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HIGHLIGHTS

1. The efficiency of CO_2 capture and conversion into biomass by cultures of Anabaena has been assessed both indoors and outdoors

2. Outdoor cultures have been simultaneously operated in different reactor types throughout a full year

3. The observed differential performance of the cultures depends upon the peculiarities of the particular reactor

4. On a per area basis, cultures in flat-panel reactor performed better than in either open pond or tubular horizontal reactor

5. The appropriate culture of Anabaena in flat-panel reactor represents a most suitable system for CO_2 capture and conversion

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

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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.

Keywords: *Anabaena*; CO_2 fixation; continuous culture; outdoor culture; flat panel reactor

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 (Fernández et al. 2012, González-López 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 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/12h dark), irradiance increasing gradually from dark until reaching a maximum on the reactor surface of 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ (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 35ºC 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 (37º24´ N, 6º0´ 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 ($g\ l^{-1}$) = $0.647 \times A_{700\ nm}$. 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.

To determine the influence of dilution rate, temperature and irradiance on the behaviour of *Anabaena sp.* ATCC 33047, continuous cultures were performed at values of dilution rate ranging from 0.016 to 0.124 h^{-1}, of temperature between 25 and 45ºC, and of irradiance from 100 to 2800 μE m^{-2} s^{-1}. After reaching the steady state condition, values for biomass productivity and CO_2 fixation rate were calculated from the standing concentration of biomass and TOC, respectively. As shown in Fig. 1, the steady-state biomass concentration decreased from 0.75 to 0.10 g l^{-1} in response to an increase in the dilution rate within the chosen range. Maximal biomass productivity (0.23 g L^{-1} d^{-1}) was achieved at 0.056 h^{-1} dilution rate, with a corresponding maximal value for the CO_2 fixation rate (0.50 g CO_2 L^{-1} d^{-1}) (Fig 1a). It is worth noting that biomass productivity values were similar within the dilution rate range 0.056-0.1 h^{-1}, although the standing concentration of biomass was significantly higher at the lower dilution rate (data not shown). Figure 1b shows that maximal biomass productivity was found at 30-35ºC. At temperature values above 35ºC, productivity decreased strongly, the decline being more moderate at temperature values below 30ºC. Similarly, the maximal value for the CO_2 fixation rate (0.40 g CO_2 L^{-1} d^{-1}) was recorded at 35ºC. No light saturation effect was

observed within the impinging irradiance (I_o) range studied (Fig 1c). Biomass productivity increased with incident irradiance, a maximal value of 0.6 g L^{-1} d^{-1} being achieved at 2800 μE m^{-2} s^{-1}, for otherwise optimal conditions of dilution rate (0.056 h^{-1}) and temperature (35ºC). Similarly, the CO_2 fixation rate also increased with the incident irradiance, being also maximal at 2800 μE m^{-2} s^{-1}. No significant changes in biomass productivity or CO_2 fixation rate were found in response to changes in neither the air flow rate (20- 80 L L^{-1} (culture) h^{-1}) nor in the pH range 7.5-9.0 (data not shown).

The information obtained in these experiments was used to generate models of the behaviour of the *Anabaena* sp. continuous cultures. The obtained models (not shown) for growth and CO_2 fixation were quite analogous to those proposed by González-López et al. (2009). From the above results, optimal conditions for our *Anabaena* sp. continuous cultures were established as: dilution rate, 0.056 h^{-1}; temperature, 30-35ºC; incident irradiance, 2800 μE m^{-2} s^{-1}; pH, 8.5; and air flow rate, 40 L L^{-1} (culture) h^{-1}. Under these conditions, a CO_2 fixation rate of 1 g CO_2 L^{-1} day^{-1} was achieved. This value is higher than those reported in the literature for *Nannochloris* or *Nannochloropsis* (Negoro et al. 1991), *Chlorella vulgaris*, *Botryococcus braunii*, *Spirulina (Arthrospira) platensis* or *Dunaliella tertiolecta* (Sydney et al. 2010), being otherwise similar to those found for *Scenedesmus*, *C. vulgaris* and *Microcystis* (Jin et al, 2006), all of them derived from batch operation. Chiang et al. (2011) have suggested the utilization of *Anabaena* sp CH1 in CO_2 mitigation, with a maximum CO_2 fixation rate of 1.01 g CO_2 L^{-1} day^{-1} in batch cultures.

