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Dynamic optimisation of a recombinant BHK-21 culture based on elementary flux analysis and hybrid parametric/nonparametric modeling

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Background

Metabolic flux analysis (MFA) and metabolic pathway analysis (MPA) are today fundamental tools to study cellular metabolism. Such tools can assist the generation of potential modifications that can alter the cell metabolic activity toward bioprocess optimisation.

Although MFA and MPA techniques have been mainly used for metabolic engineering [1], they may also be useful in other phases of the bioprocess development cycle, namely for advanced bioreactor monitoring and control [2,3]. A number of methods have been developed to study the structure of biochemical networks. From a process optimisation and control point of view, the elementary flux modes (EFMs) method is particularly attractive since it reduces network complexity to a minimal set of reactions. EFMs are unique for a given network and can be considered as nondecomposable steady state flux distributions using a minimal set of reactions.

In previous studies [4], an iterative batch-to-batch optimization scheme was developed and applied to the optimization of recombinant BHK-21 expressing the fusion glycoprotein IgG1-IL2 used in cancer therapy [5]. The main objective of the present study is complementing the previous batch-to-batch scheme with knowledge of the metabolic network of the biological system under consideration. The incorporation of reliable mechanistic knowledge in the batch-to-batch optimisation scheme, namely of the metabolic network in the form of EFMs, may increase the 'extrapolation' capacity and thus may contribute to increase the rate of success of the proposed technique.

Results

The metabolic network adopted (Fig. 1) is first decomposed into EFMs using the *FluxAnalyser* program [6]. The system has seven EFMs. The hypothesis of balanced growth allows the elimination of the intermediate metabolites resulting in a simplified set of reactions (Table 1) connecting extracellular substrates with end-products.

The resulting set of reactions is the basis for the formulation of the following hybrid model structure:

$$\frac{d}{dt}\begin{bmatrix} X_v \\ Glc \\ Gln \\ Lac \\ Amm \\ Ala \\ IgG \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & -1 & 0 & 0 & -2 & 0 \\ 0 & 0 & 0 & -1 & -1 & -5 & 0 \\ 0 & 2 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 2 & 2 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \rho_1 \times (X_V) \\ \rho_2 \times (X_V Glc) \\ \rho_3 \times (X_V Glc) \\ \rho_4 \times (X_V Gln) \\ \rho_5 \times (X_V Gln) \\ \rho_6 \times (X_V Glc Gln) \\ \rho_7 \times (X_V) \end{bmatrix} - D \begin{bmatrix} X_v \\ Glc \\ Gln \\ Lac \\ Amm \\ Ala \\ IgG \end{bmatrix} + \begin{bmatrix} o \\ F_{Glc} \\ F_{Gln} \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad (1)$$

An artificial neural network was used to identify the reaction kinetics from data: the apparent specific growth rate

