

## Optimal Operation of Hybridoma Cell Fed-Batch Cultures Using the Overflow Metabolism Model: Numerical and Analytical Approach

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**Abstract:** The maximization of biomass productivity in fed-batch cultures of hybridoma cells is analyzed based on the overflow metabolism model. Due to overflow metabolism, often attributed to limited oxygen capacity, lactate and ammonia are formed when the substrate concentrations (glucose and glutamine) are above a critical value, which results in a decrease in biomass productivity. Optimal feeding rate, on the one hand, for a single feed stream containing both glucose and glutamine and, on the other hand, for two separate feed streams of glucose and glutamine are determined using a Nelder-Mead simplex optimization algorithm. The optimal multi exponential feed rate trajectory improves the biomass productivity by 10% as compared to the optimal single exponential feed rate. Moreover, this result is validated by the one obtained with the analytical approach in which glucose and glutamine are fed to the culture such as to control the hybridoma cells at the critical metabolism state, which allows maximizing the biomass productivity.

**Keywords:** hybridoma cultures, overflow metabolism, optimal feeding profiles, biomass productivity, bioprocess optimization.

### 1. INTRODUCTION

Monoclonal antibodies (MAb) are currently used for many diagnostic and therapeutic applications (Rodrigues *et al.*, 2010; Wurm *et al.*, 2004). The high demand for these biopharmaceuticals has led to the development of large-scale manufacturing processes.

Industrial MAb production is usually achieved using fed-batch cultures of mammalian cells, which can express different kinds of recombinant proteins. From an operational point of view, the main goal is to maximize the recombinant protein production and, consequently, the biomass production, all in a minimum of time (i.e., to maximize the biomass productivity). This requires the determination of an optimal feeding strategy, that is, the optimal time evolution of the input flow rate to the fed-batch culture.

However, few studies (Tremblay *et al.*, 1992; Dhir *et al.*, 1999) have been reported on the optimization of hybridoma cell cultures, mainly because of the lack of mathematical models describing adequately their growth and production kinetics.

The main problem encountered comes from the metabolic changes of such strains in the presence of feeding overflow. This "overflow metabolism", is a metabolic phenomenon that is induced when the rate of substrate consumption exceeds a critical value, leading to a by-product formation which inhibits the oxidative capacity and the cell growth. It occurs for instance in *Saccharomyces cerevisiae* cultures with aerobic ethanol formation, in *Pichia pastoris* with aerobic

methanol formation, in *Escherichia coli* cultures with aerobic acetate formation or in mammalian cell cultures with the aerobic lactate and ammonia formation.

From a metabolic point of view, continuous mammalian cell lines and yeast *Saccharomyces cerevisiae* share some common features (Diaz-Ruiz *et al.*, 2009) though in this study the definition of hybridoma cell respiratory state is not identical to that of yeast. Until now there has been a considerable amount of research about yeast cell culture optimization (Valentinoti *et al.*, 2003; Dewasme *et al.*, 2010; Santos *et al.*, 2012), and the general idea is to avoid ethanol formation (overflow metabolism) while maximizing the biomass productivity in the process. Therefore, controlling hybridoma cells at the edge of overflow metabolism is firstly recommended for the design of optimal feeding profile. This strategy should allow for avoiding the waste of substrates (glucose and glutamine) via the overflow metabolism and for maintaining maximum biomass productivity.

In this work, the overflow metabolism model developed in (Amribt *et al.*, 2012) from hybridoma HB-58 cell fed-batch cultures at constant feed rate is used to determine an optimal feeding profile aiming at maximizing biomass productivity.

The antibodies produced by the hybridoma cell line HB-58 are secreted directly by the cells in the culture medium. The growth-associated product formation occurs during the lag and exponential phases of growth, while non-growth associated product formation occurs during the stationary phase, when the growth rate is zero. In our case the viable biomass time profiles display no clear stationary phase,

therefore, the assumption that all product formation is growth-associated is considered.

