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Abstract. This study presents an asymptotic stability analysis of a model of a bioreactor converting carbon monoxide (CO) gas into ethanol through a *C. autoethanogenum* biocatalyst. The configuration is a bubble column reactor with co-current gas-liquid flows where gas feed is introduced by a gas distributor placed at the bottom of the column. A pure culture of *C. autoethanogenum* is subsequently injected at the bottom of the column; therein, cells are dispersed in the liquid and consume the dissolved gas and release by-products such as ethanol and acetic acid. Cellular growth and byproduct secretion are affected by spatially varying dissolved gas concentrations due to advection-diffusion mass transports which are induced by the effect of the injection pressure and gravitational force. The model accounts for four species representing the biomass, the CO substrate in the liquid phase, and two by-products - ethanol and acetic acid. Substrate dynamics is described by an advection-diffusion equation.

We investigate the asymptotic stability of the biomass dynamics that is a requirement for the system's controllability, i.e. for the possibility to steer a dynamical system from an arbitrary initial state to an arbitrary final state using a set of controls. The concept of stability of the controls is extremely relevant to controllability since almost every workable control system is designed to be stable. If a control system is not stable, it is usually of no use in practice in industrial processes. In the case of a bioreactor, the control is the biomass and controllability is the possibility of modulating through this control the ethanol production. We present a test for asymptotic stability, based on the analysis of the properties of the dynamic function defining its role as storage function.

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1. Introduction

Biomanufacturing is a growing trend across countless sectors such as the production of commodities in the chemical industry [1], the production of high-value biologics in the healthcare sector [2, 3], and the wastewater treatment in the environmental protection sector [4, 5]. Albeit attracting increasing attention, biomanufacturing is confronted with several challenges. Consistency in the performances of bioreactors, which are the operation units hosting the biotechnological production processes, is determined by multiple factors, which encompass not only productivity but also process and thus product quality [6]. Industrial control systems largely depend on expensive process analytical technologies with integrated feedback mechanisms to ensure near real-time consistent and adequate process supervision, fault detection and correction during manufacturing [7, 8, 9]. It is advisable to design the control process as efficient as possible and to reduce the scope of application of near real-time process analytical technology. Assessing *a priori* the controllability of the process and/or designing the controllability itself offer solid instruments for this purpose.

Controllability is strongly related to asymptotic stability [10, 11, 12]. Although asymptotic stability does not imply controllability, asymptotic stability (an in particular global asymptotic stability) of internal dynamics is a property of the system that can be exploited to implement control procedures over the system. Recently, asymptotic stability property has in fact been exploited to implement control procedures for micro-algae growth in chemostat [13]. A theoretical study of asymptotic stability has recently been carried out also on ecological models for biodegradation of toxic substances in aquatic and atmospheric biotic systems [14]. Mathematical analysis in these contexts is useful to outline the parameters, or to design or to implement *a priori* control of parameters domain ensuring stable operations of microbial processes in a continuous mode. The property of asymptotic stability of the equilibria of the *control variable* is so important for the purpose of controllability design that, if it is not inherently present in the physical process, it is induced, for example through delayed feedback mechanisms, as also demonstrated by the work of Borisov et al. [15], which is focused on an anaerobic biological treatment of organic wastes in a continuously stirred tank bioreactor. The authors proposed an output feedback including a discrete delay for the asymptotic stabilization of the model. They motivated their study by pointing out that, in operating a plant, feedback control of bioreactor models provides many advantages among which the most important is the increment of the efficiency. Along this line of thought, we found very recent works such as that of Dimitrova et al. [16], which studies the asymptotic stability and bifurcations of a model for phenol and p-cresol mixture degradation in a continuously stirred bioreactor with respect to a practically important parameter.

Almost all studies of stability, stabilizability, and control of physical processes use the theoretical basis and computational models of Lyapunov theory [17]. The application of this theory to control is mainly based on the Lyapunov stability theorem. There are two Lyapunov methods for testing stability, of which the most popular one is the Direct Method of Lyapunov (DML). According to this method, the stability of an equilibrium point $\overline{x}$ of a dynamical system in the form $\dot{x} = f(x)$, where $f : D \subseteq \mathbb{R}^n_+ \longrightarrow \mathbb{R}$, and D is an open set, requires the flow associated with the dynamical system being decreased on a function $V : D \subseteq \mathbb{R}^n_+ \longrightarrow \mathbb{R}$ for which $\overline{x}$ is an isolated minimum. This function is known as *Lyapunov function*.

It is well known that the main drawback of the DML is the lack of a systematic method to find a Lyapunov function. The common approach consists in proposing a function and then checking if this candidate satisfies the conditions to be a valid Lyapunov functions. We note that the Lyapunov function is a special case of the storage function [18] and, with respect to the traditional error-and-trial search methodology for this function, we note that the function f can be tested for being a valid storage function. From Megretski and Megretski co-authored scientific, perspective, editorial and didactical works [19, 20, 21, 18], as well as from the results of

Mondragón et al. [22], we formulated a theorem stating the sufficient conditions that a storage function $f : D \subseteq \mathbb{R}^n_+ \longrightarrow \mathbb{R}^n$ has to satisfy in order for an equilibrium point $\overline{x}$ solution of the non-linear system $\dot{x} = f(x)$ to be asymptotically stable. According to our theorem, if f is a storage function, the Hessian matrix of f, Hf evaluated at the equilibrium point, is positive definite, and $\partial f/\partial x < 0$, $x \in D$, then the equilibrium point $\overline{x}$ is asymptotically stable. In order to be a valid storage functions f has to satisfy five conditions: (i) D has to be open, (ii) f has to be continuously differentiable, (iii) the supply rate $\sigma(x)$ has to be continuous, (iv) V has to be continuously differentiable, and (v) the dissipation inequality $\nabla V \dot{f}(x) \leq \sigma(x)$ has to be true.

