Analysis of productivity in raceway photobioreactor using computational fluid dynamics particle tracking coupled to a dynamic photosynthesis model

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HIGHLIGHTS

- Raceway PBR analysis based on dynamic photosynthesis model and real strain data.
- Coupling CFD cell trajectories to dynamic photosynthesis model to analyze yield.
- Lomb-Scargle frequency analysis shows periodic patterns in unevenly sampled data.
- Increasing circulation velocities do not promote a favorable light regime.

GRAPHICAL ABSTRACT
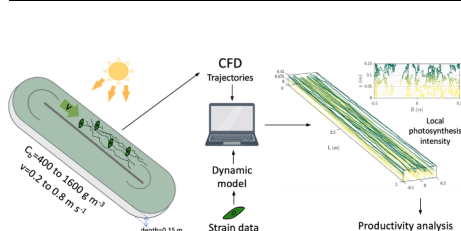
ABSTRACT

Raceway photobioreactors (RWPs) are the most common and affordable device for the mass culture of microalgae but due to geometry and the requirement of low input power, its photosynthetic performance is low. The fluid dynamics of RWPs have been studied for information such as energy dissipation and shear rate, CFD has never been used to analyze photosynthesis efficiency by coupling dynamic photosynthesis models with microalgae trajectories. In this work, we investigate by CFD simulation the effect of circulation velocities between 0.2 and 0.8 m s^{-1} in a 0.15 m^{-1} deep RWPs under standard outdoor conditions to shows that in all circumstances the RWP from the point o view of photosynthesis operates as a perfectly segregated device (no mixing) and that the average growth rate is the result of the integration of the local growth rates at different depths (integration factor $\Gamma = 0$).

1. Introduction

Microalgae are photosynthetic organisms that can be used in a wide range of applications of high and low added value. For high-value products such as ω3 fatty acids or carotenoids such as lutein or astaxanthin, the efficiency of the use of energy in the culture of microalgae is not critical since the most relevant cost in these processes is recovery and purification. On the other hand, microalgae can be also used to implement low-cost applications such as wastewater purification. Microalgal cultures show unique characteristics in this field because, in contrast with processes based only on bacteria, microalgae can take up and assimilate the different species of nitrogen and phosphor present in wastewater. This is a great difference from the classic processes that mostly eliminate organic matter but fail to recover any other nutrient.

The removal of inorganic nitrogen species such as ammonium or nitrate prevents the eutrophication of natural water bodies and saves a relevant amount of energy when this recovered nitrogen is turned into bio-fertilizers that can be reused in agriculture instead of nitrates obtained by the energy-intensive Haber process.

In spite of all those advantages, it is not easy to implement low-value microalgal-based processes since microalgal cultures require adequate mixing to attain high photosynthetic efficiency. Dark and light zones coexist inside photobioreactors (PBRs) because microalgal cultures are optically dense and impede light penetration. This situation divides the PBRs into light-saturated and light-starved zones causing low productivity. To make efficient use of light, PBRs must be well mixed from the standpoint of photosynthesis. This means that the microalgal cells must move between light and dark zones at a frequency that is high enough to support efficient growth as cells are allowed to harvest enough light in the light zones without reaching oversaturation and that they do not remain in the dark zones enough time to suffer a decrease in photosynthesis (Richmond, 2004). But mixing consumes energy which costly and balancing costs is essential for the economical feasibility of low-cost processes and thus must be designed very carefully.

Raceway photobioreactors (RWPs) for microalgal culturing are well known in many aspects the influence of mixing in pH, CO_2 transfer and O_2 has been studied (Costache et al., 2013) as well as the influence of such parameters on microalgal productivity. It is also well known how the power supplied for mixing affects culture circulation, mass transfer, and, to a certain extent, fluid dynamics behavior (de Godos et al., 2014; Mendoza et al., 2013), but the relationship with light regime and its influence on microalgal productivity remains unclear. Rigorous characterization of how the fluid dynamics in RWPs determine the light regime affecting the microalgal cells has not been attempted.

To properly assess the growth potential of a microalgal strain in dense cultures it is necessary to take into account the dynamics of photosynthesis to explore if a "light-integrating" situation, as described by Terry (1986), is possible. The characterization of the dynamic photosynthetic response is most comprehensively attained with the use of dynamic photosynthesis models, such as those proposed by Eilers and Peeters (1988) or Camacho Rubio et al. (2003). The use of such models requires knowing the precise "light history" (information of irradiance vs. time) of a microalgal cell population of significant size. Since the irradiance a microalgal cell is receiving inside a dense culture depends on its position, obtaining the light history requires a description of the light distribution inside the RWPs and of the position of microalgal cells with time.

For this last purpose, CFD (Computational Fluid Dynamics) is a tool that brings about the possibility of studying the flow of fluids in any particular system including tracking the position of virtual particles resembling microalgae ((Pruvost et al., 2002; Papáček et al., 2012; Fernández-Del Olmo et al., 2017). Thus, coupling CFD particle tracking with irradiance distribution and a dynamic photosynthesis model allows evaluating the photosynthetic performance of RWPs culturing a specific microalgal strain.

In this work, we present a study of a standard RWP in which the operating conditions (fluid circulation velocity) are connected to biomass productivity by a combination of CFD and the use of a dynamic photosynthesis model as a proof of concept of how such a combination can help advance the development of RWP by fine-tuning its design and operating conditions in order to attain economic feasibility. This will provide some clear insight on how good the light regime of current RWPs is and how its design could be improved to increase the photosynthetic yield.

2. Materials and methods

2.1. Computational fluid dynamics setup and validation.

The Raceway pond used in this study (standard raceway) is a 1.0 m wide 0.15 m deep unit. The simulation is limited to a length of 5 m in the straight part of the channel due to computing limitations (Chiaramonti et al., 2013). Additionally, to reduce the computational cost, the paddlewheel section is disregarded as its mixing effect from the point of view of photosynthesis are very limited because the turbulence dissipates in a very short distance (Prussi et al., 2014; Amini et al., 2016; Leman et al., 2018; Kubar et al., 2020).

The computational domain is discretized using ANSYS Meshing 12.1. Due to the characteristics of the geometry, a structured hexahedral grid was used for a suitable discretization of the computational domain. The variable y+, termed as the dimensionless wall distance, was used to find a suitable correct grid size near the walls. A grid-sensitivity analysis was performed to ensure mesh-independent results, reduce the discretization errors and the computational time. For this, three mesh densities were evaluated: 1,096,250, 1,656,875 and 2,096,875 cells. The mesh with 1,656,875 cells was the optimal in terms of computational time and accuracy.

