Bioresource Technology 280 (2019) 404–411

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluation of photosynthetic light integration by microalgae in a pilot-scale raceway reactor

M. Barceló-Villalobos[a], P. Fernández-del Olmo[c], J.L. Guzmán[a], J.M. Fernández-Sevilla[b], F.G. Acién Fernández[b,*]

[a] Department of Informatics, Universidad de Almería, ceiA3, CIESOL, E04120 Almería, Spain
[b] Department of Chemical Engineering, Universidad de Almería, Spain
[c] Institute for Research in Agriculture and Fisheries, Junta de Andalucía, E04720 Almería, Spain

ARTICLE INFO

Keywords:
Microalgae
Raceway
Biomass production
Light frequency
CFD

ABSTRACT

The improvement of photosynthetic efficiency in a 100 m^2 raceway reactor by enhancement of light regime to which the cells are exposed is here reported. From Computational Fluid Dynamics it was calculated that the light exposure times ranged from 0.4 to 3.6 s while the exposure times to darkness were much longer, from 6 to 21 s. It was demonstrated that these times are too long for light integration, the cells fully adapting to local irradiances. This phenomenon was validated in the real outdoor raceway at different seasons. Simulations allows to confirm that if total light integration is achieved biomass productivity can increase up to 40 g/m^2·day compared to 29 g/m^2·day obtained considering local adaptation, which is close to the experimental value of 25 g/m^2·day. This paper provides clear evidence of microalgae cell adaptation to local irradiance because of the unfavourable cell movement pattern in raceway reactors.

1. Introduction

Raceway reactors are the most extended technology for microalgae growth; more than 90% of the worldwide microalgae production is performed using this technology. The design of raceway reactors was first proposed by Oswald and Golueke in the 1960's (Oswald and Golueke, 1960). This design has recently been revised to increase microalgae production, and especially to integrate these reactors into large applications such as wastewater treatment. Therefore, the energy consumption, mixing and mass transfer of these reactors are currently the subject of research (Barceló-Villalobos et al., 2018; de Godos et al., 2014; Labatut et al., 2007; Liffman et al., 2013; Mendoza et al., 2013b). In spite of the recent improvements regarding these aspects, the overall performance of whichever microalgae culture is always limited by the light utilization efficiency; that is to say, by how the microalgae use the light impinging on the reactor surface.

In raceway reactors, as in any microalgae culture system, light gradients exist due to the light attenuation caused by the cells. According to the culture movement (vertical mixing), the cells are exposed to different light regimes as a function of the existing light gradients. The relevance of the light regime (to which the cells are exposed) on microalgae culture performance has been widely discussed, and is still a hot topic in the microalgae biotechnology field given its importance in improving the biomass productivity of real systems (Brindley et al., 2016, 2011; Grobbelaar, 1989; Schulze et al., 2014; Ugwu et al., 2005; Vejrazka et al., 2011). Moreover, it is not only light intensity and frequency that matter - the actual "shape" of the irradiance variation pattern also influences the response of microalgae cells (Brindley et al., 2016). These authors showed that pure flashing light (neat dark/light changes represented by square-wave functions) elicit a lower photosynthetic response than other light patterns, calling into question the idea that any light regime is a mere transition from light to dark. On the basis of the photosynthesis mechanism, the time requested for reactions to take place in PSI-PSII is much lower than the time required to use the energy produced by the cells. High frequency fluctuating light (< 100 ms) has been reported to lead to higher growth rates and higher photosynthesis rates than continuous light (Matthijs et al., 1996; Nedbal et al., 1996). These values are a function of light intensity and other variables such as the strain and status of the cells, among others; thus it is a complex phenomenon. To summarize, it is generally accepted that to allow complete light integration, dark cycles at frequencies higher than 1 Hz are required (Brindley et al., 2011).

However, applying these results to large-scale reactors is difficult because the real patterns in these systems have barely been analysed.

* Corresponding author.
E-mail address: facien@ual.es (F.G. Acién Fernández).

https://doi.org/10.1016/j.biortech.2019.02.032
Received 11 December 2018; Received in revised form 4 February 2019; Accepted 5 February 2019
Available online 06 February 2019
0960-8524/

Recently, the utilization of Computational Fluid Dynamics (CFD) tools, such as ANSYS Fluent, have allowed us to analyse the hydrodynamics in real-scale reactors (Bitog et al., 2011; Fernández-Del Olmo et al., 2017; García et al., 2012; Soman and Shastri, 2015). The studies performed to date are mainly focused on the reactor's energy consumption and on how to minimize the pressure drop caused by the liquid circulation. Other papers are focused on the elucidation of the light pattern to which the cells are exposed as a function of the design, operational and environmental parameters. There is, however, an underlying inconsistency in the literature dealing with the light integration effect in microalgae reactors. While numerous studies provide converging evidence for enhanced photosynthetic efficiency at a higher frequency of on/off signals (Perner-Nochta et al., 2007), there is no clear experimental evidence that more mixing actually enhances growth. Overall, studies that demonstrate productivity enhancement through increased mixing are scarce compared to studies restricted to the flashing effect, and they are rarely supported by experimental evidence (Demory et al., 2018) However, most of these studies have been performed on small reactors. Moreover, no clear figures were provided regarding the light regimes to which the cells were exposed in these reactors. The relevance of the reactor size must be emphasized because the circulation pattern is significantly modified according to the scale of the reactors. Consequently, when using small raceway reactors, the contribution of bends, paddlewheel and other structural elements, such as baffles, greatly modifies the liquid pattern in the channel, with these sections increasing the vertical mixing. Conversely, in large reactors, the contribution of these sections is minimal so the vertical mixing is very low (Demory et al., 2018; Mendoza et al., 2013a).

Accordingly, this paper focuses on quantifying the light utilization phenomenon taking place in real raceway reactors. For this, the light exposure times in a real pilot-scale reactor were determined and their influence on the cells' photosynthesis rate was measured under laboratory conditions. The objective was to determine if the biomass productivity of real-scale raceway reactors can be improved by enhancing the light regime to which the cells are exposed; and how it would be possible to achieve this objective. Clarifying this question is highly relevant for microalgae biotechnology, not only in terms of the potential improvements in the productivity of real production systems, but also because it would affect all the models and design tools currently used in this field.

2. Materials and methods

2.1. Microorganism, raceway reactor and culture conditions

The microalgae strain used was *Scenedesmus almeriensis* (CCAP 276/24). Inoculum for the raceway reactor was produced in a 3.0 m^3 tubular photobioreactor under controlled conditions (pH 8 by on-demand CO_2 injection and a temperature ranging from 18 to 22 °C) using freshwater and Mann & Myers medium prepared with fertilizers (0.14 $g{\cdot}L^{-1}$ KH_2PO_4, 0.18 $g{\cdot}L^{-1}$ $MgSO_4$ $7H_2O$, 0.9 $g{\cdot}L^{-1}$ $NaNO_3$, 0.02 $mL{\cdot}L^{-1}$ Welgro, and 0.02 $g{\cdot}L^{-1}$ Kalentol).

