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Hybridoma cell culture optimization using nonlinear model predictive control

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Abstract: This work addresses the application of control systems to the optimization of a monoclonal antibodies (MAb) production chain. The attention is focused on the maximization of hybridoma fed-batch culture productivity. The proposed model presents kinetics showing strong nonlinearities through min-max functions expressing overflow metabolism. A nonlinear model predictive control (NMPC) algorithm, choosing the best trajectory over a moving finite horizon among different sequences of inputs, is suggested in order to optimize productivity. Sensitivities of selected objective functions are considered in a minimax robust version of the NMPC in order to choose the best configuration with respect to practical operating conditions.

Keywords: hybridoma cultures, overflow metabolism, nonlinear model predictive control, bioprocess optimization

1. INTRODUCTION

Biopharmaceuticals spare today a lot of attention to any mean or device improving bioprocess yields. In a production chain of monoclonal antibodies (MAb), different key-operations are managed and optimized (i.e., cultivation, purification, filtration, capture, polishing steps,etc.). This work is focused on the productivity (compound production in a minimum of time) optimization of hybridomas expressing these MAb. In de Tremblay et al. (1992) and Dhir et al. (2000), optimisation studies are conducted on the basis of simple macroscopic mass balance models established from experimental data. In Amribt et al. (2013), a kinetic model taking into account metabolic changes is suggested for the strain HB-58 that could be used for process optimization. Indeed, hybridoma, as cells like yeast or bacteria, presents metabolic changes in presence of feeding overflow. This metabolic phenomenon, induced when the rate of glycolysis exceeds the cell respiratory or oxidative capacity (i.e., the capacity to oxidize substrates), is called "Overflow metabolism" or "short-term Crabtree effect". The cell metabolism is consequently divided in two pathways: the oxidative or respirative regime, and the respiro-fermentative regime when substrate is in excess, leading to the formation of by-products generally inhibiting the oxidative capacity. To avoid this undesirable effect, a closed-loop optimizing strategy is required, which could take various forms including nonlinear closed-loop strategies based on adaptive, probing, robust or predictive control respectively as in Pomerleau (1990), Akesson (1999), Dewasme et al. (2010) and Santos et al. (2012).

Nonlinear model predictive control (NMPC) is suitable especially for nonlinear fed-batch processes where a trajectory needs to be followed from the prediction of a nonlinear model. For these processes NMPC uses the nonlinear dynamic model to predict the effect of sequences of control steps on the controlled variables. There is a vast and rich literature with overviews on NMPC developments, research, and applications (e.g., Qin and Badgwell (2003), Lee (2011)). Some of these works address the problem of robust nonlinear model predictive control of fed-batch processes (e.g. Nagy and Braatz (2003), Nagy and Braatz (2004)). An overview of more recent developments on NMPC can be found in Magni et al. (2009) and references therein.

In this work, a NMPC algorithm comparable to Santos et al. (2012) is designed in order to optimize the HB-58 culture productivity taking model uncertainties and practical laboratory conditions into account.

In section 2, the mathematical model of HB-58 (Amribt et al. (2013)) is briefly described and optimal closed-loop control objectives are suggested and discussed. The proposed NMPC algorithm is presented and performance is assessed in simulation comparing previously selected objective functions. The robustness facing parameter uncertainties is discussed in section 4. Conclusions and perspectives end this paper in section 5.

2. HYBRIDOMA CULTURE MODEL AND CONTROL OBJECTIVES

In the following subsection, the hybridoma HB-58 model of Amribt et al. (2013) is considered. Control objectives are derived from this model in the next subsection.

