A ROBUST HYBRID OBSERVER FOR ESTIMATING STATES, REACTION RATES, AND AN EXTERNAL INPUT DISTURBANCE FOR A CONTINUOUS BIOREACTOR

VÍCTOR REZA, JORGE TORRES AND JESÚS GUERRERO

The controlling and monitoring of bioprocesses very often requires the estimation of certain biological concentrations that are difficult to measure, usually assuming some structure of the reaction rates which might be barely known. Although many algorithms have been designed to estimate these reaction rates, they are not robust against input disturbances and cannot be updated to treat them. This paper addresses the problem of estimating unmeasurable states, reaction rates, and input disturbance by applying a hybrid observer in a continuous bioreactor. The proposed algorithm uses an extended super-twisting algorithm coupled with an adaptive observer to exponentially estimate the reaction rates and input disturbance provided the persistent excitation condition is fulfilled. Later, an asymptotic observer estimates the unmeasurable states with the previous estimations. The hybrid observer is tested through simulations in a continuous sulfate-reducing bioprocess. Finally, the advantage of estimating the external disturbance is highlighted through its use in a disturbance rejection control to counteract its undesirable effect.

Keywords: hybrid observer, super twisting algorithm, adaptive observer, asymptotic observer, continuous bioreactor

Classification: 93B11, 93D11

1. INTRODUCTION

Currently, any bioprocess can be improved by monitoring and controlling its related bioreactors, where its key biochemical concentrations must be measured. However, some of these variables are difficult to measure, such as the dissolved oxygen, total nitrogen or phosphorous, and volatile fatty acids [30], due to these states being linked with sensors characterized by being discontinuous, high-priced, invasive, sensitive to noise, with a limited range of applications, and requiring constant maintenance [36].

Therefore, it is necessary to design an observer that estimates these unmeasurable states under uncertain bioreactor models due to external disturbances and unknown dynamics. First, the dilution rate is the most used input in fed-batch and continuous bioreactors, whose actuators are adjusted manually or automatically. Unfortunately, due to imperfections, these devices can add input disturbances such that bioreactor operating conditions can change notably, which may lead to inadequate or risky bioreactor dynamical behavior when optimal biomass production is required, for instance, [56]. Second, it is challenging to understand and describe the reaction rates, essential in bioreactor monitoring, control, and scale-up design, as shown in [11, 23, 31, 47]. For example, many models describe the specific growth rate of photosynthetic oxygenic microorganisms, a particular reaction rate that describes the kinetics growth rate. Still, these models are unsuitable in real operation conditions [18]. Furthermore, many reaction rates are not necessarily represented by specific reaction rates, such as chemical reaction rates [16], chlorophyll degradation, nitrogen uptake, photosynthesis, and respiration rates in marine phytoplankton growth dynamics [45], and the oxidation of ammonium and nutrient uptake for non-growth maintenance rates in cyanobacteria [24].

Previous results exist in estimating unmeasurable biochemical concentrations by applying a single observer without the presence of external disturbances in the dilution rate. When the bioreactor model is fully known, including the structure of the reaction rates, Luenberger-based observers can be applied as the extended Kalman filter [27, 37] and nonlinear observers [2, 28]. Although these algorithms can be robust under parameter uncertainties in the yield coefficients [23], these algorithms require perfect knowledge of the reaction rate structure. Another approach is to assume that the unknown reaction rates and external disturbances are unknown inputs of the bioreactor. For example, asymptotic and interval observers were applied for estimating nonmeasurable states under unknown reaction rates and external disturbances in the influent feeding rates in [3, 49], with the disadvantage that their convergence time cannot be modified. It is worth saying that an unknown input observer can be designed to estimate reaction rates, input disturbances non-related to the dilution rate, and the not-measurable states, as described in [35, 38, 44, 52]. However, applying a hybrid observer is a desirable option to estimate unmeasurable states under unknown dynamics and external disturbances, such as the unknown reaction rates and additive disturbance in the dilution rate. As described in [4], a hybrid observer combines more than one algorithm that overcomes the main limitations of a single observer, which applications in bioprocesses have increased in recent years. For example, in [58], a H_{∞} Luenberger observer coupled with a supertwisting algorithm (STA) was designed for estimating biomass, glucose, and its influent feeding rate. As shown in [11, 48], a standard hybrid observer for bioprocess applies an asymptotic observer for estimating the not-measurable states and an observer-based estimator, which is an algorithm similar to an observer that estimates unknown dynamics, uncertain parameters, or external disturbances [11, 46, 50].

Many observer-based estimators were designed for bioprocesses without additive disturbances in the dilution rate. The first one is described in [10], where an adaptive Luenberger observer was designed to estimate the specific growth rate and was extended for estimating many reaction rates [11, 46]. Another method applies artificial neural networks or fuzzy techniques for the rough estimation of reaction rates, as shown in [8, 22]. Unfortunately, deep knowledge of neural networks and enough data are necessary to train them [3, 22]. Also, high gain observers (HGO) have been applied for estimating reaction rates, robust to discrete noisy measurements, as described in [13, 26, 51]. How-

ever, in [48], a novel algorithm was designed based on sliding mode techniques by adding discontinuous terms in the adaptive observer designed in [10]. As a result, the specific growth rate estimation converges to the actual growth in finite time, except for a very high-frequency discontinuous error (known as the chattering phenomenon), which is the main drawback. In this vein, further research efforts were focused on improving this sliding mode observer (SMO) and estimating several reaction rates. In [19], a second-order SMO was designed to estimate the specific growth rate in fed-batch bioreactors while reducing the chattering phenomena compared to a conventional SMO. Subsequently, in [20], two super-twisting algorithms were designed for specific growth rate estimation in finite time for batch, fed-batch, and continuous bioreactors. At the same time, chattering is substantially reduced for conventional second-order SMO. Afterward, in [43], an extended STA for estimating several specific reaction rates was designed, provided the observer has enough measurable outputs. Moreover, in [55], an STA-based observer with weighted variable gains was designed for specific reaction rate estimation in continuous bioreactors. Later, in [40], a generalized STA coupled with an asymptotic observer was designed for reaction rate and state estimation.

Although many observer-based estimators were proposed for bioreactors, these algorithms are inadequate under an external disturbance in the dilution rate. The main reason is that the proposed sliding variable or the observer's general structure depends on biochemical concentrations coupled with specific reaction rates, such as biomass concentration and specific growth rate. Hence, this is quite limiting if there is only a monoculture of microorganisms in a bioreactor [19, 20, 48] or the reaction rates do not have this structure [16, 24, 45]. Furthermore, only some results consider the estimation of a disturbance in the dilution rate. In [15], an HGO coupled with an adaptive observer was designed for estimating a constant additive disturbance in the dilution rate and unmeasurable states when the reaction rate structure is known. While in [50], an extended STA was designed for simultaneously estimating reaction rates and an input disturbance, which guarantees that the estimation error is stable.

In this work, a hybrid observer was designed to estimate not-measurable states, unknown reaction rates, and an additive disturbance in the dilution rate for continuous bioreactors. This observer consists of an extended STA coupled with an adaptive and asymptotic observer. First, the STA jointly estimates the reaction rates and an input disturbance in a finite time. Later, the adaptive observer decoupled them and converged exponentially to their nominal values under the persistent excitation condition. Finally, the asymptotic observer uses the previous estimations to estimate the desired states. Even though this idea looks similar to mechanical and electrical systems, the proposed hybrid observer estimates the unknown internal dynamics, the external disturbances, and the unmeasurable states separately. In contrast, sliding mode techniques coupled with adaptive laws are used in non-biological systems to estimate the total uncertainty and improve the controller-based observer behavior. There are many examples of this situation, as described in hydraulic-electrical systems [9], mobile robots [34], permanent magnet synchronous motors [33, 57], lithium-ion batteries [53], wind turbines [25], and unmanned aerial vehicles [6, 14, 17]. It is worth saying that there are successful real applications of sliding mode observers in bioreactors, such as shown in [5]. In summary, the main contributions of this work are the following:

- 1. A hybrid observer was designed and analyzed to estimate the key biochemical variables of continuous bioreactors: the unmeasurable states, unknown reaction rates, and an additive disturbance in the dilution rate.
- 2. It was proven that the reaction rates and input disturbance are observable, while the unmeasurable states are detectable.
- 3. An observer-based estimator, whose structure is based on sliding mode observers and adaptive laws with projection, was designed and analyzed to estimate the reaction rates and additive disturbance in the dilution rate with the property of exponential convergence.
- 4. An asymptotic observer was designed and analyzed to estimate the nonmeasurable states, so their estimates converge near their nominal values.
- 5. A preliminary observed-based disturbance rejection control was numerically evaluated to motivate further use of the proposed hybrid observer.