The governing flow field equations are the continuity and the Reynolds averaged Navier–Stokes equations, which are given by:

$$\frac{\partial u_i}{\partial x_j} = 0 \tag{1}$$

where u_i is the Cartesian velocity component (i = 1, 2 and 3), x_i are the coordinate axis, and repeated index implies summation from 1 to 3.

$$\frac{\partial u_i u_j}{\partial x_j} = \frac{-1}{\rho}\frac{\partial p}{\partial x_i} + \frac{\partial}{\partial x_j}\left(\nu S_{ij} - \overline{u_i' u_j'}\right) \tag{2}$$

where S_{ij} is the main strain rate and calculated by:

$$S_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right) \tag{3}$$

and $\overline{u_i' u_j'} = \tau_{ij}$ is the unknown turbulent or Reynolds-stress tensor where u_i' represents the velocity fluctuation in i-direction. These equations are not a closed set and turbulence models are required to model the turbulent or Reynolds-stress tensor.

In order to exclude the dependence of the turbulence model on the results, a sensitivity study was carried out. The realizable $k - \varepsilon$ model provided the best compromise between accuracy and calculation time. Therefore, it was chosen to simulate all design models.

The transport equations are solved using the commercial software package ANSYS FLUENT 12.1. The numerical model is based on the finite volume method. The flow solution procedure is the Semi-Implicit Method for Pressure-Linked Equation SIMPLE routine, developed by Patankar (1980). The momentum equations are discretized using both first and second-order upwind scheme options, and second-order upwind for other transport equations.

The convergence criterion consisted of monitoring mass flow rate on inlet and outlet and variation of velocity profiles with iteration, reduction of several orders of magnitude in the residual errors.

To validate the CFD model (Fig. 1), the pressure drop results of the raceway pond for different circulation velocities (0.2, 0.4, 0.6 and 0.8 ms^{-1}) were verified by comparison with the empirical equation (Eq (4)) formulated by Manning, (1891). This gives an estimate for the circulation velocity in a channel with given hydraulic radius R_h and slope S (in %). The roughness of pond lining material is defined by n. In this work, n was assumed to be 0.01 (Barr et al., 1993), corresponding to polyethylene (smooth surface)

$$V = \frac{1}{n}R^{\frac{2}{3}}S^{\frac{1}{2}} \tag{4}$$

2.2. Individual cell trajectories.

The cell trajectories inside the RWP were calculated in a Lagrangian frame of reference applying the Discrete Random Walk Model (DRW)
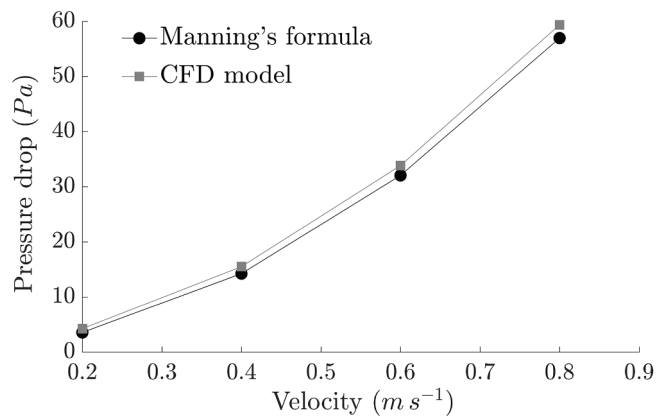
Fig. 1. CFD model validation. The pressure drop calculated with CFD in the raceway photobioreactor for different circulation velocities is compared to the Manning equation.

provided for ANSYS FLUENT. All model parameters were adapted to the properties of the algal cells (5 μm particle diameter of 1000 kg m^{-3} density) and 50 cell trajectories were computed. The DRW model is a stochastic tracking model that accounts for the eddy lifetime and can predict the dispersion of particles due to turbulence in the fluid phase.

In a DRW model, each eddy is characterized by a Gaussian distributed random velocity fluctuation, u', v', and w' a time scale, τ_e

The values of u', v', and w' that prevail during the lifetime of the turbulent eddy are sampled by assuming that they obey a Gaussian probability distribution, so that

$$u' = \zeta\sqrt{\overline{u'^2}} \tag{5}$$

where ζ is a normally distributed random number, and the remainder of the right-hand side is the local RMS value of the velocity fluctuations. Since the kinetic energy of turbulence is known at each point in the flow, these values of the RMS fluctuating components for Realizable $k - \varepsilon$ model used in this work can be defined (assuming isotropy) as

$$\sqrt{\overline{u'^2}} = \sqrt{\overline{v'^2}} = \sqrt{\overline{w'^2}} = \sqrt{\frac{2k}{3}} \tag{6}$$

The characteristic lifetime of the eddy is defined either as a constant:

$$\tau_e = 2T_L$$

For small "tracer" particles that move with the fluid (zero drift velocity), the integral time becomes the fluid Lagrangian integral time T_L and is given by

$$T_L = C_L\frac{k}{\epsilon} \tag{7}$$

for the Realizable $k - \varepsilon$ model this time scale can be approximated as

$$T_L \approx 0.30\frac{k}{\epsilon}$$

2.3. Frequency analysis

For performing the quantitative analysis of simulated particle trajectories, a signal analysis with Lomb-Scargle periodogram was used.

Among the most used Digital Signal Processing (DSP) techniques is the Discrete Fourier Transform (DFT) or its computationally efficient version, Fast Fourier Transform (FFT). Several authors have used this to analyze the particle trajectories obtained from CFD simulations (Perner-Nochta and Posten, 2007; Gómez-Pérez et al., 2015). FFT algorithm to obtain the spectrum for the data requires evenly sampled data. However, the data obtained in the simulations on the position of each particle are unevenly spaced time.

The Lomb-Scargle method for computing a periodogram offers several benefits over padding the data with zeroes. This method computes the frequency content at measured values, removing the possible error introduced by interpolation. The Lomb-Scargle periodogram is equivalent to least-squares fitting a sinusoid of frequency to the given unevenly spaced data (Lomb, 1976) and has the advantage of allowing the processing of unevenly sampled position data.

The fundamental equation for the Lomb-Scargle Periodogram is

$$P_N(\omega) \equiv \frac{1}{2\sigma^2}\left\{\frac{\left[\sum_N\left(h_n - \overline{h}\right)cos\omega(t_n - \tau)\right]^2}{\sum_N cos^2\omega(t_n - \tau)} \right.$$
$$\left. + \frac{\left[\sum_N\left(h_n - \overline{h}\right)sin\omega(t_n - \tau)\right]^2}{\sum_N sin^2\omega(t_n - \tau)}\right\} \tag{8}$$

where the N data points are unevenly sampled events at times t_i arranged as

$h_i \equiv h(t_i)$, $i = 0, \cdots, N-1$. The mean $\left(\overline{h}\right)$ and variance $\left(\sigma^2\right)$ are calculated as

$$\overline{h} \equiv \frac{1}{N}\sum_{i=0}^{N-1}h_i \tag{9}$$

$$\sigma^2 \equiv \frac{1}{N-1}\sum_{i=0}^{N-1}\left(h_i - \overline{h}\right)^2 \tag{10}$$

$$tan(2\omega\tau) = \frac{\sum_j sin2\omega\tau_j}{\sum_j cos2\omega\tau_j} \tag{11}$$

Eq. (8) then renders the Lomb-Scargle normalized periodogram where spectral power is a function of angular frequency. The relationship $\omega \equiv 2\pi f > 0$ allows the conversion into Hz.

