Transient behavior of biological loop models, with application to the Droop model

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Abstract. In this paper we study the transient behavior of a class of non-linear differential systems verifying sign conditions, through the succession of extrema of the state variables. This analysis does not depend, for the main part, on the analytical formulation of the model. The possible scenarii of sequences for the extrema are represented on a graph, and can be compared with the experimental data to validate the model. An application to the Droop model illustrates this method ; we obtain as a result the global stability of the equilibrium and the possible successions of the extrema.

1 Introduction

The validation of models is one of the main problems in biological modelling: how is it realistically possible to compare the results given by the model and those given by experiments ? In fact, the simplest method is to validate a model in a static way, i.e. at (one of) the equilibrium. It is often reasonably simple to make measures at equilibrium, by varying the inputs and measuring the outputs. Let us take, for clarity, the example of the well-known chemostat used for the growth of microorganisms([1, 2]): the biologist can vary the dilution rate or the input concentration in nutrient, wait for the equilibrium, and then measure the number of cells at equilibrium. Then he plots the data, and compares with the curves given by the model.

This validation at the equilibrium is a first step towards the validation of a model. However it is clear that it is often insufficient: one forgets the entire transient behavior of the model with respect to time. Two models of the same phenomena are often equivalent at the equilibrium; for instance, for the chemostat, the Monod model with two variables gives the same result as the more complex Droop model ([2]). But it is difficult to use the information contained in the transient behavior of the model: this behavior can be complex, because of the non-linearity of almost all biological models ; moreover, some of the parameters are often not known precisely. Some studies deal with the complete dynamical behavior of the model, but the lack of theoretical tools makes them rare when the number of state variables is greater than two. In some cases, it is possible to simplify the system by considering logical terms as approximations of non-linear functions ([3, 4]), by using singular perturbations methods, like the quasi steady-state assumption ([5, 6]), or the existence of first integrals, ... (cf. [7]). In some interesting cases, it has been possible to establish links between a graph and the behavior of the system ([8, 9]), for example for chemical systems ([10, 11]) or compartmental systems ([12]), by using the particular features of these systems and Lyapunov functions.

Of course, it is often impossible to study exhaustively the transient global behavior of a model: it is more interesting and simpler to extract some features of this behavior; these features must be easy to measure from the experiments. One of the most immediate and visible information that the biologist can extract from more or less noisy data points is the tendency of each (observed) variable, i.e. whether the variable increases or decreases, and the existence of an extremum, minimum or maximum, with respect to time. In many cases, he can also detect the convexity (or concavity) of a variable as a function of time.

In this paper, we derive a simple tool for studying in a systematic way, (for a given deterministic non-linear model described by an ordinary differential equation in n variables), the existence and sequence of the extrema of each variable with respect to time. This sequence, of course, depends on the initial conditions. It provides a set of necessary conditions that has to agree qualitatively with the data. These conditions can be complemented by specific studies giving more accurate predictions on the possible transient behavior of the model.

The basis of the method is the study of the model in the velocity space, and the study of the possible transitions between the regions of this space. The major hypotheses are the monotonicity of the interactions between the state variables; more precisely, we suppose that the off-diagonal elements of the jacobian matrix are of fixed sign in the considered domain of the state space. It is a reasonable hypothesis in biology, where the variables are very often positive and the interactions between two variables monotonous. The analysis of the sequence of extrema relies only on the signs of the jacobian matrix, and not on the analytical formulation of the functions; it can therefore be considered as qualitative, in the sense that it applies to a class of qualitatively defined models. The regions of the velocity space are represented by the nodes of a graph, and the possible transitions by the arrows of this graph. The transition between two nodes corresponds to the passage through an extremum.

This analysis could be made in the general case; but the results are simpler when the model has a loop structure, i.e. the velocity of the variable x_i depends only on x_i and x_{i+1}. We will restrict ourselves to this case, for clarity of presentation, but emphasize that the same kind of analysis is possible in the case of a full jacobian matrix. In this case, the graph is generally more complex and less exploitable. The loop structure is very frequent in biological models; many authors have, for example, studied the possibility of sustained oscillations in negative or positive feedback loop systems ([13, 14]). We will study the possible sequence of transition for such models, and take as an example a model of growth of micro-organisms in a chemostat : cell growth may be considered as the result of substrate uptake followed by its metabolic use. Both of these processes are described by the Droop model ([2]) with three variables (the external substrate, the total biomass, and the internal stock of the cell). For this model, we are able to exhibit in an exhaustive way the possible scenarii of succession of maxima or minima for the three variables (figure 14), which can be compared with the data. The figures show, for instance, that, depending on the initial state, there are at most two possible extrema for each state variable: one is a maximum, one is a minimum, and there is an extremum of another variable between the two.

Notations: For x in $\mathbf{R}^n$, we write $x > 0$ if $x_i > 0$ $(i = 1, \ldots, n)$ and $x \geq 0$ if $x_i \geq 0$ $(i = 1, \ldots, n)$. The closed non-negative orthant is $\mathbf{R}^n_+ = \{x \in \mathbf{R}^n ; x \geq 0\}$. We will also use the open positive orthant $\mathbf{P}^n = \{x \in \mathbf{R}^n ; x > 0\}$. The other orthants are defined similarly by varying the signs of the x_i.

If $V \subset \mathbf{R}^n$ is open, $h : V \to \mathbf{R}^n$ is C^1, and $x \in V$, we denote by $Dh(x)$ the jacobian $n \times n$ matrix $\partial h_i / \partial x_j(x)$.

If $V \subset \mathbf{R}^n$ is open, $h : V \to \mathbf{R}^n$ is C^1, and $x_0 \in V$, for the differential system $\dot{x} = h(x)$ ($\dot{x}$ is the derivative with respect to time t), we denote by $x(t, x_0)$ or sometimes by $x(t)$ the (maximally defined) solution in V with initial value x_0 for $t = 0$.

