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GLOBAL SENSITIVITY ANALYSIS IN METABOLIC NETWORKS

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Abstract. In this work, we have performed a global sensitivity analysis through variance-based techniques to identify which parameters have the largest impact on model output and which of them account for most of the uncertainty in that output. Sensitivity indices have been calculated for each parameter, based on Sobol's approach (2001), which makes use of Monte Carlo methods. 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. The model comprises eighteen dynamic mass balance equations for extracellular glucose and intracellular metabolites, twenty nine kinetic rate expressions and seven additional algebraic equations to represent the concentration of co-metabolites. The model involves around one hundred parameters. Each parameter has been considered to have a normal probability distribution centered on its nominal value and sample sizes of one thousand scenarios have been considered. The preceding analysis has allowed identification of less than twenty parameters as the most influential ones on the complex metabolic network under study.

1 INTRODUCTION

The interest to develop new products and processes using renewable resources through the discovery and optimization of new strains has been growing since the past few years. At this point, metabolic engineering plays an important role because it deals with the improvement of cells, considering the possibility of introduce a new pathway, delete or modify existing ones in cells, using genetic tools to achieve a specific goal (Stephanopoulos 2002). Nowadays it is possible to obtain data characterizing the status of microorganisms over time at genomic, proteomic, metabolomic and physiological levels. It means that intracellular and extracellular metabolites concentrations, measurements of protein levels and activity are available, in most cases. 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. They comprise a non-linear 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 the first step to solve the inverse problem is to carry out a sensitivity analysis, which provides knowledge about the parameters that have the largest impact on model outputs. There are local and global sensitivity analysis methodologies. The first ones study the effect of small changes of parameters on model outputs assuming linearity of variables around the nominal trajectory (Pastres and Ciavatta, 2004; Borgonovo and Peccati, 2007). Global methods are based on exploring the whole range of variation of model parameters and on performing repeated simulations to obtain the output distributions. Therefore, computational cost is much higher in global sensitivity than in local sensitivity methods. Mauch et al. (1997) proposed a local sensitivity 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. Also, Noack et al. (2008) applied local sensitivity analysis to a metabolic network. However, to our knowledge, there is no global sensitivity analysis report on metabolic networks in the literature.

In this work, we have performed a global sensitivity analysis for a large-scale differential algebraic (DAE) system representing a complex metabolic netwok. Sensitivity indices have been calculated for each parameter based on Sobol's method (2001), which is a variance-based method. The global sensitivity analysis has been performed 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).

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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. In this work the dynamic model for the Embden-Meyerhof-Parnas pathway, the pentose-phosphate pathway and phosphotranferase system of *Escherichia coli* K-12 W3110 (Chassagnole et. al. 2002) has been studied. The model consists of 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. The set of differential equations is shown by equations 1 to 18, and algebraic equations (for co-metabolites) are shown by equations 19 to 25.

$$\frac{dC_{glc}^{ext}}{dt} = D(C_{glc}^{a\lim} - C_{glc}^{ext}) + f^{pulso} - \frac{C_X r_{PTS}}{\rho_X} \tag{1}$$

$$\frac{dC_{g6p}}{dt} = r_{PTS} - r_{PGI} - r_{G6PDH} - r_{PGM} - \mu C_{g6p} \tag{2}$$

$$\frac{dC_{f6p}}{dt} = r_{PGI} - r_{PFK} + r_{TKb} + r_{TA} - 2r_{Mur\text{Sint}} - \mu C_{f6p} \tag{3}$$

$$\frac{dC_{fdp}}{dt} = r_{PKF} - r_{ALDO} - \mu C_{fdp} \tag{4}$$

$$\frac{dC_{gap}}{dt} = r_{ALDO} + r_{TIS} - r_{GAPDH} + r_{TKa} + r_{TKb} - r_{TA} + r_{Trp\text{Sint}} - \mu C_{gap} \tag{5}$$

