Ecological Complexity (Editor ID: EC04-47)
Original Research Article
Received 5 February 2004; received in revised form 18 August 2004; accepted 24 August 2004

A biochemically based structured model for phytoplankton growth in the chemostat [1]

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Abstract

In this paper, a new model of phytoplankton growth in the chemostat is proposed. First, we give a description of the chemostat and we recall the main models: the simple Monod model, experimentally validated for bacteria growth and the Droop model, which is validated for phytoplankton growth and takes into account the possible nutrient storage. Though our model is quite similar to the Droop one, our approach is based on biochemical mechanisms and not on empirical observations. We study two versions of the model: one taking into account cell mortality; the other not. The main result is the global asymptotic stability of the equilibrium, ensuring survival of the cells under some hypotheses. The paper ends with some illustrative simulations and a comparison with the dynamic energy budget modelling approach.

Key words: biochemical mechanisms, structured model, ordinary differential equations, chemostat, loop dynamical systems.

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[1] Paper presented at the 2nd Alcalá International Conference on Mathematical Ecology, Alcalá de Henares (Madrid), 5-9 September 2003

1 Introduction

A chemostat is a laboratory apparatus, composed of a reservoir and crossed by a constant liquid flow. The inflow feeds the culture with chemicals called substrates. In the vessel, microorganisms grow consuming these nutrients, then the outflow retrieves substrates and cells present in the reservoir. Usually, only one substrate is limiting in order to evaluate its influence on cells growth.

Unstructured mathematical models, meaning that only one variable is used to represent the microorganisms, are often employed to describe cell growth in chemostat. The most representative one has been proposed by (Monod, 1942). His approach is based on the interaction between microorganisms, more precisely bacteria, and substrates dissolved in the liquid medium. Although this model could be successfully used to fit steady state data when bacteria are considered, its predictions, regarding phytoplankton, are far from being satisfactory. A new modelling approach is therefore required.

Structured models, meaning that the whole cell population is described by several variables usually representing some physiological states, seems very efficient for this purpose. One of the most classical remains the one proposed by (Droop, 1968). His approach describes more precisely the substrate ingestion in two steps: first, nutrient storage and then its metabolization. The resulting model is more accurate than Monod's and is able to reproduce experimental data obtained for phytoplankton.

In this paper, we propose another *structuring* approach, based on mechanistic biochemical explanations. Our main goal is to describe biochemical phenomena: respiration, growth and mortality. Since cell respiration and cell growth are taken into account, cell population is described by its nitrogen and carbon, organic and inorganic component. Then two substrates are limiting for the growth. Moreover, cell mortality, meaning the degeneration of some mechanisms as cell division which implies premature death of the cell, is described.

First, we present the chemostat paradigm recalling the two aforementioned classical models and their main properties. Then we explain the basis of our modelling approach and we compare the resulting model to the Droop one. We study its mathematical properties and we show the existence of a global asymptotic equilibrium (survival of the cells) under some hypotheses. Finally, we compare our approach with the dynamical budget energy based model (Kooijman, 1993) and we show some illustrative simulations.

2 The chemostat paradigm

2.1 The chemostat

The chemostat is a vessel crossed by a constant flow where microorganisms grow. The nutrient is provided by a constant inflow F_{in} and a blend of nutrient and of microorganisms is retrieved in the constant outflow F_{out}. In the continuous cultures we consider here, the input flow rate and the output flow rate are the same ($F_{in} = F_{out} = F$).

The physically based mathematical modelling of a component dynamics P in concentration p with respect to this passing flow is very simple; the variation of the total mass pV in the volume V (constant), is the difference between the inflow mass Fp_{in} and the outflow mass Fp:

$$\dot{pV} = Fp_{in} - Fp \Leftrightarrow \dot{p} = dp_{in} - dp \tag{1}$$

where d the dilution rate is equal to $\frac{F}{V}$. This elementary law (1) means that there is no mass creation and no mass disappearance in the chemostat. This law is referenced as the *mass conservation principle*.

2.2 The Monod model

The most classical chemostat model was introduced by (Monod, 1942) to describe the bacteria growth. Only two variables are chosen to describe the reaction occurring in the reactor vessel: the biomass X in concentration x and the limiting substrate S in concentration s. This biological part can be formulated as a *biochemical reaction*:

$$\alpha S \xrightarrow{\mu(s)x} X$$

Reactant (S) and *product* (X) are considered; the *reaction kinetics* ($\mu(s)x$) depends on biological mechanisms.

The mathematical model is divided in two parts: the previously mentioned physical part due to the flow and the biological part, which describes the reactions in the vessel. Then the following model is obtained:

$$\begin{array}{rc|l} \dot{s} = & -\alpha\mu(s)x & -ds + ds_{in} \\ \dot{x} = & \mu(s)x & -dx \\ & \text{Biological part} & \text{Physical part} \end{array} \tag{2}$$

Let us remark some qualitative biological properties of the specific growth rate $\mu(s)$. The more substrate there is, the more cells grow (*i.e.* $\mu(0) = 0$, $\mu(s)$ increasing); this implies that cells do not "lose biomass" (*i.e.* $\mu(s) > 0$). Moreover, cells cannot absorb more than a given quantity of substrate during a given time (*i.e.* $\mu(s)$ bounded).

We will call such a function with there qualitative features **(H2.2.1)** a *"Monod like function"* (*e.g.* Holling type II). This specific growth rate $\mu(s)$ is often defined by the classical function $\mu(s) = \frac{\mu_m s}{k+s}$.

Hypothesis H 2.2.1 $\mu(0) = 0$ *and* $\mu(.)$ *is* $\mathcal{C}^1$*, increasing and bounded.*

This system (2) has been extensively studied, see for example (Smith and Waltman, 1995). Let us recall, first, that $\mathbb{R}^2_+$, which is of biological interest, is invariant under (2). Furthermore, if $\mu(s_{in}) > d$ **(H2.2.2)**, two steady states exist: an equilibrium, such that all the microorganisms in the device disappear, referred to as the washout point (denoted $(s_{in}, 0)$); another such that a biomass population remains alive in the vessel called the non trivial point (designated (s^*, x^*) with $x^* > 0$). Moreover, the *mass conservation principle* holds. Indeed, take the variable $z = \alpha x + s$ (the total mass concentration in the chemostat), then the dynamical equation of z follows equation (1).

