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A New Insight into the Main Metabolic Regulation of *Escherichia coli* Based on Systems Biology Approach

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Abstract: The systems biology approach was considered for the analysis of metabolic regulation in *Escherichia coli*. In particular, it was shown that the feed-forward and feed-back regulations are formed in the metabolic regulation by enzymatic and transcriptional regulations, where these contribute to the robustness of the cell system. Moreover, the effects of the specific pathway gene knockout such as Δ*pgi*, Δ*zwf*, and Δ*pyk* on the metabolic regulations were clarified based on systems biology approach. Mathematical modeling with computer simulation was also made for the main metabolism.

Keywords: Systems biology, Modeling and simulation, Metabolic engineering, Catabolic regulation, *pgi* mutant, *zwf* mutant, *pyk* mutant, *Escherichia coli*.

1. INTRODUCTION

The living organisms must survive in response to the varieties of environmental conditions by sensing external and/or internal state and by modulating transport and metabolism. The main part of these functional responses concerns the metabolic regulation (Shimizu, 2013a). The enzymes which form the metabolic pathways are subject to multiple levels of regulation, where the enzyme level regulation and the transcriptional regulation by global regulators play important roles in the metabolic regulation (Matsuoka and Shimizu, 2011; Shimizu, 2013a, b). Undoubtedly, it is quite important to understand the regulatory processes which govern the cellular metabolism, and this is one of the key focuses in the recent microbial systems biology. Although transcriptional regulation is typically analyzed by measurement of mRNA abundance, the intracellular reaction rates or metabolic fluxes must also be quantified, in particular ^{13}C-metabolic flux analysis is quite useful to assess the metabolic function in the network context (Shimizu, 2004, 2013b). In order to clarify the overall picture of the metabolic regulation, it is important to integrate different levels of information, and this is the main topic for systems biology. Perhaps the most comprehensive data set containing the different levels of omics information for *E. coli* grown at different growth rate and upon deletion of 24 main metabolic pathway genes (Ishii et al., 2007) may provide an unprecedented opportunity for computational analyses to extract biological insights (Sauer et al., 2007).

The cell's metabolism comprises thousands of reactions that are involved in the degradation of available nutrient sources for biosynthesis of cellular constituents such as proteins, lipids, carbohydrates, DNA and RNA etc. Note that those are formed from several key building blocks such as amino acids for protein synthesis, fatty acids for lipids synthesis, nucleotide for DNA and RNA synthesis, and sugar moieties for carbohydrate synthesis. Moreover, these building brocks are formed from only the limited number of precursor metabolites generated in the main metabolism (Nielsen, 2003; Varma and Palsson, 1993). Since the central carbon metabolism is tightly connected with overall cell function, it is quite important to understand the regulation mechanism of the central metabolism for metabolic engineering (Nielsen, 2011). Recently, intracellular metabolic flux was proposed as a novel impetus for metabolic regulation, and the mathematical models for the glycolysis and the TCA cycle were developed in view of transcription factors (Kotte et al., 2010). This concept of flux sensors have recently been verified by experiments (Kochanowski et al., 2013).

The central metabolic pathways are controlled by a number of transcription factors or the so-called global regulators depending on the culture condition (Gama-Castro et al., 2011). Biological systems are known to be robust and adaptable to the environmental perturbation. It is apparent that such robustness is inherent in the biochemical and genetic networks. Here, we consider systems biology approach for understanding the metabolic regulation of *E. coli* and its specific gene knockout mutants.

The central players in carbon catabolite regulation in *E. coli* are the transcriptional activator Crp (cyclic AMP (cAMP) receptor protein), the signal metabolite cAMP, adenylate cyclase (Cya), and the phosphotransferase systems (PTSs), where these systems are involved in both transport and phosphorylation of carbohydrates. The PTS in *E. coli* consists of two common cytoplasmic proteins, EI encoded by *ptsI* and HPr encoded by *ptsH*, as well as carbohydrate-specific EII complexes. The glucose-specific PTS in *E. coli* consists of the cytoplasmic protein $EIIA^{Glc}$ encoded by *crr* and the

