Nonlinear qualitative signal processing for biological systems: application to the algal growth in bioreactors

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Abstract. We present in this paper a qualitative method to validate and monitor the structure of a nonlinear model with respect to experimental data, under some hypotheses. This method is broadly independent of the analytical formulation of the model, and depends only on the qualitative structure (signs of the Jacobian matrix). The temporal sequences of the extrema of a filtered experimental signal are compared with the transitions allowed by a graph. In particular, we show that the usual moving average of the outputs follows this transition graph. We apply this method to compare models of algal growth in a bioreactor (in dimension three), with experimental data.

Keywords: qualitative behavior, non linear systems, moving average, algal growth, biotechnology.

1 Introduction

The validation of models with respect to experimental data is a fundamental problem in biological modeling. In the same way, for specific systems, it is important to check on-line whether the model remains valid. For example, monitoring of biological reactors in order to detect a change of the system from its standard operating mode is one of the main problems of biotechnology [2]. But the obstacles are numerous. At first practical difficulties appear when we deal with experimental data in the biological field. The high level of noise that jam data and the low sampling rate give rise to high uncertainty. The direct comparison between a model and experimental measurements is therefore delicate; even if the parameters of the model can be obtained by identification (i.e. least squares methods [19], the decision to accept or reject the model can be difficult.

From a theoretical point of view, biological models are generally nonlinear, so that their dynamical behavior is often complex [14]. Moreover this behavior can be very sensitive to the uncertain mathematical functions used in the model, so as to the value of unknown or ill-known parameters.

In this context, the development of a qualitative tool which would be robust to the parameters uncertainties, to compare the dynamical behavior of a model with experimental data, is very important [18]. The authors have presented [4] a method which determines the possible temporal sequences of some qualitative events from considerations based on the signs of the Jacobian matrix. These qualitative features are either the extrema of state variables, or their crossing of some reference values (equilibrium, other experiments), which can be derived from experimental data.

In this paper, we extend this method on two points:

- first, we consider a differential system with inputs $u(t)$ [12], that can represent external forcing terms of the environment (light, temperature...), or controlled actions on the system (nutrient input for a bioreactor [2],...). The mathematical system is thus non-autonomous.
- secondly, if the data are very noisy, the succession of extrema is corrupted and it is difficult to differentiate extrema from noise. We show here that the above qualitative approach is robust to a mobile averaging of the signals on a time interval $[t-\tau, t]$, for any input of period τ. This qualitative signal processing (using a classical tool as the moving average, see [19]) enables to validate the current model or to detect a possible change in the structure of the system.

We illustrate this methodology by considering the growth of phytoplankton in a continuously stirred tank reactor (CSTR). We use a class of phytoplankton growth models (PGM) which describes the growth of micro algae limited by a nutrient. Experimental data on the phytoplanktonic algae *Dunaliella tertiolecta* are compared with the theoretical transient behavior of the PGM.

2 Qualitative behavior of loop-structured systems

We define the differential systems we are considering, and give the main theorems on their qualitative behavior (see [4] for details).

2.1 Definitions and notations.

Let Ω be an open convex domain of $\mathbf{R}^n$, and f a C^1 mapping on Ω into $\mathbf{R}^n$. Let $\mathcal{U}$ be an open domain of admissible C^1 controls (inputs) $u : \mathbf{R} \longrightarrow \mathbf{R}^m$.

We consider on $\Omega \times \mathcal{U}$ the differential system with input:

$$(\Sigma) \left\{ \; \dot{x}(t) = f(x(t), u(t)) \right.$$

Definition 1 *The system* (Σ) *has monotonous interactions on* $\Omega \times \mathcal{U}$ *if the sign of each partial derivative* $\partial f_i / \partial x_j(x, u)$ *for* $i \neq j$ *is fixed on* $\Omega \times \mathcal{U}$.

Thereby the extra-diagonal terms of the Jacobian matrix are of fixed sign on $\Omega \times \mathcal{U}$.

Definition 2 *A system* (Σ) *has a loop structure if* $f_i(x, u) = f_i(x_i, x_{i+1}, u)$ *for* $i = 1..n$.

The velocity of each variable depends only on the variable itself and on the next one (the indices are counted modulo n).