The rest of the paper is organized as follows. Section 2 describes the bioreactor dynamics and the observation problem, and we recall some helpful theories before to show the main result. Later, in Section 3, the desired hybrid observer is designed. Section 4 applies the proposed algorithm in a sulfate-reducing process for monitoring and controlling tasks. Finally, in Section 5, we give the conclusions of this work.

NOTATION AND ABBREVIATIONS

The following notation is used throughout the manuscript. Symbols \mathbb{R} , $\mathbb{R}_{>0}$, $\mathbb{R}_{\geq 0}$ will represent the sets of real numbers, positive or strict real numbers, respectively. Vectors or matrices with real number entries are denoted by \mathbb{R}^n and $\mathbb{R}^{n \times m}$, respectively. Given a vector $v = (v_1, v_2, ..., v_n)^T \in \mathbb{R}^n$ the notation v > 0 will mean that $v_i > 0$, for all i = 1, ...n. For a given $M \in \mathbb{R}^{n \times n}$, its eigenvalues are denoted by $\lambda(M)$, with $\lambda_{\min}(M)$ and $\lambda_{\max}(M)$ being the minimum and maximum elements of $\lambda(M)$, respectively. Finally, \mathcal{L}^p or \mathcal{L}^∞ will stand for the usual normed spaces, namely, $(\mathbb{R}^k, ||u||_p)$, $(\mathbb{R}^k, ||u||_\infty)$, respectively. Finally, for $\sigma \in \mathbb{R}^m$, the next functions are defined:

$$\operatorname{ABS}(\sigma)^{1/2} \triangleq \operatorname{diag}\left(\sqrt{|\sigma_1|}, \cdots, \sqrt{|\sigma_m|}\right), \quad \operatorname{SIGN}(\sigma) \triangleq \left[\operatorname{sign}(\sigma_1) \quad \cdots \quad \operatorname{sign}(\sigma_m)\right]^T$$

Abbreviations glossary:

HGO	high gain observer
SMO	sliding mode observer
PE	persistent excitation property
STA	super-twisting algorithm
ESTA	extended super-twisting algorithm
UUB	uniformly ultimately bounded
HB-STA+AAO	hybrid observer based on super-twisting plus adaptive
	and asymptotic observers
HB-HGO+AO	hybrid observer based on high gain observer plus asymptotic
	observer.

2. PROBLEM FORMULATION

Consider the continuous bioreactor model [11]:

$$\dot{\xi}(t) = K\varphi(t) + (D(t) + \delta(t))(\xi^{in}(t) - \xi(t)) - Q(t)$$
(1a)

$$y(t) = h(\xi) \tag{1b}$$

where $\xi \in \mathbb{R}^n_{\geq 0}$ is the state, $K \in \mathbb{R}^{n \times m}$ is the matrix yield coefficient, $\varphi \in \mathbb{R}^m_{\geq 0}$ is the reaction rate vector, $D \in \mathbb{R}_{\geq 0}$ is the nominal dilution rate², $\delta \in \mathbb{R}$ is the input disturbance, $\xi^{in} \in \mathbb{R}^n_{\geq 0}$ is the mass transfer rate for each ξ in the liquid phase, $Q \in \mathbb{R}^n_{\geq 0}$ is the mass transfer rate for each ξ in the output, and $h(\xi) \in \mathbb{R}^q$ is a known continuous function.

From the dynamic system (1), it is required to estimate the complete state ξ . However, the primary objective is to estimate the reaction rates φ , as they are poorly known, complex, nonlinear functions, and present a high parameter uncertainty. In addition, determining reaction rates is also helpful for monitoring and controlling continuous bioreactors. Several techniques have been proposed to observe ξ when the reaction rate vector is partially or not known at all, see, for instance, [10, 19, 20, 43, 48]. Unfortunately, in all these cases, one faces high sensitivity to input disturbances δ because it can drastically change the bioreactor dynamics [11]. Motivated by the depicted scenario, the main goal of this paper is to design a robust state observer for the bioreactor system (1) when reaction rates φ are not known, and the system is also subjected to external disturbances δ . For such an end, a hybrid observer strategy is applied. Firstly, as discussed later, reaction rates and external disturbance are analyzed using an observer-based estimator. Then, provided that one disposes of reasonable estimates of φ and δ , the estimation of ξ can be considered.

2.1. General assumptions

In order to simplify the analysis, system (1) can be separated as follows [11]:

$$\dot{\xi}_a = K_a \varphi + (D+\delta)(\xi_a^{in} - \xi_a) - Q_a \tag{2a}$$

$$\dot{\xi}_e = K_e \varphi + (D+\delta)(\xi_e^{in} - \xi_e) - Q_e \tag{2b}$$

$$\dot{\xi}_b = K_b \varphi + (D+\delta)(\xi_b^{in} - \xi_b) - Q_b \tag{2c}$$

$$y = h(\xi) = \begin{bmatrix} \xi_a^T & \xi_e \end{bmatrix}^T$$
(2d)

where $K = \begin{bmatrix} K_a^T & K_e^T & K_b^T \end{bmatrix}^T$, $K_a \in \mathbb{R}^{m \times m}$ is a nonsingular matrix, $K_e \in \mathbb{R}^{1 \times m}$ and $K_b \in \mathbb{R}^{(n-m-1) \times m}$. In turn, (ξ_a, ξ_e, ξ_b) , $(\xi_a^{in}, \xi_e^{in}, \xi_b^{in})$ and (Q_a, Q_e, Q_b) are the partitions of ξ , ξ^{in} and Q induced by (K_a, K_e, K_b) , similar as described in [11]. Henceforth, one shall assume that the dynamic model (2) accomplishes the following.

¹The term (t) will be introduced when necessary.

 $^{^{2}}$ The dilution rate is considered the system control input and describes the relation between the influent or effluent flow rate dynamics and the volume of the bioreactor. In a continuous bioreactor, influent and effluent flow rates are strictly positive and have the same value to guarantee a constant bioreactor volume.

Assumption 2.1. The matrix $K = \begin{bmatrix} K_a^T & K_e^T & K_b^T \end{bmatrix}^T$ is known.

Assumption 2.2. All the elements of ξ^{in} and Q are positive, continuously bounded, and known, that is, for any $\xi_i^{in}(0)$ and $Q_i(0)$ with $i = \{1, \ldots, n\}$, $\exists \bar{\xi}_i^{in}, \bar{Q}_i \in \mathbb{R}_{\geq 0}$ such that $0 \leq \xi_i^{in} \leq \bar{\xi}_i^{in}$ and $0 \leq Q_i \leq \bar{Q}_i$. Furthermore, all the elements of ξ , $\dot{\xi}$ and $\dot{\xi}^{in}$ are continuously bounded, that is, $\exists \bar{\xi}_i, \bar{\xi}_{di}, \bar{\xi}_{di}^{in} \in \mathbb{R}_{\geq 0}$ such that $0 \leq \xi_i \leq \bar{\xi}_i, |\xi_i| \leq \bar{\xi}_{di}$, and $|\dot{\xi}_i^{in}| \leq \bar{\xi}_{di}^{in}$.

Assumption 2.3. All the elements of φ and $\dot{\varphi}$ are continuously bounded, that is, $\exists \bar{\varphi}_i \in \mathbb{R}_{>0}$ with $i = \{1, \dots, m\}$ such that $|\dot{\varphi}_i| < \bar{\varphi}_i$ and $\bar{\varphi}_i$ are known.

