Modeling and Simulation of Metabolic Networks for Estimation of Biomass Accumulation Parameters

U. Kaplan [a], M. Türkay [b,*], L. Biegler [c], B. Karasözen [d]

[a] *Department of Industrial Engineering, Koç University, 34450 Istanbul, Turkey*

[b] *Department of Industrial Engineering, Koç University, 34450 Istanbul, Turkey*

[c] *Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213, USA*

[d] *Department of Mathematics and Institute of Applied Mathematics, Middle East Technical University, 06531 Ankara, Turkey*

Abstract

Metabolic networks are defined as the collection of biochemical reactions within a cell that define the functions of that cell. Due to the growing need to understand the functions of biological organisms for industrial and medical purposes, modeling and simulation of metabolic networks has attracted a lot of attention recently. Traditionally, metabolic networks are modeled such as flux-balance analysis that considers steady-state nature of the cell. But it is important to consider the dynamic behavior of a cell since the environmental conditions continuously change. Sometimes due to the critical changes in the environment some of the reactions exhibit completely different behavior leading to discrete changes in the metabolic network. Therefore, a cell exhibits discrete-continuous behavior in continuous time. Since hybrid systems exhibit the same characteristics, modeling a cell as a hybrid system gives an accurate representation. The aim of this paper is to develop a simulation framework to model the evolving structure of the cell metabolism under changes in the environment. The metabolic responses that cell gives, against multiple changes in the environment are not fully understood. Therefore, in this study, a cell is modeled as a hybrid system that is composed of a system of differential and algebraic equations. The changes in the concentration of metabolites in the environment are represented by Ordinary Differential Equations and intracellular cell metabolism is represented by a set of algebraic equations. To understand the feedback relationship between intracellular and extracellular changes, the system is solved considering the effects of extra cellular stresses on the metabolic responses.

Key words: metabolic networks, hybrid systems, parameter estimation, optimization, dynamic optimization

1 Introduction

Recent years have witnessed dramatic changes in the cellular biology. One of the main problems in the cell biology was the lack of enough dependable information. However, the sequencing of the first bacterial genome changed the biology from a data-poor science to a data-rich science [1,12]. In this data-rich environment, an entire metabolic map representing all metabolic reactions that take place in the cell are determined [11]. The cellular organisms can be modeled using this information to understand its behavior in certain environmental conditions. The prediction of the behavior of the cellular organism gives valuable opportunities for using these organisms for industrial and medical purposes. In this work, we use the fermentation of wine as a case study. Because of highly variable environmental conditions during fermentation, determination of behavior of yeast and content of wine during fermentation is a very challenging task. Due to that unpredictability, many fermentations can be problematic. In some cases, the fermentation process takes too long and in some cases the process finishes very quickly without consuming all of the sugar. In these cases, the main problem is the inhibition of the some pathways in the metabolism of the yeast rather than cell death [5]. The main reason for the inhibition of certain pathways is the changing environmental conditions such as excessive temperature or lack of nitrogen. Therefore, if the behavior of the cell is known under different environmental conditions, the problems during fermentation process can be handled and huge economic loss can be prevented in wine making industry.

There are a number of approaches to model metabolic networks. One of the most used modeling techniques is the Flux Balance Analysis (FBA) [17,18]. In FBA, models are built with respect to stoichiometry of the reactions that take place in the cellular organism and predictions are made using linear programming (LP) whose objective is the maximization of certain products or the minimization of consumption of certain metabolites. Burgard and Maranas [3] proposed a bi-level optimization model to determine the objective function in FBA. Sainz et al. [14] proposed a two stage model to simulate yeast metabolism and its interaction with the environment. The internal metabolism of the yeast is modeled as a linear programming problem and variations in the environment are modeled with a set of Ordinary Differential Equations (ODE). In the LP part, the biomass is maximized with respect to flow bounds that depend on the environmental conditions. Raghunathan et al. [10] describe dynamics of fermentation process using Differential Variational Inequalities (DVIs). The solution of the problem is accomplished by discretization of the differential equations.

* Corresponding author.
Email address: mturkay@ku.edu.tr (M. Türkay).

In this paper, the internal metabolism of yeast is modeled as a LP problem and variations in environmental metabolic concentration are modeled by a set of Ordinary Differential Equations (ODE). In the LP part, the biomass accumulation is maximized with respect to flow bounds that depends on the environmental conditions. The relationship between environmental conditions and flow constraints are obtained based on experimental data that are represented with piecewise smooth functions of environmental metabolite concentrations. Instead of using two separate models, an LP that represents intracellular activities and a set of ODEs that represents extracellular metabolite concentration; we apply an integrated approach. This model leads to a Differential Algebraic Equation (DAE) system to predict the important parameters in the fermentation process. The rest of the paper is organized as follows. We describe the theoretical background in section 2. Section 3 is devoted to explanation of the model. In section 4, a solution procedure and result of the model will be discussed. Conclusions are presented in section 5.

