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Nonlinear control of the dissolved oxygen concentration integrated with a biomass estimator for production of *Bacillus thuringiensis* δ-endotoxins

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ABSTRACT

Bacillus thuringiensis is a microorganism that allows the biosynthesis of δ-endotoxins with toxic properties against some insect larvae, being often used for the production of biological insecticides. A key issue for the bioprocess design consists in adequately tracking a pre-specified optimal profile of the dissolved oxygen concentration. To this effect, this paper aims at developing a novel control law based on a nonlinear dynamic inversion method. The closed-loop strategy includes an observer based on a Bayesian Regression with Gaussian Process, which is used for on-line estimating the biomass present in the bioreactor. Unlike other approaches, the proposed controller leads to an improved response time with effective disturbance rejection properties, while simultaneously prevents undesired oscillations of the dissolved oxygen concentration. Simulation results based on available experimental data were used to show the effectiveness of the proposal.

1. Introduction

Bioprocesses control strategies are important for reducing production costs and increasing productivity while simultaneously maintaining the product quality. *Bacillus thuringiensis* (*Bt*) is an aerobic bacterium used for the production of δ-endotoxins; and the obtained bioinsecticide is of great interest and importance on account of its low environmental impact. Since *Bt* is an aerobic bacterium, the dissolved oxygen concentration (C_{DO}) in the culture medium is an important production variable. Inadequate levels of oxygen could inhibit or limit the microorganism growth. The control of biotechnological processes that include aerobics microorganisms normally involves the feed of high oxygen flow rates, in order to ensure an excess of the dissolved oxygen concentration with respect to its nominal value (Rocha-Valadez et al., 2007).

Dissolved oxygen as a nutrient is often a growth-limiting substrate. An agitator is used to improve the bioreactor productivity by minimizing spatial gradients of substrate and cell concentrations. The agitation speed is chosen to render a proper mixing while avoiding excessive shear forces that might cause cells rupture (Henson, 2006). Several articles have studied the effect of the dissolved oxygen in medium for various microorganism cultures. For example, the aeration and agitation rates as well as the type and size of the fermenter and its accessories directly affect the volumetric oxygen transfer coefficient in the production of exopolysaccharides from *Enterobacter cloacae* WD7 in (Bandaiphet and Prasertsan, 2006). For an *E. coli* fed-batch culture, (de Maré and Hagander, 2006) developed a continuous-time phenomenological model for the oxygen dynamics. In that work, some model parameters have been estimated off-line from empirical formulas while others were assumed to be known.

Most research articles on *Bt* processes suggest that C_{DO} must be kept above the minimal critical value for the microorganism growth. Data on critical oxygen concentration in *Bt* fermentations (Moraes et al., 1980) indicate that high levels of C_{DO} promote the formation of toxic compounds for *Bt*, inhibiting this way microbial growth and product formation (Onken and Liefke, 1989). On the other hand, a C_{DO} lower than the minimum critical value for the microorganisms produces the well-know “oxygen limitation” effect. Besides, high and low C_{DO} as well as oxygen fluctuations, can be detrimental for protein expression (Konz et al., 1998). For

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most microorganisms, the critical C_{DO} ranges between 0.1 mg L^{-1} and 1 mg L^{-1}. These values are relatively low when compared with the O_2 solubility in a diluted aqueous medium at 30 °C (around 7.5 mg L^{-1}). Therefore, an appropriate O_2 flow should continuously be fed into the bioreactor to approximately compensate for its consumption rate. This is a key factor that must be taken into account when designing bioreactors for aerobic microorganism culture.

In some fermentations, the C_{DO} can also be used for estimating the microorganisms concentration in the medium when the O_2 consumption is known (Amicarelli et al., 2014; di Sciascio and Amicarelli, 2008). In particular, the *Bt* life has two phases: a vegetative phase and a sporulated phase. In batch *Bt* fermentations, the oxygen concentration is critical during a large part of the microorganisms growth (the vegetative phase), but later the oxygen demand decreases during the sporulation phase. Some typical C_{DO} profiles are available for fed-batch fermentations (Li et al., 2009; Wu et al., 2002). Moreover, in (Ribbons, 1969) data regarding breathing and C_{DO} is also reported. Some experimental results have shown a significant effect of the agitation speed on the *Bt* production during the stationary phase (Wu et al., 2002).

For the *Bt* process it has been verified (Ghribi et al., 2007) that: (i) a high C_{DO} increases the cell density while reduces the δ-endotoxins synthesis; and (ii) a C_{DO} profile that maximizes the microorganism productivity is achieved by maintaining the reactor aeration near to 60% or 70% of oxygen saturation during the first 5–7 h of fermentation, while a 40% of oxygen saturation should be maintained at the sporulation phase, until the end of fermentation and regardless of the carbon source used as substrate (Ghribi et al., 2007). All these features allow, in principle, a large-scale production of proteins insecticides with low production costs.

