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Optimal control of a continuous perfectly mixed hybridoma bioreactor

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Abstract

Production of industrial scale quantities of monoclonal antibodies (MAbs) is an expensive task and the current focus is on cutting down the operating costs. One of the pertinent challenges is reducing the formation of large amounts of lactate and ammonia (secondary metabolic products of the cellular metabolism of glucose and glutamine), which are waste materials that have also inhibitory effects on cell growth and production rates. It is therefore important to maintain the cells in a physiological state characterized by a minimal production of waste metabolites and a maximum production of biomass and hence antibodies. This goal implies the development of an optimal nutrients supplying strategy which modifies the growth medium in such a way that the cells alter their metabolism to produce as much MAbs as possible, with minimal waste.
In the present study, the optimal control of a continuous perfectly mixed hybridoma bioreactor with cell recirculation from a separator was sought, using genetic algorithms. The results were compared against the optimal control of a fed batch animal cell bioreactor in order to establish which of the two performs better in similar conditions. An improved result can be obtain by combining the two ways of operating, thus reducing the drawbacks of both methods and increasing the performance of the system.

Keywords: hybridoma cell, continuous culture, optimal control, genetic algorithms

1. Introduction

Besides the discover and development of new products useful for humans, in the last period there is a constant preoccupation for optimizing the biochemical processes, in order to increase their performance with lower costs (Betlem et al., 2002; Sarkar and Modak, 2003; Zuo and Wu, 2000). One powerful tool, with proven efficiency in many other domains, is optimal control theory (Smets at al., 2002). Assuming that a proper mathematical representation of the process and a suitable performance criterion are available, optimal control theory provides a systematic and direct approach to solve time and/or distributed control problems,
A mathematical method recognized as a valuable optimization tool is Genetic Algorithms (GA). They started to be used more extensively especially in the last two decades, after the progress in computational capacities (Biegler and Grossman, 2004). Inspired from the natural selection of biological organisms (survival of the fittest), GA

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have several specific features: (a) the representation of parameters on chromosome-like structure, either binary encoded or directly stored for a faster retrieval and processing, such being the case in the present study; (b) the optimal value is searched for within a group of candidate solutions, assimilated to a population; (c) the objective function is used to compute a fitness value, for each individual, measuring its departure from the best-so-far; there is no need to employ derivatives or other additional information to the problem. Several probabilistic rules, the genetic operators (selection, crossover, mutation and cloning), are used to create new offspring from best fitted individuals. Taking into account these advantages, an improved version of GA was used in this study.

1.1 The continuous perfectly mixed hybridoma bioreactor

One of the most dynamic parts of animal cell biotechnology is the production of MAbs by hybridoma cells. *In vitro* MAbs are used as components of immunodiagnostic assays, biosensors, in affinity purification etc. *In vivo* they are used in the treatment of some diseases (to reduce the rejection of a transplanted organ, to destroy the malignant tissues). Large number but small quantities of MAbs are used in research, due to their singular high specificity, although in clinical use the actual trend is to employ larger doses (Leist, 2001). This requires much larger quantities, which implies increasing and diversifying the production methods. This is a difficult task, mainly due to the slow metabolism of hybridoma cells, reflected in their slow growth. The nutritional requirements of the animal cells are complex, making their media costly. 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, both inhibiting cell growth and product formation. Therefore, it is important to developed optimal control strategies for the culture of hybridoma cells.

Hybridomas are anchorage-independent cells, and thus can be grown and maintained in either stationary, suspension or perfusion cultures. The two dominant operating modes for suspension culture are fed batch and continuous, which ensures large amounts of antibodies production (Hu and Aunis, 1999).

The fed batch technique involves periodically adding fresh media (the limiting nutrient or the whole growth medium) making thus possible the work at higher cells concentration than in a batch culture. Culture duration is limited not by nutrients but by the accumulation of both metabolic waste products and dead cells. In continuous culture, fresh media is constantly added, while spent media with or without cells is removed. As neither nutrient supply nor waste accumulation is limiting, continuous cultures may remain viable for longer periods than fed batch ones (Griffiths, 1992). One of the major disadvantages of a continuous mode of operation is the loss of expensive medium components, but this can be attenuated through recycling. Unfortunately, by recycling the cells, there might appear changes in their physiology.

