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METABOLIC FLUX ANALYSIS : AN APPROACH FOR SOLVING NON-STATIONARY UNDERDETERMINED SYSTEMS

A. Provost and G.Bastin

Cesame, Université Catholique de Louvain
av. Georges Lemaître 4, 1348 Louvain-la-Neuve, Belgium
Phone:+32 10 478032 Fax: +32 10 47 21 80
email: (provost, bastin)@auto.ucl.ac.be

Abstract. The aim of this paper is to present an approach to analyse time-varying metabolic fluxes. The traditional tool to perform such analysis is called Metabolic Flux Analysis (MFA) and provides a flux distribution which is a constant vector, solution of a linear system constructed from the stoichiometric matrix. A basic limitation is that MFA is usually stated for a given and fixed configuration of the metabolic network that does not account for the cell adaptation to its environment and particularly to the substrate availability.
Our concern in this paper is to consider the situation where the cells adapt their metabolism in such a manner that the metabolic network structure is changing during the cell life.
This is achieved by decomposing the cell life in a succession of phases during each of which the metabolism is described by a metabolic network. Then for each phase, the flux distribution is described as a combination of Elementary Flux Modes. Finally, the complete flux distribution is obtained by smoothly switching from phase to phase. The approach is illustrated with batch cultures of Chinese Hamster Ovary (CHO) cells.

1. Introduction

Metabolic Flux Analysis is a popular tool for the modelling of metabolic networks. A metabolic network generally represents a part of the cell metabolism. The nodes of such a network represent the internal metabolites, the inputs and outputs of the network are respectively the substrates and the products and the metabolic reactions are represented by the edges of the network.
The basic idea of Metabolic Flux Analysis is that with the help of a metabolic network and with some flux measurements, it is possible to find the values of the rates at which each reaction operates. A flux distribution is a vector which entries are these rate values and that gives some insight on the mechanism of the cells (see e.g [1]). For example, it can help to detect which of several pathways is preferred by the cells to consume a particular substrate.
A basic limitation is that MFA is usually stated for a given and fixed configuration of the metabolic network that does not account for the cell adaptation to its environment and particularly to the substrate availability.
Our concern in this paper is to consider the situation where the cells adapt their metabolism in such a manner that the metabolic network structure is changing during the cell life. Therefore, several different metabolic network structures are necessary to capture the mechanism of the cells during their life time.
In our example of Chinese Hamster Ovary cells cultivated in batch mode, the measured extracellular species are Glucose, Glutamine, Lactate, Ammonia and Alanine. The experimental data collected during three experiments are shown in Figure 1. We shall use three successive metabolic networks corresponding to the three phases of the cell life: the growth phase, the transition phase and the death phase. These three phases are illustrated in Figure 1 :the growth phase marked by + , the transition phase marked by o and the death phase marked by x.
A complete MFA will then be obtained by using the three separate flux distribution models in their respective time interval, smoothly switching from network to network on the basis of the availability of the substrates.

2. Classical Metabolic Flux Analysis

Metabolic Flux Analysis derives from the pseudo-steady state assumption which states that the intracellular metabolites do not accumulate in the cell, or equivalently that around each metabolite, the net balance between the consumption and production fluxes is equilibrated. This is represented by the algebraic equation:

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

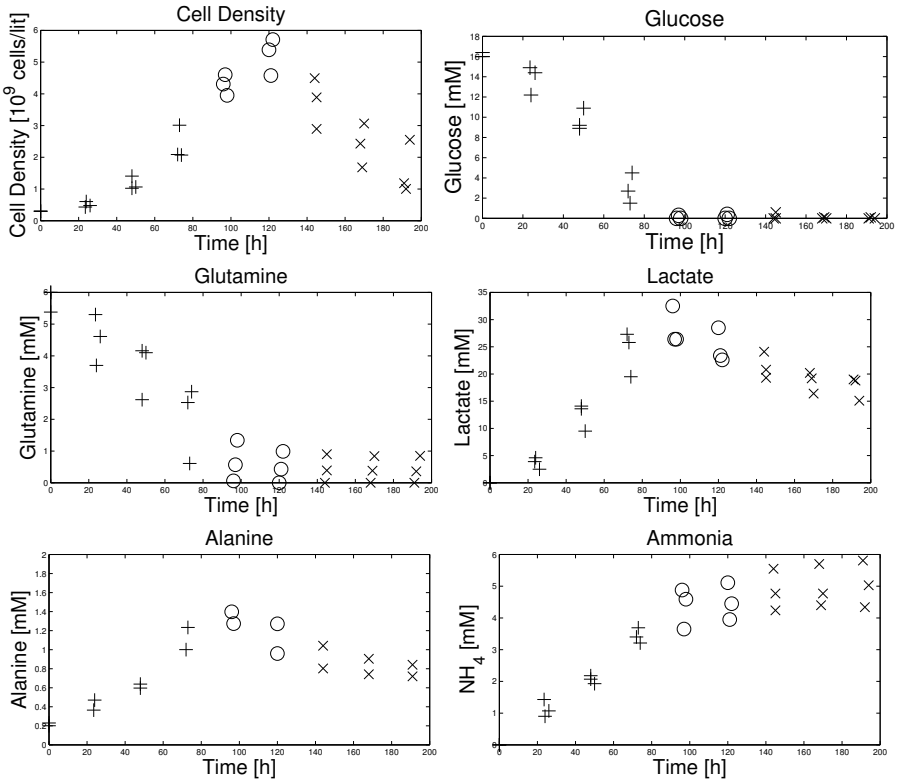

Figure 1: Biomass production and measured extracellular species for three CHO-320 batch cultures. Growth, transition and death phases data are respectively represented by +, o and x marks.

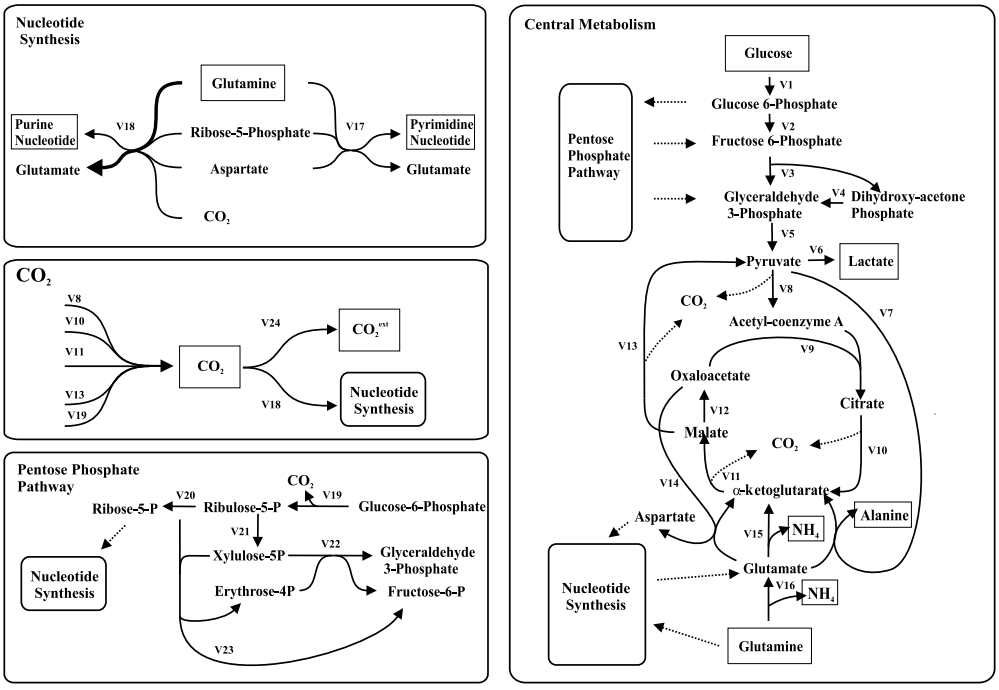

Figure 2: Metabolic network for the growth of CHO-320 cells. Bold arrows stand for double arrows.

