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Effect of plate distance on light conversion efficiency of a *Synechocystis* culture grown outdoors in a multiplate photobioreactor

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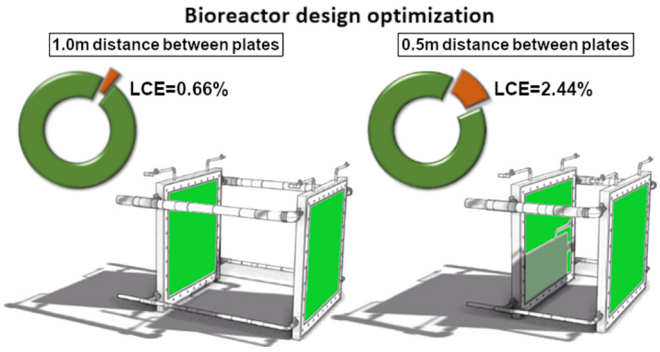
HIGHLIGHTS

- A novel vertical PBR design for efficient production of microalgae is proposed.
- The light conversion efficiency was higher when the plates were spaced 0.5 m apart.
- The photobioreactor was successfully tested outdoors with a *Synechocystis* culture.
- The photobioreactor can be easily converted for outdoor photobiological H_2 production.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, the performance of a vertical multiplate photobioreactor is analyzed and presented. The photobioreactor consisted of 20 vertical plates (1 m^2 each) connected by manifolds and a working volume of 1300 L. The total area occupied (footprint) was 10 m^2, while the illuminated area was 40 m^2, therefore the ratio of illuminated area to volume ratio was about 30 m^{-1}. The performance of the photobioreactor was evaluated using a culture of *Synechocystis* PCC 6803, circulated by a centrifuge pump. The results showed that the amount of light captured by the photobioreactor at a plate spacing of 0.5 m was 90.2 % of the light incident on the horizontal surface, while at a plate spacing of 1.0 m, 50.3 % was captured. The corresponding biomass yield, calculated based on the ground area occupied by the reactor, was 26.0 g m^{-2} day^{-1} and 7.2 g m^{-2} day^{-1}, when the plates were spaced at 0.5 m and 1.0 m respectively. Therefore, the light conversion efficiency calculated based on the ground area was significantly higher in the configuration with a plate spacing of 0.5 m, reaching 5.43 % based on PAR (photosynthetically active radiation), and 2.44 % based on solar radiation, giving a value 3.7 higher than when the plates were spaced 1.0 m apart. It was concluded that the light conversion efficiency might be further improved by reducing the plate spacing while also reducing the culture light path.

Abbreviations: DO, dissolved oxygen; DW, dry biomass weight; LCE, light conversion efficiency; MPL-PBR10, multiplate photobioreactor with 10 plates set at 1.0 m distance; MPL-PBR20, multiplate photobioreactor with 20 plates set at 0.5 m distance; MPL-PBR, multiplate photobioreactor; PBRs, photobioreactors; PFD, Photon Flux Density; PMMA, poly (methyl methacrylate); PVC, polyvinylchloride; PSII, photosystem II.

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1. Introduction

The need to increase light conversion efficiency (LCE) from the current levels of <1 % on solar energy basis, is mandatory for the use of microalgae as source of food, feed, and bioenergy. For producing biofuels, it is desirable to employ microalgae cultivation systems with much higher productivity than that achieved with the actual facilities to make the process feasible (Shekh et al., 2021; Sharma et al., 2022). Another important goal is to increase the stability of long-term, continuous photobioreactors (PBRs) applications and to develop novel process control systems (Vo et al., 2019; Fuchs et al., 2021). Compared to open ponds, closed reactors minimize the land surface they occupy, reduce freshwater requirements, and reduce dependence on seasonal fluctuations. However, designing efficient large scale closed PBRs is not straightforward (Torzillo et al., 2003; Torzillo and Chini Zittelli, 2015; Lindblad et al., 2019; Touloupakis et al., 2021a; Díaz et al., 2021).

The main factor that reduces the LCE outdoors is light saturation. Throughout the day, the amount of absorbed light dissipated by non-photochemical quenching, mainly heat, and fluorescence, can reach as much as 80 % of the daily irradiance, especially in the middle of day (Masojidek et al., 2004; Cuaresma et al., 2011). The result is that although the photosynthesis rate of a culture increases linearly with light irradiance in the morning hours, after reaching one fourth of the full solar irradiance the increase in photosynthesis decreases with further increase in light irradiance, and there is often no further increase beyond half of the full solar irradiance (2000 $\mu mol_{photons}\ m^{-2}\ s^{-1}$). Various systems have been proposed to submit cells to an ordered light-dark (L/D) cycle rather than turbulent flow, and many sophisticated PBRs have been developed for this purpose (Torzillo et al., 2003; Fuchs et al., 2021). In most cases however, the benefit achieved in terms of productivity is annulled by the increased energy consumption or by the high cost of the PBR. One of the promising ways to reduce the "*saturation effect*" of photosynthesis is to design PBRs in which "*light dilution*" can be achieved. This goal can be attained by greatly increasing the cross-section of the reactor (i.e., the illuminated part of the reactor) with respect to the ground area it occupies. This idea, which was first proposed by Morita et al. (2000) and more recently by Wijffels and Barbosa (2010), postulates that it is theoretically possible to reduce the incident light intensity on the surface of the reactor by a factor equal to the ratio between the illuminated area (A_R) of the PBR and ground area (A_G) occupied by the reactor (A_R/A_G) (Posten, 2009). The optimal value of this ratio ultimately depends on the specific microalgal species, as photosynthesis saturation irradiance varies according to the strain used. Inclined or vertical PBRs intercept sunlight at larger angles and thus "dilute" the light compared to horizontal PBRs. For this reason, vertical PBRs are expected to be more efficient than horizontal ones in terms of solar energy utilization (Hu and Richmond, 1996; Cuaresma et al., 2011). In close proximity, higher productivity of PBRs per land area unit can be achieved at the expense of higher installation costs (Tredici, 2010). However, to achieve high photosynthetic efficiency, it is important to consider various factors at the same time, such as uniform illumination of the culture, an efficient mixing system that won't stress the cells, a low mixing time in the PBR, and full computerized and automated control of the various parameters and processes (Torzillo and Chini Zittelli, 2015; Fu et al., 2021; Xuyang et al., 2021). Hydrodynamics, is another key parameter as it affects not only the energy demand of the reactor and the mixing capability in terms of dissolved gases, but also cell physiology, as it determines the movement of the cells through the strong light gradients. In the implementation of any PBR, it would be desirable to ensure sufficient mass transfer capacity to prevent excessive accumulation of photosynthetically produced oxygen. Dissolved oxygen (DO) in high levels of saturation is toxic to microalgae, and oxygen stripping is required (Masojídek et al., 2021). Maximizing mass transfer of oxygen is therefore an important issue to control oxygen-related cell inhibition and maximize microalgal growth. Oxygen stripping rate is limited by the overall mass transfer coefficient (K_La) and by the saturated DO concentration.