In a previous study (Woinaroschy et al., 2004) a hybridoma fed batch bioreactor, having both substrates (glucose and glutamine) repeatedly fed to attain the initial set-point concentrations, was optimally controlled. Both the duration of the running culture and the glutamine set-point value for each feed were considered as commands. The optimization procedure was the two-level GA approach: the search for optimum overall process time in the outer stage and for the optimum command profile in the inner stage.

The key factor for the best profile was the capacity of keeping the bioreactor at glutamine levels as high as possible on the late stages of the bioprocess.

In this study, a system composed by a continuous perfectly mixed bioreactor, with cell recirculation, a cell separator, a mixer and a purge was modelled then subjected to optimal control. The Nielsen kinetic model (Ryszczuc and Emborg, 1997) was used, such as in the aforementioned study for the optimal control of the fed batch bioreactor. This kinetic is a one-compartment model assuming amino acids as a limiting factor and saturated glucose metabolism. The substrates are glucose and glutamine, and cells produce antibody, lactate, ammonia and alanine. Glutamine is the source of all non-essential amino acid nitrogen, and alanine and ammonia are the only nitrogen products other than biomass and MAb. The production rate for MAb was modified (Ofițeru et al., 2003) to be computed the same way the specific growth rate is.

2. Mathematical model

The representation of the process with cells separation and recirculation is given in Figure 1, together with the main notations. Since the concentration of the cells is rather low, the recirculation fraction, α, was set to 0.15, while the purge fraction, β, to 0.005.

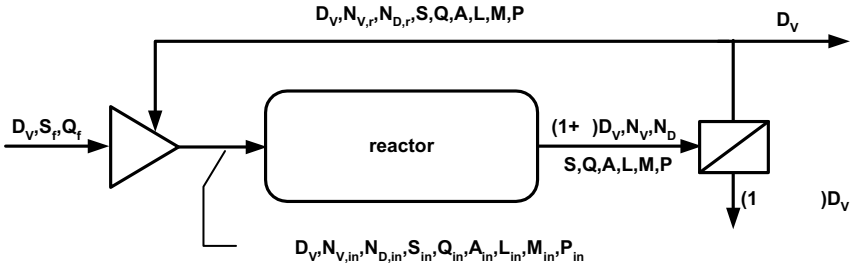

Figure 1. Representation of the process

The complete mathematical model of the system under study is given by the equations (1) ÷ (16):

$$\frac{dN_V}{dt} = \left[\mu\left(t-\tau_B^{\min}\right) - k_D\right]N_V + \frac{D_{V,in}\cdot N_{V,in}}{V} - \frac{D_{V,out}\cdot N_V}{V} \quad (1)$$

$$\frac{dN_D}{dt} = k_D N_V + \frac{D_{V,in}\cdot N_{D,in}}{V} - \frac{D_{V,out}\cdot N_D}{V} \quad (2)$$

$$\frac{dQ}{dt} = -r_Q N_V - k_Q Q + \frac{D_{V,in}\cdot Q_{,in}}{V} - \frac{D_{V,out}\cdot Q}{V} \quad (3)$$

$$\frac{dS}{dt} = -r_S N_V + + \frac{D_{V,in}\cdot S_{in}}{V} - \frac{D_{V,out}\cdot S}{V} \quad (4)$$

$$\frac{dA}{dt} = Y_{QA} r_Q N_V + \frac{D_{V,in}\cdot A_{in}}{V} - \frac{D_{V,out}\cdot A}{V} \quad (5)$$

$$\frac{dL}{dt} = Y_{SL} r_S N_V + \frac{D_{V,in}\cdot L_{in}}{V} - \frac{D_{V,out}\cdot L}{V} \quad (6)$$

$$\frac{dM}{dt} = r_M N_V + \frac{D_{V,in}\cdot M_{in}}{V} - \frac{D_{V,out}\cdot M}{V} \quad (7)$$