The optimization problem consists in determining, on the one hand, the optimal feeding rate for a single feed stream containing both glucose and glutamine and, on the other hand, two separate feed streams of glucose and glutamine, such that biomass production is maximized in a minimum culture time. To do this, the feed flow rate is first represented by a finite set of control parameters and a numerical optimization method is used for parameter selection.

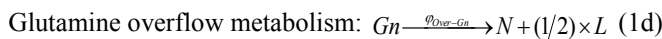
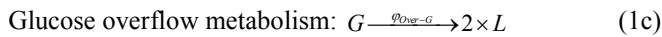
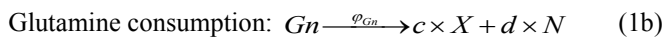
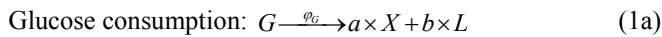
Two different objective functions (performance criterion) are considered for optimization; the first criterion to be maximized is the biomass productivity obtained at the end of the fed-batch culture, the second criterion to be minimized is the difference between global substrate consumption and the maximum respiratory capacity. The comparison between performances is conducted.

The optimal feeding profile obtained with the numerical optimization approach is validated with an analytical approach which consists to determine an analytical expression of the feeding time profile which maintains the substrate concentration at the critical value such as to control the hybridoma cells at the critical metabolism state (limit of overflow metabolism).

The paper is organized as follows. The overflow metabolism model of hybridoma cell fed-batch cultures is briefly presented in Section 2. The optimal control problems are formulated in Section 3 and numerical results obtained for different feeding policies are given. Section 4, provides the implementation and results of the analytical approach as well as a comparison between performances. Final conclusions and future work directions are pointed out in Section 5.

## 2. OVERFLOW METABOLISM MODEL

The metabolism network is described by the following macroscopic reactions linking cells ( $X$ ), glucose ( $G$ ), glutamine ( $Gn$ ), lactate ( $L$ ) and ammonia ( $N$ ):



where  $a$ ,  $b$ ,  $c$  and  $d$  are the stoichiometric coefficients, and  $\varphi_G$ ,  $\varphi_{Gn}$ ,  $\varphi_{\text{Over-G}}$  and  $\varphi_{\text{Over-Gn}}$  are the nonlinear growth rates given by:

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

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

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

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

The kinetic models associated with the global glucose consumption  $\varphi_{G1}$ , the global glutamine consumption  $\varphi_{Gn1}$ , the maximum respiratory capacity for glucose  $\varphi_{G\max}$  and the

maximum respiratory capacity for glutamine  $\varphi_{Gn\max}$  are given by:

$$\varphi_{G1} = \mu_{G\max1} \frac{G}{K_G + G} \frac{Gn}{K_{Gn1} + Gn} X_V \quad (3a)$$

$$\varphi_{Gn1} = \mu_{Gn\max1} \frac{Gn}{K_{Gn} + Gn} \frac{K_N}{K_N + N} X_V \quad (3b)$$

$$\varphi_{G\max} = \mu_{G\max2} X_V \quad (3c)$$

$$\varphi_{Gn\max} = \mu_{Gn\max2} X_V \quad (3d)$$

During a culture, the cells are likely to change their metabolism because of their limited respiratory capacity. At low substrate uptake rate ( $\varphi_{G1} < \varphi_{G\max}$  and  $\varphi_{Gn1} < \varphi_{Gn\max}$ ), glucose and glutamine are consumed with biomass growth and metabolites (lactate and ammonia) production without overflow metabolism, which is defined as **respiratory metabolism**. At high substrate uptake rates ( $\varphi_{G1} > \varphi_{G\max}$  and/or  $\varphi_{Gn1} > \varphi_{Gn\max}$ ), there is a limitation of respiratory capacity, resulting in **overflow metabolism** towards excess metabolites production. The state at which overflow metabolism is initiated ( $\varphi_{G1} = \varphi_{G\max}$  and  $\varphi_{Gn1} = \varphi_{Gn\max}$ ) is referred to as **critical metabolism**.

