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# Global sensitivity analysis in dynamic metabolic networks

J. Di Maggio [a], J.C. Diaz Ricci [b], M.S. Diaz [a,*]

[a] Planta Piloto de Ingeniería Química (PLAPIQUI), Universidad Nacional del Sur-CONICET, Argentina
[b] Instituto Superior de Investigaciones Biológicas (INSIBIO), Universidad Nacional de Tucumán-CONICET, Argentina

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ABSTRACT

In this work, we have performed global sensitivity analysis on a large-scale dynamic metabolic network through variance-based techniques. Time profiles for sensitivity indices have been calculated for each parameter, based on Sobol' approach (2001). The global sensitivity analysis has been carried out on a dynamic model for the Embden–Meyerhof–Parnas pathway, the phosphotransferase system and the pentose-phosphate pathway of *Escherichia coli* K-12 strain W3110 (Chassagnole et al., 2002). The model comprises eighteen dynamic mass balance equations for extracellular glucose and intracellular metabolites, thirty kinetic rate expressions and seven additional algebraic equations that represent concentration profiles for co-metabolites. Each parameter has been considered to have a normal probability distribution centered on its nominal value and sample sizes of two thousand and five hundred scenarios have been considered. The preceding analysis has allowed identification of eleven parameters as the most influential ones on the complex metabolic network under study.



## 1. Introduction

During the last decade, there has been increasing interest in developing new products and processes using renewable resources through the discovery and optimization of new strains. At this point, metabolic engineering plays an important role because it deals with the improvement of cells, considering the possibility of introducing new pathways, delete or modify existing ones in cells, using genetic tools to achieve a specific goal (Bailey, 1991; Stephanopoulos & Stafford, 2002). Nowadays it is possible to obtain data characterizing the status of microorganisms at genomic, proteomic, metabolomic and physiological levels, which can be used for metabolic network models development (Burgard & Maranas, 2001; Ghosh, Zhu, Grossmann, Ataai, & Domach, 2005; Lee, Palakornkule, Domach, & Grossmann, 2000; Majewski & Domach, 1990; Namjoshi & Ramkrishna, 2005; Varma & Palsson, 1994). It means that intracellular and extracellular metabolites concentrations, measurements of protein levels and activity are available, in most cases. Furthermore, the advances on experimental techniques and the consequent increase on the amount of accessible data on the dynamics of functioning cells allow the building of dynamic models for metabolic networks, which can predict the microbial behavior and constitute important tools in metabolic engineering.

Dynamic models provide time profiles for the concentration of metabolites involved in the metabolic network under study (Diaz Ricci, 1996, 2000; Diaz Ricci, Hitzmann, & Bailey, 1991; Rizzi, Baltes, Theobald, & Reuss, 1997). They comprise a nonlinear differential algebraic system of equations which arise from mass balances of metabolites and have a large number of kinetic parameters that must be estimated for a specific growth condition. However, uncertainty in input parameters has different effect on model outputs. Thus, prior to solving the inverse problem of estimating model parameters, a sensitivity analysis is required to determine which of them have the largest impact on model outputs.

There are local and global sensitivity analysis methodologies. Local methods compute sensitivity indices as the first partial derivative of model variables with respect to the parameter of interest; i.e. they compute the effect of small changes of parameters on model outputs assuming linearity of variables around the nominal trajectory, varying one parameter at a time. Global sensitivity analysis methods (Saltelli, Tarantola, Campolongo, & Ratto, 2004; Saltelli, Tarantola, & Chan, 1999; Sobol', 1990, 2001; Sobol' & Levitan, 1999) are based on exploring the entire range of variation of model parameters through the application of sampling techniques such as Monte Carlo simulations (MCS) or Latin Hypercube (LHS). Although the disadvantage of these methods is its higher computational cost, the increasing computational power of computers allows their application even in complex models. Furthermore, they are model-independent because neither the assumption of linearity nor additivity is required (Saltelli et al., 2004).

Regarding sensitivity of small dynamic metabolic networks, Mauch, Arnold, and Reuss (1997) proposed a local sensitiv-

* Corresponding author at: Planta Piloto de Ingeniería Química (PLAPIQUI), Universidad Nacional del Sur-CONICET, Chemical Engineering, Camino La Carrindanga Km 7, 8000 Bahia Blanca, Argentina. Tel.: +54 2914861700; fax: +54 2914861600.
*E-mail address:* sdiaz@plapiqui.edu.ar (M.S. Diaz).

