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Full Length Research Paper

Modeling metabolic response to changes of enzyme amount in yeast glycolysis

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Based on the work of Hynne et al. (2001), in an *in silico* model of glycolysis, *Saccharomyces cerevisiae* is established by introducing an enzyme amount multiple factor (*a*) into the kinetic equations. The model is aimed to predict the metabolic response to the change of enzyme amount. With the help of *a*, the amounts of twelve enzymes were altered by different multiples from their initial values. Then twenty metabolite concentrations were monitored and analyzed. The prediction of metabolite levels accord with the experimental result and understanding of bioprocess. It also suggested that the metabolic response to dropping enzyme amounts was stronger than the increasing concentrations. Besides, two different trends of change in the metabolite levels appeared apparently, which are correspond with the network structure. Therefore, for regulating the metabolite levels through changing enzyme amount, not only biochemical characteristic but also location of enzymes in the network should be considered.

Key words: Glycolysis, yeast, metabolites level, enzyme perturbation, kinetic model.

INTRODUCTION

In order to enhance the yield and productivity of metabolite production, researchers have, over the years, focused on enzyme amplification or other modifications of the product pathway (Hauf et al., 2000). However, the result of genetic operation is not as good as expected if it could only identify the targets simply by experience. For instance, over expression of some or all the enzymes involved in glycolysis yielded only limited increases in its fluxes because of metabolic rigidity and globe regulation of system. Although, the control mechanisms of regulating glycolysis at the metabolic level-such as products, substrates and allosteric effectors-have been extensively studied (Druvefors et al., 2005; Elbing et al., 2004; Jurica et al., 1998; Kaplan and Kupiec, 2007; Reibstein et al., 1986; Yoshino and Murakami, 1982), its regulation at the protein level is, so far, poorly understood. It is necessary to develop a model which could predict directly the metabolic response to enzyme amplification or attenuation in theory. Accordingly, the selection of object for genetic modification would be more rational while the outcome would satisfy the research more easily.

Here we present a novelty kinetic model based on the work of Hynne et al. (2001), in which enzyme amount multiple factor (*a*), an enzyme amount parameter, was particularly introduced into the kinetic equations to formulate an *in silico* model system which is presented as a steady state. It is employed to transmit the enzymatic

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Abbreviations: EtOH, Ethanol; **HK,** hexokinase; **GAPDH,** glyceraldehyde-3- phosphate dehydrogenase; **ADH,** alcohol dehydrogenase; **TIM,** triose phosphate isomerase; **lpGlyc,** lumped glycerol formation reaction; **PDC,** pyruvate decarboxylase; **ALD,** aldolase; **PFK,** phosphofructokinase; **PK,** pyruvate kinase; **PGI,** phosphoglucose isomerase; **lpPEP,** phosphoenol pyruvate formation reaction.

quantitative properties into a mathematical context capable of predicting metabolic response to enzymatic amplification or attenuation. Traditionally, that genetic manipulation of a metabolic network is considered to be the impetus for shifts in network functionality, that is, in enzyme levels as well as activity. Thus, this development provides a method to show straight-forwardly, how the enzyme alternation affects metabolite levels. In fact, the simulation result alleviates the difficulties of *in vivo* metabolome determination which restrict the exploration of their biochemical properties and domain metabolic process. Otherwise, it is also a useful approach to assess the rigidity through investigating metabolic response, without the disturbance by low molecular weight effectors triggered by enzyme alternation.

METHODS

Model establishment

To accomplish the connection between enzymes and metabolites, a parameter was introduced into the kinetic equation, which is represented by α (Equation 1). Therefore, the value of α is the changed multiple of enzymes from their original concentrations. The framework of metabolites, reactions and rate expressions are obtained from the work of F. Hynne's, as well as the experimental data set.

$$V = \alpha \cdot f(V, K, k...) \quad (1)$$

The new kinetic equation after importing the α.