The raceway reactor is located at the "Las Palmerillas" Research Centre, 36° 48′N–2° 43′W, part of the Cajamar Foundation (Almería, Spain). The reactor consists of two 50 m long channels (0.46 m high × 1 m wide), both connected by 180° bends at each end, with a 0.59 m^3 sump (0.65 m long × 0.90 m wide × 1 m deep) located 1 m along one of the channels (Barceló-Villalobos et al., 2018). A paddlewheel system was used to recirculate the culture through the reactor at a regular velocity of 0.2 $m{\cdot}s^{-1}$, although this could be increased up to 0.8 $m{\cdot}s^{-1}$ by manipulating the frequency inverter of the engine. The pH, temperature and dissolved oxygen in the culture were measured using appropriate probes (5083 T and 5120, Crison Instruments, Barcelona, Spain), connected to an MM44 control-transmitter unit (Crison Instruments, Spain), and data acquisition software (Labview, National Instruments, USA) providing complete monitoring and control of the system. The culture's pH was maintained at 8.0 by on-demand CO_2 injection whereas temperature was not controlled – this varied by ± 5 °C with respect to the daily mean air temperature, which, in turn, varied from 12 °C in winter to 28 °C in summer. The raceway reactor was inoculated and operated in batch mode for one week, after which it was operated in semi-continuous mode at 0.2 day^{-1} at a culture depth of 0.15 m. Only samples from steady-state conditions were used. Evaporation inside the reactor was compensated for by the daily addition of fresh medium.

2.2. CFD model

The ANSYS Meshing 12.1 pre-processor was used to generate the numerical grid for a suitable discretization of the computational domain. Hexahedral mesh was used because of its capabilities in providing a high-quality solution with fewer cells for simple geometry. To obtain the mesh-independent result, three different mesh densities (1,096,250, 1,656,875 and 2,096,875 cells) were evaluated. The optimal mesh density (1,656,875), in terms of computational time and accuracy, was implemented in the numerical analysis. In order to exclude turbulence model dependence on the results, a sensitivity study on the turbulence settings was performed on the optimal mesh. The realizable k-ε model was used. The numerical model was based on the finite-volume method. The transport equations were solved using the ANSYS FLUENT 12.1 CFD commercial software package. The flow solution procedure used was the Semi-Implicit Method for Pressure-Linked Equation (SIMPLE) routine. The momentum equations were discretized using both first and second-order upwind scheme options, and second-order upwind for the other transport equations. The convergence criterion consisted of monitoring the mass flow rate at the inlet and outlet, and the variation in velocity profiles with iteration, a reduction of several orders of magnitude in the residual errors. The results were validated by comparing them with the experimental measurements. 3D simulations were performed on a workstation with two Six-Core Intel Xeon X5650 2.66 GHz 12 MB/1333 processors, and HP 48 GB RAM (6 × 8 GB) DDR3-1333 (Hewlett Packard, USA).

2.3. Photosynthetic rate measurement

The photosynthesis rate of the microalgae samples was determined using a photo-respirometer built by the Chemical Engineering Department at the University of Almería. The system basically consists of a magnetically-stirred jacketed vessel (0.03 m diameter, 80 mL total volume), in which the pH (Crison pH5010, Spain), dissolved oxygen (Crison OD6050, Spain), temperature (Crison PT1000, Spain) and light (Walz US-SQS/L, Germany) probes are submerged; the light being provided by two LED lamps (SMD Bridgelux Pro 200W, Germany). The entire system was computer controlled. Sensors were connected to an MM44 control-transmitter unit (Crison Instruments, Spain) and data acquisition software (DAQFactory, Labjack, USA) to provide complete experiment monitoring and control. The LED lamps were controlled both in intensity and on/off period duration. The photosynthesis rate was measured by providing the requested light conditions and evaluating the oxygen accumulation rate, also providing air between cycles to always perform the measurements close to 100 %Sat; thus avoiding the influence of this variable. A minimum of three cycles were used to obtain a mean value under the conditions assayed.

2.4. Experimental design and accuracy of measurements

A total of 172 samples were taken from the raceway reactor at different times throughout the year (from February to July) and at different times of the day (8:00 h, 10:00 h, 12:00 h and 14:00 h). The photosynthesis rate (PO_2, mg L^{-1} h^{-1}) was calculated by diluting the culture and providing the target light conditions, measuring the oxygen production over time. The performance of the microalgae cells under

continuous light conditions was evaluated first as a control stage. Following this, the performance of the cells under different light regimes was determined. In the first set of experiments, the irradiance without cells was kept at 500 μE m^{-2} s^{-1} and the light and dark times were modified from 1 to 10 s. In the second set of experiments, the irradiance was adjusted to that existing in real outdoor reactors whereas the light and dark times were adjusted close to those experimentally determined by CFD, thus ranging from 1 to 24 s. In each experiment, the irradiance without cells (I_0), the irradiance with cells (I), the light time, and the dark time were fixed. From these values, we calculated the different parameters. The average irradiance, Iav, was calculated as the light inside the jacketed vessel multiplied by the proportion of time that it was illuminated (Eq. (1)). The illuminated cycle proportion, Φ, was calculated as the illuminated time to total time ratio (Eq. (2)). The light exposure frequency, υ, was calculated as the inverse of the total cycle time (Eq. (3)).

$$Iav = I\frac{t_{light}}{t_{light} + t_{dark}} \quad (1)$$

$$\Phi = \frac{t_{light}}{t_{light} + t_{dark}} \quad (2)$$

$$\upsilon = \frac{1}{t_{light} + t_{dark}} \quad (3)$$

2.5. Statistical analysis

Data were processed using Microsoft Excel 2016, and the statistical analysis was performed using Statgraphics Centurion 18. Data from the outdoor reactors were obtained at steady state whereas measurements under laboratory conditions were performed in triplicate (as a minimum).

3. Results and discussion

To study the influence of the light/dark cycles taking place in a 100 m^2 raceway reactor on the performance of microalgae cells, the first step was to model the photosynthesis rate for continuous light under laboratory conditions. A classical P-I curve was obtained for the *Scenedesmus almeriensis* cells taken directly from the reactor. The curve was obtained at three different biomass concentrations, 0.1, 0.2 and 0.4 g/L, by diluting the initial culture, 1.2 g/L, with fresh culture broth to minimize the variations in culture conditions. The results show that the photosynthesis rate increased hyperbolically with the irradiance, the system behaviour being equal at biomass concentrations of 0.1–0.2 g/L whereas at 0.4 g/L, a lower photosynthesis rate was observed for the same irradiance values (Fig. 1). This can be attributed to the light gradients inside the test cultures. In spite of the small size of the glass reactor used (0.03 m diameter), light gradients always exist in microalgal cultures due to light attenuation by the cells; these are more intense when the biomass concentration in the culture is high. In diluted microalgae cultures, the cells are almost continuously exposed to light and, under such conditions, photosynthetic performance is optimal (0.1–0.2 g/L). In contrast, when intense light gradients are present (0.4 g/L), the cells remain at low irradiance for a significant amount of time and thus the photosynthesis rate is reduced.