Assumption 2.4. The input disturbance δ is a continuously bounded function with a very slow dynamic, that is, $\dot{\delta} \approx 0$, and for any $\delta(0) > 0 \exists \underline{\delta}, \overline{\delta} \in \mathbb{R}$ such that $\underline{\delta} \leq \delta \leq \overline{\delta} \forall t$.

Assumption 2.5. The dilution rate D is strictly positive, bounded, and known, that is, $\exists \underline{d}, \overline{d} \in \mathbb{R}_{>0}$ such that $0 < \underline{d} \leq D \leq \overline{d} < \infty$.

Assumption 2.6. The states ξ_a and ξ_e are measurable.

Remark 2.1. The previous assumptions are similar to the ones in [10, 19, 20, 43, 46, 48]. Assumption 2.1 is feasible if these parameters are previously estimated by parametric identification, such as in [12]. Assumptions 2.2 and 2.3 are realistic due to the limited resources in the bioreactor. Furthermore, Q can be known by measuring the input and output of a gas flow rate, as described in [11]. Moreover, Assumption 2.4 says that the external disturbance in the dilution rates behaves almost as a parameter, so an adaptive observer can be used, as described in further sections. Also, Assumptions 2.4 and 2.5 are expected due to the limitations in the equipment that adjusts the dilution rate. Finally, Assumption 2.6 is necessary for the observability and detectability of the reaction rates φ , the input disturbance δ , and the unmeasurable states $\xi_b \in \mathbb{R}^{n-m-1}$, as further discussed in the next section.

Remark 2.2. The parameters previously described, such as $\bar{\varphi}_i$, $\bar{\delta}$, $\underline{\delta}$, $\underline{\xi}_i$ or \bar{Q}_i are assigned heuristically or from the bioprocess knowledge. However, adaptive laws can be applied to robustly estimate $\psi_i = |K_{a_i}|\bar{\varphi}_i + \max(|\underline{\delta}|, |\bar{\delta}|)(\bar{\xi}_{a_{d_i}} + \bar{\xi}_{a_{d_i}})$, as described for an STA in [54].

2.2. Proposed hybrid observer

A hybrid observer can now be proposed to estimate the desired key biochemical variables, whose structure is shown in Figure 1. From the start, a super-twisting algorithm can be used to estimate the total uncertainty $\omega = K_a \varphi + \delta(\xi_a^{in} - \xi_a)$, which can be seen as the sum of the unknown reaction rates and the additive disturbance in the dilution rate. Although this idea looks similar to mechanical and electrical systems, it is necessary to separate the estimation of $\hat{\varphi}$ and $\hat{\delta}$ from $\hat{\omega}$, which is done with an adaptive observer with projection. Finally, the estimations of the reaction rates and external disturbances are applied with an asymptotic observer to estimate the remaining unmeasurable biochemical concentrations.



Fig. 1: Structure of the proposed hybrid observer for estimating φ , δ , and ξ_b by using the available measurements ξ_a and ξ_e .

2.3. Background theory

The rest of this section recalls helpful definitions and theorems for the further observer's analysis and design³. Let's recall first the notion of uniformly ultimately bounded (UUB) trajectories which play a key role when a system faces bounded external disturbances.

Definition 2.1. It is said that all trajectory solutions of a nonlinear system $\dot{x} = f(x)$ are UUB with ultimate bound b if there exist constants $b, c \in \mathbb{R}_{>0}$ independent of $t_0 \ge 0$ and $\forall a \in (0, c)$, exist $T_u = T_u(a, b)$, independent of t_0 , such that:

$$||x(t_0)|| \le a \to |||x(t)|| \le b \quad \forall t \ge t_0 + T_u.$$

Theorem 2.1. Let $\dot{x} = f(x,t)$ be a nonlinear time-variant system. Suppose a continuously differentiable function $V : D \times \mathbb{R}_{\geq 0} \to \mathbb{R}_{\geq 0}$ with $D \subset \mathbb{R}^n$, and positive definite functions $\omega_1(\cdot), \omega_2(\cdot)$ and $\omega_3(\cdot)$, such that the following is satisfied:

$$\omega_1(||x||) \le V(x,t) \le \omega_2(||x||)$$

 $\dot{V}(x,t) \le -\omega_3(x), \qquad ||x|| \ge \mu > 0.$

If for a number r > 0 it is true that $r \in \mathbf{B} = \{r \in \mathbf{B} \subset D \mid r > \omega_2(\mu)\}$, then all trajectory solutions of $\dot{x} = f(x)$ are UUB with ultimate bound $b = \omega_1^{-1}(\omega_2(\mu))$.

³For more details about Lyapunov stability and adaptive observers, see [29, 32].

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Proof. See Corollary 4.2 in [29].

Additionally, parameter uncertainty can drastically modify a system's dynamic. Suppose that $\delta_V \in \mathbb{R}^m$ is a vector of parameter uncertainties associated with the nonlinear system $\dot{x} = f(x, \delta_V)$. It is said that \dot{x} is linearly parameterized if $\dot{x} = g(x) + \delta_V \phi$, where $g(x) \in \mathbb{R}^n$ is a known vector and $\phi \in \mathbb{R}^m$ is a known bounded regression vector, which determines the convergence of the parametric error $\tilde{\delta}_V = \delta_V - \hat{\delta}_V$. Applying an adaptive law to the system is an excellent choice to increase its robustness against parameter uncertainties. Hence, the following definition recalls the persistent excitation (PE) property. At the same time, Barbalat's lemma is described, which is instrumental for the theorem about the exponential convergence of δ_V to its nominal value, given below.

Definition 2.2. Let $\phi \in \mathbb{R}^p$ be an integrable and bounded function. It is said that ϕ satisfies the PE condition if there exist constants $\alpha_1, \alpha_2, T_0 \in \mathbb{R}_{>0}$ such that:

$$\alpha_2 I \ge \int_t^{t+T_0} \phi(s)^T \phi(s) \, \mathrm{d}s \ge \alpha_1 I$$

where $I \in \mathbb{R}^{p \times p}$ is the matrix identity.

Lemma 2.1. (Barbalat's Lemma) If $f(t) : \mathbb{R}_{\geq 0} \to \mathbb{R}$ is uniformly continuous and $\lim_{t\to\infty} \int_0^t |f(s)| \, ds$ exists and is finite, then $\lim_{t\to\infty} f(t) = 0$.

Proof. See Lemma 2.12 in [42].

Theorem 2.2. Let be the general adaptive observer:

$$\dot{E} = -A_E E + B\phi \tilde{\delta}_V \tag{3a}$$

$$\tilde{\delta}_V = M\phi C^T E \tag{3b}$$

$$y = C_E^T E \tag{3c}$$

where $M \in \mathbb{R}^p$ is an adaptive gain, $E \in \mathbb{R}^n$ is the measurement error vector, $y \in \mathbb{R}^n$ is the output, and $A_E \in \mathbb{R}^{n \times n}$ and $B, C_E \in \mathbb{R}^n$ form the transference function $H(s) = C_E(sI - A_E)^{-1}B$. If ϕ accomplishes the PE condition and H(s) is a strictly real positive and proper function that accomplishes $H(\infty) = 0$, then $\hat{\delta}_V \to \delta_V$ exponentially.

Proof. See Theorem 2.3 in [7].

Later, an observer's existence can be guaranteed if a dynamical system is observable or detectable. However, even though there are many criteria for evaluating this task, it is challenging to conclude if the state of a nonlinear system is observable or detectable when unknown inputs are present. Before ending this section, the following definitions of observability and detectability for nonlinear systems with unknown inputs are described [39, 41].

