Large-Scale of Metabolic Network of *Escherichia Coli* using MATLAB

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ABSTRACT
In this study, we performed local sensitivity analysis on a large-scale kinetic dynamic metabolic network. Time profile for sensitivity indices has been calculated for each kinetic parameters based on highest variance. The dynamic model of *E. coli* used in this study contain Glycolysis, Pentose Phosphate, TCA cycle, Gluconeogenesis and Glycoxylate pathways in addition to Acetate formation PTS system. The model implicates twenty-four dynamic mass balance for extracellular glucose and intracellular, thirty kinetic rate expressions. We test all the kinetics in 10% and 20 % increasing one by one at steady state condition. The former analysis in 20%, has allowed identification of eight kinetic parameters as the most effective on this model.

Keywords
Metabolic network, dynamic modeling, sensitivity analysis.

1. INTRODUCTION
In the last a few years, there has been increasing in developing or exploring new products and process depend on the available data that using renewable resource. In this way, metabolic engineering is development of targeted methods to improve the metabolic capabilities through the measurement and understanding the control of flux in vivo [1]; for that is required to develop and verify a mathematical model which can describe the dynamic behavior of the cell in response to the changes in the culture environment to how match the model are really build correspond to the behavior of the system represented by real experiments through software programing [2].

There have been several models describing the dynamic behavior of the cells [3] [4] [5] [6]; moreover large-scale integration has been performed, glycolysis and PP pathways [7] integrated with TCA cycle [2]. However the kinetic modeling of *E. coli* contains a large number of parameters inclusive kinetic constants and initial metabolites and enzymes concentration.The huge number of kinetics need to be reduced for optimization task, for that sensitivity analysis is best methods to reduce the kinetic numbers, which allows us to assess the effect of the changes in a certain parameters will have on the model conclusion. In contrast, many authors have been working in the sensitivity analysis methods, such as [8] the proposed a new method that combing the dynamic flux control coefficients and concentration control coefficients for determine the steady-state and time independent control coefficients to be obtained from dynamic models. In [7] applied a Stepwise Internalization method through analytical function to fit the time course of unbalanced metabolites concentrations. [6] the analysis of diauxic growth in E. coli is achieved by extend FBA using two formulations for dynamic FBA through Glycolysis, pentose phosphate pathways, TCA cycle with the Glyoxulate bypass, anapleurotic reaction, and redox metabolism. [9] The method of reoder-able matrix is applied into glycolysis and PP pathways for the visual exploration of the time varying matrix. [10] A variance-based method is applied to achieve a new steady state after glucose pulse injection in Embden-Meyerof-Paranas, PP pathways and PTS system. [11] The metabolic control analysis is used for calculating the time-dependent to simplified alternative kinetics of large-scale of E. coli. [12] They scaling all the kinetic parameters individually into their allowable range from 0.0 up to 2.0.

In the present work, therefor we investigate [2010] model which contain 194 kinetic parameters by increasing each kinetic parameters in 10% and 20% and quantify the changes using the highest variance between the actual model and the simulation, was found eight kinetic parameters are sensitive in the model output.

2. METHODES
In this work, we consider the main metabolic pathway of *E. coli* formulated byKadir [2010] as a benchmark. This model, describe the dynamic metabolic behavior of five pathways in addition to Acetate formation and PTS system, the model input are thirty enzymatic reactions with ten co-factors (e.g., nad, coa, atp) and the outputs are twenty four metabolites. The corresponding metabolic network is shown in Figure (1). The general mass balance for extracellular glucose and intracellular metabolites based on the following equation:

