Mathematica
Balkanica

New Series Vol.25, 2011, Fasc. 4

Sensitivity Analysis of *E. coli* Fed-batch Cultivation Local Models [1]

Olympia N. Roeva

Presented by P. Boyvalenkov

In this paper the sensitivity analysis of *E. coli* fed-batch cultivation process model is studied. Non-linear mathematical model includes a system of five ordinary differential equations to model state variables: biomass, glucose, acetate, dissolved oxygen, as well as the bioreactor volume. Various local models structures for specific rate functions are examined. Sensitivity analysis of the parameters with respect to the state variables is performed. Based on an experimental data set the sensitivity analysis has allowed drawing conclusions about which parameters will be most easily estimated. As a result a by-stage parameter identification procedure is proposed. The identification procedure is tested for model identification of an *E. coli BL21(DE3)pPhyt109* fed-batch cultivation process. The proposed identification procedure leads to easy and accurate estimation of local models parameters. The procedure effectiveness is confirmed with a model verification.

1. Introduction

Cultivation processes are characterized by a complicated structure of organization and independent characteristics, which determines their non-linear and non-stationary nature. Model formulation for these processes is traditionally performed under conditions of a well-defined medium with single-substrate limitations, conditions that are not applied to most industrial cultivations, typically running in a complex medium. On the other hand, the globally valid unstructured numeric models cannot be used in on-line monitoring and control, either because they do not reflect metabolic changes or contain too many poorly

[1]Partially supported by the National Science Fund Grants No DMU 02/4.

known parameters [3, 18, 29]. Model predictions could be improved using structured models, but these models incorporate too many equations and unknown parameters and provide a qualitative, rather than quantitative description of the process. The structured models are usually so complicated that it is difficult to use them for industrial scale production. Another alternative is model construction using functional state concept [24, 28, 29]. Based on this concept the process is divided into macrostates, called functional states (FS), according to certain metabolic pathways that dominate the overall process behaviour. In each FS the process is described by a conventional type of model, called a local model, which is valid only in this FS. Thus, more precise mathematical description is achieved and time-variation of the process parameters is taken into account.

Once, the functional state local models are defined the next essential step for the successful model development is the parameter identification. The choice of a certain optimization procedure is not a trivial task. The model parameter identification is a big challenge for the traditional optimization methods. Although various meta-heuristics are used as an alternative to surmount the parameter identification difficulties the problem with simultaneously estimating a large number of parameters connected with highly non-linear process dynamics is of present interest.

Sensitivity analysis could give some transperancy to the parameter influence on the process behaviour and could contribute to more precise parameter identification. Sensitivity analysis is a valuable tool for investigating the practical identifiability of the model parameters [2, 7, 20, 27]. A work fully dedicated to the identification of biological models, concerning wastewater treatment is presented in [11]. Sensitivity analysis can be included in parameter estimation procedures [14, 19, 26], as well as for model reduction [2, 19, 20]. Sensitivity analysis has found application also in [4-6, 9-10, 15-17]. Knowledge of sensitivity may also help to identify the driving mechanisms of a process without having to fully understand the detailed mechanistic interconnections in a complex system.

The present study focuses on the model parameters sensitivity analysis to elucidate a parameter's behaviour for use in a model identification procedure. A set of local models based on the functional state approach is examined. As a case study a fed-batch cultivation of bacteria *E. coli BL21(DE3)pPhyt109* for bacterial phytase extracellular production is considered. *E. coli* is still the most important host organism for recombinant protein production. Cultivation of recombinant micro-organisms e.g. *E. coli*, in many cases is the only economical way to produce pharmaceutical biochemicals such as interleukins, insulin, interferons, enzymes and growth factors.

2. Local models for *E. coli BL21(DE3)pPhyt109* fed-batch cultivation process

Phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8 for 3-phytase and 3.1.8.26 for 6-phytase) has become an important feed additive in the nutrition of monogastric animals since it is able to enhance plant phosphorus utilization and to eliminate the negative effects of phytic acid. For all organisms screened for the enzyme so far the phytase from *E. coli* had the highest specific activity - eight times more than the commercially used Aspergillus niger phytase [25].

The mathematical description of the *E. coli BL21(DE3)pPhyt109* is commonly presented according to the mass balance as follows [21-22, 28]:

$$\frac{dX}{dt} = \mu X - \frac{F}{V}X \tag{0.1}$$

$$\frac{dS}{dt} = -q_S X + \frac{F}{V}(S_{in} - S) \tag{0.2}$$

$$\frac{dA}{dt} = q_A X - \frac{F}{V}A \tag{0.3}$$

$$\frac{dPh}{dt} = q_{Ph} X - \frac{F}{V}Ph \tag{0.4}$$

$$\frac{dpO_2}{dt} = -q_{pO_2} X + k_L a(pO_2^* - pO_2) - \frac{F}{V}pO_2 \tag{0.5}$$

$$\frac{dV}{dt} = F \tag{0.6}$$

where: X is the concentration of biomass, g/l; S - concentration of substrate (glucose), g/l; A - concentration of product acetate, g/l; Ph - concentration of product phytase, g/l; pO_2 - concentration of dissolved oxygen, %; F - feeding rate, 1/h; V - bioreactor volume, l; S_{in} - concentration of substrate in the feeding solution, g/l; pO_2^* - saturation concentration of dissolved oxygen, %; $k_L a$ - volumetric oxygen transfer coefficient, 1/h. Local models structures and parameters of specific rate functions q_S, q_A, q_{Ph} and q_{pO_2} vary for different FS [22, 28-29].

There is a strong intuitive appeal in building systems which operate robustly over a wide range of operating conditions by decomposing them into a number of simpler modelling or control problems. The concept of functional state modelling is already applied for description of several fed-batch cultivations of *E. coli* [22, 24, 28]. In each FS a simple local model is use and the considered local models are then combined in a way to yield a global model.