Note that the *yield* coefficient α of substrate conversion into biomass (*i.e.* the quantity of substrate to be consumed to produce one quantity of biomass) is constant.

Proposition 1 (see Smith and Waltman (1995))

Under the hypotheses (H2.2.1) and (H2.2.2), the washout steady state is unstable and the non-trivial steady state is globally asymptotically stable in the positive orthant.

This simple unstructured model is very intuitive and reproduces most of bacteria population dynamics. The main problem is that the whole physiological description is contained in the sole variable x. For example, this model predicts that there is no growth if there is no more substrate ($s = 0$), which contradicts experimental observations for phytoplankton (Nisbet and Gurney, 1982). Then more variables have to be used to describe the population: the classical Droop model illustrates this idea.

2.3 The Droop model

The Droop model has been proposed to describe phytoplankton growth under limiting vitamin B_{12} in the chemostat (Droop, 1968). The main goal is to

reproduce the obtained experimental data. Then the variables chosen to describe the phytoplankton depend on what are easily measured quantities that do not always have clear biological meaning. Hence, as in the Monod model, biomass X in concentration x and limiting substrate S in concentration s are considered. Physiological chemical tests (for example, detection of radioactively labeled nutrient (Droop, 1973)) allows measurement of a quantity that is the amount of substrate components present in the solid phase after sedimentation (here the cell population); this quantity is called the intracellular limiting substrate QX in concentration qx. The variable Q is referred to as the *intracellular quota* of limiting nutrient.

Thus considering the biological phenomena and the physical part due to the passing flow, the following model is obtained:

$$\begin{array}{rc|l} \dot{s} = & -\rho(s)x & -ds + ds_{in} \\ \dot{\overline{qx}} = & \rho(s)x & -d(qx) \\ \dot{x} = & \mu(q-q_m)x & -dx \\ & \text{Biological part} & \text{Physical part} \end{array} \tag{3}$$

The equation of QX is often replaced by the equation of Q (deduced from (3)):

$$\dot{q} = \rho(s) - \mu(q-q_m)q \tag{4}$$

The phenomenon of metabolization cannot be described very precisely using the variables Q, X, S: this is the main problem of this model. Indeed, the variable Q does not differentiate between the stored nutrient and biomass.

As in the Monod model, Droop proposed some qualitative hypotheses about the specific growth rate $\mu(q-q_m)$ and storage rate $\rho(s)$ functions based on biological experiments. If there is some extracellular substrate, then the cell takes it up to make some stored nutrient. Furthermore, a parameter q_m, minimum concentration cell quota, is defined such that if $q < q_m$, there is not enough internal nutrient for the cell to grow. For example, $\mu(q-q_m) = \mu_m \dfrac{q-q_m}{K+(q-q_m)}$ or $\mu(q-q_m) = \mu_m \left(1 - \frac{q_m}{q}\right)$ (Smith and Waltman, 1995).

Hypotheses H 2.3.1 *$\rho(.)$ and $\mu(.)$ are both "Monod like" functions (e.g. $\mu(q-q_m)$ as a function of q is a translated "Monod like" function).*

The mathematical study of this model has been done by (Lange and Oyarzun, 1992; Oyarzun and Lange, 1993). First, the set $\Omega = \{s \geq 0, q \geq q_m, x \geq 0\}$, which is of biological interest, is invariant by the system (3).

If $\lim_{q\to\infty} \mu(q) = \mu_m > d$ and $\rho(s_{in}) > d\mu^{-1}(d)$ **(H2.3.2)**, there exists two steady

states: the washout point $(s_{in}, q_1, 0)$ corresponding to the disappearance of the culture from the vessel and the non-trivial steady state (s^*, q^*, x^*). Moreover, the *mass conservation principle* is verified; indeed, take the variable $z = qx + s$ (the total mass concentration in the chemostat), the dynamical equation of z verifies equation (1).

The Droop model is often referenced as a *variable yield* model as opposed to the Monod model that is a *constant yield* model. Indeed, the *yield* coefficient of substrate conversion into biomass, corresponding to α in the Monod model, is $\dfrac{\mu(q - q_m)}{\rho(s)}$ in the Droop model, which depends on time.

Proposition 2 (Lange and Oyarzun, 1992; Oyarzun and Lange, 1993)

Under all the hypotheses above, the washout equilibrium is unstable and the non-trivial steady state is globally asymptotically stable in the positive orthant.

This model, built to fit experimental data, describes stored nutrient and nutrient metabolization without making any distinction between the two. The qualitative hypotheses on the function $\mu(q)$ and the existence of the minimum quota do not rest on biochemical arguments, but on experimental results (Droop, 1968). Moreover, other biological phenomena such as cell mortality and cell respiration are not considered.

We now propose a structured model in which biochemical phenomena are explicitly taken into account.

3 Structured phytoplankton growth model

In this section, we consider phytoplankton growth limited by the two major components of photosynthetic cells: nitrogen and carbon. Moreover, we assume that the chemostat is constantly lighted.

3.1 Biological phenomena

It is well known that the phytoplanktonic cells grow via absorption of extracellular nitrogen (denoted S in concentration s) and carbon (referred to as C in concentration c). The two input limiting components are denoted S_{in} and C_{in} in concentration s_{in} and c_{in}.

First, we model the complex absorption of extracellular nitrogen S in two steps: storage and metabolization. The stored nutrient is denoted R in con-

centration r and the organic nitrogen is called B in concentration b. The reaction kinetics of nitrogen storage is $\rho(s)x$; the reaction kinetics of nitrogen metabolization is $\mu(\frac{r}{x})x$. Indeed, cell growth depends on the variable $\frac{r}{x}$ representing the available stored nitrogen par organic biomass unit since, in biology, the usual measure unit of organic biomass is the organic carbon.