Many biological systems have this structure: let us cite for example the "cellular control systems" with positive or negative feedback, that modelize the control of gene expression. the basis was given by the Goodwin model:

$$\left\{ \begin{array}{l} \dot{M} = v/(k + P^\rho) - \alpha M \\ \dot{E} = aM - \beta E \\ \dot{P} = bE - \gamma P \end{array} \right. \tag{1}$$

where M, E, P are respectively the concentration in messenger RNA, enzyme, and product. One can consult [9, 1] for further examples and references. For positive feedback, see the references in [13].

Models of interacting populations have also this structure; see the examples of predator-prey systems in [7]. Age or stage-structured models may also exhibit these features [20], just as hypercycle systems, describing the evolution of a set of polynucleotides in a flow reactor [10]. From a mathematical point of view, these systems have been deeply investigated (cf. [13]).

Notations. For $x \in \mathbf{R}^n$, we denote by $\text{sign}(x)$ the vector of signs of each coordinate (the function sign takes values in $\{+1, 0, -1\}$).

We will consider the vector of signs of the interactions:

$$\sigma = {}^t\text{sign}(\partial f_1/\partial x_2(x, u), ..., \partial f_i/\partial x_{i+1}(x, u), ..., \partial f_n/\partial x_1(x, u))$$

its components will be denoted σ_i.

The family of loop structured systems with monotonous interactions σ on $\Omega \times \mathcal{U}$ will be denoted Σ^L_σ.

2.2 The transition theorem

We want to compare the behavior of the real system with the theoretical behaviour of a model in order either to validate the model or, if the model has still been validated in normal operating conditions, to detect a qualitative change in the real system.

We state now a theorem giving the possible qualitative behavior of the model. This theorem has been presented in ([4]), its consequences for the qualitative behavior of loop systems are very important; let us remark that here the input $u(t)$ has to be constant.

But first, we define the transition rules for a state vector $x(t)$, associated with a vector of interactions σ:

Definition 3 *We say that the state vector x(t) satisfies the transition rules $\mathcal{T}_\sigma$ associated with σ if for all the indexes i we have:*

- *During the time when* $\mathrm{sign}(\dot{x}_{i+1}) = \sigma_i$*, the state variable* x_i *has at the most one extremum, which is a minimum.*
- *During the time when* $\mathrm{sign}(\dot{x}_{i+1}) = -\sigma_i$*, the state variable* x_i *has at the most one extremum, which is a maximum.*

Theorem 1 (Transition theorem) *For a loop structured system with monotonous interactions* $\Sigma \in \Sigma_\sigma^L$*, for the time interval where the input u is constant, the state vector x(t) satisfies the transition rules* $\mathcal{T}_\sigma$*.*

Sketch of the proof: Let us suppose that $\mathrm{sign}(\dot{x}_{i+1}) = \sigma_i$ *i.e.* $\partial f_i/\partial x_{i+1}(x,u)\dot{x}_{i+1}$ is positive. Then, by differentiation of the differential equation for x_i with respect to time, we obtain:

$$\ddot{x}_i = \partial f_i/\partial x_i(x,u)\dot{x}_i + \partial f_i/\partial x_{i+1}(x,u)\dot{x}_{i+1}$$

Suppose that x_i admits an extremum for t_0, then $\dot{x}_i(t_0) = 0$, thus

$$\ddot{x}_i(t_0) = \partial f_i/\partial x_{i+1}(x,u)\dot{x}_{i+1}(t_0)$$

According to the hypothesis, this means that $\ddot{x}_i(t_0)$ is positive, and thus that $\dot{x}_i$ is increasing around t_0. As $\dot{x}_i(t_0) = 0$, we conclude that $\dot{x}_i(t) \leq 0$, for $t \leq t_0$ (for $t_0 - t$ small) and $\dot{x}_i(t) \geq 0$ for $t \geq t_0$ (for $t - t_0$ small). We have the symmetrical result when $\partial f_i/\partial x_{i+1}(x,u)\dot{x}_{i+1}$ is negative.

Remark : For the loop structured system with constant input, it can be shown that it is impossible to have simultaneously $\dot{x}_i(t) = 0$ and $\dot{x}_{i+1}(t) = 0$ ([4]).