Data from the three experiments were fitted to the hyperbolic model (Molina-Grima et al., 1996) (Eq. (4)); the model's characteristic parameter values being shown in Table 1. The results show that the maximum photosynthesis rate, PO_{2max}, was 101 $mgO_2/g_{biomass}$·h at the optimal biomass concentration of 0.1–0.2 g/L but this reduced to 63 $mgO_2/g_{biomass}$·h when using the higher biomass concentration of 0.4 g/L. Regarding the irradiance at half-saturation, Ik, a value of 82–90 μE/m^2·s was obtained at the optimal biomass concentration of 0.1–0.2 g/L whereas at the higher biomass concentration of 0.4 g/L, this

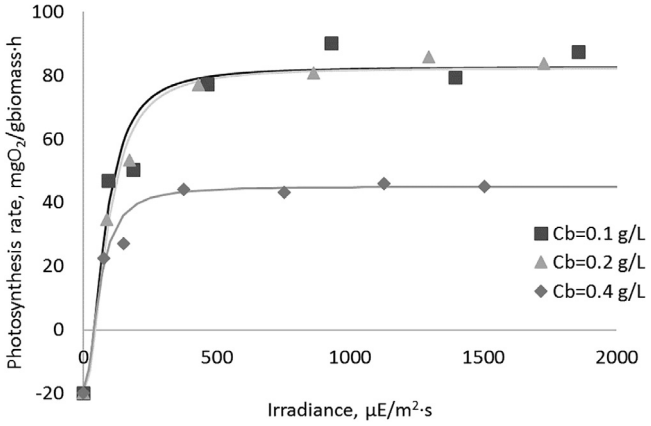

Fig. 1. Variation in the photosynthesis rate/of *Scenedesmus almeriensis* cells as a function of the average irradiance for continuous light under laboratory conditions.

Table 1
Characteristic parameter values of the hyperbolic model determined from the different samples studied: samples from the same reactor at different biomass concentrations; samples from the same reactor in different seasons.

Cb g/L	PO_{2max}, $mgO_2/g_{biomass}$·h	n	Ik μE/m2·s	RO2 $mgO_2/g_{biomass}$·h	Ic μE/m2·s	Φ %
0.1	101.3	2.01	82.2	−18.7	40.0	0.93
0.2	101.4	1.99	90.5	−18.9	43.0	0.86
0.4	63.5	2.00	61.3	−18.5	39.0	0.75
Season	PO_{2max}, $mgO_2/g_{biomass}$·h	n	Ik μE/m2·s	RO2 $mgO_2/g_{biomass}$·h	Ic μE/m2·s	Φ %
Winter	130.0	2.00	120.0	−10.0	35.0	0.86
Spring	180.0	2.00	70.0	−20.0	26.0	0.86
Summer	160.0	2.00	90.0	−20.0	34.0	0.86

value dropped to 61 μE/m^2·s. The value of form parameter, n, remained constant at 2.0. What is also highly relevant is that the respiration rate was the same regardless of the biomass concentration; a value of −18 $mgO_2/g_{biomass}$·h being measured. This confirms that the respiration rate is independent of biomass concentration; it being only a function of the culture conditions under which the cells are produced. Concerning the minimum irradiance for photosynthesis, Ic, values from 39 to 43 μE/m^2·s were obtained for the biomass concentrations assayed, with no tendency being observed of this characteristic parameter with regard to the biomass concentration. The maximum photosynthesis rate measured agreed with previously reported values of 270 $mgO_2/g_{biomass}$·h (Brindley et al., 2016). From these figures, the duty cycle can also be calculated (that is to say, the percentage of time at which the cells were exposed to light) as the ratio between the irradiance with the culture present, I, and the initial irradiance with water only, Io (Eq. (5))- a value of 0.75 was obtained when using 0.4 g/L whereas this parameter increased to 0.86 and 0.93 when using 0.2 and 0.1 g/L, respectively. These figures anticipate the relevance of the duty cycle on culture performance - the higher this parameter is, the higher the photosynthesis rate for the same average irradiance results.

$$PO_2 = \frac{PO_{2max} \times I^n}{I_k^n + I^n} + RO_2 \quad (4)$$

$$\Phi = \frac{I}{Io} \quad (5)$$

Once the photosynthesis model was known, the light/dark cycles existing in the 100 m^2 raceway reactor were studied, a critical parameter that needs to be defined is the minimum irradiance required for the microalgal cells to perform photosynthesis, Ic. On the basis of this

