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Abstract

In this paper, the problem of state estimation of a bioreactor containing a single substrate and several competing species is studied. This scenario is well-known as the *competition model*, in which multiple species compete for a single limiting nutrient. Considering the total biomass to be the only available measurement, the challenge is to estimate the concentration of the whole state vector. To achieve this goal, the estimation scheme is built by the coupling of two estimation techniques: an asymptotic observer, which depends solely on the operating conditions of the bioreactor, and a finite-time parameter estimation technique, which drops the usual requirement of the persistence of excitation. The presented methodology achieves the estimation of each competing species and a numerical example illustrates the intended application.

Keywords: state estimation, chemostat, monitoring, adaptive

1. Introduction

Biological processes have drawn the attention of both academic and industrial fields over the last decades, due to a wide range of applications that emerge from such processes. These processes consist of chemical reactions involving microbes (like bacteria, algae, and yeast) that play a certain role (such as degradation or production of some compound) and they take place in devices called *bioreactors* [1].

The interest of using a bioreactor is due to the fact that it allows controlled operational conditions. More specifically, the *chemostat* [2] is a tank bioreactor operated in a continuous mode, *i.e.*, in which fresh media might be added and removed proportionally and thus having a constant working volume.

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Monitoring biological and biochemical processes is a known issue in biotechnology. Indeed, measuring variables inside a bioreactor arises as an important question, since it is crucial to have real-time information about variables such as the concentration of biomass, dissolved products or reactants, gaseous outflows or growth, death and production rates of living organisms. Nevertheless, many difficulties are originated by the lack of available sensors for such variables, their cost, their physical set-up or even their sampling time of measurements.

To overcome these challenges, a well-known option is the use of *software sensors* [3]. These sensors, as viewed from the control community point of view, consist basically of state observers/estimators. Throughout the years, different types of observers have been presented, for instance, asymptotic observers [4], adaptive observers [5], interval observers [6][7], sliding-mode observers [8], and hybrid observers [9]. The reader is invited to an exhaustive discussion on software sensors in the survey presented by [10].

However, to the best of our knowledge, the estimation of the concentration of independent species in a competitive scenario (i.e., having independent estimates for each species present in the culture) has not received much attention. Nevertheless, this scenario is very interesting for new complex applications involving heterogeneous microbial communities. Indeed, synthetic (or bio-engineered) microbial *consortia* might allow deeper studies on the interaction of different species and promote enhanced productivity in some applications (see [11]).

The design of observers for biological processes is often challenging due to uncertainties (such as parameter uncertainties and measurement noise) in the nonlinear models and their lack of observability.

In this paper, we consider a bioreactor containing a single limiting substrate and several competing species, having the total biomass inside the bioreactor as available for measurement. The objective is then to estimate the concentration of each microbial sub-population in real-time. Then, to address this challenge, a new approach is proposed: we first design an asymptotic observer (which depends solely on the operating conditions of the bioreactor to estimate the substrate concentration) and build an adaptive estimation scheme that identifies the initial conditions of each species. The evolution of each concentration is then computed using the solution of a time-varying state equation.

To accomplish these objectives, the adaptive estimation scheme is based on a result presented in [12], where the *dynamical regressor extension and mixing* method (DREM) [13] was applied and different algorithms possessing certain robustness against external perturbations and measurement noises were proposed. These algorithms do not require the usual condition on the persistence of excitation, which is of great interest for applications like microbial growth (since trajectories obtained by highly excited inputs might not be possible in real-life experiments). In addition, finite-time converging algorithms become less dependent of observability after a finite interval of convergence. This means that, if the system approaches unobservable regions, the estimation will not be affected (it is the scenario considered for illustration in this work).

Structure of the paper: the problem statement is presented in Section 2. Observability analysis and preliminaries on numerical differentiation and parameter identification are introduced in Section 3. The state estimation scheme is discussed in Section 4 and numerical examples illustrate its application in Section 5. Concluding remarks and future directions are discussed in Section 6.

This paper is an extension of a previous conference paper [14], presenting novel results (such as the use of a fixed-time algorithm for the estimation problem), proofs on the convergence of the estimation and boundedness of the error, and substantial improvements.

Notation:

- A continuous function $\alpha : \mathbb{R}_+ \to \mathbb{R}_+$ belongs to the class $\mathcal{K}$ if $\alpha(0) = 0$ and the function is strictly increasing. A function $\beta : \mathbb{R}_+ \times \mathbb{R}_+ \to \mathbb{R}_+$ belongs to the class $\mathcal{KL}$ if $\beta(\cdot, t) \in \mathcal{K}$ for each fixed $t \in \mathbb{R}_+$ and $\beta(s, \cdot)$ is decreasing and $\lim_{t\to+\infty} \beta(s, t) = 0$ for each fixed $s \in \mathbb{R}_+$, a function $\beta : \mathbb{R}_+ \times \mathbb{R}_+ \to \mathbb{R}_+$ belongs to the class $\mathcal{GKL}$ if $\beta(s, 0) \in \mathcal{K}$, $\beta(s, \cdot)$ is decreasing and for each $s \in \mathbb{R}_+$ there is $T_s \in \mathbb{R}_+$ such that $\beta(s, t) = 0$ for all $t \geq T_s$;
- For a Lebesgue measurable and essentially bounded function $x : \mathbb{R} \to \mathbb{R}^n$, denote $|x|_\infty = \operatorname{ess\,sup}_{t\in\mathbb{R}} \|x(t)\|$, where $\|\cdot\|$ is a usual Euclidean norm, and define $\mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^n)$ as the set of all such functions with finite norms $|\cdot|_\infty$;
- Denote $\lceil x \rfloor^\alpha = |x|^\alpha \text{sign}(x)$, where $|\cdot|$ is the absolute value of $x \in \mathbb{R}$ and $\alpha \geq 0$.

2. Problem Statement

Consider the following non-linear system describing microbial growth of n species inside a chemostat, with dimensionless yield coefficients (see [15]):

$$\begin{aligned} \frac{dS(t)}{dt} &= (S_{in}(t) - S(t))D(t) - \sum_{i=1}^{n} \mu_i(S(t))x_i(t) \\ \frac{dx_i(t)}{dt} &= (\mu_i(S(t)) - D(t))x_i(t), \ i = 1 \dots n \end{aligned} \tag{1}$$

where S and x_i are, respectively, the concentration of the substrate and the i^{th} species, S_{in} and D are the control inputs (nutrient inflow concentration and dilution rate, respectively). Functions $\mu_i(S)$, called *specific growth rates*, describe the kinetics of nutrient uptake by each species. Although many forms have been proposed for such functions, in this work we consider it to be given by the Monod's law, as follows:

$$\mu_i(S(t)) = \mu_{i_{max}} \frac{S(t)}{a_i + S(t)}, \ \mu_{i_{max}} > 0 \tag{2}$$

where the index represents the *i-th* species. For readability in the following, if this index is omitted, it is assumed that μ is a component-wise vector, i.e., $\mu = [\mu_1 \dots \mu_n]^\top$.