Bearing in mind these fundamental aspects, we have designed, constructed, and tested a vertical PBR in which it is possible to achieve a light dilution factor close to 4, thus reducing the light saturation effect. The performance of the reactor outdoors was evaluated using a culture of *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) which is a promising candidate for photobiological hydrogen production (Touloupakis et al., 2016a,b; Lindblad et al., 2019; Mona et al., 2020). Indeed, the culture system can be completely sealed to be used for experiments on photobiological hydrogen production. This work represents the first attempt to cultivate the cyanobacterium *Synechocystis* at a large scale outdoors.

2. Materials and methods

2.1. Organism and culture conditions

All experiments were carried out in Sesto Fiorentino, Florence, Italy (latitude 43.8 N, longitude 11.3 E), on warm sunny days. To prepare the inoculum for the outdoor experiments, cells were grown in BG11 medium (Rippka et al., 1979), in glass tubes (5-cm light path, 400 mL working volume), immersed in a 35 °C thermostated water bath; they were subsequently scaled up to 5 L, and thereafter to 10 L Pyrex bottles (20 and 30 cm light path respectively), illuminated on both sides (Photon Flux Density - PFD, ~300 $\mu mol_{photons}\ m^{-2}\ s^{-1}$) and bubbled with a mixture of air and CO_2 (97/3 v/v). Dense *Synechocystis* cultures were used to inoculate the PBR.

2.2. Analytical procedures

Dry biomass weight (DW) and chlorophyll content were calculated according to Touloupakis et al. (2015). Total carbohydrate content was measured using the phenol-sulfuric acid method, using D+ glucose as standard (Dubois et al., 1956). Protein determination was performed in triplicate according to Lowry et al. (1951) and total lipids were analyzed according to Bligh and Dyer (1959). Ash content was determined after heating the biomass at 450 °C for 24 h. Biomass elemental composition was performed on lyophilized samples using a CHNOS Analyzer, Flash EA, 1112 series (Thermo Electron Corporation).

2.3. Fluorescence measurements

Chlorophyll fluorescence measurements were performed with a pulse-amplitude-modulation fluorometer (PAM-2100, H. Walz, Effeltrich, Germany) operated with PamWin (version 2.00f) software. The ratio between variable and maximum fluorescence, F_v/F_m, was used to determine the maximum photochemical quantum yield of photosystem II (PSII). For this purpose, samples were taken from the PBR and incubated in the dark for 15 min to remove any energy-dependent quenching. Right before the measurement, a far-red light pulse (above 700 nm) with a duration of 10 s (10 W m^{-2}), supplied by PAM-2100 was applied to oxidize the plastoquinone pool. Oxygen evolution and dark respiration rates were measured as previously reported (Touloupakis et al., 2021b). The effective photochemical quantum yield of PSII $\Delta F/F_m' = (F_m' - F_s) / F_m'$, which is the number of electrons generated per absorbed photon, was measured using F_s and F_m', which represent the steady-state and maximum fluorescence measured in the light, respectively. F_s and F_m' were measured in situ by inserting the fiber-optic probe of the fluorometer into the culture plate. Chlorophyll *a* fluorescence transients were recorded using a Handy PEA (Hansatech Instruments) in 2 mL dark adapted samples illuminated with continuous light (650 nm peak wavelength, 3500 $\mu mol_{photons}\ m^{-2}\ s^{-1}$) provided by light-emitting diodes. Each chlorophyll *a* fluorescence induction curve was analyzed using "BiolyzerHP3" software.

2.4. Photobioreactor description

The multiplate photobioreactor (hereafter MPL-PBR) (maximum working volume 1300 L) consists of 20 vertical parallel plates 50 L,

1150 × 1150 × 50 mm (*W* × *H* × *D*), directly connected to each other (Fig. 1). The culture inlet was achieved from the lower part of the plate through two manifolds (i.d. 9 cm), from which depart twenty DN15 (i.d. = 15 mm), fully open valves, through which the culture enters in the plates (Fig. 1). The outlet of the culture from the plates was achieved through twenty DN32 valves (10 on the right and 10 on the left) connected to two manifolds located in the upper part of the plates, through which the culture flows back into the degasser (Fig. S1). Therefore, the culture is circulated in parallel mode (i.e., parallel independent compartments). Both the inlet and outlet culture manifolds were made of transparent Plexiglass™. The inner frame is made of polyvinylchloride (PVC), resistant to culture media salts and chemicals used for sterilization. The inner frame in the upper part is provided with 4 ports for access to the culture (i.e., culture sampling, pH, temperature, oxygen probes). The glass windows of the MPL-PBR (1150 × 1150 mm) are made of tempered glass and attached to the frame with 32 metal screws 10 mm in diameter. Fig. 1C, shows an overview of the cross-section of the plates. Two 4 mm Ø nitrile O-ring seals placed in the dedicated tracks of the inner frame ensure operation without leakage. The plates are connected by pipes made of poly (methyl methacrylate) (PMMA) and PVC. The degasser (50 cm diameter, 90 L) was made of transparent PMMA (Fig. 1A insert). It was closed with a transparent PMMA cover, on which there are several ports for housing of probes and culture sampling. Culture O_2 degassing of culture occurs mainly in the cylindrical degasser itself, and in each single plate which are in contact with atmosphere through 2 valves. CO_2 supply was performed on demand and is injected in the culture degasser. The volume of the culture in darkness (pump, degasser, pipes, cooling system) is <5 % of total volume since most of the pipes used to connect the different units/plates are made of transparent material. The main characteristics of the PBR are listed in Table S1.

