A photobioreactor model in nitrogen limited conditions

Olivier Bernard[1], Pierre Masci[1], Antoine Sciandra[2]
[1]COMORE-INRIA, BP93, 06902 Sophia-Antipolis Cedex, France
[2]LOV, UMR 7093, Station Zoologique, B.P. 28 06234, Villefranche-sur-mer, France

Corresponding author: Olivier Bernard
BP93, 06902 Sophia-Antipolis, France
Email: olivier.bernard@inria.fr

Abstract. We propose a new photobioreactor model that deals both with nitrogen limitation and light attenuation. On the basis of the Droop model we include the light effect, and then we relate the nitrogen status to the chlorophyll content, for a given photoadaptation light. In a second step, we compute the light distribution thanks to a Beer-Lambert model. It results in a model where biology (growth in nitrogen limited conditions) and physics (radiative transfer) are deeply coupled. The model is validated with experimental data of *Isochrysis galbana*.

Microalgae and cyanobacteria use light as energy source to fix carbon dioxide (CO_2). These microorganisms (abusively gathered under the name "microalgae") have recently received a specific attention in the framework of renewable energy. Their high actual photosynthetic yield compared to terrestrial plants (whose growth is limited by CO_2 availability) lead to large potential algal biomass productions of several tens of tons per hectare and per year. After a nitrogen limitation, this biomass can reach a very high lipid content (up to 80% of dry weight under some stress conditions [17]. These possibilities have led some authors to consider that microalgae could be one of the main biofuel producers in the future [14, 7].

On top of this, the ability of microalgae to fix CO_2 in a controlled way has recently involved them in the race for mitigation systems [1, 18]. Thus microalgal biofuel production systems could be associated to industrial powerplants with a high CO_2 production. In the same spirit, microalgae could be used to consume inorganic nitrogen and phosphorus, and thus avoid expensive wastewater treatment plants.

These advantages put microalgae in a good position for renewable energy production at large scale [7]. This means that in the coming years there might be large scale industrial plants to produce microalgae. However, the culture of algae is not straightforward and suffers from many limitations [22, 6]. First, high biomass concentration leads to an increase in light absorption. A trade-off between high density cultures (where light is attenuated after a few centimetres) and a diluted culture where light is not completely absorbed must be achieved. Moreover, CO_2 must be supplied in a concentration that is optimal for biomass synthesis, without dropping the pH to deleterious levels. Finally, the production of oxygen and its storage in the medium may be highly inhibitory, and a low concentration should be maintained to achieve high productivities. Microalgae production is thus a complex process that should be strongly monitored and controlled to manage these tradoffs and guarantee an optimal working mode. Several photobioreactor models have been proposed, especially to deal with light transfer property in the culture medium [26, 20, 11]

In the specific case of biodiesel production, nitrogen limitation is known to increase the lipid content of phytoplankton. But, by changing the pigment composition and concentration [27, 12, 23, 25, 13], it also strongly affects the radiative transfer properties in the culture medium

[25]. The objective of our modelling approach is to propose a new model that can predict the behaviour of a photobioreactor in conditions of nitrogen limitation.

The first point that we have to solve in the development of a model is the level of complexity that must be chosen in order to find an optimal tradeoff between model accuracy and ability to properly analyse and calibrate the model. A too complicated model will have a complex behaviour that could not be analysed and lead to calibration procedures of poor quality, especially if the measurements of some of the model variables are not available at high frequency. Moreover, a high dimensional model is generally not mathematically handable, so that it is hardly possible to derive control and optimisation algorithms. On the contrary, a too simplistic model will not catch the main dynamics of the involved processes.

In order to propose models that keep a level of complexity compatible with the requested mathematical analyses, we will consider the simplest model that contains the elements to reproduce both the ability of microalgae to adapt their pigments to a given light level (photoadaptation) and the reduction of the pigments contents in case of nitrogen limitation. The basis of our development is the Droop model [8, 9] which has been deeply investigated [15, 3, 28] and proved to accurately reproduce situations of nitrogen limitations [9, 24, 4]. A link between cellular nitrogen and chlorophyll will then be introduced, so that a simplified light distribution model within the reactor can be proposed.

