Large-scale kinetic parameters in metabolic network of *Escherichia coli* using local sensitivity analysis

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Abstract— The usefulness of any dynamic model depends on the accuracy and reliability of its outputs, because a precise input data are rarely available. So, to optimize a large kinetics dynamic model becomes complicated, because it requires the sensitivity analysis to reduce the numbers of kinetics. Moreover; the rapid development and application of sensitivity analysis techniques have a great impact on a kinetic parameter metabolic network model of *E. coli*. For that, we apply one-at-a-time sensitivity measures for large-scale kinetic parameters which contain more than 100 kinetics and the model output are 53 metabolites and fluxes represent five pathways with acetate formation and PTS system and quantify our result by using the Mean for each kinetics. The formal analysis shows that, there are seven kinetic parameters affected on the model output.

Key words— Metabolic engineering, dynamic modeling, sensitivity analysis.

1 INTRODUCTION

One of the very major challenges to metabolic network model of *E. coli* formulated by Kadir [3] it contains a large-scale kinetic parameters. The kinetics which involve in enzyme pathways are usually subject to multiple changes of regulation, and the kinetic changes regulation plays an important role in metabolic regulation [6] [7]. However, some of these kinetic parameters are affected in the model output, making it necessary to find a way to understand and study this kinetics. In fact, the number of kinetics is very large for an optimization algorithm task, which requires sensitivity analysis to reduce the number of kinetics. On the other hand, the sensitivity analysis calculated the rate of change in the output variables of a system which result from small perturbation in the input parameter [4]. However, kinetic modeling demands a large number of parameters including kinetic constants and initial metabolites as well as enzyme concentration [2]. For that, many authors have been working on the sensitivity analysis for large-scale kinetic parameters; Chassagnole investigated the model of glycolysis and pentose phosphate pathways which contain 85 kinetic parameters by applying a Stepwise Internalization method for the sensitivity analysis through analytical function to fit the time course of unbalanced metabolite concentrations [1]. Twelve kinetic parameters were identified as the most effective parameters for Embden-Meyerhof and pentose phosphate pathways with phosphortransferase system using Monte Carlo simulation and Sobol method to calculate the times profile's for identifying the sensitivity for each parameter [5]. They apply the sensitivity analysis to 100 kinetics by scaling each kinetic parameter individually one by one from 1% to 100% of the kinetic parameters concentration; the kinetic targeting is V^{max} where seven kinetic parameters was stated as the most significant from [1] for the metabolic network of phosphotransferase system, glycolysis and pentose phosphate pathways [2]. Also, another researcher used different sensitivity analysis methods for different tasks like mass and energy balance for developing a steady-state kinetic model [11]. To this, all the authors applyied different methods to their on models and the number of kinetic parameters are less than the model formulated by Kadir [3].

In this study, therefor the model as stated in [3] are used, which consists of Glycolysis, Pentose Phosphate, TCA cycle, Gluconeogenesis, Glycoxylate pathways, phosphotransferase system and Acetate formation. The one-at-a-time sensitivity measures in large-scale kinetic parameters, was used to identify the most effective parameters that gave the most significant changes on the model output metabolites and fluxes which the target is all the kinetics, it was been found that, seven kinetic parameters are affected highly in the model output.

2 METHOD

2.1 Metabolic Structure

In the present study, we consider the main metabolic pathway of *E. coli* formulated by Kadir [3] as a benchmark. This model describes dynamic metabolic behavior of Glycolysis, Pentose Phosphate, TCA cycle, Gluconeogenesis, Glycoxylate pathways and Acetate formation containing 24 metabolites and 29 enzymatic reactions with 10 co-factors (e.g., nad, coa, atp). The corresponding metabolic network is shown in Figure 1.