2 Theoretical Background

The complete genomes of cellular organisms can be sequenced in a short time with currently available experimental methods [4]. However, the real challenge begins after sequencing. Because abundance of biological data requires a new and revolutionary understanding of biology focusing on how chemical and biological functions of organism are realized, a new and interdisciplinary field appeared: systems biology [11],[13]. In systems biology, the main concern is the determination of emergent properties of interconnected nodes of the data rather than determination of properties of a single object or node of data. In this paper, the emergent property that we are looking for is the fermentation dynamics of the yeast during wine formation [16].

Intracellular Representation: With today's technology the metabolic network and the set of reactions that take place in the cell can be determined easily [11]. We can acquire knowledge of components that comprise cells and how they interact using metabolic networks. A sample metabolic network is illustrated in Fig. 1. In Fig. 1 only the reactants and products of each reaction are shown without explicitly showing the stoichiometry of network.

In this simple metabolic network, there are 5 reactions (v_1 to v_5) and 4 metabolites (A, B, C, D). In the reaction set, the network converts 2 moles of A to 1 mole of B and the remaining reactions makes similar effect in the network. The stoichiometry of the network in Fig. 1 is represented in Table 1. In this representation each row corresponds to a metabolite and columns correspond

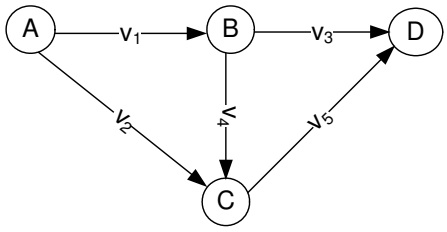

Fig. 1. Sample Metabolic Network

to reactions that the metabolites participate. If the matrix element for a particular reaction and metabolite is positive, then the metabolite is consumed by the reaction. When the matrix element is zero, then the metabolite is not involved in the reaction.

Table 1
Stoichiometric Matrix representation of Metabolic Network in Fig. 1

S_{ij}	v_1	v_2	v_3	v_4	v_5
A	-2	-2	0	0	0
B	1	0	-1	-1	0
C	0	1	0	1	-1
D	0	0	2	0	2

The matrix in the Table 1 is called a stoichiometric matrix. According to the matrix in Table 1, for instance, reaction 3 takes 1 unit of metabolite B and produces 2 units of metabolite D. We can model internal flux of a cell. If we represent the rate of change of concentration metabolites with the differential equations, the corresponding set of reactions for the network in the example will be as follows:

$$\begin{aligned}
\frac{dx_A}{dt} &= -2\nu_1 - 2\nu_2 \\
\frac{dx_B}{dt} &= \nu_1 - \nu_3 - \nu_4 \\
\frac{dx_C}{dt} &= \nu_2 + \nu_4 - \nu_5 \\
\frac{dx_D}{dt} &= 2\nu_3 + 2\nu_5
\end{aligned} \tag{1}$$

Comparing the stoichiometric matrix in Table 1 and the set of ODE in Eq. (1), the ODEs can be written as:

$$\frac{dx}{dt} = S\nu \tag{2}$$

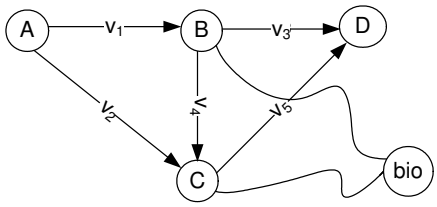

Fig. 2. Sample Metabolic Network with Biomass

where S is the stoichiometric matrix, $\nu = (\nu_1, \nu_2, \nu_3, \nu_4, \nu_5)^T$ is the flux vector, and $x = (x_A, x_B, x_C, x_D)^T$ is the concentration of metabolites. Within the cell at steady state Eq. (2) can be converted to set of homogenous linear equations:

$$0 = S\nu \tag{3}$$

To determine the dynamic behavior in an organism, Eq. (3) does not give enough information. From Eq. (3) we can only model the flux cone that includes all possible fluxes of a cell. However, the main problem here is which flux set will be carried out by the cellular organism under different conditions. One of the main tools to answer to this question is the constraint based approach [9]. In this approach an LP is solved to predict the future behavior of organisms. First, the constraints based on stoichiometry and thermodynamics' of system are determined and an objective function is included in the system to find the most promising flux distribution. Different objective functions are then applied to mimic the behavior of the organisms; these include maximization of biomass or ATP production. The suitability of these objectives are tested with experiments. However, despite existence of many objective functions, the most promising one with respect to the experimental results is the maximization of the biomass. Also it was determined that with the objective of optimal biomass formation the prediction of internal flux distributions matches the experimental results better than any other objective [9]. Nevertheless it should be noted that in the cellular organism, there is no metabolite called biomass. To add it to our metabolic network, we have to add an artificial reaction that takes some of the existing metabolites from the system and produce biomass as illustrated in Fig. 2. For the wine fermentation process, the biomass for yeast includes Carbohydrate, DNA, RNA, Lipids, and Protein. The compositional coefficient of these metabolites in the artificial biomass metabolites is determined by solving a parameter estimation problem that minimizes the difference between the experimental results and the model predictions.