2.3 Graphical representation

The transition rules $\mathcal{T}_\sigma$ defining the possible succession of extrema for a state vector $x(t)$ can be graphically represented by transition graphs (see [4]).

Each closed orthant of the space defined by the signs of the derivative of $x(t)$ is represented by a vector of signs; graphically, it is the node of a graph. For instance, the orthant in $\mathbf{R}^3$ $(\dot{x}_1 \geq 0, \dot{x}_2 \geq 0, \dot{x}_3 \leq 0)$ will be represented by the node on fig. 1.

The possible transition (given by Theorem 2) 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 explains if the transition is a minimum or a maximum of x_i. We associate therefore to the system Σ_σ^L a transition graph. Figure 2 shows an example of transition graph for the class of phytoplanktonic growth models studied further.

2.4 Extension

If the input u is not constant (and not piecewise constant), it is possible to extend the method. For a single input (*i.e.* $u \in \mathbf{R}_+$), if we denote $\rho_i = \text{sign}(Df_i/Du)$, the following result holds:

- During the time when $\text{sign}(\dot{x}_{i+1}) = \sigma_i$ and $\text{sign}(\dot{u}) = \rho_i$, the state variable x_i can have at the most one extremum, which is a minimum.
- During the time when $\text{sign}(\dot{x}_{i+1}) = -\sigma_i$ and $\text{sign}(\dot{u}) = -\rho_i$, the state variable x_i can have at the most one extremum, which is a minimum.

Indeed, the differentiation of the equation of $\dot{x}_i$ has now an additional term:

$$\ddot{x}_i = \partial f_i/\partial x_i(x,u)\dot{x}_i + \partial f_i/\partial x_{i+1}(x,u)\dot{x}_{i+1} + \partial f_i/\partial u(x,u)\dot{u}$$

and we can conclude only when the sign of $\partial f_i/\partial x_{i+1}(x,u)\dot{x}_{i+1} + \partial f_i/\partial u(x,u)\dot{u}$ can be determined.

2.5 Application to the comparison between a model structure and the actual system behaviour

Theorem 1 of qualitative behavior can be used to examine whether each experimental extremum is compatible with the transition rules $\mathcal{T}_\sigma$ *i.e.* with the structure of the model. If an experimental extremum does not fit one of the transition rules, this means that the considered model structure is not or no more suitable for the real system. In the framework of a validation study, this means that the model is not valid. In the framework of the monitoring of a system, where the structure of the system in normal operating conditions is supposed to be known, this means that the system behaves no more "normally". This is then a way to detect that a non-standard event has appeared.

Nevertheless, when measurements are very noisy, it becomes difficult to distinguish the extrema from the noise and this analysis becomes inapplicable. A procedure to smooth the data is then required. In the following paragraph, we show that the results presented in Theorem 1 still holds when filtering data in a very simple way, even for a non constant input.

3 Qualitative signal processing

We wish to apply the same method to the filtered measurements of a real system. Moreover we want to extend the analysis to the case when the system is excited by a periodic input $u(t)$.

Qualitative behavior of an averaged signal. Our main theorem is the following:

Theorem 2 *Let Θ be a diagonal matrix with, on its diagonal, positive functions $\theta_{i,i}(t)$. If x is a solution of a loop structured system $\Sigma \in \Sigma_\sigma^L$, and if the mapping $t \longrightarrow (u(t), \Theta(t))$ is periodic with period $\tau_1 + \tau_2$, then z defined as:*

$$z(t) = \frac{1}{\tau_1 + \tau_2} \int_{t-\tau_1}^{t+\tau_2} \Theta(s) x(s) ds \tag{2}$$

satisfies the transition rules (cf. Theorem 1) $\mathcal{T}_\sigma$.