The metabolite concentration rate of the changes in this metabolic network is given by the following equation:

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

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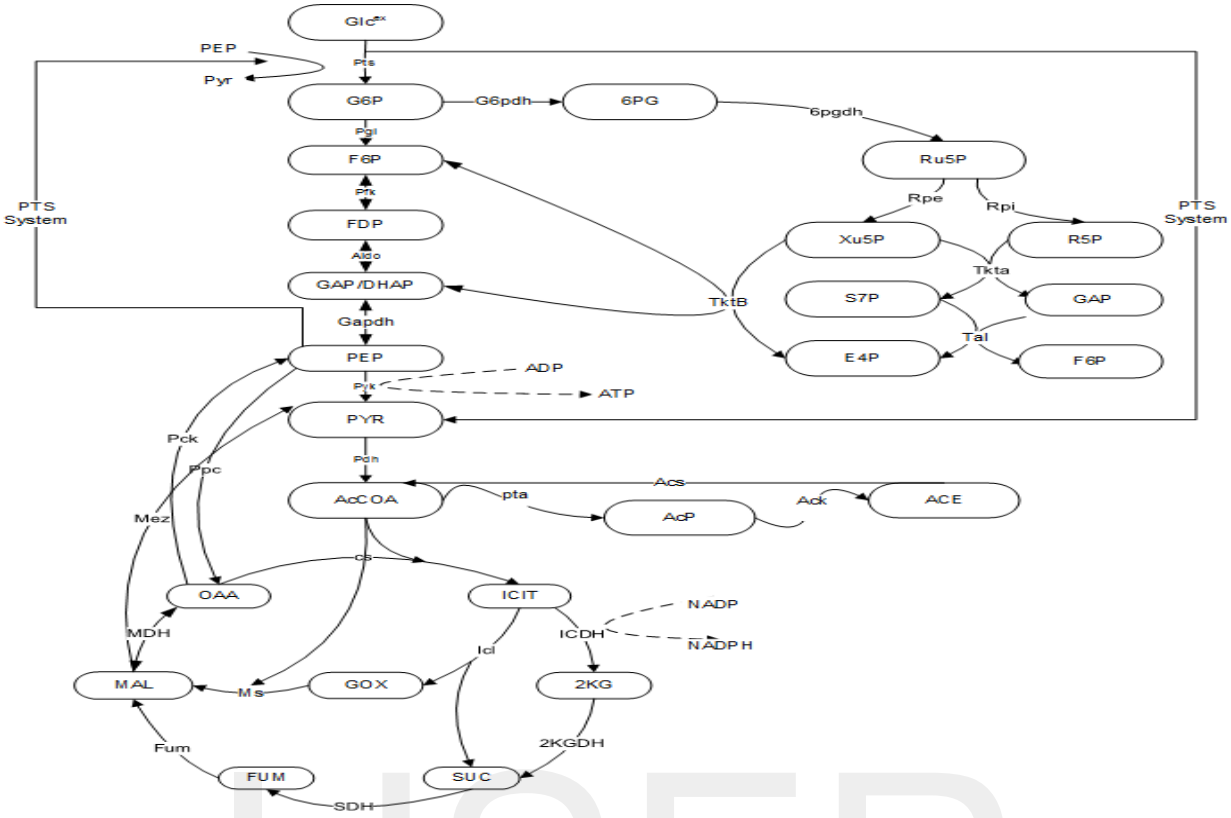

Figure. 1. Metabolic network structure.

Where, C_i is the concentration of metabolite i, R_{ii} is stoichiometric coefficient of metabolite i in the reaction j, v_i is the rate of the reaction j and μC_i is the growth rate on the dilution effect. All the formulas and the mass balance in this dynamic model are taken from [3].

2.2 Sensitivity Analysis

Numerous kinetic parameters of dynamic modeling from different laboratories in different conditions may require to fitting closely the model result to experimental data if used for

From kinetic inputs
Do
For perturbation choose randomly one point at steady state for all kinetics;
Increase the kinetics into their percentage;
Account the variance between the simulation result and the original result at that point;

Account the Percentage Changes for each kinetic often apply the increasing in each kinetics using the Mean equation below $PC = \frac{|\Sigma X_1 + X_2 \ldots X_i|}{N}$ where X_i is the model output and N the total number of the model output;
End