Table 1: Kinetics equations, used for description of specific growth rate μ

No	μ	$q_S = \frac{1}{Y_{S/X}}\mu$	$q_A = \frac{1}{Y_{A/X}}\mu$	$q_{Ph} = \frac{1}{Y_{Ph/X}}\mu$	$q_{pO_2} = \frac{1}{Y_{pO_2/X}}\mu$
1	$\mu_{\max}\frac{S}{k_S+S}$				
2	$\mu_{\max}\frac{S}{kX+S}$				
3	$\mu_{\max}\frac{1}{k_S+S+\frac{S^2}{k_i}}$				
4	$\mu_{\max}\frac{S}{k_S+\frac{S}{X}}$				
5	$\mu_{\max}\frac{S}{k_S+S}$		$\mu_{\max}\frac{A}{k_A+A}$	$\mu_{\max}\frac{Ph}{k_{Ph}+Ph}$	$\mu_{\max}\frac{pO_2}{k_{pO_2}+pO_2}$
6			$\mu_{\max A}\frac{A}{k_A+A}$	$\mu_{\max}\frac{S}{k_S+S}$	
7			$\mu_{\max}\frac{A}{k_A+A}$		$\mu_{\max pO_2}\frac{pO_2}{k_{pO_2}+pO_2}$
8	$\mu_{\max}\frac{S}{k_S+S}$			$\mu_{\max}\frac{Ph}{k_{Ph}+Ph}$	$\mu_{\max}\frac{pO_2}{k_{pO_2}+pO_2}$
9	$\mu_{\max}\frac{S}{k_S+\frac{S}{X}}$				
10	$\mu_{\max}\frac{S}{k_S+S}$		$\mu_{\max A}\frac{A}{k_A+A}\frac{S}{k_S+S}$	$\mu_{\max}\frac{S}{k_S+S}$	

The following assumptions are made in the local models' development:

– The bioreactor is completely mixed.

– The suspension viscosity in the reactor remains constant during the experiment.

– Variations in the growth rate and in substrate consumption do not significantly change the elemental composition of biomass, thus balanced growth conditions are only assumed.

– Parameters, e.g. pH and temperature, are controlled to certain constant values.

The local models' structures, discussed here, are proposed in [21]. The kinetics equations, used for description of specific rates of cell growth, substrate consumption, acetate and phytase production and dissolved oxygen utilization are listed in Table 1. Kinetic equations are selected to be able to describe the metabolic specificity in the different recognized FS during the considered cultivation process.

In Model 1 for description of main state variables Monod kinetics is used. In Model 2 Contois kinetics is considered. In the case of growth inhibition at high substrate concentration the Andrews growth kinetics is proposed (Model 3). In Model 4 for description of the specific rates Fujimoto kinetics is applied. Models 5-10 are defined as a combination of different kinetics based on results obtained from the investigations carried on the first four models [21]. In contrast to Model 1, in Model 5, it is assumed that variation of acetate production rate, phytase production rate and dissolved oxygen consumption rate follow Monod

kinetics according to the respective variable - acetate, phytase and dissolved oxygen. Acetate has a critical role as it functions as both a product and a reactant. In the presence of glucose, *E. coli* can utilize acetate [8]. Due to this fact, in Models 5-7 a saturation constant k_A that depends only on acetate concentration is proposed [13, 21]. Moreover, in the description of the dissolved oxygen consumption rate a saturation constant k_{pO_2} is applied for Models 5-10. In Model 6, a description of acetate concentration in accordance with Monod kinetics is proposed. Here specific bacteria growth rate $\mu_{\max_A}$ is introduced. Phytase production rate proportional to cell growth rate is accepted in Models 5, 6, 9 and 10. In Model 7, the accent is on the description of dissolved oxygen dynamics. Here specific bacterium growth rate $\mu_{\max_{pO_2}}$, derived from dissolved oxygen dynamics, is introduced [8]. Model 8 is a simplified version of Models 5-7. Here only the accent on phytase production rate and on dissolved oxygen consumption rate is kept. The rest specific rates (μ, q_S and q_A) are described with Monod kinetics. In Model 9 the specific rates μ, q_S, q_A and q_{Ph} are described by Fujimoto kinetics. In Model 10 an acetate production rate that depends on both acetate and substrate concentrations is introduced. For the proposed Models 1-10 parameters sensitivity analysis is performed.

3. Local models' sensitivity analysis

Sensitivity analysis is an important tool when analyzing model characteristics. The sensitivity coefficients describe the change in the system's outputs due to variations in the parameters that affect the system dynamics. High sensitivity to a parameter suggests that the system's performance can drastically change with small variations in the parameter. Vice versa, low sensitivity suggests little change in the performance. Sensitivity analysis of *E. coli BL21(DE3)pPhyt109* fed-batch cultivation local models is performed using the sensitivity functions considered in [19, 27]. The mathematical model from (0.1)-(0.6) is presented as:

$$\frac{dx_j}{dt} = f_j\left(x_1, ..., x_m, t, p_1, ...p_n\right) \tag{0.7}$$

According to [6, 8], the sensitivity functions are defined as:

$$s_{ji} = \left.\frac{\partial x_j\left(p,t\right)}{\partial p_i}\right|_{p=p_0}, \quad i = 1 \div n, j = 1 \div m \tag{0.8}$$

where s_{ji} are the sensitivity functions of i-th parameter according j-th variable, x_j - state variables, p_i - model parameters.

