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Research Article

Identifying necessary and sufficient conditions for the observability of models of biochemical processes

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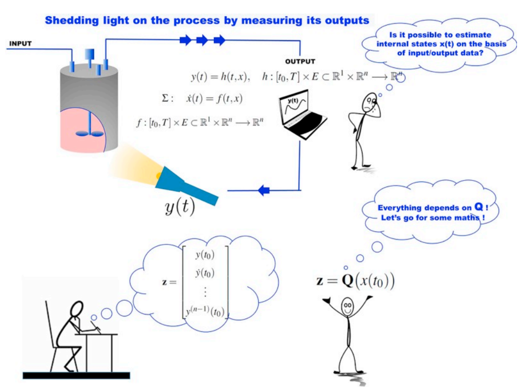
HIGHLIGHTS

- System observability is the possibility of estimating the internal states from the input/output.
- Observability is a topic of continuous research in control theory because of the duality observabilitycontrollability.
- Observability is pivotal in mathematical non-linear models for engineering biology in bio-based production applications.
- A theorem defining the necessary and sufficient conditions for a non-linear system to be observable is proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

The notions of observability and controllability of non-linear systems are a cornerstone of mathematical control theory and cover a wide scope of applications including process design, characterization, monitoring and control. Synthetic biology - which aims to (re)-program living functionalities - and bio-based process engineering - which aims to develop biotechnological manufacturing processes based on industrial and natural living agents - remarkably benefit of methodological improvements inspired to control theory for countless reasons including the huge variety of control mechanisms in living organisms, experimental limitations in terms of measurement feasibility, design of controllers - at single cell or population level - of synthetic production processes and process optimization purposes. Many fundamental problems of control theory such as stabilisability of unstable systems and optimal control may be solved under the assumption that the system is observable/controllable. Observability and controllability are mathematical duals, that means that the observability property can be determined analysing the controllability of the dual system and vice versa. Given this duality, we focus on observability. In this work, we revisit a generalization of the Fujisawa and Kuh theorem as a tool to explore the possibility that a system is observable. We show that the theorem, when applicable, is a sufficient but not necessary condition for observability. We revisit the theorem to propose a necessary and sufficient condition for observability for non-linear systems. Finally, we show how it is possible to identify regions of the phase space of the model in which the model is observable.

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1. Introduction

Understanding observability and its relevance is made easier if we underline the implications of observability assessment for controllability. For this reason we consider it appropriate in this study to introduce the reader to observability by first making some reference to controllability. Observability and controllability are two fundamental concepts appearing in the earlier studies on linear control under the impulse of Kalman [1,2]. In particular, linear controllability turns out to be useful in many applicative contexts concerning networked systems, such as the design of predictive observer models and feedback controllers [3]. A system is said to be controllable if for *any* given pair of initial and final states there exists an input function that drives the system from the initial to the final state. Non-controllable mathematical models of real systems have subspaces that influence model behaviour, but cannot be controlled by an input. Whereas calculating these subspaces in linear systems could be accomplished without difficulty, in general, such subspaces can be difficult to determine in complex non-linear networks [4]. Analysing systems observability is an effective mathematical means for gauging the controllability of the systems. A system is said to be observable if its state at any instant can be determined by measuring its outputs over a finite time interval. Mathematically, a system with an observed output map is said to be observable if for a given input function (if any), the output map uniquely determines the state of the system at any instant. Controllability and observability are duals, both for linear and non-linear networks. For the linear systems, the duality between controllability and observability is formalized as the duality between a vector space and its dual. For non-linear system, the duality between controllability and observability can be formalized in various manners. We refer the reader to the two examples of Sontag [5] that proposes two ways of the abstract and the sampling duality. The existence of this duality allows to study the observability and to draw conclusions also on controllability. The importance of observability was recognized in parameter estimation and state reconstruction problems. Indeed, the direct implications on the feasibility of reconstructing the unmeasured states of a system determine the prominence of observability in process design.

Nowadays, a huge literature on controllability and observability of systems is available. These concepts are currently basic in the canonical description of linear systems, so that the main references are textbooks [5,6] rather than papers [7–10].

In the early 1970s, the motivating insight drawn from the theory developed on linear (time-invariant) systems stimulated the undertaking of theoretical and computational scholars to extend the study of controllability and observability towards non-linear systems as well.

It soon turned out that this goal was ambitious. Apart from a few particular generalizations such as linear time-varying systems and bilinear control systems [11,12], a completely parallel theory on non-linear controllability and observability is not feasible. Therefore various weaker notions of non-linear controllability and observability have been developed in the 1970 and 1980s [13], all with an emphasis on the sufficient conditions, their computational characterizations, and implications on the system structure [14,15,16,17].

The dynamics of many processes of any kind (e.g. electrical, chemical, mechanical, biological) that occur in the real world are dominated by non-linear factors and are increasingly exploited in science and technological applications. Consequently the need to assess non-linear systems observability and controllability makes increasingly urgent to acquire formal tools including analytical techniques and software, aimed at defining the necessary and sufficient conditions for observability and at testing their validity on the data collected during the experimental observation of these systems, respectively. The interest in the formal aspects of observability has therefore rekindled in many disciplines, as evidenced by recent publications [18,19,20,21,22,8,23,24,7].

The ability to assess the observability and controllability of bio-based process models - which could be developed across multiple scales - could be a key enabler in many applications, such as fermentation and biocatalysis [20,21,22]. However, the achievement of controllable processes is challenging. In the scenario of microbial cell factories engineering, for instance, the adoption of some basic operating parameters in bio-process modelling showed deviations from observability [25]. In spite of the difficulties, taking control of bio-based processes is one of the main drivers in the development of biotechnological applications vigorously tending towards process intensification, continuous manufacturing [26] and quality control of bio-based end products [27,28].

In this paper we determine and formalize the necessary and sufficient conditions for the observability of a non-linear continuous-time system defined by a dynamic model based on differential equations of the first order. Our starting point is a theorem proposed by Kuh et al. [17] which, however, defines only the sufficient condition for observability and therefore cannot be used to determine whether a system is observable or not. Inspired by the ideas of Kuh et al., we have revisited their theorem by focusing on the concept of *observability mapping* **Q**, that is the function that links what is directly accessible to measurement (y) to what is not (x), such that $y = \mathbf{Q}(x)$. In particular, we define as observable a system in which the mapping of observability **Q** is invertible, and we therefore ultimately define the necessary and sufficient conditions for this mapping to be bijective.

2. The conditions for global observability

We consider a time-continuous non-linear input-free system Σ, whose dynamics if described by the following general equations

$$\Sigma: \quad \dot{x}(t) = f(t, x), \quad f: [t_0, T] \times E \subset \mathbb{R}^1 \times \mathbb{R}^n \to \mathbb{R}^n \tag{1}$$

and whose experimentally observed output is

$$y(t) = h(t, x), \quad h: [t_0, T] \times E \subset \mathbb{R}^1 \times \mathbb{R}^n \to \mathbb{R}^n \tag{2}$$

In this system $x(t)$ is not available for direct measurement. The initial state $x(t_0) \equiv x_0$ is unknown and belongs to the set $E_0 \subseteq E$. The observability problem consists of determining whether there exist relations binding the state-variables $x(t)$ to outputs $y(t)$ and their time-derivatives $\dot{y}(t)$ and thus locally defining them uniquely in terms of measurable quantities without the need for knowing the initial conditions [24,29,21]. If no such relations exist, the initial state of the system cannot be deduced from observing its output behaviour. This implies that there are infinitely many parameter sets that produce exactly the same output for different values of x_0 and thus the model parameters cannot be estimated from any experimental measurements. The lack of observability implies the lack of identifiability and, consequently no chance to control the system.