The paper is organized as follows. In a first part, we recall the Droop model. Then we introduce the light influence in this model. In a third part we propose a model to infer chlorophyll concentrations. Then we represent oxygen and CO_2. Finally the radiative transfer is examined, and the photoadaptation equation is proposed. The model is then validated using data from *Isochrysis galbana*.

1 The Droop model

The Droop model, initially established to represent the effect of B_{12} Vitamin internal quota on the growth rate of phytoplankton [8], has been shown appropriate to represent also the effect of macronutrients , such as nitrogen, on growth rate [9]. Contrarily to Monod model in which the growth is related to the external concentration of inorganic nitrogen s, Droop model considers that growth is related to the nitrogen cell concentration, or quota q, implying that nutrient uptake and growth are uncoupled processes. The substrate uptake leads to an increase of this internal quota which is used to sustain growth.

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

In this model the absorption rate $\rho(s)$ and growth rate $\mu(q)$ are generally taken as Michaelis-Menten and Droop functions:

$$\begin{aligned} \rho(s) &= \bar{\rho}\frac{s}{s+K_s} \\ \mu(q) &= \bar{\mu}(1-\frac{Q_0}{q}) \end{aligned} \tag{2}$$

where K_s is the half saturation constant for substrate uptake and Q_0 the minimal cell quota: at this quota level, no algal growth can take place. This model is more accurate than Monod model for algal growth modelling [28], but it is more complex and has been less studied.

Property 1 *The internal quota will stay between two bounds:*

$$Q_0 \leq q \leq Q_m \tag{3}$$

Where $Q_m = Q_0 + \frac{\bar{\rho}}{\bar{\mu}}$ *represents the maximum cell quota obtained in conditions of non limiting nutrient. The growth rate is also bounded :*

$$0 \leq \mu(q) \leq \mu_m = \frac{\rho_m \bar{\mu}}{Q_0 \bar{\mu} + \rho_m}) \tag{4}$$

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

As a corollary of this property, most of Droop model parameters can be straightforwardly identified from the measurements of the internal quota during exponential growth and at the end of a batch phase, when growth rate is zero. In this situation $q = Q_0$. The internal quota in non-limited growth conditions (when $q = Q_m$) together with the maximum growth rate provides then the values of $\bar{\mu}$ and $\bar{\rho}$. Parameter K_s can be deduced (together with $\bar{\rho}$) from a batch experiment where both s and x are measured.

The Droop model has been widely studied [15, 3, 28] and validated [9, 24, 4, 28]. However, it cannot directly be used in the case of photobioreactors for two main reasons:

- In its rough form it does not include the effect of light intensity
- It does not account for light attenuation due to the cell density

2 Improvement of Droop model to deal with light limitation

The Droop model does not take light into account. Adding light is however not straightforward. Indeed, an idea would be to introduce the light effect into the maximum growth rate $\bar{\mu} = \bar{\mu}(I)$:

$$\mu(q,I) = \bar{\mu}(I)(1 - \frac{Q_0}{q}) \tag{5}$$

where

$$\bar{\mu}(I) = \tilde{\mu} \frac{I}{I + K_{sI} + \frac{I^2}{K_{il}}} \tag{6}$$

With such a model, the maximum cell quota would be:

$$Q_m(I) = Q_0 + \frac{\bar{\rho}}{\bar{\mu}(I)} \tag{7}$$

During night period, $\bar{\mu}(0) = 0$ and thus equation (7) leads to an infinite maximal quota. Indeed, with such a formulation no growth occurs at night, so that the substrate can be indefinitely taken up into the quota without being consumed for growth. If an increase of the maximum cell quota in the absence of light is acceptable [16], obviously it cannot become infinite.

We impose therefore that the uptake rate stops as cells become replete [13]:

$$\rho(s,q) = \bar{\rho} \frac{s}{s + K_s}(1 - q/Q_l) \tag{8}$$

with $Q_l > Q_0$.