Problem 1: Estimate the concentrations $S(t)$ and $x_i(t)$, without knowledge of initial conditions and using $y(t) = \sum_{i=1}^{n} x_i(t) + w(t)$ (i.e., the total biomass concentration inside the bioreactor) as measurement, we also consider the presence of measurement noise, given by $w(t) \in \mathcal{L}_\infty(\mathbb{R}_+, \mathbb{R})$.

In real experiments, this kind of measurement is usually obtained by optical density methods (such as spectrometry). Investigation of this specific measurement set-up is the main feature of the paper, due to the mathematical complexity that it imposes on the problem.

3. Preliminaries

3.1. Robust exact differentiator

In this section, we present results from [16] for the design of an arbitrary-order, robust and exact differentiator. These exact differentiators demonstrate a finite-time convergence and also good sensitivity to input noise.

Let a measured signal $f(t) = f_0(t) + w(t)$ be defined for $t \in [0, \infty)$, where $f_0(t)$ is an unknown base signal, whose *n-th* derivative has a known Lipschitz constant $L > 0$, and w is an unknown noise signal such as $w \in \mathcal{L}_\infty(\mathbb{R}_+, \mathbb{R})$. The objective is then to have a robust and exact estimation of $f_0, \dot{f}_0 \dots f_0^{(n)}$. The following scheme offers such an estimate:

$$\begin{cases} \dot{z}_0 = -\lambda_n L^{\frac{1}{n+1}} |z_0 - f(t)|^{\frac{n}{n+1}} \mathrm{sign}(z_0 - f(t)) + z_1 \\ \dot{z}_1 = -\lambda_{n-1} L^{\frac{1}{n}} |z_0 - f(t)|^{\frac{n-1}{n}} \mathrm{sign}(z_0 - f(t)) + z_2 \\ \dots \\ \dot{z}_{n-1} = -\lambda_1 L^{\frac{1}{2}} |z_0 - f(t)|^{\frac{1}{2}} \mathrm{sign}(z_0 - f(t)) + z_n \\ \dot{z}_n = -\lambda_0 L \mathrm{sign}(z_0 - f(t)), \end{cases} \tag{3}$$

where λ_i are tuning parameters for $i = 1, \dots, n$. Although an infinite sequence λ_i can be built, it has been shown that $\{\lambda_0, \lambda_1\} = \{1.1, 1.5\}$ suffice for the zero- and first-order derivatives.

According to [16], the differentiation ensured accuracy satisfies the following inequality:

Theorem 3.1. *Let the input noise satisfy $|w(t)| \leq \epsilon$ for almost all $t \geq 0$. Then the following inequalities are established in finite-time $T > 0$, for some positive constant ϱ_i depending exclusively on the parameters $\lambda_1, \dots, \lambda_n$ of the differentiator:*

$$|z_i(t) - f_0^{(i)}(t)| \leq \varrho_i L^{\frac{1}{n+1}} \epsilon^{\frac{n-i+1}{n+1}}, \ \forall t \geq T, \ i = 0, 1, \dots, n \tag{4}$$

Also, all solutions of this scheme are Lyapunov stable. For proofs, see [16].

3.2. DREM and robust FT parameter estimation

In this section, we will recall some preliminaries on parameter estimation. In this sense, let us consider a usual estimation problem in the static linear regression model [17] as follows:

$$y(t) = \omega^\top(t)\theta + w(t), \quad t \in \mathbb{R} \tag{5}$$

where $\theta \in \mathbb{R}^n$ is the vector of unknown constant parameters which are to be estimated, $\omega : \mathbb{R} \to \mathbb{R}^n$ is the regressor function (supposedly known and bounded) and $y(t) \in \mathbb{R}$ is the available measurement signal with measurement noise $w \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R})$.

Assumption 1. Let $\omega \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^n)$ and $w \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R})$.

It is well-known that problem (5) has a solution (if $\omega(t)$ is persistently excited, see [17]) given by:

$$\dot{\hat{\theta}}(t) = \gamma\omega(t)\left(y(t) - \omega(t)^\top\hat{\theta}(t)\right), \gamma > 0 \tag{6}$$

where $\hat{\theta}$ is the estimate of θ.

In this sense, [13] proposed a method – called *dynamic regressor extension and mixing method* (hereafter abbreviated as DREM) – which aims to decouple model (5) into n one-dimensional regressions and to allow each parameter θ_i, $i = 1 \dots n$, to be evaluated under another condition than the persistence of excitation of $\omega(t)$, at the same time providing acceleration of convergence rate and monotonic decay for the parameter estimation error.

For that, under assumption that $\omega \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^n)$ and $w \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R})$, one designs $n-1$ linear operators $H_j : \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}) \to \mathcal{L}_\infty(\mathbb{R}, \mathbb{R})$.

Remark 1. According to [18], the linear operator H_j can be any stable linear time invariant filter or delay (described, for instance, by transfer functions).

As consequence of the assumptions above, one gets that $y(t) \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R})$. Then, the superposition principle induces

$$\tilde{y}_j(t) = H_j(y(t)) = \tilde{\omega}_j^\top(t)\theta + \tilde{w}_j(t), \quad j = 1, \dots, n$$

where $\tilde{y}_j(t) \in \mathbb{R}$ is *j-th* operator output, $\tilde{\omega}_j : \mathbb{R} \to \mathbb{R}^n$ is the *j-th* filtered regressor function and $\tilde{w}_j$ is the filtered *j-th* noise signal. Defining new vector variables:

$$\tilde{Y}(t) = [y(t)\ \tilde{y}_1(t) \dots \tilde{y}_{n-1}(t)]^\top \in \mathbb{R}^n,$$
$$\tilde{W}(t) = [w(t)\ \tilde{w}_1(t) \dots \tilde{w}_{n-1}(t)]^\top \in \mathbb{R}^n$$

and a time-varying matrix

$$M(t) = [\omega(t)\ \tilde{\omega}_1(t) \dots \tilde{\omega}_{n-1}(t)]^\top \in \mathbb{R}^{n\times n}, \tag{7}$$

and rewriting (5) using the above $n-1$ regressor models, one has

$$\tilde{Y}(t) = M(t)\theta + \tilde{W}(t).$$

Multiplying both sides of the above equation by the adjoint matrix of $M(t)$ (see [18] for further details), one has that the n scalar decoupled regressor models are given by

$$Y_i(t) = \phi(t)\theta_i + W_i(t) \tag{8}$$

where $\phi(t) = det\,(M(t))$, $Y(t) = adj(M(t))\tilde{Y}(t)$ and $W(t) = adj(M(t))\tilde{W}(t)$.