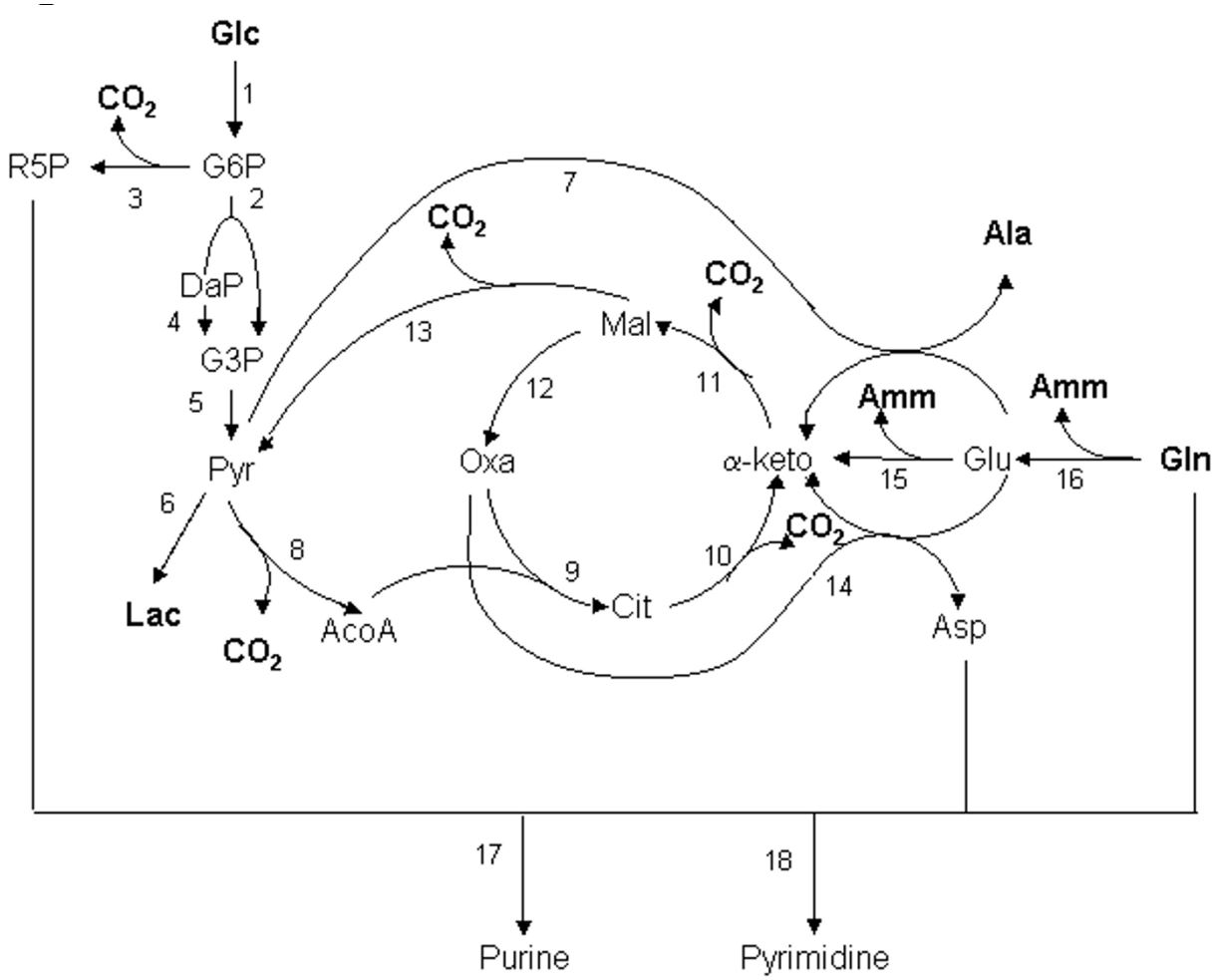

Figure 1
Animal cells metabolic network [2, 7] From the reactions of table 1, it was further assumed that $r_{e6}=r_{e7}$ since DNA and RNA are made up of equal parts of purine and pyrimidine. Therefore, these two reactions were substituted by their sum:
$2Glc+5\ Gln \rightarrow Pur+Pyr+4CO_2+2Amm$
Furthermore, it was considered that the 4th elementary mode has negligible flux, since lactate is mainly produced from glucose.

(μ-k_d), the specific protein synthesis rate (q_{IgG}), and the EFM kinetics (ρ_i functions in eq. 1). Measured data of one batch and four fed-batch runs was used. Figure 2 presents the identified intracellular flux distribution for one of the fed-batch runs.