Metabolites	v_1	v_2	v_3	v_4	v_5	v_6	v_7	v_8	v_9	v_{10}	v_{11}	v_{12}	v_{13}	v_{14}	v_{15}	v_{16}	v_{17}	v_{18}	v_{19}	v_{20}	v_{21}	v_{22}	v_{23}	v_{24}
Glucose 6-P	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	0	0	0	0	0
Dihydroxy-acetone P	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glyceraldehyde 3-P	0	0	1	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Pyruvate	0	0	0	0	1	-1	-1	-1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Acetyl-coenzyme A	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Citrate	0	0	0	0	0	0	0	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
α-ketoglutarate	0	0	0	0	0	0	1	0	0	1	-1	0	0	1	1	0	0	0	0	0	0	0	0	0
Malate	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0	0	0	0	0	0	0	0	0	0	0
Glutamate	0	0	0	0	0	0	-1	0	0	0	0	0	0	-1	-1	1	1	2	0	0	0	0	0	0
Oxaloacetate	0	0	0	0	0	0	0	0	-1	0	0	1	0	-1	0	0	0	0	0	0	0	0	0	0
Aspartate	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	-1	-1	0	0	0	0	0	0
Ribose 5-P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0	0	0
Ribulose 5-P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	-1	0	1	0	0	-1	0
Erythrose-4-P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-1	1	0
Xylulose-5-P	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1	-1	0
Fructose-6-P	0	1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
CO_2	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	-1	1	0	0	0	0	-1

Table 1: The stoichiometric matrix for the growth phase.

where $N = [n_{ij}]$ is the $m \times n$ stoichiometric matrix and v is the flux distribution vector which entries are the reaction rates, also called fluxes, of the reactions of the network.
The stoichiometric matrix translates the structure of the network in the following manner:

- in each row the non-zero entries represent production and consumption fluxes of a particular metabolite,
- in each column the non-zero entries indicate the metabolites that are involved in a particular reaction.

More precisely, the flux v_j denotes the rate of reaction j and a non-zero entry n_{ij} is the stoichiometric coefficient of metabolite i in reaction j.
As a matter of illustration, the metabolic network chosen to describe the growth phase of the CHO cells is depicted in Figure 2 and the corresponding stoichiometric matrix network appears in Table 1 (see [6,7,8]).
Some metabolite measurements are available and describe in some sense the cells under study. Here we will work with the hypothesis that only measurements of some substrates and products are available. The specific uptake and excretion rates of these measured substrates and products are denoted v_s and v_p. By definition they are linear combinations of some of the metabolic fluxes. This is expressed by defining two appropriate matrices $N_s^{s\times n}$ and $N_p^{p\times n}$ such that:

$$v_s = N_s v \qquad v_p = N_p v.$$

The purpose of MFA is to compute the unknown flux distribution v from the measurements of v_s and v_p. The goal is thus to find a non-negative solution to the linear system:

$$\begin{pmatrix} N \\ N_s \\ N_p \end{pmatrix} v = \begin{pmatrix} 0 \\ v_s \\ v_p \end{pmatrix} \text{ with } v \geq 0 \tag{2}$$

In the case of CHO cells, during the growth phase, Glucose and Glutamine are the measured substrates and Lactate, Alanine and Ammonia are the measured products (see Fig. 1). As a consequence, matrices N_s and N_p are written

as follows:

$$N_s = \begin{array}{c} \begin{matrix} v_1 & & v_{16} & v_{17} & v_{18} & & v_{24} \end{matrix} \\ \begin{pmatrix} 1 & \dots & 0 & 0 & 0 & \dots & 0 \\ 0 & \dots & 1 & 1 & 2 & \dots & 0 \end{pmatrix} \end{array} \begin{matrix} \text{Glucose} \\ \text{Glutamine} \end{matrix}$$

$$N_p = \begin{array}{c} \begin{matrix} v_1 & & v_6 & v_7 & & v_{15} & v_{16} & & v_{24} \end{matrix} \\ \begin{pmatrix} 0 & \dots & 1 & 0 & \dots & 0 & 0 & \dots & 0 \\ 0 & \dots & 0 & 1 & \dots & 0 & 0 & \dots & 0 \\ 0 & \dots & 0 & 0 & \dots & 1 & 1 & \dots & 0 \end{pmatrix} \end{array} \begin{matrix} \text{Lactate} \\ \text{Alanine} \\ \text{NH}_4 \end{matrix}$$

