dioxide for the Rubisco involved in carbon dioxide fixation. The carbon dioxide and O_2 concentrations define carboxylation (photosynthesis) and oxygenation (photorespiration) prevalence. Low carbon dioxide/O_2 ratios reduce the photosynthetic rate and, therefore, carbon dioxide fixation and favor photorespiration and carbon dioxide release (Costache et al. 2013). To avoid dissolved oxygen accumulation, both design and operational issues must be considered. They include the establishment of maximum tube length in photobioreactors and promotion of oxygen release to the gas phase by degassing the liquid and promoting turbulence of the medium (Grobbelaar 1994; Camacho-Rubio et al. 1999).

METHODOLOGICAL EVALUATION OF THE EFFECT OF ENVIRONMENTAL VARIABLES (carbon dioxide, TEMPERATURE, PH AND IRRADIANCES)

The experimental assessment of the effect of key factors reviewed above is vital to determine the optimal operation conditions to maximize growth rates and carbon dioxide capture. Data on the effects on photosynthetic activity are generally gathered by cell growth or oxygen production rates. Microalgal growth is normally measured in photobioreactors by biomass accumulation during the cultivation time and results from the combination of biological, environmental and mass transfer effects. This approach does not allow the independent evaluation of key variables and depends on the changing operational conditions and photobioreactor configuration. Furthermore, it requires a time scale of days to obtain significant results (Butterwick, Heaney and Talling 2005; Breuer et al. 2013). On the other hand, the photosynthetic activity can be determined at different culture conditions through dynamic oxygen measurements (Bitaubé, Caro and Peréz 2008; Brindley, Acién and Fernández-Sevilla 2010; Fernández et al. 2012; Costache et al. 2013). This approach yields results on a time scale of minutes and is proportional to the growth rate according to the stoichiometry of the overall photosynthetic reaction (Brindley, Acién and Fernández-Sevilla 2010). Oxygen response to changing conditions also provides information about the photo-acclimation phenomena generated in

photosystem II as a response to fluctuations in light intensity (Satoh *et al.* 2002; Avendaño and Shubert 2005). Furthermore, the time needed for determination of the photosynthetic irradiance curves and the associated kinetic parameters is reduced and some models have been developed for predicting O_2 production (Camacho-Rubio *et al.* 1999; Rebolloso *et al.* 1999; Concas and Pisu 2010; Fernández *et al.* 2012; Hu *et al.* 2012; Béchet, Shilton and Guieysse 2013).

Dynamic dissolved O_2 measurements coupled to mass balance and transfer models have been used to evaluate the effect of irradiance, temperature and pH on the photosynthetic activity for the microalga *Scenedesmus obtusiusculus* (Cabello, Morales and Revah 2015). Here, thermodynamic and intrinsic kinetic parameter data were evaluated in a mini-photobioreactor, where self-shading, mass transfer effects and carbon dioxide limitation were minimized. Results with the optimized conditions were further tested successfully in controlled flat-panel photobioreactors to produce biomass and lipids (Morales, Cabello and Revah 2015).

Dynamic methods were also used to evaluate the photosynthetic activity of the same microalga to different light intensities in an air-lift photobioreactor through step changes under N-replete and N-starved conditions (Cabello, Morales and Revah 2014), as well as the effect of variations of carbon dioxide concentration (Cabello, Morales and Revah 2017). In this case, during the experiments on-line monitoring of pH, carbon dioxide and

Table 3. Common optimum conditions for microalgae growth.

Microalgae	Temperature (°C)	Irradiance (μmol m^{-2} s^{-1})	pH tolerance	CO_2 tolerance	Photobioreactor conditions	Growth rate (day^{-1})	Biomass Productivity (g L^{-1} day^{-1})	CO_2 biofixation rate (mg L^{-1} day^{-1})	Reference
Chlorella sp.	27	100	7	40%	Conical flask, 8 days continuous aeration, air + CO_2; horizontal bubble column, 11 days, continuous aeration, N_2 + CO_2	0.38	0.09	7.2	Hanagata *et al.* (1992), Kim *et al.* (2012)
Dunaliella sp.	25	100	8	5%	Horizontal bubble column, 11 days, continuous aeration, N_2 + CO_2	0.25	0.12	10.4	Kim *et al.* (2012)
Dunalliella salina DCCBC2	27	80	8	10%	Horizontal bubble column, 11 days, continuous aeration, N_2 + CO_2	0.42	0.13	8.2	Kim *et al.* (2012)
Haematococcus pluvialis	25–28	90	7	34%	Bubble column, aeration air + CO_2	1.29			Fan, Vonshak and Boussiba (1994), Sarada, Tripathi and Ravishankar (2002), Huntley and Redjalje (2007)
Euglena gracilis	27–31	100		45%	Photobioreactor, 28 days, flue gas 11% CO_2, continuous operation	0.31	0.29	74	Nakano *et al.* (1996), Kitaya, Azuma and Kiyota (2005)
Phaeodactylum tricornutum	20.4	10	7.8	15%	Erlenmeyer flask, aeration; Roux bottles, 11 days, continuous aeration air + CO_2		0.15	280[a]	Bitaubé, Caro and Peréz (2008)
Scenedesmus almeriensis	35	200	7–8	10%	Tubular photobioreactor, outdoor conditions, flue gas (pure CO_2) on demand, operated continuously	0.34	0.42	790	Costache *et al.* (2013)
Scenedesmus obtusiusculus	35	300	7–8	10%	Bubble column, 14 days, continuous aeration air + CO_2	0.34	0.52	970[a]	Cabello, Morales and Revah (2015), Toledo-Cervantes *et al.* (2013)
Spirulina platensis	30	330	9–10	10%	Bubble column, 25 days, continuous aeration, greenhouse conditions	0.65	0.15	280[a]	Kebede and Ahlgren (1996), Oliveira *et al.* (1999), Richmond (2004), Colla *et al.* (2007), Kumar *et al.* (2010)
Spirulina maxima	35		9–10		Container of glass, 15 days, mechanical agitation	0.6	0.15	280[a]	Oliveira *et al.* (1999), Richmond (2004)

[a]Calculated from the biomass productivity according to the equation: CO_2 fixation rate (P_{CO_2}) = 1.88 × biomass productivity (g L^{-1} day^{-1}), which is derived from the molecular formula of microalgal biomass, $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$.