$$\frac{dC_{dhap}}{dt} = r_{ALDO} - r_{TIS} - r_{G3PDH} - \mu C_{dhap} \tag{6}$$

$$\frac{dC_{pgp}}{dt} = r_{GAPDH} - r_{PGK} - \mu C_{pgp} \tag{7}$$

$$\frac{dC_{3pg}}{dt} = r_{PGK} - r_{PGGluMu} - r_{SerSynth} - \mu C_{3pg} \tag{8}$$

$$\frac{dC_{2pg}}{dt} = r_{PGluMu} - r_{ENO} - 2\mu C_{2pg} \tag{9}$$

$$\frac{dC_{pep}}{dt} = r_{ENO} - r_{PK} - r_{PTS} - r_{PEPCiclasa} - r_{DAHPS} - \mu C_{pep} \tag{10}$$

$$\frac{dC_{pyr}}{dt} = r_{PK} + r_{PTS} - r_{PDH} + r_{Met\text{Sint}} + r_{Trp\text{Sint}} - \mu C_{pyr} \tag{11}$$

$$\frac{dC_{6pg}}{dt} = r_{G6PDH} - r_{PGDH} - \mu C_{6pg} \tag{12}$$

$$\frac{dC_{ribu5p}}{dt} = r_{PGDH} - r_{Ru5P} - r_{R5PI} - \mu C_{ribu5p} \tag{13}$$

$$\frac{dC_{xyl5p}}{dt} = r_{Ru5P} - r_{TKa} - r_{TKb} - \mu C_{xyl5p} \tag{14}$$

$$\frac{dC_{sed7p}}{dt} = r_{TKa} - r_{TA} - \mu C_{sed7p} \tag{15}$$

$$\frac{dC_{rib5p}}{dt} = r_{R5PI} - r_{TKa} - r_{RPPK} - \mu C_{rib5p} \tag{16}$$

$$\frac{dC_{e4p}}{dt} = r_{TA} - r_{TKb} - r_{DAHPS} - \mu C_{e4p} \tag{17}$$

$$\frac{dC_{glp}}{dt} = r_{PGM} - r_{GIPAT} - \mu C_{glp} \tag{18}$$

$$C_{ATP} = 4.27 - 4.163\frac{t}{0.657 + 1.43t + 0.364t^2} \tag{19}$$

$$C_{ADP} = 0.582 + 1.73(2.731^{-0.15t})(0.12t + 0.000214t^3) \tag{20}$$

$$C_{AMP} = 0.123 + 7.25\frac{t}{7.25 + 1.47t + 0.1t^2} + 1.073\frac{t}{1.29 + 8.05t} \tag{21}$$

$$C_{NADPH} = 0.062 + 0.332(2.718^{0.0464})(0.0166t^{1.58} + 0.000166t^{4.73} + 1.13\cdot10^{-10}t^{7.89} + 1.36\cdot10^{-13}t^{11} + 1.23\cdot10^{-16}t^{142}) \tag{22}$$

$$C_{NADP} = 0.159 + 0.00554\frac{t}{2.8 + 0.271t + 0.01t^2} + 0.182\frac{t}{4.81 + 0.526t} \tag{23}$$

$$C_{NADH} = 0.0934 + 0.0011(2.371^{-0.123t})(0.844t + 0.104t^3) \tag{24}$$

$$C_{NAD} = 1.314 + 1.314\,(2.73^{(-0.0435\,t - 0.342)}) - \frac{(t + 7.871)(2.73^{(-0.0218\,t - 0.171)})}{8.481 + t} \tag{25}$$

Equations 26 to 29 show kinetic expressions for phosphotransferase system, pyruvate dehydrogenase, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase respectively, which are involved in the above mentioned differential equations system (there are thirty kinetic equations included in the present model). A large amount of experimental data is required to estimate all parameters included in the model. However, uncertainty in some parameters may cause a slight variation in process variables and, on the other hand, uncertainty in others, can produce a large variation in model outputs. Therefore, determination and ranking of the most influential parameters in model outputs and their subsequent estimation are fundamental in developing a reliable predictive model. In this sense, global sensitivity analysis plays an important role in appropriately ranking input parameters, allowing the reduction of the number of parameters to be estimated through experimental data.

