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Hurdles and challenges for modelling and control of microalgae for CO_2 mitigation and biofuel production

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ABSTRACT

Oleaginous microalgae are considered to be a potential major biofuel producer in the future since, under conditions of nitrogen deprivation, they are capable of containing high amounts of lipids, while consuming industrial CO_2. These photosynthetic microorganisms are, however, rather different from the microorganisms usually used in biotechnology. In particular, predicting the behaviour of microalgal based processes is delicate because of the strong interaction between biology (microalgal development and respiration), and physics (light attenuation and hydrodynamics). This paper reviews existing models, and in particular the Droop model which has been widely used to predict microalgal behaviour under nutrient limitation. It details a model for raceways or planar photobioreactors, when both light and nutrients are limiting. The challenges and hurdles to improve microalgal culture process modelling and control in order to optimise biomass or biofuel production are then discussed.

1. Introduction

Autotrophic microalgae and cyanobacteria use photons as energy source to fix carbon dioxide (CO_2). These microorganisms (abusively called "microalgae") have recently received specific attention in the framework of renewable energy. Their high actual photosynthetic yield compared to terrestrial plants leads to large potential algal biomass productions of several tens of tonnes per hectare and per year [23]. After nitrogen starvation, this biomass can reach a very high lipid content (more than 60% of dry weight under certain stress conditions [75]). These possibilities have led some authors to consider microalgae as one of the main biofuel sources for the future [56,23,116].

In addition, the ability of microalgae to fix CO_2 in a controlled way has recently involved them in the race for mitigation systems [8,79]. Microalgal biofuel production systems could also contribute to mitigate industrial CO_2 emitted from power plants, cement plants, etc. In the same spirit, microalgae could be used to consume inorganic nitrogen and phosphorus in urban or industrial effluents, and thus limit expensive wastewater post-treatment [64].

Algal growth technologies for biofuel production and CO_2 mitigation were first developed from the mid-1970s to the mid 1990s in the framework of the Aquatic Species Program (ASP) funded by the US Department of Energy [100]. In Japan, the Research for Innovative Technology of the Earth program (RITE) from the New Energy Development Organization (NEDO) has carried out an extensive program in the 1990s for microalgal CO_2 utilisation, focusing on closed photobioreactors (PBR). These research activities reemerged at the worldwide scale at the end of 2000s, in the context of the strong increase of the oil barrel price, the critical necessity to secure energy supply and the clearer symptoms of the global climatic changes.

The advantages of microalgae put them in a good position for renewable energy production at large scale [23,116]. This explains the explosion of publications on this topic, and the optimistic claims of start-ups, which foresee, in the near future, industrial production of microalgal biofuel. However, microalgae have been so far only marginally used for biotechnological applications. To date, the main domains of application are focused on innovative processes to produce vitamins, proteins, cosmetics, and health foods [89,103]. Microalgae are grown in a broad range of Microalgae Culture Processes (MCP) ranging from rudimentary open ponds to high technology closed photobioreactors. They are, however, still cultivated at small scale: the total worldwide microalgal production is in the range of 10 000 tonnes of dry biomass per year to be compared to 105 million m^3 of biofuel (biodiesel and bioethanol) worldwide produced in 2010 [43].

In the perspective of large scale microalgal cultivation, new techniques both from biotechnology and from the control field

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must be deployed to ensure robustness, durability and optimisation of these new processes. Indeed, microalgae have some specificities compared to microorganisms more currently used in biotechnology, such as bacteria or yeasts. The main difference, when solar light energy conversion into chemical energy is targeted, is that each cell must have access to light in order to sustain its growth. Increasing biomass concentration leads to more light absorption. Therefore, maximal reachable biomass is bounded by a limit biomass concentration, for which all the impinging photons are absorbed. But this limit is not straightforward since cells adapt their pigments to the impinging light to optimise light harvesting [3,67], and therefore light attenuation (which is deduced from pigment concentration) is light dependant. Moreover, in conditions of nitrogen starvation (applied to stimulate lipid synthesis [93]), the pigment composition and concentration decrease [112,98,45], leading to reduced light attenuation [106]. When targeting outdoor cultivation of microalgae, these organisms grow in permanently unsteady conditions since they are submitted to daily light (and temperature) variations. As a consequence, populations are often synchronised and divide at preferential times, making their behaviour more complex.

Microalgal based processes therefore involve several new challenges for modelling and control. In addition to the classical nonlinear and complex features, which characterize most of the biotechnological processes, the permanent unstationary behaviour together with a strong feedback from the population level to the cell level through light attenuation make the domestication of these processes very challenging.

Optimising such complex processes can be much more efficient if accurate models can be developed. The recently highlighted potential of these microorganisms explains why, so far, only limited attention was paid to microalgal modelling and control. So far, a large part of the modelling studies were carried out in relation to the development of phytoplankton in the natural environment.

In this paper, I first review microalgae dynamic modelling and present some microalgal models, based on the classic Droop model. I analyse the ability of microalgae to adapt to a given light intensity, and present photoacclimation models. The modification of these models when considering outdoor high density algal cultivation is then discussed, introducing a coupling between biology and physics (mainly via light gradient). Some studies on PBR optimisation and control are then presented. Finally, the challenges for optimising such complex processes are synthesised.

2. More than 60 years of microalgae dynamics modelling

Microalgal dynamic models (mainly under the form of ordinary differential equations) have been developed in three distinct domains: oceanography, ecology and biotechnology. The first phytoplankton dynamic model was proposed by Riley [92] to account for populations on the Georges Bank. Riley represented both the effect of light (including an exponential decrease along depth) and of a limiting nutrient on growth. Several kinetic models dealing with photosynthesis have then been proposed, starting from the hyperbolic expression [4] up to more complex representations including photoinhibition by light excess [104,115,86,84]. In parallel to improvement of light impact representation, the effect of a suboptimal nutrient was described by kinetic models for uptake [32] and growth [30,31], later reformulated by [18]. The resulting Droop model will be detailed in the next section. Models which proposed a combination of limitation by light and nutrients came later on, with a necessary higher complexity to deal with the nonlinear interaction between photosynthesis and nutrient limitation (especially for nitrogen) [45,37,81].

The first dynamic model of a microalgal production process was proposed by [109] for a raceway pond in the framework of the ASP [100]. The model was later consolidated by including time-discrete photoacclimation dynamics [110]. In parallel, other less elaborated models where proposed [9,48,50].

