Growth Rate Estimation of algae in Raceway Ponds: A novel Approach

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Abstract: Microalgae mass cultiviation is a promising future source of biomass for energy and food production. In order to optimize productivity of large scale plants and to make them environmentally and economically sustainable, energy requirements have to be minimized. In particular, mixing of the growth medium is a major energy input, and its effect on overall productivity should be better understood. Several dynamic models have been developed to represent the effect of a rapidly varying light on the photosynthesis process especially for the effect of photoinhibition on growth. In order to assess the mixing effects in a complex hydrodynamic regime, we propose to reconstruct the light profile received by a single cell. A multi-layer Saint-Venant approach is used to simulate the hydrodynamics of the system. It allows for the computation of Lagrangian trajectories, and finally, when knowing the light distribution, the light pattern perceived by a cell. This pattern is then used with the dynamical model for photosynthesis. In a last step, the growth rate of the whole system is estimated as the average over a set of trajectories.

Keywords: modelling, biofuel, raceway, hydrodynamics, microalgae, optimization

1. INTRODUCTION

Microalgae are considered a new source of biomass and renewable energy since the 70s (Sheehan et al., 1998). Advantages of microalgae cultivation compared to terrestrial plants are a high photosynthetic yield, independence from fertile soil and a possibly reduced freshwater consumption (Williams and Laurens, 2010). These advantages can lead to a large algal biomass production which is not in direct competition with food production. Improving biofuel production and energy generation from microalgae receives increasing attention over the last years. Increased Lipid productivity can be obtained in special conditions of nutrient limitation and high light illumination. It has been reported that after a nitrogen limitation microalgae can reach a very high lipid content (more than 50% of dry weight) (Metting, 1996).

In a wider perspective, the consumption of CO_2 during photosynthesis can contribute to carbon mitigation (Talec et al., 2013) . There are also combined approaches for wastewater treatment technologies (Sialve et al., 2009) and for a production of high value products such as cosmetics and pharmaceuticals (Wijffels and Barbosa, 2010). All these possibilities have put microalgae in a favorable position for the integration in biorefinery concepts.

For large scale production of microalgal biofuel, many problems have to be addressed. Light conditions and nutrient supply affect the photosynthetic yield, and two key processes are involved. Among them, photoinhibition diminishes of photosynthetic yield due to an excess of irradiance which destroys some key proteins of the photosynthetic apparatus. This can be represented by models that take into account the dynamics of the photosynthetic reaction centers which are represented in resting (or open) state or active (closed) state. The dynamics of open/closed transition occurs at a fast time scale (within seconds), while photoinhibition occurs in the time scale of minutes. In order to achieve optimal biomass productivities, it is very important to understand the effects of dynamical light patterns on photosynthesis dynamics, and especially on the mechanisms which lead to photoinhibition.

In this work, we propose an integrated approach for the estimation of growth rate of an open-channel raceway microalgae culture. In a first step, numerical integration of an approximation of the Navier-Stokes equation by a multilayer Saint-Venant approach is carried out in order to

predict the trajectories of single algae cells in the medium (Bernard et al., 2013). In a second step, a dynamical model for photoinhibition (Han, 2001) is calibrated using experimental data. This model gets numerically integrated using light profiles corresponding to different trajectories and calculate the average among them. This average value is considered to be an estimation of the growth rate of the complete raceway pond.

2. METHODS & RESULTS

The presented approach for the estimation of the growth rate of raceway ponds consists of three steps:

(1) Computation of the trajectories and light profiles of individual algae cells within the raceway volume
(2) Growth rate estimation for each trajectory using a photosynthesis model
(3) Averaging of the growth rate among individual trajectories resulting in an estimation for the entire system

In the following paragraph we present the hydrodynamic model presented by Bernard et al. (2013), the Photosynthesis model of Han (2001) and how the average growth rate for the entire system is calculated. We finally present structural properties of the model of Han leading to a nonlinear growth response to dynamic light signals.

2.1 Hydrodynamics and single cell trajectories computation

An approximation of the Navier-Stokes equations has been proposed by Audusse et al. (2011a), which is well adapted to the conditions in an open raceway pond. Compared to former multilayer models that are adapted for non miscible fluids, the so-called multilayer Saint-Venant system with mass exchanges is a good approximation of the Navier-Stokes equations. The accuracy and the stability properties of the multilayer approach have been demonstrated in Audusse et al. (2011a,b).

