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State estimation and predictive control of fed-batch cultures of hybridoma cells

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ABSTRACT

Fed-batch cultures of hybridoma cells are commonly used for the production of monoclonal antibodies (MAb). In this study, a simple macroscopic model of the cell culture is used, which is based on the overflow metabolism paradigm. This allows to specify optimal culture conditions, and the natural formulation of a nonlinear model predictive control strategy (NMPC). As not all the component concentrations are available for measurement, system observability is analyzed, and an unscented Kalman filter (UKF) is designed, which provides satisfactory estimates of glucose and glutamine concentrations. Robustness of the NMPC scheme is investigated, as well as the combined UKF+NMPC scheme, through a minimax robust version and the closed-loop system.

1. Introduction

Hybridoma cells are important vectors for the production of monoclonal antibodies, and the pharmaceutical sector is paying more and more attention to Process Analytical Technologies (PAT) and process control for improving bioprocess yield or productivity. Earlier optimization studies, such as in [1,2], were conducted on the basis of simple macroscopic mass balance models established from experimental data. In this study, we proceed with the same philosophy, but using a slightly more elaborate kinetic model suggested in [3], which takes metabolic changes and especially overflow metabolism into account. This metabolic phenomenon is induced when the rate of glycolysis exceeds the cell respiratory capacity, i.e., the capacity to oxidize substrates. Depending on the substrate concentration, the cell metabolism is divided in two pathways: the respirative mode below a certain critical substrate level, and the respiro-fermentative mode when substrate is in excess, leading to the formation of inhibitory by-products (lactate and ammonia).

To drive the bioprocess close to an optimum, while avoiding this undesirable effect, a closed-loop optimizing strategy proposed in [4] is used. Various forms of optimizing control of bioprocesses have been proposed in the literature, including adaptive, probing, robust or predictive control as discussed for instance in [5–8], respectively. In this study, we give preference to nonlinear model predictive control, which offers a natural and widely accepted framework for the formulation of an optimal control under constraints. There is a vast and rich literature with overviews on NMPC developments, research, and applications (e.g., [9,10]). Some of these works address the problem of robust NMPC of fed-batch processes (e.g. [11,12]). An overview of recent developments can be found in [13] and references therein.

A simple and efficient approach to maintain hybridoma cultures in the optimal operating conditions is to regulate the glucose and glutamine concentrations at the critical levels [14]. However, reliable glucose and glutamine probes are currently rare and/or very expensive on the market and an interesting alternative is to design software sensors, which are at the same time cheap and reliable, and can be used for online measurement, as in [15–19]. In this study, an unscented Kalman filter is designed for online estimation of glucose and glutamine in hybridoma cell fed-batch cultures based on the considered available measurements, e.g., biomass, lactate and ammonia, and the robustness of the scheme is investigated.

This paper is organized as follows. In Section 2, the mathematical model of HB-58 [3] is briefly described, whereas in Section 3 optimal operating conditions are devised. A NMPC algorithm is

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presented in Section 4 and robustness to parametric uncertainties is assessed. State estimation and the design of an unscented Kalman filter is addressed in Section 5. Finally, NMPC is coupled with the state estimation scheme and tested in situations involving parametric uncertainties and measurement noise in Section 6. Conclusions and perspectives end this paper in Section 7.

2. Overflow metabolism of hybridoma cells

The following macroscopic reactions are based on the reduced metabolic network of HB-58 [3]:

$$\text{Glucose consumption:} G \xrightarrow{\varphi_G} a\,X + b\,L \tag{1a}$$

$$\text{Glutamine consumption:} Gn \xrightarrow{\varphi_{Gn}} c\,X + d\,N \tag{1b}$$

$$\text{Glucose overflow:} G \xrightarrow{\varphi_{Over-G}} 2\,L \tag{1c}$$

$$\text{Glutamine overflow:} Gn \xrightarrow{\varphi_{Over-Gn}} N + \frac{1}{2}L \tag{1d}$$

where X, G, Gn, L and N are, respectively, the concentrations of biomass, glucose, glutamine, lactate and ammonia. a, b, c and d are the stoichiometric coefficients, and φ_i ($i = G, Gn, Over-G, Over-Gn$) the reaction rates given by the discontinuous overflow kinetic model recalling the bottleneck of [20]:

$$\varphi_G = \min(\varphi_{G1}, \varphi_{G\ \max}) \tag{2a}$$

$$\varphi_{Gn} = \min(\varphi_{Gn1}, \varphi_{Gn\ \max}) \tag{2b}$$

$$\varphi_{Over-G} = \max(0, \varphi_{G1} - \varphi_{G\ \max}) \tag{2c}$$

$$\varphi_{Over-Gn} = \max(0, \varphi_{Gn1} - \varphi_{Gn\ \max}) \tag{2d}$$

where each rate is the product of Monod-type specific consumption rates r_i ($i = G1, Gn1, G_{max}, Gn_{max}$) and the concentration of viable biomass X_v as in:

$$\varphi_{G1} = r_{G1}\ X_v = \mu_{Gmax1}\frac{G}{K_G + G}\frac{Gn}{K_{Gn1} + Gn}X_v \tag{3a}$$

$$\varphi_{Gn1} = r_{Gn1}\ X_v = \mu_{Gnmax1}\frac{Gn}{K_{Gn} + Gn}\frac{K_N}{K_N + N}X_v \tag{3b}$$

$$\varphi_{G\ \max} = r_{G\ \max}\ X_v = \mu_{Gmax2}X_v \tag{3c}$$

$$\varphi_{Gn\ \max} = r_{Gn\ \max}\ X_v = \mu_{Gnmax2}X_v \tag{3d}$$

where μ_{imaxj} ($i = G, Gn, j = 1, 2$) are the maximum values of the specific rates and K_G, K_{Gn1} and K_{Gn} are the saturation coefficients. K_N is the ammonia inhibition constant over the oxidation of glutamine.

