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Macroscopic modelling of baker's yeast production in fed-batch cultures and its link with trehalose production

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ABSTRACT

A macroscopic model describing the influence of nitrogen on a fed-batch baker's yeast production process is proposed. First, on the basis of a set of biological reactions, inspired by the model of Sonnleitner and Käppeli (1986), a model in which the nitrogen and glucose consumption are coordinated is proposed. Second, an attempt of estimating trehalose concentration in yeast cells through an extension of this model is presented. The model parameters are obtained via a non-linear least squares identification. It is validated with experimental data and successfully predicts the dynamics of growth, substrate consumption (nitrogen and carbon sources) and metabolite production (ethanol and trehalose). This model allows, on the one hand, quantitatively describing the link between nitrogen and glucose consumption in yeast cultures and, on the other hand, will be valuable for the determination of culture conditions aiming at maximizing yeast productivity while guaranteeing the accumulation of a required amount of trehalose.

1. Introduction

Yeast production in fed-batch bioreactor is used for the production of a wide range of products and is an essential bioprocess in many food industries. Baker's yeast is the most prominent product for which fed-batch technique is used to reduce catabolite repression of sugar by incremental addition of carbon source molasses to the fermenter (Karakuzu, Türker, & Öztürk, 2006; Ringbom, Rothberg, & Saxén, 1996).

These last decades, quality requirements of products from food industry as well as performance and productivity in an industrial and increasingly competitive context led to an evolution in the way of controlling these processes. The control system design for a bioreactor is not an easy task due to the lack of accurate models describing cell growth and product formation, the non-linear and time-varying nature of the systems, the slow response of the process and a deficiency of reliable on-line sensors for the measurement and quantification of key state variables (Karakuzu et al., 2006; Ringbom et al., 1996).

In an industrial context, the quality parameters of commercial baker's yeast are often improved by optimizing empirically the culture conditions based on a method of trial and error. This process is long and tedious and usually leads to sub-optimal solutions. But nowadays, there is a growing need for tools to optimize and control industrial processes of yeast production. However, control tools for yeast fermenter developed at academic level mainly focus on the determination of a feeding profile of carbon source over time, to maximize performance and productivity, without taking into account other nutrient supplementation, such as nitrogen, that can influence quality parameters of the yeast as final product (Karakuzu et al., 2006; Reyman, 1992; Ringbom et al., 1996). Moreover, despite the extensive literature available on the topic of *Saccharomyces cerevisiae* production, quantitative data on the dynamics of adaptation to industrially relevant fed-batch cultures conditions are rare (van den Brink, Daran-Lapujade, Pronk, & de Winde, 2008).

It is well known that yeast growth requires coordinated uptake of carbon and nitrogen, the primary substrates for biomass production. Although the mechanisms that balance carbon and nitrogen uptake are industrially significant, as biotechnology processes can take advantage of nitrogen poor-conditions to force carbon into pathways not needed for biomass production, little understanding has been gained regarding the carbon and nitrogen coordination. Indeed, the question of this coordination during baker's yeast production is especially relevant, as nitrogen limitation has been often used to induce overproduction of intracellular carbohydrates such as trehalose that is commonly used as an indicator of a good yeast fermentation capacity and viability (Doucette, Schwab, Wingreen, & Rabinowitz, 2012).

This study proposes a macroscopic model describing the influence of carbon and nitrogen sources on the main physiological phenomena observed during the fed-batch baker's yeast