2 Definitions

Let Ω be the open connected domain of "biological interest" of the system in $\mathbf{R}^n$. "Biological interest" means that the solutions have a biological meaning only in Ω, but the mathematical system can be defined outside. Let us remark that, a priori, a solution of the system can leave Ω. In the following, one can often imagine Ω as the open positive orthant (the usual domain of interest in biology). It is also often interesting to study the behavior at the boundary of Ω (typically, the faces $x_i = 0$) where specific features may appear. For simplicity, we suppose that, from a mathematical point of view, the system is defined in an open domain O strictly containing Ω. We consider in O a C^1 differential system $\dot{x} = f(x)$ having a loop structure, that is:

$$\begin{cases} \dot{x}_1 = f_1(x_1, x_2) \\ \dot{x}_2 = f_2(x_2, x_3) \\ \dots \\ \dot{x}_n = f_n(x_n, x_1). \end{cases} \tag{1}$$

The graph of interactions between variables (graph of the jacobian matrix) is represented figure (1).

To simplify the notations, we will count the indices cyclically modulo n (that is, $n + 1 \equiv 1$).

Definition 1 *A system with a loop structure has monotonous interactions if each partial derivative $\partial f_i / \partial x_{i+1}(x)$ is positive or negative on Ω for $i = 1, \dots n$ (that is, the off-diagonal elements of the jacobian matrix are of fixed sign and non zero).*

For the sake of simplicity, we have supposed that the off-diagonal elements do not cancel. If one element cancels at time t, the discussion becomes more technical, but the results remain the same. If one element is always zero, the system becomes trivial.

Definition 2 *The variable x_i admits a strict extremum at time t if there exists an even $p \geq 2$ such that $x_i^{(k)}(t) = 0$ for $k < p$ and $x_i^{(p)}(t) \neq 0$.*

Definition 3 *For $x(t, x_0) \in \Omega$, let $z(t, x_0) = \dot{x}(t, x_0) = f(x(t, x_0))$. We will call the space of z the velocity space. The vector (x, z), for a given initial condition $(x_0, f(x_0))$ with $x_0 \in \Omega$, is solution of the differential system:*

$$\begin{cases} \dot{x} = f(x(t, x_0)) \\ \dot{z} = Df(x(t, x_0))z(t, x_0) \end{cases} \tag{2}$$

Remark: A strict extremum of x_i corresponds to intersecting and crossing the face $z_i = 0$ in the z-space.

Definition 4 *In the z-space, a closed orthant is said transient if, for all $x_0 \in \Omega$ but a set of measure zero, every solution $z(t, x_0)$ starting in this orthant eventually leaves this orthant or $x(t, x_0)$ leaves Ω. An orthant is said final if there exists a set of non-zero measure of solutions $z(t, x_0)$ with $x(t, x_0) \in \Omega$ that remains in this orthant.*

3 Analysis of the extrema

For brevity, let us denote by d_i the diagonal elements of the jacobian matrix of f, and by s_i the off-diagonal elements. We have therefore, considering the structure of (1):

$$\dot{z}_i = d_i z_i + s_i z_{i+1} \quad (i = 1 \dots n) \tag{3}$$

The fixed signs of the s_i in Ω enable us to determine the possible transitions between the orthants of the z-space.

Lemma 1

- *If $s_i > 0$, then:*
  - *If $z_{i+1}(t) > 0$, the only possible sign transition for z_i at time t is from an orthant where $z_i < 0$ to an orthant where $z_i > 0$. The variable x_i admits thus a strict minimum at time t.*
  - *If $z_{i+1}(t) < 0$, the only possible transition for z_i at time t is from an orthant where $z_i > 0$ to an orthant where $z_i < 0$. The variable x_i admits thus a strict maximum at time t.*
- *If $s_i < 0$, one has to reverse all the above inequalities.*

Indeed, when z_i crosses a face $z_i = 0$ in the z-space, we have:

$$\dot{z}_i = s_i z_{i+1}$$

and if $s_i > 0$ and $z_{i+1} > 0$, then z_i increases, and the only possible transition is from negative to positive.

This lemma applied to the z_i gives a set of constraints determining the possible transitions between the orthants of a trajectory in the z-space.

Lemma 2 *A trajectory in the z-space cannot cross simultaneously two consecutive faces $z_i = 0$ and $z_{i+1} = 0$.*

Proof: If $z_i = 0$ for all i, then we are at an equilibrium. We can therefore suppose that there exists some j and some k such that:

$$z_k \neq 0, z_{k+1} = 0, \dots, z_{j-1} = 0, z_j \neq 0$$

for a given time t. But because of the equations (3), we have:

$$\dot{z}_{k+1} = 0, \dots, \dot{z}_{j-2} = 0, \dot{z}_{j-1} = s_{j-1} z_j \neq 0$$

We can deduce that the face $z_{j-1} = 0$ is crossed. To see how we intersect the remaining faces, we have to compute the second derivative $z^{(2)}$ at time t. We obtain for z_{j-2}:

$$z_{j-2}^{(2)} = \dot{s}_{j-2} z_{j-1} + s_{j-2} \dot{z}_{j-1} = s_{j-2} s_{j-1} z_j$$

therefore the second time derivative at time t of z_{j-2} is different from zero, and the trajectory in the z-space is tangent to the face but does not cross it. The reasoning can be pursued for z_{j-3}, by showing that the second time derivative cancels, but not the third; so we cross the face .... and so on until we attain the index k. We conclude that it is impossible to cross two consecutive faces.

Corollary 1 *In dimension three, it is impossible to cross simultaneously two (or three) faces.*

Of course, for a dimension greater than three, it is possible that a trajectory in the z-space crosses simultaneously two non-consecutive faces or more. Let us remark that we are interested by the modelling of biological systems, subject to noise and perturbations. It is thus reasonable, from a practical point of view, to consider that almost every trajectory never intersects two faces at the same time, and to reject the set of trajectories (of measure zero) not satisfying this constraint. We will proceed this way in the rest of the paper.

Lemma 3 *Lemma of ejection: If the state variables x_i are bounded, then every trajectory of the z-space starting in one closed orthant eventually leaves this orthant or goes towards the equilibrium (in the z-space) $z = 0$.*

By bounded, we mean that $x(t, x_0)$ stays in O and is bounded (the bounds may depend on x_0). Now the proof is simple: indeed, in each closed orthant of the z-space, the z_i have a fixed sign or cancel, and therefore the x_i are monotonous; because they are bounded with their bounds in O, they have to converge towards an equilibrium if they stay in this orthant; let us remark that the x-trajectory may also leave the domain Ω (the z-space is only defined for $x(t, x_0) \in \Omega$).