The theorem offers a test for the asymptotic stability of equilibria and can be carried out numerically, but it is advisable that the test implementation avoids sources of numerical instability, especially those due to numerical integration procedures. We show the application of the theorem to the advection-diffusion model of a bubble column bioreactor where the control variable is represented by the biomass of the biocatalyst which operates the biological capture and conversion of carbon monoxide into by-products such as acetate and ethanol.

The paper is organized as follows: Section 2 reviews the Lyapunov stability analysis, reports the relevant literature, highlights the limitations and presents our theorem; Section 3 describes the bubble columns bioreactor of our case study; Section 4 summarizes the advection-diffusion model for this type of bioreaction, that we developed in a previous study [23], and the results of the computational test implementing the theorem of asymptotic stability applied to the advection-diffusion equation for the biomass. Finally, Section 5 draws some conclusions.

2. Stability analysis

The trajectories of a non-linear dynamical system of the form

$$\dot{x} = f(t, x) \tag{1}$$

where $f : D \to \mathbb{R}^N$ is a $\mathcal{C}^n$ is a map and $D \subset \mathbb{R}^n$ is an open set, are *asymptotically stable* if nearby solutions not only remain that way but also may converge to the equilibrium.

According the *Direct Method of Lyapunov* (DML) an equilibrium point $\overline{x}$ is said *stable* if the flow associated with the dynamical system (1) is decreased on some scalar function V for which $\overline{x}$ is an isolated minimum. This function is known as *Lyapunov function*, and the following is the Lyapunov stability theorem [24].

Theorem 1. *Let $\overline{x} \in D$ be an equilibrium point of (1). Let $V : B \to \mathbb{R}$ be a continuous function defined on a neighbourhood $B \subset D$ of $\overline{x}$, differentiable on $B - \overline{x}$, such that*

(i) $V(\overline{x}) = 0$ and $V(x) > 0$ if $x \neq \overline{x}$

(ii) the orbital derivative $\dot{V} : D \subseteq \mathbb{R}^n \to \mathbb{R}$ defined as

$$\dot{V} := \frac{\partial V}{\partial t}(t, x) + \frac{\partial V}{\partial x}(t, x) \cdot f(t, x)$$

is $\dot{V}(x) \leq 0$ in $B - \overline{x}$

then $\overline{x}$ is stable.

Furthermore, if $\dot{V}(x) < 0$ in $B - \overline{x}$, then $\overline{x}$ is asymptotically stable.

The DML has been the principal tool to analyse global stability of dynamical systems applied to basic sciences and engineering in the twentieth century [25]. If it is possible to find a Lyapunov function for a dynamic system operating around a state $\overline{x}$, then $\overline{x}$ is a stable state that is approached asymptotically. The main issue of the DML is to find a suitable Lyapunov function [26], as there is not a systematic method for finding. Commonly, finding a Lyapunov function

is a artisanal work, i.e. a function is proposed and then it is checked whether it satisfies the stability conditions, as suggested by Perko [27].

We note that Lyapunov functions are special cases of *storage* functions [18]. Here we adopt a notation similar to that of Megretski [18] and consider a system whose dynamics is defined by the behaviour set $Z = \{z\}$ of functions $z : [0, \infty) \to \mathbb{R}^p$, i.e. the elements of Z are functions returning all possible outputs for autonomous systems, and all possible input/output pairs for systems with an input [18]. Consider the case in which $z(t) = [v_i(t), v_o(t)]$ where $v_i(t)$ is the input and $v_o(t)$ the output. Assuming that the composition $\sigma \circ z$ (where $\sigma : \mathbb{R}^p \to \mathbb{R}$) is integrable over every interval $(t_1, t_2) \in \mathbb{R}^+$, $\forall z \in Z$, we can calculate the integral

$$I(z, t_1, t) = \int_{t_1}^{t} \sigma(z(\tau))d\tau.$$

Megretski [18] proved that the following conditions are equivalent

(a) $\forall z_0 \in Z$ and $t_1 \in \mathbb{R}^+$, the set of values of $I(z, t_1, t)$, taken for all $t \geq t_0$ and for all $z \in Z$ defining the same state as z_0 at time t_0, has a lower bound

(b) there exist a non-negative storage function $V_s : Z \times \mathbb{R}^+ \to \mathbb{R}^+$ (with supply rate σ) such that

$$V_s(z_1, t) = V_s(z_2, t)$$

whenever z_1 an z_2 define same state of Z at time t.

Moreover, when condition (a) is satisfied, a storage function V from (b) can be defined by

$$V_s(z_0(\cdot), t_0) = -\inf I(z, t_0, t) \tag{2}$$

where the lower extreme is taken over all $t \geq t_0$ and over all $z \in Z$ defining same state as z_0 at time t_0.