membrane-bound protein EIICB^{Glc} encoded by *ptsG*, which transport and concomitantly phosphorylate glucose. The phosphoryl groups are transferred from PEP via successive phosphorelay reactions in turn by EI, HPr, EIIA^{Glc} and EIICB^{Glc} to glucose. It has been shown that unphosphorylated EIIA^{Glc} inhibits the uptake of other non-PTS carbohydrates by the so-called inducer exclusion (Gorke and Stulke, 2008), while phosphorylated EIIA^{Glc} activates Cya, which generates cAMP from ATP and leads to an increase in the intracellular cAMP level (Park et al., 2006) (**Fig. 1**). Note that if the concentration ratio between PEP and PYR (PEP/PYR) is high, EIIA^{Glc} is predominantly phosphorylated, whereas if this ratio is low, then EIIA^{Glc} is predominantly dephosphorylated (Bettenbrock et al., 2007; Hogema et al., 1998). EIIA^{Glc} is preferentially dephosphorylated when *E. coli* cells grow rapidly with glucose as a carbon source.

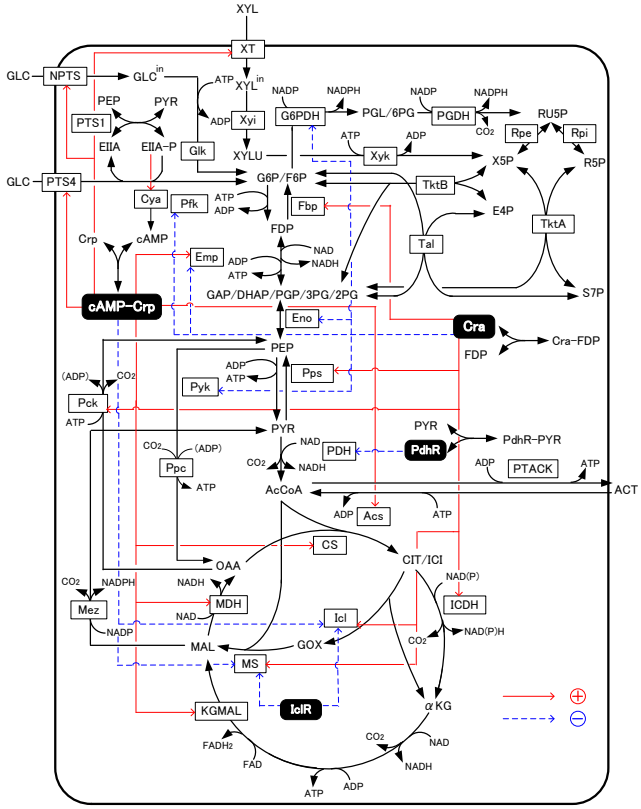


Fig. 1. Metabolic regulation system for the main metabolism of *E. coli*.

In addition to cAMP-Crp, which acts depending on the level of glucose concentration, the catabolite repressor/activator protein (Cra) originally characterized as the fructose repressor (FruR) plays an important role in the control of carbon flow in *E. coli* (Gama-Castro et al., 2011). The glycolysis genes are repressed, while gluconeogenic pathway genes are activated by Cra (Appendix A) (**Fig. 1**).

2. METABOLIC REGULATION OF THE MAIN METABOLISM OF E. coli

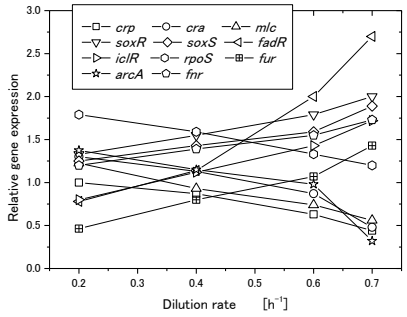
Let us consider how the specific growth rate (dilution rate in the chemostat culture) affects the global regulators and metabolic pathway genes of wild type *E. coli*. It was shown that the specific acetate production rate and the specific CO_2 evolution rate (CER) increased as the dilution rate was increased (Yao et al., 2011). **Fig. 2(a)-(c)** shows the effect of the dilution rate on gene transcript levels, where it indicates that in accordance with the increased dilution rate (or the specific glucose consumption rate), the transcript levels of *ptsG*, *ptsH*, and *pfkA* are increased, where *cra* transcript level decreased and *crp* as well as *mlc* decreased accordingly. Note that as the specific glucose consumption rate or the glycolysis flux increases, FDP concentration increases (Kochanowski et al., 2013). The increased FDP allosterically enhances the activity of Pyk (and also Ppc) by feed-forward control (**Fig. 3**). The PEP concentration tends to be decreased due to activation of Pyk (and Ppc). It has been known that PEP molecule allosterically inhibits Pfk activity by feed-back regulation. The decrease in PEP concentration thus causes Pfk activity to be increased, and the glycolysis flux, and in turn FDP concentration will increase. On the other hand, the decrease in PEP and increase in PYR make PEP/PYR ratio to be decreased. This causes phosphorylated EIIA (EIIA-P) concentration to be decreased, and in turn less activates Cya, and thus cAMP is less formed. As a result, cAMP-Crp level decreases, which decreases the expression of *ptsG*, and this causes the decrease in glucose uptake rate. This forms a negative feed-back loop for the initial increase in the glucose uptake rate (**Fig. 3**). This indicates that PTS plays an essential role from the robustness point of view.