Remark 1: For simplicity, we will suppose in the rest of this paper that the variables are bounded in O (the bounds may depend on the initial conditions); it is a rather reasonable hypothesis for biological systems.

Remark 2: If the sign of some of the d_i in (3) is known in the domain Ω, then it is possible to obtain complementary information on the curvature of $x_i(t)$ in some orthants of the z-space. Let us take an example: if for some j , $d_j > 0$ and $s_j < 0$, then in the set of orthants such that $z_j > 0$ and $z_{j+1} < 0$, we compute that $\dot{z}_j > 0$ and deduce that in this set of orthants, $x_j(t)$ is a convex function of time.

Lemma 4 *It is impossible for a trajectory to stay inside a face $z_i = 0$, except at the equilibrium $z = 0$.*

Indeed, if a trajectory belongs to $z_i = 0$ for $t \in [t_0, t_1]$, $t_0 < t_1$, then all the derivatives of z_i cancel in this interval. But $\dot{z}_i = 0 = s_i z_{i+1}$ implies that $z_{i+1} = 0$; then (see lemma 2) $\dot{z}_i^{(2)} = 0 = s_i s_{i+1} z_{i+2}$ implies that $z_{i+2} = 0$ and so on; finally it proves that $z = 0$.

Lemma 5 *Suppose that $d_i > 0$ in Ω. For a given i, let S_i be the set of orthants of the z-space such that $d_i z_i$ and $s_i z_{i+1}$ have the same sign. Then the set S_i is transient.*

Proof: Then z_i and $\dot{z}_i = d_i z_i + s_i z_{i+1}$ have the same sign. But in an orthant of S_i, a trajectory cannot converge towards the equilibrium $z = 0$, because if z_i is positive, it is also increasing, and if z_i is negative, it is also decreasing ; moreover, because of the preceding lemma, z_i cannot cancel on a time interval. Because of the lemma of ejection, this orthant is transient.

Lemma 6 *Lemma of local behavior: Suppose that there exists an asymptotically stable equilibrium x^* and that, at this point, the slowest eigenvalue λ is real, of multiplicity one, and has an eigenvector v_λ with no zero components. Then for every trajectory in the z-space associated with an initial condition in the basin of attraction of x^*, the corresponding final orthants in the z-space contain v_λ.*

By the slowest eigenvalue, we mean the closest eigenvalue to the imaginary axis. Let us take a trajectory in the x-space converging towards the equilibrium x^*. Then, except for the trajectories belonging to the other stable eigenspaces (this set is of measure zero), this trajectory will converge asymptotically along the eigenvector v_λ of the slowest eigenvalue. Because all its components are non-zero, v_λ belongs to a set of two orthants in the z-space. Of course, because our system is defined only qualitatively (we have not specified the analytical expression of the velocity), the eigenvector can belong to different orthants, depending on this analytical expression and on the value of the parameters.

Graphic representation of the possible transitions:

Each closed orthant of the z-space is represented by a vector of signs; graphically, it is the node of a graph. For instance, the orthant in $\mathbf{R}^3$ ($z_1 \geq 0, z_2 \geq 0, z_3 \leq 0$) will be represented by the node on fig. (2).

The possible transition between two orthants is represented by an arrow between the two corresponding nodes. For more clarity, the letter m_i or M_i above the arrow between two nodes will denote the transition by a minimum or a maximum of x_i. For a loop system with monotonous interactions like (1), the only possible transitions are the following (cf. Lemma 1):

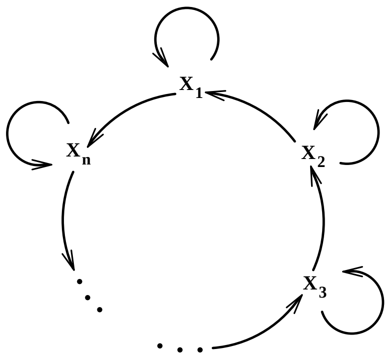

Figure 1: Graph of interactions for loop models

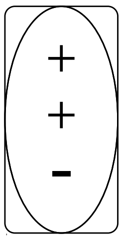

Figure 2: Representation of the $(z_1 \geq 0, z_2 \geq 0, z_3 \leq 0)$ orthant

- for $s_i < 0$

$$\begin{array}{c} \\ i \\ i{+}1 \\ \\ \end{array} \begin{pmatrix} \vdots \\ + \\ + \\ \vdots \end{pmatrix} \xrightarrow{M_i} \begin{pmatrix} \vdots \\ - \\ + \\ \vdots \end{pmatrix} \quad \text{and} \quad \begin{array}{c} \\ i \\ i{+}1 \\ \\ \end{array} \begin{pmatrix} \vdots \\ + \\ - \\ \vdots \end{pmatrix} \xleftarrow{m_i} \begin{pmatrix} \vdots \\ - \\ - \\ \vdots \end{pmatrix}$$

- for $s_i > 0$

$$\begin{array}{c} \\ i \\ i{+}1 \\ \\ \end{array} \begin{pmatrix} \vdots \\ + \\ + \\ \vdots \end{pmatrix} \xleftarrow{m_i} \begin{pmatrix} \vdots \\ - \\ + \\ \vdots \end{pmatrix} \quad \text{and} \quad \begin{array}{c} \\ i \\ i{+}1 \\ \\ \end{array} \begin{pmatrix} \vdots \\ + \\ - \\ \vdots \end{pmatrix} \xrightarrow{M_i} \begin{pmatrix} \vdots \\ - \\ - \\ \vdots \end{pmatrix}$$

We define moreover particular types of orthants that we associate graphically with different kind of nodes:

- transient orthants (see the definition 4 and fig. (3))
- final orthants (see the definition 4 and fig. (4))
- sink orthants (a positively invariant orthant, i.e. every solution starting in this orthant in the z-space stays in it)(fig. (5))
- source orthants (a negatively invariant orthant, i.e. a positively invariant orthant for the time reversed system)(fig. (6))
- unreachable orthants : there is no initial condition starting in this orthant (not represented on the graph)
- we will also indicate if (Remark 2), in a given orthant, the variable x_i is convex (concave) by a $\smile$ ($\frown$) upon the sign of the variable. The figure (7) shows an example when x_1 is convex and x_3 is concave.