2.1 Bioreactor model

The following macroscopic reactions are extracted from the reduced metabolism network of HB-58 (Amribt et al. (2013)):

$$\text{Glucose consumption}: \quad G \overset{\varphi_G}{\rightarrow} aX + bL \tag{1a}$$
$$\text{Glutamine consumption}: \quad Gn \overset{\varphi_{Gn}}{\rightarrow} cX + dN \tag{1b}$$
$$\text{Glucose overflow}: \quad G \overset{\varphi_{Over-G}}{\rightarrow} 2L \tag{1c}$$
$$\text{Glutamine overflow}: \quad Gn \overset{\varphi Over-Gn}{\rightarrow} N + \frac{1}{2}L \tag{1d}$$

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 following discontinuous overflow kinetic model recalling the bottleneck of Sonnleitner and Käppeli (1986):

$$\varphi_G = \min(\varphi_{G1}, \varphi_{G\ max}) \tag{2a}$$
$$\varphi_{Gn} = \min(\varphi_{Gn1}, \varphi_{Gn\ max}) \tag{2b}$$
$$\varphi_{Over-G} = \max(0, \varphi_{G1} - \varphi_{G\ max}) \tag{2c}$$
$$\varphi_{Over-Gn} = \max(0, \varphi_{Gn1} - \varphi_{Gn\ max}) \tag{2d}$$

where each rate corresponds to the following Monod-type consumption rates r_i ($i = G1, Gn1, G2, Gn2$) times 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 $\mu_{imax\,j}$ ($i = G, Gn,\ j = 1, 2$) are the maximum values of the specific rates and K_G, K_{Gn1} and K_{Gn} are the saturation coefficients. K_N is the ammonium inhibition constant over the oxidation of glutamine.

Sonnleitner's kinetic model was first applied to the baker's yeast strain called *Saccharomyces cerevisiae* and is based on the idea that the strain metabolism is ruled by its respiratory capacity. Indeed, during a culture, the cells are likely to change their metabolism because of their limited oxidative capacity. When the substrate is in excess (for instance, glucose concentration is above a critical level $G > G_{crit}$ and the respective consumption rate, $r_{G1} > r_{G_{max}}$), the cells produce a byproduct (here, L) through the fermentative pathway, and the culture is said to be in (respiro-) fermentative (RF) regime. 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.

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 Amribt et al. (2013) led to negligible values as compared to oxidation and overflow), V the reactor volume, $D = \frac{F_{in}}{V}$ 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 used by Amribt et al. (2013) using the same data sets and can be considered as extended results of Amribt et al. (2013). The only difference with previous results comes from the combination of sets used for simple and cross validations.

2.2 Control objectives

In Santos et al. (2010) and Santos et al. (2012), practical aspects behind the choice of the best optimizing criterion are discussed considering, of course first, the simple formulation of the current productivity optimization with respect to the product of interest (here, we speak about biomass productivity optimization represented by $P = \frac{X_v V}{t_f}$ where t_f represents the culture time) but then also the number of measurements, the existence

Table 1. Parameter values of Amribt et al. (2013)'s model

μ_{Gmax1}	1.0006 h^{-1}
μ_{Gmax2}	0.0283 h^{-1}
μ_{Gnmax1}	0.1992 h^{-1}
μ_{Gnmax2}	0.0203 h^{-1}
μ_{dmax}	0.0111 h^{-1}
K_G	23.235 mM
K_{Gn}	0.0004 mM
K_N	0.9931 mM
K_{Gn1}	0.0005 mM
a	1.1462 10^5 cells/mM of G
b	1.2939 mM of L/mM of G
c	0.1186 10^5 cells/mM of G
d	0.3000 mM of N/mM of G
m_G	0.0367 mM mL/10^5 cells
K_{Gd}	2.1862 mM
K_{Gnd}	0.0020 mM

of the corresponding probes, their price and, eventually, the possibility to express the optimizing criterion.

In this work, 4 optimizing criteria are considered:

$$\phi_1 = -\sum_{i=1}^{p} X_{k+i} \tag{6a}$$

$$\phi_2 = \sum_{i=1}^{p} \left| r_{G1,k+i} - r_{Gmax,k+i} \right| + \sum_{i=1}^{p} \left| r_{Gn1,k+i} - r_{Gnmax,k+i} \right| \tag{6b}$$

$$\phi_3 = \sum_{i=1}^{p} \left| r_{Gn1,k+i} - r_{Gnmax,k+i} \right| \tag{6c}$$

$$\phi_4 = \sum_{i=1}^{p} \left| r_{G1,k+i} - r_{Gmax,k+i} \right| \tag{6d}$$

calculated over a time horizon of length p starting at k.