$$r_{PTS} = \frac{r_{PTS}^{\max} c_{glc}^{extracellu\ lar} \dfrac{c_{pep}}{c_{pyr}}}{\left(K_{PTS,a1} + K_{PTS,a2}\dfrac{c_{pep}}{c_{pyr}} + K_{PTS,a3}c_{glc}^{extracellu\ lar} + c_{glc}^{extracellu\ lar}\dfrac{c_{pep}}{c_{pyr}}\right)\left(1 + \dfrac{c_{g6p}^{nPTS,g6p}}{K_{PTS,g6p}}\right)} \tag{26}$$

$$r_{PDH} = \frac{r_{PDH}^{\max} c_{pyr}^{nPDH}}{K_{PDH,pyr} + c_{pyr}^{nPDH}} \tag{27}$$

$$r_{G6PDH} = \frac{r_{G6PDH}^{\max} c_{G6PDH} c_{nadp}}{\left(c_{g6p} + K_{G6PDH,g6p}\right)\left(1 + \frac{c_{nadph}}{K_{G6PDH,nadph,g6pinh}}\right)\left(K_{G6PDH,nadp}\left(1 + \frac{c_{nadph}}{K_{G6PDH,nadph,g6pinh}}\right) + c_{nadp}\right)} \tag{28}$$

$$r_{PGDH} = \frac{r_{PGDH}^{\max} c_{6pg} c_{nadp}}{\left(c_{6pg} + K_{PGDH,6pg}\right)\left(c_{nadp} + K_{G6PDH,nadp}\left(1 + \frac{c_{nadph}}{K_{PGDH,nadph,inh}}\right)\left(1 + \frac{c_{ATP}}{K_{PGDH,ATP,g6pinh}}\right)\right)} \tag{29}$$

3 GLOBAL SENSITIVITY ANALYSIS

Sensitivity analysis methods can be classified into two categories: local and global ones. Local techniques study the behavior of model outputs when input parameters vary one at a time, keeping the remaining ones constant at their nominal value. These methods are based on a Taylor series expansion around the nominal trajectories. The assumption of linearity is valid only within a narrow range of variation of the parameter, around its nominal value. Therefore, when the whole range of variation of the model parameters are considered and for nonlinear models, results obtained with local sensitivity analysis are not representative (Pastres et. al. 2004). Local methods compute sensitivity indices as the first partial derivative of the outputs respect to the parameter of interest and the advantage of these techniques is that estimation of the indices has a low computational cost.

On the other hand, global sensitivity analysis is based on exploring the total range of variation of model parameters, sampling from the distribution function associated to each input parameter and on performing repeated simulations of the model taking into account the sampled values of input parameters. These methods have a higher computational cost than local techniques, but they provide more realistic results, since parameter interactions can be identified. Besides global methods do not require the assumption of linearity or additivity of the model, so they are model-independent methods. There are many techniques for global sensitivity analysis, such as Morris method, Fourier Amplitude Sensitivity Test (FAST), Sobol' method and others.

3.1 Variance-based methods: Sobol's method

Given a function Y= *f*(X), where Y is a model output and X is a vector of *k* model input parameters, this function may be decomposed into terms of increasingly dimensionality (Sobol', 2001):

$$f = f_0 + \sum_i f_i(x_i) + \sum_i \sum_{j>i} f_{ij}(x_i, x_j) + \ldots + f_{12\ldots k}(x_i, x_j, \ldots, x_k) \tag{30}$$

Equation (30) is called ANOVA representation of the function Y = *f*(X) if

$$\int f_{i_1 \ldots i_s}(x_{i_1} \ldots x_{i_s}) dx_n = 0 \qquad \text{for} \qquad n = i_1 \ldots i_s \tag{31}$$

From (31), the summands in equation (30) are orthogonal and can be expressed as integrals of f(X).