System (2) is typically underdetermined because there are fewer metabolites than reactions in a metabolic network and the number of measurements is usually too small to make the system a completely determined one. This means that, except if additional constraints are added, the solution v of the MFA problem (2) is not unique but constitutes a set of solutions that we denote $\mathcal{S}$. One of our goals is to give a geometrical characterization of the set $\mathcal{S}$ with a sensible biological interpretation.

3. Elementary Flux Modes

Obviously the nonnegative solution vector v lies in the kernel of N. Convex Analysis provides for a further characterization of the solutions. In fact, any solution of (2) can be written as a nonnegative combination of vectors e_i that constitute the so-called convex basis of the solution space:

$$v = \sum_i e_i w_i \text{ with } w_i \geq 0 \quad \forall i$$

or equivalently:

$$v = Ew \text{ with } w \geq 0$$

where $E^{n \times k}$ is a matrix which columns are the convex basis vectors and $k \geq null(N) = n - m$. The convex basis is by definition constituted of nonnegative vectors:

$$e_i^j \geq 0 \text{ for } j = 1..n \quad \forall i.$$

In biochemical terminology a convex basis vector is an Elementary Flux Mode (EFM) (see [3,4,5]). The EFMs for the growth phase of CHO cells are displayed in Table 2. From a geometric viewpoint, the convex basis defines a pointed convex polyhedral cone $\mathcal{P}$ which involves all the nonnegative solutions v of $Nv = 0$. But it is evident that the solution set $\mathcal{S}$ is only a strict subset of $\mathcal{P}$. We shall see hereafter that the set of $\mathcal{S}$ can also be geometrically described as a pointed polyhedral cone in the k-dimensional nonnegative orthant.
The Elementary Flux Modes allow for a different formulation of system (2) as follows:

$$\begin{pmatrix} N \\ N_s \\ N_p \end{pmatrix} Ew = \begin{pmatrix} 0 \\ v_s \\ v_p \end{pmatrix} \text{ with } w \geq 0 \tag{3}$$

Since $NE = 0$ the problem is reduced to find w such that:

$$\underbrace{\begin{pmatrix} N_s E \\ N_p E \end{pmatrix}}_{M^{(s+p) \times k}} w = \begin{pmatrix} v_s \\ v_p \end{pmatrix} \text{ with } w \geq 0 \tag{4}$$

Usually, k is larger than the total number of measurements $s + p$. System (4) is therefore underdetermined and the solution set $\mathcal{S}$ can be obtained from the solution set of (4) by a linear operation encoded in E. System (4) can also be written in the following form:

$$\begin{pmatrix} N_s E & -v_s \\ N_p E & -vp \end{pmatrix} \begin{pmatrix} w \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \\ 0 \end{pmatrix} \text{ with } w \geq 0 \tag{5}$$

which in turn can be analyzed in the framework of convex analysis.
For each nonnegative solution w of the nonhomogeneous system (4), there obviously exists a unique nonnegative solution $\begin{pmatrix} w \\ 1 \end{pmatrix}$ of the homogeneous system (5). This means that the solution set $\mathcal{S}$ is isomorphic to the polyhedral cone generated by the convex basis of the solution space of system (5). As a matter of illustration, system (5) for the growth phase of CHO cells is written as follows:

$$\begin{pmatrix} 1 & 1 & 3 & 3 & 0 & 0 & 0 & 1 & 1 & 1 & 1 & -0.187 \\ 0 & 0 & 0 & 0 & 1 & 1 & 1 & 2 & 1 & 1 & 1 & -0.050 \\ 2 & 0 & 5 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 1 & -0.344 \\ 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & -0.008 \\ 0 & 0 & 0 & 0 & 2 & 1 & 2 & 0 & 0 & 1 & 1 & -0.045 \end{pmatrix} \begin{pmatrix} w \\ 1 \end{pmatrix} = \begin{pmatrix} 0 \end{pmatrix} \tag{6}$$