Definition 2.3. Let be the following dynamical system:

$$\dot{x} = f(x, u, \omega), \ x(0) = x_0 \tag{4a}$$

$$y = h(x) \tag{4b}$$

$$\dot{\tilde{x}} = f(x, u, \omega) - f(x + \tilde{x}, u, \omega + \tilde{\omega}), \ \tilde{x}(0) = \tilde{x}_0 \tag{4c}$$

$$\tilde{y} = h(x) - h(x + \tilde{x}) = 0 \tag{4d}$$

where $x, \tilde{x} \in \mathbb{R}^n$ is the state and its error estimation, respectively, $u \in \mathbb{R}^m$ is the input, $\omega, \tilde{\omega} \in \mathbb{R}^q$ is the unknown input and its error estimation, respectively, $y, \tilde{y} \in \mathbb{R}^p$ is the output and its error estimation. Furthermore, $f(\cdot) \in \mathbb{R}^n$ and $h(\cdot) \in \mathbb{R}^p$ are smooth. Then:

- If $\tilde{x} \neq x$ is such that $y(t, x, u, w) = y(t, \tilde{x}, u, \tilde{w}) \ \forall t \in \mathbb{R}_{>0}$ and for some $\omega, \tilde{\omega} \in \mathbb{R}^{q}$, then \tilde{x} is a strongly u-indistinguishable state from x. Denote by $\mathcal{I}_{(u,x)}^{UI}$ the set of strongly u-indistinguishable states from x.
- The nonlinear system (4) is strongly u-observable if for every $x, \mathcal{I}_{(u,x)}^{UI} = \{x\}$.
- The nonlinear system (4) is strongly u-detectable if for every x and every $\tilde{x} \in \mathcal{I}_{(u,x)}^{UI}$ and any couple of signals ω and $\tilde{\omega}$ that renders \tilde{x} indistinguishable, it follows that $x(t, \tilde{x}, u, \tilde{\omega}) \to x(t, x, y, w)$ at $t \to \infty$.

Remark 2.3. From the previous definition, the nonlinear system (4) is strongly uobservable if and only if this system is trivial, that is, the only solution is $\tilde{x} = 0$. Moreover, this nonlinear system is strongly u-detectable if and only if it has $\tilde{x} = 0$ as an attractive equilibrium point for every $\tilde{\omega}$, that is, for every y(t, x(0), u, w) solution of (4), every \tilde{x}_0 and every \tilde{w} such that (4) is satisfied, then $\tilde{x} \to 0$.

3. ANALYSIS AND DESIGN OF THE PROPOSED HYBRID OBSERVER

Before the analysis and design of the proposed hybrid observer, its existence is studied by checking the observability and detectability properties of the bioreactor model (2). Due to φ and δ being structurally equal to an unknown input, the following result shows that this observer exists by applying the same methodology described in [41].

Proposition 3.1. Suppose that the continuous bioprocess (2) accomplishes Assumptions 2.1-2.6. Then, the reaction rates φ and external disturbance δ are strongly u-observable, while the unmeasurable state ξ_b is strongly u-detectable.

Proof. One can define a copy of system (2) by substituting the state, reaction rates, and external disturbance with their estimation, that is, $\hat{\xi}_a$, $\hat{\xi}_e$, $\hat{\xi}_b$, $\hat{\varphi}$, and $\hat{\delta}$. Hence, defining the estimation errors $\tilde{\xi}_a \triangleq \xi_a - \hat{\xi}_a$, $\tilde{\xi}_e \triangleq \xi_e - \hat{\xi}_e$, $\tilde{\xi}_b \triangleq \xi_b - \hat{\xi}_b$, $\tilde{\varphi} \triangleq \varphi - \hat{\varphi}$, and $\tilde{\delta} \triangleq \delta - \hat{\delta}$, it can be obtained the following nonlinear system:

$$\dot{\tilde{\xi}}_{a} = K_{a}\tilde{\varphi} - (D+\delta)\tilde{\xi}_{a} + \tilde{\delta}\left(\xi_{a}^{in} - \xi_{a} + \tilde{\xi}_{a}\right)$$
(5a)

$$\dot{\tilde{\xi}}_e = K_e \tilde{\varphi} - (D+\delta)\tilde{\xi}_e + \tilde{\delta} \left(\xi_e^{in} - \xi_e + \tilde{\xi}_e\right)$$
(5b)

$$\dot{\tilde{\xi}}_b = K_b \tilde{\varphi} - (D+\delta)\tilde{\xi}_b + \tilde{\delta} \left(\xi_b^{in} - \xi_b + \tilde{\xi}_b\right)$$
(5c)

by hypothesis, $\tilde{y} = \begin{bmatrix} \tilde{\xi}_a^T & \tilde{\xi}_e \end{bmatrix}^T = \begin{bmatrix} 0_{1 \times m} & 0 \end{bmatrix}^T$, that is, $\tilde{\xi}_a = 0_{m \times 1}$ and $\tilde{\xi}_e = 0$. Therefore, $\tilde{\varphi} = -\tilde{\delta}K_a^{-1}(\xi_a^{in} - \xi_a)$ and $\tilde{\delta}\phi_e = 0$, where $\phi_e = \xi_e^{in} - \xi_e - K_e K_a^{-1}(\xi_a^{in} - \xi_a)$. Hence, $\tilde{\delta} = 0$ if $\phi_e \neq 0$ and consequently, $\tilde{\varphi} = 0_{m \times 1}$. From Remark 2.3, ξ_a , ξ_e , φ and δ are strongly u-observable. Lastly, notice that:

$$\dot{\tilde{\xi}}_b = -(D+\delta)\tilde{\xi}_b$$

So, $\tilde{\xi}_b \to 0_{(n-m-1)\times 1}$ if $D + \delta > 0$ and from Remark 2.3, ξ_b is strongly u-detectable. \Box

It turns out that φ , δ , and ξ_b can be estimated using the measurements of ξ_a and ξ_e . To address the estimation of ξ_b , it seems logical to first determine φ and δ . Note that the dynamics of ξ_a and ξ_e given in (2) are algebraically dependent, so it is not possible to dissociate them by a diffeomorphic transformation. Moreover, it is impossible to estimate φ robustly without knowing δ , [50]. Therefore, the design of an extended STA is proposed for first estimating φ and δ in finite time and later decoupling these estimates with an adaptive observer. Finally, for the observation of ξ_b , an asymptotic observer based on the estimates $\hat{\varphi}$ and $\hat{\delta}$ is proposed.

3.1. Extended super-twisting algorithm

Firstly, it will be used the measurable state ξ_a to estimate the total uncertainty $\omega = K_a \varphi + \delta(\xi_a^{in} - \xi_a)$. Hence, the dynamic of ξ_a can be expressed with respect to ω :

$$\dot{\xi}_a = \omega + f_a(\xi_a, D) \tag{6}$$

where $f_a(\xi_a, D) = D(\xi_a^{in} - \xi_a) - Q_a$. Therefore, the next theorem shows the extended STA designed in [50] can estimate ω in finite time.

Theorem 3.1. Consider the dynamic model (6) with the Assumptions 2.1-2.6 and the following dynamical system:

$$\dot{\hat{\xi}}_{a} = \Psi \left(\eta + L_{1} \text{ABS}(\sigma)^{1/2} \text{SIGN}(\sigma) \right) + f_{a}(\xi_{a}, D)$$
(7a)

$$\dot{\eta} = L_2 \text{SIGN}(\sigma) \tag{7b}$$

$$\hat{\omega} = \Psi \eta \tag{7c}$$

$$\sigma = \Psi^{-1}(\xi_a - \hat{\xi}_a) \tag{7d}$$

where $\hat{\xi}_a, \hat{\omega} \in \mathbb{R}^m$ are the estimation of ξ_a and ω , respectively, $\eta \in \mathbb{R}^m$ is a vector linked with $\hat{\omega}, \sigma \in \mathbb{R}^m$ is a sliding vector, $L_1 > 0, L_2 > 1$ are algorithm gains, while $\Psi = \operatorname{diag}(\psi_1, \cdots, \psi_m)$ is a positive definite matrix such that

$$|\dot{\omega}_i| \le \psi_i = |K_{a_i}|\bar{\varphi}_i + \max(|\underline{\delta}|, |\bar{\delta}|) \left(\bar{\xi}_{a_{d_i}} + \bar{\xi}_{a_{d_i}}\right).$$

Then, the observer (7) is finite time stable and exists a positive constant ε_{ω} such that $||\hat{\omega} - \omega|| \leq \varepsilon_{\omega}$ before the finite time convergence.