The graph will be called the transition graph. A cycle is a closed oriented path in the graph. The finite set of all possible transition graphs when varying the signs of the s_i will be called the transition graph family. Even without knowing these signs, this family puts some constraints on the possible sequences of extrema; for instance, for a member of this family, if the transition is possible

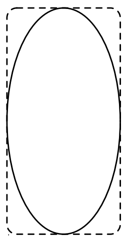

Figure 3: Representation of transient orthants

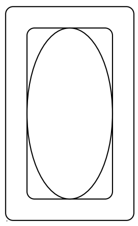

Figure 4: Representation of final orthants

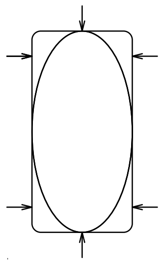

Figure 5: Representation of positively invariant orthants

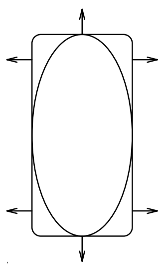

Figure 6: Representation of negatively invariant orthants

from the orthant i to the orthant j, then it is impossible from j to i; it can act as a first filter for experimental data (cf. conclusion).

We can now state some theorems making relationship between the graph of transition in the z-space and the dynamical behavior of the system. We still suppose that all the state variables are bounded in O.

Theorem 1 *If there is no cycle in the graph, then almost any trajectory (in the x-space or the z-space) converges towards an equilibrium or leaves Ω.*

Corollary 2 *If moreover Ω is positively invariant, then every trajectory converges towards an equilibrium in the closure of Ω.*

Corollary 3 *The system can have stable sustained oscillations in Ω only if the graph of transition has a cycle.*

Proof of the theorem: The lemma of ejection tells us that, in the z-space, if a trajectory does not go towards $z = 0$, it does not remain in the orthant. But because there is no cycle in the graph, the trajectory (if it does not leave Ω) will be locked in a (final) orthant, where it will go towards an equilibrium. We still exclude the trajectories intersecting simultaneously several faces.

Moreover, if the system verifies the hypotheses of the lemma of local behavior, then we can know the final orthants; all the other orthants are transient. The trajectory in the x-space admits a finite number of maxima and minima, and we can describe their sequences. These results do not depend on the analytical formulation of the system.

It is not yet excluded that some solutions go towards an equilibrium on the boundary of Ω. Because we wish to establish results of global stability for the interior equilibria, we will make some hypotheses on the stability of the boundary equilibria. For simplicity, we suppose that Ω is positively invariant.

Lemma 7 *Suppose that the equilibria on the boundary are isolated, hyperbolic and unstable; then, if there is no cycle in the graph, almost any trajectory converges towards an equilibrium in Ω.*

Indeed, any trajectory not belonging to the local stable manifold of an equilibrium on the boundary has to converge in Ω or to leave the orthant according to the lemma of ejection. As there is no cycle in the graph, it is eventually locked in a final orthant. Of course, if the stable manifold of each boundary equilibrium does not intersect Ω, then every trajectory converges in Ω. As before, we have rejected the set of trajectories intersecting simultaneously several faces.

We are able now to give a result on global stability:

Theorem 2 *Suppose that the equilibria on the boundary are isolated, hyperbolic and unstable and that there exists a single stable equilibrium in Ω ; then, if there is no cycle in the graph, almost every trajectory converges towards this equilibrium.*

Corollary 4 *Under the hypotheses of the above theorem and if the equilibrium point verifies the hypotheses of the lemma of local behavior, then the orthants in the z-space not associated with the slowest eigenvector v_λ are transient for almost every trajectory.*

Corollary 5 *Suppose that all the equilibria of the closure of Ω are isolated, hyperbolic and unstable; if there is a cycle in the graph, then almost every trajectory exhibits sustained oscillations.*

Moreover, we are able to describe the possible sequences of oscillations by the transition graph.

4 Study of an example : the Droop Model

4.1 Introduction

We take now a well known model as an example ; this model is given by an analytical formulation, but it is clear that we are studying, in fact, a qualitatively defined class of models to which this particular model belongs. This class of models has a graph of interactions given fig.(8).

Cell behavior in a chemostat, with growth controlled by a limiting nutrient, is described by the Droop model developed by Droop ([15]) and Burmaster ([2]). We call Q the concentration of nutrient per biomass unit, S the concentration of limiting substrate, and X represents the concentration of biomass in the chemostat. The model represented as a set of nonlinear ordinary differential equations has the following form :

$$
\left\{
\begin{array}{l}
\dot{S}(T) = D[S_i - S(T)] - \rho_{max} \dfrac{S(T)X(T)}{K_\rho + S(T)} \\
\dot{X}(T) = \mu_m (1 - \dfrac{K_Q}{Q(T)}) X(T) - DX(T) \\
\dot{Q}(T) = \rho_{max} \dfrac{S(T)}{K_\rho + S(T)} - \mu_m (Q(T) - K_Q)
\end{array}
\right. \tag{4}
$$

ρ_{max} is the maximum uptake rate of nutrient and K_ρ the associated half-saturation constant. μ_m is the hypothetical maximum growth rate of cells, K_Q represents the minimum cell quota. The dilution rate D of the chemostat is defined by the flow rate through the chemostat over the volume of the chemostat. We will limit this study to the parametric region that is of interest to the biologist, i.e. with a non trivial stable equilibrium. Therefore we will suppose that $D < \mu_m$;this means that the culture of microorganisms is not systematically wiped out.

Lange and Oyarzun ([16]) studied this model and proposed the following change of variables which leads to nondimensional variables,

$$
t = DT \quad x_1 = \tfrac{S}{S_i} \quad x_2 = \tfrac{\rho_{max} X}{D S_i} \quad x_3 = \tfrac{Q}{K_Q} \tag{5}
$$

Let us denote by

$$
a_1 = \tfrac{K_\rho}{S_i} \quad a_2 = \tfrac{\mu_m}{D} \quad a_3 = \tfrac{\rho_{max}}{D K_Q} \tag{6}
$$

Remarks:

- According to the hypothesis on D we have $1 < a_2$.
- According to the definition of K_Q, Q must be greater than K_Q to have a biological meaning. It means that the biologist has to choose $x_3(0) \geq 1$. We will show later that in this case, $x_3(t) \geq 1$ for all $t \geq 0$.
- This change of variables preserves the extrema.

