New Results on Energy Balance Analysis of Metabolic Networks

Qinghua Zhou¹, Simon C.K. Shiu², Sankar K. Pal³, and Yan Li¹

¹ College of Mathematics and Computer Science, Hebei University,
Baoding City 071002, Hebei Province, China

² Department of Computing, The Hong Kong Polytechnic University,
HungHom, Kowloon, Hong Kong

³ Indian Statistical Institute, Kolkata 700 108, India
{qinghua.zhou@gmail.com, csckshiu@comp.polyu.edu.hk
sankar@isical.ac.in, hbuliyan@gmail.com}

Abstract. A central and long-standing objective of cellular physiology has been to understand the metabolic capabilities of living cells. In this paper, we perform optimization of metabolic networks in *E. coli* under both flux and energy balance constraints. The impact of the energy balance is investigated on the behavior of metabolic networks. Different from the existing work, the preliminary results showed that the impact of imposing energy balance constraints on the biological metabolic systems is very limited.

Keywords: Metabolic networks, Flux Balance Analysis, Energy Balance Analysis, Constraint optimization.

1 Introduction

Cellular metabolism, the integrated interconversion of thousands of metabolic substrates through enzyme-catalysed biochemical reactions, is the most investigated complex intracellular web of molecular interactions. In particular, the ability to quantitatively describe metabolic fluxes through metabolic networks has been long desired (Reich & Sel'kov [1]). However, this endeavor has been hampered by the need for extensive kinetic information describing enzyme catalysis within the living cell. Detailed information about all the enzymes in a specific metabolic network is not available. This dilemma has recently been partially resolved by the development of flux balance-based metabolic models (Savinell & Palsson [2], Varma & Palsson [3]).

Flux Balance Analysis (FBA) assumes that metabolic networks will reach a steady state constrained by the stoichiometry. It only requires information regarding the stoichiometry of metabolic pathways along with known metabolic requirements for growth to describe metabolic flux distributions and cell growth. The stoichiometric constraints lead to an underdetermined system and the network's behavior can be studied by optimizing the steady-state behavior with respect to some objective function (Segre et al. [4]). FBA has been shown to provide meaningful predictions in *Escherichia coli* (Varma & Palsson [5], Edwards et al. [6]).

In 2002, Daniel et al. [7] introduced Energy Balance Analysis(EBA)-the theory and methodology for enforcing the laws of thermodynamics-on the base of constraints for FBA. They want to get more physically realistic results by considering of the energy balance and thermodynamics of the network reactions. Actually, the authors provided very promising results. In this paper, we test the FBA and EBA methods to analyze their difference and quality. Our results showed that the impact of imposing energy balance constraints on the biological metabolic systems is very limited. There is not only obvious improvement but the cpu running time extended from seconds to several hours for the large-scale metabolic networks. Therefore, there is no need to simply consider energy balance constraints when to deal with the FBA models of biologically metabolic networks.

The paper is organized as follows: In section 2, we introduce some basic concepts of FBA and EBA models. After that we give the detailed computational results in section 3. Finally, we give our conclusions in section 4.

2 Basis Concepts

FBA models assumed that there is a metabolic steady state, in which the metabolic pathway flux leading to the formation of a metabolite and that leading to the degradation of the metabolite must balance, which generates the flux balance equation (Savinell et al. [8])

$$S \cdot v = b \quad (2.1)$$

where S is a matrix comprising the stoichiometry of the catabolic reactions, v is a vector of metabolic fluxes, and b is a vector containing the net metabolic uptake by the cell. Typically, equation (2.1) is underdetermined, since the number of fluxes normally exceeds the number of metabolites. Then a particular solution may be found using linear optimization by stating an objective and seeking its maximal or minimal value within the stoichiometrically defined domain. In special, the ability to meet growth requirements of the cell is of central importance. It is represented by a single reaction(Varma & Palsson[5]):

$$\sum_{all\ M} d_M \cdot M \xrightarrow{V_{gro}} Biomass \quad (2.2)$$

where d_M represents the requirements in millimoles per gram of biomass of the M biosynthetic precursors and cofactors for biomass production. V_{gro} is the growth flux(grams of biomass produced), which with the basis of 1g(dry weight) per h reduces to the growth rate(grams of biomass produced per gram per hour). Minimizing the objective function $-V_{gro}$, then we get the particular solution.

What FBA lacks is the explicit consideration of the energy balance and thermodynamics of the network reactions(Daniel et al. [7]). So, they consider the following constraints in addition to the ones used in FBA models:

$$K \cdot \Delta\mu = 0 \quad (2.3)$$

$$v_i \cdot \Delta\mu_i < 0 \quad (2.4)$$

where K is a matrix which stores the null space vectors of S' , and S' is a matrix converted from S by combining redundant fluxes and removing the columns that correspond to boundary columns. Where $\Delta\mu$ is the vector of chemical potential differences associated with the reaction fluxes.