In this equation, average values of the substrate uptake rates v_s and the production rates v_p have been computed from the experimental data given in Figure 1.
The vectors of the convex basis of (6) are given in Table 3. They are normalised to have a unit last entry. Let us consider the submatrix F obtained by removing the last row. The dimension of the matrix F is $k \times l$ where k is the number of elementary flux modes and l is the size of the convex basis. Each line of the matrix corresponds to an EFM. An important and critical observation is that in each column of F, there are exactly $(s + p)$ nonzero entries and $k - (s + p)$ zero entries. From a biological viewpoint, each column of F can then be interpreted as a particular solution w of (4) that uses a minimal number of $(s + p)$ EFMs. The nonzero entries of a vector f of F can be interpreted as the weights of the respective contributions of the different EFMs in the computation of the corresponding solutions w.
Finally, since each flux distribution v satisfying (2) is defined as $v = Ew$, it follows that the columns of the matrix product EF, presented in Table 4, can be interpreted as a convex basis of the solution set $\mathcal{S}$ of the MFA problem (2).
Some additional knowledge could be used to discard some of these convex basis vectors as acceptable flux distributions. Let us illustrate this with the growth phase of CHO cells. In our example, measures of Purine and Pyrimidine nucleotides are not available but these nucleotides are necessary simultaneously for the production of biomass. Since the measures of Purine and Pyrimidine nucleotides are not involved in the calculations of the convex basis F, this is a constraint that has to be taken into account a posteriori when choosing the columns of EF to represent an acceptable flux distribution. The fluxes related to Purines and Pyrimidines respectively are noted v_{17} and v_{18} in the metabolic network (see Figure 2). Hence, the vectors of EF that possess a zero at one of these two entries (see Table 4) are discarded because they are not compatible with the growth metabolism of the CHO cells. However, they should not be excluded from the convex basis. Indeed, their combination with the other convex basis vectors, the biomass-producing flux distributions, are valid flux distributions.

4. Time-Varying Metabolic Flux Analysis

The results of previous section show how we can obtain a nonnegative flux distribution which in addition is interpretable in terms of Elementary Flux Modes.
Now that a flux distribution is available for the growth, a different metabolic network is depicted in Figure 3 for the transition phase which occurs in the time period just after Glucose is exhausted (from about 80 to 120h). As we can see in Figure 1, during this period the cell keeps growing while Lactate and Alanine start to be consumed. Our assumption is then to consider Lactate and Alanine as new substrates, in addition to Glutamine whose consumption is slowed down. Therefore, some of the reactions of the previous network are inverted in order to be in agreement with this assumption and, in particular, to still have a production of the nucleotide precursors. During the death phase, it is assumed that the nucleotides are no longer produced. The metabolic network (Figure 4) has then been modified to satisfy that assumption.

With these metabolic networks, the same calculations can be performed for the transition and the death phases.

First, the Elementary Flux Modes and afterwards the convex basis H of system (4) are computed . An acceptable flux distribution is obtained for each phase by picking one or a convex combination of the vectors of EF, provided

	e_1	e_2	e_3	e_4	e_5	e_6	e_7	e_8	e_9	e_{10}	e_{11}
v_1	1	3	1	3	0	0	0	1	1	1	1
v_2	1	0	1	0	0	0	0	0	0	0	0
v_3	1	2	1	2	0	0	0	0	0	0	0
v_4	1	2	1	2	0	0	0	0	0	0	0
v_5	2	5	2	5	0	0	0	0	0	0	0
v_6	2	5	0	0	1	0	0	0	0	0	1
v_7	0	0	0	0	0	1	0	0	1	0	0
v_8	0	0	2	5	0	0	1	0	0	1	0
v_9	0	0	2	5	0	0	1	0	0	1	0
v_{10}	0	0	2	5	0	0	1	0	0	1	0
v_{11}	0	0	2	5	1	1	2	1	2	3	2
v_{12}	0	0	2	5	0	0	1	1	1	2	1
v_{13}	0	0	0	0	1	1	1	0	1	1	1
v_{14}	0	0	0	0	0	0	0	1	1	1	1
v_{15}	0	0	0	0	1	0	1	0	0	1	1
v_{16}	0	0	0	0	1	1	1	0	0	0	0
v_{17}	0	0	0	0	0	0	0	1	0	0	0
v_{18}	0	0	0	0	0	0	0	0	1	1	1
v_{19}	0	3	0	3	0	0	0	1	1	1	1
v_{20}	0	1	0	1	0	0	0	1	1	1	1
v_{21}	0	2	0	2	0	0	0	0	0	0	0
v_{22}	0	1	0	1	0	0	0	0	0	0	0
v_{23}	0	1	0	1	0	0	0	0	0	0	0
v_{24}	0	3	6	18	2	2	5	2	3	6	3

