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A Comparative Analysis of Dynamic Models of the Central Carbon Metabolism of *Escherichia coli*

Ana Patrícia Lima*. Vítor Baixinho.*Daniel Machado. Isabel Rocha ***

Centre of Biological Engineering
University of Minho, Campus de Gualtar
4710-057 Braga - Portugal

* *These authors have contributed equally to this work*

Abstract: Dynamic models of metabolism have been developed for a variety of systems and can be applied in metabolic engineering design and to understand the time-varying characteristics of the systems when exposed to different stimuli. Hereby we analyse and compare the most used and complete kinetic models available for the central carbon metabolism of *E. coli*. Stoichiometric and kinetic comparisons showed several differences, discrepancies and incoherence especially regarding the kinetic mechanisms assumed, parameters and units. Time course and steady-state simulations and also comparison with an experimental dataset put in evidence major differences regarding responses to the same stimulus. The results presented raise important questions regarding the need of using standard methodologies in dynamic model construction as well as in using experimental data for model validation.

Keywords: Kinetic Models; *Escherichia coli*; Time course simulation; Steady-State

1. INTRODUCTION

Mathematical models help understand, predict, and optimize the properties and behaviour of cell factories. For that reason, they assume a great importance in industrial biotechnology. In order to study cellular metabolism, there are two main different modelling approaches based on different assumptions: kinetic and stoichiometric modelling (Machado et al. 2012) .

Kinetic models describe the temporal behaviour of all biochemical species in a metabolic system. They specify the details of interactions at metabolite and enzyme levels, such as allosteric regulation, therefore assuming a crucial role to a more explicit study of metabolic responses to perturbations at time-scales before a steady state is reached **(**Shmulevich 2011**)**.

Over the years, several dynamic models have been developed for different metabolic systems. Here four important dynamic models of the central metabolism of *Escherichia coli* are analysed (Chassagnole et al. 2002; Peskov et al. 2012; Kadir et al. 2010; and Khodayari et al. 2014).

The Chassagnole model is the oldest one and only describes kinetic equations for the glycolysis and pentose phosphate pathways; however, it is still widely utilized (Theobald et al. 1997; Vaseghi et al. 1999; Rizzi et al. 1997; Chassagnole et al. 2002). Meanwhile, three recent models have been published, covering other metabolic pathways, such as the tricarboxylic acids (TCA) cycle. The main goal of the Kadir model was to simulate the time profiles of batch and continuous cultures (Kadir et al. 2010). The Peskov model describes some metabolic regulations of *E. coli* central carbon metabolism (Peskov et al. 2012). The Khodayari model is the largest detailed *E. coli* kinetic model, accounting for a total of 138 reactions (1474 elementary reactions) and 93 metabolites (830 complexes and metabolites). This model was parameterized, with multiple *omics* data, using the ensemble modelling method (Toya et al. 2007; Tran et al. 2008)**.**

Besides the different pathway coverage, these models have been constructed with different applications in mind and with different assumptions and levels of experimental validation. However, a comparison of the coverage and performance of these models under the same conditions has not been performed so far, limiting any critical comparison between them.

Constraint-based methods can be used to determine intracellular metabolic fluxes based on mass balances over intracellular metabolites and the assumption of a pseudo-steady state. Contrary to the kinetic models, these models do not require the determination of kinetic equations and associated kinetic parameters, even though they are important to understand the capabilities of the metabolic network and to perform structural analysis (Szallasi 2006; Kuepfer 2014). In this work the *i*AF1260 genome-scale stoichiometric model of *E. coli* K-12 MG1655 was used for structural comparison purposes. This model encompasses 1260 genes, 2077 reactions and 1039 metabolites (Feist et al. 2007).

2. METHODS

2. 1 Dynamic models and standardization

The kinetic models used were the ones introduced by (Chassagnole et al. 2002); (Peskov et al. 2012); (Kadir et al. 2010) and (Khodayari et al. 2014).

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To allow comparing model predictions, all models were set to the same experimental conditions and prepared for simulation of a glucose pulse, as described by Chassagnole et al., 2002 with a dilution rate of 0.1 h^{-1} and a glucose concentration in the feed of 110.96 mM. For that purpose, the initial concentration of extracellular glucose was set to 2 mM. Considering a biomass concentration of 8.7 gDW/L and a cellular density of 564 gDW/L one obtains a ratio of 65 L of extracellular volume per 1 L of biomass.

