Multiplicity and Global Parametric Sensitivity of Structured Biological Models

A. Tušek, Ž. Kurtanjek

University of Zagreb, Faculty of Food Technology and Biotechnology Pierottijeva 6, 10000 Zagreb, CROATIA atusek@pbf.hr zkurt@pbf.hr

Abstract. For a paradigmatic biologically structured model multiplicity and global parameter sensitivity of a chemostat are investigated. The modeling assumptions are bisubstrate growth limitation by carbon and nitrogen sources, presence of two essential intracellular compartments, active protein synthesis system (PSS) and inactive genetic compartment. Variance based global sensitivity of the kinetic parameters is evaluated by the Extended Fourier Amplitude Sensitivity Test (FAST).

Computer simulations reveal that multiplicity can be induced by carbon source product synthesis inhibition. Results of the FAST analysis show dominant sensitivity on the maximum specific rate coefficients for assimilation of carbon and synthesis of PSS compartment.

Keywords. structured chemostat, PSS, multiplicity, FAST sensitivity

1. Introduction

Biologically structured models are essential for engineering applications in process control, optimization and reactor scale-up. Structured models based on genomic data have a very high dimension (several hundreds or thousands variables) and are difficult for estimation of kinetic parameters needed for a process control. The problem could be resolved by a "reductionism" approach by which the most essential biological processes are "lumped" into individual "synthetic" variables. This approach has been introduced in theoretical biology [6] and in engineering is associated with modeling methodology of biological compartments. The concept of biological compartments has led to a distinction between dynamic regimes at experimental (bioreactor) level, and dynamics at a cell level (intracellular processes). It enables modeling of cell adaptation to external perturbations, such as appearance of "lag phase", modeling of exponential and stationary phases, and eventually modeling

of a cell apoptosis. From applicative engineering view, such models are highly applicable for the main tasks in bioreactor design, process control and scale-up covering wide range of bioreactor configurations and mode of operations [1-2].

This work is focused on investigation if the paradigmatic structured models are able to predict appearance of steady state multiplicity in a chemostat, and consequently bifurcation to a limit cycle behavior. Also, investigated is sensitivity of the chemostat steady state to the main kinetic parameters by the Fourier Amplitude Sensitivity Test method.

2. Model

Investigated are properties of the simplest biologically structured model which take in account the main engineering aspects. The model is derived from the assumption of the two rate limiting substrate assimilation reactions, from external sources of carbon (c) to intracellular (C) and external nitrogen n to intracellular (N, protein):

$$\begin{array}{c} c \to C\\ n \to N \end{array} \tag{1}$$

The intracellular state is approximated by two internal dynamic variables (compartments), an active catalytic compartment, named protein synthesis system (PSS) and an inactive genetic (G) compartment containing DNA and storage substances [1-2]. Production of an industrially important metabolite is included into the model as a reaction in which intracellular carbon (C) substrate is transformed into intracellular product (P). The corresponding stoichiometric reactions are: intracellular reaction for product formation, bisupstrate reaction for PSS synthesis, and transformation of PSS into the inactive G compartment:

$$C \to \alpha_P \cdot P$$

$$\alpha_S \cdot C + N \to \alpha_{XA} \cdot PSS \qquad (2)$$

$$PSS \to \alpha_{XG} \cdot G$$

Produced metabolite is excreted from a cell by diffusion or on active enzymatic reaction step:

$$P \to p$$
 (3)

The model equations are derived from the assumption on bioreactor configuration and a mode of operation. Here is assumed perfectly mixed flow through reactor with constant feed composition (chemostat). At a steady state physical processes of transport rates through a reactor and the rates due to activity of a cell population are balanced. A simple concept of biomass (assuming a population of identical cells) is applied and is accounted by a concentration (x) of biomass dry matter per volume of a reactor. The extracellular carbon source balance is given by:

$$D \cdot (c_f - c) = (r_1 + \alpha_s \cdot r_2) \cdot PSS \cdot x \qquad (4)$$

where a flow rate per reactor volume, termed dilution rate (D), is the key physical (controllable) parameter. The balance of carbon substrate is accounted by two rates, product synthesis (r_1) and the substrate assimilation (r_2). The balance of the co-substrate does not involve in a product formation and is only accounted by the assimilation reaction. Hence, extracellular nitrogen source balance is:

$$D \cdot (n_f - n) = r_2 \cdot PSS \cdot x \tag{5}$$

Extracellular biomass balance accounts for cell loss by flow through and cell growth:

$$D \cdot x = \mu \cdot x = [\alpha_{XA} \cdot r_2 - (1 - \alpha_{XG}) \cdot r_3] \cdot PSS \cdot x$$
(6)