Table 2: The eleven elementary flux modes for the growth phase

	h 1	h 2	h 3	h 4	h 5	h 6	h 7	h 8	h 9	h 10	h 11	h 12	h 13	h 14	h 15	h 16	h 17
e 1	0.16	0.16	0.17	0.16	0.16	0.17	0.16	0.16	0.17	0.17	0.17	0.16	0.17	0.16	0.17	0.10	0.12
e 2	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
e 3	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02
e 4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
e 5	0.00	0.01	0.01	0.01	0.02	0.00	0.02	0.02	0.00	0.01	0.00	0.02	0.00	0.02	0.00	0.02	0.02
e 6	0.01	0.01	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00
e 7	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.02	0.02	0.00	0.02	0.00	0.02	0.00	0.00
e 8	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
e 9	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01
e 10	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
e 11	0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
e 12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Table 3: Matrix H containing the convex basis vectors of (4) and F, the matrix obtained by suppressing the last row of H

v 1	0.19	0.19	0.19	0.19	0.19	0.19	0.01	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19
v 2	0.16	0.16	0.17	0.16	0.16	0.17	0.01	0.17	0.17	0.17	0.17	0.16	0.18	0.16	0.17	0.10	0.12
v 3	0.17	0.16	0.17	0.16	0.16	0.17	0.01	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.17	0.15	0.15
v 4	0.17	0.16	0.17	0.16	0.16	0.17	0.01	0.17	0.17	0.17	0.17	0.17	0.18	0.17	0.17	0.15	0.15
v 5	0.34	0.31	0.33	0.31	0.33	0.33	0.01	0.34	0.34	0.34	0.34	0.35	0.35	0.34	0.35	0.33	0.32
v 6	0.34	0.34	0.34	0.34	0.34	0.34	0.01	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
v 7	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
v 8	0.02	0.00	0.01	0.00	0.01	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.00	0.00
v 9	0.02	0.00	0.01	0.00	0.01	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.00	0.00
v 10	0.02	0.00	0.01	0.00	0.01	0.01	0.00	0.02	0.02	0.02	0.02	0.02	0.03	0.01	0.02	0.00	0.00
v 11	0.06	0.07	0.07	0.07	0.07	0.07	0.00	0.07	0.07	0.07	0.07	0.06	0.06	0.06	0.06	0.04	0.05
v 12	0.03	0.03	0.03	0.03	0.03	0.03	0.00	0.03	0.04	0.03	0.04	0.03	0.04	0.03	0.04	0.01	0.02
v 13	0.03	0.04	0.03	0.04	0.04	0.03	0.00	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
v 14	0.01	0.03	0.02	0.03	0.02	0.02	0.00	0.02	0.01	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.02
v 15	0.02	0.03	0.02	0.03	0.03	0.03	0.00	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
v 16	0.03	0.01	0.02	0.01	0.02	0.02	0.00	0.02	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.03	0.02
v 17	0.00	0.03	0.01	0.03	0.02	0.01	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.01	0.00	0.00	0.01
v 18	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
v 19	0.03	0.03	0.02	0.03	0.02	0.02	0.00	0.02	0.01	0.02	0.01	0.02	0.01	0.03	0.01	0.09	0.07
v 20	0.02	0.03	0.02	0.03	0.02	0.02	0.00	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.04	0.03
v 21	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.05	0.03
v 22	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02
v 23	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.02
v 24	0.15	0.12	0.13	0.12	0.13	0.13	0.01	0.14	0.15	0.14	0.15	0.15	0.15	0.14	0.15	0.15	0.14

Table 4: Matrix EF containing the convex basis vectors of (2). The vectors EF_1, EF_7, EF_9, EF_{11}, EF_{12}, EF_{13}, EF_{15} and EF_{16} are not acceptable flux distributions.

