Large-scale kinetic modeling for the central metabolism in *Escherichia coli* grown on glucose or glycerol

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1 Introduction

Energy metabolism in *Escherichia coli* is the general process by which energy molecules such as ATP and NADH are synthesized. They are required for cell growth and maintenance of *E. coli*. Glucose is catabolized to acetate through glycolysis, thus contributing to cell growth. After glucose is depleted, the tricarboxylic acid (TCA) cycle consumes acetate, further contributing to cell growth [1]. In the absence of glucose, glycerol, an energy-poor carbon source, enhances the glycerol, glucogenic, Entner−Doudoroff (ED), and aromatic amino acid (AroAAs) pathways, and is coutilized with acetate as a carbon source [2]. Although the growth of *E. coli* is slower with glycerol than with glucose, the final cell density in glycerol medium is observed to be higher than that in glucose medium. Analysis of the differences in gene expression, enzyme activities, and metabolite concentrations between cells grown in one carbon condition or the other is important to elucidate the mechanism of environmental response in metabolic pathways. Large-scale kinetic models of central metabolic pathways, including glycolysis, the pentose-phosphate (PP) pathway, and the TCA cycle, in *E. coli* grown on glucose have already been developed [3,4,5]. However, a large-scale kinetic model of glycerol consumption in *E. coli* has not yet been generated. In this poster, we develop a kinetic model for the central metabolism of *E. coli* that includes not only the glucose transport system, but also the glycerol transport system.

2 Methods and Results

We extended a kinetic model of the central metabolism, including glycolysis, the PP pathway, and TCA cycle [3], by adding the glycerol transport system and the glucogenic, ED, and AroAAs pathways [4,5] (Fig. 1). The specific growth rate (μ) was expressed as a function of ATP production (v_{ATP}) and substrate concentration (S), such as those of glucose, acetate and glycerol (Eqs. (1) and (2)).

$$\mu = \mu_{\max,S} \frac{S}{K_s + S}\left(1 - \frac{X}{X_m}\right) k_{ATP} v_{ATP} \tag{1}$$

$$v_{ATP} = v^{ATP}_{glycolysis} + v^{ATP}_{TCAcycle} + v^{ATP}_{acetatemetabolism} + v^{ATP}_{anaplerotic\,pathway} + v^{ATP}_{glycerol\,pathway} + v^{ATP}_{aromatic\,pathway} \tag{2}$$

Here, $\mu_{\max,S}$ ($\mu_{\max,\mathrm{Glc}} = 0.69$, $\mu_{\max,\mathrm{Gly}} = 0.49$, $\mu_{\max,\mathrm{Ace}} = 0.9$)[2,3], K_s ($K_{\mathrm{Glc}} = 0.1$, $K_{\mathrm{Ace}} = 0.01$, $K_{\mathrm{Gly}} = 0.1$)[3] and k_{ATP} are the model parameters depending on each carbon sources, X is the cell concentration, X_m is the final value of X in the batch culture, and v_{ATP} is the sum of all the ATP produced in six metabolic pathways.

The initial metabolite concentrations were taken from literature [4,5]. The parameters ($v^{\max}$, k_{ATP}) were optimized using experimental data of cell, extracellular carbon source, acetate, and indole concentrations in *E. coli* K-12 (JM101) growing in mineral medium with glucose and glycerol [2] by Real-coded Genetic Algorithm (RCGA) [6]. The contribution rate of individual pathways to overall growth ($v^{ATP}_{pathway} / v_{ATP}$) was calculated.

The calculated rates demonstrate that cell growth, carbon source consumption, and acetate concentrations corresponded with experimental results previously obtained for cells in both glucose and glycerol media (Fig. 2). The contribution rates revealed that the ATP produced through glycolysis accounts for approximately 90% of all ATP generated during the exponential growth phase of *E. coli* grown on glucose medium. On the other hand, cell growth was first inhibited on glycerol medium as ATP was consumed by glycerol kinase (GlpK) to phosphorylate intracellular glycerol until 6 h after culture, and then activated by ATP production through glycolysis in the exponential growth phase from 6–8 h. Although the contribution of the TCA cycle to cell growth increases gradually as cells switched from the exponential growth phase to the stationary phase on glycerol medium, it remained to low contribution rates for growth (Fig. 3).

