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Regulation of lactic acid concentration in its bioproduction from wheat flour

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ABSTRACT

Lactic acid is an important molecule for biopolymer production that can be obtained by biological processes. This work deals with the control of the lactic acid concentration in its production bioprocess using wheat flour as substrate. An adaptive control strategy for the simultaneous saccharification, proteins hydrolysis and fermentation (SSPHF) continuous process of lactic acid production is proposed in order to regulate the lactic acid concentration to the target value. The latter is determined so that the lactic acid productivity is maximized. The control strategy effectiveness and robustness are illustrated by means of experimental results.

1. Introduction

Due to the use of lactic acid as the monomer for the PLA (Poly Lactic Acid) production and the increasing need of manufacturing green plastics, lactic acid production has attracted a great interest recently. However, in contrast to petroleum-derived plastics, PLA production is still considered as an immature technology at the industrial scale. This is mainly due to the cost of the used raw material, lactic acid, which is highly dependent on the substrates and the fermentation process used (Abdel-Rahman, Tashiro & Sonomoto, 2013). Employing alternative low cost substrates, improving and optimizing the fermentation process are therefore of utmost importance to reach a cost effective lactic acid production process. In this context, process control is required to improve the process production operation. The improvement of operational stability and production efficiency are the main goals when applying control methods to this type of biotechnological processes (Ben Youssef, Goma & Olmos-Dichara, 2005). Nevertheless, three main obstacles have hampered the development of modern control strategies in this field. First, since bioprocesses involve living organisms, their dynamics, strongly nonlinear and non-stationary, are often poorly understood; in addition, the replicability of experimental results is not guaranteed. Secondly, the microorganisms can be subjected to metabolic variations and physiological modifications over long operation periods, resulting in a change in the model parameters values over time. Finally, reliable sensors for real-time monitoring of key variables and control strategies implementation are often lacking (Bastin & Dochain, 1990). Prior modeling and online estimation become then necessary for the development of control strategies.

Despite these difficulties, several works in the literature on bioprocess control were reported during the last three decades. Proposed control techniques have been applied to various biotechnological processes such as biomass production, fermentation, anaerobic digestion, yeast and penicillin productions, microalgae cultures, *etc.* (Pons, 1991; Schubert, Simutis, Dors, Havlik & Lübbert, 1994; Roux, Dahhou & Queinnec, 1996; Hilgert, Harmand, Steyer & Vila, 2004; Mailleret, Bernard & Steyer, 2004; Marcos, Guay & Dochain, 2004; Ramaswamy, Cutright & Qammar, 2005; Jenzch, Simutis & Luebbert, 2006; Selișteanu, Petre & Răsvan, 2007; Sbarciog, Coutinho & Vande Wouwer, 2014).

In the case of lactic acid production, only few control strategies were proposed in the literature. Most of them concern fed-batch cultures (Choi, Al-Zahrani & Lee, 2014), while others deal with continuous cultures using glucose as substrate (Ben Youssef, Guillou & Olmos-Dichara, 2000). An adaptive on-line optimizing control strategy for maximizing lactic acid productivity from glucose has been proposed in Shi, Shimizu, Iijima, Morisue, and

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Kobayashi (1990). For continuous fermentation process, an adaptive predictive control strategy for regulating the biomass concentration was proposed by Dahhou, Chamilothoris, and Roux (1991). This predictive control scheme calculates the dilution rate from the on-line estimation of the specific growth rate (considered as a time varying parameter). The efficiency of the developed control strategy was evaluated by simulations. Moreover, a system with two bioreactors in cascade developed to maximize lactic acid production was developed in Ben Youssef et al. (2000) using glucose as substrate. The control approach regulated the substrate using an adaptive predictive control structure and online measurements of the substrate concentration. The specific growth and lactic acid production rates were estimated online using an asymptotic observer. This approach was not validated experimentally, but simulations were encouraging. Petre, Selişteanu, and Şendrescu (2011) further studied the previous system and proposed an indirect adaptive controller based on a dynamical neural network. The effectiveness of this control approach was proven by simulations.

Most of works presented previously consider glucose as substrate for fermentation. Nevertheless, the development of processes that use bioresources as substrate for biological conversions is of utmost importance. A control strategy for the fed-batch Simultaneous Saccharification and Fermentation process from Starch to Ethanol (SSFSE) was proposed in Ochoa, Lyubenova, Repke, Ignatova, and Wozny (2008). The goal of this control strategy was to maintain the glucose concentration at a quasi-steady state by feeding starch into the process. An adaptive approach was considered that estimates the glucose consumption and ethanol production rates in the bioreactor from starch and glucose concentrations, the latter being assumed to be measured. This approach was not experimentally validated due to the lack of online sensors to determine sugars concentrations.