The LP model developed to predict the internal dynamics of the system is the following:

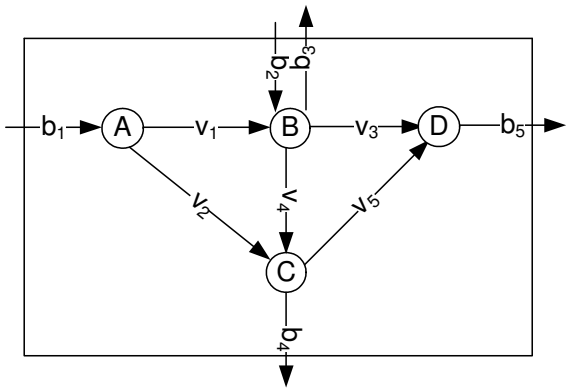

Fig. 3. Sample Metabolic Network with Biomass and Cell Exterior

$$\begin{aligned} &\max\ biomass \\ &\quad \text{st}\ S\nu = 0 \\ &\qquad \nu \geq 0 \end{aligned} \tag{4}$$

In the LP problem (Eq. (4)) all fluxes are greater than or equal zero. The main reason for this is the irreversibility of all reactions. If a reversible reaction exists in the system, we can still put the non-negativity constraint for each flux by decomposing it into two irreversible reactions.

Extracellular Representation: In the LP represented in Eq. (4), the main assumption is the steady-state of the system. At steady state, the concentration of metabolites remains constant. However, here *constant* concentrations do not imply that all reactions stop. Instead, all reactions continue to be active, but there exists a balance between each reaction. Therefore, consumption and production of each metabolite are equal to each other, and that is the main reason for the steady state. Moreover, these concentrations do not stay constant, because of the environmental stress changes. Although in Figures 1 and 2 the reactions are represented independent from cell exterior, there is a close relationship between cell interior and exterior. A more complete representation of the cell can be seen in Fig. 3.

As seen in the Fig. 3 , the metabolite A cannot be produced in the cell, therefore it should be supplied by the environment. In the case that A does not exist, most probably all of the metabolic reactions will stop. The metabolite B can be both taken from and emitted to the environment. Therefore non-existence of B will not terminate the system, but may decrease the performance of the system. If the system has to produce more B, then this may affect performance of ν_3 and ν_4 which effects biomass creation. In this study, metabolite accumulation in the extracellular medium is modeled with ODEs. For the network in Fig. 3, the ODE system is the following:

$$\begin{aligned}
\frac{dA}{dt} &= b_1 C_{bio} \\
\frac{dB}{dt} &= (b_3 - b_2) C_{bio} \\
\frac{dC}{dt} &= -b_4 C_{bio} \\
\frac{dD}{dt} &= -b_5 C_{bio}
\end{aligned} \tag{5}$$

where, b_i is external flux value of the corresponding reaction, and C_{bio} is the biomass concentration. Another important point is, as the reactions in the cell continue, certain metabolite concentrations in the environment can be depleted. For instance, after a while the concentration of the metabolite A may drop to zero if it is not supplied to the cell. For the case of wine fermentation, the glucose level will drop to zero, as fermentation continues. Therefore, extracellular concentration changes and stresses occur due to these changes. This behavior of the organism is a response of the cell to the environmental stresses. This response is mainly given by changing the flux values of some reactions that take place in the organism. In the model, this behavior is represented by making changes in the upper and lower bounds of fluxes. Therefore, for different environmental conditions different flux cones are formed for the reactions that take place inside a cell. For instance, in Fig. 3, increase of metabolite C may affect the upper bounds of ν_2 and ν_4.

3 Model

The yeast metabolism that we model includes 42 metabolites and 48 reactions (see Appendix). In the model, there are 6 external metabolites, (glucose, fructose, glycerol, ethanol, biomass, and ammonium). The LP model for intracellular fluxes is the following:

$$\begin{aligned}
&\max\ biomass \\
&\quad \text{st}\ S\nu = 0 \\
&\qquad \nu^l \leq \nu \leq \nu^r \\
&\qquad \nu \geq 0
\end{aligned} \tag{6}$$

where

$$\theta_{Pro}[Protein] + \theta_{carb}[carbonhydrate] + \theta_{DNA}[DNA] + \theta_{lipids}[lipids] + \theta_{RNA}[RNA] \rightarrow 1g.biomass$$

where θ values are the parameters that should be estimated in the biomass composition.