The equivalence between conditions (a) and (b) has an important consequence for the existence of a storage function for the solution of an ordinary differential equation model. Consider a model

$$\dot{x}(t) = f(x(t), w(t)) \tag{3}$$

where function $f : X \times W \to \mathbb{R}^n$, $X \subset \mathbb{R}^n$ and $W \subset \mathbb{R}^m$. Here, for the sake of simplicity, and without loss of generality, and following Megretski [18], we consider a function f whose domain has only two components. Then consider all functions $z(t) = [x(t); w(t)]$ where $x : [0, \infty) \to X$ is a solution of Eq. (3). In this case, two signals $z_1 = [x_1; w_1]$ and $z_2 = [x_2; w_2]$ define same state of Z at time t_0 if and only if $x_1(t_0) = x_2(t_0)$. For a given supply rate $\sigma : X \times W \to R$, stating the existence of a storage function $V_s : X \times \mathbb{R}^+ \to \mathbb{R}^+$ such that

$$V_s(x(t_2), t_2) - V_s(x(t_1), t_1) \leq \int_{t_1}^{t_2} \sigma(x(t), w(t))dt \tag{4}$$

is equivalent to stating that the lower extreme of the integrals

$$\int_{t_0}^{t} \sigma(x(\tau), w(\tau))d\tau \tag{5}$$

fits over all solutions of (3) with a fixed $x(t_0) = \overline{x}_0$ which can be extended to the time interval $[0, \infty)$. Note that when $\sigma = 0$ the storage function becomes a Lyapunov function [18]. In Appendix A, we report the version of Eq. (4) for a discrete system.

In several applications σ is a function comparing the instantaneous values of input and output [18], e.g.

$$\sigma(z(t)) = |v_o(t)|^2 - |v_i(t)|^2.$$

The search for the storage function (and the Lyapunov function) is not an easy task. However, in dynamic models, the function f is a possible candidate storage function. The following proposition lists the necessary and sufficient conditions for a function $f : X \times W \to \mathbb{R}^n$ to be a storage function [18].

Proposition 1. *A function $f : X \times W \to \mathbb{R}^n$ is a storage function iff*

(i) *X is an open set*
(ii) *f is continuously differentiable*
(iii) *σ is continuous*
(iv) *V is continuously differentiable*
(v) *this inequality holds*

$$\nabla V \cdot f(\overline{x}, \overline{w}) \leq \sigma(\overline{x}, \overline{w}). \tag{6}$$

Proof. See [18, 28, 29] for a proof and for a description of the properties of a storage function. □

The set of storage functions for a controllable system is a convex bounded polytope [30]. For an uncontrollable system the set of storage functions is unbounded because the set of Lyapunov functions for the autonomous linearized system is unbounded. The stabilizability of a system results in this unbounded set becoming bounded from below. Storage function is related to the energy stored by the system. It is easy to understand the concept of storage function in dissipative systems. In such a system, the energy depends on the supply rate and a storage function [31]. The supply rate is the rate at which energy flows into the system, and a storage function is a function measuring the amount of energy stored inside the system at any time. These functions are related via the dissipation inequality, which states that, along time trajectories of the dynamical system, the supply rate is bigger than the increase in storage [32], i.e.

$$\frac{\partial V}{\partial t} \leq \sigma(x(\tau), w(\tau)). \tag{7}$$

The dissipation inequality states that a system cannot store more energy than is supplied to it from the outside, and is equivalent to inequality (6) since

$$\frac{\partial V}{\partial t} = \nabla V \cdot f(\overline{x}, \overline{w}).$$

The difference between the supplied and the internally stored energy is the dissipated energy. When a storage function is positive definite the maximum stored energy that can be drawn out is bounded [30, 33]. In order to test the asymptotic stability of an equilibrium, once it is possible to prove that the function f in Eq. (3) is a valid storage function, all that remains is to check if this function is positive definite. The positive definiteness of the storage function is a requirement for the global asymptotic stability of the equilibrium. We then state the following theorem.

Theorem 2. *Let $\overline{x} \in X$ be an equilibrium solution of non-linear system (3). If*

(i) *the Hessian matrix of $f : X \times W \to \mathbb{R}^n$ at $x = \overline{x}$, $Hf(\overline{x})$ is positive definite*
(ii) *f is a storage function with supply rate σ*
(iii) *$\frac{\partial f}{\partial x_i} < 0$ for $i = 1, 2, , \ldots, n$*

then $\overline{x}$ is globally asymptotically stable.

Proof. Since the Hessian matrix of f at $x = \overline{x}$ is positive definite (hypothesis (i)), then $\overline{x}$ is a relative minimum. Since $f : X \times W \to \mathbb{R}^n$ is a storage function with supply rate σ (hypothesis (ii)), then, according to Proposition 1, X is an open set, and f satisfies the dissipation inequality (6) (or equivalently the dissipation inequality (7)), stating the existence of a Lyapunov function V, such that $-\dot{V}(x)$ is positive definite. Consequently, since by hypothesis (i) $Hf(\overline{\overline{x}})$ is positively definite, then also the the Hessian matrix of the product $-\dot{V}(\overline{x}) \cdot f(\overline{x})$ is positive definite. Given the hypothesis (iii), by the Corollary 4 of Theorem 3 of Mondragón et al. [22] (see also the Appendix B), we then have that $\overline{x}$ is globally asymptotically stable. □

The Theorem 2 has a straightforward generalization to any function $f : D \subseteq \mathbb{R}^n \to \mathbb{R}$.