Table 1: Elementary flux modes of the metabolic network considered.

r_{e1}:	Glucose → 2 Lactate
r_{e2}:	Glucose → 6 CO_2
r_{e3}:	Glutamine → 2 CO_2 + Ammonia+ Alanine
r_{e4}:	Glutamine → Lactate + 2 CO_2 + 2 Ammonia
r_{e5}:	Glutamine → 5 CO_2 + 2 Ammonia
r_{e6}:	Glucose + 3 Glutamine → Purine + 2 CO_2 + Ammonia
r_{e7}:	Glucose + 2 Glutamine → Pyrimidine + 2 CO_2 + Ammonia

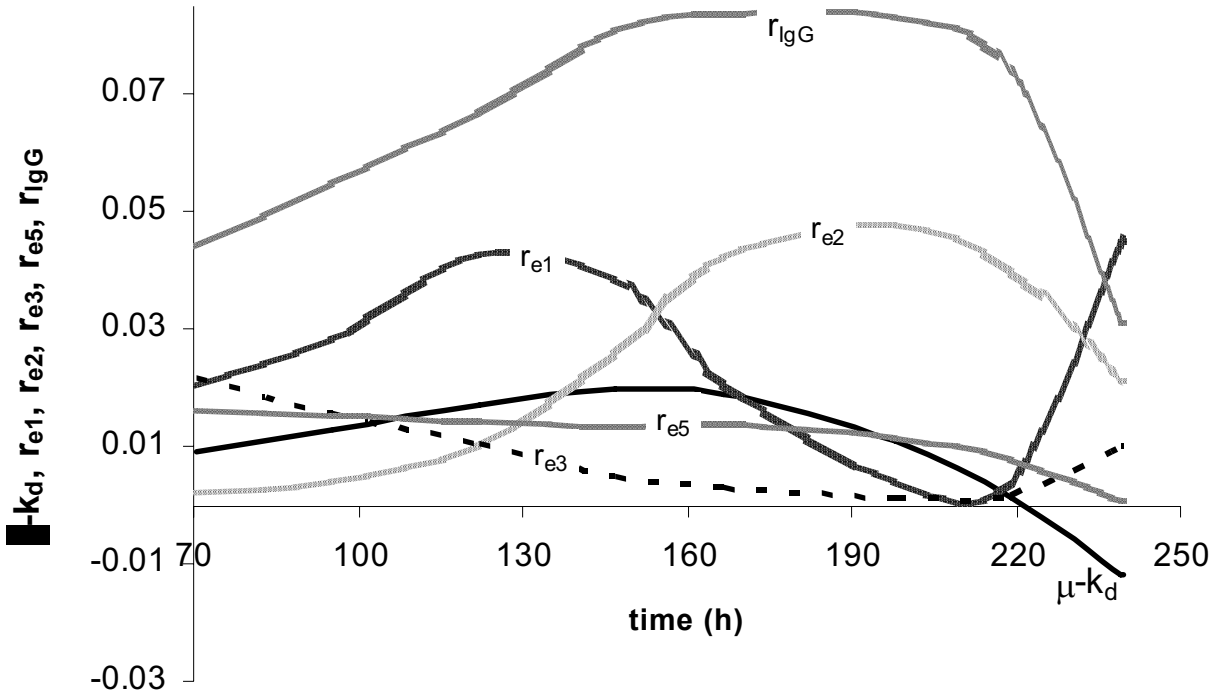

Figure 2
Apparent specific growth rate (μ-k_d), specific protein synthesis rate (rIgG) and elementary flux modes kinetics identified by the hybrid model.

Analyzing such patterns we can take some conclusions. The most energetic EFM involving glucose and glutamine are r_{e2} and r_{e5}, respectively. Looking at these two EFMs in figure 2 we can verify that glutamine seems to be the major source of energy during the growth phase since r_{e5} is almost constant, while the metabolism of glucose gradually changes from a state where it is mostly converted to lactate (r_{e1}, a poor energetic pathway), to a state of complete oxidation of glucose via TCA cycle (r_{e2}). Zielke et al. (1984) have already reported that glutamine becomes the predominant source of energy at low glucose concentration. On the other hand, in the death phase (μ-kd<0) there is a shut down in the most energetic EFMs (r_{e2} and r_{e5}) and the overflow metabolism takes place i.e., the production of lactate (r_{e1}) and alanine (r_{e3}) starts to increase. These metabolic particularities of animal cells were well captured by the hybrid model which confirms its potentialities.