The mass balance equations for the system in fed-batch mode are:

$$\frac{dX_V}{dt} = a\varphi_G + c\varphi_{Gn} - \mu_d X_V - \frac{F}{V} X_V \quad (4a)$$

$$\frac{dX_d}{dt} = \mu_d X_V - \frac{F}{V} X_d \quad (4b)$$

$$\frac{dG}{dt} = -\varphi_G - m_G X_V - \varphi_{\text{Over-G}} + \frac{F}{V} (G_{in} - G) \quad (4c)$$

$$\frac{dGn}{dt} = -\varphi_{Gn} - \varphi_{\text{Over-Gn}} + \frac{F}{V} (Gn_{in} - Gn) \quad (4d)$$

$$\frac{dL}{dt} = b\varphi_G + 2\varphi_{\text{Over-G}} + \frac{1}{2}\varphi_{\text{Over-Gn}} - \frac{F}{V} L \quad (4e)$$

$$\frac{dN}{dt} = d\varphi_{Gn} + \varphi_{\text{Over-Gn}} - \frac{F}{V} N \quad (4f)$$

$$\frac{dV}{dt} = F \quad (4j)$$

where  $m_G$  is the maintenance coefficients of glucose,  $V$  (L) is the reactor volume,  $F$  (L/h) the volumetric feed rate,  $G_{in}$  and  $Gn_{in}$  are the concentrations of glucose and glutamine in the feed stream.  $\mu_d$  is the specific death rate given by:

$$\mu_d = \mu_{d\max} \frac{K_{Gd}}{K_{Gd} + G} \frac{K_{Gnd}}{K_{Gnd} + Gn} \quad (5)$$

Additionally, an indicator of overflow is proposed for each substrate (glucose and glutamine) as follows:

$$\text{Indi.over.}\xi = \frac{\varphi_{\xi1} - \varphi_{\xi\max}}{\varphi_{\xi\max}} \quad \text{with } \xi = G, Gn \quad (6a)$$

These two metabolism indicators of overflow are positive if culture is operated at the state of overflow metabolism.

The model parameters values are:

$$\mu_{G\max1}=1.0006, \mu_{G\max2}=0.0283, \mu_{Gn\max1}=0.1992, \mu_{Gn\max2}=0.0203, \mu_{d\max}=0.0111, K_G=23.2350, K_{Gn}=0.0004, K_N=0.9931, K_{Gn1}=0.0005, a=1.1462, b=1.2939, c=0.1186, d=0.3000, m_G=0.0367, K_{Gd}=2.1862, K_{Gnd}=0.0020.$$

### 3. PROCESS OPTIMIZATION BASED ON NUMERICAL APPROACH

#### 3.1 Problem Formulation

For the case studied here, the goal of the optimization consists in determining, on the one hand, the optimal parameters of the feeding rate for a single feed stream containing both glucose and glutamine and, on the other hand, two separate feed streams of glucose and glutamine, such that biomass production is maximized in a minimum culture time.

Two different objective functions (performance criterion) are considered for optimization;

The **first criterion** to be maximized is the biomass productivity obtained at the end of the fed-batch culture, which leads to the minimization of:

$$\phi_1 = -\frac{X_V(t_f) \times V(t_f)}{t_f} \quad (7)$$

The **second criterion** to be minimized is the difference between global substrate consumption ( $\phi_{G1}$  and  $\phi_{Gn1}$ ) and the maximum respiratory capacity ( $\phi_{Gmax}$  and  $\phi_{Gnmax}$ ):

$$\phi_2 = \alpha \times \sum |\phi_{G1} - \phi_{Gmax}| + \sum |\phi_{Gn1} - \phi_{Gnmax}| \quad (8)$$

where  $\alpha$  is the weight of the glucose overflow with respect to glutamine overflow.

For the second criterion the final time  $t_f$  must be fixed to obtain realistic solutions. In this case, the final time is decided to be equal to the optimal final time identified with the first criterion.

The constraints on the control variable and the culture volume are:

$$\begin{aligned} 0 \leq F \leq F_{max} \quad \text{with} \quad F_{max} &= 0.5L/day \\ V(t_f) \leq V_{max} \quad \text{with} \quad V_{max} &= 0.7L \\ t_{fmin} \leq t_{fed} \leq t_f \leq t_{fmax} \quad \text{with} \quad t_{fmin} &= 1h \text{ and } t_{fmax} = 500h \\ 0 \leq G_{in} \leq 100mM \\ 0 \leq G_{nin} \leq 25mM \end{aligned} \quad (9)$$

where  $t_{fed}$  is the time at which the feeding starts.