3. The bubble column bioreactor

Gas fermentation by means of autotrophic acetogenic bacteria is a technologically appealing option to valorize single-carbon (C1) gas resulting from manifold industrial processes into chemicals [34, 35, 36, 37]. One of the most challenging problems in gas fermentation is to establish culture conditions which offer favourable gas-liquid mass transfer characteristics such that the gas components are readily dissolved in the medium and thus available for microbial conversion. The key objective of bioreactor design is thus to provide a high gas-liquid mass transfer efficiency of gas into the fermentation broth, while allowing process scale-up and low operational costs [38]. Commercial development efforts are currently focused on bubble column reactors due to the low specific power input per gas-liquid mass transfer unit, operational flexibility, scalability, and low maintenance and operational costs [39, 40]. Bubble column reactors, in their simplest form, are vertically arranged, cylindrical, stainless steel vessels, filled with a low viscosity liquid. Gas is injected into the liquid by means of a distributor (sparger), placed at the bottom of the column. Although bacterial cells would constitute a third phase within the bioreactor, they are considered to occupy a negligible volume and to be homogeneously distributed within the liquid phase. The mechanisms at the core of bubble column reactors functionality are gas bubbles' formation, bubbles' rise and resulting circulation patterns. The fluid dynamics of a bubble column reactor, in the absence of any draught apparatus, is controlled mainly by the gas flow. The gaseous substrate dynamics, which results from the compound intervention of gas injection thrust and hydrostatic pressure due to the column height, is described by an advection-diffusion equation with no source/sink terms. In our study, advection-diffusion processes were assumed to occur only in the axial direction of the column such that the spatial variation could be captured by a single variable z. A pure culture of *C. autoethanogenum* is injected at the bottom of the column; therein cells are dispersed in the liquids and consume the dissolved gas and release by-products such as ethanol and acetic acid. Uniform biomass concentration profile within the reactor was used as the initial condition. The uptake kinetics for dissolved carbon monoxide were specified to follow the Michaelis-Menten equation and accounted for carbon monoxide inhibition, which experimental studies suggest are important at high dissolved CO levels [41]. Cellular growth and by-products' secretion are affected by spatially varying dissolved gas concentrations due to advection-diffusion mass transports induced by the effect of the injection thrust and gravitational force. The model accounts for four species representing the biomass, the CO gaseous substrate, and two by-products - ethanol and acetic acid. The operation in industrially relevant environment is typically continuous with respect to the gas feeding whereas here we assume a batch-wise operation mode.

4. Advection-diffusion equations for a bubble column bioreactor

We examine a model for a bioreactor converting carbon monoxide (CO) gas into ethanol and acetate through a *C. autoethanogenum* biocatalyst. The model and its observability analysis

were developed by the authors in a recent study available in [23]. We use here the same notation used in [23] to report a brief description of the model.

The bioreactor is a bubble column reactor[2] with co-current gas-liquid flows where gas feed is introduced by a gas distributor placed at the bottom of the column. A pure culture of *C. autoethanogenum* is subsequently injected at the bottom of the column; therein cells are dispersed in the liquid, consume the dissolved gas and release by-products such as ethanol and acetic acid. Due to the liquid advection and to the gas diffusion processes, the column has spatially varying dissolved gas concentrations available to the bacteria. The model accounts for four species representing the biomass BM, the CO substrate in the liquid phase S, and two by-products - ethanol E and acetic acid A.

4.1. Modelling diffusion-advection of substrates

We model two transport mechanisms for substrate, biomass and products: (i) advective transport with the mean flow of the liquid in the reactor, and (ii) the diffusive transport due to concentration gradients. The advection is due to the convection motions of the liquid that results from the antagonism between the thrust of the pressure and the force of gravity. For the substrate, we assume there are neither sources nor sinks, and the velocity field describes an incompressible flow. Under these conditions, the equation for the substrate is linear diffusion-advection equation

$$\frac{\partial S}{\partial t} + c\frac{\partial S}{\partial z} = \nu\frac{\partial^2 S}{\partial z^2} \tag{8}$$

where c is the advection velocity (here assumed to be constant) and ν is the fluid velocity. As in the procedure of Mojtabi et al. [42], we impose homogeneous Dirichlet boundary conditions $S(0,t) = S(L,t) = 0$ and the initial condition $S(z,0) = \sin \pi z$.

