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CHARACTERIZATION OF THE DYNAMIC BEHAVIOR OF NONLINEAR BIOSYSTEMS IN THE PRESENCE OF MODEL UNCERTAINTY USING SINGULAR INVARIANCE PDES: APPLICATION TO IMMOBILIZED ENZYME AND CELL BIOREACTORS

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Abstract. A new approach to the problem of characterizing the dynamic behavior of nonlinear biosystems in the presence of model uncertainty using the notion of slow invariant manifold is proposed. The problem of interest is addressed within the context of singular partial differential equations (PDE) theory, and in particular, through a system of singular quasi-linear invariance PDEs for which a general set of conditions for solvability is provided. Within the class of analytic solutions, this set of conditions guarantees the existence and uniqueness of a locally analytic solution which represents the system's slow invariant manifold exponentially attracting all dynamic trajectories in the absence of model uncertainty. An exact reduced-order model is then obtained through the restriction of the original biosystem dynamics on the slow manifold. The analyticity property of the solution to the invariance PDEs enables the development of a series solution method that can be easily implemented using MAPLE leading to polynomial approximations up to the desired degree of accuracy. Furthermore, the aforementioned attractivity property and the transition towards the above manifold is analyzed and characterized in the presence of model uncertainty. Finally, examples of certain immobilized enzyme bioreactors are considered to elucidate aspects of the proposed context of analysis.

1. **Introduction.** A notable research objective in nonlinear systems analysis is undeniably the existence of invariant manifolds and the associated problem of finding/computing them [1, 10, 11, 30]. This problem has been traditionally motivated by efforts to develop systematic methods for the simplification of the analysis of

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the behavior of nonlinear dynamical systems through a reduction of the dimensionality of the original problem and the explicit computation of a reduced-order, yet accurate, description of the system dynamics [1, 2, 3, 5, 7, 9, 10, 11, 13, 14, 18, 19, 20, 21, 22, 23, 24, 28, 29, 30, 32, 33]. In particular, complex biosystems and bioprocesses that are also inherently nonlinear represent a special class of systems whose dynamic behavior could be reliably analyzed and characterized within the aforementioned framework. However, in all the above approaches, appropriate a priori information is needed for their practical application. Indeed, quasi-steady-state approximation and quasi-equilibrium-manifold methods and their variants require the explicit physical identification of the system's "fast" state variables, whereas approaches based on singular perturbation theory presuppose the explicit physical identification of a function of the system's parameters which is considered to be "small" in a certain sense, and its "smallness" is indicative of an underlying time-scale multiplicity. Please notice that in addition to relying on the above a priori knowledge, all of the above methods are inherently inexact, since they can not mathematically generate and characterize exactly the system's slow invariant manifold, inevitably resulting in long-term inaccuracies in the dynamic behavior offered by the reduced-order model that describes the on-manifold dynamics. On the other hand, a mathematically rigorous treatment of the problem of characterizing the behavior of a nonlinear dynamical system ought to be founded on the explicit characterization and computation of the system's exact slow invariant manifold, as well as the system's dynamic approach towards it [1, 10, 11, 30]. Furthermore, it should be pointed out, that dynamic models of biosystems can not fully capture and accurately describe the actual biosystem's behavior in practice, due to the inevitable modeling errors and/or model uncertainty pertaining for example to unknown or poorly known parameter values or unmodeled dynamics [1, 10, 11, 30]. It is therefore quite important to carefully analyze the behavior of a nonlinear biosystem, its approach towards the slow manifold and the on-manifold dynamics in the presence of model uncertainty and/or modeling errors [1, 10, 11, 30]. The present research study proposes a new approach to this problem by mathematically characterizing the associated system of singular invariance PDEs that offers the nominal biosystem's exact slow manifold, as well as analyzing the system's dynamic approach towards and evolution on the slow manifold in the presence of model uncertainty.

The present paper is organized as follows: Section 2 contains some conceptual and mathematical preliminaries that are necessary for the ensuing theoretical developments. The paper's main results are presented in Section 3. The proposed method is evaluated in an illustrative example focusing on the characterization of the nonlinear dynamic behavior exhibited by certain immobilized enzyme bioreactors in the presence of enzymatic activity degradation and model uncertainty. Finally, a few concluding remarks are provided in Section 5.

2. **Conceptual and mathematical preliminaries.**

Consider the nonlinear dynamics of a biosystem mathematically represented by:

$$\frac{dx}{dt} = f(x) \tag{1}$$

where $x \in X$ is the state vector and $X \subset R^n$ a compact subset of state space. It is assumed that $f(x)$ is a real analytic vector function $f : X \longrightarrow R^n$, and without

loss of generality, the origin $x^0 = 0$ is an equilibrium point of (1): $f(0) = 0$. Furthermore, it is assumed that the Jacobian matrix $A = \frac{\partial f}{\partial x}(0)$ has eigenvalues with negative real parts (Hurwitz matrix), and more specifically, its eigenspectrum $\sigma(A)$ consists of two distinct subsets of "fast" eigenvalues $\sigma_f(A)$ and "slow" eigenvalues $\sigma_s(A)$: $\sigma(A) = \sigma_f(A) \cup \sigma_s(A)$, for which the real parts of the "fast" eigenvalues are a few orders of magnitude larger than the real parts (in absolute value) of the "slow" ones.

The following definition is essential:

Definition 1 [30]: A set

$$\Omega = \{x \in R^n | \phi(x) = 0\} \tag{2}$$

where $\phi : R^n \to R^n$ is a map with $\phi(0) = 0$, is said to be invariant under the flow of dynamics (1) if for each $\phi(x(0)) \in \Omega$, the integral curve $\{x(t)\}$ of (1) satisfying $x(t = 0) = x(0)$, is such that $\phi(x(t)) \in \Omega$ for all $t \in R^+$. An invariant set $\Omega \subset R^n$ passing through the origin $x^0 = 0$ is said to be a real analytic local invariant manifold, if ϕ is real analytic and Ω has the local topological structure of an analytic manifold around the origin.

One can easily show that for Ω to be rendered invariant under the flow of (1), the map ϕ ought to satisfy the following invariance PDE:

$$\frac{\partial \phi}{\partial x}(x) f(x) = 0 \tag{3}$$

Notice, that the above invariance PDE condition is satisfied by all possible invariant manifolds of dynamics (1), and therefore, it admits multiple solutions. One of the key issues that the present study aims at addressing is the development of a method that allows the construction of the system's slow manifold out of the above multitude of invariant manifolds. Within such a context, this method would allow the explicit mathematical characterization of the biosystem's behavior that corresponds both to the "fast" eigenmodes that govern the rapid transition of the system towards the slow manifold, and the "slow" eigenmodes that determine the system's dynamic evolution on the slow manifold embedded in state space. Moreover, the restriction of the biosystem dynamics (1) on the above slow manifold would represent a reduced-order description of the original nonlinear dynamics (1).

