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Control of the production of *Saccharomyces cerevisiae* on the basis of a reduced metabolic model ⋆

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Abstract: *Saccharomyces cerevisiae* is a species of yeast with a long tradition in human history and a growing demand in industry and research. The yeast cells are produced in a series of fed batch reactors which are fed with oxygen and glucose as the main carbon source. One problem during the production process is that the cell culture can switch to the undesired production of ethanol leading to a lost batch. For improving the production process a suitable modeling and control strategy is needed that should cover the switch to ethanol production and should be able to describe the growth of the cell culture so that the operating policies can be optimized. This work presents a novel method that uses dynamic flux balance analysis to derive a reduced metabolic model from a full biochemical stoichiometric network which is then used within a model predictive control. The reduced metabolic model covers the gene regulation by using the redox metabolites as key regulators. It is shown that this modeling approach is very flexible and can be used to control and to monitor the process.

Keywords: Yeast, *Saccharomyces cerevisiae*, Biosystem, Dynamic Flux Balance Analysis, Crabtree effect, Model Predictive Control, Metabolic engineering, Switched model

1. INTRODUCTION

Saccharomyces cerevisiae is a species of yeast, which are eukaryotic microorganisms which belongs to the fungus kingdom. *S. cerevisiae* is usually known as Baker's yeast or Brewer's yeast according to its traditional application for baking, winemaking and brewing. Other applications of *S. cerevisiae* are the production of recombinant proteins or the production of bioethanol and it is an important model organism in biological research. This high variety of application ranges leads to an increasing demand of yeast cells with an annual production of 900,000 tons in Europe of which 600,000 are consumed in the European Union (www.lesaffre.com).

The production process of yeast cells starts with a sterilized culture, which is placed in several small flasks where the culture grows before it is transferred to a series of reactors, which increase in size. The reactors are semi-batch fermenters with glucose as the main carbon source where usually molasses, a byproduct of the refining of sugar canes and sugar beets, are used. Each fermenter is supplied continuously with oxygen such that aerobic conditions are guaranteed. The final step in the production process is to harvest and to dry the cells before they are packed and sold.

One problem during the production is that the cells can switch to produce ethanol, although the process is aerobic. The observation that the available glucose is then degraded via respiration and fermentation is called after its discoverer as the "Crabtree effect" (de Deken (1965)). The Crabtree effect causes not only a reduced biomass yield but it also leads to high costs because the produced batch cannot be sold.

To control the production process, a suitable control strategy is needed that both maximizes the biomass yield and avoids that the cells switch to ethanol production. The work reported here is part of the project "YeastScent" that aims at using an ion mobility spectrometry (IMS) based analytical technique for the measurements of volatile metabolites in the off-gas of yeast fermentations and integrating IMS analytics into the feed control of yeast fermentations to improve product yield and quality. The control structure is shown in figure 1 **A.** The cells are grown in a fed batch reactor with the dilution rate D at which fresh glucose is fed to the reactor. The process is continuously monitored and the measurements of the volatile metabolites are sent to the IMS. The IMS determines the concentrations of the volatile metabolites, which are used to update the plant model. The model is used for a model predictive control that determines an optimal sequence of inputs to control the process. Figure 1 **B.** shows the principle of the control. The green point means when the concentration of ethanol is in a tolerable range and the controller should optimize the dilution rate such that the yield of yeast cells is maximized. The yellow point means when the concentration of ethanol is in a critical

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range. This indicates when control may needed to steer the culture back to a tolerable range. At the red point the controller should change the dilution rate such that the cells start to metabolize the ethanol. In the worst case the model predictive control should give the information that the fermentation should be aborted at this point in time because it is not possible anymore to reduce the concentration of ethanol to a tolerable range.
Current mathematical models that describe the Crabtree

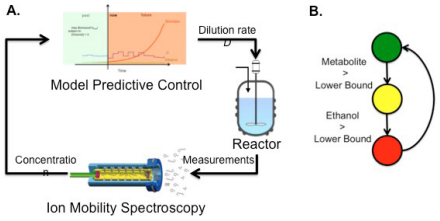

Fig. 1. **A.** Control of the process. **B.** Principle of the control.