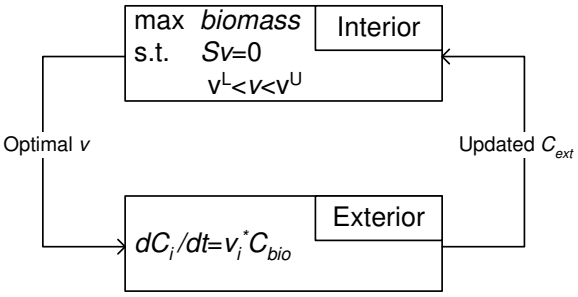

Fig. 4. Feedback relation between interior and exterior

From the result of the LP problem in Eq. 6, the optimal flux values that maximize the biomass are obtained under the assumption of steady-state. However, this steady-state characteristic of the system is not constant. In other words, as the concentration of extracellular medium changes, a new steady-state is formed. The concentration changes of the external metabolites are represented by system of ODEs:

$$\frac{dC_i}{dt} = \nu_i C_{bio} \tag{7}$$

where $i \in EXMET(glucose, fructose, glycerol, biomass, ammonium)$ and C_i is the concentration of the corresponding metabolite.

The reaction sets Eq. (6) and (7) affect each other as the fermentation process continues. In the yeast cell, as a steady-state condition is formed, a new flux of intracellular reactions changes the concentration level of the environment. The environmental changes create a stress on the cellular organism, to change its previous steady-state. As the previous steady-state of cell changes, a new flux distribution appears which changes the environment. This feedback relation continues until cell death or inhibitation of metabolism is reached (Fig. 4).

The missing piece in Fig. 4 is the lack of connection between exterior and interior of the cell. Specifically, to determine the new flux cone of the metabolic network, we have to know the kinetic relationship between cell exterior and interior. This can be done by finding kinetic parameters that determines the flow bounds in certain environmental conditions. This information is given by look-up tables [5,6], (Chapter *Heat Stress Response*, [8]) that specify bounds on reaction rates for given extracellular metabolite concentrations. The bounded reactions by these parameters are the following:

- *Bounding the ammonium (NH_4) uptake rate:* The upper bound of the ammonium uptake rate depends on the ammonium concentration in the extracellular medium. The corresponding value can be seen in the Figure 5-a.
- *Glucose uptake rate:* The uptake rate of the glucose is determined experimentally by the following equality:

$$\nu_{glu} = K_{glu,1}(C_{ammon})K_{glu,2}(C_{ammon})\nu_{glu,3}(C_{glu}, C_{eth}) \tag{8}$$

where C_{glu} and C_{eth} are the concentrations of glucose and ethanol, respectively, in g/l. As seen from Fig. 5, $K_{glu,1}$ and $K_{glu,2}$ depends on the ammonium concentration and $\nu_{glu,3}$ depends on the C_{glu} and C_{eth} values. The value of $K_{glu,2}$ can be determined from Figure 5-b. The remaining coefficients are determined from the following equalities:

$$K_{glu,1}(C_{ammon}) = \begin{cases} K^{hi}_{glu,1}, C_{ammon} > \epsilon \\ K^{lo}_{glu,1}, C_{ammon} < \epsilon \end{cases} \tag{9}$$

$$\nu_{glu,3}(C_{glu}, C_{eth}) = \begin{cases} \nu^1_{glu,3}(C_{glu}, C_{eth}),\ 0 < C_{glu} \leq 5 \\ \nu^2_{glu,3}(C_{glu}, C_{eth}),\ 5 < C_{glu} \leq 20 \\ \nu^3_{glu,3}(C_{glu}, C_{eth}),\ 20 < C_{glu} \end{cases} \tag{10}$$

The calculation of $\nu_{glu,3}$ is done using the following:

$$\begin{aligned} \nu^1_{glu,3}(C_{glu}, C_{eth}) &= \sum_{j=\{3,4,7,8\}} \frac{\nu^{\max,j}_{glu,3} C_{glu}}{k^j_{glu} + C_{glu}\left(1 + \frac{C_{eth}}{k^1_{eth}}\right)} \\ \nu^2_{glu,3}(C_{glu}, C_{eth}) &= \sum_{j=\{2,5,6\}} \frac{\nu^{\max,j}_{glu,3} C_{glu}}{k^j_{glu} + C_{glu}\left(1 + \frac{C_{eth}}{k^2_{eth}}\right)} \\ \nu^3_{glu,3}(C_{glu}, C_{eth}) &= \sum_{j=\{1,5\}} \frac{\nu^{\max,j}_{glu,3} C_{glu}}{k^j_{glu} + C_{glu}\left(1 + \frac{C_{eth}}{k^3_{eth}}\right)} \end{aligned} \tag{11}$$

where the values of the parameters in these equations can be found in Table 2.

Table 2
Values of Glucose update constants

i	$v^{max,i}_{glu,3}$	k^i_{glu}	k^i_{eth}		i	$v^{max,i}_{glu,3}$	k^i_{glu}	k^i_{eth}
1	7.45	18	17.24		5	2.7	10.8	17.24
2	1.9	1.8	46.03		6	1.08	1.67	17.24
3	1.05	0.27	46.03		7	1.31	0.18	17.24
4	4.86	10.8	46.03		8	1.31	0.36	17.24

- *Bounding ATP consumption:* The minimal ATP update rate is determined by the following equation:

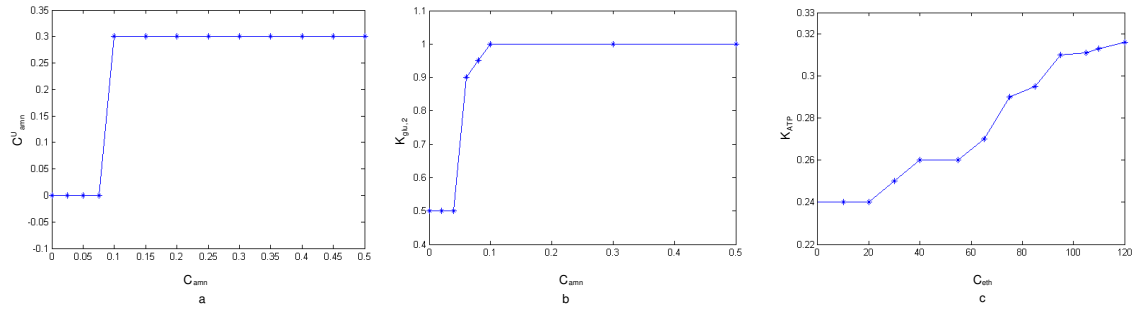

Fig. 5. Glucose update rates.

$$\nu_{ATP}^{L}(C_{ammon}, C_{eth}, C_{glu}) \leq \nu_{ATP} \tag{12}$$

$$\nu_{ATP}^{L}(C_{ammon}, C_{eth}, C_{glu}) = K_{ATP}(C_{eth})K_{glu,1}K_{ATP}(C_{ammon})K_{glu,2}K_{ammon}(C_{eth})K_{glu,3}(C_{glu}, C_{eth})$$

where the dependence between K_{ATP} and ethanol concentration in the medium found in the Fig. 5

As indicated before, the corresponding flux bounds of the internal reactions are determined with respect to external metabolite concentrations, in Eq. (8)-(12). After a while these reactions and the resultant concentrations reach steady-state. As this new steady-state is formed, the external metabolite concentration is updated. To mimic this behavior, Eqs. (6) and (7) are solved iteratively as shown in the Fig. 4, and the connections between these two equations are formed by using the look-up tables and Eq. (8-12).

Formulation integrating intra and extra-cellular reactions: In this paper, instead of decomposing the intra and extra-cellular reactions, an integrated approach is used to simulate the fermentation process. To carry this out, we write the Karush-Kuhn-Tucker (KKT) conditions for Eq. (6) in Eq. (13).

$$\begin{aligned} d + S^T\lambda + \mu^u - \mu^l &= 0 \\ S\nu &= 0 \\ (\mu^u)^T(\nu^u - \nu) &= 0 \\ (\mu^l)^T(\nu - \nu^l) &= 0 \\ \mu^u, \mu^l, \nu^u - \nu, \nu - \nu^l &\geq 0 \end{aligned} \tag{13}$$

where μ^u, μ^l, λ are the Lagrange multipliers. The two equalities correspond to complementarity conditions in Eq. (13) include non-linearities and the following changes in these constraints are made for simplification:

$$(\mu^u)^T(\nu^u - \nu) = 0, \mu^u, (\nu^u - \nu) \geq 0 \Leftrightarrow \mu_i^u - \max(0, \mu_i^u - (\nu_i^u - \nu_i)) = 0 \quad i \in MET$$
$$(\mu^l)^T(\nu - \nu^l) = 0, \mu^l, (\nu - \nu^l) \geq 0; \Leftrightarrow \mu_i^l - \max(0, \mu_i^l - (\nu_i - \nu_i^l)) = 0 \quad i \in MET \tag{14}$$

With the modification in Eq. (14) and addition of the differential equations that illustrate extracellular concentration change, the final DAE formulation is the following:

$$\begin{aligned} \frac{dC_i}{dt} &= \nu_i C_{bio} \\ d + S^T\lambda + \mu^u - \mu^l &= 0 \\ S\nu &= 0 \\ \mu_j^l - \max(0, \mu_j^l - (\nu_j - \nu_j^l)) &= 0 \quad j \in MET \\ \mu_j^u - \max(0, \mu_j^u - (\nu_j^u - \nu_j)) &= 0 \quad j \in MET \end{aligned} \tag{15}$$

where $i \in EXMET$. The set MET includes all of the metabolites in the network and $EXMET$ is the set of external metabolites that make up the biomass.

The system in Eq. (15) is classified as a DAE system. If one instance of Eq. (15) is solved for a given time-span value, a steady-state for the given extracellular conditions will be observed. However, during the whole fermentation process, there are dynamic changes in the extracellular conditions and intracellular steady-state. Therefore, to model, these dynamic characteristics of the intra- and extra- cellular conditions, the whole fermentation process is divided into discrete time steps, with lengths equal to the time-span. To model the intracellular and extracellular relation, the look up tables in Eq. (8-12) are used. After one instance of Eq. (15) is solved, a new intracellular steady-state and extracellular metabolite concentration is observed. By using the lookup tables in Eq. (8-12) and extracellular concentration for the determination of new ν^u and ν^l values, a new steady-state is established for the following time step, whose length is equal to the time-span. This computational process is executed till the end of fermentation process.