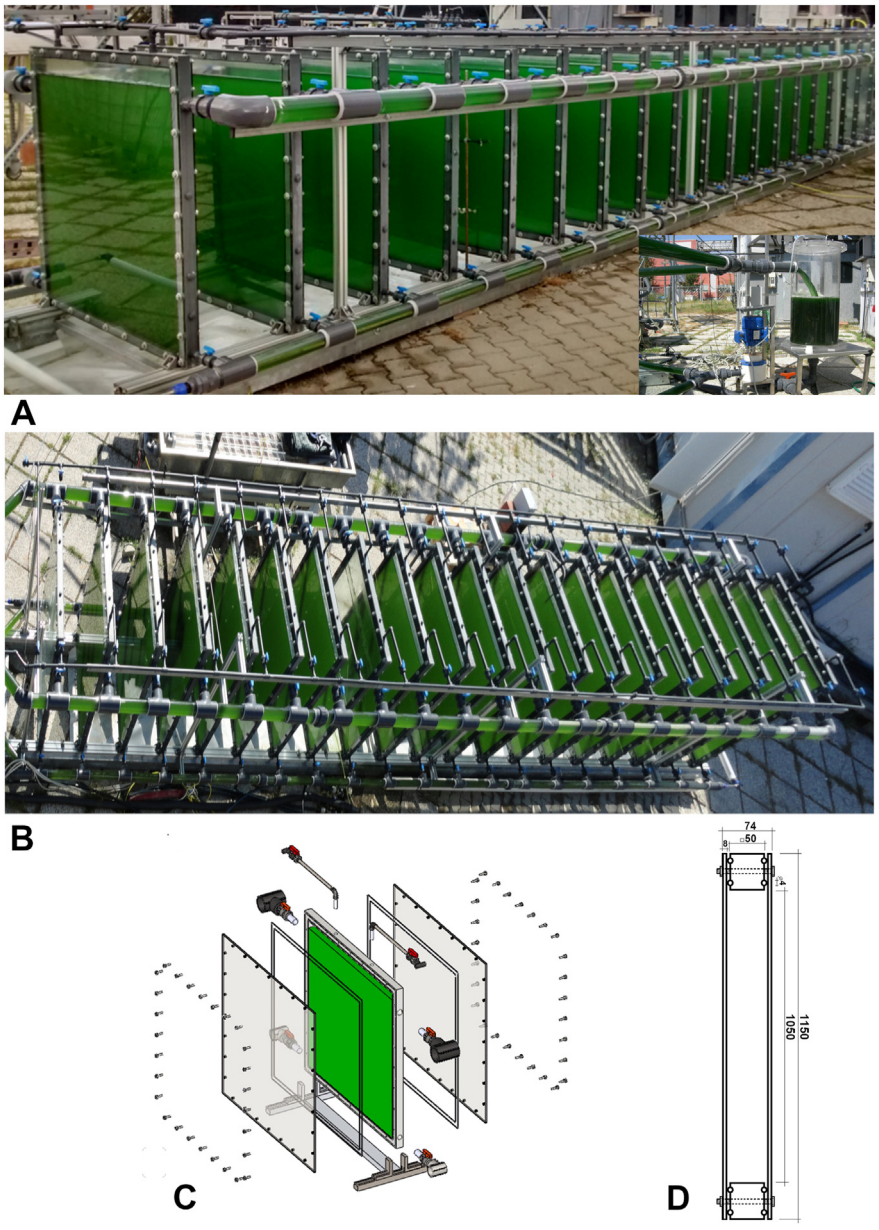

Fig. 1. The MPL-PBR installed at the CNR-IBE outdoor experimental area (Florence). (A) Lateral view; (B) view from the top. The inlet of the culture is achieved from the bottom part of the plate through two manifolds connected to the plates through two holes on the lower opposite sides, while the outlet by two manifolds located in the upper part of the plates through which the cultures flow back to the degasser (insert); (C) Glass windows and inner frame of the plate, showing the position of the 28 holes used for holding the inner frame and the two glass windows together (M2M, Naples Italy); (D) Cross section of the plate showing dimensions of the frame, O-ring seal, and glass windows (numbers in mm).

The design of the PBR allows to choose different reactor configurations, by excluding or including one or more plates from the operations and the experiments. In this work, two different reactor set-ups were tested, one referred to as MPL-PBR10 with 10 plates set at 1 m distance from each other (800 L working volume), and one called MPL-PBR20 with all the 20 plates active and operating, set 0.5 m apart (1300 L working volume). In case of accident or damage, single plates can be individually disconnected and removed from the PBR without interrupting culture circulation and with an acceptable loss of culture (about 50 L). The damaged plate can then be replaced with a spare plate to restore the original configuration. This design also allows the culture volume in the MPL-PBR to be increased gradually, greatly simplifying the inoculation of the reactor.

About 25 L of culture inoculum from the laboratory were enough to inoculate a first plate and start operating the reactor (50 L plus the volume of culture circulating in the 4 manifolds, pump and degasser, totalling 300 L), then after a couple of days of acclimation and growth, a second plate can be added providing culture medium; subsequently another four plates, and so on according to a log scale. In our experience, once the culture was acclimated to solar light, it was possible to duplicate the culture at time intervals of 2 days. Cooling of the culture was performed by the circulation of cold water provided by a cooling system (DAIKIN Hydrocube, model: EUWAN12KAZW1) within a stainless-steel tube running coaxially within the lower manifolds.

Culture mixing was provided by a centrifugal pump, designed by CNR-IBE and constructed by M2M Engineering (Naples, IT). The pump's propeller has three flat stainless-steel blades placed at equal angles of 120°. To minimize cell damage, especially when culturing flagellates or filamentous species, the distance between the blades and the inner casing is set by design at 1.0 cm. The internal diameter of the casing is 16 cm, while the height is 6.5 cm; the net volume of the pump without the impeller is about 1.1 L. The maximum flow rate is 20 $m^3\ h^{-1}$. However, to ensure sufficient turbulence of the culture without causing shear stress to the cells, the flow rate was set to about half the maximum. Before the start of each experiment, the PBR was cleaned and sterilized with a NaClO solution (0.04 % v/v). To eliminate residual sodium hypochlorite traces, the reactor was washed with sterile deionized water. The MPL-PBR was then inoculated with the culture from the 25 L bottles and filled up with fresh medium BG11, prepared with sterile water from a custom-made water pasteurizer. During cultivation, a high pH close 11 was maintained by automatic addition of CO_2 which proved to be effective to avoid the risk of contamination of the culture with *Poteriochromonas* (Touloupakis et al., 2016c).

2.5. Light irradiance distribution assessment on the reactor surface

The incident PFD on the MPL-PBR surface at different times of day was measured using a LI-250A Light Meter (LI-COR Biosciences). The PFD was determined, in the presence of the *Synechocystis* culture, on the front and back surfaces of the plates at five symmetrical points, by averaging the light intensity values and taking into consideration the shaded surface (Fig. S2).

2.6. Hydrodynamic measurements

2.6.1. Measurement of the mixing time of the PBR (T_{mix})

The mixing time was measured by the signal response technique using an acidic tracer and a pH electrode as detector. The mixing time is defined as the time needed to reach 95 % complete culture homogeneity which is determined by measuring the acid traces. For this purpose, a concentrated solution (2.0 L) of HCl (37 %) was injected just behind the circulation pump while the PBR was filled with tap water.

2.6.2. Measurement of liquid flow rate (QL $m^3\ h^{-1}$)

The flow rate of the MPL-PBR at different frequencies of the pump was measured in both MPL-PBR20 and MPL-PBR10. Each pump frequency was tested in triplicate, and at least three sets of measurements were performed for each reactor configuration.

2.6.3. Mass transfer of oxygen

The mass transfer of oxygen was evaluated in MPL-PBR20 according to Babcock et al. (2002, 2016). It was analyzed by determining the overall volumetric mass transfer coefficient for oxygen ($K_L a\ O_2$) at 25 °C in tap water. Air–bubbling was provided by spargers positioned at the bottom of the degasser station and the oxygen probe was inserted at the inlet of the degasser (Fig. 1A insert). Measurements were performed at two different liquid flow rates (5.2 and 10.8 $m^3\ h^{-1}$) and two different air sparging rates (20 and 40 L min^{-1}) in the multiplate configuration with 20 plates placed at 0.5 m. The gas-liquid mass transfer coefficient for oxygen ($K_L aO_2$) was measured by continuously recording the decrease in oxygen concentration after supersaturation of the reactor with tap water. The reactor was sparged with pure oxygen until the DO concentration in the water reached 300 % of air saturation; sparging oxygen for 10–40 min was required, depending on liquid flow rate adopted. Then air-bubbling at the operating flow rate was started. The decrease in oxygen concentration was monitored using a DO meter (model μACP 4082, Chemitec, Italy) equipped with an electrochemical (amperometric) oxygen sensor (OxySens 120, Hamilton, USA). Values were recorded every 30–60 s and the $K_L aO_2$ was estimated using the following equation:

$$C = C^*_\infty - \left(C^*_\infty - C_0\right)e^{-\mathrm{K_L}a*t}$$

where C is the DO (mg L^{-1}) at time t, $C^*\infty$ is the saturation DO (mg L^{-1}), C_0 is the DO at time 0, t is time (h), and $K_L a$ is the overall gas-liquid mass transfer coefficient (h^{-1}).