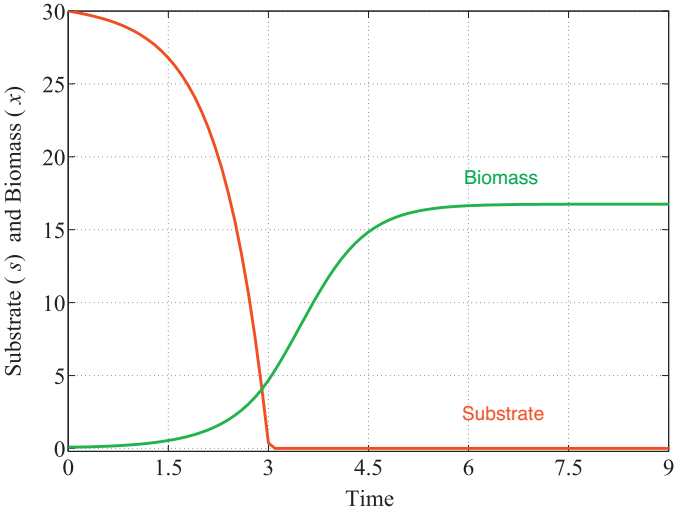

Fig. 1. Typical growth curve for microalgae: biomass continues to grow a few days after nutrient exhaustion.

The theory of a light limited chemostat was elaborated by [55] who proposed the fundamental concepts to understand the dynamic behaviour of photolimited MCP [54]. Models integrating the geometry of the photobioreactor (making the light distribution computation much more tricky) and, at the same time, able to predict the transient behaviour were proposed by [77,65,42]. Extensive literature exists on steady state photobioreactors [26,38,28,25], but dynamic models forecasting transient evolution of biomass, its photosynthetic and optical characteristics are still scarce. Most of these models rely on photolimited cultures at equilibrium, where nutrients are in excess to avoid any growth reduction. They are thus unable to properly describe the mineral starvation conditions that lead both to growth reduction and lipid accumulation.

In this paper, I use Droop model to illustrate the complexity of microalgae dynamics and present a simplified, but mathematically tractable, theory of MCP when both light and nutrients are limiting growth.

3. The Droop model: basics of microalgal growth modelling

Microalgae are known for their ability to uncouple uptake of nutrients (inorganic nitrogen, phosphorus, vitamins, etc.) from growth [97]. A classical growth curve is presented in Fig. 1, showing that biomass continues to grow during a few days after nutrient exhaustion. As a consequence, the Monod model where nutrient uptake and growth are proportional is unable to accurately reproduce this phenomenon. The Droop model, initially established to represent the effect of vitamin B_{12} internal quota on the growth rate of phytoplankton [30], has been shown appropriate to represent also the effect of macronutrients, such as nitrogen or phosphorus, on the growth rate [31]. The growth rate (denoted μ) of the biomass x is thus assumed to be related to the internal concentration of the limiting element. In the sequel, we will consider that nitrogen is the limiting nutrient (a nitrogen limitation induces lipid synthesis). The concentration of the limiting dissolved inorganic nitrogen (nitrate or ammonium) is denoted s. As a consequence, the internal nitrogen cell quota, denoted q, is defined by the amount of nitrogen per biomass unit. The Droop model equations, in a perfectly mixed continuous bioreactor (chemostat), with

dilution rate D and influent inorganic nitrogen concentration s_{in}, writes:

$$\begin{cases} \dot{s} = Ds_{in} - \rho(s)x - Ds \\ \dot{q} = \rho(s) - \mu(q)q \\ \dot{x} = \mu(q)x - Dx \end{cases} \quad (1)$$

In this model, the absorption rate $\rho(s)$ is represented by a Michaelis–Menten kinetics [18]:

$$\rho(s) = \rho_m \frac{s}{s + K_s} \quad (2)$$

where K_s is the half saturation constant for substrate uptake, associated with the maximum uptake rate ρ_m.

The growth rate $\mu(q)$ is based on a Droop function:

$$\mu(q) = \bar{\mu}\left(1 - \frac{Q_0}{q}\right) \quad (3)$$

Parameter $\bar{\mu}$ is defined as the growth rate at hypothetical infinite quota, while Q_0 is the minimal cell quota, for which no algal growth can take place. As it will be shown in Property 1, the Droop model guarantees that, for a consistent initial condition, the internal quota q stays larger than Q_0 and, therefore, μ is always non negative. This model is more accurate than the Monod model for algal growth modelling [113]. The Droop model is, however, sufficiently simple to allow a detailed mathematical analysis [60,11,13].

Property 1. *The Droop model guarantees that, for an initial condition $Q_0 \le q(0) \le Q_m$, the internal quota stays at any time t between two bounds:*

$$Q_0 \le q(t) \le Q_m \quad (4)$$

where

$$Q_m = Q_0 + \frac{\rho_m}{\bar{\mu}} \quad (5)$$

represents the maximum cell quota obtained in conditions of non limiting nutrient. The growth rate is also bounded:

$$0 \le \mu(q) \le \mu_m = \frac{\rho_m \bar{\mu}}{Q_0 \bar{\mu} + \rho_m} \quad (6)$$

where μ_m is the maximum growth rate reached in non limiting conditions.

Proof. See *e.g.* [11]. □

As a corollary of this property, most of the Droop model parameters can be straightforwardly identified from the measurements of the internal quota during starvation and during unlimited growth.

- In a starvation period, *e.g.* at the end of a batch phase when nutrients are exhausted, the growth rate becomes zero and the internal quota reaches its minimal value $q = Q_0$.
- During an unlimited exponential growth period, after a transient, the internal quota reaches its maximum value Q_m for a growth rate $\mu(Q_m) = \mu_m$. During this period, for a batch experiment, nutrients disappear at a rate proportional to biomass growth. In other terms, $s/\rho_m + x/\mu_m$ is a constant: the s versus x graph has a slope $-\rho_m/\mu_m\,(= -Q_m)$.

Now that we have experimentally estimated Q_0, Q_m, μ_m and ρ_m/μ_m, parameter $\bar{\mu}$ is derived from (6):

$$\bar{\mu} = \frac{\mu_m}{1 - Q_0\mu_m/\rho_m} \quad \text{or} \quad \bar{\mu} = \frac{\rho_m}{Q_m - Q_0} \quad (7)$$

Parameter K_s is deduced *e.g.* from dedicated batch experiments where the exhaustion of s is monitored. Then, the uptake rate with respect to s can be recomputed and fitted to a Michaelis–Menten kinetics.

Remark 1. Note that there is often a confusion in the literature between $\bar{\mu}$ and $\mu_{\max}$ which does not explicitly appear as a model parameter.

The Droop model has been widely validated [31,99,12,113]. This model, despite its simplicity, has turned out to accurately reproduce dynamics of microalgae evolving in a constant environment.

However, the Droop model cannot be used in the case of photolimited cultures, and the effect of light on the growth rate must be introduced into the model. When dealing with high density cultures, despite the homogeneity of the biochemical compounds, there is a light gradient due to light absorption and diffusion. This leads to a gradient in photosynthesis activity, which must be represented.

4. Microalgal models dealing with light limitation

4.1. Photoacclimation models

In the past decade, several models have been proposed in order to account for the response of microalgal pigment density to both light intensity and available nutrients. The most difficult case is when nitrogen is limiting growth, since nitrogen strongly interferes with pigment synthesis. Geider et al. [45] were the first to propose a simple model introducing chlorophyll (denoted Chl) as a model variable (in addition to microalgal carbon and nitrogen). This model integrates the known response of photosynthesis to both light and nitrogen status in the cell. Other models have been proposed [81,37], but they have been, so far, less used. More complex models have also been developed [122,40], but being more accurate in the detail of the described mechanisms, they involve more parameters and state variables, which makes their calibration, validation and use for control purposes more difficult.