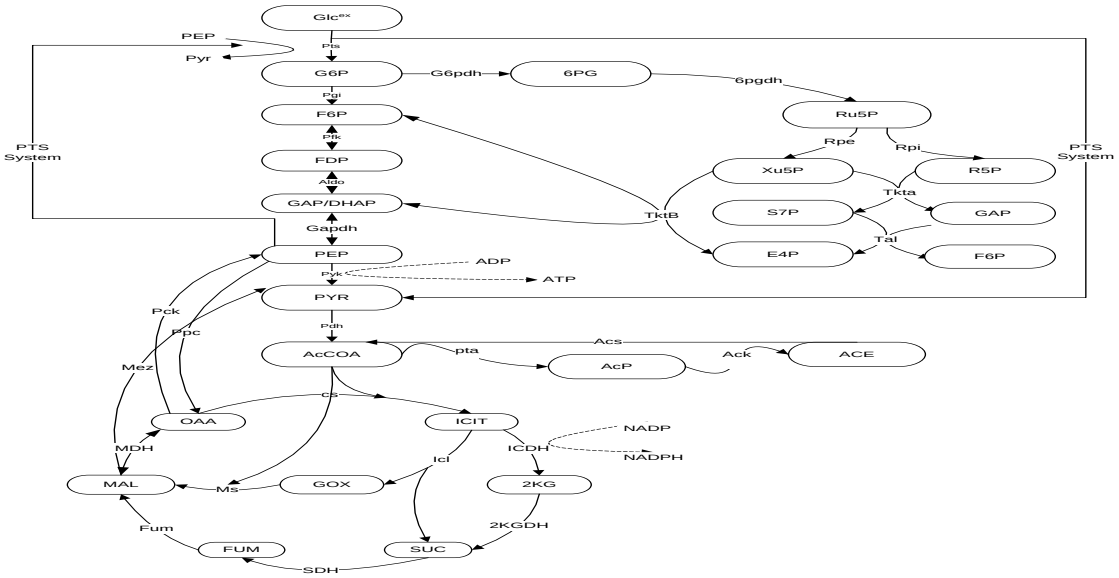

Fig 1: Metabolic of E. coli structure

$$\frac{dC_i}{dt} = \sum_j R_{ij} V_j - \mu C_i \quad (1)$$

Where C_i the concentration of the metabolite$i$,R_{ij} is the stoichiometric coefficient of metabolite i in the reaction j , V_j is the rate of reaction j and μC_i is the growth rate on the dilution effect. The mass balance for the model may describe as following below:

$$\frac{dx}{dt} = \mu[X]2$$

$$\frac{d[GLC^{ex}]}{dt} = -V_{pts}[X] \quad (3)$$

$$\frac{d[G6P]}{dt} = V_{pts} - V_{g6pdh} - V_{pgi} - \mu[G6P] \quad (4)$$

$$\frac{d[F6P]}{dt} = V_{pgi} + V_{tktb} - V_{pfk} - \mu[F6P] \quad (5)$$

$$\frac{d[FDP]}{dt} = V_{pfk} - V_{aldo} - \mu[FDP] \quad (6)$$

$$\frac{d[GAP]}{dt} = 2V_{aldo} - V_{gapdh} + V_{tkta} + V_{tktb} - V_{tal} - \mu[GAP]7$$

$$\frac{d[PEP]}{dt} = V_{pck} - V_{pyk} - V_{ppc} - V_{pts} - \mu[PEP] \quad (8)$$

$$\frac{d[PYR]}{dt} = V_{pyk} + V_{ppc} + V_{pts} - V_{pdh} - \mu[PYR] \quad (9)$$

$$\frac{d[AcCOA]}{dt} = V_{pdh} + V_{acs} - V_{cs} - V_{pta} - \mu[AcCOA] \quad (10)$$

$$\frac{d[ICIT]}{dt} = V_{cs} - V_{icdh} - V_{icl} - \mu[ICIT] \quad (11)$$

$$\frac{d[2KG]}{dt} = V_{icdh} - V_{2kgdh} - \mu[2KG] \quad (12)$$

$$\frac{d[SUC]}{dt} = V_{2kgdh} + V_{icl} - V_{sdh} - \mu[SUC] \quad (13)$$

$$\frac{d[FUM]}{dt} = V_{sdh} - V_{fum} - \mu[FUM] \quad (14)$$

$$\frac{d[MAL]}{dt} = V_{fum} + V_{ms} - V_{mdh} - V_{mez} - \mu[MAL] \quad (15)$$

$$\frac{d[OAA]}{dt} = V_{mdh} + V_{ppc} - V_{mez} - V_{pck} - \mu[OAA] \quad (16)$$