2.7. Power required for culture recycle

The power required for recycle of the culture in the MPL-PBR was calculated using the following equation:

$$\text{Power}\left(\text{W m}^{-3}\right) = 9804 \times h\, f/V \times \eta$$

where 9804 is the conversion factor required to convert m kg s^{-1} to watts, h is the head loss (m), and f is the flow rate ($m^3\ s^{-1}$), V is the volume of the culture (m^3), and η, pump efficiency, assumed to be 0.7. Head loss were provided by manufacturer of the valves, both for the DN15 valves (15 mm i.d.) located in the lower part of the plate for culture inlet (i.e., 10 valves on the right side of the PBR, and 10 on the left one), and for the 20 DN32 valves (32 mm i.d.), placed in the upper part of the plates. Local losses were also calculated for the 4 manifolds, through which the culture is delivered into the degasser (Fig. 1A), and for the 20 plates. However, the local losses recorded in the valves were much more relevant than those in the manifolds and plates in determining the global head loss, sore these losses were not included in the calculation of the power required for culture circulation.

2.8. Thermal simulations engineering

Thermal simulation was performed to estimate the energy required for temperature control of the culture grown in the MPL-PBR. The simulations were performed by using a database of solar irradiation and climatic conditions in GPS coordinates of the location where the PBR was installed, that is, the CNR experimental area in Sesto Fiorentino (Florence, Italy). The simulations were carried out by using heat transfer equations applied to each part of the system, and they are reported in the Supplementary information.

2.9. Statistics

Statistical analyses were performed using GraphPad Prism software (version 5.1 and 9.3 for Windows, GraphPad Software, La Jolla, California, USA). All statistical tests were carried out using a statistical software package (Stat graphics Plus, version 5.1 for Windows). a two-way ANOVA using

Tukey's test of variance at a 95 % confidence interval was used to evaluate the statistical differences in mass transfer.

3. Results

3.1. Mixing time

Changes in the pH between plates were recorded continuously in plates located at different distances from the pump. As expected, an increasing delay in reaching a constant pH value was observed the more distant the tested plate was from the pump. In plate 2, constant pH was reached in <50 s while in the last plate (n. 20) it took about 2.5 min (Fig. 2). However, the 95 % response time, T_{mix} (average of 3 experiments), was 2 min in all plates. This T_{mix} is adequate for efficient management of the reactor, i.e., efficient pH control of the culture, CO_2 and nutrient supply, and oxygen degassing.

3.2. Liquid flow rate

A significant linear relationship between liquid flow rate and pump frequency was found for the MPL-PBR 20 (Fig. S3). At the maximum frequency tested (40 Hz) the liquid flow rate was 16.2 $m^3\ h^{-1}$, while a minimum of 3.5 $m^3\ h^{-1}$ was obtained at a pump frequency of 10 Hz.

3.3. Mass transfer coefficient for oxygen

Two different liquid flow rates (7.6 and 16.2 $m^3\ h^{-1}$) were tested at two different air flow rates (20 and 40 L min^{-1}). Doubling both the liquid flow rate and the air flow rate, always resulted in an increase of the mass transfer coefficient for O_2, however the effect of the liquid flow was more significant (two-way ANOVA test $p < 0.0001$ vs $p = 0.001$). Indeed, doubling the liquid flow a 67 % and 82 % increase in $K_La O_2$ was observed at 20 and 40 L min^{-1} air-bubbling, respectively, while when the air flow rate was doubled, a 17 % and 30 % increase in $K_La O_2$ was observed at liquid flow rates of 7.6 and 16.2 $m^3\ h^{-1}$, respectively (Fig. 3). In Table 1 are reported the $K_La\ O_2$ recorded in different culture systems. The mass transfer attained in the MPL-PBR20 resulted lower than bubble column reactors, and much higher than that attained in open ponds.

3.4. Power required for culture recycle

Fig. 4 shows the relationship between the flow rate of the pump and the power required to circulate 1 cubic meter of culture. It increased exponentially. At the maximum pump flow rate, the power required per cubic meter was about 214 W m^{-3}.

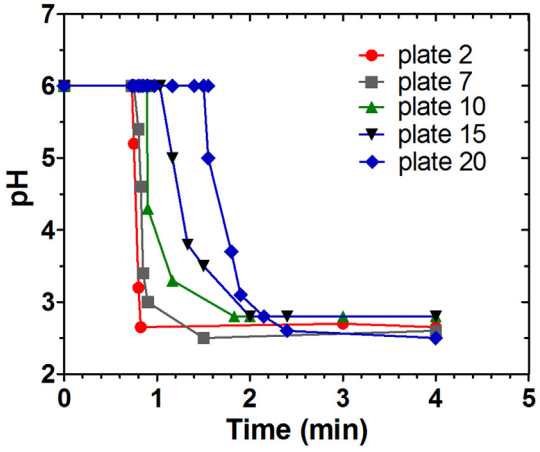

Fig. 2. Changes in pH values recorded in different plates of the PBR, after the injection of a concentrated HCl solution at the entrance of the liquid into the pump.

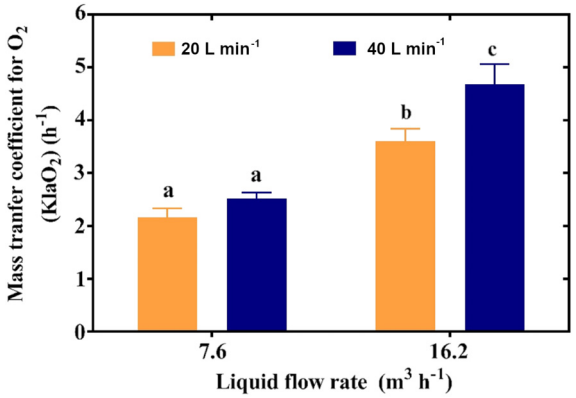

Fig. 3. Effect of the liquid flow rate and aeration rate on volumetric mass transfer coefficient for O_2 ($K_La\ O_2$) measured in the MPL-PBR20. Different letters indicate statistically significant differences ($p < 0.001$) of multiple comparisons (two-way ANOVA, Tukey's test).