Absorption of extracellular carbon C is quite simple: it is directly metabolized into intracellular organic carbon X in concentration x. The reaction kinetics of carbon metabolization is the same that nitrogen metabolization since these phenomena occur together ($\mu(\frac{r}{x})x$). These phenomena can be represented by the reactions:

$$\text{nitrogen storage}$$
$$S \xrightarrow{\rho(s)x} R$$

$$\text{growth and respiration}$$
$$\alpha R + (1+\gamma)C \xrightarrow{\mu(\frac{r}{x})x} \alpha B + X + \gamma C$$

Note that γC is eliminated by the respiration of the cell after the carbon metabolization. Furthermore, let us remark that the specific growth rate $\mu(.)$ could depend on light. We do not consider this case in this paper since the chemostat is assumed to be constantly lighted (see Lemesle (2004) for more details).

Moreover, since the premature mortality of the cells has to be represented, we represent the proportion in R, B and X of dead cells. These quantities are denoted M_R,M_B, M_X. Cells mortality describes possible cell death before division or disappearance due to the outflow. The dynamics of (M_R, M_B, M_X) are not modelled: in the literature, it can be chosen to add a delay phenomenon in the extracellular inorganic components for the recycling of the "dead cells" since the conversion of organic components into inorganic component is not immediate (Beretta and Takeuchi, 1994). The rate of this cell mortality denoted m is constant. This phenomenon is given by the following biological diagram:

$$\text{mortality}$$
$$R \xrightarrow{mr} M_R, \quad B \xrightarrow{mb} M_B, \quad X \xrightarrow{mx} M_X$$

The conversion of atmospheric carbon into dissolved carbon C is taken into account with a rate $q(c)$ (this rate can be negative). This must be then added to the physical part due to the passing flow.

3.2 Model formulation

Using this biochemical description, we obtain the model denoted ($\mathcal{S}$):

$$\begin{aligned}
\dot{s} = & \quad -\rho(s)x & & - ds + ds_{in} & (5)\\
\dot{r} = & \quad \rho(s)x - \alpha\mu\left(\frac{r}{x}\right)x - mr & & - dr & (6)\\
\dot{b} = & \quad \alpha\mu\left(\frac{r}{x}\right)x - mb & & - db & (7)\\
\dot{x} = & \quad \mu\left(\frac{r}{x}\right)x - mx & & - dx & (8)\\
\dot{c} = & \quad -\mu\left(\frac{r}{x}\right)x & & + q(c) - dc + dc_{in} & (9)\\
& \quad \text{Biological part} & & \text{Physical part} &
\end{aligned}$$

We make the same hypotheses as in the Monod model (H2.2.1) on the qualitative behaviour of the growth rates.

Hypotheses H 3.2.1 *$\mu(.)$ and $\rho(.)$ are both "Monod like" functions.*

Let us remark that the function $\mu(\frac{r}{x})$ is not defined for $x = 0$. This is not a problem since this function describes the evolution of the intracellular inorganic components, then $x = 0$ does not have a biological meaning.

3.3 Meaning of the constant α and model reduction

In this section, we explain the meaning of the constant α and why equations (5), (6), (8) are sufficient to compute the dynamics of the whole model ($\mathcal{S}$).

Firstly consider the variable $V = -\alpha x + b$. Its dynamical equation is:

$$\dot{V} = -(m + d)V$$

The equilibrium $V^* = 0$ (*i.e.* $b = \alpha x$), is globally asymptotically stable. We deduce that, *asymptotically*, the organic nitrogen b and the organic carbon x are linked by a fixed ratio $b = \alpha x$. This phenomenon has been observed in different biological experiments especially in Droop's ones.

Then let us remark that the dynamical equations (9) and (7) depend on the dynamics of x, r, c and b. Moreover, none of the other equations depend on the carbon c and the intracellular inorganic nitrogen b dynamics. So the two equations (9) and (7) are decoupled from the other three and these state

variables can be computed after solving the remaining system of three ordinary differential equations. Then in the sequel, we only consider the reduced system:

$$\begin{aligned} \dot{s} &= -\rho(s)x - ds + ds_{in} \\ \dot{r} &= \rho(s)x - \alpha\mu(\frac{r}{x})x - mr - dr \\ \dot{x} &= \mu(\frac{r}{x})x - mx - dx \end{aligned} \tag{10}$$

3.4 Interpretation of the limiting nutrient quota

The intracellular quota of limiting nutrient is proposed by Droop to fit experimental data. This variable does not have a clear biological meaning. It represents the whole intracellular limiting nutrient quantity per unit of biomass.

With our variables, the quota is the total nitrogen quantity in the cell per biomass unit given by $q = \frac{r+b}{x}$. The dynamical equation of q is then as follows:

$$\dot{q} = \rho(s) - \mu\left(q - \frac{b}{x}\right)q \tag{11}$$

Note that this equation is quite similar to the Droop equation (4). The Droop model (3) has been built under equilibria experimental conditions (*i.e.* as time has gone to ∞). Considering our system $(\mathcal{S})$ under this assumption $(t \to \infty)$, we obtain that $\frac{b^*}{x^*} = \alpha$. Thus the quota equation (11) becomes:

$$\dot{q} = \rho(s) - \mu(q - \alpha)q$$

Though we have built the model $(\mathcal{S})$ in a different manner than Droop, we obtain the same formulation for the intracellular quota. Moreover, with our modelling approach, we are now able to explain the clear biological meaning of the Droop minimum intracellular quota q_m.

Remember that q_m is the minimum stored nutrient of a cell to grow and is estimated from data; with our approach, it is the constant α defined in the previous subsection. This constant is the limit when the time tends to infinity of the variable $\frac{b}{x}$; during the transients, we can have more information on the behaviour of the minimum intracellular quota by considering the variable $\frac{b}{x}$. We find that this minimum quota varies with time, as opposed to the Droop model. Moreover, the Droop growth function $\mu(q-q_m)$ (obtained by empirical considerations) gives less information than the growth function $\mu(q-\frac{b}{x})$ where $\frac{b}{x}$ is a dynamically varying minimum quota.

4 Model analysis

We study in this section the asymptotic behaviour of two versions of the model (10): one without mortality phenomena, the other with a fixed positive mortality rate.