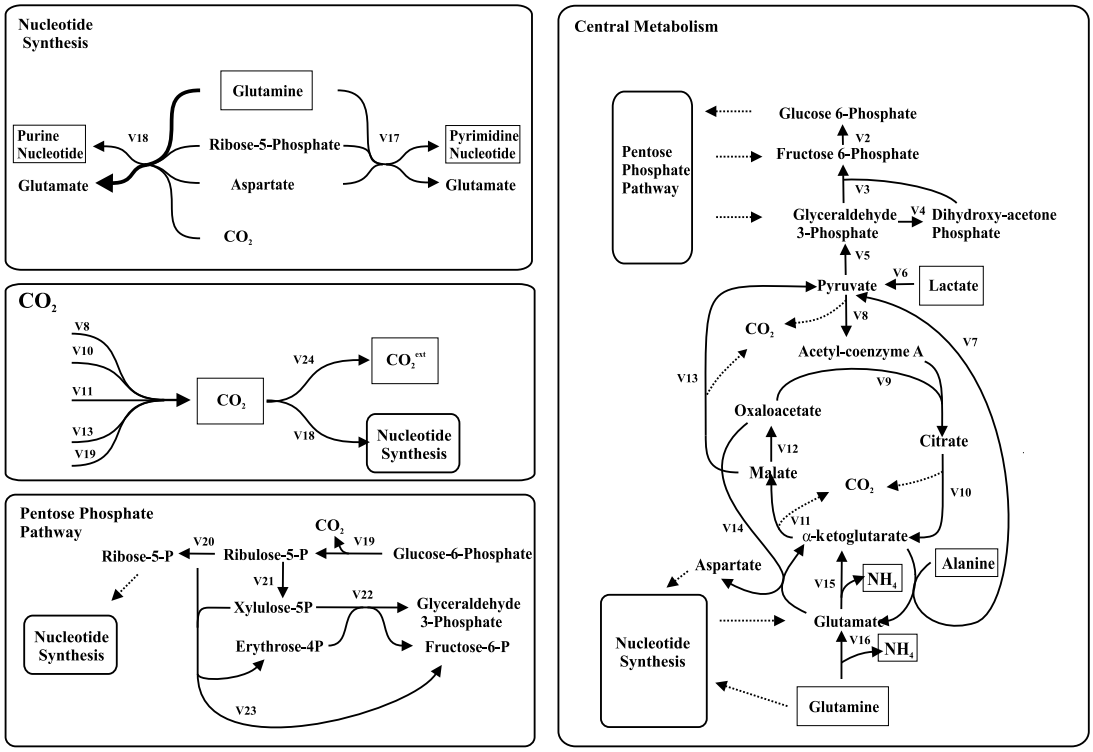

Figure 3: Metabolic network proposed for the transition phase.

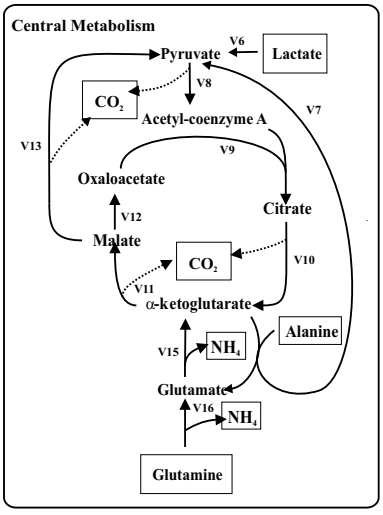

Figure 4: Metabolic network for the death phase.