The analysis may be further extended to the regulation of the TCA cycle. As mentioned above, the increase in glucose uptake rate causes PYR concentration to be increased. Note that the increase in Ppc activity caused by the increase in FDP affects OAA concentration to be increased. Moreover, the increase in PYR concentration causes AcCoA to be increased, and thus acetate formation is enhanced by Pta-Ack reaction. The increase in AcCoA also enhances Ppc activity (Yang et al., 2003). The increases in AcCoA and OAA may cause TCA cycle to be activated. On the other hand, as mentioned above, if PTS was furnished, cAMP-Crp decreases as the glucose uptake rate increases, which in turn represses the expressions of TCA cycle genes, and thus TCA cycle activity is repressed by transcriptional regulation (**Fig. 2d and Fig. 3**).

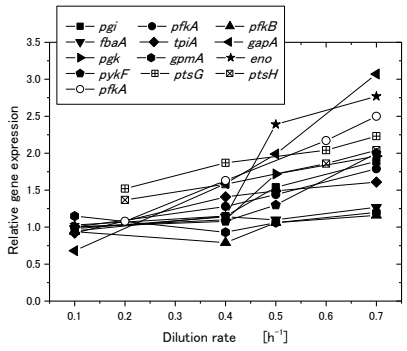
In fact, there is another global regulator Cra, where Cra detects FDP concentration, and Cra activity decreases with the increase of FDP concentration. In the above example, the increase in glucose uptake rate increases FDP concentration, and thus Cra activity decreases. This causes the increase of the expressions of glycolysis genes such as *pfkA* and *pykF* genes, while it represses the expressions of gluconeogenic pathway genes such as *ppsA* and *pckA* genes, which implies acceralation of increased glycolysis fluxes. The decrease in Cra activity also affects TCA cycle genes such that *icdA* and *aceA* gene expressions are repressed, and thus TCA cycle is further repressed by this mechanism (**Fig. 2c, d and Fig. 3**). The increase in glycolysis activity and the decrease in TCA cycle activity cause more acetate production. This is the overflow metabolism, which has been the subject of bacterial cultivation (Valgepea et al., 2010). Note also that RpoS tends to decrease due to the increase in glucose concentration as the

dilution rate increases, where *rpoS* transcript level decreased (**Fig. 2a**). The decrease in RpoS repressed *acs* expression, and this also caused acetate accumulation. Although the acetate formation is the waste formation from the fermentation point of view in *E. coli*, acetate formation is a kind of safety valve by adjusting the imbalance between glycolysis and the TCA cycle activity.

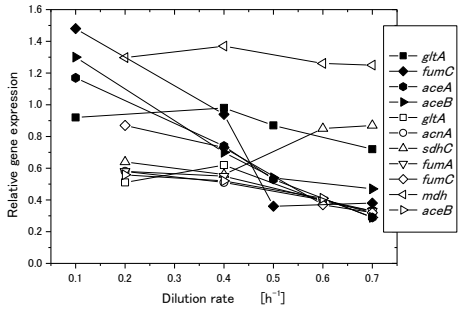
(a) Global regulatory genes



(b) Glycolysis gene expressions



(c) TCA cycle gene expressions



(d) TCA cycle fluxes and acetate formation rate

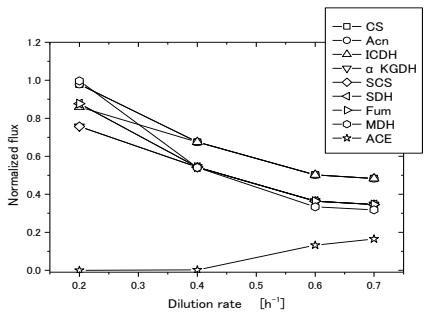


Fig. 2. Effect of the growth rate (dilution rate) on gene expressions and fluxes: (a) global regulators, (b) glycolysis genes, (c) TCA cycle genes, (d) TCA cycle fluxes and acetate formation rate (Open symbol from Yao et al., 2011; filled symbol from Ishii et al., 2007).