4.1 Existence of steady states and an invariant set

First we give some general properties of system (10) with $m \geq 0$. For this system, $x = 0$ is not acceptable since the function $\mu(\frac{r}{x})$ is not well defined at this point. Then to make a complete study, we consider the change of variables:

$$s, \quad u = \frac{r}{x}, \quad x$$

which defines a diffeomorphism from the set $D = \{s \geq 0, r \geq 0, x > 0\}$ to D. We obtain the following system, that is now well defined in the set $\Omega = \{s \geq 0, u \geq 0, x \geq 0\}$:

$$\begin{cases} \dot{s} = -\rho(s)x - ds + ds_{in} \\ \dot{u} = \rho(s) - \alpha\mu(u) - \mu(u)u \\ \dot{x} = \mu(u)x - dx - mx \end{cases} \tag{12}$$

Note that it can be seen as an extended system of (10) since it is defined for $x = 0$. Thus we only consider initial conditions for the state variables belonging to the set Ω which is also of biological meaning for (12). Moreover, let us remark that system (12) is a cyclic feedback monotone system (Mallet-Paret and Smith, 1990):

$$\dot{\xi}_i = f_i(\xi_i, \xi_{i-1}) \text{ for } i = 1, \dots \ n \ mod(n) \text{ and } \frac{\partial f_i}{\partial \xi_{i-1}} \text{ fixed sign}$$

To ensure the existence of a non-trivial equilibrium, we make the following hypotheses.

Hypotheses H 4.1.1
$\lim_{u\to\infty} \mu(u) = \mu_M > d + m.$
$\lim_{s\to\infty} \rho(s) = \rho_M > \rho(s_{in}) > (d + m)(\alpha + \mu^{-1}(d + m)).$

Proposition 3

Under the hypotheses (H3.2.1) and (H4.1.1), there exists two admissible stationary points for the system (12): the washout point $(s_{in}, u_1, 0)$ and the non-trivial equilibrium (s^*, u^*, x^*).

PROOF. The stationary points are the solutions of:

$$\begin{cases} 0 = -\rho(s)x - ds + ds_{in} \\ 0 = \rho(s) - \alpha\mu(u) - \mu(u)u \\ 0 = \mu(u)x - mx - dx \end{cases}$$

The admissible solutions in the domain Ω are:

- $(s_{in}, u_1, 0)$ with u_1 defined such that

$$\rho(s_{in}) = \alpha\mu(u_1) + \mu(u_1)u_1 \equiv \Phi(u_1)$$

which is unique since $\Phi(u)$ is an increasing function defined such that $\Phi(u) = 0$ and $\lim_{u\to\infty} \Phi(u) = +\infty$, thanks to the hypotheses (H3.2.1), and

- (s^*, u^*, x^*) defined such that

$$\begin{cases} \rho(s^*)x^* = ds_{in} - ds^* \\ \rho(s^*) = (d+m)(\alpha + u^*) \\ \mu(u^*) = d + m, \end{cases}$$

where $u^* = \mu^{-1}(d+m)$ is unique since $\mu(u)$ is an increasing function and $\mu_M > d + m$ (hypotheses (H4.1.1));

$\rho(s^*) = (d+m)(\alpha+\mu^{-1}(d+m))$ defines a unique s^* since $\rho(s)$ is an increasing function and $\rho_M > \rho(s_{in}) > (d+m)(\alpha+\mu^{-1}(d+m))$ (hypotheses (H4.1.1)). □

Proposition 4

Under the hypotheses (H3.2.1), $\Omega = \mathbb{R}^3_+$ is invariant for the system (12).

PROOF. The dynamical equations $\dot{s}$, $\dot{u}$, $\dot{x}$ computed at $s = 0$, $u = 0$ and $x = 0$ respectively, are as follows:

$$\dot{s}_{s=0} = ds_{in} > 0 \Rightarrow s = 0 \text{ is repulsive.}$$

$$\dot{u}_{u=0} = \rho(s) \geq 0 \Rightarrow u = 0 \text{ is repulsive or invariant.}$$

$$\dot{x}_{x=0} = 0 \Rightarrow x = 0 \text{ is invariant.}$$

Then Ω is invariant for the system (12) $\square$

Remark 5

Consider the subset $\{0 \leq s \leq s_{in}, u \geq 0, x \geq 0\}$. *We can prove that this set is invariant for the system (12). Indeed, computing the dynamical equation* $\dot{s}$ *at* $s = s_{in}$, *we obtain:* $\dot{s}_{s=s_{in}} = -\rho(s_{in})x \leq 0$.

4.2 Local analysis

For the local behaviour study of system (12), we use classical first order linearization techniques. Thus we compute the associated Jacobian J of system (12) at a generic point (s, u, x):

$$\begin{pmatrix} -d - \rho'(s)x & 0 & -\rho(s) \\ \rho'(s) & -\alpha\mu'(u) - \mu(u) - \mu'(u)u & 0 \\ 0 & \mu'(u)x & \mu(u) - d - m \end{pmatrix}$$

Let us remark that as the number of non-positive off-diagonal terms is odd; the system is then similar to a competitive system (*i.e* the off-diagonal jacobian terms are all non positive) taking $u = -v$ (see proposition 5.1 p.48 in (Smith, 1995)).

Firstly we prove that the washout point is unstable (saddle point) and we make a detailed study of its stable and unstable manifolds.