Finally, relating equations (8) and the estimation algorithm (6) yields

$$\dot{\hat{\theta}}_i(t) = \gamma_i\phi(t)\left(Y_i(t) - \phi(t)\hat{\theta}_i\right), \gamma_i > 0. \tag{9}$$

Now, this decoupled configuration allows enhanced estimation algorithms to be applied, such as the finite-time converging (see Appendix 1 for a recall on the definitions of such kind of convergence and stability) estimation algorithm proposed by [19, 20]:

$$\dot{\hat{\theta}}(t) = \phi(t)\left\{\gamma_1\lceil Y(t) - \phi(t)\hat{\theta}(t)\rfloor^{1-\alpha} + \gamma_2\lceil Y(t) - \phi(t)\hat{\theta}(t)\rfloor^{1+\alpha}\right\} \tag{10}$$

where $\gamma_1 > 0$, $\gamma_2 > 0$, $\alpha \in [0, 1)$.

Theorem 3.2. *[12] Let assumption 1 be satisfied and, for given $T^0 > 0$ and $T_f > 0$,*

$$\int_t^{t+\ell} \min\{|\phi(s)|^{2-\alpha}, |\phi(s)|^{2+\alpha}\}ds \geq \upsilon > 0 \tag{11}$$

for all $t \in [-T^0, T^0 + T_f]$ and some $\ell \in (0, T_f/2)$. Take

$$\min\{\gamma_1, \gamma_2\} > \frac{2^{2+\frac{\alpha}{2}}}{\alpha\upsilon\left(\frac{T_f}{2\ell} - 1\right)}$$

then the estimation error $e(t) = \theta - \hat{\theta}(t)$ dynamics for (10) with $\hat{\theta}(t_0) = 0$ is short-fixed-time ISS for T^0 and T_f.

Remark 2. The persistence of excitation in a finite-time interval given by (11) is a sufficient condition for observability of the system (5).

4. Estimating microbial sub-populations

In this section, we will discuss the estimation problem for system (1), as stated in Problem 1. For the sake of readability throughout this section, let us introduce the following notation:

$$\varphi_i(t) = e^{\int_0^t \mu_i(S(\tau)) - D(\tau)d\tau}, \quad \hat{\varphi}_i(t) = e^{\int_0^t \mu_i(\hat{S}(\tau)) - D(\tau)d\tau}$$

where the index represents the *i-th* species and $\hat{S}$ is an estimate of S. If this index is omitted, it is assumed that φ is a component-wise vector, i.e., $\varphi = [\varphi_1 \dots \varphi_n]^\top$. Also, as it will be often used in the following, let us recall the time-varying solution for each species concentration in (1) as follows:

$$x_i(t) = x_i(0)\varphi_i(t) \tag{12}$$

where $x_i(0)$ is the initial condition of each state x_i.

4.1. Observability analysis

Before designing the proposed observer, let us discuss the observability of system (1) in the point of view of the concentration of the species x_i. First, let us present the dynamics of $x_i(t)$ in (1) as a time-varying autonomous system (*i.e.*, under the form $\frac{dx(t)}{dt} = A(t)x(t)$):

$$\frac{dx_i(t)}{dt} = \begin{bmatrix} \mu_1(S(t)) - D(t) & 0 & 0 \\ 0 & \ddots & 0 \\ 0 & 0 & \mu_i(S(t)) - D(t) \end{bmatrix} x_i(t) \tag{13}$$

The observability of such systems can be evaluated applying the following result:

Theorem 4.1. *[21] Let $A(t)$ and $C(t)$ be $n-1$ times continuously differentiable. Then, the $n-$dimensional pair $(A(t), C(t))$ is observable at t_0 if there exists a finite $t_1 > t_0$ such that*

$$rank\left(\begin{bmatrix} N_0(t_1) \\ N_1(t_1) \\ \vdots \\ N_{n-1}(t_1) \end{bmatrix}\right) = n \tag{14}$$

where $N_{m+1}(t) = N_m(t)A(t) + \frac{d}{dt}N_m(t)$, for $m = 0, \dots, n-1$ and with $N_0 = C(t)$.

For an illustration on how this approach works in our setting, let us investigate the observability of (13) for $n = 3$. For the considered measurement, we have that $C = [1, \dots, 1]$ is a constant vector. Then, applying Theorem 4.1, we obtain the following requirement:

$$\text{rank}\left(\begin{bmatrix} 1 & 1 & 1 \\ \mu_1(S) - D & \mu_2(S) - D & \mu_3(S) - D \\ f_1 & f_2 & f_3 \end{bmatrix}\right) = 3 \tag{15}$$

where $f_i = (\mu_i(S) - D)^2 + \frac{\partial \mu_i(S)}{\partial S}\dot{S} - \dot{D}$. Although it is not expected that all $\mu_i(S)$ intersect at the same point (the same for their derivative with respect to S), computing a region in which the system is observable depends heavily on the parameters of $\mu_i(S)$ (it is hard to derive an analytical condition, but (15) can be effectively checked numerically in applications).

However, for $n = 2$, the rank of the first 2×2 block of the matrix in (15) should be 2, which is easily translated to the requirement that $\mu_1(S) \neq \mu_2(S)$. Hence, the system is observable out of the domain in which this condition is transgressed.

4.2. Using the total biomass as measurement

If compared to other possible measurements (for instance, the substrate or a single biomass concentration), the measurement of the total biomass, i.e., $y(t) = \sum_{i=1}^{N} x_i(t) + w(t)$, addresses a more difficult task (from a mathematical point of view) to the problem.

The core idea of this section is to design an observer for species populations $x_i(t)$ using (12). However, this solution requires knowledge of the state component $S(t)$ and the initial conditions of each species, i.e., $x_i(0)$. As none of these variables are known, an option is to design a hybrid estimation scheme, consisting of an asymptotic observer for $S(t)$, a (finite-time) differentiator, and a (finite-time) parameter estimator to identify $x_i(0)$. The overall scheme is illustrated in Figure 1.