Note that although the TCA cycle activity may be repressed based on gene expression data, the specific glucose consumption rate increases, and thus the absolute TCA cycle fluxes tends to increase on the mmol basis by taking into account the specific glucose consumption rate (Yao et al., 2011). This means that the respiration is rather activated with the increased production of NADH in the glycolysis and the TCA cycle, and more radical oxygen derivatives are generated, where this is reflected in the increase in *soxR/S* and *sodA* gene transcript levels (**Fig. 2a**), where SoxR/S is the global regulator system for the oxidative stress regulation. Fur has been also shown to be related to the oxidative stress regulation. The increased activity of SoxS causes transcriptional activation of *zwf* gene expression (Appendix A). It has been shown that 6PGDH activity is the growth rate dependent (Wolf et al., 1979), where it has been believed that the activation of the oxidative PP pathway is due to NADPH production for biosynthesis. The present analysis gives additional viewpoint for the activation of PP pathway.

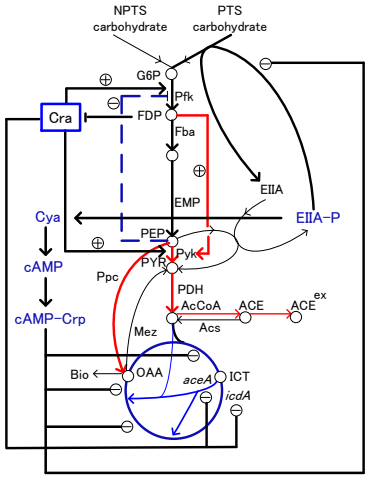


Fig. 3. Overall regulation mechanism for the main metabolism by enzyme level and transcriptional regulations.

3. EFFECT OF THE SPECIFIC PATHWAY MUTATION ON THE METABOLIC REGULATION

The proper understanding of the metabolic regulation of the central carbon metabolism is critical for the efficient metabolic engineering. For this, it may be useful to understand the effect of the specific pathway mutation on the metabolic changes based on ^{13}C-metabolic flux distribution together with different levels of information (Shimizu, 2004, 2009, 2013b). While the specific-gene knockout mutations preclude growth on glucose, a majority of such mutations seem to be potentially compensated by the use of alternative enzymes or by rerouting of the carbon fluxes through alternative pathways, resulting in a robust phenotype such as little effect on the cell growth rate etc. (Matsuoka and Shimizu, 2012).

If *pgi* gene was knocked out, the glucose catabolism occurs exclusively through the oxidative PP pathway. As a result, NADPH is overproduced, and inhibits allosterically the activity of G6PDH, thereby reducing the glucose uptake rate, resulting in the low growth rate (Toya et al., 2010). In this mutant, the ED pathway is activated, thus reducing NADPH production at 6PGDH (Hua et al., 2003). The NADPH can be converted to NADH by trans-hydrogenase Udh, or the overproduced NADPH can be utilized for heterologous protein production such as PHB production etc. (Kabir and Shimizu, 2003). The growth rate can be recovered to some extent with such manipulations. Although EMP pathway can be backed up by rerouting PP pathway, its flux becomes low, resulting in low PEP concentration, and thus the anaplerotic flux through Ppc becomes low. This causes the activation of another anaplerotic pathway, glyoxylate pathway for compensating OAA concentration (**Fig. 4**). Based on the consideration as discussed in the previous section, the decrease in the glucose uptake, or the decreased activity of PTS, causes phosphorylated EIIA (EIIA-P) to be increased, and thus cAMP-Crp increases, which in turn activates TCA cycle. Moreover, the decreased FDP concentration (Toya et al., 2010) activates Cra activity, which in turn activates *icdA* and *aceA/B*, and thus acetate formation is significantly reduced (Toya et al., 2010; Hua et al., 2003). Since cAMP-Crp increases in this mutant, the catabolite repression is relaxed, and co-consumption of multiple sugars becomes possible with the decreased cell growth rate. Co-consumption of multiple sugars can be also attained by *pts* mutants (Nichols et al., 2001), where the cell growth can be enhanced by amplifying the non-PTS sugar transporter such as GalP together with Glk (Escalante et al., 2012; Lu et al., 2012).