Proposition 6

Under the hypotheses (H3.2.1) and (H4.1.1), the washout point $(s_{in}, u_1, 0)$ *is a saddle point.*

PROOF. We compute the associated Jacobian J_{wo} matrix at $(s_{in}, u_1, 0)$:

$$\begin{pmatrix} -d & 0 & -\rho(s_{in}) \\ \rho'(s_{in}) & (-\alpha\mu'(u_1) - \mu(u_1) - \mu'(u_1)u_1) & 0 \\ 0 & 0 & \mu(u_1) - d - m \end{pmatrix}$$

The three eigenvalues of J_{wo} are $-d < 0$, $-\alpha\mu'(u_1) - \mu(u_1) - \mu'(u_1)u_1 < 0$, and $\mu(u_1) - d - m$. Then to prove the instability of the washout point, we

must find the sign of $\mu(u_1) - d - m$. Remember the definition of u_1 and u^*:

$$\rho(s_{in}) = \alpha\mu(u_1) + \mu(u_1)u_1 = \Phi(u_1)$$

$$\begin{cases} \rho(s^*) = \alpha\mu(u^*) + \mu(u^*)u^* = \Phi(u^*) \\ \mu(u^*) = d + m \end{cases}$$

Using hypotheses (H3.2.1) ($\rho(s)$, $\mu(u)$ and $\Phi(u)$ are increasing functions), we have:

$$\rho(s_{in}) > \rho(s^*) \Leftrightarrow \Phi(u_1) > \Phi(u^*) \Leftrightarrow u_1 > u^* \Leftrightarrow \mu(u_1) > \mu(u^*) = d + m$$

Then $\mu(u_1) - d - m > 0$ and the washout point is a saddle point since two non negative eigenvalues exist. □

Next we make a detailed study of the local stable and unstable manifolds of the washout point. Indeed, we want to ensure the invariance of the set Ω even asymptotically.

Proposition 7

The only eigenvector that intersects the domain Ω is the vector associated to the positive eigenvalue.

PROOF. We compute the coordinates of the eigenvectors associated to the eigenvalues of J_{wo} as follows:

$$J_{wo}v_i = \lambda_i v_i \text{ for } i = 1, 2, 3$$

We find after this simple computation that the local stable manifold of the washout point is contained in the plane $x = 0$ and the local unstable manifold intersects the domain $\Omega = \mathbb{R}^+_3$. □

Remark 8

This property ensures the invariance of the set Ω even asymptotically (for more details, see the definition of persistence and the theorem of Butler and MacGehee in (Smith and Waltman, 1995)). Moreover, we can prove with simple arguments using the deviation of the initial conditions from the equilibrium and the trend of the variables (increasing or decreasing) that all the positive orbits beginning in a set $U_0 \subset \Omega$ are bounded and do not reach the washout point (Lemesle, 2004).

Finally, we prove that the non-trivial equilibrium is locally stable.

Proposition 9

Under the hypotheses (H3.2.1) and (H4.1.1), the non-trivial equilibrium (s^*, u^*, x^*) *is locally asymptotically stable.*

PROOF. Recall that (s^*, u^*, x^*) is defined such that:

$$\begin{cases} \rho(s^*)x^* = ds_{in} - ds^* \\ \rho(s^*) = (d+m)(\alpha + u^*) \\ \mu(u^*) = d + m \end{cases}$$

Computing the Jacobian matrix J in (s^*, u^*, x^*) denoted J^*, we look for the number of positive eigenvalues using the Routh criterion for the J^* characteristic polynomial, see Hofbauer and Sigmund (1988). We compute the number of sign changes in the first Routh column. This first column is given by

$$1, \quad a_1, \quad \frac{a_1 a_2 - a_3}{a_1}, \quad a_3$$

with $a_1 = -\mathrm{tr}(J^*)$, a_2 the sum of the three principal 2×2 minors of J^* and $a_3 = -\det(J^*)$. We obtain:

$$\begin{aligned} a_1 &= -\mathrm{tr}(J^*) \\ &= \underbrace{(\alpha + u)\mu'(u)}_{a} + (d+m) + d + \rho(s^*)x^* > 0 \\ a_3 &= -\det(J^*) \\ &= (d+m)(\alpha+u)\rho'(s^*)\mu'(u)x^* > 0 \\ a_2 &= (\alpha\mu'(u) + \mu'(u)u)(d + \rho'(s^*)x^*) + d(d+m) \\ &\quad + \underbrace{(d+m)\rho'(s^*)x^*}_{a'} > 0 \end{aligned}$$

Then $a_1 > 0$, $a_3 > 0$. To conclude we must know the sign of $a_1 a_2 - a_3$. We can prove that $a_1 a_2 - a_3 > 0$. Indeed, one can see that:

$$aa' - a_3 = 0$$

and the other terms of the product $a_1 a_2$ are non negative. There is no sign change in the first Routh column then there is no eigenvalues with non negative real part. Thus the non-trivial equilibrium is locally stable. □

First, we show that the state variables are bounded. Let us remark that in this case the *mass conservation principle* is not verified. Indeed to have a conservative form, it is necessary to describe all the possible physiological states of the biomass. In this model, the dynamical behaviour of the "dead cells" components' concentration is not described.

Proposition 10

Under the hypotheses (H3.2.1) and (H4.1.1), the state variables are bounded.

PROOF. Consider the auxiliary variable $z = ux + \alpha x + s$ which is a positive definite function. The dynamical equation of z is defined such that:

$$\begin{aligned}\dot{z} &= -mux - \alpha mx - dz + ds_{in} = -(d+m)z + ms + ds_{in} \\ &\leq -(d+m)z + (d+m)s_{in},\end{aligned}$$

meaning that $z(t) < (z(0) - s_{in})e^{-(d+m)t} + s_{in}$. Thus the non negative state variables are bounded: indeed, if $z(0) > s_{in}$ then $z(t) < z(0)$ and if $z(0) \leq s_{in}$ then $z(t) < s_{in}$. $\square$

To clarify the global stability of the non-trivial stationary point, we consider the change of variables s, u, $ln(x) = y$. With these new variables, the washout point goes towards infinity. We obtain the new system:

$$\begin{cases} \dot{s} = -\rho(s)e^{y} - ds + ds_{in} \\ \dot{u} = \rho(s) - \alpha\mu(u) - \mu(u)u \\ \dot{y} = \mu(u) - d - m \end{cases} \tag{13}$$

We can easily prove that the *closed convex* domain $U = \{s \geq 0, u \geq 0, y \in \mathbb{R}\}$ is invariant for the system.

Using a theorem given by (Mallet-Paret and Smith, 1990), we can conclude that the non-trivial equilibrium (s^*, u^*, y^*) is globally stable or it exists a non-trivial periodic orbit. Indeed, this theorem is a generalization of the Poincaré Bendixson theorem for $n-$dimensional cyclic feedback monotone systems.