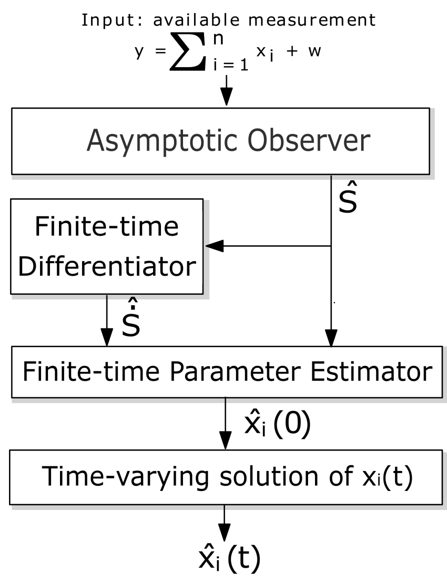

Figure 1: Overview of the proposed estimation scheme. These steps are concomitant in time.

Estimating $S(t)$

First, let us investigate the estimation of $S(t)$. As it can be seen in (1), equations for the species concentrations $x_i(t)$ and the substrate concentration $S(t)$ are coupled by the kinetic rates $\mu_i(S(t))$ (indeed, this term describes the substrate consumption by the *i-th* species). Due to this coupling, the first step is to obtain an observer for $S(t)$ that does not depend on these kinetics. This problem has been addressed in [22] and this (asymptotic) observer can be designed by introducing the change of variables $z(t) = S(t) + \sum_{i=1}^{n} x_i(t)$, which admits the dynamics

$$\dot{z}(t) = DS_{in} - Dz(t)$$

and results in the following observer equations:

$$\dot{\hat{z}}(t) = DS_{in} - D\hat{z}(t) \tag{16}$$

and thus $\hat{S}(t) = \hat{z}(t) - y(t)$.

The following theorem [22] states the convergence condition and properties of observer (16):

Theorem 4.2. *: If the following condition is satisfied*

$$\lim_{T\to+\infty} \int_0^T D(s)ds = +\infty$$

then $\lim_{t\to\infty} |z(t)-\hat{z}(t)| = 0$*, implying that* $|S(t)-\hat{S}(t)| \leq |z(0)-\hat{z}(0)|e^{-\int_0^t D(\tau)d\tau} + |w(t)|$.

Proof. The proof relies on analysis of the observation error $e_z(t) = z(t) - \hat{z}(t)$, whose dynamics is given by

$$\tfrac{d}{dt}e_z(t) = -De_z(t) \tag{17}$$

meaning that, if the condition stated in the above theorem holds, it implies an asymptotic convergence to zero of the discrepancy $z(t) - \hat{z}(t)$.

Following the error dynamics given by (17), one has that the time evolution of the discrepancy $S(t) - \hat{S}(t)$ is upper-bounded by

$$|S(t) - \hat{S}(t)| \leq |z(0) - \hat{z}(0)|e^{-\int_0^t D(\tau)d\tau} + |w(t)| \tag{18}$$

where $\hat{z}(0)$ is the initial condition of the asymptotic observer (16) and $z(0)$ is supposedly unknown but upper bounded. □

Estimating $\dot{S}(t)$

As it will be needed in the sequel, we will now design an observer for $\dot{S}$. This observer can be obtained by means of the considered measurement $y(t)$ and the differentiator (3) as follows:

$$\begin{aligned} \dot{z}_0(t) &= z_1(t) - \lambda_0 L^{\frac{1}{2}}|z_0(t) - y(t)|^{1/2} sign(z_0(t) - y(t)) \\ \dot{z}_1(t) &= -\lambda_1 L sign(z_0(t) - y(t)) \end{aligned} \tag{19}$$

where $z_0, z_1 \in \mathbb{R}$ are the states of the differentiator, $\lambda_0 > 0$ and $\lambda_1 > 0$ are tuning parameters, and $L > 0$ is an upper bound for the Lipschitz constant of the derivative of $y_0(t) = \sum_{i=1}^n x_i(t)$. Therefore, as $\dot{y}_0 = \sum_{i=1}^n (\mu_i(S(t))-D)x_i(t)$ and $\ddot{y}_0 = \sum_{i=1}^n [(\mu_i(S(t)) - D)^2 + \mu_i'(S)\dot{S}(t) - \dot{D}]x_i(t)$, where $\mu_i'(S) = \frac{d\mu_i(S(t))}{dS}$, it implies that

$$\left| \sum_{i=1}^n \left[(\mu_i(S(t)) - D)^2 + \mu_i'(S(t))\dot{S}(t) - \dot{D} \right] x_i(t) \right| \leq L, \quad \forall t \geq 0 \tag{20}$$

is the condition to be satisfied.

Remark 3. Condition (20) can be verified by knowing the domain of operation of the bioreactor. Indeed, since D is specified by the user and the upper-bound of $\mu_i(S(t))$ is known, the domain of operation regarding variables $S(t)$ and $x_i(t)$ can be estimated, leading also to an estimate of L.

Then, according to Theorem 3.3, $z_1(t) \to \dot{y}(t)$ in a finite-time in the noise-free case. Recalling (16) and that $\hat{\dot{z}}(t) = \hat{\dot{S}}(t) + \dot{y}(t)$, we use the output of the differentiator (19) to derive an observer for $\dot{S}$ as

$$\hat{\dot{S}}(t) = DS_{in} - D(\hat{S}(t) + y(t)) - z_1(t) \tag{21}$$

Estimating initial conditions $x_i(0)$

Let us now investigate the design of an estimator for $x_i(0)$. Considering the solution (12), we can rewrite the differential equation for $S(t)$ as given by (1) as the well-known linear regressor model (5):

$$\dot{S}(t) + D(S(t) - S_{in}) = \omega^\top(t)\theta \tag{22}$$

where $\omega(t)$ and θ are, respectively, the regressor function and the constant parameter vector, given by

$$\begin{aligned} \omega &= [-\mu_1(S)\varphi_1, \dots, -\mu_n(S)\varphi_n]^\top \\ \theta &= [x_1(0), \dots, x_n(0)]^\top \end{aligned}$$

By comparing (22) and (5), one readily realizes a similarity and thus we can apply the aforementioned finite-time estimation methods, i.e., DREM and algorithm (10). Hence, it is clear that we can use this approach to estimate $\hat{x}_i(0)$.