Simulation and analysis

The simulation was executed by CellDesigner™ v3.2 (Kitano et al., 2005) (http://www.celldesigner.org/), SBW (Systems Biology Workbench) (Sauro et al., 2003; http://sbw.kgi.edu/) and SBML ODE Solver Library (SOSlib) (Machne et al., 2006; http://www.tbi.univie.ac.at/%7Eraim/odeSolver/). The simulation data were analyzed with Statistical 6.0.

RESULTS

Model establishment

In this work, we first introduced the enzyme parameter (α) into the kinetic equations and consequently established an *in silico* glycolysis model of *Saccharomyces cerevisiae* in XML format (Figure 1), based on the work of Hynn et al. (2001). Equation 1 shows how the α is introduced into the kinetic equation. After introducing it, enzyme amounts can be changed by regulating α. Here we changed α from 0 to 1 and then changed it from 1 to 100, which denotes that enzyme amount was separately decreased and increased 100 times from initial concentration. The model was run until all the metabolites arrived at the steady state. All the metabolites concentrations in the steady state were summarized and analyzed by statistical 6.0.

Model predictions of metabolic response to different enzyme with different concentrations

The simulation result of our model which involved twenty metabolites, twenty four reactions, and twelve enzymes, including cofactor and energy factor, are shown in Figure 1 and Table 1. The initial metabolome data and kinetic parameter like the maximum velocities of enzymes come from the full-scale model established by Hynn et al. (2001). Certainly, they presented the initial metabolites levels as being in a steady state, as well as lumping some reactions together and ignoring some metabolites in their model. Using the modeling tool CellDesigner developed by Kitano et al. (2005), we successfully built up our graphical model and executed the simulation after employing all kinds of parameters, experiment data and mathematical expressions of kinetics. In order to observe the response of metabolite levels to enzyme amounts, we set the corresponding α value of twelve enzymes at eight different levels, including 100, 10, 5, 1, 0.5, 0.1, 0.01 and 0. Accordingly, the increasing or decreasing corresponding amount of enzyme was realized.

The qualitative state of twenty species over 10,000 virtual minutes was simulated by *in silico* modeling. Ten thousand steps were computed during the period being simulated, while 10,000 data points. For each metabolite, the mean value was computed using data from 2,000 to 10,000 steps, which is considered the final concentration of this metabolite. Thus, eight final concentrations of each metabolite in the steady state were produced while the α was varied from 0 to 100. At the end, twenty metabolites' concentrations, corresponding to enzyme amount change in eight levels, were simulated and illustrated (Figures 1 and 2), which represented the relationship between metabolite levels and enzyme amounts.

DISCUSSION

Metabolite levels respond to the changes in single enzyme amounts differently. Actually, the metabolic response to decreasing enzyme amounts was more dramatic than to increasing concentrations, which is consistent with experimental results found in the literature (Flikweert et al., 1999; Pearce et al., 2001; Piper et al., 1986; Schaaff et al., 1989). For instance, Flikweert et al. (1999) showed that the formation of ethanol and acetate was reduced by 60 to 70 percent when pyruvate decarboxylase (PDC) activity was decreased three to four fold. In fact, similar results showed up when PDC was down regulated in the present work (Figure 2G). Redundancy was one possible reason for these phenomena. Therefore, it is inefficient to improve the yield of production by over expressing a single enzyme's concentration.