Theorem 11 (Mallet-Paret and Smith, 1990)

Consider the cyclic feedback monotone system

$$\dot{\xi}_i = f_i(\xi_i, \xi_{i-1}) \text{ for } i = 1, \ldots, n \ mod(n)$$
$$\text{with } \frac{\partial f_i}{\partial \xi_{i-1}} \text{ fixed sign}$$

in a positively invariant closed convex invariant domain U containing a single equilibrium ξ^*. If $U \supset \gamma^+(\xi_0)$, then either (i) $\omega(\xi_0) = \xi^*$, (ii) $\omega(\xi_0)$ is a non constant periodic orbit, or (iii) $\omega(\xi_0)$ consists of ξ^* together with a collection of orbits homoclinic to ξ^*. If

$$\Delta \det(-J^*) < 0$$

with $\Delta = \delta_1\delta_2\delta_3$ (δ_i defined such that $\delta_i \frac{\partial f_i}{\partial \xi_{i-1}} > 0$), then (iii) cannot occur.

Proposition 12

Under the hypotheses (H3.2.1) and (H4.1.1), the non trivial equilibrium (s^*, u^*, y^*) is globally asymptotically stable or the system (13) has a non trivial periodic orbit.

PROOF. We check the hypotheses of the theorem 11.

The domain U is an invariant closed convex domain for the system (13) and it contains the non-trivial equilibrium $p^* = (s^*, u^*, y^*)$.

Moreover, it can be proved by simple arguments that every positive orbit starting at p_0 is bounded and cannot go towards $y = -\infty$ (see remark 8).

We can say then either

(i) $\omega(p_0) = p^*$,

(ii) $\omega(p_0)$ is a non constant periodic orbit,

(iii) $\omega(p_0)$ consists of p^* together with a collection of orbits homoclinic to p^*.

We can rule out the contingency (iii) by a condition given in the theorem 11. Indeed, we have:

$$\Delta = \delta_1\delta_2\delta_3 = -1$$

and then as $\det(-J^*) = (-1)^3 \det(J^*)$, we obtain:

$$\Delta \det(-J^*) = \det(J^*) < 0$$

Thus (iii) cannot occur.

We can conclude that if we could rule out case (ii), then the non-trivial equilibrium would be globally attractive; thus since it is locally asymptotically stable, it would be globally asymptotically stable. □

This result is not very strong but arguments from structural stability (see next section) and numerical simulations show that for a small enough m, the equilibrium is globally asymptotically stable.

4.4 Global analysis without a mortality rate

We consider the system (12) in the case $m = 0$. We obtain the system:

$$\begin{cases} \dot{s} = -\rho(s)x - ds + ds_{in} \\ \dot{u} = \rho(s) - \mu(u)(\alpha + u) \\ \dot{x} = \mu(u)x - dx \end{cases} \tag{14}$$

Proposition 13

Under hypotheses (H3.2.1) and (H4.1.1), the mass principle conservation is verified and the state variables are bounded.

PROOF. Consider $z = ux + \alpha x + s$, the total mass concentration in the chemostat. The dynamical equation of z verifies equation (1):

$$\dot{z} = -dz + ds_{in} \Leftrightarrow z(t) = (z(0) - s_{in})e^{-dt} + s_{in}$$

This means that $\lim_{t\to\infty} z(t) = s_{in}$. Thus the mass principle conservation holds and the variables are bounded. □

To prove the global stability of this equilibrium, we use the same techniques as (Lange and Oyarzun, 1992; Oyarzun and Lange, 1993) for the study of the classical Droop model and a lemma given by (Viel et al., 1995; Vidyasagar, 1993).

Lemma 14

Consider the non linear triangular system:

$$\begin{cases} \dot{x} = f(x, y) \\ \dot{y} = \quad g(y) \end{cases} \tag{15}$$

with $y \in \mathbb{R}^k$ and $x \in \mathbb{R}^{n-k}$. Assume:
(i) $y^* \in \mathbb{R}^k$ is globally asymptotically stable for $\dot{y} = g(y)$,
(ii) $x^* \in \mathbb{R}^{n-k}$ is globally asymptotically stable for $\dot{x} = f(x, y^*)$,
(iii) all the orbits of the system (15) are bounded.

Then $(y^*, x^*) \in \mathbb{R}^n$ is globally asymptotically stable for (15).

Proposition 15

Under hypotheses (H3.2.1) and (H4.1.1), the non-trivial equilibrium (s^*, u^*, x^*) is globally asymptotically stable in the positive orthant.

PROOF. Consider the change of variables s, x, $z = ux + \alpha x + s$. The system (14) becomes:

$$\begin{cases} \dot{s} = -\rho(s)x - ds + ds_{in} = f_1(x, s, z) \\ \dot{x} = \mu\left(\frac{z - \alpha x - s}{x}\right) x - dx = f_2(x, s, z) \\ \dot{z} = -dz + ds_{in} = g(z) \end{cases}$$

To prove the global asymptotic stability of the non-trivial equilibrium, we must show that the hypotheses of the lemma 14 are verified, for more details see (Lemesle, 2004).

• First, hypothesis (iii) is fulfilled using proposition 13 and remark 8.

• The stationary point $z^* = s_{in}$ for the system $\dot{z} = g(z)$ is globally asymptotically stable. Then hypothesis (i) holds.

• Consider the two dimensional system

$$\dot{\xi} = f(\xi, s_{in}),$$

with $\xi = (s, x)^t$ and $f = (f_1, f_2)^t$; we want to show that the non-trivial stationary point $\xi^* = (s^*, x^*)$ is globally asymptotically stable. Note that this system is defined for the state variables in the manifold

$$z = ux + \alpha x + s = s_{in}.$$

Next we only consider the variables x and s in this set, denoted M.

To prove the global attractivity of $(s^*, x^*) \in M$, it suffices to prove that the $\omega-$limit set of all initial conditions $(s^0, x^0) \in M$ is reduced to (s^*, x^*).