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Nomenclature

X	biomass concentration (g/L)
G	glucose concentration in bioreactor (g/L)
G^{in}	glucose concentration in feeding medium (g/L)
N	inorganic nitrogen concentration in bioreactor (g/L)
N^{in}	inorganic nitrogen concentration in feeding medium (g/L)
E	ethanol concentration in bioreactor (g/L)
O	oxygen concentration in bioreactor (g/L)
A	α-ketoglutarate concentration in cells (g/gX)
V	culture volume (L)
F	feeding rate (L/h)
k_i	pseudo-stoichiometric coefficients (g/g)
K_G	Monod constant of glucose (g/L)
K_O	Monod constant of oxygen (g/L)
K_E	Monod constant of ethanol (g/L)
K_I	ethanol inhibition constant for respiration of glucose (g/L)
K_N	Monod constant of nitrogen (g/L)
K_A	Monod constant of α-ketoglutarate (g/L)
K_{IA}	α-ketoglutarate inhibition constant for uptake rate of glucose (g/L)
K_{IA2}	α-ketoglutarate inhibition constant for uptake rate of nitrogen (g/L)
K_{I2}	nitrogen inhibition constant for uptake rate of nitrogen (g/L)
μ_{Omax}	maximum specific respiration rate (g/gX/h)
μ_{Gmax}	maximum specific uptake rate of glucose (g/gX/h)
μ_{Nmax}	maximum specific uptake rate of nitrogen (g/gX/h)

production process. The model equations, inspired from Sonnleitner and Käppeli (1986), consist of 6 ordinary differential equations containing 15 parameters, and describe the dynamics of cell growth, substrate consumption (nitrogen and carbon) and metabolite production (ethanol and trehalose). The first part of this study presents the strategy used for the determination of experimental culture conditions and the mathematical formulation of the carbon and nitrogen source coordinated uptake (Section 3). In a second part, the proposed model is validated with experimental data of yeast fed-batch cultures and numerical results obtained for the parameter estimation are presented (Section 4). Finally, in a third step, an extension of this model establishing the link with trehalose production is proposed (Section 5). Note that Section 3 (Model development) and Section 4 (Model identification and validation) are a typical illustration of a systematic methodology for mathematical modelling and parameter estimation. The workflow (comparable to standard ones as presented by Heitzig, Sin, Sales-Cruz, Glarborg, and Gani (2011)) is made of the following steps: modelling objective (process simulation and optimization, as described in this Section 1), realization of an experimental database (model-based designed as explained in Section 3.2), model structure determination (extension of the Sonnleitner and Käppeli model proposed in Section 3.3), parameter identification, sensitivity analysis, model reduction and, finally, direct and cross validation of the model (Section 4).

2. Materials and methods

2.1. Microorganism

The microorganism used in this work was a *S. cerevisiae* commercial strain (*Bruggeman*). The microorganism was maintained on Petri dishes (glucose 20 g/L, yeast extract 10 g/L, agar–agar 20 g/L) at 4 °C.

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 litre of solution): glucose, 20 g; $(NH_4)_2SO_4$, 13.5 g; yeast extract (Sigma), 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 cultures were performed during 21 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 determination of the feeding time profile is presented in Section 3.2. The glucose concentration of the feeding was 300 g/L and the concentration of $(NH_4)_2SO_4$ was varied between the different experiments: without ammonium sulfate (Experiment 1), with 33 g/L (Experiment 2), with 16.5 g/L (Experiment 3) and for Experiment 4 the concentration of $(NH_4)_2SO_4$ was 33 g/L during the first 15 h and then was switched to a feed without $(NH_4)_2SO_4$. The composition of the feeding has been chosen to mimic industrial conditions of production. The cultures were 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.

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 (three, 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).

2.3.3. Nitrogen

The nitrogen concentration was determined by the phenol-hypochlorite method (Solorzano, 1969). 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).

2.3.5. Trehalose

The determination of trehalose content was made according to the method of Parrou and François (1997) with the following modifications. Samples (triplicates) were centrifuged, and each cell pellet was stored at −20 °C. Cells hydrolysis (under alkaline conditions with Na_2CO_3 0.25 M at 95 °C during two hours; neutralized with acetic acid/acetate, resulting in 0.2 M Na acetate[pH 5.2]) and trehalose hydrolysis were done as described in Parrou and François (1997) using enzyme concentrations 0.05 U/mL of trehalase (pig kidney; Sigma) at 37 °C overnight. The released glucose was determined using an enzymatic kit assay (Biomérieux) in 96-well plates with a spectrophotometric microplate reader (Epoch, BioTek).