$$\frac{d[GOX]}{dt} = V_{icl} - V_{ms} - \mu[GOX] \quad (17)$$

$$\frac{d[ACP]}{dt} = V_{pta} - V_{ack} - \mu[ACP] \quad (18)$$

$$\frac{d[ACE]}{dt} = V_{ack} - V_{acs} - \mu[ACE] \quad (19)$$

$$\frac{d[6PG]}{dt} = V_{g6pdh} - V_{6pgdh} - \mu[6PG] \quad (20)$$

$$\frac{d[Ru5P]}{dt} = V_{6pgdh} - V_{rpi} - V_{rpe} - \mu[Ru5P] \quad (21)$$

$$\frac{d[Xu5P]}{dt} = V_{rpe} - V_{tkta} - V_{tktb} - \mu[Xu5p] \quad (22)$$

$$\frac{d[R5P]}{dt} = V_{rpi} - V_{tkta} - \mu[R5P] \quad (23)$$

$$\frac{d[S7P]}{dt} = V_{tkta} - V_{tal} - \mu[S7P] \quad (24)$$

$$\frac{d[E4P]}{dt} = V_{tal} - V_{tktb} - \mu[E4P] \quad (25)$$

We performed the sensitivity analysis on large-scale dynamic kinetic model under continuous culture with dilution rate 0.2 at steady-state condition by scaling all the kinetic parameters in increasing one by one in percentage start from 10% to 20% in this enzymes pts, pgi, pfk, aldo, gapdh, pyk, pdh, pta, aces, ask, cs, icdh, 2kgdh, sdh, fum, mdh, icl, ms, ppc, pck, mez, g6pdh, 6pgdh, rpe, rpi, tkta, tktb, tal using MATLAB software program.

3. RESULT AND DISCUSSION

The formal analysis shown that in 10% increasing no affection while in 20% increasing shown that there are eight kinetic parameters affected in the model output, the

kinetic parameters are V_ALDOmax, n_PK, Ki_PDH, ICDH, Kf_ICDH, V_SDH, V_FUM and V_ICLmax represent the reaction rates of ($V_{aldo}, V_{pyk}, V_{pdh}, V_{icdh}, V_{icl}, V_{sdh}$ and V_{fum}) with concentration of the metabolites which are substrate and products of that reaction rates ($C_{FDP}, C_{GAPDHAP}, C_{PEP}, C_{PYR}, C_{AcCOA}, C_{ICIT}, C_{2KG}, C_{SUC}$, $C_{GOX}, C_{FUM}, and\ C_{MAL}$).

The affections of the eight kinetic parameters are analyzed below with some Figures.

The interaction of ***V_ALDOmax*** in the model cause high changes in glycolysis pathway specially in metabolites of *FDP, GAPDAHP, PEP and PYR*; and the enzymes of pts, *pgi, pfk, aldo, gapdh and pyk*; also in acetate formation in *ACP* metabolite and *pta, ask, acs* enzymes. The interaction of ***n_PK*** in the model cause high changes in glycolysis, TCA cycle pathways and acetate formation specially in metabolites of Cell, FDP, PEP, PYR, 2KG and ACP; and the enzymes ofpyk, pita, ask and acs respectively. The interaction of ***Ki_PDH*** in the model cause changes in glycolysis pathway specially in metabolites of PEP, PYR, ACCOA; and gapdh, pdh enzymes;TCA cycle pathway the metabolites of 2KG and SUC was affected; moreover in Acetate formation the metabolites of ACE and ACP; and enzyme of aces Figure (2)& (3).