$$N_{V,in} = \frac{\alpha}{\alpha+\beta}\cdot N_V \quad (9)$$

$$N_{D,in} = \frac{\alpha}{\alpha+\beta}\cdot N_D \quad (10)$$

$$Q_{in} = \frac{Q_f + \alpha\cdot Q}{\alpha+1} \quad (11)$$

$$S_{in} = \frac{S_f + \alpha\cdot S}{\alpha+1} \quad (12)$$

$$A_{in} = \frac{A_f + \alpha\cdot A}{\alpha+1} \quad (13)$$

$$L_{in} = \frac{L_f + \alpha\cdot L}{\alpha+1} \quad (14)$$

$$M_{in} = \frac{M_f + \alpha\cdot M}{\alpha+1} \quad (15)$$

$$\frac{dP}{dt} = r_{PM} N_V + \frac{D_{V,in} \cdot P_{in}}{V} - \frac{D_{V,out} \cdot P}{V} \quad (8) \qquad P_{in} = \frac{\alpha}{\alpha + 1} \cdot P \quad (16)$$

where N_V and N_D are the living and dead cell concentrations, V, the bioprocess volume, and Q, S, A, L, M and P, respectively the concentrations of glutamine, glucose, alanine, lactate, ammonia, MAb. The flow through the bioreactor is $D_{V,in} = D_V \left(1 + \alpha\right)$.

2.1 The objective function

The objective function should encode the search for the maximum MAb production through an optimum flowrate profile, *Dv(t)*, for a given operating period. In order to compare the performance of this system with the fed batch case, this time was kept the same, t_f=*120 h* (Ofițeru et al., 2003). Thus, the objective function used in this work is:

$$Fob = \int_0^{t_f} D_V\left(t\right) \cdot P\left(t\right) \cdot dt \Big/ D_V^{\max} \cdot P^{\max} \cdot t_f \quad (17)$$

In the equation (17), the product $D_V^{\max} \cdot P^{\max} \cdot t_f$ represents the hypothetical maximum production of the system, provided that the flow rate is at its highest value, such as the MAb concentration, during the whole process.

3. Results and discussions

3.1 Solving procedure

The operating period was divided in 30, 40 and 50 intervals, each interval being divided into 40, 30 and 24 subdivisions, respectively. This way, the overall number of subintervals was kept the same, 120. For each interval, a chromosome encoding the feeding flow is assigned, all being assembled in one gene programming the control variable profile. Accordingly, there are three possible genes, having 30, 40 and 50 chromosomes, thus increasing the refinement of the command profile description. The feeding flow which is the command variable can be modified either in a step like manner, therefore keeping its value constant during an interval of time, or in a linear fashion, although keeping its value constant during a subinterval. So, we can see the subinterval as the smallest temporal unit with a constant command profile.

Eighteen runs were executed, three replicas for each pare of gene encoding profile and flow modification rule (step or piece-wise linear) for an interval, in order to test the convergence of the procedure. Each run was started from the same concentrations of the living and dead cells as those used as initial concentrations in the fed batch cases (Ofițeru et al., 2003; Woinaroschy et al., 2004). The fractions of the recirculation flow and purge, respectively, were kept constant for the whole runs of this virtual experiment. All the runs were sufficiently close, in the limits of ± 1.5 mg, to the total mean quantity of MAb (around 68 mg), obtained after 120 h of system functioning. Irrespective of the number of intervals or the way the feed flow is modified (step or piece-wise linear), there is a clear optimal control profile for the command variable, as can be seen from Figure 2.

During the first period, the feed flow is high to ensure the increase of the living cells concentration to compensate the dilution produced by the free-cells input. As the living cells concentration in the whole system increases, the feed flow is decreased to its

minimum value, in order to keep as low as possible the dead cells concentration. The concentration of the MAb increases exponentially, up to its maximum value, following the raise of the concentration of the living cells. Immediately after the time when MAb reaches its highest value, the feed flow is progressively increased in order to maintain the MAb concentration at its maximum level. This is ensured through the raise of the living cells concentration and maintenance of the dead cells concentration as low as possible. It is worth mentioning, however, that the rate at which the dead cells accumulate is greater then the rate the living cells concentration increases (see Figure 2(a), left, the slopes of N_V and N_D curves, after t = 45 h. It should be noticed that the maximum value of the MAb concentration is attained when the glucose is practically completely consumed as it enters the reactor. Since the glucose and glutamine are partially complementary-partially substitutable, after the complete consumption of glucose its role is taken by glutamine, whose concentration is kept slowly decaying for the rest of time.