2.4. Data processing

MATLAB version R2017a was used to process all the data and compute and display the periodogram results. The built-in graphics processing ability makes it appropriate for this research.

2.5. Evaluation of photosynthetic efficiency

The movement of microalgal cells inside a dense culture determines the efficiency in the use of light (Brindley et al., 2011). This can be analyzed by using dynamic models of photosynthesis such as the proposed by Eilers and Peeters (1988) and Camacho Rubio et al. (2003). Both were conceived as black-box models that assume that light is captured and transferred to the so-called "excited centers", which subsequently fuel photosynthesis and become deactivated during this process. The main difference between these two models is that Eilers and Peeters (1988) assumed that the deactivation rate (r) is proportional to the concentration of excited PSFs (γ·x_2) while Camacho Rubio et al. (2003) proposed that this step is controlled by an enzyme-mediated reaction that can become saturated:

$$r = \frac{r_m^*\cdot a^*}{K_s^* + a^*} \tag{12}$$

The model proposed by Camacho Rubio et al. (2003) encompasses the Eilers-Peeters model because Eq. (12) can show linearity between the specific deactivation rate (r) and the concentration of excited centers (represented by a* in the Camacho-Rubio model) is greater than the saturation constant of the enzymatic step ($K_s^*\gg a^*$) as well as a saturation behavior typical of enzymatic reactions for larger concentrations a*.

The balance of activated centers for the Camacho–Rubio model is:

$$\frac{da^*}{dt} = k_a \cdot I \cdot (a - a^*) - \frac{r_m^* \cdot a^*}{K_s^* + a^*} \tag{13}$$

This equation expresses that new activated centers are generated when a non-activated center absorbs a photon, contributing to increasing the concentration of activated centers (a*), which are in turn consumed during the enzyme-controlled reaction.

The photosynthesis rate - expressed here as the specific oxygen generation rate (PO₂, mol O₂ (g biomass)$^{-1}$s^{-1}) - is proportional to the deactivation rate of a*:

$$PO_2 = k \cdot \frac{r_m^* \cdot a^*}{K_s^* + a^*} \tag{14}$$

The constant k expresses the proportionality between the deactivation of excited intermediates and the production of oxygen. Photosynthesis stoichiometry would suggest a theoretical maximum of one mole of O_2 generated per eight moles of activated centers consumed (Emerson and Arnold, 1932; Nelson and Cox, 2017), but this proportion can vary when other deactivation mechanisms are at work.

Camacho Rubio et al. (2003) proposed a dimensionless model by dividing each parameter by the total concentration of photosynthetic centers. Thus, the concentration of activated centers (a*) becomes a fraction (x*, the fraction of activated centers). In the original work the authors conveniently organized the parameters of the model into the following groups:

$$\alpha = \frac{r_m^*}{k_a \cdot a^*}; \ \beta = \frac{r_m^*}{a^*}; \ \kappa = \frac{K_s^*}{a^*} \tag{15}$$

Where k_a is the absorption coefficient of the photosynthetic centers. For continuous irradiance (I = constant) Eq. (13) reaches a steady state and the following equation can be worked out (Camacho Rubio et al., 2003):

$$\frac{P_{cont}}{P_{max}} = \frac{I}{2 \cdot \alpha} \left[\left(1 + \kappa + \frac{\alpha}{I} \right) - \sqrt{\left(1 - \kappa - \frac{\alpha}{I} \right)^2 + 4 \cdot \kappa} \right] \tag{16}$$

This equation allows obtaining the values of the parameters α and κ by nonlinear regression of P-I data obtained under continuous light. Such data are widely available for a large number of microalgae species. If I_{av} is used in place of I (Molina Grima et al., 1997) the values of P given by Eq. (16) correspond to a regimen of complete light integration. On the other hand, for a perfectly segregated light regime (local P integration), the following equation must be used:

$$\frac{P_{cont}}{P_{max}} = \frac{1}{D} \int_0^D \frac{I(x)}{2 \cdot \alpha} \cdot \left[\left(1 + \kappa + \frac{\alpha}{I(x)} \right) - \sqrt{\left(1 - \kappa - \frac{\alpha}{I(x)} \right)^2 + 4 \cdot \kappa} \right] dx \tag{17}$$

Where D is the total depth of the RWP and I(x) is an equation describing the light attenuation with depth. In our case, the Lambert-Beer law is used.

Eqs. (14), (16) and (17) are used to obtain respectively the results of P given by the dynamic model, the equivalent continuous light response (light regime integration) and the integration of local rates. In this study the photosynthetic response is presented as the ratio P/P$_{max}$ in order to make the results as species-independent as possible. The values chosen for the other model parameters are α = 100 μmole photons m^{-2} s^{-1}, κ = 0.1 and β = 5 Hz as they are representative of the behavior of the most frequent strains used in RWPs for the reasons explained in section 3.4.

3. Results and discussion

Microalgal cells cultured for mass production are grown in industrial photobioreactors usually under sunlight and with cell densities as high as possible in order to maximize productivity. This causes intense light gradients inside the cultures that hinder the global photosynthetic efficiency of the culture due to the heterogeneity of the light distribution. In dark zones, the microalgae growth may slow to a halt or even net

respiration can be observed while in the most intensely illuminated zones the microalgae become oversaturated and thus a large proportion of the light energy harvested is dissipated through non-productive processes.

This situation can be ameliorated or even avoided with adequate mixing. There is currently clear and ample evidence that when in this situation microalgae are cycled several times per second between illuminated and dark zones, the photosynthetic efficiency of the cultures is increased as all cells seem to "see" an average irradiance and thus saturation and growth stall are both avoided and instead an average growth rate is observed throughout the culture.

Raceways are the most common and most widely used type of photobioreactor for mass production of microalgae or similar processes such as water depuration or bioremediation. As in any other type of PBR, light and dark zones coexist in the photosynthetic efficiency of microalgal cultures growing in a raceway photobioreactor is the result of the complex interaction of the microorganism population with the light pattern that arises from the movement of the microalgal cells inside the light gradient caused by the mutual cell shading. Thus, three elements interact to produce the global photosynthetic response: the movement of the microalgal cells inside the photobioreactor, the light distribution and the growth characteristics of the particular microalgal strain being cultured represented by a dynamic growth model.