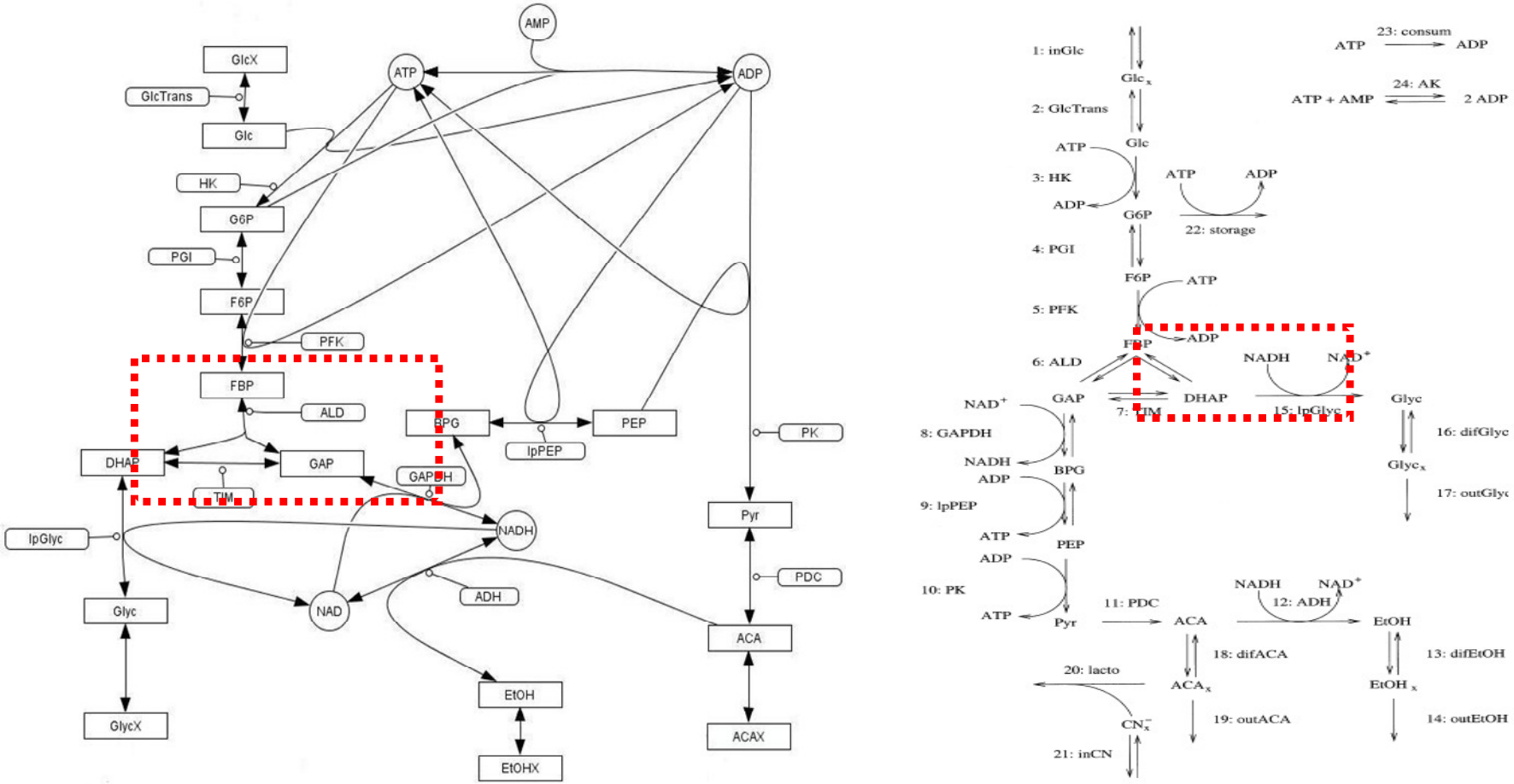

Figure 1. Schematic representation of saccharomyces cerevisiae glycolysis pathway in silico model. a) Glycolysis pathway in silico model was established by CellDesignerTM v3.2. Kinetic equations with *a* were available for simulation. b) Reaction network of the model (reproduced from Hynne.et al, 2001). The special triangle structure in the network which envelop by dashed line was emphasized in later discussion.

The predicted metabolite levels changed in accordance with the classic understanding of biochemical reaction and flux analysis. For instance, ethanol (EtOH), a very important industrial product which many researchers and environmentalists are concerned with, will be increased if Glutrans, hexokinase (HK), glyceraldehyde-3- phosphate dehydrogenase. (GAPDH), triose phosphate isomerase (TIM), and alcohol dehydrogenase (ADH) were up regulated. Obviously, ADH has a deep impact to EtOH level, compared to other enzymes. As for another significant product Glycerol, increasing Glutrans, HK, PFK, lpGLyc (lumped glycerol formation reaction) and decreasing PDC (pyruvate

Table 1. List of enzymes and metabolites abbreviations.