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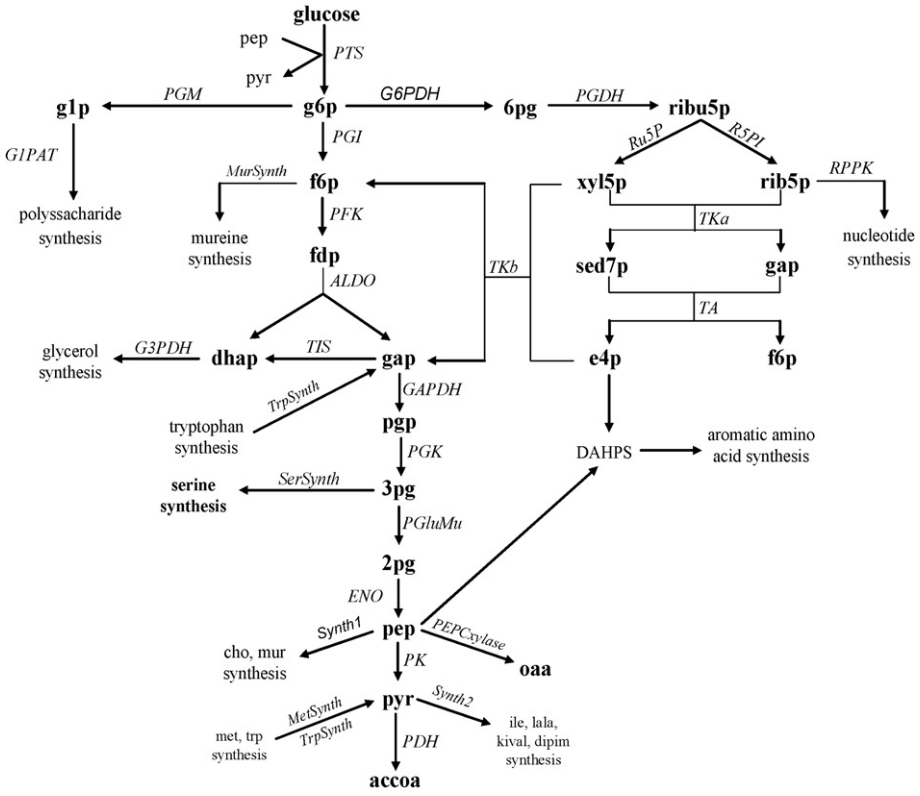


**Fig. 1.** Metabolic network for the Embden–Meyerhof–Parnas pathway, pentose-phosphate pathway and phosphotransferase system for *Escherichia coli* K-12 W3110 (Chassagnole et al., 2002).

ity method to determine stationary and time-dependent flux control coefficients and concentration control coefficients for a generic metabolic network and applied it to a metabolic network represented by two ordinary differential equations, with twelve parameters. Noack et al. (2008) applied local sensitivity analysis to an *Escherichia coli* dynamic metabolic network and developed animations as visualization techniques to present time varying sensitivities from the initial steady state up to the glucose pulse injection. Di Maggio, Diaz Ricci, and Diaz (2008a, 2008b) and Di Maggio, Diaz Ricci, and Diaz (2009) addressed sensitivity analysis through global techniques in a kinetic model of a metabolic network for reduced sets of parameters.

In this work, we have performed global sensitivity analysis for a large-scale differential algebraic (DAE) system representing the complex dynamic metabolic network corresponding to the Embden–Meyerhof–Parnas pathway, the phosphotransferase system and the pentose-phosphate pathway of *E. coli* K-12 strain W3110 (Chassagnole, Noisommit-Rizzi, Schimd, Mauch, & Reuss, 2002). Monte Carlo simulations have been performed for the calculation of times profiles for main effect indices in twenty input parameters, for main state differential and algebraic variables. Normal probability distributions have been associated to each parameter with media values taken from the literature. First and total sensitivity indices have been calculated for each parameter based on Sobol' method (2001). Global sensitivity analysis results have allowed determination of parameters describing specific enzyme properties, which have high influence on the variability of the system output, i.e. metabolite concentrations or reaction rates, as well as non-significant model parameters and value ranges of parameters within which model outputs show extreme values.

## 2. Mathematical modeling of metabolic networks

Dynamic models for metabolic networks comprise a nonlinear differential algebraic system of equations that arises from mass balances for extracellular and intracellular metabolites and co-metabolites involved in the metabolic pathways (van Riel, 2006).

In this work we have studied the dynamic model for the Embden–Meyerhof–Parnas pathway, the pentose-phosphate pathway and phosphotranferase system of *E. coli* K-12 W3110 (Chassagnole et al., 2002). The corresponding metabolic network is shown in Fig. 1. The model comprises eighteen differential equations that represent dynamic mass balances of extracellular glucose and intracellular metabolites, thirty kinetic rate expressions and seven additional algebraic equations for co-metabolites and involves around one hundred parameters. Dynamic mass balances have been formulated at each node, corresponding to intracellular metabolites, in the metabolic network; a general expression is shown in Eq. (1), where $C_i$ is the concentration of metabolite $i$, $r_j$ stands for rate of reaction $j$, $\nu_{ij}$ is the stoichiometric coefficient for metabolite $i$ in reaction $j$ and $\mu$ is the specific growth rate.

$$\frac{dC_i}{dt} = \sum_j \nu_{ij} r_j - \mu C_i \quad \forall i \tag{1}$$

$i$ = *g6p*, *f6p*, *fdp*, *gap*, *dhap*, *pgp*, *3pg*, *2pg*, *pep*, *pyr*, *6pg*, *ribu5p*, *xyl5p*, *sed7p*, *rib5p*, *e4p*, *g1p*.

$j$ = *PTS*, *PGI*, *PFK*, *ALDO*, *TIS*, *GAPDH*, *PGK*, *PGluMu*, *ENO*, *PK*, *PDH*, *PEPCxylase*, *PGM*, *G1PAT*, *RPPK*, *G3PDH*, *SerSynth*, *MurSynth*, *DAHPS*, *TrpSynth*, *MetSynth*, *G6PDH*, *PGDH*, *Ru5p*, *R5PI*, *TKa*, *TKb*, *TA*, *Synth1*, *Synth2*.