A valid verification of the performance of *Anabaena* cultures and assessment of their efficiency in CO_2 capture and utilization has to be performed outdoors. In a first approach, *Anabaena* sp. continuous cultures in the 1.8 L photochemostats used in indoor experiments were tested under the conditions established above as optimal,

except for the incident light, which was that naturally available. Biomass productivity values of 0.31-0.42 and 0.66 g dry biomass $L^{-1}d^{-1}$ were obtained in spring (irradiance, 14-17 MJ m^{-2} d^{-1}) and summer (irradiance, 21 MJ $m^{-2}d^{-1}$), respectively. This means that the behaviour of the cultures inside the laboratory and outdoors is analogous, provided that both the reactor design and the operation regime are maintained. Thus, maximal productivity and CO_2 fixation values observed indoors were reproduced outdoors, offering relevant information on maximal attainable performance in outdoor operation.

In a second and more thorough endeavour, the potential of the *Anabaena* sp. cultures was assessed under conditions closer to those under which outdoor production systems operate. To this end, semi-continuous cultures were performed in parallel using different pilot scale photobioreactors, namely open ponds, flat-panel and closed horizontal tubular systems. The reduced light path of the tubular reactor (3 cm) allowed the highest cell density (1.8 g L^{-1}), in comparison to 0.55 g L^{-1} in the open ponds (10 cm) and 0.65 g L^{-1} in the flat-panel reactor (7 cm).

Productivity values can be expressed on a per volume basis or as a function of land surface, which can in turn correspond to either photosynthetically active area, or total area occupied by the system. The first two parameters refer to the energy efficiency of a system with regard to the conversion of light energy into algal biomass, while the third is used to refer to the total surface occupied by an industrial facility. On a volumetric basis, the photochemostats were more effective, with mean dry biomass productivity values of 0.66 g L^{-1} d^{-1} versus 0.55 g L^{-1} d^{-1} for the tubular photobioreactor, 0.17 g L^{-1} d^{-1} for the flat-panel reactor and 0.05 g L^{-1} d^{-1} for the open pond. Nevertheless, when considering areal productivity, the photochemostat exhibited the highest biomass productivity, 29.7 g m^{-2} d^{-1}, higher than the 23.8 g m^{-2} d^{-1} of the flat-panel reactor (Table 1). The same considerations apply to the CO_2 fixation rate on a per

volume basis, with values of 0.10, 0.25, 0.96 and 1.1 g L^{-1} d^{-1} for the open pond, flat-panel, tubular reactor and photochemostat, respectively. Although the data included in Table 1 correspond to experiments carried out in summertime, the superiority of the flat-panel reactor, compared with open ponds and tubular reactor, was evident throughout the year, regardless the season considered. In all instances, values for both biomass productivity and CO_2 fixation capacity on a surface basis for cultures in the flat-panel reactor were at least 3.4 and 1.5 fold higher than those obtained for the open pond and horizontal tubular reactor, respectively. Furthermore, values obtained with the flat-panel reactor were not much lower than those recorded for the small photochemostats, when the latter were operated outdoors under continuous regime and optimal, fully controlled conditions (Table 1). These results underline the efficiency of the flat panel reactor in outdoor operation.

The validity of vertical flat-panel reactors in CO_2 abatement and concomitant conversion into organic carbon by *Anabaena* sp. cultures has been further examined, since scarce information is available concerning outdoor experiences with such systems (Rodolfi et al. 2009; Zemke et al. 2013; Zhang et al. 2001a,b). Semi-continuous cultures were performed, removing daily a portion of the culture and replacing with fresh medium to recover the minimal cell density of 0.35 g L^{-1} (Fig. 2). Continued operation of these cultures could be maintained for longer than 3 months of experimentation, thus confirming the stability of the system.

In order to optimize the operation conditions outdoors, the effect of the minimal cell density (i.e. the value of biomass concentration to be established following removal of a culture aliquot and addition of fresh medium) on the performance of the culture has been analyzed throughout the year (Table 2). The optimal values found for minimal cell density varied according to the irradiance impinging on the cultures, being of 0.4 g L^{-1}

in spring and summer (above 14 MJ $m^{-2}d^{-1}$), 0.3 in Autumn and 0.25 in Winter. The highest values of both productivity (0.17 g biomass $L^{-1}d^{-1}$) and CO_2 fixation yield (0.25 g $L^{-1}d^{-}1$) were obtained for a combination of 0.4 g L^{-1} minimum cell density and the highest irradiance value recorded (Summer, 20 MJ $m^{-2}d^{-1}$). Table 2 also contains data concerning the efficiency of solar energy conversion by the cultures of *Anabaena* sp. in flat-panel reactors. Photosynthetic efficiency has been calculated for different seasons of the year under optimized conditions for each experimental period. The lowest value - about 2.6%- was found for the higher irradiance periods (Summer), and conversely, the highest efficiency (over 4%) was recorded in Winter. In any case, these high efficiency values underline the effectiveness of the flat-panel reactor system for maximizing photosynthetic CO_2 fixation. These results are similar those of outdoor experiences in closed photobioreactor with the cyanobacteria *Arthrospira* (*Spirulina*) (Chini Zittelli et al. 1996) in which photosynthetic efficiency was higher in winter than in summer.