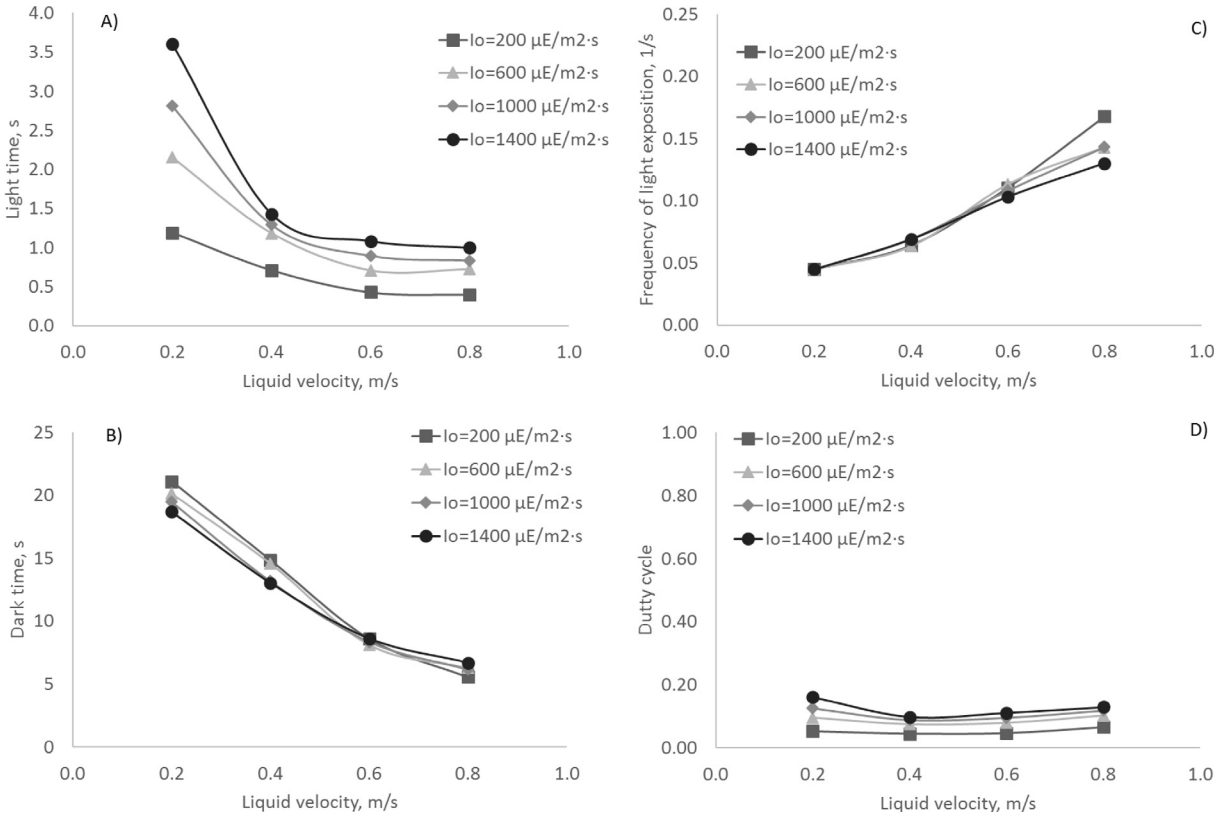

Fig. 2. Variation in the light time (A), dark time (B) and light exposure frequency (C) of Scenedesmus almeriensis cultures in a raceway reactor as a function of the liquid velocity/and solar irradiance/on the reactor surface. Data obtained from CFD analysis of a real raceway reactor operated in continuous mode.

value, the entire volume of any reactor can be divided into light volume (a zone where there is enough light for photosynthesis to take place) and a dark volume (a zone where no photosynthesis is possible). Considering an Ic value equal to 40 µE/m^2·s (Table 1), the time that the cells spend in the illuminated volume and in the dark volume of the real raceway reactor was calculated by CFD (Fig. 2). The results show that the time the cells were exposed to light, when close to the surface of the raceway reactor, was much shorter than the time that the cells remained in the dark, inside the reactor, where maximum values of 3.6 and 28 s were obtained, respectively (Fig. 2A and B). Conversely, the minimum time the cells were exposed to light was 0.4 s compared to a minimum dark time of 5.6 s. All of these figures are mean values, obtained considering the average times for a total of 50 cells/particles moving along the raceway reactor for 50 m in a straight channel. The data show that the vertical movement of the liquid in the raceway reactor was minimal so displacement of the cells/particles from the reactor's light to dark zones was very slow, with some of them never moving between the light and dark zones; however, on average, the times calculated correspond to the expected average behaviour of a single cell. The results also show how the light time increased when increasing the solar irradiance onto the reactor surface due to deeper light penetration into the culture whereas this reduced when increasing the liquid velocity because of the higher light/dark cycle frequency taking place. Regarding the dark time, this was reduced when the solar irradiance was greater, again due to the increased light penetration into the reactor. Likewise, higher liquid velocity in the reactor brought about an increase in the light/dark transition frequency to which the cells were exposed. Analysing the data on the frequency of light exposure, one observes how this increased when the liquid velocity increased; the solar irradiance influence being minimal in spite of the maximum frequencies measured at 0.17 Hz (Fig. 2C). It has been indicated that, to maximize the performance of microalgae cultures, an approximate mixing frequency of 15 Hz is needed, which would require an increase in liquid velocity up to 7.2 m/s in a 0.09 m tube diameter tubular photobioreactor (Brindley et al., 2016). Such a high liquid velocity would be very difficult to achieve in real reactors, and it would cause undesirable side-effects; for example, severe damage to the cells by shear-related stress phenomena (Alías et al., 2004). In raceway reactors, increasing the velocity above 0.8 m/s is almost impossible; in addition, when the velocity is increased above 0.2 m/s, the power consumption required for circulating the liquid is greatly increased (Mendoza et al., 2013a). Regarding the duty cycle, the results show how, in real raceway reactors, the proportion of time that the cells received light was very low, with maximum values of 0.16, and where no relevant variations were observed with liquid velocity or solar irradiance (Fig. 2D). Duty cycle values in the 0.05 range are typical for concentrated cultures whereas in diluted cultures, values up to 0.4 can be found; values up to 0.9 being measured only in really diluted cultures (Brindley et al., 2016). Higher duty cycle values favour light integration by the cells although this is also influenced by other variables such as the irradiance and frequency under those conditions (Brindley et al., 2011). In any case, the most relevant insight from the data obtained is that the times to which the cells are exposed to light or dark conditions are very large, especially when compared to that recommended for light integration, which is less than 1 s (Brindley et al., 2011). Therefore, in order to evaluate the performance of microalgae cells based on the light/dark variations taking place inside real raceway reactors, experiments must be performed under these conditions, thus providing light and dark periods in the range of seconds.

Consequently, the first set of experiments was performed taking culture from the raceway reactor and evaluating its photosynthesis rate under laboratory conditions yet simulating those outdoors, thus providing light and dark times based on the experimental raceway reactor results. For this, a complete factorial experimental design was performed that considered light and dark times of 1, 2, 3, 4, 5 and 10 s with the photosynthesis rate under these conditions being determined

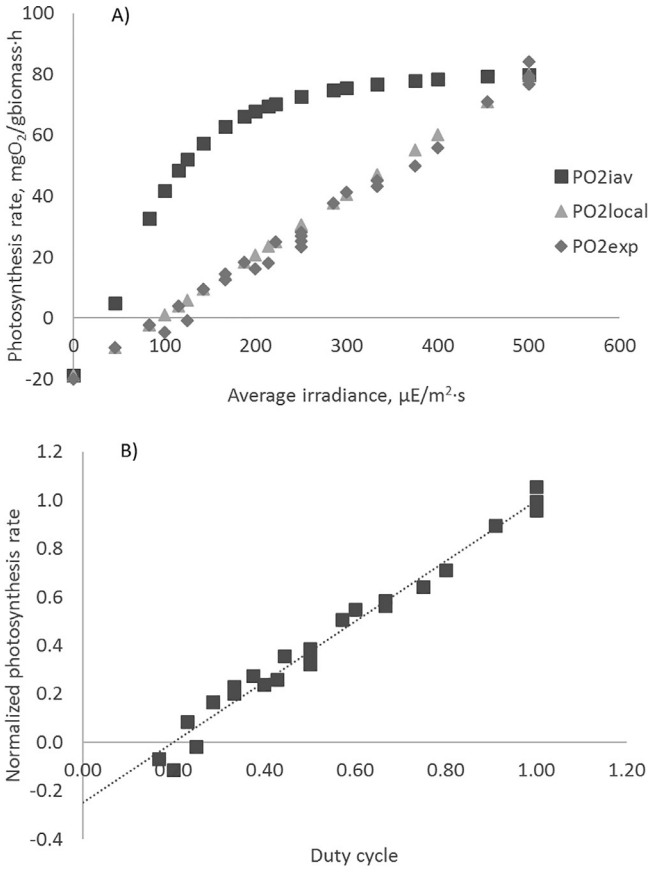

Fig. 3. (A) Variation in the photosynthesis rate (mg O_2/g biomass h) of Scenedesmus almeriensis cultures in a raceway reactor as a function of the average irradiance (µE/m^2 s) to which the cells were exposed, under different light/dark cycles ranging from 1 to 10 s. PO2iav corresponds to the modelled photosynthesis rate if complete light integration takes place; whereas PO2local corresponds to the modelled photosynthesis rate with null light integration. (B) Variation in the normalized photosynthesis rate with the duty cycle for the same experiments.