Moreover the multilayer model has been successfully confronted with analytical solutions of the hydrostatic Euler and Navier-Stokes systems with free surface (Boulanger and Sainte-Marie (2013)). The multilayer model consists in Galerkin type approximation along the vertical axis of the Navier-Stokes sytem, it results in a set of partial differential equations with hyperbolic features and written over a fixed mesh. The system is numerically solved by considering a 2D triangular mesh of the ground surface, the layers defined by the water depth giving the third spatial dimension. The discretization technique used a finite volume scheme based on a kinetic interpretation of the equations. A specific forcing term mimicking the effect of the paddle wheel was added (Bernard et al., 2013). The impact of the wheel on the fluid is considered as a normalized force applied by the wheel's blade and equal to the square of the velocity at each point. Here, a 3D extension to the model presented in Bernard et al. (2013) was used. The used meshing represents a real raceway pond of the Environmental Biotechnology Laboratory of INRA Narbonne (see Figure 1).

Finally, Lagrangian trajectories of several particles are reconstructed by integrating the velocity field for various initial conditions of the particles. In addition, a Brownian
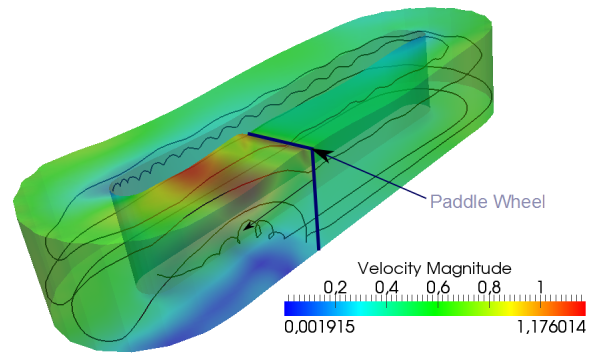

Fig. 1. 3D representation of the water volume and its velocity field in the numerical simulation. A particular trajectory is also represented.
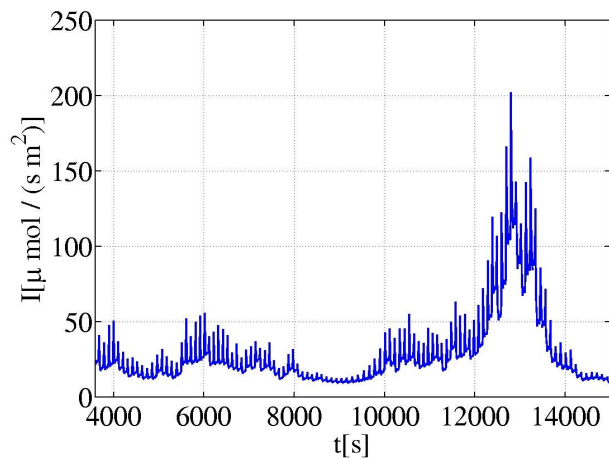

Fig. 2. Light signal received by a single cell as a function of time

motion model is applied to better represent the local diffusion effect.

The raceway simulation has been run for 15 000 seconds. The simulation starts with static non agitated medium, and the paddle wheel is immediately set on. Consequently, the first 3600 seconds of simulation were disregarded, in order to reach a stabilisation period.

Light intensity profiles are calculated from the trajectories by applying the law of Beer-Lambert for light attenuation. The attenuation coefficient was chosen to be a fixed constant which leads to 5 % of the incident light intensity at the bottom of the raceway. Consequently, the light intensity as a function of the water depth z above the particle is:

$$I(z) = I_0 \cdot e^{-\gamma \cdot (z)} \qquad (1)$$

Taking into account the depth of the culture of $0.3m$, we can deduce a value of $15.35m^{-1}$ for the parameter γ. A typical light pattern is presented on fig. 2.

2.2 The Han Model

Han (2001) presented a mechanistic model for photosynthesis which includes dynamical effects of photoinhibition. This model represents the dynamics of the photosystems depending of light flux. Photosystems are the first units involved in photosynthesis, they collect photons and transfer electrons to the rest of the system. They can have three possible states: open (A) and relaxed, closed while they are processing photons (B) or inhibited (C) when they received an excess of photons.

Dynamic processes are represented by the transition between the states and depend on the photon flux. Primary production is described by the transition between A and B. Excitation is assumed to occur at a rate of σI, with σ being the functional cross section of the photosynthetic unit (PSU). De-excitation is assumed to occur at a rate of $\frac{1}{\tau}$, with τ being the turnover time of the electron transport chain. At higher irradiation photoinhibition occurs: PSII's pass over from closed to inhibited state at a rate $k_d \sigma I$. At the same time a repair mechanism compensates this effect at a constant rate k_r.