Using the developed hybrid model, the process performance (described as the glycoprotein titre at the end of the bioreaction, eq. 2) is optimized with respect to glucose and glutamine feeding using a micro-genetic algorithm [9].

$$\max_{u} \quad J = C_{IgG1-IL2}(t_f)V(t_f) \qquad (2)$$

The final optimization results are presented in Fig. 3. The optimized strategy suggests to control glucose and glutamine at low levels while cells are growing (fig. 3a). During this period cells use both nutrients in an increasingly efficient way: complete oxidation of both glucose (r_{e2}) and glutamine (r_{e5}) increases while glucose converted into lactate (r_{e1}) and glutamine converted into alanine (r_{e3}) decreases. As shown in figure 3b, the ratios between the respective EFM and total glucose and glutamine consumption rates corroborates this metabolic efficiency improvement. When cells start dying (probably because ammonia reached toxic levels) the best strategy seems to be to increase the glutamine concentration. By doing so, a redistribution in the intracellular fluxes occurs that favours product formation. The process productivity may be considerably increased applying the proposed nutrients feeding strategy. The final product titre predicted by the model is 25 mg/l against the 15 mg/l that had been obtained in the fed-batch experiments.

Conclusion

In this work we present a novel bioreactor optimisation method that incorporates detailed metabolic knowledge

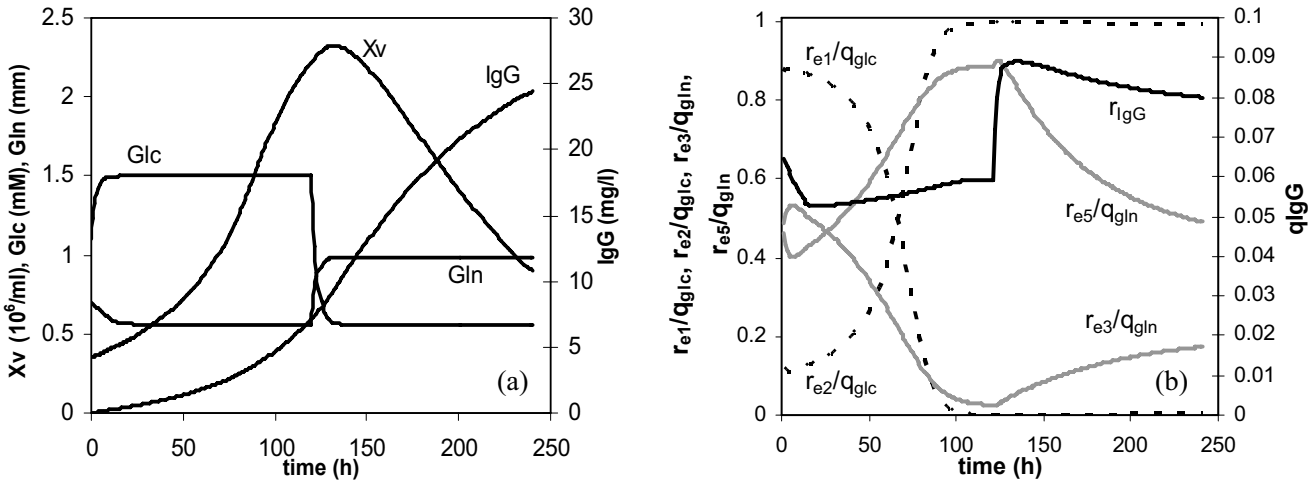

Figure 3
Optimization results.

of the biological system under consideration. The method was applied to a recombinant BHK-21 cell line expressing a fusion glycoprotein. The method allows to identify metabolic fluxes over the runtime of a bioprocess. Such knowledge allows to better understand metabolic structural changes by the analysis of the relative importance of elementary flux modes. The final hybrid model was used to optimise the flux distribution towards maximising the final product titre. It was concluded that the process productivity can be substantially improved by increasing the glutamine concentration during the cells death phase

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