We suppose that the n-th order derivatives of f and h exist for every $x \in E$ and every $t \in [t_0, T]$, and that $y(t)$ is smooth, so that we can approximate $y(t)$ by a truncated Taylor series as follows

$$y(t) = y(t_0) + \dot{y}(t_0)(t - t_0) + \frac{\ddot{y}(t_0)}{2!}(t - t_0)^2 + \ldots + \\ + \frac{y^{(n-1)}(t_0)}{(n-1)!}(t - t_0)^{n-1} + \frac{y^{(n)}(t^*)}{n!}(t - t_0)^n \tag{3}$$

where $t^* \in (t_0, T)$, and

$$\begin{aligned} y(t_0) &= h(x(t_0), t_0) \equiv h_0(x(t_0), t_0) \\ \dot{y}(t_0) &= \frac{\partial h_0}{\partial t}(x(t_0), t_0) + \left(\frac{\partial h_0}{\partial x(t_0)}(x(t_0), t_0)\right) f(x(t_0), t_0) \equiv h_1(x(t_0), t_0) \\ &\vdots \\ y^{(n-1)}(t_0) &= \frac{\partial h_{n-2}}{\partial t}(x(t_0), t_0) + \left(\frac{\partial h_{n-2}}{\partial x(t_0)}\left(x(t_0), t_0\right)\right) f(x(t_0), t_0) \equiv h_{n-1}(x(t_0), t_0). \end{aligned} \tag{4}$$

These equations can be written as a non-linear map [17].

$$\mathbf{z} = \mathbf{Q}(x(t_0)) \tag{5}$$

where

$$\mathbf{z} = \begin{bmatrix} y(t_0) \\ \dot{y}(t_0) \\ \vdots \\ y^{(n-1)}(t_0) \end{bmatrix} \tag{6}$$

and $\mathbf{Q}$, called the *observability mapping* of Σ, is

$$\mathbf{Q}(x(t_0)) = \begin{bmatrix} h_0(x(t_0), t_0) \\ h_1(x(t_0), t_0) \\ \vdots \\ h_{n-1}(x(t_0), t_0) \end{bmatrix}. \tag{7}$$

The system Σ is observable if $\mathbf{Q}$ is a one-to-one mapping whose codomain is restricted to its image. If the codomain of $\mathbf{Q}$ is restricted to its image, $\mathbf{Q}$ becomes also surjective and therefore invertible. By definition, a system described by Eq. (1) is completely observable in set E_0 of initial states on time interval $[t_0, T]$ if there exist a one-to-one correspondence between the set E_0 and the set of observed outputs $y(t)$ for $t \in [t_0, T]$. If $\mathbf{Q}$ is a one-to-one mapping from E_0 to $\mathbf{Q}(E_0)$, since $\mathbf{Q}(E_0) = Im(\mathbf{Q})$, $\mathbf{Q}$ is also surjective, so that $x(t_0)$ can be uniquely determined from $y(t)$. Finally, given $x(t_0)$, $x(t)$ can be determined by recursion at any t.

The following theorem (see [17] for the complete proof) defines a condition for $\mathbf{Q}$ to be a one-to-one mapping.

Theorem 1. *Given* $\mathbf{Q}: E^n \to E^n$ *differentiable of class* C^n *with Jacobian matrix* $J(\mathbf{x})$, *if there exists a constant* $\varepsilon > 0$ *such that the absolute value of the leading principal minors*[1] $M_1, M_2, \ldots, M_n$ *of* $J(\mathbf{x})$ *satisfy the ratio condition*

$$|M_1| \geq \varepsilon, \quad \frac{|M_2|}{|M_1|} \geq \varepsilon, \quad \ldots, \frac{|M_n|}{|M_{n-1}|} \geq \varepsilon$$

for all $\mathbf{x} \in E^n$, then $\mathbf{Q}$ is a one-to-one mapping from E^n to E^n.

Theorem 1 is the Kou et al. generalization [17] of the Fujisawa and Kuh theorem [30] on the existence and uniqueness of solutions of non-linear networks, and can be demonstrated by induction on n. It states that if the ratio conditions are satisfied, then $\mathbf{Q}$ is a one-to-one mapping. We note that the respect of the ratio condition of the Theorem 1 is only a sufficient condition for $\mathbf{Q}$ to be a one-to-one mapping. We also note that the existence of a one-to-one mapping $\mathbf{Q}: E^n \to E^n$ is only a sufficient condition for observability. Indeed the fact that $\mathbf{Q}$ is injective does not imply that $\mathbf{Q}$ is invertible. It is equally important to note that the ratio condition is not always applicable, as it may not always be defined. In this case, Theorem 1 cannot be used as a tool to evaluate the possible observability of the model. We can prove this by reporting as a counterexample, the Rössler model:

$$\begin{cases} \dot{x}_1 = -x_2 - x_3 \\ \dot{x}_2 = x_1 + ax_2 \\ \dot{x}_3 = b + x_3(x_1 - c), \qquad a, b, c \in \mathbb{R} \end{cases} \tag{8}$$

that is a minimal model for the mass action law model of the following system of chemical reacions [31]:

$$R_1: A_1 + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} 2X \qquad R_2: X + Y \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} 2Y$$
$$R_3: A_5 + Y \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} A_2 \qquad R_4: X + Z \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} A_3$$
$$R_5: A_4 + Z \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} 2Z,$$

where reactions R_1 and R_5 are two auto-catalytic steps involving the species X and Z coupled to another catalytic step, that is reaction R_2 involving species Y.

Assume that $y = x_2$. Then, the observability mapping is

$$\mathbf{Q}_{\text{Rössler}} = \begin{bmatrix} x_2 \\ x_1 + ax_2 \\ ax_1 + (a^2 - 1)x_2 - x_3 \end{bmatrix} \tag{9}$$

and the Jacobian matrix is

$$\mathbf{J}_{\text{Rössler}} = \begin{bmatrix} 0 & 1 & 0 \\ 1 & a & 0 \\ a & a^2 - 1 & -1 \end{bmatrix} \tag{10}$$

Since $det\ (\mathbf{J}_{\text{Rössler}}) \neq 0\ \forall a \in \mathbb{R}$, the Rössler systems is observable from x_2. The rationale for this is the following. Consider the map

$$\psi: \mathbb{R}^n \to \mathbb{R}^n$$
$$\mathbf{x} \to y(t), \dot{y}(t), \ddot{y}(t), \ldots, y^{n-1}(t). \tag{11}$$

If ψ is invertible (and consequently injective), it is possible to reconstruct $\mathbf{x} = \{x_1, x_2, \ldots, x_n\}$ from y. The map ψ is invertible at x_0 if its Jacobian is non null (or, equivalently, if the Jacobian matrix has full rank).

Despite the fact that the Rössler system is observable from the coordinate x_2, the ratio condition of the Theorem 1 is not verified, since $|M_1| = 0$. In summary, observability does not imply that the ratio condition holds.

2.1. On the invertibility of observability mapping

The observability of a non-linear dynamic system from an observable $y(t)$ is related to the characteristics of the map ψ we have defined in (11). If ψ is injective but not surjective, it is not invertible. In seeking a necessary and sufficient condition for observability. it is therefore convenient to consider possible implications between the characteristics of the Jacobian matrix of the $\mathbf{Q}$ observability mapping and the $\mathbf{Q}$ invertibility. For this purpose, we consider the following properties:

Property 1 Each positive (negative) definite matrix is invertible.

Property 2 For the Sylvester criterion, a symmetric matrix is positive (negative) definite if and only if its leading principal minors are all positive (negative) [32,33].

Property 3 If A and B are two positive (semi)-definite matrices, AB is positive (semi)-definite if and only if AB is normal [34], i.e.

$$(AB)^T AB = AB(AB)^T.$$

It follows that the product of two positive definite matrices A and B is a positive definite matrix if and only if $AB = BA$.

Furthermore, we observe that we can express a real coefficient matrix as the product of two symmetric matrices [35]. Consider a matrix M, whose characteristic polynomial has a non-negative discriminant. It admits a Jordan's canonical form F_{Jordan}, i.e. there exist an invertible matrix P such that M can be expressed as $PM_{\text{Jordan}}P^{-1}$.

In this work we consider the possibility to factor the Jacobian matrix into two symmetric matrices for two reasons. The first is that in this way the computational analyses on the Jacobian matrix are moved to the symmetric matrices for which numerical linear algebra software dedicate special efficient libraries.