Proof: differentiating equation (2) we find:

$$\dot{z}(t) = \frac{1}{\tau_1 + \tau_2} [\Theta(t + \tau_2) x(t + \tau_2) - \Theta(t - \tau_1) x(t - \tau_1)] \tag{3}$$

Because of the periodicity of Θ, it reduces to

$$\dot{z}(t) = \frac{1}{\tau_1 + \tau_2} \Theta(t - \tau_1)[x(t + \tau_2) - x(t - \tau_1)] \tag{4}$$

Moreover, if we apply the generalized Taylor formula to f between (x_1, u_1) and (x_2, u_2), there holds:

$$\begin{aligned} f(x_2, u_2) - f(x_1, u_1) = \; & [\textstyle\int_0^1 D_x f(\alpha x_2 + (1-\alpha) x_1, \alpha u_2 + (1-\alpha) u_1) d\alpha](x_2 - x_1) \\ & + [\textstyle\int_0^1 D_u f(\alpha x_2 + (1-\alpha) x_1, \alpha u_2 + (1-\alpha) u_1) d\alpha](u_2 - u_1) \end{aligned} \tag{5}$$

Let us denote

$$A(t) = \int_0^1 D_x f(\alpha x(t + \tau_2) + (1-\alpha) x(t - \tau_1), \alpha u(t + \tau_2) + (1-\alpha) u(t - \tau_1)) \, d\alpha \tag{6}$$

With this notation, the periodicity of the input implies from (5):

$$f(x(t + \tau_2), u(t + \tau_2)) = f(x(t - \tau_1), u(t - \tau_1)) + A(t)(x(t + \tau_2) - x(t - \tau_1)) \tag{7}$$

differentiating (4), and using (2) and (7) we then have

$$\ddot{z}(t) = B(t) \dot{z} \tag{8}$$

with $B(t) = \dot{\Theta}(t - \tau_1) \Theta^{-1}(t - \tau_1) + \Theta(t - \tau_1) A(t) \Theta^{-1}(t - \tau_1)$

Since the Jacobian matrix $D_x f$ is of fixed off-diagonal sign on $\Omega \times \mathcal{U}$, (6) proves that it has the same offdiagonal signs σ as $A(t)$. These signs are not affected by the multiplication by the positive diagonal matrix Θ or Θ^{-1} or the sum with the diagonal matrix $\dot{\Theta}(t - \tau_1)\Theta^{-1}(t - \tau_1)$. Hence, $B(t)$ has the same offdiagonal signs σ as $D_x f$, and therefore its associated qualitative behavior is the same: it follows the same transition rules $\mathcal{T}_\sigma$.

Remark : For constant input, Theorem (2) is valid for any τ_1 and τ_2.

Application to the moving average $\bar{x}(t)$. Let us take $\Theta = I_n$ (identity matrix of dimension n), $\tau_1 = \tau$ and $\tau_2 = 0$, then $z(t)$ represents the moving average $\bar{x}(t)$ of $x(t)$, between time $t - \tau$ and t:

$$\bar{x}(t) = \frac{1}{\tau} \int_{t-\tau}^{t} x(s) ds \tag{9}$$

Corollary 1 (Qualitative behavior of the moving average) *If x is a solution of a loop structured system Σ_σ^L, and if the mapping $t \longrightarrow u(t)$ is periodic with period τ, then the moving average $\bar{x}(t)$ satisfies the transition rules $\mathcal{T}_\sigma$ (cf. Theorem 1).*

The moving average is a classical low-pass filter which removes the high frequencies of noise. This simple signal processing has the very strong advantage that it does not require any knowledge of the system, and particularly it does not need any calibration.

Now we can write a graphical version of our theorem. For the sake of simplicity, we give it only for the moving average.

Corollary 2 (Qualitative behavior of the moving average - graphical version) *If x is a solution of a loop structured system Σ_σ^L, and if the mapping $t \longrightarrow u(t)$ is periodic with period τ, the sequence of extrema of the average $\bar{x}(t)$ follows the transition graph of Σ_σ^L.*

We can now verify if the experimental qualitative extrema (after filtering) are compatible with the transition rules $\mathcal{T}_\sigma$ defined by Theorem 2 and represented by the transition graph. Each experimental extremum of $\bar{x}(t)$ can be compared with this graph in order to see if it is allowed by the assumed structure of the model. If not, it proves that the structure of the model does not or no more correspond with the real system. This structure does not depend on the values of the parameters. In fact, we can monitor the "qualitative structure" of the model.

Note that a change can appear which may not be detected by this analysis (abrupt change in parameters values, modification of the expression of the kinetics with unchanged interactions,...) if it does not affect the structure.

