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FROM MFA TO FBA: LEGITIMATING OBJECTIVE FUNCTION AND LINEAR CONSTRAINTS

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Abstract: A careful analysis of the admissible metabolic flux intervals (determined from linear programs) obtained with detailed (input and output) or limited (only input) flux measurements is proposed in a case study made of a fed-batch culture of hybridoma cells. It is shown how a cost function in FBA and additional linear constraints on the fluxes can be legitimated and efficiently introduced so as to recover admissible flux intervals based on limited external measurements which are similar to the ones based on all the available measurements. A way to model overflow metabolism on both glucose and glutamine thanks to only two inequality constraints is proposed. *Copyright © 2016 IFAC*

Keywords: metabolic network, mathematical modelling, underdetermined systems, Metabolic Flux Analysis, Flux Balance Analysis, flux-spectrum approach, overflow metabolism, mammalian cells.

1. INTRODUCTION

There is an ever increasing use of cell lines in biotechnological applications such as in biopharmaceutical industries (production of vaccines and monoclonal antibodies), food industry, bioresources and environment management, etc. Metabolic networks provide a valuable information about the cell metabolism and, more specifically, allows determining what are the key fluxes (and their amplitude) involving internal metabolites and/or involving external substrates and metabolites. Metabolic Flux Analysis (MFA) is the general approach aiming at determining the flux values based on the knowledge of the metabolic network, some measured fluxes and the basic assumption that internal metabolites do not accumulate in cells (Stephanopoulos et al., 1998). This requires solving a system of linear equations (where the equations are the mass balances of the internal metabolites and the unknowns are the fluxes) under linear equality or inequality constraints (measured fluxes, non-negativity of some fluxes). As the above mentioned biotechnological processes are usually dynamical ones, Dynamic Metabolic Flux Analysis techniques have been introduced (Antoniewicz, 2013) for dealing with the time dependency of the metabolic fluxes.

A major difficulty in MFA is that the fluxes are solutions of a generally underdetermined system of equations. Many solutions to that problem have been proposed, among which the determination of additional constraints (Haag et al., 2005), the determination of flux intervals based on convex analysis and elementary flux modes (Zamorano et al., 2013) or based on linear programs in the flux-spectrum approach (Llaneras and Picó, 2007). Another way to circumvent the underdetermination problem is Flux Balance Analysis (FBA) which consists in assuming that the cell metabolism is optimal in the sense of an objective cost function made of a linear combination of the fluxes (Orth et al., 2010). A frequent choice of objective cost function is the maximization of the cell growth. The choice of such an objective cost function and/or of additional constraints on the fluxes (except for the ones corresponding to measurements) is always tricky and potentially controversial. For instance, Simeonidis et al. (2010) discuss several possible choices of an objective cost function for describing yeast fermentation and overflow metabolism with FBA.

In this contribution, we show how to legitimate the choice of an objective cost function for FBA and of additional linear constraints on the fluxes for determining intervals of admissible fluxes, based on a limited number of external measurements, which are consistent with the ones obtained based on more detailed external measurements. To this end we illustrate on a case study made of a fed-batch culture of hybridoma HB-58 cells how admissible flux intervals (deduced from linear programs) can be analysed when the information content about external fluxes measurements is reduced (from 2 inputs and 3 outputs to only 2 inputs). Based on this analysis, appropriate objective function and additional linear constraints are proposed based on some biological assumptions (maximization of cell growth, glucose and glutamine overflow metabolism, repartition between two anaplerotic fluxes). They successfully allow reaching the above mentioned goal of obtaining admissible flux intervals consistent with the ones derived from more detailed external measurements.

The text is organized as follows. Section 2 describes the case study on hybridoma HB-58 fed-batch cultures and the metabolic network under consideration. Section 3 explains the methodology for obtaining MFA results and their discussion. Section 4 turns to FBA and introduces the

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motivation and impact of choosing the objective cost function (section 4.1) and additional linear constraints (sections 4.2 and 4.3). Final conclusions and perspectives are given in Section 5.

2. CASE STUDY: HYBRIDOMA HB-58 FED-BATCH CULTURES AND METABOLIC NETWORK

Fed-batch cultures of hybridoma cell line HB-58 (American Culture Collection – ATCC) were performed at the State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology (ECUST), Shanghai. The experiment used in the present case study has been realized in a 1L bioreactor, in controlled environment (37 °C, 50% DO, pH 7.0, stirring rate 120 RPM). After a batch phase of 35h, the culture was switched to a fed-batch mode with constant feeding (0.1 L/day) containing 9.3 mM glutamine and 15 mM glucose. Samples (10 mL) were taken about every 12h for measuring viable cell density and glucose, glutamine, lactate, ammonium and alanine concentrations. These measurements are represented in Fig. 1 together with their smoothing splines (functions *spaps* and *fnval* in Matlab) which will be used later for estimating the corresponding input (glucose, glutamine) and output (lactate, ammonium, alanine) fluxes. More details about the material and experiments can be found in Amribt et al. (2013). Note that in this former contribution, a macroscopic simulation model of fed-batch cultures was proposed without using any MFA or FBA approach.