The equations describing the rates of change in the fractions of open, closed and inhibited states are given by:

$$\dot{A} = -I\,\sigma\,A + \frac{B}{\tau}$$

$$\dot{B} = I\,\sigma\,A - \frac{B}{\tau} + k_r\,C - k_d\,\sigma\,I\,B \qquad (2)$$

$$\dot{C} = -k_r\,C + k_d\,\sigma\,I\,B.$$

Expressions giving the fractions of open, closed and inhibited states at steady state as a function of the irradiance I can be derived explicitly from 2. The steady-state expression A^∞ for the open state A is given by:

$$A^\infty(I) = \frac{1}{1 + \tau\,\sigma\,I + k_d/k_r\,\tau\,\sigma^2\,I^2}, \qquad (3)$$

Finally, growth rate is described by a function proportional to IA. At steady state, this corresponds to a Haldane type curve:

$$\mu^\infty(I) = \bar{\mu}\frac{I}{1 + \tau\,\sigma\,I + k_d/k_r\,\tau\,\sigma^2\,I^2}, \qquad (4)$$

2.3 Parameters identification

In order to identify the parameters of the Han model for the species *Dunaliella salina* completely it was necessary to use data from different studies. The light response curve was taken from Park et al. (2013) describing the photosynthetic response of microalgae (measured by oxygen evolution) to different light intensities. With this data, we fitted a well-parametrized form of eq. 4 by Bernard and Rémond (2012) In a second step, experiments consisting of measuring the growth rate for different crenellated light/dark alternation frequencies have been done. For such caricatural light signals varying at high frequencies, approximation of the response of the Han model can be obtained (Hartmann et al., 2013). Such approximations responses were used to fit the model to data measured by Combe et al. (2013). It leads to a value for τ and the respiration rate R. The increase of the Quantum yield

Parameter	Value	Unit
k_r	$2 \cdot 10^{-4}$	s^{-1}
k_d	$1.82 \cdot 10^{-16}$	.
τ	0.667	s
σ	0.0143	$m^2/(\mu mol)$
R	4.9250	mol 10^{-6} cells min^{-1}

Table 1. Estimated model parameters based on experimental data by (Park et al., 2013) and data by Combe et al. (2013)
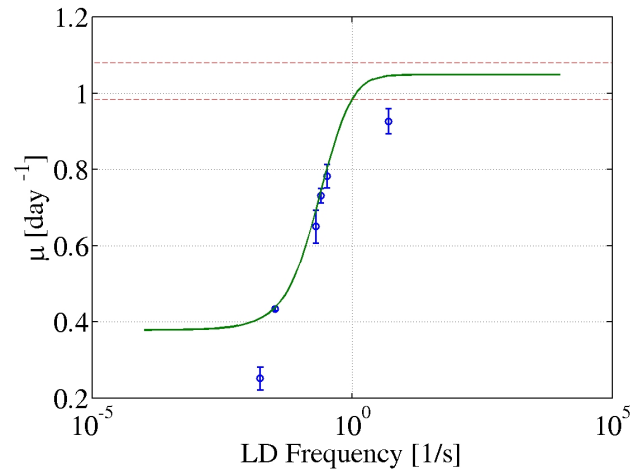

Fig. 3. Dependency between Frequency and Growth rate. Frequency on a logarithmic scale, Circles show measured values of data by Combe et al. (2013), lines show model predictions according to Hartmann et al. (2013)

after light to dark tranistion as published by Neidhardt et al. (1998) imposes a time scale for the repair process of the photocenters. From this we deduced the value for k_r. However, according to the data of Park et al. (2013), inhibition plays a minor role within natural values for the light intensity. Thus the values for k_d and k_r have negligible impact on the model behaviour in real-life conditions.

After a least square fit on the data presented in paragraph 2.3, the parameters turned out as in tab. 1. The fit of the experimental data is presented in figures 3 and 4.