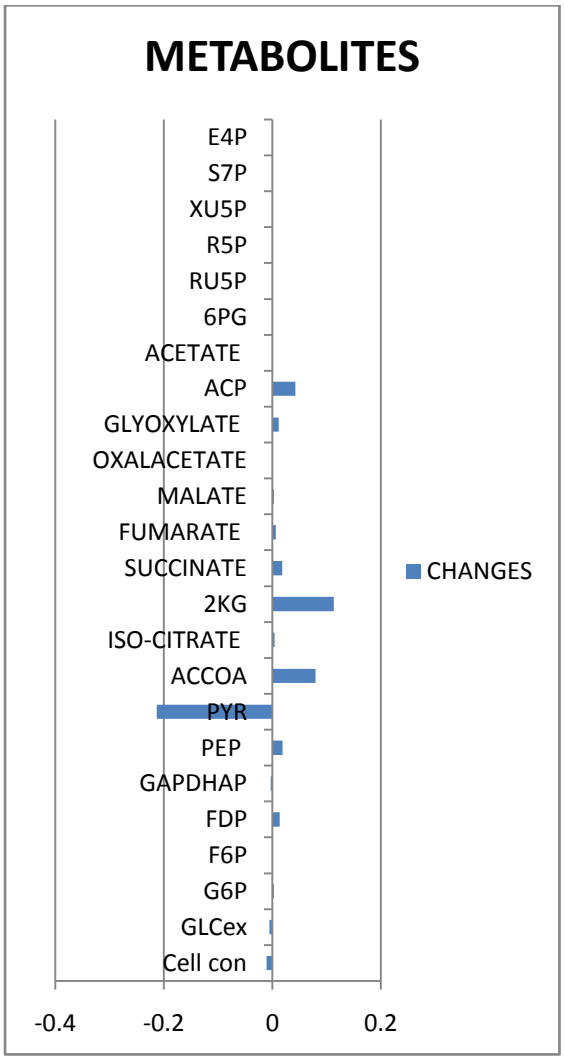

Fig. 2. The affection of Ki_PDH in the Metabolites

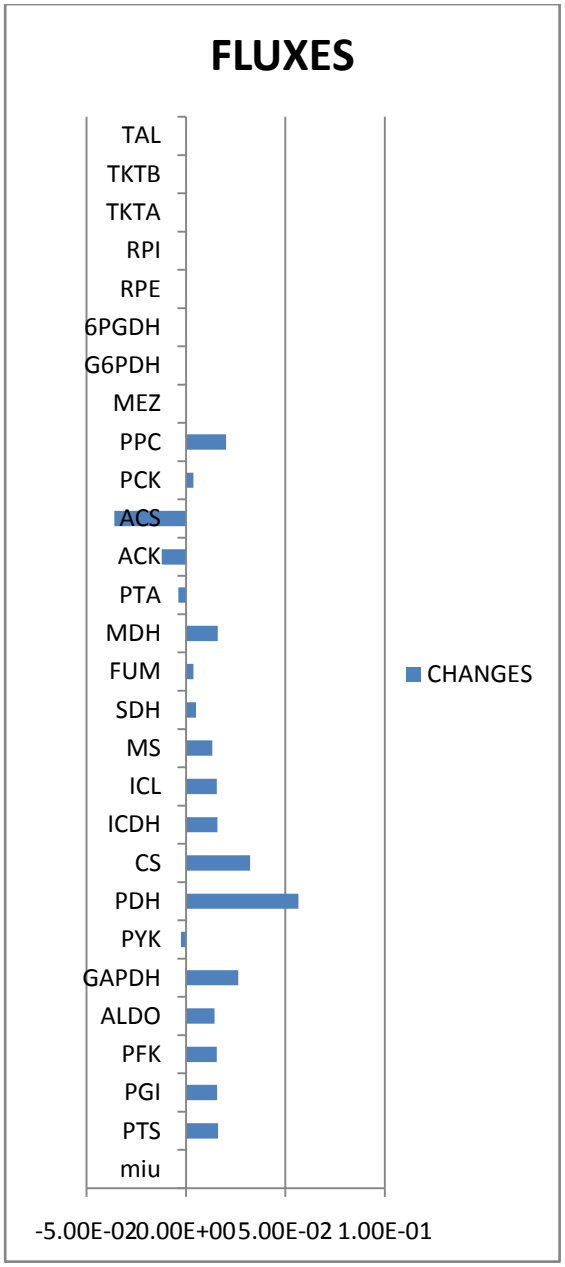

Fig. 3. The affection of Ki_PDH in the Fluxes

The interaction of ***ICDH*** in the model cause high changes in glycolysis, TCA cycle and Acetate formation in these metabolites of FDP, PYR, AcCOA, ACE, ICIT, 2KG, SUC, FUM and MAL; and the enzymes of pdh, pita, acs, sdh, fum, mdh, icl and ms. The interaction of ***Kf_ICDH*** in the model cause high changes in glycolysis, TCA cycle and Acetate formation in these metabolites of FDP, PYR, AcCOA, ACE, ICIT, 2KG, SUC, FUM and MAL; and the enzymes of pdh, pita, acs, sdh, fum, mdh, icl and ms. Theinteraction of ***V_SDH*** in the model cause high changes in glycolysis, TCA cycle and Acetate formation in these metabolites FDP, PYR, ACP, ICIT, 2KG, SUC, FUM, MAL and GOX; and the enzymes of pdh, cs, icl, sdh, fum, mdh, pta and acs. The interaction of ***V_FUM*** in the model cause high changes in glycolysis, TCA cycle and Acetate formation in these metabolites FDP, PYR, 2KG, FUM and ACP; and the enzymes of pdh, cs, icdh, icl, ms, sdh, fum, mdh, pta and aces Figure (4) & (5).

