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Microbial production of enzymes: Nonlinear state and kinetic reaction rates estimation

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ABSTRACT

The nonlinearity of the biotechnological processes and the absence of cheap and reliable instrumentation require an enhanced modelling effort and estimation strategies for the state and the kinetic parameters. This work approaches nonlinear estimation strategies for microbial production of enzymes, exemplified by using a process of lipase production from olive oil by *Candida rugosa*. First, by using a dynamical mathematical model of this process, an asymptotic observer which reconstructs the unavailable state variables is proposed. The design of this kind of observers is based on mass and energy balances without the knowledge of kinetics being necessary; only minimal information concerning the measured concentrations is used. Second, a nonlinear high-gain observer is designed for the estimation of imprecisely known kinetics of the bioprocess. An important advantage of this high-gain estimator is that the tuning is reduced to the calibration of a single parameter. Numerical simulations in various scenarios are provided in order to test the behaviour and performances of the proposed nonlinear estimation strategies.

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1. Introduction

Enzymes are biological molecules that are produced by a living organism, which act as catalysts for specific biochemical reactions. More precisely, they are highly selective catalysts that can significantly accelerate the rate and specificity of metabolic reactions, which range from the digestion of food to the synthesis of DNA [1]. Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are enzymes which are responsible for the hydrolysis of triglyceride ester bonds into diglycerides, monoglycerides, fatty acids, and glycerol using a complex phenomenon of interfacial activation [2]. The most commercially important fields of application of lipases in the last three decades comprise the industry of additives, food industry, fine chemistry, detergents, cosmetics and perfumery, wastewater treatment, pharmaceutical applications, and medical area [1–4]. Microbial lipases have gained special industrial attention due to their stability, selectivity, and broad substrate specificity [5,6]. *Candida rugosa* has been called the most frequently used organism for lipase synthesis [1,6]. A comprehensive study regarding the lipase production processes can be found, for example, in [6], and a particular review about the lipases production by *C. rugosa* is given in [7].

Mathematical modelling is an important tool for optimization and control of bioprocesses, particularly for the microbial production of enzymes. Despite the major interest for lipase production, few works dealt with the modelling of this enzyme production [8–11]. For example, in [10] it is proposed a reaction scheme for lipase production that uses the diauxic growth of *C. rugosa* on olive oil. A structured mathematical model for lipase production by *C. rugosa* in batch fermentation is elaborated in [12]. Montesinos et al. [13] simulated the best conditions to produce lipase. The conclusion was that best lipase productivity was obtained in continuous culture, whereas the highest predicted lipase activity was obtained in fed-batch cultures. Continuous production of lipase is a complex process because of the tetraphasic nature of culture broth: a highly dynamic system of four phases (biomass, gas phase, aqueous phase, and organic phase) is required [7].

The advanced monitoring and control of microbial production of enzymes is a difficult task. Often, on-line measurements of some biological variables involved in these processes (such as biomass, substrate, and product concentrations) are not easily available. The

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indirect measurement or estimation of state variables and kinetic parameters, which constitutes the so-called software sensors, is a valuable alternative [8,14–16]. In fact, a software sensor is a combination between a hardware sensor and a software estimator, which can be used not only for the estimation of concentrations (states), but also for the estimation of kinetic parameters. During the last decades, several estimation strategies have been developed to provide accurate on-line estimations of state variables. Two main classes of state observers for bioprocesses were developed [15–19]. The first class (including classical observers like extended Luenberger and Kalman observers, nonlinear observers) is based on the knowledge of model structure. Such state observers were used in some applications concerning lipase production processes (extended Kalman observers [20,21], nonlinear observers [12,22]). A drawback of this class is that the uncertainty in the model parameters can generate possibly large bias in the estimation of the unmeasured states. A second class, called asymptotic observers, is based on the idea that the process uncertainty lies in the kinetics models. The design is based on mass and energy balances without the knowledge of kinetics being necessary. Some studies regarding the design of asymptotic observers for simple prototype lipase production processes were reported [21,23]. The potential weakness of asymptotic observers is a low rate of estimation convergence, which depends on the operating conditions. However, the key advantage is the independence of the kinetics. Besides these two classes, another approach, used in the last decade for bioprocesses without a complete knowledge of inputs, is represented by the interval observers, which allows the reconstruction of a guaranteed interval on unmeasured states instead of reconstructing their precise numerical values [24–27].