• Firstly let us remark that the washout point cannot be reached using the same arguments that in remark 8. Thus $(s_{in}, 0)$ is not in the $\omega-$limit set.

• According to the Poincaré - Bendixson theorem (Guckenheimer and Holmes, 1983; Hirsch and Smale, 1974), as the positive orbit beginning at (s^0, x^0) is bounded, the $\omega-$limit set of $(s^0, x^0) \in M$ either contains the non-trivial equilibrium or reduces to a closed orbit.

• We can rule out the second case by Dulac criterion (Hirsch and Smale, 1974). This entails showing that

$$\frac{\partial h f_1}{\partial x} + \frac{\partial h f_2}{\partial s} < 0$$

where $h(x, s) = \frac{1}{x}$, a real $\mathcal{C}^1$ function defined on $\{x > 0\} \subset \Omega$. We obtain for $\frac{\partial h f_1}{\partial x} + \frac{\partial h f_2}{\partial s}$:

$$-\rho'(s) - \frac{d}{x} + \frac{s - s_{in}}{x^2}\mu'\left(\frac{s_{in} - \alpha x - s}{x}\right) \tag{16}$$

since the variables are on the manifold M

$$ux + \alpha x + s = s_{in} \Rightarrow s - s_{in} = -ux - \alpha x \leq 0$$

Thus the Dulac criterion (16) is uniformly negative. We can conclude that the $\omega-$limit set of all initials conditions (s^0, x^0) contains the non trivial stationary point. This point is locally stable, so it cannot be an homoclinic orbit; thus, the $\omega-$limit set is reduced to this point and the global attractivity of the non-trivial point is proven. Using the the local stability, we prove the global asymptotic stability of the non-trivial equilibrium. Then hypothesis (ii) of lemma 14 is fulfilled.

Thus applying the lemma 14, the non-trivial equilibrium (s^*, r^*, x^*) is globally asymptotically stable. □

5 Comparison with the DEB model

(Hanegraaf, 1997; Kooi and Kooijman, 1994) built a model taking into account the dynamic energy budget theory coupled with mass balance modelling and are able to obtain the Droop model after simplification. In a continuous culture, the following model is obtained:

$$\dot{s} = -\mu_m \frac{sx}{k+s} - ds + ds_{in}$$

$$\dot{e} = \nu\left(\frac{s}{k+s} - e\right)$$

$$\dot{x} = \left(\frac{\nu e - mg}{e+g} - d\right) x$$

To make a comparison with our model, we have changed the authors notation: s is the substrate concentration, x the biomass concentration, e the scaled energy density. Moreover, to simplify we have denoted $[\dot{I}_m] = \mu_m$, ν the specific energy conductance, g the cost for growth and m the metabolic maintenance cost. The description of micro-organisms growth depends on the energy density and not on the intracellular quota as in the Droop model.

Thus this model is based on a mechanistic variable (the energy) as in our model. Furthermore, it becomes the Droop model when there are no costs for maintenance ($m = 0$), taking $q - q_m = Ce$ ($C = \frac{\mu_m}{\nu}$) and $g = \frac{q_m \nu}{\mu_m}$ (see (Kooi and Kooijman, 1994)). Indeed, under these assumptions, the DEB model becomes:

$$\dot{s} = -\mu_m \frac{sx}{k+s} - ds + ds_{in}$$

$$\dot{q} = \mu_m \frac{sx}{k+s} - \nu(q - q_m)$$

$$\dot{x} = \nu \frac{q - q_m}{q} - dx$$

We recognize the classical Droop model (3) and a particular form of our model (11), choosing the following particular specific growth rate functions: $\mu(q - q_m) = \nu\left(1 - \frac{q_m}{q}\right)$ and $\rho(s) = \mu_m \frac{s}{k+s}$. Hence the energy density can be seen as the difference between the intracellular quota and the minimum intracellular quota necessary for the cell to grow. This point of view is particularly interesting and seems to conform with the biology.

What are the main differences between the DEB model and ours? Our model seems to give more information on the dynamics of the minimum intracellular quota q_m, which is fixed in the DEB model as in the Droop one. Moreover, let us remark that we do not specify growth and uptake functions analytically and we have a general form of the variable yield model. Indeed, with the DEB approach, a particular version version of the Droop model (*i.e.* $\mu(q - q_m) = \nu(1 - \frac{q_m}{q})$) is obtained. Furthermore, our approach gives the structural variable yield model for any value of the parameters: we do not need to assume additional relations between these constant terms (*i.e.* g is constrained in the DEB model).

Nevertheless, let us remark that the DEB theory is a very general approach to model biological processes; our approach is very specific to phytoplankton growth. Further investigation will be necessary to make a complete comparison with this model, particularly taking into account the premature mortality of the cell; moreover, comparison using simulations or experimental data in both approaches could be a good way to determine their main differences.

6 Simulations

In these simulations, we show our model (Fig. 1) and the Droop one (Fig. 2). For our model, we take $\alpha = 0.5$ (*i.e.* the limit of the minimum intracellular quota), $\rho(s) = \frac{s}{15+s}$, $\mu(\frac{r}{x}) = \frac{r}{x+r}$, $s_{in} = 10$, $d = 0.1$ and $m = 0$. Moreover, we take particular initial conditions: in dash line, we choose $b_0 = 0.5x_0$ (steady state of $\frac{b}{x}$); in dash-dot line, we choose $b_0 = 0.2x_0$ meaning that there is a lack of intracellular nutrient ($0.2 < \alpha = 0.5$ the limit of the minimum intracellular quota); in solid line, we choose $b_0 = 2x_0$ meaning that the cell is in good conditions to grow ($2 > \alpha = 0.5$).

For the Droop model, we take the same analytical form for $\rho(s)$, the same values for s_{in} and d; we choose $q_m = 0.5$ the minimum intracellular quota and $\mu(q - q_m) = \frac{q-0.5}{1+q-0.5}$. We choose the same corresponding values for the initial conditions.