The second reason is that the possibility of factoring the Jacobian matrix into two symmetric matrices requires that the matrix satisfies some assumptions indispensable to define the necessary and sufficient invertibility conditions of $\mathbf{Q}$. We will see which assumptions we are dealing with later on after the statement of a second theorem (Theorem 2) which we propose as a theorem for the oservability of non-linear systems.

In general, let M be the matrix we want to express as a product of two symmetric matrices M_1 and M_2, and let M_{Jordan} be Jordan's canonical form of M. We express F_{Jordan} block by block (of eigenvalue λ) in the following way:

[1] The i-th leading principal minor of a square matrix $M \in \mathbb{K}^{n-m}$, denoted by M_i, is defined to be the determinant of the matrix obtained by deleting the last $n - i$ columns and rows of M.

$$\begin{bmatrix} \lambda & 1 & \\ & \lambda & 1 \\ & & \lambda \end{bmatrix} = \begin{bmatrix} & & 1 \\ & 1 & \\ 1 & & \end{bmatrix} \begin{bmatrix} & & \lambda \\ & \lambda & 1 \\ \lambda & 1 & \end{bmatrix}.$$

Then, we have that

$$M = PF_{\text{Jordan}}P^{-1} = PM_1M_2P^{-1} = PM_1P^T(P^T)^{-1}M_2P^{-1} = [PM_1P^T][(P^{-1})^TM_2P^{-1}]. \quad (12)$$

This result known in the complex field, can be extended to the real case because a real square matrix M is $\mathbb{R}$-similar to its real Jordan form, whose Jordan blocks for conjugate pairs of non-real eigenvalues $a \pm ib$ are block-triangular matrices of the form

$$\begin{bmatrix} C_2 & I_2 & & & \\ & C_2 & I_2 & & \\ & & \ddots & \ddots & \\ & & & C_2 & I_2 \\ & & & & C_2 \end{bmatrix} = \begin{bmatrix} & & & D_2 \\ & & D_2 & \\ & & & \\ D_2 & & & \end{bmatrix} \begin{bmatrix} & & & H_2 \\ & & H_2 & D_2 \\ & & & \\ H_2 & D_2 & & \end{bmatrix}$$

where

$$C_2 = \begin{bmatrix} a & -b \\ b & a \end{bmatrix}, \qquad D_2 = \begin{bmatrix} 1 & \\ & -1 \end{bmatrix}$$

and I_2 is the 2×2 identity matrix [44,45].

Proposition 1. *Given* $\mathbf{Q}: E^n \to E^n$, $E^n \subset \mathbb{R}^n$, *differentiable with Jacobian matrix* $J(\mathbf{x})$, *expressed as product of two matrices* J_1, *and* J_2, *such that* $J_1J_2 = J_2J_1$, $\mathbf{Q}$ *is invertible if*:

1. the principal leading minors of J_1 and J_2 are all positive
2. J_1J_2 is normal.

Proof. If the principal leading minors of J_1 and J_2 are all positive and J_1J_2 is normal, then $J = J_1J_2$ is positive definite, and for the Property 1, J is invertible.

Proposition 1 is only a sufficient condition for $\mathbf{Q}$ to be invertible, because $\mathbf{Q}$ invertible does not imply that $\mathbf{J}$ is invertible, from which would follow that J_1 and J_2 are invertible, and then positive definite. Furthermore, Proposition 1 requires a rather restrictive and rare condition to occur in real systems, namely that J_1J_2 is normal, i.e. that J_1 and J_2 commute and consequently that J is positive definite. We propose the Theorem 2 that extends the Proposition 1 to more realistic cases in which the Jacobian is not a positive definite matrix. On the other hand it is very rare that in real biochemical systems the Jacobian matrix issymmetric.

Theorem 2. Given $\mathbf{Q}: E_1 \subset E^n \to E^n$, where $E^n \subset \mathbb{R}^n$ and E_1 is an open set, such that $\mathbf{Q}$ is continuously differentiable[2] of class C^n with Jacobian matrix $J(\mathbf{x})$, different from the identity matrix and expressed as product of two symmetric matrices J_1 and J_2. $\mathbf{Q}$ is invertible if and only if J_1 and J_2 are both positive (negative) or one of them is positive definite and the other is negative definite.

Proof. We prove the theorem in the two directions.

($\Leftarrow$) Assume that $\mathbf{Q}$ is invertible, then $\mathbf{Q}$ is injective, that implies that $\mathbf{Q}^{-1}: \mathbf{Q}(E) \to E$ is also differentiable, that implies that J is invertible. Therefore $0 \neq \det(J) = \det(J_1)\det(J_2) \neq 0$. Since J_1 and J_2 are two symmetric matrices, a non-singular Jacobian matrix implies that J_1 and J_2 are both positive (negative) or one of them is positive definite and the other is negative definite.

($\Leftarrow$) The proof of this direction uses the inverse function theorem. If J_1 and J_2 are both positive (negative) or one of them is positive definite and the other is negative definite, then $\det(J) \neq 0$. Therefore there is an open set $E_2 \subset \mathbb{R}^n$ such that $\mathbf{Q}: E_1 \to E_2$ has a continuous inverse $\mathbf{Q}^{-1}: E_2 \to E_1$ which is differentiable for all $y \in E_2$.

[2] A function is continuously differentiable if it is differentiable and its derivative is continuous.

Theorem 2 introduces the following new assumptions with respect to Theorem 1.

1. $\mathbf{Q}$ is *continuously* differentiable.
2. The Jacobian matrix J of Q is such that $j \neq I$. Suppose to have n of variables $x_1, x_2, \ldots, x_n$. Systems whose matrix Q gives a Jacobian matrix equal to the identity matrix are linear homogeneous systems as the following one.

$$\Sigma_1: \begin{cases} \dot{x}_1 = x_2 \\ \dot{x}_2 = x_3 \\ \cdots = \cdots \\ \dot{x}_i = \dot{x}_{(i+1)} \\ \cdots = \cdots \\ \dot{x}_{n-1} = x_n \\ \dot{x}_n = x_n, \end{cases}$$

If, for example, Σ_1 is observed from x_1 (i.e. the measurement equation is $y = x_1$), it has a matrix

$$Q = \begin{bmatrix} \dot{x}_1 \\ \ddot{x}_1 \\ \cdots \\ x_1^{(n-1)} \end{bmatrix} = \begin{bmatrix} x_1 \\ x_2 \\ \cdots \\ x_n \end{bmatrix}$$

that gives as Jacobian matrix the $n \times n$ identity matrix. In this example, the function $Q: \mathbb{R}^n \to \mathbb{R}^n$ is

$$Q: \mathbb{R}^n \to \mathbb{R}^n$$
$$(x_1, x_2, \ldots, x_n) \to Q(x_1, x_2, \ldots, x_n)$$

where the components of Q are

$$Q_1(x_1, x_2, \ldots, x_n) = x_1$$
$$Q_2(x_1, x_2, \ldots, x_n) = x_2$$
$$\cdots = \cdots$$
$$Q_n(x_1, x_2, \ldots, x_n) = x_n.$$

Note also that the n solutions of the system Σ_1 are all equals, i.e. $x_1(t) = x_2(t) = x_3(t) = \cdots = x_n(t)$, $\forall t$. This is the case of a trivial system.

In systems Σ_1, x_n is self-sustaining, it sustains the rate of variation of x_{n-1}, that in $n-2$ steps sustains the rate of variation of x_1. A schematic representation of this situation is shown in Fig. 1.

Given a measurement equation, obtaining a Jacobian matrix equal to the identity matrix is â€‹â€‹indicative of the fact that the coordinate chosen as a measurement is the final output of a “sequential” process like that of this example, for which the problem of observability is trivial. From x_1 the system is observable. Σ_1 is a linear system, so that the full rank condition of the Jacobian matrix is a necessary and sufficient condition for observability.