The average annual productivity of *Anabaena* cultures outdoors with the flat-panel reactor was about 19 g biomass m^{-2} d^{-1}, with a corresponding CO_2 fixation yield of 33 g m^{-2} d^{-1}. This represents only about one fourth of the corresponding values obtained indoor under tightly controlled conditions. In the flat-panel reactor, the length of the light path could be modified to improve yield of the cultures. In fact, when the width of the reactor was reduced from 7cm to 5 cm, volumetric biomass productivity and CO_2 fixation increased to 0.25 and 0.36 g L^{-1} d^{-1}, respectively. On a per area basis, the CO_2 fixation yield obtained in summer was 43.2 g m^{-2} d^{-1} for an irradiance value of 20 MJ m^{-2} d^{-1} and a minimal cell density of 0.6 g L^{-1}. The shaking system and inclination degree of the reactor could be also optimized (Slegers et al. 2011). Thus, it seems that there is stillroom for improvement of efficiency of the plat-panel reactor.

4. **Conclusions**

The culture of N_2-fixing *Anabaena* in vertical flat-panel reactor represents a highly suitable system for CO_2 capture and utilization coupled to the production of organic matter. In addition to a higher productivity on a per area basis, the flat-panel option has further advantages over alternative reactor types, such as low energy consumption for better heat transfer, efficient mixing, suitability to scale-up, low cost of construction and simplicity of handling.

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Figure Captions

Figure 1. Influence of dilution rate (a), temperature (b) and irradiance (c) on biomass productivity (open circles) and CO_2 fixation rate (closed circles) of continuous cultures of *Anabaena* sp. ATCC 33047 in photochemostats. General culture conditions were as described in Material and Methods. Values for temperature and irradiance in (a) were 35ºC and 1000 $\mu E \cdot m^{-2} \cdot s^{-}1$, respectively; in (b), dilution rate was 0.056 h^{-1} and irradiance 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$; in (c), temperature was 35ºC and dilution rate 0.056 h^{-1}.

Figure 2. Outdoor culture of *Anabaen*a sp. ATCC 33047 in vertical flat-panel reactor operated under semicontinuous regime. The culture was diluted daily, in the early morning, as to recover the prefixed minimal cell density (ca. 0.35 g l^{-1}); solar irradiance 20.9±1.7 MJ m^{-2} d^{-1}. Mean productivity was 0.17 g (dry weight) per liter and day.