Figure. 2. Sensitivity analysis algorithm

the optimization algorithms purpose, which needs the sensitivity analysis to reduce the kinetic numbers [4]. Thus, the Sensitivity analysis concern on mathematical equations to represent a model system; which assesses the sensitivity of the model results in variation of model input given by variables or parameters and variation assumption [10]. Sensitivity analysis can be represented by different mathematical prospective which give access to different numerical methods, this method is divided into local and global methods; the local methods consider the small changes in the model inputs whereas the global methods consider input values as random variables [8]. For that, we apply the local sensitivity analysis of large-scale dynamic metabolic network of *E. coli* [3] using the method of *one-at-a-time sensitivity measures* [9] under the continuous culture at the steady state condition, by scaling all the kinetics of V^{max} and K one by one into percentage increasing 10%, 20% and 40% with dilution rate 0.1. All the tests were applied in these enzymes *pts, pgi, pfk, aldo, gapdh, pyk, pdh pta, acs, ack, cs, icdh, 2kgdh, sdh, fum, mdh, icl, ms, ppc, pck, mez, g6pdh, 6pgdh, rpe, rpi, tkta, tktb and tal.* In Figure 2, shows the sensitivity analysis algorithm, that used in order to achieve our target, this began by increasing each kinetics into their allowable range at a steady state point, and then calculate the variance between the original model output and the simulation model, after which the changes were figure out using the Mean for

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each kinetics.

3 RESULT

Twenty nine algebraic equations for kinetic expression and co-metabolites concentration and twenty nine differential equations, was targeted to perform sensitivity analysis on the large-scale dynamic metabolic network under steady-state condition of *E. coli* by increasing each kinetic parameters in 10%, 20% and 40% from 40% we found that seven kinetic parameters are affected in the model output, which the kinetic is *V_PYKmax, n_PK, ICDH, Kf_ICDH, Kd_ICDHnadp, Km_ICDHnadp* and *V_ICLmax* represent the reaction rate of V_{pvk}, V_{icdh} and V_{icl} With a concentration of the metabolite which are substrates and products of that reaction rate C_{PEP}, C_{PYR}, C_{ICIT}, C_{2KG}, C_{GOX}, and C_{SUC}. Match more, the changes caused by four kinetic parameters in the reaction rate of V_{icdh} are important due to the changes in *ICIT* and *2KG* during execution time.

More than 180 kinetics have been investigated, by considering the highest Mean of percentage for all kinetics will be described with percentages result in table 1. The deviations in ***V_PYKmax*** result shown that, the metabolites of *FDP* and *ICIT* are highly increased and *ACE* is highly degreased and the enzyme of *ALDO* is highly increased due to highly decreasing in *GLcex*, which in turn is regulated by its effectors *ATP, ADP* and *PEP*. The deviations in ***n_PK*** shows that, the metabolites of *FDP, GAPDHAP, PEP, ICIT* and *E4P* are highly increased and *ACP*, *ACE* and S7P highly degreased; the enzymes of Aldo are highly increased and the, Ack and Pck are highly decreased due to highly decreasing in GLCex which in turn is regulated by the same V_PYKmax effectors. The deviations in ***ICDH***, ***Kf_ICDH*** and ***Kd_ICDHnadp*** results has shown that, highly increasing in metabolite of *ICIT* and deviation in ***Km_ICDHnadp*** result cause highly decreasing in ICIT also; which in turn is regulated by its effectors *NADP*, *NADPH* and *2KG*. Moreover, the kinetics of ***ICDH*** and ***Kf_ICDH*** have the same results may be due to highly increase in *ICIT* metabolites. The deviation in ***V_ICLmax*** result shows that, the metabolites of *SUC* and *GOX* are decreased, which in turn is regulated by its effector ICIT. Table 1, show the percentage changes.

