

Dynamic modeling of central metabolism and regulatory network of *Escherichia coli* for amino acid production simulation

Yoshihiro Usuda^{1,*}, Yosuke Nishio¹, Shintaro Iwatani¹, Stephen J. Van Dien^{1,2}, Akira Imaizumi¹, Kazutaka Shimbo¹, Naoko Kageyama¹, Daigo Iwahata¹, Hiroshi Miyano¹, Kazuhiko Matsui¹

1. Life Science Institute, Ajinomoto Co., Inc., Kawasaki, Japan

2. Present address: Genomatica Inc., San Diego, USA

*email: yoshihiro_usuda@ajinomoto.com

Primary metabolites are important industrial products, which can be produced by fermentation process. Among amino acids used as seasoning, feed additives, and fine chemicals and mainly produced by bacteria such as *Escherichia coli*, glutamate is produced all over the world in the greatest amount, over one million tons. Thus, fermentation process of primary metabolites is of industrial importance and has been one of promising target for metabolic engineering and metabolic flux analysis. Dynamic simulation of cell metabolism is believed to become a useful method for bioprocess development. However, it is well known that this kind of simulation is challenging mainly because the kinetic parameters that describe the enzymatic reactions and regulatory events are difficult to obtain and sometimes different from physiological ones inside the cell. Our motivation is to construct a practical simulation system which can describe the transient metabolic and regulatory states of amino acid production process. Recently, we have developed stoichiometric model for *E. coli* (Van Dien et al. 2006) and a practical method for metabolic flux analysis using ¹³C-substrate (¹³C-MFA), in which metabolites inside the cell from initially added labeled substrate can be analyzed in industrial fermentation process (Iwatani et al. 2007).

The metabolic part of *E. coli* central metabolism model based on Michaelis-Menten kinetics includes phosphotransferase (sugar uptake) system, glycolysis, pentose-phosphate pathway, TCA cycle, glyoxylate shunt, and anaplerotic pathways and the regulatory part includes regulation by transcription factors, CRP, Mlc, Cra, PdhR, and IclR. The over 180 differential equations for concentrations of metabolites, mRNA of genes, enzymes, and transcription factors were solved by ode15s of MATLAB. We tried to explain transient batch-cultivation of wild-type and glutamate producing strain by this model. A wild-type *E. coli* MG1655 and a glutamate producing strain MG1655 Δ sucA were cultivated in 1L-scale jar fermentor with 222 mM glucose. The metabolic fluxes were measured using ¹³C-MFA. We adjusted several enzyme and expression parameters of the *E. coli* wild-type model using metabolic fluxes which are the most reliable data of enzyme activities *in vivo*. The representative results of simulation are shown in Fig.1. According to experimental cell growth, cell volume is increased (Fig. 1(A)) and glucose consumption and CO₂ exhaust show good agreement with measured profiles (Fig. 1 (B) and (F)). Although major products of a wild-type strain culture, acetate and formate, are not in good agreement with experimental

results, we succeeded to simulate all the gene expression and enzyme reaction within allowable range of metabolite concentrations.

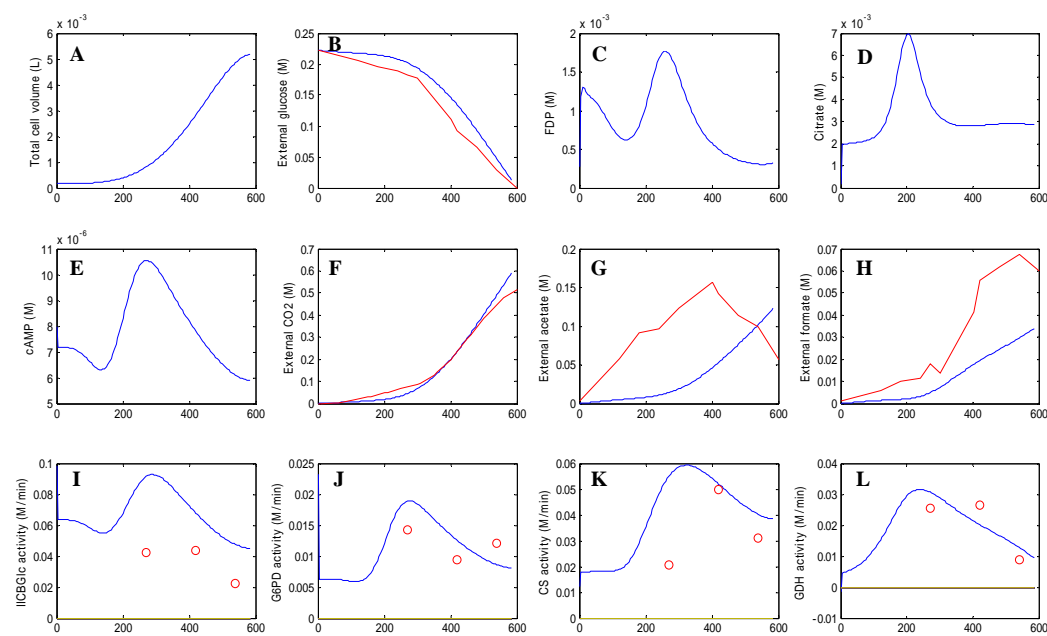


Figure 1: Simulation of cultivation of *E. coli* wild-type strain (MG1655) in batch cultivation. (A) Cell volume. (B) external glucose. (C) fructose-1,6-diphosphate. (D) citrate. (E) cAMP. (F) external CO₂. (G) external acetate. (H) external formate. (I) IICB^{Glc} activity. (J) glucose-6-phosphate dehydrogenase activity. (K) citrate synthase activity. (L) glutamate dehydrogenase activity. Experimental results are shown in red line and enzyme activities converted from metabolic fluxes are plotted by red circle.

