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Application of Dynamic Metabolic Flux Convex Analysis to CHO-DXB11 Cell Fed-batch Cultures

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Abstract: In this work, a dynamic metabolic flux analysis based on convex analysis (DMFCA) is applied to CHO-DXB11 cell fed-batch cultures. This approach exploits all the available knowledge of the metabolic network and the time evolution of extracellular component concentrations, to determine bounded intervals for the fluxes continuously over time. Smoothing splines and mass balance differential equations are used to estimate the time evolution of the uptake and excretion rates from experimental data. Furthermore, the method is suitable for underdetermined systems, and does not require the definition of ad-hoc objective functions to be optimized. Moreover the metabolic network considered in this work allows an estimation of the carbon dioxide flux.

Keywords: CHO cells, Metabolic network, Dynamic metabolic flux analysis, Underdetermined system, Convex analysis, Mathematical modeling

1. INTRODUCTION

Over the past years, classical Metabolic Flux Analysis (MFA) has been extensively used to determine intracellular fluxes from extracellular measurements, such as cell density, substrate and product concentrations in, among others, mammalian cell cultures. This tool has been widely applied to investigate static metabolic states of cells, corresponding to intracellular fluxes which do not change over time. This assumption is supported by the observation that intracellular dynamics are much faster than extracellular dynamics. Therefore, it makes sense to neglect the fast dynamics and consider that intracellular fluxes are in pseudo-steady state (Stephanopoulos et al., 1998). The main disadvantage of classical MFA is that it does not provide information on metabolic transient. To overcome this weakness, dynamic metabolic flux analysis (DMFA) techniques have been proposed (Leighty and Antoniewicz, 2011; Lequeux et al., 2010; Llaneras et al., 2012; Niklas et al., 2011; Vercammen et al., 2014).

DMFA is also based on stoichiometric metabolite balancing within an assumed metabolic model. Most of the proposed DMFA approaches are dedicated to exactly determined or overdetermined systems. However, due to the complexity of the metabolic networks, measurable and available extracellular data is usually insufficient, leading to an underdetermined system of algebraic equations, whereby a unique solution cannot be computed. When an underdetermined system is considered, the literature suggests the use of dynamic flux balance analysis (DFBA) (Mahadevan et al., 2002) and isotopic tracer approaches for non-steady state flux analysis (Antoniewicz et al., 2007). The former approach implies the determination of an appropriate objective function, which remains valid over the whole culture, and involves large computational expenses. Detailed dynamic models including information on the kinetics have been introduced (Dorka et al., 2009; Ghorbaniaghdam et al., 2014; Robitaille et al., 2015), but those dynamic models require more experimental data for their validation. The identification of a priori unknown reaction kinetics is a critical task due to the model nonlinearity, relatively large number of parameters, and scarcity of informative experimental data.

In the present study, an alternative DMFA method is presented, which is suitable for underdetermined systems, and does not require the definition of ad-hoc objective functions. The method is based on convex analysis, and builds upon the methodology introduced in Provost and Bastin (2004) and further exploited in (Zamorano et al., 2010; Fernandes et al., 2015). In these former works the specific uptake and production rates are assumed constants and are determined using linear regression. In this

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study, 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). Dynamic Metabolic Flux Convex Analysis (DMFCA) 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. In this work, DMFCA is applied to experimental data collected from CHO fed-batch cultures.

This paper is organized as follows. The next section describes the experimental data. The considered metabolic reaction network is 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. Section 5 is devoted to the numerical results and section 6 draws some conclusions.

2. EXPERIMENTAL DATA

Our study is based on a set of the experimental data from CHO-DXB11 cell line, producing a chimeric heavy chain monoclonal antibody (EG2-hFc). The experimental work has been performed at the Research Laboratory in Applied Metabolic Engineering, University of Montréal, Quebec, Canada (Robitaille et al., 2015). This set of experimental data results from a fed-batch culture and 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 limitations (see figure 1). Mathematically speaking, this type of fed-batch, with punctual injections, is characterized as a succession of batch cultures.