Differentiation of the right-hand side (0.7) with respect to p leads to:

$$\frac{\partial f_j(p,t)}{\partial p_i} = \sum_{j=1}^{m} \frac{\partial f_j(x,p,t)}{\partial x_j} \frac{\partial x_j}{\partial p_i} + \frac{\partial f_j(x,p,t)}{\partial p_i} \tag{0.9}$$

and further the derivatives $\frac{\partial x_j}{\partial p_i}$ are obtained:

$$\frac{d}{dt}\frac{\partial x_j}{\partial p_i} = \sum_{j=1}^{m} \frac{\partial f_j}{\partial x_j} \frac{\partial x_j}{\partial p_i} + \frac{\partial f_j}{\partial p_i} \tag{0.10}$$

or

$$\frac{ds_{ji}}{dt} = \sum_{j=1}^{m} \frac{\partial f_j}{\partial x_j} s_{ji}(t,p) + \frac{\partial f_j}{\partial p_i} \tag{0.11}$$

The mathematical model (0.7) and the sensitiyity equations (0.11) together form the sensitivity model of the considered system. For all ten models, the state variable vector is presented as $x = [X\ S\ A\ Ph\ pO_2]$. For example, the model parameters vector p is:

Model 5: $p = [\mu_{\max}\ k_S\ k_A\ k_{Ph}\ k_{pO_2}\ Y_{S/X}\ Y_{A/X}\ Y_{Ph/X}\ Y_{pO_2/X}\ k_La]$;

Model 8: $p = \left[\mu_{\max}\ k_S\ k_{Ph}\ k_{pO_2}\ Y_{S/X}\ Y_{A/X}\ Y_{Ph/X}\ Y_{pO_2/X}\ k_La\right]$;

Model 10: $p = \left[\mu_{\max}\ \mu_{\max_A}\ k_S\ k_A\ k_{pO_2}\ Y_{S/X}\ Y_{A/X}\ Y_{Ph/X}\ Y_{pO_2/X}\ k_La\right]$.

Solving the sensitivity model of the system, the following parameter values are used [1, 12]: $t_0 = 6.69$, $X(t_0) = 1.25$, $S(t_0) = 0.81$, $A(t_0) = 0.03$, $Ph(t_0) = 3.00$, $pO_2(t_0) = 21.08$, $\mu_{\max} = 0.46$, $\mu_{\max_A} = 0.21$, $\mu_{\max_{pO_2}} = 0.35$, $k_S = 0.012$, $k = 0.03$, $k_A = 0.012$, $k_{Ph} = 0.10$, $k_{pO_2} = 0.012$, $Y_{S/X} = 0.49$, $Y_{A/X} = 0.015$, $Y_{Ph/X} = 0.20$, $Y_{pO_2/X} = 0.043$, $k_La = 290.0$.

Based on these parameter values, two parameter groups +15% parameter variation (Group 1) and -15% parameter variation (Group 2), respectively, are formed. For all ten models, both sensitivity models are analytically worked out and the sensitivity functions of all 78 parameters are calculated. For Model 8 the simulated dynamics of X, S, A, Ph and pO_2 is presented on Fig. 1. On Figs. 2-4, some simulation curves from sensitivity analysis of Model 8 are presented. For the rest of the models, the results are similar. Results for the parameter sensitivity, according to the considered state variables X, S, A, Ph and pO_2, for three models (Models 5, 6 and 8), are summarized in Table 2. Due to similarity the sensitivity patterns for the rest of the models are not presented.

The results of the sensitivity analysis of both sensitivity models (Groups 1 and 2) for Models 1-10 could be generalized as follows:

(i) The highest sensitivity is featured by parameter $\mu_{\max}$;

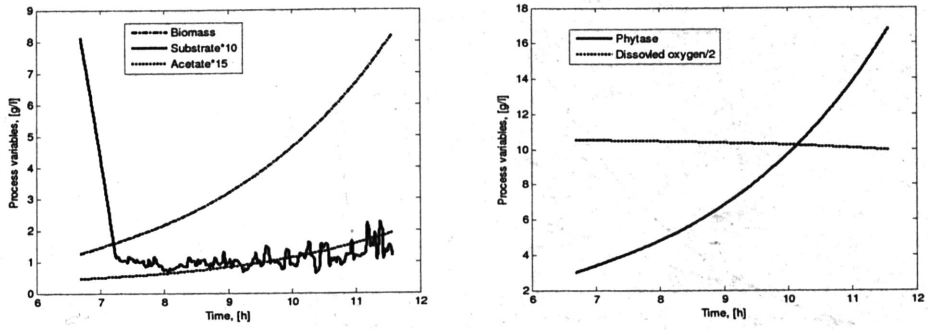

Figure 1: Simulated dynamics of process variables for Model 8

(ii) The following parameters exhibit decreasing degrees of sensitivity in a similar range:

- $Y_{S/X}$ - according to variables biomass and substrate (considering Models 1-10),
- $Y_{A/X}$ (considering Models 1-10) and $\mu_{\max_A}$ (considering Model 6) - according to variable acetate,
- $Y_{Ph/X}$ - according to variable phytase (considering Models 1-10),
- $Y_{pO_2/X}$ (considering Models 1-10) and $\mu_{\max_{pO_2}}$ (considering Model 7) - according to variable dissolved oxygen;

(iii) The influence of the rest of the parameters (k_S, k, k_A, k_{Ph}, k_{pO_2} and k_La) is lower, compared to the parameters sensitivity classified in (i) and (ii).

As a result from the sensitivity analysis a by-stage parameter identification procedure is proposed. The procedure is conformable to parameter sensitivity and global model structure. The parameters with high sensitivity will be estimated on the first step based on data of the variable which is affected most. The parameter division into groups is limited of the global model structure. So, on the **first step** of the identification procedure (concerning Models 1-10) three parameters ($\mu_{\max}$, k_S and $Y_{S/X}$) are estimated. The system (0.1), (0.2) and (0.6) is considered and experimental data set for dynamics of X and S is used.

On the next three steps, consequently the parameters with higher sensitivity according to variables acetate, phytase and dissolved oxygen are identified.

