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Global stabilisation of continuous bioreactors: Tools for analysis and design of feeding laws☆

Hernán De Battista [a,*], Martín Jamilis [a], Fabricio Garelli [a], Jesús Picó [b]

[a] Grupo de Control Aplicado (GCA), Instituto LEICI, UNLP-CONICET, Facultad de Ingeniería, Universidad Nacional de La Plata, Argentina
[b] SB²C Lab, Institut d'Automàtica i Informàtica Industrial, Universitat Politècnica de València, Spain

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ABSTRACT

This work revisits the dynamic behaviour of stirred continuous reactors in which a single bioreaction with unknown kinetics occurs. Conditions on the feeding strategy to avoid washing out the biomass and falling in batch operation are obtained. These conditions derive in a closed positively invariant region including the desired operating point. It is stated that no closed orbits may exist in this region and, furthermore, that no fixed point exists but on one of its borders. Therefore, global stability is achieved by finding a feeding law that fulfils the aforementioned invariant conditions and gives a single equilibrium for a first-order dynamics. These results are useful to determine the stability properties of different control laws and, more importantly, to design new ones. The main advantages of the proposed approach are its simplicity and that, differing from previous results, input saturation does not affect stability results. The potentiality of the developed tools is illustrated by means of classical and novel feeding laws.

1. Introduction

A continuous bioreactor is a vessel where a biochemical reaction takes place while fresh medium is continuously supplied and an effluent stream is withdrawn to keep volume constant. We focus on continuous stirred-tank bioreactors in which biomass is suspended in the liquid medium and the composition of the effluent is supposed to be the same as in the vessel.

This type of bioreactors has been widely used in industry during the last decades with several purposes: either to produce chemical compounds, to cultivate biomass, for extraction of intracellular products and in bioremediation. They are also receiving a renewed interest in research. Since microbial growth occurs in an unchanging environment, continuous bioreactors are a source for large volumes of uniform cells or protein. This is fundamental for low noise characterisation of engineered microorganisms and biological circuits in synthetic biology (Canton, Labno, & Endy, 2008; Saldanha, Brauer, & Botstein, 2014; Scott, Gunderson, Mateescu, Zhang, & Hwa, 2010).

Continuous bioreactors are long-term processes along which set-points, control objectives, physicochemical variables and dynamic behaviour can be changed by the operator. For instance, in research applications it is interesting to grow cultures under different conditions in order to properly characterise the microorganisms and to find optimum productivity (Paalme, Elken, Kahru, Vanatalu, & Vilu, 1997; Takahashi, Miller, Ekness, Dunham, & Klavins, 2015). Therefore, global stability is essential for their successful control. To make most profit of the process, it is also convenient to minimise the transient between consecutive steady state operating points.

Control of continuous bioprocesses has been addressed using different design tools. Linear control theory has been applied in Dunn, Heinzle, Ingham, and Přenosil (2003). Exact feedback linearisation has been exploited, for instance, in Bastin and Van Impe (1995), Perrier and Dochain (1993) and Proll and Karim (1994), but input flow saturation impacts directly on the controller stability and performance. Lyapunov theory has also been used both for stability analysis and control design (Mailleret, Bernard, & Steyer, 2004; Mazenc, Harmand, & Malisoff, 2016; Sbarciog, Loccufier, & Noldus, 2005). In Sbarciog et al. (2005), Lyapunov functions are used to determine domains of attraction of stable equilibria for open-loop processes in which the dilution rate is constant. In Mazenc et al. (2016), sampled measurement of substrate concentration is used for feedback control but it requires a model

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* Corresponding author.
E-mail addresses: deba@ing.unlp.edu.ar (H. De Battista), martin.jamilis@ing.unlp.edu.ar (M. Jamilis), fabricio@ing.unlp.edu.ar (F. Garelli), jpico@ai2.upv.es (J. Picó).

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of the growth kinetics. In Karafyllis, Malisoff, and Krstic (2015), the control of an age-structured continuous bioreactor modelled by a partial differential equation is addressed, but the effect of substrate on growth rate is not considered. In Mailleret et al. (2004) globally stable feedback laws are designed, being the feedback gain dynamically adapted in such a way that control never saturates. In this control, speed of convergence is not an issue, so it is not set by controller tuning. There are other global control approaches (Rapaport & Harmand, 2002), but they often do not explicitly consider the constraints on the input flow.

Here we propose a simple and systematic methodology to design globally stabilising controllers for continuous bioreactors involving a pure culture growing on one limiting substrate. The trade-off between convergence speed and global stability becomes clear. One of the main advantages of the proposed approach is that input flow saturation does not affect stability, so the designer should not care about it. From a bioengineering viewpoint, this paper offers a mathematical analysis to re-think and understand trade-offs and limitations of empirical feeding laws.

We derive our results from a mass balance model of a continuous bioprocess in which a single bioreaction occurs, a common approach in the literature (Bastin & Van Impe, 1995; Mailleret et al., 2004). Since no particular expression for the reaction kinetics is considered, this model is suitable to describe the growth of a broad variety of microorganisms. The method presented here is also a basis for analysing and designing controllers for more complex bioreactions.