The underlying key feature of these models is the photoacclimation process. This acclimation mechanism allows the algae to adapt pigment (and especially chlorophyll) synthesis to light intensity. Fig. 2 represents experimental data extracted from [3], where the CO_2 uptake rate appears as a function of light for two different microalgal cultures, which were first photoacclimated at two different light intensities. It is worth noting that, when photosynthesis is normalised by Chl, the initial slope of the response curve is independent of the photoacclimation light [67] and, thus, of the initial $\theta = \text{Chl}/x$ ratio. This fact supported the development of kinetic models where the growth rate was a function of both light and θ. Fig. 2 also highlights the photoinhibition process, which takes place at high irradiance. The classical photosynthesis models do not represent this feature. It is however an important problem in practice since this mechanism leads to reduced yields at high light. One way of modelling the light effect consists in including light in parameter $\bar{\mu} = \bar{\mu}(I)$ [51] and potentially in parameter $Q_0 = Q_0(I)$ (this second feature is not considered here for sake of simplicity). We consider the kinetic model of [33,34] to represent the typical curves of Fig. 2:

$$\bar{\mu}(I) = \bar{\mu}\frac{I}{I + K_{sI} + I^2/K_{iI}} \quad (8)$$

Here, an inhibition coefficient K_{iI} is considered, together with parameter K_{sI}, they define the light intensity $I_{\text{opt}} = \sqrt{K_{sI}K_{iI}}$, for which $\bar{\mu}(I)$ is maximal.

To account for the photoacclimation mechanisms in Eq. (8), parameter K_{sI} is computed from θ as follows:

$$K_{sI} = \frac{K^*_{sI}}{\theta} \quad (9)$$

With this expression, the initial slope of the inorganic carbon uptake rate normalised by chlorophyll is constant, whatever the pre-acclimation light (and its associated θ), and yields $\bar{\mu}/K^*_{sI}$.

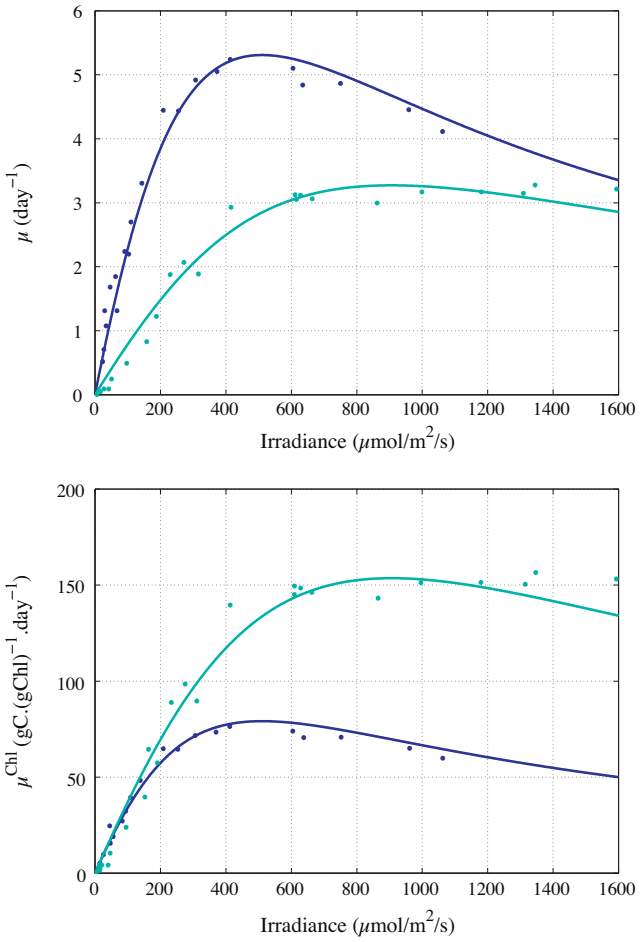

Fig. 2. Model and data of the photosynthetic response of the diatom *Skeletenonema costatum* photoadapted at low ($I_L = 50\ \mu mol\ m^{-2}\ s^{-1}$, dark points and lines) and at high irradiance ($I_H = 1200\ \mu mol\ m^{-2}\ s^{-1}$, light grey points and lines). The top graph is normalised by carbon and the lower graph is normalised by chlorophyll. Data from [3].

4.2. Modelling pigment dynamics

The chlorophyll concentration must be represented in the model in order to be able to predict the light field throughout the culture. With the same spirit of presenting a very simple model, we assume that chlorophyll is proportional to the cellular proteins, *i.e.* linearly correlated to particulate nitrogen xq [62]. More specifically, for a culture photoacclimated at an irradiance I^*, we have [15]:

$$\mathrm{Chl} = \gamma(I^\star)xq \tag{10}$$

where

$$\gamma(I^\star) = \gamma_{\max}\frac{k_{I^*}}{I^\star + k_{I^*}} \tag{11}$$

This expression results from experimental observations of photoadapted cultures obtained at various irradiances and nitrogen conditions.

Fig. 3 shows data for cultures of *Rhodomonas salina* with various levels of nitrogen limitation (*i.e.* dilution rates) and various light intensities. Relationship (10) accurately represents the Chl per unit of algal nitrogen.

One of the key originalities of the model proposed by [15] is that it uses a conceptual variable, denoted $I^\star$, which is the irradiance at which the cells are photoacclimated. In a light homogeneous (low biomass density) steady state culture, this variable is exactly the mean irradiance. It is related to average light intensity for denser

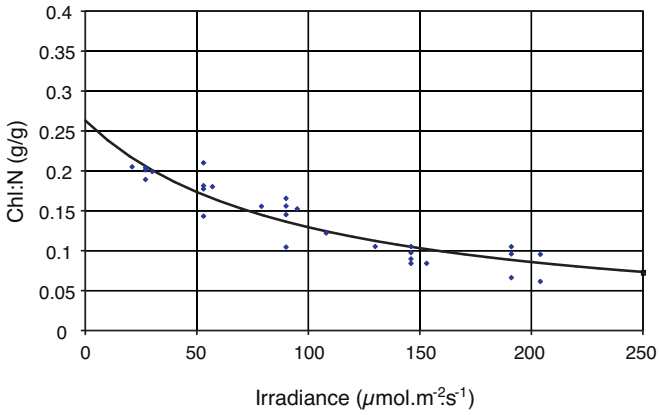

Fig. 3. Representation of the ratio Chlorophyll *a* over particulate nitrogen for various light conditions for *R. salina* with different levels of nitrogen limitation in chemostat experiments (data from [83]).

cultures [110]. To represent this light adaptation dynamics, we use the following formulation:

$$\dot{I}^\star = \delta\mu(q, I)(\bar{I} - I^\star) \tag{12}$$

where $\bar{I}$ is the average irradiance along the culture volume, and δ is the photoacclimation rate. Nevertheless, a more subtle computation of $\bar{I}$ can be considered accounting for the hydrodynamics of denser cultures. Indeed, at the scale of the cell, depending on the hydrodynamic regime, the cell successively experiences high light intensity (at the surface) and darkness (at the bottom). The question of the light, for which cells are photoadapted, is therefore crucial, and it is clearly an open problem [119,88,85,94].

