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Off-line optimization of baker's yeast production process

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HIGHLIGHTS

- An optimization of biomass produced during baker's yeast production is presented.
- Two different optimization approaches are presented.
- The two approaches lead to the determination of similar optimal operation conditions.
- The optimal solutions are in agreement with the industrial operations.
- The optimal solutions are validated with numerical and experimental data.

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ABSTRACT

A macroscopic model describing the influence of nitrogen on a fed-batch baker's yeast production process was used for the determination of optimal operating conditions in the sense of a production criterion. To this end, two different approaches were used: a control vector parameterization approach with mesh refinement and an approach based on the mathematical analysis of optimal operating policy (semi-analytical approach). The results of the two approaches lead to the determination of similar optimal operation conditions, which have been implemented for a new experimental phase. Moreover, these optimal conditions are in agreement with the profiles obtained by industrial manufacturers through an empirical optimization of the process (trial and error method). The model predictions are in good accordance with experimental data. This conclusion was supported by an uncertainty analysis on the model outputs with respect to the parameter estimation errors.

1. Introduction

In recent decades, many efforts have been devoted to the dynamic optimization and control of bioprocesses, and more specifically, of cell cultures performed in fed-batch bioreactors. The fed-batch operation mode is largely used in industry since it allows the control of the biological phenomena taking place within the bioreactor by manipulating the quantity of substrate available throughout the culture (Chen, 2005; Dewasme et al., 2010; Komives and Parker, 2003; Modak et al., 1986; Pomerleau, 1990).

In a fed-batch production context, the determination of optimum operating conditions consists of the definition of a feeding time profile optimizing a cost function (optimization criterion) while taking into account all the constraints of the process (working volume of bioreactor, maximum feeding rate of the pumps, etc.). It should be noted that industrial practice is often used to determine such a profile, at the stage of the process development, based on a method of trial and error. Dynamic optimization allows the computation of this profile by solving an optimization problem formulated as a pre-defined performance index (optimization criterion) that underlines the wishes of a given industry (e.g. production, yield, productivity, or an economical index derived from the industrial operation) (Alford, 2006; Amribt et al., 2014; Banga et al., 2005; Berber et al., 1998; Betts, 2010; Chen, 2005; Hunag et al., 2012; Modak et al., 1986; Renard, 2006; Valentinotti et al., 2003; van Impe and Bastin, 1995).

When the process model is known and relatively simple, this problem can be solved analytically by applying the principle of the Pontryagin minimum. But for more complex models, the solution is difficult to obtain in an analytical form given the highly nonlinear characteristics of the model used and the constraints often present on both the system states and control variables.

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However, based on the analytical solutions obtained for simple models, it is often possible to restrict the number of parameters characterizing the optimization problem. (Banga et al., 2005; Berber et al., 1998; Betts, 2010; Chen, 2005; Renard, 2006).

The dynamic optimization problems for complex models continue to present a challenge to researchers today. In this context, nonlinear programming (NLP) is the simplest methodology for solving this kind of optimization problem. The problem is defined by a finite set of variables, by some constraints (system of equalities and/or inequalities) and by an objective function to be maximized or minimized, where all these can have nonlinear characteristics (Banga et al., 2005; Betts, 2010; Chen, 2005).

However, in reality, optimal control problems involve, most of time, continuous functions such as the feed rate (often chosen as the control variable), which appears linearly in the system of differential equations. Hence, the problem has an infinite dimension (singular problem), which is in opposition with the requirement of the finite dimension of the set of variables characterizing the optimization problem for NLP resolution methodology. Therefore, the conversion of the infinite-dimensional problem into a finite-dimensional approximation can be convenient in order to view this singular optimization problem as an infinite-dimensional extension of a NLP problem (Banga et al., 2005; Betts, 2010; Chen, 2005).

The numerical methods for the solution of dynamic optimization that transform the original dynamic optimization problem into a nonlinear programming (NLP) problem are often classified as direct methods (as opposed to indirect methods). Direct approaches seem to be the currently preferred way for solving dynamic optimization problems (Banga et al., 2005; Betts, 2010; Chen, 2005).