Also for this bioprocess, (di Sciascio and Amicarelli, 2008) proposed a biomass estimator based on Bayesian Regression with Gaussian Process (Kocijan, 2016; Rasmussen, 2006). In this case, biomass concentration is considered a stochastic process i.e. an uncertain dynamic system perturbed by a process noise where its evolution for a particular fermentation is a realization of the stochastic process. This same estimator is considered again in this work but now integrated in closed loop with the new controller proposal. Since the *Bt* life span exhibits highly different dynamics along its vegetative growth and sporulation phases, then the time evolution of the biomass concentration resembles a non-stationary stochastic process.

Bayesian Regression with Gaussian Process is a non-parametric technique based on both, experimental data and qualitative knowledge of the bioprocess (di Sciascio and Amicarelli, 2008). The Bayesian non-parametric framework is flexible enough to represent a wide variety of bioprocess data. It makes possible interpreting the prior distribution, computing the posterior and full predictive distributions, as well as evaluating the mean predictions and the predictive uncertainties. The estimation algorithm consists of three parts. At first, we need to choose a particular form of the covariance function, then we obtain the posterior distribution of the Bayes' rules from the likelihood and prior distributions, and finally, we integrate all the information contained in the observed data. For simplicity the prior mean function was set to be zero, without loss of generality provided that a constant term is included in the covariance function (Kuß, 2006; Williams and Rasmussen, 1996). In this way, it is possible to analytically express the posterior distribution (see e.g. (Mardia and Marshall, 1984)). The hyper-parameters are the "parameters" of the likelihood and prior distributions. In contrast, hyper-parameters of the covariance function are unknown, and must be determined using the training data. The maximization of the posterior distribution is known as the maximum at posterior (MAP) approach, which is a Bayesian version of the maximum likelihood estimate. The MAP estimate was found by using the mentioned analytical expression of the posterior distribution in a gradient descent, or conjugate-gradient optimization technique, to calculate a local maximum of the posterior distribution. The number of hyper-parameters of the covariance function linearly increases with *n*, the dimension of the input space.

As stated in (Ali et al., 2015), the trend in the use of observers for chemical process has changed from single-based observer design to hybrid observers design. For instance, the combination of a reduced-order observer with a sliding mode observer. This is in spite that single-based observers, as for example Bayesian Estimators and asymptotic or exponential observers, will still be applicable in many applications involving chemical process systems.

In other previous work, the C_{DO} control in the *Bt* process has been solved by using a Lyapunov-based controller (Amicarelli et al., 2010). However, relatively poor performances were detected in several simulated cases for changes in the C_{DO} set point. Motivated by this, in the present work, a novel controller based on nonlinear dynamic inversion is designed to track an available optimal C_{DO} profile (Ghribi et al., 2007) for the *Bt* production process in order to improve the control system performance. The derived control law requires the biomass on-line knowledge, provided by the already mentioned biomass estimator. The proposed methodology is evaluated on the original *Bt* process model (Amicarelli et al., 2010) integrated with the biomass observer (di Sciascio and Amicarelli, 2008). All fermentation data considered corresponds to experiments that were carried out with glucose-based substrates (Amicarelli et al., 2010; Ghribi et al., 2007).

The paper is organized as follows. Section 2 describes the microorganism and the mathematical phenomenological model for the *Bt* process. The methodology for the controller design is presented in Section 3. Through several simulated experiments, Section 4 compares the performances of the new control law, the Lyapunov-based controller (Amicarelli et al., 2010), and a classical PID controller. Main conclusions and remarks are summarized in the last section.

2. Materials and methods

2.1. Microorganism and bioprocess characteristics

After the *Bt* vegetative growth, the beginning of the sporulation phase is induced by a substrate deficiency, called the mean exhaustion point. Normally the sporulation phase is accompanied by the production of crystal proteins known as δ-endotoxins. After sporulation, the process is completed with the cellular wall rupture (cellular lysis), and the subsequent liberation of spores and crystals to the culture medium (Aronson, 1993; Liu and Tzeng, 2000; Starzak and Bajpai, 1991).

In a previous work, several *Bt* fermentations experiments have been used for modeling and validating the bioprocess, and particularly the C_{DO} dynamics (Amicarelli et al., 2010; Atehortúa et al., 2007); and such conditions were adopted for the current work. The microorganisms were *Bacillus thuringiensis serovar. kurstaki* strain 172-0451, isolated and stored in the culture collection of the *Unidad de Biotecnología y Control Biológico* (Colombia) (Amicarelli et al., 2010). Growth experiments of the *Bt* fermentation process were performed in a reactor of 20 L of nominal volume, with an effective cultivation medium of 11 liters. The pH of the medium was adjusted to 7.0 with KOH before heat sterilization. Culture conditions at harvest are typified by 90% free spores and δ-endotoxins crystals. The temperature was kept around 30 °C by using an ON/OFF controller; whereas the pH was automatically controlled between 6.5 and 8.5 through a PID controller. The air flow and the agitation speed rate were set up at 22 L/min and 400 rpm, respec-