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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 seen as a potential major biofuel producer in the future since, under conditions of nitrogen deprivation, they can contain high amounts of lipids, while they consume CO_2 from power plants. 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 Droop Model which has been widely used to predict microalgal behaviour under nutrient limitation. It details a model for photobioreactors or raceways, when both light and nutrients are limiting. The challenges and hurdles to improve photobioreactor modelling and control in order to optimise biomass or biofuel production are then discussed.

Keywords: microalgae, photobioreactors, raceways, modelling, optimization, biofuel, CO_2 mitigation

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 tons per hectare and per year (Chisti, 2007). After a nitrogen starvation, this biomass can reach a very high lipid content (up to 80% of dry weight under certain stress conditions (Metting, 1996)). These possibilities have led some authors to consider that microalgae could be one of the main biofuel sources in the future (Huntley and Redalje, 2007; Chisti, 2007).

In addition, the ability of microalgae to fix CO_2 in a controlled way has recently involved them in the race for mitigation systems (Benemann, 1997; Olaizola, 2003). Microalgal biofuel production systems could also contribute to mitigate CO_2 from industrial power plants. In the same spirit, microalgae could be used to consume inorganic nitrogen and phosphorus, and thus limit expensive wastewater post-treatment.

These advantages put microalgae in a good position for renewable energy production at large scale (Chisti, 2007). This explains the explosion of publications on this topic, and the optimistic speech of start-ups which foresee, in the near future, industrial production of microalgal biofuel. However, microalgae were so far rarely used for biotechnological applications. To date, the main domains of application are focused on innovative processes to produce vitamins, proteins, cosmetics, and health foods (Pulz and Gross, 2004; Spolaore et al., 2006). Microalgae are thus still cultivated at small scale: the total worldwide microalgal production is in the range of 10 000 tons of dry biomass per year. In the perspective of large scale microalgal cul-

tivation, new techniques both from biotechnology and from the control field must be deployed to ensure optimisation of these new processes. Indeed, microalgae have some specificities compared to more current microorganisms, such as bacteria or yeasts. The main difference, when 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 influent photons are absorbed. But this limit is not straightforward since cells adapt their pigments to the influent light to optimize light harvesting, and therefore the light attenuation coefficient (which is deduced from pigment concentration) is light dependant. Moreover, in conditions of nitrogen starvation, which increase the lipid content, the pigment composition and concentration decrease (Turpin, 1991; Sciandra et al., 1997; Geider et al., 1998), leading to a reduced light attenuation coefficient (Stramski et al., 2002). When targeting outdoor cultivation of microalgae (in photobioreactors (PBR) or high rate ponds (raceways)), these organisms grow in permanent unsteady conditions since they are submitted to daily light (and temperatures) variations. It results that 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 unstationnary behaviour together with a strong feedback from the population level to the cell level through light attenuation make these processes more challenging.

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

In this paper we first present some microalgal models, based on the classical Droop model. We analyse the ability of microalgae to adapt to a given light intensity, and present photoadaptation 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 photobioreactor optimisation and control are then presented. Finally the challenges for optimizing such complex processes are synthesised.

2. DROOP MODEL: BASICS OF MICROALGAL GROWTH MODELLING

Microalgae are known for their ability to uncouple uptake of nutrients (inorganic nitrogen, phosphorus, vitamins, ...) with growth. A classical growth curve is presented in Figure 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. Droop

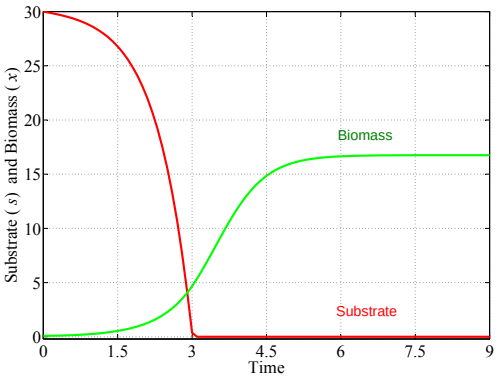

Figure 1. Typical growth curve for microalgae: biomass continues to grow a few days after nutrient exhaustion.