At this point, please notice that one can always triangularize the linear part of the system dynamics (1) by transforming the system's Jacobian $A = \frac{\partial f}{\partial x}(0)$ into a block-triangular form. In particular, one can always find a linear coordinate transformation such that the Jacobian $A = \frac{\partial f}{\partial x}(0)$ becomes transformed into a block-triangular form where the eigenvalues of the diagonal blocks are exactly the slow and fast eigenvalues of A [30]. As a result, in the new coordinate system the original system dynamics is represented via the following form:

$$\begin{aligned} \frac{dx_f}{dt} &= F_f(x_s, x_f) \\ \frac{dx_s}{dt} &= F_s(x_s, x_f) \end{aligned} \tag{4}$$

with $F_f(x_s, x_f)$ and $F_s(x_s, x_f)$ being real analytic vector functions with: $F_f(0, 0) = 0$, $F_s(0, 0) = 0$, $\frac{\partial F_s}{\partial x_f}(0, 0) = 0$ and $\sigma_s(A) = \sigma(\frac{\partial F_s}{\partial x_s}(0, 0))$, $\sigma_f(A) = \sigma(\frac{\partial F_f}{\partial x_f}(0, 0))$ are

the set of the slow and fast eigenvalues of the Jacobian A as they surface once the block-triangularization of the system's linear part is performed.

However, special attention should be drawn with respect to broad classes of bioprocesses or biosystems that exhibit the exact triangular structure shown below (also known as a skew-symmetric system):

$$\begin{aligned} \frac{dx_f}{dt} &= F_f(x_s, x_f) \\ \frac{dx_s}{dt} &= F_s(x_s) \end{aligned} \tag{5}$$

where the second dynamic equation describes the "slow" motion and the first the "fast" one. Within this context, the first dynamic equation may correspond to a biosystem or bioprocess whose own dynamics is driven by:

(i) either a time-varying process parameter vector $x_s(t)$ that follows the "slow" dynamics of the second dynamic equation and models phenomena such as enzymatic deactivation or loss of viablity of cells in immobilized enzyme or cell bioreactors respectively [10, 16], or

(ii) the "slow" reaction-invariant dynamics encountered in numerous theoretical and applied studies involving biological reactor dynamics [28], or

(iii) a "slowly" varying input/disturbance dynamics mathematically realized by the second dynamic equation (where input or disturbance changes are modeled and generated as "outputs" of the autonomous nonlinear dynamics associated with the second equation) [2, 16], or finally

(iv) by an "upstream" bioprocess with slow dynamics modeled through the second dynamic equation in (5) [16].

As it is often the case in practice, the above dynamic models can not adequately capture and faithfully describe the behavior of the actual biosystem due to model uncertainty that is inevitably introduced at the modeling stage. For example, one may envision cases where kinetic parameters in biochemical reaction systems are unknown or poorly known, or certain dynamics has not been captured by modeling, and therefore, cases where an element of uncertainty and/or error is introduced in the dynamic description of the biosystem under consideration [1, 10, 11, 30]. Mathematically, this uncertainty is very often represented in the following fashion:

$$\begin{aligned} \frac{dx_f}{dt} &= F_f(x_s, x_f) + \epsilon_1 G_f(x_s, x_f) \\ \frac{dx_s}{dt} &= F_s(x_s, x_f) + \epsilon_2 G_s(x_s, x_f) \end{aligned} \tag{6}$$

where the terms: $F_s(x_s, x_f), F_f(x_s, x_f)$ represent the "known" part of the dynamic model (or equivalently its nominal part), whereas the terms: $G_s(x_s, x_f), G_f(x_s, x_f)$ represent the model uncertainty or modeling error or some "unmodeled dynamics". Please notice, that $\epsilon_1, \epsilon_2 > 0$ are typically small numbers (perturbation parameters) and even though we do not know $G_s(x_s, x_f), G_f(x_s, x_f)$ exactly, we do have some knowledge about them, for example some type of bound can be established. Indeed, it is often assumed that the perturbation terms $G_s(x_s, x_f), G_f(x_s, x_f)$ are bounded on X satisfying the following condition:

$$\begin{aligned} ||G_s(x_s, x_f)|| &\leq M_s \\ ||G_f(x_s, x_f)|| &\leq M_f \end{aligned} \tag{7}$$

where $M_s, M_f > 0$ and $(x_s, x_f) \in X$.

Let us now focus on the "known" nominal dynamic model (4) (or (5)). One can easily infer that:

$$\Omega = \{(x_f, x_s) \in R^n | x_f - \pi(x_s) = 0\} \tag{8}$$

represents an invariant manifold for system (4), if the map π satisfies the system of invariance PDEs shown below:

$$\frac{\partial \pi}{\partial x_s} F_s(x_s, \pi(x_s)) = F_f(x_s, \pi(x_s)) \tag{9}$$

Typically the above systems of invariance PDEs is accompanied by the condition: $\pi(0) = 0$, which reflects the fact that the system's equilibrium point lies on the invariant manifold. From a mathematical standpoint, attention should be drawn to the fact that the above system of first-order PDEs is of particular structure and admits a common principal part consisting of the components of the vector function $F_s(x_s, x_f)$. Furthermore, notice that the principal part vanishes at $x = 0$ due to the equilibrium condition, and thus, the origin becomes a characteristic (singular) point for the system of PDEs (9) [6, 8]. As a consequence, the well-known existence and uniqueness Cauchy-Kovalevskaya theorem can not be invoked because the pertinent conditions are not satisfied for the singular system of PDEs (9) [6, 8], and inevitably one needs to resort to methods and results from singular PDE theory. Specifically, the following results can be derived for the nominal system in the absence of model uncertainty [14, 15]:

Theorem 2.1 *Consider the nonlinear system (4) and let all the aforementioned assumptions hold true. Moreover, assume that the eigenvalues k_i of matrix $A_s = \frac{\partial F_s}{\partial x_s}(0,0)$ are not related to the eigenvalues λ_i of matrix $A_f = \frac{\partial F_f}{\partial x_f}(0,0)$ through any equations of the type:*

$$\sum_{i=1}^{m} m_i k_i = \lambda_j$$

$(j = 1, ..., p)$, where all the m_i are non-negative integers that satisfy the condition:

$$\sum_{i=1}^{m} m_i > 0$$

Then, the set Ω (8) is a real analytic invariant manifold of (4), where $\pi(x_s)$ is the unique locally analytic solution of the singular invariance PDEs (9).