Abbreviation	Name
Enzyme	
GlcTrans	Glucose transport reaction
HK	Hextose kinase
PGI	Phosphoglucose isomerase
PFK	Phosphofructokinase-1
ALD	Aldolase
TIM	Triose phosphate isomerase
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
lpPEP	Lumped Phospho-glycerate kinase reaction, Phospho-glycerate mutase reaction, Enolase reaction
PK	Pyruvate kinase
PDC	Pyruvate decarboxylase
ADH	alcohol dehydrogenase
lpGlyc	Lumped glycerol 3-phosphate dehydrogenase reaction, glycerol phosphatase reaction
Metabolite	
GlcX	Extracellular glucose
Glc	Intracellular glucose
G6P	Glucose-6-phosphate
F6P	Fructose-6-phosphate
FBP	Fructose-1,6-bisphosphate
DHAP	Dihydroxyacetone phosphate
GAP	Glyceraldehyde-3-phosphate
BPG	Glycerate-1,3-diphosphate
PEP	Phosphoenolpyruvate
Pyr	Pyruvate
ACA	Intracellular acetic acid
ACAX	Extracellular acetic acid
EtOH	Intracellular ethanol
EtOHX	Extracellular ethanol
Glyc	Intracellular glycerol
GlycX	Extracellular glycerol

decarboxylase), TIM, and GAPDH help to enhance the yield. Although, the effect is limited, it reinforces glucose transport and branch pathway or weaken competitive pathway that is apparently beneficial for increasing target metabolite level. Besides, glucose accumulates when mostenzymes are down-regulated because of the reduction of fluxes. Some enzymes exhibit little influence to metabolite concentration like aldolase (ALD). In contrast, the change of phosphofructokinase (PFK) impacts the metabolite remarkably, as a rate limiting enzyme.

Two types of trends of metabolite levels were presented. In the first type, when the enzyme amount was down-regulated, most metabolite concentrations showed a continual decrease (Figures 2A, B, C, and E). In the second type (Figures 2F, G, K, H, I, J, K, and L), they accumulated to the maximum and then descended rapidly. Interestingly, the two group of enzymes triggered different trends of metabolic response, which happen to distribute upstream and downstream of triangle structure (Figure 1) separately. That is, HK, Glutrans, phosphor-glucose isomerase (PGI, and PFK, which are located before the triangle structure (Figure 1) in the network, influence the system similarly. Also, the second group of enzymes which are located after the triangle structure in the network (Figure 1), including PK, PDC, ADH, lpGlyc, GAPDH and lpPEP, affected the system metabolism similarly either. An implication of metabolic response to enzyme change exists between biochemical properties and network structure.