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

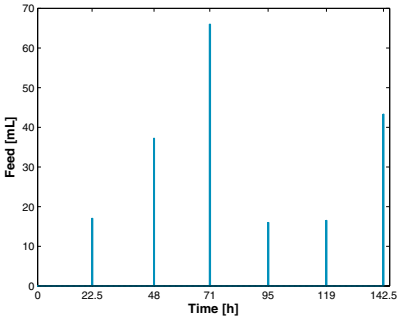

Fig. 1. Feeding strategy over CHO-DXB11 fed-batch culture.

3. METABOLIC NETWORK MODEL

The metabolic network considered in this work contains 70 biochemical reactions, 45 internal metabolites and 21 extracellular metabolites present in the culture medium, which are either substrates or products. It embraces the major reactions of central metabolism such as glycolysis, Tricarboxylic Cycle Acid (TCA), Penthose Phosphate Pathway (PPP) and amino acids metabolism (see Table 2). Furthermore, 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 authors emphasize that 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. However, special care has to be exercised to preserve the stoichiometry while lumping and/or combining reactions.

Also, note that convex analysis provides positive intervals (solutions). Therefore the flux direction of the biochemical reactions is fixed a priori in agreement with the metabolic state of the cells.

Table 2. Metabolic network of CHO cells.

Flux	Reactions
	Glycolysis
v_1	$Glc_{ext} + ATP \rightarrow G6P + ADP$
v_2	$G6P \leftrightarrow F6P$
v_3	$F6P + ATP \rightarrow DHAP + G3P + ADP$
v_4	$DHAP \leftrightarrow G3P$
v_5	$G3P + NAD^+ + ADP \leftrightarrow 3PG + NADH + ATP$
v_6	$3PG + ADP \rightarrow Pyr + ATP$
	Tricarboxylic Acid Cycle
v_7	$Pyr + NAD^+ + CoASH \rightarrow AcCoA + CO_2 + NADH$
v_8	$AcCoA + Oxal + H_2O \rightarrow Cit + CoASH$
v_9	$Cit + NAD(P)^+ \rightarrow \alpha KG + CO_2 + NAD(P)H$
v_{10}	$\alpha KG + CoASH + NAD^+ \rightarrow SucCoA + CO_2 + NADH$
v_{11}	$SucCoA + GDP + Pi \leftrightarrow Succ + GTP + CoASH$
v_{12}	$Succ + FAD \leftrightarrow Fum + FADH_2$
v_{13}	$Fum \leftrightarrow Mal$
v_{14}	$Mal + NAD^+ \leftrightarrow Oxal + NADH$
	Pyruvate Fates
v_{15}	$Pyr + NADH \leftrightarrow Lac_{ext} + NAD^+$
v_{16}	$Pyr + Glu \leftrightarrow Ala + \alpha KG$
	Pentose Phosphate Pathway
v_{17}	$G6P + 2NADP^+ + H_2O \rightarrow R5P + 2NADPH + CO_2$
v_{18}	$R5P \leftrightarrow X5P$
v_{19}	$2X5P + R5P \leftrightarrow 2F6P + G3P$
	Anaplerotic Reaction
v_{20}	$Mal + NAD(P)^+ \leftrightarrow Pyr + CO_2 + NAD(P)H$
	Amino Acid Metabolism
v_{21}	$Glu + NAD(P)^+ \leftrightarrow \alpha KG + NH_4^+ + NAD(P)H$
v_{22}	$Oxal + Glu \leftrightarrow Asp + \alpha KG$
v_{23}	$Gln \rightarrow Glu + NH_4^+$
v_{24}	$Thr + NAD^+ + CoASH \rightarrow Gly + NADH + AcCoA$
v_{25}	$Ser \leftrightarrow Gly$