Theorem 2.2 *Let all assumptions of Theorem 1 hold true. Furthermore, let Ω (8) be an invariant manifold of (4), where $\pi(x_s)$ is the unique locally analytic solution of the invariance PDEs (9) and $\{x_s(t), x_f(t)\}$ a solution curve of (4). There exists a neighborhood U^0 of the origin and real numbers $M > 0$ and $K > 0$ such that, if $(x_s(0), x_f(0)) \in U^0$, then:*

$$||x_f(t) - \pi(x_s(t))||_2 \leq M \exp(-Kt) ||x_f(0) - \pi(x_s(0))||_2 \tag{10}$$

Furthermore, the rate of decay of the dynamics of the off-manifold coordinate: $z = x_f - \pi(x_s)$ is governed by the fast eigenvalues of matrix $A_f = \frac{\partial F_f}{\partial x_f}(0,0)$.

Theorems 2.1 and 2.2 imply that for the nominal biosystem and in the absence of model uncertainty, Ω represents exactly the system's slow invariant manifold that exponentially attracts all system trajectories once the fast transients die out.

Therefore, a reduced-order description of the biosystem dynamics is the following one:

$$\begin{aligned} \frac{dx_s}{dt} &= F_s(x_s, \pi(x_s)) \\ x_f &= \pi((x_s)) \end{aligned} \tag{11}$$

The above reduced-order model represents exactly the system's dynamics on the slow manifold Ω (restriction of the system's flow on the slow manifold), and can be used in practice since the fast transients could be justifiably ignored. Indeed, the proposed reduced-order model implies that almost instantaneously the fast state x_f jumps from its initial condition $x_f(0)$ to $\pi(x_s(0))$ on the manifold Ω where the system is bound to evolve and the relation $x_f(t) = \pi(x_s(t))$ holds true for every $t > 0$.

In order to be able to make practical use of the proposed method, one must provide a solution scheme for the associated system of singular invariance PDEs (9). Notice that the method of characteristics is not applicable because the aforementioned system of PDEs (9) is singular [6, 8]. However, since all functions involved are locally analytic around the origin, it is possible to calculate the solution $x_f = \pi(x_s)$ in the form of a multivariate Taylor series around the origin. The method involves expanding all functions involved, as well as the unknown solution $x_f = \pi(x_s)$ in a Taylor series and equating the same order Taylor coefficients of both sides of the PDEs (9). This procedure leads to linear recursion formulas, through which one can calculate the N-th order Taylor coefficients of the unknown solution $x_f = \pi(x_s)$, given the Taylor coefficients of $x_f = \pi(x_s)$ up to the order $N-1$ [15].

In the derivation of the recursion formulas, it is convenient to use the following tensorial notation:

a) The entries of a matrix A are represented as a_i^j, where the subscript i refers to the corresponding row and the superscript j to the corresponding column of the matrix.

b) The partial derivatives of the μ-th component $F_\mu(x_s, x_f)$ of the vector function $F(x_s, x_f)$ with respect to the state variables x_s evaluated at $(x_s, x_f) = (0, 0)$ are denoted as follows:

$$\begin{aligned} F_\mu^i &= \frac{\partial F_\mu}{\partial x_{s,i}}(0,0) \\ F_\mu^{ij} &= \frac{\partial^2 F_\mu}{\partial x_{s,i}\partial x_{s,j}}(0,0) \\ F_\mu^{ijk} &= \frac{\partial^3 F_\mu}{\partial x_{s,i}\partial x_{s,j}\partial x_{s,k}}(0,0) \end{aligned} \tag{12}$$

etc., where $i, j, k, ..{=}1, ..., n$.

c) The standard summation convention where repeated upper and lower tensorial indices are summed up.

Under the above notation the l-th component $\pi_l(x_s)$ of the unknown solution $\pi(x_s)$ can be expanded in a multivariate Taylor series as follows [19]:

$$\begin{aligned} \pi_l(x_s) &= \frac{1}{1!}\pi_l^{i_1} x_{s,i_1} + \frac{1}{2!}\pi_l^{i_1 i_2} x_{s,i_1} x_{s,i_2} + ... + \\ &+ \frac{1}{N!}\pi_l^{i_1 i_2 ... i_N} x_{s,i_1} x_{s,i_2} ... x_{s,i_N} + ... \end{aligned} \tag{13}$$

Similarly one expands the components of the vector functions $F_s(x_s, x_f)$, $F_f(x_s, x_f)$ in multivariate Taylor series. Substituting the Taylor expansions of $\pi_l(x_s)$ and $F_s(x_s, x_f)$, $F_f(x_s, x_f)$ into the system of PDEs (9) and matching the Taylor coefficients of the same order, the following relation for the N-th order terms may be obtained [15]:

$$\sum_{L=0}^{N-1} \sum_{\binom{N}{L}} \pi_l^{\mu i_1 \ldots i_L} F_{s,\mu}^{i_{L+1} \ldots i_N} = F_{f,l}^{\mu} \pi_{\mu}^{i_1 \ldots i_N} + f_l^{i_1 \ldots i_N}(\pi^{i_1 \ldots i_{N-1}}) \tag{14}$$

where $f_l^{i_1 \ldots i_N}(\pi^{i_1 \ldots i_{N-1}})$ is a function of Taylor coefficients of the unknown solution $\pi_l(x_s)$ calculated in the previous recursive steps. Note that the second summation symbol in (14) should be regarded as summing up the relevant quantities over the $\binom{N}{L}$ possible combinations of the indices $(i_1, ..., i_N)$. Furthermore, equations (14) represent a set of linear algebraic equations in the unknown coefficients $\pi_{\mu}^{i_1,\ldots,i_N}$, and this is the mathematical reason that allows the series solution method to be accomplished in an automated fashion by exploiting the computational capabilities and commands of a symbolic software package such as MAPLE. Finally, it should be also pointed out, that occasionally the Taylor series solution method for the invariance PDEs (9) exhibits slow convergence. In these cases, significant improvement of the convergence properties of the invariance PDE solution scheme can be achieved if direct Newton-type methods as described in [5, 10] are employed, or relaxation methods such as the ones reported in [5, 12].

In light of the above remarks, an important question naturally arises: Would the aforementioned invariant manifold-based approach to the problem of characterizing the biosystem's dynamic behavior still offer reliable results in the presence of model uncertainty, and therefore the associated properties be "robust" to modeling errors and uncertainty? Mathematically stated, under what conditions within the above framework, the biosystem dynamics is structurally stable and does not significantly deviate from the nominal one in the presence of perturbation terms representing model uncertainty/error? Before proceeding with the study of this particular problem, we present the following Lemma which is an extension of Gronwall-Bellman's inequality [17].