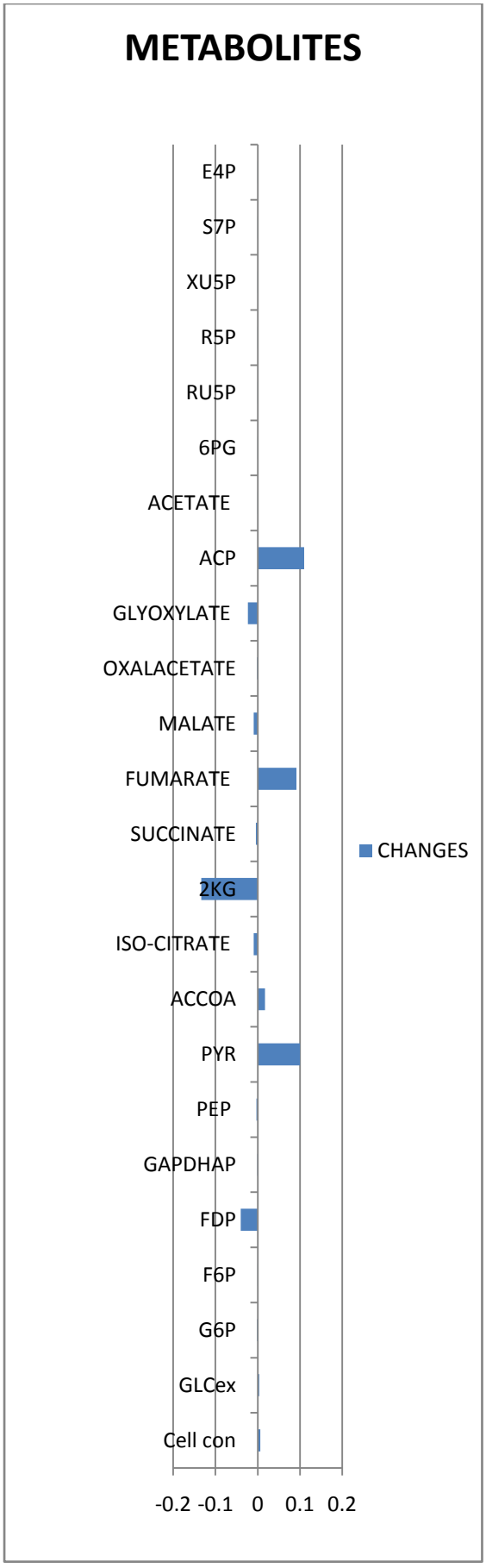

Fig. 4. The affection of V_FUM in the Metabolites

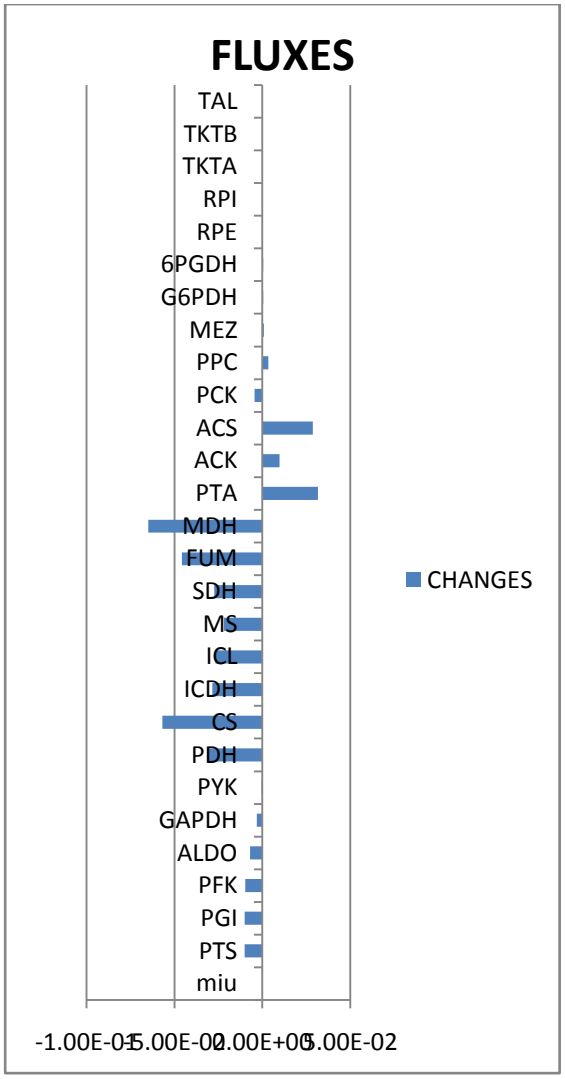

Fig. 5. The affection of V_FUM in the Fluxes

The interaction of ***V_ICLmax*** in the model cause high changes in glycolysis, TCA cycle and Acetate formation in these metabolites FDP, PYR, 2KG, SUC, FUM, AcCOA and ACP; and the enzymes of cs, icl, ms and mdh.

From the analyzation there are three pathways affected in the model outputs which are glycolysis, TCA cycle and Acetate formation especially in these metabolites FDP, PYR, 2KG and ACP. For that we should consider these pathways and their affection in the model output in the optimization of the kinetic parameters of this model.

4. CONCLUSION

More than 190 kinetic parameters as a target study for investigate their affection in dynamic kinetic model experimentally using software program, the perturbation we mad in 10% and 20% increasing concentrations of each kinetics show that in 20% increasing they are eight kinetics are affect in the model output. It will be consider to be optimized to re-correct this model.

Nomenclature

Metabolites

GLCex glucose; G6P: Glucose-6-phosphate; F6P: Fructose-6-phosphate; FDP: Fructose 1,6-bisphosphate, GAP: Glyceraldehyde 3-phosphate; DHAP: Dihydroxyacetone