Since we have computed estimates $\hat{S}(t)$ and $\hat{\dot{S}}(t)$ previously, we are able to design an estimation scheme for the initial conditions $x_i(0)$. Recalling (22), we have that

$$\hat{\dot{S}}(t) + D(\hat{S}(t) - S_{in}) = \hat{\omega}^\top\theta + w_e \tag{23}$$

where we would like to design an algorithm for calculation of $\hat{\theta} = [\hat{x}_1(0), \dots, \hat{x}_n(0)]$ as an estimate of the vector of unknown parameters θ, $\hat{\omega} = -\mu(\hat{S})\hat{\varphi}$, $\mu(\hat{S}) = \operatorname{diag}[\mu_1(\hat{S}), \dots, \mu_n(\hat{S})]$ is a diagonal matrix, and w_e is the noise caused by the measurement disturbance and the differentiation algorithm, being an essentially bounded function of time.

In order to better characterize the error w_e in (23), let us rewrite (22) as follows

$$\begin{aligned} 0 &= \dot{S}(t) + D(S(t) - S_{in}) - \omega^\top\theta \\ &= \hat{\dot{S}}(t) + (\dot{S}(t) - \hat{\dot{S}}(t)) + D(\hat{S}(t) - S_{in}) + D(S(t) - \hat{S}) - \omega^\top\theta \end{aligned} \tag{24}$$

As aforementioned, $\omega = -\mu(S)\varphi$ and $\hat{\omega} = -\mu(\hat{S})\hat{\varphi}$, then after simple algebraic manipulations, one can write the following equality:

$$\begin{aligned} \omega &= -\mu(\hat{S})\varphi - \Big(\mu(S) - \mu(\hat{S})\Big)\varphi \\ &= -\mu(\hat{S})\hat{\varphi} - (\varphi - \hat{\varphi})\mu(\hat{S}) - (\mu(S) - \mu(\hat{S}))\varphi \\ &= \hat{\omega} + w_\omega \end{aligned} \tag{25}$$

Referring back to (23), one may write the following inequality:

$$|w_e(t)| \leq |w_\omega(t)||\theta| + |\dot{S}(t) - \dot{\hat{S}}(t)| + D\Big(|w(t)| + |z(0) - \hat{z}(0)|e^{-\int_0^t D(\tau)d\tau}\Big) \tag{26}$$

Finally, in order to apply algorithm (10) in this set-up, the regressor function $\phi(t)$ is computed by constructing the time-varying matrix (7) as $M(t) = [\hat{\omega}\ \tilde{\omega}_1 \ldots \tilde{\omega}_n]^\top$, where $\tilde{\omega}_j = H_j\hat{\omega}$, for $j = 1 \ldots n$ and H_j being a properly chosen (stable) linear filter.

Assumption 2. Suppose that conditions (11) and (20) hold, and $\int_0^t |w(\tau)|d\tau < +\infty$ for all $t > 0$.

Assumption 3. There exists a constant $R \geq 0$ such that $|\varphi(t)| \leq R$, for all $t \geq 0$.

Our main result is as follows:

Theorem 4.3. *Let assumptions 2–3 hold and the estimates of concentrations $\hat{x}_i(t)$ to be computed by*

$$\hat{x}_i(t) = \hat{\theta}_i\hat{\varphi}_i(t). \tag{27}$$

Then, the estimation error is bounded by

$$|x(t) - \hat{x}(t)| \leq \sigma_1(|z(0) - \hat{z}(0)|) + \sigma_2(|w|_\infty) + \sigma_3\left(\int_0^t |w(\tau)|d\tau\right)$$

for all $t \geq \overline{T} = \max\{T_f, T_d\}$, where $\sigma_1, \sigma_2, \sigma_3 \in \mathcal{K}$ and T_f and T_d are the time of convergence of (10) *and* (19)*, respectively.*

Proof. First, it is worth noticing that, although impossible for the asymptotic converging terms, algorithms (10) and (19) have sharp finite-time estimates of convergence (the dependence of initial deviations is canceled after a finite time). Using this feature, we will compute the upper bound of the error after a finite-time $\overline{T}$, which guarantees that both algorithms will have converged.

In this sense, consider the estimation error given in terms of solution (12) and estimates (27) as follows:

$$|x_i(t) - \hat{x}_i(t)| = |\theta_i\varphi_i - \hat{\theta}_i(t)\hat{\varphi}_i| \tag{28}$$

Adding and subtracting $\hat{\theta}_i(t)\varphi_i$ on (28) and applying the triangular inequality, we have that

$$|x(t) - \hat{x}(t)| \leq |\varphi - \hat{\varphi}||\hat{\theta}| + |\theta - \hat{\theta}(t)||\varphi| \tag{29}$$

Let us consider the first right-hand side term of (29). Taking into account the error from observer (16), given by (18), while profiting on properties of the exponential function and the fact that it is a Lipschitz function locally (with a Lipschitz constant L_{exp} in the domain of interest), one has that

$$|\varphi - \hat{\varphi}| \leq L_{exp} \Big| \int_0^t \mu(S) - \mu(\hat{S}) d\tau \Big|$$

Concerning the term inside the integral, we recall that $\frac{d\mu(S)}{dS} = \frac{\mu_{max}}{(a+S)^2}$ to state the following upper bound:

$$|\mu(S) - \mu(\hat{S})| \leq \frac{\mu_{max}}{a} |S - \hat{S}| \tag{30}$$

and hence, combining (30) and the error estimate (18), one has that

$$\begin{aligned} |\varphi - \hat{\varphi}| &\leq L_{exp} \frac{\mu_{max}}{a} \int_0^t |S - \hat{S}| d\tau \\ &\leq L_{exp} \frac{\mu_{max}}{a} \left[|z(0) - \hat{z}(0)| \int_0^t e^{-\int_0^\tau D(s)ds} d\tau + \int_0^t |w(\tau)| d\tau \right] \\ &\leq L_{exp} \frac{\mu_{max}}{a} \left[|z(0) - \hat{z}(0)| A_2 + \int_0^{+\infty} |w(\tau)| d\tau \right] \end{aligned} \tag{31}$$

where, for brevity in the following, $A_2 = \int_0^{+\infty} e^{-\int_0^\tau D(s)ds} d\tau$.