2.4 Simple factors in light signal affecting productivity

In a preliminary study, the effect of the paddle wheel on the light received by a cell was studied by a caricatural light/dark signal with equal periods of light (at intensity I_0) and darkness. In the work of Hartmann et al. (2013), the effect of the light/dark frequency ω is studied. It is shown that the average growth rate $\mu(I_0, \omega)$ is an increasing function of ω, and that the growth rate is bound within:

$$\frac{1}{2}\mu^\infty(I_0) \le \mu(I_0, \omega) \le \mu(\frac{I_0}{2}, +\infty) = \mu^\infty(\frac{I_0}{2}) \qquad (5)$$

This means that for very low frequencies, the system can be approximated by a system where half of the time growth rate is obtained for a continuous light at I_0, while there is no growht the rest of the time. For a very fast frequency, much faster that the time scales of the Han model, the light signal is averaged into a light $\frac{I_0}{2}$.
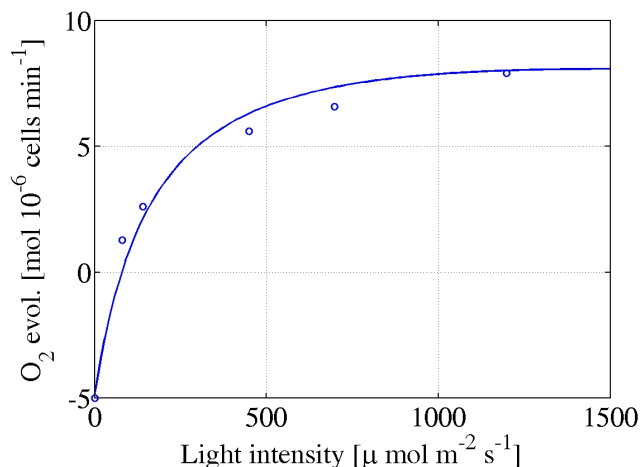
Fig. 4. Steady state Light response curve for the utilized parameters

The next step is to test this effect with a more realistic light pattern, derived from a trajectory computed with our hydrodynamical approach.

Firstly, the effect of the signal variance on the growth rate at steady state is analysed. Given different signals with alternation of constant light periods of same length, between $I - \delta I$ and $I + \delta I$; The previously considered case is a specific case for $\delta I = I$.

Hypothesis 1. (H1) Assuming that $I + \delta I$ is in the convex part of the Han equation (4). In other words:

$$\frac{d^2 \mu^\infty}{dI^2}(I + \delta I) < 0 \qquad (6)$$

In order to optimize productivity of large scale plants and to make them environmentally and economically sustainable, energy requirements have to be minimized.

Property 1. Using Hypothesis H1, we have

$$\frac{\mu^\infty(I + \delta I) + \mu^\infty(I - \delta I))}{2} \le \mu^\infty(I)$$

Proof: This Property is easily proven when considering the decreasing function (Hypothesis (H1)) $\frac{d\mu^\infty}{dI}\mu^\infty(I)$. We have

$$\frac{d\mu^\infty}{dI}(I) \le \frac{d\mu^\infty}{dI}(I - \delta I)$$

Integrating this expression between I and $I + \delta I$, we get

$$\mu^\infty(I + \delta I) - \mu^\infty(I) \le \mu^\infty(I) - \mu^\infty(I - \delta I)$$

This provides the result.

As a consequence, any light signal varying at very low frequency between $I - \delta I$ and $I + \delta I$ would lead to an average growth rate lower than the growth rate for the average light I.

The same reasoning holds for δI: increasing δI (provided that μ^∞ stays in a region where it is convex) leads to a reduced growth rate. This reasoning highlights the two effects affecting growth:

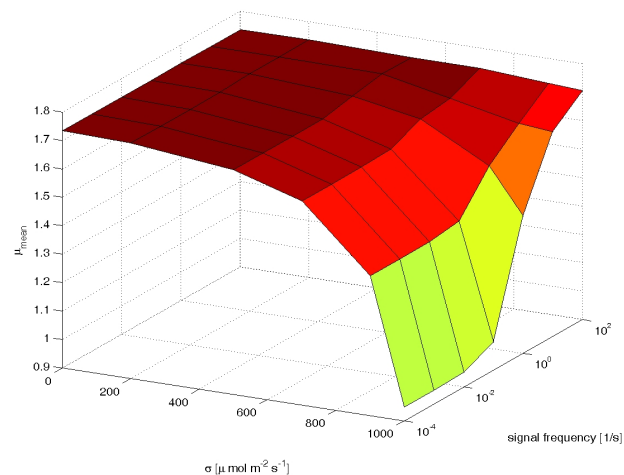- Signal frequency which tends to increase growth rate

Fig. 5. Average growth rate based on the Han model using rectangular light signals with different variance and frequency

- Variance of the Light signal which, at low frequency, tends to decrease the growth rate

Fig. 5 shows results for numerical integrations of the Han model based on a rectangular signals with different signal variance and frequency. Coherently with the analytical prediction, it can be observed that the growth rate increases with frequency and decreases with increasing variance.