A problem of great importance is the estimation of kinetic rates, i.e. of the so-called kinetics of the bioprocess. One of the first approaches from historically point of view is based on Kalman filter which leads to complex nonlinear algorithms. Another classical technique is the Bastin and Dochain approach based on adaptive systems theory [15,17]. The strategy consists in the estimation of unmeasured states with asymptotic observers, and after that, the measurements and the estimates of state variables are used for on-line estimation of kinetic rates. This technique was applied for numerous bioprocesses, including lipase production processes [21]. This approach is useful, but in some cases, when many reactions are involved, the implementation requires the calibration of too many parameters. To overcome this problem, a possibility is to design an estimator using a high gain approach [21,28,29]. The gain expression of these observers involves a single tuning parameter whatever the number of components and reactions. High gain observers have evolved as a valuable tool for the design of output feedback control of nonlinear systems [30,31]. The early work on high gain observers emerges in the late 1980s, and later the technique was developed mainly by French researchers (Gauthier, Hammouri, Farza and others [28,29]) and by U.S. researchers (see, for example, Khalil [31]). Other kinetic parameters estimation techniques include recursive prediction error algorithms, neural networks and hybrid approaches [12,32,33].

However, all the estimation strategies need a better understanding of biotechnological processes and a complex mathematical support. Generally speaking, due to specificity and nonlinearity of the lipase production processes, there is no universal solution to the estimation problem, and good solutions are given only by studying each particular bioprocess.

In this paper, two correlated estimation issues concerning the processes used for the production of microbial enzymes are studied. The reported results can be applied to all microbial productions of enzymes which hydrolyze primary substrate and use products of hydrolysis for microbial growth, for example various glysosidases. As exemplification, the proposed estimation techniques are implemented for a process of lipase production from olive oil by *C. rugosa* [34,35]. First, by using a dynamical mathematical model of this process, an asymptotic observer which reconstructs the unavailable state variables is proposed. More precisely, by using three measured concentrations (states) of the bioprocess, other four concentrations are estimated. This design is possible due to the structure of the dynamical nonlinear model. Second, because the kinetic rates of the process are nonlinear and highly uncertain, an on-line estimation strategy is designed. A nonlinear high-gain observer is proposed for the estimation of three imprecisely known kinetic rates. This parameter estimator possesses certain advantages concerning the robustness against disturbances and simple tuning (which consists in the calibration of a single parameter). The high gain estimation scheme does not require any model for the kinetics. The performances and the behaviour of the proposed estimation techniques are studied by using extensive numerical simulations. All these simulations are achieved by using the development, programming and simulation environment MATLAB (registered trademark of The MathWorks, Inc., USA).

2. Materials and methods

2.1. Lipase production by C. rugosa: a mathematical model

In this paper, we will consider a lipase production from olive oil by *C. rugosa*, which takes place into a continuous reactor [34,35]. More precisely, there is a growth of microorganisms on two substrates that are produced by the hydrolysis of a primary complex organic substrate. The following three-step reaction network of this complex bioprocess has been assumed in the literature [34,35]:

(a) The hydrolysis:

$$k_1S_1 + E \xrightarrow{\varphi_1} S_2 + k_2S_3 + E \tag{1}$$

(b) The growth on substrate S_2 (glycerol):

$$k_3S_2 + k_4O \xrightarrow{\varphi_2} X + k_5P \tag{2}$$

(c) The growth on substrate S_3 (fatty acids):

$$k_6S_3 + k_8O \xrightarrow{\varphi_3} X + k_7E + k_9P \tag{3}$$

In the above reaction scheme S_1 is the primary substrate, i.e. the olive oil (which is made of several compounds, especially triglycerides), S_2 and S_3 (the secondary substrates) are the glycerol and the fatty acids, respectively, E is the enzyme (lipase), X is the biomass (*C. rugosa*), O is the dissolved oxygen and P is the dissolved carbon dioxide. φ_1, φ_1 and φ_3 are the reaction rates corresponding to the three reactions of the lipase production process, and k_i, $i = \overline{1, 9}$ are the yield coefficients.

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