model, initially established to represent the effect of B_{12} Vitamin internal quota on the growth rate of phytoplankton (Droop, 1968), has been shown appropriate to represent also the effect of macronutrients, such as nitrogen or phosphorus on growth rate (Droop, 1983). Growth of the biomass (denoted 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). 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:

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

In this model the absorption rate $\rho(s)$ is represented by a Michaelis-Menten kinetics (Burmaster, 1979):

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

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:

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

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. This model is more accurate than Monod model for algal growth modelling (Vatcheva et al., 2006). Droop model is however sufficiently simple to allow a detailed mathematical analysis (Lange and Oyarzun, 1992; Bernard and Gouzé, 1995, 2002).

Property 1. Droop model guarantees that the internal quota stays between two bounds:

$$Q_0 \leq q \leq Q_m \quad (4)$$

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 :

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

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

Proof: See *e.g.* (Bernard and Gouzé, 1995).

Droop model has been widely validated (Droop, 1983; Sciandra and Ramani, 1994; Bernard and Gouzé, 1999; Vatcheva et al., 2006). This model, despite its simplicity turned out to accurately reproduce dynamics of microalgae evolving in a constant environment.

However, Droop model cannot be used in the case of high density cultures and must be modified in order to:

- Include the effect of irradiance on microalgal growth
- Account for the light gradient due to light absorption by the microalgal biomass
- Represent the modification of light attenuation due to pigment adaptation

3. MICROALGAL MODELS DEALING WITH LIGHT LIMITATION

3.1 Photoadaptation models

In the past decade, several models have proposed to account for the response of microalgal pigment density to both light intensity and available nutriments. The most difficult case is when nitrogen is limiting growth, since nitrogen strongly interferes with pigment synthesis. Geider et al. (1998) 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, but they have been, so far, less used (Pahlow, 2005; Faugeras et al., 2004). More complex models have also been developed (Zonneveld, 1998; Flynn, 1991), 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 adaptation mechanisms allows the algae to adapt pigment (and especially chlorophyll) synthesis to light intensity. Figure 2 represents experimental data extracted from Anning et al. (2000), where the CO_2 uptake rate appears as a function of light for two different microalgal cultures which were grown at two different light intensities at which they photoadapted. It is worth noting that, when photosynthesis is normalised by Chl, the initial slope of the response curve is independent of the photoacclimation light (MacIntyre et al., 2002). This fact supported the development of kinetics models where the growth rate was a function of both light and the ratio $\theta = \frac{\text{Chl}}{x}$. Figure 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

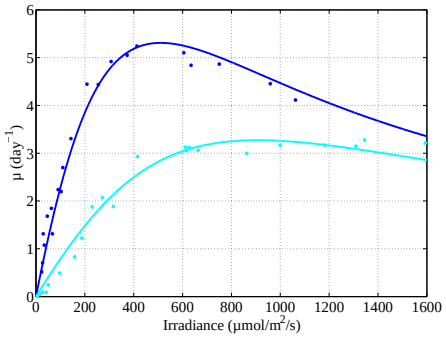

Figure 2. Model and data of the photosynthetic response of the diatom *Skeletenonema costatum* photoadapted at low ($I_L = 50\mu mol.m^{-2}.s^{-1}$, dark points and lines) and at high irradiance ($I_H = 1200\mu mol.m^{-2}.s^{-1}$, light grey points and lines). Data from Anning et al. (2000).

light effect consists in including light in parameter $\bar{\mu} = \bar{\mu}(I)$ (Han, 2001). We consider the kinetics model of Eilers and Peeters (1988, 1993) to represent the typical curves of Figure 2 :

$$\bar{\mu}(I) = \tilde{\mu}\frac{I}{I + K_{sI} + \frac{I^2}{K_{iI}}} \tag{7}$$

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 photoadaptation mechanisms in Equation (7), parameter K_{sI} is computed from θ as follows:

$$K_{sI} = K^*_{sI}/\theta \tag{8}$$

With this expression, the initial slope of the inorganic carbon uptake rate normalized by chlorophyll is constant and yields $\frac{\tilde{\mu}}{K^*_{sI}}$.