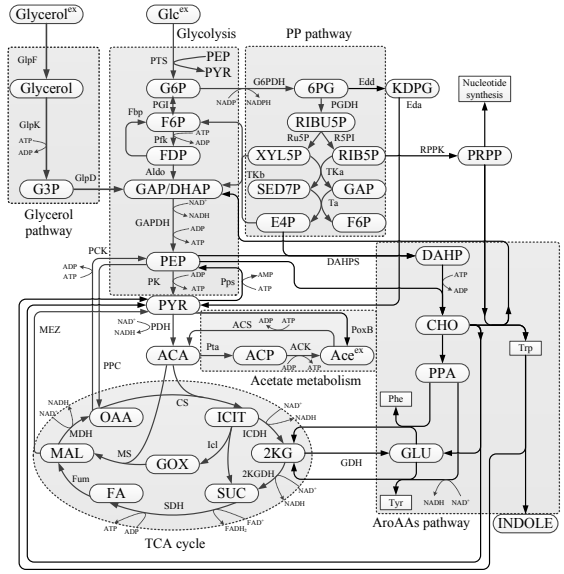

Figure 1: Model of energy metabolism in *E. coli* including glycolysis, the TCA cycle, gluconeogenesis, and the PP, ED, and AroAAs pathways.

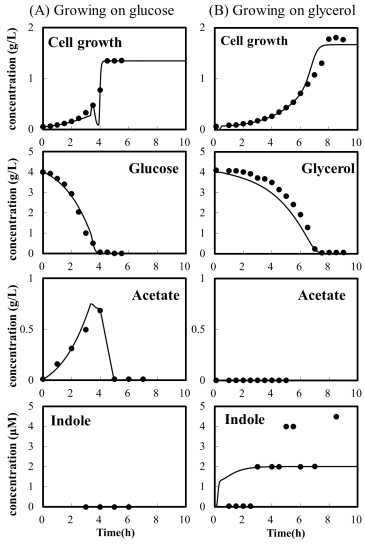

Figure 2: Cell growth and metabolite concentrations determined from our study (solid lines) and from previous experiments (dots).

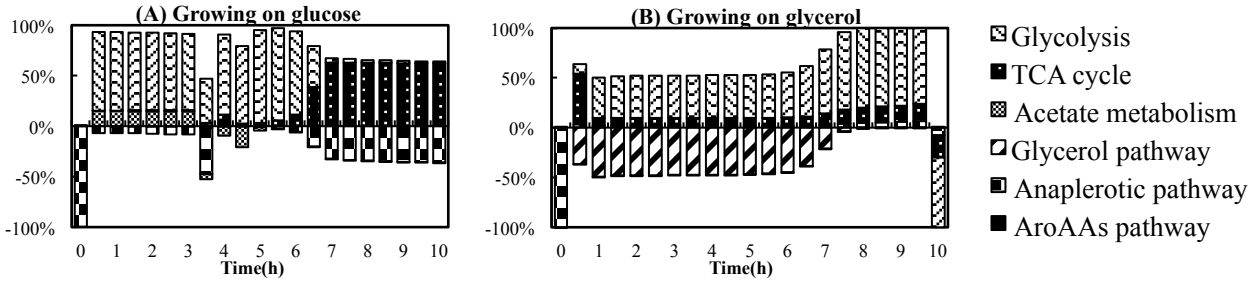

Figure 3: Changes in the contribution rates of individual pathways for growth with (A) glucose and (B) glycerol.

3 Conclusion and Future Works

In this study, the parameters set in the model were optimized for each carbon source by RCGA. Data from our simulation corresponded well with experimental data for cell growth and metabolite concentration in both growth conditions, except for data pertaining to indole concentrations. Differences of flux activities in the TCA cycle and in phosphoenolpyruvate synthetase between the glucose and glycerol media were indicated by calculating the contribution rates. To further investigate the metabolic response of *E. coli* to environmental changes, we need to implement indole degradation and glycerol catabolite repression by glucose in our model.

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