3.5. Energy required for culture cooling

The temperature simulation, depicted in Fig. 5, was carried out for a representative day of cultivation of *Synechocystis* (June 25) during which culture temperature was not controlled, (i.e. in free running without cooling). According to the thermal model used in this study, culture reached a maximum temperature of 41 °C in the absence of cooling (Fig. 5).

The optimal temperature for *Synechocystis* is close to 35 °C, so it is desirable not to exceed such an upper limit. It was concluded that in order to maintain the culture temperature within the optimum, the energy required for cooling was close to 9.2 kWh m^{-3} of culture. Detailed information on the main thermal components of the heat transfer of the system which were included in the thermal simulations are reported in supporting information.

3.6. Light irradiance distribution assessment

The two different reactor configurations MPL-PBR10 and MPL-PBR20 entail a different total culture volume in the PBR, as well as a difference in light uptake (Fig. S2). The time course of solar irradiation on the PBR's surface was recorded at 1-hour intervals on sunny days (see Table 2). With the vertical plates at a distance of 0.5 m from each other, the average light irradiance intercepted by each plate reached about 44.6 % of that falling on horizontal surface, and it increased to 49.4 % with a plate spacing of 1.0 m. The corresponding average light irradiance impinging on the plates was 550 $\mu mol_{photons}\ m^{-2}\ s^{-1}$ at 0.5 m spacing and 618 $\mu mol_{photons}\ m^{-2}\ s^{-1}$ at 1.0 m spacing.

3.7. Assessment of the performance of the PBR with a Synechocystis culture

3.7.1. Biomass productivity

The MPL-PBR was inoculated with different volumes of *Synechocystis* to obtain two different cell concentrations (0.35 g L^{-1} and 0.67 g L^{-1} in the PBR with 0.5 m and 1.0 m spacing between plates respectively). The culture temperature was maintained within 35 °C during the light period in summer by using a chiller unit, and it dropped to ambient temperature (20–22 °C) at night. In Fig. 6, are shown the volumetric productivities and the corresponding areal yields achieved with the two PBR configurations (0.5 m and 1.0 m plate spacing). Volumetric productivity was 45 % higher with a plate distance of 0.5 m. The difference in performance of the two PBR configurations increased further (73 %) when comparison was done in term of areal yield (Fig. 6). The higher difference was due to the higher volume per m^2 attained with the MPL-PBR-20 (that is with plate set at 0.5 m distance).

Table 1
Comparison of mass transfer coefficients K_La O_2 among different culture systems. Some data from the originally reported results were converted to obtain consistent units for this comparison.

PBR type	Air flow rate (L min^{-1})	Liquid flow (m^3 h^{-1})	K_La O_2 (h^{-1})	Reference
MPL-PBR 20 (1300 L)	40	10.8	4.68	This work
Green Wall Panel (GWP) (1.0 m × 0.05 m × 2.5 m)	30	–	21.6	Tredici et al., 2010
Vertical Flat Plate (1.5 m × 0.07 m × 2.5 m)	80	–	25	Sierra et al., 2008
Near-Horizontal Tubular Reactor (NHTR) 220 L (20 m long tubes, slope 4°)	12.5	–	7	Babcock et al., 2002
ENHTR 220 L (20 m long tubes with enhancements)	23	–	18	Babcock et al., 2016
	13		11	
Horizontal serpentine PBR			6.2–7.2 overall	Camacho Rubio et al., 1999
Open pond (100 m^2 pond, 0.2 m depth)	6 m^3 h^{-1}	0.22 m s^{-1} (culture velocity)	0.87	Mendoza et al., 2013
Corrugated cascade	–	66	21.96	Moroni et al., 2021

3.7.2. Chlorophyll fluorescence and photosynthesis changes

During the day chlorophyll fluorescence and photosynthesis rates were monitored at one-hour intervals (Fig. 7). In both the cultures the F_v/F_m ratio, decreased continuously with a clear drop after 12.00 pm; it declined by 57 % compared to the initial morning value at 03:00 pm, indicating that both cultures were subject to photoinhibition. After 04:00 pm, with the decrease in light intensity, the cultures began to recover their initial value, which was different for the two cultures, indicating that the culture grown in the MPL-PBR10 configuration (plate spacing 1.0 m) was subjected to a permanent type of stress (Fig. 7).

The physiology of the culture was further investigated by measuring the chlorophyll fluorescence transient. A significant modification of the chlorophyll fluorescence transient was recorded in both cultures around 13:00, when PFD reached its maximum (Table S2). J-phase increased, indicating an accumulation of reduced Q_A^-. Around noon, cultures showed increased M_0 and V_J values, indicating an increased rate of closure of reaction centers and an increase in the net rate of Q_A reduction. The Φ_{E0} value decreased by 60 % compared to the initial one. During the experiment M_0 and V_J values increased continuously indicating an increased rate of closure of the reaction centers and an increment in the net rate of Q_A reduction. At noon hours Φ_{E0} value and Ψ_0 decreased by 60 % and 90 %, respectively, compared to the initial values (Table S2).

3.7.3. Photosynthesis performance of the cultures

In MPL-PBR20 (0.5 m plate spacing) net oxygen evolution was 1.6 times higher than in MPL-PBR10 (1.0 m spacing) (Table 3). The values recorded at time intervals of 1 h were more constant in the MPL-PBR20 (average 332 ± 3.9 μmol O_2 mg chl^{-1} h^{-1}) as compared to the other 206 ± 28.9 μmol O_2 mg chl^{-1} h^{-1}. The lowest value was observed in the MPL-PBR10 at the beginning of the day, most likely due to some photoinhibition because of the combination of high light and low morning temperature (about 20 °C). Indeed, at 08.00 am the MPL-PBR10 captured about 52 % of the solar light incident on the horizontal surface, while the MPL-PBR20 captured 37 % of the incident light on a horizontal surface. The respiration rate was about 2 times higher in the MPL-PBR20. On average, dark respiration was 11 % and 15 % (1.0 m and 0.5 m spacing respectively) of the total oxygen evolution rate.

3.7.4. Light conversion efficiency attained with a culture of Synechocystis

Table 4 summarizes the most important variables affecting the productivity and LCE obtained with a culture of *Synechocystis* and operated in the two configurations (10 and 20 plates, spacing 0.5 and 1.0 m). The concentration of the cultures was set by considering the distance of the plates, therefore in the MPL-PBR10 it was double than that in the other. However, both MPL-PBR arrangements had approximately the same areal density (DW m^{-2}). Biomass yield differed greatly between the two MPL-PBR set-ups. It reached 26 g m^{-2} day^{-1} in the MPL-PBR20, while it was 7.2 g m^{-2} day^{-1} in the MPL-PBR10 (Fig. 6). As a result, the calculated LCE differed strongly between the two MPL-PBR designs, reaching 2.44 % (solar basis) with 0.5 m spacing, and 0.67 % with 1.0 m spacing.