4.3. Inorganic nitrogen uptake rate

When including light effect in the growth rate, the maximum inorganic nitrogen uptake rate must be adapted to limit cell quota increase, especially in the dark. Indeed, with the Droop model and a constant maximum uptake rate, Eq. (5) becomes:

$$Q_m(I) = Q_0 + \frac{\rho_m}{\bar{\mu}(I)} \tag{13}$$

As a consequence of such a formulation, no growth occurs at night ($\bar{\mu}(0) = 0$), so that the substrate can be indefinitely taken up into the cell without being consumed for growth, leading to an infinite maximal quota. To account for the down regulation of nutrient uptake when the nitrogen quota reaches a maximal level, expression (2) is revisited as proposed by [63]. The uptake rate stops as cells become nutrient saturated:

$$\rho(s, q) = \bar{\rho}\frac{s}{s + K_s}\left(1 - \frac{q}{Q_l}\right) \tag{14}$$

with $Q_l > Q_0$. As for Property 1, it is straightforward to show that if $Q_0 \le q(0) \le Q_l$, then for any time $Q_0 \le q(t) \le Q_l$.

4.4. Respiration

The last phenomenon, which must be embedded in a high density microalgal model, is the respiration process. Respiration is usually hidden in the "net" growth rate of the Droop model. However, for high density cultures where a fraction of the culture is in the dark, respiration must be considered. Indeed, the domains of the culture where light is so low that the growth rate is lower than the respiration rate are areas of the reactor where the net carbon balance is negative in the sense that more CO_2 is released than taken up. Respiration is the sum of a basal respiration proportional to biomass and a term proportional to the cost of biosynthesis, and

thus to the growth or to the uptake rates [45,95]. A simple way of including it in a model consists in assuming that the biosynthesis cost is already included in the "net growth rate" and that the basal respiration is a term proportional to biomass. Note that, in most of the models [45,81], nitrogen is assumed to be released at the same rate as carbon, which also means that respiration terms in the models also account for cell mortality.

5. Dealing with light gradient

5.1. Average light

We investigate here a simple representation of light distribution inside a high density MCP. For sake of simplicity, we assume a planar geometry with illumination perpendicular to the plane (corresponding to a planar PBR or a raceway) of thickness L. For more complex geometries, it is still possible to compute light distribution [38,27,108], but it is more difficult to obtain analytical results. We still assume that all the concentrations are homogeneous and, therefore, that only light has a spatial distribution.

The theory of the "light-limited chemostat" has been first developed by [55,54]. Here, we revisit and extend it in the framework of photoinhibition.

With such hypotheses, irradiance distribution in the MCP can be represented to a first approximation by a Beer–Lambert exponential decrease with a rate linearly related to biomass concentration. When I_0 is the irradiance at the surface, and $I(z)$ the irradiance at depth z, we have thus, for a MCP where cells are photoacclimated at light $I^\star$:

$$I(z) = I_0 e^{-\xi z} \tag{15}$$

where ξ is the light attenuation rate, mainly dependent on the chlorophyll content, and on the biomass: $\xi = a\,\mathrm{Chl} + bx + c$. The coefficients a, b are the specific light attenuation coefficients due to chlorophyll and biomass, respectively, and c is the background turbidity.

Note that the Beer–Lambert approximation, which does not account for the light backscattering in a dense culture, can be improved by using more accurate radiative transfer models based on the inherent optical properties of microalgae [58,27,26,108,87,42]. Indeed, such light transfer models in the culture medium can be used provided that the pigment concentration and composition are predicted at any time. This variable pigment prediction (together with cell size) must, however, be related to light in the reactor and available nutrients [106].

The light attenuation coefficient ξ is used to compute the optical depth $\lambda = \xi L$, corresponding to:

$$\frac{I(L)}{I_0} = e^{-\lambda} \tag{16}$$

This key parameter reflects how efficiently light energy is absorbed by the MCP.

The average irradiance received by the cell culture between 0 and L is therefore:

$$\bar{I} = \frac{I_0}{L}\int_0^L e^{-\xi z}\,dz = \frac{I_0}{\lambda}[1 - e^{-\lambda}] = \frac{I_0 - I(L)}{\ln(I_0/I(L))} \tag{17}$$

5.2. The average growth rate

Experimental evidence that the microalgal cells respond to all light intensities within the gradient can be found in [54] who showed by means of fluorescence techniques that photosynthetic efficiency increased with decreasing light intensity and thus with culture depth.

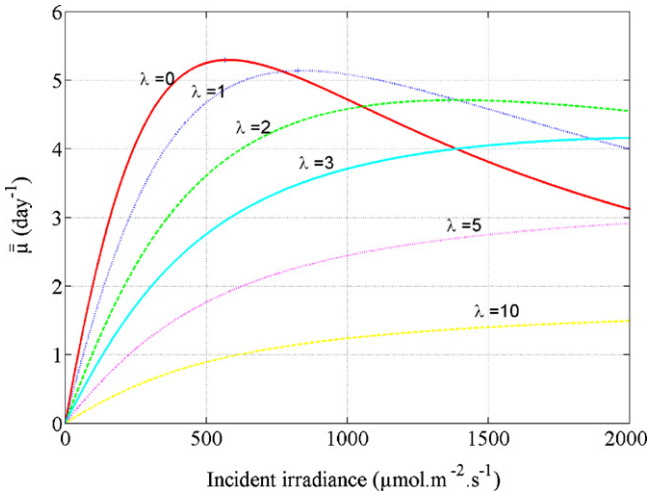

Fig. 4. Average growth rate computed in the MCP with respect to impinging light and optical depth λ.

Now, the average growth rate $\bar{\mu}(I(z))$ through the light gradient can be computed:

$$\bar{\bar{\mu}}(I_0) = \frac{1}{L}\int_0^L \bar{\mu}(I(z))\,dz \tag{18}$$

Property 2. *The average growth rate is*

$$\mu(I_0, q, \xi) = \bar{\bar{\mu}}(I_0, \xi)\left(1 - \frac{Q_0}{q}\right)$$

considering that $K_{il} < 2K_{sl}$ [1]:

$$\bar{\bar{\mu}}(I_0, \xi) = \tilde{\mu}\frac{2K_{il}}{\lambda\sqrt{\Delta}}\arctan\left(\frac{I_0(1 - e^{-\lambda})\sqrt{\Delta}}{2I_0^2 e^{-\lambda} + I_0(1 + e^{-\lambda})K_{il} + 2I_{\mathrm{opt}}^2(\theta)}\right) \tag{19}$$

where $\Delta = 4I_{\mathrm{opt}}^2(\theta) - K_{il}^2$. *Function* $\bar{\bar{\mu}}(I_0)$ *is an increasing function of* I_0 *up to an irradiance* $\tilde{I}_0 = I_{\mathrm{opt}}(\theta)e^{\lambda/2}$, *then it is decreasing* ($I_{\mathrm{opt}}(\theta)$ *is the irradiance providing maximal rate of photosynthesis, as given by* Eq. (8)).