To obtain a closed form solution, we apply the change of variables suggested in [42], i.e.

$$S(z,t) = Y(z,t)e^{\alpha z+\beta t}, \tag{9}$$

where α and β are free parameters. Substituting the Eq. (9) in Eq. (8) and simplifying by the exponential, we obtain that governing equation for $Y(z,t)$, that is

$$\frac{\partial Y}{\partial t} = -[\beta + \alpha(c - \alpha\nu)]Y - (c - 2\alpha\nu)\frac{\partial Y}{\partial x} - (c - 2\alpha\nu)\frac{\partial Y}{\partial z} + \nu\frac{\partial^2 Y}{\partial z^2}. \tag{10}$$

In order to obtain the standard heat equation from the Eq. (10), we set

$$\begin{aligned} \beta + \alpha(c - \alpha\nu) &= 0 \\ c - 2\alpha\nu &= 0 \end{aligned}$$

that gives $\alpha = \frac{c}{2\nu}$ and $\beta = -\frac{c^2}{4\nu}$. Therefore, the equation for Y becomes

$$\frac{\partial Y}{\partial t} = \nu\frac{\partial^2 Y}{\partial z^2} \tag{11}$$

subject to the homogeneous conditions $Y(0,t) = Y(L,t) = 0$ and the initial condition

$$Y(z,0) = -\sin \pi x e^{-\alpha z}$$

[2] A column bioreactor has a cylindrical shape with the radius R much smaller than the height L.

The solution of the Eq. (11) is

$$Y(z,t) = \sum_{k=0}^{\infty} \left(A_k \sin \frac{k\pi z}{2} + B_k \cos \frac{k\pi z}{2} \right) e^{-\frac{\nu k^2 \pi^2}{4} t} \tag{12}$$

where the boundary conditions impose that $A_{2p+1} = B_{2p} = 0$ for $p = 0, 1, 2, \ldots$. Therefore the Eq. (12) becomes

$$Y(z,t) = \sum_{p=0}^{\infty} \left(A_{2p} \sin(p\pi z) e^{-\nu p^2 \pi^2 t} + B_{2p+1} \cos \left(\frac{2p+1}{2} \pi z \right) e^{-\nu \frac{2p+1)^2}{4} \pi^2 t} \right). \tag{13}$$

Mojtabi et al. [42] showed that the coefficients A_{2p} and B_{2p+1} can be calculated using the orthogonality properties of the Fourier polynomials, and the standard trigonometrical relations, and that, when the viscosity goes to zero, the final solution becomes

$$\begin{aligned} S(z,t) &= 8\pi^2 \left(\frac{\nu}{c} \right)^3 \exp \left[\frac{c}{4\nu} \left(z + 1 - \frac{c}{2} t \right) \right] \\ &\times \left[\sum_{h=0}^{\infty} (-1)^h \left(2h \sin(h\pi z) + (2h+1) \cos \left(\frac{2h+1}{2} \pi z \right) \right) \right]. \end{aligned} \tag{14}$$

For very small values of the kinematic viscosity the calculation of the solution according the Eq. (14) produces a numerical overflow due to the exponential term. In literature, we find a number of methods to treat this ill-behaviour (see for example [43, 44, 45, 46, 47, 48, 49, 50]). We adopted the solution of Mojtabi et al. [42]. Assuming periodic boundary conditions, the solution of the diffusion-advection equation is the Fourier solution, that in case of low kinematic viscosity is approximated as follows

$$S(z,t) = -\sin(\pi(z - ct)) e^{-\nu \pi^2 t} \approx -\sin(\pi(z - ct))(1 - \pi^2 t + \mathcal{O}(\nu^2)). \tag{15}$$

The solution given in Eq. (15) will be used in the equation for the dynamics of biomass, as we will see in the next section.

4.2. Biomass and product equations

We consider the model we developed in [23], that describes the evolution of the biomass concentration M with the following equation

$$\frac{\partial M}{\partial t} = \nu \frac{\partial^2 M}{\partial z^2} - c \frac{\partial M}{\partial z} + \mu(\tilde{S}) M \tag{16}$$

where

$$\mu(\tilde{S}) = \frac{\mu_{\max} \, \tilde{S}}{K_S + \tilde{S}} \tag{17}$$

is the bacterial growth rate and $\tilde{S} = \nu_{\text{uptake}} S$. The term ν_{uptake} is the rate of uptake of the substrate, therefore $\mu(\tilde{S})$ is the growth rate expressed as a function of the actual portion of substrate consumed. Assuming a n-product inhibited Monod kinetics, the uptake rate has the following expression

$$\nu_{\text{uptake}} = \frac{\nu_{\text{uptake\_max}} \, S}{\left(K_M + S \right) \left(1 + \frac{\sum_{j=1}^{n} P_j}{K_I} \right)} \tag{18}$$

where $\nu_{\text{uptake\_max}}$ is the maximum uptake rate, K_M the saturation constant and K_I the inhibition constant. From the Eq. (18) in Eq. (17), we obtain that

$$\mu(\tilde{S}) = \frac{\mu_{\max}\ \nu_{\text{uptake\_max}}\ S^2}{\nu_{\text{uptake\_max}}\ S^2 + K_S(K_M + S)\left(1 + \frac{\sum_{j=1}^{n} P_j}{K_I}\right)}. \tag{19}$$

From the Eq. (19) we see that the inhibitory effect of the products becomes preponderant as $\sum_{j=1}^{n} P_j \geq S$, because the infinity order of the denominator reaches the infinity order of the numerator. Based on this observation, in Eq. (19), we set $\frac{\sum_{j=1}^{n} P_j}{K_I} \approx \frac{nS}{K_I}$. This choice is justified by the fact that the closed form of the spatio-temporal dynamics of the products is the same as that of the substrates when the dynamics of the substrate is controlled mainly by advection and diffusion, and no source/sink term is present.