Parameter Estimation: The biomass is actually a collection of different substances. Therefore, to quantitatively represent the growth in the cell, biomass is created artificially from sum of the metabolites which are expected to be the main ingredients of growth. However, determination of the molecular composition and its coefficients is the main difficulty of this procedure.

In this paper, the biomass is represented by composition of protein, carbohydrate, DNA, RNA and lipid. Therefore Eq. (16) is added to represent the

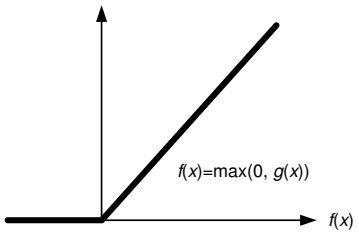

Fig. 6. Graphical representation of the $f(x) = max(0, g(x))$.

biomass accumulation:

$$\theta_{Carb}[carbohydrate]+\theta_{Pro}[Protein]+\theta_{Lip}[Lipid]+\theta_{DNA}[DNA]+\theta_{RNA}[RNA] \rightarrow 1g.Biomass \tag{16}$$

Besides determination of composition of the biomass, the coefficients of these metabolites is another important issue that should be determined. In this paper, a parameter estimation scheme is applied to estimate the θ values in the Eq. (16) and value of $K^{hi}_{glu,1}$ in (9).
To estimate these parameters, the Eq. (17) should be minimized.

$$\sum_j \sum_t (C_j(t) - C_j^{meas}(t))^2 \tag{17}$$

where $C_j(t)$ is the concentration level found by the model, $C_j^{meas}(t)$ is the concentration of measured coefficients by the experiments, $\forall j \in MEAS$ and $\forall t \in T$. In Eq. (17) $MEAS$ represents the set of the measured metabolites in the experimental setup and t represents the time interval of the simulation takes place.

4 Numerical Results

The DAE system in the Eq. (15) is solved with MATLAB Version 7.2. However without any preprocessing, above system cannot be solved by most commercial solvers. The system in Eq. (15) has a DAE index greater than 1 therefore; with this form, MATLAB cannot solve the system. The main reason of above situation is the singularity of the algebraic part. To deal with that singularity we approximate the max operator in the last two equalities of the system [2].

The function $f(x) = \max(0, g(x))$ behaves like in Fig. 6. As seen from Fig. 6, there is a nondifferentiability at 0. We can smooth this nondifferentiability by using the approximation given in Eq. (18).

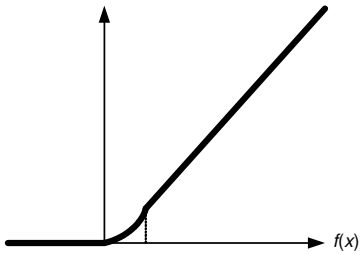

Fig. 7. Smooth approximation of the $f(x) = max(0, g(x))$.

$$\bar{f}(x) = 0.5[g(x)^2 + \varepsilon^2]^{\frac{1}{2}} + \frac{1}{2}g(x) \tag{18}$$

where ε is a sufficiently small number. After the implementation of smoothing in Eq.(18), the resulting approximated function behaves as shown in Fig. 7.

After the foregoing change the updated Eq. (15) will be the following:

$$\begin{aligned}
\frac{dC_i}{dt} &= \nu_i C_{bio} \\
d + S^T\lambda + \mu^u - \mu^l &= 0 \\
S\nu &= 0 \\
0.5[(\mu_j^u - (\nu_j^u - \nu_j))^2 + \varepsilon^2]^{\frac{1}{2}} + \frac{1}{2}(\mu_j^u - (\nu_j^u - \nu_j)) &= 0 \quad j \in MET \\
0.5[(\mu_j^l - (\nu_j - \nu_j^l))^2 + \varepsilon^2]^{\frac{1}{2}} + \frac{1}{2}(\mu_j^l - (\nu_j - \nu_j^l)) &= 0 \quad j \in MET
\end{aligned} \tag{19}$$

To solve DAE system in Eq. (19), the θ parameters in the Eq. (16) and value of $K_{glu,1}^{hi}$ in the Eq. (9) should be estimated. To estimate these parameters, we have used $fminsearch$ solver of the MATLAB during the minimization of Eq. (17). This solver is DFO (Derivative Free Optimization) solver which implements a Nelder-Mead (NM) method. The solution of the parameter estimation is schematically represented in Fig. 8.

At the end of the solution of the parameter estimation problem, the resulting parameter values are shown in Table 3.

The DAE system in Eq. (19) can be solved by MATLAB's DAE solver, ode15s. The program simulated with the initial conditions in Table 4.