3.1. Cell trajectories inside a raceway photobioreactor

The first element needed in the analysis of raceway photobioreactors is a detailed description of the cell trajectories. This has been repeatedly done by CFD (Perner-Nochta and Posten, 2007; Prussi et al., 2014; Nikolaou et al., 2016; Gao et al., 2017) but it is important that the simulation and position sampling are done taking into account the characteristics of the photosynthetic response. Microalgae have been shown by different authors (Phillips and Myers, 1954; Terry, 1986; Camacho Rubio et al., 2003) to respond to irradiance fluctuations between 1 and 50 Hz, so in the present work, the simulations have been programmed to record 50 position samples per second for 5 s which allows detecting such movements of frequencies between 0.2 and 50 Hz. This is the range where the transition from local growth rates to full light integration occurs. This sample rate allows a clear characterization of the shape of the irradiance fluctuation, which is important as Brindley et al. (2011) shown because it is not only the light-to-dark change what determines the occurrence of light integration but the precise way the transition occurs. It is also essential that the movement analyzed is representative of the whole PBR volume and for this, not a single trajectory but a representative population must be studied. This is shown in Fig. 2, where the trajectories of 10 representative particles out of a total of 50, are depicted at four circulation velocities, 0.2, 0.4, 0.6 and 0.8 m s^{-1} chosen in the range attainable in a raceway PBR.

As Fig. 2 shows, the vertical mixing observed in the raceway PBR using the CFD simulations is poor from the point of view of microalgal growth for all the circulation velocities. This was also observed by Amini et al. (2016). From the results obtained it is obvious that there is no complete movement between the bottom of the PBR and the surface and thus no complete transitions from saturating conditions that take place at the surface to the darkness at the bottom, concurring with the observations of Chiaramonti et al. (2013), Prussi et al. (2014) and Kubar et al. (2020). Although it is difficult to draw conclusions from the microalgal cell movements depicted in Fig. 2, some points are clear. First, it is obvious that a significant number of particles linger near the bottom or the surface for the full elapsed period of five seconds at al the circulation velocities tested which is in agreement with the literature (Prussi et al., 2014; Amini et al., 2016; Leman et al., 2018). This means that there is a significant population of cells severely deprived of light while another population staying near the surface for seconds will be oversaturated. Those populations will be mixed sooner or later during the raceway operation (Prussi et al., 2014; Leman et al., 2018) either in
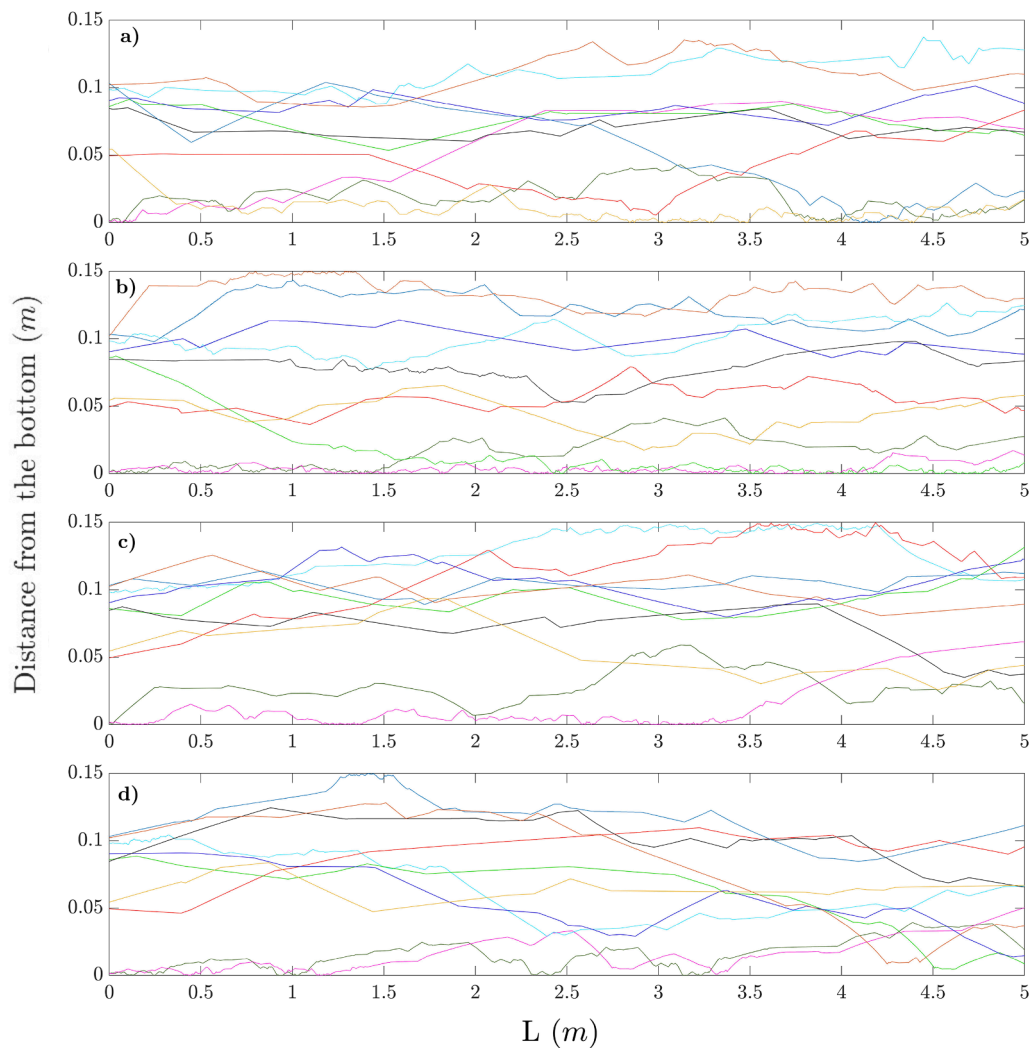
Fig. 2. Vertical movement of individual cells inside the photobioreactor at different circulation velocities a) 0.2, b) 0.4, c) 0.6 and d) 0.8 m s^{-1}. Only 10 trajectories out of 50 are shown for clarity.

bends, sumps or by the paddle wheel action, but the 5 s period with no transitions will lead to a nearly perfect adaptation to local growth rates and thus to a situation that is contrary to the most photosynthetically efficient light integration. Secondly, it is important to note that the circulation velocity v does not significantly influence the light–dark transitions. It may induce more frequent smaller moves but does not promote the surface-to-bottom movements needed for light integration and thus efficient photosynthesis. The velocities tested here are in the range 0.2 to 0.8 m s^{-1} which spans the range of the most commonly used circulation velocities 0.2–0.4 m s^{-1} (Lundquist et al., 2010; Sompech et al., 2012) and goes well above to test if an increase of the circulation velocity could bring about any improvement. Thus, as a preliminary conclusion, raceways PBRs could be operated at lower circulation velocities from the point of view of light efficiency as higher circulation velocities in a feasible range do not seem to promote any substantial light regime improvement. This will be analysed later in more detail.

The third observation that can be done from Fig. 2 is that a faster circulation velocity might improve local mixing. This is, that although there are no complete light-to-dark transitions, the amplitude and frequency of the movement might be locally greater at faster circulation velocities. This is difficult to see in this representation type and will be analyzed later in a frequency analysis.