3.2 Modelling pigment evolution

The chlorophyll concentration must be represented in the model in order 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 (Laws and Bannister, 1980). More specifically, for a culture photoacclimated at an irradiance $I^\star$, we have Bernard et al. (Submitted):

$$\text{Chl} = \gamma(\text{I}^\star)\text{xq} \tag{9}$$

where

$$\gamma(I^\star) = \gamma_{max}\frac{k_{I^*}}{I^\star + k_{I^*}} \tag{10}$$

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

One of the key originalities of the model proposed by Bernard et al. (Submitted) 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 cultures. To represent this light adaptation dynamics, we use the following formulation:

$$\dot{I}^\star = \delta\mu(q, I)(\bar{I} - I^\star) \tag{11}$$

where $\bar{I}$ is the average irradiance, and δ is the photoadaptation 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 hydrodynamical regime, the cell successively perceives 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 (Yoshimoto et al., 2005; Pruvost et al., 2006; Perner-Nochta and Posten, 2007; Rosello Sastre et al., 2007).

3.3 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. Indeed, with Droop model and a constant maximum uptake rate, equation (5) becomes:

$$Q_m(I) = Q_0 + \frac{\rho_m}{\bar{\mu}(I)} \tag{12}$$

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, the expression proposed by Lehman et al. (1975) is used. It stops uptake rate as cells become nutrient replete:

$$\rho(s,q) = \bar{\rho}\frac{s}{s+K_s}(1-q/Q_l) \qquad (13)$$

with $Q_l > Q_0$.

3.4 Respiration

The last phenomenon which must be embedded in a PBR model is the respiration process. Indeed, respiration is hidden in the "net" growth rate of Droop model. However, for high density cultures where a fraction of the culture is in the dark, respiration must be considered. Indeed, the domains where light is so small that growth rate is lower than 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 cell activity, and thus to the growth rate. A simple way of including it in a model consists in assuming that the proportional respiration is included in the "'net growth rate". Note that, in most of the models (Geider et al., 1998; Pahlow, 2005), nitrogen is assumed to be released at the same rate as carbon, which also means that respiration terms in the models also accounts for cell mortality.

4. DEALING WITH LIGHT GRADIENT

4.1 Average light

We investigate here a simple representation of light attenuation inside a PBR (or a raceway) of thickness L, due to high biomass. We still assume that all the concentrations are homogeneous and that only light has a spatial distribution. For sake of simplicity, we consider a planar geometry with illumination perpendicular to the plane, so that irradiance distribution in the PBR can be represented with a good accuracy by a Beer-Lambert exponential decrease with a rate linearly related to chlorophyll concentration. When I_0 is the irradiance

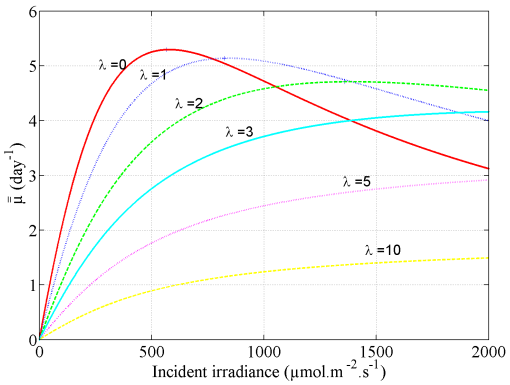

Figure 3. Average growth rate computed in the PBR with respect to influent light and optical depth λ.

at the surface, we have thus, for a PBR where cells are photoacclimated at light $I^\star$:

$$I(z) = I_0 e^{-\xi z} \qquad (14)$$

where ξ is the light attenuation rate $\xi = a\text{Chl} + \text{b} = \text{a}\gamma(\text{I}^\star)\text{qx} + \text{b}$.