Another example in which the Jacobian matrix is equal to the identity matrix is given by the system Σ_2 observed from x_1.

$$\Sigma_2: \begin{cases} \dot{x}_1 = x_2 \\ \dot{x}_1 = x_2 \\ \cdots = \cdots \\ \dot{x}_i = \dot{x}_{(i+1)} \\ \cdots = \cdots \\ \dot{x}_{n-1} = x_n \\ \dot{x}_n = c, \quad c \in \mathbb{R} \end{cases}$$

The solutions of Σ_2 are real polynomials in t: x_n is a polynomial of degree 1, x_{n-1} is a polynomial of degree 2, …, and x_1 is a polynomial of degree n in t. Also Σ_2 as Σ_1 can be visually represented as a linear graph, since, also in this case

$$x_i = \int x_{i-1}dt \Bigg]$$

The only difference is that for Σ_2 there is not the self-loop on x_n.

In summary, a Jacobian Matrix equal to the identity matrix obtained from a certain variable of observation can therefore be index that the

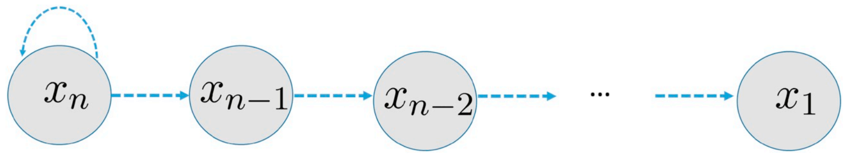

Fig. 1. A representation indicative of system Σ_1.

variable is a sort of collector of the outputs of all the variables connected sequentially as shown by the linear graph. The assumption of Theorem 2 excludes the case in which $J = I$ that is indicative of a linear "chain" system and of a choice of a measurement equation involving a (non-transformed) variable representing the last node of the chain connecting the other variables, as in the examples illustrated here. By means of the assumption of Theorem 2 we have excluded the trivial case of a system and a measurement equation for which the problem of observability does not actually arise.

3. The Jacobian matrix admits Jordan's canonical form in $\mathbb{R}$, which is equivalent to require that its characteristic polynomial has positive or null discriminant. Since $\mathbb{R}$ is not algebraically closed, only real valued matrices whose characteristic polynomial has null discriminant admit a Jordan canonical form. The possibility to express the Jacobian matrix J through its Jordan canonical form allows to factorize J into two symmetric matrices J_1 and J_2 which, depending on whether they are defined as positive or negative or undefined, cause the determinant of J to be different from zero or zero. We note that the condition of non-negative discriminant, is equivalent to say that the eigenvalues of the Jacobian matrix all real.

Note that the occurrence of these two assumptions allows us to state that $\mathbf{Q}$ is invertible if and only if the determinant of its Jacobian matrix is different from zero. Otherwise, if assumptions are not verified, then, in non-linear systems the fact that the $det(J) \neq 0$ is different from zero is only a sufficient condition for the invertibility of $\mathbf{Q}$.

2.2. Observability regions of the phase space

The formal definition of observability and the theorems that define the necessary and sufficient condition give a categorical answer. However, in practice, it is useful to be able to establish for which parameters, or initial conditions, the phase space of the system is observable. Observability is a property that the system could possess only in certain intervals of time, and the duration of these intervals may depend on the initial conditions or model parameters. In systems describing real biochemical processes, local observability is more frequent than global observability. In the next subsections we give some examples of use of the Theorem 1 and Theorem 2 to explore the local observability of a system. In particular we show four examples, as follows:

Example 1: the system is locally observable. The sufficient condition of Theorem 1 is satisfied only in a short interval of time. According to Theorem 2 it is not observable on the whole time domain, as the observability mapping is not bijective over the whole time domain.

Example 2: the system is not observable (not even on some time intervals), because the determinant of its Jacobian matrix is null over the whole time domain. It becomes observable only on some intervals of time according to both Theorems 1 and 2 if redundant equations are removed from the system definition.

Example 3–4: according to Theorem 1 the systems are locally observable. Non-observability regions are those in which either the ratio condition is not defined or the ratio condition is not satisfied, even if singularity points of the Jacobian matrix are excluded from the analysis. Also according to Theorem 2 the system is observable only on some time intervals, as the observability mapping is bijective only over some time intervals.

As we will see better in the section dedicated to the case study (Section 3), the result of the Theorem 2, in terms of presence/absence of local observability, does not contradict that of Theorem 1 (assumed that the ratio condition is definite) if

1. the Jacobian matrix has all the real eigenvalues, that is if it is a real Jordan shape factorizable in two symmetric matrices
2. the observability mapping is continuously differentiable on $\mathbb{R}$.

The accordance of the two theorems is expected except that in these cases, because if the ratio condition is locally satisfied, $\mathbf{Q}$ is a one-to-one mapping whose image is equal to its codomain, hence $\mathbf{Q}$ is bijective, therefore invertible. In the case in which the Jacobian matrix has complex eigenvalues, the systems is oscillatory, the system has at least one equilibrium point the nature of which determines the differentiability and/or the continuous differentiability or not of the observability mapping. This situation may lead to two cases: (i)Theorem 1 is not usable to investigate the observability of the system, or (ii) the ratio condition is not satisfied, although the system could be observable.

2.3. Example 1

We consider a model of batch bioreactor as in [36,37] for the culture of micro-organisms on inhibitory substrates. After initial charge of substrate s and biomass m in the bioreactor, there is no inflow or outflow of the medium. The rate of growth of the biomass is modelled as a function $\mu(s)$, the inhibition function of the substrate. The system Σ is

$$\Sigma\colon \begin{cases} \dot{m} = \mu(s)m & \text{biomass rate equation} \\ \dot{s} = -\mu(s)m & \text{substrate rate equation}' \end{cases} \tag{13}$$

where we suppose that the biomass is the measurable output. Therefore, we introduce y, such that $y = m$. The vector state of the system is

$$\mathbf{x} = \begin{bmatrix} m \\ s \end{bmatrix}. \tag{14}$$

Since $\dot{y} = \mu(s)m$, the observability mapping is

$$\mathbf{Q} = \begin{bmatrix} m \\ \mu(s)m \end{bmatrix}, \tag{15}$$

whose Jacobian matrix is[3]

$$J(\mathbf{x}) = \begin{bmatrix} 1 & 0 \\ \mu(s) & m\dfrac{\partial \mu(s)}{\partial s} \end{bmatrix}, \tag{16}$$

and its minors are

$$|M_1| = 1 \geq 1, \qquad |M_2| = |m\frac{\partial \mu(s)}{\partial s}|.$$

Assuming as in Andrews et at. [37] the following model for the inhibition function

$$\mu = \frac{\hat{\mu}}{1 + \frac{K_s}{s} + \frac{s}{K_i}}, \quad s > 0 \tag{17}$$

we obtain that

[3] Note that $\frac{\partial m}{\partial s} = 0$ because, if only $y = m$ is measurable, s is not measurable and therefore the dependence of m from s is not observable.

$$\frac{\partial \mu(s)}{\partial s} = \frac{\hat{\mu}\left(\frac{K_s}{s^2} - \frac{1}{K_i}\right)}{\left(1 + \frac{K_s}{s} + \frac{s}{K_i}\right)^2} \tag{18}$$

where $\hat{\mu}$ is the maximum specific growth rate (in time^{-1}) in the absence of inhibition, s is the limiting substrate concentration (in units of mass/volume), K_s is the saturation constant (in units of mass/volume), i.e. the lowest value of s at which μ is equal to $\frac{\hat{\mu}}{2}$, K_i is the inhibition constant (in units of mass/volume), i.e. the highest value of s at which μ is equal to $\frac{\hat{\mu}}{2}$. We note that

$$\frac{\partial \mu(s)}{\partial s} = 0 \quad \text{iff} \quad s = \sqrt{K_s K_i}$$

that means that for all values of s equal to $\sqrt{K_s K_i}$ the system is non-observable. Furthermore, we say that Σ is not completely observable, if $\frac{|M_2|}{|M_1|}$ is not greater or equal than 1 for all $\mathbf{x} \in \mathbf{R}^2$.

In order to find the observability singularities of this system we simulated it for $t \in [0, 42]$ h, with the following values for the initial conditions and parameters: $m(t_0) = 0.005$ g/l, $s(t_0) = 10$ g/l, $\hat{\mu} = 1$ h^{-1}, $K_s = 0.03$ g/l, $K_i = 2$ g/l). Since this model is input-free, and in the model of [37] μ is not defined for $s = 0$, the simulation has been stopped for values of $t > 42$ h, that is the time at which the substrate zeroes. The results of the simulation showed in Table 1 indicate that the system is observable almost at the end of the process, when the substrate is almost all consumed, i.e. for $s \leq 1.951$ g/l, and $t \geq 40.3$ h. The Jacobian is null for $s = \sqrt{K_s K_i} = 0.245$ g/l at time $t = 40.6$ h.