Suppose, f(X) is squared integrable in its domain of existence, then squaring (30) and integrating, the following relation is obtained

$$\int f^2(x) dx - f_0 = \sum_{s=1}^{k} \sum_{i_1 < \ldots < i_s}^{k} f^2_{i_1 \ldots i_s} dx_{i_1} \ldots dx_{i_s}$$

where

$$V(Y) = \int f^2(x) dx - f_0^2 \qquad V_{i_1 \ldots i_s} = \int f^2_{i_1 \ldots i_s} dx_{i_1} \ldots dx_{i_s}$$

the constants $V(Y)$ and $V_{i_1 \ldots i_s}$ are called unconditional and conditional variance respectively.

The unconditional variance can be written as the variance-decomposition scheme, as proposed by Sobol' (Sobol' 1990), as follows:

$$V(Y) = \sum_i V_i + \sum_i \sum_{j>i} V_{ij} + \ldots + V_{12 \ldots k} \tag{32}$$

Equations (30) and (32) are unique if the input parameters are orthogonal and the summands in (30) are square integrable in the domain of existence.

Despite the input factors are orthogonal or not, the unconditional variance can be decomposed as

$$V(Y) = V\left(E\left(Y|x_i\right)\right) + E\left(V\left(Y|x_i\right)\right) \tag{33}$$

$$V(Y) = V\left(E\left(Y|x_{-i}\right)\right) + E\left(V\left(Y|x_{-i}\right)\right) \tag{34}$$

where

- $V\left(E\left(Y|x_i\right)\right) = V_i$ computes the effect on the model output when all parameters except x_i vary and it is called the first-order effect of the parameter x_i.
- $V\left(E\left(Y|x_{-i}\right)\right) = V_{-i}$ compute the effect on the model output when x_i varies and the other parameters are kept constant.
- $E\left(V\left(Y|x_{-i}\right)\right) = V_i^{TOT}$ compute the effect on the model output when all parameters except x_i are fixed, i.e., it takes into account all the terms in equation (32) that include x_i, for the case of orthogonal input factors.

If equations (33) and (34) are divided by the unconditional variance, the following expressions are obtained

$$1 = \frac{V\left(E\left(Y|x_i\right)\right)}{V(Y)} + \frac{E\left(V\left(Y|x_i\right)\right)}{V(Y)} \tag{35}$$

$$1 = \frac{V\left(E\left(Y|x_{-i}\right)\right)}{V\left(Y\right)} + \frac{E\left(V\left(Y|x_{-i}\right)\right)}{V\left(Y\right)} \tag{36}$$

The first-order sensitivity index, S_i is defined as follows:

$$S_i = \frac{V\left(E\left(Y|x_i\right)\right)}{V\left(Y\right)} = \frac{V_i}{V(Y)} \tag{37}$$

S_i gives the reduction on the unconditional variance that is possible to obtain if x_i can be fixed. Sobol's (2001) has proposed a methodology to compute sensitivity indices, based on Monte Carlo simulations, with a minimum amount of function evaluation. Main steps are as follows:

1. Two different random sets of model parameters are generated: $\xi = (\eta, \zeta)$ and $\xi' = (\eta', \zeta')$, which could be called sample and re-sample matrices respectively. Each matrix has dimensions $N \times k$, where N is the sample size for the Monte Carlo method and k is the number of parameters. In the previous nomenclature η is a vector of dimensions $N \times 1$, which contains the N random values of the parameter x_i whose sensitivity indeces want to be calculated and ζ is a matrix of dimensions $N \times (k-1)$ and contains the random values of the $k-1$ remaining input parameters.
2. Two new matrices are generated combining ξ and ξ', which are required for the computation of the variances, as follows (f_0 stands for $E(Y|x_i)$):

$$\frac{1}{N}\sum_{i=1}^{N} f(\xi_i) \xrightarrow{P} f_0 \tag{38}$$

$$\frac{1}{N}\sum_{i=1}^{N} f^2(\xi_i) \xrightarrow{P} V + f_0^2 \tag{39}$$

$$\frac{1}{N}\sum_{i=1}^{N} f(\xi_i) f(\eta_i, \zeta_i') \xrightarrow{P} V_i + f_0^2 \tag{40}$$

$$\frac{1}{N}\sum_{i=1}^{N} f(\xi_i) f(\eta_i', \zeta_i) \xrightarrow{P} V_{-i} + f_0^2 \tag{41}$$