This light attenuation coefficient is used to compute the optical depth $\lambda = \xi L$, defined as follows:

$$\frac{I(L)}{I_0} = e^{-\lambda} \qquad (15)$$

This key parameter reflects how efficiently light energy is absorbed.

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

$$\bar{I} = \frac{I_0}{L}\int_0^L e^{-\xi z}dz = \frac{I_0}{\lambda}[1-e^{-\lambda}] \qquad (16)$$

Note that this approximation could be improved by using more accurate models of the radiative transfer that would take the detailed pigment composition into account. Indeed, several light transfer models in the culture medium exist and can be used (Franco-Lara et al., 2006; Pottier et al., 2005; Suh and Lee, 2003; Pottier et al., 2005; Pruvost et al., 2006) provided that the pigment concentration and composition are known at any time.

4.2 Average growth rate

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

$$\bar{\bar{\mu}}(I_0) = \frac{1}{L}\int_0^L \bar{\mu}(I(z))dz \qquad (17)$$

Property 2. The average growth rate is

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

considering that $K_{iI} < 2K_{sI}$:

$$\bar{\bar{\mu}}(I_0, \xi) = \tilde{\mu}\frac{2K_{iI}}{\lambda\sqrt{\Delta}} \arctan\left(\frac{I_0(1-e^{-\lambda})\sqrt{\Delta}}{2I_0^2 e^{-\lambda} + I_0(1+e^{-\lambda})K_{iI} + 2I_{\text{opt}}^2(\theta)}\right) \qquad (18)$$

where $\Delta = 4I_{\text{opt}}^2(\theta) - K_{iI}^2$ The function $\bar{\bar{\mu}}(I_0)$ is an increasing function of I_0 up to an irradiance $\tilde{I}_0 = I_{\text{opt}}(\theta)e^{\lambda/2}$, and is then decreasing after ($I_{\text{opt}}(\theta)$ is the irradiance providing maximal rate of photosynthesis, as given by equation (7)).

Proof: see Bernard et al. (Submitted).

Remark 1: Property 2 shows that a PBR with high biomass or large thickness won't show any inhibition behaviour. Indeed, the maximum of $\bar{\bar{\mu}}$ is reached at a value which is much higher than $I_{\text{opt}}(\theta)$. Figure 3 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 $\lambda = 3$ corresponds to a PBR where 95% of the light is absorbed. Of course this does not mean that photobioreactors do not photoinhibit, but it means that photoinhibition effect disappears in the averaging process. However photoinhibition clearly induces a loss of productivity. As a key result, the behaviour of high density PBR can be approximated with a good accuracy with Monod type responses.

4.3 Model validation

Model simulations from Bernard et al. (Submitted) are shown on Figure 4 with *Isochrysis galbana*. The good adequation obtained with the experimental data illustrates the facts that the model calibration is rather straightforward, and demonstrates the ability of the model to properly reproduce such data set. These results can be compared with those obtained by Smith and Yamanaka (2007) that use both biological models of Geider et al. (1998) and of Pahlow (2005), where the light distribution was added as an extra layer in the model. The predictions are of comparable quality, while the presented

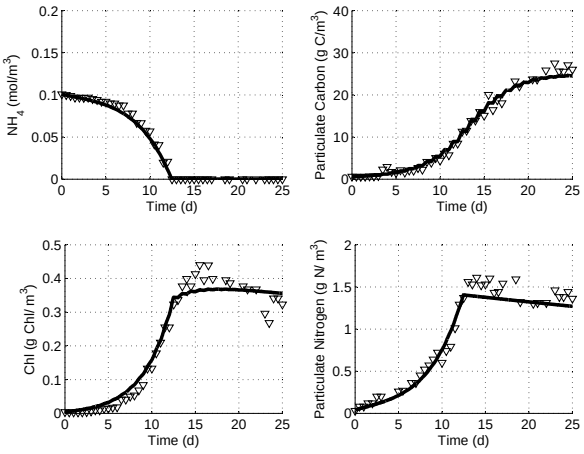

Figure 4. Simulation of the PBR model and comparison with experimental data from Flynn et al. (1994).