In Fig. 2 we show how the time after which the system in Eq. (13) becomes observable (t_{obs}) changes by changing the initial conditions $m(t = 0)$ ans $s(t = 0)$. We observe that *tobs* is most influenced by the initial concentration of the substrate than by the initial biomass (see Fig. 2). In this simple input-free system, where *tobs* depends almost exclusively on one variable, the observability property is a scale-invariant, i.e. as $s(t = 0)$ grows, the time of non-observability expands in such a way t_{obs} corresponds to the time at which the available substrate concentration zeroes (see Fig. 3).

Table 1
Observability singularities of system Σ as Eqs. (13).

Type of singularity	s (in g/l)	Time (hours)
$\varepsilon = 1$ (rate condition)	1.951	40.3
$s = \sqrt{K_s K_i}$ (null Jacobian)	0.245	40.6

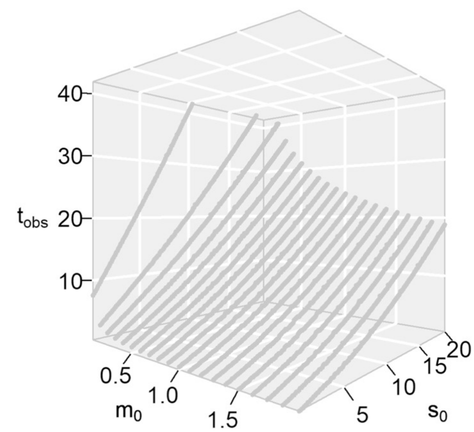

Fig. 2. Time after which the system in Eqs. 13 becomes observable as function of the initial conditions $m(t = 0)$ ans $s(t = 0)$.

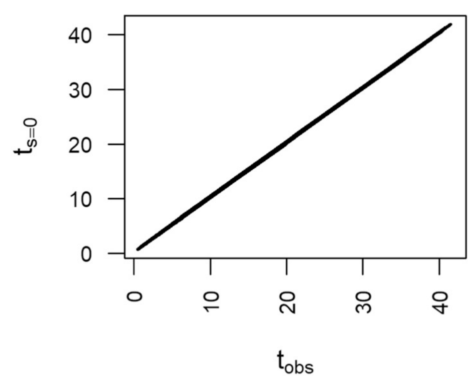

Fig. 3. For the system in Eq. (13), t_{obs} is linearly dependent on the time t at which $s = 0$.

2.4. Example 2

To show an example of non-observable system according both to Theorem 1 and Theorem 2, we consider a model of continuous culture of *Lactobacillus delbrueckii*, a homofermentative lactic acid producer, on a substrate of glucose. In this model proposed by [38] both substrate and product play the role of inhibitor of the kinetics of biomass growth. In this model Σ is as follows

$$\Sigma: \begin{cases} \dot{m} = \mu(s,p)m & \text{biomass} \\ \dot{p} = \alpha\dot{m} + \beta m & \text{lacticacid} \\ \dot{s} = -\frac{1}{Y_{p/s}}\dot{p} & \text{product} \end{cases} \tag{19}$$

where s_m and p_m are the maximum substrate concentration and product concentration (in g/l), respectively, above which the growth rate is zero, α and β are kinetic constants (in units of g of lactic acid / g of cells), n_1 and n_2 model constants, and $Y_{p/s}$ is the product yield (in units of g of product/ g of substrate). In this model the specific growth rate depends both on s and p as follows:

$$\mu(s,p) = \hat{\mu}\left(1 - \frac{s}{s_m}\right)^{n_1}\left(1 - \frac{p}{p_m}\right)^{n_2} \tag{20}$$

The vector state of the system is

$$\mathbf{x} = \begin{bmatrix} p \\ m \\ s \end{bmatrix}, \tag{21}$$

and assuming that the experimental outputs if the concentration of lactic acid we set $y = p$, so that the observability mapping is

$$\mathbf{Q} = \begin{bmatrix} p \\ \alpha\dot{m} + \beta m \\ \alpha\ddot{m} + \beta\mu(s,p)m \end{bmatrix}. \tag{22}$$

The second time derivative of m is

$$\ddot{m} = \mu(s,p)\left[\dot{m} - \frac{mn_1}{s_m}\left(1 - \frac{s}{s_m}\right)^{-1}\dot{s} - \frac{mn_2}{p_m}\left(1 - \frac{p}{p_m}\right)^{-1}\dot{p}\right] \tag{23}$$

The Jacobian matrix of $\mathbf{Q}$ is then

$$J(\mathbf{x}) = \begin{bmatrix} 1 & 0 & 0 \\ \alpha m\frac{\partial \mu(s,p)}{\partial p} & \alpha\mu(s,p) + \beta & \alpha m\frac{\partial \mu(s,p)}{\partial s} \\ \beta m\frac{\partial \mu(s,p)}{\partial p} & \alpha\frac{\partial \ddot{m}}{\partial m} + \beta\mu(s,p) & \beta m\frac{\partial \mu(s,p)}{\partial s} \end{bmatrix}, \tag{24}$$

and its principal minors are

$$|M_1| = 1 \tag{25}$$

$$|M_2| = |\alpha\mu(s,p) + \beta| \tag{26}$$

$$|M_3| = m\left|(\beta^2 - \alpha\mu^2(s,p))\left(\frac{\partial\mu(s,p)}{\partial s} - Y_{p/s}\frac{\partial\mu(s,p)}{\partial p}\right)\right|. \tag{27}$$

Finally, the conditions of Theorem 1 are

$$|M_1| = 1 \geq 1 \tag{28}$$

$$\frac{|M_2|}{|M_1|} = |\alpha\mu(s,p) + \beta| \geq 1 \tag{29}$$

$$\frac{|M_3|}{|M_2|} = \frac{m\left|(\beta^2 - \alpha\mu^2(s,p))\left(\frac{\partial\mu(s,p)}{\partial s} - Y_{p/s}\frac{\partial\mu(s,p)}{\partial p}\right)\right|}{|\alpha\mu(s,p) + \beta|} \geq 1. \tag{30}$$

By simulating Σ in (19) with $\hat{\mu} = 0.58\ \mathrm{h}^{-1}$, $s_m = 401.8$ g/l, $p_m = 81$ g/l, $\alpha = 2.36$ g lactic acid/ g cell, $\beta = 0.816$ g lactic acid/ (g cell $\times$ h), $n_1 = 0.71$, $n_2 = 2.1$, $Y_{p/s} = 0.9$, and $m(t_0) = 0.03$ g/l, $s(t_0) = 100$ g/l, $p(t_0) = 0$ g/l as in [38], we obtained that the condition (29) is satisfied for $t < 15.3$ h (corresponding to $s > 46.2$ g/l and $p < 48.4$ g/l), whereas the condition (30) is never satisfied. Furthermore we found the $M_3 \approx 0$ (i.e. the Jacobian matrix is rank deficient) at $t \approx 5.8$ h. Consequently Σ in (19) would result not observable. It would have been completely observable on the first order of derivation in p. We hypothesize that the absence of observability on the third order of derivation is due to the introduction in the sigma definition of an equation for the substrate proportional to the equation for the product. It is therefore a redundant system, completely definable by a state vector of dimension 2, for which it would not be necessary therefore to consider orders of observability greater than the first.