The final concentration of lactate is constrained to be less than 30 mM (Ozturk *et al.*, 1991) to avoid the problem of lactate inhibition at high concentration, which has not been taken into account in the model formulation.

The initial culture conditions are:

$X_{v0}=1.85 \times 10^5$  cells/mL,  $X_{d0}=0.25 \times 10^5$  cells/mL,  $G_0=17.17$  mM,  $G_{n0}=2.41$  mM,  $L_0=0.36$  mM,  $N_0=0.23$  mM and  $V_0=0.35L$ .

The fed concentrations  $G_{in}$  and  $G_{nin}$  are included in the optimization problem with the constraints given in (9).

Based on results obtained with different feeding policies in (Amribt *et al.*, 2012), we considered only the optimization with an exponential feed rate (Table 1).

**Table 1. Considered feeding policies and corresponding parameters**

|                            | Expression   | Parameters  |
|----------------------------|--|---|
| Single exponential feed    | $F = \beta \times e^{\lambda(t-t_{fed})}$  | $t_{fed}, t_f, \beta, \lambda, G_{in}, G_{nin}$   |
| Multiple exponential feeds | $F_G = \beta_G \times e^{\lambda_G(t-t_{fed-G})}$<br>$F_{Gn} = \beta_{Gn} \times e^{\lambda_{Gn}(t-t_{fed-Gn})}$ | $t_{fed-G}, t_{fed-Gn}, t_f, \beta_G, \lambda_G, \beta_{Gn}, \lambda_{Gn}, G_{in}, G_{nin}$ |

The Nelder–Mead simplex optimization algorithm (function `fminsearchcon`) is used for optimal parameter selection.

#### 3.2 Single Feed Optimization

Fig. 1 shows the comparison between the optimal feed flow rate and optimal concentration profiles of viable cells, substrates (glutamine and glucose) and metabolites (lactate and ammonia), obtained by optimization of criterion 1 (blue line) and criterion 2 ( $\alpha=1$ , red line).

It is shown that the optimization of the two criterions gives the same performances. The optimal feeding profile corresponds to  $6 \times 10^{-4} \times e^{0.038(t-52)}$ . After the batch period (about 52 h) wherein culture is operated in overflow metabolism, the feeding rate started when cell culture is at the edge of overflow metabolism and the concentrations of substrates (glutamine and glucose) remain critical. The final culture time and maximum biomass productivity obtained are 134 h and  $4.55 \times 10^7$  cells/h. The concentrations of glucose and glutamine in the feed stream ( $G_{in}$  and  $G_{nin}$ ) correspond to 38 mM and 10 mM respectively.

#### 3.3 Extension to Multiple Feeds

Two separate feed streams  $F_G$  and  $F_{Gn}$  of glucose and glutamine are considered here.

Then the dynamic mass balance equations (4a-4j) can be written as:

$$\frac{dX_V}{dt} = a\phi_G + c\phi_{Gn} - \mu_d X_V - \frac{F_G + F_{Gn}}{V} X_V \quad (10a)$$

$$\frac{dX_d}{dt} = \phi_d - \frac{F_G + F_{Gn}}{V} X_d \quad (10b)$$

$$\frac{dG}{dt} = -\phi_G - m_G X_V - \phi_{Over-G} + \frac{F_G}{V} G_{in} - \frac{F_G + F_{Gn}}{V} G \quad (10c)$$

$$\frac{dGn}{dt} = -\phi_{Gn} - \phi_{Over-Gn} + \frac{F_{Gn}}{V} G_{nin} - \frac{F_G + F_{Gn}}{V} Gn \quad (10d)$$

$$\frac{dL}{dt} = b\phi_G + 2\phi_{Over-G} + 0.5\phi_{Over-Gn} - \frac{F_G + F_{Gn}}{V} L \quad (10e)$$

$$\frac{dN}{dt} = d\phi_{Gn} + \phi_{Over-Gn} - \frac{F_G + F_{Gn}}{V} N \quad (10f)$$

$$\frac{dV}{dt} = F_G + F_{Gn} \quad (10j)$$

The optimal feeding policies corresponding to two separate feed streams of glucose and glutamine obtained by optimization of criterion 1 (blue line) and criterion 2 ( $\alpha=1$ , red line) are shown in Fig. 2. It can be observed that the feeding of glucose and glutamine started almost at the same time (between 50h and 65h).