Conclusions

In the present work, *in silico* model with an importing

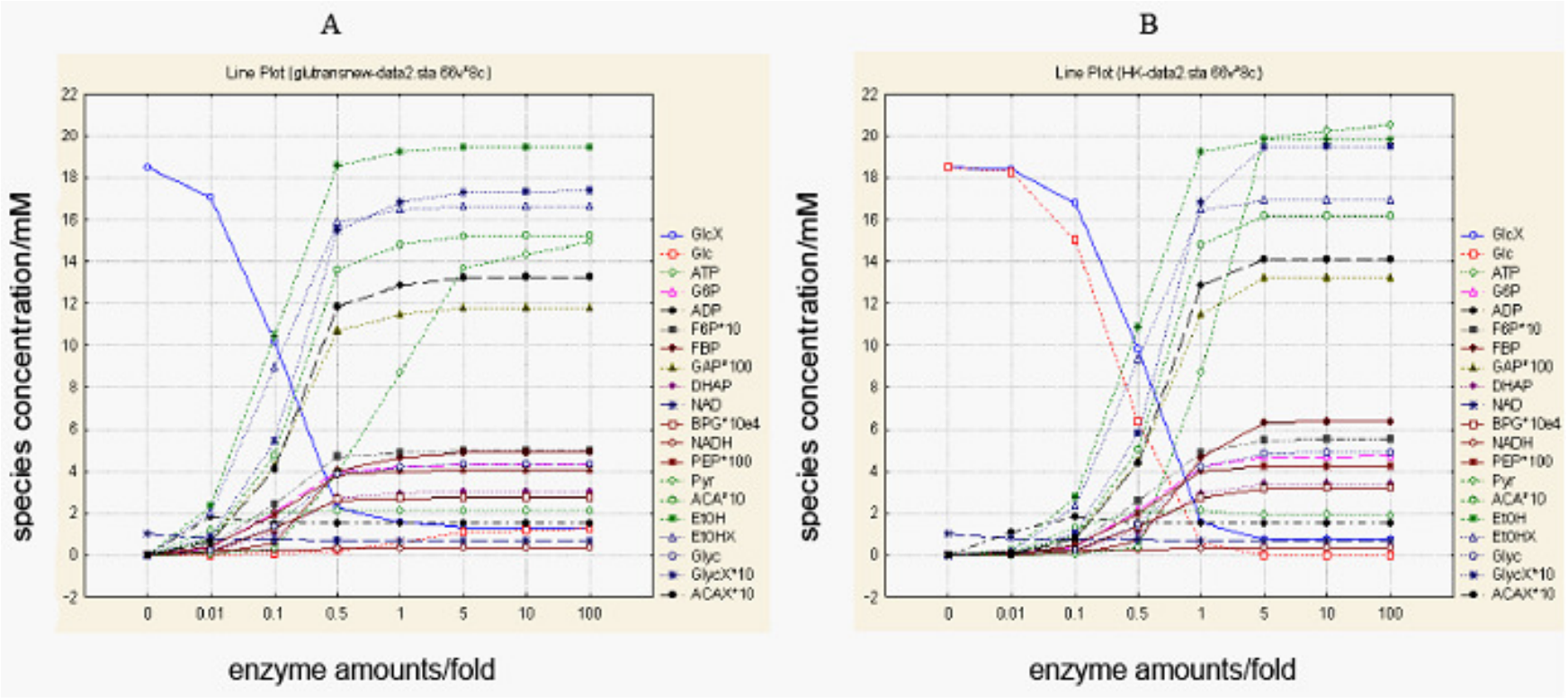

Figure 2. Metabolite levels corresponding to changes in the enzymes concentrations in glycolysis model. 20 metabolites concentration corresponding to changes in 12 enzyme amount were showed. Every single enzyme was set at 8 different concentrations, including 0, 0.01, 0.1, 0.5, 1, 5, 10 and 100. Metabolites are represented by different shapes and colors, which are listed on the right-hand side of the figure. Metabolite levels corresponding to the changes in (A) Glutrans, (B) Hk, (C) PGI, (D) PFK, (E) ALD, (F) TIM , (G) GAPDH, (H) lpPEP, (I) PK, (G) PDC, (K) ADH and (L) LpGlyc.

enzyme amount multiple factor (*a*) is established. The dynamic model aims at making a direct connection between metabolite levels and enzyme levels, which enables the study to predict steady state metabolites concentration, as a function of enzyme concentration.The results showed that the response of metabolite levels to down-regulation of enzyme amounts were stronger than their up-regulation. Basically, metabolite levels remained stable when they were set higher than some certain levels. Moreover, the prediction of metabolic response is consistent with the results of the experiment and basic understanding of biochemistry. Besides, most of the metabolite concentrations decreased continually during the regulation of some enzyme amounts, while they accumulated until a peak was shown and then descended rapidly during the changes in other enzyme amounts. Considering those enzymes' location in the network, the metabolic response relates not only biochemical characteristics but also the structure of network.

