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Assessment of the CO_2 fixation capacity of *Anabaena* sp. ATCC 33047 outdoor cultures in vertical flat-panel reactors

Marta E. Clares, José Moreno, Miguel G. Guerrero, Mercedes García-González*

Instituto de Bioquímica Vegetal y Fotosíntesis, Centro de Investigaciones Científicas Isla de la Cartuja, Universidad de Sevilla-Consejo Superior de Investigaciones Científicas, Avda. Américo Vespucio 49, 41092-, Sevilla, Spain

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ABSTRACT

The extent of biological CO_2 fixation was evaluated for outdoor cultures of the cyanobacterium *Anabaena* sp. ATCC 33047. Culture conditions were optimized indoors in bubble-column photochemostats operating in continuous mode, subjected to irradiance cycles mimicking the light regime outdoors. Highest values achieved for CO_2 fixation rate and biomass productivity were 1 and 0.6 g L^{-1} day^{-1}, respectively. The comparison among different reactors operating simultaneously – open pond, horizontal tubular reactor and vertical flat-panel – allowed to assess their relative efficiency for the outdoor development of *Anabaena* cultures. Despite the higher volumetric CO_2 fixation capacity (and biomass productivity) exhibited by the tubular photobioreactor, yield of the flat-panel reactor was 50% higher than that of the tubular option on a per area basis, reaching values over 35 g CO_2 fixed m^{-2} d^{-1}. The flat-panel reactor actually represents a most suitable system for CO_2 capture coupled to the generation of valuable biomass by *Anabaena* cultures.

1. Introduction

During the past two centuries the atmospheric concentration of greenhouse gasses increased significantly (Solomon et al., 2007). It has been projected that a CO_2 reduction of 50–85% is required by 2050 in order to stabilize the CO_2 level in the air within the "safe zone" of 450 ppm. Compatible mitigation strategies are required to neutralize the excess CO_2. Higher plants, microalgae and cyanobacteria hold a potential as bio-based system for CO_2 capture and utilization associated to sustainable production of bioproducts through photosynthesis, key process in which, at the expense of sunlight, energy-rich compounds are synthesized from CO_2 and other oxidized low-energy inorganic substrates. The utilization of microalgae for carbon dioxide capture and utilization is not a new concept (Oswald, 1988; Kurano et al., 1995), although recently has emerged as a hot issue (Acién Fernández et al., 2012; Gonzalez-Lopez et al., 2009, 2012; Sánchez Fernandez et al., 2012). In fact, almost ten years ago we pioneered the use of cultures of the cyanobacterium *Anabaena* sp. ATCC 33047 for the purpose of CO_2 capture and utilization (Guerrero et al., 2005, 2006).

Anabaena sp. is a filamentous marine cyanobacterium, which can use atmospheric nitrogen as the sole source of this essential element, thus not requiring combined nitrogen as a nutrient. Besides lowering the cost of the culture medium, this ability restricts the problems of contamination by other microorganisms. Moreover, in batch cultures, *Anabaena* sp. exhibits a high growth rate and a wide optimum range of temperature and pH, as well as tolerance to salinity and high irradiance (Moreno et al., 1995). The ability of *Anabaena* sp. for vigorously growing outdoors has been verified (Moreno et al., 2003). Besides, its biomass can be easily harvested by autoflocculation, which represents an important advantage, since harvesting is a relevant economic issue in microalgal biomass production (Fontes et al., 1987). Under certain conditions, this organism releases into the medium significant amounts of an exopolysaccharide, as well as it can accumulate phycocyanin and allophycocyanin at high levels (Moreno et al., 1998). Thus, cultures of *Anabaena* might be suitable for the combined objective of capturing CO_2 and producing valuable organic matter (phycobiliprotein and carbohydrate-rich biomass and exopolysaccharide) simultaneously. Different applications of such organic materials can be envisaged, including the use of the carbohydrate-rich fraction as a feedstock for bioethanol.

Different systems for the photoautotrophic production of algae biomass at large-scale have been deployed that are based on open pond and closed photobioreactor technologies. Within the open systems, the best choice seems to be the open shallow pond running as simple loops or as meandering systems, covering an area

* Corresponding author. Tel.: +34 954489520; fax: +34 954460165.
E-mail addresses: meclares@gmail.com (M.E. Clares), jmfernan@ibvf.csic.es (J. Moreno), mgguerrero@us.es (M.G. Guerrero), mggonza@us.es (M. García-González).

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of several hundred square meters. Open systems cannot ensure a contamination-free monoalgal operation. The culture conditions are poorly controlled and only a few resistant microalgal strains can grow under the extreme conditions (high pH, salinity or temperature) that normally take place in open systems. The adequate supply of carbon dioxide is very critical and it is usually controlled through a pH-stat. Temperature fluctuation due to diurnal cycles and seasonal variations are difficult to control. These drawbacks limit the applications of these reactors to a few strains. The more technologically advanced closed systems provide better options to grow virtually every microalgal strain, protecting the culture from invasion of contaminating organism and allowing exhaustive control of operation modes. These systems consist of an array of straight glass or plastic tubes to capture sunlight and can be aligned horizontally, vertically, inclined or as a helix. They offer higher productivity and better quality of the generated biomass, although they are more expensive to build and operate than the open systems (Brennan and Owende, 2010).

Some of the earliest forms of microalgal culture systems are flat-panel photobioreactors (Tredici and Zittelli, 1998). These reactors are suitable for mass cultures of algae due to low accumulation of dissolved oxygen and its high photosynthetic efficiency. They offer efficient mixing, high volumetric mass transfer rates and are low-cost, compact and easy to operate (Sanchez-Mirón et al., 2003). Vertical flat panel photobioreactors consisting of a plastic bag enclosed between two iron frames (Sierra et al., 2008; Tredici and Rodolfi, 2004) offer advantages over the classical rigid wall option.