Proof. see Appendix 1. □

Remark 2. Property 2 shows that a MCP with high biomass or large thickness will not show any inhibition behaviour. Indeed, the maximum of $\bar{\bar{\mu}}$ is reached at a value which is much higher than $I_{\mathrm{opt}}(\theta)$. Fig. 4 illustrates this, considering values of λ ranging from 0 (limit case where no shading effect occurs) to 10 (obtained when light is completely attenuated by a high biomass or a large reactor thickness). For example, λ = 3 corresponds to a MCP where 95% of the light is absorbed. Of course this does not mean that MCP do not photoinhibit, but it means that the photoinhibition effect is no more directly visible in the averaging process. However photoinhibition clearly induces a loss of productivity. As a key result, the behaviour of high density MCP can be approximated with a good accuracy with Monod type responses, keeping in mind that the Monod half saturation constant should be biomass related.

[1] An equivalent formulation can be obtained in the other case [15].

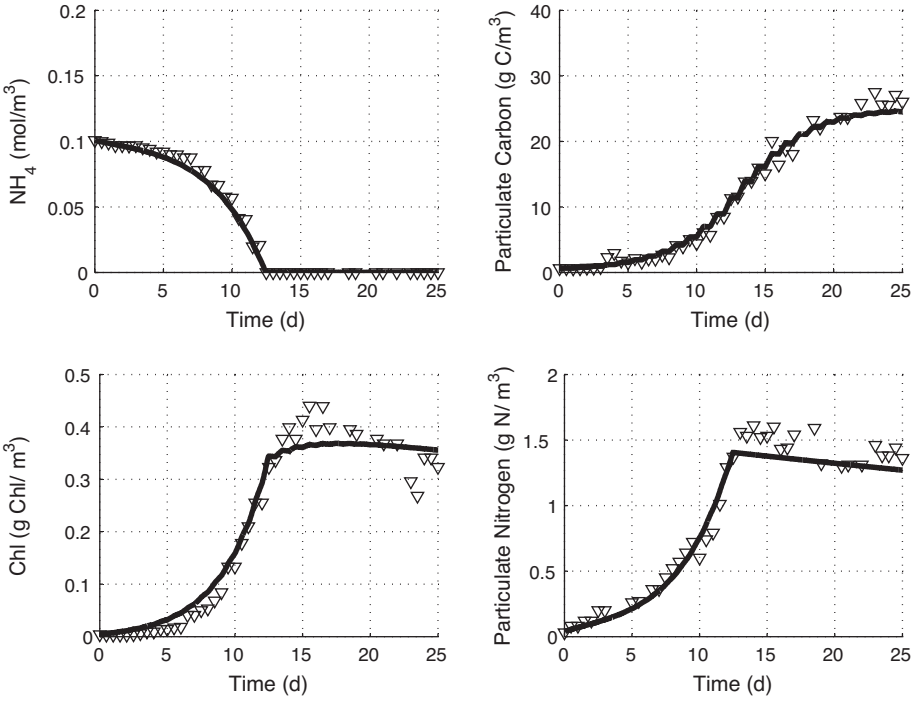

Fig. 5. Simulation of the MCP model and comparison with experimental data from [41].

5.3. The nitrogen limited MCP model

Synthesising the results of the previous sections, the resulting model in a light gradient field writes now, for an incident irradiance I_0:

$$\begin{cases} \dot{s} = Ds_{in} - \tilde{\rho}\dfrac{s}{s+K_s}\left(1-\dfrac{q}{Q_l}\right)x - Ds \\ \dot{q} = \tilde{\rho}\dfrac{s}{s+K_s}\left(1-\dfrac{q}{Q_l}\right) - \bar{\tilde{\mu}}(I_0, I^\star, x, q)(q-Q_0) \\ \dot{x} = \bar{\tilde{\mu}}(I_0, I^\star, x, q)(1-\dfrac{Q_0}{q})x - Dx - Rx \\ \dot{I}^\star = \bar{\tilde{\mu}}(I_0, I^\star, x, q)(1-\dfrac{Q_0}{q})(\bar{I}-I^\star) \end{cases} \tag{20}$$

where the irradiance, at which cells photoacclimate ($\bar{I}$), can be, depending on the species, computed on the basis of the average irradiance (see Eq. (17)).

5.4. Model validation

Model simulations are shown in Fig. 5 with *Isochrysis galbana* (parameter values are presented in Table 1). The good agreement of simulation results with the experimental data illustrates that the model calibration is rather straightforward, and demonstrates the ability of the model to properly reproduce such a data set. These results can be compared with those obtained by [102] that use both biological models of [45,81], where the light distribution was added as an extra layer in the model. The predictions are of comparable quality, while the presented model explicitly represents the coupling between microalgae physiology and light transfer properties on the MCP. The structure of the models in [45,81] makes the analysis and computation much more difficult.

Table 1
Parameter values used for the simulation of the MCP model.

Parameter	Value	Unit
$\tilde{\mu}$	1.7	day^{-1}
Q_0	0.050	$gN\,gC^{-1}$
Q_l	0.25	$gN\,gC^{-1}$
K^*_{sl}	1.4	$\mu mol\,m^{-2s-1}$
K_{il}	295	$\mu mol\,m^{-2}\,s^{-1}$
$\tilde{\rho}$	0.073	$gN\,gC^{-1}\,day^{-1}$
K_s	0.0012	$gN\,m^{-3}$
R	0.0081	day^{-1}
γ_{max}	0.57	$gChl\,gN^{-1}$
k_{I^*}	63	$\mu mol\,m^{-2}\,s^{-1}$
a	16.2	$m^2\,gChl^{-1}$
b	0.087	m^{-1}

6. Extensions

6.1. Dealing with cell synchronisation

Microalgae are photosynthetic microorganisms, and they have adapted their division, along the evolution processes, to the periodic light forcing. Indeed, when observing a population of microalgae under a diurnal light signal, it turns out that most of the cells are synchronised. This has two main consequences. First, they divide mainly at the same time period, leading to a strong increase (almost a doubling) in cell numbers within a small time interval. Second, the mitosis effect acts on the nitrogen acquisition [52], since nitrogen uptake stops during some specific phases of the cell cycle.