2.5. Example 3

Here as an example we consider a system defined by the following equations.

$$\begin{cases} \dot{x}_1 = \frac{1}{2}x_1^2 + be^{x_2} - x_2x_3 \\ \dot{x}_2 = x_1 \end{cases}. \tag{31}$$

Assume that the observation is $y = e^{x_1}$. The observability mapping is

$$\mathbf{Q} = \begin{bmatrix} e^{x_1} \\ (ax_1^2 + be^{x_2} - x_2)e^{x_1} \end{bmatrix}, \tag{32}$$

and its Jacobian matrix is

$$J(x) = \begin{bmatrix} e^{x_1} & 0 \\ (ax_1^2 + x_1 + be^{x_2} - x_2)e^{x_1} & e^{x_1}(be^{x_2} - 1) \end{bmatrix}, \tag{33}$$

whose determinant is different from zero for all $x_2 \neq \ln\frac{1}{b}$. The leading principal minors of J are

$$|M_1| = e^{x_1} \geq \varepsilon \tag{34}$$

$$\frac{|M_1|}{|M_2} = |e^{x_1}(be^{x_2} - 1)| \geq \varepsilon \tag{35}$$

We consider $a = 5 \times 10^{-4}$ and $b = 10^{-3}$, and $\varepsilon = \ln 2$. This value of ε is obtained by intersecting $|M_1|$ with $\frac{|M_1|}{|M_2}$. In Fig. 4 we show that the system is not continuously observable, but only in some intervals of times.

2.6. Example 4

As another example of non-continuously observable system we consider again the Rössler system from the coordinate x_1. The system is not continuously observable from the observation $y = x_1$. The observability mapping $\mathbf{Q}$ is

$$\mathbb{H} = \begin{bmatrix} x_1 \\ -x_2 - x_3 \\ -\dot{x}_2 - \dot{x}_3 \end{bmatrix}, \tag{36}$$

and the Jacobian matrix is

$$J(x) = \begin{bmatrix} 1 & 0 & 0 \\ 0 & -1 & -1 \\ -1 - \dot{x}_3 & -a & -x_1 - c \end{bmatrix}. \tag{37}$$

The Jacobian matrix has full rank for all $x_1 \neq -a - c$. Simulations excluding this value for x_1 are reported in Fig. 5 and show that the Rössler systems is not continuously observable from x_1. In this case, $\varepsilon = 1$, as

$$|M_1| = |M_2| = 1$$

$$|M_3| = |x_1 + c - a|.$$

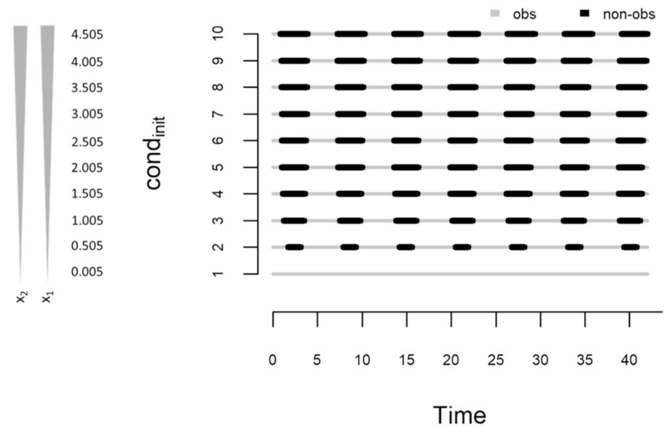

Fig. 4. Application of Theorem 1 to the system described by Eq. (31). Shown is the fulfilment of the ratio condition by the absolute value of the leading principal minors at each simulated time point. The outcome along the simulation time is colour-coded (observability = grey, non-observability = black). We performed the test at multiple initial condition where x1 and x2 are allowed to increase from 0.005 to 5 by a step equal to 0.5.

3. A real case study: the mechanisms of action and the metabolism of Dichloroacetate

Oncogenic transformations cause a tumour cell proliferation greater than vascular capacity, thus generating hypoxia [39]. Hypoxia within the tumour microenvironment enhances glycolytic metabolism through the activation of the hypoxiainducible factor 1 (HIF1) transcription factor. The increment of the glycolysis increases the production of lactate, which contributes to an acidic extracellular pH and further changes in gene expression. Both hypoxia and acidosis contribute to increase the number of somatic mutations that can further drive tumour progression. A scarce concentration of oxygen within the tumour induces the metabolic program of HIF1 transcription factor that reduces oxygen demand by decreasing mitochondrial function. This response to low levels of oxygen is mediated through the HIF1-dependent induction of PDKs (pyruvate dehydrogenase kinases) within the tumour cells and a reduction in pyruvate oxidation within the mitochondria. This is an

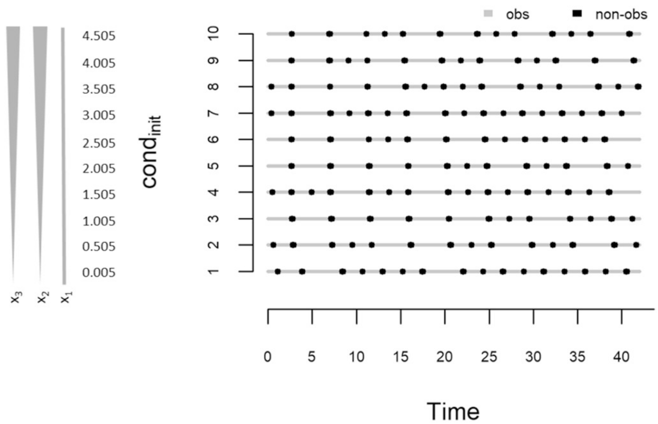

Fig. 5. Application of Theorem 1 to the Rössler system, with observations $y = x_1$. Shown is the fulfilment of the ratio condition by the absolute value of the leading principal minors at each simulated time point. The outcome along the simulation time is colour-coded (observability = grey, non-observability = black). We performed the test at multiple initial condition where x_1 is pre-set and x_2 and x_3 are allowed to increase from 0.005 to 5 by a step equal to 0.5.

adaptive response to hypoxia responsible for bringing the demand for oxygen closer to the limited supply. Dichloroacetate (DCA) interferes with this adaptation to tumour hypoxia by inhibiting the function of the PDKs [39–42]. Fig. 6 shows a scheme of the kinetics and catabolism of DCA.

In vitro, DCA activates PDC (pyruvate dehydrogenase complex) by inhibition of PDK at concentration of 10–250 μM or 0.15–37.5 μg/ml in a dose-dependent fashion [41]. DCA acts like a pyruvate-mimetic. When pyruvate concentration is high, it acts as an allosteric regulator stimulating pyruvate metabolism in the mitochondria. This means that when pyruvate concentration is high, PDC activity is high and the inhibitory kinases are turned off.Â PDC phosphorylation is low (i.e. PDC-P are present in low concentration), PDC activity is high and the excess pyruvate is metabolized. When the pyruvate is metabolized, there is no longer need for PDC activity, and, consequently, the kinases are turned back on to add inhibitory phosphorylations. This is a self regulating system when pyruvate is driving the regulation.

Finally, Stacpoole et al. [40] postulated two principal in vivo routes of DCA catabolism: (i) by reduction of MCA and (ii) by oxidation to glycoxilate. Glycoxilate and its catabolites are naturally occurring endogenous molecules. Glycine provides a route of entry of DCA into the carbon pool of the host.

3.1. The mathematical model

The following set of first-order differential equations models the kinetics and catabolism of DCA as shown in Fig. 6. In this specification, the activation and inhibition action of DCA on PDP1-2 and PDK1-4, respectively, are modelled as first order kinetics.

$$\frac{d\,[\text{Glucose}]}{dt} = -k_1\,[\text{Glucose}] + F_1(t) \tag{38}$$

$$\frac{d\,[\text{Pyruvate}]}{dt} = k_1\,[\text{Glucose}] + k_2\,[\text{Lactate}] - k_3\,[\text{Pyruvate}] - k_4\,[\text{Pyruvate}][\text{PDC}] \tag{39}$$

$$\frac{d\,[\text{Lactate}]}{dt} = k_3\,[\text{Pyruvate}] \tag{40}$$

$$\frac{d\,[\text{PDK}]}{dt} = -k_6\,[\text{PDC}][\text{PDK}] - k_5\,[\text{DCA}] \tag{41}$$

$$\frac{d\,[\text{PDC}]}{dt} = -k_4\,[\text{Pyruvate}][\text{PDC}] + k7[\text{PDC} - \text{P}] * [\text{PDP}] \tag{42}$$

$$\frac{d\,[\text{PDC} - \text{P}]}{dt} = -k_7\,[\text{PDC} - \text{P}][\text{PDP}] + k_6\,[\text{PDC}][\text{PDK}] \tag{43}$$

$$\frac{d\,[\text{PDP}]}{dt} = -k_7\,[\text{PDC} - \text{P}][\text{PDP}] + k_8\,[\text{DCA}] \tag{44}$$

$$\frac{d\,[\text{DCA}]}{dt} = F_2(t) - (k_9 + k_{10})[\text{DCA}] \tag{45}$$

$$\frac{d\,[\text{Glyoxylate}]}{dt} = k_9\,[\text{DCA}] - (k_{11} + k_{12} + k_{14} + k_{15})[\text{Glyoxylate}] \tag{46}$$

$$\frac{d\,[\text{CO}]_2}{dt} = k_{15}\,[\text{Glyoxylate}] - k_{16} * [\text{CO}]_2 \tag{47}$$