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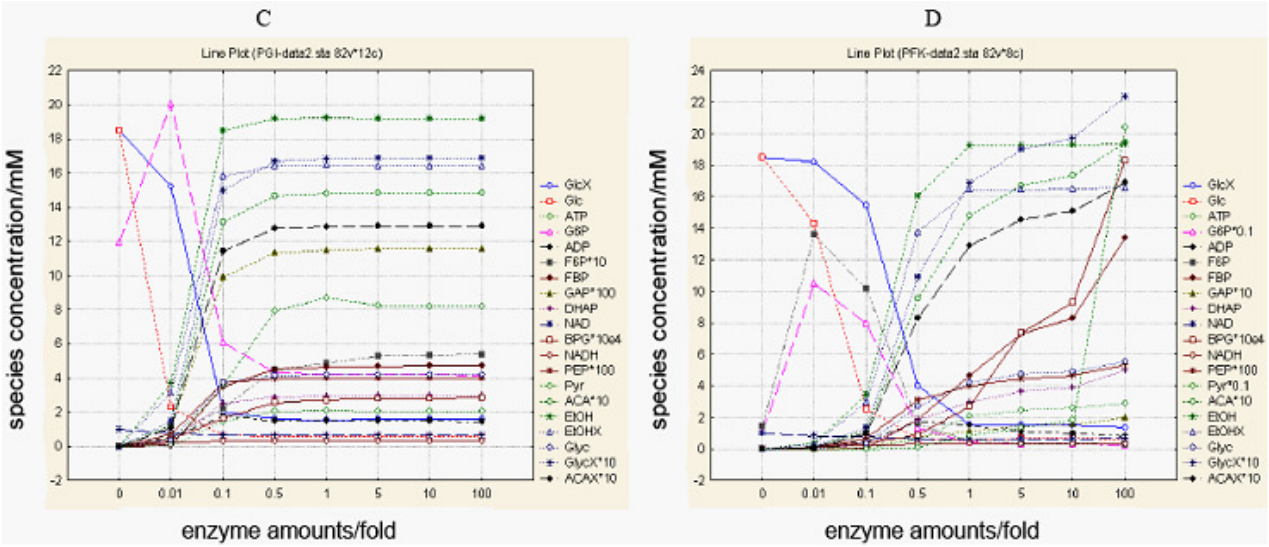

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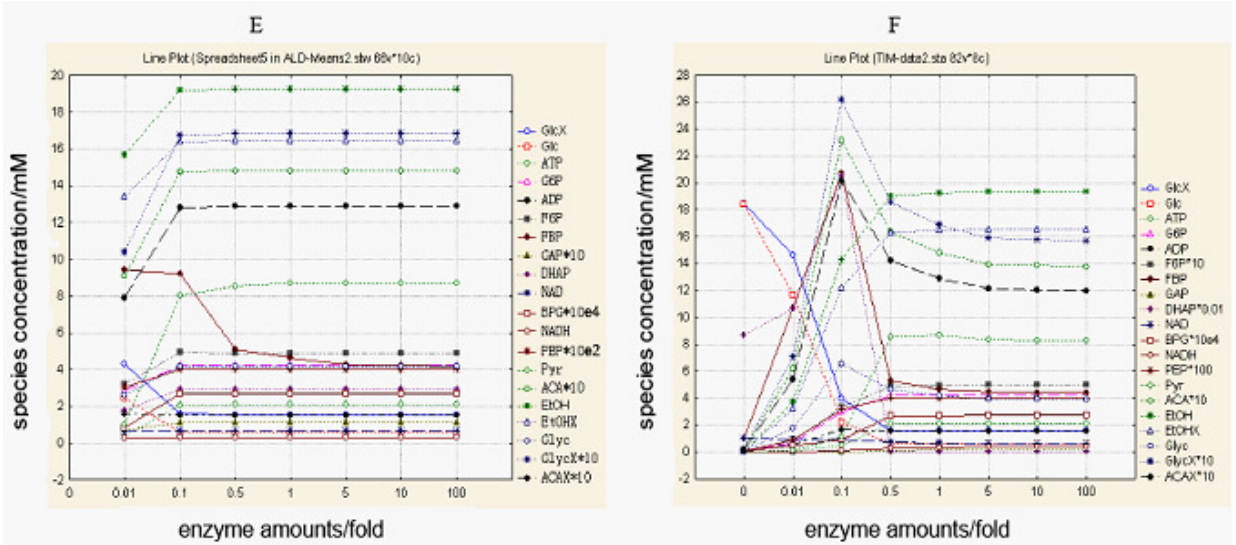