These aspects turn out to be crucial when algae are cultivated under natural illumination, and especially, when a nitrogen stress must be applied, as in the cases of biodiesel or *e.g.* astaxanthin [1] production. The response to the nitrogen stress can, thus, be very different depending on the cell position in its cycle. To cope with these aspects, and optimise these complex nonlinear

processes, modelling is required. A few models have been developed to represent the cell cycle [114,82], but none represents the cell cycle dynamics with a simple enough manner that allows straightforward calibration from experimental data. In [76], a model was derived from the Droop model, introducing the cell cycle and relating the transition from one cell phase to another with light or nitrogen content in the cell. Three main states are considered within the cell cycle: G1, G2 and M. The dynamics of each phase are represented by a Droop model. The transition rate from one state to another is assumed to depend on the nutrient status (from G1 to G2) or on the light dose (from G2 to M). The model was calibrated with experiments performed in various conditions of light and nitrogen limitation. The model turns out to accurately represent the cell cycle dynamics, and the carbon fluxes, however it introduces a significant degree of complexity.

6.2. Lipid and sugar modelling

Recently, a model has been proposed by [69] to represent the lipid production process by microalgae as a response to nitrogen limitation under continuous light, in the perspective of biodiesel production. In this model, intracellular carbon is divided between a functional pool and two storage pools (sugars and neutral lipids). The various intracellular carbon flows between these pools lead to complex dynamics with a strong discrepancy between accumulation and mobilisation of neutral lipids. This model has been designed such that the dynamics of the total biomass (including functional and storage carbon) is described by a Droop model, and therefore it inherits from the properties of the Droop model and from its fairly validation in transient conditions. The model has therefore a cascade structure, where the dynamics of lipid and functional quota depend on the Droop model variables. As a consequence, this model has a simpler structure than other models, which also represent carbon storage in the cell [95,80].

An interesting point is the ability of the model from [69] to reproduce the experimentally observed hysteresis in the dynamics of lipid accumulation. This hysteretic behaviour contributes to make the biolipid optimisation strategy complex. Model validation from experimental data is shown in Fig. 6. Other experimental works, considering simultaneous lipid production in periodic light conditions, have shown that the dynamics can become very complicated. Neutral lipids accumulate at a much lower rate than in continuous light: after nitrogen starvation, the produced lipids are consumed (probably through a respiration process) during the night, maintaining the lipid pool at a low level [59]. These observations result from the superposition of cell synchronisation and lipid accumulation hysteretic behaviour, and many works remain to be carried out in this direction.

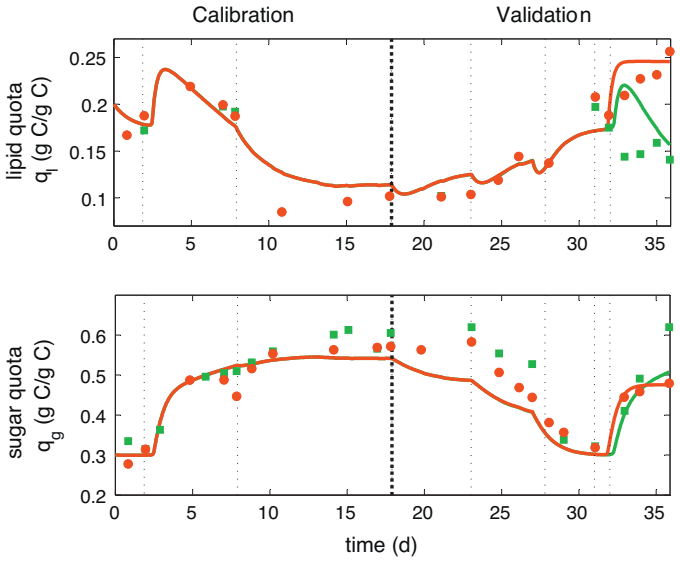

Fig. 6. Measurements and simulations of the neutral lipid (q_l) and sugar (q_g) quota as a response to a succession of nitrogen limitation rates. From [69].

6.3. Anaerobic digestion of microalgae

Anaerobic digestion is a biological process based on an anaerobic bacterial ecosystem, which converts an organic substrate into a biogas made of a mixture of CO_2 and CH_4. When applied to microalgae, this process not only recovers the energy stored in biomass (or in the biomass residual after lipid extraction), it also leads to ammonium and phosphate release, which can be source of nutrients for the microalgae culture [101,120]. Coupling microalgae culture and anaerobic digestion is, therefore, a promising process to recover energy into methane and recycle nitrogen and phosphorus. Nevertheless, the anaerobic digestion of microalgae faces several hurdles [101,78]:

- High ammonium concentration resulting from the high nitrogen content of microalgae can inhibit bacterial growth, especially for the methanogenic bacteria [57,22].
- For some species, the nature of the cell wall can lead to a low biodegradability.
- The potential toxicity of sodium (for marine microalgae digestion) can reduce the digester performance.

Because of the unstable character of anaerobic digestion and since it is a complex and nonintuitive process, a dynamic model is crucial for apprehending the process complexity and for identifying optimal working strategies.

Modelling of anaerobic digestion has been widely developed since the seventies [66], from simple models (*i.e.* considering one limiting reaction [47,29] or two reactions [14]) to more realistic representations (*e.g.* ADM1 [5] with 19 biochemical reactions). A modified version of the ADM1 model has been proposed in order to cope with microalgae digestion [71]. This model successfully fits a 140 day experiment of *Chlorella vulgaris* digestion [90]. A simpler 3 reaction model proposed by [70] also achieves a good fit with the same data. With a lower level of complexity, it can be mathematically tractable and suitable for optimisation and control.

7. MCP control and optimisation

7.1. Optimising MCP productivity

In order to produce biofuel or to mitigate CO_2, it is of key importance to maximize MCP surface productivity. Here, we focus on biomass productivity (*i.e.* CO_2 mitigation), and the problem is even more complex for lipid productivity. In this paragraph, we give some ideas on MCP optimisation, in the simple case of constant impinging light and assuming a simplified light attenuation model with a light attenuation rate linear with respect to biomass x. With this approximation, the optical depth λ depends on the biomass per surface unit $X = xL$.

Our aim here is to provide some ideas on how to compute and optimise biomass surface productivity at steady state. From Eq. (19), $\bar{\tilde{\mu}}$ is a function of X. Hence productivity turns out to depend on q and X as follows:

$$P(I_0, q, X) = (\mu(I_0, q, X) - r)X \tag{21}$$

At equilibrium, productivity is the product between dilution rate ($D = \mu(I_0, q, X) - r$) and surface biomass.

Remark 3. Productivity is a function of the nitrogen/carbon quota q and surface biomass. According to this model, a thin culture (L small) with high biomass concentration x is equivalent to a deep culture (L high) with low biomass concentration x, if they have the same surface biomass X.

The reactor can be seen as a solar panel whose main parameter is X, associated to an energy yield. A low X (low biomass and/or thin culture) indicates that only a small fraction of light is absorbed by the culture: the panel has a low energy yield. A high X indicates that, at the bottom of the culture, there is almost no remaining light and only respiration losses occur. Thus, there exists an optimal value for the biomass per surface unit X in order to maximize the panel's efficiency P.

Since we consider the case of high density PBR or raceways (*i.e.* $\lambda > 3$), we can reasonably assume that the average response to light can be deduced from a simplified Monod model: $\bar{\mu}(I) = \tilde{\mu}I/(I + K_I)$. Indeed, this results from Remark 2, which shows that, for high optical depth, a MCP has a Monod type response (see also Fig. 4).