On the **second step**, for acetate variable the following local models parameters are identified:

(i) $Y_{A/X}$ for Models 1-4, 8 and 9;

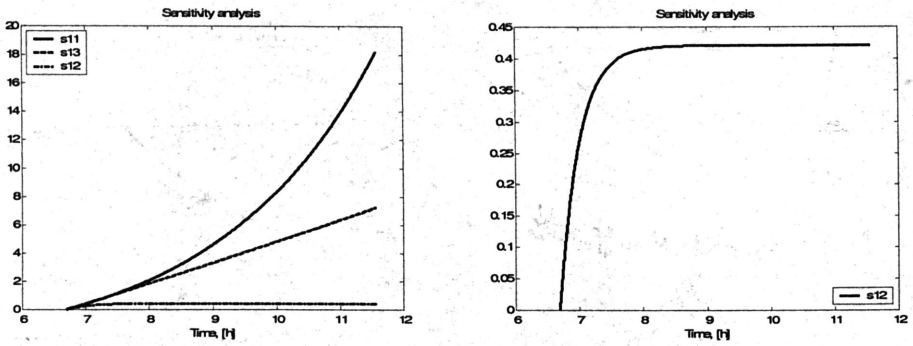

Figure 2: Model 8 sensitivity of Group 1 in relation to variable biomass

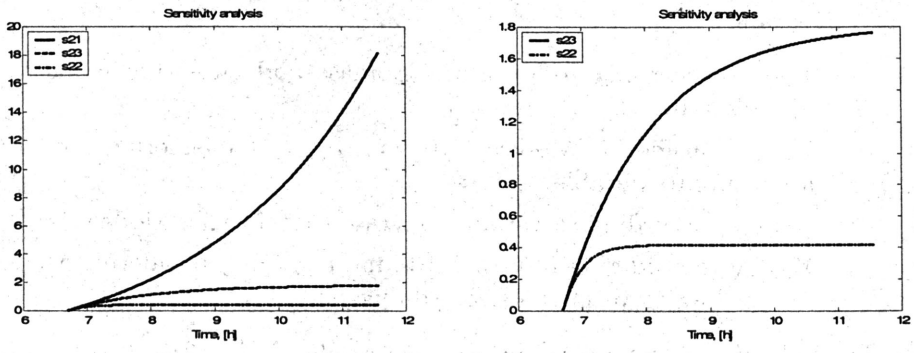

Figure 3: Model 8 sensitivity of Group 1 in relation to variable substrate

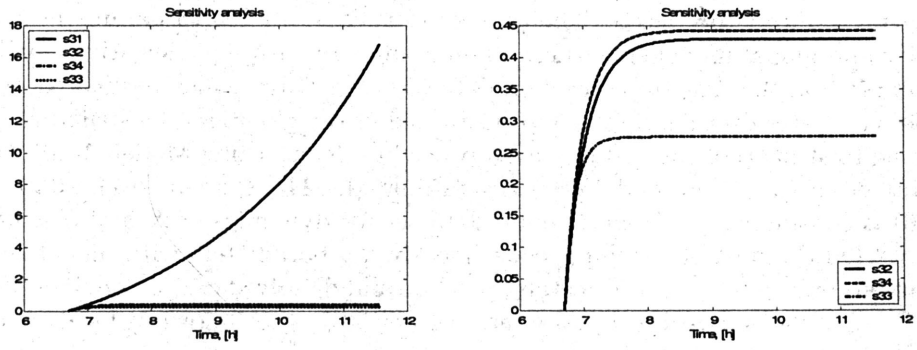

Figure 4: Model 8 sensitivity of Group 1 in relation to variable acetate

Table 2: Parameter sensitivity

Model	State variable	Parameter sensitivity the highest → higher → lower → the lowest
Model 5	X	$\mu_{\max} \rightarrow Y_{S/X} \rightarrow k_S$
	S	$\mu_{\max} \rightarrow Y_{S/X} \rightarrow k_S$
	A	$\mu_{\max} \rightarrow Y_{A/X} \rightarrow k_S,\ k_A \rightarrow Y_{S/X}$
	Ph	$\mu_{\max} \rightarrow Y_{Ph/X} \rightarrow k_S,\ k_{Ph} \rightarrow Y_{S/X}$
	pO_2	$\mu_{\max} \rightarrow Y_{pO_2/X} \rightarrow k_La \rightarrow Y_{S/X} \rightarrow k_S$
Model 6	X	$\mu_{\max} \rightarrow Y_{S/X} \rightarrow k_S$
	S	$\mu_{\max} \rightarrow Y_{S/X} \rightarrow k_S$
	A	$\mu_{\max},\ \mu_{\max_A} \rightarrow Y_{A/X} \rightarrow k_S,\ k_A \rightarrow Y_{S/X}$
	Ph	$\mu_{\max} \rightarrow Y_{Ph/X} \rightarrow k_S \rightarrow Y_{S/X}$
	pO_2	$\mu_{\max} \rightarrow Y_{pO_2/X} \rightarrow k_La \rightarrow k_S,\ k_{pO_2} \rightarrow Y_{S/X}$
Model 8	X	$\mu_{\max} \rightarrow Y_{S/X} \rightarrow k_S$
	S	$\mu_{\max} \rightarrow Y_{S/X} \rightarrow k_S$
	A	$\mu_{\max} \rightarrow Y_{A/X} \rightarrow k_S \rightarrow Y_{S/X}$
	Ph	$\mu_{\max} \rightarrow Y_{Ph/X} \rightarrow k_S,\ k_{Ph} \rightarrow Y_{S/X}$
	pO_2	$\mu_{\max} \rightarrow Y_{pO_2/X} \rightarrow k_La \rightarrow Y_{S/X} \rightarrow k_S,\ k_{pO_2}$

(ii) $Y_{A/X}$ and k_A for Model 5 and 7;

(iii) $\mu_{\max_A}$, $Y_{A/X}$ and k_A for Model 6 and 10.

The system (0.1), (0.2), (0.3) and (0.6), estimated parameters ($\mu_{\max}$, $Y_{S/X}$ and k_S) and experimental data set for acetate dynamics are considered.

On the **third step**, for phytase variable the following local models parameters are identified:

(i) $Y_{Ph/X}$ for Models 1-4, 6, 7, 9 and 10;

(ii) $Y_{Ph/X}$ and k_{Ph} for Model 5 and 8;

The system (0.1), (0.2), (0.4) and (0.6), estimated parameters ($\mu_{\max}$, $Y_{S/X}$ and k_S) and experimental data set for phytase dynamics are considered.