The trivial "wash out" solution is excluded from analysis. The intracellular protein, PSS compartment balance, involves cell growth and build up of the genetic compartment:

$$\alpha_{XA} \cdot r_2 = r_3 + \mu \tag{7}$$

The most intricate part of the modeling involves kinetic parameters. Use of Michaels-Menten ki-

netics is well theoretically founded and generally accepted, but values of the kinetic parameters, in principle, must be estimated from experimental data. Uncertainty of the kinetic parameters is the main obstacle in a model validation [4-5]. In this work are considered the following kinetic parameters: for the specific rate of carbon assimilation:

$$r_1 = k_1 \cdot \frac{c}{K_1 + c + \left(\frac{c}{K_1}\right)^2}$$
(8)

for the specific rate of protein (nitrogen) assimilation:

$$r_2 = k_2 \cdot \frac{c}{K_2 + c} \cdot \frac{n}{K_N + n} \tag{9}$$

and for the specific rate of protein system synthesis (PSS):

$$r_3 = k_3 \cdot \frac{c}{K_3 + c} \cdot \frac{n}{K_N + n} \tag{10}$$

Assumed values of the parameters are given in Table 1. These parameters are adapted from [1-2] which are estimated from numerous experiments in chemostat and fed-batch reactors.

Table 1. Parameters of the structured chemostat model.

Model parameters		
$k_1 = 5.23$	$k_2 = 1.71$	$k_3 = 1.28$
$K_1 = 10^{-2}$	$K_2 = 3.19 \ 10^{-2}$	$K_3 = 2.56 \ 10^{-2}$
$K_{\rm N} = 0.21$	$c_u=10$	$n_f = 1.4$
Stoichiometric parameters		
$\alpha_{XA}=1.08$	$\alpha_{\rm XG} = 0.97$	$\alpha_{\rm S} = 0.14$

Importance of individual parameters is analyzed by general parameter sensitivity theory based on variance contribution [3-5]. Parameters are sampled from assumed range of values and uniform probability density function. The values are sampled along piece wise linear functions defined by:

$$k_i = \frac{1}{2} + \frac{1}{\pi} \cdot \arcsin(\sin(\omega_i \cdot s + \varphi_i)) \quad (11)$$

where s is a sampling parameter in the range of [-1,1], and ω_i are selected frequencies, and φ_i are randomly selected phase angels. Results (chemostat steady states) of the output function are evaluated for each drawn sample and are decomposed into Fourier series with the coefficients:

$$A_{\omega} = \frac{1}{2 \cdot \pi} \cdot \int_{-\pi}^{\pi} f(s) \cdot \cos(\omega \cdot s) \cdot ds \qquad (12)$$

$$B_{\omega} = \frac{1}{2 \cdot \pi} \cdot \int_{-\pi}^{\pi} f(s) \cdot \sin(\omega \cdot s) \cdot ds \qquad (13)$$

The total dispersion D_{T} is determined from the Fourier coefficients

$$D_{T} = 2 \cdot \sum_{\omega=1}^{\infty} \left(A_{\omega}^{2} + B_{\omega}^{2} \right)$$
(14)

And individual parameter contribution in the total dispersion is calculated by the corresponding parameter harmonics.

$$D_{i} = 2 \cdot \sum_{\omega=k:\omega_{i}}^{\infty} \left(A_{\omega}^{2} + B_{\omega}^{2}\right)$$
(15)

The first and second order sensitivity coefficients are calculated by:

$$S_{i} = \frac{D_{i}}{D_{T}}$$
 $S_{i}^{tot} = 1 - \frac{D_{-i}}{D_{T}}$ (16)

3. Results

Firstly, the steady state balances (4-7) are transformed into a single polynomial equation of the fifth order. For the symbolic and numerical



Figure 1. Carbon source and biomass concentration profiles versus dilution rates at steady states in a chemostat.

computation Wolfram Research "*Mathematica*" is applied [7]. The solutions of the polynomial equation are scanned by the variable dilution rate in the range from 0.01 - 0.45 h⁻¹. The obtained steady state profiles of the substrate concentrations, biomass and the protein synthesis PSS compartment are depicted in Fig. 1-2.



Figure 2. Nitrogen source concentration and intracellular protein synthesis system compartment fraction profiles versus dilution rates at steady states in a chemostat.



Figure 3. Multiplicity of steady states (•) induced by the carbon source inhibition.

