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Optimal control of a hybridoma bioreactor. Changes induced by considering by-products in the objective function

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Abstract

The main target in the production of monoclonal antibodies (*MAbs*) is reduction of operating costs. One of the pertinent challenges is improving the yield of *MAbs* through the reduction of secondary metabolic products. Therefore searching for an optimal nutrients supplying strategy becomes mandatory.
This study is about the influence of by-products together with the dead cell concentration upon the performance of a bioreactor for *MAbs* production. The by-products and the dead cell concentration are considered in the objective function for optimal control of the system. Three cases were studied: fed batch, continuous and the sequence fed batch – continuous. The optimization procedure was based upon genetic algorithms, which are applied either for the optimal glutamine set point computation for the fed batch operating mode, or the determination of the time inlet flow profile for the continuous mode, both guaranteeing the optimum monoclonal antibody production.

Keywords: hybridoma cell, combined culture, optimal control, genetic algorithms, by-products influence

1. Introduction

The *MAbs* are produced in the last years in large quantities. Their unique specificity and high binding strength lead to a wide range of potential applications both *in vitro* and *in vivo*. The potential growth of the market is

more than 25% per year (Sommerfeld and Strube, 2005). The nutritional requirements of the animal cells are complex and the current production costs are very high. The main efforts are focused on cutting down the operating costs.
Hybridoma cells utilize glutamine and glucose as their primary nitrogen, carbon and energy sources. The metabolism of glucose and glutamine leads to the accumulation of lactic acid and ammonia. These are waste materials that have also inhibitory effects on cell growth and production rates. It is therefore important to maintain the cells (which should be at high concentration) in a physiological state characterized by a minimum production of waste metabolites and a maximum production of antibodies. This goal is achieved searching for an optimal nutrients supplying strategy that modifies the growth medium in such a way that the cells alter their metabolism to produce as much *MAbs* as possible, with minimal waste.
Optimization studies have been made both for fed batch (Dhir *et al.*, 2000; Sarkar and Modak, 2003; Woinaroschy *et al.*, 2004) and continuous processes (Ofițeru *et al.*, 2005). In our previous studies (Woinaroschy *et al.*, 2004; Ofițeru *et al.*, 2005&2006) we have addressed the problem of hybridoma cell bioreactor optimal control, considering three operating strategies: fed batch, continuous and respectively the sequence fed batch – continuous. In all cases, the objective function (final production of *MAb*) took into account only the influence of viable cells or of both viable and dead cells. The optimal control profiles for the command variable assured a high monoclonal antibody production. Nevertheless, since no restrictions have had been placed on the by-products, which were not considered in the objective function, the results indicated that in the optimal cases the by-products formation is also favored. Despite the augmentation of *MAb* quantity, the increased concentration of by-products affects the subsequent processes of separation, and also the physiological state of the cells.
By this study we re-evaluate all three cases with a more extend and restrictive objective function. A throughout comparison with the base case will be made to quantify the influence of by-products on the performance obtained (in terms of monoclonal antibody mass).

2. The mathematical model

The system used to produce *MAb,* whose representation is given in Figure 1 together with the main notations, is fully presented elsewhere (Lavric *et al.*, 2006). Following some preliminary test-runs, the recirculation fraction, α, was set to 0.15, while the purge fraction, β, to 0.005. When the system is operated under the fed batch strategy, there is no recirculation, and the process is formed only by the reactor, together with the feeding.
The Nielsen kinetic model (Ryszczuc and Emborg, 1997) was used, such as in the aforementioned studies. This kinetic is a one-compartment model assuming amino acids as a limiting factor and saturated glucose metabolism. The cells

produce monoclonal antibody (*P*) and the by-products lactate (*L*), ammonia (*M*) and alanine (*A*) using a medium which has glucose (S) and glutamine (Q) as substrates. The detailed mathematical model used for each operating strategy or stage of the process together with the description of the objective functions and solving procedures are given in Woinaroschy *et al.* (2004), Ofițeru *et al.* (2005 and 2006) and Lavric *et al.* (2006), respectively.