The present study was undertaken to determine the potential of *Anabaena* sp. cultures in different outdoor systems (open, tubular closed and vertical flat-panel reactors) for CO_2 capture and utilization. Beforehand, optimal culture conditions for biomass production and carbon dioxide fixation were determined.

2. Materials and methods

Anabaena sp. ATCC 33047 from the American Type Culture Collection, Rockville (USA), was grown photoautotrophically on the medium described by Moreno et al. (1995). Indoor experiments were carried out in 2 L capacity jacketed photochemostats (bubble column type), containing 1.8 L of cell suspension (Del Río et al., 2008) with a volume/surface ratio of 45 L m^{-2}. The photochemostats were continuously bubbled with air at a flow rate between 20 and 80 L (per L culture) h^{-1} at the bottom of the column. Temperature of the culture was maintained at the indicated values by flowing water through the jacket. The pH was controlled by on-demand injection of CO_2 into the air stream entering the cultures. The photochemostasts were illuminated by means of six surrounding Osram ecopack-FQ24W/840HO white-light lamps. The irradiance impinging on the reactor surface was regulated by an automated system to simulate a circadian cycle (12 h light/12 h dark), irradiance increasing gradually from dark until reaching a maximum on the reactor surface of 1000 μE m^{-2} s^{-1} (unless otherwise indicated) after 6 h, decreasing thereafter progressively until reaching the new dark period.

Indoor experiments were carried out in continuous mode, at dilution rates ranging from 0.02 to 0.12 h^{-1}. In general, four photochemostats, each at a given combination of pH, temperature and dilution rate were operated simultaneously to optimize culture conditions. Each reactor was inoculated with batch-growing cells and operated on discontinuous mode for 5 days, to reach a density of 0.6 g biomass L^{-1}. Henceforth, the reactor was switched to operate in continuous mode, by feeding continuously fresh medium to the reactor at the selected dilution rate only during the 12 h of illumination. Overnight the culture was operated as a batch to avoid culture washout. Dilution rate values thus correspond to the daylight period. Samples for analytical determinations were collected once a steady state was reached by the continuous culture.

For outdoor operation, three different designs of photobioreactors were compared: open ponds without temperature control (Moreno et al., 2003), as well as closed horizontal tubular reactors (Del Campo et al., 2001) and flat-panel reactors (Sierra et al., 2008), both temperature-controlled. In all of the experiments, *Anabaena* cultures were operated under semi-continuous regime, supporting a pre-established cell density by removal of part of the cell suspension in the morning and replacement with fresh medium, with either daily dilutions or every 2–3 days for the case of open ponds. The increase in biomass between two consecutive dilutions was taken as a measurement of productivity. Culture pH was maintained at 8.5–9 by on-demand CO_2 injection.

Cultures were performed in 1 m^2 open ponds at 10 cm in deep (Moreno et al., 1995) with a volume/surface ratio of 100 L m^{-2}, in tubular reactors of 55 L capacity occupying 2.2 m^2 (Del Campo et al., 2001) and 25 L m^{-2}, and in flat-panel reactors with a volume/surface ratio of 140 L m^{-2}. The latter type of reactor consisted of a disposable plastic bag located between two iron frames 0.070 m apart. Frames and plastic bag are 1.5 m high and 2.5 m long. The plastic bag is made of free-dispersant 0.75 μm polyethylene, with a 65% transmittance in the photosynthetically active spectrum. The bag, holding 350 L when completely filled, can be easily replaced when convenient, excessive fouling or contamination being the most common factors demanding replacement. A gas sparger (20 mm PVC tube with 1 mm holes every 3 cm length) was placed from side to side at the bottom of the plastic bag for aeration (8.6 L air L^{-1} h^{-1}), and a heat exchanger consisting of four 2 m long, 25 mm diameter stainless steel tubes was located 0.5 m above the gas sparger inside the bag for temperature control. Temperature of the culture was maintained between 25 and 35 °C by circulating water through the heat exchanger. pH and temperature probes were located in the upper part of the photobioreactor. Experiments were performed during one year in Seville, Spain (37°24′N, 6°0′W).

Growth rate and productivity were estimated on a dry weight basis. Dry weight and total organic carbon (TOC) determinations in outdoor cultures were performed twice a day, by early morning and following dilution of the culture. To this end, the absorbance at 700 nm was measured and dry biomass weight estimated from a linear correlation: dry biomass (g L^{-1}) = 0.647 × $A_{700\,nm}$. A TOC analyzer (Shimadzu V-CPH) was used to determine total organic carbon concentration in culture samples, the extent of CO_2 fixation being calculated from the TOC values, taking into account that every gram of organic carbon corresponds to 3.66 g of fixed CO_2. In indoor experiments, dry weight and TOC values were average of two independent daily determinations throughout four consecutive days after cultures had reached the steady state. For photosynthetic efficiency determinations, a value of 22.44 kJ g^{-1} was used (Del Campo et al., 2001) for the heat of combustion of dry *Anabaena* sp. ATCC 33047 biomass. Statistical analysis of data was carried out by using software SPSS.

3. Results and discussion

Previous data concerning the influence of culture conditions on productivity of *Anabaena* sp. ATCC 33047 in either batch or continuous culture were obtained using a single photochemostat subjected to continuous illumination at low irradiance level (Moreno et al., 1998). For the present study, at least four photochemostats operating under continuous regime and subjected to a circadian rhythm of illumination have been used simultaneously. The data obtained were most accurate and reproducible, the information derived being straightforward and applicable to the operation of outdoor production systems.