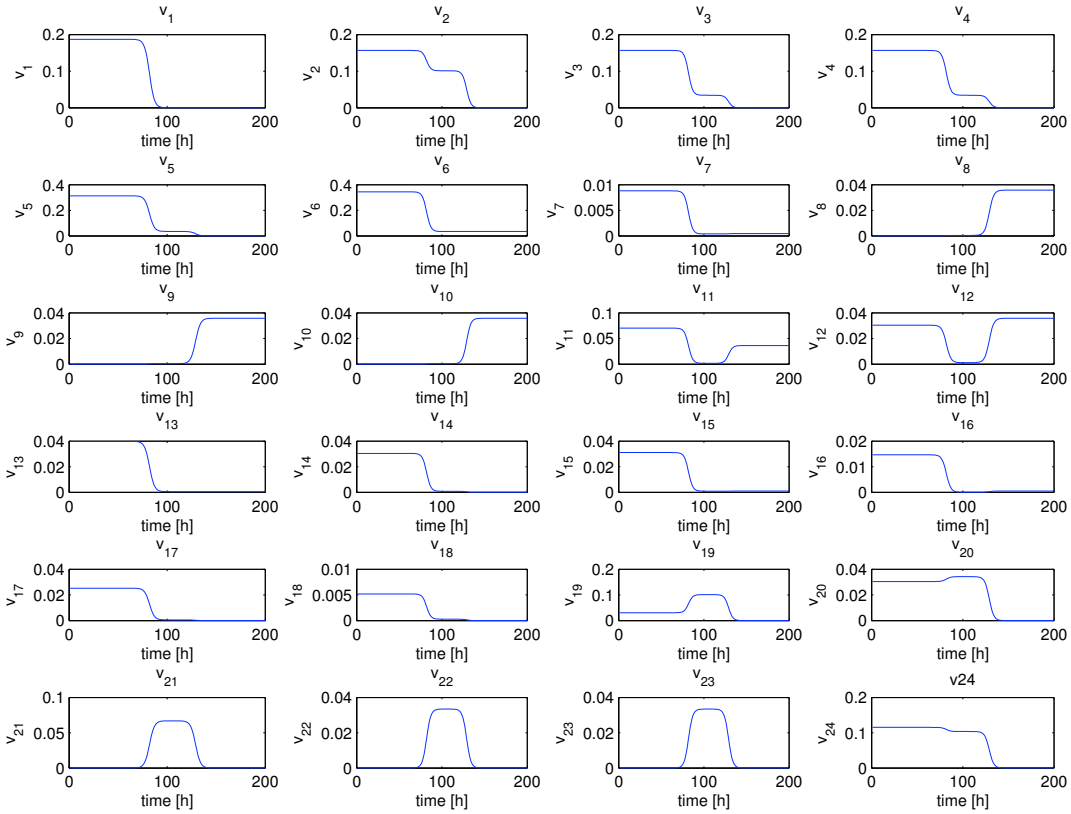

Figure 5: Time-varying flux distribution for the entire life of CHO cells. In particular, the growth flux distribution is obtained by using f_2.

that it is compatible with the metabolism of the corresponding phase of the cells. Then a time-varying flux distribution for the entire cell life is obtained by switching from each phase flux distribution to the next. In the case of CHO cells, the following time-varying flux distribution is obtained:

$$v(t) = E_{growth}\ f_{growth}\ \phi_{growth}(t) + E_{transition}\ f_{transition}\ \phi_{transition}(t) + E_{death}\ f_{death}\ \phi_{death}(t)$$

where $\phi(t)$ are smooth switching functions and the f_i are selected columns of matrix F for each phase. For example, the second column of F has been used for the growth phase of CHO cells. A similar choice has been made for the consecutive phases. It yields the resulting time-varying flux distribution presented in Figure 5.

Conclusion

In this paper, we presented an approach to compute time-varying flux distributions. This is achieved by first decomposing the cell life in a succession of phases during each of which the metabolism is described by a metabolic network. Then for each phase, the flux distribution is described as a combination of Elementary Flux Modes. The admissible weights of this combination are given by the entries of a selected convex basis vector which solves a problem involving the measurements and the Elementary Flux Modes. Finally, the complete flux distribution is obtained by smoothly switching from phase to phase.

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