Lemma 2.1 *Let $y : [0, T] \to R$ be a continuous function satisfying the inequality shown below:*

$$y(t) \leq l + m \int_0^t y(s)ds + h(t) \tag{15}$$

for $0 \leq t \leq T$, where l, m are positive scalar constants and $h : [0, T] \to R$ a continuous function. Then, on the same interval, the following inequality holds true:

$$y(t) \leq l \exp(mt) + m \int_0^t \exp(m(t-s))h(s)ds + h(t) \tag{16}$$

Proof. Denote: $z(t) \equiv m \int_0^t y(s)ds$ and $v(t) \equiv z(t) + h(t) + l - y(t) \geq 0$. Notice that:

$$\frac{dz}{dt} = my(t) = mz(t) + m(h(t) + l - v(t)) \tag{17}$$

with $z(0) = 0$, and therefore:

$$z(t) = m \int_0^t \exp(m(t-s))(h(s) + l - v(s))ds \tag{18}$$

Since $v(t) \geq 0$, Equation (18) yields:

$$\begin{aligned} z(t) &\leq m \int_0^t \exp(m(t-s))(h(s) + l)ds \\ &= ml \int_0^t \exp(m(t-s))ds + m \int_0^t \exp(m(t-s))h(s)ds \\ &= l \exp(mt) - l + m \int_0^t \exp(m(t-s))h(s)ds \end{aligned} \tag{19}$$

or:

$$\begin{aligned} y(t) &\leq l + l \exp(mt) - l + m \int_0^t \exp(m(t-s))h(s)ds + h(t) \\ &= l \exp(mt) + m \int_0^t \exp(m(t-s))h(s)ds + h(t) \end{aligned} \tag{20}$$

thus completing the proof. □

The following theorem provides the analytical means to address the key question stated above, namely the characterization of the actual system's behavior in the presence of model uncertainty.

Theorem 2.3 *Let all assumptions of Theorem 2 be satisfied. Furthermore, let* Ω *(8) be a slow manifold of the "known" nominal system (4), where* $\pi(x_s)$ *is the unique locally analytic solution of the invariance PDEs (9). Then, in the presence of model uncertainty and for the perturbed system (6), the following holds true asymptotically as* $t \longrightarrow \infty$*:*

$$||x_f(t) - \pi(x_s(t))||_2 \approx_{t \to \infty} O(\epsilon_1, \epsilon_2) \tag{21}$$

Proof. Taking into account that: $\dfrac{\partial F_s}{\partial x_f}(0,0) = 0$, the dynamic equations of the original system (4) may be rewritten as follows:

$$\begin{aligned} \frac{dx_f}{dt} &= F_f(x_s, x_f) = A_f x_f + A_{fs} x_s + f(x_s, x_f) + \epsilon_1 G_f(x_s, x_f) \\ \frac{dx_s}{dt} &= F_s(x_s, x_f) = A_s x_s + g(x_s, x_f) + \epsilon_2 G_s(x_s, x_f) \end{aligned} \tag{22}$$

where A_f, A_s, A_{fs} are constant matrices with appropriate dimensions, and $f(x_s, x_f)$, $g(x_s, x_f)$ are real analytic functions of (x_s, x_f) with Taylor series expansions beginning with terms of degree greater than one: $\dfrac{\partial f}{\partial x_f}(0,0) = \dfrac{\partial f}{\partial x_s}(0,0) = \dfrac{\partial g}{\partial x_f}(0,0) = \dfrac{\partial g}{\partial x_s}(0,0) = 0$. Furthermore, in the nominal case (of unmodeled dynamics), the singular invariance PDEs (9) attain the following form:

$$\frac{\partial \pi}{\partial x_s}(A_s x_s + g(\pi(x_s), x_s)) = A_f \pi(x_s) + A_{fs} x_s + f(\pi(x_s), x_s) \tag{23}$$

Denote now by z the "off-manifold" coordinate:

$$z(t) = x_f(t) - \pi(x_s(t)) \tag{24}$$

whose dynamics is described by the following nonlinear differential equation:

$$\begin{aligned}
\frac{dz}{dt} &= \frac{dx_f}{dt} - \frac{\partial \pi}{\partial x_s}\frac{dx_s}{dt} = A_f x_f + A_{fs}x_s + f(x_f, x_s) + \epsilon_1 G_f(x_f, x_s) - \\
&- \frac{\partial \pi}{\partial x_s}(A_s x_s + g(x_f, x_s) + \epsilon_2 G_s(x_f, x_s)) \\
&= A_f(z + \pi(x_s)) + A_{fs}x_s + f(z + \pi(x_s), x_s) + \epsilon_1 G_f(z + \pi(x_s), x_s) - \\
&- \frac{\partial \pi}{\partial x_s}(A_s x_s + g(z + \pi(x_s), x_s) + \epsilon_2 G_s(x_f, x_s)) \\
&= A_f z + \{f(z + \pi(x_s), x_s) - f(\pi(x_s), x_s) + \frac{\partial \pi}{\partial x_s} g(\pi(x_s), x_s) - \\
&- \frac{\partial \pi}{\partial x_s} g(z + \pi(x_s), x_s)\} + \epsilon_1 G_f(z + \pi(x_s), x_s) - \\
&- \epsilon_2 \frac{\partial \pi}{\partial x_s} G_s(z + \pi(x_s), x_s) \\
&= A_f z + H(z, x_s) + \epsilon_1 h_1(z, x_s) + \epsilon_2 h_2(z, x_s) \qquad (25)
\end{aligned}$$

where:

$$H(z, x_s) = f(z + \pi(x_s), x_s) - f(\pi(x_s), x_s) + \frac{\partial \pi}{\partial x_s} g(\pi(x_s), x_s) - \frac{\partial \pi}{\partial x_s} g(z + \pi(x_s), x_s)$$

$$h_1(z, x_s) = G_f(z + \pi(x_s), x_s)$$

$$h_2(z, x_s) = -\frac{\partial \pi}{\partial x_s} G_s(z + \pi(x_s), x_s)$$

Notice that $H(z, x_s)$ is a real analytic vector function with: $H(0, 0) = \frac{\partial H}{\partial z}(0, 0) = 0$, and $||h_1(z, x_s)|| = ||G_f(z + \pi(x_s), x_s)|| \leq M_1 \equiv N_1$, $||h_2(z, x_s)|| = ||\frac{\partial \pi}{\partial x_s} G_s(z + \pi(x_s), x_s)|| \leq NM_2 \equiv N_2$, since $||\frac{\partial \pi}{\partial x_s}|| \leq N$ in the compact set X. Consequently, in the domain: $||z||_2 < \rho_1$, $||x_s||_2 < \rho_2$ the following inequality holds true:

$$||H(z, x_s)||_2 < L||z||_2 \qquad (26)$$

where the positive constant L can be made arbitrarily small by choosing ρ_1, ρ_2 small enough. Furthermore, since A_f is Hurwitz, there exist positive constants β, γ such that [18, 30]:

$$||\exp(A_f t)y||_2 \leq \gamma \exp(-\beta t)||y||_2 \qquad (27)$$

for all $y \in R^n$. From equation (25), one obtains:

$$\begin{aligned}
z(t) &= \exp(A_f t)z(0) + \int_0^t \exp(A_f(t - \tau))\{H(z(\tau), x_s(\tau)) + \\
&+ \epsilon_1 h_1(z(\tau), x_s(\tau)) + \epsilon_2 h_2(z(\tau), x_s(\tau))\}d\tau \qquad (28)
\end{aligned}$$

and therefore:

$$||z(t)||_2 \leq \gamma \exp(-\beta t)||z(0)||_2 + \int_0^t \gamma \exp(-\beta(t - \tau))\{L||z(\tau)||_2 + \epsilon_1 N_1 + \epsilon_2 N_2\}d\tau \qquad (29)$$

or:

$$\begin{aligned}\exp(\beta t)||z(t)||_2 &\leq \gamma||z(0)||_2 + \int_0^t \gamma L \exp(\beta\tau)||z(\tau)||_2 d\tau + \\ &+ \frac{\epsilon_1\gamma N_1}{\beta}(\exp(\beta t)-1) + \frac{\epsilon_2\gamma N_2}{\beta}(\exp(\beta t)-1) \qquad (30)\end{aligned}$$

Using Lemma 2.1 one obtains:

$$\begin{aligned}\exp(\beta t)||z(t)||_2 &\leq \gamma\exp(\gamma L t)||z(0)||_2 + \\ &+ \gamma L\int_0^t \exp(\gamma L(t-\tau))\{\frac{\epsilon_1\gamma N_1}{\beta}(\exp(\beta\tau)-1)\}d\tau + \\ &+ \gamma L\int_0^t \exp(\gamma L(t-\tau))\{\frac{\epsilon_2\gamma N_2}{\beta}(\exp(\beta\tau)-1)\}d\tau + \\ &+ \frac{\epsilon_1\gamma N_1}{\beta}(\exp(\beta t)-1) + \frac{\epsilon_2\gamma N_2}{\beta}(\exp(\beta t)-1) \Rightarrow \\ ||z(t)||_2 &\leq \gamma\exp(-(\beta-\gamma L)t)||z(0)||_2 + \\ &+ \gamma L\exp(-\beta t)\int_0^t \exp(\gamma L(t-\tau))\{\frac{\epsilon_1\gamma N_1}{\beta}(\exp(\beta\tau)-1)\}d\tau + \\ &+ \gamma L\exp(-\beta t)\int_0^t \exp(\gamma L(t-\tau))\{\frac{\epsilon_2\gamma N_2}{\beta}(\exp(\beta\tau)-1)\}d\tau + \\ &+ \frac{\epsilon_1\gamma N_1}{\beta}(1-\exp(-\beta t)) + \frac{\epsilon_2\gamma N_2}{\beta}(1-\exp(-\beta t)) \qquad (31)\end{aligned}$$

An analytical calculation of the integrals of the right-hand side of (31) leads to:

$$\begin{aligned}||z(t)||_2 &\leq \gamma\exp(-(\beta-\gamma L)t)||z(0)||_2 + \\ &+ \frac{\epsilon_1\gamma^2 L N_1}{\beta}\{\frac{1}{(\beta-\gamma L)}(1-exp(-(\beta-\gamma L)t)) + \\ &+ \frac{1}{\gamma L}(\exp(-\beta t)-exp(-(\beta-\gamma L)t))\} + \frac{\epsilon_1\gamma N_1}{\beta}(1-\exp(-\beta t)) + \\ &+ \frac{\epsilon_2\gamma^2 L N_2}{\beta}\{\frac{1}{(\beta-\gamma L)}(1-exp(-(\beta-\gamma L)t)) + \\ &+ \frac{1}{\gamma L}(\exp(-\beta t)-exp(-(\beta-\gamma L)t))\} + \frac{\epsilon_2\gamma N_2}{\beta}(1-\exp(-\beta t)) \qquad (32)\end{aligned}$$

Since L can be made arbitrarily small, let us denote: $K = \beta - \gamma L > 0$ and also let $t \longrightarrow \infty$. The above inequality yields:

$$\begin{aligned}||z(t)||_2 &\leq \frac{\epsilon_1\gamma^2 L N_1}{\beta K} + \frac{\epsilon_1\gamma N_1}{\beta} + \\ &+ \frac{\epsilon_2\gamma^2 L N_2}{\beta K} + \frac{\epsilon_2\gamma N_2}{\beta} \Longrightarrow \\ ||z(t)||_2 &\leq \frac{\epsilon_1\gamma N_1}{\beta}(\frac{\gamma L}{K}+1) + \frac{\epsilon_2\gamma N_2}{\beta}(\frac{\gamma L}{K}+1) \qquad (33)\end{aligned}$$

and the proof is complete. □

On the basis of result (33) the following remarks can be made:

i) In the absence of model uncertainty: $G_s(x_s, x_f) = G_f(x_s, x_f) \equiv 0$, Theorem 2.3 naturally reproduces the result of Theorem 2.2. In this case, Ω represents exactly

the system's slow invariant manifold exponentially attracting all system trajectories once the fast transients die out, and the biosystem dynamics ultimately evolves on Ω until it reaches the reference equilibrium point.

ii) Notice that due to the presence of the model uncertainty terms $G_s(x_s, x_f)$, $G_f(x_s, x_f)$ the off-manifold coordinate $z(t) = x_f(t) - \pi(x_s(t))$ does not converge to zero asymptotically (even under "zero initial condition", i.e. starting on the slow manifold of the nominal system (4) : $x_f(0) = \pi(x_s(0))$). However, as can be easily inferred from (21) or (33), the inevitable offset from Ω is of order $O(\epsilon_1, \epsilon_2)$. Equivalently stated, the distance from the nominal system's slow manifold will be ultimately bounded if the perturbation or model uncertainty term is itself bounded. In particular, results (21) or (33) derived as bounds (in an asymptotic sense) on the off-manifold coordinate $z(t) = x_f(t) - \pi(x_s(t))$, and therefore a measure of boundedness of the distance from Ω, suggest that it is directly proportional to the magnitude of the model uncertainty terms.

iii) The above results suggest that the slow manifold-based analysis is capable of providing an accurate and robust quantitative characterization of the biosystem's dynamic behavior in the presence of suitably modeled and rather broad classes of modeling errors and/or model uncertainties. The associated issues will be illustrated in the next Section's bioreactor examples.