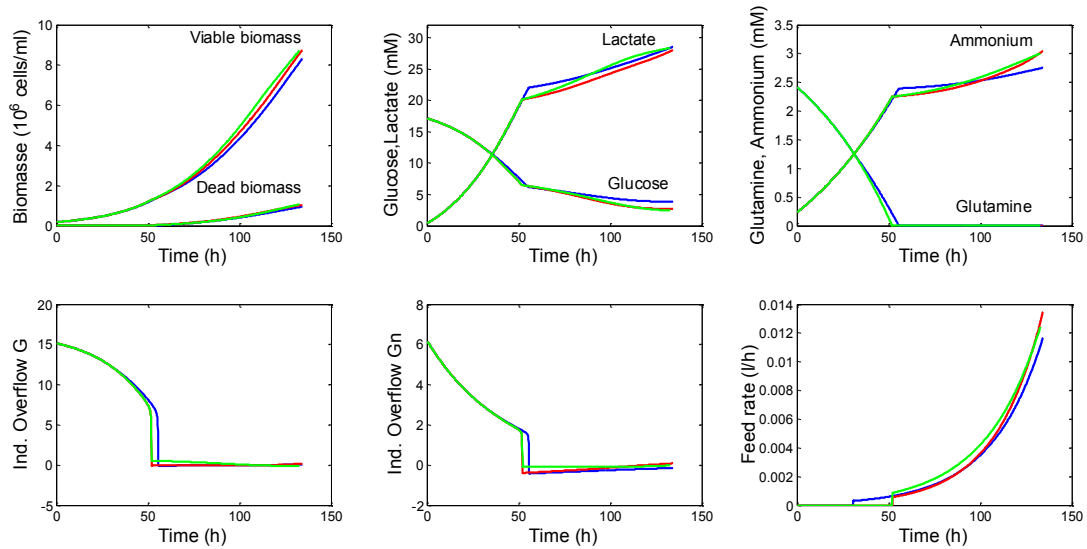


Fig. 1. Optimal feeding rate for a single feed stream and corresponding optimal concentration profiles of viable cells, substrates (glutamine and glucose) and metabolites (lactate and ammonia) obtained by numerical approach optimization of criterion 1 (red line), criterion 2 ( $\alpha=1$ , blue line) and analytical approach (green line).

The biomass productivity yielded by the optimal multiple feed rates is found to be  $4.94 \times 10^7$  cells/h, an improvement of 10% as compared to the optimal single feed exponential rate. The final culture time, the concentrations of glucose and glutamine in the feed stream ( $G_{in}$  and  $G_{nin}$ ) correspond to 137 h, 91 mM and 22 mM respectively.

As illustrated in Fig. 2, in the multiple feeds, the optimal solution correspond to add more glutamine than glucose (the feed rate of glutamine is more important than the one of glucose). From the indicators of overflow it can be seen that the glucose and glutamine are fed to the culture such as to control the hybridoma cells at the critical metabolism state (edge of overflow metabolism), which allows to maximize the biomass productivity. The optimal feeding trajectory

obtained by optimization of criterion 2 ( $\alpha=1$ ) confirmed this strategy.

Fig. 3 shows the effect of  $\alpha$  (the weight of the glucose overflow in the second optimization criterion) on the biomass productivity. It appears that when  $\alpha$  becomes lower than 0.3 the biomass productivity decreases, this indicates that glucose overflow is more important than glutamine overflow. Therefore, we conducted another optimization with exponential feed rates based only on minimizing the difference between global glucose consumption ( $\phi_{GI}$ ) and the maximum respiratory capacity for glucose ( $\phi_{Gmax}$ ) in the second criterion. The optimal feeding policies and optimal concentration profiles are similar to those obtained by optimization of criterion 2 ( $\alpha=1$ , red line) in Fig. 2.