Proof. See Theorem 1 in [50].

The previous result shows that ω can be estimated in finite time, which contains the joint estimation of φ and δ . In the next section, we used an adaptive observer to decouple the estimation of φ and δ from ω .

3.2. Adaptive observer

Secondly, once the total uncertainty ω is estimated, decoupling φ and δ from this estimated variable is necessary. For this purpose, notice that δ is a scalar function. So, the measurement of ξ_e can be used to decouple the desired dynamics, which dynamic model can be expressed with respect to the total uncertainty ω , that is:

$$\dot{\xi}_e = K_e K_a^{-1} \omega + \delta \phi_e(\xi_a, \xi_e) + f_e(\xi_e, D) \tag{8}$$

where $\phi_e(\xi_a, \xi_e) = \xi_e^{in} - \xi_e - K_e K_a^{-1} (\xi_a^{in} - \xi_a)$ and $f_e(\xi_e, D) = D(\xi_e^{in} - \xi_e) - Q_e$. Due to δ slowly changes in time, an adaptive observer can be designed to separate φ and δ from ω and ξ_e . For such an end, the PE condition for ϕ_e is discussed in the following.

Proposition 3.2. If $\xi_e^{in} - \xi_e - K_e K_a^{-1} (\xi_a^{in} - \xi_a) > 0 \ \forall t$, then $\phi_e(\xi_a, \xi_e)$ satisfies the PE condition given in Definition 2.2.

Proof. It is a directed consequence because all the elements of $\phi_e(\xi_a, \xi_e) > 0$ are continuously bounded (Assumption 2.2).

Even though there are different adaptive laws [32], a gradient algorithm with projection is proposed for estimating φ and δ with the advantage of avoid high overshoots in their estimates, as described in the following.

Theorem 3.2. Let the next algorithm be:

$$\hat{\xi}_e = K_e K_a^{-1} \hat{\omega} + \hat{\delta} \phi_e(\xi_a, \xi_e) + f_e(\xi_e, D) + \alpha_1 \tilde{\xi}_e$$
(9a)

$$\dot{\hat{\delta}} = \begin{cases} \alpha_2 \tilde{\xi}_e \phi_e(\xi_a, \xi_e) & \text{if } \hat{\delta} \in \mathcal{A}_\delta \\ 0 & \text{otherwise} \end{cases}$$
(9b)

$$\mathcal{A}_{\delta} = \left\{ \hat{\delta} \in \mathbb{R} \mid \left(\hat{\delta}^2 < \gamma^2 \right) \text{ or } \left(\left(\hat{\delta}^2 = \gamma^2 \right) \text{ and } \left(2\alpha_2 \tilde{\xi}_e \phi_e(\xi_a, \xi_e) \hat{\delta} \le 0 \right) \right) \right\}$$
(9c)

where $\alpha_1, \alpha_2 \in \mathbb{R}_{>0}$ are algorithm gains, $\gamma > \max(|\underline{\delta}|, |\overline{\delta}|)$, while $\tilde{\xi}_e = \xi_e - \hat{\xi}_e$ is the estimation error of ξ_e . Therefore, $\tilde{\xi}_e$ converges asymptotically to the origin while $\hat{\delta}$ and $\hat{\varphi} = K_a^{-1}(\hat{\omega} - \hat{\delta}(\xi_a^{in} - \xi_a))$ converge in a neighborhood near their nominal values. Furthermore, if $\phi_e(\xi_a, \xi_e)$ accomplishes Lemma 3.2, then $\hat{\delta}$ and $\hat{\varphi}$ converge exponentially to δ and φ , respectively.

Proof. The differentiation of $\tilde{\xi}_e$ with respect to time is:

$$\dot{\tilde{\xi}}_e = -\alpha_1 \tilde{\xi}_e + \tilde{\delta}\phi_e(\xi_a, \xi_e) + K_e K_a^{-1}(\omega - \hat{\omega})$$

Now, let be the Lyapunov candidate function $V = \chi^T P_{\alpha} \chi$ where $\chi = \begin{bmatrix} \tilde{\xi}_e & \tilde{\delta} \end{bmatrix}^T$ and $P_{\alpha} = 0.5 \cdot \text{diag}(1, \alpha_2^{-1})$. Applying Rayleigh-Ritz inequality:

$$\lambda_{\min}(P_{\alpha})||\chi||^{2} \le V \le \lambda_{\max}(P_{\alpha})||\chi||^{2}$$
(10)

where $\lambda_{\min}(P_{\alpha}) = 0.5 \min(1, \alpha_2^{-1})$ and $\lambda_{\max}(P_{\alpha}) = 0.5 \max(1, \alpha_2^{-1})$, it is concluded that V is a positive definite and decreasing function. Differentiation of V with respect to time gives:

$$\dot{V} = -\alpha_1 \tilde{\xi}_e^2 + \tilde{\delta} \left(\tilde{\xi}_e \phi_e(\xi_a, \xi_e) + \alpha_2^{-1} \dot{\tilde{\delta}} \right) + K_e K_a^{-1} (\omega - \hat{\omega}) \tilde{\xi}_e.$$

For Theorem 3.1, it follows that:

$$|K_e K_a^{-1}(\hat{\omega} - \omega)\tilde{\xi}_e| \le \rho_{\omega} |\tilde{\xi}_e|, \quad 0 \le t < \tau$$

where $\rho_{\omega} = \lambda_{\max}(K_a^{-1})||K_e||\epsilon_{\omega}$. Otherwise, it is concluded that $K_e K_a^{-1}(\omega - \hat{\omega})\tilde{\xi}_e = 0$ since $\hat{\omega} \to \omega$ when $t \ge \tau$. Now it is necessary to analyze two different cases.

Case I: $\tilde{\delta} = -\alpha_2 \tilde{\xi}_e \phi_e$. For this case:

$$\dot{V} \le -\alpha_1 \tilde{\xi}_e^2 + \rho_\omega |\tilde{\xi}_e|.$$

During the interval $0 \leq t < \tau$, notice that $\dot{V} < 0$ if $|\tilde{\xi}_e| > \rho_\omega/\alpha_1$ and for Theorem 2.1 it is proved that all trajectories of V are UUB with positive definite functions $\omega_1 = \lambda_{\min}(P_\alpha)||\chi||^2$, $\omega_2 = \lambda_{\max}(P_\alpha)||\chi||^2$ and ω_3 equal to the right term of \dot{V} . Now, in the interval $t \geq \tau$, it is easy to show that $\tilde{\delta}, \tilde{\xi}_e, \dot{\tilde{\xi}}_e \in \mathcal{L}_\infty$ and $\tilde{\xi}_e \in \mathcal{L}_2$. Therefore, ξ_e is uniformly continuous and $\lim_{t\to\infty} \int_0^t |\xi_e(s)| \, ds$ exists and is finite, so for Barbalat's Lemma 2.1 it is proved that $\tilde{\xi}_e$ converge asymptotically to origin. Lastly, as a consequence of $\tilde{\delta}$ is bounded and $\hat{\omega} = \omega$ in finite time, $\hat{\delta}$ and $\hat{\varphi}$ are also bounded.