TABLE 1
PERCENTAGE CHANGES WITH 40%

Metabolites and Fluxes	Original values	V_PYKmax	n_PK	ICDH	Kf_ICDH	Kd_ICDHnadp	Km_ICDHnadp	V_ICLmax
Cell Con	1.5783	-11.98%	-27.87%	-3%	-3.00%	-4.59%	4.12%	3.99%
GLCex	0.022105	-45.95%	-263.06%	-10.98%	-10.98%	-17.82%	9.51%	12.16%
G6P	0.20345	12.24%	25.45%	3.35%	3.35%	5.05%	-4.92%	-4.77%
F6P	0.21311	9.99%	21.27%	2.71%	2.71%	4.09%	-3.89%	-3.81%
FDP	1.4621	60.42%	90.18%	13.44%	13.44%	20.09%	-17.84%	-20.37%
GAPDHAP	0.31094	34.37%	75.73%	4.63%	4.63%	7.28%	-4.3%	-5.76%
PEP	1.4914	33.71%	75.80%	3.9%	3.9%	6.21%	-2.92%	-4.67%
PYR	2.8117	-4.42%	9.74%	-8.92	-8.92	-13.96	10.19%	10.96%
AcCOA	1.0018	-0.43%	5.77%	-6.69%	-6.69	-10.46%	7.81%	8.26%
ICIT	0.21101	63.16%	90.59%	87.88%	87.88%	93.11%	-1155.72%	-3.95%
2KG	5.3724	9.47%	36.54%	8.99%	8.99%	15.77%	28.99%	-14.74%
SUC	0.57217	-4.57%	-4.87%	15.68%	15.68%	22.94%	-18.63%	-28.49%
FUM	0.35609	-2.74%	-2.16%	11.69%	11.69%	17.53%	-11.9%	-18.69%
MAL	0.14263	-0.27%	4.25%	11.94%	11.94%	18.37%	-8.07%	-17.49%
OAA	0.029637	8.35%	29.87%	11.99%	11.99%	18.98%	5.55%	-18.53%
GOX	0.34577	-7.06%	-11.61%	23.07%	23.07%	34.97%	-27.13%	-33.55%
AcP	2.0199	-30.76%	-127.28%	-10.24%	-10.24%	-16.42%	9.72%	11.6%
ACE	0.000209	-55.5%	-408.78%	-12.96%	-12.96%	-21.18%	11.07%	14.04%
6PG	0.017832	-0.17%	-0.87%	-1.41%	-1.41%	-2.22%	0.73%	1.84%
Ru5P	0.02134	4.38%	8.52%	0.33%	0.33%	0.47%	-0.79%	-0.5%
R5P	0.07617	4.75%	9.36%	0.46%	0.46%	0.68%	-0.92%	-0.69%
Xu5P	0.026436	5.11%	9.98%	0.57%	0.57%	0.84%	-1.02%	-0.82%
S7P	0.004747	-28.5%	-185.29%	-2.3%	-2.3%	-3.89%	0.68%	2.13%
E4P	0.027433	33.67%	70.4%	5.88%	5.88%	9.07%	-6.41%	-7.8%
Miu	0.099617	-0.16%	-0.33%	-0.1%	-0.1%	-0.16%	-0.07%	0.1%
Pts	1.4003	10.93%	22.93%	2.99%	2.99%	4.48%	-4.36%	-4.22%
Pgi	1.3	11.58%	24.35%	3.23%	3.23%	4.87%	-4.65%	-4.57%
Pfk	1.3402	11.18%	23.54%	3.08%	3.08%	4.64%	-4.47%	-4.36%
Aldo	0.52536	80.38%	141.15%	14.52%	14.52%	22.49%	-17.7%	-18.48%