In the next paragraph, a reference trajectory is used so that the second effect is eliminated and the result depends only on the frequency of light dark succession.

2.5 Effect of the paddle wheel velocity

In a simple approach, in order to understand the effect of the paddle wheel on productivity, it is assumed that increasing mixing intensity is equivalent to contraction and dilatation of the time axis of the cell trajectory. This corresponds to a linearisation of the paddle wheel effect. Considering one reference trajectory, means that different mixing intensities are represented by dilation factors of the time axis. Increased paddle wheel mixing thus corresponds to a time-contracted reference trajectory.

Based on the light signals the mean photosynthetic productivity is calculated from the Han model over the full trajectory. The travel time has been contracted and dilated down by a factor of 100. Moreover, together with the travel time, we simultaneously considered the effect of incident light irradiation I_0 on productivity. The results are presented in fig. 6.

As it can be observed, a reduced travelling time yields only about 70 % of the productivity at the original traveling time for 1000 $\mu mol/s^{-1}m^{-2}$) surface light intensity. There is only an insignificant difference between the result for the light profile passing a 100 times faster and the original speed.
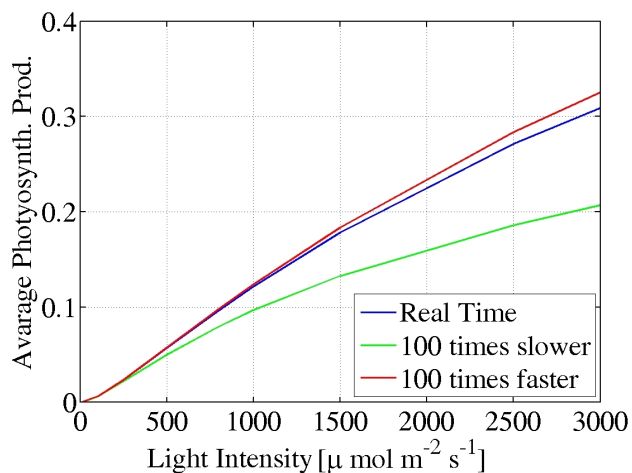
Fig. 6. Steady state Light response curve for the utilized parameters, exemplary trajectory
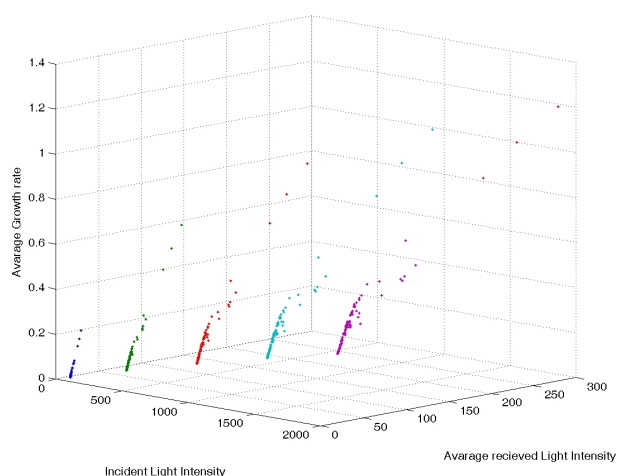

Fig. 7. Average received light intensity and growth rate as a function of surface light intensity. Each color of points refers to one surface light intensity.

2.6 Growth rate prediction for the mixed Culture

In order to estimate the growth rate of the entire culture, we calculated the average growth rate of all 460 trajectories for different light intensities at the surface. For the total average growth rate μ_{av} we get consequently:

$$\mu_{av} = \frac{\sum_{i=1}^{n} \bar{\mu}(z_i)}{n} \qquad (7)$$

With z_i being the time dependent depth coordinate for the ith trajectory. The resulting average photosynthetic productivity as a function of incident light intensity of the culture is presented in Fig. 8. The individual growth rate of all single trajectories are presented in Fig. 7. The growth rate and the average received light intensity of each trajectory is represented by one point in the 3D Coordinate system.