Case II: $\tilde{\delta} = 0$. For this case:

$$\dot{V} \le -\alpha_1 \tilde{\xi}_e^2 + \left(|\phi_e(\xi_a, \xi_e)| |\tilde{\delta}| + \rho_\omega \right) |\tilde{\xi}_e|.$$
(11)

For Assumptions 2.2-2.5, $\phi_e(\xi_a, \xi_e)$ is a bounded function, that is, exists a constant $\sigma_1 \in \mathbb{R}_{>0}$ such that $|\phi_e| \leq \sigma_1$. Also, as a consequence of $\dot{\tilde{\delta}} = 0$, it is clear that $|\tilde{\delta}| = |\delta - \hat{\delta}| \leq \sigma_2$. Therefore:

$$\dot{V} \le -\alpha_1 \left| \tilde{\xi}_e \right| \left(\left| \tilde{\xi}_e \right| - \frac{\kappa + \rho_\omega}{\alpha_1} \right)$$

where $\kappa = \sigma_1 \sigma_2$. Thus, $\dot{V} < 0$ if $|\tilde{\xi}_e| > (\kappa + \rho_\omega)/(\alpha_1)$ and for Theorem 2.1 it is proved that $\forall t \in \mathbb{R}_{\geq 0}$ all trajectories of V are UUB with positive definite functions $\omega_1 = \lambda_{\min}(P_\alpha) ||\chi||^2, \omega_2 = \lambda_{\max}(P_\alpha) ||\chi||^2$ and ω_3 equal to right term of \dot{V} . Later, observe that V is UUB when $0 \leq t < \tau$. However, it is different once $\hat{\omega}$ converges to its nominal value when $t > \tau$. Even though case II shows that V is UUB, notice that $\hat{\delta}$ is in the convex region \mathcal{A}_{δ} which includes all possible values of δ described in Assumption 2.4. In addition, this set is bigger enough to include the maximum absolute values of δ without $\hat{\delta}$ being in the convex region limits. Hence, in long term $\hat{\delta}$ always belong in \mathcal{A}_{δ} and as a result, $\tilde{\xi}_e$ converges asymptotically to origin. Furthermore, as mentioned before, if $\hat{\delta}$ and $\hat{\varphi}$ are bounded, it means they converge in a neighborhood near their nominal values δ and φ .

Now, let be defined the next dynamical system

$$\dot{\tilde{\xi}}_e = -\alpha_1 \tilde{\xi}_e + \tilde{\delta} \phi_e, \qquad \qquad \dot{\tilde{\delta}} = -\alpha_2 \phi_e \tilde{\xi}_e, \qquad \qquad y = \tilde{\xi}_e.$$

This system has the same structure as (3). Moreover, for this system, $H(s) = 1/(s + \alpha_1)$ is a strictly real positive and proper function that satisfies $H(\infty) = 0$ and suppose that $\phi_e(\xi_a, \xi_e)$ accomplishes Lemma 3.2. Therefore, for Theorem 2.2, it is proved that $\hat{\delta}$ converges exponentially to δ . Finally, because $\hat{\omega} = \omega$ in finite time, it is concluded that $\hat{\varphi}$ converge exponentially to φ .

Remark 3.1. Due to the projection defined in the adaptive law (9b), the estimations of φ and δ are defined inside the set \mathcal{A}_{δ} , which is constructed using the input disturbance properties described in Assumption 2.4. Therefore, φ and δ estimations are defined near their possible values.

3.3. Asymptotic observer

Lastly, it is clear that ξ_b can be estimated using the previous estimations of φ and δ decoupled from ω . However, due to $\hat{\omega}$ converging to its nominal value in finite time, it is feasible to use $\hat{\omega}$ instead of $\hat{\varphi}$ to estimate ξ_b , as will be shown. Therefore, notice that ξ_b can be expressed with respect to ω , that is:

$$\dot{\xi}_b = K_b K_a^{-1} \omega + \delta \phi_b(\xi_a, \xi_b) + f_b(\xi_b, D)$$
(12)

where $\phi_b(\xi_a, \xi_b) = (\xi_b^{in} - \xi_b - K_b K_a^{-1} (\xi_a^{in} - \xi_a))$ and $f_b(\xi_b, D) = D(\xi_b^{in} - \xi_b) - Q_b$. Hence, an asymptotic observer can be used with $\hat{\omega}$ and $\hat{\delta}$ to estimate ξ_b , as described in the following theorem.

Theorem 3.3. Let the next algorithm be:

$$\dot{\hat{\xi}}_b = K_b K_a^{-1} \hat{\omega} + \hat{\delta} \phi_b(\xi_a, \hat{\xi}_b) + f_b(\hat{\xi}_b, D).$$
(13)

If $\hat{\omega} = \omega$ in a finite time, $|\delta - \hat{\delta}| \leq \varepsilon_{\delta} \in \mathbb{R}_{\geq 0}$, and $D + \delta - \varepsilon_{\delta}$ accomplishes the PE condition, then $\hat{\xi}_b$ converges asymptotically to a neighborhood near ξ_b , that is, exists a constant $\kappa > 0$ such that:

$$\lim_{t \to \infty} ||\xi_b(t) - \hat{\xi}_b(t)|| \le \frac{2\sqrt{2m_1}}{\kappa}$$
$$m_1 = \varepsilon_\delta \sum_{k=1}^{n-m-1} \left(\bar{\xi}_{b_k}^{in} + \bar{\xi}_{b_k} + \left| K_{a_k}^{-1} \right| \sum_{j=1}^m |K_{b_{kj}}| \left(\bar{\xi}_{a_j}^{in} - \bar{\xi}_{a_j} \right) \right).$$

Robust hybrid observer for estimating key biochemical variables in continuous bioreactors

Proof. The differentiation of error $\tilde{\xi}_b = \xi_b - \hat{\xi}_b$ with respect to time is:

$$\dot{\tilde{\xi}}_b = -(D+\delta)\tilde{\xi}_b - \tilde{\delta}\left(\varsigma - \tilde{\xi}_b\right)$$

where $\varsigma = \xi_b^{in} - \xi_b - K_b K_a^{-1} \left(\xi_a^{in} - \xi_a\right)$. Now, defining the candidate Lyapunov function $V = \frac{1}{2} \tilde{\xi}_b^T \tilde{\xi}_b$, which is a positive definite and decrescent function. The differentiation of V with respect to time is:

$$\dot{V}(\xi_b(t)) \le -m_0(t)||\tilde{\xi}_b(t)||^2 + m_1||\tilde{\xi}_b(t)||$$

where $m_0(t) = D(t) + \delta(t) - \varepsilon_{\delta}$. Using the comparison lemma $(u = \sqrt{V})$, it is concluded that:

$$V(\xi_b(t)) \le V(\xi(0))\rho(t,0) + \frac{\sqrt{2}m_1}{2} \int_0^t \rho(t,\tau) \,\mathrm{d}\tau$$
$$\rho(t_1,t_2) = \exp\left(-\int_{t_2}^{t_2+T} m_0(s) \,\mathrm{d}s\right)$$

where $T = t_1 - t_2$. Due to $m_0(t)$ accomplishes PE condition and for Cauchy-Schwarz inequality, then:

$$-\int_{t}^{t+T} m_0(s) \,\mathrm{d}s \le -\sqrt{\kappa T} < 0$$

where $\kappa, T > 0$. Therefore $\rho(t_1, t_2) \leq e^{-\sqrt{\kappa(t_1 - t_2)}}$ and from the previous results, one gets:

$$||\tilde{\xi}_{b}(t)|| \leq 2||\tilde{\xi}_{b}(0)||e^{-\sqrt{\kappa t}} + \frac{2\sqrt{2m_{1}}}{\kappa}\rho_{0}(t)$$
$$\rho_{0}(t) = 1 - e^{-\sqrt{\kappa t}} \left(1 + \sqrt{\kappa t}\right).$$

Due to $\lim_{t\to\infty} \rho_0(t) = 1$, then $\lim_{t\to\infty} ||\tilde{\xi}_b(t)|| \leq \frac{2\sqrt{2}m_1}{\kappa}$ and the main result is proved.

Remark 3.2. Notice that the asymptotic observer has an indirect output injection term due to the observer-based estimators previously designed.

Remark 3.3. Due to the STA guarantees that $\hat{\omega} \to \omega$ in finite time, the adaptive observer can be studied directly, as similarly done in [48]. The asymptotic observer uses $\hat{\omega}$ instead of $\hat{\varphi}$ to avoid carrying additional estimation errors. Furthermore, observe that ξ_b do not affect ξ_a and ξ_b dynamics, and consequently, the previous estimation of φ and δ are independent of ξ_b estimation.