- ϕ_1 considers live biomass concentration and not production ($X_v\,V$), assuming small changes in volume during the culture. In practice, a 1 liter bioreator is indeed initiated at a level around 30 % of its maximum volume and the fed-batch culture ends around 70 to 80 % in order to avoid possible tank overflows. The final volume is then generally twice or three times the initial one while biomass concentration goes through different orders, starting, for instance, at order 0.1 and ending at order 1 or even 10, depending on the feeding density. In this work, predictive control horizons strictly smaller than the culture time are used, inducing very small volume variations and essentially biomass concentration variations, justifying the choice of ϕ_1.
- ϕ_2 represents the distance between any value of r_{G1} and r_{Gn1} and their optimal values r_{Gmax} and r_{Gnmax}, also limiting the production of inhibitory byproduct N. ϕ_2 is then the expression of the productivity optimum, comparable to what was obtained in Santos et al. (2012) for *E. coli* but extended to two substrates saturating the respiratory capacity.

Even if this productivity optimum appears as evident as long as μ_d remains relatively low (i.e., there are enough glucose and glutamine in the bioreactor following (5)) in (4a), it is interesting to see how this optimum is evolving with the ammonium concentration following (3b). Indeed, this optimum is not unique as there exists an infinity of pairs (N_{crit}, Gn_{crit}) which guarantee that $(3b) = (3d)$ while G_{crit} depends on Gn_{crit} as shown in (3a). Following (4f), ammonium is a metabolic byproduct which is actively produced during glutamine overflow but also during glutamine oxidation, which is an essential reaction for cell survival. This implies that the ammonium quantity will increase all along the culture and, consequently, that the optimum productivity will change.

Expressions of the critical substrate concentrations can be obtained by equating (3a) and (3b) respectively with (3c) and (3d), providing:

$$G_{crit} = \frac{K_G \mu_{Gmax2}(K_{Gn1} + Gn_{crit})}{\mu_{Gmax1} Gn_{crit} - \mu_{Gnmax2}(K_{Gn1} + Gn_{crit})} \tag{7a}$$

$$Gn_{crit} = \frac{K_{Gn} \mu_{Gnmax2}(K_N + N_{crit})}{\mu_{Gnmax1} K_N - \mu_{Gnmax2}(K_N + N_{crit})} \tag{7b}$$

From (7b) and the general positivity of a concentration, it is obvious that there is an existence condition of Gn_{crit} on N. Indeed, $Gn_{crit} > 0$ implies the following upper bound N_U:

$$N < N_U = 8.75\ mM \tag{8}$$

which can be interpreted as a limit of ammonium inhibition on the cell glutamine oxidative capacity. In other words, if this limit is exceeded, the cells become unable to reach the critical rate of glutamine oxidation (3d). Moreover, two critical concentrations G_{crit} and Gn_{crit} lead to two optimal feeding profiles. Considering that G and Gn are constant, we obtain respectively from (4c) and (4d):

$$F_G = \frac{\mu_{Gmax2} + m_G}{G_{in} - G_{crit}} V X_v \tag{9a}$$

$$F_{Gn} = \frac{\mu_{Gnmax2}}{Gn_{in} - Gn_{crit}} V X_v \tag{9b}$$

As model (4) only considers an inhibition of ammonium on the oxidative capacity, any glucose concentration $G \geq G_{crit}$ is sufficient to reach (3c). One can now wonder if applying F_{Gn} could be sufficient to obtain $G \geq G_{crit}$, which, rewritten in terms of feeding profiles, corresponds to:

$$F_{Gn} \geq F_G \tag{10}$$

Using (9) in (10), we obtain $G_{crit} \geq 2.37\ 10^{-4}\ mM$ which, complemented by (8) leads to a lower bound $N_L = 2.63\ mM$ such that:

$$N_L < N < N_U \tag{11}$$

It appears anyway that even if the upper bound is independent of the feed medium composition and is therefore fixed by the model parameter values, it is not the case for the lower bound. It is then of interest to consider the evolution of this lower bound with respect to G_{in} and Gn_{in}. Starting from (10), and considering the parameter values defined in Table 1, an inequation of the second order in Gn_{crit} parameterized by G_{in} and Gn_{in} is obtained and solved in Figure 1.