A glutamate producer can be obtained by disruption of *sucA* gene encoding α -ketoglutarate dehydrogenase (KGDH) from wild type strain. We performed cultivation experiment and simulation of glutamate production by batch-culture. The parts of results of simulation are shown in Fig.2. Although acetate excretion and CO₂ exhaust are not in good agreement with experimental results (Fig. 2 (G) and (F)), glucose consumption and glutamate production showed excellent agreement with measured profile (Fig. 2 (B) and (H)).

The modified parameters are very important, because we can know the discrepancies between *in vitro* and *in vivo*. One example is that enzymatic parameters of triosephosphate isomerase, forward k_{cat} for forward reaction and k_{cat} for reverse reaction, were changed to be 3,000-fold and 1/3,000, respectively. Some great gaps may mean the existence of unknown regulation, activation or inhibition by metabolite(s). We have also performed sensitivity analyses for glutamate excretion. In a wild-type strain model, the parameters of expression and enzyme which negatively affected

intracellular glutamate concentration were translation efficiency and k_{cat} of KGDH, respectively. In a glutamate-producing model, the parameters of expression and enzyme which negatively affected extracellular glutamate concentration by are decay constant of *gltA* mRNA and k_{cat} of isocitrate lyase, respectively. These imply the next targets for improvement of glutamate producing strain.

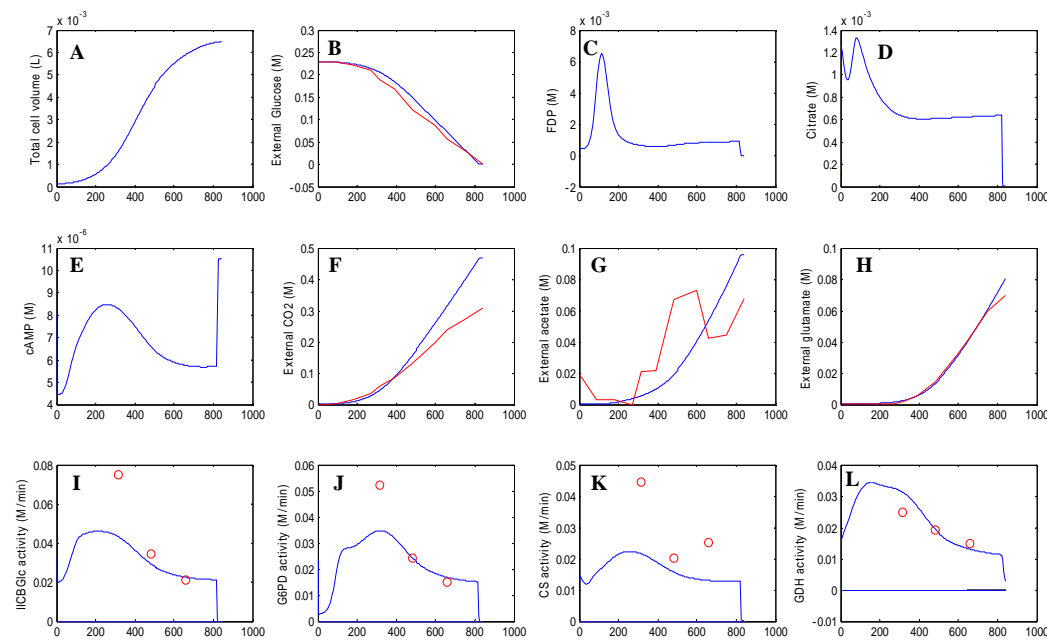


Figure 2: Simulation of glutamate production process of *E. coli* MG1655 Δ *sucA* in batch cultivation. (A) Cell volume. (B) external glucose. (C) fructose-1,6-diphosphate. (D) citrate. (E) cAMP. (F) external CO₂. (G) external acetate. (H) external glutamate. (I) IICB^{Glc} activity. (J) glucose-6-phosphate dehydrogenase activity. (K) citrate synthase activity. (L) glutamate dehydrogenase activity. Experimental results are shown in red line and enzyme activities converted from metabolic fluxes are plotted by red circle.

Acknowledgements

We thank Hiroshi Matsui, Yusuke Nakazawa, and Daisuke Shimazaki of Kyoto Prefectural University for kinetic data of enzymes. The authors are also grateful to Mai Shimazaki, Fumiko Yamamoto, and Hiroyuki Aoyagi for excellent technical assistance.

References

- Van Dien SJ, Iwatani S, Usuda Y, Matsui K (2006) Theoretical analysis of amino acid-producing *Escherichia coli* using a stoichiometric model and multivariate linear regression. *J Biosci Bioeng* **102**: 34-40
- Iwatani S, Van Dien S, Shimbo K, Kubota K, Kageyama N, Iwahata D, Miyano H, Hirayama K, Usuda Y, Shimizu K, Matsui K (2007) Determination of metabolic flux changes during fed-batch cultivation from measurements of intracellular amino acids by LC-MS/MS. *J Biotechnol* **128**: 93-111