3.2. Irradiance patterns at different biomass concentration

The vertical movement of the microalgal cells inside the raceway PBR is only one of the interacting factors affecting the performance of a microalgal culture. The photosynthetic intensity of individual cells depends on the local irradiance that in a raceway PBR is given by the attenuation of the incident photosynthetically active radiation (PAR) I_o by the culture layer that is above the considered microalgae. If a Lambert-beer type attenuation is considered the local irradiance at a depth x, represented as I(x), is given by the following equation

$$I(x) = I_o \cdot e^{-k_a \cdot C_b \cdot x} \tag{18}$$

Where k_a is the extinction coefficient of the microalgae (Molina Grima et al., 1994) that represents the intensity with which a given strain of microalgae absorbs PAR light, C_b is the biomass concentration in the culture being analyzed and the depth x× (distance from the culture surface) is easily worked out from the data presented in Fig. 2 by subtracting the distance from the raceway bottom from the total depth L = 0.15 m. The calculation in Eq. (2) assumes that I_o impinges perpendicularly on the culture surface and ignores wall effects but is otherwise representative of the actual attenuation of light in a generic microalgal culture.

In this way, it is possible to obtain temporal irradiance patterns I(t) at different circulation velocities for the individual cell trajectories

obtained from CFD as shown in Fig. 3. These I(t) can be obtained for a variety of circumstances. In order to be able to carry out an analysis representative of mass culture production conditions, we have chosen operating conditions of $I_o = 1500$ µmole photons m^{-2} s^{-1}, which is representative of the year-long average of the sun PAR irradiation impinging on our location at noon (Morillas-España et al., 2020) and biomass concentrations of 400 and g m^{-3} which are representative of the conditions that can be found in raceways working in continuous mode in a pseudo steady state.

Fig. 3 shows the irradiance patterns that single microalgal cells "see"

in the conditions described above using the CFD trajectories shown in Fig. 2, obtained for different circulation velocities and a 5 m circulation length in every case. The irradiance varies from a maximum of 1500 at the culture surface (x = 0) and 41.0 and 1.2 µmole photons m^{-2} s^{-1} at the bottom respectively for $C_b = 400$ and 800 g m^{-3}. Thus, at 400 g m^{-3} the microalgal cells can move from 41.0 to 1500 µmole photons m^{-2} s^{-1} oscillating around an average irradiance of 405 µmole photons m^{-2} s^{-1} although, as can be seen in Fig. 3, most of the population remains all the time under the average that is attained because a small part of the population reaches significantly higher, oversaturating irradiances. This
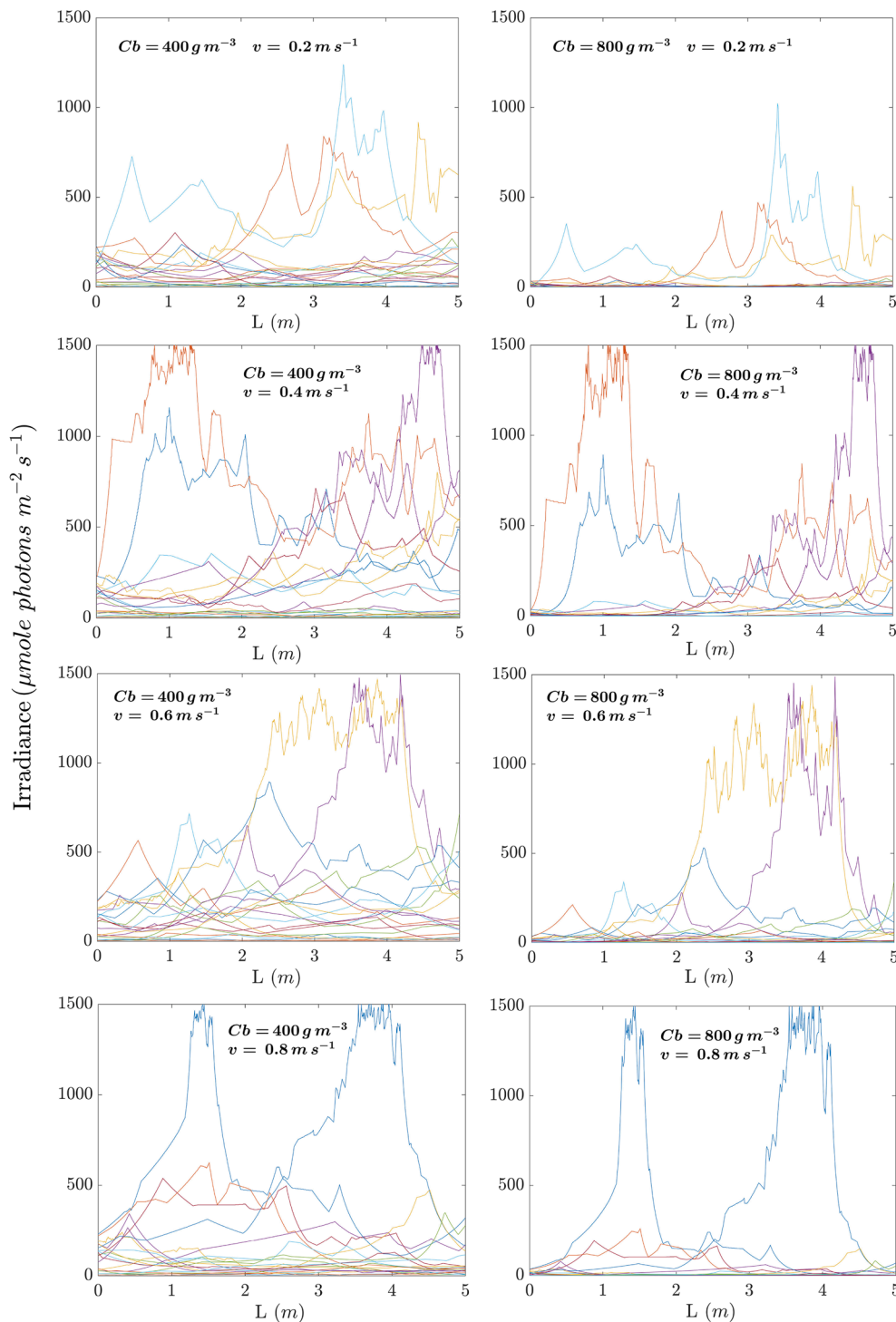

Fig. 3. Irradiance vs. time pattern at different circulation velocities (v) and biomass concentrations ($C_b = 400$ and 800 g m^{-3}) calculated at $I_o = 1500$ µmol m^{-2} s^{-1} incident irradiance. Only 10 trajectories out of 50 are shown for clarity.

representation emphasizes how unfavorable the mixing pattern of RWP is as it practically behaves as a completely segregated culture device from the point of view of light utilization. Increasing circulation velocity does not bring about much improvement. At v = 0.2 m s^{-1} it takes 25 s for the simulated trajectory to complete the 5 m channel length. Thus, the scarce light-to-dark changes are incomplete (do not reach the maximum I$_o$) and far too slow to attain anything close to a light integration situation (Terry, 1986; Nedbal et al., 1996; Brindley et al., 2011) as they take 10–15 s to attain a significant irradiance change and this translates to light regime frequencies under 0.1 Hz. At this 0.2 m s^{-1} circulation velocity the increase in biomass concentration only decreases light availability. Although this biomass concentration should be attainable, in reality they are never obtained in RWP unless a thin layer device is used.