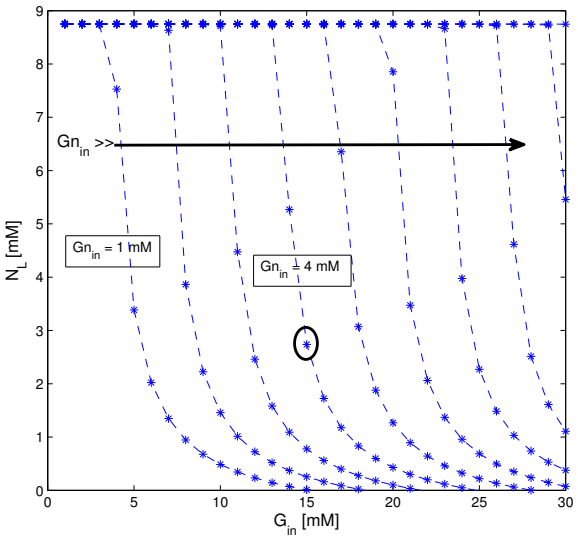

Fig. 1. Evolution of N_L with respect to the medium composition, i.e., G_{in} and Gn_{in}. The arrow indicates the increasing concentrations of Gn_{in} while the circle indicates the N_L value of the current operating conditions (i.e. $G_{in} = 15\ mM$ and $Gn_{in} = 4\ mM$).

Figure 1 shows the solutions of (10) when coupled to (7b) in order to get the corresponding N_L values. This graph represents the evolution of N_L with respect to different values of G_{in} and Gn_{in}. It appears that the media composition plays an important role in the possibility to optimize the cell growth as there exist couples (G_{in},Gn_{in}) such that $N_L = N_U$, i.e., such that (11) is not respected.

An important conclusion on the way to design the experiments is drawn and the following theorem holds:

Theorem 2.2.1 *Provided that there is a significative but not excessive ammonium concentration in the bioreactor respecting (11), $F_{Gn}(N_{crit})$ as defined in (9b), is always an optimal feeding profile.*

- ϕ_3 (6c) and ϕ_4 (6d) are therefore proposed in order to validate theorem 2.2.1. Indeed, if (11) is respected when finishing the batch phase, the profile tracking the minimization of ϕ_3 should lead to the same solution as the profile minimizing ϕ_2 while the one generated by the minimization of ϕ_4 should be sub-optimal, following (10), in terms of biomass productivity over a fixed culture time.

3. NONLINEAR MODEL PREDICTIVE CONTROL

A nonlinear model predictive control (NMPC) strategy comparable to Santos et al. (2012) is applied to maximize the production of biomass within a defined time, using the minization of one of the objective functions defined in (6), together with a penalization of the control moves in order to avoid too important feeding variations eventually leading to local minima as in:

$$\psi_i = \phi_i + \sum_{j=1}^{m} (F_{\text{in},k+j-1} - F^{\text{ref}}_{\text{in},k+j-1}) \qquad (12)$$

where $i = 1,2,3$, p is the prediction horizon, and m is the control horizon, with $m \leqslant p$. F_{in} is the manipulated variable, $F^{\text{ref}}_{\text{in}} = F_{in,k+j-m}$ is the feed rate of reference.

Table 2. Productivities obtained for different objective functions

Objective function	Productivity $[10^7 cells/h]$
ψ_1	3.50
ψ_2	3.59
ψ_3	3.59
ψ_4	3.40

The NMPC problem is then stated as

$$\min_{\boldsymbol{u}} \psi \qquad (13a)$$

$$\text{s.t.} \quad \dot{x} = f(x,u,\theta) \qquad (13b)$$

$$u(t) = u(t_{j-1+m}),\ t \in [t_{j+m}, t_{j+p}] \qquad (13c)$$

$$\boldsymbol{x}_{\text{L}} \leqslant \boldsymbol{x} \leqslant \boldsymbol{x}_{\text{U}} \qquad (13d)$$

$$\boldsymbol{u}_{\text{L}} \leqslant \boldsymbol{u} \leqslant \boldsymbol{u}_{\text{U}} \qquad (13e)$$