3.4. A summary of the hybrid observer design procedure

In this part, the design steps to be followed for the sequential estimation of φ , the external perturbation δ and/or the nonmeasurable states ξ_b of system (2) are suggested:

- 1. First, the implicit estimation of φ and δ is carried out by means of the estimation of $\omega := K_a \varphi + \delta(\xi_a^{in} \xi_a)$ using the ESTA algorithm (7). Next, one can go to steps 2 or 3 according to the desired estimation objective.
- 2. Suppose that, starting from $\hat{\omega}$, one wants to estimate φ and δ independently. To this end, it is proposed here to use the adaptive asymptotic observer (8) to estimate δ and consequently also determine φ , taking into account that $\hat{\omega} := K_a \hat{\varphi} + \hat{\delta}(\xi_a^{in} - \xi_a).$
- 3. Finally, suppose it is wanted to estimate the nonmeasurable states ξ_b . For this, it is proposed here to use the asymptotic observer (12), which only requires the finite-time estimate of ω provided in step 1.

4. SIMULATION RESULTS

Consider sulfate-reducing bioprocess of *Desulfovibrio alaskensis* 6SR described in [1]:

$$\dot{X} = \varphi - (D + \delta)X - \beta X \tag{14a}$$

$$\dot{L} = -Y_L \varphi + (D+\delta)(L^{in} - L)$$
(14b)

$$\dot{A} = Y_A \varphi - (D + \delta)A \tag{14c}$$

$$\dot{S}_1 = -Y_{S_1}\varphi + (D+\delta)(S_1^{in} - S_1)$$
(14d)

$$\dot{S}_2 = Y_{S_2}\varphi - (D+\delta)S_2 \tag{14e}$$

where X is bacteria biomass, L, A, S_1 , and S_2 are lactate, acetate, sulfate, and sulfide concentration, respectively. While L^{in} and S_1^{in} are the influent concentration of L and S_1 , respectively. Moreover, Y_L , Y_A , Y_{S_1} and Y_{S_2} are lactate-biomass, acetate-biomass, sulfate-biomass, and sulfide-biomass yield coefficients, respectively, while β is the specific mortality rate and φ is the growth rate of microorganisms. Notice that the system (14) can be expressed as the dynamic model (2), where:

$$\begin{aligned} \xi_a &= X & \xi_a^{in} &= 0 & K_a &= 1 \\ \xi_e &= L & \xi_e^{in} &= L^{in} & K_e &= -Y_L \\ \xi_b &= \begin{bmatrix} A \\ S_1 \\ S_2 \end{bmatrix} & \xi_b^{in} &= \begin{bmatrix} 0 \\ S_1^{in} \\ 0 \end{bmatrix} & K_b &= \begin{bmatrix} Y_A \\ -Y_{S_1} \\ Y_{S_2} \end{bmatrix}. \end{aligned}$$

Remark 4.1. The dynamic model (14) describes the reduction of sulfate to sulfide by *Desulfovibrio alaskensis 6SR*. This species is an anaerobic bacterium that produces energy by reducing sulfate, and it is commonly used for the bioremediation of heavy metals. In this case, lactate is used as a carbon source, while acetate is a secondary metabolite from the bacteria. Sulfide is another secondary metabolite that, in high concentrations, inhibits the reproduction of the bacteria. For definiteness, let us summarize the context under which the experimental results will be presented.

• The main objective is to monitoring the unmeasurable states A, S_1 , and S_2 under the external disturbance δ and without knowing the structure of φ , which is described for simulation purposes as:

$$\varphi = \mu_{\max} \left(\frac{L}{K_L + L}\right) \left(\frac{S_1}{K_{S_1} + S_1}\right) \left(1 - \frac{S_2}{P_s}\right)^n X$$

where $\mu, K_L, K_{S_1}, P_s, n \in \mathbb{R}_{>0}$ are kinetic parameters.

- It is assumed that the input disturbance δ has four different behaviors: δ is null (before 80 h), is negative (from 80 h to 160 h), is positive and greater to the nominal dilution rate (from 160 h to 240 h), and its continuously bounded (after 240 h).
- Two cases will be studied for the sulfate reducing process. In case one, the monitoring performance of δ , φ , and the unmeasurable states (A, S_1, S_2) will be shown. In case two, a compensation action based on δ -estimated $(\hat{\delta})$ will be presented to alleviate the undesirable effects of δ on the bioreactor dynamics. This case will illustrate the advantages of having a good estimate of δ and φ .

Case	Initial State Values (g/L)				
	Biomass (X)	Lactate (L)	Acetate (A)	Sulfate (S_1)	Sulfide (S_2)
System	0.125	2.75	0.025	5.25	0.05
Observer	0.5	1.0	0.5	4.0	0.3

Tab. 1: Initial conditions used in the simulations.

Algorithm	RMSE			
	φ	A	S_1	S_2
HB-STA+AAO	0.0037	0.1932	0.1426	0.0299
HB-HGO+AO	0.0168	0.2491	0.2374	0.0464

Tab. 2: RMSE results by estimating φ , A, S_1 , and S_2 with the HB-STA+AAO (7), (9) and (13) and the HB-HGO+AO (15) during $t \ge 25$ h to avoid the high-frequency discontinuous behavior for the HB-STA+AAO and the peaking phenomena for the HB-HGO+AO.

Now, to compare estimation dynamics of φ , δ , and ξ_b with the designed hybrid observer based on super-twisting plus adaptive and asymptotic observers (HB-STA+AAO), suppose that $\delta \approx 0$. Defining $Z = [\xi_a^T \varphi^T K_a]^T$, then ξ_a and φ can be expressed as following:

$$\dot{Z}(t) = AZ(t) + f(Z, D) + g(t)$$
$$y(t) = CZ(t)$$



Fig. 2: Simulation results for monitoring φ with the HB-STA+AAO (7), (9), and (13) and the HB-HGO+AO (15) in the sulfate-reducing bioprocess (14).

where

$$A = \begin{bmatrix} 0_{m \times 1} & I_{m \times m} \\ 0 & 0_{1 \times m} \end{bmatrix} \qquad \qquad C = \begin{bmatrix} I_{m \times m} & 0_{m \times m} \end{bmatrix}$$
$$f(Z, D) = D(\xi_a^{in} - Z_1(t)) - Q_a \qquad \qquad g(t) = K_a \dot{Z}_2(t).$$

Hence, the following hybrid observer can be used:

$$\dot{Z} = A\hat{Z} + f(\hat{Z}, D) + \theta \Delta_{\theta}^{-1} K(y - C\hat{Z})$$
(15a)

$$\dot{\hat{\xi}}_b = K_b \hat{\varphi} + D(\xi_b^{in} - \xi_b) - Q_b \tag{15b}$$

where $\hat{\varphi} = K_a^{-1}Z_2$, $K \in \mathbb{R}^{2m \times 1}$ is an algorithm gain, θ is a high gain term and $\Delta_{\theta} = \text{diag}(I_{m \times m}, \frac{1}{\theta}I_{m \times m})$. This hybrid observer is based on a high gain observer plus an asymptotic observer (HB-HGO+AO). The HGO guarantees that $\hat{Z}(t)$ converges exponentially to its nominal value [13], while the asymptotic observer guarantees that $\hat{\xi}_b$ converges near its nominal value. Additionally, the root mean squared error (RMSE) will be used to compare the performance estimation of φ and ξ_b between both observers, that is:

$$\text{RMSE}(F_i, \hat{F}_i) = \left(\frac{1}{T_R} \sum_{i=1}^T \left(F_{ij} - \hat{F}_{ij}\right)^2\right)^{1/2}$$
(16)

where $F = \begin{bmatrix} \varphi & A & S_1 & S_2 \end{bmatrix}$ and $T_R > 0$ is the number of observations.

All simulations were done in MATLAB with Simulink (2023b) using Euler solver which step solution size is fixed as 1 miliseconds. While the numerical values of model parameters are equal to the ones described in [1], except for $\mu_{\text{max}} = 1 \text{ h}^{-1}$ and $\beta =$ 0.01 h^{-1} . Also, the input values of the bioprocess are $D = 0.1 \text{ h}^{-1}$, $L^{in} = 10 \text{ g/L}$, and $S_{1}^{in} = 6 \text{ g/L}$, while the initial conditions of the system are described in Table 1. Additionally, the states X and L are measured discontinuously each 0.25h (equivalent to 15 minutes). Lastly, the gain values of both observers are $L_1 = 1$, $L_2 = 0.2 K_1 = 1$, $K_2 = 2$, $\Psi = 0.02$, $\alpha_1 = \alpha_2 = 1$, $\theta = 1.5$, and $\gamma = 0.25$. In both cases, the observers' performance was compared with simulated data obtained by simulating the bioprocess model (14).