There are basically two strategies for the optimization problem formulation in direct approaches (Banga et al., 2005):

- *Control vector parameterization* (*CVP*): Only the control variables (e.g. feeding time profile) are parameterized by using appropriate function approximations, resulting in a NLP problem for which dimensionality is directly related to the discretization level chosen for the control variables;
- *Complete parameterization* (*CP*), *also called simultaneous strategy*: Both the controls and the states are parameterized by using appropriate function approximations, resulting in a NLP problem with a larger number of parameters which may be computationally intensive to solve.

The control vector parameterization (CVP) approach is one of the most-widely used techniques for the dynamic optimization of fed-batch processes and is one of the two methods chosen in the framework of this work (Banga et al., 2005).

After a brief introduction on the model of Richelle et al. (2014) (Section 3.1), the optimization problem and the procedure to solve it will be presented (Section 3.2). To this end, two different approaches will be presented: a control vector parameterization approach (Section 3.2.1) and an approach based on the mathematical analysis of optimal operation (semi-analytical approach) (Section 3.2.2). The two approaches will be compared with numerical and experimental data (Section 4).

2. Materials and methods

2.1. Microorganism

The microorganism used in this work was a *Saccharomyces cerevisiae* commercial strain. The microorganism was maintained on Petri dishes (glucose 20 g/L, yeast extract 10 g/L, agar-agar 20 g/L) at 4 °C. Periodic inoculations were made in new Petri dishes every 4 months.

2.2. Inoculum development, medium composition and experimental conditions

Inoculum was grown at 30 °C and 250 rpm overnight in a 1 L flask containing 250 mL of a medium having the following composition (per liter of solution): glucose, 20 g; $(NH_4)_2SO_4$, 13.5 g; yeast extract, 13.5 g; KH_2PO_4, 3.5 g; $MgSO_4 \cdot 7H_2O$, 1.7 g; $CaCl_2 \cdot 2H_2O$ 1.7 g. Fed-batch culture was performed during 20 h in a 20 L bioreactor (Biostat C-DCU3, Sartorius B. Braun Biotech International) using an initial biomass concentration of 0.1 g/L dry weight and a start volume of 6.5 L with the same medium composition than for flask but without glucose and ammonium sulfate. The glucose concentration of the feeding was 300 g/L and the concentration of $(NH_4)_2SO_4$ was 33 g/L. The composition of the feeding has been chosen to mimic industrial conditions of production. The culture was performed at 30 °C at a stirrer speed of 750 rpm and an air flow of 20 slpm in order to ensure purely aerobic conditions. The pH was maintained at 5 with KOH 5 M. Samples of 0.1 L are taken every 2 h until at the 10th hour of culture. During the next 3 h, samples of 0.075 L are taken every hour. Thereafter and until the end of culture, the volume of samples taken every hour is 0.05 L. These samples were used to measure biomass, glucose, nitrogen and ethanol concentration in the medium. All measurements were made in triplicate.

2.3. Analytical methods

2.3.1. Biomass

The yeast growth was followed by measuring the optical density of the culture at 650 nm with an UV–Vis spectrophotometer (Genesys 10, Thermo Electron Corporation) and by dry weight determinations. Samples (1 mL each) were centrifuged for 5 min at 10,000 rpm, washed twice with deionized water, dried for 24 h at 105 °C, and stored in a desiccator before being weighted. A correlation between dry weight and optical density was established.

2.3.2. Glucose

The glucose concentration was determined by the glucose oxidase method using an enzymatic kit assay (Glucose-RTU, Biomérieux) and the absorbance was read at 505 nm in 96-well plates with a spectrophotometric microplate reader (Epoch, BioTek).

2.3.3. Nitrogen

The nitrogen concentration was determined by the phenol-hypochlorite method. The blue color of indo-phenol was formed by the reactions of ammonia with a hypochlorite-alkaline and a phenol-nitroprusside solutions. The absorbance was read at 550 nm in 96-well plates with a spectrophotometric microplate reader (Epoch, BioTek).

2.3.4. Ethanol

The ethanol concentration was measured using an enzymatic kit assay (K-ETOH, Megazyme).

3. Problem statement and model presentation

3.1. Macroscopic modelling of baker's yeast production

On the basis of a set of biological reactions, inspired by the model of Sonnleitner and Käppeli (1986), Richelle et al. (2014)