4 Example: algal growth in bioreactors

4.1 The modelling of phytoplankton growth

The models used to describe the growth of phytoplanktonic cells (biomass x_2) on a substrate (of concentration x_1) assume usually [8] that the growth is a function of a variable (x_3), called internal quota, representing the nutrient stored in the cells:

$$(\Sigma_{PGM}) \begin{cases} \dot{x}_1 = u(t)(1 - x_1) - \rho(x_1)x_2 \\ \dot{x}_2 = (\mu(x_3) - u(t))x_2 \\ \dot{x}_3 = \rho(x_1) - \mu(x_3)x_3 \end{cases} \tag{10}$$

The input $u(t)$ is the dilution rate of the continuously stirred bioreactor (we suppose $u(t) \geq \underline{u} > 0$). The functions ρ and μ represent the absorption rate and the growth rate. Note that for sake of simplicity (Σ_{PGM}) has been adimentionalized by dividing the substrate and the biomass concentration by the influent concentration of the substrate.

Among these models (Σ_{PGM}), the Droop model ([8], [6]) is widely used. For this model we have:

$$\rho(x_1) = a_1 \frac{x_1}{a_2 + x_1}; \;\; \mu(x_3) = a_3(1 - \frac{a_4}{x_3}) \tag{11}$$

This model forced by a periodic input has been studied in [16, 17].

Very often, it is difficult to specify so precisely the expression of the functions ρ and μ, and a qualitative description is more appropriate. In the sequel we shall consider the general class of models verifying the following hypotheses, corroborated by the experiments:

- **(H1)**: The absorption rate ρ is a non negative and increasing function of x_1 with $\rho(0) = 0$.
- **(H2)**: The growth rate μ is an increasing function of x_3.

The class of models (Σ_{PGM}) verifying hypotheses (H1)-(H2) in the considered domain is called the class of Phytoplanktonic Growth Models (PGM).

Property 1 *The PGM are loop structured with monotonous interactions $\sigma = {}^t(-1, +1, +1)$; their qualitative behavior can be represented by the transition graph of figure 2.*

It is easy to verify (cf. [15]) that, with hypotheses (H1)-(H2), the PGM verify our definitions in the positively invariant domain $x_1 > 0, x_2 > 0, x_3 > 0$.

From Theorem 2, the qualitative behavior (i.e., the sequence of extrema) of the moving average $\bar{x}$ on a time interval $[t - \tau, t]$, has to follow this graph for any input $u(t)$ of period τ.

4.2 Simulation and detection of a qualitative change

In this section we simulate a qualitative change at time t_0 in a culture of microalgae in bioreactor by changing the structure of the model at this instant. Then we show how this structural change can be detected by a qualitative analysis of the transitions.

We take the Droop model (equations (10) and (11)) to simulate the growth of the phytoplankton in normal conditions. This model will then provide for the time interval $[0, t_0]$ a standard simulation of the growth process. We consider here a case where the dilution rate is periodic; it is a sinusoid of period 24 hours, and we take $t_0 = 3$ days.

Now we want to simulate a qualitative change in the cellular growth for time interval $]t_0, t_1]$. We suppose for example that during this period the algae are stressed and thus that their growth is inhibited by the substrate instead of being enhanced. To simulate this inhibition effect, we take the following function for the uptake rate: $\rho(x_1) = \frac{a_4}{a_1 + x_1}$ which represents an hyperbolic substrate inhibition.

For the time interval $]t_1, t_f[$ we assume that the stress phase disappeared, and we take again the "reference model".

The generated data have been corrupted with an additive white noise to get closer to real data. These data are represented on Figure 3a.

In order to detect the change we will now process these data. We apply then the moving average with a 24 hours time interval to filter the noise (see Figure 3b). To do this, we compute a numerical approximation of the following integral at the measurement instants t_i:

$$I(t_i) = \int_0^{t_i} x(s)ds \tag{12}$$

The obtained values of $I(t_i)$ are then interpolated (using cubic spline interpolation) to estimate the value of $I(t)$ for all time t. Finally the moving average can be estimated at any time $t \geq \tau$:

$$\bar{x}(t) = \frac{1}{\tau}[I(t) - I(t-\tau)] \tag{13}$$

These smoothed data can now be matched to the transition graph associated with the standard structure of the PGM (fig. 2).