Table 4. Seasonal growth of *Scenedesmus obtusiusculus* in air-lift extended loop photobioreactor.

Conditions	Productivity ($g\ L^{-1}\ day^{-1}$)	carbon dioxide fixation[a] ($g\ L^{-1}\ day^{-1}$)	Temperature range (°C)	I_{max} ($\mu mol\ m^{-2}\ s^{-1}$)/mol photons	PE[b] (%)
Winter	0.19	0.36	5–31	1536/92.9	4.11
Spring	0.29	0.55	11–47	2035/98.8	5.34
Summer	0.21	0.40	5–26	2055/71.2	4.13
Autumn	0.22	0.42	7–45	2100/88.5	4.21
Optimum laboratory conditions[c]	0.97	1.84	35.2	300/15.6[d]	4.4

[a]Calculated from theoretical value of 1.8 g carbon dioxide g biomass^{-1}. [b]Photosynthetic efficiency (PE) is calculated as $PE = (X_t V \Delta H E_N)/(EAI_t)$ where X_t is the biomass at time t, A is the illuminated area of the reactor, V is the rector volume, ΔH is the combustion heat of the microalgal biomass with values between 20.6 and 26.7 kJ g^{-1} for a microalgal biomass with a lipid content of 54.7% and 18.1%, respectively, I_t is the impinging irradiance at the time t, E_N is the coefficient related to the energy required for nitrogen assimilation with values of 1.25 for nitrate assimilation (Williams and Laurens 2010), E is the energy from photosynthetically active radiation (210 KJ mol photons^{-1}) (Dillschneider *et al.* 2013).[c] Evaluated in a flat panel photobioreactor. [d]Continuous illumination.

dissolved O_2 was performed. The dynamic experiments allowed a rapid analysis of the short-term carbon dioxide consumption rates in response to changes in inlet gas carbon dioxide concentration.

OUTDOOR OPERATION

The impact of environmental conditions on carbon dioxide capture and biomass growth is clearly observed in outdoor cultivation. The microalgal growth rate is drastically lower than that obtained under controlled conditions in the laboratory and many microalgal strains do not grow reliably outdoors as a consequence of diurnal or seasonal fluctuations in irradiance and temperature (Moheimani 2013; Eustance *et al.* 2016).

Excellent reviews on algal physiology and large-scale outdoor cultures of microalgae have been published, including those on evaluation of the effect of photobioreactor configuration (see Table 3) on microalgal productivity and photosynthetic efficiency (de Vree *et al.* 2015). However, limited information exists about large-scale production and long-term operation of outdoor cultivation systems for most microalgal species (Van Bergeijk, Salas-Leiton and Cañavate 2010; Chiu, Soong and Chen 2016), and performance comparisons of a strain at optimum conditions in laboratory and outdoor environments are scant. Therefore, here we will take the case of *Scenedesmus obtusiusculus* as an example (Sánchez *et al.* 2013; Cabello, Morales and Revah 2015).

Scenedesmus obtusiusculus was grown in a 35 L airlift tubular photobioreactor, based on the design proposed by Molina-Grima *et al.* (2001). It was operated outdoors during a yearlong cultivation in Mexico City on a building roof located at N 19°21′32.466″, W 99°4′25.192″ in 2013. Table 4 shows results of biomass productivity and carbon dioxide fixation, including data under controlled optimal conditions in a flat panel photobioreactor. As can be seen, the highest biomass productivity, 0.29 g L^{-1} day^{-1}, and carbon dioxide fixation rate, 0.55 g L^{-1} day^{-1}, were obtained during the spring when the highest temperatures and irradiances (photon exposition) were recorded. However, these values were lower than those obtained in laboratory controlled conditions, where the biomass productivity was 0.97 g L^{-1} day^{-1} and a carbon dioxide fixation rate of 1.84 g L^{-1} day^{-1} was achieved (Toledo-Cervantes *et al.* 2013). This difference could be attributed to the fact that the above-mentioned conditions (temperature, irradiance) change dynamically making it difficult to clearly isolate the impact of any single factor.

Finally, few works have evaluated the effect of outdoor conditions on the carbon dioxide capture, biomass composition and biofuel potential. Recently, Ho *et al.* (2017) have found that *Scenedesmus obliquus* CNW-N exhibited the optimal carbon dioxide fixation rate and carbohydrate productivity during the summer with an average irradiance around 200 $\mu mol\ m^{-2}\ s^{-1}$ and temperatures close to 31°C. They recognize the importance of understanding the seasonal changes and conclude that it is possible to combine carbon dioxide fixation and bioethanol production using microalgae grown outdoors.

CONCLUSION

There is a common consensus in the scientific community about the need to address the significant difficulties involved in the large-scale production of microalgae and their products. Furthermore, to exceed the economic break-even point and make microalgal technologies a relevant contributor to carbon dioxide sequestration, the range of products should be extended, production should be maximized under the constraints (the selected strain) and the environmental conditions set in accordance with the cultivation method and the location of the facilities.

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