model explicitly represents the coupling between microalgae physiology and light transfer properties on the PBR. The structure of models of Geider et al. (1998) and Pahlow (2005) make the analysis and computation much more difficult.

5. EXTENSIONS

5.1 Dealing with cell synchronisation

Microalgae are photosynthetic microorganisms, and they have adapted, along the evolution processes, their division 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 synchronized. 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 (Hildebrand and Dahlin, 2000), since nitrogen uptake stops during some specific phases of the cell cycle (Mocquet et al., 2010).

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 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 optimize these complex nonlinear processes, modelling is required. A few models have been developed to represent the cell cycle (Vaulot and Chisholm, 1987; Pascual and Caswell, 1997), but none represent the cell cycle dynamics with a simple enough manner that allows straightforward calibration from experimental data. In Mocquet et al. (2010), a model was derived from 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.

5.2 Lipid and sugar modelling

Recently, a model has been proposed by Mairet et al. (Submitted) 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 mobilization of neutral lipids. An interesting point is the ability of the model to generate an hysteresis in the dynamics of lipid accumulation. This hysteretic behaviour was observed experimentally, and contributes to make the biolipid optimisation strategy complex. Model validation from experimental data is shown in Figure 5. Other experimental works, considering simultaneously 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 respirated) during the night, maintaining the lipid pool at a low level. 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.

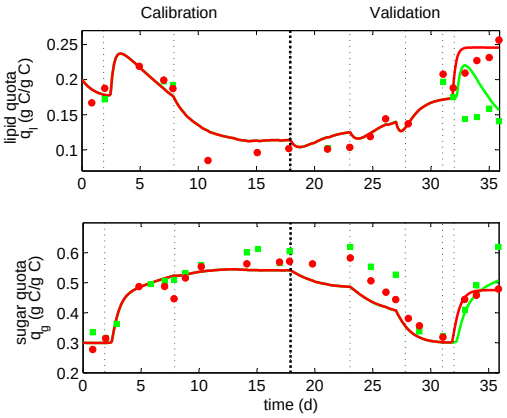

Figure 5. Measurements and simulations of the neutral lipid (q_l) and sugar (q_g) quota as a response to a succession of nitrogen limitation rates. From Mairet et al. (Submitted).

6. PBR CONTROL AND OPTIMIZATION

6.1 Optimizing PBR productivity

In order to produce biofuel or to mitigate CO_2, it is of key importance to maximize PBR 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 PBR optimisation, in the simple case of constant influent light and assuming a simplified light attenuation model with a light attenuation rate linear with respect to biomass. 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 optimize biomass surface productivity at steady state. From Equation (18), $\bar{\bar{\mu}}$ is a function of X, so that productivity turns out to depend on q and X as follows:

$$P(I_0, q, X) = (\mu(I_0, q, X) - r)X \qquad (19)$$

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

Remark 2: 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: $\bar{\mu}(I) = \tilde{\mu}\frac{I}{I+K_I}$. Indeed, this results from Remark 1, which shows that, for high optical depth, a PBR has a Monod type response (see also Figure 3).

The following theorem (Masci et al., 2010) has been established when photoadaptation 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 productivity (19) is such that growth rate at depth L is equal to the respiration rate:

$$\mu^L(q, I(q, X_{opt})) = r \tag{20}$$

This optimal surface biomass concentration can thus be computed and is equal to

$$X_{opt}(q) = \frac{1}{a\gamma q} \ln\left(\frac{I_0}{K_I}\left(\frac{\bar{\mu}(1-\frac{Q_0}{q})}{r} - 1\right)\right) \tag{21}$$

Proof: See Masci et al. (2010).