2. Operating modes and dynamic model

2.1. Modes of operation

In industry, continuous reactors are often operated as chemostats, that is the pump feeds fresh medium into the vessel at a constant rate. Chemostats reach their steady state when the dilution of the culture equals the microbial growth. Thus, the experimenter manipulates the specific growth rate of microorganisms by setting the set-point of the feeding pump. However, chemostats suffer from some limitations. They are not reliable to regulate specific growth rates close to maximum since an unexpected or unmodelled growth inhibition may lead to biomass wash-out. The culture might be lost during transient from one steady state to another one unless set-point changes are made slow enough. Additionally, multiplicity occurs when, as usual, the growth is inhibited by a nutrient in excess.

Different closed-loop strategies have been developed as alternative to chemostats. For instance, nutristats regulate substrate concentration at a given set-point. This operating mode avoids multiplicity and allows driving the biochemical reaction to maximum specific growth rate conditions. This operation is restricted to those processes in which the nutrient can be reliably measured on-line in low concentrations like in Kleman, Chalmers, Luli, and Strohl (1991) and Rutgers, Breure, and Andel (1994). On the other hand, turbidostats regulate cell density at a prescribed value (de Vree, Bosma, Wieggers, Gegic, Janssen, Barbosa, & Wijffels, 2016; Lee, Boccazzi, Sinskey, & Ram, 2011). Cell density is continuously monitored using a spectrophotometer/turbidometer to measure the optical density for control purposes (Bolic, Larsson, Hugelier, Lantz, Krahne, & Gernaey, 2016), or other methods based on dielectric permittivity (Downey, Graham, Breit, & Glutting, 2014). Other operating methods use on-line measurement of other variables such as pH, dissolved oxygen (DO), oxygen uptake rate (OUR), oxygen transfer rate (OTR), chemical oxygen demand (COD) to indirectly regulate a key variable of the biochemical reaction (Nakano, Lee, Yoshida, Matsumoto, Shiomi, & Katoh, 2006; Simova, Beshkova, Angelov, & Dimitrov, 2008).

In all these modes of operation, the dilution rate is manipulated to drive the process towards the desired operating point. Typically, set-point step and ramp changes are implemented to study the effects of the specific growth rate on the production rate and other issues. However, arbitrary set-points for both biomass and substrate concentrations cannot be achieved by only manipulating the dilution rate because of reaction constraints. To overcome them, a piece-wise constant inlet substrate concentration profile is often implemented, limited by maximal biomass concentration or OTR constraints. The switching times are chosen separated enough so that the process is mostly operated at steady-state. A time of five generations of microorganisms in the new macroscopical steady-state is considered enough to assure internal steady-state in the cell metabolism.

2.2. Mass balance dynamic model

We consider continuous bioprocesses in which a single species of microorganisms grows in a perfectly stirred vessel. It is assumed that the growth is limited by a single carbon and energy source (CES) whereas other required nutrients are in excess or suitably regulated. It is important to remark that no particular model for the kinetics of the reaction is considered. In fact, bioreactions obeying different types of kinetics could be: monotonic kinetics like Monod, Teissier and Moser, inhibitory kinetics by excessive substrate like Haldane, inhibitory kinetics by excessive biomass like Contois, etc. (Bellgardt, 2000). Therefore, the results presented below apply to a very wide range of bioprocesses. Further, the extension to even more general processes involving dual substrates will be briefly discussed too.

Let us consider the bioreaction mass balance model:

$$\dot{X} = \mu X - D(t)X \qquad X \in \Re_+ \tag{1a}$$

$$\dot{S} = -y\mu X + D(t)(S^{in}(t) - S) \qquad S \in \Re_+ \tag{1b}$$

where X and S are the biomass and substrate concentrations in the reactor, respectively, μ is the specific growth rate, D is the manipulated dilution rate, y is the substrate-to-biomass yield and $S^{in}(t)$ is the substrate concentration in the inlet flow. In the mass balance model (1), endogenous metabolism and cell maintenance are neglected.

Mass balance model (1) fulfils the dynamic restriction

$$\dot{Z} = D(S^{in}(t) - Z) \tag{2}$$

where $Z = yX + S$ is the amount of CES per volume that was supplied into the reactor and is currently part of the cells or diluted in the liquid medium.

Assumption 2.1. Let $\mu(X, S, q)$ be a globally Lipschitz function satisfying $\mu(X, 0, \cdot) \equiv 0, \quad \mu(X, S, \cdot) > 0 \ \forall S > 0$. where $q \in \mathcal{Q}$ gathers uncertain parameters and other variables (DO, temperature, pH, etc.) affecting the kinetics.

Note that Assumption 2.1 is not restrictive from the bioprocess viewpoint since it is verified by most of the kinetic models used in biotechnology. The extension to other kinetic functions modelling complete inhibition is discussed in Section 5.

Assumption 2.2. Let $S^{in}(t)$ be positive and piece-wise constant. That is, given a set of instants $\{t_j\}$ that partitions the process time into intervals $I_j = [t_j, t_{j+1})$ and a set of positive constants $\{S_j^{in}\}$, then $S^{in}(t) = S_j^{in} \ \forall t \in I_j$.

Recall that the metabolic steady-state is achieved some time after the transient in the reactor media vanishes. Therefore, time intervals I_j are supposed to be much longer than the settling time of the closed-loop responses.