3.8. Biochemical composition of the biomass

Biomass composition was strongly dependent on the design of the MPL-PBR (Table S3). In the culture grown in MPL-PBR with plates spaced 1.0 m apart, the biomass composition was very unbalanced and characterized of a high carbohydrate content, reaching 31 ± 5.6 % (mean) of the total DW. In contrast, carbohydrates in the culture grown in PBR with 0.5 m plate spacing were substantially lower 25.6 ± 6.2 % (mean value). The differences in the amount of carbohydrates between the two cultures were

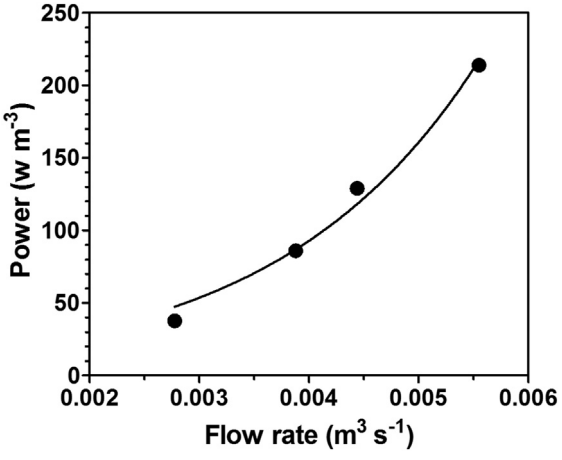

Fig. 4. Power required per cubic meter of culture for circulation at different pump flow rates.

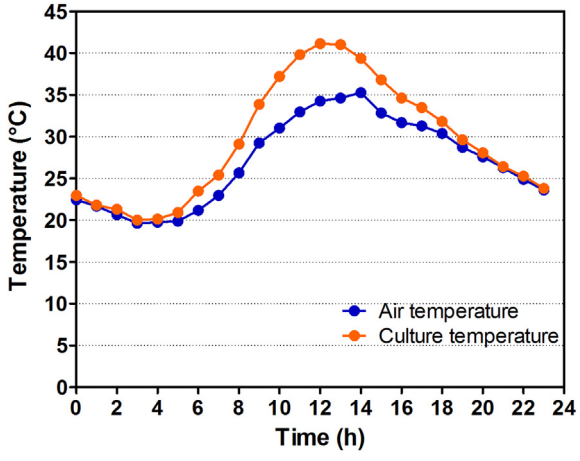

Fig. 5. Culture temperature in PBR-MPL20 simulated by the thermal calculation model (in red). The external air temperature is indicated in blue.

Table 2
Daily variation of irradiation (%) received by a single PBR's plate in August. PFD is expressed as ($\mu mol_{photons}\ m^{-2}\ s^{-1}$).

August (time of day-legal)	Distance between plates 0.5 m				Distance between plates 1.0 m			
	Horizontal PFD	Captured PFD (South)	Captured PFD (North)	% PFD Captured	Horizontal PFD	Captured PFD (South)	Captured PFD (North)	% PFD Captured
08:00	342	36	92	37.4 ± 3.4	490	70	187	52.4 ± 3.8
09:00	792	146	61	26.1 ± 1.8	830	159	108	32.2 ± 3.0
10:00	1115	435	72	45.5 ± 3.3	1150	393	135	45.9 ± 3.1
11:00	1447	592	88	47.0 ± 2.5	1414	618	140	53.6 ± 4.0
12:00	1646	717	94	49.3 ± 2.4	1585	738	158	56.5 ± 4.5
13:00	1779	601	87	38.7 ± 2.2	1725	659	171	48.1 ± 3.2
14:00	1773	675	97	43.5 ± 2.3	1685	686	171	50.9 ± 3.7
15:00	1580	639	78	45.4 ± 3.3	1550	679	146	53.2 ± 4.9
16:00	1313	570	88	50.1 ± 3.3	1335	569	147	53.6 ± 3.6
17:00	965	479	57	55.5 ± 4.5	1032	425	123	53.1 ± 5.1
18:00	655	295	49	52.5 ± 2.5	732	225	93	43.4 ± 5.4

much more evident in the middle of day. An opposite behavior was observed for protein content, which reached 53.7 ± 6.0 % and 45.6 ± 1.3 %, respectively, in the culture grown in PBR with plates 0.5 m and 1.0 m apart. Chlorophyll *a* content was not significantly different between the two cultures (Table S3).

The elemental composition (% of DW) of the biomass produced in the two PBR configurations, sampled at the beginning and the end of the experiment, is shown in Table S4. Nitrogen content decreased by 16 % and 18 % in MPL-PBR20 and MPL-PBR10 respectively, same as for sulfur content. No significant changes were observed in the oxygen, carbon, and hydrogen contents. Based on the elemental composition of the biomass we determined the chemical composition of the biomass harvested in the morning (08.00 am) and evening for *Synechocystis* cultures grown in both PBR configurations (Table S5). A sizeable reduction (about 4 %) in the molecular weight of the biomass was observed between cells harvested in the morning and evening due to a reduction in nitrogen content, while no significant difference was observed between cells grown at different plate distances.

4. Discussion

We tested two geometries of vertical PBR, configured with plates distant 0.5 m (MPL-PBR20) and 1.0 m (MPL-PBR10) from each other, to compare two different light dilution conditions.

The light irradiance measurements performed on the plate surfaces, showed a consistent deviation of the measured light intensity received by the plates from that predicted by the calculations, indicating that the reduction in intensity by a factor of 4, (0.5 m spacing), as expected considering the ratio between the illuminated area of the reactor (A_R) and the surface area occupied by the reactor, i.e., the ground area (A_G), was not attained (Fig. S4). Indeed, the light irradiance incident on the southern face of the reactor during the central hours of the day (between 11.00 am 4.00 pm), in summer, was reduced by a factor of 2.5, which may still not be enough to avoid photo saturation and thus dissipation of light. Therefore, considering these findings, we must conclude that the light dilution factor, that is, the ratio A_R/A_G, indicates a mere geometric reduction of incident solar light. However, other factors may also affect the light uptake of the plates, such as the reflection by the narrow panel, and albedo. In our case, an important role may have been played by the light reflection from the ground, since a white sheet was placed on the ground below the plates to harness the light falling on the bottom.

Another important fact to be highlighted is the strong light inhomogeneity observed on the surface of the plates, as demonstrated by the measurements made on the different parts of the plate at different hours of the day (Fig. S4). The measurements showed that the light irradiance in the upper part of the plate reached as high as 1100 $\mu mol_{photons}\ m^{-1}\ s^{-1}$ in the middle of the day, while in the lower part it was below the saturation irradiance (about 120 $\mu mol_{photons}\ m^{-1}\ s^{-1}$). Such high light irradiance recorded on the plate surface could be the reason for the decrease in the F_v/F_m recorded, particularly when growing a more dilute *Synechocystis* culture suspension in the PBR (Fig. 7).

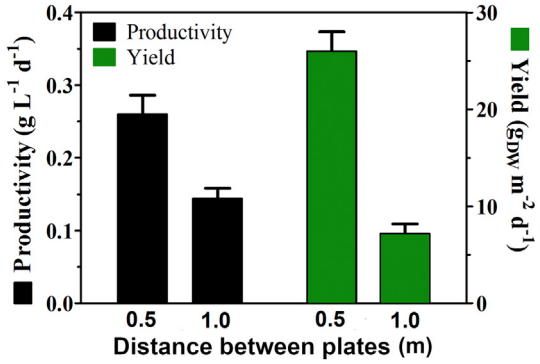

Fig. 6. Volumetric productivity and areal yield comparisons between the two PBR configurations. Volumetric productivity (left Y axis) and areal yield (right Y axis) comparisons between the two PBR configurations. For each distance, experiments were carried out for 14 days during the months of August.