The equations for the product P_i, templated according to Chen et al. [40] are:

$$\frac{\partial P_i}{\partial t} = M_w^{(i)} \nu_i M - c\frac{\partial P_i}{\partial z} + \nu \frac{\partial^2 P_i}{\partial z^2}, \tag{20}$$

where ν_i is the flux of the product and $M_w^{(i)}$ is its molecular weight, and i = Ethanol, Acetate.

4.3. Remarks

We note that we modelled advection-diffusion processes occurring in water, and in conditions in which the advection velocity (c) is four orders of magnitude higher than the kinematic viscosity of water (μ). This disproportion makes the process of advection preponderant over that of diffusion and consequently the model particularly sensitive to the value of c. In addition, the model predicts that the substrate S periodically cancels out whenever $z = ct$, condition implausible realistically, but which can be corrected by including in the model also the convection processes of the liquid in which the advection processes of the gaseous bubbles of the substrate take place. Some literature referring to bubble column bioreactors (as for example [51, 52]) report in detail the effects of convective liquid movements on the gas dispersion, that should be integrated in the current model. We point out, however, that, according to this literature, the values of the velocity of advection, convection are parameters also related to the geometry of the bioreactor, for which in this model we did not want to be specific in order to let the model be a working system adaptable to the specific needs of its users.

4.4. Software

We implemented the advection-diffusion model in Python 3.7.0. The code is publicly available at

https://gitlab.inf.unibz.it/Paola.Lecca/advection-diffusion-simulation

The integration method used to obtain the numerical solution of the partial differential equations is the Forward Euler method [53].

The parameters c, $\mu_{\max}$, K_S, $\nu_{\text{uptake\_max}}$, K_M, K_I, the molecular weights $M_w^{(i)}$, ν_A and ν_E are reported in the code together with the literature references [40, 54] from which the values were extracted and adapted at our simulation framework.

4.5. Computational test for asymptotic stability

For the bioreactor model we consider the function $f : \mathbb{R}^4 \to \mathbb{R}$ for the control M:

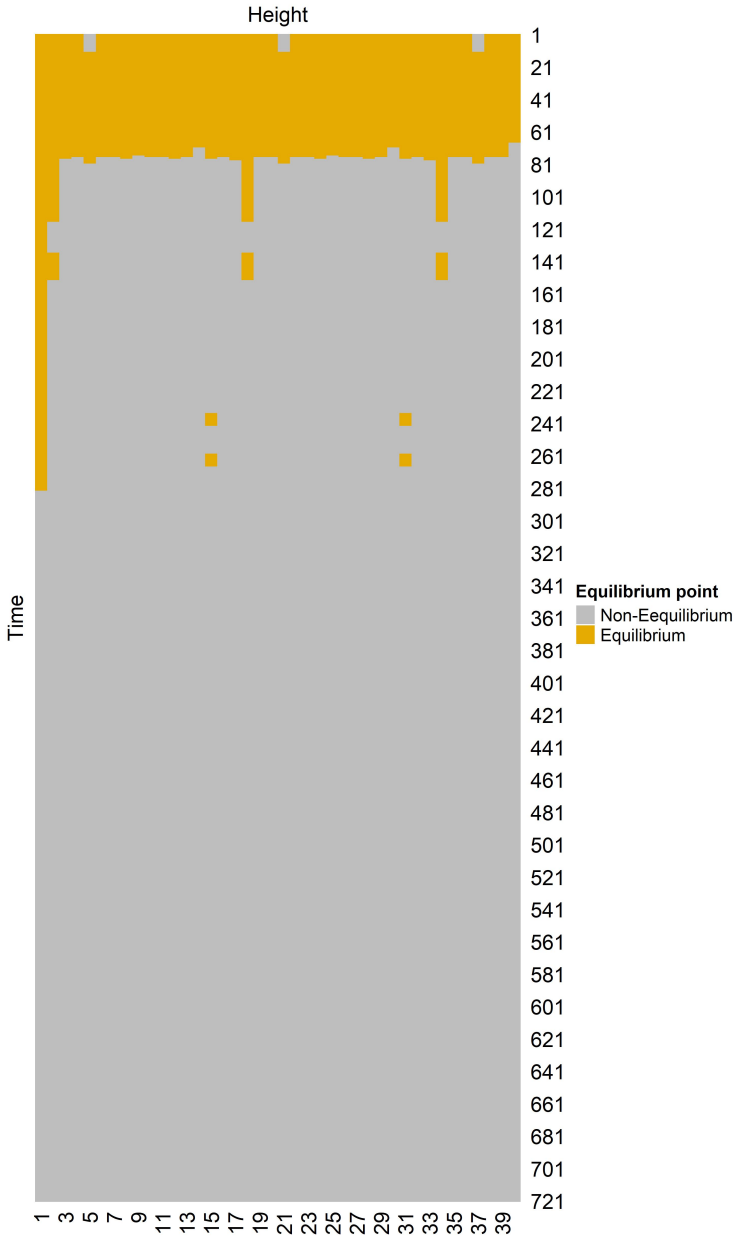

Figure 1. The heatmap shows the values of the derivative of biomass with respect to time along the temporal and reactor's vertical axial dimensions.

$$(M, S, P_{\text{ethanol}}, P_{\text{acetate}}) \longrightarrow \nu \frac{\partial^2 M}{\partial z^2} - c\frac{\partial M}{\partial z} + \mu(S, P_{\text{ethanol}}, P_{\text{acetate}})M. \tag{21}$$