This model is inspired from Sonnleitner's kinetic model [20], which was first applied to the baker's yeast strain called *Saccharomyces cerevisiae* and which is based on the idea that the strain metabolism is ruled by its respiratory capacity. When the substrate is in excess (for instance, glucose concentration is above a critical level $G > G_{crit}$ and the consumption rate $r_{G1} > r_{G_{\max}}$), the cells produce lactate through the fermentative pathway, and the culture is said to be in respiro-fermentative (RF) mode. On the other hand, when substrate becomes limiting (for instance, glucose concentration is below a critical level $G < G_{crit}$ and the substrate consumption rate $r_{G1} < r_{G_{\max}}$), the available substrate, and possibly the byproduct (as a substitute carbon source), if present in the culture medium, are oxidized (if the strain is able to oxidize it, which is not the case for HB-58). The culture is then said to be in respirative (R) regime. This metabolic mechanism is also applicable in parallel to glutamine and ammonia. However, it is important to note that oxygen is not represented as the system is assumed to be perfectly oxygenated and, consequently, metabolic switches are essentially due to substrate variations.

Table 1
Parameter values and their respective variation coefficients obtained using data sets from [3].

μ_{Gmax1}	1.0006 h^{-1}	8.23%
μ_{Gmax2}	0.0283 h^{-1}	5.71%
μ_{Gnmax1}	0.1992 h^{-1}	7.92%
μ_{Gnmax2}	0.0203 h^{-1}	3.64%
μ_{dmax}	0.0111 h^{-1}	10.73%
K_G	23.235 mM	11.99%
K_{Gn}	0.0004 mM	18.21%
K_N	0.9931 mM	11.86%
K_{Gn1}	0.0005 mM	21.81%
a	1.1462×10^5 cells/mM of G	6.46%
b	1.2939 mM of L/mM of G	6.90%
c	0.1186×10^5 cells/mM of Gn	21.52%
d	0.3000 mM of N/mM of Gn	6.39%
m_G	0.0367 mM mL/10^5 cells	1.80%
K_{Gd}	2.4888 mM	25.60%
K_{Gnd}	0.0020 mM	117.19%

Mass balances on each component yield the following differential equations:

$$\frac{dX_v}{dt} = a\varphi_G + c\varphi_{Gn} - \mu_d X_v - DX_v \tag{4a}$$

$$\frac{dX_d}{dt} = \mu_d X_v - DX_d \tag{4b}$$

$$\frac{dG}{dt} = -\varphi_G - m_G X_v - \varphi_{Over-G} + D(G_{in} - G) \tag{4c}$$

$$\frac{dGn}{dt} = -\varphi_{Gn} - \varphi_{Over-Gn} + D(Gn_{in} - Gn) \tag{4d}$$

$$\frac{dL}{dt} = b\varphi_G + 2\varphi_{Over-G} + \frac{1}{2}\varphi_{Over-Gn} - DL \tag{4e}$$

$$\frac{dN}{dt} = d\varphi_{Gn} + \varphi_{Over-Gn} - DN \tag{4f}$$

$$\frac{dV}{dt} = DV \tag{4g}$$

where m_G is the maintenance coefficient of glucose (note that maintenance on glutamine is not considered as identification results obtained in [3] led to negligible values as compared to oxidation and overflow), V the reactor volume, $D = F_{in}/V$ the dilution rate, F_{in} the inlet feed rate and G_{in} and Gn_{in} are the substrate concentrations in the feed medium. X_d represents the dead biomass concentration and μ_d the corresponding rate given by:

$$\mu_d = \mu_{dmax}\frac{K_{Gd}}{K_{Gd} + G}\frac{K_{Gnd}}{K_{Gnd} + Gn} \tag{5}$$

The substrate inhibition terms of (5) simply mean that cell death is limited as long as there are enough glucose and glutamine in the bioreactor.

The parameter values listed in Table 1 were obtained following an identification procedure comparable to the one in [3] using the same data sets. The only difference with these previous results comes from the combination of sets used for direct and cross validations. Moreover, Table 1 also provides the variation coefficients of the estimated parameters, i.e., the square roots of the diagonal components in the inverse Fisher information matrix [21], normalized by the respective parameter values. During the identification procedures, the operating parameters, i.e. G_{in} and Gn_{in} have been respectively fixed to 15 and 4 mM. These values will be used throughout the next sections.

3. Optimal feed trajectory

The productivity optimum is easy to determine when μ_d is low, i.e., when there is enough glucose and glutamine in the bioreactor,