(Fig. 3). Based on the irradiance provided, the maximum photosynthesis rate, PO_{2io}, was calculated using the hyperbolic model previously described (Eq. (6)). By employing the average irradiance to which the cells were exposed, calculated as the irradiance provided multiplied by the percentage of time for which it was provided, the theoretical maximal photosynthesis rate with full light integration (PO_{2iav}) was determined (Eq. (7)). Conversely, the theoretical minimum photosynthesis rate, if null light integration took place, PO_{2local}, was calculated as the sum of the photosynthesis rate during light and dark periods, both calculated using the same hyperbolic model previously determined (Eq. (8)). The experimental photosynthesis rate, PO_{2exp}, must be midway between these two limit values, the higher the frequency at which the light was provided, the greater the light integration with the experimental values of the photosynthesis rate approaching the PO_{2iav} values.

$$PO_{2io} = \frac{PO_{2max} \times Io^n}{I_k^n + Io^n} + RO_2 \quad (6)$$

$$PO_{2iav} = \frac{PO_{2max} \times Iav^n}{I_k^n + Iav^n} + RO_2 \quad (7)$$

$$PO_{2local} = (PO_{2io}\hat{A}\cdot\Phi)_{light} + (RO_2\hat{A}\cdot(1-\Phi))_{dark} \quad (8)$$

The results show how the maximal photosynthesis rate, PO_{2iav}, increased hyperbolically with the average irradiance; as expected from the hyperbolic model used to calculate these values (Fig. 3). These values correspond to the maximal values in the event that full light integration of the cells took place. Regarding the photosynthesis rate, if null light integration took place, PO_{2local}, values increased linearly with the average irradiance because these experiments were always performed at the same external irradiance of 500 µE/m^2·s using diluted cultures. Hence, the average irradiance increased linearly with the proportion of light in the illumination cycle assayed. What is more interesting is that the experimental measurements performed, PO_{2exp}, completely fitted the trend of PO_{2local}; thus indicating that light integration does not occur under long light/dark times. The results show that, only when the average irradiance was really high, did the experimental photosynthesis rate approximate the maximal one although this was because, under these conditions, the minimum dark times assayed were of 1 s. In contrast, it was also observed that, when providing long light/dark times, the minimum average irradiance required to start the photosynthesis process increased up to 100 µE/m^2·s. Analysing the variation in the normalized photosynthesis rate (PO_{2exp}/PO_{2iav}) with the proportion of light time in the cycle, a clear linear correlation was observed (Fig. 3B). This confirms that, at the time scale used, there is no light integration and the cells perform photosynthesis according to the irradiance that they receive during the "seconds-scale" times provided. It has been reported that, in commercial photobioreactors, medium-frequency fluctuations prevail, the behaviour being different for medium frequency fluctuations than for high frequency fluctuations (Barbosa et al., 2003). Light/dark cycles in the 6–87 s range lead to similar or lower growth rates and biomass yields on light energy compared to those obtained under continuous light of the same light intensity as that during the light period of the light/dark cycles (Janssen et al., 2000a,b, 1999). No influence of light/dark cycles in the 1–263 s range was found in terms of the volumetric productivity, specific oxygen production, or carbon dioxide fixation (Grobbelaar, 1991, 1989). On the other hand, it has been reported that maximal carbon dioxide fixation is achieved under 4 s light/dark cycles (Bosca et al., 1991) whereas maximal growth rates are achieved under light/dark cycles with a dark period of 9 and 6 s (Lee and Pirt, 1981; Merchuk et al., 1998).

To demonstrate this fact, a large set of experiments was performed over six months, maintaining the raceway reactor in continuous mode and taking samples from the reactor to evaluate the photosynthesis rate in the laboratory yet simulating the real light/dark cycles at which the cells were exposed to inside the outdoor reactor. In this case, the external irradiance provided was that found in the outdoor reactor at different daylight times, where several samples were measured each day at different hours of the day, with light times ranging from 1 to 8 s while dark times ranged from 1 to 24 s. To take into account the variation in culture conditions in different seasons, we periodically evaluated the performance of the cells taken directly from the reactor under continuous light. In this way, the P-I curve of the culture was obtained as a control curve. The results confirmed that the hyperbolic model is always suitable to fit the light response of the *Scenedesmus almeriensis* cells, including from real outdoor reactors in different seasons (Fig. 4). However, the results clearly show how the behaviour was not the same for all of the seasons evaluated. The cultures grown in spring performed better than those grown in summer and winter. By fitting the experimental data to the hyperbolic model, we calculated the characteristic parameter values for the cultures obtained in each season (Table 1). The values were similar to the previous ones not only in terms of the maximal photosynthesis rate and the half-saturation irradiance but also in terms of the respiration rate and the minimum irradiance required to start photosynthesis. The major difference was for the maximum photosynthesis rate, which was notably high for the culture grown in spring; thus indicating the adequacy of the operating conditions during this period.

To consider the variation in cell performance in the different seasons, the same analysis as previously performed to study the influence of average irradiance and light/dark cycles on the performance of

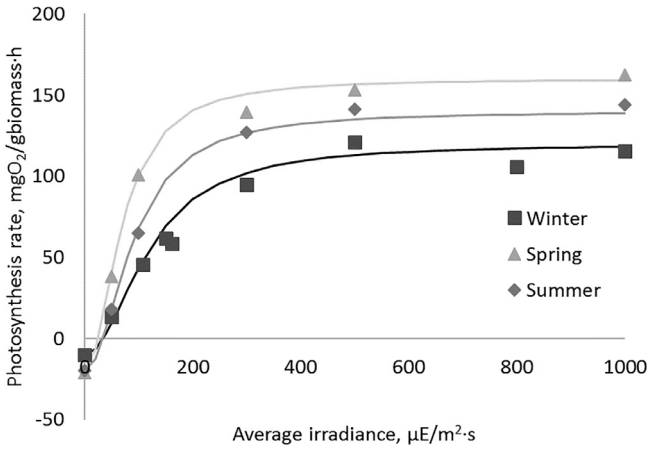

Fig. 4. Variation in the photosynthesis rate with average irradiance for three Scenedesmus almeriensis samples produced in a 100 m^2 raceway reactor in continuous mode at 0.2 day^{-1} for different seasons.

outdoor cultures was applied to the entire set of data from the six-month operation of the raceway reactor. Because different irradiances, and different light and dark times were used, it was not possible to provide a single figure summarizing all of the results. However, the results did confirm that the behaviour of the cultures completely fitted the local irradiance available. Hence, Fig. 5 demonstrates how a linear relationship exists between the experimental photosynthesis rate determined in the laboratory simulating the light/dark cycles taking place in real raceway reactors with that predicted by the hyperbolic model and the estimated local utilization of light. The correlation is slightly better in the winter time yet it is also highly acceptable for spring and summer time, especially considering the large variations in culture conditions that take place in outdoor reactors and the large number of measurements taken (up to 172). In a previous study, the effect of a medium-frequency cycle time (10–100 s) and light fraction (0.1–1) on the growth rate and the biomass yield of the microalgae Dunaliella tertiolecta was studied. The biomass yield and growth rates were mainly affected by the light fraction while the cycle time had little influence (Barbosa et al., 2003).