A similar result was also demonstrated by Cornet and Dussap (2009); Takache et al. (2009) using other approaches.

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 high potential growth rate $\bar{\mu}(q)$, but also to higher light attenuation, so that an optimal intermediate value exists, and is unique in certain conditions (see Masci et al. (2010)).

6.2 Optimizing PBR productivity for a periodic light

So far, theoretical studies optimizing biomass productivity (*i.e.* CO_2 fixation rate) in fluctuating light are still rare (Akhmetzhanov et al., 2010). This objective is made very challenging by two aspects which have been, so far, neglected. First, the microalgal photoadaptation to a fluctuating light intensity should be better understood and taken into account in order to optimize the process. Second, 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 synchronization, 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 optimizing biofuel production under periodic light. It is therefore a difficult challenge from a mathematical point of view.

6.3 PBR monitoring and control

There are a few studies aiming at designing observers to predict non measured variables. In Bernard et al. (1998), a high gain observer is developed in order to estimate both internal quota and remaining nutrients. In Goffaux et al. (2009), an interval observer provides these estimates with a confidence interval, taking into account the discrete nature of the measurements. Other authors use inorganic carbon (Becerra-Celis et al., 2008) or oxygen production (Su et al., 2003) to estimate microalgal production.

The studies aiming at controlling microalgal cultures are rare. Turbidostats (using a turbidity regulating algorithm) are often used to grow microalgae (Sandnes et al., 2006; Masci et al., 2008), and pH is generally regulated through CO_2 injection (Berenguel et al., 2004; Buehner et al., 2009), on the basis of standard linear algorithms (Sandnes et al., 2006). The higher

complexity induced by a Droop-like model is probably the main reason why this challenging problem has not been further considered, and why nonlinear controllers are scarce (Mailleret et al., 2005).

7. CHALLENGES

7.1 Improving photoadaptation modelling in dynamical regimes

Photoadaptation is a key phenomenon through which the algae adapts its photon harvesting system to light intensity. However, depending on the hydrodynamical regime, a cell can have a significantly different perception of light signal (Luo and Al-Dahhan, 2004; Rosello Sastre et al., 2007; Pruvost et al., 2006). The average light received, together with the frequency of commutation between reactor dark zone and high light can significantly differ from reactor and hydrodynamical regimes. The way microalgae respond to these variable light regimes (flashing effect) and the resulting pigment adaptation is still partially known and is probably strongly species dependent. Better predicting the productivity for such a population submitted to high frequency variations of light is a key issue to improve photobioreactor optimisation.

7.2 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 mechanisms involved in the metabolic Carbon flux from CO_2 to protein, carbohydrate and lipid is limiting efficient applications at a massive scale. There exists a few metabolic models (Yang et al., 2000; Chang et al., 2007; Cogne et al., 2003), but they don't 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 experience permanent accumulation and reuse of energy, carbon, nitrogen, ... It is therefore crucial to develop metabolic models which are valid even in unsteady growth 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 (Stephanopoulos, 2002). The natural dynamical aspect of the microalgal population will be the main challenge that will have to be tackled.

8. 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 (Pulz and Gross, 2004; Spolaore et al., 2006). However, such photosynthetic organisms are more difficult to manage and use than bacteria, yeasts or fungus. First they have a strong aptitude to store nutrients, which induces the use of quota models (typically Droop model) which are more complex than the classical Monod model. Second, their pigments attenuate the light, which is their source of energy and this generates a strong coupling between biology (microalgae growth) 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 with 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 can realistically predict the behaviour of photobioreactor or a raceway, especially in the framework of bioenergy production from solar energy. Such models will sup-

port process monitoring and optimisation and help the development of these new, promising technologies. They may also help to more realistically quantify the possible productivities, and improve the assessment (Lardon et al., 2009) of 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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