effect are based on the observation that a part of the glucose is reduced to ethanol above a certain specific growth rate. At this rate it can be observed that the respiration rate exceeds its maximum and remains constant, as observed in Sonnleitner and Käppeli (1986) or drops as observed in van Hoek et al. (1998). These types of model are hybrid models where the specific growth rate changes when a certain threshold is exceeded (see e.g. Dantigny (1995)). These types of models do not represent the biochemistry of the cell in detail and it is difficult to consider further metabolites. Also stress reactions cannot be described with these models which can also be a reason for switching to ethanol production what may be a possible explanation that cells in some batches produce ethanol also when the feeding rates for each batch are the same.
In this work a novel approach is presented that uses Dynamic Flux Balance Analysis to derive a model and to simulate the change between different metabolic states. The model is derived from a full stoichiometric network, which is extended by nonlinear kinetics to describe the uptake of the substrates. The switch between the metabolic states is realized by using the redox metabolites as key regulators. The extended model is then analyzed and reduced by determining the elementary modes such that the reduced model depends only on the external metabolites and the regulators. The stoichiometric coefficients in the biomass equation are chosen as additionally degrees of freedom for validating the model to experimental data. This has also the advantage that stress reactions can be detected if the stoichiometric coefficient of ATP increases significantly. The model predictive control is then tested in simulations to show that with the reduced model the production process can be monitored and controlled.

2. METHODS

The modeling approach follows a dynamic optimization of the fluxes, which is known in the literature as Dynamic Flux Balance Analysis, an extension of metabolic flux analysis. Both methods are explained briefly in section 2.1 and 2.2. A detailed stoichiometric network was analyzed and reduced by determining the elementary modes of the network. The principles of elementary mode analysis are explained in section 2.3. The result is a reduced stoichiometric model which is then used for model predictive control which is explained in section 2.4.

2.1 Metabolic Flux Analysis

Metabolic Flux Analysis (MFA) is an important tool in metabolic engineering that provides the quantification of all intracellular fluxes in the central metabolism of a microorganism. MFA models the stoichiometry of a biochemical network within the cell and it can be compared with a roadmap that summarizes all possible routes from a defined start point to a final point. In MFA mostly 13C-labeled substrates are used to model the stoichiometric network by analyzing the path of the labeled C-atoms. A detailed description can be found in e.g. Wiechert (2001). One important and basic tool for MFA is the use of the stoichiometric matrix S where the coefficients of S are the stoichiometric coefficients of the internal metabolites in the reactions of the stoichiometric network. The basic relation in MFA is given by,

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \underline{S}\,\underline{v}. \tag{1}$$

with $S \in \mathbb{R}^{m\times n}$ and $v \in \mathbb{R}^n$ where m is the number of metabolites and n is the number of reactions. x is the vector of the concentrations with $x \in \mathbb{R}^m$. In MFA it is assumed that the system is in steady state, i.e.

$$\underline{S}\,\underline{v} = 0. \tag{2}$$

which is the fundamental relation in MFA. MFA has the advantage that the problem is linear and a detailed knowledge of the usually nonlinear reaction rates is not necessary. In the most cases S is underdetermined and by choosing a suitable objective function linear programming can be applied to calculate the unknown fluxes.

2.2 Dynamic Flux Balance Analysis

Dynamic Flux Balance Analysis (DFBA) is an extension of MFA. In DFBA also the change of the concentrations of the external metabolites are taken into account. For a typical yeast producing batch reactor the external concentrations are described by:

$$\frac{\mathrm{d}G}{\mathrm{d}t} = -v_G X \tag{3}$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = v_P X \tag{4}$$

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \mu X \tag{5}$$

where G is the concentration of the subtrate, P is the concentration of the product and X is the concentration of the yeast biomass, which reflects the growth of the cell culture. v_G, v_P are the reaction rate for the substrate or the product respectively and μ is the specific growth rate. It is assumed that the internal metabolites equilibrates rapidly in the presence of external disturbances (Stephanopoulos et al. (1998)) such that the rates can be determined by equation 2. The rate v_G is usually calculated by assuming a Monod kinetic and the unknown rates, here v_P and μ, are calculated by solving the linear optimization problem,