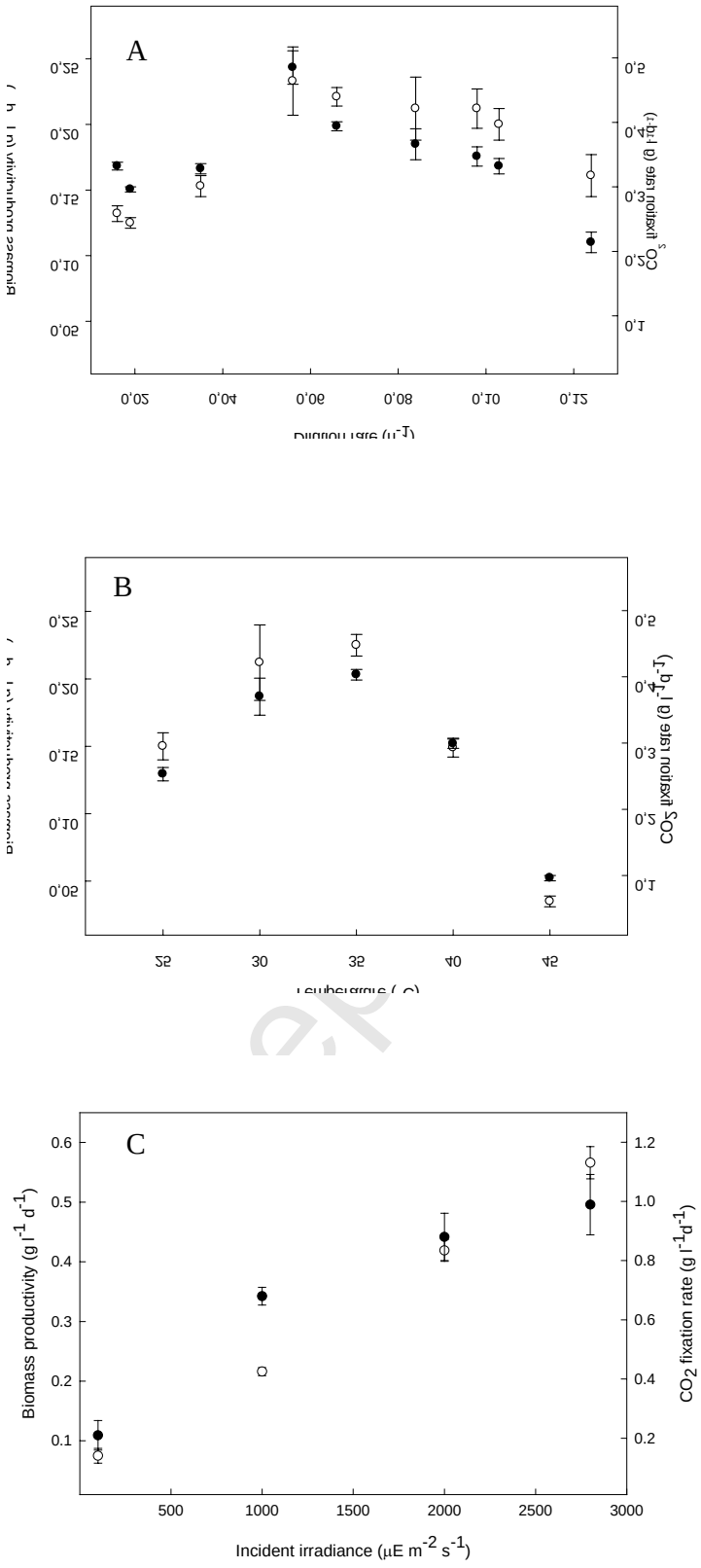

Figure 1

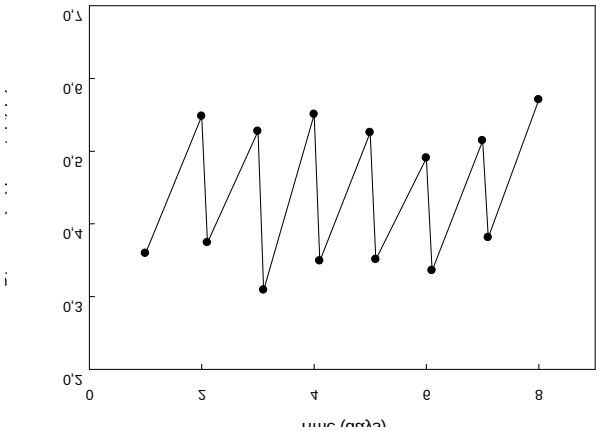

Figure 2

Table 1. Biomass productivity and CO_2 fixation capacity of *Anabaena* sp. ATCC 33047 in different culture systems in summer

	Biomass productivity		**CO_2 fixation**	
	(g l^{-1} d^{-1})	**(g m^{-2} d^{-1})**	**(g l^{-1} d^{-1})**	**(g m^{-2} d^{-1})**
Photochemostat[1]	0.66±0.05	29.7	1.1±0.08	49.5
Open pond[2]	0.05±0.01	5.3	0.10±0.02	10.4
Horizontal Tubular[3]	0.55±0.08	13.8	0.96±0.10	24.0
Vertical Flat Panel[4]	0.17±0.02	23.8	0.25±0.06	35.6

The systems were operated throughout June, July, August and September. Mean solar irradiance: 20.4±3.2 MJ m^{-2} d^{-1}. Culture temperature ranged between 20 and 26 ºC, 25-35 ºC and 26-32 ºC in the open pond, horizontal tubular and vertical flat panel, respectively, and was 30ºC in the photochemostat.

[1] continuous culture, dilution rate, 0.056 h^{-1}

[2] semi-continuous culture, dilution every second day until minimal cell density of 0.2 g l^{-1}

[3] semi-continuous culture, daily dilutions until minimal cell density of 1 g l^{-1}

[4] semi-continuous culture, daily dilutions until minimal cell density of 0.35 g l^{-1}

Table 2. Optimal cell density, biomass productivity, CO_2 fixation rate, ratio of biomass yield to incoming energy (R) and photosynthetic efficiency of *Anabaena* sp. ATCC 33047 in outdoor semi-continuous culture in vertical flat-panel reactor throughout the year

Season	Minimal cell density ($g\ l^{-1}$)	Solar irradiance ($MJ\ m^{-2}\ d^{-1}$)	Biomass productivity ($g\ l^{-1}\ d^{-1}$)	CO_2 fixation ($g\ l^{-1}\ d^{-1}$)	Photosynthetic efficiency (%)
Spring	0.40	14.3 ± 2.1	0.14 ± 0.04	0.18±0.05	3.07
Summer	0.40	20.4 ± 3.2	0.17 ± 0.01	0.25±0.04	2.60
Autumn	0.30	9.9 ± 2.6	0.11 ± 0.04	0.16±0.03	3.48
Winter	0.25	5.9 ± 2.3	0.08 ± 0.03	0.12±0.05	4.26