$$\Delta u_{\text{L}} \leqslant \Delta u_{j-1} \leqslant \Delta u_{\text{U}},\ j = 1,\cdots,m \qquad (13f)$$

where (13b) is the process model, x and u are respectively the vector of state and control variables, and θ is the vector of parameters. The subscripts $_{\text{L}}$ and $_{\text{U}}$ stand respectively for lower and upper. In this case study $\boldsymbol{u} = \{F_{\text{in},k}, \cdots, F_{\text{in},k+m-1}\}$ is the feed rate policy over a control horizon of m sampling time intervals, and $\boldsymbol{x}$ is the augmented vector of the state predictions $\{x_{k+1}, \cdots, x_{k+p}\}$. Linear inequalities (13f) enforce control move rate constraints over the control horizon.

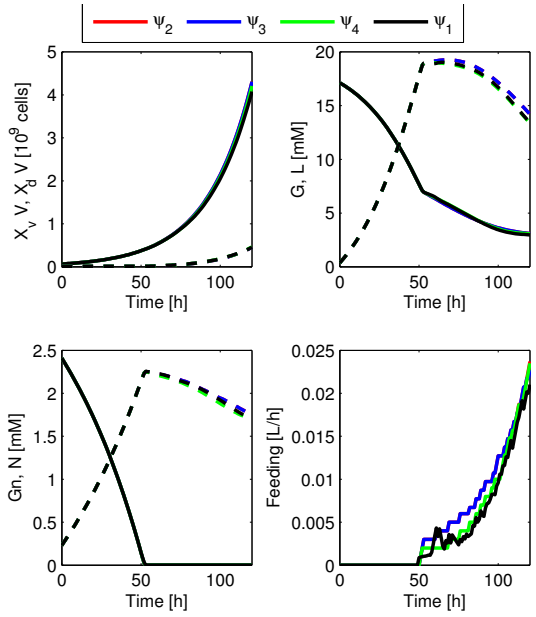

Fig. 2. Comparison of the NMPC performances using ψ_i ($i = 1,2,3,4$) as objective functions. X_vV (alive biomass production), G and Gn are in solid line and X_dV (dead biomass production), L and N are in dashed line.

In order to compare the performances of the cost functions (12), simulations of a batch starting with the following initial and operating conditions, are performed:

$X_{v0} = 1.85\ 10^5\ cells/mL$, $X_{d0} = 0.25\ 10^5\ cells/mL$, $G_0 = 17.17mM$, $Gn_0 = 2.41mM$, $L_0 = 0.36mM$, $N_0 = 0.23mM$, $V_0 = 0.35L$, $G_{in} = 15mM$, $Gn_{in} = 4mM$, $p = 3$, $m = 1$ and the final culture time is fixed to $t_f = 120\ h$. For a limited computation and gain of time, the sampling period is chosen equal to $t_s = 1\ h$.

Results are shown in Figure 2 and biomass productivity values are listed in Table 2. Initial conditions force the controller to

remain at zero until the glutamine is nearly depleted (around 50 h). At this moment, the batch phase ends and the first strictly positive inputs are calculated by the controller in order to catch an exponential trajectory, function of the criterion used among (6). The best values are obtained for ψ_2 and ψ_3 confirming theorem 2.2.1. However, performances of ψ_1 are less than 5 % lower than the best case while less than 10 % for ψ_4, which means that these criteria, failing to lead to the optimum, remain in a close neighborhood.

4. PRACTICAL CONDITIONS - MINIMAX ROBUST NMPC

The use of NMPC assumes a perfect knowledge of the model parameters and the availability of all the state measurements. Even if having all the probes is achievable in practice (or by means of observers as in Dewasme et al. (2013)), a perfect model parameter identification is impossible to achieve and a more robust version of the NMPC algorithm is then required. Taking into account results of section 3, only ψ_1 and ψ_3 are considered in the following (ψ_4 presents the lowest productivity and ψ_2 is comparable to ψ_3). Performances of the NMPC algorithm using ψ_1 and ψ_3 in the presence of parameter uncertainties are now assessed. This new study could be lead for all model parameters but following the structure of (6c), it is legitimate to think that sensitivity of the optimization with respect to model uncertainties is essentially due to μ_{Gnmax1}, μ_{Gnmax2}, K_{Gn} and K_N. To justify this last sentence, note that even if the general model used to calculate state trajectories is not perfect, its influence on the states is attenuated by the reinitialization performed by the new measurements at each sampling period t_s while the influence on the objective function is direct and never damped.