3. Sensitivity indices are subsequently calculated by the corresponding definitions given above.

4 DISCUSSION OF RESULTS

We have implemented a large scale metabolic network model consisting of eighteen differential equations and thirty seven algebraic ones that stand for kinetic expressions and co-metabolite concentrations, in g-PROMS (PSE Enterprise, 2007), in which the differential algebraic system of equations is solved with DASSL (Brenan et al., 1996). In this environment, two different sets of random parameters, ξ and ξ', have been generated for

k=twenty parameters, with sample size of *N*=2500 scenarios. Normal distribution has been assumed for each parameter and their mean values have been taken from the literature (Chassagnole et al., 2002), with a 10% standard deviation. Table 1 shows uncertain input parameters and their mean values.

Table 1. Uncertain input factors and their distribution parameters

Enzyme	Parameter	Nominal value
PTS (Phosphotranferase system)	$K_{PTS,1}$	3082.3 mM
	$K_{PTS,g6p}$	2.15 mM
	$N_{PTS,g6p}$	3.66
	$r_{PTS,max}$	389696.028 mM/sec
PGI (Glucose-6-phosphate isomerase)	$K_{PGI,eq}$	0.1725
PFK (Phosphofructokinase)	$K_{PFK,f6ps}$	0.325 mM
	N_{PFK}	11.1
	$r_{PFK,max}$	0.406 mM/sec
GAPDH (Glyceraldehyde-3-phosphate dehydrogenase)	$K_{GAPDH,gap}$	0.683 mM
	$K_{GAPDH,pgp}$	0.0000104 mM
	$r_{GAPDH,max}$	705.917 mM/sec
PGK (Phosphoglycerate kinase)	$K_{PGK,eq}$	1934.4
PGluMu (Phosphoglycerate mutase)	$K_{PGluMu,eq}$	0.188
ENO (Enolase)	$K_{ENO,eq}$	6.73
PDH (Pyruvate dehydrogenase)	N_{PDH}	3.68
	$r_{PDH,max}$	4.596 mM/sec
PEPCxylase (PEP carboxylase)	$K_{PepCxylase,fdp}$	0.7 mM
G6PDH (Glucose-6-phosphate dehydrogenase)	$r_{G6PDH,max}$	1.063 mM/sec
PGDH (6-phosphogluconate dehydrogenase)	$K_{PGDH,6pg}$	37.5 mM
	$r_{PGDH,max}$	12.491 mM/sec

We have performed the *N(2k+1)* Monte Carlo simulations in g-Proms and temporal profiles for the eighteen state variables have been exported for subsequent conditional and unconditional variances calculation according to equations 38 to 41. Temporal profiles of S_i were calculated within a Fortran 90 environment using the calculated variances profiles, with equation (37).

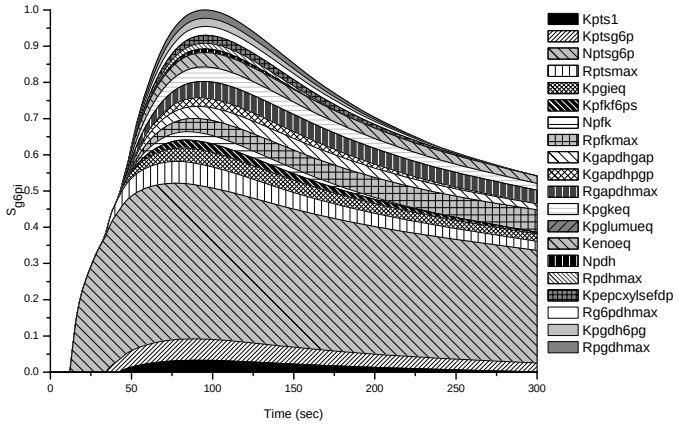

Figure 1. Profiles of S_i for glucose-6-phosphate concentration.