Increasing circulation velocities bring about significant changes in the vertical mixing with regard to light but they are in no way enough to produce a significant light integration and thus the photosynthetic efficiency is bound to remain low. An increase to 0.4 m s^{-1} circulation velocity increases noticeably mixing and a larger fraction of the population is exposed to significant light/dark cycles. It can be seen that even for the higher biomass concentration C$_b$ = 800 g m^{-3} 2 out of 10 trajectories (20%) go through a complete cycle (1500 to virtually zero μmole photons m^{-2} s^{-1}) although it still takes around 6 s to complete. Even at the higher circulation velocity v = 0.8 m s^{-1}, the fastest cycle measured takes 2 s which represents a maximum light-to-dark frequency of 0.5 Hz, still far from the conditions needed for a light integration situation even for the most favorable microalgae reported (Camacho Rubio et al., 2003; Brindley et al., 2011).

Still, Fig. 3 shows that the mixing situation is clearly improved with a higher circulation velocity as it gives rise to faster, more frequent albeit incomplete light-to-dark cycles. Given the variability of these cycles, the best way to ascertain if they bring about any improvement to the efficiency of photosynthesis is to analyze the light pattern I(x) for every simulated trajectory by using a dynamic photosynthesis model and calculating how the average population performs in such situations. This

is done in section 3.4.

3.3. Lomb-Scargle spectral analysis

Although it is clear from Figs. 2 and 3 that RWPs are far from being well-mixed with regard to photosynthetic response, it is difficult to infer values of exposition frequencies and the rate of significant light-to-dark changes from single trajectories or light histories. The Fourier Transform (FT) has been frequently used to derive the existence of periodic patterns in flow systems including the analysis of fluid dynamics in PBR. This type of transformation turns the position-versus-time history of a fluid element into a distribution of movement amplitude that allows for a clearer analysis of the repetitive patterns that are beneficial for the enhancement of photosynthesis.

FT is particularly popular since the advent of the Fast Fourier Transform (FFT), an algorithm that allows a very efficient calculation of the Discrete Fourier Transform that demands little computing resources even for large sets of data. The problem with FT and specifically with the FFT is that it requires that the data set to be transformed be regularly spaced in time, a question that is frequently overlooked. On the other hand, the FFT algorithm also requires 2^n data points, a condition that is not always easy to meet. In particular, when FFT is used to process a data set sampled at irregular intervals, the frequency information can be severely altered.

In this sense, the Lomb-Scargle method is a class of least-squares spectral analysis (LSSA) that permits the obtention of a periodogram from unevenly sampled data and also mitigates other defects of the FFT method. The Lomb-Scargle periodogram was used to analyze the vertical flow of the trajectories population presented in this work by evaluating the periodic behavior of individual simulated particles.

The spectral analysis by the Lomb-Scargle method presented in Fig. 4 shows the amplitude of the vertical movement as a function of the frequency in the conditions tested in this work. In all four cases, linear velocities v = 0.2 to 0.8 m s^{-1}, it is clear that the rate at which significant depth changes take place (and thus significant changes in irradiance) is
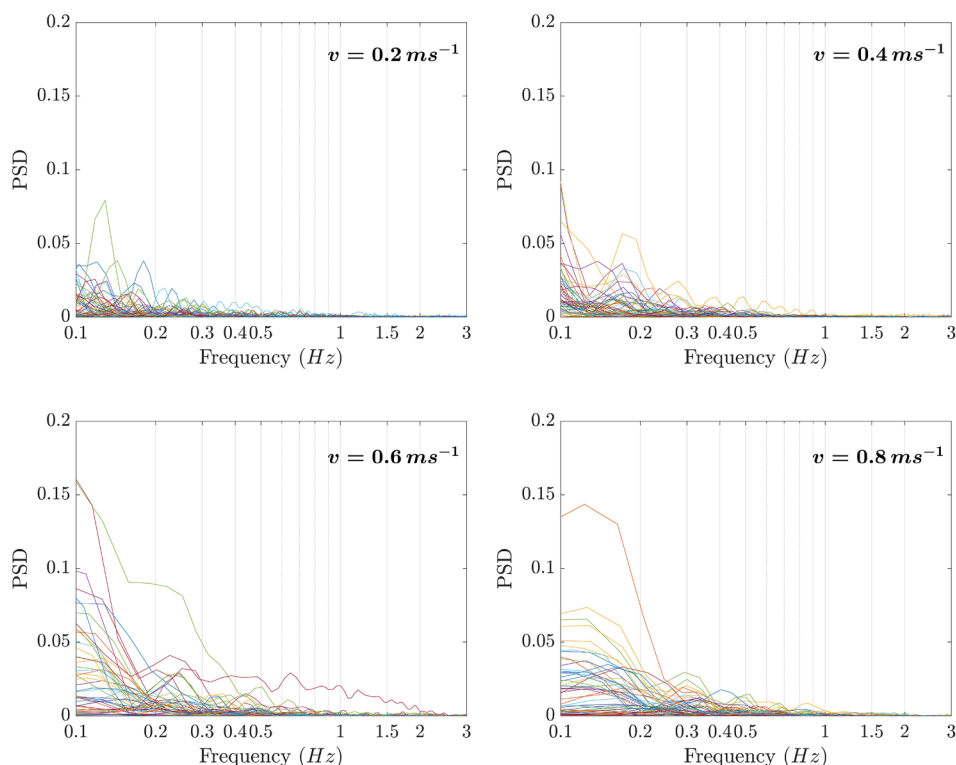

Fig. 4. PSD (Power spectral density) versus frequency in the raceway photobioreactor at different circulation velocities.

slow and completely insufficient to have any effect of light integration. At velocities v = 0.2 and 0.4 m s^{-1}, the changes that happen with a frequency over 1 Hz are practically nil while at the highest velocities tested there are modest vertical movements at 1 and 2 Hz that span only 10% of the total RWP depth for 0.6 and 0.8 m s^{-1}. At the lowest velocity (v = 0.2 m s^{-1}), mixing is very poor. Vertical movements of only 1 cm take seconds to complete. This is in agreement with the simulations carried out by Quiroz-Arita et al. (2020) with pilot-plant 20 cm deep RWPs that report mixing frequencies between 0.27 and 0.35 Hz from top to bottom for mixing energy inputs varying from 0.1 to 2.1 W m^{-3}. This corroborates the range of mixing frequencies obtained in our study and the little variation in vertical movement with increasing mixing intensities.

Although mixing is slightly enhanced with circulation velocity, even at the highest v, the vertical velocities remain insufficient for a good light regime as there are not significant L/D transitions at the frequencies that enhance microalgal growth. The microalgae reported with a lower characteristic frequency of 5 Hz (this is, the most appropriate to be grown under low frequency flashing light) is *Scenedesmus almeriensis* (Brindley et al., 2011). This means that to attain a 63% light integration regime (integration factor Γ = 0.63, see Fig. 5) the microalgal population must experience complete L/D transitions (from maximum to minimum irradiance) 5 times a second. The results presented here indicate that even in the most favorable conditions (a microalga with β = 5 and a high linear velocity v = 0.8 m s^{-1}) most of the microalgal population would be experimenting significant L/D transitions of a frequency ν under 0.5 Hz (ν/β < 0.1) and thus an integration factor under 0.1 should be expected.