3. **Illustrative examples of immobilized enzyme bioreactors.** The use of immobilized cell and enzyme bioreactors in the food, pharmaceutical and nutraceutical industries, as well as in the field of medicine is rather wide. Examples of such processes for food or nutraceutical production include the production of flavor volatiles such hexanal from linoelic acid using the immobilized enzymes lipoxygenase and hydroperoxide lyase [4], and the production of the food grade linoleic acid from corn oil using immobilized lipase [26]. Furthermore, the number of biomedical applications in which immobilized enzyme or cell bioreactors assume a key role is growing. Notable examples include implantable bioreactors consisting of immobilized phospholipase A_2 that have been proposed for the reduction of serum levels of low density lipoprotein [27], and immobilized hepatocyte bioreactors that have been examined for use as extracorporeal bioartificial livers, and have been shown to be quite effective at clearing harmful toxins from a medium (blood) stream [31]. It should be pointed out that in all the above biosystems/bioprocesses, the short term behavior of the bioreactor depends primarily on the (typically nonlinear) kinetics of the immobilized enzymes or cells participating in the associated (bio)chemical reaction schemes. However, the long term behavior of the bioreactors depends on either the stability of the immobilized enzymes or the viability of the immobilized cells employed in specific applications. It should be also emphasized that while the short term behavior of these systems is important in determining the conversion of a nutraceutical or degradation of a toxin (parameters associated with bioreactor performance), the long term behavior of the bioreactor typically determines when the enzyme or cell catalyst needs to be replaced in order to maintain conversions at acceptable levels. Therefore, being able to accurately estimate bioreactor performance degradation over time (below the acceptable levels) has important implications and consequences for the health/safety of a patient as well as the profitability of the bioprocess.

Actual kinetic data on enzyme performance and enzyme degradation are considered in the present study for an immobilized enzyme bioreactor that is used for

the production of food grade linoleic acid from corn oil [26]. In the case study considered, we assume that the enzymatic bioreactor behaves as an ideal continuous stirred tank reactor (CSTR). It is also assumed that the enzyme involved converts substrate into product, in this case corn oil into linoleic acid, via a ping-pong bi bi mechanism, as reported in [26]. Under a set of standard assumptions, the following nonlinear dynamic process model can be developed:

$$\begin{aligned} \frac{dS}{dt} &= f^{(1)}(S,E) = \frac{k_1 ES}{1-k_2 S} + \frac{v_0}{V}(S_0 - S) \\ \frac{dE}{dt} &= g^{(1)}(E) = -k_{d1} E \end{aligned} \tag{34}$$

The above dynamic equations describe the change in substrate concentration in the reactor as a function of time, and the degradation of activity of the enzyme. S, S_0 and E represent the concentrations of substrate, substrate in the feed stream and enzyme respectively. k_1, k_2 represent kinetic parameters describing the rate of the enzymatic reaction and k_{d1} is a kinetic parameter describing the rate of deactivation of the enzyme. v_0 is the flow rate of the substrate and V is the reactor volume. In Table 1, kinetic parameters used in the example, as well as initial substrate and enzyme concentrations are provided. It is worth mentioning that under these parameter values, the above bioreactor dynamics is characterized by a latent two-time-scale multiplicity attributed to the slow degradation of the enzyme when compared to the much faster bioprocess dynamics. Let us now assume that the value of the k_1 kinetic constant is not fully known, but uncertain. It is assumed that: $k_1 = k_1^0 + \epsilon\Delta k$, where $k_1^0 = 8.2E-2$ $(h^{-1}g^{-1})$ is a known nominal value and $\epsilon\Delta k$ with $|\Delta k| < M$ represents a bounded term reflecting the uncertainty that characterizes the numerical value of k_1, and $\epsilon > 0$ being a small positive constant. Given the structure of the above dynamic model (34), it can be easily deduced that the perturbation term is given by the following expression:

$$h(S,E) = \epsilon \frac{(\Delta k) ES}{1-k_2 S} \tag{35}$$

Furthermore, it can be easily proven that the nominal system is asymptotically stable around the equilibrium point of interest $(S^0, E^0) = (3.4, 0)$ using standard Lyapunov stability arguments, and that the above perturbation term is indeed bounded. In order to conform to the theory presented in the previous Section, the following set of deviation variables relative to the equilibrium point $(S^0, E^0) = (3.4, 0)$ are introduced:

$$\begin{aligned} x &= S - S^0 \\ w &= E - E^0 \end{aligned} \tag{36}$$

Let us also denote: $\bar{F}^{(1)}(x,w) = F^{(1)}(x+S^0, w+E^0)$, $\bar{G}^{(1)}(w) = G^{(1)}(w+E^0)$. Notice that for the bioreactor model (34) all conditions of Theorems 2.1, 2.2 and 2.3 are satisfied. Therefore, for the nominal bioreactor model there exists a unique and locally analytic slow manifold: $x = \pi(w)$, with $\pi(w)$ being the solution to the following invariance equation:

$$\begin{aligned} \frac{\partial \pi}{\partial w} \bar{G}^{(1)}(w) &= \bar{F}^{(1)}(\pi(w), w) \\ \pi(0) &= 0 \end{aligned} \tag{37}$$

A series solution to the above invariance equation is sought around the origin. The Taylor coefficients of the unknown solution $x = \pi(w)$ can be automatically computed using a simple MAPLE code. A finite-order series truncation N is considered leading to a Taylor polynomial approximation $x = \pi^{[N]}(w)$ of the actual solution of the invariance equation (37). In particular, with the aid of the aforementioned MAPLE code a 5-th order series truncation was considered: $N = 5$. In the absence of uncertainty $\epsilon = 0$ and for $N = 5$, Figure 1 depicting the phaseportrait of the bioreactor dynamics suggests that the actual approach to the slow invariant manifold (solid line) can be quite satisfactorily approximated by the computed one (dotted line) obtained using the proposed series solution method. If one increases the truncation order to $N = 8$, they become almost indistinguishable as demonstrated in the phaseportrait of Figure 2. This is certainly not surprising since it follows from the uniform convergence of the series solution of the invariance equation (37). Please notice the underlying two-time-scale multiplicity that manifests itself quite explicitly in Figures 1 and 2 and how a familiar dynamic pattern naturally emerges: the transition of the system from the initial state to the slow manifold is depicted through the vertical constant-E lines since the enzymatic concentration remains practically unchanged due to the much slower enzymatic dynamics, while the substrate concentration changes rather rapidly until the system reaches the slow manifold, upon which the bioreactor dynamics is bound to evolve (for large times). In the presence of model uncertainty: $\epsilon\Delta k = 0.002$ and in the case of $N = 8$, one can observe in Figure 3 the inevitable offset and deviation from the system's actual approach to the slow manifold (solid line) exhibited by the one computed on the basis of the nominal system (dotted line) and through the solution of the invariance equation (37). As uncertainty increases: $\epsilon\Delta k = 0.0045$, $N = 8$, and in agreement with the theoretical results obtained in the previous Section, the above deviation becomes more pronounced as shown in Figure 4 and the distance/offset from the system's actual slow manifold becomes larger.