A robust formulation of the predictive controller is therefore proposed based on the solution at each sampling period of a minimax problem:

$$\min_{\boldsymbol{u}} \max\ \omega_{i1},\cdots,\omega_{i\eta} \tag{14a}$$

$$\text{s.t.}\quad (13b-13f) \tag{14b}$$

where each cost function, ω_{ij}, $j=1,\cdots,\eta$, defined as in (12), is evaluated at one of the vertices of the uncertainty parameter polytope.

μ_{Gnmax1}, μ_{Gnmax2}, K_{Gn} and K_N nominal values are assumed to be influenced by uncertainties of maximum 15 percents (i.e., parameter identification is assumed to be well achieved but naturally tarnished by uncertainties). Table 3 shows the productivity means, maxima and standard deviations obtained for parameter variations applied first independently and then, in combination with others following a series of 14 times 50 runs.

Globally, the sensitivities of the algorithms using different objective functions reach the same order as the number of uncertain parameters increases ($O(10^6 cells/h)$). Anyway, ψ_3 still presents higher productivities and also more robustness with respect to parameter sets in which μ_{Gnmax2} does not appear.

The algorithm is mainly sensitive to μ_{Gnmax1} and μ_{Gnmax2} (see Table 3) independently of the criterion. Reminding that these parameters define the metabolic threshold separating both pathways, it appears clearly that if μ_{Gnmax1} is underestimated, the productivity will decrease as the true critical level is never reached, while if μ_{Gnmax1} is overestimated, more ammonium will be produced generating an increase of G_{ncrit} (following (7b)) and a faster drift of the optimum which is, however, not so detrimental for the productivity as this optimum (G_{ncrit},N_{crit}) is never reached (as always moving) but correctly tracked. As all vertices of the polytope defined by the maximum parameter variations are considered before selecting the worst productivity level as in (14), Figure 3 shows consequently that the minimax algorithm using ψ_1 as objective function is likely to always reach a good productivity level if μ_{Gnmax1} is overestimated.

On the other hand, K_{Gn} and K_N do not severely alter the final productivity when using ψ_3 as standard deviations concerning these parameters alone never exceed 5 10^4 cells/h.

The algorithm using ψ_1 as objective function presents standard deviations of an order of 10^6 cells/h (i.e., from 0.5 to 1 10^6 cells/h), regardless of the uncertain parameter set. This means that a good identification of μ_{Gnmax2} is a sufficient condition of robustness if ψ_3 is used as objective function while ψ_1 requires a more accurate identification of all the model parameters. Moreover, considering that ψ_3 basically reaches higher productivities than ψ_1, the choice of the user should be oriented towards ψ_3 in realistic experimental conditions.

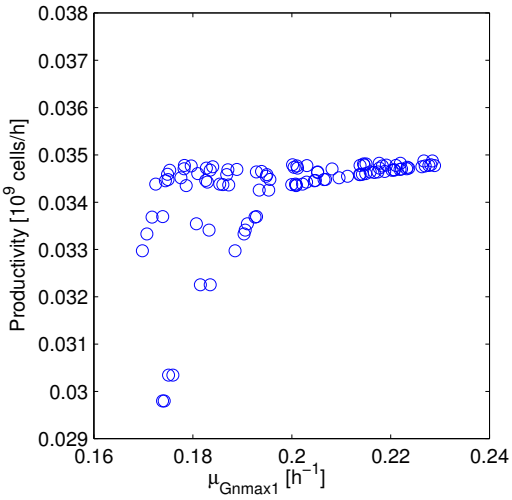

Fig. 3. Productivities obtained during 50 runs where μ_{Gnmax1} undergoes variations using ω_1.