On the **fourth step**, for dissolved oxygen variable the following local models parameters are identified:

(i) $Y_{pO_2/X}$ and k_La for Models 1-4;

(ii) $Y_{pO_2/X}$, k_La and k_{pO_2} for Models 5, 6, 8-10;

(iii) $\mu_{\max_{pO_2}}$, $Y_{pO_2/X}$, k_La and k_{pO_2} for Model 7.

Table 3: Initial conditions of the cultivation parameters

Cultivation	t_0	$X(t_0)$	$S(t_0)$	$A(t_0)$	$Ph(t_0)$	$pO_2(t_0)$
S_{sp} = 0.2 g/l	4.30 h	3.20 g/l	0.84 g/l	0.086 g/l	5.84 g/l	31.28 %
S_{sp} = 0.1 g/l	3.10 h	3.20 g/l	0.50 g/l	0.087 g/l	2.39 g/l	33.04 %

The system (0.1), (0.2), (0.5) and (0.6), estimated parameters ($\mu_{\max}$, $Y_{S/X}$ and k_S) and experimental data set for dissolved oxygen dynamics are considered.

With the proposed identification procedure the number of simultaneous estimated parameter is reduced. For example, for Model 10 instead of the estimation of the ten parameters in one step, the four groups within three, three, one and three parameters, respectively, are identified. Parameters reduction is a precondition for good performance assessment of the used optimization method and for obtaining adequate local models with higher degree of accuracy. The proposed identification procedure is tested for parameter estimation of local models describing recognized FS in the *E. coli BL21(DE3)pPhyt109* fed-batch cultivation process.

4. Local models parameter estimation of fed-batch cultivation process of *E. coli BL21(DE3)pPhyt109*

For modelling of *E. coli BL21(DE3)pPhyt109* cultivation experimental data sets of two different runs are used. The cultivation conditions are presented in details in [25, 28]. Each experimental data set includes data for the dynamics of biomass (X), substrate (S), acetate (A), phytase (Ph) and dissolved oxygen (pO_2) during the cultivation process. In the first set, used for local models parameter identification, the substrate concentration is kept at set point S_{sp} = 0.2 g/l. The second set of experimental data, where the substrate concentration is kept at set point S_{sp} = 0.1 g/l, is used for model verification. The initial parameters of the considered cultivation processes are presented in Table 3. For both cultivation processes substrate concentration in the feeding solution is 500 g/l and initial volume is 2.70 l [25, 28].

A discussion about recognized FS and the rules for recognition is presented in details in [22, 28]. For the first experimental data set three FS are recognized [28]:

FS VI: dissolved oxygen limitation state;

FS V: second acetate production state;

FS I: first acetate production state.

Corresponding local models, according to [22, 28] are: Model 8 for FS I;

Model 6 for FS IV and Model 10 for FS V. The local models parameters that have to be estimated are:

FS I: $p = \left[\mu^I_{\max}\ k^I_S\ k^I_{Ph}\ k^I_{pO_2}\ Y^I_{S/X}\ Y^I_{A/X}\ Y^I_{Ph/X}\ Y^I_{pO_2/X}\ k_La^I\right]$;

FS IV: $p = \left[\mu^{IV}_{\max}\ \mu^{IV}_{\max A}\ k^{IV}_S\ k^{IV}_A\ k^{IV}_{pO_2}\ Y^{IV}_{S/X}\ Y^{IV}_{A/X}\ Y^{IV}_{Ph/X}\ Y^{IV}_{pO_2/X}\ k_La^{IV}\right]$;

FS V: $p = \left[\mu^V_{\max}\ \mu^V_{\max A}\ k^V_S\ k^V_A\ k^V_{pO_2}\ Y^V_{S/X}\ Y^V_{A/X}\ Y^V_{Ph/X}\ Y^V_{pO_2/X}\ k_La^V\right]$.

Proposed by-stage identification procedure is applied. Due to similarity only the identification procedure of the local models parameters in the Model 10 is described.

On the first step the parameters $\mu_{\max}$, $Y_{S/X}$ and k_S are estimated. The system (0.1), (0.2) and (0.6), including the specific rates kinetics, proposed in Model 10 is considered. Experimental data set for dynamics of X and S are used. On the second step the local models parameters $\mu_{\max_A}$, $Y_{A/X}$ and k_A are identified. The system (0.1), (0.2), (0.3) and (0.6), including the specific rates kinetics for acetate production rate, accepted in Model 10 is considered. The experimental data set for acetate dynamics and the values of the parameters ($\mu_{\max}$, $Y_{S/X}$ and k_S), estimated on the first step, are used. On the third step the local models parameter $Y_{Ph/X}$ is identified. The system (0.1), (0.2), (0.4) and (0.6), including the specific rates kinetics for phytase production rate, proposed in Model 10 is considered. The experimental data set for phytase dynamics and the values of the parameters, estimated on the first step, are used. On the final step the local models parameters $Y_{pO_2/X}$, k_La and k_{pO_2} are identified. The system (0.1), (0.2), (0.5) and (0.6), already estimated parameters ($\mu_{\max}$, $Y_{S/X}$ and k_S) and experimental data set for dissolved oxygen dynamics are considered.

The parameter estimation problem was stated as the minimization of a distance measure J between experimental and model predicted values of the state variables (X, S, A, Ph and pO_2):

$$J = \sum_{i=1}^{n}\sum_{j=1}^{m}\left\{\left[\gamma_{\text{exp}}(i) - \gamma_{\text{mod}}(i)\right]_j\right\}^2 \to \min$$

where: J is the optimization criterion; γ_{exp}, γ_{mod} - experimental and model data vectors; n - number of measurements for each state variable; m - number of state variables.