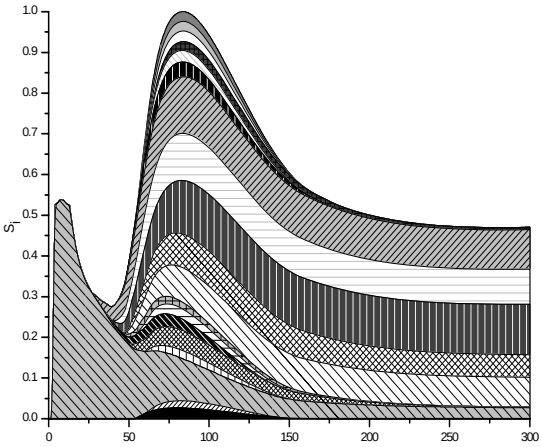

Figure 2. Profiles of S_i for phosphoenolpyruvate concentration.

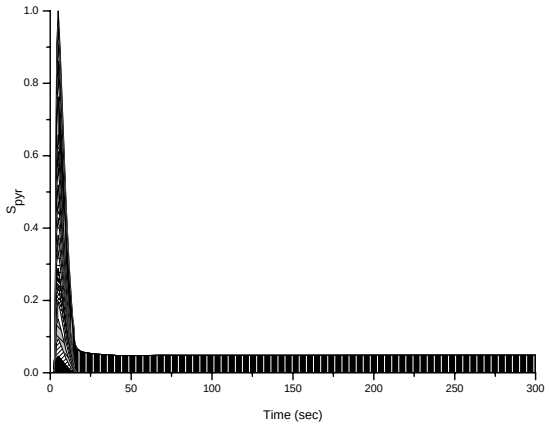

Figure 3. Profiles of S_i for pyruvate concentration.

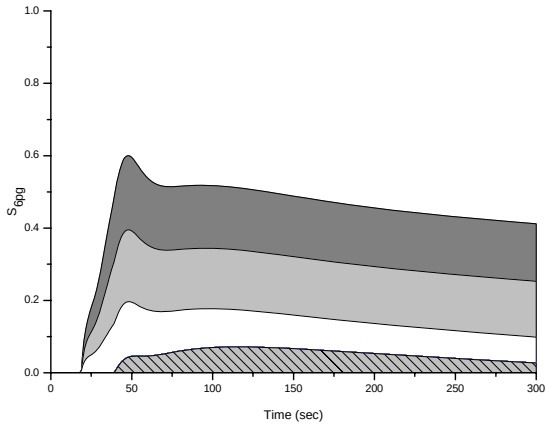

Figure 4. Profiles of S_i for 6-phosphogluconate concentration.

Figures 1 to 4 show profiles for sensitivity indices for glucose-6-phosphate (g6p), pyruvate (pyr), phosphoenolpyruvate (pep) and 6-phosphogluconate (6pg), respectively. Figure 3, shows that most of the parameters affect the concentration of pyruvate in the first twenty seconds, and then Npdh (the exponent for pyruvate concentration in the r_{PDH} kinetic expression) is the only parameter which produces variations in this model output. In the case of the concentration of 6pg, this model output is sensitive to only four parameters, Nptsg6p, RG6PDHmax, KPGDH6pg and RPGDHmax, which are involved in the kinetic expressions for phosphotransferase system (r_{PTS}), the glucose-6-phosphate dehydrogenase (r_{G6PDH}) and 6-phosphogluconate dehidrogenase (r_{PGDH}), as it can be seen in Figure 4.

5 CONCLUSIONS

Global sensitivity analysis has been performed to a large-scale metabolic network model**,** which comprises a differential algebraic system of equations. To our knowledge, this is the first time a large-scale DAE representing biological systems is studied through this methodology. It has allowed both a reduction of the input parameter set and a ranking of most influential ones to pave the way to formulation and solution to the dynamic parameter

estimation problem for the main parameters ranked in this study.

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