The following theorem [74] has been established when photoacclimation is neglected, *i.e.* when $\gamma(I^\star)$ is assumed to be constant.

Theorem 1. *For given constant I_0 and q, the optimal surface biomass X for maximizing the productivity (21) is such that the growth rate at depth L is equal to the respiration rate. This optimal surface biomass concentration can, thus, be computed:*

$$X_{opt}(q) = \frac{1}{a\gamma q} \ln\left(\frac{I_0}{rK_I}\left(\bar{\mu}\left(1 - \frac{Q_0}{q}\right) - r\right)\right) \tag{22}$$

Proof. See [74]. □

In other words, it means that the remaining light at the bottom of the MCP should be the compensation light [55], *i.e.* the light, for which respiration compensates growth.

A similar result was also demonstrated by [25,111] using other approaches in the case when only photolimitation is considered.

We are then left with the choice of an optimal nitrogen/carbon value q, which can be controlled by adjusting D and s_{in}. High quota values lead to a high potential growth rate $\bar{\mu}(q)$, but also to higher light attenuation, so that an optimal intermediate value of the quota exists, and is unique in certain conditions (see [74]).

7.2. Optimising MCP productivity for a periodic light

So far, theoretical studies optimising biomass productivity (*i.e.* CO_2 fixation rate) in fluctuating light are still rare. In [2], the authors optimise productivity on a daily basis, while, in [20] high frequency fluctuations resulting from algae transportation through light gradient are considered. This objective is made very challenging by two aspects which have been, so far, neglected. Firstly, the microalgal photoacclimation to a fluctuating light intensity should be better understood and taken into account in order to optimise the process. Secondly, the light periodicity induces an additional mathematical complexity: optimisation of nonlinear dynamical systems of dimension higher than two is a tricky problem, especially if it is non autonomous. Moreover, as it was already discussed, periodic light generates population synchronisation, which makes the system response more complex. A key challenge for the coming years will clearly consist in better understanding and modelling the effects of light variation on population synchronisation. The next stage consists in optimising biofuel production under periodic light. It is therefore a difficult challenge from a mathematical point of view.

7.3. MCP monitoring and control

There are a few studies aiming at designing observers to predict non measured variables. In [16], a high gain observer is developed in order to estimate both internal quota and remaining nutrients. In [46], an interval observer provides these estimates with a confidence interval, taking into account the discrete nature of the measurements. Other authors use inorganic carbon [6] or oxygen production [107] to estimate microalgal production.

The studies aiming at on-line controlling microalgal cultures are rare, even if the first work dealing with computer driven on-line control of a MCP was carried out in the 1980s [7,49], where a static criterion was on-line optimised through PID controllers.

Turbidostats (using a turbidity regulating algorithm) are often used to grow microalgae [96,73], and pH is generally regulated through CO_2 injection [10,17], on the basis of standard linear algorithms [96]. In [44], a predictive controller is developed in order to reduce the fraction of CO_2, which is lost by the MCP. The higher complexity induced by a nonlinear Droop-like model is probably the main reason why this challenging problem has not been further considered, and why nonlinear controllers are still scarce [68,53].

8. Challenges

8.1. Improving photoinhibition and photoacclimation modelling in dynamical regimes

Photoacclimation is a mechanism which adapts the photon harvesting system to light intensity by modifying the efficiency and capacity of the light reactions (light absorption and photosynthetic electron transport). Photoinhibition results from irreversible damage (termed photodamage) mainly caused by an excess of photons to a key protein involved in the photosystem II reaction centres in the chloroplasts [36]. Photoinhibition and photoacclimation are tightly coupled and have motivated the design of specific dynamic models [33,34,121,72,19]. However, depending on the hydrodynamical regime, a cell can have a significantly different perception of the light signal [65,85,94,88]. The average light received, together with the frequency of commutation between reactor dark zone and high light can highly differ for reactors and hydrodynamic regimes. Fig. 7 extracted from [85] presents the estimated light intensity seen by a cell in a tubular PBR. The way microalgae respond to these variable light regimes (flashing effect) and the resulting photodamages and pigment adaptation is still partially known and is probably strongly species dependent. How the independent history of each cell impacts the global working of the MCP is a difficult, yet crucial question. Another difficulty is that considering photoinhibition involves three very different time scales ranging from milliseconds (light reaction), minutes (photodamage) to days (growth and acclimation), which should be included within the same model, as in [35], where a coupled Han-Geider model is embedded in a Large Eddy Simulation framework. The fastest time scale can be managed by singular perturbation arguments [91,20], when considering some typical periodic forcing [117]. But when coupled with a hydrodynamic model that represents strong light variation at the scale of the cell (down to 10 ms in PBR [85]), these mechanisms may play a significant role and the model becomes more tricky to simplify. Managing these very different time scales is very challenging, and the difficulty can begin with numerical issues when computing several weeks at a time-step of the millisecond. Better predicting the productivity for such a population submitted to high frequency variations of light is a key issue to improve MCP optimisation.

8.2. Metabolic modelling

The models discussed so far describe cell behaviour at a general macroscopic level and do not take into account more refined knowledge of the fluxes of carbon in the cell and the fate of this carbon in the cell. The current working comprehension of the intricate

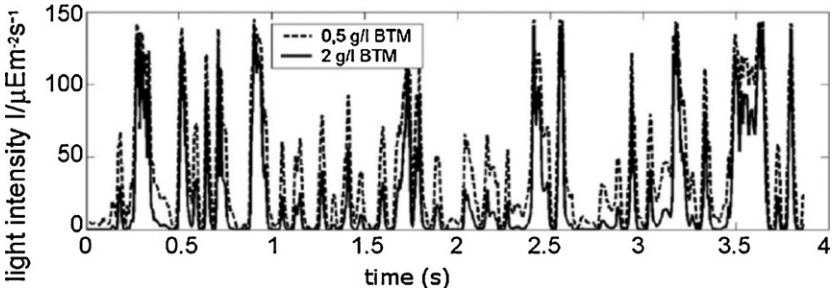

Fig. 7. Numerical evaluation of the light intensity seen by a cell in a tubular PBR for two different biomass concentrations (dashed: $0.5\,g\,l^{-1}$ and plain: $2\,g\,l^{-1}$). Figure reproduced from [85].

mechanisms involved in the metabolic carbon flux from CO_2 to protein, carbohydrate and lipid is limiting efficient applications at a massive scale. There exist a few metabolic models [118,21,24] (see Fig. 8 for an illustration), but they do not include all the microalgal metabolic pathways. For example, the details of the lipid pathway are not provided. But the strong bottleneck with such an approach is that they are generally limited to balanced growth conditions. The natural solar light/dark cycles maintaining a forcing signal on the cell cycle makes the notion of balanced growth not relevant. Indeed, we have seen that microalgae can rarely be considered in the steady situation of balanced growth, while cells experiment permanent accumulation and reuse of energy, carbon, nitrogen, etc. Moreover, during specific phases of the cell cycle some metabolic functions are modified (*i.e.* nitrogen uptake during mitosis [52]). When cells are synchronised, some metabolism kinetics should, therefore, be related to the cell cycle. It is, thus, crucial to develop metabolic models, which are valid even in unsteady growth conditions and which can take into account the position of the cell in its cycle. The model proposed by [39] is a first step to deal with nonsteady conditions.