Obtained results from the model parameters identification are presented in Table 4. As it could be seen, parameters values in the different FS are different. As it is well known, the parameters of the cultivation processes models, and particularly in the cultivation of *E. coli BL21(DE3)pPhyt109*, are time-varying. The use of global process models could not reflect this fact, while the functional state approach allows taking into account time-varying of parameters.

Table 4: Numerical values of the local model parameters

FS I		FS IV		FS V	
Parameter	Value	Parameter	Value	Parameter	Value
$\mu^I_{\max}$	0.52 1/h	$\mu^{IV}_{\max}$	0.58 1/h	$\mu^V_{\max}$	0.54 1/h
$\mu^I_{\max_A}$	-	$\mu^{IV}_{\max_A}$	0.10 1/h	$\mu^V_{\max_A}$	0.14 1/h
k^I_S	0.076 g/l	k^{IV}_S	0.006 g/l	k^V_S	0.04 g/l
k^I_A	0.10 g/l	k^{IV}_A	0.51 g/l	k^V_A	0.10 g/l
$k^I_{pO_2}$	0.10 %	$k^{IV}_{pO_2}$	0.006 %	$k^V_{pO_2}$	0.04 %
$Y^I_{S/X}$	0.16 g/g	$Y^{IV}_{S/X}$	0.45 g/g	$Y^V_{S/X}$	0.18 g/g
$Y^I_{A/X}$	0.56 g/g	$Y^{IV}_{A/X}$	0.51 g/g	$Y^V_{A/X}$	0.62 g/g
$Y^I_{Ph/X}$	0.23 g/g	$Y^{IV}_{Ph/X}$	0.54 g/g	$Y^V_{Ph/X}$	0.28 g/g
$Y^I_{pO_2/X}$	0.45 g/g	$Y^{IV}_{pO_2/X}$	0.49 g/g	$Y^V_{pO_2/X}$	0.42 g/g
k_La^I	178.01 1/h	k_La^{IV}	179.21 1/h	k_La^V	179.88 1/h

Thus, an adequate and more precise model is obtained.

Both the real cultivation trajectories and the simulated ones are presented in Fig. 5. The figure shows the dynamics of the biomass, glucose, acetate, phytase and dissolved oxygen concentrations for all recognized FS (FS I, FS IV and FS V). The initial values for the simulation in the new functional state are the last simulated values in the previous functional state so that the trajectories were continuous.

The obtained results clearly showed that the developed local models described the process dynamics with high degree of accuracy. The proposed local models structures for each specific rate, related to the corresponding FS, fit quite well the experimental data.

Model verification

The second independent data set of *E. coli BL21(DE3)pPhyt109* fed-batch cultivation with $S_{sp} = 0.1$ g/l was used for the model verification. Here two FS were recognized. In the beginning of the cultivation, from 3.1 h to 9.08 h cultivation time the FS I was identified, from 9.08 h to 11.12 h cultivation time - FS V. The developed local models for FS I and FS V were tested for a prediction of the corresponding FS behavior in the second data set. The simulation results from the model verification are presented in Fig. 6.

As it can be seen the results from the verification are good. The present mismatch between the experimental data used for verification and the developed model outputs is explained with the fact that the local models were developed