The average growth rate (fig. 7) of the culture increases linearly with the surface light intensity. However, due to a special characteristics in the hydrodynamic simulation the
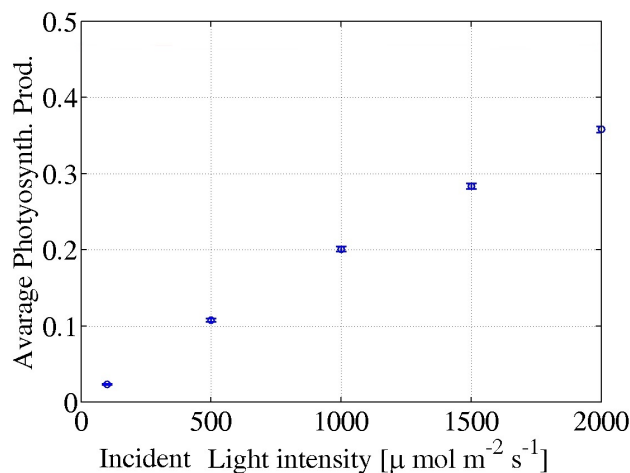

Fig. 8. Average Value of gross Growth rate between the 460 trajectories for different incident illuminations.

average received light intensity has significant variability among the trajectories (compare fig. 7) for the same surface intensity.

3. DISCUSSION

With the presented approach, we can estimate the growth rate of the raceway pond based on the growth response of individual algae cells. Contrary to similar approaches, we did not rely on rectangular light signals, but on light profiles which have been calculated from the hydrodynamics of the system.

For the calibration of the Han Model for *Dunaliella salina*, data from three different studies have been used. The increasing productivity as a function of cycle frequency in the data of Combe et al. (2013) has been identified as an effect of the open/closed dynamics, which is directly linked to the value of the parameter τ. Since this increase can be observed at frequencies of about 1 Hz, the parameter τ has been estimated as 0.67 s. This value, unusually high, is slower than what is considered a typically in the literature (cf. Falkowski and Raven (2007)). Nevertheless, the study follows an experimental protocol presented by Falkowski and Raven (2007), which allows for direct determination of this physiological parameter. This result might therefore be taken as a hint for an unusual behaviour of *Dunaliella salina* in flashing light conditions.

Structural properties of the Han model show that the growth response depends not only on the average light intensity, but also on the frequency and the shape of the received light profile. This leads to the conclusion that considering only the frequency of the light signal is not enough to estimate the photosynthetic efficiency. Contrarily to several works studying the growth response as a function of flash frequency, predictions of actual productivity should also be based on the variance of the signal. With the proposed approach we suggest a new paradigm to treat this problem by reconstructing realistic trajectories. In order to predict the growth rate within the aggregate system, the average growth rate has been deduced from 460 trajectories. A linear relation between surface light intensity and aggregated growth

rate is found. This could be interpreted similarly to the average growth rate computed in Bernard (2011), when averaging the Han static response over the depth of the culturing system. Analytical computation in this simplified case shows that this is similar to shifting the optimal light intensity towards higher light, and reducing the maximum growth rate. Shifting the maximum of the curve on fig. 4 is similar to rescaling the response curve for low light intensity. As a consequence, the response to light becomes approximatively linear.

When regarding the individual values for average growth rate and depth for different trajectories, there is a significant spread for the average depth of the particles. This indicates, that the simulation time is not sufficient in order to show the ergodicity of the system.

At the current state of this study, we suspect that a numerical artefact in the integration of the hydrodynamics leads to an overestimated spatial probability for the particles at the bottom of the raceway. This imposes consequently an offset for the average received light intensity of the particles and leads to growth rates which are underestimated. In the context of the high respiration – which was found for our calibration – the growth rates are even below respiration. It should be pointed out that – to our best knowledge – at present no studies exist which evaluate the spatial distribution of particles in a hydrodynamic raceway simulation for sufficiently long simulation time.

4. CONCLUSION

This work investigates how hydrodynamics impacts biology in microalgae culture ponds. The actual effect on photosynthetic efficiency is assessed by coupling the Lagrangian trajectories computed from a multi-layer Saint-Venant hydrodynamical model, to a dynamical model representing the fast time scales of photosynthesis. Such study highlights the idea that experiments conducted to assess the effect of light/dark frequency on photosynthesis are not sufficient to characterize the mixing effect in a raceway. More work is required to better study the impact of the paddle wheel, by ensuring that basics statistical properties of Lagrangian trajectories (average depth, equirepartition of the particules) are not affected by the wheel velocity. This approach is also the foundation for the development of a computer driven approach able to optimize the design of raceways and increase the ratio between the recovered biofuel from the microalgae and energy consumption for mixing.

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