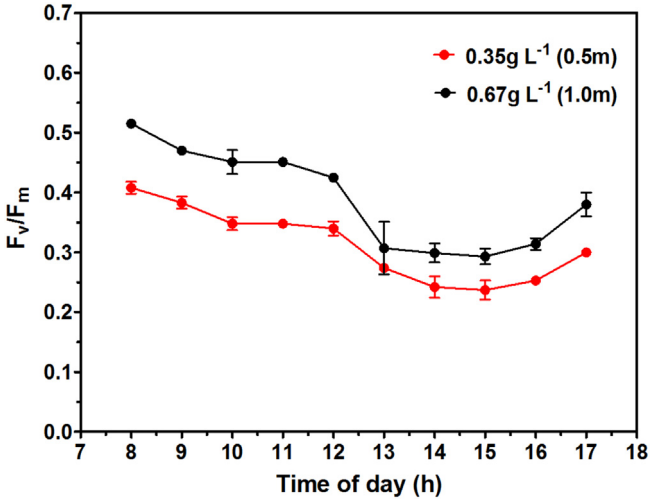

Fig. 7. Daily variation of maximum quantum efficiency of PSII photochemistry (F_v/F_m) of the two experimental conditions (distance between plates 1.0 m and 0.5 m). Daily variation of Photon Flux Density (PFD) of the single experiments is reported in Table 2.

Table 3
Daily variation of Photon Flux Density (PFD), net O_2 evolution, and dark respiration of *Synechocystis* cultures in PBR with two distance settings (1.0 m and 0.5 m) between the plates.

Daytime	PFD ($\mu mol_{photons}\ m^{-2}\ s^{-1}$)		Net O_2 evolution ($\mu mol\ O_2\ mg\ chl^{-1}\ h^{-1}$)		Respiration ($\mu mol\ O_2\ mg\ chl^{-1}\ h^{-1}$)	
	1.0 m	0.5 m	1.0 m	0.5 m	1.0 m	0.5 m
08:00	490	342	160 ± 3	357 ± 7	30.3 ± 2.1	43.7 ± 3.9
09:00	830	792	–	–	–	–
10:00	1150	1115	223 ± 17	327 ± 2	22.1 ± 2.8	49.5 ± 2.3
11:00	1414	1447	–	–	–	–
12:00	1585	1646	246 ± 12	322 ± 12	23.3 ± 1.1	54.7 ± 8.3
13:00	1725	1779	–	–	–	–
14:00	1685	1773	211 ± 11	338 ± 8	20.7 ± 2.2	54.7 ± 2.2
15:00	1550	1580	–	–	–	–
16:00	1335	1313	194 ± 8	326 ± 5	19.4 ± 0.6	45.7 ± 3.5
17:00	1032	965	–	–	–	–
18:00	732	655	204 ± 6	321 ± 31	20.6 ± 1.8	48.6 ± 9.9

4.1. Assessment of the light conversion efficiency of the PBR with a Synechocystis PCC 6803 culture

The purpose of the vertical PBR experiments with two plate distances, was to assess whether LCE could be improved by the light dilution effect. For this purpose, the two aforementioned reactor configurations were compared. Positioning the plates at 0.5 m distance resulted in a much higher LCE than that obtained using 1.0 m distance. Indeed, the LCE at 0.5 m was almost 3.7 times higher than that at 1.0 m distance. However, as previously pointed out, the outdoor cultures were grown at the suboptimal pH of 11 to mitigate the grazing of *Synechocystis* cells by the golden alga *Poterioochromonas*. Indeed, under laboratory conditions, it was found a linear reduction in the LCE from 12.5 % (PAR basis) at pH 7.5 to 8.9 % at pH 11.0 (Touloupakis et al., 2016c). Therefore, it is conceivable that under optimal pH, the attainable LCE could be significantly higher.

Another question that arises is whether a better LCE (and thus a higher areal yield) can be achieved by further reducing the distance between plates. According to Slegers et al. (2011) modelling, optimal distance between plates is about 0.2 m. However, no dramatic reduction in yield is expected (<20 %) if the distance is increased to 0.5 m when the plates are oriented North-South. They concluded that the optimal yield is obtained using 0.3 m for the plate spacing and 5 cm for the light path. Obviously, narrow spacing between plates minimizes loss of light to the ground surface, but if it not combined with the light path reduction, it may result in large volume PBRs with low volumetric productivity and low biomass concentrations. Similar conclusions were reached by Endres et al. (2016). According to these authors, the captured sunlight per unit of ground area increases with the reduction of the distance between plates (5 cm light path) until it peaks at a distance ranging between 0.2 and 0.4 m. In our case, reducing the distance from 1.0 m to 0.5 m almost doubled the light captured. This is quite intuitive since the number of plates on a fixed surface, in our case 10 m^2, doubled (20 plates instead of 10 plates).

One would expect that greater distance would translate into greater volumetric productivity, since it leads to an increased surface area to volume ratio of the reactor, and thus more light available per unit of volume. However, in our case, the amount of light captured by each plate was essentially the same (near 50 % of the horizontal one). Therefore, we can conclude that the concentration used with plates at a 1.0 m distance, although almost double that the one set in the PBR with plates at 0.5 m, was still not optimal. The fluorescence measurements support this hypothesis. The maximum oxygen mass transfer coefficient in the MPL-PBR reactor resulted approximately 4.7 h^{-1} which resulted significantly lower than that recorded in bubble column reactors, but much higher than that in open ponds. However, it should be noted that the MPL-PBR is different from a "bubble column reactor" and degassing occurs mainly in the degassing station that represents about 5 % of the total volume of the reactor. The circulation of the culture carried out in parallel strongly reduces the time cycle of the reactor; on the other hand, increasing the degasser volume leads to an increased volume of the culture in the dark. Enhancing the volume of air fed into the degasser or providing the plates (or some of them) with valves for bubbling air at the bottom even just for a few hours a day are other possibly viable options to increase the mass transfer.

Table 4
Productivity and light conversion efficiency (LCE) achieved in August in MPL-PBR10 and MPL-PBR20 arrangements.