The main conclusion of this frequency analysis is that RWPs are very badly mixed from the point of view of light regime and photosynthesis yield. Increasing linear velocity over 0.8 m s^{-1} is energetically inefficient, technically difficult and, in view of the results presented, unlikely to be a feasible method to enhance photosynthetic yield in RWPs.

3.4. Consequences on the photobioreactor productivity

The influence of mixing on the efficiency of PBRs has been repeatedly discussed but always from the point of view of fluid dynamics terms, disregarding that the photosynthetic response in PBRs is a consequence of the exposition of microalgae to an actual irradiance temporal pattern I(t). The influence of fluid dynamics in photosynthesis in dense cultures has frequently been assessed using partial approaches such as the consideration of residence times in light and dark zones chosen arbitrarily or with no homogeneous criteria for the irradiance levels that mark the threshold. One common approach is to assimilate the transitions between light and dark zones to a simple flashing light regime
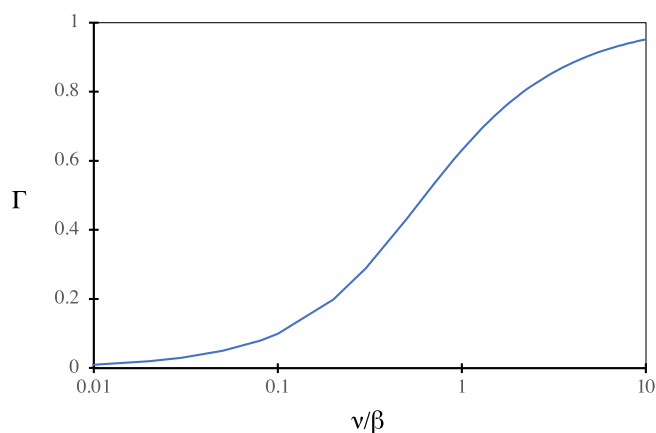
Fig. 5. Integration factor (Γ) vs. adimensional frequency (ν/β) calculated with the Camacho-Rubio et al. model (2003).

(Merchuk et al., 1998) as those studied by Phillips and Myers (1954) or Terry (1986). In this sense, Brindley et al. (2011) demonstrated that this simplified approach can lead to large discrepancies with the actual photosynthetic response. Not only is the assimilation of a real light regime in PBR to a flashing light of a given frequency (ν) and duty cycle (φ) incorrect, but also the "shape" of the light pattern influences the photosynthetic.

Thus, the only reliable way to estimate the photosynthetic response is to use the actual irradiance pattern I(t) coupled to a dynamic photosynthesis model. I(t) arises from the coupling of the irradiance distribution and the cell population trajectories obtained by CFD. In this work, the attenuation of light has been described by the Lambert-Beer model as a sole function of depth as the dynamic variable (Eq. (18)). The CFD trajectories x(t) calculated in the former sections have been coupled to the light distribution in the RWP to obtain the light history I(t) of the 50 particles population. These were then used to calculate the photosynthetic response under different operating conditions in the simulated RWP in order to determine to which extent variables such as the biomass concentration can be manipulated to enhance the photosynthetic yield. To calculate the photosynthetic response of the rapidly varying light pattern I(t), the dynamic photosynthesis model of Camacho Rubio et al. (2003) has been used. This model is given in the form of differential equations that take I(t) as input and render a photosynthetic productivity history P(t). The Camacho-Rubio model has been used because of the availability of dynamic parameters for different strains made available by the original work and others (Camacho Rubio et al., 2003; Brindley et al., 2011; 2016;; Fernández-Sevilla et al., 2018) and in particular the convenience of the parameter β "characteristic frequency" that allows an intuitive visualization of the relationship between frequency (ν) and the integration factor (Γ) as shown in Fig. 5.

Bearing this in mind, the photosynthetic performance (P/P$_{max}$) of a 0.15 m deep RWP has been studied under similar conditions to those that can be found operating outdoors. In Fig. 6 is shown the result of the simulation of the photosynthetic response under an external irradiance I$_o$ of 1500 μmole photons m^{-2} s^{-1} operating at two biomass concentration C$_b$ of 400 and 1600 g m^{-3} that have been chosen to demonstrate the influence of the light distribution and its interaction with the fluid dynamics of the population studied. The kinetic parameters were used (saturation α = 100 μ mole photons m^{-2} s^{-1}, shape factor κ = 0.1 and characteristic frequency β = 5 Hz) are representative of the microalgal strain *Scenedesmus almeriensis* (Brindley et al., 2011).

As Fig. 6 shows, the calculations performed with the dynamic model confirm that RWPs are very badly mixed in all the circumstances tested. The photosynthetic response, shown in Fig. 6 as P/P$_{max}$, is always stratified, being higher at the top, where light availability is higher and low at the bottom. The circulation velocity does not appear to have any influence, either at low or high biomass concentration. This means that the modest amplitude and frequency increases that a higher circulation velocity brings about, as shown in the frequency analysis, do not have any visible effect improving the light regime in the range of velocities tested (v = 0.2 to 0.8 m s^{-1}). This is coherent as the circulation velocities cause increases in the 0.5–1 Hz in the most favorable situation, which is completely insufficient to promote light-averaged growth for a microalga with a characteristic frequency β = 5. Moreover, *S. almeriensis* has a particularly low β, which means that it is easier for this strain to elicit a light-averaged response (Γ near 1) with low mixing frequency. Thus, other strains with β of 15 or 25 such as *Murielopsis* or *Nannochloropsis* are even more difficult to grow in a light-integrating situation and it can be concluded that for most of these strains Γ = 0 in RWP.