The Droop equations now become:

$$
\left\{
\begin{array}{l}
\dot{x}_1(t) = 1 - x_1(t) - \dfrac{x_1(t)x_2(t)}{a_1 + x_1(t)} \\
\dot{x}_2(t) = a_2 (1 - \dfrac{1}{x_3(t)}) x_2(t) - x_2(t) \\
\dot{x}_3(t) = a_3 \dfrac{x_1(t)}{a_1 + x_1(t)} - a_2 (x_3(t) - 1)
\end{array}
\right. \tag{7}
$$

For clarity of notation we will now denote x for $x(t)$.

5 Basic properties of the Droop model

Property 1 *The open domain $\Omega = \{x \in \mathbf{R}^3; x_1 > 0, x_2 > 0, x_3 > 1\}$ is positively invariant.*

Proof:

If $x_1 = 0, \dot{x}_1 = 1 > 0$ and therefore the face $x_1 = 0$ is repulsive,

If $x_2 = 0, \dot{x}_2 = 0$, the face $x_2 = 0$ is invariant, and then the domain $\{x \in \mathbf{R}^3; x_2 > 0\}$ is positively invariant,

If $x_3 = 1, \dot{x}_3 = a_3 \frac{x_1}{a_1+x_1} > 0$ so that $x_3 = 1$ is repulsive.

Remark: Because it is not interesting to take initial conditions outside the open domain Ω, we shall take this open positively invariant domain for our domain of biological interest.

Property 2 *The Droop model is a loop structured model with monotonous interactions in the open domain Ω.*

Proof: The jacobian matrix is given by :

$$\begin{pmatrix} -1 - \frac{a_1 x_2}{(a_1+x_1)^2} & -\frac{x_1}{a_1+x_1} & 0 \\ 0 & a_2(1-\frac{1}{x_3}) - 1 & a_2 \frac{x_2}{x_3^2} \\ \frac{a_1 a_3}{(a_1+x_1)^2} & 0 & -a_2 \end{pmatrix} \tag{8}$$

which is of the following signs in Ω (* means that the sign can change over Ω):

$$\begin{pmatrix} - & - & 0 \\ 0 & * & + \\ + & 0 & - \end{pmatrix} \tag{9}$$

Property 3 *If u represents the total nutrient concentration in the chemostat divided by the concentration of inflow nutrient,*

$$u = x_1 + \frac{x_2 x_3}{a_3} \tag{10}$$

then

$$\dot{u} = 1 - u. \tag{11}$$

This simple law - easy to verify - describing total concentration of nutrient in the chemostat was first identified by Burmaster ([2]).

Corollary 6 *Depending on initial conditions u is increasing or decreasing, and is bounded between the initial value $u(0) = x_1(0) + \frac{x_2(0)x_3(0)}{a_3}$ and 1.*

Property 4 *x_3 is bounded by $1 \leq x_3 \leq max(x_3(0), 1 + \frac{a_3}{a_2})$, x_1 and x_2 are also bounded.*

See Lange and Oyarzun ([16]) to find a justification of this property.

6 Study of the transition graph

6.1 The transition graph

Applying the theory developed in the first part, with the jacobian matrix (9) we obtain the basis graph of transition (figure 9).

This graph depends only on the sign of the extradiagonal terms s_i of the jacobian matrix, and not on their precise formulation. The biologist can therefore use this graph as a first filter to test if the basis structure of his model matches the experiments. He can indeed compare the sequences of maxima and minima he has obtained experimentally with the possible sequence in the graph.

To obtain complementary information on the previous graph, we will now use more specific features of the model.

6.2 Unreachable orthants

We have seen that $u = x_1 + \frac{x_2 x_3}{a_3}$ is a monotonous function of time and, depending on initial conditions, is either increasing or decreasing. When u increases the orthant $\begin{pmatrix} - \\ - \\ - \end{pmatrix}$ is unreachable due to the fact that $\dot{u} = z_1 + z_2 \frac{x_3}{a_3} + z_3 \frac{x_2}{a_3} > 0$. Analogously, the orthant $\begin{pmatrix} + \\ + \\ + \end{pmatrix}$ is unreachable for $\dot{u} < 0$. The basis graph reduces now to two transition graphs (cf figure 10 and figure 11), depending on the initial conditions on u.

6.3 Stability of the Droop model

The transition graphs show that cycling is not permitted, for the graphs contain no cycle. The domain Ω is positively invariant, thus according to theorem (2) the trajectories have to go toward an equilibrium in the closure of Ω.

Lange and Oyarzun ([16]) have shown that depending on parametric values we can have one stable equilibrium or two equilibria, one stable and one unstable, as shown in table (1).

In the case where there exist two equilibria, the equilibrium on the boundary is unstable, and theorem (2) proves finally that the stable equilibrium is globally stable for the qualitatively defined class of models having the Droop model as an element.

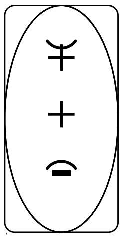

Figure 7: Representation of convexity or concavity

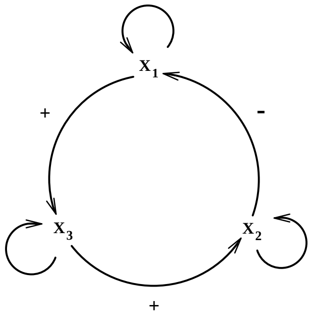

Figure 8: Graph of interactions for the Droop model.