It leads to the following model including light effect, where we have also added a term dealing with respiration. As in [13], we assume that the rate of nitrogen loss (due both to mortality and remineralization) is the same than the respiration rate.

$$(D_L)\begin{cases} \dot{s} = Ds_{in} - \bar{\rho}\frac{s}{s+K_s}(1-q/Q_l)x - Ds \\ \dot{q} = \bar{\rho}\frac{s}{s+K_s}(1-q/Q_l) - \bar{\mu}\frac{I}{I+K_I}(q-Q_0) \\ \dot{x} = \tilde{\mu}\frac{I}{I+K_{sI}+I^2/K_{iI}}(1-\frac{Q_0}{q})x - Dx - Rx \end{cases} \tag{9}$$

Now, with uptake rate (8), it is straightforward to show that the absence of light prevents growth, but that the maximum cell quota is lower than parameter Q_l:

Property 2 *The internal quota stays between two bounds:*

$$Q_0 \leq q \leq Q_m(I) \leq Q_l \tag{10}$$

Where $Q_m(I) = \frac{\bar{\rho}+\bar{\mu}(I)Q_0}{\bar{\rho}+\bar{\mu}(I)Q_l}Q_l$ *represents the maximum cell quota obtained in replete nutrient conditions. In these conditions, the associated maximum growth rate, at irradiance* I *is now given by :*

$$0 \leq \mu(q,I) \leq \mu_M(I) = \bar{\mu}(I)(1-\frac{Q_0}{Q_m(I)}) \tag{11}$$

Proof: The equation of the quota, in conditions of abundance of nutrients in the environment ($\bar{\rho}\frac{s}{s+K_s} \simeq \bar{\rho}$), becomes:

$$\dot{q} = \bar{\rho}(1-q/Q_l) - \bar{\mu}(I)(q-Q_0) = (\mu(I) + \frac{\bar{\rho}}{Q_l})(Q_m(I)-q) \tag{12}$$

where

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

It is thus clear from (12) that q tends towards $Q_m(I)$ at a rate $(\mu(I) + \frac{\bar{\rho}}{Q_l})$.

3 Relationship between chlorophyll and particulate nitrogen

With the same spirit of keeping the model very simple, we propose a direct relationship to compute chlorophyll from particulate nitrogen xq, following the work of [19], for a culture photoacclimated at a light intensity I^*, we have:

$$\mathrm{Chl} = \gamma(\mathrm{I}^\star)\mathrm{xq} \tag{14}$$

where

$$\gamma(I^\star) = \gamma_{max}\frac{K_I}{I^\star + K_I} \tag{15}$$

This expression results from experimental observations obtained at steady state. Figure 1 presents data obtained at various light/dilution rate experiments.

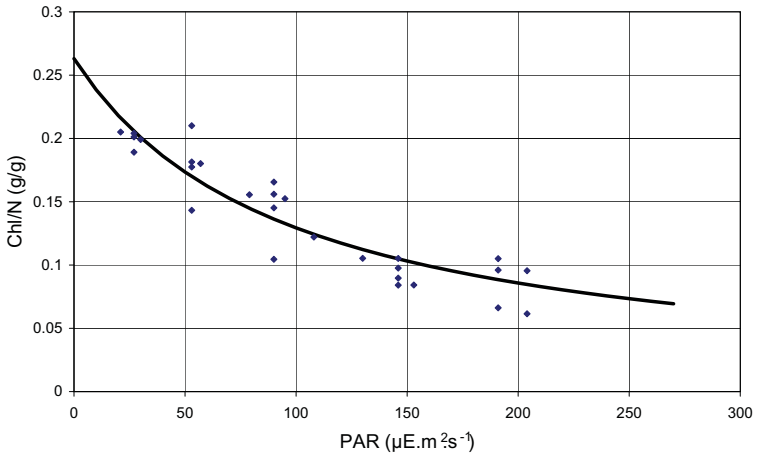

Figure 1: Representation of the ratio Chlorophyll *a* over particulate nitrogen for various light conditions and dilution rates (data from [19]).

Finally, since photoadaptation is not instantaneous after a light change, we represent the light adaptation dynamics, as follows:

$$\dot{I}^\star = \mu(q,I)(I - I^\star) \tag{16}$$

where we assume that the chlorophyll adaptation rate is the growth rate.