Fig. 9 and 10 compares the experimental data and result of the model for *biomass* and *glucose* respectively. As shown, although there is not one to one

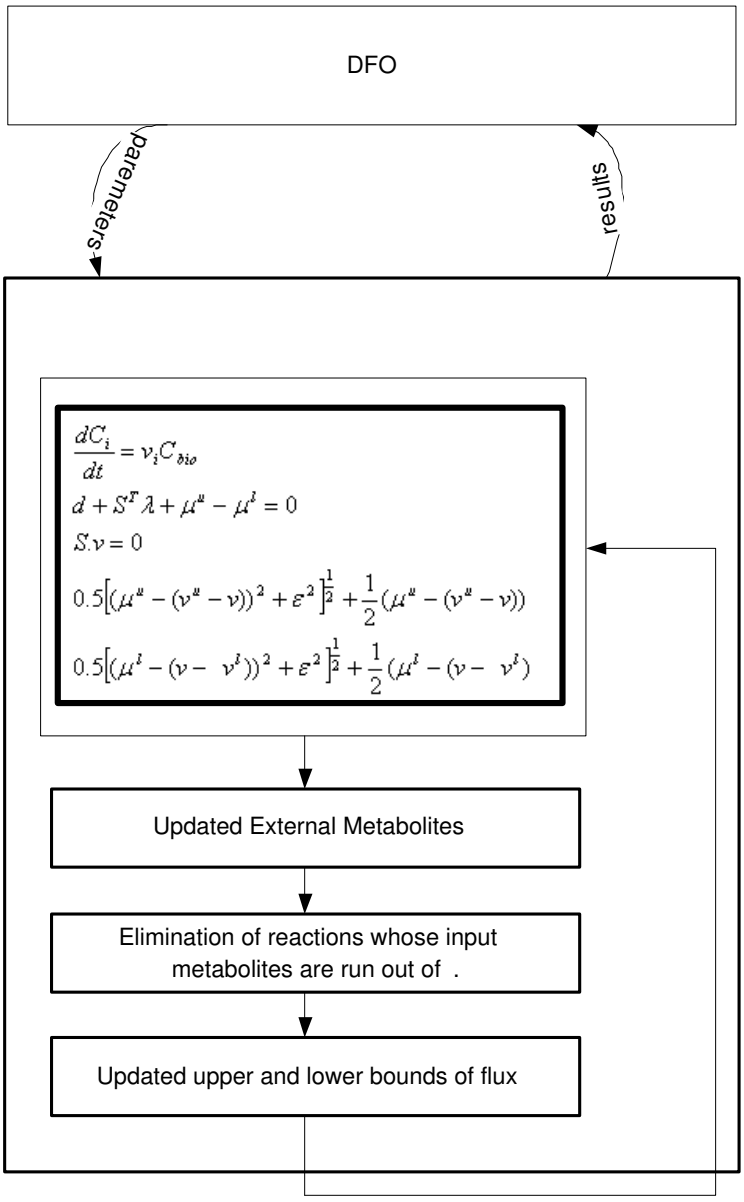

Fig. 8. Parameter estimation problem.

correspondence between model and experimental data, the trends are same. The the result of the simulation follows the experimental model very closely.

5 Conclusions

We have presented a new framework to determine the behavior of yeast during the fermentation process. Many fermentation processes during wine produc-

Table 3
Result of Parameter Estimation Problem.

Parameter	Value
θ_{Carb}	0.0134
θ_{DNA}	0.00014
θ_{RNA}	0.000469
θ_{Pro}	0.0164
θ_{Lip}	0.000169
$K^{hi}_{glu,1}$	0.35

Table 4
Initial Metabolite Concentration

Metabolite	Concentration
Biomass	0.1
Glucose	225
Fructose	115
Ethanol	1.25
Ammonium	1.24
Glycerol	0

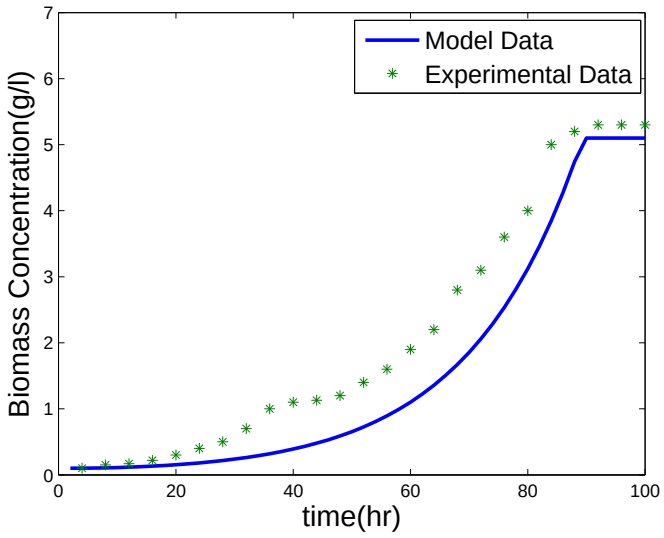

Fig. 9. Comparison of biomass profile.

tion are problem fermentations and the main reason is due to inconvenient environmental conditions. In this study, we represent the fermentation dynamics with a set of DAEs. We model both interior and exterior of the cell. The kinetic parameters in the system formulation are estimated by using a