First, let us remark some qualitative properties highlighted in the simulations of our model (see Fig. 1). When there is a lack of intracellular nutrient (dash-dot line), the convergence speed is the lowest. Indeed, a nutrient-deficient cell needs more time to make sufficient stored nutrient to be within growth conditions. Moreover, we obtain more information on the dynamics of the minimum intracellular quota. When we begin at the steady state, this variable remains constant since the variable $\frac{b}{x}$ is decoupled from the others. We can see also the lower convergence rate of the quota $\frac{r+b}{x}$ when there is a lack of intracellular nutrient (dash-dot line).

Compare now the simulations of our model to the Droop ones (see Fig. 2). The qualitative behaviour of both models Fig. 2 is the same. We can see than the biomass equilibrium (x) is the same for both. Indeed, in both models, the intracellular carbon is described. When there is a lack of intracellular nutrient ($q_0 < 0.5$), the convergence rate of the intracellular quota q is lower than the quota $\frac{r+b}{x}$ (see Fig. 1, $\frac{r_0+b_0}{x_0} < 0.5$). But when the cell is in good conditions to grow ($q_0 > 0.5$), the convergence rate of q is faster than $\frac{r+b}{x}$. Then, for nutrient-deficient cell our model seems to be better than Droop's one.

7 Conclusion

The classical unstructured Monod model is not able to explain all biological observations of continuous cultures of phytoplankton cells. Various modifications have been proposed to improve its accuracy by introducing new descriptive variables: for example, structuration as in the Droop model. This structure has

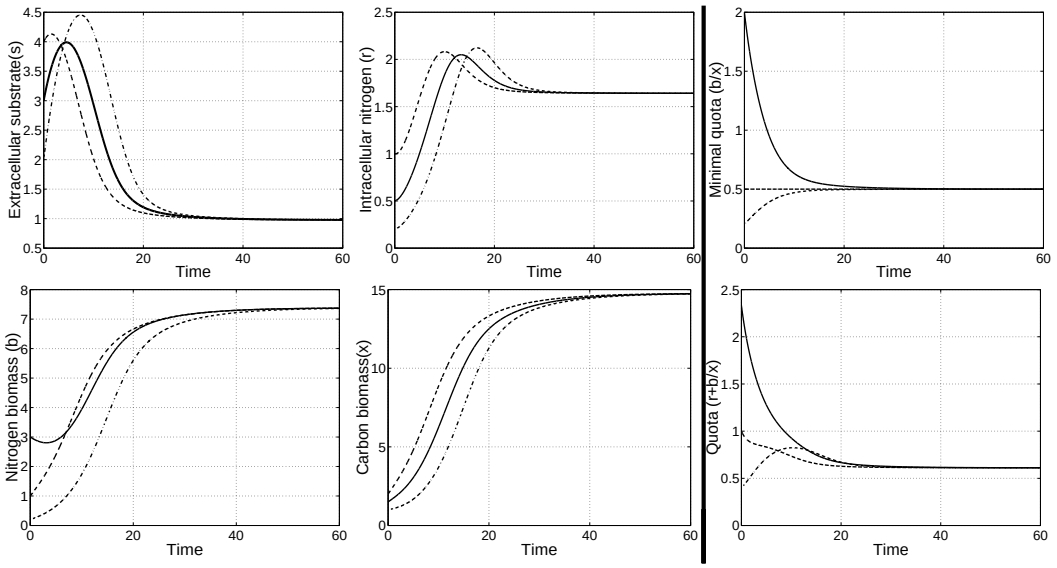

Fig. 1. Left tabular part: Extracellular nitrogen, stored nitrogen, nitrogen biomass, carbon biomass without mortality $m = 0$. Right tabular part: Minimum quota $\frac{b}{x}$. Quota $\frac{r+b}{x}$ without mortality $m = 0$.

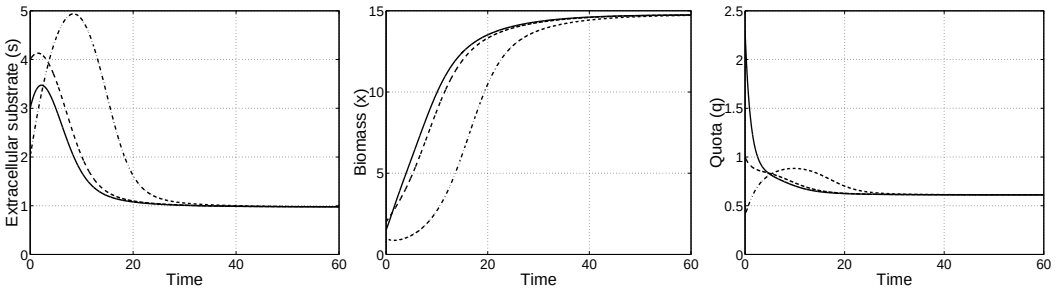

Fig. 2. Droop model: substrate, biomass, intracellular quota.

been proposed to fit biological data and has introduced an intracellular quota. In this paper a different approach has been considered. The new structured model is built on biochemical mechanisms describing stored and metabolized nutrient. Moreover, cell respiration and possible cell mortality, due to premature death of some cells, are taken into account. Thus we obtain a descriptive model using biological variables. However, if Droop's experimental conditions are considered (equilibrium experimental conditions, no mortality rate), it should be noted that the same formulation as for the intracellular quota is obtained. Indeed, since the goal of our model is to represent biological phenomena, we are able to find under some assumptions, the variable yield model, constructed to fit steady state data only. Since this structured model contains more information (*e.g.* we consider the dynamics of the minimum intracellular quota) than the Droop one, no contradictions exist between the two.

The mathematical study of this model demonstrate the global asymptotic stability of the non-trivial equilibrium under hypotheses depending on the as-

sumed cell mortality. Finally, comparison with the dynamics energy budget based (DEB) model shows some similarities: a change of variables to recover again the Droop model, the mechanistic approach and the description of the premature cell mortality. However our model seems to give more information on the dynamics of the minimum intracellular quota and does not assume precise analytical forms for the growth and uptake functions.

Acknowledgements

The authors gratefully acknowledge the very constructive comments of the reviewers, and thank L. Mailleret (Nice University, France) for several very interesting discussions on the subject.

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