4 Prediction of pH, O_2 and CO_2 transfer

CO_2 availability is a key issue to guarantee that phytoplanktonic growth is not limited by the inorganic carbon resource. At the industrial scale, most of the efficient production systems inject CO_2 in the medium, and the risk of medium acidification is high if the influent gas flow rate is not closely regulated. For an efficient control it is therefore necessary to have a predictive model both for dissolved CO_2 and pH. In the same spirit, high concentrations of O_2 can lead to growth inhibition and it is thus also important to monitor and control oxygen concentration.

The gaseous flow rates are computed as follows:

$$Q_{CO_2} = K_L a_{CO_2}(CO_2 - CO_2^{\text{sat}}) \text{ and } Q_{O_2} = K_L a_{O_2}(O_2 - O_2^{\text{sat}}) \tag{17}$$

where CO_2^{sat} and O_2^{sat}, respectively the saturating concentration of CO_2 and O_2, are linearly related to the partial pressure in the gas phase. The transfer coefficient $K_L a$ for carbon dioxide is assumed to be proportional to that of oxygen ($K_L a_{CO_2} = 0.93\, K_L a_{O_2}$), due to the low difference in aqueous diffusivity of the two gases [5].

In order to compute the carbon dioxide concentration, we assume that, for non-calcifying species, the water alkalinity (Alk) is hardly affected by microalgal growth and is thus constant [29]. As a consequence the bicarbonate concentration is kept to constant level: $[HCO_3^-] = Alk$. Note that this hypothesis has to be revisited if ammonium is used as a nitrogen source: it should be

replaced by $Alk = Alk_0 + [NH_4^+]$ (where Alk_0 is constant), inducing thus a decrease in alkalinity. It follows that

$$CO_2 \simeq c - Alk$$

Where c is the inorganic carbon concentration. We finally propose a pH computation formula from the model state variables [2]. We use the affinity constant of the acid base couple CO_2/HCO_3^-:

$$K_a = \frac{[HCO_3^-]h}{[CO_2]}$$

where $h = [H^+]$ is the proton concentration.

$$h = (\frac{c}{Alk} - 1)K_a$$

pH is thus computed by $pH = -\log_{10}(h)$.

The evolution of dissolved inorganic carbon and oxygen are then directly obtained:

$$\begin{array}{l} \dot{c} = D(c_{in} - c) - \mu(q,I)x + R_r x - K_L a_{CO_2}(c - Alk - CO_2^{sat}) \\ \dot{o} = D(o_{in} - o) + R_q \mu(q,I)x - R_r x - K_L a_{O_2}(o - O_2^{sat}) \end{array} \quad (18)$$

Where R_q is the photosynthetic quotient, c_{in} and o_{in} the influent inorganic carbon and oxygen, respectively.

5 Modelling light gradient in the photobioreactor

We investigate here a simple representation of light attenuation inside a photobioreactor of thickness L, due to high biomass. As a result, the light decrease in the photobioreactor can be represented by a Beer-Lambert exponential decrease with a rate proportional to qx. When I_0 is the irradiance at the surface, we have thus:

$$E(z) = I_0 exp(-(a\gamma(I^\star)q.x + b)z) \quad (19)$$

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

$$\bar{I} = I_0 \frac{\int_0^L exp(-(a\gamma(I^\star)q.x + b)z)dz}{L} = \frac{I_0}{(a\gamma(I^\star)q.x + b)L}[1 - exp(-(a\gamma(I^\star)q.x + b)L)] \quad (20)$$

Note that this approximation could be improved by using more accurate models of the radiative transfer that would take the pigment composition into account [20, 21]. In order to get a more workable equation, we may replace (20) by a rational expression with equivalent behaviour:

$$\bar{I} \simeq I_0 \frac{K_g}{(a\gamma(I^\star)qx + b)L + K_g} \quad (21)$$

with $K_g = 1.25$. This expression is a good approximation of (20) since $\frac{1-exp(-x)}{x} \simeq \frac{K_g}{K_g+x}$. As a consequence, the influence of light should not be represented from the intensity of the light source, as was the case in the term $\frac{I_0}{I_0+K_I}$; it should be replaced by a similar term that takes the average light intensity reaching the microorganisms:

$$\begin{array}{rl} \bar{\mu}(I_0, qx) & = \tilde{\mu} \frac{I_0}{I_0 + K_{sI} + \frac{I_0^2}{K_{iI}}} \\ & = \tilde{\mu} \frac{I_0 K_g((a\gamma(I^\star)qx+b)L+K_g)}{I_0 K_g((a\gamma(I^\star)qx+b)L+K_g) + K_{sI}((a\gamma(I^\star)qx+b)L+K_g)^2 + I_0^2 K_g^2/K_{iI}} \end{array}$$