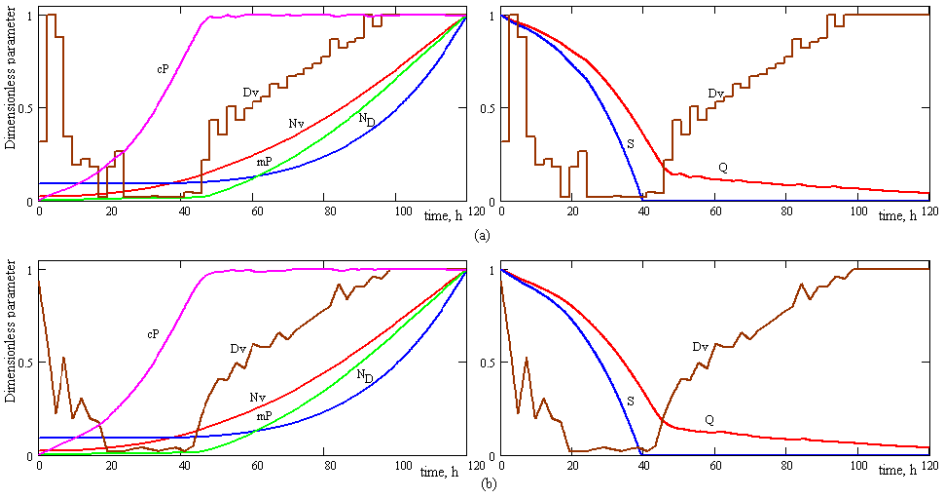

Figure 2. Normalized concentrations and input flow profiles for the optimized solution for the 50 intervals case. (a) step like feeding; (b) piece-wise linear feeding; cP is the concentration of the MAb, while mP is the mass of MAb

We can compare the performance of this optimal controlled continuous bioreactor against the one of the optimal fed-batch bioreactor (Woinaroschy et al., 2004) two ways: in terms of the total MAb produced in the same working time, or in terms of productivity, such as the mass of the monoclonal antibody produced per unit of working bioreactor volume. The maximum MAb production resulted from the aforementioned experiments with an optimally controlled continuous bioreactor was 75.6 mg, several times larger than the 10.3 mg which was the best production of an optimally controlled fed-batch bioreactor (Woinaroschy et al., 2004). But, in terms of productivity, the continuous bioreactor, with its average 1.51 mg/l MAb is clearly surpassed by the 5.97 mg/l MAb obtained in the fed-batch bioreactor. This is the result of the period of about 45 h from the beginning of the process, when the continuous bioreactor worked under its maximum output MAb concentration (see Figure 2, cP profiles).

4. Conclusions

For the final value of MAb mass does not matter very much the number of intervals, although the piece-wise linear feeding gives slightly better productions, with a mean of 0.3 mg larger than for the step case. After reaching the maximum concentration of the MAb, the input flow rises for maintaining this value. In all cases, the glucose concentration becomes nearly zero after approximately 40 to 45 working hours, but since the production of MAb depends only on glutamine concentration in the adopted kinetic model, this depletion does not alter severely the results and is not considered as a key factor in optimization.

Compared with the fed-batch bioreactor, in the continuous case viable cells concentration is growing constantly, with a bigger slope after increasing the input flow. The influence of the by-products is reduced, but still the Nielsen kinetic model is not suitable for long time runs as it does not consider the cell lysis. Although the dead cells concentration starts to rise earlier compared with fed-batch case, by recycling and constantly diluting the medium, they never overcome the living cells concentration in the last period of the culture.

As a future research direction, to avoid waste of valuable substrate, the bioreactor should be run fed-batch until reaching a critical dead cell concentration, and then switched to the continuous mode, till the first sign of changes in viable cells physiology.

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