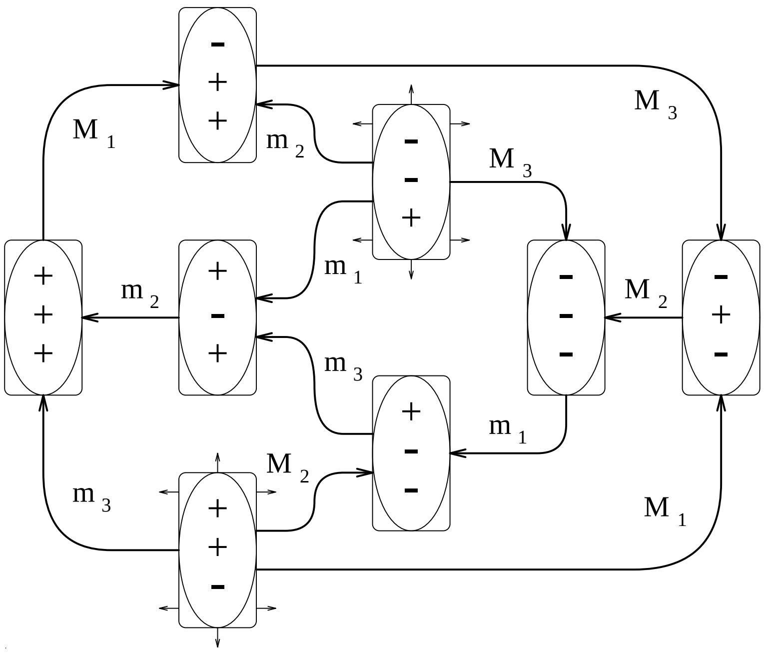

Figure 9: Basis transition graph

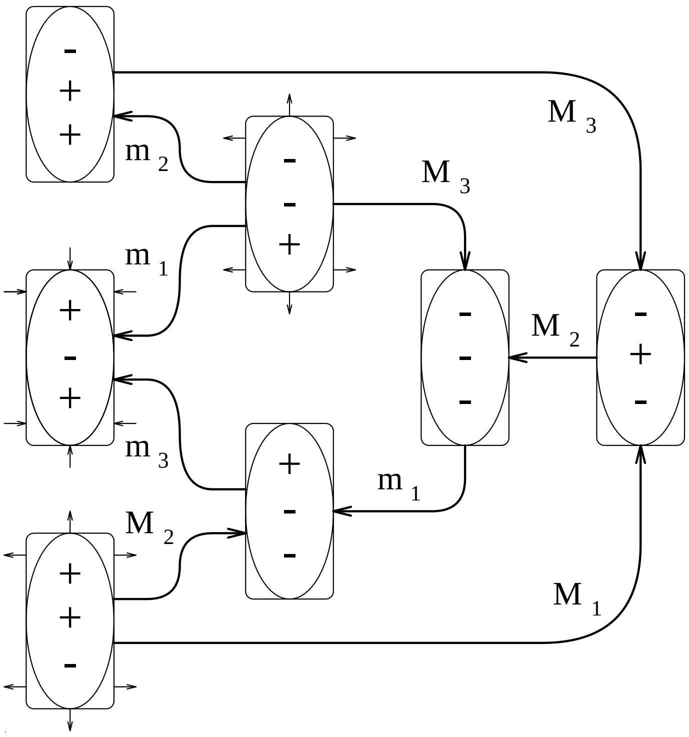

Figure 10: Transition graph for $\dot{u} < 0$

parametric region	$a_2a_3 - a_2 - a_3 - a_1a_2 \leq 0$	$a_2a_3 - a_2 - a_3 - a_1a_2 > 0$
stable equilibrium	$\begin{cases} x_1^* = 1 \\ x_2^* = 0 \\ x_3^* = \frac{a_2+a_3+a_1a_2}{a_2(a_1+1)} \end{cases} \quad (12)$	$\begin{cases} x_1^* = \frac{a_1a_2}{a_2a_3-a_2-a_3} \\ x_2^* = \frac{(a_2a_3-a_2-a_3-a_1a_2)(a_2-1)a_3}{a_2(a_2a_3-a_2-a_3)} \\ x_3^* = \frac{a_2}{a_2-1} \end{cases} \quad (13)$
unstable equilibrium	/	$\begin{cases} x_1^\diamond = 1 \\ x_2^\diamond = 0 \\ x_3^\diamond = \frac{a_2+a_3+a_1a_2}{a_2(a_1+1)} \end{cases} \quad (14)$

Table 1: Equilibria of the Droop model

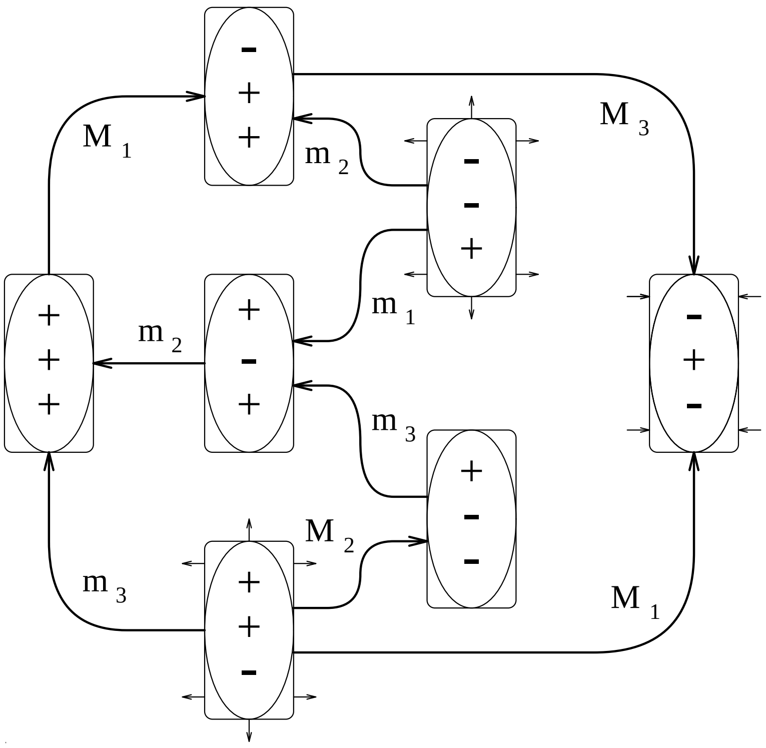

Figure 11: Transition graph for $\dot{u} > 0$

From now on we will focus our attention to the non trivial stable equilibrium (13) in the case $a_2a_3 - a_2 - a_3 - a_1a_2 > 0$.

6.4 Information derived from the signs of diagonal terms

- **Concavity and convexity**

  Since $d_1 < 0$ and $d_3 < 0$ from remark (2) we know that

  - For the two orthants $\begin{pmatrix} - \\ - \\ * \end{pmatrix}$, x_1 is convex. In the symmetric orthants it is concave.
  - In the same way, for the two orthants $\begin{pmatrix} + \\ * \\ - \end{pmatrix}$, x_3 is convex and in the symmetric orthants it is concave.
  - Remark that d_2 and z_2 are of the same sign. Therefore in the four orthants $\begin{pmatrix} * \\ * \\ + \end{pmatrix}$, x_2 is convex.