However, [20] showed that a more accurate way of estimating the growth rate consists in computing the average value of $\mu(I_0, qx)$.

Property 3 *Assuming that $K_{sI} > 4K_{sI}$, there exists two positive constants I_a and I_b (with $I_b > I_a$), such that: $(I+I_a)(I+I_b) = I^2 + K_{iI}I + K_{iI}K_{sI}$.*

The average value of the growth rate $\bar{\mu}(I)$ can then be computed as follows:

$$\bar{\tilde{\mu}}(I_0) = \frac{\tilde{\mu}}{I_b - I_a} \frac{1}{\gamma(I^\star)xqL} \ln\left(\frac{I_0 + I_a}{I_0 + I_b} \frac{I_0 \exp(-\gamma(I^\star)xqL) + I_b}{I_0 \exp(-\gamma(I^\star)xqL) + I_a}\right) \tag{22}$$

The function $\bar{\tilde{\mu}}(I_0)$ is increasing up to a light intensity $\bar{I}_0 = \sqrt{K_{iI}K_{sI}\exp(\gamma(I^\star)xqL)}$, and is then decreasing

It can be approximated with an excellent accuracy by an Haldane function.

$$\bar{\tilde{\mu}}(I_0, I^\star, x, q) = \tilde{\mu} \frac{\bar{I}}{\bar{I} + K_{sI} + \frac{\bar{I}^2}{K_{iI}} \exp(\gamma(I^\star)xqL/2)} \tag{23}$$

Proof: The first point is straightforward computation. The second point results from extensive numeric tests.

6 The nitrogen limited photobioreactor model

This section synthesises the results of the previous sections, and gathers the various model elements together.

The simplified reduced model writes now, for an incident light intensity I_0:

$$(PDM) \begin{cases} \dot{s} = Ds_{in} - \tilde{\rho}\frac{s}{s+K_s}(1 - q/Q_l)x - Ds \\ \dot{q} = \tilde{\rho}\frac{s}{s+K_s}(1 - q/Q_l) - \bar{\mu}(I_0, I^\star, x, q)(q - Q_0) \\ \dot{x} = \bar{\mu}(I_0, I^\star, x, q)(1 - \frac{Q_0}{q})x - Dx - Rx \\ \dot{I}^\star = \bar{\mu}(I_0, I^\star, x, q)(1 - \frac{Q_0}{q})(\bar{I} - I^\star) \end{cases} \tag{24}$$

with the average light intensity $\bar{I} = I_0 \frac{K_g}{(a\gamma(I^\star)qx+b)L+K_g}$. The average growth function is given by equation (23).

The model has been validated using the experimental data deriving from [10], as it is shown on figure 2. Parameter values are given in table 1.

7 Conclusion

We have developed a dynamical model that accurately represents the dependence of microalgae growth on nitrogen and light. This model takes into account the light gradient into the photobioreactor and predicts both carbon and oxygen fluxes. It strongly represents the coupling between biology (microalgae growth) and physics (radiative transfer properties).