The following qualitative state transition is to be seen from the smoothed data a little bit after 3 days:

$$\begin{pmatrix} + \\ - \\ + \end{pmatrix} \rightarrow \begin{pmatrix} + \\ - \\ - \end{pmatrix}$$

This transition is not permitted by the transition graph: the structural change in the process is detected.

4.3 Real experiments

These qualitative monitoring technics have been used to study the response of the phytoplankton when it is fed with a periodic nutrient source. The experiment consists in supplying the phytoplankton with a sinusoid pattern for the dilution rate. Different supplying periods have been studied. These experiments reproduce some real nutrient supply patterns which appear in the sea under some specific hydrodynamic conditions ([11]).

The green microalgae *Dunaliella tertiolecta* has been grown in a periodic nitrate depleted environment ([3]). The phytoplanktonic biomass has been estimated with the cellular concentration (using a particle counter (HIAC/ROYCO with laser sensor HRLD 400)). Nitrate has been measured by colorimetric methods (Auto-analyzer Technicon). Precise indications on the computer controlled environment controlling the culture so as on the conditions of experiments can be found in [5].

Slow inputs. Figure 4 shows an example of slow nutrient fluctuation period: the nitrogen source oscillates with a daily period. After filtering the data with a 24h moving average, the oscillations disappear, so that all the averaged variables become monotonous. Some narrow oscillations remain nevertheless for the substrate x_1, but they are not significant because they are in a range lower than the variability of the measurements (0.02). For this period, no incompatibilities with the transition graph (Figure 2) was noticed: we have no reason to reject the class of models.

Fast inputs. Figure 5 presents another experiment with a faster nutrient supplying period: the dilution rate changes with a period of 8 hours. The smoothed data (cf Figure 5) can then be compared with the transition graph. The first transition ($t \simeq 1.25$ days) that can be noticed is the following:

$$\begin{pmatrix} - \\ + \\ - \end{pmatrix} \rightarrow \begin{pmatrix} + \\ + \\ - \end{pmatrix}$$

This transition is not compatible with the transition graph of the PGM (Figure 2). Moreover, it can be noticed from the transition graph that the states $\begin{pmatrix} + \\ + \\ - \end{pmatrix}$ and $\begin{pmatrix} - \\ - \\ + \end{pmatrix}$ can be reached only once, for the initial conditions. In the fast varying experiment, these qualitative states are observed several times. It seems therefore that the structure of the PGM is not valid to represent the growth of phytoplankton with a fast varying nitrogen source.

This particular behaviour of the algae for fast input variations may be due to a synchronisation effect of the cellular division on the nutrient source ([5]).

5 Conclusion

This method of qualitative analysis of the dynamical transitions can be used to validate the structure of a model with the measurements performed on the real system. Once the structure of the model in normal operating condition has been validated, this can be used to monitor the structure of the actual system, in order to detect a qualitative change.

This method can be applied to a wide range of biological systems (bioprocess, ecosystem, ...). The main points of this method are the following:

- the data are filtered using a low pass filter: the moving average. This signal processing remains compatible with a qualitative analysis of the data.
- the change in the structure of the system can be detected, even if only a qualitative model for this structure is available.
- the inputs $u(t)$ can be non constant, if they are periodic (or otherwise see extension in section 2.4).

The method has been applied to data of phytoplankton fed in chemostat with a periodic nutrient source. It turns out that the qualitative behaviour of the algae change when the variation of the nutrient is too fast: the class of model usually used to represent the phytoplankton growth is then no more suitable.

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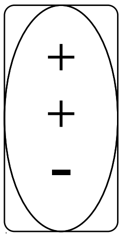

Figure 1: Representation of the orthant defined by the signs of the derivatives of $x(t)$.