Now, let us consider the term $|\theta - \hat{\theta}|$ in (29), whose dynamics is governed by fixed-time converging algorithm (10) and that is perturbed by the noise (26). First, from results (30) and (31), one has that

$$\begin{aligned} |w_\omega| \leq\ & L_{exp} \frac{\mu_{max}^2}{a} \left[|z(0) - \hat{z}(0)| A_2 + \int_0^{+\infty} |w(\tau)| d\tau \right] \\ & + \frac{\mu_{max}}{a} \left[|z(0) - \hat{z}(0)| e^{-\int_0^t D(\tau)d\tau} + |w| \right] R \end{aligned} \tag{32}$$

The term $|\dot{S} - \dot{\hat{S}}|$, as readily seen in (21), includes the estimation errors of both observer (16) and differentiator (19). However, according to Theorem 3.3, the differentiation error $|\dot{y} - z_1|$ is upper bounded by (4), i.e., there exists $\eta > 0$ such that $|\dot{y} - z_1| \leq \eta\sqrt{|w|}$ after a finite-time T_d. Hence, for all $t > T_d$, we can state that

$$|\dot{S} - \dot{\hat{S}}| \leq D \left(|z(0) - \hat{z}(0)| + |w| \right) + \eta\sqrt{|w|} \tag{33}$$

Then, from (18), (32) and (33), we have that:

$$\begin{aligned} |w_e| \leq & \left(L_{exp} \frac{\mu_{max}^2}{a} \left[|z(0) - \hat{z}(0)| A_2 + \int_0^{+\infty} |w(\tau)| d\tau \right] + \frac{\mu_{max}}{a} \left[|z(0) - \hat{z}(0)| + |w| \right] R \right) |\theta| \\ & + D \left(|z(0) - \hat{z}(0)| + |w| \right) + \eta\sqrt{|w|} + D\Big(|z(0) - \hat{z}(0)| + |w| \Big) \end{aligned}$$

which, after rearranging terms, can be rewritten as

$$|w_e| \leq |z(0) - \hat{z}(0)| \left(L_{exp} \frac{\mu_{max}^2}{a} A_2 |\theta| + \frac{\mu_{max}}{a} R|\theta| + 2D \right)$$
$$+ \left(L_{exp} \frac{\mu_{max}^2}{a} \int_0^{+\infty} |w(\tau)| d\tau + \frac{\mu_{max}}{a} |w| R \right) |\theta| + 2D|w| + \eta\sqrt{|w|} .$$

Note that the first two terms in this last result are essentially bounded functions by definition, and, thanks to Assumption 2, the same holds for the two last, this implies that $w_e \in \mathcal{L}_\infty$. Thus, as this satisfies Assumption 1, Theorem 3.4 allows us to conclude that the estimation error $|\theta - \hat{\theta}|$ is short-fixed-time ISS, i.e.,

$$|\theta - \hat{\theta}| \leq \beta(|\theta(0) - \hat{\theta}(0)|, t - t_0) + \sigma(|w_e|)$$

and, furthermore, $\beta(|\theta(0) - \hat{\theta}(0)|, t - t_0) = 0$ for $t > T_f$.

Considering that $|\theta| \leq \bar{\theta}$, we can finally conclude that, for all $t > \overline{T}$, (29) is upper-bounded by

$$|x(t) - \hat{x}(t)| \leq \sigma(|w_e|)R + L_{exp} \frac{\mu_{max}}{a} (\bar{\theta} + \sigma(|w_e|)) \Bigg(|z(0) - \hat{z}(0)| A_2 + \int_0^{+\infty} |w(\tau)| d\tau \Bigg) \tag{34}$$

and taking into account the obtained upper bound for $|w_e|$ and Assumption 2, there exist σ_1, σ_2 and σ_3 from the class $\mathcal{K}$ ensuring the desired estimate. □

Remark 4. The last condition in Assumption 2, which regards the profile of the measurement noise, is the main restriction of the proposed approach. It shall be noticed that, due to the asymptotic convergence of (16), this term is unavoidable and represents a flaw for long periods of estimation.

Final form of the observer

The final form of the proposed estimator can summarized as follows:

$$\dot{z}_0(t) = -1.1L^{\frac{1}{2}} |z_0(t) - y(t)|^{\frac{1}{2}} \mathrm{sign}(z_0(t) - y(t)) + z_1(t) \tag{35a}$$
$$\dot{z}_1(t) = -1.5L\mathrm{sign}(z_0(t) - y(t)) \tag{35b}$$
$$\dot{\hat{S}}(t) = DS_{in} - D(\hat{S}(t) - y(t)) - z_1(t) \tag{35c}$$
$$\dot{\hat{\theta}}_i(t) = \phi(t) \left\{ \gamma_1 \lceil Y(t) - \phi(t)\hat{\theta}_i(t) \rfloor^{1-\alpha} + \gamma_2 \lceil Y(t) - \phi(t)\hat{\theta}_i(t) \rfloor^{1+\alpha} \right\} \tag{35d}$$
$$\dot{\hat{x}}_i(t) = \hat{\theta}_i(t)\hat{\varphi}_i(t) \tag{35e}$$

where (35a) and (35b) are related to the differentiation of the measurement $y(t)$, (35c) is related to the asymptotic observer (16), (35d) is the finite-time parameter estimator (10) (note that $Y(t)$ is obtained by applying DREM to the linear regression (5) with input a $y_e(t) = \dot{\hat{S}}(t) + D(\hat{S}(t) - S_{in})$) and, finally, (35e) related to the solution (27).

5. Numerical Example

In this section, we present a numerical example in order to illustrate the usefulness of the proposed methodology. Considering (1) with $n = 2$, and the following kinetic rates for microbial growth:

$$\mu_1(S) = \frac{4S}{20 + S}, \;\; \mu_2(S) = \frac{2S}{6 + S}$$

we simulate the chemostat having $S(0) = 10$, $x_1(0) = 15$ and $x_2(0) = 7$ as initial conditions (which are unknown to the designer), $S_{in} = 15$ and a periodic dilution rate, given by

$$D = \mu_1(S_i) + 0.1 \sin(0.05t)$$

where S_i is the intersection point of $\mu_1(S)$ and $\mu_2(S)$ given above (this is the point at which both species can be stabilized simultaneously, and where, unfortunately, the system looses its observability). This set-up will generate periodic trajectories on both species' concentrations.

In the following, we present simulation results of the aforementioned estimation scheme. Also, throughout this section it is assumed that all measurements are corrupted by a white noise w, generated with a power spectral density $P = 1 \times 10^{-4}$ and a sampling time $\tau_s = 0.01$.

The observer setup is given by (35a)–(35e). The part related to (16) (*i.e.*, estimation of S and $\dot{S}$) is initialized with $\hat{z}(0) = 23$. The differentiator is initialized with $z_0 = 0, z_1 = 0$ and $L = 0.1$.