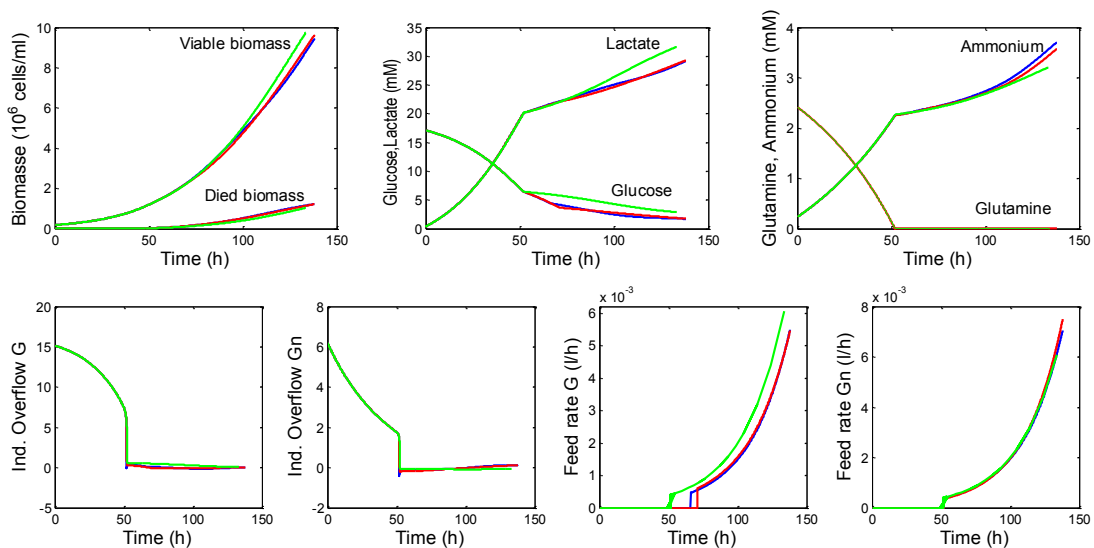


Fig. 2. Optimal feeding rate for two separate feed streams of glucose and glutamine and corresponding optimal concentration profiles of viable cells, substrates (glutamine and glucose) and metabolites (lactate and ammonia) obtained by optimization of criterion 1 (red line), criterion 2 ( $\alpha=1$ , blue line) and analytical approach (green line).

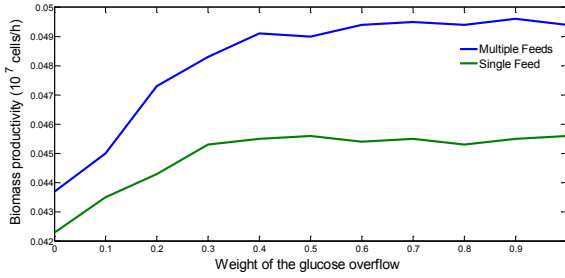


Fig. 3. Effect of  $\alpha$  (the weight of the glucose overflow in the second optimization criterion) on the biomass productivity.

### 3. PROCESS OPTIMIZATION BASED ON ANALYTICAL APPROACH

The analytical determination of optimal feed rate profiles for fed batch bioreactors is a difficult problem especially with multiple control variables. In this section, we propose a simplified solution strategy based on the mass balance equations of the overflow metabolism model, for the determination of optimal feeding rate in single and multiple feed cases.

#### 3.1 Single Feed Optimization

Based on the previous research about yeast cell culture optimization (Valentinoti *et al.*, 2003; Dewasme *et al.*, 2010; Santos *et al.*, 2012), and the results of numerical optimization, it is clear that controlling hybridoma cells at the edge of overflow metabolism is recommended to maximize biomass productivity. Therefore, the substrate concentrations must be maintained at the critical values ( $G=G_{crit}$  and  $Gn=Gn_{crit}$ ) such as to control the hybridoma cells at the critical metabolism state ( $\phi_{Gl}=\phi_{Gmax}$  and  $\phi_{GnI}=\phi_{Gnmax}$ ).