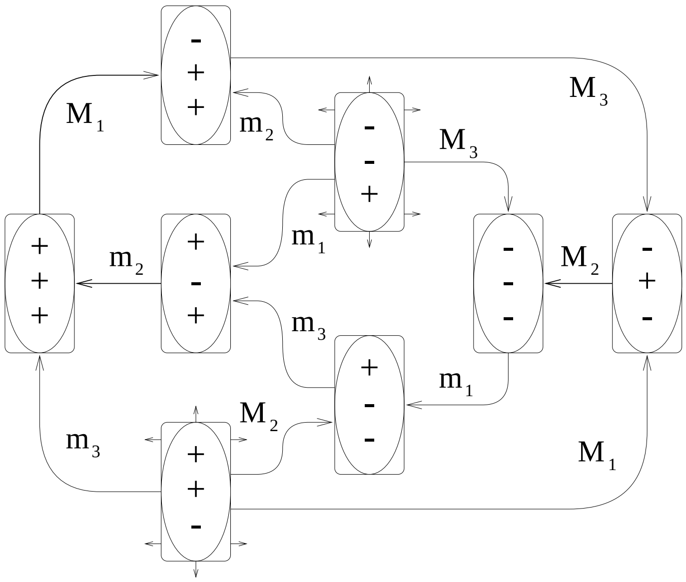

Figure 2: The transition graph for the PGM family. The nodes represent the tendency of the variables, the arrows between the nodes synthesize rules of theorem (2): m_i [resp. M_i] denotes a minimum [resp. a maximum] of $\bar{x}_i$.

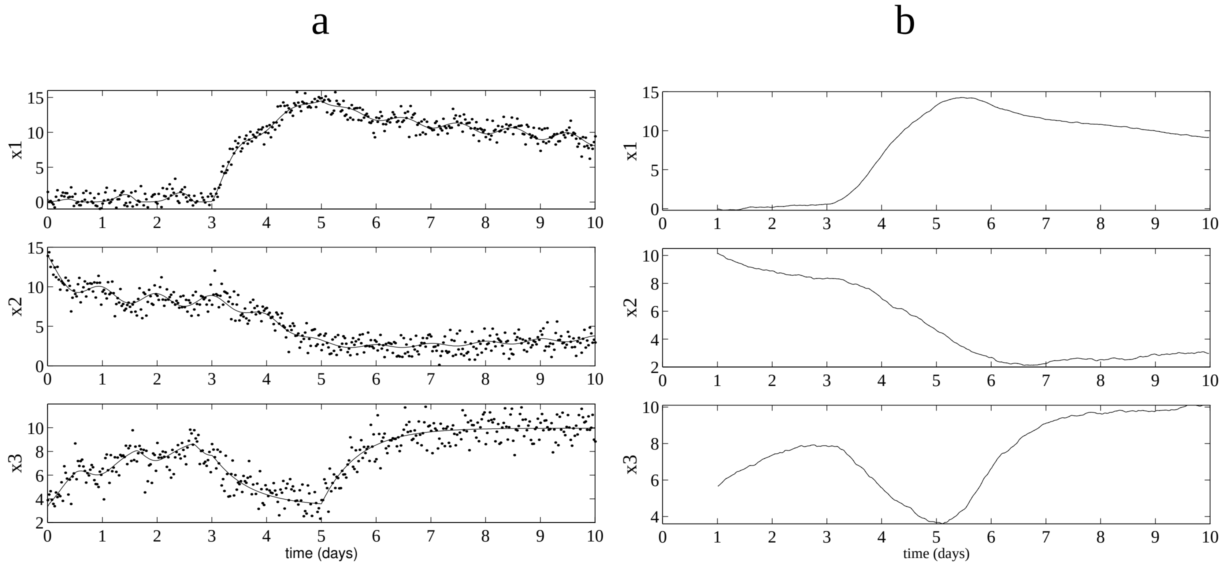

Figure 3: a. Simulation corrupted by noise (phytoplankton in the bioreactor). b. Data processed by moving average.