Now, in order to apply DREM as described in section 3.2, we choose $T = 25$ and $\alpha = 0.5$ for algorithm (10). As it is needed for this procedure, the linear operator is chosen as a composition of a linear filter and a delay, given by:

$$H(s) = \frac{s + 2.5}{s^2 + 0.5s + 1} e^{-20s}$$

Then, as results of the simulations, Figure 3 illustrates the estimation of $x_1(0)$ and $x_2(0)$. Finally, by means of (12), Figure 4 shows the final estimates $\hat{x}_1(t)$ and $\hat{x}_2(t)$, where the red curves correspond to the trajectories of the designed algorithm, and the green curves are obtained by stopping the output injection (*i.e.*, stopping the integration of (10)) after $t = 25\,\text{min}$ in order to avoid depreciation of the estimates close to the unobservable region.

Remark 5. The identified initial conditions, as shown in Figure 3, are related to the moment in which (10) is integrated, *i.e.*, when the estimation of $\hat{x}_i(0)$ starts.

Remark 6. Figure 4 evidences the issue discussed in section 4.1. When $S = S_i$, observability is depreciated due to rank deficit, and an accentuated effect of noise is observed. However, by stopping the estimation after a fixed-time (which is upper estimated, see section 3.2), the monitoring is no longer depreciated by noise in the unobservable regions (compare the green and red curves in Figure 4), confirming the intended features of this observer.

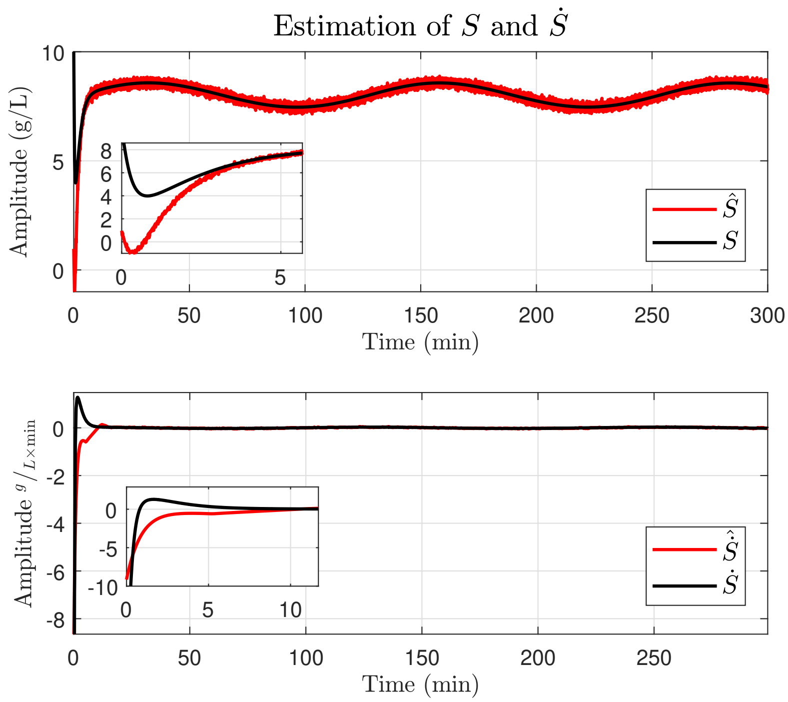

Figure 2: Computation of $\hat{S}$ (above) and $\hat{\dot{S}}$ (below). Time scale is zoomed in the small boxes to highlight the transient phase.

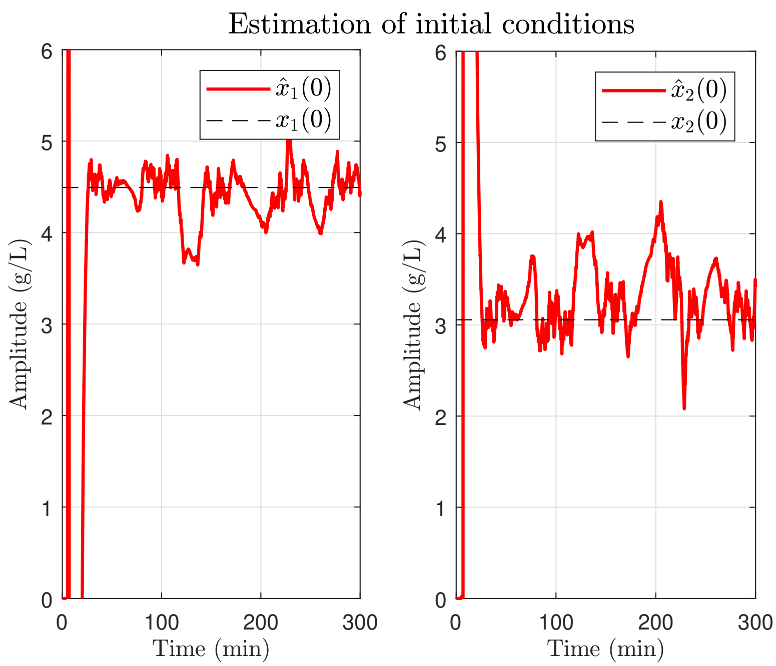

Figure 3: Estimation of $x_1(0)$ and $x_2(0)$

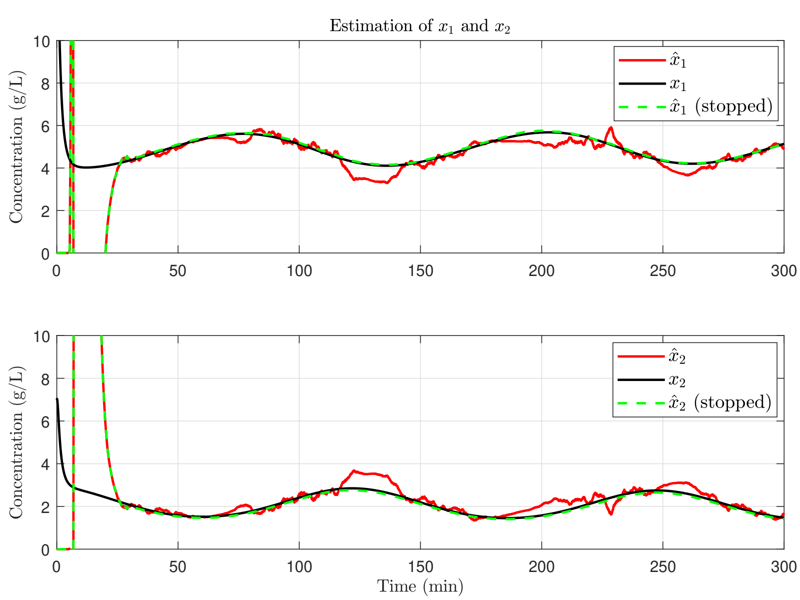

Figure 4: Computation of $\hat{x}_i(t)$

In a possible control problem in which each species have to be stabilized at a certain level (which can only be attained by setting $S = S_i$, see [23]), the feature discussed in Remark 6 is of great interest.