3 Computational Results

We tested FBA and EBA models on examples selected from Daniel et al. [7] and [9], Also on a real biological metabolic systems of E.Coli. Details are as follows:

Example 1

Reaction network:

which contains 5 reactions acting on 4 metabolites. And where $v_1, \dots, v_5$ represents the flux through the relative reaction. Following the ideas of Daniel et al. [7], we consider the same problem: Determine the maximum steady-state production of reactant D , for a given set of maximal input fluxes of reactants A, B , and C . (Three related problems are considered below. The first problem assumes that reactant A is the only available input substrate. For this case, the maximal input fluxes of A, B and C are set to 1, 0, and 0 mmol sec⁻¹, respectively. The remaining two cases use B only and C only as input substrates.)

Optimal fluxes are obtained by using the MATLAB software package. In particular, for FBA we use the procedure *linprog* in the optimization toolbox of MATLAB. For EBA, we choose the procedure *fmincon*. Further, for all the cases tested, we select the zeros vector as initial point needed by the procedure *fmincon*. Details are reported in Table 1. Where the variable A_{in} means the input flux of metabolite A , D_{out} means the output flux of metabolite D . Where the symbol *FBA* means flux balance constraints only and *EBA* means both flux and energy balance constraints.

From Table 1, we can see that for all the three cases, the computational results by FBA and EBA procedures are similar. That is to say, the impact of imposing energy balance constraints on Flux balance equation is very limited and we did not get more biologically meaningful results. (It is different with the ones in Daniel et al. [7]).

Table 1. Computational results of Example 1

	A input		B input		C input	
	FBA	EBA	FBA	EBA	FBA	EBA
v_1	-0.0284	0	0.1477	0.1250	-0.1660	-0.125
v_2	-0.3440	-0.3333	0.2134	0.2083	0.4300	0.4583
v_3	0.0249	0	0.3449	0.3750	0.6219	0.6250
v_4	0.3156	0.3333	-0.657	-0.0833	0.4041	0.4167
v_5	-0.3405	-0.3333	0.7208	0.7083	0.9741	0.9583
A_{in}	1	1	0	0	0	0
B_{in}	0	0	1	1	0	0
C_{in}	0	0	0	0	1	1
D_{out}	1	1	1	1	3	3

Example 2

Reaction network:

Which is similar with the ones in Daniel et al. [9] except every reaction is reversible for convenience. Further, we perform the same procedure as in Example 1 and the results are shown in Table 2.

As we can see, the results between FBA and EBA is quite similar and we got the same outcomes in all the cases. It has proved that there is no actual differences when consider the energy balance constraints in addition to the flux balance ones.

Table 2. Computational results of Example 2

	A input		B input		C input	
	FBA	EBA	FBA	EBA	FBA	EBA
v_1	0.5	0.5	-0.1296	-0.1250	0.1296	0.1250
v_2	0	0	0.2592	0.2500	-0.2592	-0.2500
v_3	-0.5	-0.5	-0.1296	-0.1250	0.1296	0.1250
v_4	0.5	0.5	0.3887	0.3750	0.6113	0.6250
v_5	-0.5	-0.5	-0.6113	-0.6250	-0.3887	-0.3750
A_{in}	1	1	0	0	0	0
B_{in}	0	0	1	1	0	0
C_{in}	0	0	0	0	1	1
D_{out}	1	1	1	1	1	1

Then, we tested the above ideas on the E.Coli metabolism systems (Edwards & Palsson [10]). The reaction network contains 953 fluxes acting on 536 metabolites (Detailed information can be found in [11]). Within the 953 fluxes, there have 214 boundary fluxes and 114 redundant fluxes, which results a 645×1578 coefficients matrix representing the scale of linear constraints, also with 625 nonlinear constraints. We reproduced the above programming and optimized the production of biomass with glucose and oxygen uptake constrained to be less than or equal to 10 and 15 mmol g/DW $\cdot$ h, respectively. For the flux of representing the non-growth related maintenance, we let it to be 7.6 mmol g/DW $\cdot$ h. For FBA algorithm, the resulting flux produces a growth rate of 0.78 mmol g/DW $\cdot$ h on glucose minimal media. And it only takes for several minutes. While for EBA algorithm, it stops after more than 9 hours and does not give optimal solution.

4 Conclusions

In this paper, we have tested FBA and EBA models respectively on two examples of little-scale metabolic networks and a real large-scale ones of E.Coli. Our initial results showed the limited impact of imposing energy balance constraints on flux balance constraints. There is no obvious improvement and the results of them are very similar for the two little-scale examples. As for the metabolic networks of E.Coli, the number of constraints nearly double the original size and the cpu running time increases from seconds to several hours. Therefore, we think there is no need to simply add the energy balance constraints to the flux balance constraints when considering the function of metabolic networks of real livings.

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