phosphate; PEP: Phosphoenolpyruvate, PYR: Pyruvate; AcCOA: Acetyl-CoA; AcP: Acetylphosphate; ACE: Acetate; ICIT: Isocitrate; 2KG: 2-Keto-Dgluconate; SUC: Succinate; FUM: Fumarate; MAL: Malate; OAA: Oxaloacetate; 6PG: 6-Phosphogluconolactone; Ru5P: Ribose 5-phosphate; Xu5P: Xylulose 5-phosphate; R5P: Ribulose 5-phosphate; S7P: Sedoheptulose 7-phosphate; E4P: Erythrose 4-phosphate.

Enzymes

Pts: Phosphotransferase system; Pgi: Phosphoglucose isomerase / Glucosephosphate isomerase; Pfk: Phosphofructokinase-1; Aldo: Aldolase; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; Pyk: Pyruvate kinase; Pdh: Pyruvate dehydrogenase; Acs: Acetylcoenzyme A synthetase; Pta: Phosphotransacetylase; Ack: Acetate kinase; cs: Citrate synthase; ICDH: Isocitrate dehydrogenase; 2KGDH: 2-Keto-D-gluconate Dehydrogenase; SDH: Succinate dehydrogenase; Fum: Fumarase; MDH: Malate dehydrogenase; Mez: Malic enzyme; Pck: Phosphoenolpyruvatecarboxykinase; Ppc: PEP carboxylase; ICL: Isocitratelyase; Ms: Malate synthase; G6pdh: Glucose-6-phosphate dehydrogenase; 6Pgdh: 6Phsophogluconate dehydrogenase; Rpi: Ribulose5phosphate 3-isomerase; Rpe: Ribulose phosphate 3epimerase; Tkta: TransketolaseI; Tktb: TransketolaseII; Tal: Transaldolase.

5. ACKNOWLEDGMENTS

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