Furthermore, note that trajectories of $x_i(t)$ are realistic for such an application. Hence, it highlights the usefulness of the proposed scheme, since it does not require a highly-excited input in order to properly compute estimates $\hat{x}_i(t)$.

6. Conclusion

In this paper we further explored the problem of estimation of microbial growth on continuous bioreactors. The proposed schemes aim to provide estimates of all unknown variables (e.g., the substrate and biomass concentration) and in a finite-time whenever possible. In fact, if the measurement signal is $y = S$, all variables can be estimated in finite-time, while if $y = \sum_{i=0}^{N} x_i$, the estimation time is delayed by an asymptotic convergence of the estimate $\hat{S}(t)$. The main key in both schemes is the coupling of different estimation techniques, like sliding-mode exact differentiators and a finite-time parameter estimation technique. It is worth noticing that, although common in many estimation techniques, this approach requires persistence of excitation only on a fixed time interval, making it very interesting for chemostat applications under observability loss.

As an object of future research, an appealing direction is to investigate the use of this estimation scheme in an observer-based control problem. Indeed, having fast estimation of each species' concentration is an interesting feature for experiments on coexistence and interaction of different species. Also, increasing the robustness against noises (both measurement and process) is an important issue to be investigated.

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Appendix 1: Definitions of (robust) stability

Consider a time-dependent differential equation [24]:

$$\frac{dx(t)}{dt} = f(t, x(t), d(t)),\ t \geq t_0,\ t_0 \in \mathbb{R}, \tag{32}$$

where $x(t) \in \mathbb{R}^n$ is the state vector, $d(t) \in \mathbb{R}^m$ is the vector of external inputs and $d \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^m)$ (where $\mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^m)$ represents the set of Lebesgue-measurable essentially bounded functions from $\mathbb{R}$ to $\mathbb{R}^m$); $f : \mathbb{R}^{n+m+1} \to \mathbb{R}^n$ is a continuous function with respect to x, d and piecewise continuous with respect to t, $f(t, 0, 0) = 0$ for all $t \in \mathbb{R}$. A solution of the system (32) for an initial condition $x_0 \in \mathbb{R}^n$ at time instant $t_0 \in \mathbb{R}$ and some $d \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^m)$ is denoted as $X(t, t_0, x_0, d)$, and we assume that f ensures definiteness and uniqueness of solutions $X(t, t_0, x_0, d)$ in forward time at least on some finite time interval $[t_0, t_0+T)$, where $T > 0$ may be dependent on the initial condition x_0, the input d and the initial time t_0.

Definitions of stability

Let Ω, Ξ be open neighborhoods of the origin in $\mathbb{R}^n$, $0 \in \Omega \subset \Xi$. Let us consider system (32) at a steady state $x = 0$ with $d = 0$, then

Definition D1: [18] The system (32) is said to be:
(a) *short-time stable* with respect to (Ω, Ξ, T^0, T_f) if for any $x_0 \in \Omega$ and $t_0 \in [-T^0, T^0]$, $X(t, t_0, x_0, 0) \in \Xi$ for all $t \in [t_0, t_0 + T_f]$;
(b) *short-finite-time stable* with respect to (Ω, Ξ, T^0, T_f) if it is short-time stable with respect to (Ω, Ξ, T^0, T_f) and finite-time converging from Ω with the convergence time $T^{t_0,x_0} \leq t_0 + T_f$ for all $x_0 \in \Omega$ and $t_0 \in [-T^0, T^0]$;
(c) *globally short-finite-time stable* for $T^0 > 0$ for any bounded set $\Omega \subset \mathbb{R}^n$ containing the origin there exists a bounded set $\Xi \subset \mathbb{R}^n$, $\Omega \subset \Xi$ and $T_f > 0$ such that the system is short-finite-time stable with respect to (Ω, Ξ, T^0, T_f);
(d) *short-fixed-time stable* for $T^0 \geq 0$ and $T_f > 0$, if for any bounded set $\Omega \subset \mathbb{R}^n$ containing the origin there exists a bounded set $\Xi \subset \mathbb{R}^n$, $\Omega \subset \Xi$ such that the system is short-finite-time stable with respect to (Ω, Ξ, T^0, T_f).

Considering system (32) at a steady-state $x = 0$ with $d \neq 0$, the following results concerning robust stability are recalled:

Definition D2: [18] The system (32) is said to be:
(a) *short-finite-time ISS* with respect to (Ω, T^0, T_f, D) if there exists $\beta \in \mathcal{GKL}$ and $\gamma \in \mathcal{K}$ such that for all $x_0 \in \Omega$, all $d \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^n)$ with $|d|_\infty < D$ and $t_0 \in [-T_0, T_0]$:

$$|X(t, t_0, x_0, d)| \leq \beta(|x_0|, t - t_0) + \gamma(|d|_\infty), \qquad \forall t \in [t_0, t_0 + T_f]$$

and $\beta(|x_0|, T_f) = 0$;
(b) *globally short-finite-time ISS* for $T^0 > 0$ if there exists $\beta \in \mathcal{GKL}$ and $\gamma \in \mathcal{K}$ such that for any bounded set $\Omega \subset \mathbb{R}^n$ containing the origin there is a $T_f > 0$ such that for all $x_0 \in \Omega$, all $d \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^n)$ and $t_0 \in [-T_0, T_0]$:

$$|X(t, t_0, x_0, d)| \leq \beta(|x_0|, t - t_0) + \gamma(|d|_\infty), \qquad \forall t \in [t_0, t_0 + T_f]$$

and $\beta(|x_0|, T_f) = 0$;
(c) *short-fixed-time ISS* for $T^0 > 0$ and $T_f > 0$, if there exists $\beta \in \mathcal{GKL}$ and $\gamma \in \mathcal{K}$ such that for all $x_0 \in \mathbb{R}^n$, all $d \in \mathcal{L}_\infty(\mathbb{R}, \mathbb{R}^n)$ and $t_0 \in [-T_0, T_0]$:

$$|X(t, t_0, x_0, d)| \leq \beta(|x_0|, t - t_0) + \gamma(|d|_\infty), \qquad \forall t \in [t_0, t_0 + T_f] \text{ and } \beta(|x_0|, T_f) = 0$$

Remark 7. The notions given in Definition D1 can also be equivalently formulated using the functions from the class $\mathcal{GKL}$ (see [24]).