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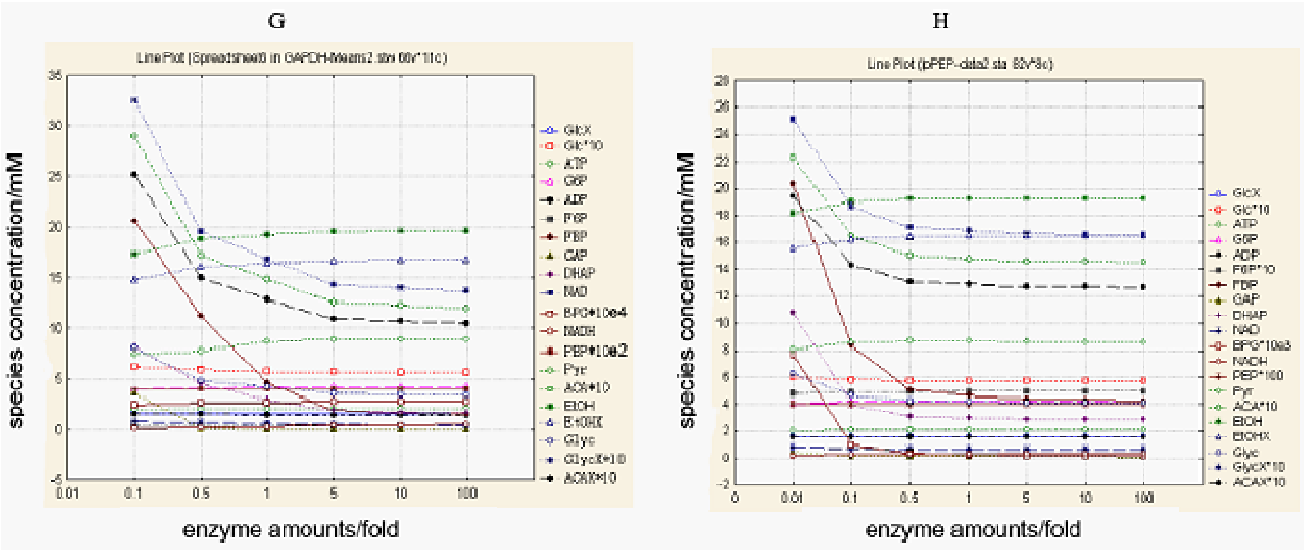

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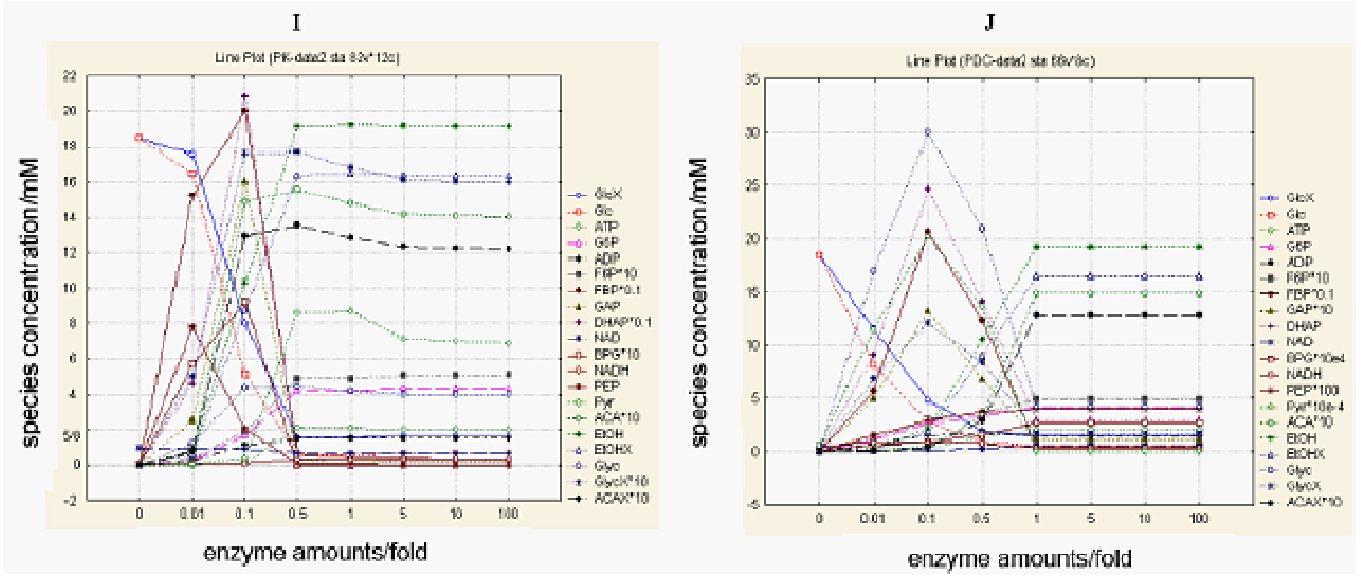