The Simultaneous Saccharification and Fermentation process from starch was also studied in (Dai, Word & Hahn, 2014). The process was operated in batch mode and the effect of the temperature on the ethanol production was modeled by an energy-balance equation. The cooling rate and the enzymes addition were further optimized in simulation, leading to about 10% increase in the ethanol yield.

The literature survey clearly underlines that most of the works on lactic acid production do not validate experimentally their control strategies, although it is of prime importance when envisaging their further application in industrial facilities. This is one of the goals of this study. More specifically, the study presented hereafter will focus on the development of a control strategy for the SSPHF (Simultaneous Saccharification and Proteins Hydrolysis Fermentation) continuous process using wheat flour as the substrate for the process. The continuous mode was preferred to batch or fed-batch modes for two main reasons: (i) to avoid the inhibition effect of lactic acid on bacteria growth (Gonzalez et al., 2016), (ii) even if higher lactic acid concentrations are reached in batch or fed-batch fermentations, higher production rates are obtained in continuous fermentation where process shut down occurs less frequently (Hofvendahl & Hahn-Hägerdal, 2000). The continuous mode is then more attractive for the industrial production of lactic acid. In the context of PLA production, this work is a pre-study for the optimization of the industrial production of lactic acid by bacteria from wheat flour using a dedicated control strategy. The proposed control law regulates the lactic acid concentration at a target value that maximizes its productivity using the feed flow rate as the control variable. It represents a first step in the development of an industrial process of lactic acid production.

The article is organized as follows. The next section describes the experimental set-up followed by the system modeling. Then, the control design is presented. A feedback linearizing control approach is considered in the first place and then modified in order to reduce the control law complexity and increase its robustness with respect to model uncertainties. An adaptive control law is then proposed using the lactic acid production rate estimation. The adaptive controller is validated experimentally and its robustness regarding operational factors disturbances is evaluated. Finally, concluding remarks and perspectives are stated at the end. In Appendix, the convergence of the estimation and control strategies are analyzed.

2. Materials and methods

2.1. Microorganism and culture conditions

Lactobacillus coryniformis subsp. *torquens* DSM 20004 is stored at −80 °C in *Lactobacilli* MRS medium with 40% glycerol. Precultures were prepared by proliferation of a stock culture to 100 mL MRS medium and cultured in an incubator shaker MAXQ 4000 (Thermo Scientific) at 30 °C for 12 h. A second proliferation was done at 30 °C in 1000 mL culture medium during 12 h. The cells were then harvested after centrifugation (3000 g, 3 min, 20 °C), resuspended in 100 mL distilled water. This suspension was then used for the fermenter inoculation (corresponding to 3% of the total working volume of the fermenter).

2.2. Bioreactor description

The studied set-up consists of a continuous stirred tank reactor (CSTR) (Global Process Concept, La Rochelle France) illustrated in Fig. 1. Five variables are controlled: temperature, pH, culture broth level in the bioreactor, agitation and feed flow rate. The temperature, pH and broth level are controlled by PID controllers using temperature, pH and foam sensors.

A mechanical overhead stirring device ensures the medium mixing and is regulated by a PID controller which adjusts the motor speed of the agitator to a setpoint value. Finally the feed flow rate is regulated by a peristaltic pump with variable rotation speed.

2.3. Bioprocess description

The whole process studied in this work is divided into three steps. First, in the liquefaction step (see below), starch is liquefied from wheat flour into maltose and glucose. In a second step, the simultaneous saccharification and wheat proteins hydrolysis (SSPH) is performed to partially hydrolyze maltose and wheat proteins into glucose and amino acids, respectively. This step is performed in order to make carbon and nitrogen sources available to the bacteria. In the final step, a simultaneous saccharification proteins hydrolysis and fermentation (SSPHF) allows hydrolyzing the remaining maltose and wheat proteins simultaneously with the fermentation. The liquefaction and SSPH steps are performed in batch mode. This paper will focus on the SSPHF process. Two types of bioreactors are used: a 5 L bioreactor (Fig. 1) where the continuous SSPHF step takes place, and a 12 L bioreactor (Fig. 2) which contains the partially hydrolyzed wheat flour solution that feeds the 5 L bioreactor. Liquefaction and SSPH steps are performed in both reactors. SSPHF step is only performed in the 5 L reactor.

2.4. Liquefaction

The whole wheat flour was suspended in water at a concentration of 260 g L^{-1}, heated to 50 °C and agitated at 400 rpm in a 5 L bioreactor. The pH was regulated at 5.5, with addition of sodium hydroxide and sulfuric acid. The liquefaction of wheat