The multiple steady state solutions appear when substrate inhibition of carbon assimilation is introduced. The evaluation of multiple steady states is based on the balance (6) where the rates involved in the specific growth rate model are expressed as a function of a carbon concentration as a single variable. The multiple solutions are found graphically by intersection of the constant dilution rate and the nonlinear growth function. For example, when the inhibition constant $K_i = 2$ is introduced, a case with three steady states appears, besides the fourth trivial wash-out state, as depicted in Fig. 3.

Since in this model kinetic parameters can not be derived from individual enzyme kinetic data, their values are determined from experimental data [1-2]. There is a large uncertainty of their true values (broad confidence interval). In order to reveal which of the parameters are most significant for the model validation, evaluated are global sensitivities of the variance between model predictions and experimental data. Applied is Extended Fourier Amplitude Sensitivity Test method. Seven parameters are simultaneously sampled from the interval of values in the range of one order of magnitude around the estimate with a uniform probability density function. Samples are generated by the transformation given by (11) and for each sample is calculated a variance. Total number of evaluated samples is 2000 with uniformly distributed sampling parameter s in the range [-1,1].



Figure 4. First order global sensitivities of the model variance on the kinetic parameters.



Figure 5. Second order global sensitivities of the model variance on the kinetic parameters.

The calculated set of discrete values of the variance is interpolated (zero order) to obtain a continuous function f(s). The Fourier expansion coefficients are determined by numerical integration provided by the adaptive method in Wolfram Research "Mathematica" [7]. The maximum frequency in the expansion series is set to $\omega_{\rm max} = 100$ which is taken as a sufficient when compared to the maximum sampling frequency $\omega_7 = 17$ (Shannon theorem). Contribution of each kinetic parameter in the total dispersion is determined by (15-16). Results are presented as first and second order sensitivity coefficients, Fig. 4-5. The first order sensitivities determine contribution of each parameter in the total variance, while the second order coefficients reflect the contributions due to variations of the parameter interactions. The results show dominant effects of $k_1 = k_{10}$, $k_3 = k_{20}$ and $k_4 = K_{20}$. These parameters are: k_1 is maximum specific rate of carbon utilization in the product formation; and k_3 and k_4 are the maximum rate and saturation constant for a carbon source in protein system synthesis (PSS).

4. Discussion

Biologically structured models involve numerous species, reactions and extensive set of model parameters. Kinetic parameters are essential in modeling of process dynamics and control. Results of this investigation show the main features of a simple and paradigmatic two compartment biomass model with the catalytically active protein synthesis and inactive genetic compartment. Steady state analysis is revealed based on root location of a 5-th order polynomial in carbon source concentration. The results given in Fig. 1-2. indicate that for small dilution rates there is a region of limited protein synthesis compartment, followed by a region of bisupstrate limitations for carbon and nitrogen sources, and for high dilution rates there is lack of substrate limitation and maximum PSS compartment is achieved (optimal growth rate conditions).

The key modeling variable is protein synthesis compartment which in simple terms reflects metabolic activity of a cell, and enables prediction of metabolism adaptation to changes in environment, and/or bioreactor configuration.

Introduction of a substrate inhibition in a product synthesis results in appearance of multiple steady states, Fig. 3. Based on simple graphi-

cal interpretation can be concluded that the first and third steady state are locally stable, while the second intermediate state is an unstable state and can be surrounded by a limit cycle. This has an important implication in prediction of complex dynamical behavior, for example such as sustained biological oscillations.

The presented results of the general sensitivity analysis, Fig. 4-5. clearly indicate dominant influence of the parameters associated with carbon source limitation in protein, PSS compartment, and a product synthesis. These results could be applied for a selection of better experimental conditions and methods by which more accurate estimation of the key parameter is possible. At the same time, this also implies which are the key factors to be improved for a better process control.

5. Conclusion

Biologically structured models are essential for modeling of microbial cell adaptation to changing environmental conditions, specifically to available substrates for cell growth and metabolite production. These are important features in process control, especially in cases of genetically modified microorganisms. Results of this work show how adaptation of cell metabolism can be modeled by change in size of protein synthesis compartment in the wide range of substrate compositions determined by dilution rate in a chemostat.

Important features of multiple steady state and existence of a necessary condition for appearance of a limit cycle is shown.

The key modeling difficulty is associated with uncertainty in model parameter predictions, and can be effectively approached by global sensitivity analysis and application of numerically effective Extended Fourier Amplitude Sensitivity Test method.

Presented results confirm the expected conclusion that parameters associated with a carbon source limitation in protein synthesis and product formation are the key model parameters.

These conclusions on parameter sensitivity can be also extended to analysis of sensitivity of input variables by which a better process control can be achieved.

6. References

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