- **Transient orthants**

  Remark that $d_2 > 0$ when $z_2 > 0$. Thus the lemma (4) demonstrates that the two orthants $\begin{pmatrix} * \\ + \\ + \end{pmatrix}$ are transient.

6.5 Study of final orthants

The jacobian matrix of the linearized system at the equilibrium (13) is :

$$\begin{pmatrix} -1 - \frac{a_1 x_2*}{(a_1+x_1*)^2} & -\frac{x_1*}{a_1+x_1*} & 0 \\ 0 & 0 & a_2\frac{x_2*}{x_3*^2} \\ \frac{a_1a_3}{(a_1+x_1*)^2} & 0 & -a_2 \end{pmatrix} \tag{15}$$

For the commodity of notations denote by

$$a_4 = a_1\frac{x_2^*}{(a_1+x_1^*)^2} = \frac{(a_2a_3 - a_2 - a_3 - a_1a_2)(a_2a_3 - a_2 - a_3)}{a_1a_2a_3(a_2-1)} \tag{16}$$

The eigenvalues of the linearized system are $(\lambda_1, \lambda_2, \lambda_3) \in \mathbf{R}_-^{3*}$, with: $\lambda_1 = -1$, λ_2 and λ_3 are the real roots of the polynomial :

$$P(\lambda) = \lambda^2 + (a_4 + a_2)\lambda + a_4(a_2 - 1) \tag{17}$$

(We denote by λ_2 the greater of these roots so that : $\lambda_2 > \lambda_3$)

The associated eigenvectors are :

$$v_1 = \begin{pmatrix} \frac{(a_2-1)x_2^*}{a_4 a_3} \\ -\frac{(a_2-1)^2 x_2^*}{a_2} \\ 1 \end{pmatrix} \quad v_i = \begin{pmatrix} -\frac{(\lambda_i+a_2-1)a_2}{a_3(a_2-1)^2} \\ 1 \\ \frac{\lambda_i a_2}{(a_2-1)^2 x_2^*} \end{pmatrix} (i = 2, 3) \tag{18}$$

respectively.

Because of the lemma (6) of local behavior the trajectories converge asymptotically along the eigenvector associated with the slowest eigenvalue. But $\lambda_3 < \lambda_2$ therefore either λ_1 or λ_2 can be the slowest eigenvalue, depending on the numerical values of the parameters.

Assume that λ_2 is the slowest eigenvalue, the system is locally equivalent to v_2 . Let us remark that the sign of $\lambda_i + a_2 - 1$ can be found by comparing $1 - a_2$ and λ_i, the roots of P :

$$P(1 - a_2) = (1 - a_2) < 0 \tag{19}$$

Therefore $1 - a_2$ is between the roots of P and then $\lambda_2 > 1 - a_2$. Thus v_2 has the signs $\begin{pmatrix} - \\ + \\ - \end{pmatrix}$, which means that the final orthants can be either $\begin{pmatrix} + \\ - \\ + \end{pmatrix}$ or $\begin{pmatrix} - \\ + \\ - \end{pmatrix}$

In the other case where λ_1 is the slowest eigenvalue, the trajectories converge asymptotically along v_1 of the sign $\begin{pmatrix} + \\ - \\ + \end{pmatrix}$. Then the final orthants can be either $\begin{pmatrix} + \\ - \\ + \end{pmatrix}$ or $\begin{pmatrix} - \\ + \\ - \end{pmatrix}$

Nevertheless, in this case where λ_1 is the slowest eigenvalue, we can show that only one orthant is a final orthant : by applying lemma (6), there exists a non zero constant K such that around equilibrium (13) :

$$z(t) \sim K \exp(\lambda_1 t) v_1 \tag{20}$$

We have for $\dot{u}$:

$$\dot{u} = z_1 + z_2 \frac{x_3}{a_3} + z_3 \frac{x_2}{a_3} \tag{21}$$

Therefore, around equilibrium (13)

$$\dot{u}(t) \sim K \exp(\lambda_1 t) \frac{x_2^*(a_2 - 1 - a_2 a_4 + 2a_4)}{a_3 a_4} \tag{22}$$

Let us remark that $P(\lambda_1) = -(a_2 - 1 - a_2 a_4 + 2a_4)$, thus

$$\dot{u}(t) \sim -K \exp(\lambda_1 t) \frac{x_2^*}{a_3 a_4} P(\lambda_1) \tag{23}$$

λ_1 is the slowest eigenvalue i.e. $\lambda_1 > \lambda_2 > \lambda_3$ so that $P(\lambda_1) > 0$.

If $\dot{u} < 0$ because of (23) we have that $K > 0$ and then equation (20) gives us the single final orthant : $\begin{pmatrix} + \\ - \\ + \end{pmatrix}$

If $\dot{u} > 0$ the final orthant is $\begin{pmatrix} - \\ + \\ - \end{pmatrix}$

6.6 Synthesis

Depending on initial conditions, and depending on parameters we can forecast the evolution of variables x_i in terms of extrema.

The two graphs (12) and (13) summarize the behavior of the system. From these graphs we can derive all the possible behaviors of the system in the region being of interest for the biologist. These behaviors -in terms of extrema - depending on the parameter values and on the initial conditions are summarized in graph (14). This graph extracted from graphs (12) and (13) represents the possible sequence of extrema. For example, suppose at $t = 0$ for $\dot{u} > 0$, that the biologist observed a minimum of x_2. The two scenarii that the model can produce are the following

- This minimum of x_2 is followed in time by a maximum of x_1, a maximum of x_3 and then no more extremum is observed until equilibrium is reached.
- The minimum of x_2 is followed by a maximum of x_3, and no more extremum is observable until equilibrium is reached.

7 Conclusion

This tool provides us with a way to validate models, by comparing the experimental sequel of extrema with those allowed by the model. It is interesting to note that models are filtered at different levels:

- **First filter:** The experimental data do not follow any graph of the transition graph family (the family of basis graphs obtained by varying all the possible signs of s_i), therefore the model cannot have a loop structure with monotonous interactions.
- **Second filter:** The experimental data do not agree with the basis transition graph. It proves that the signs of s_i chosen in the model (i.e the signs of interactions between the state variables) are wrong (this step does not depend on analytical expression of the model).