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PERCENTAGE CHANGES WITH 40%

Metabolites and Fluxes	Original values	V_PYKmax	n_PK	ICDH	Kf_ICDH	Kd_ICDHnadp	Km_ICDHnadp	V_ICLmax
Pyk	0.62509	-28.6%	-56.26%	0.7%	0.7%	1.13%	-0.49%	-0.78%
Pdh.	1.766	-0.7%	-3.15%	4.16%	4.16%	6.39%	-5.27%	-5.52%
Cs	1.4682	3.47%	13.52%	6.91%	6.91%	10.72%	-8.20%	-8.85%
ICDH	0.93296	7.42%	25.01%	-2.01%	-2.01%	-2.1%	24.63%	0.53%
2KGDH	0.40201	5.4%	10.6%	-16.47%	-16.47%	-25.65%	19.16%	20.34%
Icl	0.51436	-6.1%	-10.45%	19.79%	19.79%	30.64%	-21.98%	-26.05%
Ms	0.47975	-6.05%	-10.37%	19.55%	19.55%	30.32%	-21.62%	-25.51%
SDH	0.85922	-0.83%	-0.96%	3.09%	3.09%	4.81%	-2.95%	-4.17%
Fum	0.8237	-0.74%	-0.91%	2.72%	2.72%	4.26%	-2.57%	-3.54%
MDH	1.2698	-2.76%	-4.6%	8.88%	8.88%	13.8%	-9.66%	-11.55%
Pita	0.2504	-35.12%	-161.62%	-10.85%	-10.85%	-17.51%	9.97%	12.29%
Ask	0.052391	-48.51%	-278.56%	-11.74%	-11.74%	-19.05%	10.23%	13%
Aces	0.15652	-48.54%	-278.48%	-11.74%	-11.74%	-19.06%	10.24%	13.01%
Pck	0.068774	-35.45%	-157.26%	8.55%	8.55%	13.82%	8.11%	-13.41%
Ppc	0.2702	22.89%	55.39%	-1.88%	-1.88%	-2.83%	2.96%	2.63%
Mez	0.019458	-0.2%	3.1%	8.91%	8.91%	13.97%	-5.7%	-12.04%
G6pgdh	0.079927	-0.15%	-0.74%	-1.2%	-1.2%	-1.88%	0.62%	1.58%
6pgdh	0.078143	-0.14%	-0.74%	-1.19%	-1.19%	-1.87%	0.62%	1.57%
Rpe	0.04516	-1.3%	-2.71%	-1.52%	-1.52%	-2.38%	0.95%	2.01%
Rpi	0.030597	1.26%	1.56%	-0.81%	-0.81%	-1.28%	0.23%	1.05%
Tkta	0.022996	0.14%	-0.97%	-1.2%	-1.2%	-1.89%	0.63%	1.6%
TktB	0.019783	-3.81%	-6.41%	-2.15%	-2.15%	-3.33%	1.65%	2.88%
TaL	0.022522	0.74%	2.92%	-1.18%	-1.18%	-1.85%	0.63%	1.59%
Mean	-	16.06%	54.87%	8.22%	8.22%	11.99%	8.85%	29.36%

5 CONCLUSION

In this paper, a large-scale kinetic model investigated through by *one-at-a-time sensitivity measures*. 185 kinetic parameters that represent all the kinetics model which seven kinetics are the most effective on the model output and all the kinetics increased individually in 10%, 20% and 40% where in 40% shown good effect often apply the Mean percentage to all the kinetics which explained in the result part, the metabolite of *ICIT* is very important because *ICIT* is substrate and the reaction is inhibited by *NADP/NADPH* for *ICDH* equation rate; since the first six kinetics cause highly decreasing or increasing on *ICIT*. The results shown that, the local sensitivity analysis can be applied.

6 FUTURE WORK

We use local sensitivity analysis method for large-scale kinetic parameters. This problem appears highly underconditional at first. However, we have shown that by applying one-at-a-time sensitivity measures. Sensible results can nonetheless be obtained; such an approach may prove in other kinetic parameters.

Most of our efforts have focused on how to apply the sensitivity analysis technique in large kinetic parameters. The result of one-at-a-time sensitivity measures showed a good result in terms of sensitivity analysis, principal, but had a lack in searching all the kinetic simultaneously. We suspect that, these methods can be blamed as long as there are many local methods can be applied. We believe that, there are significant methods for a complex biological model by applying global sensitivity analysis technique to large-scale kinetic parameters.

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: Phosphoenolpyruvate carboxykinase; Ppc: PEP carboxylase; ICL: Isocitrate lyase; Ms: Malate synthase; G6pdh: Glucose-6-phosphate dehydrogenase; 6Pgdh: 6Phsophogluconate dehydrogenase; Rpi: Ribulose 5phosphate 3-isomerase; Rpe: Ribulose phosphate 3epimerase; Tkta: TransketolaseI; Tktb: TransketolaseII; Tal: Transaldolase.

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