From  $\phi_{Gl}=\phi_{Gmax}$  and  $\phi_{GnI}=\phi_{Gnmax}$ , the critical values  $G_{crit}$  and  $Gn_{crit}$  are given by:

$$G_{crit} = \frac{K_G \mu_{Gmax2} (K_{GnI} + Gn_{crit})}{\mu_{Gmax1} Gn_{crit} - \mu_{Gmax2} (K_{GnI} + Gn_{crit})} \quad (11a)$$

$$Gn_{crit} = \frac{K_{Gn} \mu_{Gnmax2} (K_N + N_{crit})}{\mu_{Gnmax1} K_N - \mu_{Gnmax2} (K_N + N_{crit})} \quad (11b)$$

The feeding profiles controlling  $G$  constant ( $=G_{crit}$ ) and  $Gn$  constant ( $=Gn_{crit}$ ) can be obtained from (4c) and (4d) by setting  $dG/dt=0$  and  $dGn/dt=0$ :

$$F = \frac{\mu_{Gmax2} + m_G}{G_{in} - G_{crit}} V X_V \quad (12a)$$

$$F = \frac{\mu_{Gnmax2}}{Gn_{in} - Gn_{crit}} V X_V \quad (12b)$$

By setting  $\phi_{Gl}=\phi_{Gmax}$  and  $\phi_{GnI}=\phi_{Gnmax}$ , (4a) can be integrated to give the exponential growth:

$$V X_V = V_e X_e e^{\theta(t-t_{fed})} \quad (13a)$$

$$\theta = a \mu_{Gmax2} + c \mu_{Gnmax2} - \mu_{dmax} \frac{K_{Gd}}{K_{Gd} + G_{crit}} \frac{K_{Gnd}}{K_{Gnd} + Gn_{crit}} \quad (13b)$$

where subscript  $e$  represent quantities at the beginning of fed-batch mode. The duration of the batch phase ( $t_{fed}$ , time when the feeding rate is started) is determined by simulation of the batch phase and corresponds to the time when the culture switches from overflow to respiratory metabolism. In (11b)

$N_{crit}$ , corresponds to concentration of ammonium simulated in the batch phase at time  $t_{fed}$ .

We consider that the concentrations of glucose and glutamine in the feed streams ( $G_{in}$  and  $Gn_{in}$ ) are equal to those obtained with the numerical approach.

If we consider the application of the feeding rate (12a) at time when critical value of glucose is reached, the glutamine in the culture medium will be depleted, because it appears that the critical value of glutamine is firstly reached. Therefore, the optimal solution consists in applying the feeding rate (12b) at time when the critical value of glutamine is reached.

Fig. 1 (green line) shows the optimal feed flow rate and optimal concentration profiles of viable cells, substrates (glutamine and glucose) and metabolites (lactate and ammonia), obtained by this analytical approach. The maximum biomass productivity obtained is  $4.42 \times 10^7$  cells/h, which is of the same order as the one obtained with the numerical approach. The final culture time is 132h.

From indicators of overflow, it can be seen that in contrast with the numerical approach, the feeding rate (12b) controls the culture at the edge of glutamine overflow, which is because the feeding rate is started exactly at time when critical value of glutamine is reached (glucose is still above its critical value).

#### 3.2 Extension to Multiple Feeds

The two separate feed streams  $F_G$  and  $F_{Gn}$  of glucose and glutamine can be derived from the mass balance equations (10a-10j) as follows:

$$F_{Gn} = \frac{\left[ \mu_{Gnmax2} + \frac{(\mu_{Gmax2} + m_G) Gn_{crit}}{(G_{in} - G_{crit})} \right]}{(Gn_{in} - Gn_{crit} - \frac{G_{crit} Gn_{crit}}{G_{in} - G_{crit}})} V X_V \quad (14a)$$

$$F_G = \frac{(\mu_{Gmax2} + m_G) V X_V + F_{Gn} G_{crit}}{(G_{in} - G_{crit})} \quad (14b)$$

From (14a) and (14b) it appears that  $F_{Gn}$  and  $F_G$  are dependent, which makes difficult to maintain simultaneously glucose and glutamine at this critical concentrations. The feeding rate (14a) starts at time when the critical value of glutamine is reached and (14b) at time when the critical value of glucose is reached.