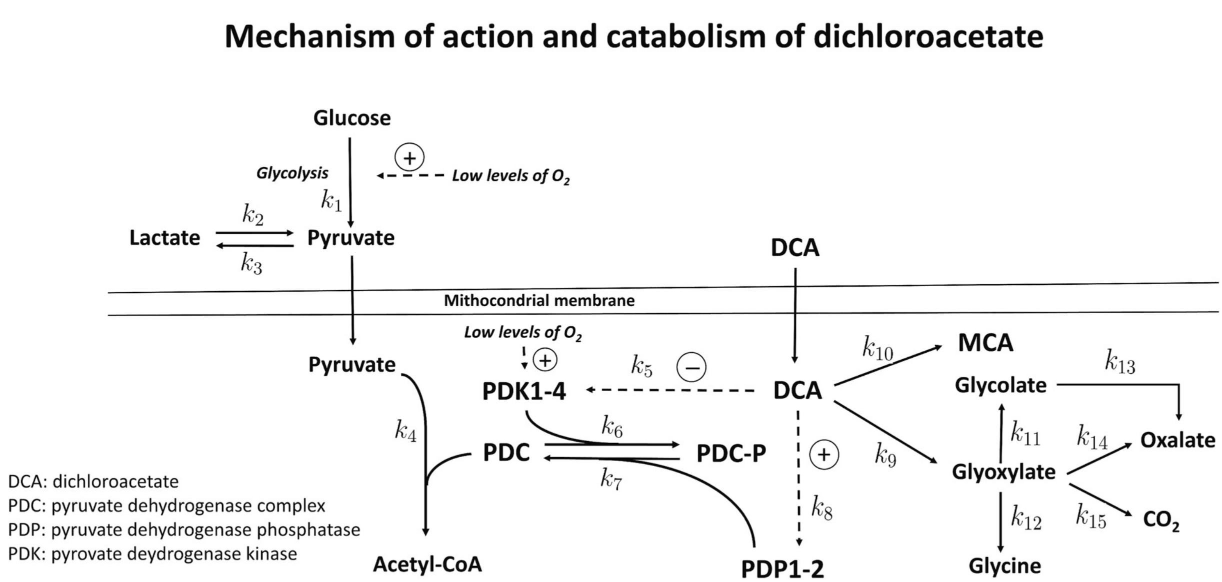

Fig. 6. The network of biotransformations of DCA [39–41]. The ks denotes the rate constants of the interactions.

Table 2
Parameters and initial conditions used to simulate the dynamics of the DCA biotransformation network. The values reported in this table are hypothetical values, useful only for the purpose of producing numerical solutions that reflect the expected qualitative trends of the various species involved in teh DCA biotransformation network.

Rate constants	Initial conditions
$k_1 = 10^{-5}\,h^{-1}$	Glucose (X_1) = 400 μM
$k_2 = 0.1\,h^{-1}$	Pyruvate (X_2) = 0 μM
$k_3 = 0.01\,h^{-1}$	Lactate (X_3) = 0 μM
$k_4 = 0.011\,\mu M^{-1}\,h^{-1}$	Lactate (X_4) = 0 μM
$k_5 = 0.01\,h^{-1}$	PDK = 100 μM
$k_6 = 0.001\,\mu M^{-1}\,h^{-1}$	PDC (X_5) = 100 μM
$k_7 = 100\,\mu M^{-1}\,h^{-1}$	PDC-P (X_6) = 0 μM
$k_8, \dots k_{16} = 1\,h^{-1}$	PDP (X_7) = 100 μM
$\alpha_1 = 1\,\mu M$	DCA (X_8) = 0 μM
$\beta_1 = 0.2\,h^{-1}$	Glyoxylate (X_9) = 0 μM
$\alpha_2 = 0.2\,\mu M$	CO_2 (X_{10}) = 0 μM
$\beta_2 = 0.4\,h^{-1}$	Glycolate (X_{11}) = 0 μM
	Oxalate (X_{12}) = 0 μM

$$\frac{d\,[\text{Glycolate}]}{dt} = k_{12}\,[\text{Glyoxylate}] \tag{48}$$

$$\frac{d\,[\text{Oxalate}]}{dt} = k_{15}\,[\text{Glyoxylate}] + k_{14}\,[\text{Glycolate}] \tag{49}$$

where $F_1(t) = \alpha_1 \cos \beta_1 t$ and $F_2(t) = \alpha_2 \cos \beta_2 t$ are functions describing the Glucose and DCA administration scheduling, respectively. The initial conditions and the values of the rate constants ks are reported in Table 2.

3.2. Observability analysis

We performed numerical simulations of the differential equations describing the DCA metabolism and mechanism of action, whose numerical solution are shown in Fig. 7. The numerical implementation of the model aimed at assessing the conditions underlying the observability of the system. Once the observation variable is chosen, the calculation of the elements of the Jacobian matrix requires the values of the derivatives of the model function at the simulated time points, which are obtained by numerical differentiation using a finite difference scheme. Numerical implementation was realized on the basis of the evoper and NbClust packages within the R computing environment [43]. We performed time-wise assessment of the Jacobian matrix determinant, rank and eigenvalues using the base and Matrix packages and the Jordan canonical form of the Jacobian matrix was performed using the mcompanion package which are available in the R environment. As we show in Fig. 8 the Jacobian matrix is constantly rank-deficient regardless of the coordinate from which we observe the system. The reasons for this are that (i) the time derivatives are defined but become zero from a certain order of differentiation onwards, or (ii) the observability mapping is not differentiable in each time domain point, or (iii) the observability mapping is not continuously differentiable. For some molecular species of the DCA network we are in the situation (i) for others in the situations (ii) or (iii), as can be seen also by looking at the numerical simulations in Fig. 7.