These results have clear consequences regarding the performance and modelling of raceway reactors. The first is that, from the photosynthetic point of view, cell adaptation to average irradiance cannot be approximated in these reactors. It has been demonstrated that cells adapt to the local irradiance present at different positions inside the reactor. The second consequence is that, in raceway reactors, the light frequency to which the cells are exposed inside the culture is about 0.05 Hz, far from that required to allow light integration (1 Hz), and thus trying to optimize the light regime in raceway photobioreactors by increasing liquid velocity is unrealistic. The third is that raceway reactor productivity is limited by the phenomenon of local irradiance adaptation.

To analyse how the local irradiance adaptation phenomenon influences raceway reactor productivity, a simple simulation exercise was performed considering the operation of the 100 m^2 raceway reactor under the culture conditions imposed (Dilution rate = 0.2 day^{-1}, Cb = 1.2 $g{\cdot}L^{-1}$). Knowing the extinction coefficient of the biomass (Ka = 0.15 $m^2{\cdot}g^{-1}$), the light profile inside the reactor at different solar hours/irradiances could be estimated using Lambert's law (Fig. 6A); this was performed on a spring day in Almeria (Spain) where the raceway reactor is located. The results show how irradiance inside the reactor exponentially decreased with culture depth regardless of the solar irradiance on the reactor surface; this is because only the first 0.04 m (approximately) of the culture receives a light intensity greater than the minimum value, of Ic = 40 $\mu E/m^2{\cdot}s$, required for photosynthesis. Therefore, approximately 73% of the culture is in total darkness,

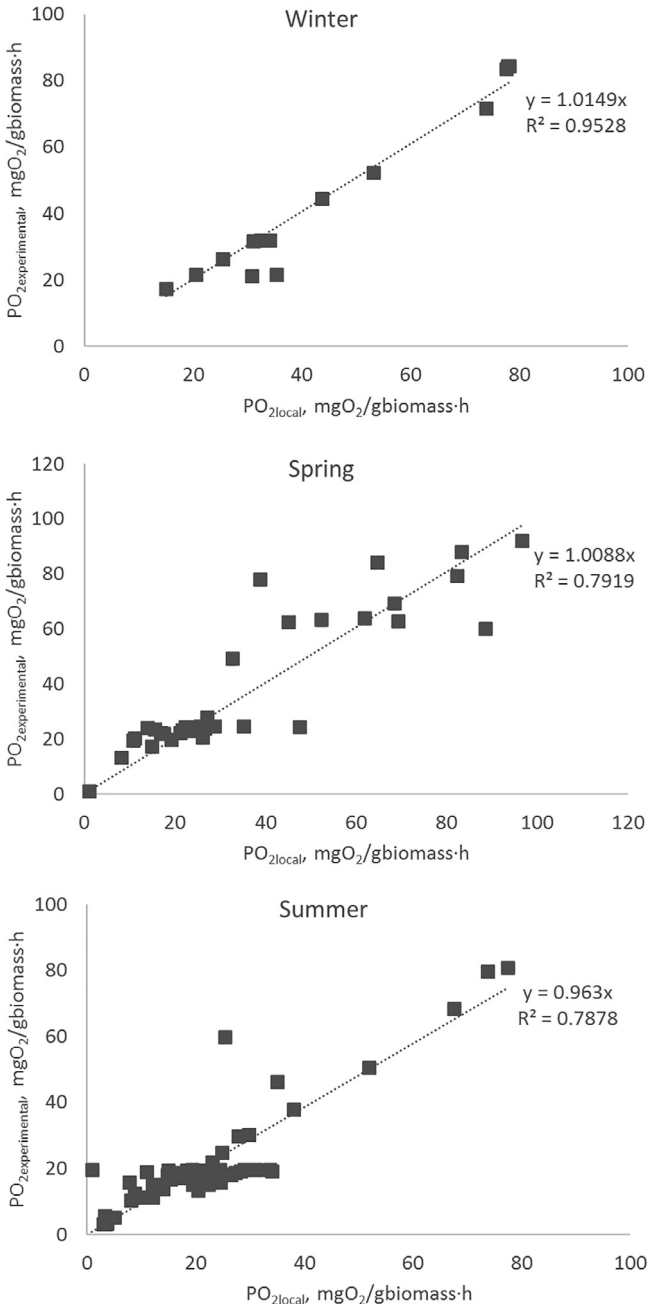

Fig. 5. Correlation between the experimental and local photosynthesis rate of Scenedesmus almeriensis samples produced in a 100 m^2 raceway reactor in continuous mode at 0.2 day^{-1} for different seasons.

receiving insufficient light for photosynthesis to take place. From this data, the maximal proportion of illuminated culture was calculated to be 27%. In terms of the photosynthesis rate, by considering the local irradiance at different culture depths and the hyperbolic growth model found in springtime, the variation in the photosynthesis rate with the culture depth at different solar irradiances can also be easily calculated (Fig. 6B). The results show how most of the photosynthesis took place in the first 0.035 m of culture; only at really high irradiances did photosynthesis take place at culture depths greater than 0.050 m. However, independent of the solar irradiance, there is always a large reactor volume performing respiration in complete darkness. In most studies focusing on the flashing effect, light is represented as an oversimplified on/off signal. A better option is to represent the light pattern, assuming that the light source switches between two intensities (Demory et al.,