In the case of *zwf* gene knockout, the cell growth phenotype is little affected, where the non-oxidative PP pathway flux is reversed (Zhao et al., 2004; Hua et al., 2003). This mutant shows significant overflow metabolism, and some of the NADPH, which cannot be produced at the oxidative PP pathway, is backed up by Mez (**Fig. 5**), and by the transhydrogenase Pnt from NADH (Hua et al., 2003). Note that *zwf* gene knockout causes the increase in glycolysis flux, and thus EIIA-P decreases and in turn cAMP-Crp decreases. This causes TCA cycle to be repressed (**Fig. 5**). Moreover, as the glycolysis flux increases, FDP concentration also increases, and in turn represses Cra activity, and thus TCA cycle is repressed by the down regulations of *icdA* and *aceA/B* genes. This is the reason why more acetate was produced as compared to wild type strain.

In the case of *pyk* gene knockout, PEP is accumulated, which causes the increase in the Ppc flux. This in turn causes the increased fluxes through MDH and Mez, and the reduced PYR (caused by Pyk disruption) could be backed up by these alternative pathway fluxes (**Fig. 6**) (Siddiquee et al., 2004; Toya et al., 2010). Note that the accumulation of PEP, and thus EIIA-P increases, and in turn cAMP increases to some extent (Cunningham et al., 2009). However, this increase is counterbalanced by the accumulation of G6P. Namely, although the accumulation of PEP causes the activation of PTS, PEP inhibits allosterically the activation of Pfk. This in turn causes the accumulation of G6P, and then causes the degradation of mRNA of *ptsG*, which causes the deactivation of PTS and the activation of the oxidative PP pathway.

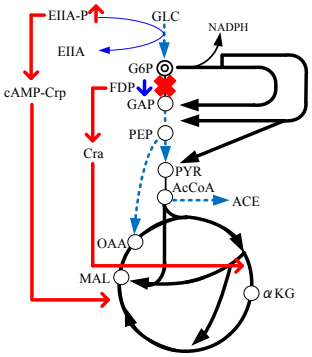


Fig. 4. Metabolic regulation in response to *pgi* gene knockout.

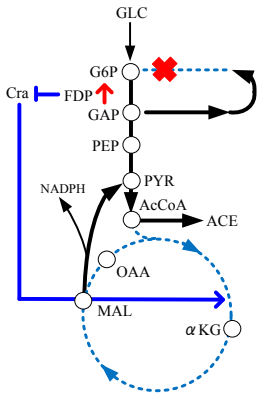


Fig. 5. Metabolic regulation in response to *zwf* gene knockout.

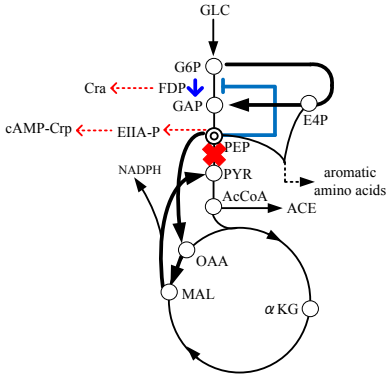


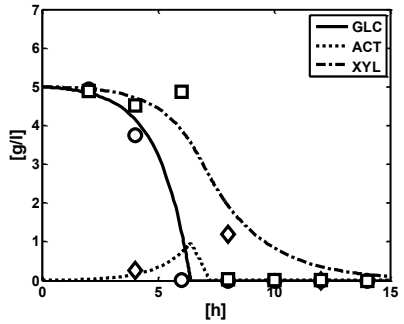
Fig. 6. Metabolic regulation in response to *pyk* gene knockout.

4. MODELING AND COMPUTER SIMULATION

The above analysis based on gene expressions, intracellular metabolite concentrations, and ^{13}C-metabolic fluxes can be extended or backed up by computer simulation using appropriate model. We have previously developed a kinetic model for the main metabolic pathways (Kadir et al., 2010). Here, we also incorporated the catabolite regulation mechanism (Kotte et al., 2010), where the effect of global regulators on the metabolic pathway reaction rates were considered. The mathematical models considered are briefly

given in Appendix B (Matsuoka and Shimizu, 2013). The simulation result for the batch culture of *E. coli* wild type and its *ptsG* mutant for the case of using a mixture of glucose and xylose as a carbon source is shown in **Fig. 7a, b**. Moreover, it was shown that the result of **Fig. 2** can be well expressed by computer simulation (Matsuoka and Shimizu, 2013).