5. CONCLUSION

In this work, optimization of hybridoma HB-58 fed-batch culture productivity using a mathematical model assuming overflow metabolism is performed. Nonlinearities and discontinuities of such biological kinetic models generally require the use of advanced controllers able to track unstable trajectories following exactly the boundary of two metabolic pathways. NMPC algorithms, optimizing sequences of trajectories over a finite horizon on the basis of objective cost functions minimization ψ, are able to solve this optimality problem as long as the minimum of ψ corresponds to the productivity optimum. To illustrate this idea, four different mathematical criterions have been chosen and compared in terms of biomass productivity over a finite culture time. It appears clearly that, even if each of them leads to a satisfactory productivity level, only two of them represent the right cost functions to reach the productivity optimum: ψ_2, representing both metabolic overflows on glucose and glutamine and ψ_3, representing the overflow of glutamine

Table 3. Productivitiy means, maxima and standard deviations for 14 series of 50 runs considering two objective functions ψ_1 and ψ_3 and possible parameter uncertainties (i.e., a total of 1400 runs)

Parameters	Prod. mean $[10^7 cells/h]$		Prod. max $[10^7 cells/h]$		Prod. stand. dev. $[10^7 cells/h]$	
	ψ_1	ψ_3	ψ_1	ψ_3	ψ_1	ψ_3
μ_{Gnmax1}	3.42	3.56	3.49	3.57	$1.00\ 10^{-1}$	$5.58\ 10^{-3}$
μ_{Gnmax2}	3.31	3.43	3.43	3.59	$6.35\ 10^{-2}$	$2.3\ 10^{-1}$
K_{Gn}	3.41	3.56	3.48	3.57	$9.94\ 10^{-2}$	$3.35\ 10^{-3}$
K_N	3.42	3.56	3.47	3.57	$7.92\ 10^{-2}$	$3.65\ 10^{-3}$
$\mu_{Gnmax1}, \mu_{Gnmax2}$	3.41	3.53	3.49	3.59	$1.30\ 10^{-1}$	$8.73\ 10^{-2}$
μ_{Gnmax1}, K_{Gn}	3.45	3.56	3.5	3.57	$7.03\ 10^{-2}$	$5.09\ 10^{-3}$
μ_{Gnmax1}, K_N	3.44	3.56	3.5	3.57	$9.74\ 10^{-2}$	$6.18\ 10^{-3}$
μ_{Gnmax2}, K_{Gn}	3.43	3.54	3.48	3.59	$6.71\ 10^{-2}$	$9.64\ 10^{-2}$
μ_{Gnmax2}, K_N	3.42	3.55	3.47	3.59	$5.90\ 10^{-2}$	$9.1\ 10^{-2}$
K_{Gn}, K_N	3.45	3.56	3.5	3.57	$6.52\ 10^{-2}$	$5.17\ 10^{-3}$
$\mu_{Gnmax1}, \mu_{Gnmax2}, K_{Gn}$	3.46	3.56	3.51	3.59	$4.55\ 10^{-2}$	$6.01\ 10^{-2}$
$\mu_{Gnmax1}, \mu_{Gnmax2}, K_N$	3.42	3.55	3.47	3.59	$7.92\ 10^{-2}$	$9.03\ 10^{-2}$
$\mu_{Gnmax1}, K_{Gn}, K_N$	3.45	3.56	3.53	3.57	$6.87\ 10^{-2}$	$4.37\ 10^{-3}$
$\mu_{Gnmax2}, K_{Gn}, K_N$	3.4	3.55	3.49	3.59	$1.00\ 10^{-1}$	$7.95\ 10^{-2}$
$\mu_{Gnmax1}, \mu_{Gnmax2}, K_{Gn}, K_N$	3.45	3.53	3.52	3.59	$7.03\ 10^{-2}$	$9.46\ 10^{-2}$

only, based on an existence condition on ammonium. Considering those productivity levels but also practical conditions, two criterions are chosen: ψ_1 and ψ_3. Interestingly, the last criterion requires a minimum of parameter knowledge (i.e., four parameters) and leads to the productivity optimum. The main drawback of predictive control is the assumption of a perfect model and available state measurements which is, in practice, difficult to set up. Therefore, a minimax algorithm considering parameter uncertainties (often encountered following identification) allows to limit the loss of productivity especially thanks to the inherent robustness due to the choice of a particular cost function: ψ_3.

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