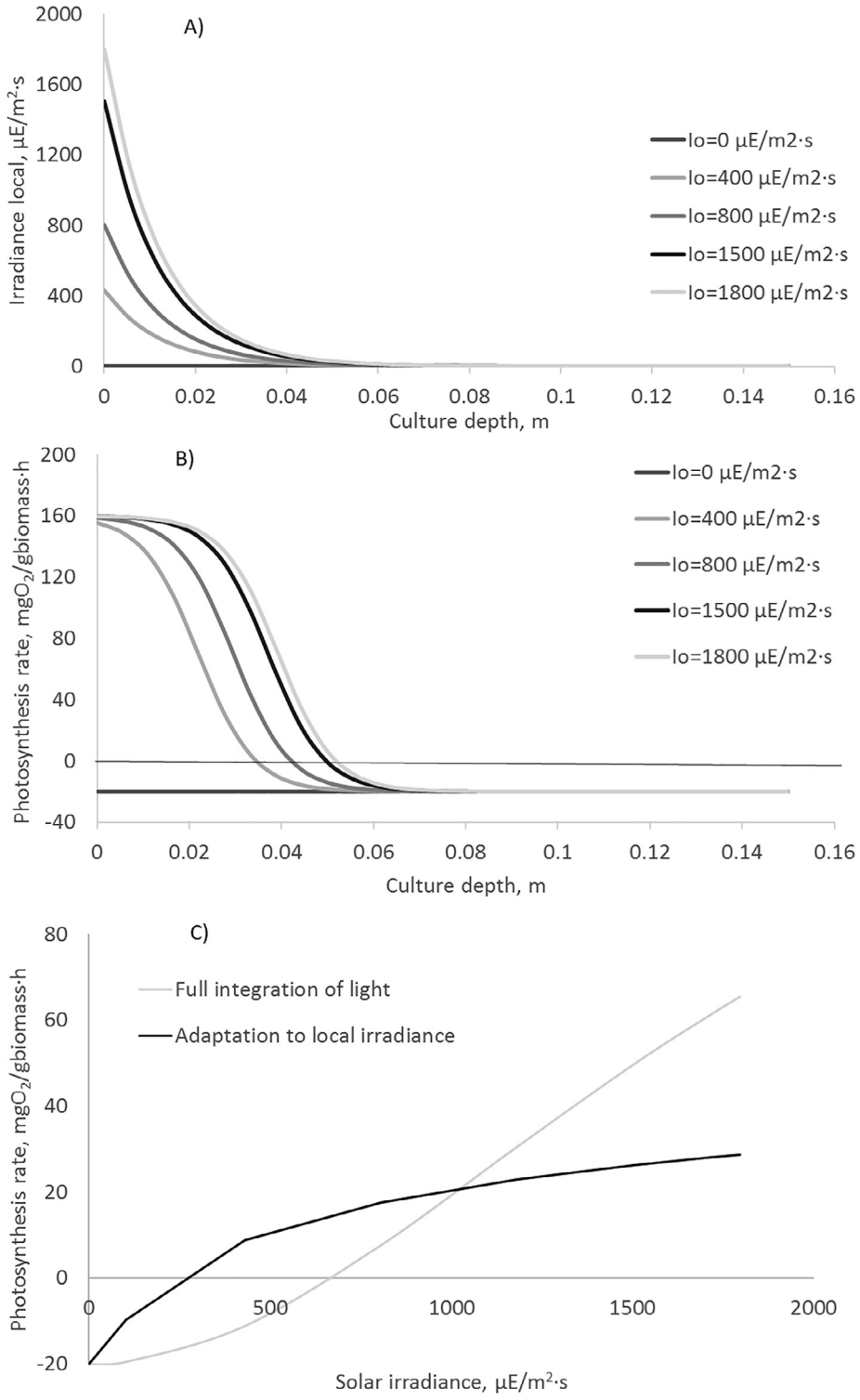

Fig. 6. Variation in the local irradiance (A) and photosynthesis rate (B) with the culture depth for a Scenedesmus almeriensis culture produced in a 100 m^2 raceway reactor in continuous mode at 0.2 day^{-1}. (C). Integrated values of the net photosynthesis rate at different solar irradiances. Data simulated on the basis of the experimental results obtained.

2018). However, an even better approach is obtained if one considers the light profile on the entire reactor, as demonstrated here, as well as using the complete optimal light regime history (although this requires more complex and time-consuming computing efforts) (Brindley et al., 2016).

To determine the net photosynthesis rate inside the reactor, the sum of all the photosynthesis rates at different culture depths must be calculated (Fig. 6C). The data show that, if light integration takes place inside the reactor, the photosynthesis rate increases almost linearly with the solar irradiance on the reactor surface. Nonetheless, the real scenario is that the photosynthesis rate adapts to local irradiance and so the curve corresponding to this behaviour fits a hyperbolic trend. It is highly relevant that at low irradiances, the model considering local adaptation provides higher photosynthesis rates than the model

considering light integration. However, the opposite occurs at high solar irradiances when the photosynthesis rate considering light integration is much higher than when adaptation to local irradiance takes place. By integrating the photosynthesis rate in the daylight period, a daily value of 303 $mgO_2/g_{biomass}$ was obtained when considering full light integration, whereas when considering adaptation to local irradiance, the daily value was 216 $mgO_2/g_{biomass}$ (Fig. 6*C*). Taking into account the basic photosynthesis equation, these values are equivalent to biomass productivities of 0.23 and 0.16 g per gram, respectively; again, this is equivalent to 40 and 29 $g_{biomass}/m^2$·day, respectively. Such values are quite reasonable; the 29 $g_{biomass}/m^2$·day value is very close to the 25 $g_{biomass}/m^2$·day value determined experimentally under these conditions. Our figures clearly show how modifying the light regime in raceway reactors would improve their performance by up to 40%. Nonetheless, this is really a difficult objective, especially when considering the greater energy input requirements to achieve it.

4. Conclusions

It has been demonstrated how the light regime at which the microalgae cells are exposed to in a raceway reactor is far from the optimal one required to optimize the performance of microalgae cultures through light integration. Photosynthesis rate measurements, performed at light/dark times in the range of that experimentally determined, have confirmed that no light integration exists, and that the cells are adapted to the local irradiance inside the reactor. This phenomenon has been validated with reactor samples over a complete annual cycle. The phenomena here described are fundamental to better understanding the potential and limitations of raceway reactors.

Acknowledgements

This study was supported financially by the Ministry of Economy and Competitiveness (DPI2014-55932-C2-1-R, DPI2017-84259-C2-1-R), (EDARSOL, CTQ2014-57293-C3-1-R), and the European Union's Horizon 2020 Research and Innovation Program under Grant Agreement No. 727874 SABANA. We are most grateful for the practical assistance given by the staff of the Cajamar Foundation's "Las Palmerillas" Experimental Station.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.02.032.

References

Alías, C.B., García-Malea López, M.C., Acién Fernández, F.G., Fernández Sevilla, J.M., García Sánchez, J.L., Molina Grima, E., 2004. Influence of power supply in the feasibility of *Phaeodactylum tricornutum* cultures. Biotechnol. Bioeng. 87, 723–733.

Barbosa, M.J.J., Janssen, M., Ham, N., Tramper, J., Wijffels, R.H.H., 2003. Microalgae cultivation in air-lift reactors: modeling biomass yield and growth rate as a function of mixing frequency. Biotechnol. Bioeng. 82, 170–179.