The f in (21) trivially satisfies the conditions (i)-(iv) of Proposition 3 (where the supply function is the output-input difference, where the input is the substrate and the output is the biomass, i.e. $\sigma(M, S, P_{\text{ethanol}}, P_{\text{acetate}}) = M - S,$) and also the dissipation inequality (condition (v)), where V is

$$V(x) = \sum_{i=1}^{4} \left[x_i - \overline{x}_i - \overline{x}_i \ln\left(\frac{x_i}{\overline{x}_i}\right)\right]$$

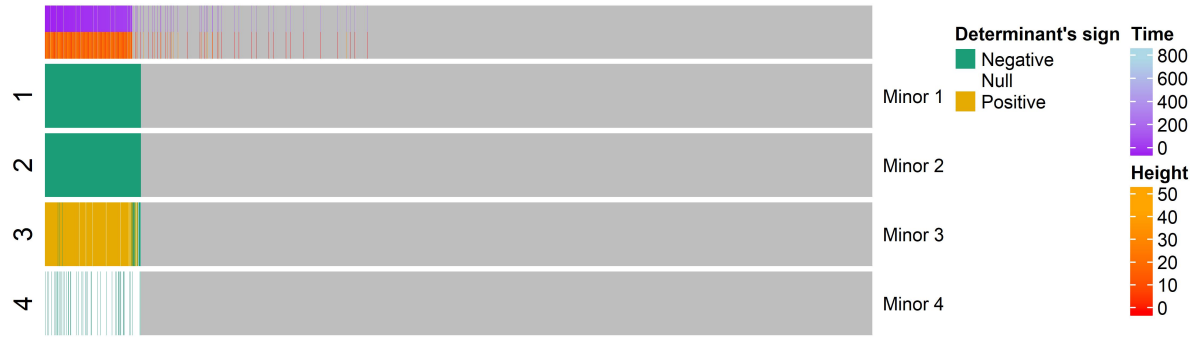

Figure 2. The heatmap shows if principal minors of Hf, are negative, null or positive in a 2D space whose coordinates are the time and height of the reactor. The Hessian is computed just at the equilibrium points. NA values corresponding to non-equilibrium points are displayed in grey. Rows correspond to the Hessian's minors and columns to each possible combination of time and reactor's height. The coordinates of the equilibria points are displayed in color code in the top panel containing columns' annotations

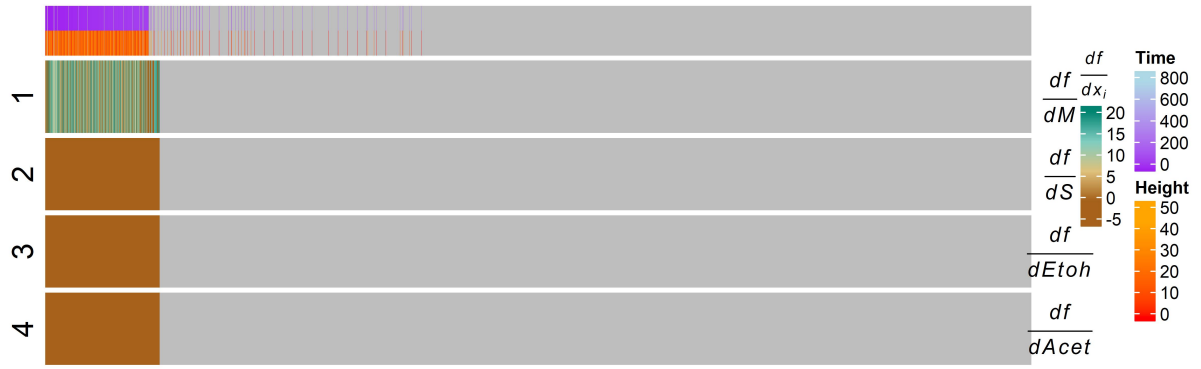

Figure 3. The heatmap shows the sign of the partial derivatives of the function: f with respect to the four state variables, namely biomass, substrate, ethanol and acetate concentration, in a 2D space whose coordinates are the time and height of the reactor. Rows correspond to the partial derivatives and columns correspond to each possible combination of time and reactor's height. NA values corresponding to non-equilibrium points are displayed in grey. The coordinates of the equilibria points are displayed in colour code in the top panel containing columns' annotations.

as in the majority of ordinary-differential-equation models of biological mass-action like models [22]. Here, and hereafter $x \equiv (x_1, x_2, x_3, x_4) \equiv (M, S, P_{\text{ethanol}}, P_{\text{acetate}})$.

We then performed the following computational step in R programming language [55]:

(i) Identification of equilibrium points $(t_{\text{eq}}, z_{\text{eq}})$ of $M(t, z)$, defined by the stationarity condition

$$\frac{\partial M}{\partial t} \approx 0, \quad S(t, z) \neq 0$$

where $\frac{\partial M}{\partial t}$ has been computed by the function `stinemanSlope` of the package `stinepack`, and the stationarity condition has been considered satisfied for $|\frac{\partial X}{\partial t}| < 0.0001$. The cases where substrate is null were neglected since they just reflect a limitation in our model that include the advection but not the convection.