Considering metabolic models valid for dynamic conditions is even more capital when dealing with transient lipid synthesis induction after a nitrogen limitation. This is not a standard framework since the balanced growth is the underlying hypothesis [105]. The natural dynamical aspect of the microalgal population will be the main challenge that will have to be tackled.

9. Conclusion

Microalgae are microorganisms which have, so far, hardly been exploited regarding their huge potential. Indeed, there is a wide diversity of innovative applications ranging from pigments, antioxidants, vitamins, proteins, cosmetics, fish food, to CO_2 capture and bioenergy [89,103]. However, such photosynthetic organisms are more difficult to domesticate and manage than bacteria, yeasts or fungi. Firstly, they have a strong aptitude to store nutrients, which motivates the use of quota models (typically the Droop model) that are more complex than the classical Monod model. Secondly, their pigments attenuate light, which is their source of energy and this generates a strong coupling between biology (microalgae growth)

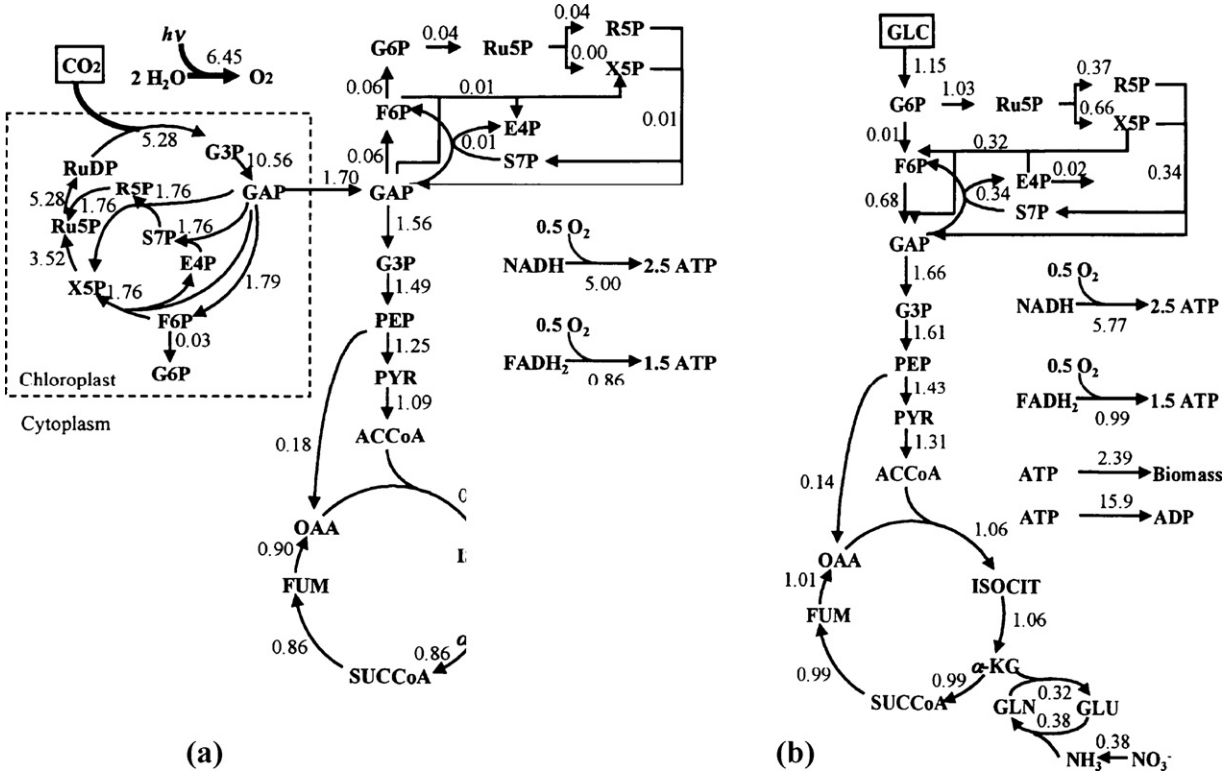

Fig. 8. Metabolic flux distribution of *Chlorella pyrenoidosa* cells in (a) autotrophic and (b) heterotrophic cultures. The flux values are expressed in mmol $g^{-1}\,h^{-1}$. These values represent the flux distributions of the exponential growth phase during the first dark period in a cyclic autotrophic/heterotrophic cultivation. Figure from [118].

and physics (radiative transfer properties and hydrodynamics). Microalgae adapt their pigments to light intensity, which makes the behaviour of a photobioreactor or a raceway difficult to understand and forecast without modelling. When growing under solar light, cell division synchronises and most of the cells divide at the same time, with consequences on the elemental acquisition at the population level. Finally, such organisms are most of the time far from the classical hypotheses (namely balanced growth) required to apply classical results in metabolic engineering.

Some models exist, which can describe separately some of these processes, but there is a clear incentive to develop new predictive models, which could realistically predict the behaviour of a MCP, especially in the framework of bioenergy production from solar energy. Such models will support process monitoring and optimisation and help the development of these new, promising technologies. They will also help to more realistically quantify the reachable productivities, depending on species, type of culture process, period of the year and location and, therefore, calibrate the corresponding investments. They will also contribute to improve the environmental impact assessment [61] by better quantifying the balance between the requested energy to maintain the algae in suspension and inject CO_2, and the recovered energy through biofuel.

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Appendix 1.

Proof or Property 2. This results from straightforward computation based on the fact that, for a planar geometry:

$$\bar{\tilde{\mu}}^b(I_0) = \frac{1}{L}\int_0^L \tilde{\mu}\,\frac{K_{il}^b I_0 e^{-\xi z}}{K_{il}^b I_0 e^{-\xi z} + I_{\text{opt}}^2(\theta) + I_0^2 e^{-2\xi z}}\,dz \tag{23}$$

with the variable change $v = I_0 e^{-\xi z}$, we get $dv = -\xi v\,dz$, and

$$\begin{aligned}\bar{\tilde{\mu}}^b(I_0) &= -\frac{\tilde{\mu}K_{il}^b}{\lambda}\int_{I_0}^{I_0 e^{-\lambda}} \frac{1}{v^2 + K_{il}^b v + I_{\text{opt}}^2(\theta)}\,dv \\ &= -\frac{2\tilde{\mu}K_{il}^b}{\lambda\sqrt{\Delta}}\left[\arctan\left(\frac{2v + K_{il}^b}{\sqrt{\Delta}}\right)\right]_{I_0}^{I_0 e^{-\lambda}} \quad \square\end{aligned} \tag{24}$$

□

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