Let us now increase the degree of complexity of the example considered by including a second enzymatic step in the associated bioprocess and use representative kinetic parameters for the second enzyme. In particular, we assume that the second enzyme converts substrate to product via a Michaelis-Menten mechanism, and both enzymes degrade via a first order decomposition process [16]. Under these assumptions the following nonlinear dynamic process model can be developed:

$$\begin{aligned}
\frac{dS}{dt} &= F^{(1)}(S, E_1, E_2) = \frac{k_1 E_1 S}{1 - k_2 S} + \frac{k_3 E_2 S}{K_{M_2} + S} + \frac{v_0}{V}(S_0 - S) \\
\frac{dE_1}{dt} &= G^{(1)}(E_1, E_2) = -k_{d1} E_1 \\
\frac{dE_2}{dt} &= G^{(2)}(E_1, E_2) = -k_{d2} E_2
\end{aligned} \tag{38}$$

As before, the above dynamic equations describe the change in substrate concentration in the reactor as a function of time, and the degradation of activity of the two enzymes. S, S_0, E_1 and E_2 represent the concentrations of substrate, substrate in the feed stream, enzyme one, and enzyme two respectively. k_1, k_2, k_3, and K_{M_2} represent kinetic parameters describing the rates of reaction of enzyme one and two, and k_{d1} and k_{d2} are kinetic parameters describing the rate of deactivation of enzymes one and two respectively. Table 2 contains the new set of kinetic parameter values, as well as initial substrate and enzyme concentrations. Let us also assume

that the value of the k_1 kinetic constant is, as in the previous case, not fully known, but uncertain: $k_1 = k_1^0 + \epsilon\Delta k$, where $k_1^0 = 8.2E-2$ $(h^{-1}g^{-1})$ is a known nominal value and $\epsilon\Delta k$ with $|\Delta k| < M$ represents the bounded uncertainty term. Given the structure of the above dynamic process model (38), it can be easily inferred that the perturbation term is the same as before: $h(S,E) = \epsilon\dfrac{(\Delta k)ES}{1-k_2S}$. The following set of deviation variables relative to the equilibrium point $(S^0, E_1^0, E_2^0) = (3.4, 0, 0,)$ is now introduced:

$$\begin{aligned} x_1 &= S - S^0 \\ w_1 &= E_1 - E_1^0 \\ w_2 &= E_2 - E_2^0 \end{aligned} \tag{39}$$

Furthermore, let us also denote: $\bar{F}^{(1)}(x_1, w_1, w_2) = F^{(1)}(x_1 + S^0, w_1 + E_1^0, w_2 + E_2^0)$, $\bar{G}^{(i)}(w_1, w_2) = G^{(i)}(w_1 + E_1^0, w_2 + E_2^0)$ $(i = 1, 2)$. Notice that for the bioreactor system (38) all conditions of Theorems 2.1, 2.2 and 2.3 are satisfied, and therefore, there exists a unique and locally analytic slow manifold: $x_1 = \pi(w_1, w_2)$, with $\pi(w_1, w_2)$ being the solution to the following singular PDE (invariance equation):

$$\begin{aligned} \frac{\partial\pi}{\partial w_1}\bar{G}^{(1)}(w_1,w_2) + \frac{\partial\pi}{\partial w_2}\bar{G}^{(2)}(w_1,w_2) &= \bar{F}^{(1)}(\pi(w_1,w_2),w_1,w_2) \\ \pi(0,0) &= 0 \end{aligned} \tag{40}$$

A series solution to the above singular PDE is sought around the origin, and the Taylor coefficients of the unknown solution $x_1 = \pi(w_1, w_2)$ are computed by using a MAPLE code. We computed both the actual response of the bioreactor by simulating the full model (38) in both the nominal and perturbed cases , as well as the long term asymptotic behavior of the bioreactor, i.e. the on-manifold dynamics using the slow-manifold approach by solving the invariance PDE (40) with $N = 3$. As it can be seen in Figure 5, the estimated substrate concentration profile (dotted line) at the outlet of the reactor obtained through the reduced-order dynamics on the slow manifold (calculated for $N = 3$) becomes indistinguishable from the actual substrate concentration profile (solid line) in the nominal case ($\epsilon = 0$) at times close to 100h, the approximate half life of the fastest decaying enzyme. In Figure 6, the estimated substrate concentration profile (depicted by the dotted line and computed using the invariance PDE in the "known" nominal case) is compared with the actual one (solid one) in the presence of uncertainty: $\epsilon\Delta k = 0.002$. One observes, that there is now a slight discrepancy or offset between the asymptotic behavior estimate based on the on-manifold dynamics and the system's actual behavior for large times. However, under the given uncertainty, the asymptotic behavior estimate remains satisfactorily close to the actual bioprocess performance at times much shorter than the half life of the fastest decaying enzyme, a very useful feature from a bioprosess monitoring standpoint. Indeed, in this example, the knowledge of when substrate conversion drops below acceptable values is certainly of importance in the profitability of the process. Furthermore, the above analysis yields estimates of drop off in bioreactor performance that are superior to intuition or "engineering judgment" based on knowledge of the half lives of enzymes in the bioreactor. Please notice that when the bioreactor serves as an artificial organ or is implanted, the benefit to understanding how bioreactor performance declines with time has real implications for the health of the patient. Within this context, the

above analysis provides a fairly accurate method for estimating the long term decline in the performance of the artificial organ, and could be used in scheduling implant replacement prior to artificial organ failure and the associated decline in health. Finally, as expected, for a larger uncertainty magnitude: $\epsilon\Delta k = 0.0045$, the discrepancy between the nominal invariant manifold-based susbstrate concentration profile estimate and the actual one becomes more pronounced as shown in Figure 7.