The Chassagnole model is available at the BioModels Database (BioModels ID: BIOMD0000000051) (Le Novère et al. 2006; Juty et al. 2015; Li et al. 2010) in the SBML format. For this model, the extracellular to intracellular volume ratio (65:1) had been implicitly incorporated in the stoichiometry of the Phosphotransferase System (PTS). To facilitate comparisons with other models and allow to easily change this parameter, the ratio was defined in the respective compartment volumes and the PTS stoichiometry was fixed. It should be emphasized that this change does not affect the model predictions.

The Kadir model was provided in MATLAB code by the authors. An SBML version was constructed using JWS Online (Olivier & Snoep 2004).

The Peskov model had been downloaded from JWS Online in SBML (but it is no longer available). An external compartment, with volume equal to 65 L, was added to the model. The extracellular glucose was redefined from a parameter to a metabolite and the Glc_{in} metabolite was removed accordingly.

The Khodayari model was downloaded in MATLAB from the author's web page, which can be accessed at http://www.maranasgroup.com/submission_models/escherichiaColiCoreMetabolism.htm (Research Goup of Costas D. Maranas. 2014). An SBML version was constructed using JWS Online (Olivier & Snoep 2004).

In all models, a common equation for the extracellular glucose kinetics (as defined in the Chassagnole model) was added:

$$V_{glcex} = Dil \times ([Glc]_{feed} - [Glc]_{ext}) \quad (1)$$

where V_{glcex} is the extracellular glucose exchange rate, *Dil* is the system dilution rate, $[Glc]_{feed}$ is the glucose concentration in the feed and $[Glc]_{ext}$ is the extracellular concentration of glucose.

2.2 Units conversion

To facilitate comparison, all models were standardized to the same units. The ones commonly used in genome-scale models were chosen for that purpose. Therefore, metabolite concentrations and reaction rates were changed from mM and mM/s to mmol/gDW and mmol/gDW/h, respectively. The parameters were also converted, while dimensionless parameters were kept unchanged. Some discrepancies regarding the kinetic parameters were found. In some cases, there were differences between the parameter values in the models and those reported in the original papers. In those cases, the values present in the SBML file prevailed since they more accurately replicated the published results.

2.3 Changes in kinetic laws

Changes in some kinetic equations were performed, due to discrepancies found during the units' conversion step. The most common case was the re-arrangement of Hill equations to make the Hill coefficient explicit for the dissociation constants (otherwise it would lead to inconsistent units). For instance, in the Chassagnole model, the parameter $K_{PTS,g6p}$, had to be re-calculated, as it had been defined incoherently (in the inhibition term it appears as $\frac{C_{g6p}^{n_{PTS,g6p}}}{K_{PTS,g6p}}$). It was recalculated as follows:

$$K'_{PTS,g6p} = \sqrt[n_{PTS,g6p}]{K_{PTS,g6p}} \quad (2)$$

The recalculated parameter was then re-introduced in the PTS kinetic equation with an explicit Hill coefficient (eq. 3).

$$r_{PTS} = \frac{r_{max} \times C_{gluc} \times \frac{C_{pep}}{C_{pyr}}}{\left(K_{PTS,a1} + K_{PTS,a2} \times \frac{C_{pep}}{C_{pyr}} + K_{PTS,a3} \times C_{gluc} + C_{gluc} \times \frac{C_{pep}}{C_{pyr}}\right) \times \left(1 + \frac{C_{g6p}^{n_{PTS,g6p}}}{\boldsymbol{Knew}^{n_{PTS,g6p}}}\right)} \quad (3)$$

A similar procedure was applied to both the DAHP synthase (DAHPS) and the pyruvate dehydrogenase (PDH) reactions. Table 1 shows the values of each recalculated parameter and the new values in both units used.

Table 1 – Results for the re-estimation of some parameters for the Chassagnole model.

Parameter	Original value (mM)	New value	
		mM	mmol/gDW
$K_{PTS,g6p}$	2.15	1.23	0.0022
$K_{DAHPS,e4p}$	0.035	0.275	0.00049
$K_{DAHPS,pep}$	0.0053	0.0924	0.0002
$K_{PDH,pyr}$	1159	6.802	0.012

In the Kadir model, the kinetic equations for the PTS, aldolase (ALDO) and acetate kinase (ACK) reactions were also modified. The PTS kinetics is equal to the one described in the Chassagnole model and thus the changes were performed in the same way. The kinetic equations for ALDO and ACK had differences between the SBML and the ones reported in original article. The formulations present in the article were chosen since this option was the only one that allowed obtaining an agreement in the validation process.

2.4 Time courses and steady-state experiments

Both the time course and the steady-state experiments were performed using the COPASI (COmplex PAthway Simulator) software (Hoops et al. 2006). Time-course simulations were performed for a total of 4 hours with a time-step of 1 second.

3. RESULTS AND DISCUSSION

3.1 Comparison of structures and kinetic laws