The optimal feeding policies corresponding to two separate feed streams of glucose and glutamine obtained with this analytical approach are shown in Fig. 2 (green line). It can be observed that if we consider the maximum culture volume ( $V=V_{max}=0.7$ ), the obtained final concentration of lactate is bigger to 30 mM, which corresponds to violation of the constraint on lactate ( $L_{max} \leq 30$  mM). If we consider that the final time of the culture corresponds to the time when the maximum concentration of lactate is reached, the maximum biomass productivity is  $4.76 \times 10^7$  cells/h, which is of the same order as the one obtained with the numerical approach. Additionally, the optimal concentration profiles of viable cells, substrates and metabolites obtained with the analytical approach are similar to those obtained with the numerical approach, as illustrated in Fig. 2.

**Table 2. The optimal biomass productivity and final culture time for the various cases**

| Case                           |             |             | Productivity<br>(10 <sup>7</sup> cells/h) | $G_{in}$ (mM) | $Gn_{in}$ (mM) | Final culture<br>time (h) |
|--------------------------------|-------------|-------------|---|---------------|----------------|---------------------------|
| <b>Numerical<br/>approach</b>  | Single feed | Criterion1  | 4.46                                      | 41            | 9              | 134                       |
|                                |             | Criterion 2 | 4.55                                      | 38            | 10             | Fixed to 134              |
|                                | Multi feed  | Criterion1  | 4.90                                      | 93            | 21             | 137                       |
|                                |             | Criterion 2 | 4.94                                      | 91            | 22             | Fixed to 137              |
| <b>Analytical<br/>approach</b> | Single feed |             | 4.42                                      | Fixed to 38   | Fixed to 10    | 132                       |
|                                | Multi feed  |             | 4.76                                      | Fixed to 91   | Fixed to 22    | 130                       |

As shown from the indicators of overflow it is clear that, in the multiple feeds case, the glucose and glutamine are fed to the culture such as to control the hybridoma cells at the critical metabolism state, on the contrary to the single feed case where the glucose is above its critical concentration.

Table 2 summarizes the results obtained with the analytical and numerical (criterion1 and criterion2) approaches.

## 5. CONCLUSIONS

The feed rate optimization of fed-batch bioreactors involving multiple control variables is a difficult problem. An optimization procedure based on the overflow metabolism model (Amribt *et al.*, 2012) is developed for the determination of substrate feeding policies in fed-batch cultures with single and multiple feeds.

The optimal control problem is formulated and solved by using two different approaches:

- ✓ **Numerical approach:** which consists in determining the optimal parameters of the feeding rate based on two different objective functions (performance criterion), such that biomass production is maximized in a minimum culture time.
- ✓ **Analytical approach:** which consists in determining an analytical expression of the feeding time profiles which maintains the substrate concentrations at the critical values such as to control the hybridoma cells at the critical metabolism state (limit of overflow metabolism).

For the numerical approach the optimal multi exponential feed rate trajectory improves the biomass productivity by 10% as compared to the optimal single exponential feed rate. Moreover, this result is validated by the one obtained with the analytical approach in which glucose and glutamine are fed to the culture such as to control the hybridoma cells at the critical metabolism state, which allows maximizing the biomass productivity.

Supplementary numerical results indicate clearly that the glucose overflow is more important than glutamine overflow and numerical optimization based only on minimizing the difference between global glucose consumption and the maximum respiratory capacity allows to maximize the biomass productivity.

As future work, the robustness analysis of optimal feeding profiles obtained with different optimization strategies will be conducted regarding the uncertainty on model parameters.

The optimization strategies developed in this work provides therefore a valuable basis for the design of on-line monitoring and robust control of animal cell cultures. This will be the subject of further investigation.

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