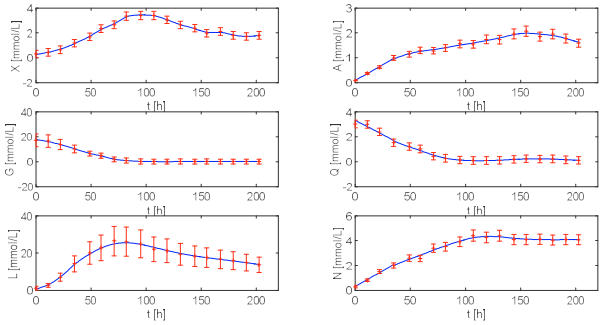

Fig. 1. Measured concentrations (with 95 % confidence intervals) of biomass, alanine, glucose, glutamine, lactate and ammonium (in red) together with their corresponding smoothing splines (in blue)

For describing the central metabolism of these hybridoma cells, a simplified metabolic network has been chosen in Provost et al. (2006). Although originally proposed for the growth phase of CHO-320 cells, this metabolic network (represented in Fig. 2) is sufficiently limited to the basic metabolism (glycolysis, citric acid cycle, pentose phosphate pathways, nucleotide synthesis) so that it can be assumed valid for other mammalian cell lines. Glucose and glutamine are external substrates entering the cells while lactate, ammonium and alanine are external metabolites produced by the cells. All the remaining 17 internal metabolites are assumed to be balanced and therefore do not accumulate in the cells.

This quasi-steady-state assumption leads to the usual set of linear equations

$$Nv = 0 \tag{1}$$

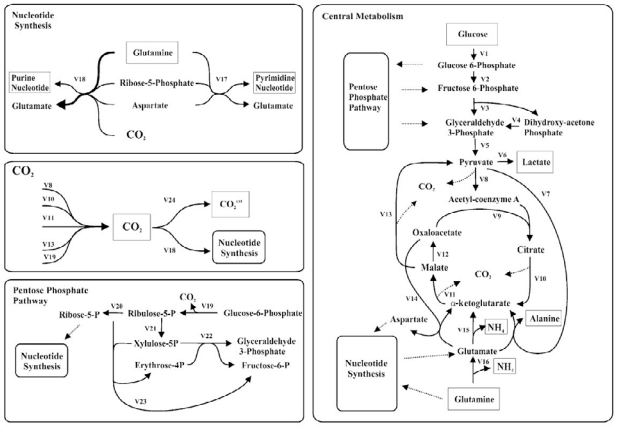

Fig. 2. Simplified metabolic network used for describing the basic metabolism of hybridoma HB-58 cells (from Provost et al. (2006))

where $N \in \Re^{18\times 24}$ is a matrix whose first 17 rows describe the contribution of the fluxes v to the mass balance of each of the 17 internal metabolites and whose last row corresponds to the additional assumption $v_{17} = v_{18}$ (equal fluxes for purine and pyrimidine nucleotide synthesis); $v \in \Re^{24}$ is the vector containing the 24 fluxes of the metabolic network. These fluxes are assumed to be non-negative $v \geq 0$. The values within the stoichiometric matrix N are immediately derived from Fig. 2 and are given in Provost et al. (2006). Note that N is a full row rank matrix and its condition number equals 9.6 showing that this metabolic network is well-conditioned.

3. DYNAMIC METABOLIC FLUX ANALYSIS

The fed-batch culture represented in Fig. 1 is of course a dynamical process which evolves with time. At the level of the metabolic network depicted in Fig. 2, this can be taken into account by considering the time profiles of the external input fluxes (glucose v_G, glutamine v_Q) and output fluxes (lactate v_L, ammonium v_N, alanine v_A). They are linked to the internal fluxes of vector v by the following relations

$$\begin{aligned} v_G(t) &= v_1(t) \\ v_Q(t) &= v_{16}(t) + v_{17}(t) + 2v_{18}(t) \\ v_L(t) &= v_6(t) \\ v_N(t) &= v_{15}(t) + v_{16}(t) \\ v_A(t) &= v_7(t) \end{aligned} \tag{2}$$

which can be written in matrix form

$$N_{in} v(t) = v_{in}(t) \tag{3}$$

$$N_{out} v(t) = v_{out}(t) \tag{4}$$

with $v_{in}^T(t) = \begin{bmatrix} v_G(t) & v_Q(t) \end{bmatrix}$ and

$v_{out}^T(t) = \begin{bmatrix} v_L(t) & v_N(t) & v_A(t) \end{bmatrix}$.

Note that the external fluxes $v_{in}(t)$ and $v_{out}(t)$ can easily be deduced from the smoothing splines of the corresponding concentrations (see Fig. 1) and mass balances of the form

$$\dot{c}_{in}(t) = -v_{in}(t)X(t) + D(t)(c_{feed} - c_{in}(t)) \tag{5}$$

$$\dot{c}_{out}(t) = v_{out}(t)X(t) - D(t)c_{out}(t) \tag{6}$$