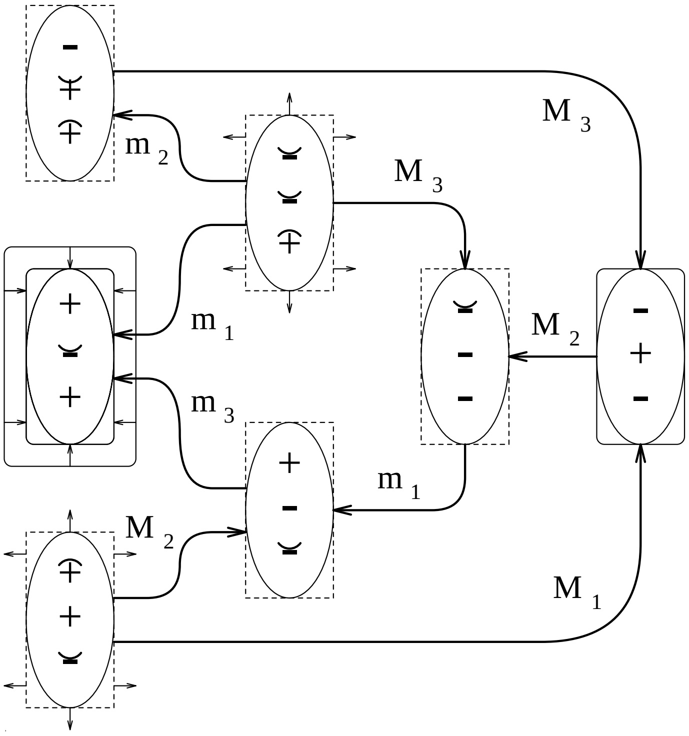

Figure 12: Final transition graph for $\dot{u} < 0$

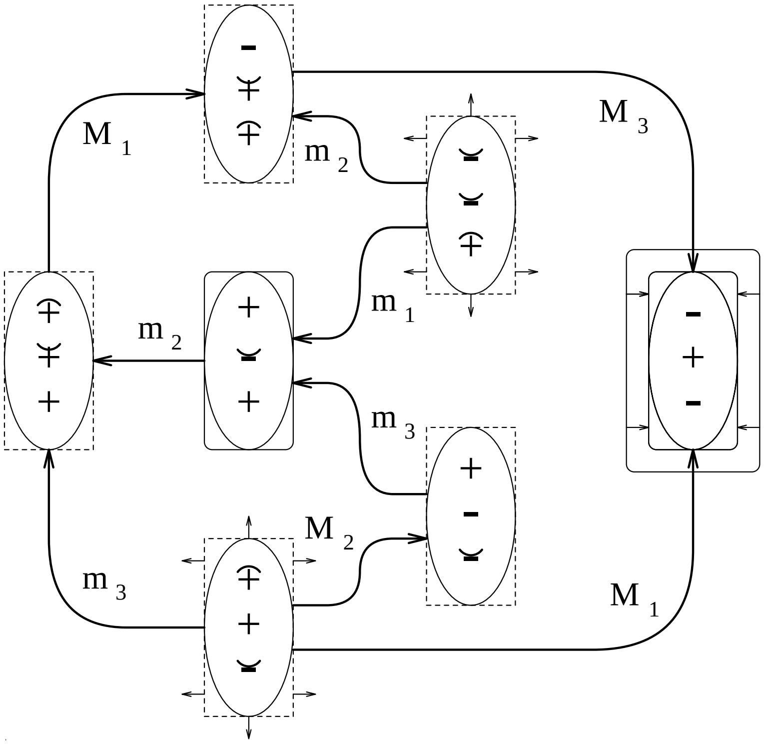

Figure 13: Final transition graph for $\dot{u} > 0$

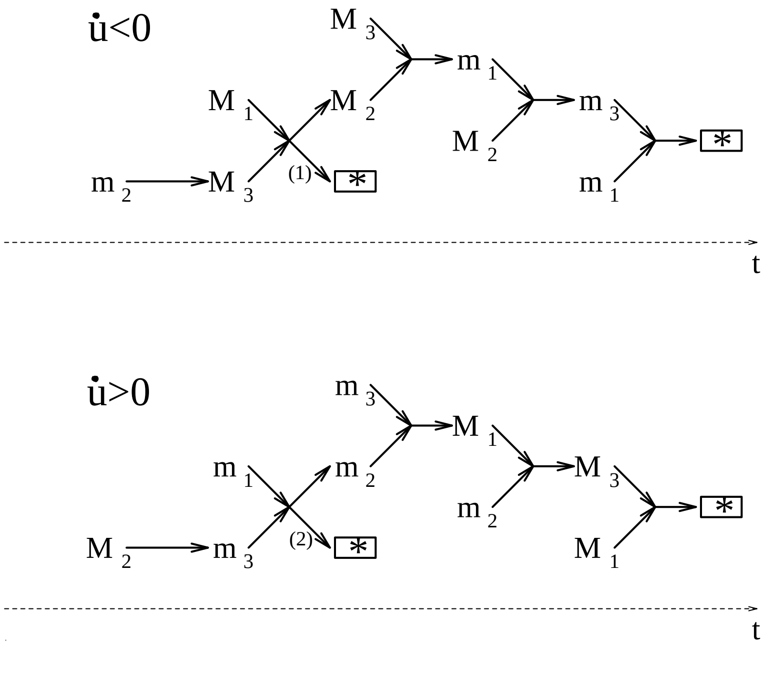

Figure 14: Summary of the possible sequences of extrema that the model can give. [*] represents the equilibrium. The paths (1) and (2) are not possible if λ_1 is the slowest eigenvalue.

- **Third filter:** In the orthants where the curvature of x_i is known, it does not match the experimental curvature of data. Then the sign of d_i is wrong (this step does not depend on analytical expression of the model).

- **Fourth filter:** The experimental data do not follow the final transition graph. One of the arguments used to simplify the basis transition graph is wrong. For example, in the Droop model, the conservation law of total nutrient is wrong if the experimental trajectories enter the unreachable orthants.

- **Fifth filter:** The forecasted final orthant(s) do not correspond with the observed data. Then the analytical expressions of the function(s) used in the model are wrong.

To validate these kind of simple models the experimentalist would have to focus his attention on the transient dynamic of the data. He would have to increase the number of experiments in order to have the maximum set of experimental scenarii to be compared with the transition graph.

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