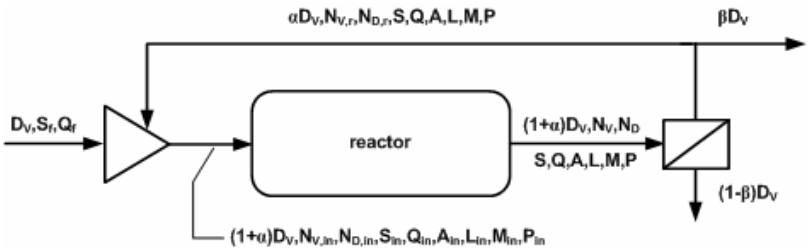

Figure 1. Sketch of the process, together with the main notations used in the mathematical model

2.1. The objective function

The specific objective function should encode the search for the maximum *MAb* production through an optimum glutamine set-point profile for the fed batch bioreactor, or an optimum flow rate profile for the continuous bioreactor.
To capture the influence of both dead cells and by-products concentration upon the final quantity of *MAb*, a composite objective function was used for the inner optimization problem. Its minimisation should imply higher *MAb* productions for lower by-products and dead cells concentrations.

$$Fob = \left(1-\omega_{ND}-\omega_{ALM}\right)\cdot\exp\left(-\frac{P_{final}}{t_{final}}\right)+\omega_{ND}\cdot\exp\left(-\frac{N_V}{N_D}\right)+\omega_{ALM}\cdot\exp\left(-\frac{N_V}{A+L+M}\right) \quad (1)$$

$$P_{final}\Big|_{Fed\ Batch} = P_{MAb}\cdot V_{Fed\ Batch}\Big|_{final} \quad (2)$$

$$P_{final}\Big|_{Continuous} = \int_0^{t_{Continuous}} D_V(\tau)\cdot P(\tau)\cdot d\tau \quad (3)$$

In Eq. (1), the first term encourages high productivities, maximising the output of *MAb* for a given unit of time. The second term favours higher ratios of productive cells while the last one strives for the production of less by-products. Although the composite objective functions like Eq. (1) take into account the weighted influence upon the process of each and every one of its terms (ω_{ND},, ω_{ALM}), this kind of rule does not guarantee that the optimality had been attaint simultaneously with respect to all terms. It could be possible to obtain a slightly lower composite objective function with one condition relatively far from its

optimality, while the rest are very close to theirs. Another possible case is when the same condition is nearer its optimum, while the rest are a little bit farer away but still acceptably close. In many situations, this drawback is solved changing the weights accordingly, but still there are cases in which what should be changed is the optimisation strategy itself.
When it comes to the sequence fed bath – continuous operating strategy, we sought after the maximum *MAb* production, so the natural choice of the objective function for the outer level of the optimisation cycle is:

$$Fob_{Process} = P_{MAb} \cdot V_{Fed\ Batch}\Big|_{final} + \int_{0}^{t_{Continuous}} D_V(\tau) \cdot P(\tau) \cdot d\tau \tag{4}$$

3. Results and discussions

3.1. The solving procedure

Two of the operating strategies (the free end-time fed batch bioreactor and the fed batch – continuous operating sequence) are a two level optimization problem. In the former case, the overall operating time is subject to the outer optimization, while the inner stage is devoted to optimum command profile search (glutamine set point). In the later case, the optimum switch time between fed batch and continuous modes is wanted in the outer optimization, while in the inner stage either the glutamine set point profile or the optimum flow rate profile are sought. The optimal control of the continuous bioreactor is a single level optimization problem, the maximum *MAb* production being sought for a given operating period, with the inlet flow rate *Dv(t)* as command.
An improved variant of the Luus and Jaakola's algorithm was used for the outer optimization, and a genetic algorithm with shrinking mating region was employed for the inner optimization. The complete description of the solving procedure for each of the envisaged cases is given in our previous papers (Woinaroschy *et al.*, 2004; Ofițeru *et al.*, 2005&2006).

3.2. The test cases

The results obtained for the three aforementioned cases are summarised in Table 1. All possible combinations of the composite objective function as given by changing the weights in Eq. (1) were used in searching for the optimal control profiles, as displayed in the four rows of Table 1.
Fed batch bioreactor The restrictions included in the objective function (the dead cells and by-products) lowered significantly both the operation time (with more than 30%) and final obtained *MAb* mass (with more than 50%), as can be seen from Fig. 1. The shape of the system variables remains the same, except

for the viable cells' concentration, which grows higher when restrictions are placed upon dead cells and by-products. The optimizer reduced the operating time, in order to observe the by-products restriction; unfortunately the productivity (g/h *MAb*) decrease is around 45% which is unacceptable.