This case study shows therefore how the hypothesis of continuous

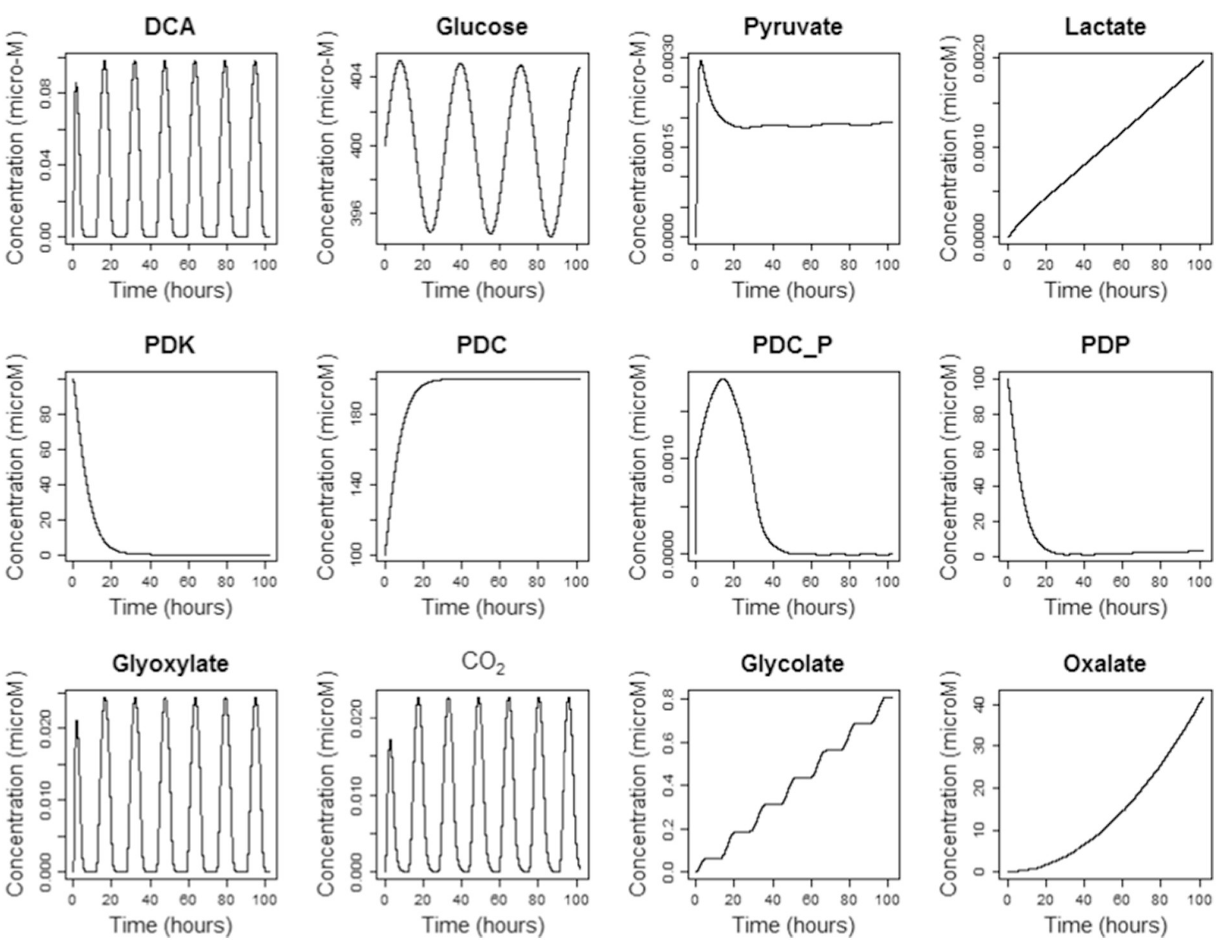

Fig. 7. Numerical simulations network of biotransformations of DCA [39–41], described by the system of differential eqs. (38)–(49) with parameters defind in Table 2.

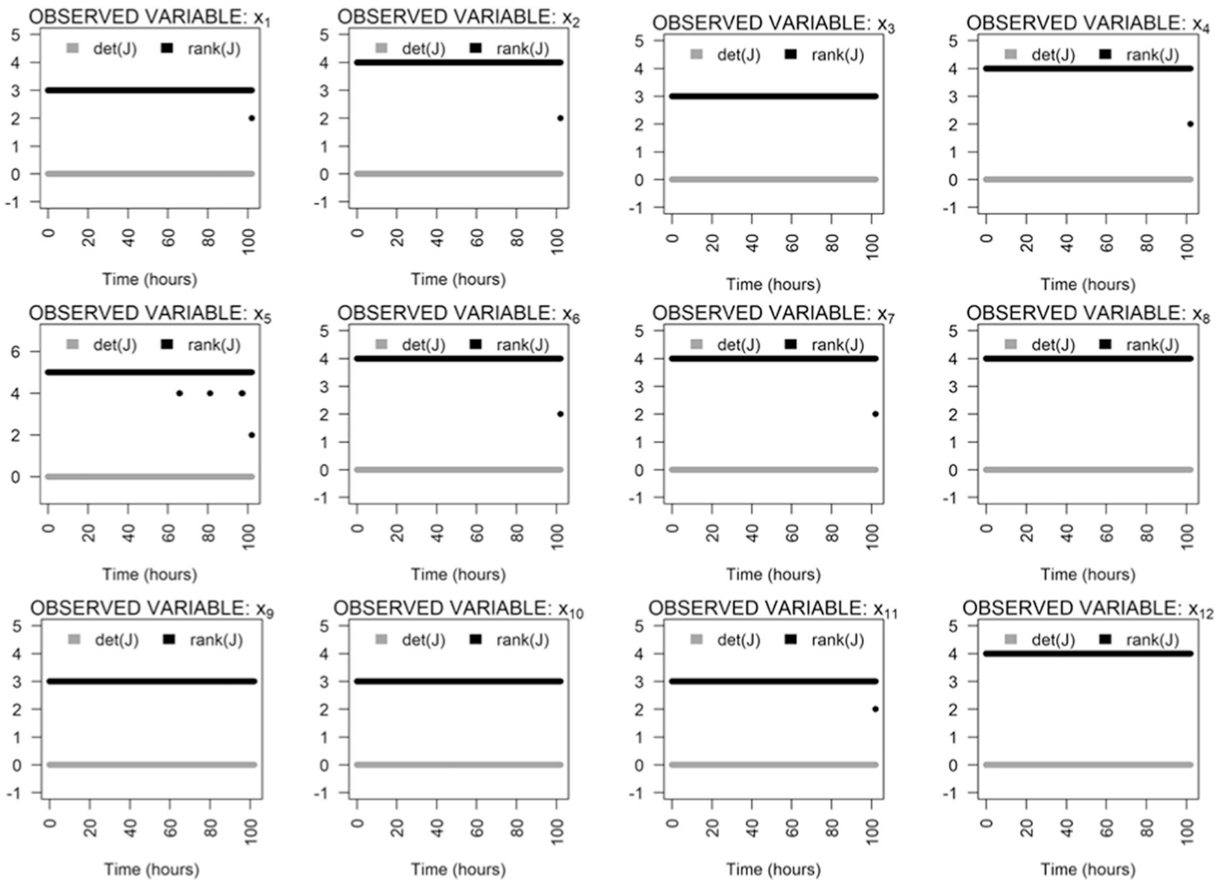

Fig. 8. Determinant and rank of the Jacobian matrix of the system of differential equations describing the dynamics of DCA metabolism and mechanism of action as time varies (Eqs. (38)–(49)). The Jacobian matrix is a 12 × 12, and never turns out to have full rank, and consequently the observability mapping is not invertible.

differentiability posed by Theorem 2 (but not by Theorem 1[4]) is indispensable in order to define the necessary and sufficient conditions for observability.

The eigenvalues of the Jacobian matrix have been found complex, meaning that the system is oscillating, i.e. it has at least one equilibrium point the nature of which could prevent the continuous differentiability. Furthermore, since a real matrix has canonical form of Jordan if and only if all the roots of the characteristic polynomial are real, we conclude that the Jacobian matrix of this system cannot be factorized by two symmetric matrices. Theorem 2 states that the system is not observable (not even locally).

4. Conclusions

Dynamical processes taking place in complex networks are ubiquitous in natural and in technological systems. A quantitative description of a complex system is inherently limited by our ability to estimate the system internal state from experimentally accessible outputs. Mathematical models are increasingly being used to infer the state trajectories of complex systems. However, the fundamental relevance of the choice of a model and measurement function that faithfully captures and predicts the system dynamics is often underestimated. As a matter of fact, observability requires us to establish a relationship between the outputs, the state space, and the inputs to afford the reconstruction of the system complete initial state. Our contribution here is to formalize necessary and sufficient conditions for the observability of non-linear continuous-time systems defined by dynamic models based on first-order differential equations. Our results could find applications in the systematic exploration of the dynamics of a wide range of biological (e.g. synthetic biology, metabolic engineering and medicine) and technological systems, helping to identify the mathematical form the dynamics of a system could be cast in along with the quantities suitable to monitor the systems internal state. Our work also raises fundamental questions concerning non-continuously observable systems worthy of future pursuit. Since an observable model of a system is identifiable, the proper choice of the observability mapping is bound to provide us with fine-grained level of detail on the kinetic parameters governing the dynamics of the system of interest. Furthermore, analysing observability represents an effective means for probing the controllability of a system. Therefore, observability assessment could provide a guideline to biotechnological applications aimed at achieving certain control objectives in terms of process intensification and accuracy of process outcome requirements.

[4] The Theorem 1 only says that **Q** has to be *differentiable*.

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