As Fig. 6 shows, lowering the operating biomass concentration to 400 g m^{-3} increases the photosynthetic activity for the sole reason that a lower biomass concentration allows better light penetration and thus increases productivity, but the circulation velocity does not appear to exert any effect at this C$_b$ either. The conclusion is that RWPs for the cultivation of microalgae are stratified devices with the microalgae growing at local growth rates and that an increase of the circulation
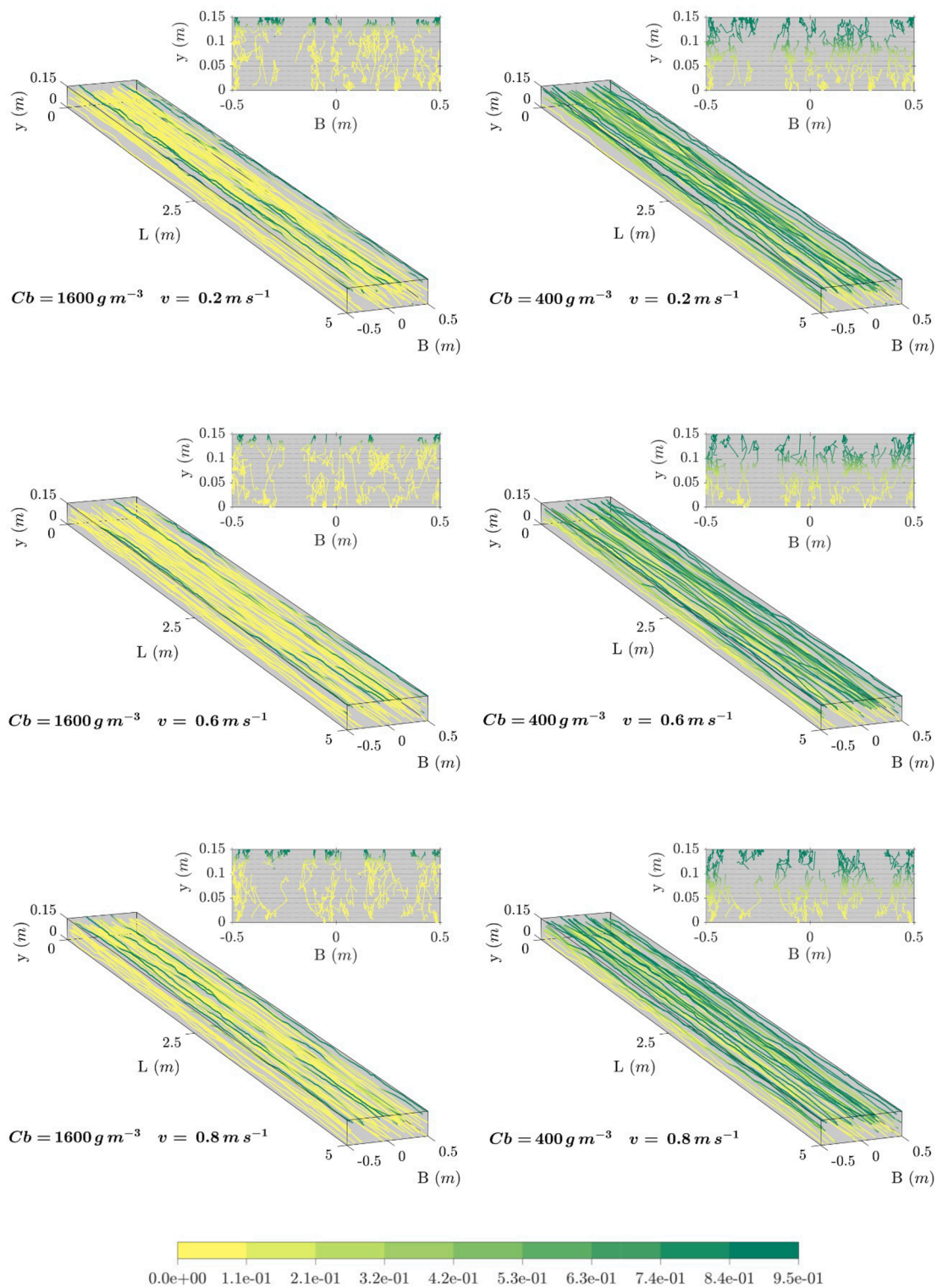
Fig. 6. Photosynthesis intensity distribution (P/P$_{max}$) with depth (y is distance from the bottom) at different circulation velocities and biomass concentrations calculated by coupling CFD trajectories to the dynamic photosynthesis model of Camacho-Rubio et al. (2003) for β = 5.

velocity alone is not effective to improve productivity. The studies of the influence of mixing on photosynthesis enhancement based only on the motion of particles can be misleading. As the mixing intensity seems to show little or no influence on photosynthesis in RWPs it is easy to jump to the conclusion that those systems are well mixed (Quiroz-Arita et al., 2020). From our research, the correct interpretation seems to be that increasing energy mixing input in the ranges tested this far is unable to

promote a vertical mixing that is sufficient to promote a light integration regime. For this, it is necessary to couple the CFD results to a dynamic photosynthesis model with reliable data from actual microalgal strains.

Still, it is important to highlight that RWPs have ample potential for productivity enhancement. Table 1 displays the population-averaged photosynthetic response for the conditions shown in Fig. 6. An intermediate biomass concentration $C_b = 800 \, g \, m^{-3}$ has been included. The

Table 1

Photosynthetic response (P/P$_{max}$) in a 0.15 m deep RWP at different circulation velocities (v) and biomass concentrations calculated by coupling trajectories calculated with a CFD model and a dynamic photosynthesis model. The rightmost column (∞) represents theoretical perfect mixing.

	v (m s^{-1})				
C$_b$ (g m^{-3})	0.2	0.4	0.6	0.8	∞
400	0.412	0.425	0.432	0.433	0.760
800	0.207	0.192	0.181	0.228	0.467
1600	0.103	0.081	0.080	0.119	0.244

P/P$_{max}$ corresponding to infinite mixing (light-averaged response, $\Gamma = 1$) has also been included in the rightmost column.

As Table 1 shows, the population-averaged response shows little variation with circulation velocity. The modest increase of 0.412 to 0.433 at 400 g m^{-3} is only a 5% and thus of little significance. For 800 and 1600 g m^{-3} the increase is 10% and 15% respectively. But the most significant conclusion that can be drawn from the calculations for theoretical perfect mixing shown in the column v=∞ ($\Gamma = 1$) is that a better mixing of RWP would have the potential of nearly doubling their current photosynthetic performance if the light regime could be improved. The data presented by Morillas-España et al. (2020) agree with this last comment. This author shows that the year-average productivity of a 0.15 deep RWP of 11.8 m^3 working volume growing *Scenedesmus almeriensis* under a maximum incident irradiance I$_o$ of 2000 μmole photons m^{-2} s^{-1} was 20–25 g m^{-2} day^{-1} while a thin-layer device operated side-by-side attained 30–35 g m^{-2} day^{-1}. This is approximately a 50% increase in productivity that, given the experimental conditions, can only be attributed to the more intense mixing and thus better light regime existing in thin-layer devices. As the authors discuss, the productivity of the thin-layer device is probably limited by the high oxygen concentrations that take place (over 400%). Otherwise, it would be possible for a well-mixed culture system (the thin-layer device) to double the productivity of an RWP.

4. Conclusions

Raceways photobioreactors for the production of microalgae (RWPs) are poorly mixed from the point of view of photosynthesis. Increasing the circulation velocity in the range 0.2–0.8 m s^{-1} does not bring about significant enhancement of the light regime. It has been shown that for a correct analysis of the photosynthetic response in RWPs essential to use a dynamic photosynthesis model coupled to CFD results as the mere consideration of estimated cycling and residence times between light and dark zones may lead to inaccurate conclusions about the light regime in these systems.

CRediT authorship contribution statement

P. Fernández del Olmo: Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Visualization. **F.G. Acién:** Conceptualization, Methodology, Formal analysis, Supervision. **J.M. Fernández-Sevilla:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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