4.1. Case 1: Robust estimation

The simulation results are shown in Figures 2–4, while the calculated RMSE is shown in Table 2. Thus, the HB-HGO+AO has the fastest and best performance for estimating the reaction rate before 80 h because $\delta = 0$. Furthermore, although the adaptive law with projection ensures that $|\hat{\delta}| \leq \gamma$, it affects the estimation performance of φ and δ with the HB-STA+AAO by producing a high-frequency discontinuous behavior during the first 10 h, as shown in Figures 2a and 3a. However, the estimation performance of φ , A, S_1 , and S_2 with the HB-HGO+AO drastically worsens under the presence of external input disturbance. Moreover, the RMSE calculated shows that the HB-STA+AAO has the best performance for estimating the key biochemical variables. Also, observe that ϕ_e accomplishes Lemma 3.2 due to $\phi_e > 0$, as shown in Figure 5a. Therefore, $\Phi(t, T)$ is strictly positive and bounded, as shown in Figure 5b. Consequently, ϕ_e accomplishes the PE condition and guarantees that the adaptive observer performs well in estimating δ .



Fig. 3: Simulation results for monitoring δ with the HB-STA+AAO (7), (9), and (13) in the sulfate-reducing bioprocess (14).

4.2. Case 2: Disturbance rejection control

For D = 0.1 h⁻¹ and $\delta = 0$, the state $\xi = [X \ L \ A \ S_1 \ S_2]^T$ of system (14) has two feasible equilibrium points:

$$\bar{\xi}_{eq_1} = \begin{bmatrix} 0 & L^{in} & 0 & S_1^{in} & 0 \end{bmatrix}^T$$
$$\bar{\xi}_{eq_2} = \begin{bmatrix} 0.3167 & 5.2518 & 2.9657 & 3.1661 & 0.5533 \end{bmatrix}^T$$



Fig. 4: Simulation results for monitoring A, S_1 and S_2 with the HB-STA+AAO (7), (9), and (13) and the HB-HGO+AO (15) in the sulfate-reducing bioprocess (14).



Fig. 5: Dynamics of $\phi_e(t)$ and $\Phi(t,T) = \int_t^{t+T} \phi_e^2(\tau) d\tau$ (T = 80 h, PE condition) in the sulfate-reducing bioprocess (14) for Case 1 and 2.

Notice that ξ_{eq_1} corresponds to the washout equilibrium condition, that is, a pathological behavior of the process, such that no more bioreactions occur. Hence, it could be reasonable that the state ξ converge to an optimal equilibrium point. However, the previous simulation shows that bioreactor dynamics and their equilibrium points can be drastically changed by the effect of the input disturbance δ . So, it is necessary to propose a disturbance compensation action to reject the impact of δ in the bioreactor dynamics. For this task, the simplest compensation is given by $D = U - \hat{\delta}$, where U may stabilize the bioreactor, guarantee the tracking of a desired dynamic reference, or drive the bioreactor's behavior to a desirable equilibrium point, for instance [47]. In order to fulfill Assumption 2.5, which avoids negative values of the dilution rate and batch or washout conditions, it is necessary that D has the following structure:

$$D = \min\left(\max\left\{\left(\hat{\delta} - U\right)\operatorname{sign}\left(\hat{\delta} - U\right), \underline{d}\right\}, \overline{d}\right)$$
(17)

where $\underline{d}, \overline{d} \in \mathbb{R} > 0$ are the minimum and maximum value of D, respectively. Although the proposed action rejects δ , notice that if $|\delta| > U$, it is impossible to deny its effect. Therefore, it is given an additional assumption usually accomplished in real applications.

Assumption 4.1. The absolute value of the input disturbance δ is never bigger or equal to U, that is, $|\delta| < U$.

Now, suppose that ${}^{4}U = Y_{S_2}\hat{\varphi}/S_r$ where $S_r = 0.9P_s$, $\underline{d} = 0.002 \text{ h}^{-1}$, $\overline{d} = 0.2 \text{ hr}^{-1}$ and D = U in the first 25 hr to avoid the high-frequency discontinuous estimation of δ . The simulation results of the sulfate-reducing bioprocess (14) coupled with the controller (17) is shown in Fig.6a for the HB-STA+AAO. At the same time, the simulated data were obtained by simulating the bioprocess coupled with the disturbance rejection control, assuming that δ is fully known and $D = U - \delta$. Notice that the proposed controller-based observer guarantees a good disturbance rejection and estimation of S_2 . Moreover, S_2 converge near $0.9P_s$ except between the 160 h and 240 h. This specific behavior happens because Assumption 4.1 is no longer valid, that is, $|\delta| > U$ and D must be negative to reject the input disturbance, as shown for the simulated case in Figure 6b. However, a negative value of D is impossible for real applications. Hence, the dilution rate needs to be strictly positive and bounded to avoid negative values, as shown in Figure 6b. Finally, notice that ϕ_e still accomplishes the PE condition for all time, as shown in Figure 5.

To summarize, the main advantage of the proposed algorithm is that it can exponentially estimate the reaction rates and additive disturbance in the dilution rate, in contrast with the convergence near their nominal values obtained in [50]. Moreover, these estimations belong to a bounded range of values due to the projection in the adaptive law. Hence, the peaking phenomena of an HGO do not happen in this algorithm. Also, the estimation of the unmeasurable states converges near their nominal values. However, the main limitation of this hybrid observer is that the external disturbance must slowly change in time, and the projection in the adaptive law can produce discontinuities for a short interval of time, as shown in the following section. Furthermore, the

⁴This controller is based on the results developed in [21], that is, under a known φ and $\delta = \beta = 0$, this algorithm guarantees that the bioreactor avoids washout and batch conditions, while $X \to 0.9P_s$, $L \to L^{in} - (0.9Y_LP_s/Y_{S_2})$, $A \to 0.9Y_AP_s/Y_{S_2}$, $S_1 \to S_1^{in} - (0.9Y_S_1P_s/Y_{S_2})$, and $S_2 \to 0.9P_s$.



Fig. 6: Dynamic of the sulfide S_2 and dilution rate D in the disturbance rejection control of the sulfate-reducing bioprocess (14).

proposed algorithm must be modified for biochemical processes under noisy or discrete measurements.

5. CONCLUSION

The problem under study is centered on the robust observation problem of continuous bioreactors. For this, and of independent interest, the paradigm of unknown reaction rate dynamics faced by input external disturbance is considered. The hybrid observer design methodology presents a systematic way based on a careful and realistic state space representation that facilitates the hybrid sequential observer design. Hence, a hybrid observer was designed to estimate unmeasurable states, unknown reaction rates, and an input disturbance in the dilution rate for a continuous bioprocess. The proposed algorithm uses a partition of the measurable state and the extended super-twisting algorithm to jointly estimate reaction rates and an input disturbance in finite time. Later, an adaptive observer with projection and the measurement of another reactive or product was used to decouple them. These estimates converge exponentially to their nominal values if the persistent excitation condition is accomplished. Lastly, using the previous estimations and an asymptotic observer, the unmeasurable states converge near their nominal value. Finally, the proposed technique may offer a way of approaching robust disturbance rejection control problems under partially known dynamics in bioreactors. The proposed observer and controller are illustrated in two simulations dealing with a sulfate-reducing process in a continuous bioreactor. The results show a good performance in monitoring and controlling different input disturbance behaviors.

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- Víctor Reza, Automatic Control Department, CINVESTAV, Mexico City. Mexico. e-mail: victor.reza@cinvestav.mx
- Jorge Torres, Automatic Control Department, CINVESTAV, Mexico City. Mexico. e-mail: jotorres@cinvestav.mx

Jesús Guerrero, Tecnológico Nacional de México/ITS de Irapuato, Irapuato, Guanajuato 36821. Mexico.

e-mail: norberto.gt@irapuato.tecnm.mx