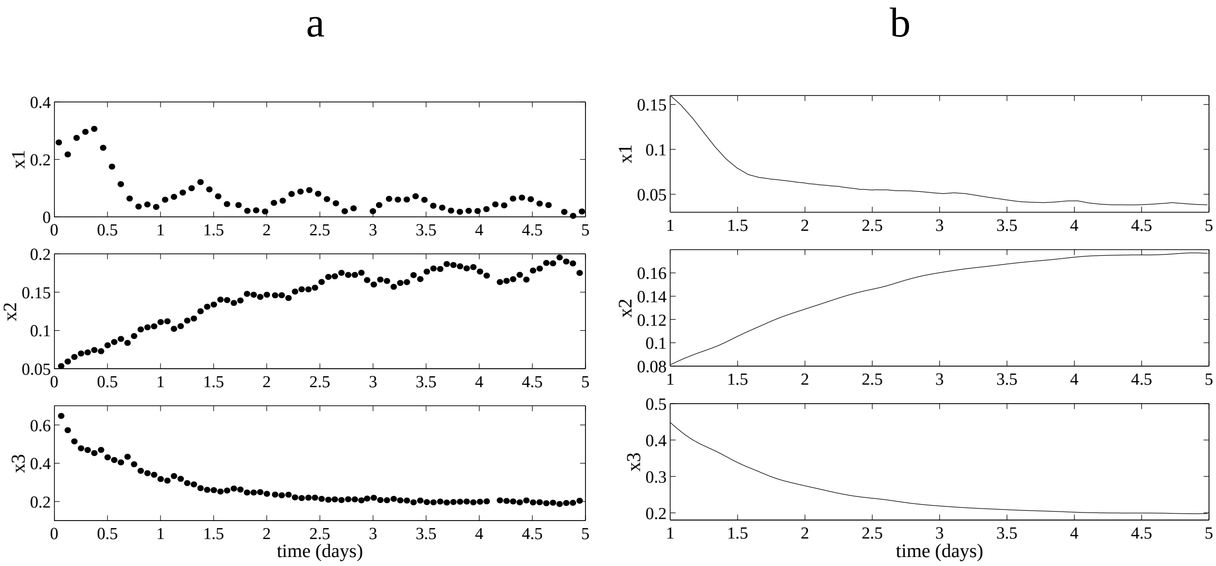

Figure 4: a. Real data of algae growing in a chemostat under nitrate limitation (period: 24 hours). b. Data processed by moving average.

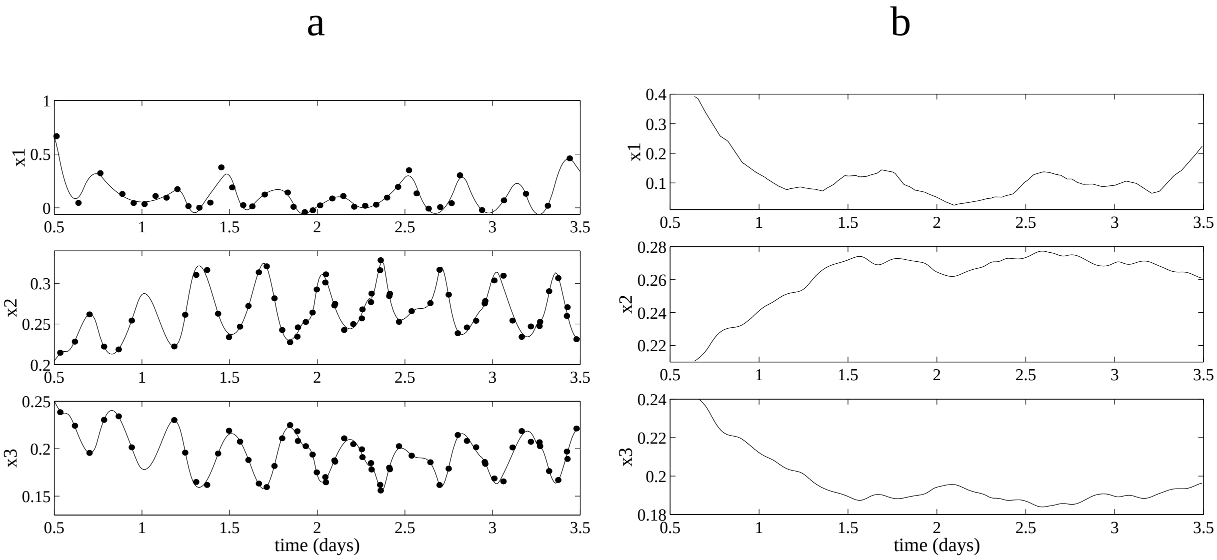

Figure 5: a. Real data of algae growing in a chemostat under nitrate limitation (period: 8 hours). b. Data processed by moving average.