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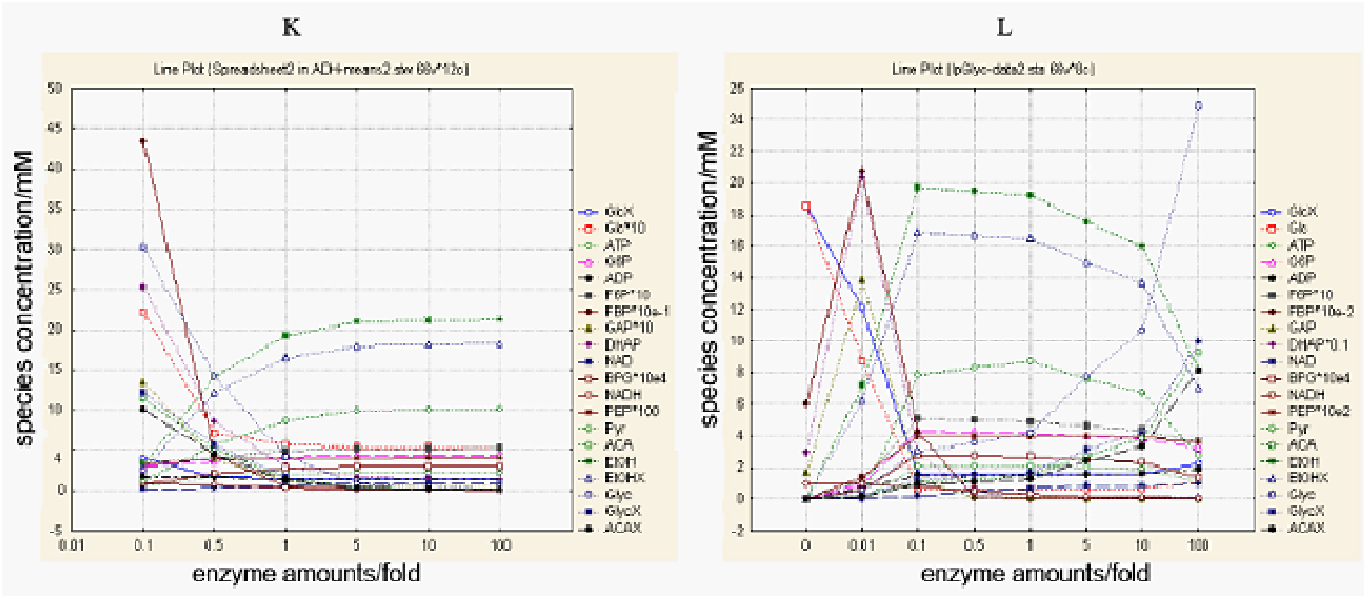

Figure 2 Cont.

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