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# Dynamic metabolic flux analysis of underdetermined and overdetermined metabolic networks

**Sofia Fernandes** * **Julien Robitaille** *** **Georges Bastin** ** **Mario Jolicoeur** *** **Alain Vande Wouwer** *

* *Automatic Control Laboratory, University of Mons, 31 Boulevard Dolez, 7000 Mons, Belgium (e-mail: sofia.afonsofernandes and alain.vandewouwer@umons.ac.be)*

** *Université catholique de Louvain, ICTEAM, Department of Mathematical Engineering, av. G. Lemaitre 4, B1348 Louvain-La-Neuve, Belgium (e-mail: Georges.Bastin@uclouvain.be)*

*** *Laboratory in Applied Metabolic Engineering, Department of Chemical Engineering, École Polytechnique de Montréal, C.P. 6079, Centre-ville Station, Montréal(Quebec), Canada (e-mail: Julien.Robitaille@nrc-cnrc.gc.ca and mario.jolicoeur@polymtl.ca)*

**Abstract:** In this work, two metabolic networks representing the metabolism of CHO cells in fed-batch cultures are considered. The first metabolic network is relatively detailed and underdetermined with the available extracellular measurements, while the second is a reduced version of the former and is overdetermined. A dynamic metabolic flux analysis based on convex analysis (DMFCA) is applied to the detailed network, which allows the computation of the time evolution of bounded intervals. On the other hand, a linear optimization problem is solved for the reduced-size network, with either positivity constraints or box constraints inferred from DMFCA. In all cases, smoothing splines and mass balance differential equations are used to infer the time evolution of the uptake and excretion rates from experimental data. The analysis allows to get insight into CHO metabolism as well as to investigate the influence of the size of the metabolic network.



*Keywords:* CHO cells, Dynamic metabolic flux analysis, Convex analysis, Underdetermined system, Overdetermined system, linear optimization.

## 1. INTRODUCTION

Metabolic flux analysis (MFA) is a useful tool to determine intracellular fluxes from extracellular measurements, such as cell density, substrates and products concentrations in, among others, mammalian cell cultures. Determining in vivo fluxes provides quantitative information on the degree of engagement of various metabolic pathways in the overall cellular metabolism. The classical MFA method is used to study systems at metabolic steady state, meaning that intracellular fluxes do not change in time. This assumption is supported by the observation that intracellular dynamics are much faster than extracellular dynamics (Stephanopoulos et al., 1998). This assumption is usually applied during the early exponential growth in batch cultures and in steady-state continuous cultures (Niklas et al., 2011). However, classical MFA does not provide information on metabolic transient. To overcome this weakness, the development of dynamic metabolic flux analysis (DMFA) techniques has been addressed (Leighty and Antoniewicz, 2011; Lequeux et al., 2010; Llaneras et al., 2012; Vercammen et al., 2014; Robitaille et al., 2015; Fernandes de Sousa et al., 2015).

A particular aspect of MFA is that depending on the information provided by extracellular measurements and on the properties of the stoichiometric matrix, the system can be determined, overdetermined or underdetermined.

In this study, two metabolic networks are considered in order to understand CHO metabolism as well as to discuss the importance of the size of the metabolic network. To this end, an underdetermined and an overdetermined metabolic networks are considered. Basically, these metabolic networks embrace the same major metabolic pathways: glycolysis, pentose phosphate pathway, TCA cycle, amino acids metabolism, nucleotides, biomass and antibody synthesis. The small-size network can be obtained by reduction of the larger, more detailed network. Of course, there is no exact metabolic network to represent cellular metabolism: a candidate metabolic network is based on available metabolic knowledge and built in a way that allows describing the consumption and production of the available extracellular metabolites in a satisfactory manner. Special care has to be exercised to preserve the stoichiometry while lumping and/or combining reactions.

To obtain the flux distribution in the larger metabolic network, a dynamic metabolic flux analysis using convex analysis (Fernandes de Sousa et al., 2015) is applied. DMFCA is an approach suitable for underdetermined


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systems, and does not require the definition of ad-hoc objective functions. Mass balance differential equations for the extracellular concentrations, together with cubic spline smoothing, are used to assess the time evolution of the uptake and excretion rates. This information is then processed by convex analysis assuming that the intracellular species are in pseudo-steady state with respect to the time evolution of the extracellular concentrations (slow-fast approximation). This method allows determining bounded intervals for each intracellular flux, and makes the most of the available information (metabolic network and available extracellular measurements) without introducing additional constraints or objective function.