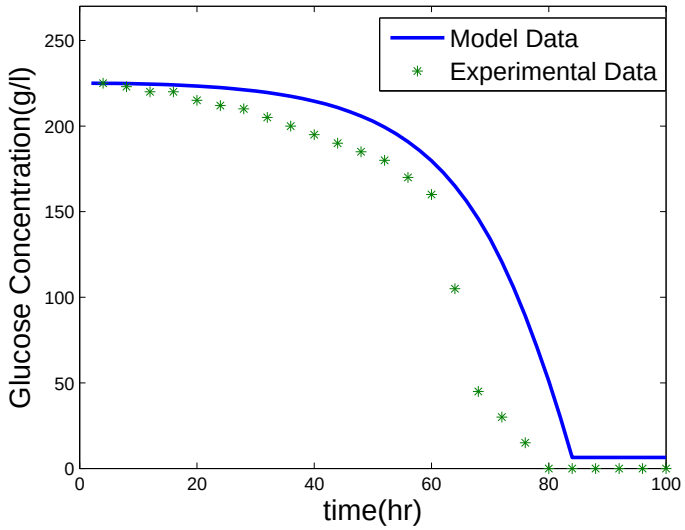

Fig. 10. Comparison of glucose profile.

DFO routine. The behavior of the yeast cell during wine fermentation is determined and compared with the experimental results. It is illustrated that the result taken from simulation study matches the results taken from experimental studies.

6 Acknowledgements

The financial support of the work by NSF-INT(CTS-0457379), TUBITAK Project TBAG-U/114(104T253) is gratefully acknowledged.

References

[1] R.D. Fleischmann, M.D. Adams, O. White, R.A. Clayton, E.F. Kirkness, A.R. Kerlavage, C.J. Bult, J.F. Tomb, B.A. Dougherty, J.M. Merrick, and et. al. ,Whole-genome Random Sequencing and Assembly of Haemophilus Influenzae Rd, 269 (1995) 496-512.

[2] L. T. Biegler, I.E. Grossmann, A.W. Westerberg, Systematic Methods of Chemical Process Design. Prentice Hall, New Jersey, 1997

[3] A.P. Burgard, C.D. Maranas, Optimization-Based Framework for Inferring and Testing Hypothesized Metabolic Objective Functions. Biotechnology and Bioengineering. 82 (2003) 670-677.

[4] C.A. Hutchison, J.C. Venter, Single-cell Genomics, Nature Biotechnology 24 (2006) 657-658.

[5] C. Martabit, C. Varela, J. Sainz, E. Agosin, A Model Combining Sugar Uptake Kinetics and Metabolic Networks for Predicting Wine Fermentations. Technical Report, Department of Chemical and Bioprocess Engineering, Pontifica Universiad Católica de Chile. 2004

[6] T. Nissen, U. Schulze, J. Nielsen, J. Villasden, Flux Distribution in Anaerobic, Glucose-Limited Continuous Cultures Cultures of Sacchromyces Cerevisiae. Microbiology. 143 (1997) 203-218.

[7] J. A. Papin, N.D. Price, B. Palsson, Extreme Pathway Lengths and Reaction Participation in Genome-Scale Metabolic Networks. <www.genome.org>

[8] P. Piper, Yeast Stress Response, Chapman & Hall, New York, 1997

[9] N.D. Price, J.A. Papin, C.H. Schilling, B. Palsson, Genome-Scale Microbial in-silico Models: The Constraints-Based Approach. Trends in Biotechnology. 21 (2003) 162-169.

[10] A.U. Raghunathan, J.R. Perez-Correa, E. Agosin, L.T. Biegler, Parameter Estimation in Metabolic Flux Balance Models for Batch Fermentation-Formulation & Solution using Differential Variational Inequalities (DVIs). Annals of Operational Research. 148 (2006) 251-270

[11] B. Pallson, Systems Biology:Properties of Reconstructed Networks. Cambridge University Press, New York, 2006

[12] N.C. Jones, P.A. Pevzner, An Introduction to Bioinformatics Algorithms. 2004, The MIT Press, Cambridge.

[13] U. Sauer, Metabolic Networks in Motion: C_{13}-based Flux Analysis, Molecular Systems Biology. 2 (2006) 1774-4292.

[14] J. Sainz, F. Pizarro, J.R. Perez-Correa, E. Agosin, Modeling of Yeast Metabolism and Process Dynamics in Batch Fermentation. Biotechnology and Bioengineering. 81 (2003) 818-828.

[15] C.H. Schiling, J.S. Edwards, D. Letscher, B. Pallson, Combining Pathway Analysis with Flux Balance Analysis for the Comprehensive Study of Metabolic Systems. Biotechnology and Bioengineering. 71 (2001) 286-306.

[16] Institute for System Biology <http://www.systemsbiology.org>

[17] A. Varma and B.O. Palsson, Metabolic Capabilities of Escherichia coli: II. Optimal growth patterns. J. Theor. Biol. 165 (1993) 503-522.

[18] S.J. Wiback and B.O. Palsson, Extreme Pathway Analysis of Human Red Blood Cell Metabolism, Biophysical Journal. 83 (2002) 808-818.