(a)



(b)

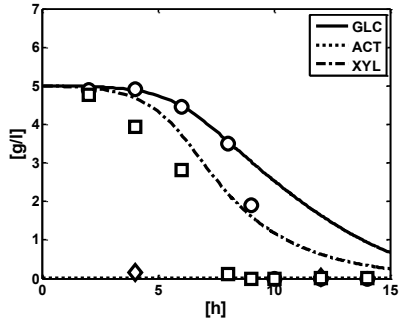


Fig. 7. Computer simulation for wild type (a) and *ptsG* mutant (b) for the case of using a mixture of glucose and xylose as a carbon source.

5. CONCLUSION

The ultimate goal of systems biology is to understand the whole cell system including cell metabolism. Here, it was shown that the systems biology approach is quite useful in getting insight into the metabolic regulation made by both enzymatic and transcriptional regulations focusing on the roles of transcription factors.

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Appendix A. REGULATION OF GLOBAL REGULATORS ON THE METABOLIC PATHWAY GENES

Global regulator	Metabolic pathway gene
Cra	+: *aceBAK, cydB, fbp, icdA, pckA, pgk, ppsA* −: *acnB, adhE, eda, edd, pfkA, pykF, zwf*
Crp	+: *acnAB, aceEF, focA, fumA, gltA, malT, manXYZ, mdh, mlc, pckA, pdhR, pflB, pgk, ptsG, sdhCDAB, sucABCD, ugpABCEQ* −: *cyaA, lpdA, rpoS*
RpoS	+: *acnA, acs, adhE, asmC, fumC, gadAB, talA, tktB, poxB* −: *ompF*
SoxR/S	+: *acnA, fumC, sodA, zwf*

Appendix B. MODELING FOR THE MAIN METABOLISM

Referring to **Fig. 1**, the mass balance equations are expressed for the glycolysis as follows:

$$\frac{d[GLC^{in}]}{dt} = v_{NPTS} - v_{Glk} - \mu[GLC^{in}] \quad (1)$$

$$\frac{d[G6P/F6P]}{dt} = v_{PTS4} + v_{Fbp} + v_{TktB} + v_{Tal} - v_{Pfk} - v_{G6PDH} - v_{Bio,G6P} - \mu[G6P] \quad (2)$$

$$\frac{d[FDP]}{dt} = v_{Pfk} - 0.5v_{Emp} - v_{Fbp} - \mu[FDP] \quad (3)$$

$$\frac{d[GAP]}{dt} = v_{Emp} + v_{TktA} + v_{TktB} - v_{Eno} - v_{Tal} - v_{Bio,GAP} - \mu[GAP] \quad (4)$$

$$\frac{d[PEP]}{dt} = v_{Eno} + v_{Pck} + v_{Pps} - v_{Pyk} - v_{Ppc} - v_{PTS1} - v_{Bio,PEP} - \mu[PEP] \quad (5)$$

$$\frac{d[PYR]}{dt} = v_{Pyk} + v_{Mez} + v_{PTS1} - v_{PDH} - v_{Pps} - v_{Bio,PYR} - \mu[PYR] \quad (6)$$

where PP pathway, TCA cycle and xylose assimilating pathway can be expressed in the similar way, and μ is the specific growth rate. The detailed model for the enzymatic reactions together with gene expressions are given elsewhere (Matsuoka and Shimizu, 2013), where $v_\bullet$ are the functions of substrate, product, metabolites which allosterically affect the reaction rate as well as the function of transcription factors. Some of the parameter values such as the maximum reaction rates were tuned based on the steady state values of our past data (Ishii et al., 2007). The MATLAB was used for simulation. The specific growth rate may be estimated based on the experimental observation that the cell growth rate is linearly correlated with the specific ATP production rate as

$$\mu = (\alpha_{GLC} + \alpha_{XYL} + \alpha_{ACT}) \cdot k_{ATP} \cdot v_{ATP}(\bullet) \quad (7)$$

where k_{ATP} is the constant parameter, and $v_{ATP}(\bullet)$ is the specific ATP production rate. Moreover, α_{GLC}, α_{XYL} and α_{ACT} may be expressed as a function of the corresponding substrate. Note that if the metabolic fluxes of the main metabolic pathways were obtained, the ATP production rate can be also estimated by considering substrate level phosphorylation or the oxidative phosphorylation, where the reducing equivalents such as NADH and $FADH_2$ can contribute in generating ATP via oxidative phosphorylation.