Light heterogeneity in the medium induces a complex process of photoadaptation resulting from two opposite feedbacks. After an incident light shift, the biomass concentration increases and so do the pigments in the medium. It results in reducing the range of average light increase

Parameter	Value	Unit
$\tilde{\mu}$	1.8	day^{-1}
Q_0	0.053	mgN. mgC^{-1}
K_{sI}	50	$\mu E.m^{-2}s^{-1}$
K_{iI}	1000	$\mu E.m^{-2}s^{-1}$
$\bar{\rho}$	1.8	$mmolN.mgC^{-1}.day^{-1}$
Q_l	0.11	mg $N.mgC^{-1}$
K_{sI}	50	$\mu E.m^{-2}s^{-1}$
γ_{max}	0.12	$mgChl.mgN^{-1}$
K_I	50	$\mu E.m^{-2}s^{-1}$

Table 1: Parameter values used for the simulation of the PDM model

compared to the incident light shift. This negative feedback is completed by a positive feedback, since photoadaptation to a higher light intensity leads to a reduction of the pigment content and thus a higher light penetration. This shows that modelling is the only possibility to qualitatively describe what happens in a photobioreactor. Such a model can be the base for the optimisation of biomass or subproducts yield.

Acknowledgements: This paper presents research results supported by the ANR-06-BIOE-014 Shamash project.

8 References

[1] J.R. Benemann. Co2 mitigation with microalgae systems. Energy Conversion and Management, pages 475–479, 1997.

[2] O. Bernard, A.Sciandra, and S. Madani. Multimodel analysis of the response of the coccolithophore *Emiliania huxleyi* to an elevation of pCO_2 under nitrate limitation . Ecol. Model., 211:324–338, 2008.

[3] O. Bernard and J. L. Gouzé. Transient Behavior of Biological Loop Models, with Application to the Droop Model. Mathematical Biosciences, 127(1):19–43, 1995.

[4] Olivier Bernard and Jean-Luc Gouzé. Nonlinear qualitative signal processing for biological systems: application to the algal growth in bioreactors. Math. Biosciences, 157:357–372, 1999.

[5] F. Camacho Rubio, F. G. Acien Fernandez, J. A. Sanchez Perez, F. García Camacho, and E. Molina Grima. Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture. Biotech. Bioeng., 62:71–86, 1999.

[6] A.P. Carvalho, L.A. Meireles, and F.X. Malcata. Microalgal reactors: A review of enclosed system designs and performances. Biotechnology Progress, 22(6):1490–1506, 2006.

[7] Y. Chisti. Biodiesel from microalgae. Biotechnology Advances, 25:294–306, 2007.

[8] M. R. Droop. Vitamin B12 and marine ecology. IV. the kinetics of uptake growth and inhibition in *Monochrysis lutheri*. J. Mar. Biol. Assoc., 48(3):689–733, 1968.

[9] M. R. Droop. 25 years of algal growth kinetics, a personal view. Botanica marina, 16:99–112, 1983.

[10] K.J Flynn, K. Davidson, and J.W. Leftley. " carbon-nitrogen relations at whole-cell and free amino-acid levels, during batch growth of isochrysis galbanna (prymnesiophyceae) under conditions of alternating light and dark ". Mar. Biol., 118:229–237, 1994.

[11] E. Franco-Lara, J. Havel, F.Peterat F, and D. Weuster-Botz D. Model-supported optimization of phototrophic growth in a stirred-tank photobioreactor. Biotech. Bioeng.,

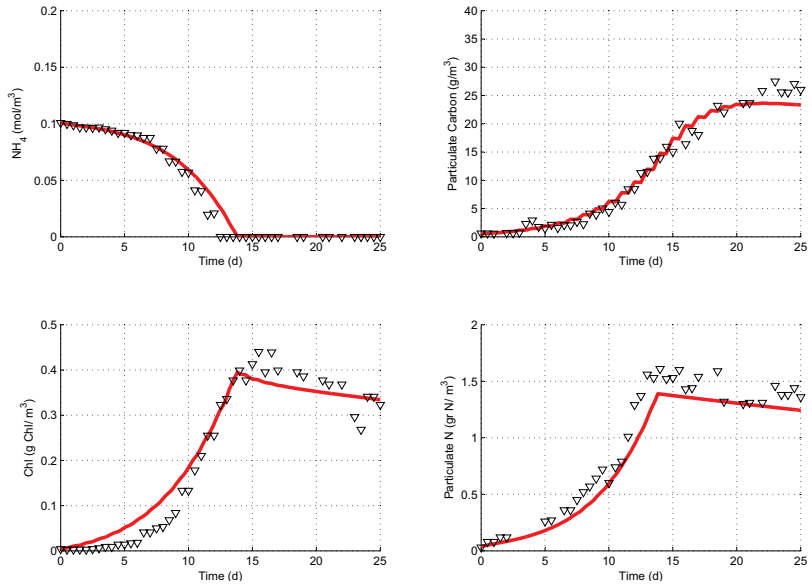

Figure 2: Simulation of the photobioreactor Droop model and comparison with experimental data from [10].