Barceló-Villalobos, M., Guzmán Sánchez, J.L., Martín Cara, I., Sánchez Molina, J.A., Acién Fernández, F.G., 2018. Analysis of mass transfer capacity in raceway reactors. Algal Res. 35, 91–97.

Bitog, J.P., Lee, I., Lee, C., Kim, K., Hwang, H., Hong, S., Seo, I., Kwon, K., Mostafa, E., 2011. Application of computational fluid dynamics for modeling and designing photobioreactors for microalgae production: a review. Comput. Electron. Agric. 76, 131–147.

Bosca, C., Dauta, A., Marvalin, O., 1991. Intensive outdoor algal cultures: how mixing enhances the photosynthetic production rate. Bioresour. Technol. 38, 185–188.

Brindley, C., Fernández, F.G.A., Fernández-Sevilla, J.M., 2011. Analysis of light regime in continuous light distributions in photobioreactors. Bioresour. Technol. 102, 3138–3148.

Brindley, C., Jiménez-Ruíz, N., Acién, F.G., Fernández-Sevilla, J.M., 2016. Light regime optimization in photobioreactors using a dynamic photosynthesis model. Algal Res. 16, 399–408.

de Godos, I., Mendoza, J.L., Acién, F.G., Molina, E., Banks, C.J., Heaven, S., Rogalla, F., 2014. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. Bioresour. Technol. 153, 307–314.

Demory, D., Combe, C., Hartmann, P., Talec, A., Pruvost, E., Hamouda, R., Souillé, F., Lamare, P.O., Bristeau, M.O., Sainte-Marie, J., Rabouille, S., Mairet, F., Sciandra, A., Bernard, O., 2018. How do microalgae perceive light in a high-rate pond? Towards more realistic Lagrangian experiments. R. Soc. Open Sci. 5, 180523.

Fernández-Del Olmo, P., Fernández-Sevilla, J.M., Acién, F.G., González-Céspedes, A., López-Hernández, J.C., Magán, J.J., 2017. Modeling of biomass productivity in dense microalgal culture using computational fluid dynamics. Acta Hortic.

García, S., Paternina, E., Pupo, O.R., Bula, A., Acuña, F., 2012. CFD simulation of multiphase flow in an airlift column photobioreactor for the cultivation of microalgae. In: ASME 2012 6th Int. Conf. Energy Sustain. Parts A and B. American Society of Mechanical Engineers, pp. 1253.

Grobbelaar, J.U., 1991. The influence of light/dark cycles in mixed algal cultures on their productivity. Bioresour. Technol. 38, 189–194.

Grobbelaar, J.U., 1989. Do light/dark cycles of medium frequency enhance phytoplankton productivity? J. Appl. Phycol. 1, 333–340.

Janssen, M., Bresser, L.De, Baijens, T., Tramper, J., Mur, L.R., Snel, J.F.H., Wijffels, R.H., 2000a. Scale-up aspects of photobioreactors: Effects of mixing-induced light/dark cycles. J. Appl. Phycol. 12, 225–237.

Janssen, M., Janssen, M., De Winter, M., Tramper, J., Mur, L.R., Snel, J., Wijffels, R.H., 2000b. Efficiency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light/dark cycles. J. Biotechnol. 78, 123–137.

Janssen, M., Kuijpers, T.C., Veldhoen, B., Ternbach, M.B., Tramper, J., Mur, L.R., Wijffels, R.H., 1999. Specific growth rate of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* under medium duration light/dark cycles: 13–87 s. J. Biotechnol. 70, 323–333.

Labatut, R.A., Ebeling, J.M., Bhaskaran, R., Timmons, M.B., 2007. Hydrodynamics of a large-scale mixed-cell raceway (MCR): experimental studies. Aquac. Eng. 37, 132–143.

Lee, Y.-K., Pirt, S.J., 1981. Energetics of photosynthetic algal growth: influence of intermittent illumination in short (40 s) cycles. J. Gen. Microb. 124, 43–52.

Liffman, K., Paterson, D.A., Liovic, P., Bandopadhayay, P., 2013. Comparing the energy efficiency of different high rate algal raceway pond designs using computational fluid dynamics. Chem. Eng. Res. Des. 91, 221–226.

Matthijs, H., Balke, H., Van Hes, U., Kroon, B., Mur, L., Binot, R., 1996. Application of light-emitting diodes in bioreactors- flashing light effects and energy economy in algal culture (*Chlorella pyrenoidosa*). Biotechnol. Bioeng. 50, 98–107.

Mendoza, J.L., Granados, M.R., de Godos, I., Acién, F.G., Molina, E., Banks, C., Heaven, S., 2013a. Fluid-dynamic characterization of real-scale raceway reactors for microalgae production. Biomass Bioenergy 54, 267–275.

Mendoza, J.L., Granados, M.R., de Godos, I., Acién, F.G., Molina, E., Heaven, S., Banks, C.J., 2013b. Oxygen transfer and evolution in microalgal culture in open raceways. Bioresour. Technol. 137, 188–195.

Merchuk, J.C., Ronen, M., Giris, S., Arad, S.(Malis), 1998. Light/dark cycles in the growth of the red microalga *Porphyridium* sp. Biotechnol. Bioeng. 59, 705–713.

Molina-Grima, E., Sevilla, J.M.F., Pérez, J.A.S., Camacho, F.G., 1996. A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. J. Biotechnol. 45, 59–69.

Nedbal, L., Tichý, V., Xiong, F., Grobbelaar, J.U., 1996. Microscopic green algae and cyanobacteria in high-frequency intermittent light. J. Appl. Phycol. 8, 325–333.

Oswald, W.J., Golueke, C.G., 1960. Biological transformation of solar energy. In: W.W. Umbreit (Ed.), Advances in Applied Microbiology. Vol. 2. New York, pp. 223–262.

Perner-Nochta, I., Lucumi, A., Posten, C., 2007. Photoautotrophic cell and tissue culture in a tubular photobioreactor. Eng. Life Sci. 7, 127–135.

Schulze, P.S.C., Barreira, L.A., Pereira, H.G.C., Perales, J.A., Varela, J.C.S., 2014. Light emitting diodes (LEDs) applied to microalgal production. Trends Biotechnol. 32, 422–430.

Soman, A., Shastri, Y., 2015. Optimization of novel photobioreactor design using computational fluid dynamics. Appl. Energy 140, 246–255.

Ugwu, C.U., Ogbonna, J.C., Tanaka, H., 2005. Light/dark cyclic movement of algal culture (*Synechocystis aquatilis*) in outdoor inclined tubular photobioreactor equipped with static mixers for efficient production of biomass. Biotechnol. Lett. 27, 75–78.

Vejrazka, C., Janssen, M., Streefland, M., Wijffels, R.H., 2011. Photosynthetic efficiency of *Chlamydomonas reinhardtii* in flashing light. Biotechnol. Bioeng. 108, 2905–2913.