Table 1. Optimal control results, in terms of two significant variables, with different forms of the composite objective function (1) for the fed batch, continuous and fed batch – continuous sequence

Objective function	Fed batch bioreactor		Continuous bioreactor (120 h)		Fed batch – continuous sequence (1848 h)	
	time, h	*MAb*, g	N_D, g/l	*MAb*, mg	τ_{FB} ratio	*MAb*, g
Base, $\omega_{ND}=\omega_{ALM}=0$	164.5	0.787	0.016	10.266	0.277	7.549
Dead cells, $\omega_{ND}=0.5$; $\omega_{ALM}=0$	110.3	0.358	0.017	10.536	0.419	7.193
By-products, $\omega_{ND}=0$; $\omega_{ALM}=0.5$	111.9	0.376	0.013	10.608	0.266	7.451
Dead cells & by-products, $\omega_{ND}=0.33$; $\omega_{ALM}=0.33$	111.3	0.364	0.013	10.211	0.419	7.174

Continuous bioreactor Contrary to the fed batch case, the optimal control policy of the continuous bioreactor seems not to be significantly affected by the presence or the absence of the supplemental restrictions, as can be seen in Table 1. It seems that the perfect mixing acts like a dampener, the command profile changes being rather small (data not shown).

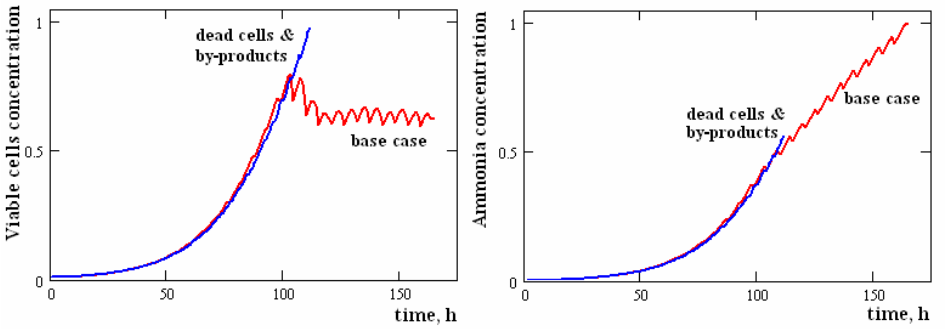

Figure 1. Fed batch bioreactor. The difference in viable cells and ammonia concentration between the base case and the case with both dead cells and by-products in the objective function *(in all figures, normalization was done with respect to the higher value from the four runs, for each state variable)*

The sequence fed batch – continuous bioreactor The most significant influence upon the switch time and mass of the obtained *MAb* is given by the dead cells. The by-products alone in the objective function gave a result similar to the base case. Important to notice that the highest *MAb* production is obtained for the base case too (Table 1). Also, the gain by lowering the ammonia production is not so important, compared with the loss of valuable *MAb*.

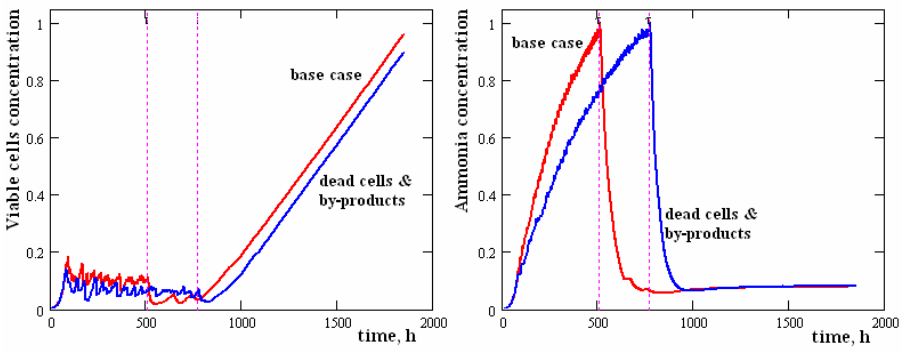

Figure 2. The sequence fed batch – continuous bioreactor. The difference in viable cells and ammonia concentration between the base case and the case of including both dead cells and by-products in the objective function

Nevertheless, the question is still open: how can we maximize the production, minimizing in the same time the formation of by-products, responsible for several inhibitory effects, and dead cells concentration? A better answer may be found using a multi objective optimization.

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