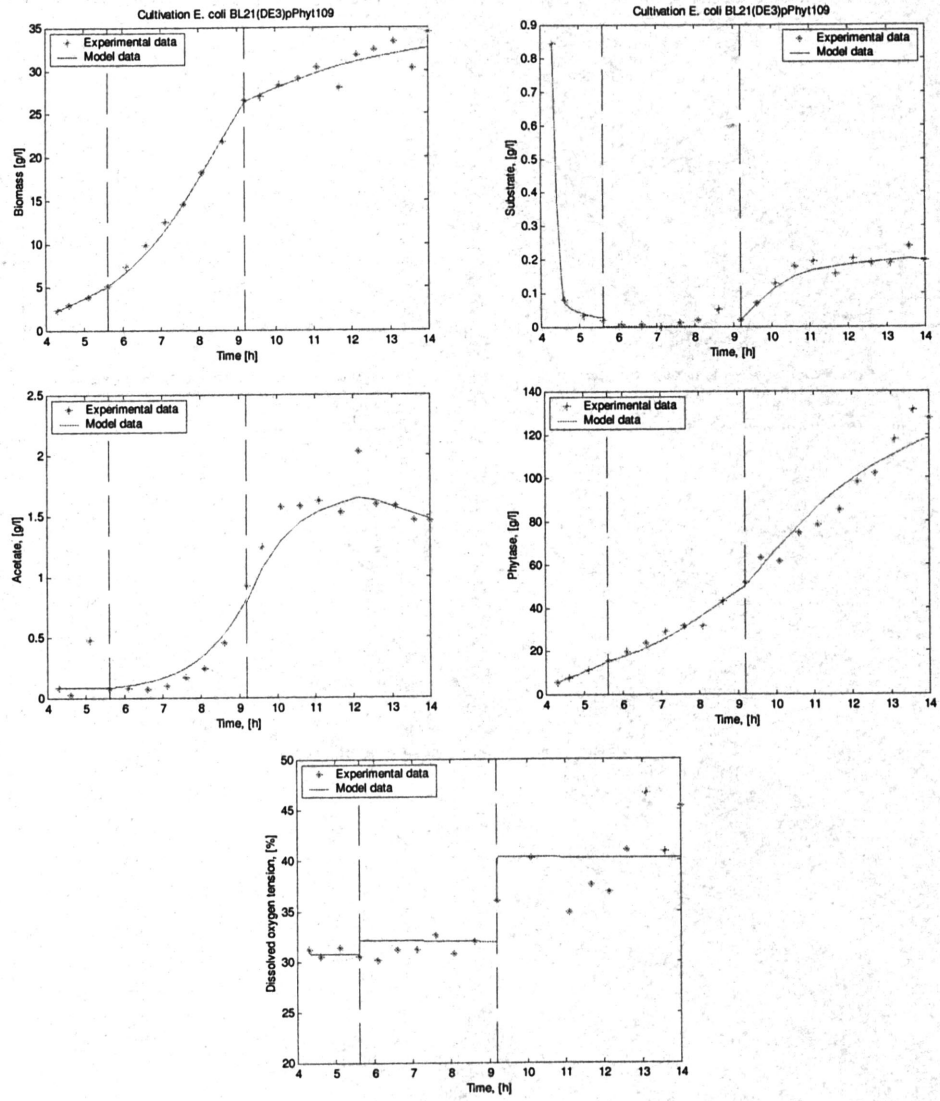

Figure 5: Simulation results and experimental data for all recognized FS

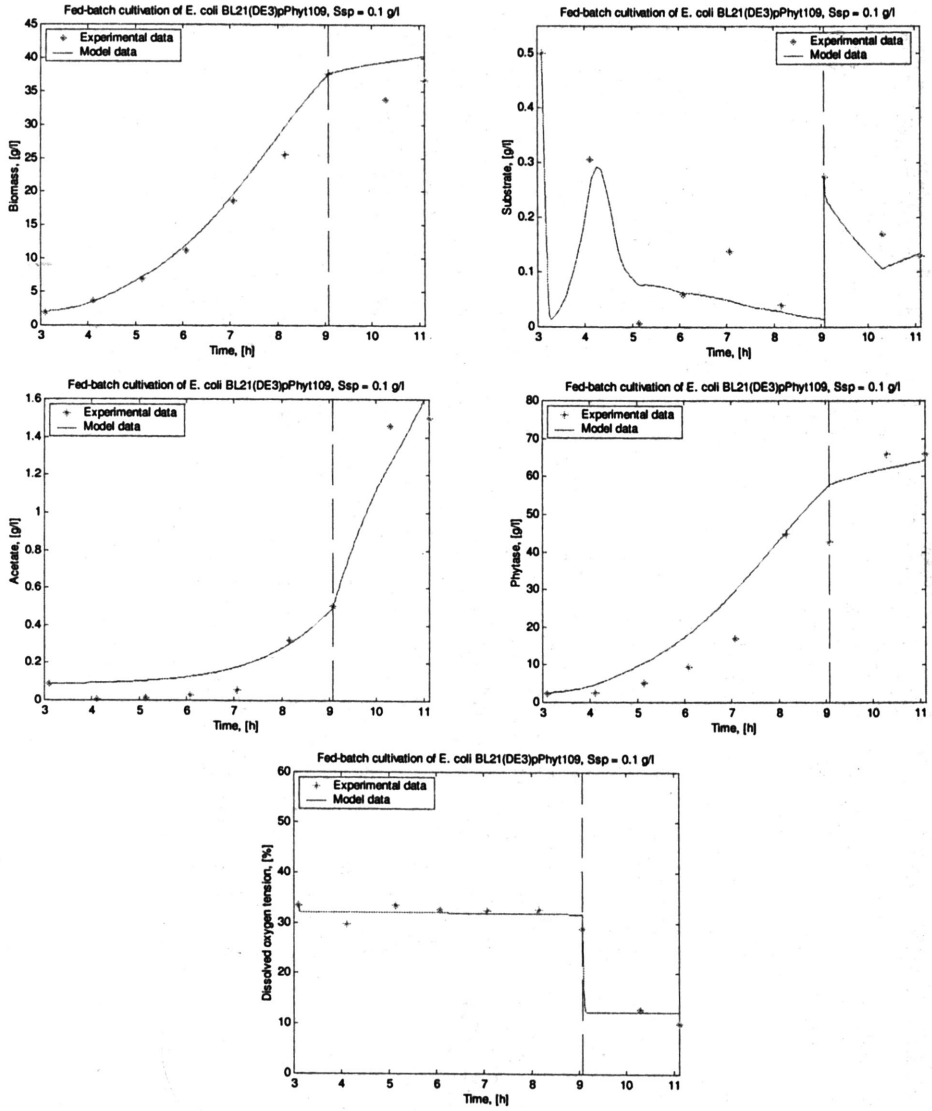

Figure 6: Simulation results from model verification for all recognized FS

based on data set from cultivation with $S_{sp} = 0.2$ g/l and the data for verification are from cultivation with $S_{sp} = 0.1$ g/l. On the other hand, failures could be consequence of inaccurate data (noise, inexact analysis, wrong measurements etc.). Mentioned above is a preposition that the model predictions are not with a high accuracy. Nevertheless, the verification results are considered as satisfactory and it could be concluded that the verification of the developed local models was successfully fulfilled.

5. Conclusions

In bioprocess modeling, mathematical structures and their parameters, used to describe microorganisms' behaviour, constitute the key problem of bioprocess modeling, in particular, the modelling of parameter estimation. In this paper, the sensitivity analysis of non-linear mathematical model of *E. coli* fed-batch cultivation process is studied. Various structures of local models describing specific rate functions, according to the concept of functional state modelling, are examined. Sensitivity analysis of the parameters with respect to the state variables is presented. As a result a by-stage identification procedure is proposed. The identification procedure is tested for model identification of an *E. coli BL21(DE3)pPhyt109* fed-batch cultivation process. The proposed identification procedure leads to easy and accurate estimation of local models parameters. The procedure effectiveness is confirmed with a model verification. The results presented here demonstrate the importance of applying the appropriate sensitivity analysis according to the dynamics of the cultivation process.

References

[1] A. Castan, S.-O. Enfors. Characteristics of a DO-controlled fed-batch culture of *Escherichia coli*, *Biopr. Eng.*, **22**, 2000, 509-515.

[2] A. Holmberg. On the practical identifiability of microbial growth models incorporating Michaelis-Menten type nonlinearities, *Math. Biosci.*, 62, 1982, 23-43.

[3] A. N. Venkat, P. Vijaysai, R. D. Gudi. Identification of complex nonlinear processes based on fuzzy decomposition of the steady state space, *J. of Proc. Cont.*, **13**, 2003, 473-488.