95:1177–1187, 2006.

[12] R.J. Geider, H.L. MacIntyre, and T.M. Kana. "a dynamic model of photoadaptation in phytoplankton. ". Limnol Oceanogr, 41:1–15, 1996.

[13] R.J. Geider, H.L. MacIntyre, and T.M. Kana. "a dynamic regulatory model of phytoplanktonic acclimation to light, nutrients, and temperature. ". Limnol Oceanogr, 43:679–694, 1998.

[14] M. Huntley and D.G. Redalje. Co2 mitigation et renewable oil from photosynthetic microbes: A new appraisal. Mitigation et Adaptation Strategies for Global Change, 12:573 – 608, 2007.

[15] K. Lange and F. J. Oyarzun. The attractiveness of the Droop equations. Mathematical Biosciences, 111:261–278, 1992.

[16] E.W. Laws and T.T. Bannister. " nutrient- and light-limited growth of thalassiosira fluviatilis in continuous culture, with implications for phytoplankton growth in the ocean. ". Limnol Oceanogr, 25:457–473, 1980.

[17] F.B. Metting. Biodiversity and application of microalgae. Journal of Industrial Microbiology & Biotechnology, 17:477 – 489, 1996.

[18] M. Olaizola. Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomolecular Engineering, 20:459–466, 2003.

[19] L. Pawlowski. Modélisation de l'incorporation du carbone photosynthétique en environnement marin contrôlé par ordinateur. PhD thesis, Université Pierre et Marie Curie, Paris VI, 2004.

[20] L. Pottier, J. Pruvost, J. Deremetz, J.-F. Cornet, J. Legrand, and C.G. Dussap. A fully predictive model for one-dimensional light attenuation by chlamydomonas reinhardtii in a torus photobioreactor. Biotechnology and Bioengineering, 91:569–582, 2005.

[21] J. Pruvost, L. Pottier, and J. Legrand. Numerical investigation of hydrodynamic and mixing conditions in a torus photobioreactor. Chemical Engineering Science, 61:4476–4489, 2006.

[22] O. Pulz. Photobioreactors: production systems for phototrophic microorganisms. Applied Microbiology et Biotechnology, 57:287–293, 2001.

[23] A. Sciandra, J. Gostan, Y. Collos, C. Descolas-Gros, C. Leboulanger, V. Martin-Jézéquel, M. Denis, D. Lefèvre, C. C. Copin, and B. Avril. Growth compensating phenomena in continuous cultures of *Dunaliella tertiolecta* limited simultaneously by light and nitrate. Limnol. Oceanogr., 46:1325–1339, 1997.

[24] A. Sciandra and P. Ramani. The limitations of continuous cultures with low rates of medium renewal per cell. J. Exp. Mar. Biol. Ecol., 178:1–15, 1994.

[25] D. Stramski, A. Sciandra, and H. Claustre. Effects of temperature, nitrogen, and light limitation on the optical properties of the marine diatom thalassiosira pseudonana. Limnol. Oceanogr., 47:392–403, 2002.

[26] I.S. Suh and S.B. Lee. A light distribution model for an internally radiating photobioreactor. Biotech. Bioeng., 82:180–189, 2003.

[27] D.H. Turpin. "effects of inorganic n availability on algal photosynthesis and carbon metabolism. ". J Phycol, 27:14–20, 1991.

[28] I. Vatcheva, Hidde deJong, Olivier Bernard, and N.J.L. Mars. Experiment selection for the discrimination of semi-quantitative models of dynamical systems. Artif. Intel., 170, 2006.

[29] Richard E. Zeebe and Dieter Wolf-Gladrow. CO_2 in seawater: equilibrium, kinetics, isotopes. Elsevier, 2003.