4. **Concluding remarks.** A new approach to the problem of characterizing the dynamic behavior of nonlinear biosystems in the presence of model uncertainty using the notion of slow invariant manifold was presented. The problem of interest was formulated and addressed within the context of singular partial differential equations (PDE) theory, and in particular, through a system of singular first-order quasi-linear invariance partial differential equations (PDEs). Within the class of analytic solutions, a set of conditions was derived that guarantees the existence and uniqueness of a locally analytic solution which was proven to represent the slow invariant manifold of the nonlinear biosystem under consideration exponentially attracting all dynamic trajectories in the absence of model uncertainty. Under these conditions, an exact reduced-order model for the nonlinear biosystem dynamics can be obtained through the restriction of the original system dynamics on the slow manifold. The aforementioned attractivity property and the fast transition towards the above manifold was then analyzed and characterized in the presence of model uncertainty. Finally, features of the proposed framework of analysis are illustrated in examples involving certain types of immobilized enzyme bioreactors.

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TABLE 1: Example I - Kinetic and Bioreactor Parameter Values

Process parameters	Values
S_0	3.4 M
$S(t=0)$	3 M
$E(t=0)$	4 g
V	50 ml
v_0	100 ml/h
k_1	8.2E-2 $h^{-1}g^{-1}$
k_2	5.9E-1 M^{-1}
k_{d1}	3.4E-3 h^{-1}

TABLE 2: Example II - Kinetic and Bioreactor Parameter Values

Process parameters	Values
S_0	3.4 M
$S(t=0)$	3 M
$E_1(t=0)$	4 g
$E_2(t=0)$	1 g
V	50 ml
v_0	100 ml/h
k_1	8.2E-2 $h^{-1}g^{-1}$
k_2	5.9E-1 M^{-1}
k_3	3.0E-1 $Mh^{-1}g^{-1}$
k_{M_2}	8 M
k_{d1}	3.4E-3 h^{-1}
k_{d2}	5.0E-4 h^{-1}

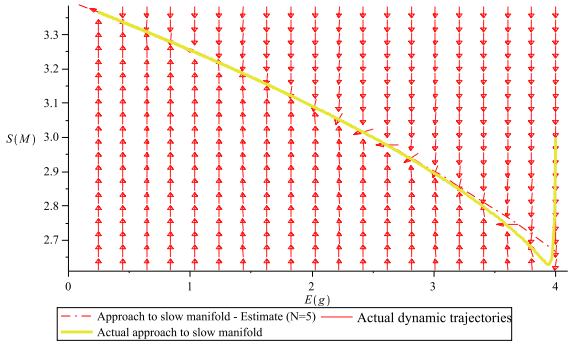

FIGURE 1. Example I: Approach to slow manifold (N=5 and no uncertainty)

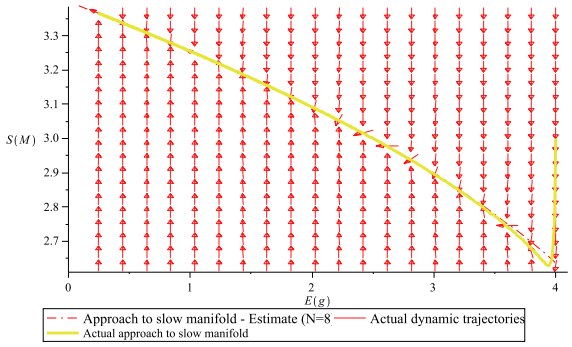

FIGURE 2. Example I: Approach to slow manifold (N=8 and no uncertainty)

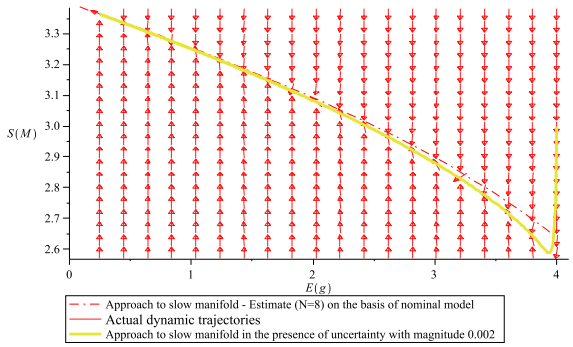

FIGURE 3. Example I: Approach to slow manifold (N=8 and uncertainty 0.002)

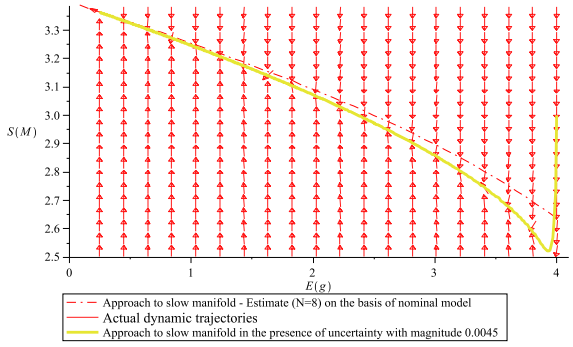

FIGURE 4. Example I: Approach to slow manifold (N=8 and uncertainty 0.0045)

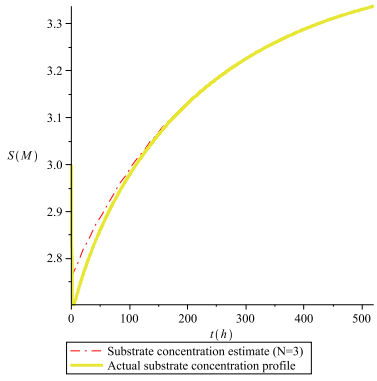

FIGURE 5. Example II: Substrate concentration profile (N=3 and no uncertainty)

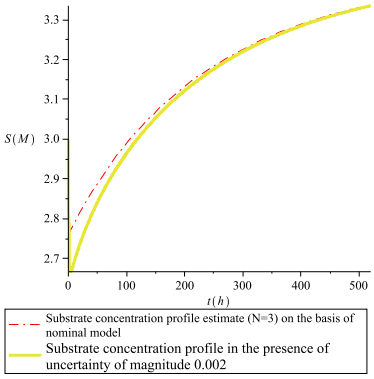

FIGURE 6. Example II: Substrate concentration profile (N=3 and uncertainty 0.002)

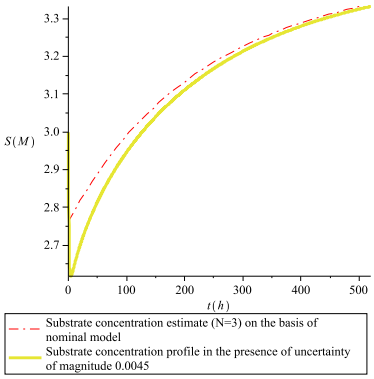

FIGURE 7. Example II: Substrate concentration profile (N=3 and uncertainty 0.0045)

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