(ii) Verification of the conditions (i), (ii) and (iii) of the Theorem 2 at the equilibrium points.

The behaviour $\frac{\partial M}{\partial t}$ is shown in Figure 1. We found that conditions (i) of Theorem 2 are not satisfied as it is shown in the Figure 2. In particular, we found no case where the minors are all positives and no case of alternate signs starting from "-" that can indicate unstable equilibria. Also condition (iii) of Theorem 2 is not satisfied (see Figure 3). We therefore conclude that the equilibria of the biomass are not asymptotically stable.

5. Conclusion

In this paper, we presented a theorem for verifying global asymptotic stability and applied it to the case study of an advection-diffusion model in a bioreactor. The theorem is the main result of this study. According to this theorem, the equilibrium points of the advection-diffusion processes are not globally asymptomatically stable. We hypothesize that the origin of the instability of the equilibrium points in this process is due to two main factors: 1. the preponderance of the advective mechanisms over the diffusive ones, 2. the effect of the physical boundary conditions of the system (i.e. at the bottom and at the top of the reactor). A strongly preponderant advective motion over diffusive motion makes the boundary conditions unstable if not undefined. This result is also corroborated by previous simulation studies that the authors have performed in [23] and in which they report the extremely steep solution trend for the biomass at the top of the reactor tends to infinity. With increasing time this trend moves more and more progressively towards the origin, so the instability of the boundary conditions propagate gradually closer and closer to the origin. We therefore also hypothesize that a different geometry and/or size of the bioreactor, coupled with a different proportion between the orders of magnitude of the advection and diffusion parameters may have an effect on the stability of the system. Further analysis, supported by numerical simulations of a model, which may also include convective effects and not only diffusive gas dispersion mechanisms, is the next step to be taken to validate these hypotheses.

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Appendix A. Discrete system

In the case of discrete time state space equations

$$x(t+1) = f(x(t), w(t)), \quad y(t) = g(x(t), w(t)), \tag{A.1}$$

where $x(t) \in D \subset \mathbb{R}^n$ is the system state, $w(t) \in W \subset \mathbb{R}^m$ is system input, $y(t) \in Y \subset \mathbb{R}^k$ is system output, and $f : X \times W \to Y$. A functional $V_s : X \to \mathbb{R}$ is a *storage function* for system (A.1) with *supply rate* $\sigma : Y \times W \to \mathbb{R}$ if

$$V_s(x(t+1)) – V_s(x(t)) \leq \sigma(y(t)) \tag{A.2}$$

for every solution of (A.1).

Appendix B. Mondragón et al. theorem

To allow a better reading and understanding of the proof of Theorem 2, we report in this appendix the results of Mondgragón et al. [22] that have been used.

Proposition 2. *Let D be an open subset of $\mathbb{R}^n_+$ and $\overline{x} = (\overline{x}_1, \overline{x}_2, \dots \overline{x}_n)$ equilibrium point belonging to D. Suppose that $f : D \longrightarrow \mathbb{R}^n_+$ is a function of class $\mathbb{C}^1$, and that $f(\overline{x}) = 0$. Let $\Delta_j(\overline{x})$ be the determinants defined as*

$$\Delta_j(\overline{x}) = (-1)^j \left| \frac{a_j}{\overline{x}_j} \frac{\partial f_j(\overline{x})}{\partial x_i} + \frac{a_i}{\overline{x}_i} \frac{\partial f_i(\overline{x})}{\partial x_j} \right|_{i=1,2,\dots,j}, \quad j = 1, 2, \dots, n$$

where a_j is a real positive constant.

(i) If $\Delta_j(\overline{x})$ for $j = 1, 2, \dots, n$ are positive, then $\overline{x}$ is globally asymptotically stable.

(ii) If $\Delta_j(\overline{x})$ for $j = 1, 2, \dots, n$ have alternate signs staring from a negative value, the $\overline{x}$ is unstable.

From this proposition Mondragón et al. [22] deduced the following corollary (Corollary 4 in the original paper of Mondragón et al.)

Corollary 1. *If the Hessian of matrix $Hg(x)$, where $g(x) = - \cdot V(x)$ evaluated at $\overline{x}$ is positive definite , then $\overline{x}$ is globally asymptotically stable on D, and unstable when $Hg(\overline{x})$ is negative.*

Using Proposition 2 and Corollary 1, the following theorem can be stated (Theorem 5 in the original paper of Mondragón et al.).

Theorem 3. *Let $\overline{x} \in D \subseteq \mathbb{R}^n_+$, an equilibrium solution of the non-linear system $\dot{x} = f(x)$. If*

(i) $\frac{\partial f_i(\overline{x}}{\partial x_i} < 0$, $i = 1, 2, \dots, n$

(ii) $l_{ij}(\overline{x}) > 2$ for $i, j = 1, 2, \dots n$ $(i \neq j)$, where

$$l_{ij}(\overline{x}) = \left(\frac{\partial f_i(\overline{x})}{\partial x_i} \right)^{-1} \frac{\partial f_i(\overline{x})}{\partial x_j} + \left(\frac{\partial f_j(\overline{x})}{\partial x_j} \right)^{-1} \frac{\partial f_j(\overline{x})}{\partial x_i}$$

then $\overline{x}$ is globally asymptotically stable.

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