[4] A. Saltelli, M. Ratto, S. Tarantola, F. Campolongo. Sensitivity analysis for chemical models, *Chem. Rev.*, **105**, No 7, 2005, 2811-2828.

[5] B. Gorry, A. Ireland, P. King. Sensitivity analysis of real-time aystems, *Engineering and Technology*, **37**, 2008, 12-19.

[6] B. Ingalls, H. M. Sauro. Sensitivity analysis of stoichiometric networks: an extension of metabolic control analysis to non-steady state trajectories, *Journal of Theoretical Biology*, **222**, 2003, 23-36.

[7] B. Petersen, K. Gernaey, M. Devisscher, D. Dochain, P. Vanrolleghem. A simplified method to assess structurally identifiable parameters in Monod-based activated sludge models, *Wat. Res.*, **37**, 2003, 2893-2904.

[8] B. Xu, M. Jahic, G. Blomsten, S.-O. Enfors. Glucose overflow metabolism and mixed-acid fermentation in aerobic large-scale fed-batch processes with *Escherichia coli*, *Appl. Microbiol. Biotechnol.*, **51**, 1999, 564-571.

[9] C. M. Silva, D. F. S. Souza, E. C. Biscaia. Multiobjective parameter estimation problems: an improved strategy, *Inverse Problems in Engineering*, **12**, No 3, 2004, 297-316.

[10] C. S. Woodward, K. E. Grant, R. Maxwell. Applications of sensitivity analysis to uncertainty quantification in variably saturated flow, *XIV Int. Conf. on Comp. Methods in Water Resources*, Amsterdam, 2002, 1-8.

[11] D. Dochain, P. A. Vanrolleghem. *Dynamical modelling and estimation in wastewater treatment processes*, IWA Publishing, UK, 2001.

[12] D. O'Beirne, G. Hamer. The utilization of glucose/acetate mixtures by *Escherichia coli W3110* under aerobic growth conditions, *Bioproc. Eng.*, **23**, 2000, 375-380.

[13] G. Bastin, D. Dochain. *On-line estimation and adaptive control of bioreactors*, Els. Sc. Publ., 1991.

[14] I. Simeonov. Methodology for parameters estimation of nonlinear models of anaerobic wastewater treatment processes in stirred tank bioreactors, *Symp. Reprints Watermatex 2000*, Grent, Belgium, 2002, 8.40-8.47.

[15] J. M. King, N. J. Titchener-Hooker, Y. Zhou. Ranking bioprocess variables using global sensitivity analysis: a case study in centrifugation, *Bioproc. Biosyst. Eng.*, **30**, No 2, 2007, 123-134.

[16] M. A. Pai, T. B. Nguyen. Trajectory sensitivity theory in non linear dynamical systems: some power system applications, Stability and Control of Dynamical Systems with Applications, D. Liu and P. J. Antsaklis (Eds), *Control Engineering Series*, Birkhauser Boston, 2003, 1-21.

[17] M. Barnsley. Environmental modelling. Parameter estimation, model validation, sensitivity analysis and model inversion, *GEG209 Environmental Modelling, Sensitivity Analysis*, 2001, 1-9.

[18] M. Feng, J. Glassey. Physiological state-specific models in estimation of recombinant *Escherichia coli* fermentation performance, *Biotech. Bioeng.*, **69**, No 5, 2000, 495-503.

[19] N. Noykova, M. Gyllenberg. Sensitivity analysis and parameter estimation in a model of anaerobic waste water treatment processes with

substrate inhibition, *Biopr. Eng.*, **23**, 2000, 343-349.

[20] N. Noykova, T. Muler, M. Gyllenberg, J. Timmer. Quantitative analyses of anaerobic waste water treatment process: identifiability and parameter estimation, *Biotech. Bioeng.*, **78**, No 1, 2002, 89-103.

[21] O. Roeva. *Functional state modelling of Escherichia coli cultivation applying genetic algorithms*, PhD Tessis, TU-Sofia, 2007. (in Bulgarian)

[22] O. Roeva, T. Pencheva, St. Tzonkov, M. Arndt, B. Hitzmann, S. Kleist, G. Miksch, K. Friehs, E. Flaschel. Multiple model approach to modelling of *Escherichia coli* fed-batch cultivation extracellular production of a bacterial phytase, *El. J. Biotech.*, **10**, No 4, 2007, 592-603.

[23] P. R. Patnaik. Transient sensitivity analysis of a cybernetic model of microbial growth on two substrates, *Biopr. Eng.*, **21**, 1999, 135-140.

[24] R. Murray-Smith, T. A. Johansen. *Multiple model approaches to modelling and control*, Taylor and Francis, 1997.

[25] S. Kleist, G. Miksch, B. Hitzmann, M. Arndt, K. K. Friehs, E. Flaschel. Optimization of the extracellular production of the bacterial phytase with *Escherichia coli* by using different fed-batch fermentation strategies, *Appl. Microbiol. Biotechnol.*, **61**, 2003, 456-462.

[26] S. T. Yordanova, N. Noykova. An investigation of the model of aerobic waste water treatment processes, *Biopr. Eng.*, **15**, No 4, 1996, 201-203.

[27] T. G. Muler, N. Noykova, M. Gyllenberg, J. Timmer. Parameter identification in dynamical models of anaerobic waste water treatment, *Math. Biosci.*, **177**, 2002, 147-160.

[28] T. Pencheva, O. Roeva, I. Hristozov. *Functional state approach to fermentation processes Modelling*, Prof. Marin Drinov Academic Publishing House, Sofia, 2006.

[29] X.-Ch. Zhang, A. Visala, A. Halme, P. Linko. Functional state modelling approach for bioprocesses: local models for aerobic yeast growth processes, *J. of Proc. Contr.*, **4**, No 3, 1994, 127-134.

Institute of Biophysics and Biomedical Engineering
Bulgarian Academy of Sciences
Sofia 1113, BULGARIA
E-MAIL: olympia@clbme.ba.bg

Received 22.02.2011