The flux distribution in the reduced-size network is determined by solving a linear optimization problem using Linear Programming (LP). The problem is first solved under positivity constraints, which is the basic assumption when no additional a priori information is available. Then, the problem is solved under box constraints inspired by the bounded intervals obtained by DMFCA.

Both DMFCA and LP methods are applied to experimental data collected from CHO fed-batch cultures.

This paper is organized as follows. The next section describes briefly the experimental data. Both metabolic networks are introduced in section 3. In section 4, the DMFCA problem is formulated, including extracellular dynamic mass balance equations, spline smoothing of the experimental data, and determination of bounded intervals for the intracellular fluxes using convex analysis. The linear optimization problem is formulated in section 5. Section 6 is devoted to the numerical results and section 7 draws some conclusions.

## 2. EXPERIMENTAL DATA

Our study is based on a set of experimental data from fed-batch cultures of CHO-DXB11 cell line, producing a chimeric heavy chain monoclonal antibody (EG2-hFc) (Robitaille et al., 2015). This set contains the time evolution of the extracellular concentrations of biomass, recombinant mAb, glucose, glutamine, lactate, alanine, ammonia and 15 amino acids (except leucine, tryptophan and cysteine). The fed-batch culture was fed daily with punctual injections of fresh medium, to avoid nutrients limitation (see figure 1). Mathematically speaking, this type of fed-batch, with punctual injections, can be considered as a succession of batch cultures with different initial conditions.

For more details about the experimental procedure and analytical methods, the reader is referred to (Robitaille et al., 2015).

## 3. METABOLIC NETWORK

Two metabolic networks are considered in this work. The first one is relatively detailed (see table 2) and contains 72 biochemical reactions, 45 internal metabolites and 21 extracellular metabolites present in the culture medium, which are either substrates or products. The second one is described in (Robitaille et al., 2015) and contains 29 reactions, 16 internal metabolites and 21 extracellular measurements. Both metabolic networks embrace the major reactions of central metabolism such as glycolysis, Tricarboxylic Cycle Acid (TCA), Penthose Phosphate Pathway (PPP) and amino acids metabolism. Biomass and antibody synthesis are also incorporated into the model. The stoichiometric coefficients of the biomass and antibody synthesis were taken from literature (Robitaille et al., 2015). The small-size network can be obtained by reduction of the larger network. If $N_u$ and $N_o$ are the stoichiometric matrices of the underdetermined and overdetermined networks, respectively, it is possible to prove that $N_o \in N_u$, through the following steps:

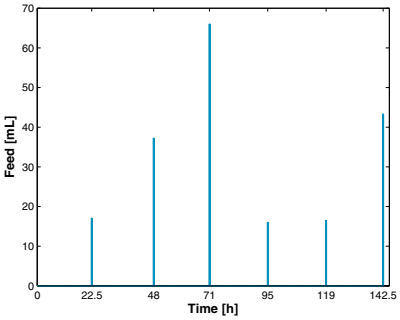


Fig. 1. Feeding strategy over CHO-DXB11 fed-batch culture.

- Reactions $v_3$ and $v_4$ are lumped into an overall reaction.
- Reactions $v_{10}$ and $v_{11}$ are lumped into an overall reaction and the metabolite Succinyl-CoenzymeA (SucCoA) is eliminated.
- Reactions $v_{12}$ and $v_{13}$ are lumped into an overall reaction and the metabolite Fumarate (Fum) is eliminated.
- Reactions $v_{30}$, $v_{31}$, $v_{32}$, $v_{33}$, $v_{34}$, $v_{35}$, $v_{36}$, $v_{37}$ and $v_{39}$ are lumped into an overall reaction (this reaction is called amino acids transamination in Robitaille's work).
- According to Robitaille's work, the amino acids threonine, phenylalanine, methionine and cysteine are only used to biomass and mAb synthesis. Therefore, reactions $v_{25}$, $v_{38}$, $v_{40}$ and $v_{41}$ are not taken into account.
- Reactions $v_{43}$, $v_{44}$, $v_{45}$, $v_{46}$ and $v_{47}$ are lumped into an overall reaction (in Robitaille's work, this reaction is called Histidine/arginine transamination).
- Reactions $v_{26}$, $v_{27}$, $v_{28}$, $v_{48}$, $v_{49}$ and $v_{50}$ are not taken into account in Robitaille's network.
- The only transport reaction considered in Robitaille's work is the one of glutamate (Glu).

Table 2. Metabolic network of CHO cells.

| Flux | Reactions |
|---|---|
| | **Glycolysis** |
| $v_1$ | $Glc_{ext} + ATP \rightarrow G6P + ADP$ |
| $v_2$ | $G6P \leftrightarrow F6P$ |