Parameter	Distance between plates	
	0.5 m	1.0 m
Volume in plates (litres)	1000[a]	500[a]
Occupied surface (footprint) (m^2)	10	10
Illuminated surface (m^2)	40	20
Surface (footprint) to volume ratio (m^{-1})	10	20
Illuminated area to surface (footprint) (−)	4	2
Biomass dry weight (mg L^{-1})	350	670
Moles of photons collected by the PBR per day (mole $PBR^{-1}\ day^{-1}$)	435.5	244.8
Percent of total PFD captured by the plates with respect to the horizontal surface	90.22	50.27
Corresponding amount of energy collected (kJ) by the PBR (assuming 218 kJ mol^{-1} within 400–700 nm).	94,929	53,366
Energy received by the horizontal surface (KJ m^{-2})	10,522	10,673
Amount of energy captured per litre of culture (kJ L^{-1})	94.9	53.3
Volumetric productivity (g $L^{-1}\ day^{-1}$)	0.260[b]	0.144[b]
Total biomass output by the reactor (g)	260	72
Biomass yield ground area (g $m^{-2}\ day^{-1}$)	26.0	7.2
Mean energy content kJ g^{-1} biomass	21.98	21.98
LCE % PAR (400–700 nm) on the ground area	5.43[c]	1.48[c]
LCE % on total light (PFD × 0.45)	2.44	0.666

[a] Considering only the volume of culture contained in the plates.
[b] Assuming that, the growth of cultures occurred only in the plates (illuminated portion of the reactor), i.e., excluding the volume in the pump, degasser and manifolds, that is, the total biomass produced (g of DW) has been divided by the illuminated culture volume contained in the plates.
[c] Referred to the energy received by the horizontal surface.

A comparison with other culture systems, such as tubular PBR, is difficult because there is no large-scale outdoor production of *Synechocystis* PCC 6803. A problem with this organism that seriously limits its diffusion in outdoor cultures is its strong susceptibility to grazing by the Chrysophyta (*Poterioochromonas*). This problem was addressed by us by increasing the pH of the culture to 11 (Touloupakis et al., 2016c). At this pH, *Poterioochromonas* cannot survive and therefore disappears completely from the culture. However, comparing the LCE with a two-plane tubular PBR for the culture of *Spirulina*, which was developed to exploit the light dilution effect (Torzillo et al., 1993), the LCEs are very comparable, i.e. 2.97 % for *Spirulina* and 2.44 % for *Synechocystis*. However, the latter had to be cultured at a high pH to avoid contamination by *Poterioochromonas*, which resulted in a reduction in productivity of almost 20 %. Moreover, *Spirulina* has a long history of phenotypical adaptation to high pH, high light, and temperature compared to *Synechocystis*, among other advantages, which explains its success in mass culture.

4.2. Energy expenditure for culture circulation

The power required for culture circulation in the MPL-PBR20, was 214 W m^{-3} at the maximum pump flow rate (0.0055 $m^3\ s^{-1}$) and it was comparable to that found in a PBR with 9 cm inner tube diameter which resulted 300 W m^{-3} (Acién Fernández et al., 2001). On the contrary, we found a power requirement that was about 3 times higher than that reported by Torzillo et al. (1993) for culture circulation in an airlift tubular PBR made with 2.6 cm inner diameter. Obviously, the specific power supply for culture circulation depends strongly on the velocity of the culture, the geometry of the reactor which strongly effect on the head loss, and particularly on the local losses as in the case of the MPL-PBR20. Regarding flat plate, a typical value of 53 W m^{-3} has been cited frequently (Sierra et al., 2008), although this value neglects

the pressure drop over the air sparger holes or the energy efficiency in the production of compressed air. Tredici et al. (2015) calculated a power requirement of about 48 W m^{-3} of culture for 1-Ha Green Wall Panel plant, where mixing was achieved by air bubbling. Similar values of power requirement (about 38 W m^{-3}) were attained in the MPL-PBR20 when the pump flow rate was half of the maximum attainable (0.00277 m^3 s^{-1}).

4.3. Energy for culture cooling

The energy required for culture cooling strongly depends on the optimal temperature for growth of the strain used for outdoor mass culture, by the efficiency of the cooling system and the geographic location. Interestingly, for the same location used for culture growth (experimental area in Sesto Fiorentino), Tredici et al. (2015) using the microalga *Tetraselmis suecica*, which has an optimal growth temperature close to 30 °C (Chini Zittelli et al., 2006), found an average energy expenditure for cooling of 0.192 kWh day^{-1}, while 9.2 kWh day^{-1} was required for cooling the *Synechocystis* culture with an optimal temperature of 35 °C (Zavřel et al., 2017) (see supporting information). This strong difference in energy requirement for culture cooling can be explained by the fact that in the case of Tredici et al. (2015) the energy consumption was limited to that of a submersible pump used to circulate seawater (temperature 20 °C) through a serpentine placed in the bottom of the panels, whereas in the case of MPL-PBR, the tap water (about 24 °C) had to be first cooled by a cooling device before being circulated through a serpentine in the MPL. The two systems differ greatly in their mixing system. In the Green Wall Panel, the culture is mixed by bubbling air from the bottom which promotes evaporative cooling, while in the MPL the culture is circulated by a pump in a closed system, which is a mandatory requirement for using the PBR for hydrogen production experiments. The efficiency of the MPL-PBR cooling can be significantly improved, for example, by replacing the dark PVC frames of the plates, with white plastic materials with a much lower heat absorption coefficient or by making the entire plate of glass.

5. Conclusions

The results show that the optimal distance between plates cannot be determined in a simple way, but for a given latitude it is the result of a proper combination of distance, light path, and biomass concentration that must be validated in the field. Under light conditions of central Italy (43° 46′ N), *Synechocystis* cultures grown in vertical reactors with plates set at 0.5 m apart resulted in a much higher yield compared to those grown at 1.0 m distance. We may conclude that a further decrease in plate spacing (e.g. 0.25 m) should be accompanied by a decrease in light path to 2.5 cm, which may lead to a further increase in yield and LCE. This configuration may result adequate for experiments on photobiological hydrogen production which are usually carried out under low irradiance. Our results provide a useful experimental basis for better refinement of models aimed at predicting the best combination between plate spacing, light path, and biomass concentration for industrial installation of flat vertical PBRs.

CRediT authorship contribution statement

Giuseppe Torzillo: fund acquisition, design of the photobioreactor, conceptualization, methodology, data analysis, writing-original draft, review and editing. **Graziella Chini Zittelli**: investigation and writing. **Bernardo Cicchi**: investigation and editing. **Ana Margarita Silva Benavides**: investigation, data analysis. **Marcello Diano**: design and construction of the photobioreactor. **Maddalena Parente**: photobioreactor thermal simulations. **Serena Esposito**: technical and investigation assistance. **Eleftherios Touloupakis**: design of the experiments, investigation, data analysis, writing, and review. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Giuseppe Torzillo reports financial support was provided by European Commission.

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Giuseppe Torzillo: fund acquisition, design of the photobioreactor, conceptualization, methodology, data analysis, writing-original draft, review and editing. Graziella Chini Zittelli: investigation and writing. Bernardo Cicchi: investigation and editing. Ana Margarita Silva Benavides: investigation, data analysis. Marcello Diano: design and construction of the photobioreactor. Maddalena Parente: photobioreactor thermal simulations. Serena Esposito: technical and investigation assistance. Eleftherios Touloupakis: design of the experiments, investigation, data analysis, writing, and review. This work was supported by the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement number 308518 (CyanoFactory). We thank Mr. Edoardo Pinzani for technical support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.156840.

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